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Chapter

# Cultivation of Artificial Algal Crust and Its Effect on Soil Improvement in Sandy Area

Jinchao Feng, Wei Li and Linna Ma

## Abstract

Algae are the pioneer species of biological soil crusts. Cyanobacteria, microschwannophyta and pseudocladophyta can form fixed quicksand algae crusts on the surface of sand surface. Through artificial culture, soil crusts can be formed in a short time. The development and succession of algeal-sand crust promoted the enrichment of nutrients in the sand surface layer, and created conditions for the reproduction of micro-soil organisms and the colonization of herbaceous plants, thus promoting the desert ecosystem to enter a virtuous cycle. This chapter will focus on the cultivation process of artificial soil crust and its effect on soil improvement (soil organic matter and nitrogen) in sandy areas. In conclusion, the application of algal solution can rapidly form algal crusts, and according to the research results, the formation of algal crusts can significantly improve the chemical and biological properties of soil.

Keywords: algal crust, soil organic carbon, microbial carbon and nitrogen

### 1. Introduction

China is one of the countries most seriously affected by desertification in the world. Desertification not only causes the imbalance of ecosystem, but also reduces the area of arable land, and brings serious impact and harm to industrial and agricultural production and people's life. The arid desert and desertified land in northwest China have become one of the main sources of sandstorms in China and even in the Asia-Pacific region, causing great losses to the country, society and economy [1]. Therefore, desertification land management is the urgent need of the country in ecological construction and environmental protection. For a long time, afforestation and grass planting have been the main ways to control the desert, and some results have been achieved in practice. However, desertification control through traditional methods such as afforestation can sometimes be difficult to achieve, for example, in areas with less than 200 mm of rainfall. Therefore, it is necessary to have new ideas to control desertification [2].

With the increase of the construction history and economic investment of artificial vegetation in sandy land in China, the research on the development mechanism of sandy soil under artificial vegetation and the effect of soil modification by plants have been gradually strengthened [1]. Especially in recent years, the research on the ecological role of Biological Soil Crusts (BSC) has attracted great attention after the mobile sandy land was fixed. Sand surface after intervention algae and algae growth

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through its metabolism, driving the growth of soil heterotrophic microorganisms, increase the biodiversity in the desert surface, thus promotes the mineralization process of sand and soil weight circulation and flow, which is beneficial to improve soil physical and chemical properties, and increase soil organic matter, total nitrogen and total phosphorus content [1].

Biological soil crust refers to the complex surface cover formed by the interaction of cyanobacteria, green algae, lichens, mosses, microorganisms and other related organisms on and below the soil surface with soil surface particles through mycelium, pseudoroots and secretions. As ground cover, it generally exists in arid and semi-arid regions of the world, and its coverage accounts for 70% of bare land area [3]. It is a component of dryland ecosystem and an important landscape feature [4, 5].

The existence of biological crust plays a crucial ecological role, for the physical character, it could improve soil pore structure, reduce soil bulk density [6], reduce rainfall infiltration rate [7, 8], effectively ease the rain splash erosion and rainfall runoff scouring effect [6], add sticky powder [9, 10]. In extreme environments, such as water shortage, malnutrition, high temperature, the biological crust has strong survival ability, and can gradually improve soil quality and the surrounding environment, plays an important role in the prevention and control of soil erosion and sand-fixation [13]. For the chemical character, it could change the content of soil pH, plant nutrients required important and effectiveness, increase soil organic matter, total nitrogen, total phosphorus and total potassium [11–14]. For the biological character, it could increase the enzyme activity such as soil urease, invertase, catalase and dehydrogenase [15–19]. It has obvious ecological functions in soil and water conservation, improvement, windbreak and sand fixation, and response to global climate change [20], which is of great significance to the sustainable development of desert landscape.

As an important part of desert ecosystem, the formation and development of biological soil crust is one of the main indicators of ecosystem health. However, the natural formation of biological soil crusts is very slow, often taking years or even decades [21]. Therefore, it is imperative for desertification control to accelerate ecological restoration and reconstruction in sandy areas by artificial cultivation and propagation techniques.

The naturally developed BSC has a good sand fixation effect, so can we use artificial cultivation of BSC as a new method to prevent and control desertification? Artificial crust sand-fixation technology is to use BSC fixed sand table and the role of resistance to wind erosion, combined with the traditional biological sand technology, will be the main organisms in the BSC (algae, mosses, and lichens) for artificial cultivation and inoculation to the sand surface, through the maintenance of survival, the surface of the formation of BSC, have the effect of windbreak and sand-fixation, improve the effect of the windbreak and sand-fixation.

