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Responses of soil fungal community to the sandy grassland restoration in Horqin Sandy Land, northern China

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Abstract Sandy grassland restoration is a vital process including re-structure of soils, restoration of vegetation, and soil functioning in arid and semi-arid regions. Soil fungal community is a complex and critical component of soil functioning and ecological balance due to its roles in organic matter decomposition and nutrient cycling following sandy grassland restoration. In this study, soil fungal community and its relationship with environmental factors were examined along a habitat gradient of sandy grassland restoration: mobile dunes (MD), semi-fixed dunes (SFD), fixed dunes (FD), and grassland (G). It was found that species abundance, richness, and diversity of fungal community increased along with the sandy grassland restoration. The sequences analysis suggested that most of the fungal species (68.4 %) belonged to the phylum of Ascomycota. The three predominant fungal species were *Pleospora herbarum*, *Wickerhamomyces anomalus*, and *Deconica Montana*, accounting for more than one fourth of all the 38 species. *Geranomyces variabilis* was the subdominant species in MD, *Pseudogymnoascus destructans* and *Mortierella alpine* were the subdominant species in SFD, and *P. destructans* and *Fungi incertae sedis* were the dominant species in FD and G. The result from redundancy analysis (RDA) and stepwise regression analysis indicated that the vegetation characteristics and soil properties explain a significant proportion of the variation in the fungal community, and aboveground biomass and C:N ratio are the key factors to determine soil fungal community composition during sandy grassland restoration. It was suggested that the restoration of sandy grassland combined with vegetation and soil properties improved the soil fungal diversity. Also, the dominant species was found to be alternative following the restoration of sandy grassland ecosystems.

Keywords Fungal community · Grassland restoration · PCR-DGGE · RDA · Horqin Sandy Land

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Introduction

Horqin Sandy Land is located in the semi-arid area of southeastern Inner Mongolia, northern China. Due to overgrazing and unsustainable cultivation, this area has become one of the most severely desertified regions in China (Zhu and Chen 1994; Wang 2003). The original landscape was dominated by grassland with scattered trees (mainly elms, *Ulmus* spp.). However, the grassland has been mostly replaced by shifting sand dunes after decades of extensive overgrazing and gathering of fuelwood (Zhao et al. 2003). Since the 1970s, the government has implemented no-grazing and fencing policy to restore the ecosystems, and many effective measures

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have been performed to stabilize sand dunes and restore vegetation in this region. As a result, mobile dunes have recovered gradually into semi-fixed and fixed dunes (Zuo et al. 2012; Zhao et al. 2015; Liu et al. 2015a, b). In recent years, studies have been carried out along gradients of vegetation restoration and dune stabilization in the Horqin Sandy Land, and most of the studies focused on soil properties (Shirato et al. 2005; Li et al. 2013), vegetation succession (Zhang et al. 2005; Zuo et al. 2009), plant growth (Li et al. 2005), soil enzyme activity (Cao et al. 2008), biological soil crusts (Guo et al. 2008), and soil macrofauna (Liu et al. 2009). However, the changes in the soil fungal community in response to grassland restoration remained unclear.

As is well known, the microbial community plays an active role in organic matter decomposition, nutrient cycling, and humus formation in association with plants and animals, and helps to regulate the development of soil structure and plant productivity (Van Der Heijden et al. 2008; Harris 2009; Van Der Wal et al. 2013). Particularly, fungi constitute up to 75 % of the soil microbial biomass and play a key role in preserving soil functioning and its ecological balance (Varela et al. 2015). Fungal mycelium ramify through soil, enmeshing and binding soil particles tighter to stabilize soil structure, accelerate decomposition, affect plant diversity, so that to enhance restoration process (Harris 2009). The estimated 1.5 million fungal species demonstrate the magnitude of fungal diversity at a global scale (Hawksworth 2001). It was reported that fungal species richness does not decline as sharply as plant species diversity with increasing latitude, and thus, fungi are a key component of total terrestrial biodiversity, and their communities play important implications on biodiversity conservation (Wardle and Lindahl 2014). It was found that plant diversity, soil pH, and soil nutrient content are the main driving forces on soil fungal diversity, and the impact of these factors varies among different ecosystems (Peay et al. 2013; Liu et al. 2015a, b). However, the role of soil fungi is complex, but appears to be critical to maintain the functionality of terrestrial ecosystems (Fracetto et al. 2013), and there was largely unknown how the soil fungal community responded to the environmental factors following sandy grassland restoration.

The former study showed that fungal abundance increased significantly with increasing restoration of sand dunes using culture-dependent methods (Wang et al. 2011). However, only 5–10 % of fungal species can be detected by such methods (Hawksworth 1997; Hawksworth 2001; Mueller and Schmit 2007). With the development of molecular biotechnology, culture-independent techniques based on direct extraction of soil microbial DNA have permitted a remarkable progress in research on the soil fungal community (Hoshino 2012; Zhang et al. 2014). In the present study, we analyzed the structure of soil fungal community along a gradient of sandy grassland restoration and described its relationship with vegetation changes and environmental factors in the Horqin Sandy Land.

