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Effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts under experimental conditions

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Soil cyanobacterial crusts occur throughout the world, especially in the semiarid and arid regions. It always encounters sand burial, which is an important feature of mobile sand dunes. A greenhouse study was conducted to determine the effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts in six periods of time (0, 5, 10, 15, 20 and 30 d after burying) and at five depths (0, 0.2, 0.5, 1 and 2cm). The results indicated that with the increase of the burial time and burial depth extracellular polysaccharides content and *Fv/Fm* decreased correspondingly and there were no significant differences between 20 and 30 burial days under different burial depths. The degradation of chlorophyll *a* content appeared only at 20 and 30 burial days and there was also no significant difference between them under different burial depths. It was also observed a simultaneous decrease of the values of the *Fv/Fm* and the content of extracellular polysaccharides happened in the crusted cyanobacterium *Microcoleus vaginatus* Gom. It may suggest that there exists a relationship between extracellular polysaccharides and recovery of the activity of photosystem II (PS II) after rehydration.

Microcoleus vaginatus Gom., man-made cyanobacterial crusts, sand burial, biomass, photosynthetic activity, extracellular polysaccharides

Soil cyanobacterial crusts are popularly distributed in various land ecosystems throughout the world, especially in semiarid and arid regions. Studies on cyanobacterial crusts have documented their pioneering and colonizing roles in these ecosystems, which include the stabilization of soils, improved nutrient status of vascular plants growing in the crusted land, and improved soil structure[1].

Sand burial is an important factor controlling the distribution and composition of vegetation in the desert ecosystem[2−9]. It affects the vegetation by altering their normal microenvironment. For example, moisture, nutrients and microorganisms in proximity to the plant are increased after sand accumulation whereas soil temperature and aeration, and light intensity are decreased[10,11]. A large number of literatures focused on the responses of angiosperms to varying degrees of burial, however, bryophytes, such as cyanobacteria were rarely reported. Campbell[12] suggested that filamentous cyanobacteria could respond to burial by moving up to 5 mm every 24 hours when soils were moist. When soils were dry, these organisms were not able to move. Burial killed non-mobile photosynthetic components of the crust. On the contrary, Potts et al.[13] found that herbarium specimens remained viable after more than a century of desiccation.

What happens to the soil cyanobacterial crusts buried by dry sand and whether the crusts will be killed or not?

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How can dry sand burial play its role on the cyanobacterial crusts? The intent of this paper is to answer the above two questions. *Microcoleus vaginatus* Gom., one of the most dominant cyanobacterial species in the microbiotic crusts in the world, was isolated and purified by the authors' team from the desert area in China and used as inoculum to make man-made soil crusts. The impacts of different sand burial depths and time periods on the man-made cyanobacterial crusts were investigated experimentally under greenhouse conditions and three physiological parameters were measured.

1 Materials and methods

1.1 Cultures and man-made soil crusts preparation

M. vaginatus Gom. was isolated from the desert algal crust of Dalateqi, Inner Mongolia, China (40°21′N, 109°51′E) by our research group. Cultures were grown in the BG-11 medium at 25±1℃, and illuminated with cool white fluorescent light at 40 $\mu E \cdot m^{-2} \cdot s^{-1}$[14] with aeration of humidified air. After 18 d, cultures (6.4 μg chlorophyll *a* /mL) were inoculated in the sand from the local dunes. Sand was put in the plastic containers ($20 \times 13.5 \times 13$ cm^3) and each container was given 250 mL cultures. The drainage outlet at the bottom of plastic containers was covered with strips of nylon mesh to prevent the loss of sand while allowing drainage of excess water[9]. To make sure the inoculum had a uniform distribution, excessive water which exceeded the saturated moisture capacity of the sand was poured to the containers and homogenized inoculums were inoculated to the water above the sand.

The containers were placed in a greenhouse for one month, the man-made cyanobacterial crusts formed and they were allowed to be treated by sand burial. Containers were watered daily in the first fifteen days with distilled water (50 mL/d). Temperature in the greenhouse was maintained at 25±1℃ and illumination was 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ all the day.

