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Variations of Microbial Community Composition in Turfgrass Soil at Different Depth Using Illumine Sequencing

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Abstract: Many studies have been carried out to assess the effects of plant on soil microorganism communities in different environments. However, little is known about whether soil depths affect soil microbial community in turfgrass ecosystems. Therefore, in the present study the microbial diversity in turf-grass soil at different depth was studied by high-throughput sequencing technique, and the corresponding analysis of microbial composition were conducted. The results showed that the bacteria and fungi in the turf soil at depths of 0-10 cm were richer than that of 10-20 cm. The difference of soil microbial community at the genera level is more significant than that at phylum level. Among bacteria, a total of 3 dominant genus and 148 non-dominant genera were shared by the two samples. Two dominant genera and 12 non-dominant genera were present only in sample CP3 (10-20 cm), while 2 dominant genera and 18 non-dominant genera present only in sample CP4 (0-10 cm). Among fungi, a total of 7 dominant genera and 79 non-dominant genera were shared by the two samples. Ten non-dominant genera were present only in sample CP3, while 6 dominant genera and 21 non-dominant genera present only in sample CP4. The results also indicated that both dominant and non-dominant microbial populations differed greatly in the two samples, as did the overall soil microbial community structure. This study provides previously unknown information regarding the impact of soil depths on microbial communities in turfgrass soil and also lays a foundation for further investigations into microbiota in turfgrass ecosystems.

Key words: High-throughput sequencing technology; soil depths; microbial community.

Introduction

Turfgrasses, including golf courses, parks and home lawns, are essential components of the urban landscape, providing both recreational and environmental benefits [3]. Turfgrasses is a unique ecosystem that consists of closely-spaced turfgrass and the subtending soil. As with any plant-soil ecosystem, turfgrass soils support abundant and diverse microbial populations [22]. By traditional microbial cultivation method, various microbes including fluorescent pseudomonads, Gram-positive bacteria, Gram-negative bacteria, Stenotrophomonas, maltophilia-like bacteria, actinomycetes, and heat-tolerant bacteria had been found in newly constructed golf course putting greens of creeping bentgrass [8]. Yao *et. al*., [25] found that the microbial community composition differed significantly between the turfgrass and pine ecosystems, and to a lesser extent in turf of different ages. Shi *et. al*.,[23] investigated vertical varia-

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tions in the turfgrass soil microbial activity and biomass.

In northwest China, the prevailing turfgrass species are cool-season grasses such as perennial ryegrass and *Festuca elata*, which are most productive at temperatures of 16°C-24°C. Some leaves of these grasses always withered up and fall off in winter, which form the ground litter. Thus it may be hypothesized that soil microorganisms existing in horizons may strongly be influenced by carbon inputs from ground litter rich in rhizogenic organic matter. Using Phospholipid Fatty-acid Analysis (PLFA) techniques, Bartlett [2] indicated that the community structure was signiûcantly different at 0-75 mm from the surface on all areas of the golf course.

Since PLFA methods can only provide a relatively coarse measure of soil microbial community composition and structure, it is possible that changes were not detectable. Recently, high-throughput sequencing technologies which can produce useful high-throughput amplicon data, offer an opportunity to understand the whole microbial community much more comprehensively than traditional approaches [4]. This technique has been successfully used in the study of the microbial composition of different type of samples [14,19]. However, relatively little research has addressed the microbial community structure in turfgrass systems using high-throughput sequencing technology.

In this study, our objective was to making an inventory of the diversity of both soil bacterial and fungal communities in turfgrass ecosystems at different depths using Illumina Miseq approaches. The data generated, particularly the differences in the distribution of particular taxonomic groups, was used to evaluate soil depths effect on soil microbial community.

Materials and methods

Sampling site

The sampling sites is turfgrass plots before No. 3 teaching building, which was established in 2013 and located in the Campus of Gansu Agricultural University, Lanzhou, Gansu Province (103.698°E, 36.091°N). Turf species in the sampling site are perennial ryegrass.

