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REGULAR ARTICLE

Development of artificially induced biological soil crusts in fields and their effects on top soil

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Abstract
Aims Biological soil crusts (BSCs) could improve severe environment ecological conditions by increasing soil moisture, soil nitrogen concentration, and so on. In order to control desertification and recover the destroyed soil fertility utilizing a new means using BSCs, the soil surface was artificially inoculated with *Microcoleus vaginatus* and *Scytonema javanicum*. Relationships between the development of the artificially induced biological soil crusts and the distribution and dynamic changes of nitrogen and phosphorus in the soil crusts have been analyzed.
Methods Crusts of different ages were investigated by measuring soil physical and chemical factors, such as moisture, pH, total and available N content, and total and available P, which were correlated with the depths of the crusts.
Results This study found that the types of color, shape, and species components of the algal crusts increased with crust development. Soil moisture, total N, available N, and available P increased gradually with crust growth. Soil with crusts was wetter than the controlled naked sandy soil, and a significant correlation was observed between biomass and total nitrogen (r=0.946, P=0.015). Soil pH was lower than that of control. The scytonemin on the soil surface was exceptionally higher than the other pigments, and all the pigments were mainly distributed at the soil surface level. Though the crusts were mainly distributed on soil surface, the available P was mainly stored below the crust layer.
Conclusions Pearson correlation tests indicated that artificially inoculated biological crusts could improve soil fertility and micro-environment of the top soil: The development of artificially induced BSCs was very well, and this was favorable to inducing the following crust succession.

Keywords Bioremediation · Biological soil crusts growth · Algal species · Nitrogen · Phosphorus

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Introduction

Biological soil crusts (BSCs) are highly specialized communities comprised of cyanobacteria, green algae, lichens, mosses, bacteria, and microfungi (West 1990). According to succession stage and dominant organism components, BSCs are classified as cyanobacteria

crusts, lichen crusts, and moss crusts (Belnap et al. 2001). BSCs can adapt themselves to extreme environment conditions, such as high temperature, pH and salinity, low precipitation, strong irradiation, and desiccation. Algal crusts, at the initial phase of BSCs succession, provide an excellent base for the following succession stages. Cyanobacteria in the crusts secrete scytonemin against strong UV radiation (Rao et al. 2011) using the air dew to grow (Rao et al. 2009) and exhibiting good tolerance to salt (Lan et al. 2010). On one hand, BSCs can fix mobile sand dunes and change top soil moisture (Galun et al. 1982) and resistant wind and water erosion, which result in an upturn of the surrounding environment and influencing seed germination (Harper and Marble 1988; Su et al. 2007). On the other hand, the soil fertility which BSCs improve through mineral chelation, dust entrapment, and metabolism are beneficial to invertebrates and reptiles, as well as vascular plants (Belnap et al. 2001; Bielders et al. 2002). For example, BSCs increase the content of soil organic matter through photosynthesis, meanwhile algal crusts and lichen crusts increase the soil nitrogen concentration via nitrogen fixation (Ettershank et al. 1978; Belnap et al. 2001).

The ecological function of BSCs mentioned above is of great help in resisting desertification (Li et al. 2003), boosting desert soil formation (Cabala et al. 2011), and changing nutrient circulation. Some studies have reported that cyanobacteria and lichens have the abilities to accelerate rock erosion by excreting organic acids (Chen et al. 2000; Brehm et al. 2005). This studies attempt to use BSCs as a bioremediation to control desertification and recover soil fertility at the southern edge of the Horbq Desert in China, where artificially induced cyanobacteria crusts had been developing over the past 9 years. To evaluate relationships between artificially induced crusts and the distribution and dynamics of nitrogen and phosphorus in crusts soil, this study investigated the soil crusts at different development time and soil depth by measuring physical and chemical factors, such as moisture, pH, total N and available N content, and total and available P. The development of quality of artificially induced BSCs was conducted through crusts biomass, components, structure, color, and thickness to make an interrelationship analysis with soil physicochemical properties.

