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Assessment of soil fungal diversity at different depth in Haibat Sultan Mountain by PCR-cloning-sequencing methods

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A B S T R A C T

Understanding the fungal community structure of soil is important for optimizing their role as decomposers in the soil food web. In order to explore the fungal composition and diversity in Haibat_Sultan Mountain soil, PCR used based cloning and sequencing of Internal Transcribed Spacer (ITS) of genome. Soil samples were collected from different depth points (1, 5 to 10 cm depth) and fungal universal primers targeting ITS2 region of the fungal genome were used to assess their diversity in soil samples. More than 1400 fungal clones were sequenced and led to detect total of 89 OTUs in all samples. The majority of the Operational Taxonomic Units (OTUs) belong to the Ascomycota (67.4%), the second most constituents of soil samples were Basidiomycota with (28.1%), and few members of Zygomycota were detected. Concluded that fungal richness and diversity were abundant at the depth of 1 cm and reached their peak at the depth of 5 cm, while at the depth of 10 cm, the fungal communities decreased. The highest Chao1 and Shannon value were obtained at the soil depth of 5 cm followed by soil depth 1cm, whereas the lowest value was detected in soil depth 10 cm. Results highlight the variations of fungal diversity at different depth points. Thus, further studies are needed to expand knowledge about fungi communities in soil through using high-throughput sequencing to improve detection fungi in soil.

1. INTRODUCTION

Fungi constitutes one of the most important and highly diverse functional groups of microbes in the soil (Bridge and Spooner, 2001). A variety of microbial communities inhabited in soil, these diverse groups play a crucial role in decomposition, nutrient cycle and flow of energy (Wardle and Giller, 1996). Particularly the role of Fungi in soil is very important in nutrient cycling, facilitating plant nutrient uptake, growth of plant and preventing disease (Christensen, 1989, Thorn, 1997).

Over the last two decades fungal diversity studies in soil and organic layers have received greater attentions (Parungao et al., 2002). This is primarily because of industrial and biotechnologi- cal roles of fungi (Lodge, 1997). Christensen 1989 (Christensen, 1989) described twenty functions of fungi including degradation role in soil. Besides of that, fungi capable of breaking down complex substances, such as lignin, cellulose, chitin, keratin and play leading role in ecological processes required for ecosystem maintaining (Subramanian, 1982, Rossman, 1994). Finally, fungi are important in organic material decomposition and ecosystem functioni-ng (Heilmann-Clausen and Christensen, 2003). Despite ecological functions of fungi in soil ecosystem, little is known of fungal functioning and community diversity in soil in comparison to soil bacterial communities, and it is not rare when studying soil microbial ecology, all bacteria received the attention of microbiologists in published articles (Hattori et al., 1997, Ogram, 2000, Kent and Triplett, 2002).

Mycologists rely on culture-based approaches to investigate diversity of fungi in soil, these methods limitations have been highlighted numerous times (Bridge and Spooner, 2001). The inability to proper identify and characterize fungi from morphological techniques led to the development of molecular-based approaches for identification of fungi (Raja et al, 2017). Molecular techniques are now standard methods in many studies dealing with phylogeny, classification, and identification. Polymerase chain reaction (PCR) greatly accelerated the molecular approaches in studying environmental material, particularly soil (Bridge and Spooner, 2001). The fungal ITS region comprise of ITS1 and ITS2 variable regions which are located between the 18S and the 5.8S subunits and between the 5.8S and the 28S subunits respectively. They have been used widely in taxonomic identification of fungal populations (White et al., 1990). In this study, we used molecular approaches such as PCR based cloning and sequencing of internal transcribed spacer (ITS2) of fungi to investigate the fungal diversity of Haibat Sultan mountain ecosystem from soil samples collected at different depths.

2. Materials and methods

2.1. Study site

Haibat Sultan Mountain has elevation of about 860 m above sea level (ASL) (Shaheen and Salam, 2011). The series of the mountain located to the east of Koya town, which is about 70 kilometers east of Erbil, Kurdistan region-Iraq (Fig S1).

2.2. Soil Samples collection and total genomic extraction

The soil samples were collected at different depths of 1, 5 and 10 cm from five different locations pots (Table S1) in May 2016. Soil samples were sieved to remove plant residue and particles larger than 1 cm, then an aliquot (5 g) of each sample were suspended in 10 ml of buffer solution and incubated at 60°C for 2 hours. The total DNA was extracted from soil suspension using the PowerSoilTM DNA Isolation Kit (https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf), following manufacturer's instructi-ons.

2.3. Fungal genomic amplification

The fungal ITS2 region were amplified using universal fungal primer pairs ITS 3 (GCATCGATGAAGAACGCAGC) (White et al., 1990) and

ITS-4R (TCCTCCGCTTATTGATATGC) (Gardes and Bruns, 1993). The amplification conducted in the 50 µL PCR reaction mixture contained 5 µL of dNTPs (2 mM of each nucleo - tide), 5 µL of DNA polymerase buffer (Qiagen) 2 µl of MgCl2 (25 mM), 0.25 µL HotStarTaq DNA polymerase (1.25 U) (Qiagen), 1 µL of each primer (Eurogentec, Liège, Belgium) and 5 µl of DNA. The PCR was performed with an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 95°C for 45 seconds, an annealing temperature 50°C for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The PCR products (190-300 bp) were analyzed using agarose gel electrophoresis (1.5%) and visualized with ethidium bromide staining. Positive PCR products were subsequently purified using the NucleoFast® 96 PCR Kit (MACHEREY-NAGEL, Hoerdt, France) according to the manufacturer's instructions.

2.4. Cloning and sequencing

PCR product was cloned using the pGEM® -T Easy Vector System Kit (Promega, Lyon, France) as described previously (Hamad et al., 2012). M13 forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-AGGAAACAGCTATGAC-3') primers were used at annealing temperature of 58°C. to confirm presence of the insert.PCR amplifications of the plasmid vector were performed as described above. Purified PCR products were sequenced in both directions using the Big Dye® Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems, Villebon-sur-Yvette, France) with the M13 forward and M13 reverse primers. These products were run on an ABI PRISM 3130 automated sequencer (Applied Biosystems). The obtained sequences were compared with a BLAST database of pre-assigned sequences in GenBank (available at the National Center for Biotechnology Information website: http://www.ncbi.nlm.nih.gov/).

2.5. Phylogenetic analysis

Fungal ITS2 sequences in current study and the pre-assigned sequences in GenBank were aligned by CLUSTAL-X, version 2.1 and imported into MEGA 6.0.6 (Tamura et al., 2013) to generate Neighbor-joining (NJ) trees. The program parameters used were p-distance with pairwise deletion. The quality of the branching patterns for NJ was assessed by bootstrap resampling of the data sets with 1,000 replications.

2.6. Statistical Analysis

Chao1 estimator and Shannon diversity were calculated by using the PAST (Version 3.15) software package (https://folk.uio.no/ohammer/past/). Statistical analyses were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, California, USA).

