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Chlorophyll and growth performance of biological sand-fixing materials inoculated on sandy desert surface

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Abstract

Desert biocrusts play an important role in the control of desertification and artificial inoculation can promote the formation and development of biocrusts. Physiological and growth responses of biocrusts inoculated on desert surfaces were investigated to assess the effect of mixture ratio, inoculation times, and water supply under laboratory conditions. The application of biological sand-fixing material prepared by cultivated algae crust and polymeric composites in a 1:1 ratio accelerated the most accumulation of chlorophyll *a* in 0.55 mg kg^{-1}, thickness in 3.06 mm, and fresh mass in 0.69 g cm^{-1}, was the most beneficial to formation and development of artificial biocrust. The water supply and cultivation time always significantly promoted the growth and accumulation of chlorophyll *a* and biomass under artificial cultivation and inoculation treatments. Artificial inoculation of biological sand-fixing material can lead to the formation of desert biocrust, which provides an engineering application method for desertification control.

Keywords: chlorophyll *a*; desert biocrust; fresh mass; inoculation; thickness.

Introduction

Desert biocrusts are highly specialized communities composed of cyanobacteria, green algae, lichens, mosses, bacteria, and microfungi (Chock *et al.* 2019). Desert biocrusts might be classified as cyanobacterial crusts, lichen crusts, or moss crusts based on their successional stage and dominant components (Kidron 2015, Antoninka *et al.* 2016, Chiquoine *et al.* 2016, Bustos *et al.* 2022). The organisms comprising biocrusts could adapt to extreme environmental conditions, such as high temperature, salinity, low precipitation, strong irradiation, and desiccation (Rao *et al.* 2012, Zhao *et al.* 2016, Felde *et al.* 2018, Ji *et al.* 2019, Hui *et al.* 2022), they could also fix mobile sand dunes as well as alter topsoil moisture and resistance to wind and water erosion to improve carbon and nitrogen fixation and nutrient cycling, resulting in improvement of the surrounding environment and regulation of soil microbe abundance and community diversity (Videla *et al.* 2021, Aranibar *et al.* 2022, Drahorad *et al.* 2022, Shi *et al.* 2023). Additionally, desert biocrusts could improve soil fertility through mineral chelation, dust entrapment, and metabolism, which is beneficial to invertebrates and reptiles, as well as vascular plants (Strong *et al.* 2013, Sinsabaugh *et al.* 2015, Zhang *et al.* 2015, Zhou *et al.* 2016, Zheng *et al.* 2018, Rajnoch *et al.* 2022, Tang *et al.* 2023). Accordingly, desert biocrusts were considered a solution for the restoration of degraded desert soil.

Natural self-recovery of desert biocrust to a stable succession state could take several decades to millennia, although the self-recovery of desert biocrust in desertification land happens all the time (Chock *et al.* 2019). The colonization of vascular plants usually occurs after biological crusts improve the topsoil environment, which could take a long time; the natural recovery of

Highlights

- The best ratio in biocrust and polymeric composites was 1:1
- Attapulgite could facilitate biocrust
- Water supply accelerated the accumulation of chlorophyll *a* and biomass

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Abbreviations: *ANOVA* – analysis of variance; BA – benzylaminopurine; BSM – biocrust sand-fixing material; Chl – chlorophyll; *MANOVA* – multivariate analysis of variance.
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desert biocrusts from disturbance takes especially long and is highly variable from place to place (Giraldo-Silva *et al*. 2019, Li *et al.* 2022). In addition, changes in climatic conditions (temperature and precipitation) impact the growth of desert biocrust. Extreme weather tends to inhibit soil respiration in biocrusts (Guan *et al.* 2021, Ayala-Niño *et al.* 2022). Many researchers have conducted experiments investigating the rapid artificial induction of biocrusts by using moss and cyanobacteria (Kidron 2015). Chen *et al.* (2006) constructed man-made desert algal crusts in Inner Mongolia, China, by inoculating *Microcoleus vaginatus* onto unconsolidated sand dunes. Wang *et al.* (2013) also tested the feasibility of mixed cyanobacterial inoculation with straw checkerboards and automatic-sprinkling micro-irrigation techniques in desert areas. The soil fertility and microenvironment of the topsoil improved as cyanobacterial crusts developed (Park *et al.* 2017). Different soil substrates also affect the colonization of artificial biocrusts. Zhao *et al.* (2021) successfully cultivated artificial biocrusts in field conditions by inoculating natural cyanobacteria and cyanobacteria–lichen crust fragments. They found that left-over soils from dredged irrigation channels and abandoned farmlands can provide a good substrate to culture desert biocrusts inoculum material. Although the above studies provide some useful information regarding artificial biocrust generated using cyanobacteria, it is still unclear how efficiently cultured cyanobacteria biomass could be used for artificial biocrust formation (Jech *et al.* 2023). Due to the fact that a large amount of artificial desert biological crust was cultivated in laboratories or other facilities, the optimal application method should be determined for large-scale applications to form a sufficiently robust biological crust biomass.

