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Soil Fungal Diversity in Turfgrass Ecosystems of Northwest China Using Illumina Sequencing of the ITS Region

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ABSTRACT

Many studies have been carried out to assess the effects of plants on soil microorganism communities in different environments. However, little is known about fungal diversity in turfgrass soil ecosystems. Therefore, in the present study the fungal diversity of turfgrass soil collected from northwest China was studied by high-throughput sequencing technique. A total of 215,321 high-quality 18S rRNA gene sequences were recovered from the five samples. The lengths of high-quality sequence mainly ranged from 180~240 bases. A total of 8 phyla were identified in the samples via taxonomic summary, with the following five being dominant: Ascomycota, Basidiomycota, Glomeromycota, Zygomycota, and Rozellomycota. Numerous fungal genera were identified in the sample, and the dominant genera were *Cryptococcus*, *Modicella*, *Alternaria*, *Guehomyces*, *Phoma* and others. The results of diversity analysis showed that season and turfgrass species have more impact on soil fungal diversity, while soil depths may result in little difference of soil fungal diversity. The fungal communities differed significantly across the samples. This study provides previously unknown information regarding the impact of turfgrass establishment on microbial diversity in turfgrass soil and also lays a foundation for further investigations into microbiota in turfgrass ecosystems.

INTRODUCTION

Soil fungi play important roles in terrestrial ecosystems and promote cycling of soil organic matter and mineral components, including cellulose, lignin, carbon, and nitrogen (Joergensen & Wichern 2008, Hollister et al. 2010, Wang et al. 2016). They also are related to taxonomic community structures, and greater functional diversity improves plant nutrient acquisition, protect plants against pathogens, and directly affects plant productivity (Deacon et al. 2006, Van Der Heijden et al. 2008). Soil fungal community structures are significantly influenced by the physical and chemical properties of the soil (Jirout et al. 2011, Wu et al. 2007). Many studies have focused on fungal abundance and structures in different terrestrial ecosystems, although most of these studies were based on indirect techniques used to measure the physiological and biochemical properties of the microorganisms (Jirout et al. 2011, Wu et al. 2007, Wang et al. 2010).

Turfgrasses, a unique ecosystem, consists of closely-spaced turfgrass and the subtending soil. In this specific ecosystem, turfgrass soils support abundant and diverse microbial populations (Shi et al. 2007). Traditional microbial cultivation methods and PLFA techniques have been used to investigate the turfgrass soil microbial activity and diversity, which can only provide a relatively coarse measure of soil microbial community composition and structure (Elliott et al. 2003, Shi et al. 2006, Yao et al. 2006). High-throughput sequencing technologies, an effective approach to understand the whole microbial communities, has been used successfully in the study of the microbial diversity and composition of different types of samples (Bulgarelli et al. 2012, Lundberg et al. 2012, Schlaeppi et al. 2014). However, relatively little research has addressed the fungal diversity in turfgrass systems using high-throughput sequencing technology.

In this study, our objective was to make an inventory of the soil fungal diversity and communities in turfgrass ecosystems using Illumina Miseq metabarcoding.

MATERIALS AND METHODS

Sampling Site

This research was conducted at Gansu Agricultural Univer-

Table 1: Information of different samples.

Sample	Turf	Time	Soil depth
CP1	Perennial ryegrass	March 5, 2016	0-10cm
CP2	Festuca elata	March 5, 2016	0-10cm
CP3	Perennial ryegrass	April 25, 2016	10-20cm
CP4	Perennial ryegrass	April 25, 2016	0-10cm
CP0	-	April 25, 2016	0-10cm

Note: - indicates no turfgrass.

sity, Lanzhou, Gansu, China (103.698°E, 36.091°N at an elevation of 1520 m above sea level). Soil samples were collected from a turfgrass plots established in 2013, which was located before No. 3 teaching building in Gansu Agricultural University. Annual mean temperature at the research site was about 10.3°C.

