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Responses of soil microbial biomass and community composition to biological soil crusts in the revegetated areas of the Tengger Desert

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ABSTRACT

As a key component of desert ecosystems, biological soil crusts (BSCs) play an important role in dune fixation and maintaining soil biota. Soil microbial properties associated with the colonization and development of BSCs may indicate soil quality changes, particularly following dune stabilization. However, very little is known about the influence of BSCs on soil microbes in sand dunes. We examined the influence of BSCs on soil microbial biomass and community composition in revegetated areas of the Tengger Desert. BSCs increased soil microbial biomass (biomass C and N), microbial phospholipid fatty acid (PLFA) concentrations and the ratio of fungal to bacterial PLFAs. The effects varied with crust type and crust age. Moss crusts had higher microbial biomass and microbial PLFA concentrations than cyanobacteria-lichen crusts. Crust age was positively correlated with microbial biomass C and N, microbial PLFA concentrations, bacterial PLFA concentrations, fungal PLFA concentrations and the ratio of fungal to bacterial PLFAs. BSCs significantly affected microbial biomass C and N in the 0–20 cm soil layers, showing a significant negative correlation with soil depth. The study demonstrated that the colonization and development of BSCs was beneficial for soil microbial properties and soil quality in the revegetated areas. This can be attributed to BSCs increasing topsoil thickness after dunes have been stabilized, creating suitable habitats and providing an essential food source for soil microbes.

1. Introduction

Biological soil crusts (BSCs) are diverse microbial associations of cyanobacteria, algae, fungi, lichens, other bacteria, and mosses (Billings et al., 2003). BSCs are widely distributed in arid and semi-arid landscapes, which account for 33–40% of the Earth's terrestrial surface (Belnap and Lange, 2003). BSCs frequently occupy the interspaces between sparse vascular plants in a variety of habitats, vary from a few millimeters to a few centimeters in depth, and are common in revegetated areas of the Tengger Desert (Belnap and Lange, 2003; Li et al., 2010). The BSCs develop through successional stages over time after sand stabilization or disturbance. Mobile cyanobacteria are the pioneers in colonizing sand dunes, owing to their ability to withstand high temperatures, radiation, and low water potential (West, 1990; Belnap, 1993; Zaddy et al., 2000). Subsequently, lichens, green algae, and mosses become established in the later stages of successional maturity (Zaddy et al., 2000; Neher et al., 2009). Numerous important ecosystem services have been ascribed to BSCs in desert ecosystems, including preventing soil erosion caused by wind and water; enhancing soil fertility and stability; increasing soil temperature, moisture, aeration, and porosity; changing local-scale hydrology; affecting vascular plant colonization; and improving soil invertebrate diversity (Evans and Johansen, 1999; Prasse and Bornkamm, 2000; George et al., 2003; Warren, 2003; Guo et al., 2008; Neher et al., 2009; Liu et al., 2011; Zhao et al., 2011).

Soil microbes are essential ecosystem components, acting as major players in carbon cycling, nutrient dynamics, and ecosystem productivity (Hackl et al., 2004; Han et al., 2007). The biomass and composition of soil microbial communities show a close relationship with soil properties such as texture, organic matter content, nutrient concentrations, pH level, moisture, and temperature (Emmerling et al., 2001). Microbial communities also respond rapidly to disturbance, recovery, or other environmental changes. Therefore, soil microbial biomass and community composition have been used as sensitive bioindicators of the impacts of environmental changes and human influences on soil quality (Fernandes et al., 2005). In recent years, the close relationship of BSCs with belowground soil organisms has attracted increasing attention. Belnap and Lange (2003) suggested that BSCs could affect belowground microbes and microfauna communities by providing food, suitable habitats, or both. Some reports suggested that

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Table 1
Topsoil (depth 0–20 cm) and crust properties in revegetated areas of the Tengger Desert.

Soil and crust properties	Years after re-vegetation (y)				
	55 (1956)	47 (1964)	30 (1981)	20 (1991)	0 (mobile sand dunes)
Sand (%)[a]	66.39	68.28	71.54	78.87	99.67
Silt (%)[a]	22.59	24.79	23.59	15.60	0.12
Clay (%)[a]	11.01	6.93	4.87	4.45	0.21
Soil water content (%)[a]	2.56	2.2	2.09	1.92	1.55
Organic carbon (g kg^{-1})[a]	7.74	7.59	4.32	1.65	0.37
Total nitrogen (g kg^{-1})[a]	1.02	0.74	0.52	0.22	0.17
Total phosphorous (g kg^{-1})[a]	0.77	0.75	0.71	0.44	0.4
pH[a]	7.99	7.95	7.90	7.82	7.42
EC (m s^{-1})[a]	0.19	0.17	0.15	0.14	0.09
Bulk density (%)[a]	1.44	1.47	1.5	1.52	1.53
The thickness of soil crust and subsoil (cm)[a]	2.5	2.2	1.4	0.72	0
Cyanobacteria-lichen coverage (%)[b]	39	31.2	11.8	6.9	0
Moss coverage (%)	56.54	57.46	54.02	49.82	0
Crustal coverage (%)[c]	95.54	88.66	65.10	56.72	0

[a] Li et al. (2007a).
[b] Su et al. (2011).
[c] Li et al. (2011).

soil microarthropod and nematode communities are significantly influenced by the successional stages of BSCs (Belnap and Eldridge, 2001; Darby et al., 2007; Neher et al., 2009; Liu et al., 2011). Yu et al. (2012) found that the structure and biomass of bacteria and fungi varied among BSCs in different development stages. Although the importance of BSCs to soil biota is being gradually recognized, relatively little is known about the impacts of BSCs, crust type, and crust age on soil microbial biomass and community composition.

In the study, we examined the responses of soil microbial biomass and community composition to BSC characteristics and quantified the function of BSC in revegetated areas of the Tengger Desert in northern China. Understanding the spatial range and types of impacts of crusts on below-ground soil processes may facilitate exploitation of BSCs in the recovery of degraded areas of the desert. We tested four hypotheses. First, BSCs increase the biomass and composition of below-ground microbial communities. This hypothesis is based on prior results showing that BSCs improved soil properties (Li et al., 2007a, 2011). Second, below-ground microbial biomass and community composition vary with crust type. Soil under late stage crusts has higher organic matter, total N, total P, moisture, and lower daytime temperatures than soil under early stage crusts (Li et al., 2011). Third, crust age is positively correlated with soil microbial biomass. Fourth, soil microbial biomass under crusts declines with soil depth.

