Original article

Effects of drought and salt stresses on man-made cyanobacterial crusts

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ABSTRACT

As a primary successional stage of biological soil crusts (BSCs), cyanobacterial crusts form firstly in the arid and semiarid areas. At the same time, they suffer many stress conditions, such as drought, salt, etc. In this study, we constructed man-made cyanobacterial crusts with *Microcoleus vaginatus* Gom. and comparatively studied the effects of drought and salt stresses on the crusts. The results showed that crust growth and photosynthetic activity was significantly inhibited by the stress conditions ($P < 0.05$), and inhibitory effect increased with the increasing stress intensity and treated time. Compared with salt stress, drought completely stopped crust metabolic activity, so the crust biomass was conserved at a higher level, which meant that drought itself might provide the crusts some protection, especially when the crusts simultaneously suffered drought and salt stresses. That is very important for the survival of crusts in the high-salt areas. In addition, to some extent the crusts could adapt to the stress conditions through metabolic adjustment. In our experiment, we found the accumulation of exopolysaccharides (EPS) increased under stress conditions within a certain threshold.

1. Introduction

The spatial coverage of arid ecosystems is approximately 33–40% of the terrestrial land surface, and also likely to increase due to climate change and desertification [11,24]. In these areas, drought is a significant limiting factor for many organisms due to the limited rainfall and high evapotranspiration. At the same time, concomitant with the evapotranspiration, salinity accumulates on the surface and high-salt becomes another stress factor. In these situations, many organisms are restricted, however biological soil crusts (BSCs) distribute widely, and even constitute up to 70% of the living cover in some areas [4,10]. Despite their unassuming appearances, BSCs play a significant role in desert ecosystems, including the process of soil formation, nutrient cycling, preventing soil erosion by water or wind, influencing the establishment and performance of vascular plants, and serving as habitats for other microorganisms and protozoa [4,10,21,37,43,47].

In order to facilitate the BSCs formation or rehabilitation and ecosystem regeneration, some authors suggested the pathway of cyanobacterial inoculation [1–3,6,21]. It has been applied in some areas, and has gained obvious effect [9,42,44]. As the colonizer and pioneer in BSCs, cyanobacteria (especially the filamentous cyanobacteria) play an irreplaceable role in the formation of BSCs. As the primitive oxygenic photosynthetic organisms, cyanobacteria have developed multi-protection mechanisms in order to adapt to the various adversities [14,22,33]. Many studies have verified that cyanobacteria were able to tolerate a certain degree of drought and salinity, including the accumulation of extracellular substances, the protection of anti-oxidative system and the adjustment of other metabolic mechanism [8,34,39]. Salinity both reduces water potential and causes direct toxicity [7,31,46]. However, little is known about the comparative effects of drought and salt stress, especially in BSCs, where the drought and salt stress are often combined. Therefore, our objectives are to comparatively study the effects of drought and salt stress on the cyanobacterial crusts; to distinguish the difference in drought stress and salt stress; and to discuss the adaptability of cyanobacterial crusts to the stress conditions through constructing man-made cyanobacterial crusts. The results may help us to better understand the adaptability of BSCs to the stress conditions, and provide some theoretical foundation for the construction of man-made cyanobacterial crusts to control desertification.

2. Methods and materials

2.1. Inoculation and treatment of man-made cyanobacterial crusts

Microcoleus vaginatus Gom., as a common dominant species, was isolated from BSCs of Dalateqi County (Inner Mongolia, China; 40°21′N, 109°51′E) for constructing man-made cyanobacterial

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crusts. The isolated *M. vaginatus* was first cultured in BG-11 medium in a greenhouse (25 ± 2 °C) [45], illuminated with cool white fluorescent light at 40 μE m^{-2} s^{-1} with aeration. After 15 d, 100 mL cultures (1.72 ± 0.16 μg chlorophyll-*a* (Chl-*a*) ml^{-1}) were spray inoculated into Petri-dishes (15 cm in diameter) containing sand from local dunes (450.00 ± 0.10 g of sand, about 1.5 cm thickness). In order to avoid the effects of salinity and microorganisms on the experimental results, the sand was previously rinsed with distilled water, and sterilized at 121 °C for 30 min. Then the Petri-dishes were placed in a greenhouse and watered daily to maintain a water content of 10%. After 18 d, when the crusts formed (1.21 ± 0.25 mm thickness; 4.66 ± 0.29 μg Chl-*a* cm^{-2}), they were treated with drought or salt as described in Table 1. Three replicates for each treatment were made and the sampling was conducted after 1, 8, 15 and 30 d. For the drought treated groups, the Petri-dishes were directly evaporated to dryness as soon as possible. For the salt treated groups, the Petri-dishes were evaporated to half-dryness (about 5% water content) and then wetted back to 10% water content with salt solutions to give 0.1%, 0.2%, 0.5% and 1% weight percent of NaCl to dry sand (as shown in Table 1). For the salt + drought treated groups, the Petri-dishes were firstly treated with NaCl solution, and then evaporated to dryness, so that the salinity in each group ultimately came to 0.2% and 0.5% NaCl.