Materials and methods

Study area

The study was conducted at the research station of Dalateqi County (40°21′ N, 109°51′ E), which is located at the South fringe of the Hopq Desert in Inner Mongolia, China. The elevation of the area is 1,040 m, and there are several mobile dunes with average height of 5 m above the ground. The annual precipitation there is between 240 and 360 mm; average annual evaporation is 2,160–2,448 mm, and the rainy season usually occurs from July to October. The climate is a typical continental monsoon pattern, with an average temperature of 6.1 °C annually and with windy days (>5 ms^{-1}) of more than 180 $daysyear^{-1}$ (Rao et al. 2009). The extreme temperatures in the area are 40.2 °C and−34.5 °C. Furthermore, the highest temperature on the sandy soil surface is up to 66 °C, whereas the lowest is −39 °C. Main vascular plants here are *Elymus dahuricus*, *Artemisia desterorum*, *Sophora alopecuroides*, *Medicago sativa*, and *Catsia tora*. Soil pH is about 8.56. The average contents of total N, organic C, and $CaCO_3$ are 0.19, 0.41, and 4.48 gkg^{-1} dry soil, respectively (Wang et al. 2008). Aeolian sandy soil and salt meadow soil are the main types of soil in this area. There are three kinds of terrain in this area: flat ground, fixed, and semi-fixed mobile dunes.

Cyanobacterial crust inoculation and cultivation

Microcoleus vaginatus and *Scytonema javanicum* were cultured on a large scale because these two filamentous cyanobacteria have important morphological and ecological functions in BSCs (Hu et al. 2002b, 2003; Rao et al. 2009), since they both bond sand grains with their filiform texture to fix the mobile dune surface. Firstly, the two species of cyanobacteria were separately cultured in BG-11 and BG-11_0 medium (*S. javanicum* fix atmospheric nitrogen) under 40 $\mu Em^{-2}s^{-1}$ light intensity and 14:10 h light/dark photoperiod in a green house (28±2 °C) for 6 days. Secondly, the two were cultured together in 60 m^3 raceway ponds for 14 days in summer natural ambient temperature and light illumination. Then, the mixture was harvested and inoculated onto the semi-fixed naked dunes surface. In the first 15 days, the inoculated area was watered 3 lm^{-2} every 3 days as the post-conservation. Barriers were set at the edge of the field inoculation

area to protect it from people, livestock tread, or fire disturbance. Since this experiment has been carried out for 10 years, algal crusts of different ages are studied in the area (Liu and Ley 1993; Hu et al. 2003; Xie et al. 2007; Wang et al. 2008).

Crusts sampling

The samplings were conducted twice in December 2009 and July 2010, the moments when biological crusts of six (0~7 years) development times were collected. The 6- and 7-year-old crusts are on the north border of the 5-year-old crusts, and the 4-year-old crusts are 300 m away from the 5-year-old crusts, and the 2-year-old crusts are 20 km away from the other aged crusts. Each area interested by the development age experiment was divided into nine subareas of equal size; five crust quadrats were taken at the same depth level in each replicate, and then the five examples were pooled into a single sample. Biological soil crusts (0 cm) and sandy soil at 0–2 cm and 2–5 cm depths below the crust were taken as three depth levels for each replicate. The crusts and sandy soil samples were placed in sterilized and dried envelopes and taken to the lab for analysis.

Crust sample analysis

According to Belnap et al. (2001), the shapes of induced BSCs were classified as flat, rugose, rolling, and pinnacled. The collected samples were air-dried in ventilated and shade conditions for 1 week and then kept in desiccators after moisture determination. Except for biomass measurements, the samples are first ground carefully with mortar and pestle and then sifted through a 0.2 mm sifter. Finally, they were kept in a desiccator for the following determination.

Pigment and biomass measurement

Three kinds of pigments from the biological crusts were determined namely chlorophyll *a* (Chl *a*), carotenoid (Car), and scytonemin (Scyt). Determination of algal crust biomass is measured by chlorophyll *a* content which is a significant presentation for algal crust biomass. Carotenoid is a kind of protective pigment which absorbs and delivers light energy for photosynthesis. And scytonemin represents the ability of crusts to resist against UV radiation (Büdel et al. 1997). The crusts samples (1.06 ± 0.03 cm^2) are ground and extracted with 10 ml 100 % acetone and kept at 4 °C for 18 h in the dark. Then, the extracts are centrifuged at 8,000 rpm for 10 min. In the end, the supernatant fluids are sucked to measure their pigment absorbance value at 384, 490, and 663 nm by a spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd.) after thin-layer chromatography. A group of trichromatic equations is used to obtain the absorbance value at 384 nm due to Scyt, the absorbance at 663 nm due to Chl *a*, and absorbance at 490 nm due to pooled Car (Garcia-Pichel and Castenholz 1991; Stephan and Richard 1997).