2.7. Nucleotide sequence accession numbers

Fungal ITS2 sequences more than 200 bp were deposited in the GenBank database with the accession numbers KY615747 to KY615766.

Table S1: Locations and GPS coordinates of soil samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain

	Soil Sample location				
Depth	Location 1	Location 2	Location 3	Location 4	Location 5
1 cm 5 cm 10 cm	Loc1(N:36°05241\E:044 °39324\634m W:158	Loc2(N:36°05223\E:0 4 4°39291\634 E:1	Loc3(N:36°06269\E:044 °39198\82 1m N:1	Loc4(N:36°06259\E:044 °39186\81 1m N:3	Loc5(N:36°06248\E:0 4 4°9189\812m SE:2

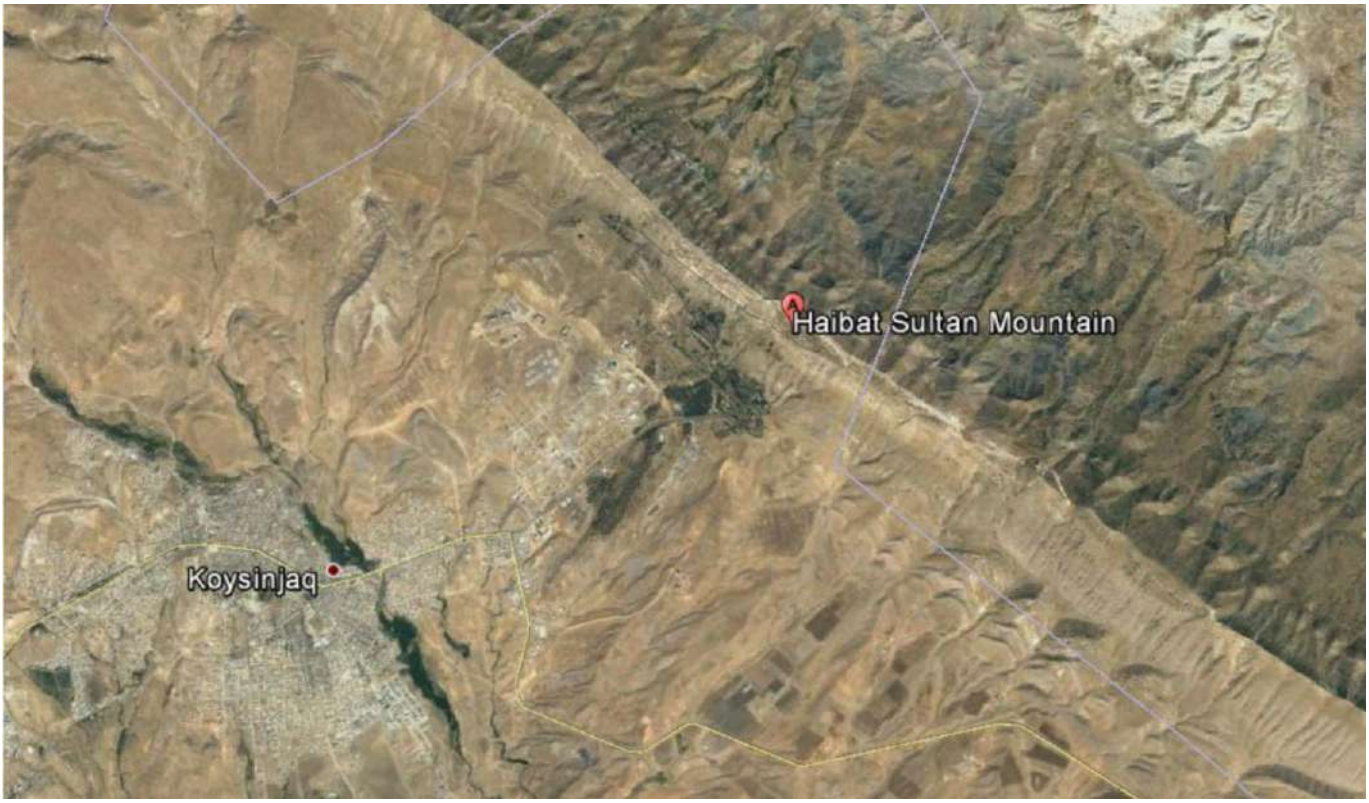

Figure S1: Satellite view of Haibat Sultan Mountain

3. Results and Discussion

3.1. Results

3.1.1. Composition of soil fungal communities

Cloning libraries were generated from DNA which amplified by fungal universal primers (Table 1). A total of 1440 clones were subjected to sequence analysis. After excluding plant DNA sequences that presented in soil, a total of 1418 fungal clone sequences were obtained (Table 1) and led to detect 89 OTUs in all soil samples (Fig1 and 2).

The Ascomycota represented 67.4% of all sequences (Table 1 and Table S2). The orders Eurotiales, Pleosporales, Chaetothyriales, Saccharomycetales and Capnodiales were the most common order in all ITS2 Cloning libraries (Table S2), while orders (Helotiales, Hypocreales and Botryosphaeriales) were less than others in ITS2 Cloning libraries (Table S2). Most of the EurotialesOTUs belong to *Aspergillus* and *Penicillium*, *Byssochlamys*, *Elaphomyces* and *Sagenomella* (Table 1 and Fig1).

Table 1: List of fungal OTUs detected in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain.

OTU	Taxa	Order	Assigned Taxa	D 1cm Loc1	D 1cm Loc2	D 1cm Loc3	D 1cm Loc4	D 1cm Loc5	D 5cm Loc1	D 5cm Loc2	D 5cm Loc3	D 5cm Loc4	D 5cm Loc5	D 10cm loc1	D 10cm loc2	D 10cm loc3	D 10cm loc4	D 10cm loc5
OTU_85	a	Botryosphaeriales	*Botryosphaeriales sp.*	0	0	0	4	0	0	3	0	0	0	0	0	0	0	0
OTU_78	a	Botryosphaeriales	*Neofusicoccumbatangarum*	0	1	9	0	23	0	5	0	11	0	2	0	0	0	0
OTU_46	a	Capnodiales	*Capnobotryella sp.*	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
OTU_55	a	Capnodiales	*Cercosporachrysanthemi*	0	0	0	0	0	0	0	0	0	0	0	0	0	12	1
OTU_21	a	Capnodiales	*Cladosporiumhalotolerans*	3	0	0	0	1	1	1	1	1	2	0	0	0	0	1
OTU_74	a	Capnodiales	*Mycosphaerellaceae sp.*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2