Material and methods

Site description

The study was conducted in a typical sandy grassland ecosystem, about 20 km from Naiman Desertification Research Station (NDRS) in the Horqin Sandy Land, in the southeastern part of Inner Mongolia, northern China (42°55'N, 120°42'E; altitude approx. 360 m asl) (Fig. 1). The area has a temperate, semiarid continental monsoonal climate, with a hot summer and cold winter. The average annual precipitation is 360 mm, with more than 75 % falling during June to September. The annual mean open-pan evaporation is around 1935 mm, which is more than five times of the precipitation. The annual mean temperature is around 6.4 °C, with monthly mean temperatures ranging from a minimum of −16.8 °C in January to a maximum of 23.5 °C in July. The annual mean wind velocity ranges from 3.5 to 4.5 m s^{-1}, and wind for sand movements (>4.5 m s^{-1}) occurs on an average of 210 days per year. The zonal soils are sandy chestnut soils, light yellow in color, and loose in structure, and are vulnerable to wind erosion when they are exposed (Zhao et al. 2003). The landscape is characterized by mobile, semi-fixed, and fixed dunes, as well as by open grassland that resembles the region's natural vegetation (Liu et al. 2009; Zuo et al. 2012). Plant distributions respond strongly to subtle variations of soil properties. *Agriophyllum squarrosum* is the dominant species in mobile dunes. The native shrub *Artemisia halodendron* is the dominant species in semi-fixed dunes. The annual forb *Artemisia scoparia* is the dominant species in fixed dunes and in grasslands. Two perennial grasses *Cleistogenes squarrosa* and *Phragmites communis* are subdominant species in grasslands.

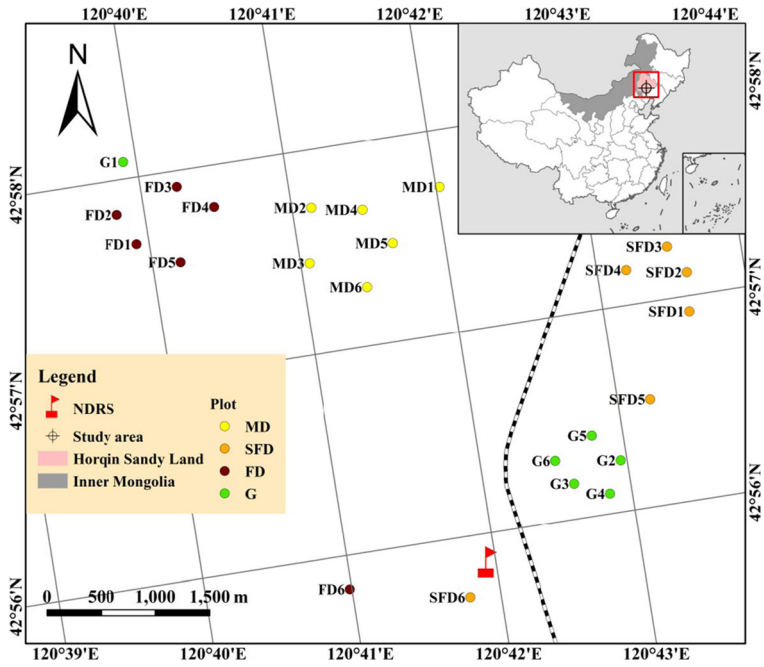

Fig. 1 Location of the study sites. *Dots* represent the sample plots. *MD* mobile dunes, *SFD* semi-fixed dunes, *FD* fixed dunes, *G* grasslands

Experimental design

Soil sampling and vegetation surveys were carried out in mid-August 2013. We selected 24 sampling sites (recorded by GPS) that represented a gradient for the restoration of sandy grassland, which served as a proxy for recovery duration; that is, we used the "space-for-time substitution" approach. Sampling was performed at four habitat types: mobile dunes (MD), with less than 10 % vegetation cover; semi-fixed dunes (SFD) with 10–60 % vegetation cover; fixed dune (FD) with more than 60 % vegetation cover and grassland (G) with more than 60 % vegetation cover, and each habitat type had six replicate sites. These sites were separated by at least 500 m (Fig. 1). Semi-fixed dunes and fixed dunes were naturally restored from mobile dunes by fencing for grazing exclusion approximately for 18 and 33 years, respectively. Grassland sites were enclosed to vegetation restoration from 1996, which represent a relatively natural vegetation with good grass quality and high soil nutrient. Fixed dunes are dominated by native annual forb of *Artemisia scoparia*, and grasslands are dominated by perennial grass of *Phragmites communis* and *Pennisetum centrasiaticum*.

At each site, we established a 20×20-m plot in a flat area (slope <5°); then, we established five 1×1 m quadrats at the four corners and the center of the plot and performed soil sampling and vegetation surveys in these quadrats. In each quadrat, we obtained a pooled sample derived from three random soil samples to a depth of 10 cm in each quadrat using a 3-cm diameter soil auger, and after removing any rocks and litter, we transformed the samples to the laboratory for analysis. The soil samples were stored in sterile plastic bags and kept in a portable icebox during transportation to the laboratory. Total DNA was extracted as soon as the soil samples arrived at the laboratory, and the extracted DNA was stored at −80 °C until analysis.

We measured the following environmental factors that supposed to influence soil fungal community in each quadrat: plant cover, species richness, and aboveground biomass represented the vegetation characteristics, and the soil water content, bulk density, particle size distribution, carbon, nitrogen, pH, and electrical conductivity represented the soil properties. Plant cover and the number of plant species were recorded in each quadrat, and aboveground plant was harvested and dried at 70 °C for 24 h to measure the biomass. Soil core was taken and dried at 105 °C for 24 h to measure soil water content in each quadrat. Soil samples were also collected for soil bulk density using a soil auger equipped with a stainless-steel cylinder (5 cm in both diameter and height). Soil particle size from international and USDA classification systems was determined by the wet sieving method. Soil total carbon (C) and total nitrogen (N) were analyzed by an elemental analyzer (vario Macro

cube, Elementar, Germany). Soil pH and electrical conductivity (EC) were measured in a 1:1 soil-water slurry and in a 1:5 soil-water aqueous extract (Multiline F/SET-3, Germany), respectively.