$$A_{384}(\text{Scyt.}) = 1.04A_{384} - 0.79A_{663} - 0.27A_{490}$$
$$A_{663}(\text{Chl } a) = 1.02A_{663} - 0.027A_{384} + 0.01A_{490}$$
$$A_{490}(\text{Car.}) = 1.02A_{490} - 0.08A_{384} - 0.026A_{663}$$

The contents of chlorophyll *a*, carotenoids, and scytonemin were calculated using specific extinction coefficients of scytonemin (112.6 lg $m^{-1}cm^{-1}$), chlorophyll *a* (92.6 lg $m^{-1}cm^{-1}$), and carotenoids (200 lg $m^{-1}cm^{-1}$) (Stephan and Richard 1997; Lan et al. 2010), expressed as μg cm^{-2} crusts.

Algae isolation and identification

The isolation and identification of algae in the artificially induced soil crusts were based on Liu and Ley (1989). Three kinds of media were used to isolate and identify the algae species in the crusts of different ages: Chu 10, BG-11, and BBM. After being pestled into powder, the crusts samples were cultured in distilled water for 2 days. Then, they were inoculated into the three solid mediums and cultured at 25 °C and under 40 $\mu Em^{-2}s^{-1}$ light intensity for 2 days. Finally, the algae were isolated, cultured in fluid mediums, and identified using electron microscope (Olympus CX 31, Japan); the algae morphological identification was referred to Hu et al. (2003) and Hu et al. (2006).

Crust moisture

Crusts samples were placed into aluminum boxes and dried at 105 °C for 24 h (Rao et al. 2009). The aluminum boxes were weighted for three times: empty, before being dried with crusts, and after being dried. Therefore, the value differences in weight

between the undried and dried boxes were considered to be the crust moisture.

Soil physico-chemical analysis

Total nitrogen (N) and total phosphorus (P) were determined by using the $CuSO_4$-Se Kjeldahl procedure and H_2SO_4-$HClO_4$ digestion colorimetry method. Available N was obtained using the alkaline hydrolization-diffusion method. Available P was gained by using the Olsen P method. When soil crusts was sampled from the soil with a cutting ring, the thickness of each sample is measured from crusts' upper surface to the lower surface with a Vernier caliper for five times, and the soil pH was determined in 1:1 soil/water slurry using a pH meter (pHS-4C$^+$). All the relevant methods are clearly described by Bao et al. (2000).

Statistical analysis

All data were analyzed by using SPSS 13.0 program. Differences among the samples of five ages and the three depths were tested using one-way ANOVA test. Pearson correlation coefficients test was used to evaluate the relationship of variances.

Results

Development factors of cultivated biological soil crusts

From the second to the seventh year, the aspect of the algal crust changed: Filamentous cyanobacteria had grown, and moss crusts emerged in the sixth year (in Fig. 1). Table 1 showed chlorophyll *a* had a significant interactive effect (r=0.885, P=0.046) on crust thickness and algae. And the thickness of the crusts could reach to 5.45 mm in the seventh year and twice the thickness of those 2-year-old crusts. Moisture of the crusts was correlated with the crusts biomass (r=0.917, P=0.028); the 7-year-old crust was 1.5 times more than control (naked sandy soil). Crust soil pH was apparently lower than the control, but it did not show any correlation with the algal chlorophyll *a* (r=−0.359, P>0.05). The color, shape, and component of the algal crusts began to have more types as crusts developed. However, the coverage of the crusts did not show any differences (P>0.05) among ages most probably because the BSCs were artificially inoculated.

Algal constitution analysis of different artificially inoculated crust development steps

In the control area (Table 2), no algae of any kind were found by using the optical microscope, whereas two main kinds of inoculated cyanobacteria were observed in the 2-year-old area crust samples. Filamentous cyanobacteria, as well as some other species belonging to Chlorophyta and Bacillariophyta, emerged in the crusts when the crusts grew to the fourth year. But *M. vaginatus* and *S. javanicum* were still the dominant algal species in the algal crusts.