2. Materials and methods

2.1. Site description

The field study was conducted in Shapotou of the Ningxia Hui Autonomous Region at the southeastern edge of the Tengger Desert in northern China (37°32′N and 105°02′E), at 1339 m above mean sea level. This area is a typical transitional zone between desertified steppe and desert (Li et al., 2004). The mean annual temperature is 10.0 °C with the coldest monthly mean temperature of −6.9 °C in January and warmest of 24.3 °C in July. The mean annual precipitation is approximately 180 mm, and 80% of the total rainfall occurs between May and September. The mean potential evaporation is about 3000 mm. The northwest wind is predominant for the area with a mean annual wind speed of 3.5 m s^{-1}. The soil consists of 71.3% fine sand with grain sizes between 0.05 and 1 mm, 21.7% silt with grain sizes between 0.002 and 0.05 mm, and 6.9% clay with grain sizes less than 0.002 mm. The loose, infertile, and mobile soil at the study site is classified as orthic sierozem and aeolian sandy soil, with very low but constant moisture content of 3–4%, and soil organic matter content of 1.5–4.0 g kg^{-1} (Li et al., 2007b; Liu et al., 2011). The major native plants include *Hedysarum scoparium* Fisch., *Agriophyllum squarrosum* Moq. and *Psammochloa villosa* Bor, with a cover of about 1–2% (Shapotou Desert Experiment Research Station, CAS, 1991).

To protect the natural desertified steppe from burial by constantly extending sand dunes, sand-binding vegetation was established along the Baotou-Lanzhou railway in 1956 by erecting 1 m × 1 m straw checkerboard sand barriers on shifting sandy surfaces, with a width of 500 m to the north side and 200 m to the south side of the railway. Parallel stabilized areas were expanded one by one along the railway line in 1964, 1981, and 1991. Once the shifting sandy surfaces were stabilized, shrubs like *Artemisia ordosia Krasch*, *Caragana korshinskii Kom. and Caragana microphylla* Lam. were planted within the checkerboard and they grew only with rain inputs. BSCs colonized and developed on these stabilized dunes once sand-binding vegetation was established. The BSCs evolved from the initial cyanobacteria-dominated crusts to lichen- and moss- dominated crusts. The common members of BSCs in this region include cyanobacteria, algae, lichens and mosses (Li et al., 2011). In our study areas, moss crusts were green under wet conditions and were 8–20 mm thick while cyanobacteria-lichen crusts were dark brown or black with a clear surface microtopography and were 2–3.5 mm thick. Currently, the average coverage of crusts is high with more than 80% of the revegetated areas (Jia et al., 2008). The area covered by crusts, and the thickness of soil crust and subsoil increase with time after sand stabilization (Table 1). We named soil crusts established in 1956, 1964, 1981, and 1991 as 55-, 47-, 30-, and 20-year-old crusts, respectively. The composition of dominant vegetation has successively changed from shrub-only, with species such as *C. korshinskii* and *H. scoparium*, to annual plants and shrubs with shallow-rooting system (*A. ordosia*) after sand stabilization. The colonization and development of BSCs on the sand surface increased soil clay and silt content, water content, soil pH, electric conductivity (EC), organic C, total N, and total P, but decreased soil bulk density in the revegetated areas compared to mobile sand dunes (Table 1).

2.2. Soil sample collection and preparation

Soil samples were collected in July 2011 from different revegetated areas stabilized in 1956, 1964, 1981, and 1991, respectively. The mobile sand dunes were used as the contrast.

Within each revegetated area, five sub-plots (10 m × 10 m) existing cyanobacteria-lichen and moss crusts were established, at least 20 m apart. In addition, five sub-plots (10 m × 10 m) without BSCs were established in the mobile sand dunes, at least 20 m apart. Within each sub-plot, soil samples under cyanobacteria-lichen crusts were collected from each soil layer at depths of 0–10 cm, 10–20 cm and 20–30 cm, respectively, with a core sampler (4 cm inner diameter × 10 cm depth). The five soil samples from five sub-plots with the same soil depth were mixed to form a composite sample. Three duplicated soil samples were taken from each plot using the same method. Soil samples under moss crusts and of mobile sand dunes were also collected using the same method as cyanobacteria-lichen crusts. Each soil sample was individually packaged in a plastic bag and brought back to the laboratory for preparation before laboratory analysis. In total, 72 soil samples from three soil layers under cyanobacteria-lichen and moss crusts and nine sand dune soil samples from three soil layers were collected.

A sieve with 2 mm apertures was used to remove large plant debris and stones before samples were stored at 4 °C until analysis. All soil samples were analyzed for soil microbial biomass C and N. Eighteen soil samples from the 0 to 10 cm depth under cyanobacteria-lichen and moss crusts and nine sand dune soil samples from the 0 to 10 cm soil depth were used in analysis of microbial community composition. Soil moisture content was determined gravimetrically using 20 g of field moist soil sample oven-dried at 105 °C for 24 h.

2.3. Soil microbial analysis

Each soil sample was divided into three equal subsamples for microbial biomass C and N determination using the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Two portions, 25 g (oven dry weight) moist soil were taken from each soil sample. One portion was fumigated for 24 h at 25 °C with ethanol free $CHCl_3$, while the other portion (serving as the control) was incubated under the same condition without $CHCl_3$. After removing $CHCl_3$, C and N were extracted from the fumigated and non-fumigated samples with 100 ml 0.5 M K_2SO_4 by shaking for 30 min in a rotatory shaker at 200 rpm. Organic C in the filtered extracts was measured by the titrimetric method of oxidation with potassium dichromate, and total N was measured by Kjeldahl digestion. The difference between C and N extracted from fumigated and non-fumigated samples was converted into C and N in the microbial biomass by using the K_{EC} and K_{EN} factors with values of 0.38 (Vance et al., 1987) and 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996), respectively.