DNA extraction and PCR-DGGE analyses

Soil fungal community was conducted by means of the polymerase chain reaction with denaturing gradient gel electrophoresis (PCR-DGGE) (Fracetto et al. 2013). Total DNA extracted from soil samples (500 mg per quadrat) using the MOBIO PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The five extracted DNA samples from each site were mixed as a targeted DNA. We conducted PCR amplification targeting fungal 18S rDNA (Hoshino and Morimoto 2008), using fungal specific primers NS1 (5'-GTA GTC ATA TGC TTG TCT C-3'), GCfung (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCC CCG CCC CAT TCC CCG TTA CCC GTT G-3') and Fung (5'- CAT TCC CCG TTA CCC GTT G-3') (White et al. 1990; May et al. 2001; Hoshino 2012). Amplification was performed in a solution (approximately 50 ng DNA), adding 5-μL buffer of PCR 10×, 1 μL of each primer, 3.2 μL of dNTP (2.5 mM), and 0.4 μL of rTaq (5 U/μL), and sterile ultrapure water was loaded to adjust the mixture to a final volume of 50 μL. PCR amplification was run on a T-gradient diversity system (Bio-Rad, Italy). The amplification was performed under the following conditions: an initial denaturation for 5 min at 94 °C, amplification denaturation (94 °C for 1 min), annealing (50 °C for 45 s), 30 cycles, extension (72 °C for 1 min), and a final extension of 10 min at 72 °C. Then, PCR samples containing amplicons were loaded onto 8 % (*w/v*) acrylamide gel with a 35–55 % (100 % denaturing solution included 7-M urea and 40 % deionized formamide) denaturant gradient and run in 1 × TAE (Tris-acetate-EDTA) buffer for 4 h and 150 V at 60 °C. After the electrophoresis, the gel was stained in silver staining solution for 20 min and photographed to get DGGE profile using a Bio-Rad transilluminator (BIO-RAD Laboratories, Segrate, Italy) under UV light. Banding patterns of the DGGE profile were analyzed by Quantity One software (version 4.6.2). The position and intensity of each band were determined automatically. The fungal composition was illustrated based on the DGGE profile bands and band intensity using Heatmap Illustrator (http://hemi.biocuckoo.org). Common bands and bands that differed among the restoration stages were carefully excised from the DGGE gel and subjected to sequencing, and the obtained sequences were determined using BLAST in NCBI. We constructed a phylogenetic tree using the neighbor-joining (NJ) method by MEGA 5 to identify closely related species and phylogenetic affiliations among the obtained sequences. Soil fungal diversity was described and evaluated based on the bands and band intensity in the DGGE profile.

Statistical analysis

The soil fungal abundance (*A*) refers to DGGE band intensity value, and species richness (*S*) was based on DGGE band number of DGGE bands in each sample. We used the Shannon-Wiener index (*H*) (Gafan et al. 2005) to determine the diversity of the fungal community.

$$H = -\sum_{i=1}^{S} (Pi)(\ln Pi)$$

where *S* is the number of species (DGGE bands) in a sample and *Pi* is the proportion of samples that contained species *i* in the total number of the samples.

We performed cluster analysis to compare the fungal DGGE patterns among the site types using the unweighted pair group method with arithmetic averages (UPGMA) (Jiang et al. 2014). Similarities were displayed graphically using a dendrogram. The cluster analysis and dendrogram generation were carried out using the Quantity One software. Diversity index data were expressed as mean±SE ($n=6$). Differences among the four restoration stages were identified using one-way analysis of variance (ANOVA) followed by least-significant-difference (LSD) tests. All differences are significant at stated at the level of $p<0.05$ unless otherwise noted.

The descriptive statistical parameters and significance tests were performed by SPSS (version 16.0). Correlations between fungal community and environmental factors were analyzed using Pearson's two-tailed tests. Ordination analysis was used to determine the relative contribution of measured environmental variables to community composition. The fungal community data were first analyzed by detrended correspondence analysis (DCA), suggesting that redundancy analysis (RDA) was an appropriate approach (length of gradient <3) (Liu et al. 2015b) to analyze

relationships of the soil fungal community with vegetation characteristics and soil properties in the sandy grassland restoration. We also used a distribution-free Monte Carlo test for the significance of species-environment correlations in RDA. The DCA and RDA analysis was performed using CANOCO software (version 4.5). Stepwise regression analyses are used to analyze associations of soil fungal diversity index with the correlated factors from RDA.

Results

Responses of soil fungal community along sandy grassland restoration

The soil fungal community was analyzed based on the DGGE profile. Discernible differences in band numbers and intensities among the 24 samples are visible in the DGGE profile (Fig. 2). We detected 38 bands in the DGGE profile, with 9–14, 11–16, 13–22, and 13–22 bands in MD, SFD, FD, and G sites, respectively. Bands 4, 11, 18, and 21 were only detected in MD sites, band 35 was only detected in FD sites, and bands 5, 7, 30, and 33 were only detected in G sites. Bands 15, 16, 17, 19, 22, 23, 24, 26, 29, 37, and 38 were present in all four stages of grassland restoration, but their intensities varied among the stages. Band 22 was present in all 24 sites.