Changes of pigments at different development times and soil depths

Figure 2 shows that there was an escalating trend in crusts biomass (expressed as chlorophyll *a*) and scytonemin from the second year to the sixth year; on the contrary, carotenoid did not increase. Chlorophyll *a* and scytonemin in the 7-year-old crusts could reach up to 24.38 and 183.1 $\mu g cm^{-2}$, respectively. In the 4-year-old crusts, the content of chlorophyll *a* was 21.5 $\mu g cm^{-2}$ which was between that of the 2-year-old and 5-year-old ones, whereas the scytonemin content was only 30.4 $\mu g cm^{-2}$ which was the least among all five development stages. But no significant correlation was found among chlorophyll *a*, scytonemin, and carotenoid (r=0.577, P>0.05).

Significant differences of all the three pigment contents were all observed (P<0.01) between the crust soil (0 cm) and soil below the biological soil crusts (0–2 and 2–5 cm, shown in Fig. 3). The content of scytonemin at the 2 cm depth under the crusts was only 0.0027 $\mu g cm^{-2}$, 1.52 % of the surface (0 cm) level crusts soil. The chlorophyll *a* content was only 21.64 %.

Comparison of N and P in soil at different development times and horizon levels

There was a progressive increase in the total N (P=0.015), available N (P<0.001), and available P (P=0.032) on the surface soil from the second to the seventh year crusts (Fig. 4). The content of total N and available P of the 7-year-old crusts was twice

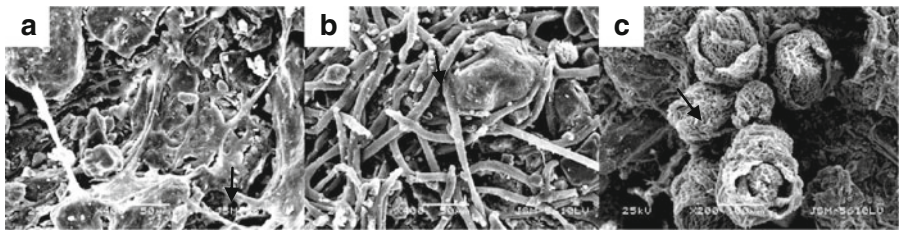

Fig. 1 Ultrastructure characteristics of BSCs at different development times; **a** 2-year-old algal crusts, and the *arrow* means the cyanobacteria filamentous adhesion with soil particles; **b** 5-year-old algal crusts, the *arrow* points at the increased cyanobacteria filaments; **c** 7-year-old moss crusts, the *arrow* indicates the moss in crusts

more than that of the 2-year-old crusts, and the available N was four times more than. The regression equations of available N was expressed as $y=0.0238x+0.0019$, $r=0.8745$, and $P=0.00$, whereas the total N was expressed as $y=0.0328x+0.1346$, $r=0.9044$, and $P=0.015$. A correlation between algal biomass and the total nitrogen ($r=0.946$, $P=0.015$) existed and also between available N and total N ($r=0.959$, $P=0.01$). However, no correlation between the crust biomass and available N ($r=0.819$, $P=0.09$) could be found. ANOVA test also indicated that no difference ($P=0.385$) of total P among the five age samples was found, whereas the available P exhibited obvious differences ($P=0.032$). However, the total P and available P did not show any correlation with Chl *a* ($P=0.680$ and $P=0.587$). The content of total N in the soil showed significant differences ($P<0.05$) between the two depth levels (Fig. 5): crust level (0 cm) and below crust layer (0–5 cm) level. So did available N, total P, and available P. As the soil depth increased, the content of available N and total N decreased. The total N and available N showed significant differences between crust level (0 cm) and 0–5 cm level ($P<0.01$), but there was no distinct difference between 0–2 and 2–5 cm levels ($P>0.05$). For both available P and total P, there was a significant difference between 0 and 0–2 cm ($P<0.05$), but not between 0–2and 2–5 cm level ($P>0.05$). The content of available P both in the 0–2 and 2–5 cm level soils was higher than at the crust surface.