Phospholipid fatty acids (PLFAs) were extracted, fractionated and methylated using the methods described by Frostegård et al. (1991) and Wu (2009). The abundance of individual fatty acids was determined as nmol per g of dry soil and standard nomenclature was used (Tunlid et al., 1989; Liu et al., 2012). Concentrations of each PLFA were calculated based on the concentration of methyl non-adecanoate fatty acid (19:0), used as an internal standard. Gram-positive bacteria were identified by PLFAs: *i*15:0, *a*15:0, *i*16:0, *i*17:0, and *a*17:0 whereas Gram-negative bacteria were identified by PLFAs: 16:1ω7*c*, 18:1ω9*c*, and 18:1ω 7*c* (Frostegård et al., 1993; Zelles, 1999). Fungi were identified by PLFA 18:2ω6, 9 (Frostegård and Bååth, 1996), and PLFA 10Me 16:0 was used as a marker for actinomycetes (Fierer et al., 2003). The ratio of fungal to bacterial PLFAs (F:B) was calculated using 18:2ω6, 9 and the sum of bacterial PLFAs (Frostegård and Bååth, 1996). Other unspecific PLFAs such as 16:0, 16:1 2OH, and 18:0 were used in conjunction with group-specific PLFAs to quantify total soil microbial biomass.

2.4. Statistical analysis

Statistical analysis was done using the SPSS 16.0 software. A repeated measures ANOVA was used to evaluate the differences in microbial biomass C and N relative to soil depth, crust age, and crust type. The correlations of soil depth and crust age with microbial biomass were examined with Pearson's correlations coefficients. Two way ANOVA followed by a Tukey's HSD post hoc test was used to analyze the effects of crust age and crust type on microbial PLFA concentrations, bacterial PLFA concentrations, fungal PLFA concentrations, and the ratio of fungal to bacteria PLFAs. Thirteen individual PLFAs (mol%) from the PLFA analysis of soil samples were subjected to principal component analysis (PCA) after standardization for equal unit variance. Differences obtained at levels of $p < 0.05$ were considered significant.

3. Results

3.1. Microbial biomass C and N

Soil crusts significantly affected soil microbial biomass C and N in the revegetated areas. In the 0–10 cm and 10–20 cm soil layers, soil microbial biomass C was significantly higher under crusts than in mobile sand dunes ($p < 0.05$), and the highest of 73.92 and 88.00 mg kg^{-1} in the topsoil layer (0–10 cm) were obtained under 55-year-old cyanobacteria-lichen and moss crusts, respectively (Fig. 1a and b). However, there were no significant differences in the 20–30 cm soil layer among moss crusts and cyanobacteria-lichen crusts or mobile sand dunes (Fig. 1a and b).

Soil microbial biomass C also varied significantly with crust type, crust age, and soil depth (Table 2, $p < 0.05$). Soil microbial biomass C was significantly higher under moss crusts than under cyanobacteria-lichen crusts (Table 2, $p < 0.05$). The age of cyanobacteria-lichen and moss crusts was significantly related with soil microbial biomass C (Table 2, $p < 0.001$) with a positive correlation ($r = 0.987$ and $r = 0.985$, respectively, $p < 0.05$). Soil microbial biomass C under cyanobacteria-lichen and moss crusts significantly decreased with soil depth (Table 2, $p < 0.001$) with a negative correlation ($r = 0.999$ and $r = 0.997$, respectively, $p < 0.05$). The interactions between crust age and soil depth on microbial biomass C were also significant ($p < 0.001$), meaning that the effect of crust age on microbial biomass C varied with soil depth.

Soil microbial biomass N was significantly higher under crusts than in mobile sand dunes in the 0–10 cm and 10–20 cm soil layers ($p < 0.05$). Microbial biomass N reached its highest value of 5.65 and 9.40 mg kg^{-1} in the 0–10 cm soil depth under 55-year-old cyanobacteria-lichen and moss crusts, respectively (Fig. 1c and d). There were no significant differences in microbial biomass N detected in the 20–30 cm soil layer under crusts compared with mobile sand dunes (Fig. 1c and d).

Soil microbial biomass N also varied significantly with crust type, crust age, and soil depth (Table 2, $p < 0.001$). Soil microbial biomass N was significantly higher under moss crusts than under cyanobacteria-lichen crusts (Table 2, $p < 0.001$) and the effect of crust type on microbial biomass N was determined by interactions with soil depth, meaning that the effect of crust type on microbial biomass N varied with soil depth. The age of cyanobacteria-lichen and moss crusts significantly influenced soil microbial biomass N (Table 2, $p < 0.001$) and was positively correlated with soil microbial biomass N in the two crust types ($r = 0.983$ and $r = 0.966$, respectively, $p < 0.05$). Soil microbial biomass N under cyanobacteria-lichen and moss crusts significantly varied with soil depth (Table 2, $p < 0.001$), showing a negative correlation with soil depth ($r = 0.946$ and $r = 0.955$, respectively, $p < 0.05$). The interactions between crust age and soil depth on microbial biomass N

