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Effect of Turfgrass Establishment on Soil Microbiota Using Illumina Sequencing

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ABSTRACT

Soil bacteria and fungi play key roles in ecosystem functioning and the maintenance of soil fertility. Many studies have been carried out to assess the effects of plant on microorganism communities in different environments. However, little is known about whether turfgrass establishment affects soil microbial community. Therefore, in the present study, the microbial diversity in turfgrass soil and vacant land soil were 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 soil of turfgrass are richer than that of vacant land. The difference of soil microbial community at the genera level is more significant than that at phylum level. Among bacteria, a total of one dominant genus and 91 non-dominant genera were shared by the two samples. Five dominant genera and 66 non-dominant genera were present only in sample CP4 (turfgrass soil), and 13 dominant genera and 17 non-dominant genera only in sample CP0 (vacant land). Among fungi, a total of 5 dominant genera and 71 non-dominant genera were shared by the two samples, 8 dominant genera and 23 non-dominant genera were present only in sample CP4, and 6 dominant genera and 20 non-dominant genera only in sample CP0. 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 turfgrass establishment on soil microbial communities and also lays a foundation for further investigations into microbiota in turfgrass soil.

INTRODUCTION

Soil microbes and plants grow in soil and have a close association (Zhang et al. 2012). Microbes can convert certain nutrients into more usable forms for plants assimilation, while plants can secrete substances that provide nutrition to the soil microorganisms (Bais et al. 2006). Beneficial soil microbes also contribute to pathogen resistance, water retention, and synthesis of growth-promoting hormones (Mendes et al. 2011, Berendsen et al. 2012, Bulgarelli et al. 2013). There are many factors that affect the soil microbial community composition and structure, such as soil type, management practice, and plant species (Bossio et al. 1998, Webster et al. 2002, Clegg et al. 2003, Girvan et al. 2003). Previous studies have also reported that plant species were important determinant of soil microbial communities (Nusslein & Tiedje 1999, Grayston et al. 2001).

Turf is a unique ecosystem that consists of closely-spaced turfgrass and the subtending soil (Shi et al. 2006, 2007). As with any plant-soil ecosystem, turfgrass soils support abundant and diverse microbial populations. 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 have been found in the newly constructed golf course, putting greens of creeping bentgrass (Elliott et al. 2003). In the further study, they found that microbial population densities were only slightly affected by temporal and spatial changes in the soil environment (Elliott et al. 2004). In the recent years, soil microbial communities of turfgrass were fingerprinted using phospholipid fatty acid (PLFA) composition. Yao et al. (2006) examined soil microbial community composition and diversity in a turfgrass chronosequence of bermudagrass (1 to 95 years old) and compared with those in adjacent native pines ecosystems. The microbial community composition differed mainly between the turfgrass and pine ecosystems, and to a lesser extent in turf of different ages. Microbial community composition in urban lawns dominated by Kentucky

bluegrass, identified using PLFA techniques, was also similar to that in the arable soils and in the shortgrass prairie ecosystem (Kaye et al. 2005). But, to our knowledge, no information is available about the effect of turf establishment on soil microbial community composition and structure.

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 (Bulgarelli et al. 2012). This technique has been successfully used in the study of the microbial composition of different type of samples (Lundberg et al. 2012, Schlaeppi et al. 2014). 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 make an inventory of the diversity of both soil bacterial and fungal communities in turfgrass ecosystems using Illumina Miseq approaches. The data generated, particularly the differences in the distribution of particular taxonomic groups, were used to evaluate turfgrasses establishment effect on soil microbial community.

MATERIALS AND METHODS

Sampling site: The sampling sites are the 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. Sample CP4 was collected from the turfgrass plots, while CP0 was collected from the vacant land adjacent to the turfgrass. The physico-chemical properties of the two soil samples were identical before turfgrass was established.