Soil collection

Soil collection was conducted on April 25, 2016 (after the grass turned green). Five soil cores were randomly collected at depth of 0-10 cm (CP4) and 10-20 cm (CP3) using quincunx sampling method and homogenized into one sample. Soil samples were passed through a 2.0–mm sieve and quickly frozen using liquid nitrogen, and stored at "80°C prior to DNA extraction.

DNA extraction

DNA was extracted from 0.2 g of the pellet soil using an E.Z.N.A. Soil DNA Kit D5625-01 (OMEGA, Norcross, GA, USA) according to the manufacturer's instructions. The extracted DNA was quantified using a Qubit 2.0 spectrophotometer (Invitrogen, Carlsbad, CA, USA), and the integrity of the extracted DNA from the soil was confirmed by electrophoresis in a 1% agarose gel.

Illumina Mi Seq sequencing

Next generation sequencing library preparations and Illumina Mi Seq sequencing were conducted at ALLWEGENE Inc. (Beijing, China). The bacterial 16S rRNA gene was amplified with the 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) primers targeting the V3-V4 region. The fungal 18S rRNA gene was amplified with the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) 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 bacterial 16S rRNA gene PCR thermal cycle profile was as follows: 5 min at 95°C followed by 25 cycles of 30 s at 95°C, 30 s at 56°C, and 40 s at 72°C and then final extension for 10 min at 72°C, after which the samples were held at 4°C. The fungal 18S r RNA gene PCR thermal cycle profile was similar to that of the bacterial profile except that it had five more cycles.

Processing of high-throughput sequencing data

Amplicons were sequenced using a pair-end method by Illumina Miseq with a six cycle index read. Raw data generated from the high-through-

put sequencing run were processed and analyzed following the pipelines of Mothur [20] and QIIME [5]. Sequence reads were trimmed so that the average Phred quality score for each read was above 20. After trimming, these reads were assembled using the Flash software [11] and reads that could not be assembled were discarded. Chimera sequences were identified and removed using UCHIME [6]. Quality sequences were subsequently assigned to samples according to their unique 7 bp barcode and sequences clustering were performed by uclust (QIIME) with a similarity cutoff of 97 %, after which samples were clustered into operational taxonomic units (OTUs) [11]. This dataset was available in the SRA at the NCBI under accession number PRJNA340319.

Diversity and statistical analysis

The relative abundance (%) of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon versus the number of total sequences obtained for that sample. 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/). The community structure was analyzed statistically at different classification levels.

Results

Diversity analyses of microbial communities

After the quality control, a total of 52,020 high-quality 16S r RNA gene sequences (20,764 for CP3 and 31,256 for CP4) were recovered from the two samples. Additionally, a total of 89,618 validated 18S r RNA gene sequences reads were recovered (38,441 for CP3 and 51,177 for CP4; Table 1). The Good's coverage of the all the samples ranged from 98.48 to 99.73 %, which indicated an adequate level of sequencing to identify the majority of diversity in the samples. In terms of OTU number, sample CP4 had the richest diversity of bacterial and fungal communities (1264 OTUs and 483 OTUs, respectively). The Shannon and Chao1 indices revealed a higher bacterial and fungal diversity in CP4, showing that the bacteria and fungi in the soil at 0-10 cm were richer than that at 10-20 cm.

Differences of bacteria at phylum level

Table 2 showed the difference of bacterial abundance in the samples at phylum level. A total of 17 phyla were identified in the two samples via taxonomic summary, with following seven being dominant and having a relative abundance >1 %: Acidobacteria, Proteobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Firmicutes and Actinobacteria. The relative abundance of 10 non-dominant phyla was lower. The phylum abundance differed between the samples. The abundance of nine phyla in sample CP4 is higher than that of CP3, and the abundance of the remaining eight phyla is lower. Among them, the difference in the relative abundance of 4 phyla was higher than 1 %, while that of 6 phyla ranged from 0.1 % to 1 %, and that of the remaining 7 was lower than 0.1 %. Chloroflexi have the biggest difference of relative abundance, while Deinococcus-Thermus have the smallest difference of relative abundance in the two samples. Deinococcus-Thermus were not present in the sample CP4.