Discussion

Effects of BSCs on the topsoil microenvironment

The present study showed that the microbes in crusts had the ability to promote soil moisture; Rao et al. (2009)'s results showed that the cyanobacterial crusts could not only use rainfall, but also use dew, and the soil moisture was positively correlated with dew. Crustal organisms could raise the soil pH to 10.5 (Garcia-Pichel and Belnap 1996), which leads to the decrease of some available nutrients and carbonate cycle rates (Nobel 1991; Belnap et al. 2001). However, some studies found soil pH was decreased due to soil microbe respiration which acted at increasing dissolved CO_2 concentrations (Bååth and Anderson 2003). These results might account for our survey where the formation of artificially induced BSCs decreased the soil pH. From scanning electron microscope observation, we inferred that, the more cyanobacterial filaments grew, the more extracellular

Table 1 Comparison of the development of artificial BSCs at different ages

Age (years)	Thickness (mm)	Color	Moisture (%)	pH	Crust shape	Coverage (%)	Dominant components
0	0	Fawn	0.33±0.09	9.1	Smooth	0	–
2	2.36±0.50	Grey	0.38±0.07	8.5±0.2	Flat	79±4	Cyanobacterial crusts
4	4.65±2.19	Grey	0.44±0.04	8.0±0.4	Pinnacled	87±7	Cyanobacterial crusts
5	6.82±1.67	Dark	0.55±0.12	8.4±0	Flat	93±8	97 % algal, 3 % lichen crusts
6	3.99±0.44	Grey	0.48±0.19	8.1±0.3	Rolling	83±4	92 % algal, 8 % moss crusts
7	5.45±1.49	Black	0.49±0.11	8.2±0.2	Rolling	74±11	92 % algal, 8 % moss crusts

Value represents the mean±SE

Table 2 Algal species components of artificial crusts at different ages

Age (years)	*M. vaginatus Gom.*	*S. javanicum*	*Phormidium tenue Gom*	*Chlorella vulgaris Beij.*	*Diatoma vulagare Bory*	*Frastrulia vulagaris Thwait*	*Chlorococcum Humicola*	*Palmella mucosa Kütz*
0								
2	+	+						
4	++	++	+	+	+		+	+
5	+	++	+	+	+	+		
6	++	++	+	+	+	+	+	+
7	++	++	+		+	+	+	+

[+] represents algal species that emerged in the crusts

[++] represents the biomass of the algae was ten times more than "+" in the crusts

polysaccharides (EPS) were released. Researches have found the EPS could absorb and retain mineral, and could bond cyanobacterial filaments with sand grains or clay particles as a whole, resulting in an increase of microbial algal crust formation and enhanced crust thickness (Metting 1981; Hu et al. 2002b, 2003).

Composition changes of algal species within the BSCs

We found that the algal crust was thin and the algal constitution was simple in the 2-year-old crusts, in which only *M. vaginatus* and *S. javanicum* could be found under the optical microscope. However, Chlorophyta and Bacillariophyta began to exist in the crusts as the crusts developed to the fourth year. The presence of diatoms and filamentous cyanobacteria enriched the sandy soil with nutrients, thus it created favorable conditions for BSC succession (Metting 1981; Zhang et al. 2009). Some species of Chlorophyta and filamentous cyanobacteria (*Scytonema* sp. and *Nostoc* sp., etc.) could associate fungi to form lichen crusts (Belnap et al. 2001; Hu and Liu 2003). The higher C and N fixation efficiency of lichen crusts made the microenvironment suitable for the colonization of mosses, which appeared more in interdune areas (Metting 1981; Belnap et al. 2001; Zhang et al. 2009). In the 6-year-old and 7-year-old crust study area, moss crusts existed, whereas lichens were not found. Taking into consideration of the spatial and time heterogeneity of soil characteristics, Bowker et al. (2005, 2006) found that lichen- and moss-dominated crusts were correlated with orthophosphates and $CaCO_3$ of soil in the Colorado Plateau. Rivera-Aguilar (2009) proved that lichen-dominated crusts were positively correlated with pH, too. Zhu et al. (2004) considered it also to be affected by soil texture and soil moisture. Thus, the present study indicated that the artificially inoculated biological crusts had a fine succession with the development time.

