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Original Research

Study of the Physiological Characteristics of *Didymodon vinealis* with the Aid of Attapulgite-Based Nanocomposite

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Abstract

Desertification is one of global environmental problems, which is faced by human beings. Finding a new sand-fixing material is an effective method to combat desertification. In desert areas, water shortage is one of problems that *Didymodon vinealis* (*D. vinealis*) grew. In this study, we built up a special-structured nanocomposite. Attapulgite had chain layered crystal structure and many active hydroxyl groups, which made them have high absorption and retention capacity of water. The optimum ratio of dried *D. vinealis* to attapulgite-based nanocomposite was proved to be 1:4 and the optimum spraying amount 4 mg DW·cm^{-2}. The obtained results provided some useful information on the formation of *D. vinealis* BSCs with the aid of attapulgite-based nanocomposite. In addition, the results would offer a method for the maintenance and management of *D. vinealis* in desertification control.

Keywords: *Didymodon vinealis*, attapulgite, physiological characteristics, nanocomposite

Introduction

Desertification is the most important environmental, economic and social problem all over the world. It makes regional ecological security threaten, economic development and social stability restrict at the national level [1]. In China, desertification is one of a serious environmental problem, which has the development of local economy restrict increasingly [2], the economic losses caused by desertification are estimated to be US $6.8 billion per year [3]. In order to control desertification, the Chinese government has implemented a series of large-scale disaster mitigation programs, such as the Three North Shelter Forest Program and the Combating of Desertification Program [4]. Without effective measures to control desertification, the externalization of degradation costs will continue, and desertification will cause serious sustainability problems for future generations [5]. The purpose of effective desertification control is to improve the well-being of local residents, reverse desertification and promote sustainable development.

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Vegetation restoration is an important measure for the control desertification, and how to select excellent sand-fixing material is the key. Therefore, the selection of pioneer sand-fixing material plays a very significant role during this process.

Under natural conditions, the coverage area of biological soil crusts (BSCs) accounts for about 12% of the earth's surface, which can effectively prevent desertification [6, 7]. BSCs are complex assemblages of minute organisms that are formed primarily by cyano-bacteria, green algae, lichens, and mosses on the surface of soils [8-10]. Many BSCs play an important role in desert vegetation [11]. According to the dominant plants, BSCs are usually divided into different types according to their constituent organisms, such as cyanobacterial crusts, algal crusts, lichen crusts, moss crusts, and a complex mix of these types [12]. *Didymodon vinealis* (*D. vinealis*) is an important moss species in the Tengger Desert of China, they are not only relatively simple in morphology and structure, but also can grow in a dry environment [13], which play an irreplaceable role in the restoration and reconstruction of degraded ecosystems [14].

Although moss crust cultivation is a new field, it has already become a research focus in the past 20 years [15], most research mainly focus on the effects of environmental factors on the development of moss crust [16], few studies have been followed with interest to the effects of polymer substances on moss crusts, which have a significant function in plant growth and formation, promote role water conservation in soil, stop the soil from desertification and keep the ecological environment balance. Herein, attapulgite-based nanocomposites were polymerized, which were used as carriers for *D. vinealis*. Attapulgite had chain layered crystal structure and many active hydroxyl groups, which made them have high absorption and retention capacity of water. Acidified attapulgite was added to different polymers and was grafted with some functional groups, which improved water absorption and water retention [17].

In order to understand the effects of polymer substances on the growth of *D. vinealis* in the Tengger Desert, in this study, *D. vinealis*, polymer and attapulgite combined at a different mass ratio was studied to find the optimal mass ratio, while investigating the physiological characteristics of the moss studied. So, this study aimed to present attapulgite-based nanocomposites used as carriers for *D. vinealis* and to determine whether they could accelerate the formation and the growth of *D. vinealis* on sand surfaces, with the hope of offering a method for the maintenance and management of *D. vinealis* in desertification control and developing an ecological reconstruction technique in arid and semiarid areas.

Material and Methods

Cultivation of *D. vinealis*

D. vinealis were collected from Zhongwei County of the Tengger Desert, China (N37°26'46.9", E104°50'11.2"). The light incubator (HPG-280B, China) for culturing *D. vinealis* was at 120 μE.m^{-2}·s^{-1} for 14 h with light and with dark for 10 h at 30ºC in Hogland medium.

