فصلنامه علمی زیست‌شناسی میکروارگانیسم‌ها
سال یازدهم، شماره ۴۴، زمستان ۱۴۰۱ ، صفحه ۱۶۸-۱۵۹
تاریخ دریافت: ۱۴۰۱/۰۵/۱۸ – تاریخ پذیرش: ۱۴۰۱/۰۷/۲۷

تعیین تنوع قارچی در یک منطقه نیمه‌خشک بیابانی با استفاده از توالی‌یابی نسل دوم

مریم تیموری: گروه میکروبیولوژی، دانشکدهٔ علوم زیستی، دانشگاه الزهرا، تهران، ایران- موسسهٔ تحقیقات جنگل ها و مراتع، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران، mteimouri@rifr-ac.ir

پریسا محمدی*: گروه میکروبیولوژی، دانشکده علوم زیستی، دانشگاه الزهرا س، تهران، ایران، مرکز تحقیقات میکروبیولوژی کاربردی و بیوتکنولوژی میکروبی، دانشگاه الزهرا، تهران، ایران p.mohammadi@alzahra.ac.ir

عادل جلیلی: استاد موسسهٔ تحقیقات جنگل ها و مراتع، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران، jalili@rifr-ac.ir

چکیده

مقدمه: اهمیت قارچ‌ها در اکوسیستم‌های خشکی بسیار درخور توجه است؛ با وجود این، دانش ما دربارهٔ انتشار و تنوع قارچ‌ها در زیستگاه‌های طبیعی به‌ویژه اکوسیستم‌های نیمه‌خشک کم است. به‌منظور داشتن اطلاعات مناسب از ساختار جوامع قارچی و تأثیرعوامل محیطی بر ترکیب جوامع قارچی چنین محیط‌هایی، تنوع قارچی در کنار ویژگی‌های شیمیایی خاک بررسی شد.

مواد و روش‌ها: نمونه‌برداری از خاک در بهار و تابستان از دو ناحیه سرد و گرم اکوسیستم نیمه‌خشک واقع در استان کرمان انجام شد. ویژگی‌های شیمیایی خاک با استفاده از روش‌های استاندارد خاک تعیین شدند. قارچ‌های ساکن در خاک با استفاده از توالی‌یابی نسل دوم ناحیه ITS (ITS1f-ITS2) تعیین شدند.

نتایج: با تجزیه و تحلیل یافته‌های حاصل از پلت‌فرم Illumina Miseq، ۱۵۴۲ واحد عملکردی تاکسونومی در نمونه‌های خاک مطالعه‌شده شناسایی شدند. تفاوت مشاهده‌شده در ترکیب جمعیت قارچی دو ناحیه سرد و گرم

* نویسندهٔ مسئول مکاتبات

10.22108/BJM.2022.134408.1478

می‌تواند ناشی از اختلاف چشمگیر شرایط محیطی این دو ناحیه ازجمله تفاوت در میزان رطوبت و کربن آلی خاک باشد. آسکومیکوتا، شاخه غالب قارچی شناسایی‌شده بود و در مرتبه بعدی بازیدیومیکوتا فراوانی بیشتری داشت.

بحث و نتیجه‌گیری: قارچ‌های متنوعی در این اکوسیستم شناسایی شدند. برای آگاهی از نقش گونه‌های غالب قارچی در اکوسیستم‌های نیمه‌خشک به مطالعات بیشتری نیاز است.

واژه‌های کلیدی: تعیین توالی جوامع قارچی، ترکیب جوامع قارچی، غناء، یکدستی

Biological Journal of Microorganism
Year 11, Vol.11, No.44, Winter 2023
Received: 2022-08-09 - Accepted:Page: 2022-10-19
P:159-168

Determination of Fungal Diversity in a Semiarid Area Desert by Next-Generation Sequencing

Maryam Teimouri
Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran- Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran, mteimouri@rifr-ac.ir
Parisa Mohammadi*
Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Research Center for Applied Microbiology and Microbial Biotechnology, Alzahra University, Tehran, Iran, p.mohammadi@alzahra.ac.ir
Adel Jalili
Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran, jalili@rifr-ac.ir

Abstract

Introduction: The fugal importance in terrestrial ecosystems is significant. However, our knowledge based on fungal distribution and its diversity in natural communities, especially in semi-arid ecosystems, seems to low. To have proper information on fungal community structure and the impact of environmental factors on the community composition, we assessed fungal diversity accompanied by soil chemical properties.