OTU_59	a	Capnodiales	*Teratosphaeriapseudocryptica*	1	0	0	0	0	0	0	0	0	0	4	0	0	0	0
OTU_60	a	Capnodiales	Uncultured *Gibberella sp.*	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
OTU_23	a	Dothideales	*Aureobasidiummelanogenum*	0	1	3	0	0	1	1	0	0	0	0	1	0	0	7
OTU_7	a	Dothideales	*Pseudoseptoriaobscura*	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1	a	Pleosporales	*Alternariaalternata*	0	1	2	2	2	0	4	0	0	1	2	8	4	0	21
OTU_26	a	Pleosporales	*Alternariainfectoria*	0	1	5	0	0	2	3	4	3	0	0	10	1	3	0
OTU_25	a	Pleosporales	*Alternariasolani*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_65	a	Pleosporales	*Epicoccum sp.*	4	0	0	5	0	2	4	2	0	3	8	1	0	0	0
OTU_41	a	Pleosporales	*Helminthosporiumsolani*	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
OTU_70	a	Pleosporales	*Phoma sp.*	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
OTU_54	a	Pleosporales	*Stagonospora sp.*	1	0	2	0	0	0	2	2	1	0	0	2	0	0	0
OTU_81	a	Pleosporales	*Stagonosporopsiscucurbitacearum*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
OTU_44	a	Chaetothyriales	*Exophialaeucalyptorum*	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
OTU_86	a	Chaetothyriales	*Chaetothyriales sp.*	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_42	a	Chaetothyriales	*Coniosporium sp.*	0	1	0	0	0	1	3	6	0	6	4	2	0	0	0
OTU_43	a	Chaetothyriales	*Coniosporium sp.*	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
OTU_53	a	Chaetothyriales	*Coniosporium sp.*	0	0	2	0	0	0	1	2	0	0	0	0	0	0	0
OTU_32	a	Chaetothyriales	*Cyphellophorapauciseptata*	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0
OTU_38	a	Eurotiales	*Aspergillusconicus*	0	0	2	0	0	0	2	2	0	1	0	0	0	0	0
OTU_8	a	Eurotiales	*Aspergillusniger*	0	14	28	2	9	0	1	6	0	4	0	33	5	0	0
OTU_66	a	Eurotiales	*Aspergillus sp.*	3	3	2	0	4	3	1	3	5	2	9	1	0	1	3
OTU_48	a	Eurotiales	*Aspergillusvitricola*	0	2	0	0	0	8	1	9	0	2	0	0	11	7	0
OTU_49	a	Eurotiales	*Byssochlamysnivea*	0	0	0	0	0	0	1	0	0	3	0	0	1	0	2
OTU_33	a	Eurotiales	*Elaphomyceslabyrinthinus*	0	0	0	0	1	2	0	0	0	0	0	0	0	3	5
OTU_	a	Eurotiales	*Penicilliumpusillum*	0	2	2	8	6	2	1	0	0	3	2	0	0	0	0

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OTU_9	a	Eurotiales	*Penicilliumsalmoniflumine*	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
OTU_52	a	Eurotiales	*Sagenomellagriseoviridis*	0	11	13	6	0	5	2	8	2	3	3	0	1	8	3
OTU_11	a	Onygenales	*Ascosphaeraapis*	0	0	0	0	2	0	2	3	0	0	0	0	0	0	1
OTU_10	a	Onygenales	*Ascosphaeranaganensis*	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
OTU_35	a	Ostropales	*Trullulamelanochlora*	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_3	a	Chaetothyriales	*Knufiapetricola*	3	0	0	1	4	2	4	0	1	6	0	10	0	2	2
OTU_31	a	Erysiphales	*Blumeriagraminis*	2	0	2	4	0	2	4	0	2	1	1	0	1	0	0
OTU_30	a	Erysiphales	*Golovinomyces sp.*	5	0	0	5	2	0	0	0	0	0	0	0	0	0	0
OTU_39	a	Helotiales	*Helotiales sp.*	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
OTU_40	a	Helotiales	*Naevalaminutissima*	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
OTU_80	a	Helotiales	*Phialocephalafluminis*	1	5	2	5	1	1	1	0	2	6	3	0	0	19	0
OTU_14	a	Helotiales	*Sclerotiniasclerotiorum*	1	0	0	0	0	0	0	0	3	1	1	0	0	0	0
OTU_6	a	Saccharomycetales	*Debaryomyceshansenii*	1	0	0	0	0	1	3	0	0	0	0	0	0	0	0
OTU_88	a	Saccharomycetales	*Debaryomyces sp.*	0	20	2	0	1	6	7	6	18	5	3	0	51	30	9
OTU_28	a	Saccharomycetales	*Hanseniasporaguilliermondii*	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
OTU_34	a	Saccharomycetales	*Lodderomyceselongisporus*	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0
OTU_29	a	Saccharomycetales	*Saccharomycetales sp.*	4	0	0	0	0	0	0	0	1	0	2	0	0	1	0
OTU_4	a	Saccharomycetales	Unassigned S*accharomyces sp.*	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_5	a	Saccharomycetales	Undesigned*Fungi sp.*	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
OTU_12	a	Diaporthales	*Phomopsis sp.*	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
OTU_89	a	Glomerellales	*Acremonium sp.*	0	0	0	0	0	0	2	0	0	0	7	0	0	0	0
OTU_37	a	Glomerellales	*Plectosphaerellacucumerina*	5	1	1	0	1	0	1	0	0	0	0	0	0	0	0
OTU_84	a	Hypocreales	*Beauveriabassiana*	1	4	0	5	0	3	1	1	16	0	1	5	0	0	0
OTU_22	a	Hypocreales	*Fusariumequiseti*	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0

OTU_57	a	Hypocreales	*Gliocephalotrichumcylindrosporum*	4	0	4	0	0	2	2	0	0	0	0	1	0	0	0
OTU_13	a	Hypocreales	*Hypocreales sp.*	0	0	0	0	2	0	0	2	0	0	0	0	0	0	2
OTU_77	a	Microascales	*Doratomyces sp.*	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_24	a	Microascales	*Wardomycesdimerus*	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_63	a	Xylariales	*Eutypellaquaternata*	0	0	0	8	2	0	0	0	0	2	0	0	0	0	4
OTU_47	b	Agaricales	*Hypholomafasciculare*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_68	b	Cantharellales	*Sistotremabrinkmannii*	0	0	0	0	3	0	0	0	0	2	0	0	0	0	2
OTU_45	b	Corticiales	*Kneiffiella sp.*	0	1	0	0	2	0	2	0	0	0	0	0	0	0	0
OTU_76	b	Corticiales	*Lyomyces sp.*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_18	b	Corticiales	*Peniophorellapraetermissa*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_58	b	Corticiales	*Phlebialivida*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
OTU_19	b	Corticiales	*Phlebiaradiata*	0	0	2	0	2	0	0	0	1	0	3	0	0	0	0
OTU_61	b	Corticiales	*Radulomycesconfluens*	0	18	0	0	0	4	3	3	0	6	7	0	11	0	0
OTU_62	b	Corticiales	Uncultured *Sistotrema sp.*	5	1	0	3	0	0	2	3	1	1	3	0	0	0	2
OTU_56	b	Hymenochaetales	*Hyphodontiacrustosa*	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_17	b	Polyporales	*Coriolopsisgallica*	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0
OTU_16	b	Polyporales	*Phanerochaetechrysosporium*	0	0	0	0	0	5	1	0	3	1	0	0	0	0	0
OTU_79	b	Russulales	*Heterobasidionirregulare*	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0
OTU_36	b	Russulales	*Peniophoracinerea*	6	1	0	0	6	2	0	0	5	3	6	0	0	0	0
OTU_27	b	Cystobasidiales	*Cystobasidiumlaryngis*	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
OTU_72	b	Erythrobasidiales	*Erythrobasidium sp.*	0	0	0	0	3	0	1	0	0	0	5	0	0	0	0
OTU_73	b		Unassigned *Cystobasidiomycetes sp.*	7	3	2	6	0	4	0	0	0	0	6	0	2	0	3
OTU_20	b	Exobasidiales	*Exobasidiumotanianum*	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0
OTU_71	b	Kriegeriales	*Phenoliferiapsychrophila*	5	0	0	4	7	9	3	6	5	3	1	2	0	0	8