Preparation of Attapulgite-Based Nanocomposite

First, nitrogen was purged with 30 minutes to remove oxygen in the solution, to use 20 ml distilled water dissolved acrylamide (7.1 g) in a 500 ml four neck flask. Attapulgite (0.71 g) was added, which was modified by 4 M H_2SO_4, the mixture was stirred half an hour. Then the initiator, potassium persulfate (0.09 mg), the crosslinker, N'-methylene-bis-acrylamide (0.06 mg) were added. The mixture was stirred constantly, and nitrogen was purged for 3 h all through the polymerization at 50ºC. Then the polymer (8 g) was added 40 mL of 1.5 M NaOH and was heated to 70ºC for 2 h. Attapulgite-based nanocomposite were washed several times and dried at 70ºC for 72 h. All samples were milled.

Attapulgite-Based Nanocomposite Used as Carriers for *D.vinealis*

D. vinealis were dried at 30ºC and water content was 85%. Dried *D. vinealis* and attapulgite-based nanocomposite were mixed with A1, A2, A3 and A4 (A1 (dried *D. vinealis* to attapulgite-based nanocomposite was 1:1, 1:2, 1:3 and 1:4; 2 mg DW·cm^{-2} mixture were sprayed on the sandy surface); A2 (the same as A1, 3 mg DW·cm^{-2} mixture were sprayed on the sandy surface); A3 (the same as A1, 4 mg DW·cm^{-2} mixture were sprayed on the sandy surface); A4 (the same as A1, 5 mg DW·cm^{-2} mixture were sprayed on the sandy surface). Tap water was sprayed three times every day and added Hogland once for seven days. Dried *D. vinealis* and sandy soil were mixed with A5 (dried *D. vinealis* to sandy soil was 1:4, 4 mg DW·cm^{-2} mixture were sprayed on the sandy surface), and all environmental conditions were the same.

Characterization

Surface features of samples were tested by scanning electron microscope (SEM300, China). Soils were taken under *D. vinealis* mixed with attapulgite-based nanocomposite, which was grown for 60 days, A1, A2, A3, A4 and A5 treatments as attapulgite-based nanocomposites used as carriers for *D. vinealis*.

Chlorophyll A (Chla) Measurements

According to Lan [18]. *D. vinealis* were extracted by 95% frozen ethanol after grinding. The absorbance was determined at 649 nm and 665 nm (UV5Nano, Switzerland).

Malondialdehyde (MDA) Measurements

According to Xie [19], fresh samples of *D. vinealis* were homogenized in 10 mL solution which contained 5% trichloroacetic acid and 0.67% thiobarbituric acid. The mixture was activated by heating in hot water for 30 min at 100ºC and then centrifuged for 10 min at 25ºC, then the supernatant was immediately assayed at 450, 532 and 600 nm (UV5Nano, Switzerland).

Soluble Protein Measurements

According to Bradford [20], fresh samples were homogenized in 5 mL PBS (50 mM), centrifuged for 10 min at 25ºC, removed 0.02 mL supernatant and added 3 mL G-250 coomassie brilliant blue in a test tube. It was determined at 595 nm for 20 min (UV5Nano, Switzerland) and bovine serum albumin as a standard.

Soluble Sugar Measurements

According to Li [21], fresh samples of *D. vinealis* were homogenized and added to 10 mL distilled water, after being heated for 30 min boil water bath. 0.5 mL the extract, 1.5 mL distilled water, 0.5 mL anthracene ketone reagent, and 5 mL H_2SO_4 were added in a test tube. Samples was cooled at 25ºC, to determine at 630 nm with a spectrophotometer (UV5Nano, Switzerland).

Statistical Analysis

Data were analysed using a general linear model with the Statistical Package for the Social Science (SPSS) version 19.0 software. Pairwise comparisons were performed using the least significant difference (LSD) procedure at the 0.05 significance level.