Materials and Methods: Soil sampling was done in two seasons, spring and autumn, from two different sites, which were both cold and warm in a semiarid ecosystem located in Kerman province, Iran. Soil chemical properties were measured according to standard methods. The fungal community was estimated by the next-generation sequencing of the ITS region (ITS1f-ITS2).

Results: By Illumina Miseq data analysis, 1542 Operational Taxonomic Units in soil samples were identified. Variation in the fungal community between the two sampling sites may be because of remarkable differences in environmental conditions, such as moisture and soil organic carbon. The dominant phylum was Ascomycota, followed by Basidiomycota.

Discussion and Conclusion: Various fungi were identified in this ecosystem. Further studies are necessary to reveal the possible roles of the dominant fungal species inhabiting semiarid soils.

Keywords: Communities Sequencing Determination, Fungal Composition, Evenness, Richness.

*Corresponding Author

10.22108/BJM.2022.134408.1478

Introduction

The dimension of the soil fungal diversity is poorly understood even though fungi are an essential group in ecosystem processes, and they have high levels of diversity (1-3). Emerging methods of direct extracting nucleic acids from soil and amplification of fungal ITS (Internal Transcribed Spacer) variable regions combined with new techniques in sequencing, permit us to extend our knowledge on fungal diversity in soil (4). In recent years, conducted research on fungal diversity by using new sequencing techniques like next-generation sequencing (NGS) has made a possible accurate estimation of soil fungal diversity and identification of novel taxa. Our information on the soil fungal diversity in terrestrial ecosystems is restricted (5-9). Most of the studies have been done on forest, tundra, compost, and grassland (10-12). It makes the situation even worse in arid and semiarid ecosystems, especially in Iran.

According to our survey, there is no study on fungal diversity in Iran, especially using next-generation sequencing techniques.

In this study, the diversity and composition of the fungal community were determined in the collected soil samples from a semiarid area. Plant and animal diversity in this area has been reported to some extent (13, 14), but according to the literature, there has been no published report on fungal diversity indices and their community structure. The present study aimed to monitor the possible variation in soil chemical properties and the fungal community, which has been affected by the site, located in cold or warm areas, and seasons (spring and autumn).

Methods and Materials

The study was carried out in Kerman province, Iran. The two selected plots were at the cold and warm sites of the Khabr National Park and Ruchun Wildlife Refuge (Table 1). Sampling was carried out in June (S, spring) and November (A, autumn). Thus, there were four treatments: (1) cold-spring (CS), (2) cold-autumn (CA), (3) warm-spring (WS), and (4) warm-autumn (WA). Soil samples were collected from 0-10 cm soil depth. Plant debris was removed from soil samples by sieving through a 2-mm sieve. Soil samples were divided into two parts: 1- was dried at room temperature and used for soil chemical analysis, and 2- was kept at -80 °C for the community analysis of the fungi by NGS.

Table 1- The General Properties of the Sampled Areas

Area	Geographical coordinates	Elevation	Dominant vegetation coverage
Cold	56◦ 22′ 59′′ N 28◦ 38′ 08 ′′E	2365 m	*Artemisia siberi* and *Stipa hassknechti*
Warm	56◦ 18′ 08′′ N 28◦ 52′ 27′′ E	1707 m	*A. siberi*

Soil Chemical Analysis: The soil pH and electrical conductivity were measured (15). The gravimetric water content (GWC) was determined after drying soil at 105 °C for 24 h (16). The soil texture was determined by the hydrometer method (17). Soil organic carbon (SOC) was measured by the wet oxidation method (18). SOC was multiplied by 1.72 to calculate soil organic matter (SOM). The sodium bicarbonate was used to extract available phosphorous (AP) from soil samples (19). Total nitrogen in soil samples was measured using a micro Kjeldahl (20). Soil samples were extracted by ammonium acetate and then their available K was measured by atomic absorption spectroscopy (21).