Soil fungal abundance (*A*), species richness (*S*), and the Shannon-Wiener index (*H*) differed significantly ($p<0.01$) among the four stages of grassland restoration (Table 1). Along with the gradient from MD to G, fungal abundance (*A*) increased by 68.0 %, species richness (*S*) increased by 50.7 %, and Shannon-Wiener index (*H*) increased by 17.0 %.

Cluster analysis separated the DGGE patterns of the fungal community for the MD sites from those of the other habitats along the terms of the DGGE patterns of the fungal community (Fig. 3). The average similarities were 65.47, 57.71, and 53.56 % between MD and SFD sites, MD and FD sites, and MD and G sites, respectively, indicating that the difference in the fungal community increased as grassland restoration increased.

Phylogenetic analysis of fungal DNA sequences

The 38 bands were excised, reamplified, and successfully sequenced, and the obtained sequences were determined by using BLAST in NCBI. BLAST search

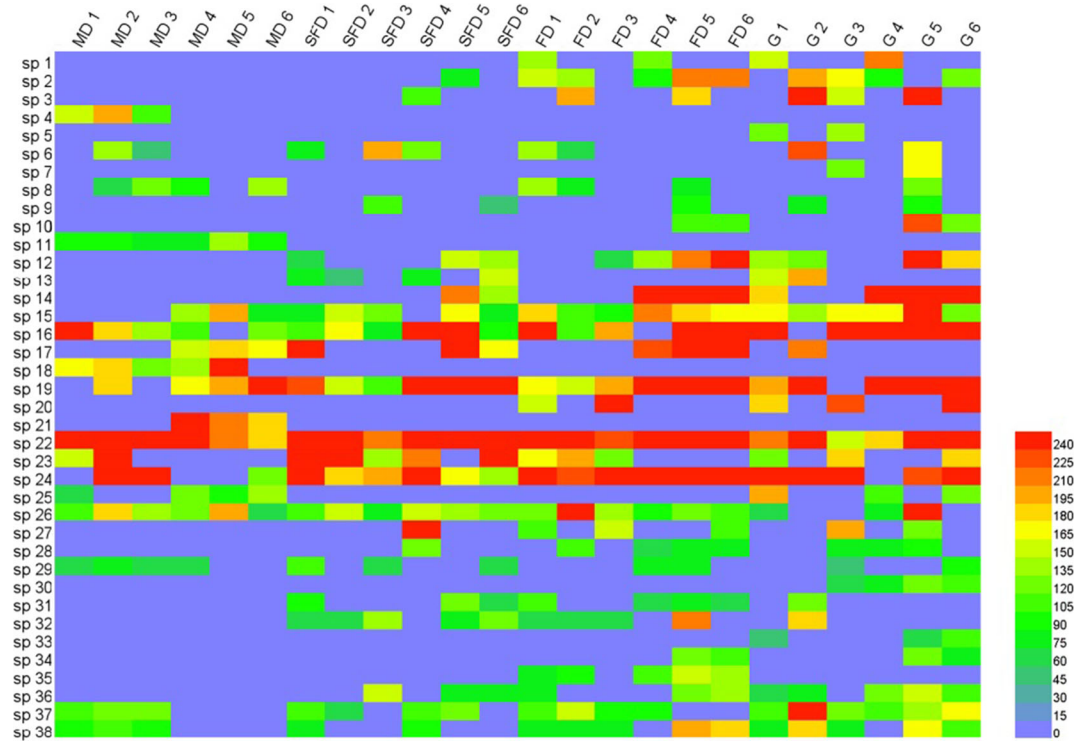

Fig. 2 Heat map of the fungal community composition based on the DGGE bands and band intensities in the 24 samples along a gradient of grassland restoration. *MD* mobile dunes, *SFD* semi-fixed dunes, *FD* fixed dunes, *G*: grasslands, *sp* fungal species number

Table 1 Diversity indices for the soil fungal community in sites at four stages of grassland restoration

	Abundance (*A*)	Species richness (*S*)	Shannon-Wiener index (*H*)
MD	1747.72±124.27a	11.50±0.67a	2.36±0.05a
SFD	2053.39±147.90a	13.50±0.95a	2.50±0.07a
FD	2750.96±275.96b	17.17±1.30b	2.74±0.08b
G	2935.32±288.97bc	17.33±1.26bc	2.77±0.08bc
F	6.445	7.064	7.276
p	0.003	0.002	0.002

MD mobile dunes, *SFD* semi-fixed dunes, *FD* fixed dunes, G grassland

indicated that most of the sequences were closely related to fungal clones or isolates in GenBank (Table 2). A phylogenetic tree was built to reflect the relationships among the obtained sequences (Fig. 4). Phylogenetic analysis clustered the fungal species into five main groups: Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and *Fungi incertae sedis*. Among the 38 species, 26 species (68.4 %) were Ascomycete, seven species (18.4 %) were Basidiomycota, one species (2.6 %) was Chytridiomycota, two species (5.3 %) was Zygomycota, and two species (5.3 %) were *Fungi incertae sedis*.

Among the 38 fungal species, *Deconica montana* (sp22), *Pleospora herbarum* (sp16), and *Wickerhamomyces anomalus* (sp19) are the three main fungal species in the four habitats, accounting for more than one fourth of all the species in each habitat. However, there was specific species in different habitats, e.g., *Geranomyces variabilis* (sp18, the relative abundance=8.3 %) was the subdominant species in MD; *Pseudogymnoascus destructans* (sp24, 10.1 %) and *Mortierella alpina* (sp23, 9.6 %) were the subdominant species in SFD; *Pseudogymnoascus destructans* (sp24) and *Fungi incertae sedis* (sp15) were the dominant species in FD and G.