Pigments analysis

Scytonemin was an important sheath pigment possessed by cyanobacteria as a kind of UV-absorbing substance to provide photoprotection against light irradiation

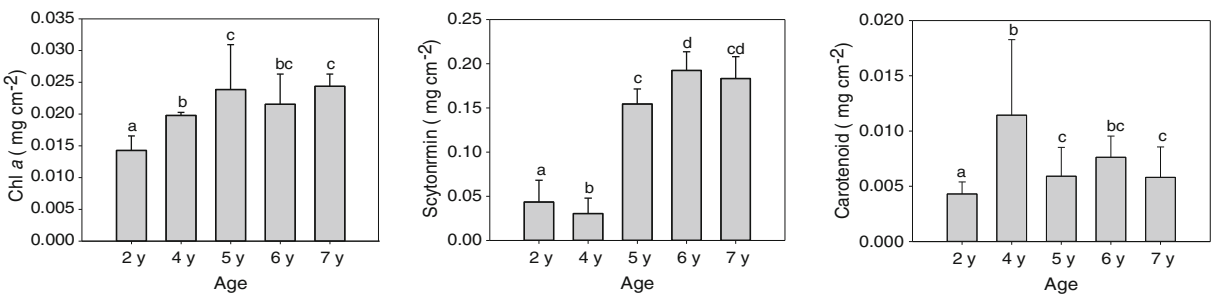

Fig. 2 Crusts pigments changes from 2 to 7 years old (*different letters* indicate $P<0.05$ and the *same letters* indicate $P>0.05$)

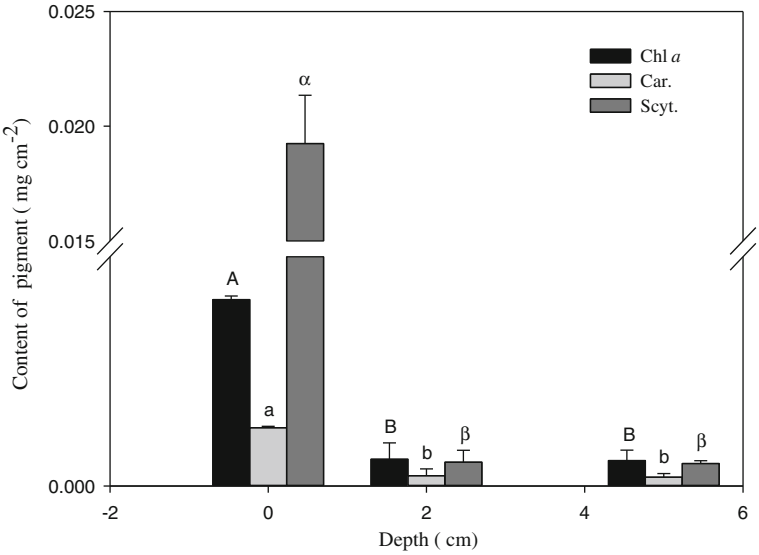

Fig. 3 Crusts pigments at different depth levels (three mean pigment contents appeared on three depth crusts (0, 0–2, and 2–5 cm crusts). *Chl a* means chlorophyll *a*, *Car* means carotenoids, and *Scyt* means scytonemin. The *different capital letters* indicate significant difference in chlorophyll *a* in different depth at $P<0.05$ level, the *different small letters* indicate significant difference in carotenoids in different depth at $P<0.05$ level, the *different Greek letters* indicate significant difference in scytonemin in different depth at $P<0.05$ level)

(Garcia-Pichel et al. 1992). Some researchers verified that the correlation between UV and scytonemin was variable or even negative (Pentecost 1993), whereas other researchers found that scytonemin functioned as a radiation shield (Stephan and Richard 1997). It was demonstrated in our study that scytonemin content of the crust soil surface was exceptionally higher than the other pigments, and scytonemin contents of cyanobacterial crusts were significantly affected by soil depth. From the analysis at the different depth level,