Results

Effects of Different Ratios of Mass on Mossbioactivity

D. vinealis grew for 60 days under the conditions of 30ºC and 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ in the illuminated incubator. Table 1 showed that pH, thickness and dry weight of *D. vinealis* had significant differences at different ratios ($p<0.05$). The surface of the soil became gradually rough and there were a lot of different sizes pores, and the dispersion effect were beneficial as the ratio was 1:4 (Fig. 1). As the optimum ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:4, exactly this ratio was used for future experiments.

Table 1. Development characteristics of *D. vinealis* (growth for 60 days).

The amounts sprayed ($mgDWcm^{-2}$)	The mixture ratio	Colour	Shape	pH	Thickness (mm)	Dry weight ($mg \cdot cm^{-2}$)
2	1:1	Grey	Flat	7.790±0.010	3.393±0.015	0.058±0.001
2	1:2	Grey	Flat	7.663±0.0058	3.420±0.010	0.062±0.001
2	1:3	Grey	Rough	7.490±0.010	3.563±0.006	0.068±0.001
2	1:4	Dark	Bit rough	7.640±0.010	3.487±0.006	0.072±0.001
3	1:1	Grey	Flat	7.610±0.010	3.387±0.006	0.064±0.001
3	1:2	Grey	Flat	7.740±0.010	3.430±0.010	0.063±0.001
3	1:3	Black	Rough	7.290±0.010	3.790±0.010	0.079±0.001
3	1:4	Dark	Rough	7.387±0.0058	3.680±0.010	0.075±0.0005
4	1:1	Grey	Flat	7.470±0.010	3.440±0.010	0.072±0.001
4	1:2	Grey	Rough	7.660±0.010	3.470±0.010	0.077±0.001
4	1:3	Dark	Rough	7.287±0.0058	3.640±0.010	0.075±0.001
4	1:4	Black	Bit rough	7.010±0.010	4.003±0.006	0.092±0.001
5	1:1	Grey	Flat	7.487±0.006	3.350±0.010	0.082±0.001
5	1:2	Grey	Flat	7.440±0.010	3.410±0.010	0.076±0.0006
5	1:3	Dark	Rough	7.230±0.010	3.693±0.006	0.081±0.001
5	1:4	Black	Bit rough	7.190±0.010	3.570±0.010	0.079±0.001