Relationship of soil fungal community with vegetation characteristics and soil properties

The diagram originated by the first two axes of the RDA (Fig. 5) showed much contribution of vegetation and soil variables on soil fungal community distribution along the gradient of sandy grassland restoration. A Monte Carlo permutation test indicated that all of the canonical axes were significant (p= 0.002). The first axis was significant positively correlated with soil bulk density and coarse sand content, but significant negatively correlated with the soil C,

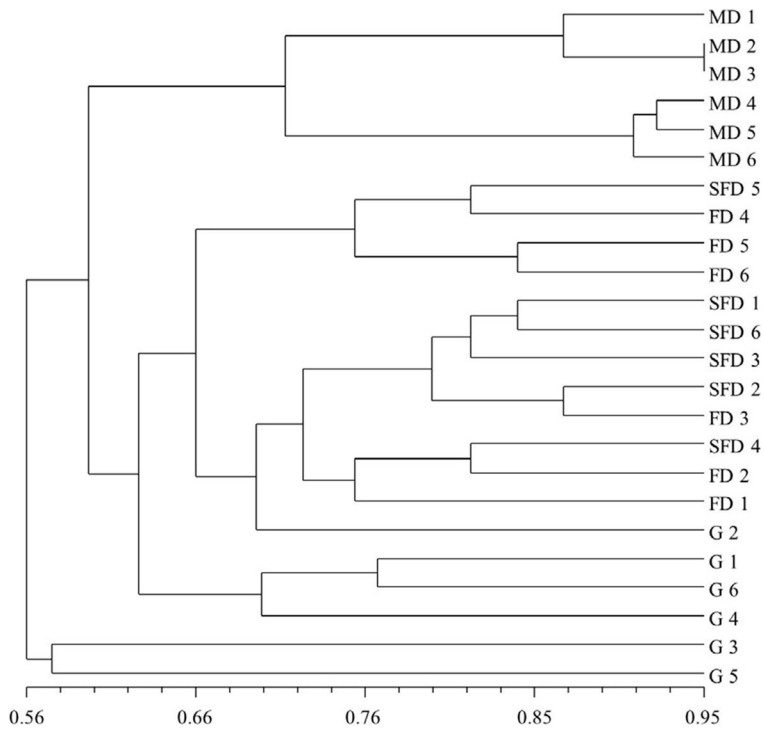

Fig. 3 Cluster analysis (UPGMA) based on the soil fungal 18 s rDNA PCR-DGGE profile

Table 2 Identity of the sequences on the selected DGGE bands by BLAST in NCBI

DGGE band	Closest strain from GenBank by blast	Accession number	Similarity (%)	Phylogenetic affiliations
1	*Parastagonospora nodorum*	KJ830777	99	Ascomycota
2	*Cochliobolus* sp.	GU190186	100	Ascomycota
3	*Arthrobotrys oligospora*	JQ809337	99	Ascomycota
4	*Trematosphaeria heterospora*	AY016354	99	Ascomycota
5	*Wallemia ichthyophaga* EXF-994	XR_657242	99	Basidiomycota
6	*Hymenoscyphus kiko*	KC164672	99	Ascomycota
7	*Cladosporium* sp. Pt-2	JQ824844	99	Ascomycota
8	*Geomyces* sp. P7	GQ214385	99	Ascomycota
9	*Cochliobolus* sp. 007 (L) 1-1	FJ235087	99	Ascomycota
10	*Myrothecium verrucaria*	EF211127	100	Ascomycota
11	*Ciboria batschiana*	KJ830799	98	Ascomycota
12	*Mortierella alpina*	AY550125	99	Zygomycota
13	*Graphium fructicola*	AB007659	91	Ascomycota
14	*Amyloporia xantha*	KJ830780	100	Basidiomycota
15	Uncultured fungus	DQ412118	99	*Fungi incertae sedis*
16	*Pleospora herbarum*	PHU43458	99	Ascomycota
17	*Salpingoeca fusiformis*	KJ631039	94	*Fungi incertae sedis*
18	*Geranomyces variabilis*	AF164241S1	98	Chytridiomycota
19	*Wickerhamomyces anomalus*	AY251638	99	Ascomycota
20	*Dioszegia hungarica*	AF314235	87	Basidiomycota
21	*Melanoleuca verrucipes*	DQ457645	99	Basidiomycota
22	*Deconica montana*	DQ465342	99	Basidiomycota
23	*Mortierella alpina*	AY546097	99	Zygomycota
24	*Pseudogymnoascus destructans*	GU999983	99	Ascomycota
25	*Saccharomyces* sp. WW-W8	DQ345290	98	Ascomycota
26	*Campanella* sp. MCA2235	AY916675	99	Basidiomycota
27	*Glyphium elatum*	AF346419	99	Ascomycota
28	*Pseudogymnoascus destructans*	GU350433	100	Ascomycota
29	*Purpureocillium lilacinum*	HQ232214	99	Ascomycota
30	*Phoma* sp. ZH2.1	FJ450059	98	Ascomycota
31	*Phoma macrostoma* var.	AB454217	99	Ascomycota
32	*Dothideomycetes* sp. LS-2013a	KC315859	98	Ascomycota
33	*Lulwoana uniseptata*	AY879034	96	Ascomycota
34	*Macrolepiota dolichaula*	AY771602	98	Basidiomycota
35	*Nanoscypha tetraspora*	AF006314	97	Ascomycota
36	*Candida glucosophila*	AB013519	99	Ascomycota
37	*Helicascus elaterascus*	AF053727	98	Ascomycota
38	*Trichoderma reesei*	JN831373	99	Ascomycota

N, C:N, pH, EC, very fine sand, silt and clay, plant cover, plant species richness and biomass. The second axis was significant positively correlated with the soil pH, EC, silt and clay and soil water content, but significant negatively correlated with soil bulk density (Table 3). The cumulative percentage variances explained by the first two axes were separately 29.8 and 17.3 %, indicating that the soil fungal community was strongly affected by the measured vegetation characteristics and soil properties.