Soil Collection

Soil sampling was conducted from March to April in 2016. Five soil cores were randomly collected using quincunx sampling method and homogenized into one sample. Four samples (CP1, CP2, CP4 and CP0) were taken at a depth of 0-10 cm, while sample CP3 was taken at a depth of 10-20 cm. All the samples were passed through a 2.0 mm sieve to eliminate large rocks and roots. Then they were quickly frozen using liquid nitrogen, and stored at -80°C prior to DNA extraction. Additional information on the samples is given in Table 1.

DNA Extraction

DNA was extracted from 0.2 g of soil sample using an E.Z.N.A. Soil DNA Kit D5625-01 (OMEGA, Norcross, GA, USA) according to the manufacturer's protocol. The extracted DNA was assessed using a Qubit 2.0 spectrophotometer (Invitrogen, Carlsbad, CA, USA), and the integrity of the extracted DNA from the soil was confirmed by agarose gel electrophoresis (Fu et al. 2015).

Illumina Mi Seq Sequencing

Illumina Mi Seq sequencing was conducted at ALLWEGENE Inc. (Beijing, China). The fungal 18S rRNA gene was amplified with the ITS1-F (CTTGGTCATTTA GAGGAAGTAA) and ITS2 (TGCGTTCTTCATCGATGC) primers targeting the ITS1-ITS2 region. PCR was conducted using specific primers with barcodes and high-fidelity Trash Start Fastpfu DNA Polymerase (Trans Gen Biotech, China). The 18S rRNA gene PCR thermal cycle profile was as follows: 5 min at 95°C followed by 25 cycles of 30s at 95°C, 30s at 56°C, and 40s at 72°C and then final extension for 10 min at 72°C, after which the samples were held at 4°C.

Processing of High-Throughput Sequencing Data

Raw data of Illumina Mi Seq sequencing were processed in the Mothur pipeline (Schloss et al. 2009). Paired sequence reads from the DNA fragments were merged using FLASH software (Caporaso et al. 2010). Quality sequences were subsequently clustered into operational taxonomic units (OTUs) by uclust (QIIME) with a similarity cutoff of 97% (Fu et al. 2015). This dataset was available in the SRA at the NCBI under accession number PRJNA340319.

Diversity and Statistical Analysis

Alpha diversity analysis, which included the Chao1, and Shannon, coverage indices were performed using the summary single command of the MOTHUR software (http://www.mothur.org/). Beta diversity was obtained using the Bray-Curtis index and weighted and unweighted UniFrac distance matrices (Lozupone et al. 2011). The community structure was analysed statistically at different classification levels. Data plotting and statistical analysis were conducted using the R program (v 2.15.3; https://www.r-project.org/).

RESULTS

Sequencing and Classification

Total DNA was extracted from the soil samples, and ITS regions were PCR amplified from each of those five DNA samples. PCR products of ITS regions were sequenced using pair-end method by Illumina Mi Seq. After quality control, a total of 215,321 high-quality 18S r RNA gene sequences were recovered from the five samples (Table 2).

Table 2: OTUs, coverage, Chao1 and Shannon's index for 18S r RNA sequencing of the samples.

Sample ID	Reads	OTU	Coverage	Chao1	Shannon
CP0_F	33679	417	99.81%	459.02	5.44
CP1_F	155362	469	99.72%	391.0	4.64
CP2_F	187380	497	99.69%	466.75	4.72
CP3_F	38441	465	99.73%	478.28	5.12
CP4_F	51177	483	99.68%	540.71	5.86