2.2. Measurements

2.2.1. Chl-a, carotenoids and scytonemin

For the Chl-*a*, carotenoids and scytonemin determination, crust samples (1.72 ± 0.03 cm^2) were gathered with a punch, then the samples were ground in 10 ml 100% acetone with mortar and pestle. The extractions were placed at 4 °C for 18 h. Extracts were then centrifuged at 900 g for 10 min to remove the suspended sediments, and the supernatant fluids were measured at 663 nm, 490 nm and 384 nm using a spectrophotometer. The contents of chl-*a*, carotenoids and scytonemin were calculated according to Garcia-Pichel [14,15]:

$$Chl\text{-}a = (1.02\ A_{663} - 0.027\ A_{384} - 0.01\ A_{490})/C_{Chl\text{-}a} \times V/S$$

$$Car = (1.02\ A_{490} - 0.08\ A_{384} - 0.026\ A_{663})/C_{Car} \times V/S$$

$$Scyt = (1.04\ A_{384} - 0.79\ A_{663} - 0.27\ A_{490})/C_{Scyt} \times V/S$$

where Chl-a, Car and Scyt represent the content of Chl-*a*, carotenoids and scytonemin respectively (μg cm^{-2}); $C_{Chl\text{-}a}$, C_{Car} and C_{Scyt} are the extinction coefficients of Chl-*a*, carotenoids and scytonemin (L g^{-1} cm^{-1}), and their values are 92.5, 250 and 112.6 respectively; A_{663}, A_{490} and A_{384} are absorbances of extracts at 663 nm, 490 nm and 384 nm; V is the extract volume (mL), S is the area of the crust sample (cm^2).

2.2.2. Chlorophyll (Chl) fluorescence

Chl fluorescence reflects the photosynthetic state of the plants and has been wildly used in ecophysiological studies. In our experiment, the Chl fluorescence was measured with a plant efficiency analyzer (PEA, Hansatech, UK). For the initial fluorescence (Fo) measure, cyanobacterial crusts were previously dark-adapted for at least 20 min, and for the maximal fluorescence (Fm) measure, the saturating light pulse (about 1500 photon·m^{-2} s^{-1}) was used. The variable fluorescence (Fv) was calculated by Fv = Fm − Fo, and the Fv/Fm ratio was also calculated. The Fv/Fm ratio of dark-adapted Chl, representing the maximum photochemical efficiency of Photosystem II (PS II), reflects the photosynthetic activity [12,27].

2.2.3. Exopolysaccharides (EPS)

Crust samples (1.72 ± 0.03 cm^2) were first gathered with the punch, ground in 2 ml distilled water, and incubated with 1 ml 98% sulfuric acid for 24 h at room temperature. Then the extracts were neutralized to pH 7.0 using 6 M NaOH and adjusted to 8 mL with distilled water. The prepared extracts were centrifuged at 900 g for 10 min to remove the suspended sediments, and the supernatant fluids were collected for future analysis. The quantification of EPS was conducted according to the phenol-sulfuric acid method as described by Li [28].

2.3. Data analysis

All the variances of Chl-*a*, photosynthetic activity, EPS and carotenoids were analyzed using One-way or Three-way ANOVA at 95%. All data analyses were carried out using SPSS 13.0 software.

3. Results

3.1. The biomass in different treatments

Chl-*a* content was taken as a measure of crust biomass, and Three-way ANOVA showed the Chl-*a* content was significantly affected by salinity ($F = 117.21$, $P < 0.01$), interaction of salinity and time ($F = 21.02$, $P < 0.01$; Table 2). From Fig. 1 we also found crust growth was markedly inhibited by salt treatment and the inhibitory effect increased with increasing NaCl concentrations and time. The biomasses of the four treatment groups were significantly lower than that of the control from the 15th day to the end, and the difference between control and 1% NaCl treatment group was visible from the 8th day ($P < 0.05$). Compared with the control, in which the biomass increased initially and then decreased from the 15th day, crust biomasses in low-salt (0.1% and 0.2% NaCl) treated groups remained steady during the experiment. While the biomasses in high-salt (0.5% and 1% NaCl) treated groups decreased markedly with experimental time.