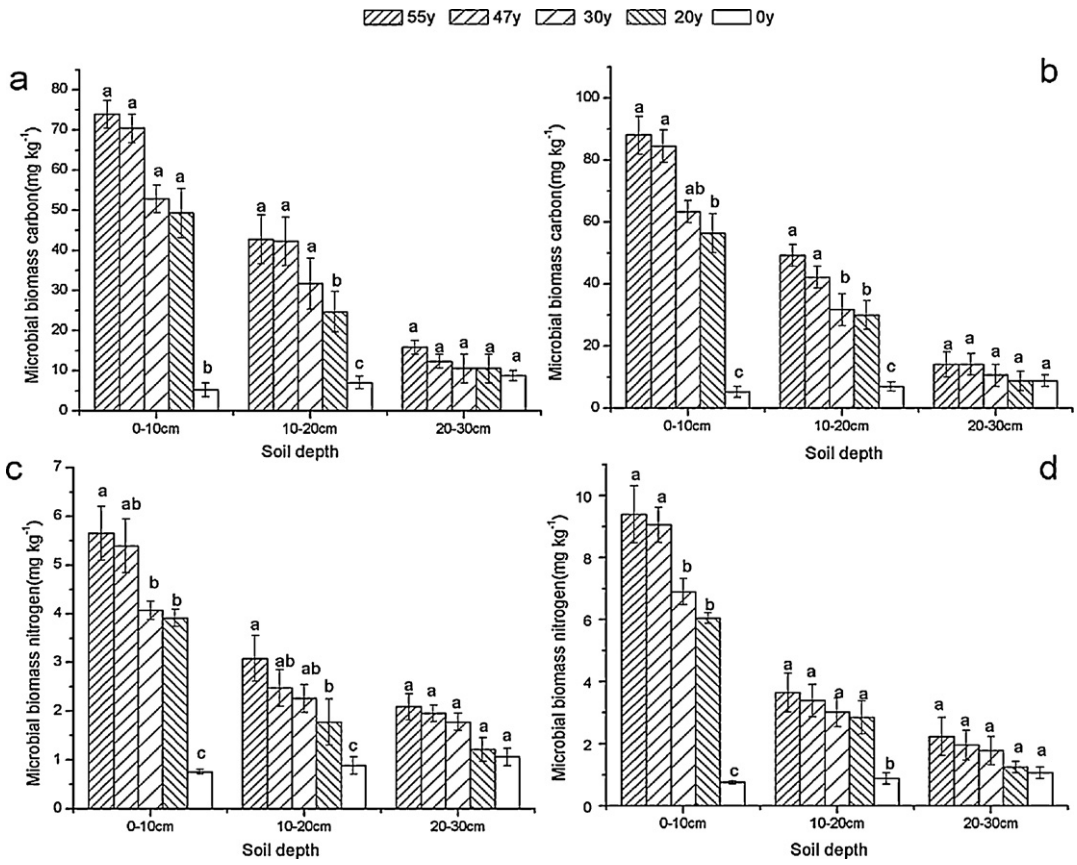

Fig. 1. Soil microbial biomass C and N under cyanobacteria-lichen and moss crusts in revegetated areas of the Tengger Desert. Panels a and c refer to microbial biomass C and N, respectively, under cyanobacteria-lichen crusts; panels b and d refer to microbial biomass C and N, respectively, under moss crusts. Crust age, or time since colonization, is indicated by 55 y, 47 y, 30 y, 20 y, 0 y. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show standard error (SE) ($n = 3$).

Table 2
Statistical analysis of microbial biomass C and N under cyanobacteria-lichen and moss crusts.

Source of variation	Type III Sum of Squares	df	Mean Square	*F*	*p*
Microbial biomass C					
Within					
Soil depth	29,509.2	1	29,509.2***	235.2	0.000
Soil depth * crust type	225.1	1	225.1	1.8	0.195
Soil depth * crust age	10,128.9	4	2532.2***	20.2	0.000
Soil depth * crust type * crust age	67.9	4	17.0	0.1	0.967
Error (soil depth)	2509.1	20	125.5		
Between					
Crust type	379.7	1	379.7*	2.1	0.017
Crust age	17,751.1	4	4437.8***	24.2	0.000
Crust type* crust age	161.1	4	40.3	0.2	0.924
Error	3667.6	20	183.4		
Microbial biomass N					
Within					
Soil depth	189.7	1	189.7***	222.8	0.000
Soil depth * crust type	22.4	1	22.4***	26.3	0.000
Soil depth * crust age	63.5	4	15.9***	18.7	0.000
Soil depth * crust type* crust age	6.8	4	1.7	2.0	0.133
Error (soil depth)	17.0	20	0.9		
Between					
Crust type	25.0	1	25.0***	39.8	0.000
Crust age	132.5	4	33.1***	52.7	0.000
Crust type * crust age	6.9	4	1.7	2.7	0.057
Error	12.6	20	0.6		

* $p < 0.05$.
*** $p < 0.001$.

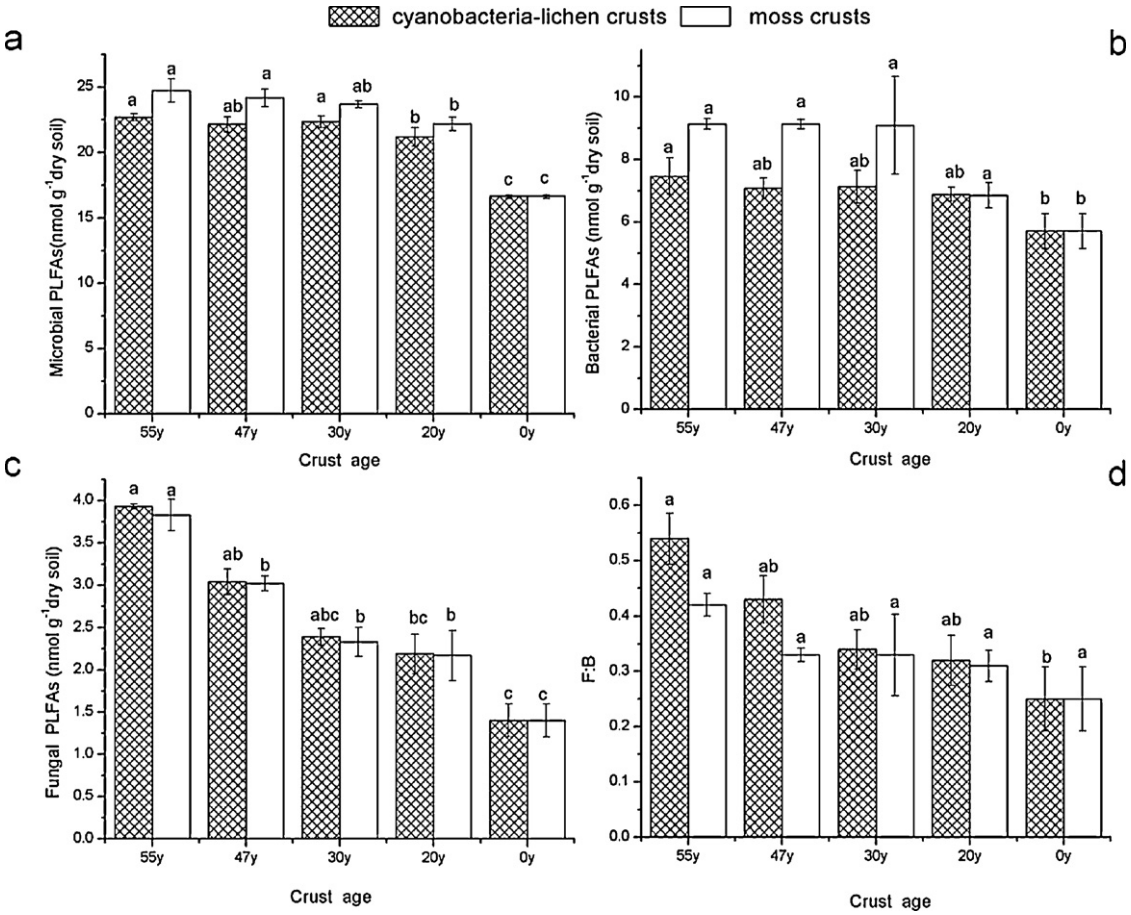

Fig. 2. Soil microbial PLFAs under cyanobacteria-lichen and moss crusts in revegetated areas in the Tengger Desert. Panel a – total microbial PLFAs. Panel b – bacterial PLFAs. Panel c – fungal PLFAs. Panel d – ratio of fungal to bacterial (F:B) PLFAs. Crust age is indicated by 55 y, 47 y, 30 y, 20 y, 0 y. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show standard error (SE) ($n = 3$).