Soil collection: Soil collection was conducted on April 25, 2016 (after the grass turned green). Five soil cores were randomly collected at a depth of 0-10 cm 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 (GGACTACH VGGGTWTCTAAT) primers targeting the V3-V4 region. The fungal 18S rRNA gene was amplified with the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (TGCGTTC TTCATCGATGC) 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 Mi Seq with a six cycle index read. Raw data generated from the high-throughput sequencing run were processed and analysed following the pipelines of Mothur (Schlosset et al. 2009) and QIIME (Caporaso et al. 2010). The 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 (Fu et al. 2015) and reads that could not be assembled were discarded. Chimera sequences were identified and removed using UCHIME (Edgar et al. 2011). Quality sequences were subsequently assigned to the 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) (Fu et al. 2015). 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 Chao 1 and Shannon coverage indices, were performed using the summary single command of the MOTHUR software (http://www.mothur.org/). The community structure was analysed statistically at different classification levels.

RESULTS AND DISCUSSION

Diversity analyses of microbial communities: After the quality control, a total of 57,127 high-quality 16S r RNA

gene sequences (31,256 for CP4 and 25,871 for CP0) were recovered from the two samples. Additionally, a total of 72,120 validated 18S r RNA gene sequences reads were recovered (38,441 for CP4 and 33,679 for CP0; Table 1). The Good's coverage of all the samples ranged from 98.48 to 99.81%, 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 turfgrass soil is richer than that of vacant land soil.

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 10 phyla in sample CP4 is higher than that of CP0, and the abundance of the remaining seven phyla is lower. Among them, the difference in the relative abundance of 4 phyla was higher than 10%, while that of 3 phyla ranged from 1% to 3%, and that of the remaining 10 was lower than 1%. Actinobacteria has the biggest difference of relative abundance, while Spirochaetae has the smallest difference of relative abundance in the two samples. Deinococcus-Thermus were not present in the sample CP4, while Chlamydiae and Elusimicrobia were absent in the sample CP0.

Difference of bacteria at genus level: The difference of bacterial community at the genera level is more significant than that at phylum level. A total of 173 and 126 bacterial genera were identified in the two samples via taxonomic summary, respectively. The abundance of dominant bacterial genus in the samples is presented in Table 3. Two dominant genera were shared by the two samples, both of which had a relative abundance higher than 1%. Additionally, 4 genera were dominant only in the sample CP4, while 13 were dominant only in sample CP0. Specifically, the abundance of five genera was higher in sample CP4 than CP0, while that of the remaining 13 genera was lower. Additionally, the difference in the relative abundance of 3 genera was higher than 5%, while that of 14 genera ranged from 1% to 5%, and the abundance of the remaining genera was less than 1%. The abundance of *Bacillus* in the two samples was 0.459% and 9.908%, which is the biggest difference in the two samples.

Table 1: OTUs, Good's coverage, Chao1and Shannon's Index for 16S r RNA and 18S r RNA sequencing of the samples.

Sample ID	Reads	OTU	Good's Coverage	Chao1	Shannon
CP4_B	31256	1264	98.48%	1424.88	9.03
CP0_B	25871	615	99.13%	750.37	6.89
CP4_F	38441	483	99.68%	540.71	5.86
CP0_F	33679	417	99.81%	459.02	5.44

Table 2: Differences in bacterial abundance at the phylum level.

Phylum	Relative abundance CP4	CP0	Difference of relative abundance
Actinobacteria	12.729%	39.352%	26.623%
Firmicutes	2.569%	12.725%	10.156%
Gemmatimonadetes	2.446%	4.696%	2.250%
Cyanobacteria	0.145%	0.389%	0.244%
Verrucomicrobia	0.468%	0.643%	0.175%
Nitrospirae	0.159%	0.276%	0.117%
Deinococcus-Thermus	0.000%	0.113%	0.113%
Spirochaetae	0.009%	0.000%	-0.009%
Chlorobi	0.055%	0.005%	-0.050%
Bacteroidetes	7.724%	7.657%	-0.066%
Chlamydiae	0.118%	0.000%	-0.118%
Elusimicrobia	0.136%	0.000%	-0.136%
Fibrobacteres	0.332%	0.005%	-0.327%
Planctomycetes	0.605%	0.009%	-0.596%
Unidentified	1.473%	0.254%	-1.219%
Chloroflexi	4.787%	3.356%	-1.431%
Proteobacteria	43.879%	28.932%	-14.947%
Acidobacteria	22.367%	1.589%	-20.777%

Table 3: Differences in the abundance of the dominant bacterial genera.