Difference of bacteria at genus level

A total of 173(CP3) and 167(CP4) bacterial genera were identified in the two samples via taxonomic summary, respectively. The abundance of dominant bacterial genus in the samples was showed in Table 3. Three dominant genera were shared by the two samples, both of which had a

Table 1. Operational taxonomic units, Good's Coverage, Chao1and Shannon's Index for 16S r RNA and 18S r RNA sequencing of the samples

Sample ID	Reads	OUT	Good's Coverage	Chao1	Shannon
CP3_B	20764	1238	98.58%	1421.78	8.79
CP4_B	31256	1264	98.48%	1424.88	9.03
CP3_F	38441	465	99.73%	478.28	5.12
CP4_F	51177	483	99.68%	540.71	5.86

Table 2. Differences in bacterial abundance at the phylum level

Phylum	Relative abundance CP3	CP4	Difference ofrelative abundance
Proteobacteria	39.67%	43.88%	4.21%
Acidobacteria	19.29%	22.37%	3.07%
Bacteroidetes	6.79%	7.72%	0.93%
Firmicutes	2.03%	2.57%	0.54%
Verrucomicrobia	0.21%	0.47%	0.26%
Planctomycetes	0.44%	0.60%	0.16%
Chlamydiae	0.03%	0.12%	0.09%
Cyanobacteria	0.08%	0.15%	0.07%
Elusimicrobia	0.11%	0.14%	0.03%
Unidentified	1.46%	1.47%	0.01%
Deinococcus-Thermus	0.01%	0.00%	-0.01%
Spirochaetae	0.02%	0.01%	-0.02%
Chlorobi	0.09%	0.05%	-0.03%
Fibrobacteres	0.41%	0.33%	-0.08%
Nitrospirae	0.39%	0.16%	-0.23%
Gemmatimonadetes	3.29%	2.45%	-0.84%
Actinobacteria	14.57%	12.73%	-1.84%
Chloroflexi	11.11%	4.79%	-6.32%

Table 3. Differences in the abundance of the dominant bacterial genus

Genus	Relative abundance CP3	CP4	Difference ofrelative abundance
Sphingomonas	2.405%	1.796%	-0.609%
Chryseolinea	1.583%	2.400%	0.817%
Pseudomonas	1.522%	2.459%	0.937%
Gaiella	1.260%	0.996%	-0.265%
Haliangium	1.163%	0.873%	-0.290%
Pedomicrobium	0.718%	1.182%	0.464%
Steroidobacter	0.651%	1.137%	0.485%

relative abundance higher than 1 %. Additionally, *Pedomicrobium* and *Steroidobacter* were dominant only in the sample CP4, while *Gaiella* and *Haliangium* were dominant only in sample CP3. Specifically, the abundance of 3 genera were higher in sample CP3 than CP4, while that of the remaining 4 genera were lower. Additionally, the difference in the relative abundance of all 7 genera ranged from 0.1 % to 1 %. The abundance of *Pseudomonas* in the two samples was 1.522 % and 2.459 %, which have the biggest difference in the two samples.

A total of 12 non-dominant genera were present only in CP3 (Table 4). Among them, the abundance of 9 genera' abundance ranged from 0.01 % to 0.1 %, while that of 3 genera showed abundances lower than 0.01 %. As is shown in Table 5, a total of 18 non-dominant genera were present only in sample CP4, and their relative abundance was lower. Among them, the abundance of 8 genera' abundance ranged from 0.01 % to 0.1 %, while that of 10 genera showed abundances lower than 0.01 %.

