Biocrusts mediate the plant community composition of dryland restoration ecosystems

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HIGHLIGHTS
• Biocrusts may be a key mediator of species performance in dryland plant communities.
• Biocrusts had significantly different effects on the germination and establishment of three plant species, respectively.
• The currently unsuitable plant species are sieved out and filtered by biocrusts.
• The species that pass the filter effect of biocrusts can coexist well with other species in the community.

ABSTRACT
Degradation and expansion are current threats associated with drylands. During natural or artificial restoration, dryland ecosystems tend to contain a unique community, namely, biocrusts. Studies have shown that biocrusts serve multiple functions and have the potential to accelerate or inhibit degradation. However, the mechanisms by which biocrusts mediate dryland plant community structure and composition remain largely unknown. In this study, we performed a series of pot experiments under greenhouse conditions at three disturbance levels (strong, moderate and no disturbance) and at three rainfall gradients (−50 %, normal, +50 %) for different biocrust types. Our objective was to examine how biocrusts influence the introduction and establishment of different functional forms of plant species during the different succession stages of dryland recovery. Our results showed that biocrusts have significantly different effects on the seed germination and survival of the tested plant species. Although strong disturbances to the biocrusts and increasing rainfall alleviated the inhibitory effects of biocrusts on the perennial herb (S. glareosa) and subshrub (C. latens) species, these factors could only promote the temporary survival of these two plants in the revegetated area. These results indicate that biocrusts in revegetated systems play important sieving functions during plant species establishment. Additionally, the survival of S. viridis, a plant species with small seeds adapted to the current habitat, was promoted in the community, but two plant species that are currently unsuitable, S. glareosa and C. latens, were filtered out by the biocrusts. Our conclusions suggest that biocrusts play an ecological filtering role in plant species introduction and establishment in revegetated ecosystems and demonstrate the scientific significance of biocrusts in maintaining the health and stability of dryland ecosystems.
1. Introduction

Drylands, which include hyperarid, arid, semiarid, and dry subhumid ecosystems, are large and important regions of the Earth (Borrelli et al., 2017; Yirdaw et al., 2017; Phillips et al., 2022). Multiple lines of evidence indicate that drylands face enormous risks for ecosystem degradation and that they are expanding, which can have unpredictable and negative impacts on global ecological systems and on the people who depend on them (Global Land Project, 2005; Phillips et al., 2022). To prevent land degradation, it is important to understand the ability of fundamental dryland organisms to resist change during disturbances (resistance) and to recover following disturbances (resilience) (Pimm, 1984; Tilman et al., 1994). Therefore, the restoration and reconstruction of degraded ecosystems has become one of the hotspots for current ecological research, and the reconstruction of biological communities is essential to restoring degraded arid ecosystems (Jones et al., 2016). Natural or artificial restoration of these ecological systems tends to produce a unique biological community, namely, biological soil crusts (biocrusts). Although often overlooked, biocrusts can be found on every continent and have the potential to accelerate or inhibit land degradation, eventually increasing or reducing the rates of desertification (Lan et al., 2015; Li et al., 2020; Wang et al., 2020; Rossi et al., 2022).

Biocrusts aggregate and occupy the soil surface, and they consist mainly of nonvascular primary producers, such as cyanobacteria, algae, mosses and lichens, in various combinations (Belnap et al., 2003a; Bu et al., 2015; Bowker et al., 2022). Biocrusts occur in the bare spaces between vegetation in drylands, and they coexist with patchy mosaic forms of bare soil and vegetation, creating habitat and soil resource heterogeneity through physical and chemical changes to the soil environment (Bowker et al., 2008; Concostrina-Zubiri et al., 2013; Belnap et al., 2016; Chen et al., 2020). Research suggests that biocrusts play a key role in the nutrient cycling of drylands; they fix approximately 15% of global terrestrial carbon (C) and fix 40% to 85% of global terrestrial nitrogen (N) (Elbert et al., 2012; Rodriguez-Caballerio et al., 2018; Su et al., 2021). Similarly, biocrusts enhance soil fertility by increasing the availability of C, N and other mineral nutrients, further altering the soil microclimate by altering soil hydrology and surface temperature (Eldridge et al., 2000; Li et al., 2012; Zhao et al., 2016; Tucker et al., 2017; Li et al., 2018; Xiao et al., 2019). Given the wide range of soil modifications they carry out, biocrusts can strongly affect the introduction and success of the plant species with which they coexist (Li et al., 2010; Zhang and Belnap, 2015; Song et al., 2017).