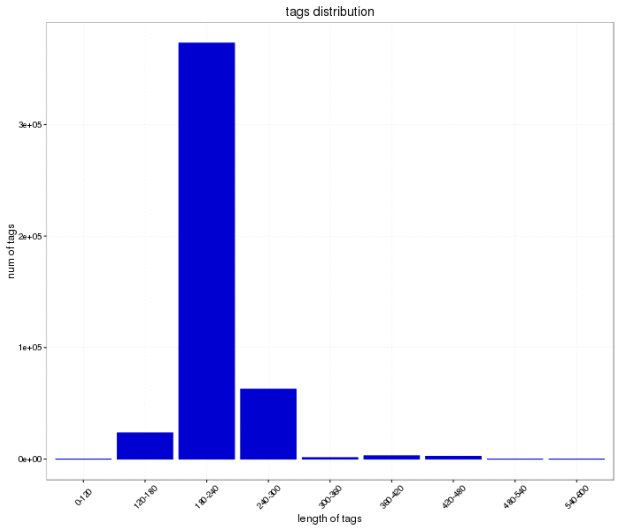

Fig. 1: The tags distribution of sequences.

The lengths of high-quality sequence mainly ranged from 180~240 bases (Fig. 1). The coverage of the all the samples ranged from 99.68 to 99.81%, which indicated an adequate level of sequencing to identify the majority of the diversity in the samples. The number of OTUs detected at 97 % sequence identity ranged from 417 to 497. Sample CP2 had the highest diversity of fungal communities (497 OTUs), while CP0 had the lowest diversity of fungal communities (417 OTUs). As shown in Fig. 2, 124 OTUs were shared by the five samples.

Analysis of Alpha Diversity

The alpha diversity demonstrated that the diversity of microbial communities was abundant in the analysed samples (Table 2). The lowest diversity was observed in CP0, while the sample CP1, CP2, CP3, and CP4 exhibited a higher diversity than CP0. It turned out that fungi in the turfgrass soil were richer than that of vacant land soil. The fungal diversity was different in CP1 and CP2, indicating that turfgrass species may influence fungi of the turfgrass soil. The fungal diversity in CP4 was higher than that of CP1, showing that season is an important factor regarding fungi diversity of the turfgrass soil. The fungal diversity in CP4 was higher than that of CP3, demonstrating that soil depths also have impact on fungi of the turfgrass soil.

Analysis of Beta Diversity

The beta diversity index is a measurement used to compare the differences between multiple groups of samples. The analysis is based on the Unifrac metric, which is a value derived from the phylogenetic tree. Phylogenetic tree is commonly used for measuring the species composition similarities. As shown in Fig. 3, colour block represents the degree distance; more blue colour indicates higher similarity. From the figure we know that the highest degree of similarity was between samples CP3 and CP4, showing that soil depth has less impact on soil fungal diversity. The lowest degree of similarity was between samples CP1 and CP4, indicating that season has more impact on soil fungal diversity. The middle degree of similarity was between samples CP1 and CP2, demonstrating that turfgrass species has some impact on soil fungal diversity. The figure also shows that soil sample collected from vacant land soil is less similar to turfgrass soil samples, indicating that turfgrass establishment has more impact on soil fungal diversity.

Differences of Fungi at Phylum Level

We examined the fungal community compositions of the five samples. A total of 8 phyla were identified in the samples via taxonomic summary, with following five being

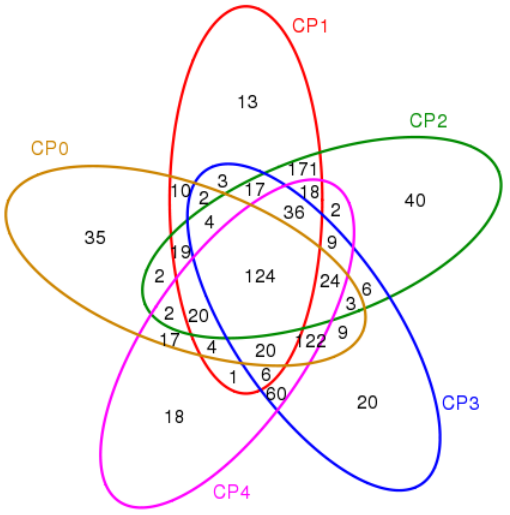

Fig. 2: The venn diagram of the samples.