A total of 148 non-dominant genera were shared

Table 4. Non-dominant bacterial genera present only in sample CP3

Genus	Relative abundance	Genus	Relative abundance
Rhodopseudomonas	0.006%	*Geodermatophilus*	0.012%
Streptococcus	0.006%	*Nitrosospira*	0.012%
Truepera	0.006%	*Rhodocytophaga*	0.012%
Anaeromyxobacter_dehalogenans	0.012%	*Sediminibacterium*	0.012%
Coxiella	0.012%	*Oscillochloris*	0.030%
Ferrovibrio	0.012%	*Herpetosiphon*	0.055%

Table 5. Non-dominant bacterial genera present only in sample CP4

Genus	Relative abundance	Genus	Relative abundance
Haloactinopolyspora	0.055%	*Microcoleus*	0.009%
Azotobacter	0.036%	*Paracoccus*	0.009%
Enterococcus	0.032%	*Rubritepida*	0.009%
Nocardia	0.018%	*Thermomonospora*	0.009%
Aquabacterium	0.014%	*Alkaliphilus*	0.005%
Methylobacterium	0.014%	*Cytophaga*	0.005%
Planifilum	0.014%	*Methylotenera*	0.005%
Wolbachia	0.014%	*Roseburia*	0.005%
Bartramia pomiformis	0.009%	*Tahibacter*	0.005%

by the two samples, all of which had a relative abundance of < 1 %. The abundance of 63 genera in sample CP4 is higher than that of CP3, while that of another 85 were lower than CP3. Among them, the difference in the relative abundance of 23 genera was higher than 0.1 %, while that of 88 genera ranged from 0.01 % to 0.1 %, and that of the remaining 37 genera was lower than 0.01 %.

Difference of fungi at phylum level

A total of seven phyla were identified in the two samples via taxonomic summary. Figure 1 showed the difference of fungal abundance between samples at phylum level. The four dominant phyla presented in the two samples were Ascomycota, Basidiomycota, Rozellomycota and Zygomycota, which had a relative abundance higher than 1 %. Glomeromycota were only dominant in sample CP4. The relative abundance of 2 non-dominant phyla was lower. The abundance of all the phyla is quite different in the two samples. Among them, the abundance of Ascomycota is the highest in the two samples, while that of Cercozoa is the smallest.

Difference of fungi at genus level

A total of 113 and 102 fungal genera were identified in the two samples via taxonomic summary, respectively. The abundance of dominant fungal genera in the samples is shown in Figure 2. Seven dominant genera were shared by the two samples, with a relative abundance higher than 1 %. Additionally, 6 genera were dominant only in the sample CP4. The abundance of the other genera was quite different between the two samples. Specifically, the abundance of 9 genera was higher in sample CP4 than CP3, while that of the remaining 4 genera was lower. The difference in the relative abundance of 2 genera was higher than 5 %, while that of 5 genera ranged from 1 % to 5 %, and the abundance of the remaining 7 genera was less than 1 %. The abundance of *Modicella* in the