OTU_51	b	Sporidiobolales	*Rhodotorulamucilaginosa*	6	2	7	3	4	0	1	6	3	5	0	5	2	1	8
OTU_87	b	Sporidiobolales	*Sporidiobolales sp.*	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
OTU_50	b	Cystofilobasidiales	*Itersoniliaperplexans*	2	0	0	0	0	4	0	0	0	0	0	1	0	0	0
OTU_69	b	Filobasidiales	*Filobasidiumwieringae*	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
OTU_15	b	Tremellales	*Tremellales sp.*	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
OTU_67	b	Wallemiales	*Wallemiamellicola*	2	0	0	0	0	0	0	0	6	0	7	0	0	0	0
OTU_2	z	Mucorales	*Mucorvelutinosus*	0	0	0	0	1	1	2	3	2	4	0	2	0	0	7
OTU_82	u	-	Unassigned *Fungi* sp.	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
OTU_64	u	-	Unassigned *Pezizomycotina*	8	0	2	1	0	3	0	0	0	1	4	2	0	9	0
OTU_75	u	-	Unassigned *Pezizomycotina sp.*	3	0	0	0	0	1	0	0	0	3	0	0	0	0	0

OTU= operational taxonomic unit, D= Depth, Loc= Location, a= Ascomycota, b= Basidiomycota, z= zygomycota, u= Unassigned

Table S2: List of fungal orders that detected in all ITS Cloning libraries.

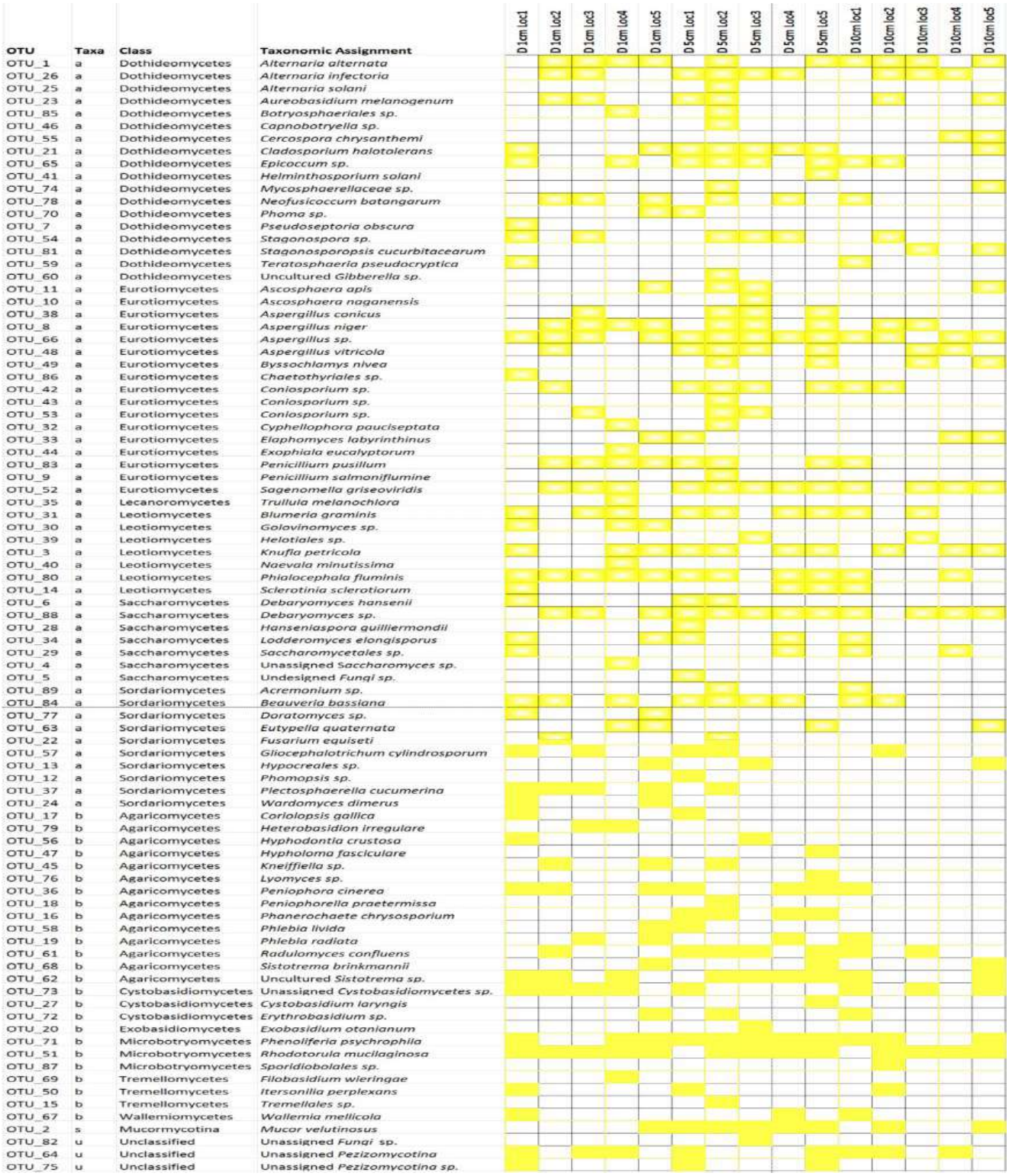
Taxa	**Order**	OTUs	Total OTUs	%
a	Eurotiales	9	60	15.0
a	Pleosporales	8	60	13.3
a	Chaetothyriales	7	60	11.7
a	Saccharomycetales	7	60	11.7
a	Capnodiales	6	60	10.0
a	Helotiales	4	60	6.7
a	Hypocreales	4	60	6.7
a	Botryosphaeriales	2	60	3.3
a	Dothideales	2	60	3.3
a	Erysiphales	2	60	3.3
a	Glomerellales	2	60	3.3
a	Microascales	2	60	3.3
a	Onygenales	2	60	3.3
a	Diaporthales	1	60	1.7

