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Diversity and distribution of soil fungal communities associated with biological soil crusts in the southeastern Tengger Desert (China) as revealed by 454 pyrosequencing

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ABSTRACT

We assessed the diversity and distribution of soil fungal communities associated with biological soil crusts (BSCs) in the southeastern Tengger Desert (China) using 454 pyrosequencing. Soil fungal communities showed high diversity, with 63,427 reads belonging to 478 operational taxonomic units (OTUs). Of these OTUs, 369 belonged to the Ascomycota, 73 to the Basidiomycota, 24 to the Chytridiomycota, one to the Glomeromycota, five to the Mortierellomycotina, and six to unassigned phyla. The most frequent genera were *Phoma*, *Fusarium*, *Alternaria*, *Dendryphion*, *Peyronellaea*, *Curvularia*, *Epicoccum*, *Embellisia* and *Pleiochaeta*. Different BSC sands (algae-crust sand, moss-crust sand and lichen-crust sand) did not harbor significantly different fungal communities. However, the fungal communities in these BSC sands were significantly different from those in non-crusted sands, with members of the Hypocreales and *Fusarium* being more frequent in the latter. The distribution of soil fungi (measured by Bray-Curtis distance) correlated with abiotic parameters, including total P ($r^2 = 0.9191$, $P = 0.001$), organic C ($r^2 = 0.7237$, $P = 0.004$), total N ($r^2 = 0.7200$, $P = 0.004$), pH ($r^2 = 0.6858$, $P = 0.002$) and electrical conductivity ($r^2 = 0.4723$, $P = 0.030$). The results suggest that BSCs in the Tengger Desert harbor diverse fungal communities that are influenced by abiotic factors.

1. Introduction

In arid and semi-arid regions, soil surfaces are often covered with highly specialized organisms such as mosses, lichens, algae, fungi, cyanobacteria and bacteria, communities of which are referred to biological soil crusts (BSCs). BSCs occur in desert regions all over the world due to their tolerance to stressful conditions, such as exposure to desiccation, extreme temperatures and high solar UV irradiances. BSCs may play important ecological roles in desert ecosystems, such as in the stabilization of sand dunes (Veste et al., 2001), in the prevention of soil erosion by wind (Zhang et al., 2006), and in the enhancement of soil fertility (Housman et al., 2006). Generally, the development of BSCs can be divided into three phases: an initial colonisation phase by soil microbes, an algae crust phase, and a lichen or bryophyte crust phase. The establishment of the former phase acts as the basis for the next phases of crust succession (Zhang and Wang, 2010). It is microbes, such as fungi (States et al., 2001), cyanobacteria (Garcia-Pichel and Wojciechowski, 2009) and algae (Hu et al., 2002), that play important roles in establishing crust communities.

Fungi associated with BSCs can occur as free-living microfungi or in symbiosis with algae or cyanobacteria (forming lichen thalli). The free-living microfungi have important roles in improving soil properties by functioning as decomposers and as major contributors to soil microbial biomass (a food source for crust-dwelling microfauna). Their hyphae can also bind sand particles together (States et al., 2001). So far, a high number of free-living soil fungi have been reported from desert regions all over the world, such as the Tengger Desert in China (Grishkan et al., 2015), the Arabian Desert in Oman (Abed et al., 2013), the Negev Desert in Israel (Grishkan et al., 2006; Grishkan and Kidron, 2013), and the Colorado Plateau, Chihuahuan Desert and Sonoran Desert in the Americas (Bates and Garcia-Pichel, 2009; Bates et al., 2010a, 2012).

Previous studies have focused on the diversity of cultured free-living soil microfungi (Grishkan et al., 2006; Abed et al., 2013; Grishkan and Kidron, 2013; Grishkan et al., 2015). However, the

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culturing method cannot reflect the actual diversity of fungi in soil because of its selectivity. A few studies have surveyed fungi in BSCs using DNA-based molecular methods such as DGGE, cloning and q-PCR (Bates and Garcia-Pichel, 2009; Bates et al., 2010a, 2012). To the best of our knowledge, only one study has hitherto surveyed BSC-associated fungi using pyrosequencing with fungal-specific primers targeting the 18S rRNA gene (Abed et al., 2013).

The high sensitivity of high-throughput pyrosequencing enables the detection of species in communities at low abundances (Zhan et al., 2013) and thereby provides more detailed information on fungal diversity than conventional culturing and molecular methods. In the present study, Roche 454 pyrosequencing was used to investigate the fungal communities associated with non-crusted sand and BSC-associated sands (algae-crust sand, moss-crust sand and lichen-crust sand) at the southeastern edge of the Tengger Desert in China. We determined: (1) the taxonomic composition of fungal communities inhabiting the desert sands, (2) whether or not the fungal communities in the non-crusted and BSC-associated sands differed significantly, and (3) which environmental factors influenced soil fungal diversity and community composition.