A large number of studies and practices have proved that BSC can be cultivated artificially. In addition, the cultured BSCs are characterized by rapid formation. The progress of natural BSC formation is slow in semi-arid and desert areas, and stable BSC can be formed in about 10 years, while artificially cultivated BSC can complete the natural process within one year [22]. In the Tengger Desert, researchers three cyanobacteria (Nostoc sp., Phormidium sp. Scytonemaarcangeli Bornet ex Flahault) were isolated and cultured from native BSCs and then inoculated in quicksand in combination with sand fixers and the superabsorbent polymers. After 1 year, soil hardness increased obviously. The carbohydrate content of newborn BSC, the biomass of cyanobacteria, microbial biomass, soil respiration, carbon fixation and effective quantum yield can be obtained 50% ~ 100% of natural BSC after 20 years of development [23].

Since the 1980s, soil algae biotechnology and microalgae metabolism physiology have been developed rapidly in the world, and a large number of breeding and preservation of fine algae species, and many new technologies have been developed. A large-scale artificial desert algal coating construction  $(3000 \text{ m}^2)$  were carried out in Shapotou area on the southeastern edge of the Tengger Desert in Ningxia Province, in which a large number of Phormidum lucidum were cultivated and directly inoculated on loose sand, and automatic sprinkling irrigation facilities of micro-irrigation were adopted for dune irrigation. The results showed that precipitation and low light intensity could significantly increase the biomass of artificial algae crusting [24]. The feasibility of inoculating cyanobacteria to accelerate soil biological recovery was verified, the results revealed that the inoculation of cyanobacteria increased soil organic carbon and total nitrogen, soil total salt, calcium carbonate and electrical conductivity [25]. The minimum light intensity suitable for the growth of microalgae was determined through indoor culture [26]. Microalgae biomass, microbial biomass and most enzyme activities increased with the development of biological soil crust were found in the Gurbantunggut Desert, Xinjiang [9]. Artificial inoculation is one of the important measures to promote the formation of algae crust in mobile sandy land. Artificial inoculation can make the crust form in quicksand in a short time, and the indoor culture cycle is generally 40–60 days [27].

Existing studies mainly focus on the cultivation process of single microbial algae, and the influence of algal crusts on soil physical and chemical properties and hydrological characteristics [9, 17–19]. However, there is still a lack of understanding on the influence of different ratios of algal crusts on soil physical and chemical and biological properties. In order to provide reference for ecological construction and desertification control in desert areas, different proportions of algal crusting inoculation were carried out in Ulan Buh Desert sample land. In this chapter, the artificial cultivation method of BSC and its effect on soil improvement in sandy areas will be described in detail.

## 2. Material and methods

#### 2.1 Study site

The research site is located in Dengkou County, Bayannur City, Inner Mongolia, with an altitude of about 1050 m. This region is located in the eastern edge of Ulan Buh Desert, which belongs to temperate continental monsoon climate. The average annual temperature is 7.6°C, and the accumulated temperature during the growing period is about 3100°C. The annual average rainfall is 144.5 mm, and the precipitation is unevenly distributed throughout the year, mainly from June to September. The annual average evaporation is 2397.6 mm, and the frost-free period is 136 days. The main soil type is eolian sandy soil, whose mechanical composition is dominated by fine sand (0.05–0.25 mm), accounting for more than 84%, with little physical clay and coarse sand. The experimental site is located at 106°50'E, 40° 30'N.

#### 2.2 Field survey, lab examination and data analysis

The indoor algae culture experiment began on November 20, 2019. The field algae culture experiment began on January 2, 2020 in Southeastern margin of Ulan Buh Desert, but due to the sand burial, the field experiment did not succeed. After the algae spraying, water was sprayed once a day to keep the surface layer moist. On January 2, 2020, the 0–5 cm soil was sampled and divided into three parts. One part of the fresh soil was sealed in a self-sealing bag and sent to the laboratory at a low

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temperature in an ice box. The fresh soil was stored in a refrigerator at  $-80^{\circ}$ C for the determination of soil microbial carbon and nitrogen. Part of the fresh soil was sealed in a self-sealing bag and brought back to the laboratory at a low temperature in an ice box. It was stored in a 4°C refrigerator for the determination of ammonium and nitrate nitrogen content. The other parts of the soil were used to determine the organic matter, total carbon, total nitrogen and total phosphorus of the soil after natural air drying, in order to quantify the effects of different ratios of algae crusting on the basic physical and chemical properties of the soil.