Genus	Relative abundance CP4	CP0	Difference ofrelative abundance
Bacillus	0.459%	9.908%	9.449%
Arthrobacter	0.336%	7.485%	7.149%
Massilia	0.086%	5.774%	5.688%
Nocardioides	0.586%	4.297%	3.711%
Xanthomonas	0.000%	2.522%	2.522%
Pontibacter	0.000%	2.051%	2.051%
Adhaeribacter	0.564%	2.540%	1.976%
Gemmatimonas	0.077%	1.897%	1.820%
Lactococcus	0.032%	1.662%	1.630%
Caenimonas	0.132%	1.589%	1.457%
Marmoricola	0.205%	1.553%	1.348%
Patulibacter	0.036%	1.259%	1.223%
Peredibacter	0.018%	1.114%	1.096%
Sphingomonas	1.796%	1.779%	-0.017%
Steroidobacter	1.136%	0.049%	-1.087%
Pedomicrobium	1.182%	0.023%	-1.159%
Pseudomonas	2.459%	0.0951%	-2.364%
Chryseolinea	2.400%	0.004%	-2.396%
Unidentified	69.214%	42.281%	-26.933%

As is given in Table 4, a total of 66 non-dominant genera were present only in sample CP4, and their relative abundance was lower. Among them, the abundance of 11 genera was higher than 0.1%, while that of 37 genera ranged from 0.01% to 0.1%, the remaining genera showed abundances lower than 0.01%. A total of 17 non-dominant genera were present only in CP0 (Table 5). Among them, the abundance of five genera was higher than 0.1%, while that of ten genera was higher than 0.01%, and the abundance of the remaining genera was less than 0.01%.

The abundance of non-dominant bacterial genera present in both the samples is given in Table 6. A total of 91 non-dominant genera were shared by the two samples, all of which had a relative abundance of < 1%. The abundance of 29 genera in sample CP4 is higher than that of CP0, while that of another 60 was lower than CP0, and the abundance of *Roseburia* and *Opitutus* is equal in two samples. Among them, the difference in the relative abundance of 47 genera was higher than 0.1%, while that of 31 genera ranged from 0.01% to 0.1%, and that of the remaining 13 genera was lower than 0.01%.

Difference of fungi at phylum level: A total of eight phyla were identified in the two samples via taxonomic summary. Table 7 shows the difference of fungal abundance between samples at phylum level. The three dominant phyla presented in the two samples were Ascomycota, Basidiomycota and Zygomycota, which had a relative abundance higher than 1%. The relative abundance of 5 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. Zygomycota have the biggest difference in the two samples, while Chytridiomycota have the smallest difference. Blastocladiomycota were only present in sample CP0.

Difference of fungi at genus level: The differences of fungal community at the genera level are more significant than that at phylum level. A total of 113 and 110 fungal genera were identified in the two samples via taxonomic summary, respectively. The abundance of dominant fungal genera in the samples is given in Table 8. Five dominant genera were shared by the two samples, with a relative abundance higher than 1%. Additionally, 8 genera were dominant only in the sample CP4, while 4 were dominant only in sample CP0. The abundance of the other genera was quite different between the two samples. Specifically, the abundance of 10 genera was higher in sample CP4 than CP0, while that of the remaining 10 genera was lower. The difference in the relative abundance of 5 genera was higher than 5%, while that of 8 genera ranged from 1% to 5%, and the abundance of the remaining 6 genera was less than 1%. The abundance of *Alternaria* in the two samples was 2.249% and 14.603%, which is the biggest difference in the two samples.

Table 4: Non-dominant bacterial genera present only in sample CP4.