During the artificial propagation of desert biocrusts, growth-favoring environmental conditions were often helpful in rapid forming of the biocrust (Hui *et al.* 2013, 2014, 2018; Park *et al.* 2017, Tao *et al.* 2021). Thus, these conditions might be used in the field where environmental factors are variable and sometimes harsh (Bu *et al.* 2017). Some artificial propagation methods of desert biocrust were developed and confirmed to be practical (Antoninka *et al.* 2016, Szyja *et al.* 2017, Zhang *et al.* 2021, Zhao *et al.* 2023). Therefore, developing artificial propagation methods for wider and larger-scale field applications was widely anticipated.

To prevent wind and water erosion in arid or semiarid areas, sand-fixing agents, such as polymerized by the monomer of vinyl acetate (PVIN), polyvinyl alcohol, polyaspartic acid, and polyacrylamide, have been used. These chemical agents were normally expensive and effective for fixing sand particles in the short term but were not reliable and sustainable in the long term due to microbial degradation (Hui *et al.* 2013, 2014; Rozenstein *et al*. 2014, Ma *et al.* 2016). Conversely, biocrust formations were more appropriate for sand-fixing and restoring damaged soil in arid areas. However, it will take several years, or even a few decades, to induce biocrust formation in nature depending on environmental conditions during the initial stage. The combined application of chemical agents and clay could accelerate biocrust formation under natural conditions compared to the application of individual chemical agents. The clay and chemical agents could physically fix fine sand particles in a short period, which is crucial to desert algae settlement for biocrust induction on bare sand soils, while also increasing water availability for desert algae crust growth due to their high water-absorbing capacity (Shi *et al*. 2016, Park *et al*. 2017, Hui *et al*. 2018, Abulimiti *et al.* 2023). Thus, the inoculation of biological sand-fixing material could enable more rapid induction and stable formation of biocrusts than the application of desert algae crust alone under natural conditions. Moreover, the combined application of sand-fixing material with desert biocrust could overcome the defects associated with sand-fixing material during field trials (Abulimiti *et al.* 2023).

The lack of water resources and precipitation and high water dissipation severely restrict the ecological model and process of the desert ecosystem (Cheng *et al.* 2022, Sun and Li 2022). Efforts to counter desertification had been initiated over the past few decades; however, only a small improvement had been reached. This was of great concern, as desertification is the leading problem inhibiting development in China, especially in northern and northwestern China. At present, research on the rapid cultivation of moss crust in China and elsewhere mainly focus on indoor tissue culture, artificial repair of wild biological crust, and influencing factors. Recently, several artificial cultivation methods for desert biocrusts were developed, confirming that the technology of rapid artificial inoculation of biocrusts is practical (Antoninka *et al.* 2016, Wu *et al.* 2013). The propagation of biocrusts often performed some particular growth responses under variable environmental conditions.

Can the artificial biocrust be prepared with different materials and methods to survive and grow under different combined inoculation applications and water supply? The present study was conducted to induce the formation and growth of biocrust by using biological sand-fixing material. The sand-fixing material added to attapulgite clay was applied as a sand-fixing and water-retaining agent under the early stage of biocrust succession. The inoculation of biological sand-fixing materials was conducted to investigate the effects of inoculation times and water supply to evaluate the feasibility of application and potential methods for promoting the establishment of desert biocrusts.