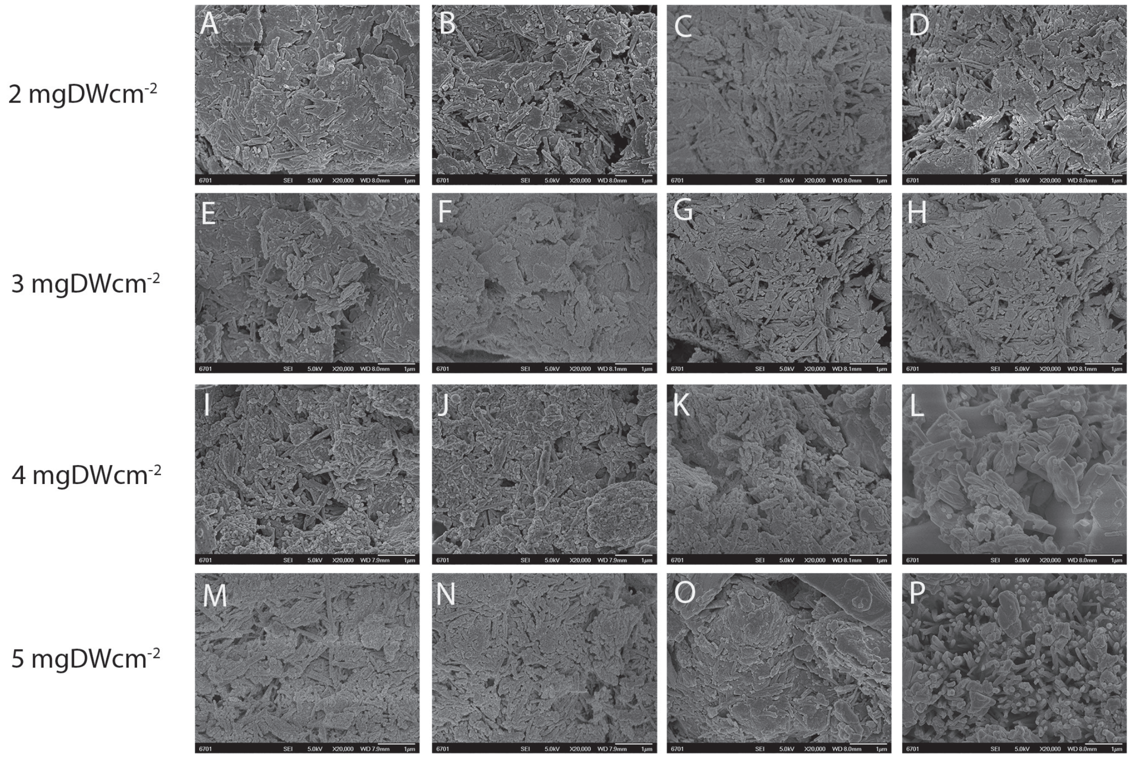

Fig.1. The surface character of the soil at a different mass ratio of *D. vinealis* with the aid of attapulgite-based nanocomposite. (A, B, C and D) the ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:1, 1:2, 1:3 and 1:4; E, F, G and H (the ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:1, 1:2, 1:3 and 1:4); I, J, K and L (the ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:1, 1:2, 1:3 and 1:4); M, N, O and P (the ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:1, 1:2, 1:3 and 1:4).

Effect on Chlorophyll a Content

Under the applied conditions of temperature and light, the sprayed amount significantly affected Chlorophyll a content of *D. vinealis* (p<0.05). As time continued, Chlorophyll a content gradually increased in the four groups. Of the four spraying levels, Chlorophyll a content at 4 mg DW·cm^{-2} was the highest (Fig. 2a). At the 4 mg DW·cm^{-2} spraying level, Chlorophyll a content of *D. vinealis* with the aid of attapulgite-based nanocomposite was higher than that of *D. vinealis* grown the sand (Table 2).

Effect on MDA Content

The amount sprayed significantly affected MDA in *D. vinealis* (p<0.05). As the time increased, MDA gradually increased in the four groups. Of the four spraying levels, MDA at 4 mg DW·cm^{-2} was the lowest (Fig. 2b). At the 4 mg DW·cm^{-2} spraying level,

Table 2. The physiological characteristics of *D. vinealis* with the aid of attapulgite-based nanocomposite.

Days		Chlorophyll a content (μg DWcm^{-2})	MDA content (μmol DWcm^{-2})	Soluble protein content (mg DWcm^{-2})	Soluble sugar content (μg DWcm^{-2})
15	A4	3.987±0.367	0.146±0.032	0.748±0.007	9.342±0.105
	A5	4.000±0.147	0.207±0.013$^{\#}$	0.680±0.006$^{\#}$	6.636±0.015$^{\#}$
30	A4	5.290±0.066	0.235±0.032	0.832±0.008	9.594±0.158
	A5	5.007±0.009$^{\#}$	0.329±0.021$^{\#}$	0.735±0.008$^{\#}$	6.931±0.057$^{\#}$
45	A4	6.733±0.112	0.254±0.032	0.893±0.013	9.779±0.198
	A5	6.186±0.218$^{\#}$	0.366±0.025$^{\#}$	0.780±0.015$^{\#}$	7.498±0.154$^{\#}$
60	A4	7.760±0.676	0.308±0.036	0.947±0.007	10.091±0.169
	A5	8.807±0.079$^{\#}$	0.517±0.025$^{\#}$	0.892±0.005$^{\#}$	8.477±0.307$^{\#}$