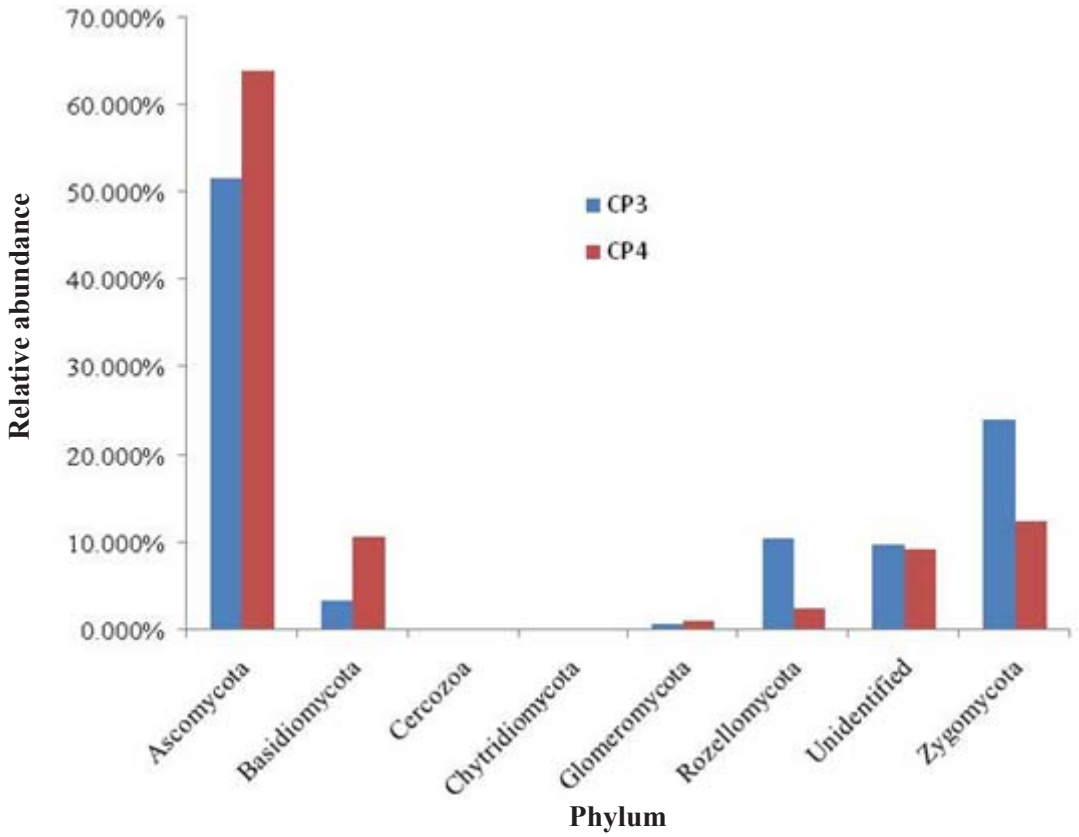

Fig. 1. Difference in fungal abundance at the phylum level

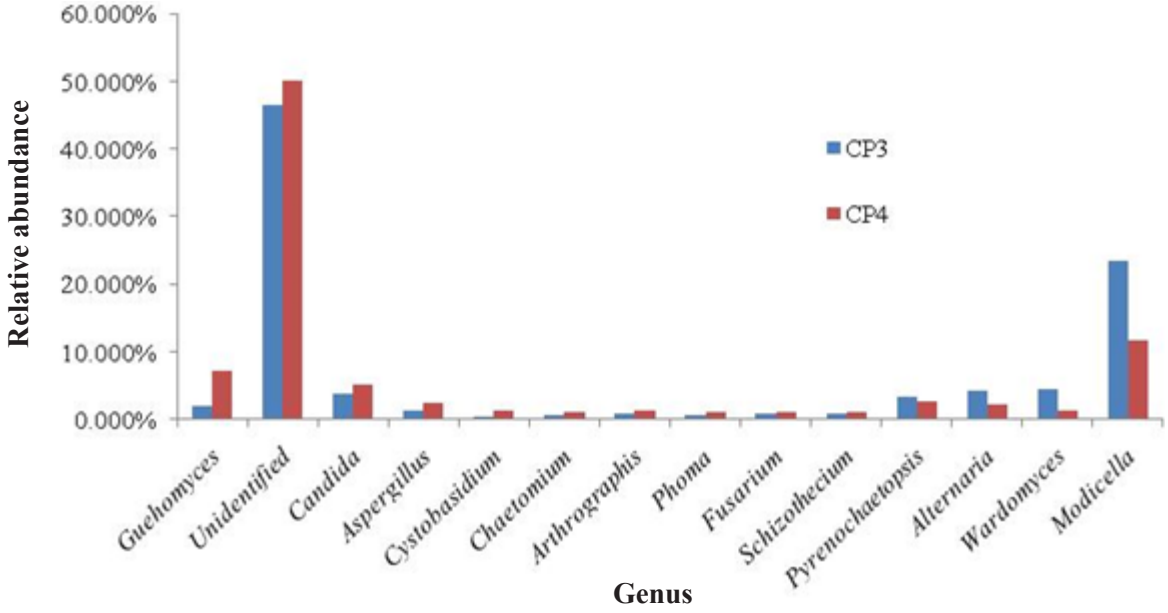

Fig. 2. Difference in abundance of the dominant fungal genera

two samples was 23.447 % and 11.693 %, which have the biggest difference in the two samples.

As is shown in Figure 3, a total of 10 non-dominant genera were present only in sample CP3, and their relative abundance was lower. The abundance of all genera' abundance was lower than

0.01 %. A total of 21 non-dominant genera were present only in sample CP4 (Figure 4). Among them, the abundance of 3 genera' abundance ranged from 0.01% to 0.1%, while that of the remaining 8 genera showed abundances lower than 0.01 %.