DNA Extraction, Illumina Sequencing, and Data Processing: PowerSoil®DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsdad, CA) was used to extract DNA. DNA was amplified by the ITS1f_ITS2 primer set (22) to make a library. ITS1f_ITS2 primer amplifies the ITS 1 region in fungi. Sequencing was done by Illumina MiSeq platforms at 2 × 150 of the paired-end reads. The obtained sequences were analyzed using QIIME, v 1.9.0 (23). After demultiplexing, individual sequences (R1 and R2) were downloaded from Illumina Base spaces. Barcodes were removed from raw Illumina fastq files of each pair R1 and R2, and combined by PANDAseq (24). The "pick-open-reference.py" for the operational taxonomic unit (OUT) was used. UNITE database v 5.0 (25) was used to assign the qualified sequences to all OTUs with 97% similarity. The Chao1 and J indices were determined using appropriate commands of QIIME.

Statistical Analysis: The data normality was done by the Shapiro-Wilk test *via* Jonson transformation in Minitab software whenever necessary. The two-way variance was used to analyze the data. The site and season effects were determined by the Mann-Whitney U test. The mean comparison was done by the Least Significant Difference (LSD) test (5%).

Results and Discussion

Soil Chemical Properties: The sampling site soil was categorized in Haplic Calcisoils. Soil particles on average were clay (10%), silt (30%), and sand (60%) with a texture of sandy loam. The results indicated a significant effect ($p<0.01$) of the season on pH value (Table 2), which a higher pH value was seen in autumn soil samples (Table 2). Soil pH as a chemical indicator has a critical role in soil processes such as the cycling of elements, the solubility of nutrients, microbial activity, and enzymatic activity (26-30). The low pH value in spring soil samples can be explained by releasing carbon dioxide through plant roots as well as microbial activities in this natural ecosystem (31).

The quantity and quality of SOC and SOM have been considered soil quality indicators (32, 33). The SOM was affected by the site treatment (Table 2), and more SOM was at the cold site (Table 2). The less SOM in the warm site can be explained by the faster transformation of organic C to inorganic C and less vegetation coverage in this site. The negative relation between temperature and soil organic matter has been previously reported by other researchers (34).

The effect of site and season was significant ($p<0.01$) for total nitrogen (TN), available P (AP), and exchangeable potassium (AK) values. In general, the presence of higher vegetation coverage reduces the loss of micro and macro nutrients explaining the greater values of NPK (Table 2) at the cold site.

Table 2- Mean Comparison (n 3) of Soil Chemical Properties between Site (Cold with Warm) and Season (Spring with Autumn) Treatments

Treatments		pH	EC mS/cm	TN %	EK mg/ Kg	AP mg/ Kg	GWC %	SOC %	SOM %
Site	Cold	8.09 ± 0.03^{a}	100.07± 2.26^{a}	0.083±.001^{a}	237.59±4.805 ^{a}	35.0±1.076^{a}	4.44±0.7^{a}	0.65±.008^{a}	1.33±0.064^{a}
	Warm	8.13± .0.04^{a}	99.18 ±1.95^{a}	0.049 ±.002^{b}	188.34±4.233^{b}	30.5±0.750^{b}	1.33±0.17 ^{b}	0.029±015^{b}	.51±0.027^{b}
Season	Spring	7.9± 0.01^{b}	100.02 ±2.41^{a}	0.061 ±0.004^{b}	224.11± 5.85^{a}	34.5± 0.85^{a}	0.93±0.52^{b}	0.55±0.006^{a}	0.94±0.044^{a}
	Autumn	8.3± 0.03^{a}	99.85± 2.85^{a}	0.074 ±0.007^{a}	220.35±5.66^{a}	31±1.08^{b}	4.83±0.75^{a}	0.51±0.012^{a}	0.87±0.057^{a}

Different letters show significant differences at $p< 0.05$ level. TN: total nitrogen, EK: exchangeable potassium, AP: Available phosphorous, GWC: Gravimetric water content, SOC: soil organic carbon, and SOM: soil organic matter.