Using concentrated sulfuric acid - distillation titration method to measure the total soil nitrogen, using elemental Analyzer to measure the total soil carbon. Soil total phosphorus was determined by alkali fusion - molybdenum antimony anti-spectrophotometry, and microbial carbon and nitrogen were determined by chloroform fumigation extraction method.

One-way ANOVA was used to study the change degree of soil chemical and biochemical indicators of soil samples sprayed with different ratios of algae under 95% confidence interval. If there were significant differences, the least significant difference method (LSD) was used for multiple comparisons. P < 0.05 indicated significant differences. Data were collected using SPSS (version 17.0, SPSS Inc., Chicago, IL, USA) and charted using Origin (version 2019, OriginLab Inc., Northampton, MA, USA).

#### 2.3 Artificial culture of algae crust

Algae are the pioneer species for the formation of BSC. As pioneer colonizers, cyanobacteria can grow and reproduce under harsh environmental conditions, such as drought, ultraviolet radiation, poor nutrition, etc. Cyanobacteria can form a fixed quicksand algae crust on the sand surface.

Sand-fixing algae crusts can be formed in a short time by artificial culture. With the development and succession of algeal-sand crusts, and the material input brought by the deposition of fine particles and the accumulation of atmospheric dust, the nutrient enrichment in the sand surface layer was promoted, which created conditions for the reproduction of micro-soil organisms and the colonization of herbaceous plants, and then promoted the desert ecosystem into a virtuous cycle [28].

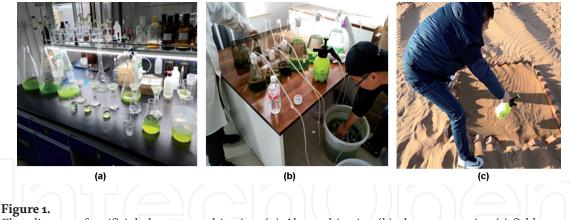
Algae crust artificial cultivation, is using the principle of algae ecology, physiology, and the theory of biological crust, separating, choosing a natural development formation of the excellent algal crust, after large-scale artificial cultivation, incubated on the surface of the sand to make the rapid formation of crust with algae, bacteria, fungi, algae. The technology mainly includes five aspects [1]:

(1) Isolation, purification and breeding of fine algae species in algae crusts; (2) the scale culture of alga species; (3) Factory/scale production; (4) Incubation in the field; (5) Management and maintenance. The main process and steps of artificial culture of algae crust are as follows (**Figure 1**).

(1) The algae crusts developed well under natural conditions were adopted. During sampling, the sampling frame is first pressed into the soil forcefully. When the sampling frame is completely put into the soil, the algae crust of about 50 cm<sup>2</sup> in the stainless steel frame is gently taken out with a shovel disinfected with 75% alcohol and put into an envelope for use.

(2) Sample sieving. Disinfect the gloves with 75% alcohol before operation, and wear gloves; The collected algae crust samples were crushed, passed through a 0.2 mm sieve, and set aside.

(3) Cleaning. 20 g of the screened algae crust sample was soaked in 50 mL distilled water and stood for 20 min. The soaked algae crust sample was placed on an ordinary medical gauze and rinsed slowly in distilled water until all the impurities and soil in the sample were removed. The remaining liquid was a mixed algal suspension.



Flow diagram of artificial algae crust cultivation. (a) Algae cultivation (b) algae propagation (c) field inoculation.

(4) The separation of algae species. The algal suspension in step (3) is examined under a microscope. If it is found that a large number of algae need to be separated, it can be separated immediately. If the quantity is small, it should be pre-cultured first, and then separated after increasing.

(5) Species of algae culture. 2 ~ 5 mL of isolated single or mixed seaweed suspension was taken and added with 200 mL BGI1. The nutrient solution (BG11 culture solution composition and dosage are shown in **Table 1** was placed in a triangular flask. Place the triangular flask on the shaker (Rpm is 140rmin-1) for initial culture, indoor temperature was controlled at 25 ~ 30°C, light intensity was controlled at 600 lx. Indoor culture after 7–10 days, the algae suspension was transferred to a 2-L volumetric flask for preliminary propagation. After propagation for 7 days, the culture medium was transferred into a 50 L plastic bucket, each bucket was equipped with 50 L BG11 culture medium and a set of oxygenation facilities (such as oxygenation pump for domestic ornamental fish); The dosage requirement of algal factory production can be reached after about 7 days of cultivation outdoors.