were significant ($p < 0.001$), meaning that the effect of crust age on microbial biomass N varied with soil depth.

3.2. Microbial community composition

Soil crusts significantly affected microbial PLFA concentrations in the 0–10 cm soil layer in the revegetated areas. Microbial PLFA concentrations were significantly higher under crusts than in mobile sand dunes ($p < 0.05$), reaching a maximal value of 22.68 and 24.74 nmol g^{-1} dry soil under 55-year-old cyanobacteria-lichen and moss crusts, respectively (Fig. 2a). Microbial PLFA concentrations also showed significant variability with crust type and age (Table 3, $p < 0.01$). Microbial PLFA concentrations were significantly higher under moss crusts than under cyanobacteria-lichen crusts (Table 3, $p < 0.01$). The age of cyanobacteria-lichen and moss crusts significantly affected microbial PLFA concentrations (Table 3, $p < 0.001$), displaying positive correlations with microbial PLFA concentrations in the two crust types ($r = 0.884$ and $r = 0.826$, respectively, $p < 0.05$).

Bacterial PLFA concentrations, fungal PLFA concentrations, and the ratio of fungal to bacterial PLFAs were higher under crusts than under mobile sand dunes, but not always statistically significant (Fig. 2b–d). The concentrations of bacterial PLFA and fungal PLFA significantly varied with crust age (Table 3, $p < 0.05$), displaying positive correlations with age of cyanobacteria-lichen ($r = 0.903$ and $r = 0.972$, respectively, $p < 0.05$) and moss crusts ($r = 0.909$ and $r = 0.974$, respectively, $p < 0.05$). The ratio of fungal to bacterial PLFAs was significantly affected by crust age (Table 3, $p < 0.05$), displaying positive correlations with age of cyanobacteria-lichen and moss crusts ($r = 0.956$ and $r = 0.910$, respectively, $p < 0.05$). However, crust type did not show any significant effect on bacterial PLFA concentrations, fungal PLFA concentrations, or the ratio of fungal to bacterial PLFAs (Table 3).

The first and second principal components (PC1 and PC2) accounted for 47.70% and 26.11% of the variation in the 13 PLFAs dataset obtained in the revegetated areas (Fig. 3a and b). The PLFAs 18:1ω9c, 18:1ω7c, 16:1ω7c, i16:0, a15:0, and i15:0 were the main components for PC1 while a17:0 and 10Me 16:0 were main components for PC2 (Fig. 3b). Soil crusts changed the composition of soil microbial communities in the revegetated areas, as indicated by the shift along the PC2 axis of crusted soil compared with mobile sand dunes (Fig. 3a). The difference was mainly caused by PLFAs a17:0 and 10Me 16:0, which represent Gram-positive bacteria and actinomycetes, respectively.

4. Discussion

4.1. Effect of BSCs on soil microbial biomass and community composition

As we hypothesized, BSCs had a strong impact on the biomass and composition of soil microbial communities in the revegetated areas of the Tengger Desert. BSCs generally increased microbial biomass C and N, microbial PLFA concentrations, bacterial PLFA concentrations, fungal PLFA concentrations, and fungal to bacterial PLFA ratios. This may be mainly attributed to the favorable

Table 3
Statistical analysis of PLFA concentrations under cyanobacteria-lichen and moss crusts.

Source of variation	Type III Sum of squares	df	Mean square	*F*	*p*
Microbial PLFA concentrations					
Corrected model	222.6	9	24.7	29.4	0.000
Crust age	207.7	4	51.9***	61.7	0.000
Crust type	10.0	1	10.0**	11.9	0.003
Crust age * crust type	4.9	4	1.2	1.4	0.257
Bacterial PLFA concentrations					
Corrected model	45.8	9	5.1	2.1	0.084
Crust age	29.6	4	7.4*	3.0	0.043
Crust type	9.6	1	9.6	3.9	0.062
Crust age * crust type	6.6	4	1.7	0.7	0.616
Fungal PLFA concentrations					
Corrected model	21.0	9	2.3	24.2	0.000
Crust age	20.9	4	5.2***	54.4	0.000
Crust type	0.1E1	1	0.1E1	0.1	0.741
Crust age * crust type	0.1E1	4	0.3E2	0.3E1	0.998
F:B					
Corrected model	0.2	9	0.2E1	2.3	0.061
Crust age	0.2	4	0.4E1*	4.2	0.013
Crust type	0.2E1	1	0.2E1	1.7	0.211
Crust age * crust type	0.2E1	4	0.5E2	0.5	0.741