Although the Chl-*a* content was not significantly affected by drought solely ($F = 2.20$, $P = 0.14$), the Chl-*a* content was significantly affected by the interaction of drought, salinity and time ($F = 10.09$, $P < 0.01$, Table 2). As in the situations in salt treatments above, crust biomasses in drought and salt + drought treatments were also significantly lower than that in control after 15 d ($P < 0.05$), but there was no significant difference among those treated groups ($P > 0.05$; Fig. 2). That was because the biomass of each treatment group was kept at a comparatively steady level during the experiment, while the biomass in control increased.

The differences of biomass between the dry and wet crusts under different salt treatments also existed (although the differences were not significant before day 15) and differed among the

Table 1
The water, NaCl content and water potential in each treatment (Mean ± S.D., $n = 3$).

Treatments	Water content (%*)	NaCl content (%*)	Water potential (MPa)
Control	10.01 ± 0.01	0	−8.27 ± 0.06 (*10^{-3})
0.1% NaCl	10.01 ± 0.02	0.10 ± 0.00	−0.64 ± 0.12
0.2% NaCl	10.00 ± 0.01	0.20 ± 0.00	−1.20 ± 0.04
0.5% NaCl	10.00 ± 0.01	0.50 ± 0.00	−2.73 ± 0.08
1% NaCl	10.01 ± 0.01	1.00 ± 0.00	−4.97 ± 0.06
Drought	0.24 ± 0.03	0	−32.87 ± 0.68
0.2% NaCl + Drought	0.42 ± 0.15	0.20 ± 0.00	−41.73 ± 3.65
0.5% NaCl + Drought	0.55 ± 0.10	0.50 ± 0.00	−38.70 ± 2.15

*Weight percent of water or NaCl to dry sand.

Table 2
Three-way ANOVA of effects of salinity, drought, treated time and their interactions on crust Chl-*a* content and Fv/Fm ratio.

Variables	Factors	Sum of Squares	df	Mean Square	F	P
Chl-*a* content	Salinity	104.07	4	26.02	117.21	<0.01
	Drought	0.49	1	0.49	2.20	0.14
	Salinity × Treated time	56.00	12	4.67	21.02	<0.01
	Drought × Treated time	2.42	3	0.81	3.63	0.02
	Drought × Salinity	9.77	2	4.89	22.01	<0.01
	Drought × Salinity × Treated time	13.44	6	2.24	10.09	<0.01
Fv/Fm ratio	Salinity	2.17	4	0.54	1781.09	<0.01
	Drought	1.94	1	1.94	6355.89	<0.01
	Salinity × Treated time	0.02	12	0.00	4.84	<0.01
	Drought × Treated time	0.02	3	0.01	22.10	<0.01
	Drought × Salinity	0.74	2	0.37	1218.55	<0.01
	Drought × Salinity × Treated time	0.01	6	0.00	4.00	<0.01

salt treated groups (Fig. 3). In the group without salt treatment, the biomass in dry crusts was significantly lower than that in wet crusts from day 15 ($P < 0.05$). However, a reverse situation was observed in the group with high-salt (0.5% NaCl) treatment. No significant difference was detected between the dry and wet crusts in the low-salt (0.2% NaCl) treated group ($P > 0.05$).

3.2. The photosynthetic activity in different treatments

As a reflection of photosynthetic activity, the Fv/Fm ratio was significantly affected by salinity ($F = 1781.09$, $P < 0.01$), interaction of salinity and time ($F = 4.84$, $P < 0.01$; Table 2). High-salt (0.5% and 1% NaCl) treatments significantly inhibited crust photosynthetic activity, and significant decreases in the Fv/Fm ratio of the two high-salt treated groups were detected after 1 d of treatment ($P < 0.05$), while the inhibition was not detected in low-salt treated groups (0.1% and 0.2% NaCl treatment; Fig. 4). Compared with the control, in which the photosynthetic activity had a slight decrease along with the experimental progress, the photosynthetic activities of low-salt treated groups initially increased and then decreased, while the activities of high-salt treated groups decreased sharply and then remained at a low level during the experiment (<0.1). Especially in the 1% NaCl treated group, almost no activity was detected from day 1.