(6) The large-scale production of algae. Add the species algae cultured in step (5) into the production pool (the fresh weight of species algae added in each pool is about 200 g, and the dry weight is about 2 g). The culture medium used in the incubator was BG11 culture medium, and the water depth was about 0.5 m. In the process of cyanobacteria culture, the growth of cyanobacteria is the best when the water temperature is 25–30°C, and cooling measures should be taken when the water temperature is >40°C (because cyanobacteria will stop growing when the water temperature exceeds

Component	Dosage(mgL <sup>-1</sup> )	Component
NaNO <sub>3</sub>	1500	the composition of As
MgSO <sub>4</sub> •7H <sub>2</sub> O	75	H <sub>3</sub> BO <sub>3</sub>
K <sub>2</sub> HPO <sub>4</sub> •3H <sub>2</sub> O	40	MnCl <sub>2</sub> •4H <sub>2</sub> O
EDTA-Na <sub>2</sub>	1	Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O
CaCl <sub>2</sub> •2H <sub>2</sub> O	36	ZnSO <sub>4</sub> •7H <sub>2</sub> O
C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	6	CuSO <sub>4</sub> •5H <sub>2</sub> O
C <sub>6</sub> H <sub>8</sub> FeNO <sub>7</sub>	6	
As	1 ml/L	
Na <sub>2</sub> CO <sub>3</sub>	20	

#### Table 1.

Composition and dosage of BG11 medium.

40°C). The light intensity is controlled at 15000 lx. Harvest will be carried out after 7 to 12 days of culture of cyanobacteria (about 7 days in July and August in summer, 10–12 days in April and June in spring and 10–12 days in September and October in autumn). Determination of harvest period: it was determined according to the growth curve of cyanobacteria. When the growth rate or increase amount of cyanobacteria began to decline (the growth amount began to decline after reaching the maximum), the harvest was carried out. Before harvest, the liquid in the culture tank was left standing for 6 ~ 8 h. The culture solution is then discharged into an empty pool. (After one culture, the algae can still grow, but the yield is reduced.)30% ~ 40%. During the culture process, green algae from the air will enter the culture pond, leading to increased competition between algae and cyanobacteria. When the water depth in the pool is about 0.5 cm, the algal fluid is collected into a plastic bucket for use.

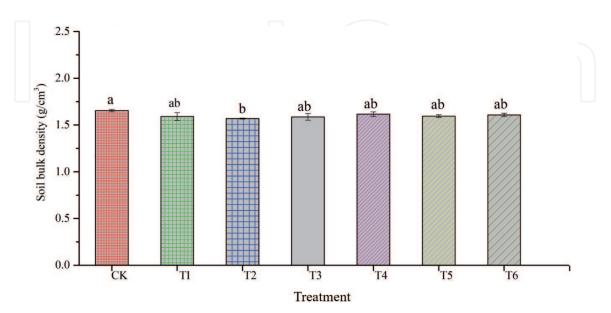
## 2.4 Field application of algae crust

Field inoculation: the collected algal solution was evenly injected into the sandy land surface with a sprayer, and the amount of algal solution was 3 g dry weight  $m^{-2}$ . For the first 10 days after inoculation, spray water every 2 days (the surface is moist), stop watering during rain. A month later, to measure the formation of algae crust, the topsoil area of 5 cm<sup>2</sup> and the thickness of about 5 mm were used for detection (due to the main algae distributed within 5 mm of the soil surface, so each sampling should be no more than 5 mm), and the content of chlorophyll a and chlorophyll b were measured.

## 3. Effect of algal crusts on soil improvement

### 3.1 Effects of different treatments on soil physical indexes

The soil bulk density after spraying algae culture solution in different proportions was shown in **Figure 2**. After spraying different proportions of algae culture solution, the bulk density of the 0–5 cm surface soil tended to decrease as a whole, among which, the soil bulk density of spraying mixed algae Microcolus vaginatus:



#### Figure 2.

Effects of different treatments on soil physical indexes. CK T1: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 1:1:1; T2: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1; T3: Microcolus vaginatus: Scytonema sinense 5:1; T4: Microcolus vaginatus; T5: Scytonema sinense; T6: Phormidum lucidum.