Genus	Relative abundance	Genus	Relative abundance
Rhodomicrobium	0.722%	*Chitinibacter*	0.023%
Hirschia	0.550%	*Lacibacter*	0.023%
Catelliglobosispora	0.468%	*Polaromonas*	0.023%
Flavobacterium	0.372%	*Crocinitomix*	0.018%
Ohtaekwangia	0.290%	*Dongia*	0.018%
Acinetobacter	0.204%	*Phaselicystis*	0.018%
Byssovorax	0.200%	*Rhizomicrobium*	0.018%
Marinicella	0.191%	*Actinocorallia*	0.014%
Leptothrix	0.182%	*Agaricicola*	0.014%
Turicibacter	0.168%	*Aquabacterium*	0.014%
Solitalea	0.127%	*Asteroleplasma*	0.014%
Chitinophaga	0.095%	*Azospirillum*	0.014%
Neochlamydia	0.091%	*Cylindrotheca closterium*	0.014%
Aquicella	0.086%	*Planifilum*	0.014%
Planosporangium	0.082%	*Wolbachia*	0.014%
Nakamurella	0.073%	*Acidiferrobacter*	0.009%
Dactylosporangium	0.068%	*Anaeromyxobacter*	0.009%
Ferruginibacter	0.064%	*Bartramia pomiformis*	0.009%
Haloferula	0.059%	*Dysgonomonas*	0.009%
Haloactinopolyspora	0.055%	*Leptolyngbya*	0.009%
Nannocystis	0.055%	*Sphingopyxis*	0.009%
Rhizobacter	0.055%	*Thermomonospora*	0.009%
Labrys	0.050%	*Turneriella*	0.009%
Legionella	0.050%	*Alkaliphilus*	0.005%
Pseudolabrys	0.046%	*Anaerotruncus*	0.005%
Mucilaginibacter	0.041%	*Cytophaga*	0.005%
Azotobacter	0.036%	*Fluviicola*	0.005%
Thermoactinomyces	0.036%	*Incertae Sedis*	0.005%
Dyadobacter	0.032%	*Litorilinea*	0.005%
Simiduia	0.032%	*Phycisphaera*	0.005%
Alistipes	0.027%	*Sandaracinus*	0.005%
Solibacillus	0.027%	*Tahibacter*	0.005%
Sphingobacterium	0.027%	*Taibaiella*	0.005%

Table 5: Non-dominant bacterial genera present only in sample CP0.

Genus	Relative abundance	Genus	Relative abundance
Hymenobacter	0.539%	*Leuconostoc*	0.041%
Streptococcus	0.204%	*Truepera*	0.027%
Sporichthya	0.190%	*Lactobacillus*	0.023%
Carnobacterium	0.140%	*Rhodocytophaga*	0.023%
Nitrosomonas	0.104%	*Bacteriovorax*	0.014%
Deinococcus	0.086%	*Pseudobutyrivibrio*	0.014%
Aciditerrimonas	0.077%	*Nitrosospira*	0.009%
Planomicrobium	0.077%	*Parasegetibacter*	0.005%
Geodermatophilus	0.049%		

As is given in Table 9, a total of 23 non-dominant genera were present only in sample CP4, and their relative abun-

Table 6: Non-dominant bacterial genera present in both the samples.