Table 3- Mean Comparison (n 3) of Soil Diversity Index between Site (Cold with Warm) and Season (Spring with Autumn) Treatments

Treatments		Chao1	Shannon
Site	Cold	378±14[a]	0.73 ± 0.02[a]
	Warm	330±28[b]	0.85 ±0.04[b]
Season	Spring	435±23[a]	0.68 ±0.03 [b]
	Autumn	380±27[b]	0.75±0.02[a]

Different letters show significant differences at p< 0.05 level. Chao1 and Shannon, H indicate the richness and evenness of soil samples, respectively

Whole Fungal Community Profile: A total of 390022 sequences were obtained *via* Illumina Miseq sequencing. Analysis was carried out on the qualified sequences (333201). The number of the detected sequences for each sample was 7002 up to 66494. There were 3057 OTUs at 97% similarity. As shown in Table 3, the effect of site, season, and their interactions was significant ($p<0.01$) on richness (chao1) and evenness (Shannon, H index). Statistical analysis showed higher richness in samples taken from cold sites (378±14) and spring season (435±23) than the warm site (330±28) and autumn season (380±27), while there was more evenness at the warm site (0.85±0.041) and autumn season samples (0.73±0.022) as compared to the cold site (0.73±0.028) and spring season samples (0.68±0.031).

Zhang, et al. (35) reported a positive correlation between soil microbial richness and moisture, supporting more richness of soil samples in the cold site and spring season samples. In addition, several studies have suggested that vegetation coverage and soil organic carbon can increase soil moisture (36- 39). Aridity has a negative impact on soil organic carbon content and the positive impact of SOC on the abundance and diversity of both bacteria and fungi has been reported in many studies (40). The reduction of microbial abundance changes negatively the function of the soil ecosystem (41). There are several studies, reporting seasonal variation in fungal richness and evenness (42, 43), which is inconsistent with our results. The more observed diversity in spring season sample soils can be related to the less pH of the soil because fungi prefer acidic pH for growth. We did not observe any difference in SOM values between the spring and autumn season samples. These observed results can be attributed to animal excreta.

The interaction effect of site and season indicated that the samples taken at the cold sites in spring (CS) and the warm sites in autumn (WA) had the highest and the lowest richness according to Chao1 values, respectively (Figure 1a).

There was higher richness and evenness at the cold site, indicating more diversity and even distribution of fungi (Figures 1a and 1b). Samples taken in the autumn season at the warm site showed fewer numbers of species, and a high evenness index clarified the uniform distribution of fungal species. A higher number of species and a low evenness index for spring season samples at the warm site indicated to more uneven distribution of species. These results were surprising for both the number of members and how evenly distributed in the community (43, 44). Thus, a community might have more species and its abundance is low.

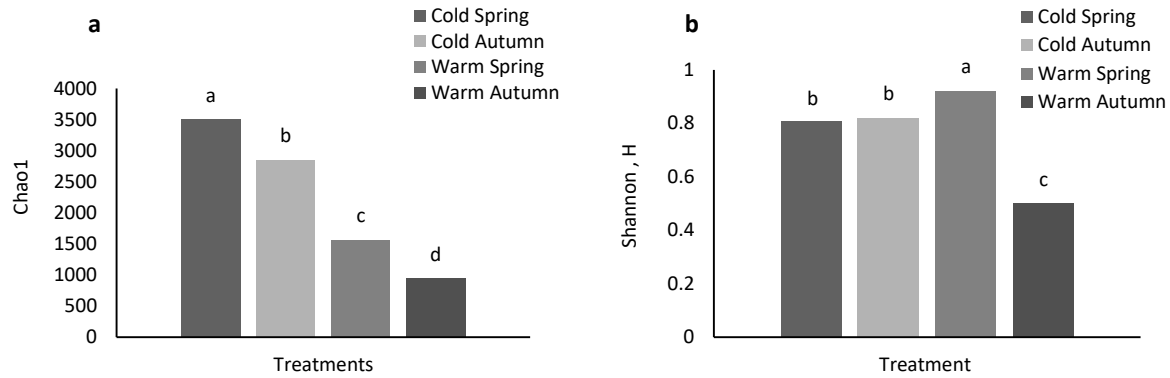

Fig. 1- Estimated values of fungal community richness (a) and evenness index (b) under interaction effect of site and season. Significant differences are indicated by different letters. Chao1 provides an estimation of the observed species