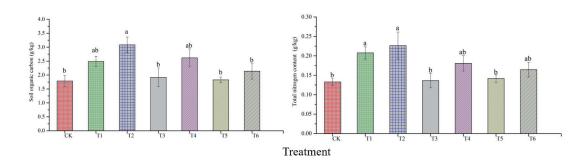
Scytonema sinense: Phormidum lucidum 3:2:1 significantly decreased (P < 0.05). In conclusion, spraying different proportion of algal culture solution can reduce the soil bulk density, and the mixed algae with the ratio of Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1 has the best culture status. In the study of biological soil crusts in the Horqin Sandy Land of Inner Mongolia, algae crusts can significantly reduce the surface soil bulk density [21].

#### 3.2 Effects of different treatments on soil chemical indexes

Accumulation of soil carbon and nitrogen is regarded as an indicator of soil fertility and productivity [29]. Algae spraying in different proportions had different effects on soil organic carbon. Among them, the ratio of single algae species Scytonema sinense and Phormidum lucidum, and mixed algae species Microcolus vaginatus: Scytonema sinense 5:1 did not change soil organic carbon content, while the ratio of single algae species Microcolus vaginatus; Scytonema sinense: Phormidum lucidum 1:1:1 showed a trend of increasing soil organic carbon content. The soil total carbon content was significantly increased by 72.6% (P < 0.05) by spraying mixed algae with the ratio of Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1(**Figure 3**).

Previous studies have shown that biological soil crusts can fix atmospheric carbon and nitrogen [30, 31]. Fixed carbon and nitrogen are released into the surrounding environment and used by other organisms such as vascular plants, fungi and bacteria [32]. The study of the algal crust under the canopy of Artemisia sphaerica vegetation and the organic matter in the underlying soil in Mu Us Sandy Land found that most of the algal crust developed stably between canopies, and most of the algal crust under the canopy was at the early or middle stage of development, and the content of organic matter in the intercanopies and underlying layer increased significantly [33]. Nitrogen fixing cyanobacteria can fix atmospheric nitrogen and increase soil organic matter content. The nitrogen-fixing activity of algal crusts in the artificial vegetation sand-fixing area and the natural vegetation area were compared, the results indicated that the nitrogen-fixing activity of algal crusts in the artificial vegetation area increased significantly with the extension of vegetation restoration time [34]. The results showed that the development of algal crusts promoted soil nitrogen content and soil development after the implementation of artificial vegetation restoration measures.

The difference in total nitrogen content is an indication of different nitrogen inputs [13]. Soil total nitrogen content and soil organic carbon content had a similar response trend. Neither single algal culture medium could significantly increase soil total nitrogen content, but the soil total nitrogen content in the ratio of Microcolus



#### Figure 3.

Effects of different treatments on soil chemical indexes. CK T1: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 1:1:1; T2: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1; T3: Microcolus vaginatus: Scytonema sinense 5:1; T4: Microcolus vaginatus; T5: Scytonema sinense; T6: Phormidum lucidum.

vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1 was significantly increased (P < 0.05), with the increase ratios of 56.2% and 70.3%, respectively.

All the algal culture media had no effect on soil total P content. Carbon input is an important ecological function of biological soil crust in arid regions, and the ability of carbon fixation is affected by the development degree of biological soil crust. In well-developed biological soil crusts, the carbon fixation rate is about two times higher than that in poorly developed soil crusts, which is mainly attributed to the increase of chlorophyll a in well-developed biological soil crusts [35].

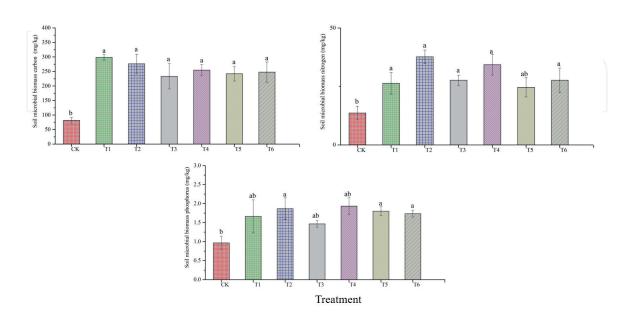
Most of the nitrogen fixed by biological soil crusts can be immediately released into the soil [36]. At low soil moisture content, biological soil crusts had no effect on soil organic matter and soil total nitrogen content, while at high soil moisture content, Biological soil crusts can significantly increase soil organic carbon and soil total nitrogen content at 0–5 cm depth [37] f. This may be because the available water under the surface of the soil is too high, and the water condition is high enough to activate carbon fixation components [38] and drive photosynthesis to produce ATP and carbohydrates for nitrogen fixation [39].