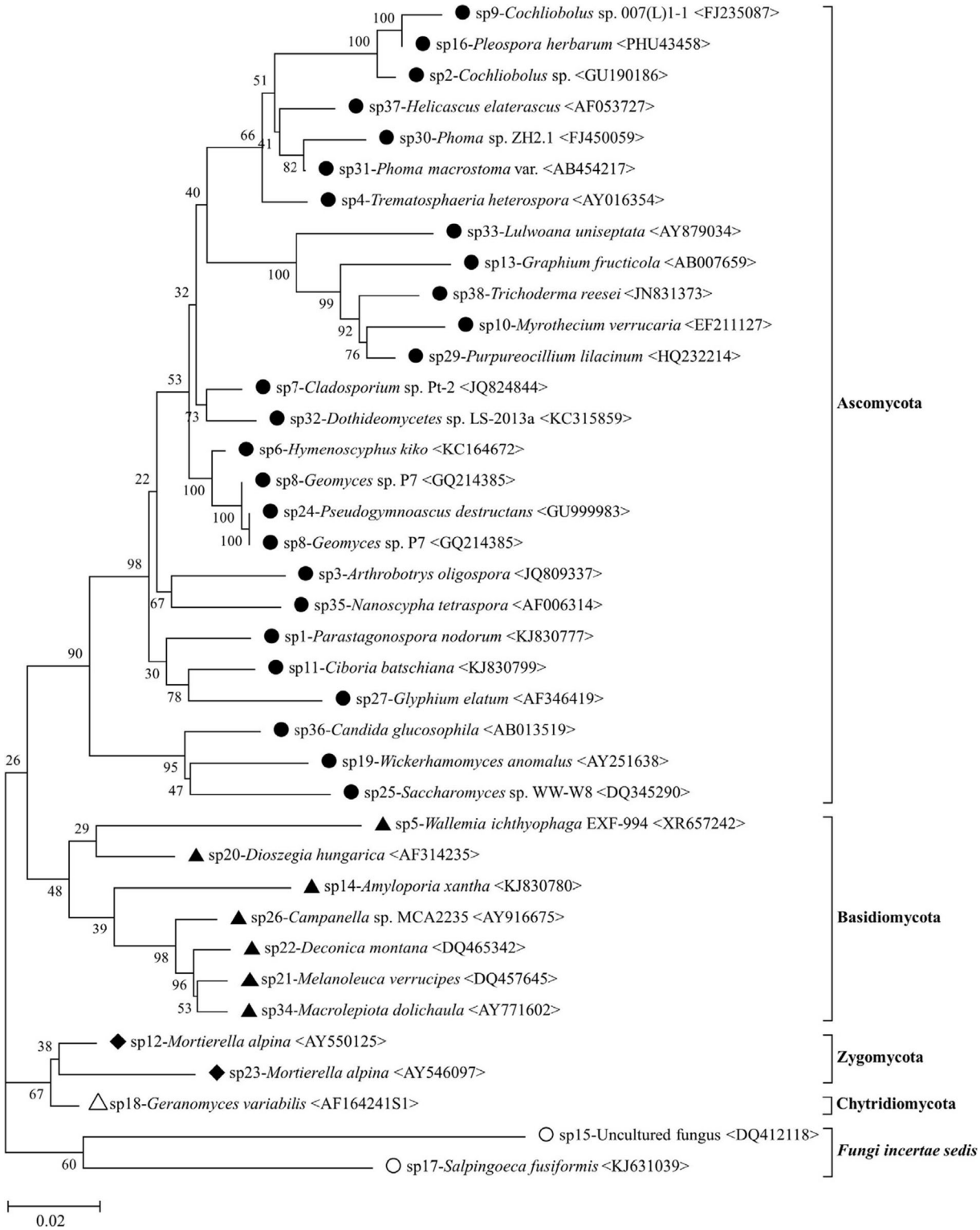

Fig. 4 Phylogenetic tree (neighbor joining) for the 18S rDNA gene sequences. Fungal species numbers (sp1-sp38) correspond to the band numbers listed in Table 2