Fungal Community Composition: The five major fungal phyla were observed in all samples with different abundance. Almost 45% of sequences were unidentified. Results have shown that sequences and OTUs belonging to Ascomycota were dominant in both cold and warm sites and spring and autumn season samples. Chytridiomycota and Zygomycota were more prevalent in cold sites, whereas more Glomeromycota was detected in warm sites (Fig 2).

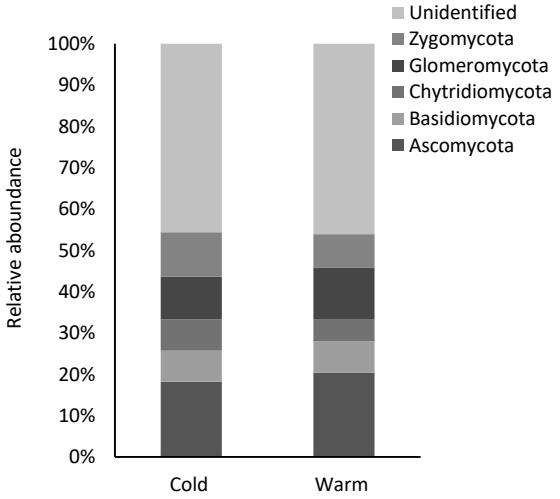

Fig. 2- Relative Abundance of Fungal Phyla Determined by Next-generation Sequencing

The fungal community composition changed with the season as Ascomycota and Glomeromycota were more frequent in spring compared to autumn, whereas the Basidiomycota and Chytridiomycota were more prevalent in autumn samples (Fig. 3).

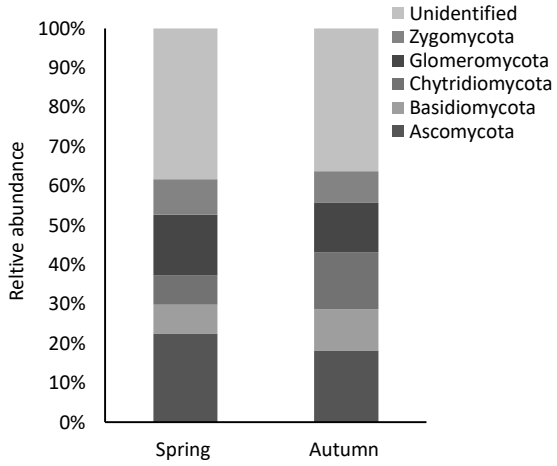

Fig. 3- Relative Abundance of Fungal Phyla Determined by Next-generation Sequencing in Different Seasons

The seasonal changes even were observed in other taxonomic levels too. The Sordariales and Pleosporales both belonging to Ascomycota were the dominant order in spring samples. However, Hypocreales and Capnodiales, which both belong to Ascomycota, had more abundance in autumn samples. There were several unique phylotypes in both sites (cold or warm) and seasons (spring or autumn), which can be used as indicators. The diversity in the Ascomycota phylum was more and it was distinguished into 6 classes, 12 orders, 18 families, and 27 genera. A total of 6 identified classes were

observed across all treatments. Dothideomycete and Incertaesedis were common to all treatments. Chytridiomycetes were exclusive to the cold site. Agaricomycetes and Leotiomycetes were observed only in spring samples at the cold site. Eurotiomycetes was one unique class in autumn samples at the warm site. We detected 12 orders in total, two of which, Glomerales and Pleosporales, were common to all treatments. The highest number of unique orders observed in spring samples at the cold sites were Corticiales, Diversisporales, Helotiales, Sordariales, and Hypocreales. Eurotiales and Pezizales were observed only in autumn season samples at the warm sites.