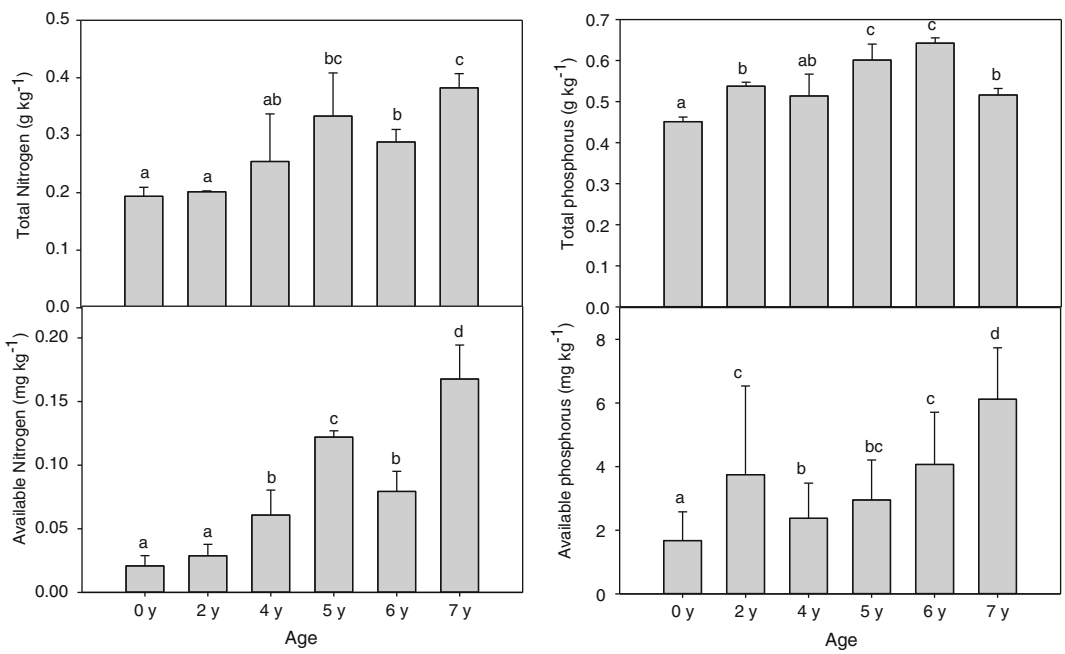

Fig. 4 Contents of N and P at different ages of crusts soil (*different letters* indicate $P<0.05$ and the *same letters* indicate $P>0.05$)