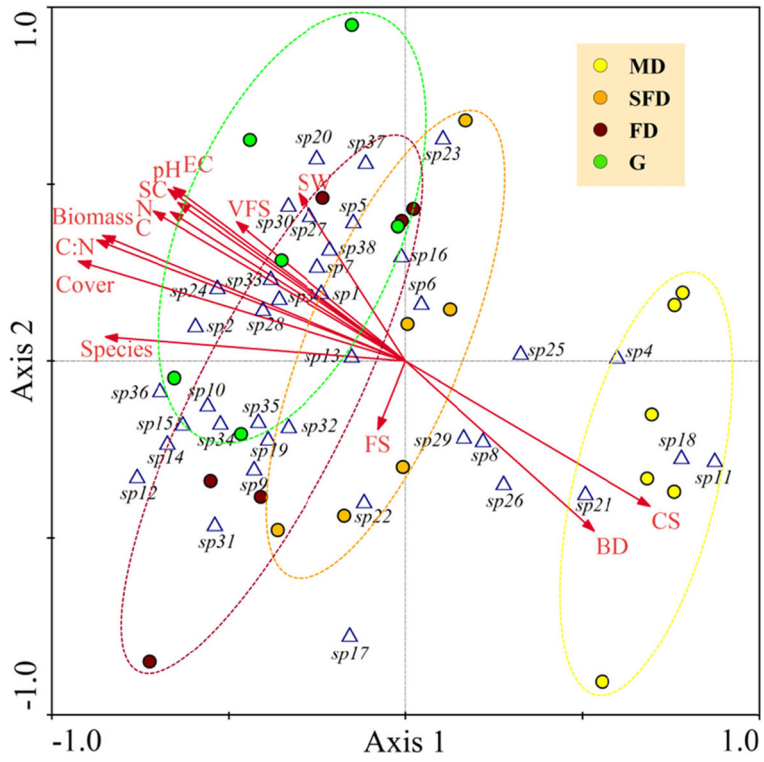

Fig. 5 The two-dimensional RDA ordination diagram for the first two axes, showing the distribution of the 24 samples (*circles*), 38 fungal species (*triangles*), and environmental variables (*vectors*). Abbreviations of the environmental variables are the same as in Table 3

From Fig. 5 bipplot, it is worthwhile to note that there is a clear separation of samples depending on habitat of sandy grassland restoration associated with vegetation and environmental factors and similar distribution of soil fungal species with vegetation and soil variables. Figure 5 showed that the vegetation characteristics (plant cover, species richness, and aboveground biomass) and soil properties (C, N, C:N, pH, EC, soil water content, silt and clay, and very fine sand) increased along the first axis from the right to the left, but soil bulk density and coarse sand content decreased. Figure 5 also shows the correlations between the soil fungal species distribution (triangles) and the environmental variables (vectors), and some certain species were clearly associated with specific variables (e.g., sp30 and sp27 with the soil water content), whereas other species (e.g., sp6, sp13, and sp25) were weakly associated with any examined environmental variables.

Stepwise regression analysis was used to quantify the relationships between the soil fungal diversity index and the correlated factors from RDA (Table 4). The final models showed that soil fungal abundance was significantly affected by aboveground biomass ($p<0.001$), and soil fungal species richness and Shannon-Wiener index were significantly affected by soil C:N ratio. Thus, aboveground biomass and soil C:N ratio are the main factors that influence soil fungal diversity in sandy land restoration.

Discussion

The number of DGGE bands represents the dominant fungal populations in most soils, and many of these bands were located at equivalent positions along the denaturing gradient, suggesting that they represented similar species (Nakatsu 2007). We detected a total of 38 bands in the 24 samples, with 18, 23, 28, and 33 fungal species in MD, SFD, FD, and G, respectively; that is, the number of fungal species increased along with the sandy grassland restoration. Plant cover, species and biomass increased along sandy grassland restoration, which increased the soil nutrient input from plant litter and root. The increased nutrient provides enough food and energy for the fungal growth and proliferation, and different litter and root was supposed to breed different fungal species (Sharma et al. 2015). Among the 38 fungal species, sp16 (*Pleospora herbarum*), sp19 (*Wickerhamomyces anomalus*), and sp22 (*Deconica montana*) are dominant fungal species that were found in

Table 3 Intra-set correlations of the environmental variables, eigenvalue, cumulative percentage variance of fungal species, and fungal species-environment correlation coefficients for the first four axes of RDA

	Axis			
	SPX1	SPX2	SPX3	SPX4
C	−0.704***	0.393	0.291	0.267
N	−0.654***	0.391	0.319	0.280
C:N	−0.861***	0.317	−0.142	0.037
pH	−0.661***	0.450*	0.321	0.214
EC	−0.646***	0.455*	0.407*	0.158
BD	0.524**	−0.447*	−0.286	−0.150
CS	0.680***	−0.382	−0.262	−0.048
FS	−0.076	−0.179	−0.645***	−0.085
VFS	−0.472*	0.364	−0.533**	−0.042
SC	−0.635***	0.415*	0.293	0.369
SW	−0.298	0.439*	0.372	0.050
Cover	−0.911***	0.261	0.012	0.018
Species	−0.837***	0.063	−0.276	0.292
Biomass	−0.844***	0.330	−0.083	−0.177
Eigenvalue	0.208	0.121	0.089	0.055
Cumulative percentage variance/%	29.8	47.1	59.9	67.8

C soil organic carbon, *N* soil total nitrogen, *pH* soil pH value, *EC* electrical conductivity, *BD* soil bulk density, *CS* coarse sand (>0.25 mm), *FS* fine sand (0.25–0.10 mm), *VFS* very fine sand (0.10–0.05 mm), *SC* silt and clay (<0.05 mm), *SW* soil water content, *Cover* plant cover, *Species* plant species richness, *Biomass* plant above ground biomass

*Correlation was significant at the 0.05 level (two-tailed)

**Correlation was significant at the 0.01 level (two-tailed)

***correlation was significant at the 0.001 level (two-tailed)

all four habitats, accounting for more than one fourth of the species. The sp18 (*Geranomyces variabilis*) is the most distinctive species, as it was only found at the MD sites, and was the only Chytridiomycota. Most of the fungal species (68.4 %) in the sandy soils of our study were Ascomycota, which was similar to previous results for an alpine grassland soil (Pellissier et al. 2014), an oak forest soil (Varela et al. 2015), and a soybean rhizosphere soil (Wang et al. 2009), but considerably different from the results for an Amazon river bank soil, where Zygomycota was dominant (Fracetto et al. 2013). Our previous research in the study area identified several fungal species with a strong ability to decompose cellulose, which were also categorized as Ascomycote (Wang et al. 2015). The soil fungal community succession was similar to vegetation succession along sandy grassland restoration, and it could be explained by the distribution of the dominant fungal species in each habitat from the phylogenetic tree obtained by the fungal 18S rDNA gene sequences (Fig. 4).