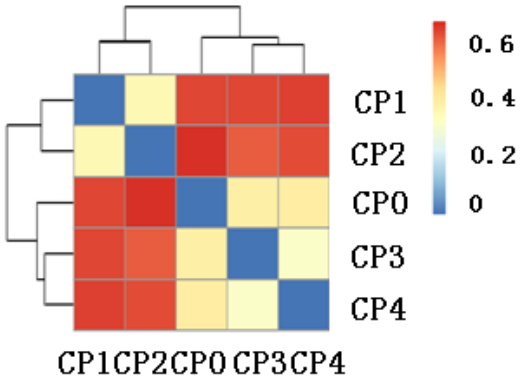

Fig. 3: Beta diversity analysis of different samples.

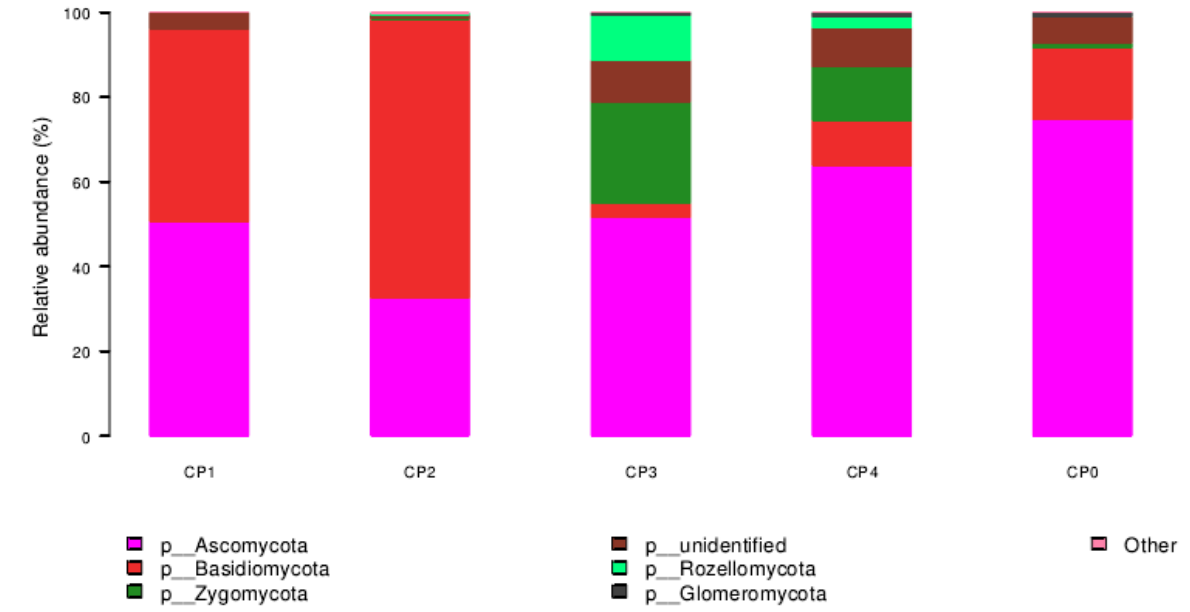

Fig. 4: Fungi-phylum level distribution.