Artificial burial treatments (additional sand depth of 0, 0.2, 0.5, 1 and 2 cm) were imposed under greenhouse conditions on the first day when the cyanobacterial crusts' chlorophyll fluorescence decreased to zero; biomass, chlorophyll fluorescence and extracellular polysaccharides were measured after 0, 5, 10, 15, 20 and 30 d. Six replicates were used for each treatment.

1.2 Algal biomass

Chlorophyll *a* content was used to estimate algal biomass. The 2×2 cm^2 crust samples were ground for 3 min in a mortar and then placed in scintillation vials. *N*, *N*-dimethylformamide (DMF) of 5 mL was added into each vial. The sample was shaken, and then incubated at room temperature in the dark overnight. Samples were resuspended in the following day and centrifuged at 2500 r/min for 10 min and the supernatant decanted into cuvettes. Absorbance was measured at 664, 647, 625, and 603 nm using a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, England). Calculations were made according to the following equation[15]:

$$\mu g\mathrm{Chl}\ a/cm^2 = (12.92 A_{664} - 2.16 A_{647} + 1.44 A_{625} - 4.91 A_{603})(\mathrm{mL\ MF})/4\ cm^2$$

1.3 Photosynthetic activity

Chlorophyll *a* fluorescence was measured with a plant efficiency analyzer (PEA, Hansatech, UK). Algal materials were dark-adapted for at least 20 min before measuring the fluorescence parameter *Fv/Fm* (Photosystem II activity). The excitation light intensity was about 1500 μ $photon \cdot m^{-2} \cdot s^{-1}$, and the recording time was 5 s. *Fv/Fm* is the ratio of the variable fluorescence to the maximal fluorescence[14].

Each sample was moistened with BG-11 medium and then measured after half an hour.

1.4 Extracellular polysaccharides content

Extracellular polysaccharides were quantified according to the phenol-sulphuric acid method of Dubios et al.[16], using glucose as standard, and modified by Pistoc chiet al.

1.5 Data analysis

All the data on algal biomass, chlorophyll *a* fluorescence and extracellular polysaccharides were analyzed using one- and two-way ANOVA at 95%. If ANOVA showed significant differences, Duncan's multiple comparison tests were used to determine differences between treatments. All data analyses were carried out using SPSS11.5 software.

2 Results

2.1 Algal biomass

The two-way ANOVA showed that chlorophyll *a* content was significantly affected by different burial time periods (F_5=76.053, P<0.0001), burial depths (F_4=17.649,

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P<0.0001) and interaction between burial depth and burial time (F_{20}=6.415, P<0.0001; Table 1). For the six burial time periods, the decrease of the chlorophyll *a* content appeared on the 20th, 30th days. There were no decreases on the 0, 5th, 10th and 15th days (Figure 1(a)). For each of the last two of five different burial times (Figure 1(b)), there was a negative correlation between chlorophyll *a* content and burial depth (20 d, $y = -0.184x+22.491$, $r^2 = 0.912$, $F_{1,5} = 104.44$, P<0.0001; 30 d, $y = -0.286x + 23.28$, $r^2 = 0.853$, $F_{1,5} = 179.40$, $P < 0.0001$).