It was very difficult for the microbes to survive in mobile dunes due to the extreme environmental conditions, such as low nutrient content and unstable cover. Thus, fewer bacterial species and lower abundance of each species were detected in the previous studies of mobile dunes (Wang et al. 2011; Jiang et al. 2014). However, we found 18 fungal species (an average species richness of 11.5) presented in mobile dunes of Horqin Sandy Land, with four species (*Trematosphaeria heterospora*, *Ciboria batschiana*, *Geranomyces variabilis*, and *Melanoleuca verrucipes*) were able to survive only in this low-nutrient environment. It was probably because fungal was more tolerant to low-nutrient environment than bacteria (Zhou 2002). Fungal species sp3 (*Arthrobotrys oligospora*), sp12 (*Mortierella alpina*), sp14 (*Amyloporia xantha*), and sp36 (*Candida glucosophila*) were found in SFD sites, and these species abundance increased with the increasing restoration. Fungal species sp5 (*Wallemia ichthyophaga*), sp7(*Cladosporium sp.*), sp30(*Phoma sp.*), and sp33(*Lulwoana uniseptata*) were only detected in the G sites (Fig. 2). Fungal species change along the gradient of grassland restoration, as is the case for plant species (Zuo et al. 2012). Our vegetation surveys showed that *Agriophyllum squarrosum* is a pioneer plant species that can survive only in mobile dunes, but that is replaced by other species, such as *Lespedeza davurica*, *Cleistogenes squarrosa*, and *Bassia dasyphylla* along sand dune stabilization. *Leymus secalinus* and *Artemisia sieversiana* only grow in grassland of the region (Zhou et al. 2015). Our result indicated that fungal community changes were similar to plant community changes along sandy grassland restoration. The investigation of soil microbial communities in restoring ecosystems is providing vivid insights into how both the original and restored ecosystems function, and as research on this aspect of restoration ecology continues to be refined and extended, will provide insights into restoring ecosystems (Harris 2009).

Soil microbial communities are sensitive to environmental changes (Chapin III et al. 2002). Soil pH is a particularly important factor in determining the composition of soil bacterial communities (Lauber et al. 2009). However, findings about fungal communities appear to differ (sometimes greatly) from those for bacterial communities. Research on fungal community in black soil explained that fungal community composition was

Table 4 Stepwise regression analysis between fungal community and the correlated environmental factors

Fungal diversity index	Independent variables	Coefficient B	*T*	*p*
Abundance (*A*)	Constant	1668.65	8.85	<0.001
	Biomass	6.66	4.53	<0.001
	A=1668.65+6.66*Biomass (R^2=0.48, F=20.50, p<0.001)			
Species richness (*S*)	Constant	5.44	2.62	=0.02
	C:N	1.31	4.70	<0.001
	S=5.44+1.31*C:N (R^2=0.50, F=22.08, p<0.001)			
Shannon-Wiener index (*H*)	Constant	1.95	14.35	<0.001
	C:N	0.09	4.92	<0.001
	H=1.95+0.09*C:N (R^2=0.52, F=24.19, p<0.001)			

mostly affected by the soil pH and carbon content (Liu et al. 2015a), and in cultivated soils, the fungal community was closely related to the soil C:N ratio and P content (Lauber et al. 2008). In the sandy soils of our study, we found that the fungal community was strongly related to aboveground biomass and soil C:N ratio.

The soil fungal diversity was reported to be strongly correlated with plant diversity in forest (Peay et al. 2013) and grassland (Brodie et al. 2003) ecosystems. Our study showed a similar pattern: higher plant species richness resulted in higher soil fungal diversity. Soil fungi and plants are tightly linked via mycorrhizal symbiosis, pathogenic interactions, and nutrient release during the organic matter decomposition process (Wardle and Lindahl 2014). Thus, it is logical to predict that higher plant diversity would result in higher soil microbial diversity (Fracetto et al. 2013). However, it is not yet scientifically clear whether manipulation of microbial communities would enhance ecosystem succession to more desirable states (Harris 2009), and the recent research casts doubt on the hypothesis that changes in major fungal groups could potentially affect plant productivity and diversity (Van Der Heijden et al. 2008; Wardle and Lindahl 2014). The cause-and-effect relationship between plants and soil microbes and their mutual associating with the soil's mineral-organic matrix, must be further studied to reveal the plant-soil feedbacks that occur in terrestrial ecosystems (Putten et al. 2013).

Conclusion

Soil fungal community changes and their relationships with vegetation characteristics and soil properties were analyzed along a habitat gradient of sandy grassland restoration in a semi-arid region. The fungal abundance, richness, and diversity increased significantly along sandy grassland restoration, which were similar with the vegetation changes. Dominant fungal species were universal in the four restoration habitat. However, distinctive fungal species were also detected to dominant in each habitat. The vegetation characteristics and soil properties explain a significant proportion of the variation in the fungal community during sandy grassland restoration, and aboveground biomass and C:N ratio are the key factors to determine soil fungal community composition. The increased fungal diversity indicates the potential restoration of sandy grasslands.

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