a	Ostropales	1	60	1.7
a	Xylariales	1	60	1.7
b	Corticiales	7	25	28
b	Polyporales	2	25	8
b	Russulales	2	25	8
b	Sporidiobolales	2	25	8
b	Agaricales	1	25	4
b	Cantharellales	1	25	4
b	Cystobasidiales	1	25	4
b	Cystofilobasidiales	1	25	4
b	Erythrobasidiales	1	25	4
b	Exobasidiales	1	25	4
b	Filobasidiales	1	25	4
b	Hymenochaetales	1	25	4
b	Kriegeriales	1	25	4
b	Tremellales	1	25	4
b	Wallemiales	1	25	4
b	Unassigned order	1	25	4

a= Ascomycota

b= Basidiomycota were

OTU= operational taxonomic unit



OTU	Taxa	Class	Taxonomic Assignment
OTU_1	a	Dothideomycetes	*Alternaria alternata*
OTU_26	a	Dothideomycetes	*Alternaria infectoria*
OTU_25	a	Dothideomycetes	*Alternaria solani*
OTU_23	a	Dothideomycetes	*Aureobasidium melanogenum*
OTU_85	a	Dothideomycetes	*Botryosphaeriales sp.*
OTU_46	a	Dothideomycetes	*Capnobotryella sp.*
OTU_55	a	Dothideomycetes	*Cercospora chrysanthemi*
OTU_21	a	Dothideomycetes	*Cladosporium halotolerans*
OTU_65	a	Dothideomycetes	*Epicoccum sp.*
OTU_41	a	Dothideomycetes	*Helminthosporium solani*
OTU_74	a	Dothideomycetes	*Mycosphaerellaceae sp.*
OTU_78	a	Dothideomycetes	*Neofusicoccum batangarum*
OTU_70	a	Dothideomycetes	*Phoma sp.*
OTU_7	a	Dothideomycetes	*Pseudoseptoria obscura*
OTU_54	a	Dothideomycetes	*Stagonospora sp.*
OTU_81	a	Dothideomycetes	*Stagonosporopsis cucurbitacearum*
OTU_59	a	Dothideomycetes	*Teratosphaeria pseudocryptica*
OTU_60	a	Dothideomycetes	Uncultured *Gibberella sp.*
OTU_11	a	Eurotiomycetes	*Ascosphaera apis*
OTU_10	a	Eurotiomycetes	*Ascosphaera naganensis*
OTU_38	a	Eurotiomycetes	*Aspergillus conicus*
OTU_8	a	Eurotiomycetes	*Aspergillus niger*
OTU_66	a	Eurotiomycetes	*Aspergillus sp.*
OTU_48	a	Eurotiomycetes	*Aspergillus vitricola*
OTU_49	a	Eurotiomycetes	*Byssochlamys nivea*
OTU_86	a	Eurotiomycetes	*Chaetothyriales sp.*
OTU_42	a	Eurotiomycetes	*Coniosporium sp.*
OTU_43	a	Eurotiomycetes	*Coniosporium sp.*
OTU_53	a	Eurotiomycetes	*Coniosporium sp.*
OTU_32	a	Eurotiomycetes	*Cyphellophora pauciseptata*
OTU_33	a	Eurotiomycetes	*Elaphomyces labyrinthinus*
OTU_44	a	Eurotiomycetes	*Exophiala eucalyptorum*
OTU_83	a	Eurotiomycetes	*Penicillium pusillum*
OTU_9	a	Eurotiomycetes	*Penicillium salmoniflumine*
OTU_52	a	Eurotiomycetes	*Sagenomella griseoviridis*
OTU_35	a	Lecanoromycetes	*Trullula melanochlora*
OTU_31	a	Leotiomycetes	*Blumeria graminis*
OTU_30	a	Leotiomycetes	*Golovinomyces sp.*
OTU_39	a	Leotiomycetes	*Helotiales sp.*
OTU_3	a	Leotiomycetes	*Knufia petricola*
OTU_40	a	Leotiomycetes	*Naevala minutissima*
OTU_80	a	Leotiomycetes	*Phialocephala fluminis*
OTU_14	a	Leotiomycetes	*Sclerotinia sclerotiorum*
OTU_6	a	Saccharomycetes	*Debaryomyces hansenii*
OTU_88	a	Saccharomycetes	*Debaryomyces sp.*
OTU_28	a	Saccharomycetes	*Hanseniaspora guilliermondii*
OTU_34	a	Saccharomycetes	*Lodderomyces elongisporus*
OTU_29	a	Saccharomycetes	*Saccharomycetales sp.*
OTU_4	a	Saccharomycetes	Unassigned *Saccharomyces sp.*
OTU_5	a	Saccharomycetes	Undesigned *Fungi sp.*
OTU_89	a	Sordariomycetes	*Acremonium sp.*
OTU_84	a	Sordariomycetes	*Beauveria bassiana*
OTU_77	a	Sordariomycetes	*Doratomyces sp.*
OTU_63	a	Sordariomycetes	*Eutypella quaternata*
OTU_22	a	Sordariomycetes	*Fusarium equiseti*
OTU_57	a	Sordariomycetes	*Gliocephalotrichum cylindrosporum*
OTU_13	a	Sordariomycetes	*Hypocreales sp.*
OTU_12	a	Sordariomycetes	*Phomopsis sp.*
OTU_37	a	Sordariomycetes	*Plectosphaerella cucumerina*
OTU_24	a	Sordariomycetes	*Wardomyces dimerus*
OTU_17	b	Agaricomycetes	*Coriolopsis gallica*
OTU_79	b	Agaricomycetes	*Heterobasidion irregulare*
OTU_56	b	Agaricomycetes	*Hyphodontia crustosa*
OTU_47	b	Agaricomycetes	*Hypholoma fasciculare*
OTU_45	b	Agaricomycetes	*Kneiffiella sp.*
OTU_76	b	Agaricomycetes	*Lyomyces sp.*
OTU_36	b	Agaricomycetes	*Peniophora cinerea*
OTU_18	b	Agaricomycetes	*Peniophorella praetermissa*
OTU_16	b	Agaricomycetes	*Phanerochaete chrysosporium*
OTU_58	b	Agaricomycetes	*Phlebia livida*
OTU_19	b	Agaricomycetes	*Phlebia radiata*
OTU_61	b	Agaricomycetes	*Radulomyces confluens*
OTU_68	b	Agaricomycetes	*Sistotrema brinkmannii*
OTU_62	b	Agaricomycetes	Uncultured *Sistotrema sp.*
OTU_73	b	Cystobasidiomycetes	Unassigned *Cystobasidiomycetes sp.*
OTU_27	b	Cystobasidiomycetes	*Cystobasidium laryngis*
OTU_72	b	Cystobasidiomycetes	*Erythrobasidium sp.*
OTU_20	b	Exobasidiomycetes	*Exobasidium otanianum*
OTU_71	b	Microbotryomycetes	*Phenoliferia psychrophila*
OTU_51	b	Microbotryomycetes	*Rhodotorula mucilaginosa*
OTU_87	b	Microbotryomycetes	*Sporidiobolales sp.*
OTU_69	b	Tremellomycetes	*Filobasidium wieringae*
OTU_50	b	Tremellomycetes	*Itersonilia perplexans*
OTU_15	b	Tremellomycetes	*Tremellales sp.*
OTU_67	b	Wallemiomycetes	*Wallemia mellicola*
OTU_2	s	Mucormycotina	*Mucor velutinosus*
OTU_82	u	Unclassified	Unassigned *Fungi sp.*
OTU_64	u	Unclassified	Unassigned *Pezizomycotina*
OTU_75	u	Unclassified	Unassigned *Pezizomycotina sp.*