Materials and methods

Biocrust, attapulgite, and polymer-mixed materials: The algae biocrust was collected from the desert region on the southeastern edge of the Tengger Desert of China (37°31'15"N, 105°2'16"E) in September 2021. This region is an ecotone between desert and oasis, with an elevation of 1,300 m, mean annual temperature and precipitation are –6.9°C and 186 mm. Biocrusts of 10 g was placed into a 250-mL Erlenmeyer flask, and 200 mL of cultivation medium with a growth regulator (6-BA, 0.5 mg L^{-1}) was added to the flask. The Erlenmeyer flask was placed in a light incubator at 25 ± 1°C with

a light intensity of 120 μE m^{-2} s^{-1}. The culture medium was refreshed every 15 d. The cultivated biocrusts were smashed after air-dried for the preparation of biological sand-fixing material.

Attapulgite was collected from Linze County of Gansu province, China. Attapulgite (10 g) was immersed in 100 mL of 4 mol L^{-1} H_2SO_4 solution at 25°C for 72 h. The attapulgite was washed with distilled water until the pH was 6 ~ 7 and then dried at 105°C for 8 h to produce acid-activated attapulgite. NaOH (10 g) was added to 10 mL of distilled water in a beaker, then, 15 mL of acrylic acid was slowly added to the beaker in an ice-water bath. Acrylamine (4 ml) was added to the beaker under vigorous stirring to produce the monomer solution. The acid-activated attapulgite and 30 mL of distilled water were added to the beaker with vigorous stirring to produce a mixture. The mass fraction of acid-activated attapulgite in the system was 10%. Then, 0.05% N,N'-methylene bisacrylamide, 0.6% persulfate, and sodium bisulfite (a molar ratio of potassium persulfate to sodium bisulfite of 1:1) were dissolved in 10 mL of distilled water in a beaker to produce the initiator-crosslinker solution. The beaker containing the mixture was put into an ultrasonic reaction device with a variable amount of ultrasonic power of 200 W and heated at 80°C. Next, the monomer solution and initiator-crosslinker solution were added to the beaker with stirring for 10 min, the mixture was washed three times with water and alcohol (1:9 in volume) to remove any unreacted reactants, then, it was dried in an oven at 90°C until the constant mass. The polymer-mixed material was crushed and grounded to allow passage through 100 mesh.

Preparation and cultivation of biological sand-fixing materials: Four biological sand-fixing materials were prepared with the biocrust and polymer-mixed material by 2:1, 0:1, 1:1, and 1:2 mass ratio, and named BSM21, BSM01, BSM11, and BSM12. Four materials were laid in the 150-mm Petri dish in 1-cm thickness respectively, three replicates**.** All dishes were placed in a light incubator for 60 d at 28 ± 1°C and 120 μE m^{-2} s^{-1}; 12 mL of distilled water and 6 mL of BG11 medium were sprayed in each Petri dish. Starting on the 20th d, the chlorophyll *a* concentration, thickness, and fresh mass of the samples were determined every 10 d.

Inoculation and water supply: The sand was sprinkled into a Petri dish in 1-cm thickness, four sand-fixing materials were sprinkled uniformly onto the surface of the sand respectively, three replicates, and distilled water was sprayed onto materials three times per day, 12-ml dosage.

The distilled water was sprayed onto the samples three times per day at four dosages: 6 ml, 9 ml, 12 ml, and 15 ml, amounting to 5.1, 7.6, 10.2, 12.7, and 15.3 mm effective precipitation during 20, 30, 40, 50, 60 d incubation periods for BSM11 treatment to investigate the performance of sand-fixing material under different water supply.

Measurements of chlorophyll (Chl) *a* and growth trait: Thickness was measured with a Vernier caliper, and fresh mass was measured with an electronic balance. Samples (0.2 g) were ground with a trace of quartz sand and calcium carbonate in a mortar, then, the extract was obtained with 1.5 mL of 95% ethanol and brought to a volume of 25 mL with 95% ethanol, the absorbance value of the extract was measured at 665 nm and 649 nm to get chlorophyll *a* concentration (*UV-300*, UK) (Lan *et al.* 2011).

Statistical analysis: Each experimental treatment had three replicates. Statistical analysis was done using *STATISTICA 14*. To test the data on significant differences, a two-way *MANOVA* and one-way *ANOVA* were used after a check of normal distribution and variance homogeneity including Chl *a*, thickness, and fresh mass. A pairwise comparisons between groups were calculated by post hoc *Tukey*'s honestly significant difference test at the test level of 0.05.