2.2 Chlorophyll *a* fluorescence

According to the two-way ANOVA chlorophyll *a* fluorescence was also significantly affected by different burial times (F_5=153.054, P<0.0001), burial depths (F_4=152.011, P<0.0001) and interaction between burial depth and burial time (F_{20}=12.471, P<0.0001, Table 1). For the six burial times, the decrease of the chlorophyll *a* fluorescence appeared on the 5th, 10th, 15th, 20th, 30th days (Figure 2(a)) and there was a negative correlation between chlorophyll *a* fluorescence and burial depth for each of those burial times (5 d, $y = -0.045x+0.503$, $r^2 = 0.65$, $F_{1,4} = 4.57$, P<0.0001; 10 d, $y = -0.133x +0.52$, $r^2 =0.86$, $F_{1,4}= 20.65$, P<0.0001; 15 d, $y = -0.19x +0.49$, $r^2 =0.86$, $F_{1,4} = 24.93$, P<0.0001; 20 d, $y = -0.20x+0.39$, $r^2 = 0.76$, $F_{1,4} = 168.52$, P<0.0001; 30 d, $y = -0.19x+0.39$, $r^2 = 0.68$, $F_{1,4} = 74.10$, P<0.0001). For the five burial depths, the decrease of the chlorophyll *a* fluorescence appeared at 0.2, 0.5, 1, 2 cm (Figure 2(b)) and there was a negative correlation between chlorophyll *a* fluorescence and burial time for each of those burial depths (0.2 cm, $y = -0.012x + 0.542$, $r^2 = 0.92$, $F_{1,5}$ =54.47, P< 0.0001; 0.5 cm: $y=-0.012x+0.540$, $r^2= 0.89$, $F_{1,5}$=77.53, P<0.0001; 1 cm, $y =-0.012x + 0.531$, $r^2 = 0.90$, $F_{1,5}$ = 50.40, $P < 0.0001$; 2 cm, $y = -0.018x+0.462$, $r^2= 0.82$, $F_{1,5} = 36.09$, $P < 0.0001$).

2.3 Extracellular polysaccharides content

Extracellular polysaccharides was also significantly affected by different burial times (F_5=236.207, P<0.0001), burial depths (F_4=426.737, P<0.0001)and interaction between burial depth and burial time (F_{20}=25.458, P<0.0001, Table 1) by the two-way ANOVA. For the six burial times, the decrease of the water-soluble sugar appeared on the 5th, 10th, 15th, 20th, 30th days (Figure 3(a)) and there was a negative correlation between water-soluble sugar and burial depth for each of those burial times (5 d, $y = -3.06x+16.30$, $r^2 = 0.966$, $F_{1,4}$ = 90.02,

Table 1 Variance comparison of effects of different burial times, depths, and their interaction on algal biomass, chlorophyll *a* fluorescence and extracellular polysaccharides content

Source of variation		SS	df.	MS	*F*	*P*
	burial time	382.103	5	76.421	76.053	<0.0001
Chlorophyll *a* content	burial depth	70.938	4	17.735	17.649	<0.0001
	burial time×burial depth	128.927	20	6.446	6.415	<0.0001
	burial time	1.160	5	0.232	153.054	<0.0001
Photosynthetic activity	burial depth	0.922	4	0.230	152.011	<0.0001
	burial time×burial depth	0.378	20	0.019	12.471	<0.0001
	burial time	674.880	5	134.976	236.207	<0.0001
Extracellular polysaccharides content	burial depth	975.402	4	243.851	426.737	<0.0001
	burial time×burial depth	290.951	20	14.548	25.458	<0.0001

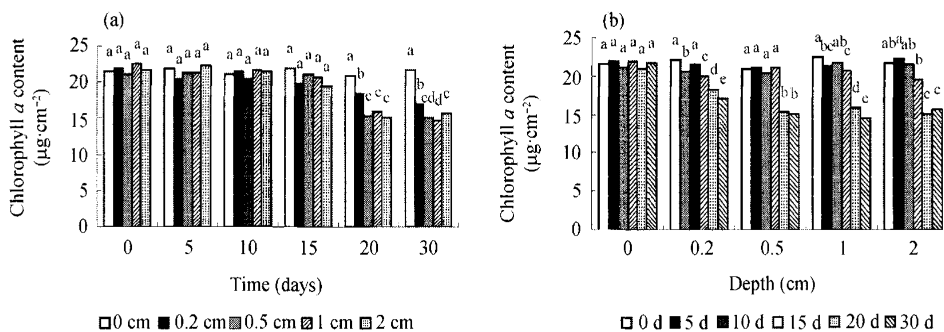

Figure 1 (a) Chlorophyll *a* content of cyanobacterial crusts for six different burial times from five different burial depths; (b) chlorophyll *a* content of five different burial depths for six different burial times. Values with the same superscript letters are not significantly different at P<0.05 according to Duncan's multiple comparison test.