Genus	Difference of relative abundance	Genus	Difference of relative abundance
Rubellimicrobium	0.874%	*Cellvibrio*	-0.027%
Microvirga	0.638%	Pseudoxanthomonas	-0.027%
Blastococcus	0.561%	*Sporosarcina*	-0.032%
Streptomyces	0.493%	*Sorangium*	-0.032%
Pedobacter	0.439%	*Lysinibacillus*	-0.041%
Enterococcus	0.349%	*Paucimonas*	-0.041%
Chthoniobacter	0.190%	*Actinomadura*	-0.041%
Hyalangium	0.181%	*Actinoplanes*	-0.050%
Flavisolibacter	0.167%	*Geminicoccus*	-0.055%
Euzebya	0.163%	*Candidatus alysiosphaera*	-0.082%
Bdellovibrio	0.131%	Candidatus *entotheonella*	-0.082%
Ornithinibacter	0.117%	*Nonomuraea*	-0.086%
Paracoccus	0.109%	*Skermanella*	-0.087%
Pseudonocardia	0.108%	*Roseiflexus*	-0.096%
Paenibacillus	0.104%	*Candidatus solibacter*	-0.100%
Roseomonas	0.104%	*Woodsholea*	-0.100%
Bosea	0.059%	*Nordella*	-0.100%
Methylotenera	0.050%	*Saccharopolyspora*	-0.105%
Bacteroides	0.045%	*Gaiella*	-0.126%
Citrobacter	0.045%	*Kineosporia*	-0.136%
Caulobacter	0.036%	*Thermomonas*	-0.137%
Cohnella	0.023%	*Agromyces*	-0.141%
Pseudospirillum	0.018%	*Bauldia*	-0.146%
Brevundimonas	0.018%	*Arenimonas*	-0.146%
Defluviicoccus	0.014%	*Amaricoccus*	-0.146%
Nocardia	0.009%	*Altererythrobacter*	-0.151%
Sporocytophaga	0.009%	*Phenylobacterium*	-0.168%
Rubritepida	0.005%	*Rhodoplanes*	-0.177%
Kribbella	0.004%	*Devosia*	-0.178%
Roseburia	0.000%	*Enhygromyxa*	-0.196%
Opitutus	0.000%	*Luedemannella*	-0.227%
Microcoleus	-0.005%	*Solirubrobacter*	-0.247%
Williamsia	-0.005%	*Bryobacter*	-0.264%
Azoarcus	-0.005%	*Terrimonas*	-0.273%
Methylobacterium	-0.009%	*Hyphomicrobium*	-0.277%
Pelagibius	-0.009%	*Reyranella*	-0.277%
Rhodococcus	-0.009%	*Iamia*	-0.292%
Flavitalea	-0.009%	*Mycobacterium*	-0.300%
Saccharomonospora	-0.014%	*Rhodobium*	-0.346%
Lautropia	-0.018%	*Ilumatobacter*	-0.391%
Niastella	-0.018%	*Blastocatella*	-0.400%
Actinomycetospora	-0.023%	*Bradyrhizobium*	-0.473%
Rhizobium	-0.023%	*Haliangium*	-0.483%
Streptosporangium	-0.023%	*Shinella*	-0.596%
Nitrospira	-0.023%	*Aquamicrobium*	-0.723%
Lysobacter	-0.024%		

dance was lower. Among them, the abundance of 7 genera was higher than 0.01%, while that of the 16 remaining genera showed abundances lower than 0.01%. A total of 20 non-dominant genera were present only in sample CP0 (Table 10). Among them, the abundance of 8 genera was higher than 0.01%, while that of the 12 remaining genera showed abundances lower than 0.01%.

Table 7: Difference in fungal abundance at the phylum level.

Phylum	Relative abundance CP1	CP2	Difference of relative abundance
Ascomycota	63.843%	74.610%	10.767%
Basidiomycota	10.657%	16.922%	6.265%
Cercozoa	0.016%	0.031%	0.015%
Blastocladiomycota	0.000%	0.006%	0.006%
Chytridiomycota	0.041%	0.038%	-0.003%
Glomeromycota	1.131%	0.895%	-0.237%
Rozellomycota	2.437%	0.063%	-2.375%
Unidentified	9.345%	6.316%	-3.029%
Zygomycota	12.529%	1.120%	-11.409%

Table 8: Difference in abundance of the dominant fungal genera.