Figure 1: List of detected fungi in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain.

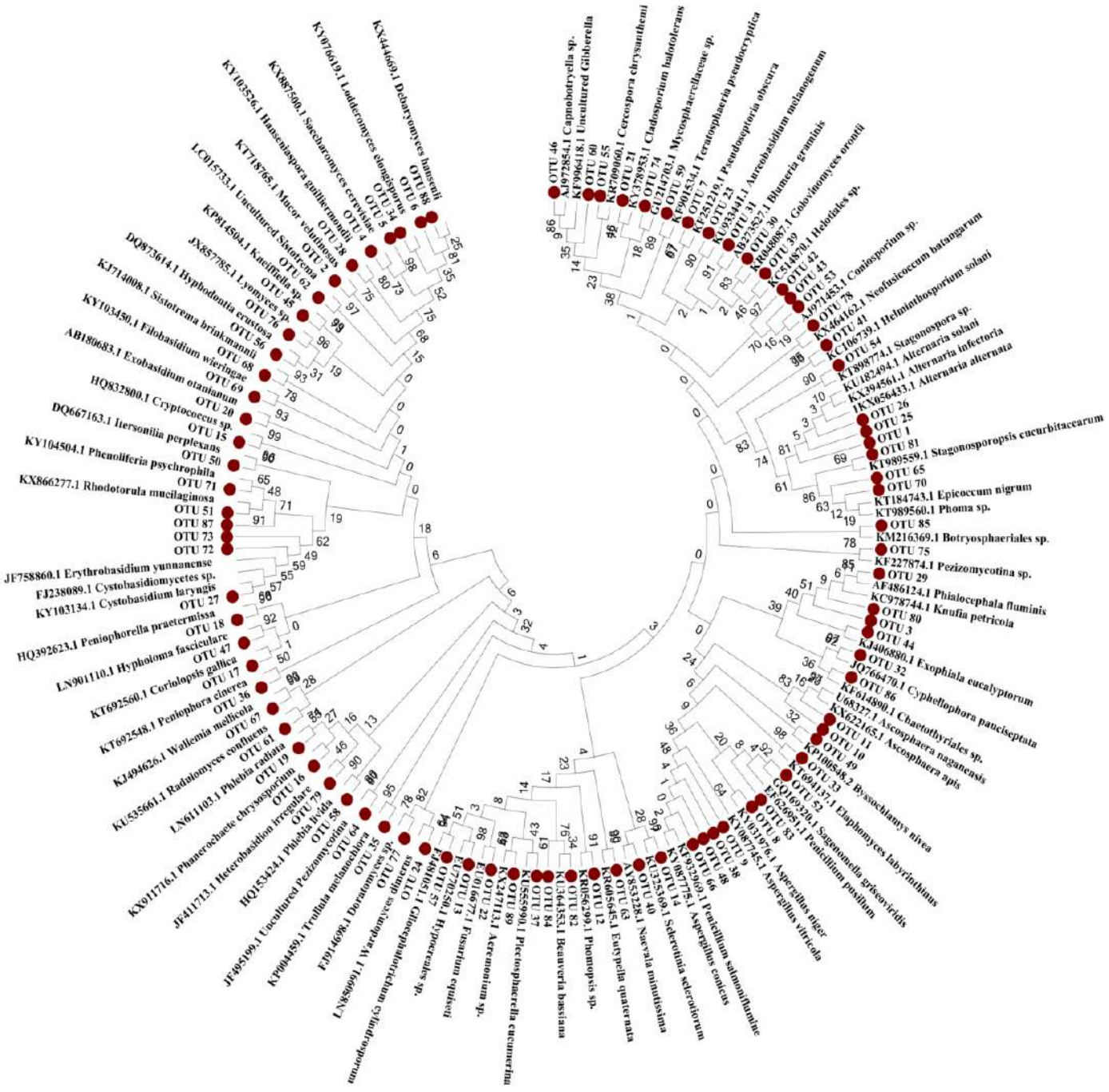


Figure 2: Phylogenetic tree of detected fungal clones based on ITS sequences. The name of the host species is followed by GenBank accession numbers [in brackets]. Numbers indicate bootstrap values (1000 replicates).

Moreover, the most common fungi from order Pleosporales were related to species in *Alternaria*, *Epicoccum*, *Stagonospora*, *Helminthosporium* and Phoma (Table 1 and Fig

1). Three different OTUs of genus *Coniosporium* which belonged to order Chaetothyriales were detected in ITS2 Cloning libraries, whereas *Debaryomyces* and *Hanseniaspora* were most common fungi from order Saccharomycetales detected in the soil samples (Table 1).

Basidiomycota represented only 28.1% of fungal diversity of soil samples. Genera which belonged to order Corticiales included *Phlebia*, *Lyomyces*, *Peniophorella*, *Kneiffiella* and *Radulomyces* were the most common Basidiomycota found in the soil samples (Table 1). Other Basidiomycota genera from Polyporales and Russulales including genera such as *Coriolopsis*, *Phanerochaete*, *Heterobasidion*and *Peniophora*were also found in the soil samples.

3.1.2. Fungal diversity at different depths

The diversity indices of the soil fungal communities from the various depths of the Haibat Sultan Mountain are illustrated in (Table 2). The Chao1 estimator and Shannon diversity indices were used to estimate the diversity of fungal communities in soil samples at different depths. The highest Chao1 value for the fungal community was obtained at the soil depth of 5 cm, followed by soil depth 1cm, whereas the lowest value was detected in soil depth 10 cm (Table 2, Fig 3 and Fig S2).

Similarly, to Chao1 estimator, the Shannon diversity index values showed relatively high fungal diversity at the soil depth of 5 cm followed by soil depth 1cm while low Shannon index was obtained at soil depth 10 cm (Table 2, Fig 3 and Fig S3).