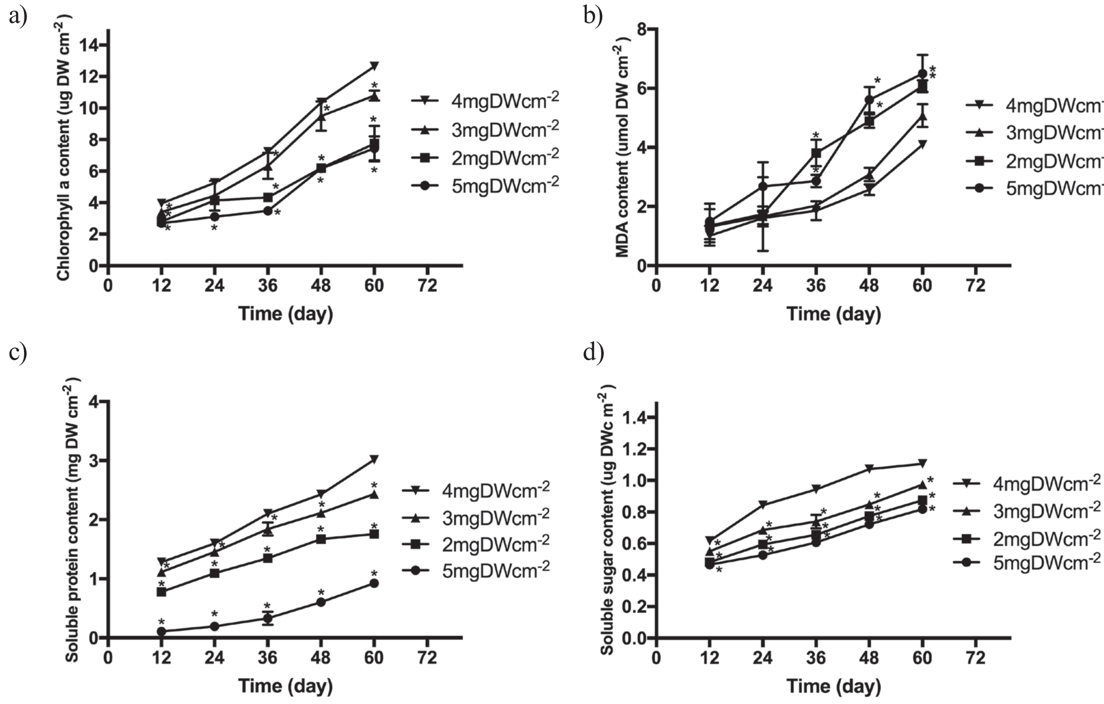

Fig. 2. The physiological characteristics of *D. vinealis* with the aid of attapulgite-based nanocomposite. *compare to 4 mg DW•cm^{-2}, $p<0.05$. a) Changes of chlorophyll a content from *D. vinealis* with the aid of attapulgite-based nanocomposite; b) Changes of MDA content from *D. vinealis* with the aid of attapulgite-based nanocomposite; c) Changes of soluble protein content from *D. vinealis* with the aid of attapulgite-based nanocomposite; d) Changes of soluble sugar content from *D. vinealis* with the aid of attapulgite-based nanocomposite.

MDA of *D. vinealis* with the aid of attapulgite-based nanocomposite was lower than that of *D. vinealis* grown the sand (Table 2).

Effect on Soluble Protein Content

Under used conditions of temperature and light, the amount sprayed significantly affected soluble protein content of *D. vinealis* ($p<0.05$). With increasing time, soluble protein content gradually increased in the four groups. Of the four spraying levels, soluble protein content at 4 mg DW·cm^{-2} was the highest (Fig. 2c). At the 4 mg DW·cm^{-2} spraying level, soluble protein content of *D. vinealis* with the aid of attapulgite-based nanocomposite was higher than that of *D. vinealis* grown the sand (Table 2).

Effect on Soluble Sugar

Under studied conditions of temperature and light, the amount sprayed significantly affected soluble sugar content of *D. vinealis* ($p<0.05$). With increasing time, soluble sugar content gradually increased in the four groups. Of the four spraying levels, soluble sugar content at 4 mg DW·cm^{-2} was the highest (Fig. 2d). At the 4 mg DW·cm^{-2} spraying level, soluble sugar content of *D. vinealis* with the aid of attapulgite-based nanocomposite was higher than that of *D. vinealis* grown the sand (Table 2).

Discussion

Although previous research proves that we can use artificial environments to culture moss crusts successfully, there are still few research that attapulgite-based nanocomposites used as carriers for *D. vinealis*, which is the key step for adequately exercising their many important ecological functions. Therefore, successfully studying and grasping the technology under the laboratory attapulgite-based nanocomposites used as carriers for *D. vinealis* is only one early step.