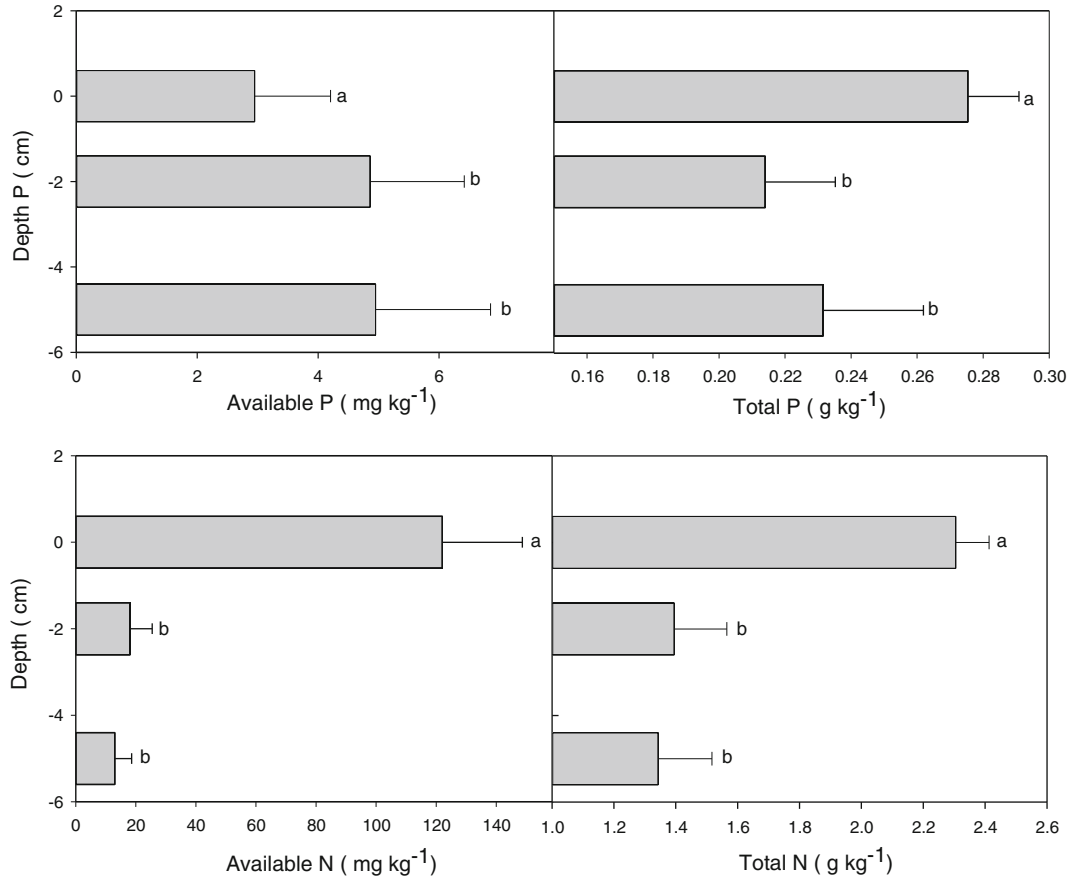

Fig. 5 Contents of N and P in soil at different depths (*different letters* indicate $P<0.05$ and the *same letters* indicate $P>0.05$)

scytonemin, chlorophyll *a,* and carotenoid were mainly distributed on the soil surface, which manifested that the crusts embraced most of soil phototrophic microorganisms in the desert soil.

Analysis of N and P in the crust soil

Soil texture and nutrients were ameliorated following the biological soil crust development (Langhans et al. 2009; Zhao et al. 2011); our studies indicated that artificially inoculated biological crusts could improve soil fertility as well. Low level of N was the main nutrient limiting factor for plants existing in arid areas (Madan et al. 2007; Zhao et al. 2010), thus the ability to fix nitrogen was important for the pioneer cyanobacteria. Some algal species in the crusts could fix nitrogen from air, such as *S. javanicum* and *Nostoc*, making the algal crusts more adaptable to barren environment. We found that the total N and available N increased gradually versus development time. Furthermore, a significant correlation between the available N and the algal crusts biomass might be observed with more study samples of the crusts. Since azotobacter in the soil could also fix N, it might share some ratio of N fixation. Total N and available N on the surface soil were more than in the 0–2 and 2–5 cm depths under the crusts ($P<0.01$), which suggested that some microbes in the crusts should have a major contribution for the soil N content.

Geographical physicochemical factors and animal activities at different dune positions mainly affect the soil total P (Chen et al. 2007), and this point of view was supported by our study. As the inoculated crusts formed, both the biomass of cyanobacteria and the diversity of algae had increased, and the distribution of other soil microbes such as fungi turned out to be a move-up trend as the crusts get older (Hu et al. 2000; Hu and Liu 2003). Cyanobacteria in the crusts, like P-solubilizing heterotrophic bacteria, were confirmed to have an ability to mobilize bound phosphates (Healey 1982; Mandal et al. 1999). Some species of cyanobacteria even have the

capacity to discharge effective phosphorus from indissoluble phosphorus (Qing et al. 2002). Tabatabai (1994) considered that the activities of phosphatase secreted by soil microorganism or released from dead cells were 2,979 times stronger in the crusts soil than that in the shift dune. Meanwhile, it was proved that artificially induced BSCs could accelerate organophosphorus compounds hydrolyzing to available phosphorus (Tang et al. 2003) and even promoting soil phosphorus circulation. When nutrients were liberated to soil due to cell decomposition (Mandal et al. 1999; Acea et al. 2003), they could be absorbed by other soil microbes immediately. Microbes, such as bacteria in and below the crust layer soil, could accelerate the metabolism of phosphate-solubilization (Nagy et al. 2005; Pérez et al. 2007), which could explain why the available P under the crust layer was higher than in the soil surface.

Conclusion

This experiment was designed to determine the possibilities of BSC-bioremediation technique in the case of desertification control as a way to recover the destroyed soil fertility. Here, we show that the artificially inoculated algae were able to develop to a complex soil crust through 7 years and could contribute to the improvement of ecological conditions in hostile environments, such as in increasing soil moisture, total nitrogen and available nitrogen, and decreasing the alkaline soil pH. Phosphorus and nitrogen were the major nutrients necessary for plants to grow. Along with the emergence and development of the biological soil crusts, soil microenvironment was improved, which was favorable for vascular plants to grow in the following stage of succession. Our results indicated that artificially induced algal crusts could develop very well and improve the environment with their biochemical functions.

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