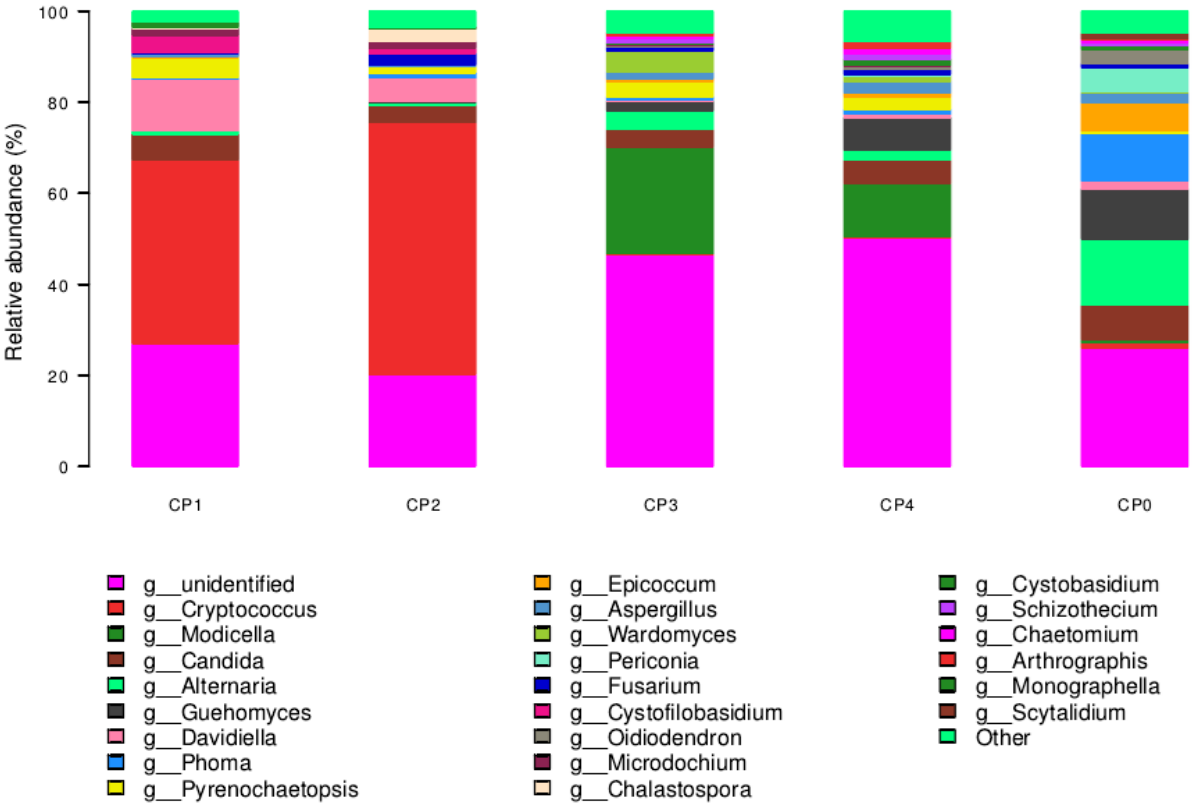

Fig. 5: Fungi-genus level distribution.