A total of 79 non-dominant genera were shared by the two samples, with a relative abundance of

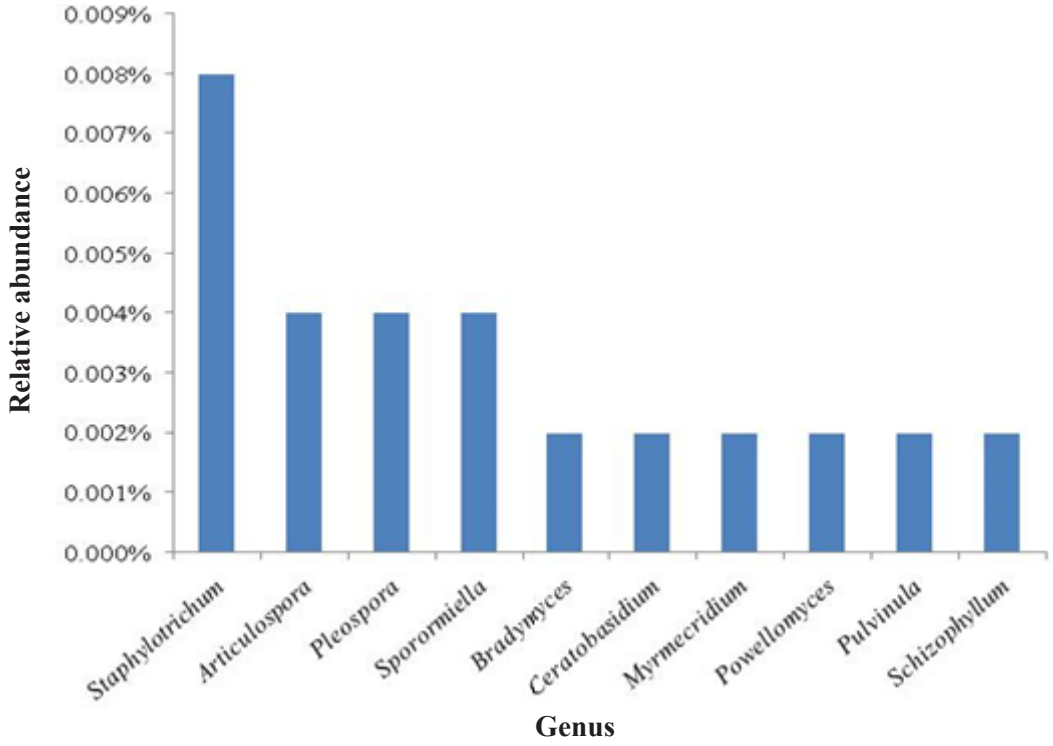

Fig. 3. Non-dominant fungal genera present only in sample CP3

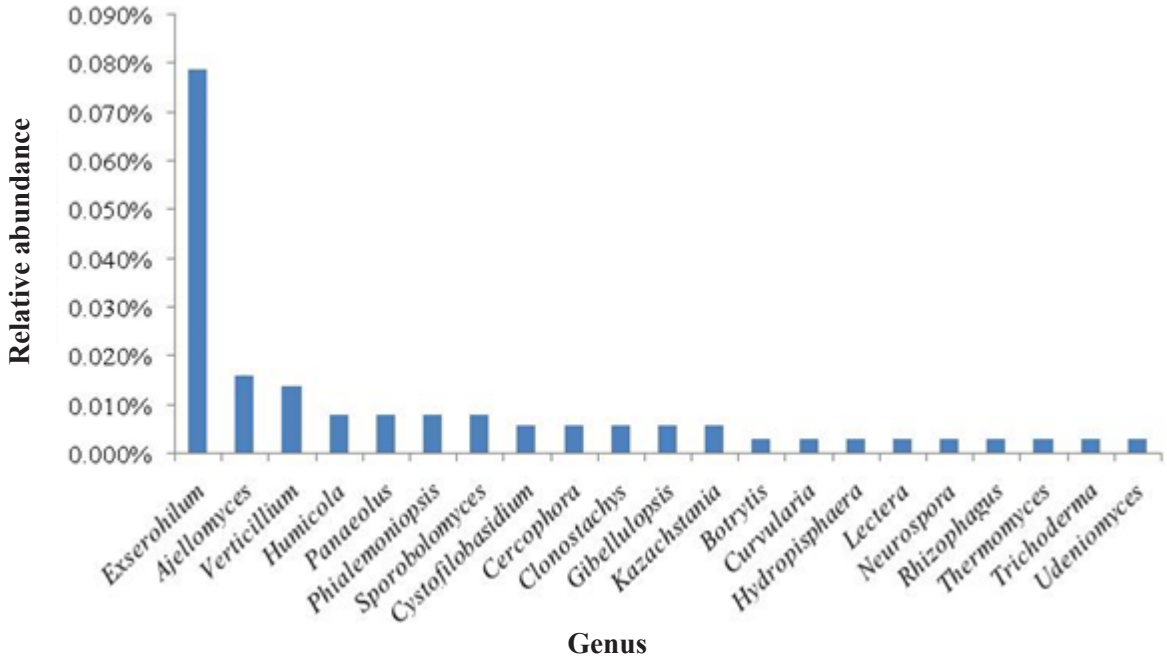

Fig. 4. Non-dominant fungal genera present only in sample CP4

lower than 1 %. The abundance of 53 genera was higher in sample CP4 than CP3, while that of 25 genera were lower, and the abundance of one genus is equal in two samples. Among them, the difference in the relative abundance of 16 genera were higher than 0.1 %, while that of 30 genera ranged from 0.01 % to 0.1 %, and that of the remaining 32 genera were lower than 0.01 %.

Discussion

Turfgrass systems are being recognized for enhancing land restoration and for protecting soil, air and water in urban, suburban and rural communities [23]. A variety of research has examined the ability of turfgrass systems to mitigate environmental pollution and reduce leaching and runoff of pesticides and fertilizers [22]. However, the focus has often been on the turfgrass plant rather than on the broader soil ecology. There is a need for basic information on soil microbial community in turfgrass systems [22]. In the present study, we detected more species of microorganisms and further analyzed the difference in microbial communities 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 the soil microbial community structure in turfgrass.

Numerous studies have been carried out about the soil microbial community of other plants such as wheat, rice, and maize [7,16,18]. Previous study emphasized on the dominant microbes in the soil, the non-dominant bacteria and fungi usually have been ignored in the analysis of the microbial community structure [12,16,21]. In this study, we found that the relative abundance of non-dominant microbes is quite different in the two samples. 