Results

Chl *a* and growth traits of biological sand-fixing materials under cultivation: The Chl *a* concentration, thickness, and fresh mass were significantly affected by biological sand-fixing materials, cultivation period, and their interaction by a two-way *ANOVA* (Table 1). The Chl *a* showed a significant difference between biological sand-fixing materials, BSM12 and BSM21 did not show a significant difference in all five cultivation periods (Fig. 1). The thickness and fresh mass of BSM12 and BSM21 treatments both were significantly higher than BSM01 and lower than BSM11 (Figs. 2, 3). The Chl *a* and both growth traits of the BSM11 always performed the largest value than other materials, the BSM01 without the addition of biocrust presented the lowest values at all cultivation periods. The BSM11 demonstrated stronger survival capacity and more effective photosynthesis under artificial cultivation conditions.

Chl *a* and growth traits after inoculation: After 60 d of the inoculation period, the four kinds of biological sand-fixing materials showed a revived state. They formed biological crusts, which were green and adhered to the sand at the bottom, indicating that the four kinds of sand-fixing materials could survive and grow on the sand surface. Among them, BSM11 material showed stronger vitality (Fig. 4).

According to the results of the two-way *ANOVA*, the biological sand-fixing materials, inoculation times, and their interaction significantly affected the Chl *a* concentration, thickness, and fresh mass after artificial inoculation (Table 2). The Chl *a* concentration was significantly different between four inoculation treatments in all five inoculation times and among five inoculating times by a one-way *ANOVA*. The Chl *a* content under BSM11 treatment always significantly performed the highest value; the lowest Chl *a* content appeared in BSM01 treatment for all inoculation periods, after 30-d inoculation period. The Chl *a* contents under BSM12 and BSM21 did not show the significant difference and they both were significantly higher than the BSM01 treatment and lower

Table 1. Two-factor analysis of variance for chlorophyll *a*, thickness, and fresh mass of biological sand-fixing material under artificial cultivation conditions.

Trait	Source of variation	df	*F*-value	*P*
Chlorophyll *a*	cultivation period	4	152.72***	<0.001
	biological sand-fixing material	3	22.26**	<0.005
	cultivation period × biological sand-fixing material	12	26.08**	<0.005
Thickness	cultivation period	4	146.28***	<0.001
	biological sand-fixing material	3	85.97***	<0.001
	cultivation period × biological sand-fixing material	12	12.36**	<0.005
Fresh mass	cultivation period	4	123.51***	<0.001
	biological sand-fixing material	3	160.87***	<0.001
	cultivation period × biological sand-fixing material	12	36.86***	<0.001

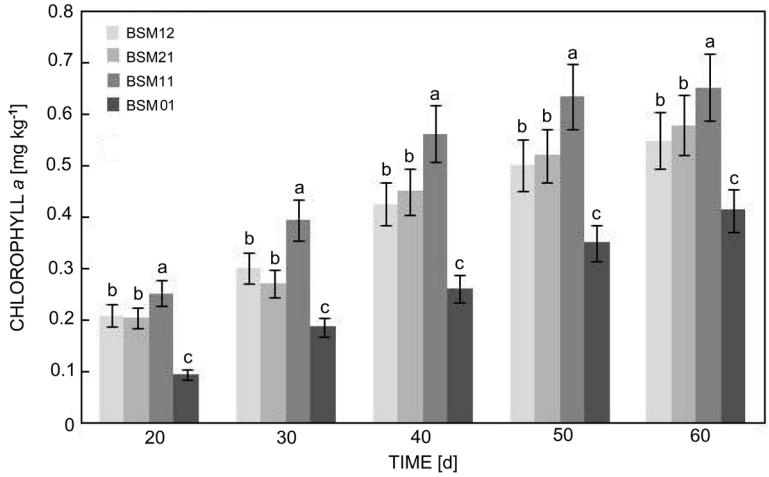

Fig. 1. Chlorophyll *a* content [mg kg^{-1}] of biological sand-fixing materials under five cultivation periods. Values with the *same lowercase letters* were not significantly different between four treatments at $p<0.05$ or according to *Duncan*'s multiple comparison tests. BSM – biocrust sand-fixing material.