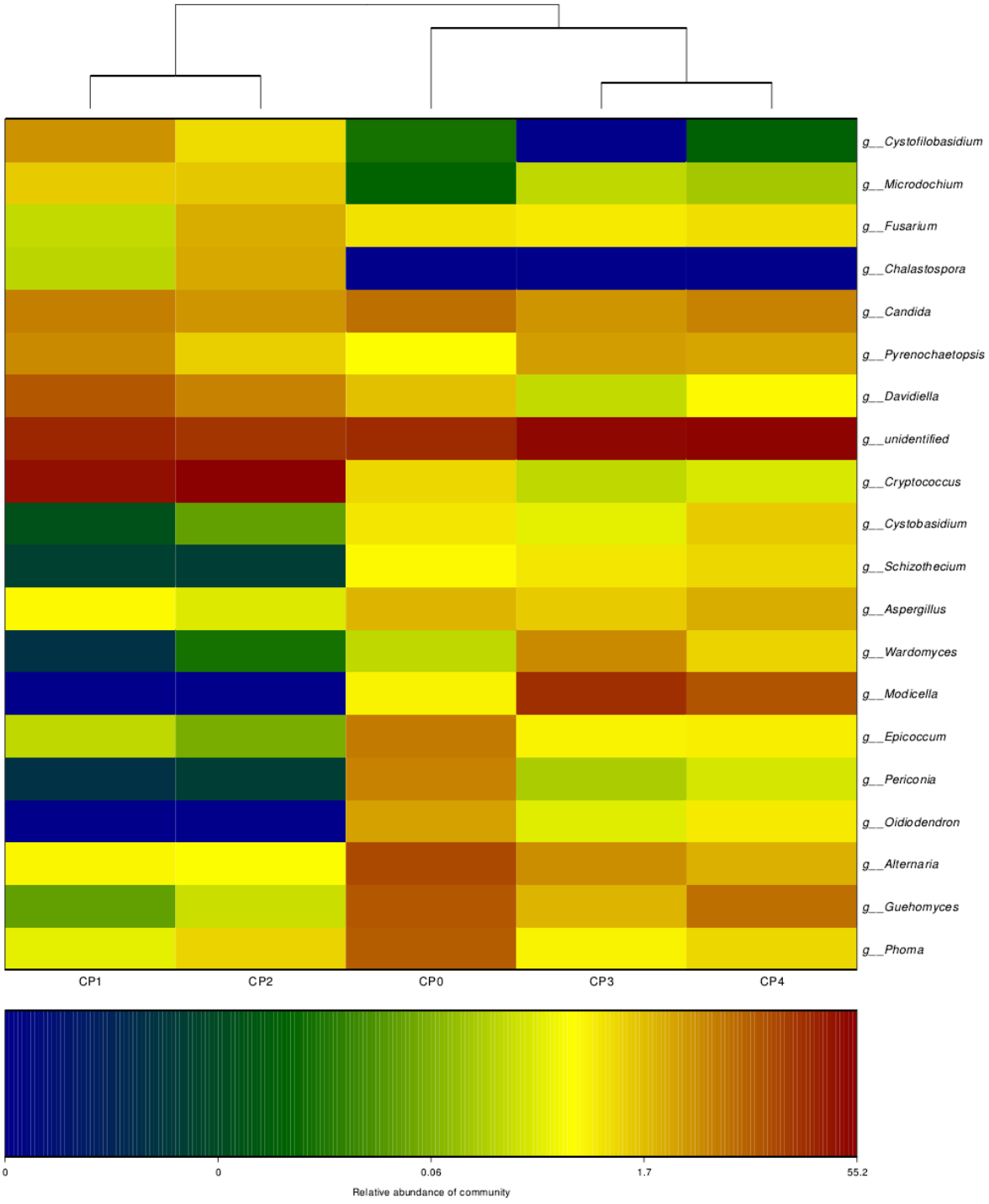

Fig. 6: Heatmap of the samples showing fungal distribution.

dominant and having a relative abundance >1%: Ascomycota, Basidiomycota, Glomeromycota, Zygomycota, Rozellomycota (Fig. 4). The relative abundance of Ascomycota, the dominant phylum in all the five samples, ranged from 32.4% (CP2) to 74.6% (CP0). Basidiomycota was the most dominant phylum in CP2 and second-dominant phylum in CP1 (39.4%), the relative abundance of which ranged from 12.7% to 39.4% in the five

samples. Zygomycota was the second-dominant phylum in CP3 and CP4, non-dominant phylum in the CP1 and CP2, whose relative abundance ranged from 0.08% to 24.01% in the five samples. The relative abundance of Glomeromycota differed between the samples, which ranged from 0.02% to 1.13%. Besides the above fungi phyla, other 3 non-dominant phyla's relative abundance was lower than 1% and differed between the samples.