12 non-dominant bacterial genera and 10 non-dominant fungal genera were present only in sample CP3. 18 non-dominant bacterial genera and 21 non-dominant fungal genera present only in sample CP4. The results indicated that the soil microbial community structure of non-dominant genera in the two samples is quite different.

Although typically grown as monoculture, turfgrasses produce a highly diverse soil envirommenf for microorganisms [22]. The changes of soil microbial community are attributed to many factors such as soil nutrients, moisture, pH, temperature soil gases and so on [9,26]. Potthoff, *et. al*., [17] reported that the vertical composition and distribution of soil microorganisms would also be impacted by root distribution. The effects of abiotic conditions on soil microbial community composition have been studied in annual cropping systems, grasslands, wetlands, and forest systems [1,10,13,15,24]. In this study, we found that the bacteria and fungi in the turfgrass soil at 0-10 cm were richer than that at 10-20 cm, showing that soil depths may result in difference of soil microbial communities. The difference of soil resource availability, soil physical and chemical characteristics, and other abiotic conditions at different depths may affect the activity of soil microorganisms.

In conclusion, the bacteria and fungi in the turfgrass soil at depths of 0-10 cm were richer than that of 10-20 cm. The difference of soil microbial community at the genera level is more significant than that at phylum level. Both dominant and non-dominant genera differed greatly in the two samples, as did the overall soil microbial community structure.

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