Table 2: Diversity index of fungi in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain.

Indice	D 1cm Loc1	D 1cm Loc2	D 1cm Loc3	D 1cm Loc4	D 1cm Loc5	D 5cm Loc1	D 5cm Loc2	D 5cm Loc3	D 5cm Loc4	D 5cm Loc5	D 10cm loc1	D 10cm loc2	D 10cm loc3	D 10cm loc4	D 10cm loc5
Taxa_S	31.00	21.00	21.00	24.00	28.00	33.00	45.00	25.00	22.00	31.00	25.00	17.00	13.00	12.00	21.00
Dominance_D	0.04	0.13	0.13	0.05	0.09	0.05	0.03	0.06	0.10	0.05	0.06	0.17	0.34	0.18	0.10
Simpson_1-D	0.96	0.87	0.87	0.95	0.91	0.95	0.97	0.94	0.90	0.96	0.94	0.83	0.66	0.82	0.90
Shannon_H	3.24	2.42	2.52	3.03	2.86	3.28	3.65	3.03	2.64	3.24	3.02	2.23	1.59	2.00	2.67
Evenness_e^H/S	0.83	0.54	0.59	0.86	0.62	0.80	0.85	0.83	0.63	0.82	0.82	0.55	0.38	0.62	0.69
Brillouin	2.82	2.14	2.22	2.67	2.49	2.83	3.08	2.66	2.33	2.81	2.66	1.99	1.42	1.83	2.37
Menhinick	3.16	2.17	2.16	2.46	2.87	3.39	4.64	2.57	2.28	3.20	2.57	1.74	1.36	1.23	2.17
Margalef	6.57	4.40	4.39	5.05	5.93	7.03	9.69	5.27	4.63	6.60	5.27	3.51	2.65	2.41	4.40
Equitability_J	0.94	0.79	0.83	0.95	0.86	0.94	0.96	0.94	0.85	0.94	0.94	0.79	0.62	0.81	0.88
Fisher_alpha	15.88	8.40	8.35	10.34	13.39	17.93	33.88	11.06	9.10	16.14	11.06	6.03	4.13	3.62	8.40
Berger-Parker	0.08	0.21	0.29	0.08	0.24	0.09	0.07	0.09	0.19	0.09	0.09	0.35	0.55	0.31	0.22
Chao-1	34.50	32.25	21.08	25.50	33.00	38.00	54.56	25.86	26.20	37.00	27.00	18.67	18.00	13.50	21.86

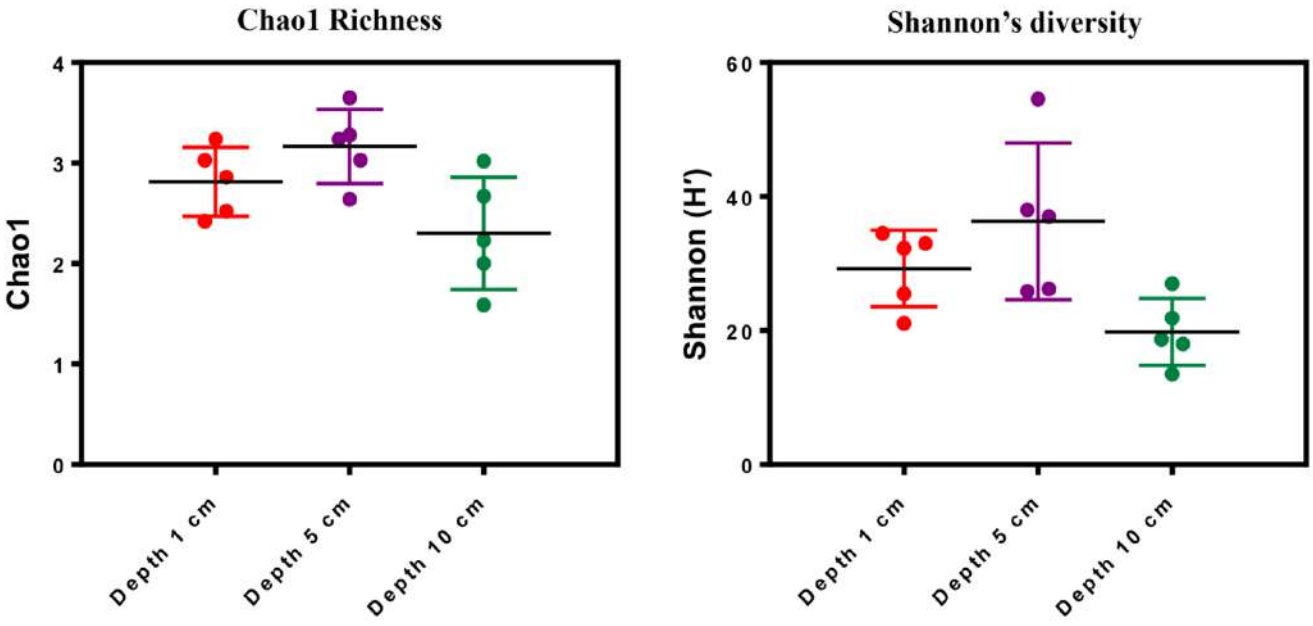


Figure 3: Chao1 estimator and Shannon diversity index of fungi in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain.