2. Material and methods

2.1. Field site and sampling

The study area is located at the southeastern edge of the Tengger Desert in Ningxia Hui Autonomous Region, close to the Yellow River. This area is an ecotone between steppified desert and desertified steppe, being also a transitional zone between sandy bare and revegetated deserts (Li et al., 1998). The mean annual precipitation, which falls primarily between May and September, is about 186.5 mm (according to meteorological records from 1956 to 2002), the mean annual temperature is 10.0 °C, and the mean monthly temperature is −6.9 °C in January and 24.3 °C in July (Li et al., 2011). The non-crusted dunes consist of 99.7% sand and 0.3% fine particles (silt and clay), whereas the BSCs contain 30%–35% fine particles (Li et al., 2006). A non-irrigated vegetation system was initially established in 1956 to protect the Baotou-Lanzhou railway line from sand burial. The vegetation in the area is dominated by psammophytes (e.g. *Hedysarum scoparium* Fisch., *Agriophyllum squarrosum* Moq., and *Pugionium calcaratum* Kom.), covering about 1% of the area (Li et al., 2004). Following surface stabilization, cyanobacteria (previously termed blue-green algae) and green algae began to colonize the dune surfaces. These algae crusts were then gradually converted to moss and lichen crusts (Li et al., 2003).

Sampling was performed on 26–27 June 2012. Fourteen samples (sand or BSC to 2–3 cm deep), including non-crusted sands, algal crusts, moss crusts and lichen crusts (Table 1), were collected directly into 100 ml sterile plastic tubes. Samples were then immediately transported to the laboratory and stored at −80 °C prior to nucleic acid extractions.

2.2. Abiotic parameters

The following abiotic parameters of sand samples were measured: organic carbon (C), total nitrogen (N), total phosphorus (P), total potassium (K), pH and electrical conductivity (EC) (Table 1). Organic C and total P and K were measured by the methods described by Liu et al. (1996). The pH was measured in water (soil:water ratio = 1:2.5) using a pH electrode. Total N was measured with a Kjeltec system 1026 Distilling Unit (Tecator AB, Sweden). EC was measured in water (soil:water ratio = 1:5) using a portable conductivity meter (Cole-Parmer Instrument Company, USA).

2.3. DNA extraction and PCR amplification

DNA was extracted from sub-samples of sand (0.25 g), from which visible moss and lichen thalli had been removed, using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA) according to the manufacturer's instructions. DNA was extracted from three replicate sub-samples from each sample. The fungal internal transcribed spacer (ITS; ITS1–5.8S–ITS2) region of the nuclear ribosomal DNA was amplified using the ITS1F and ITS4 primer set (White et al., 1990; Gardes and Bruns, 1993). The PCR amplification was performed using the amplicon fusion primers 5′-A-x-ITS1F-3′ and 5′-B-ITS4-3′, where A and B represent the pyrosequencing adaptors and x represents an 8 bp-tag for sample identification. The 20 μl reaction mixture contained the template DNA (10 ng), 4 μl of 5 × buffer, 2 μl of 2.5 nM dNTP, 0.8 μl of FastPfu Polymerase, 2 μM of each primer and ddH_2O. PCR started with an initial denaturation at 95 °C for 2 min, then 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min.

2.4. 454 pyrosequencing and data treatment

The PCR products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). The purified PCR

Table 1
Locations and abiotic properties of the 14 sand samples studied.

Sample code	Type (dominant species)	Latitude and longitude	Altitude (m)	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	pH	EC (μS cm^{-1})
S1	Non-crusted sand	37°25′ N; 104°34′ E	1701	1.36	0.11	0.48	17.0	9.37	215.5
S34	Non-crusted sand	37°28′ N; 105°00′ E	1345	0.87	0.09	0.26	15.0	9.04	60.3
S45	Non-crusted sand	37°28′ N; 105°00′ E	1259	0.79	0.09	0.23	15.0	9.21	54.3
S4	Algae crust sand	37°25′ N; 104°34′ E	1701	9.37	0.84	1.04	18.0	8.97	173.5
S28	Algae crust sand	37°28′ N; 105°00′ E	1333	7.32	0.59	0.49	16.0	8.69	139.9
S38	Algae crust sand	37°28′ N; 105°00′ E	1259	12.74	0.86	1.02	17.0	8.48	229
S2	Moss crust sand (*Didymodon* spp.)	37°25′ N; 104°34′ E	1701	18.42	1.26	1.10	17.0	8.77	204.9
S10	Moss crust sand (*Didymodon* spp.)	37°28′ N; 104°40′ E	1698	48.30	2.09	1.13	16.0	8.23	228
S14	Moss crust sand (*Didymodon* spp.)	37°26′ N; 104°46′ E	1619	27.24	1.35	0.95	16.0	8.12	323
S21	Moss crust sand (*Didymodon* spp.)	37°28′ N; 105°00′ E	1329	27.16	2.09	0.92	16.0	8.29	307
S27	Moss crust sand (*Didymodon* spp.)	37°28′ N; 105°00′ E	1335	35.62	1.45	0.93	16.0	8.02	385
S6	Lichen crust sand (*Endocarpon* spp.)	37°28′ N; 104°40′ E	1698	33.84	1.83	1.14	17.0	8.41	249
S12	Lichen crust sand (*Endocarpon* spp.)	37°26′ N; 104°46′ E	1619	21.94	1.52	1.01	16.0	8.31	223
S16	Lichen crust sand (*Endocarpon* spp.)	37°26′ N; 104°46′ E	1619	39.81	2.29	1.18	16.0	8.10	254