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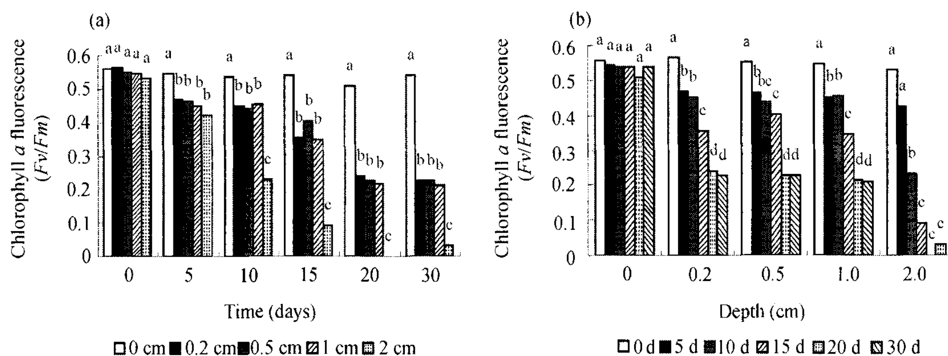

Figure 2 (a) Chlorophyll *a* fluorescence of cyanobacterial crusts for six different burial times from five different burial depths; (b) chlorophyll *a* content of five different burial depths for six different burial times. Values with the same superscript letters are not significantly different at P<0.05 according to Duncan's multiple comparison test.

P<0.0001; 10 d, $y = -4.263x+15.50$, $r^2 = 0.91$, $F_{1,4}$= 15.78, P<0.0001; 15 d, $y = -2.99x+19.07$, r^2=0.99, $F_{1,4}$ = 757.84, P< 0.0001; 20 d, y=$-6.448x+13.66$, r^2= 0.89, $F_{1,4}$ = 1413.35, P<0.0001; 30 d, $y = -6.56x+13.60$, r^2= 0.89, $F_{1,4}$ = 5765.41, P < 0.0001). For the five burial depths, the decrease of the chlorophyll *a* fluorescence appeared at 0.2, 0.5, 1, 2 cm (Figure 3(b)) and there was a negative correlation between water-soluble sugar and burial time for each of those burial depths (0.2 cm: $y = -0.135x + 16.16$, r^2 = 0.81, $F_{1,5}$ =130.19, P<0.0001; 0.5 cm: $y = -0.286x + 15.497$, r^2 = 0.89, $F_{1,5}$ =91.26, P<0.0001; 1 cm: $y = -0.394x + 14.93$, r^2 = 0.885, $F_{1,5}$ = 30.96, P< 0.0001; 2 cm: $y = -0.465x + 13.13$, r^2 = 0.83, $F_{1,5}$ = 203.26, P < 0.0001).

3 Discussion

In this study, we examined the effects of sand burial on the cyanobacterial crust of *M. vaginatus* Gom. under controlled conditions. In general, the gradient in decrease of content of the extracellular polysaccharides and *Fv/Fm* concurred with the changes of burial time and depth. It means that, with the increase of the burial time and burial depth, extracellular polysaccharides content and *Fv/Fm* decreased correspondingly. Exceptionally, there was no significant difference between 20 and 30 burial days under different burial depths. The degradation of chlorophyll *a* content appeared only on 20 burial days and 30 burial days and there was also no significant difference between them under different burial depths.

It was supposed that dry sand burial played its role on man-made soil crusts through creating a comparatively suitable environment for the microorganisms beneath the soil crusts, and not directly affected the metabolizability of the man-made soil crusts. Soil crusts became dry and dormant when the value of *Fv/Fm* got to zero[17]. However, microorganisms that lived on the extracellular polymeric substances of the cyanobacteria still kept active due to the imperfectly desiccant sand beneath the