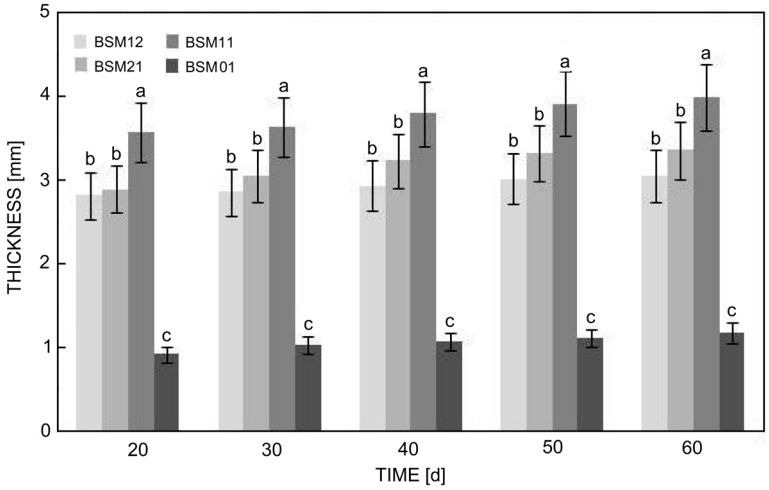

Fig. 2. The thickness of biological sand-fixing materials under five cultivation periods. Values with the *same lowercase letters* were not significantly different between four treatments at $p<0.05$ or according to *Duncan*'s multiple comparison tests. BSM – biocrust sand-fixing material.

than the BSM11 treatment. The Chl *a* contents under four treatments significantly increased with the extension of inoculation time (Table 3). It indicated that the sand-fixing materials were in a state of survival and continuous growth after inoculation.

A one-way *ANOVA* suggested that the thickness significantly differed between four inoculation treatments at all inoculation periods. At 20 and 30 d, the thickness of the BSM11 treatment was significantly higher than other treatments, the thickness of the BSM12 treatment was significantly higher than the BSM01 treatment and lower than the BSM21 treatment. At 40, 50, and 60 d, there was no significant difference in thickness between the BSM12 and BSM01 treatments, and the thickness of BSM11 was always significantly higher than in all the other treatments. For all inoculation treatments, the thickness of inoculated

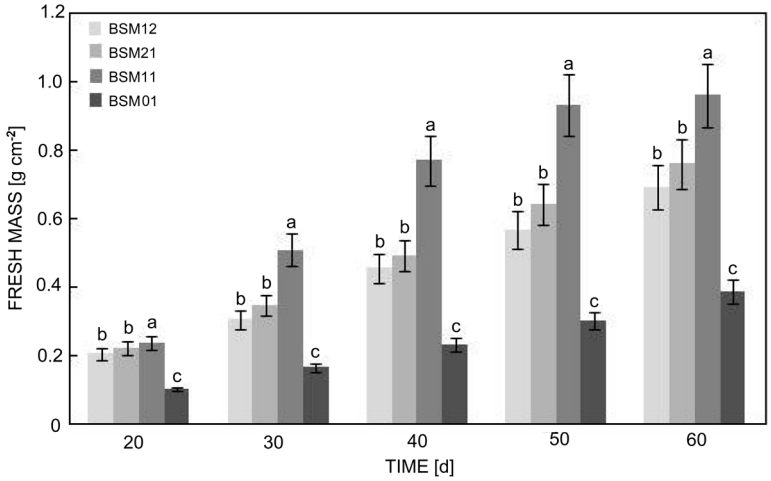

Fig. 3. The fresh mass [g cm^{-2}] of biological sand-fixing materials under five cultivation periods. Values with the *same lowercase letters* were not significantly different between four treatments at $p<0.05$ or according to *Duncan*'s multiple comparison tests. BSM – biocrust sand-fixing material.

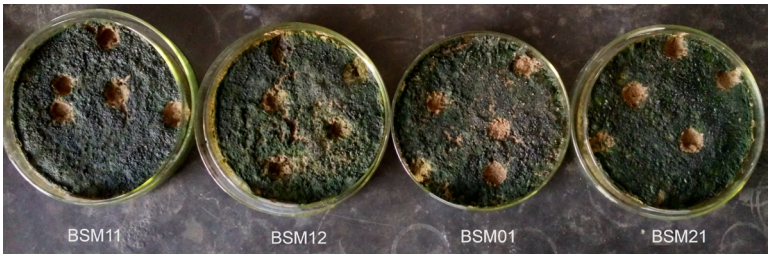

Fig. 4. The appearance of biological sand-fixing materials cultivated for 60 d. BSM – biocrust sand-fixing material.