Genus	Relative abundance CP1	CP2	Difference of relative abundance
Alternaria	2.249%	14.603%	12.355%
Phoma	1.131%	10.380%	9.249%
Epicoccum	0.787%	6.182%	5.395%
Periconia	0.284%	5.362%	5.078%
Guehomyces	7.198%	10.984%	3.786%
Candida	5.107%	7.514%	2.407%
Oidiodendron	0.809%	3.031%	2.223%
Scytalidium	0.087%	1.283%	1.195%
Davidiella	0.650%	1.724%	1.073%
Cryptococcus	0.306%	1.142%	0.836%
Fusarium	1.052%	0.923%	-0.129%
Aspergillus	2.552%	2.187%	-0.365%
Cystobasidium	1.413%	0.920%	-0.493%
Schizothecium	1.142%	0.648%	-0.495%
Chaetomium	1.230%	0.666%	-0.563%
Wardomyces	1.252%	0.181%	-1.070%
Arthrographis	1.347%	0.053%	-1.294%
Pyrenochaetopsis	2.765%	0.601%	-2.165%
Modicella	11.693%	0.704%	-10.989%
Unidentified	50.074%	25.953%	-24.120%

The abundance of non-dominant bacterial genera present in both the samples is presented in Table 11. A total of 71 non-dominant genera were shared by the two samples, with a relative abundance of lower than 1%. The abundance of 40 genera was higher in sample CP4 than CP0, while that of 28 genera was lower, and the abundance of 3 genera is equal in two samples. Among them, the difference in the relative abundance of 14 genera was higher than 0.1%, while that of 42 genera ranged from 0.01% to 0.1%, and that of the remaining genera was 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 (Shi et al. 2006). A variety

Table 9: Non-dominant fungal genera present only in sample CP4.

Genus	Relative abundance	Genus	Relative abundance
Efibulobasidium	0.057%	*Clonostachys*	0.005%
Corynascus	0.041%	*Kazachstania*	0.005%
Zymoseptoria	0.030%	*Purpureocillium*	0.005%
Olpidium	0.025%	*Clavaria*	0.003%
Faurelina	0.016%	*Curvularia*	0.003%
Gymnascella	0.016%	*Geomyces*	0.003%
Roussoella	0.014%	*Hydropisphaera*	0.003%
Cyphellophora	0.008%	*Neurospora*	0.003%
Humicola	0.008%	*Phaeosphaeria*	0.003%
Phialemoniopsis	0.008%	*Rhizophagus*	0.003%
Slopeiomyces	0.008%	*Thermomyces*	0.003%
Chrysosporium	0.005%		

Table 10: Non-dominant fungal genera present only in sample CP0.

Genus	Relative abundance	Genus	Relative abundance
Pleospora	0.038%	*Myrmecridium*	0.009%
Schizophyllum	0.022%	*Plenodomus*	0.009%
Aureobasidium	0.019%	*Chromelosporium*	0.006%
Ilyonectria	0.019%	*Rhexocercosporidium*	0.006%
Neocamarosporium	0.016%	*Rhodosporidium*	0.006%
Hypocrea	0.013%	*Volvariella*	0.006%
Paraphaeosphaeria	0.013%	*Bradymyces*	0.003%
Powellomyces	0.013%	*Dothiorella*	0.003%
Articulospora	0.009%	*Phaeococcomyces*	0.003%
Bullera	0.009%	*Sarcinomyces*	0.003%

of research has examined the ability of turfgrass systems to mitigate environmental pollution and reduce leaching and runoff of pesticides and fertilizers (Shi et al. 2007). However the focus has often been on the turfgrass plant rather than on the broader soil ecology. Based on this review of the literature, it is apparent that knowledge of turfgrass soil ecology is lagging compared to arable soils, grasslands and forests. There is a need for basic information on soil microbial community in turfgrass systems (Shi et al. 2007). In the present study, the microbial diversity in two kinds of soil was studied by high-throughput sequencing technique, the results showed that bacteria and fungi in the soil of turfgrass were richer than vacant land. Because the sampling turf and the vacant land were in close proximity and had similar or identical soils, turfgrass establishment may result in the difference of soil microbial communities. The growth of turfgrass may promote the activity of microorganisms in soil, which consist with previous reports of other plants (Grayston et al. 1998, O'Donnell et al. 2001).

Soil is considered to be the most diverse natural environment on the Earth (Daniel 2005). The soil microbial communities harbour thousands of different organisms that contain a substantial number of genetic information (Vogel et

Table 11: Non-dominant fungal genera present in both the samples.