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

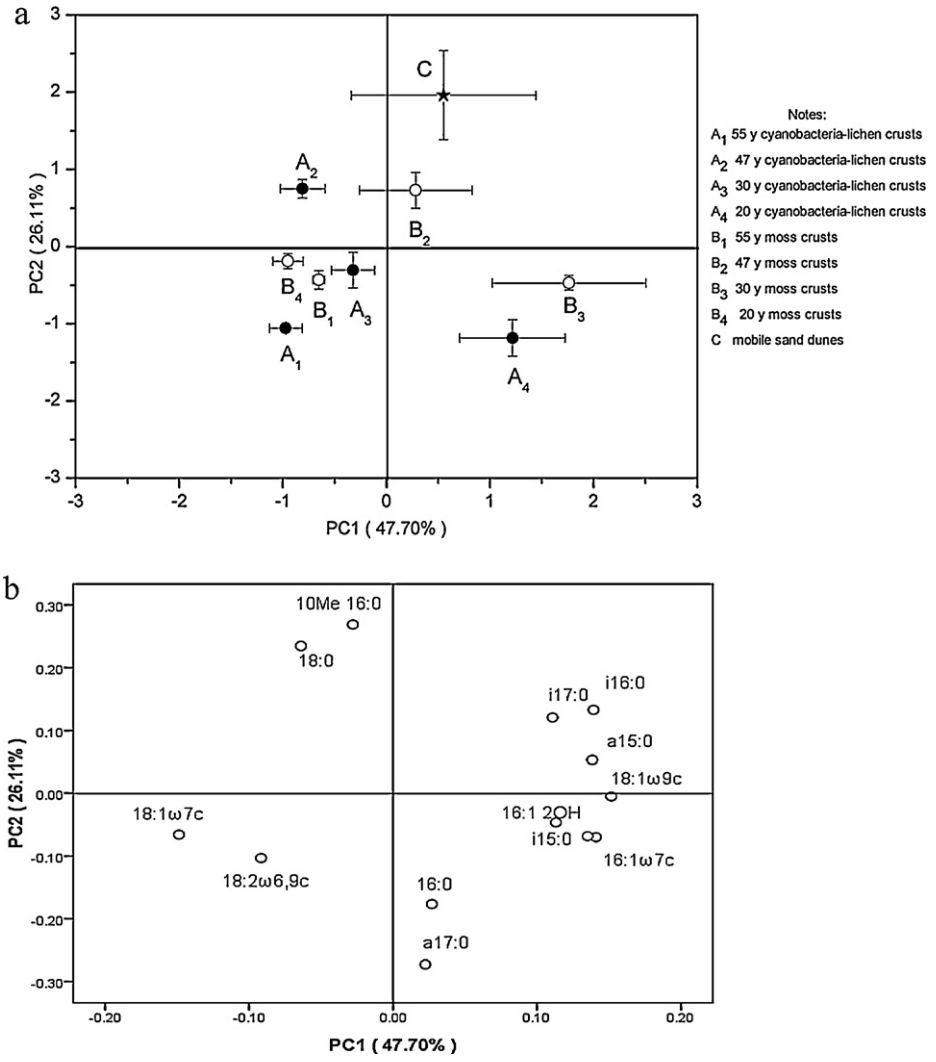

Fig. 3. (a) Score plot of principal component analysis (PCA) showing the separated soil under cyanobacteria-lichen and moss crusts as well as mobile sand dunes along PC1 and 2 in revegetated areas of the Tengger Desert and (b) loading values for the PLFAs. Error bars show standard error (SE) ($n = 3$).

environment created by BSCs with higher soil moisture and lower daytime temperatures than uncrusted soil and relatively abundant food sources for soil microbes, as suggested by Belnap and Lange (2003). This speculation is supported by our previous finding that soil under BSCs had 11.3-fold higher organic matter, 10.4-fold higher total N, 3.25-fold higher C:N ratio, 1.46-fold higher total P, 1.57-fold higher soil moisture, and 1.13-fold lower daytime temperatures than uncrusted soil in the study area (Li et al., 2011). These improved soil properties contribute positively to the activity and reproduction of soil microorganisms, enhancing bacterial and fungal biomass, and changing microbial community composition (Zogg et al., 1997; Li and Sarah, 2003; Steenworth et al., 2003; Drenovsky, 2004). Chen et al. (2009) reported similar findings, wherein BSCs significantly enhanced soil microbial biomass C and N in copper mine tailings in Tongling, China. Our data showed that soil bacterial biomass was higher than soil fungal biomass under BSCs in the revegetated areas. This is consistent with Belnap and Lange (2003) and Yu et al. (2012), who found that soil bacterial biomass dominated under crusts in some arid areas. Based on these results, dominance of bacteria over fungi may be a general characteristic of crusted soils in arid ecosystems.

4.2. Effect of crust type on soil microbial biomass and community composition

Late stage moss crusts had higher microbial PLFA concentrations and microbial biomass C and N than early stage cyanobacteria-lichen crusts, implying that late stage crusts can harbor considerably more microbes than early stage crusts as suggested by Belnap and Lange (2003) and Bates et al. (2010). Similarly, Yu et al. (2012) found that late stage crusts had higher soil microbial biomass than the early stage crusts in the western Negev Desert. Late stage crusts (moss crusts) may provide more abundant and diverse sources of food, suitable soil temperature, higher moisture and organic matter, and more stable food-webs than early stage crusts (cyanobacteria-dominated crusts), stimulating higher microbial metabolic activity or biomass accumulation (Evans and Lange, 2001; Housman et al., 2006; Li et al., 2010; Liu et al., 2011). For example, we showed that soil under late stage crusts had 1.26-fold higher organic matter, 1.88-fold higher total N, 1.77-fold higher total P, 1.29-fold higher soil moisture, and 1.15-fold lower daytime temperatures than soil under early stage crusts in revegetated areas of the Tengger Desert (Li et al., 2011). Our current study clearly showed that bacterial PLFA concentrations were higher under moss crusts than under cyanobacteria-lichen crusts. Higher soil fertility under moss crusts may contribute to increased bacterial biomass. In fact, Gu et al. (2009) suggested that soil fertility was the leading factor for increased bacterial biomass under moss crusts. The higher microbial PLFA concentrations under moss crusts relative to cyanobacteria-lichen crusts may be attributed mainly to bacteria groups instead of fungi because the dominant bacteria under moss crusts could achieve higher rates of growth and reproduction, possibly owing to more suitable food sources from moss crusts.