The BSCs thickness was related to the ecosystem production analogous to biomass and proportion of *D. vinealis* in the BSC complex [22]. Soil pH was usually thought to be an important factor, which affected soil ecosystems [23]. Soil particle size distribution reflected nutritious content of the soil, the soil texture and the structure of the top-layer soil, and so on [24]. In the present study, the top-layer soil characteristics and the biological activities of *D. vinealis* with the aid of attapulgite-based nanocomposite showed that the optimum ratio of dried *D. vinealis* to attapulgite-based nanocomposite was 1:4, it is exactly the one used in the results.

Chlorophyll a is a photosynthetic pigment, which exists in all Eukaryotic photosynthetic organisms, and its ratio is relatively stable, which is used to display photosynthetic biomass [25]. In our study, at 4 mg DW·cm^{-2}, Chlorophyll a content was the highest

(Fig. 2a). The results suggested that *D. vinealis* could grow well as attapulgite-based nanocomposites used for carriers. It was also evident that Chlorophyll a was more abundant, indicating faster formation of *D. vinealis* with the aid of attapulgite-based nanocomposite on sand surfaces indoors.

MDA is a product of lipid peroxidation, and it is used as a mark of oxidative damage in the cell membrane, which made metabolic function disrupted and cell integrity lost. Therefore, lipid peroxidation has been related to the damage caused by various environmental factors [26]. In our study, at 4 mg DW·cm^{-2}, MDA content was the lowest (Fig. 2b). The results showed that *D. vinealis* with the aid of attapulgite-based nanocomposite had little lipid peroxidation and oxidative damage. It is also suggested that *D. vinealis* could protect themselves by inhibiting lipid peroxidation when *D. vinealis* formed and grew as attapulgite-based nanocomposites used for carriers.

Substances responsible for osmotic adjustments in plants, including free proline, soluble sugars and soluble protein, are often used as indicators for stress tolerance [27, 28]. The accumulation of osmoregulatory substances within plant cells is the key process in improving their osmoregulatory ability [29]. Soluble protein and sugar are the main components of osmoregulation materials.

Soluble protein content, which is an important regulatory factor for plant, reflects plant metabolism to a certain extent. The higher the content, the higher the level of plant metabolism [30]. In our study, at 4 mg DW·cm^{-2}, soluble protein content was the highest (Fig. 2c). The results implied that *D. vinealis* with the aid of attapulgite-based nanocomposite had high osmotic adjustment ability, especially when water was limited in the later stage of growth and could maintain water absorption [31].

When plants were suffered from stress, soluble sugar could regulate cellular osmosis, detoxicate reactive oxygen species, protect membrane integrity and stabilize proteins or enzymes to protect them [32]. Therefore, the response of plant to environmental stress can be reflected by the change of soluble sugar. In our study, at 4 mg DW·cm^{-2}, soluble sugar content was the highest (Fig. 2d). The results showed that *D. vinealis* with the aid of attapulgite-based nanocomposite accumulated more soluble sugar. The results suggested that osmotic potential of *D. vinealis* with the aid of attapulgite-based nanocomposite increased with the accumulation of soluble sugar, which decreased osmotic pressure, enhanced water-holding capacity of cell and maintained water needed for normal growth of *D. vinealis*.

In this study, we built up a special-structured nanocomposite. Attapulgite-based nanocomposites were polymerized and used as carriers for *D. vinealis*, which could offer a proper microenvironment in the soil. Attapulgite-based nanocomposites had abundant hydrophilic groups that could effectively increase the absorption and retention ability of the soil and was favorable for the formation of *D. vinealis*.

Conclusions

By investigating the growth status and some physiological characteristics of *D. vinealis* sporogons mixed at different ratios with inoculum of attapulgite-based nanocomposites used as their carriers, it was found that the optimal ratio of dried biomass of *D. vinealis* to the nanocomposite should be 1:4, and the optimal sputtering amount should be 4 mg DW·cm^{-2}. The attapulgite-based nanocomposites, which contained a lot of –COOH and –OH group, could effectively increase the soil retention capacity and promote the crust formation with *D. vinealis*. Therefore, the results obtained can provide a theoretical basis for the development of a new sand binding technology for environmental sustainability. In conclusion, the present study succeed in developing a soil crust formation using in desert conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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