Table 2. Two-factor analysis of variance for chlorophyll *a* concentration, thickness, and fresh mass of BSM11 applied with four inoculation methods under different incubation times with three replicates.

Trait	Source of variation	df	*F*-value	*P*
Chlorophyll *a*	cultivation period	4	56.23	<0.001
	biological sand-fixing material	3	25.66	<0.005
	cultivation period × biological sand-fixing material	12	29.68	<0.005
Thickness	cultivation period	4	117.26	<0.001
	biological sand-fixing material	3	76.94	<0.001
	cultivation period × biological sand-fixing material	12	14.46	<0.005
Fresh mass	cultivation period	4	156.42	<0.001
	biological sand-fixing material	3	94.32	<0.001
	cultivation period × biological sand-fixing material	12	38.96	<0.001

biocrusts showed a significant increase with the extension of inoculation time, especially in the later period, the increase was more obvious (Table 3). These four artificial biological sand-fixing materials had significant signs of survival and reproductive ability when inoculated on the desert surface.

The fresh mass exhibited significant differences between four inoculation treatments at all inoculation periods according to the one-way *ANOVA*. In all inoculation periods, the fresh mass of the BSM11 treatment was significantly higher than other treatments, and the fresh mass of the BSM12 treatment was significantly higher than the BSM01 treatment and lower than the BSM21 treatment. For each inoculation treatment, the fresh mass always demonstrated a significant rising tendency with the extension of inoculation time, the accumulation of biomass significantly correlated with the increase of inoculating time (Table 3).

Influence of water supply on inoculated biocrusts: The Chl *a* concentration, thickness, and fresh mass of BSM11 after artificial inoculation were significantly affected by the water supply, inoculation time, and their interaction according to the results of the two-way *ANOVA* (Table 4). The contents of Chl *a*, thickness, and fresh mass were significantly different between four kinds of water supply in all five inoculation periods, and among five inoculation periods under water supply by a one-way *ANOVA*. At all inoculation periods, the contents of Chl *a*, thickness, and fresh mass under 12 ml/time and 15 ml/time water supply treatments always were significantly higher than other treatments, the most water supply

Table 3. Chlorophyll *a* content [mg kg^{-1}], thickness [mm], and fresh mass [g cm^{-2}] of BSM11 applied with four inoculation methods under different incubation times with three replicates. Values with the *same lowercase letters* were not significantly different among four materials, and those with the *same capital letters* were not significantly different among five incubation times at $p<0.05$ or according to *Duncan*'s multiple comparison tests. * significant difference at 0.05 level, ** at 0.01 level, *** at 0.001 level according to *ANOVA*.

	20 days	30 days	40 days	50 days	60 days	*F*-value
Chlorophyll *a*						
BSM12	0.122^{cE}	0.239^{bD}	0.349^{bC}	0.416^{bB}	0.490^{bA}	61.17^{***}
BSM21	0.180^{bE}	0.271^{bD}	0.364^{bC}	0.465^{bB}	0.511^{bA}	58.56^{***}
BSM11	0.210^{aE}	0.302^{aD}	0.427^{aC}	0.503^{aB}	0.552^{aA}	67.39^{***}
BSM01	0.098^{dE}	0.184^{cD}	0.295^{cC}	0.379^{cB}	0.468^{cA}	127.33^{***}
F-value	46.41^{***}	255.93^{***}	52.01^{***}	153.64^{***}	170.50^{***}	
Thickness						
BSM12	2.36^{cD}	2.42^{cCD}	2.46^{cC}	2.56^{cB}	2.68^{cA}	28.35^{***}
BSM21	2.56^{bD}	2.64^{bC}	2.80^{bB}	2.88^{bA}	2.92^{bA}	64.28^{***}
BSM11	2.80^{aC}	2.86^{aB}	2.94^{aA}	3.02^{aA}	3.06^{aA}	14.60^{***}
BSM01	2.26^{dC}	2.22^{dC}	2.44^{cB}	2.56^{cA}	2.60^{cA}	100.50^{***}
F-value	113.80^{***}	152.73^{***}	186.40^{***}	89.94^{***}	75.27^{***}	
Fresh mass						
BSM12	0.1412^{cE}	0.2018^{cD}	0.3220^{cC}	0.4490^{cB}	0.5985^{cA}	42.64^{***}
BSM21	0.1779^{bE}	0.2643^{bD}	0.3578^{bC}	0.4945^{bB}	0.6213^{bA}	152.43^{***}
BSM11	0.2066^{aE}	0.3069^{aD}	0.4562^{aC}	0.5683^{aB}	0.6913^{aA}	167.53^{***}
BSM01	0.1334^{dE}	0.1605^{dD}	0.2465^{dC}	0.3827^{dB}	0.5244^{dA}	85.15^{***}
F-value	36.43^{***}	61.80^{***}	20.23^{*}	20.83^{*}	17.20^{**}	