There were 11 identified families, with one common family, Pleosporaceae, which was observed across all treatments. Chaetomiaceae, Corticiaceae, Diversisporaceae, Helotiaceae, Nectriaceae, and Olpidium were observed only in samples of the cold sites in spring. There was only one unique family in the warm site which was taken in autumn and named Pezizaceae.

There were no common genera (11 genera) among treatments. The detected genera belonged to different groups of fungi with different functions in soil. Mortierella and Phoma were observed in spring samples, taken from both cold and warm sites. There were seven unique genera in spring samples, which were taken from cold sites and identified as Chaetomium, Chalastospora, Diversispora, Gibberella, *Olpidium brassicae*, Rhizoctonia, and Rhizoscyphus. Only one unique genus was observed in autumn samples of the warm sites called Terfezia. The observed differences in fungal composition between the site and season have been reported by other researchers (45). The season has a special impact on fungal composition, as more fungi were observed during the wet season (46). The diversity of fungal communities depends on environmental factors such as temperature, precipitation, snow coverage, and nutrient availability (47, 48). The number of observed classes, orders, families, and genera related to each phylum has been presented in Table 4.

Table 4- Total Phylotypes at Five Taxonomic Levels in Soil Samples

Phylum	Class	Order	Family	Genus
Ascomycota	6	11	18	27
Basidiomycota	4	5	4	3
Chytridiomycota	1	1	1	1
Glomeromycota	1	2	1	1
Zygomycota	1	1	1	1

Conclusion

The richness, evenness, and community composition of fungi were determined in a semiarid area. The impact of site and season treatments on fungal communities was studied by next-generation sequencing. The soil fungal communities are sensitive to soil chemical properties, especially elements concentration, soil organic matter, and moisture. We observed the most richness in the cold sites with higher element concentration, more organic matter, and higher moisture if compared to the warm sites. In addition, we observed seasonal changes in the soil fungal diversity due to alteration of soil chemicals such as

the amount and kind of soil organic matter, moisture, and others.

References

(1) Bahram M., Netherway T. Fungi as mediators linking organisms and ecosystems. *FEMS Microbiology Reviews* 2022; 46 (2): fuab058.

(2) Blackwell M. The fungi: 1, 2, 3 … 5.1 million species?. *American Journal of Botany* 2011; 98 (3): 426-38.

(3) Tedersoo L., Bahram M., Põlme1 S., Kõljalg U., Yorou NS., Wijesundera R., Ruiz LV., Vasco-Palacios AM., Thu PQ., Suija A., Smith ME. Global diversity and geography of soil fungi. *Science* 2014; 346 (6213): 1078.

(4) Lee OO., Wang Y., Yang J., Lafi FF. Al-Suwailem A., Qian PY. Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *The ISME Journal* 2011; 5 (4): 650–64.

(5) Bahram M., Netherway T., Frioux C., Ferretti P., Coelho LP., Geisen S., Bork P., Hildebrand F. Metagenomic assessment of the global diversity and distribution of bacteria and fungi. *Journal of Environmental Microbiology* 2021; 23 (1): 316-26.

(6) Oliveira LG., Cavalcanti MA., Fernandes MJ., Lima DM. Diversity of filamentous fungi isoated from the soil in the semiarid area, Pernambuco, Brazil. *Journal of Arid Environment* 2013; 95: 49-54.

(7) Porras-Alfaro A., Herrera J., Natvig DO., Lipinski K., Sinsabaugh RL. Diversity and distribution of soil fungal communities in a semiarid grassland. *Mycologia* 2011; 103 (1): 10-21.

(8) Romero-Olivares AL., Baptista-Rosas RC., Escalante AE., Bullock SH., Riquelme M. Distribution patterns of Dikarya in arid and semiarid soils of Baja California, Mexico. *Journal of Fungal Ecology* 2013; 6 (1): 92-101.

(9) Vargas-Gastelum L., Romero-Olivares AL., Escalante AE., Rocha-Olivares A., Brizuela C., Riquelme M. Impact of seasonal changes on fungal diversity of a semi-arid ecosystem revealed by 454 pyrosequencing. *FEMS Microbiology Ecology* 2015; 91 (5): 1-13.