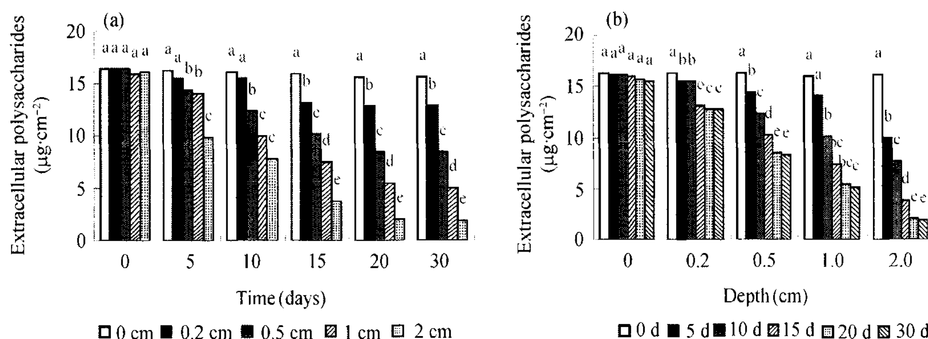

Figure 3 (a) Extracellular polysaccharides of cyanobacterial crusts for six different burial times from five different burial depths; (b) extracellular polysaccharides of five different burial depths for six different burial times. Values with the same superscript letters are not significantly different at P<0.05 according to Duncan's multiple comparison test.

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soil crusts. Subsequent sand burial increased the moisture while decreased aeration[10,11]. Comfortable microenvironment promoted the activity of microorganisms in proximity to the soil crusts[11] and accelerated the degradation of extracellular polysaccharides of the soil crust.

Extracellular polysaccharides have been proved to fulfill a variety of different roles such as protecting algae from harmful effects of toxic substances or unfavorable factors (e.g. desiccation, antibiotics, ultraviolet illumination etc.) and directly attach to solid surface and other matrix[18–20]. As early as in 1964, Moore and Tischer[21] suggested that algal EPS affected life-support systems[20]. In recent years, Wright et al.[22] suggested that cyanobacterium *Nostoc commune* tolerated the simultaneous stresses of desiccation, UV irradiation, and oxidation through modulation of the structure and function of the three-dimensional extracellular matrix. In our study, we observed a simultaneous decrease of the value of the *Fv/Fm* and the content of extracellular polysaccharides. We speculated that there existed a relationship between extracellular polysaccharides and recovery of the activity of photosystem II (PS II) after rehydration.

Twenty days after burial, the invariability of the chlorophyll *a* content, *Fv/Fm* and extracellular polysaccharides showed that both the microorganisms and the cyanobacterial crusts got into the dormant condition. Till then soil crusts could be conserved without being easily damaged.

In natural conditions in China, burial by wind-deposited sand often occurs in the dry seasons in which air usually keeps dry and dew was sparse. In most of the time the microenvironments around the biological soil crusts are dry and the microorganisms and the cyanobacterial soil crust can hardly keep active. Though microorganisms can react through occasional rainfall and dew precipitation with the rehydration of cyanobacterial soil crusts[23–25], it will soon become dry again. From the above we conclude that cyanobacterial soil crusts might be less influenced while being buried in natural conditions than in the experimental conditions. This might be a favorite message for the usage of mass-cultured cyanobacterial inoculants to stabilize mobile dunes.

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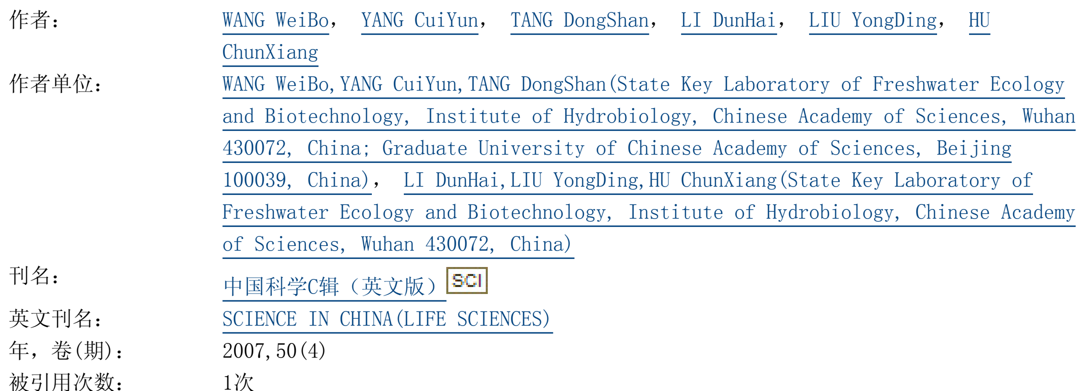

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