Table 4. Two-factor analysis of variance for chlorophyll *a* content, thickness, and fresh mass of BSM11 inoculated on sandy desert under different water supply.

Trait	Source of variation	df	*F*-value	*P*
Chlorophyll *a*	inoculation period	4	135.21	<0.001
	water supply	3	24.13	<0.005
	inoculation period × water supply	12	25.37	<0.005
Thickness	inoculation period	4	136.57	<0.001
	water supply	3	84.26	<0.001
	inoculation period × water supply	12	13.27	<0.005
Fresh mass	inoculation period	4	134.26	<0.001
	water supply	3	86.34	<0.001
	inoculation period × water supply	12	44.12	<0.001

(15 ml/time) significantly led to the highest increase of Chl *a* contents, thickness, and fresh mass. Under each water supply treatment condition, the Chl *a* contents and fresh mass of artificial biocrusts showed a very obvious growth trend with the extension of inoculation time, and there was a significant difference between the two inoculation periods. The thickness of inoculated biocrusts did not show any significant difference between the five inoculation periods but performed a less obvious increase with the extension of the inoculation period (Table 1S, *supplement*).

Discussion

The chlorophyll *a*, thickness, and biomass commonly were considered important factors for the evaluation of the survival, establishment, and propagation of artificial biocrusts (Lan *et al.* 2010, Wu *et al.* 2013, Zhang *et al.* 2013, Chiquoine *et al.* 2016). In this research, the influences of mixture ratio on the performances of biological sand-fixing materials were explored by the investigation of Chl *a* content, thickness, and fresh mass. The biological sand-fixing materials with different constituents showed significant differences. The cultivated biological sand-fixing material in a 1:1 mixture ratio presents the best performance based on the production of the Chl *a*, thickness, and fresh mass after incubation. The addition of attapulgite clay in sand-fixing materials promoted the survival and growth capacity and more effective photosynthesis according to the performance of Chl *a*, this may be due to the powerful capacity of water retention and replenishment of clay (Abulimiti *et al.* 2023).

Different proportions of biological sand-fixing materials and quantitative water supply could significantly affect the Chl *a* content, thickness, and fresh mass of biological sand-fixing materials inoculated on the sand surface. It had been determined that the addition of cultivated biocrusts in biological sand-fixing materials usually had a positive impact on the survival and propagation of artificial biocrusts; these biological sand-fixing materials may form the more suitable physical structure for the survival and growth of biocrust (Strong *et al.* 2013, Wang *et al.* 2015, Zhou *et al.* 2016). Water supply usually was a vital factor in the desert region, the inoculated biological sand-fixing materials showed higher Chl *a* content and biomass under more water supply (Bu *et al.* 2017), natural desert biocrusts always presented consistent requirement for water supply (Zaady *et al.* 2014, Zhao *et al.* 2014, Zheng *et al.* 2018, Sun and Li 2022).

The addition of attapulgite clay promoted the growth capacity and photosynthesis of biological sand-fixing material. The biological sand-fixing material prepared cultivated biocrust and sand-fixing material in 1:1 ratio always presented the best performances. More water supply usually improved the production of chlorophyll *a*, addition of thickness and biomass. The investigation results suggested that the optimal preparation methods of biological sand-fixing materials could promote the propagation of desert biocrust. The artificial biological sand-fixing materials had significant signs of survival and developing ability when inoculated on the desert surface. These results will be significant for desertification control.

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