#### 3.3 Effects of different treatments on soil biological indexes

Soil microbial biomass fluctuated between 0.29 and 2.02 g C m<sup>-2</sup> in soil biological crust, and its value was significantly correlated with the development degree of soil crust [40]. Increasing organic matter and polysaccharides offers a plentiful carbon source for microorganisms and invertase, resulting in an increase of microbial biomass C [41, 42]. Increase of microalgal biomass is helpful to improve organic C and available P. The release of carbonic acid by the algal cells can accelerate the weathering of minerals, hence make improvement of inorganic ions [43].

All medium significantly increased the content of soil microbial biomass carbon (P < 0.05), but there was no significant difference among different medium (P > 0.05).

Except for Scytonema sinense, other algal culture media significantly increased the soil single species Scytonema sinense and Phormidum lucidum significantly increased soil microbial biomass phosphorus (P < 0.05), and other species had an increasing trend, but the statistical difference was not significant (**Figure 4**).



#### Figure 4.

Effects of different treatments on soil biological indexes. CK T1: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 1:1:1; T2: Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1; T3: Microcolus vaginatus: Scytonema sinense 5:1; T4: Microcolus vaginatus; T5: Scytonema sinense; T6: Phormidum lucidum.

#### 3.4 Existing problems and suggestions

As a new method to prevent and control desertification, artificial cultivation of algae crust has made substantial progress and breakthrough in the practice of preventing and controlling desertification. However, the following two aspects need to be further discussed: sand burial is one of the most common disturbance factors of desert ecosystem in sand area, especially in arid desert ecosystem with frequent eolian sand activities. Sand burial affected the growth and survival of BSC by changing the light, temperature and soil physical and chemical properties of BSC habitat. Especially in the early stage of the construction of artificial algae crust, the survival ability of algae is relatively weak, and the existence of sand crust seriously threatens the further development and formation of algae crust. Artificial crust is more fragile, and its resistance to adversity is weak. Therefore, after the construction of artificial algae crust is completed, how to prevent and control the damage of sand burial to it. It is a problem to be faced in constructing artificial algae crust. The key links of technology still need further optimization and innovation, still need to be improved and perfected through a lot of scientific research and production practice. In addition, the early artificial hydration measures after inoculation are conducive to the normal synthesis and metabolism of extracellular polysaccharide, and the accumulation of extracellular polysaccharide is helpful to restore the drought-tolerant ability of algae and adapt to the external drought environment, thus increasing the biomass of algae, improving the drought-tolerant ability of algae and promoting the formation of artificial algae crust. Therefore, early water acquisition is the key factor for the successful formation of artificial algae crust. However, water is the most important ecological limiting factor in arid areas. If water is replenished continuously in the early stage, it cannot be realized in largescale desertification prevention and control practices. Therefore, how to cultivate the algae with stronger drought resistance and more suitable for the formation of artificial algae crust is the key technology to be broken through in the construction of artificial algae crust.

#### 4. Conclusions

This study shows that different proportions of algae crusts can improve the physical, chemical and biological properties of desert soil to different degrees. The results showed that the soil bulk density in the 0-5 cm surface layer had a decreasing trend after spraying different proportion of algae culture solution, and the soil bulk density in the mixed algae Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1 was significantly decreased (P < 0.05). The soil organic carbon content was increased with the ratio of single species Microcolus vaginatus, Microcolus vaginatus: Scytonema sinense: Phormidum lucidum: 1:1:1. The soil total carbon content was significantly increased by 72.6% (P < 0.05) when the ratio of mixed species Microcolus vaginatus: Scytonema sinense: Phormidum lucidum: 3:2:1. The soil total nitrogen content was significantly increased with the ratio of Microcolus vaginatus: Scytonema sinense: Phormidum lucidum 3:2:1 (P < 0.05), increasing by 56.2% and 70.3%, respectively; All medium significantly increased the soil microbial biomass carbon content (P < 0.05). And artificial cultivation of algae crusts can greatly shorten the formation time of algae crusts. The results in this study highlight the significant role of BSCs for soil improvement in semiarid and arid areas. Therefore, when carrying out ecological construction in arid desert, it is necessary to fully consider the difference of effects of different ratio of algal solution, so as to achieve the optimal soil nutrient improvement effect of biological crusts.

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## **Conflict of interest**

The authors declare no conflict of interest.

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