Three-way ANOVA showed crust photosynthetic activity was also significantly inhibited by the interaction of drought, salinity and time ($F = 4.00$, $P = 0.02$; Table 2). The crusts under drought (both in the drought treated group and in the salt + drought treated groups) had no photosynthetic activity (the Fv/Fm ratios were 0).

3.3. The accumulation of scytonemin, carotenoids and EPS

During the whole experiment, there was no accumulation of scytonemin in the crusts (data not shown). The content of carotenoids in the control remained at a comparatively steady level, at a range of 0.32–0.35 g g^{-1} Chl-*a* (Table 3). Similarly, the carotenoid contents were maintained at constant levels in the low-salt (0.1% and 0.2% NaCl) and drought treated groups, while the contents decreased in the high-salt (0.5% and 1% NaCl) and salt + drought treated groups. Compared with the control, the contents of carotenoids decreased ($P < 0.05$) in the 1% NaCl treatment from day 1; 0.5% NaCl and 0.5% NaCl + drought treatments from day 8; 0.2% NaCl + drought and drought treatments from day 30.

Crust EPS content in the control increased with time (Table 4). While in the treated groups, the situations were different from each other. In the low-salt (0.1% and 0.2% NaCl) and 0.5% salt treated groups, the EPS accumulation increased along the temporal sequence. However, the EPS contents remained constant in the 1% NaCl and salt + drought treated groups. Compared with the control, low-salt treatments (0.1% NaCl and partial 0.2% NaCl) promoted the accumulation of EPS, while high-salt (0.5% and 1% NaCl), salt + drought and drought treatments reduced the accumulation.
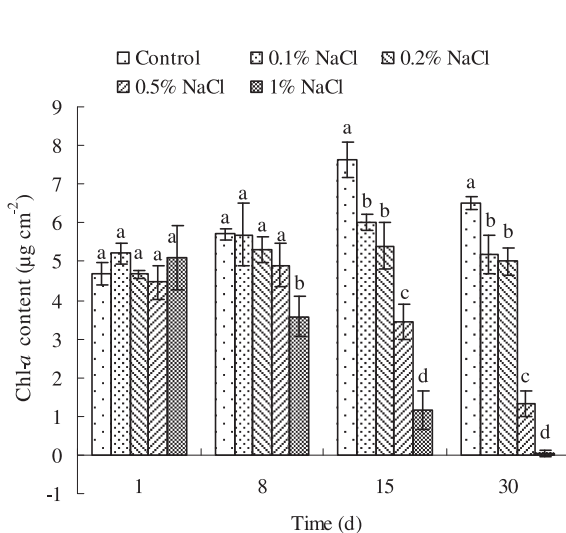

Fig. 1. Effects of different NaCl treatments on crust biomass. Values with different letters are significantly different ($P < 0.05$).
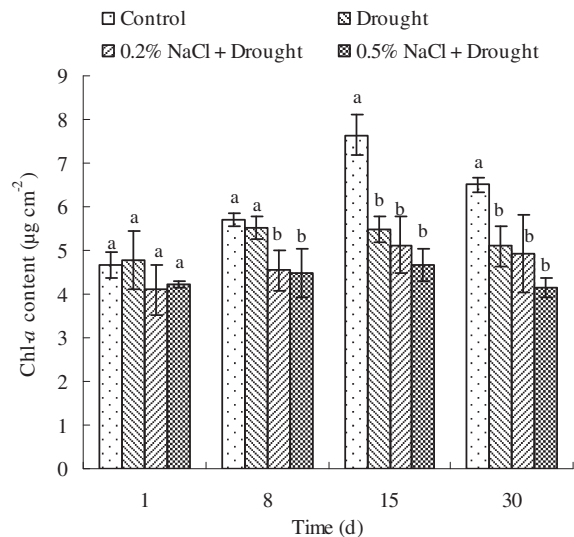