Substantial evidence suggests that biocrusts may be key mediators of species performance in dryland plant communities. For example, biocrusts can enhance the performance of native plant species while inhibiting the performance of nonnative species. This observation has promoted great interest in the potential of biocrusts to facilitate biological resistance in plant communities (Hernandez and Sandquist, 2011; Briggs and Morgan, 2011; Havrilla and Barger, 2018; Song et al., 2020). The composition of the biocrust community may also determine its impact on plant species because biocrust composition largely determines the contribution of the biocrusts to soil hydrology and to carbon and nitrogen cycling (Bowker et al., 2014; Barger et al., 2016). According to community theory, with changes in resource constraints, biotic interactions can have different effects on species performance and trait organizations along environmental gradients (Cornwell and Ackerly, 2009; Maestre et al., 2010; He et al., 2013). Therefore, the importance of niche-based processes increases with increasing abiotic stress (Bruno et al., 2003; Liancourt et al., 2005; Gross et al., 2010). Thus, we suggest that the magnitude and trends in plant responses to biological crusts can be mediated by biogenic factors and by disturbances.

Theoretically, dispersal limitations and barriers caused by abiotic factors are of primary importance in predicting species distribution and abundance patterns in plant communities (Cornwell and Ackerly, 2009; Kraft et al., 2015). However, understanding how local biotic interactions restrict or improve species performance remains a challenge for ecologists. Species abundance, their distribution in a community and the range of species traits are key attributes of an ecosystem, which can be determined by positive or negative species interactions in the community (Michalet et al., 2006; Boulangeat et al., 2012; Wisz et al., 2013). Therefore, biotic interactions are increasingly being incorporated into community theory and into predictions of how communities respond to global change (Bruno et al., 2003; Brooker et al., 2008; McCluney et al., 2012; HillerisLambers et al., 2012; He et al., 2013; Staniczenko et al., 2017). However, most of the advances in the study of biotic interactions in plant community ecology have come from the study of vascular plants, and ecologists have tended to ignore the importance of soil biotic communities in determining plant species performance; however, evidence indicates that plant community structure and productivity are strongly influenced by biocrusts (Tilman, 2004; Bowker et al., 2018; Raggio et al., 2018). Therefore, in this study, our objective was to examine how biocrusts influence the introduction and establishment of different functional forms of plant species during the different succession stages of dryland recovery. We hypothesized that biocrusts have a biological sieving effect, allowing some plants to enter and become established and others to be filtered out from the current processes of dryland restoration. We believe it is critical and timely to examine the relationship between biocrusts and plant species to better understand how dryland vegetation communities recover and to clarify the scientific significance of biocrusts in maintaining the health and stability of revegetated dryland ecosystems.

2. Materials and methods

2.1. Study site

Our study site is located on the southeast edge of the fourth largest desert, the Tengger Desert, in China. In the early nineteenth century, large areas of lush desert grasslands were present at the southern edge of the Tengger Desert. However, due to overgrazing over the past 200 years, the dominance of shrub species in the community composition has increased dramatically, converting the primary vegetation type from herbs to shrub-dominated species, and some regions have even become desert dunes (Li et al., 2004; Shapotou Desert Research Experiment Station, 1991; Qiu et al., 2000). Fortunately, recognizing the importance of ecological restoration, the Chinese government has carried out a series of ecological restoration projects, including the Baotou-Lanzhou Railway vegetation protection system. This is one of the earliest and most successful cases of vegetation restoration, and it served as a historical precedent for desertification control and vegetation restoration without irrigation (Li et al., 2014). Over the past 60 years, the vegetation protection system has ensured the smooth operation of the Baotou-Lanzhou railway, and the stability of the ecosystem has also greatly improved. In particular, the biocrusts restored naturally, and their coverage exceeded the natural vegetation areas and even exceeded 90% in some areas (hilly lowlands) (Li et al., 2012).