Genus	Difference of relative abundance	Genus	Difference ofrelative abundance
Corticium	0.159%	*Gibellulopsis*	0.004%
Myrothecium	0.123%	*Cystofilobasidium*	0.004%
Chloridium	0.121%	*Gibberella*	0.004%
Kurtzmanomyces	0.101%	*Talaromyces*	0.002%
Sporobolomyces	0.098%	*Aphanoascus*	0.000%
Didymella	0.073%	*Botrytis*	0.000%
Penicillium	0.056%	*Udeniomyces*	0.000%
Ophiostoma	0.049%	*Torula*	-0.002%
Monodictys	0.039%	*Cyathus*	-0.005%
Melanochaeta	0.037%	*Cladosporium*	-0.006%
Verticillium	0.036%	*Scedosporium*	-0.008%
Simplicillium	0.034%	*Phaeoacremonium*	-0.010%
Monographella	0.032%	*Nectria*	-0.012%
Rhodotorula	0.029%	*Ajellomyces*	-0.013%
Trichosporon	0.026%	*Mucor*	-0.014%
Pestalotiopsis	0.026%	*Zygopleurage*	-0.019%
Hyphopichia	0.024%	*Mrakiella*	-0.026%
Trametes	0.024%	*Monocillium*	-0.027%
Sarocladium	0.023%	*Rhizopus*	-0.032%
Leptosphaeria	0.022%	*Exserohilum*	-0.042%
Trichoderma	0.022%	*Pseudeurotium*	-0.043%
Exophiala	0.022%	*Volutella*	-0.048%
Preussia	0.022%	*Mycothermus*	-0.052%
Sphaerobolus	0.021%	*Podospora*	-0.065%
Wickerhamomyces	0.021%	*Wallemia*	-0.075%
Mrakia	0.020%	*Microdochium*	-0.111%
Bipolaris	0.020%	*Remersonia*	-0.135%
Phaeomycocentrospora	0.020%	*Pseudallescheria*	-0.170%
Apodus	0.018%	*Glomus*	-0.242%
Lectera	0.016%	*Stachybotrys*	-0.251%
Paecilomyces	0.010%	*Lecythophora*	-0.259%
Arnium	0.010%	*Acremonium*	-0.287%
Boothiomyces	0.009%	*Oliveonia*	-0.383%
Cercophora	0.007%	*Mortierella*	-0.392%
Sphaerodes	0.007%	*Coniochaeta*	-0.532%
Panaeolus	0.004%		

al. 2009). Based on traditional approaches for cultivating and isolating soil microorganisms, early studies have focused on culturable bacteria which only account for less than 1% of soil microbial populations (Delmont et al. 2011). Numerous studies have been carried out about the soil microbial community of other plants such as wheat, rice and maize (Peiffer et al. 2013, Edwards et al. 2015, Qin et al. 2016). In the present study, we found a large amount of bacteria and fungi in the samples. Among the bacteria, a total of one dominant genus was shared by the two samples. 5 dominant genera and 66 non-dominant genera were present only in sample CP4 (turfgrass soil) and 13 dominant genera and 17 non-dominant genera present only in sample CP0 (vacant land). Among fungi, a total of 5 dominant genera were shared by the two samples, 8 dominant genera were

present only in sample CP4 and 6 dominant genera present only in sample CP0. The results indicated that the soil microbial community structure of dominant genera in the two samples is quite different. Previous studies 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 (Gottel et al. 2011, Peiffer et al. 2013, Shakya et al. 2013). In this study, we found the relative abundance of the non-dominant microbes is quite different in the two samples. Sixty six non-dominant bacterial genera and 23 non-dominant fungal genera were present only in sample CP4 and 17 non-dominant bacterial genera and 20 non-dominant fungal genera present only in sample CP0. The relative abundance of 91 non-dominant bacterial genera and 71 non-dominant fungal genera shared by two samples was quite different. The results indicated that the soil microbial community structure of non-dominant genera in the two samples is quite different. In conclusion, microbial species increased significantly after turfgrass establishment and soil microbial community structure became more complex.

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