(10) Liu D., Liu G., Chen L., Han W., Wang D. Plant diversity is coupled with soil fungal diversity in a natural temperate steppe of northeastern China. *Journal of Soil Ecology Letters* 2021; 4 (4): 454–69

(11) Kazeeroni EA., Al-Sadi AM. 454-Pyrosequencing reveals variable fungal diversity across farming systems. *Journal of Frontiers in Plant Science* 2016; 7: 314.

(12) Yang RH., Su JH., Shang JJ., Wu YY., Li Y., Bao DP., Yao YJ. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PLoS ONE* 2018; 13 (10): e0206428.

(13) Shirvani A. Noctuidae (Lepidoptera) species sampled from Khabr national park, Kerman, Iran part I. *Journal of the Lepidopterists Society* 2012; 66 (3): 121-32.

(14) Sharafatmandrad M., Khosravi A. Ethnopharmacological study of native medicinal plants and the impact of pastoralism on their loss in arid to semiarid ecosystems of southeastern Iran. *Journal of Scientific Reports* 2020; 10 (1): 1-8.

(15) McLean EO. Soil pH and lime requirement. In: Page AL, editor. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Madison: American Society of Agronomy (ASA) and Soil Science Society of America (SSSA); 1982: 199-224.

(16) Schlichting E., Blumer HP. *Methods of soil analysis*. Hamburg: Springer; 1994.

(17) Toogood JA. A simplified textural classification diagram. *Canadian Journal of Soil Science* 1958; 38 (1): 54–5.

(18) Walkley A., Black IA. An examination of Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science* 1934; 63 (1): 29-38.

(19) Olsen SR., Sommers LE. Phosphorus. In:

Page AL, editor. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Madison: American Society of Agronomy (ASA) and Soil Science Society of America (SSSA); 1982: 416-422.

(20) Bremner C., Mulvaney JM. Total nitrogen, Methods of Soil Analysis. In: Page AL, editor. Chemical and Microbiological Properties. Madison: American Society of Agronomy (ASA) and Soil Science Society of America (SSSA); 1982: 595-624.

(21) Warncke D., Brown, JR. Potassium and other basic Cations. In: Brown JR editor. Recommended chemical soil test procedures for the North Central Region. Columbia: North Central Regional Research Publication Bull; 1998: 33-40.

(22) White TJ., Bruns TD., Lee SB., Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White, TJ editors. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press; 1999: 315-322.

(23) Caporaso JG., Kuczynski J., Stombaugh J., Bittinger K., Bushman FD., Costello EK., Fierer N., Peña AG., Goodrich JK., Gordon JI., Huttley GA. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 2010; 7 (5): 335–6.

(24) Masella AP., Bartram AK., Truszkowski JM., Brown DG., Neufeld JD. PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics* 2012; 13 (1): 31-7.

(25) Abarenkov K., Nilsson RH., Larsson KH., Alexander IJ., Eberhardt U., Erland S., Høiland K., Kjøller R., Larsson E., Pennanen T., Sen R. The UNITE database for molecular identification of fungi–recent updates and future perspectives. *The New Phytologist* 2010; 186 (2): 281–5.

(26) Jackson K., Meetei TT. Influence of soil pH on nutrient availability: A review. *Journal of Emerging Technologies and Innovative Research* 2018; 5 (12): 707-13.

(27) Gramss G., Bergmann, H. Microbial competition, lack in macronutrients, and acidity as main obstacles to the transfer of basidiomycetous ground fungi into (organically or heavy-metal contaminated) soils. *Journal of Basic Microbiology* 2007; 47 (4): 309-16.

(28) Holland JE., Bennett AE., Newton AC., White PJ., McKenzie BM., George TS., Pakeman RJ., Bailey JS., Fornara DA., Hayes RC. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of the Total Environment* 2018; 610–611: 316-32.

(29) Pietri JA., Brookes PC. Nitrogen mineralization along a pH gradient of a silty loam UK soil. *Journal of Soil Biology and Biochemistry* 2008; 40 (3): 797-802.