Fig. 2. Effects of different treated conditions on crust biomass. Values with different letters are significantly different ($P < 0.05$).
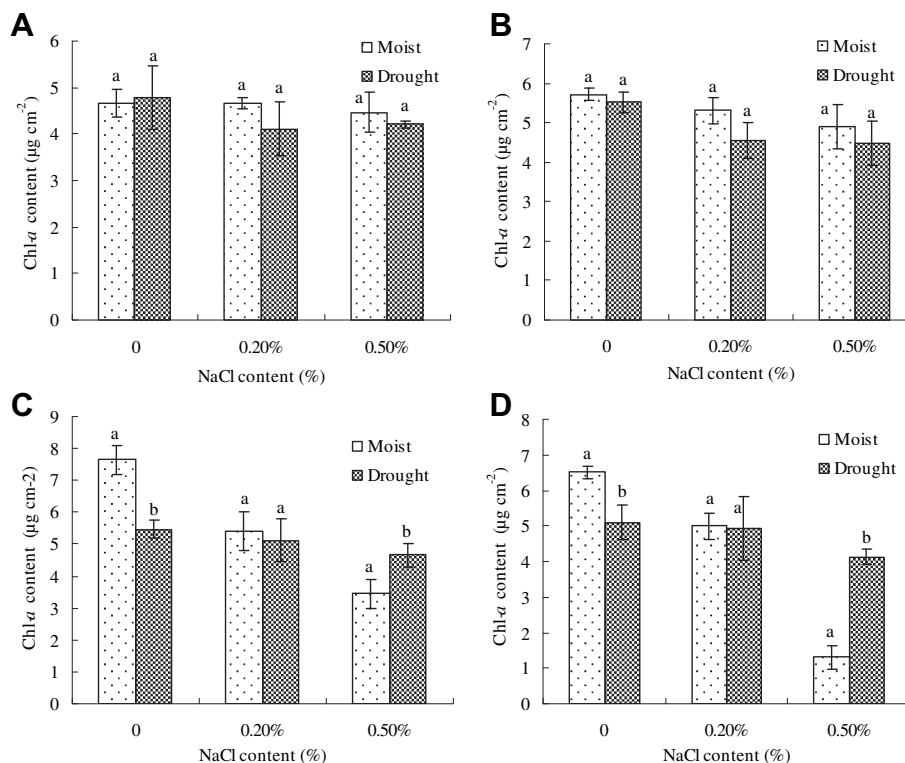
Fig. 3. The differences of biomasses between the dry and wet crusts under different salt treatments. (A), (B), (C) and (D) were the results at day 1, 8, 15 and 30 after treatments. Values with different letters represent the significant difference of biomass in moist and drought conditions ($P < 0.05$).

4. Discussions

4.1. The effects of stresses on the cyanobacterial crusts

As a common dominant species in BSCs, the *M. vaginatus* has been chosen as an inoculation in constructing or regenerating BSCs in some researches [9,42,44]. Therefore, we constructed the man-made cyanobacterial crusts with *M. vaginatus* in our experiment. The strain used in the current study was isolated from the BSCs in Qubqi Desert at Dalateqi region of Inner Mongolia Autonomous Region. After inoculation, the crusts form about a week later, crust

Fig. 4. Effects of different NaCl treatments on crust photosynthetic activity (Fv/Fm). Values with different letters are significantly different at 0.05 level ($P < 0.05$).

biomass increases with the crust formation and up to 7.65 ± 0.45 µg Chl-*a* cm^{-2} about a month later. Then the biomass decreased, which might be due to the restriction of nutriments or other resources in the culture media. While in the practical application, the crusts often suffer many stress factors, which certainly would affect the crust growth. In our experiment the crust growth slowed and even stopped under stress treatments, which might be caused by the decrease of water or nutritional availability, ionic imbalance and also a combination of those factors both at physiological and biochemical levels [31,39].

The Fv/Fm ratio of dark-adapted Chl indicates the maximum photochemical yield, the potential and greatest photosynthetic activity of PS II, reflecting the largest solar energy conversion efficiency in PSII reaction center [12,27]. Under the unstressed conditions, the Fv/Fm ratio remains at a constant value, >0.8 for higher plants [5], and about 0.65 for algae [26]. While in the crusts, the ratios were lower than 0.6 in most cases, indicating that the crust microenvironment itself is a "stress" condition for the algae compared with the aqueous medium. Furthermore when the crusts are under other stress conditions (eg. drought and salt stresses), their photosynthetic activities would further decline, which may be due to the damage to PS II complex and decline in overall activity of the electron transport chain of PS II [29].