Differences of Fungi at the Genus Level

At the genus level, unclassified fungi in the four samples accounted for 26.95, 20.23, 46.45, 50.07 and 25.95% for samples CP1, CP2, CP3, CP4 and CP0, respectively (Fig. 5). *Cryptococcus* was the most abundant genus in samples CP1 (40.23%) and CP2 (55.20%), but had low relative abundance in CP3, CP4 and CP0. *Modicella* was the most abundant in sample CP3 (23.45%) and CP4 (11.69%), but had low relative abundance in other samples. *Alternaria*, the most abundant genus in sample CP0 (vacant land soil), had low relative abundance in turfgrass soil (CP1, CP2, CP3 and CP4). Besides *Alternaria*, *Guehomyces* and *Phoma* were dominant in CP0 and had a relative abundance >10%. Besides the above genus, there were large differences in the abundance of *Candida*, *Wardomyces*, *Pyrenochaetopsis*, *Aspergillus*, *Schizothecium*, *Fusarium*, *Cystobasidium*, *Davidiella*, *Microdochium*, *Periconia*, and *Scytalidium*.

We also compared the microbial community across the different samples. A heatmap (Fig. 6) was plotted to estimate the similarities of the samples. An inspection of the tree reveals that sample CP3 and CP4 cluster together, showing that the highest degree of similarity was between the two samples. The heatmap also indicated that the highest degree of similarity was between samples CP1 and CP2.

DISCUSSION

Gene sequencing is a great way to interpret life, and high-throughput sequencing technology is a revolutionary technological innovation in gene sequencing research. This technology is characterized by low cost and high-throughput data. Currently, metabarcoding technology has been widely applied in the field of ecosystem exploration, which offers an opportunity to understand the whole microbial community much more comprehensively than traditional approaches (Bulgarelli et al. 2012). Natural environments as diverse as air, soil, water, plants, as well as various human microbiota have been thoroughly explored by this approach (Peiffer et al. 2013, Edwards et al. 2015, Qin et al. 2016). In the present study, we analysed the difference in soil fungal diversity between samples by Illumina Mi Seq sequencing using specific primers. The results showed that Illumina Mi Seq sequencing is an effective method for analysis of fungal diversity in turfgrass systems.

Soil microbes and plants grow in soil, and thus have a close association (Zhang et al. 2009). Microbes can convert certain nutrients into more usable forms for plants assimilation, while plants can secrete substances that can serve as nutrition to the soil microorganisms (Bais et al. 2006). The changes of soil microbial community are attributed to many factors such as soil nutrients, moisture, pH, temperature soil gases and so on (Zvyagintsev 1994, Fierer et al. 2003, Potthoff et al. 2005, Bardgett et al. 1999, Fritze et al. 2000, Kelley & Hentzen 2001, Pankhurst et al. 2002, Steenwerth et al. 2002). In turfgrass ecosystems, soil is the important component and is useful for mitigating environmental pollution and reducing leaching and runoff of pesticides and fertilizers (Shi et al. 2006, Shi et al. 2007). However, the focus has often been on the turfgrass plant rather than on the broader soil ecology. In the present study, we analysed the soil fungi in turfgrass ecosystem by Illumina Mi Seq sequencing and found that the relative abundance of all the dominant fungal phyla and genera was quite different in the samples, indicating that the soil fungal community structure in the samples is quite different. The results also showed that season (period of turning green), turfgrass species, and soil depths may result in difference in the soil fungal diversity, which is in accordance with our previous studies (Zhang et al. 2016, Zhang & Yao 2017, Zhang et al. 2017, Zhu & Zhang 2017).

CONCLUSIONS

Metabarcoding has been shown to be a powerful tool in exploring a large diversity of natural environments; however, few studies have considered microbiota in turfgrass soil ecosystems until recently. In this study, fungal diversity and communities in turfgrass soil from northwest China were studied by high-throughput sequencing. This is the first study to apply this technology to study microbiota in turfgrass soil. The results presented herein provide insight into the impact of turfgrass on fungal diversity in turfgrass soil and lay a foundation for further investigations into microbiota in turfgrass ecosystems.

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