4.3. Effect of crust age on soil microbial biomass and community composition

Microbial PLFA concentrations, bacterial PLFA concentrations, fungal PLFA concentrations, the ratio of fungal to bacterial PLFAs, and microbial biomass C and N increased linearly with crust age as we hypothesized. This indicates that successional processes in the microbial communities and changes in soil quality may be ongoing under crusts in the revegetated areas. This is the first study to show that soil bacterial and fungal biomass positively correlated with crust age in an arid ecosystem, although a similar trend was observed in a study of reclaimed mine soils (Chodak et al., 2009). McKinley et al. (2005) reported that bacterial and fungal PLFA concentrations both increased with prairie age in prairie-restoration sites in North America. In our study, bacterial and fungal PLFA concentrations as well as the ratio of fungal to bacterial PLFAs indicated that the bacterial biomass increased less than fungal biomass with crust age. The positive linear correlation between crust age and soil microbial properties suggested that crust age was a significant factor in determining soil microbial biomass abundance and community composition. Crust age likely affected soil microbes in several ways. First, older crusts were thicker, which created more suitable habitat (more soil water and suitable temperature) with better food sources for soil fauna and microbes. Benefits to soil fauna can, in turn, stimulate soil microbial biomass. Second, soil physical and chemical properties (e.g. structure, pH, organic matter, N and P concentrations) improved with crust age, and the improved soil properties would favor microbial activity (Fierer et al., 2003; Hamman et al., 2007; Kara et al., 2008).

4.4. Effect of soil depths on soil microbial biomass C and N

Microbial biomass C and N under crusts showed a significant negative correlation with soil depth, consist with other reports (Fernandes et al., 2005; Yu and Steinberger, 2012). The significant differences in microbial biomass C and N between crusts and mobile sand dunes in the 0–20 cm soil layers suggested that BSCs could only significantly alter soil microbial parameters in the upper soil layers. There are mainly two reasons for this. First, BSCs may provide more food to soil microbes in the topsoil layer than in deeper soil layers. Higher organic matter and nutrients in the top soil (Jia et al., 2007) can support higher microbial biomass while the quantity and quality of substrates in deeper soil may limit microbial activity (Fierer et al., 2003). Secondly, BSCs provide a more favorable environment (i.e. soil physical, chemical, and biological properties) to microbes in the topsoil layer than in deeper soil layers (Li et al., 2007a). However, we found significant interactions between crust age and soil depth influencing microbial biomass C and N, which may imply that BSCs can influence soil microbes and improve soil quality not only in the upper soil layers (0–20 cm soil layers) but also in the deeper soil layers as crust age continues to increase. The result indicated that BSCs gradually promoted soil recovery in the vertical direction with the increasing of crust age in revegetated areas. Therefore, BSCs following sand stabilization could facilitate the recovery of degraded areas of the Tengger Desert.

5. Conclusion

The present study showed that BSCs increased soil microbial biomass, microbial PLFA concentrations and the ratio of fungal to bacterial PLFAs in revegetated areas of the Tengger Desert. Crust type and crust age could affect soil microbial communities. Moss crusts had higher microbial biomass than cyanobacteria-lichen crusts because moss crusts could provide more abundant and diverse sources of food, suitable soil temperature, higher moisture and organic matter content, and more stable food-webs for soil microbes compared with cyanobacteria–lichen crusts. Our findings show that soil quality improves with crust age, although the improvements are confined at present to the 0–20 cm soil layer. The collective effects of BSCs aid soil recovery in degraded areas in the Tengger Desert.

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References

Bates, S.T., Nash III, T.H., Sweat, K.G., Garcia-Pichel, F., 2010. Fungal communities of lichen-dominated biological soil crusts: diversity, relative microbial biomass, and their relationship to disturbance and crust cover. J. Arid Environ. 74, 1192–1199.

Belnap, J., 1993. Recovery rates of cryptobiotic crusts: inoculant use and assessment methods. Great Basin Nat. 53, 89–95.

Belnap, J., Eldridge, D., 2001. Disturbance and recovery of biological soil crusts. In: Belnap, J., Lange, O.L. (Eds.), Biological Soil Crusts: Structure, Function, and Management. Ecological Studies Series 150. Springer-Verlag, Berlin, pp. 363–383.

Belnap, J., Lange, O.L., 2003. Biological Soil Crust: Structure, Function and Management. Springer-Verlag, Berlin, 3–30.

Billings, S.A., Schaeffer, S.M., Evans, R.D., 2003. Nitrogen fixation by biological soil crusts and heterotrophic in an intact Mojave Desert ecosystem with elevated CO_2 and added soil carbon. Soil Biol. Biochem. 35, 643–649.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842.

CAS, Shapotou Desert Research and Experiment Station, 1991. Study on Shifting Sand Control in Shapotou Region of Tengger Desert (2). Ning xia people's publishing house, Yinchuan, 507 pp. (in Chinese).

Chen, Z., Yang, G.D., Sun, Q.Y., 2009. Effects of bio-crust on soil microbial biomass and enzyme activities in copper mine tailings. Chin. J. Appl. Ecol. 20 (9), 2193–2198 (in Chinese).

Chodak, M., Pietrzykowski, M., Niklińska, M., 2009. Development of microbial properties in a chronosequence of sandy mine soils. Appl. Soil Ecol. 41, 259–268.

Darby, B.J., Neher, D.A., Belnap, J., 2007. Soil nematode communities are ecologically more mature beneath late- than early-successional stage biological soil crusts. Appl. Soil Ecol. 35, 203–212.

Drenovsky, R.E., 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb. Ecol. 48, 424–430.

Emmerling, C., Udelhoven, T., Schröder, D., 2001. Response of soil microbial biomass and activity to agricultural de-intensification over a ten year period. Soil Biol. Biochem. 33, 2105–2114.

Evans, R.D., Johansen, J.R., 1999. Microbiotic crusts and ecosystem processes. Crit. Rev. Plant Sci. 18, 183–225.

Evans, R.D., Lange, O.L., 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In: Belnap, J., Lange, O.L. (Eds.), Biological Soil Crusts: Structure, Function and Management. Springer-Verlag, Berlin, pp. 203–279.

Fernandes, S.A.P., Bettiol, W., Cerri, C.C., 2005. Effect of sewage sludge on microbial biomass, basal respiration, metabolic quotient and soil enzymatic activity. Appl. Soil Ecol. 30, 65–77.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fert. Soils 22, 59–65.

Frostegård, A., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163.

Frostegård, A., Bååth, E., Tunlid, A., 1993. Shift in the structure of soil microbial communities in limed forest as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.

George, D.G., Roundy, B.A., St Clair, L.L., Johansen, J.R., Schaalje, G.B., Webb, B.L., 2003. The effects of microbiotic soil crusts on soil water loss. Arid Land Res. Manage. 17, 113–125.

Gu, Y.F., Zhang, X.P., Tu, S.H., Lindström, K., 2009. Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. Eur. J. Soil Biol. 45, 239–246.