4.2. Drought and salt stresses

The crust cyanobacteria under drought stress will be exposed to the gas phase, while under salt stress they still may be in an aqueous environment, albeit one in which the water activity is diminished. Therefore, some studies described the drought stress as water matric stress or physical water stress, and the salt stress as osmotic stress or physiological water stress [17,18,33]. Crust response to salt and drought stress has much in common. Salinity
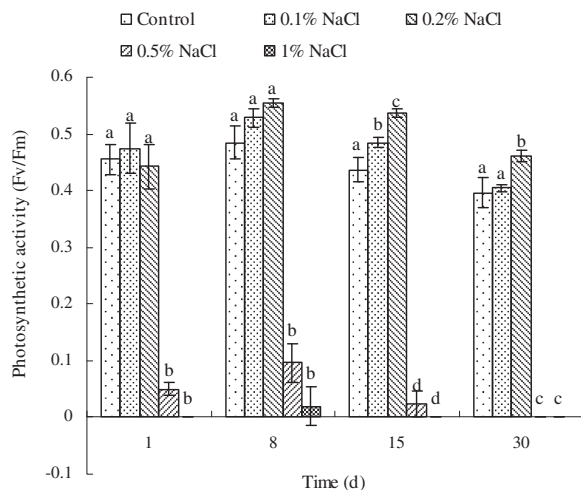
Table 3
The contents of carotenoids (g g^{-1} Chl-a) in crusts with different treatments (Mean ± S.D., n = 3).

Day	Control	0.1% NaCl	0.2% NaCl	0.5% NaCl	1% NaCl	Drought	0.2% NaCl + Drought	0.5% NaCl + Drought
1	0.34 ± 0.03 A	0.36 ± 0.04 A	0.30 ± 0.02 A	0.32 ± 0.01 A	0.26 ± 0.02** A	0.33 ± 0.03 A	0.37 ± 0.03 A	0.36 ± 0.01 A
8	0.32 ± 0.02 A	0.35 ± 0.02 A	0.32 ± 0.05 A	0.28 ± 0.03** A	0.23 ± 0.03** A	0.31 ± 0.03 A	0.32 ± 0.01 B	0.31 ± 0.03*B
30	0.35 ± 0.02 A	0.38 ± 0.01 A	0.36 ± 0.05 A	0.20 ± 0.04**B	0.14 ± 0.00**B	0.30 ± 0.01* A	0.31 ± 0.02*B	0.26 ± 0.01**C

*, ** Significant difference between treatment and control at $P < 0.05$ or 0.01 level respectively. Different letters represent significant differences between days ($P < 0.05$).

in the crusts reduces the soil water potential, and this quickly causes the decline in crust photosynthetic activity and growth rate, which is identical to those effects caused by drought stress. Especially in the initial stress stage, salt stress responses are not salt-specific, while they are the same as the stress responses caused by other solutes, such as KCl, mannitol and polyethylene glycol [7,46]. However, there is a salt-specific effect that has impacts on crust photosynthetic activity and growth, because once the excessive amounts of sodium enters the cell, salinity would eventually rise to toxic levels [31]. Compared with the salt stress, drought response is more rapid and direct [33,41]. Once losing the water, the crusts quickly enter the non-active state, which to some extent is a protection against the stress factor. Although the crusts have more water availability under salt stress (from −4.965 ± 0.058 to −0.637 ± 0.116 MPa in the salt treated groups) than that under drought stress (−32.873 ± 0.681 MPa), the crust biomass under drought stress keeps at a comparatively steady level, and is higher than that under salt stress. That means the decrease of biomass in crusts is mainly caused by the salinity toxicity instead of decrease of water activity.

In the desert environment, crusts may suffer a variety of stress factors. Garcia-Pichel and Castenholz [14] and Potts [33] found the UV stress effect could be enhanced by other environmental variables, such as cold and, in particular, drought. On the other hand, some "stress" factors also may reduce the stress effects caused by other stress factors. In our experiment, when the crusts under salt stress were in a dry state, crust biomass was conserved at a higher level, which might be because the drought stopped the crust metabolic activity, and provided the crusts some protection against salinity toxicity. That could be very important for the survival of crusts in the high-salt areas. During a rainfall event, rainwater leaches the salt into the soil substrate, which would reduce the salt stress effects and may provide an opportunity for crust metabolism and growth. While later with the evaporation, as well as the accumulation of salinity in the crust layer, crusts would revert into the dry state again.