(30) Sinsabaugh RL., Lauber CL., Weintraub MN., Ahmed B., Allison SD., Crenshaw C., Contosta AR., Cusack D., Frey S., Gallo ME., Gartner TB. Stoichiometry of soil enzyme activity at global scale. *Journal of Ecology Letters* 2008; 11 (11): 1252-64.

(31) Subba Rao NS. *Soil microbiology*. New Delhi: Science Pub Inc; 2009.

(32) Islam KR., Weil RR. *Permanganate reactive C field test for soil quality. Agronomy Abstracts*. Madison: American Society of Agronomy; 1999.

(33) Jangir CK., Kumar S., Meena RS. Significance of soil organic matter to soil quality and evaluation of sustainability. *Sustainable Agriculture Scientific Publishers*, 2019; 357-81.

(34) Conant RT., Ryan MG., Ågren GI., Brige HE., Davidson EA., Eliasson PE., Evans SE., Frey SD., Giardina CP., Hopkins FM., Hyvönen R. Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward. *Journal of Global Change Biology* 2011; 17 (11): 3392-404.

(35) Zhang XF., Zhao L., Xu Jr SJ., Liu YZ., Liu HY., Cheng GD. Soil moisture effect on bacterial and fungal community in Beilu River (Tibetan Plateau) permafrost soils with different vegetation types. *Journal of Applied Microbiology* 2013; 114 (4): 1054-65.

(36) Zhang YW., Shangguan, ZP. The change

of soil water storage in three land use types after 10 years on the Loess Plateau. *Catena* 2016; 147: 87–95.

(37) Genxu W., Yuanshou L., Yibo W., Qingbo W. Effects of permafrost thawing on vegetation and soil carbon pool losses on the Qinghai–Tibet Plateau, China. *Geoderma* 2008; 143 (1-2): 143–52.

(38) Wu X., Zhao L., Chen M., Fang H., Yue G., Chen J., Pang Q., Wang Z., Ding Y. Soil organic carbon and its relationship to vegetation communities and soil properties in permafrost areas of the Central Western Qinghai-Tibet Plateau, China. *Permafrost Periglac Process* 2012; 23 (2): 162–9.

(39) Signori CN., Thomas F., Prast AE., Ricardo CG., Pollery RC., Sievert SM. Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. *Journal of Frontiers in Microbiology* 2014; 5: Article 647.

(40) Maestre FT., Delgado-Baquerizo M., Jeffries TC., Singh BK. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *The Proceedings of the National Academy of Sciences* 2015; 112 (51): 15684-9.

(41) Maestre FT., Delgado-Baquerizo M., Jeffries TC., Eldridgec DJ., Ochoaa V., Gozalo B., et al. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the United States* 2015; 112 (51): 1-6.

(42) Burke DJ. Effects of annual and interannual environmental variability on soil fungi associated with an old-growth, temperate hardwood forest. *FEMS Microbial Ecology* 2015; 91 (6): fiv053.

(43) Pereira e Silva MC., Dias AC., van Elsas JD., Salles JF. Spatial and temporal variation of archaeal, bacterial and fungal communities in agricultural soils. *Plos One* 2012; 7 (12): e51554.

(44) Gosselin F. An assessment of the dependence of evenness indices on species richness. *Journal of Theoretical Biology* 2006; 242 (3): 591-7.

(45) Wilsey B., Stirling G. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. *Journal of Plant Ecology* 2007; 190 (2): 259-73.

(46) Tokumasu S. Fungal succession on pine needles fallen at different seasons: The succession of interior colonizers. *Mycoscience* 1998; 39 (4): 409-16.

(47) Zhou DQ., Hyde KD. Fungal succession on bamboo in Hong Kong. *Journal of Fungal Diversity* 2002; 10: 213-27.

(48) Davey ML., Heegaard E., Halvorsen R., Ohlson M., Kauserud, H. Seasonal trends in the biomass and structure of bryophyte associated fungal communities explored by 454 pyrosequencing. *New Phytologist* 2012; 165 (4): 844-56.

\