Guo, Y.R., Zhao, H.L., Zuo, X.A., Drake, S., Zhao, X.Y., 2008. Biological soil crust development and its topsoil properties in the process of dune stabilization, Inner Mongolia, China. Environ. Geol. 54, 653–662.

Hackl, E., Bachmann, G., Zechmeister-Bolternstern, S., 2004. Microbial nitrogen turnover in soils under different types of natural forest. Forest Ecol. Manage. 188, 101–112.

Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol. Biochem. 39, 1703–1711.

Han, W.Y., Kemmitt, S.J., Brookes, P.C., 2007. Soil microbial biomass and activity in Chinese tea gardens of varying stand age and productivity. Soil Biol. Biochem. 39, 1468–1478.

Housman, D.C., Powers, H.H., Collins, A.D., Belnap, J., 2006. Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. J. Arid Environ. 66, 620–634.

Jia, X.H., Li, X.R., Li, Y.S., 2007. Soil organic carbon and nitrogen dynamics during the re-vegetation process in the arid desert region. Chin. J. Plant Ecol. 31, 66–74 (in Chinese).

Jia, R.L., Li, X.R., Liu, L.C., Gao, Y.H., Li, X.J., 2008. Responses of biological soil crusts to sand burial in a revegetated area of the Tengger Desert, Northern China. Soil Biol. Biochem. 40, 2827–2834.

Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass:calibration of the K_{EN} value. Soil Biol. Biochem. 28, 33–37.

Kara, Ö., Bolat, ì., Çakıroğlu, K., Öztürk, M., 2008. Plant canopy effects on litter accumulation and soil microbial biomass in two temperate forests. Biol. Fert. Soils 45, 193–198.

Li, X.Z., Sarah, P., 2003. Arylsulfatase activity of soil microbial biomass along a Mediterranean-arid transect. Soil Biol. Biochem. 35, 925–934.

Li, X.R., Zhang, Z.S., Zhang, J.G., Wang, X.P., Jia, X.H., 2004. Association between vegetation patterns and soil properties in the southeastern Tengger Desert, China. Arid Land Res. Manage. 18, 369–383.

Li, X.R., He, M.Z., Duan, Z.H., Xiao, H.L., Jia, X.H., 2007a. Recovery of topsoil physicochemical properties in revegetated sites in the sand-burial ecosystems of the Tengger Desert, northern China. Gemorphology 88, 254–265.

Li, X.R., Kong, D.S., Tan, H.J., Wang, X.P., 2007b. Changes in soil and in vegetation following stabilisation of dunes in southeastern fringe of the Tengger Desert, China. Plant Soil 300, 221–231.

Li, X.R., He, M.Z., Zerbe, S., Li, X.J., Liu, L.C., 2010. Micro-geomorphology determines community structure of biological soil crusts at small scales. Earth Surf. Proc. Land 35, 932–940.

Li, X.R., Jia, R.L., Chen, Y.W., Huang, L., Zhang, P., 2011. Association of ant nests with successional stages of biological soil crusts in the Tengger Desert, Northern China. Appl. Soil Ecol. 47, 59–66.

Liu, Y.M., Li, X.R., Jia, R.L., Huang, L., Zhou, Y.Y., Gao, Y.H., 2011. Effects of biological soil crusts on soil nematode communities following dune stabilization in the Tengger Desert, Northern China. Appl. Soil Ecol. 49, 118–124.

Liu, L., Gundersen, P., Zhang, T., Mo, J.M., 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. Soil Biol. Biochem. 44, 31–38.

McKinley, V.L., Peacock, A.D., White, D.C., 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol. Biochem. 37, 1946–1958.

Neher, D.A., Lewins, S.A., Weicht, T.R., Darby, B.J., 2009. Microarthropod communities associated with biological soil crusts in the Colorado Plateau and Chihuahuan deserts. J. Arid Environ. 73, 672–677.

Prasse, R., Bornkamm, R., 2000. Effect of microbiotic soil surface crusts on emergence of vascular plants. Plant Ecol. 15, 65–75.

Steenworth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R., Scow, K.M., 2003. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 35, 489–500.

Su, Y.G., Li, X.R., Zhang, Z.S., Chen, Y.W., Lu, Y., 2011. Time effect of photosynthetic carbon fixation of cyanobacterial crusts in arid artificially revegetated areas. Acta Pedologica Sin. 48, 570–577 (in Chinese).

Tunlid, A., Hoitink, H.A.J., Low, C., White, D.C., 1989. Characterization of bacteria that suppress rhizoctonia damping-off in bark compost media by analysis of fatty-acid biomarkers. Appl. Environ. Microbiol. 55, 1368–1374.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Warren, S.D., 2003. Synopsis: influence of biological soil crusts on arid land hydrology and soil stability. In: Belnap, J., Lange, O.L. (Eds.), Biological Soil Crusts: Structure, Function, and Management. Springer, Berlin/Heidelberg/New York, pp. 349–360.

West, N.E., 1990. Structure and function of microphytic soil crusts in wild land ecosystem of arid and semi-arid regions. Adv. Ecol. Res. 20, 179–223.

Wu, Y.P., 2009. Studies on soil microbial community structure based on phospholipid fatty acid (PLFA) analysis. Zhejiang University, Zhejiang, pp. 32-33 (in Chinese).

Yu, J., Steinberger, Y., 2012. Vertical distribution of soil microbial biomass and its association with shrubs from the Negev Desert. J. Arid Environ. 78, 110–118.

Yu, J., Kidron, G.J., Pen-Mouratov, S., Wasserstrom, H., Barness, G., Steinberger, Y., 2012. Do development stages of biological soil crusts determine activity and functional diversity in a sand-dune ecosystem? Soil Biol. Biochem. 55, 66–72.

Zaddy, E., Kuhn, U., Wilske, B., Sandoval-Soto, L., Kesselmeier, J., 2000. Patterns of CO_2 exchange in biological soil crusts of successional age. Soil Biol. Biochem. 32, 959–966.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fert. Soils 29, 111–129.

Zhao, H.L., Guo, Y.R., Zhou, R.L., Drake, S., 2011. The effects of plantation development on biological soil crust and topsoil properties in a desert in northern China. Geoderma 160, 367–372.

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Sci. Soc. Am. J. 61, 475–481.