4.3. The response of cyanobacterial crusts to stresses

As a primary successional stage of BSCs, cyanobacterial crusts can adapt to a variety of stress conditions and develop in the arid and semiarid areas due to the characteristics of the filamentous cyanobacteria, including relatively rapid growth and migration, their extraordinary abilities to survive drought and salinity [16,19,22,23,43,48]. According to the viewpoint of Kidron et al. [25] and Mazor et al. [30], most of the cyanobacterial carbohydrates are EPS, so we therefore determined crust EPS as a measure of

carbohydrate. For the cyanobacteria, EPS play an important role in resisting all kinds of stresses: 1) the secreted EPS accumulate and form a buffer zone out of cells, which adsorb some ions and water molecules, thereby reducing the entrance of hypertonic ions into cells and the loss of intracellular water [8,31,35]; 2) EPS provide a matrix for many other extracellular substances such as sucrose and trehalose, which lower the phase transition temperature (Tm) of plasma membrane, as well as form a carbohydrate glass with a relatively high glass transition temperature, and prevent fusion of membrane vesicles [20,34]; 3) some specific proteins or polypeptides accumulated in EPS (such as water stress proteins, WSPs) have a structural role in cell stability in view of their abundant content of hydroxylated amino acids [34]; 4) UV-absorbing substances accumulated in EPS (such as scytonemin) could alleviate the radiation damage to the organisms and protect the PS II [14]. However, in our experiment no any scytonemin was accumulated in the crusts, which might be due to the absent of UV radiation in our experiment, because it had been demonstrated that the UV radiation was essential for the synthesis of scytonemin [14]. On the other hand, once the stress exceeds the tolerance capability of the organisms (including the stress intensity and time), the synthesis of EPS is also affected. In our experiment, when the crust treated with 1% NaCl, the EPS content declined after 1 d, and after 30 d under 0.2% NaCl stress treatment.

Many stresses can also lead to oxidative damage, while organisms develop a complex anti-oxidative system to alleviate the damage [32,39]. In our experiment, the decline of crust photosynthetic activity was the stress response; but it might also reduce the electron transfer rates and accumulation of reactive oxidative species (ROS) sequentially, ultimately alleviating the stress damage. In addition, many studies suggested that carotenoids were not only the light-harvesting pigments transporting excitation energy to Chl-a, but also an anti-oxidation protective agents quenching ROS and dissipating excess excitation energy, which could prevent the oxidative damage to PS II [13,40]. However, some other studies found when plants (including algae and higher plants) were under stress conditions, their carotenoid content decreased significantly [36,38]. In our experiment, we also found that crust carotenoid content decreased under stresses, but the specific mechanism was still not clear yet, and needed further studies and discussions.

In the previous studies, it has been demonstrated that the $M.$ $vaginatus$ could adapt to a variety of stress conditions, including drought, salinity and so on [8,9,22,23,45]. In the present work, we further know the man-made crusts constructed with $M.$ $vaginatus$ also could adapt the drought and salt stress to a certain extent. Especially in the drought and high-salt areas, there is some protection of the crusts from salinity toxicity by drought, which

Table 4
The contents of exopolysaccharides (μg cm^{-1}) in crusts with different treatments (Mean ± S.D., n = 3).

Day	Control	0.1% NaCl	0.2% NaCl	0.5% NaCl	1% NaCl	Drought	0.2% NaCl + Drought	0.5% NaCl + Drought
1	5.73 ± 0.26 A	6.79 ± 0.41** A	6.66 ± 0.30* A	5.17 ± 0.55 A	4.78 ± 0.56* A	7.34 ± 0.54** A	9.09 ± 0.16** A	6.30 ± 0.06 A
8	9.13 ± 0.13B	10.35 ± 0.52*B	9.58 ± 0.29 B	6.06 ± 0.66** A	5.20 ± 0.55** A	8.27 ± 0.38 A	8.16 ± 0.49* A	6.04 ± 0.92** A
30	15.97 ± 0.37 C	19.49 ± 2.19**C	10.95 ± 1.31**C	7.32 ± 0.82**B	5.84 ± 1.03** A	8.31 ± 0.84** A	9.11 ± 1.48** A	6.90 ± 1.87** A

*, ** Significant difference between treatment and control at $P < 0.05$ or 0.01 level respectively. Different letters represent significant differences between days ($P < 0.05$).

helps us to understand the adaptability of BSCs to the stress conditions, and provides some theoretical support for constructing man-made crusts in drought and high-salt areas.

Acknowledgements

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