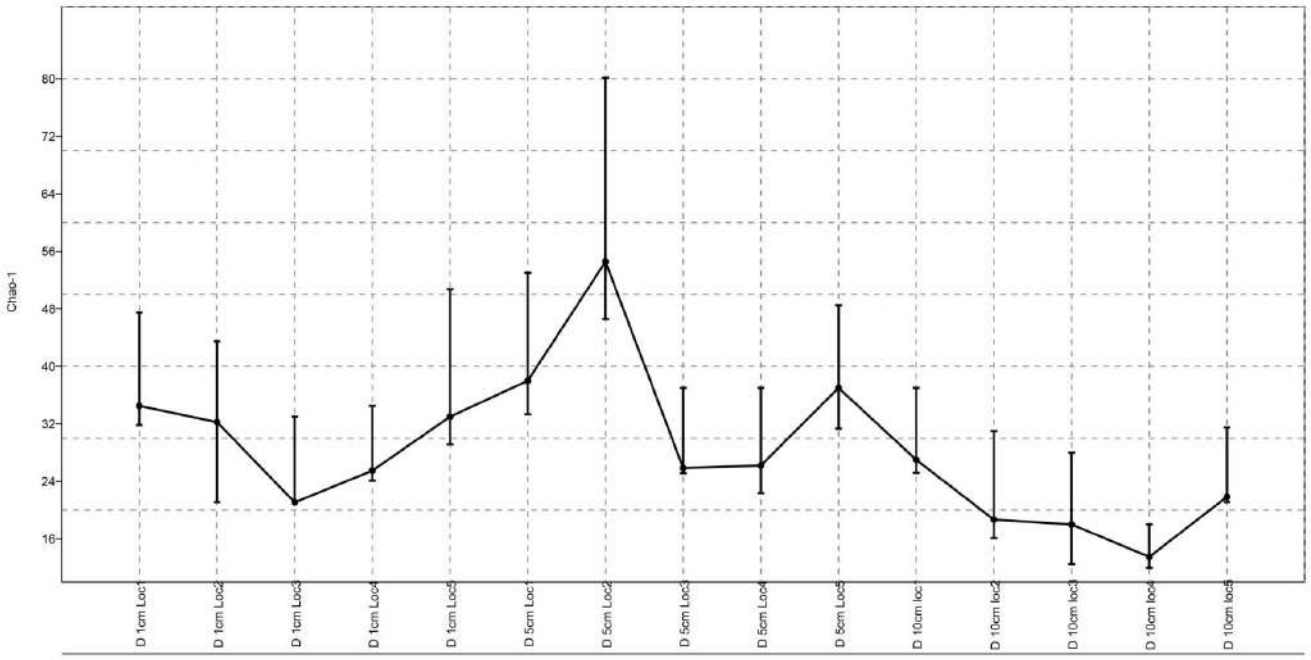


Figure S2: Chao1 estimator of fungi in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat-Sultan Mountain.

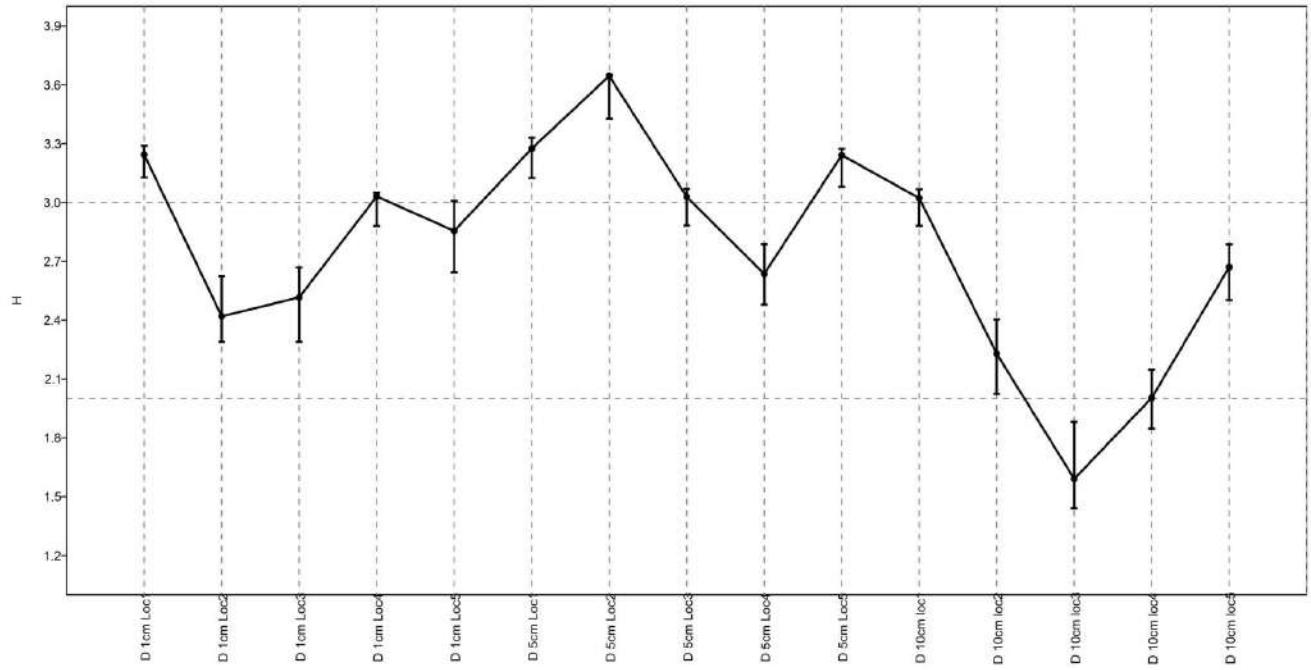


Figure S3: Shannon diversity index of fungi in soils samples that collected at different depths of 1, 5 and 10 cm in Haibat Sultan Mountain.

3.2. Discussion

Fungi constitute a diverse microbial community in soil and have a significant role in decomposition and nutrient cycling (Bailey et al., 2002, Buée et al., 2009). However only a small fraction of the estimated 1.5 million fungi have been described (Hawksworth, 2001), studying these fungal communities was difficult, because of their diversity, and substrate complex (Bridgen and Spooner, 2001), therefore the ecological roles of fungi are poorly understood and we have limited ability to distinguish individual taxa (McGuire and Treseder, 2010).

The ITS2 region is generally more variable (Iwen et al., 2002) and lacks the problem of co-amplification of the 5'-end small subunit intron (5'SSU intron) as well as it has more availability in the reference database than ITS1 (Nilsson et al., 2009).

In our study, we found out that fungal richness and diversity were abundant at the depth of 1 cm and reached their peak at the depth of 5 cm, while at the depth of 10 cm, the fungal communities decreased. We attribute this difference in fungal community richness to many factors, such as abiotic conditions of soil including PH, moisture content and temperature and availability of nutrient and carbon source (Zachow et al., 2009). Grantia L. *et. al*(Grantina et al., 2011) found that the number of filamentous fungi species and the total number of cultivable microorganisms showed a tendency to decrease with increasing depth. The diversity of fungi obtained with amplified ribosomal DNA (rDNA) gene restriction analysis was similar for all studied land use groups (forest, former agricultural land, meadow and arable land), diversity decreased with soil depth (Grantina et al., 2011).

Study investigated different depths of dry and sandy soils vary from shallow alkaline to deep acid podsol have resulted in the identification of 148 species, among them genera *Penicilliunm* and *Mortierella* were the most common, it's also been proven that number of fungal species and colonies decrease with depth (Warcup, 1951).

Even thought that using molecular methods can offer a better insight into the genetic heterogeneity of microbial communities in soil and identify particular organisms without isolation, the molecular methods have also some limitations. Limitation factors such as successful isolation of DNA from soil, presence of DNA amplification or restriction inhibitors, choice of primers, and the limited number of tested clones and discriminating power of analysis must be considered (Kowalchuk et al., 2006, Hamad et al., 2014).

4. Conclusions

This study describes an attempt at assessing the fungal diversity in the soil in Haibat Sultan Mountain soil by using PCR based cloning and sequencing of fungal ITS amplicon. It is well established that fungal abundance decreases with soil depth. Further efforts are needed to use

extensive molecular methods with different sets of fungal universal primers targeting different region of ribosomal unit in parallel to detect more fungi communities in soil. Moreover, using high-throughput sequencing will expand the fungal diversity in soil.

Disclosure

The authors declare no conflicts of interest.

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