The vegetation protection system was established in 1956, 1973 and 1991, forming three natural succession stages of biocrusts. Therefore, this study area can be used as an ideal place to study the effect of biocrusts on artificial vegetation succession on degraded lands. The current plant community is dominated by Setaria viridis, Eragrostis poaeoides, Artemisia capillaris, Caragana korshinskii, Atriplex korshinskii and Bassia dasyphylla (Li et al., 2010; Song et al., 2017). Among these plants, the shrub species were artificially planted for sand fixation, and the herbaceous species occurred naturally.

2.2. Plant species selection and seed collection

We selected three typical plant species, Setaria viridis (L.) Beauv., Stipa glareosa P. Smirn., and Ceratoides latens (J. F. Gmel.) Reveal et Holmgren, to test the influence of biocrusts in an artificially vegetated system on the establishment of plants from the adjacent desert steppe ecosystem. The three plants belong to different functional types. Among them, S. viridis is an annual herb that established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been established early, while the perennial herb S. glareosa is a relatively recent arrival, and the subshrub C. latens has never been
found in the revegetated region (Li et al., 2014). The seeds of *S. viridis*, *S. glareosa* and *C. latens* were collected from August to September, May to June and October to November in 2014, respectively. They were collected in the revegetated area (*S. viridis*) and in the Hongwei desert-steppe area (*S. glareosa* and *C. latens*) on the southeastern edge of the Tengger Desert (Fig. S2). The collected plant seeds were dried and stored in seed bags for future use.

2.3. Sample collection

In April 2015, cyanobacterial crust, lichen crust and moss crust were collected from the 1991, 1973 and 1956 revegetation areas in Shapotou, the southeastern edge of the Tengger Desert (Table 1). In this study, a specific type of crust was defined by the dominant species with >70% coverage. For example, when the coverage of cyanobacteria in a biocrust was >70%, the biocrust was called a cyanobacterial crust.

Prior to sampling, the soil was slightly wetted with water to ensure that the biocrusts were intact and not easily broken during collection. Then, we used a cylindrical PVC sampler with a diameter of 20 cm and a depth of 20 cm to collect the biocrust samples. We collected 135 samples of cyanobacterial crusts, lichen crusts and moss crusts. Since the soils in the areas that were not artificially restored were sandy soils, we collected 45 sand samples using the same method as the control. Therefore, a total of 450 samples were collected. Near each biocrust sampling point, a 5-cm soil surface layer sample without biocrust was collected for soil nutrient analysis (soil organic matter content, total nitrogen and total phosphorus content, as well as available nitrogen and phosphorus content, \( n = 5 \), in April 2015). At the same time, a Vernier caliper was used to measure the thickness of the biocrusts (Table 1).

Then, each biocrust sample was placed into a pot with a diameter of 20 cm and a height of 20 cm and was cultured in an automated greenhouse. The light transmittance was 89%, the indoor temperature was controlled at 25–33 °C, and the relative humidity was in the range of 40% ~ 60%. Before the experiment, distilled water was sprayed into the pots every day to keep the biocrust surface moist and to promote seed germination from the soil seed bank. Seed germination was observed regularly until no new seedlings appeared for 6 weeks. The germinated seedlings were removed to eliminate disturbances from the soil seed bank to the experiment.

2.4. Experimental design

Before the greenhouse experiment was conducted, the seeds of each plant species were placed in separate petri dishes with moist filter paper, each containing 100 seeds, to test the germinability of the plant seeds. The final germination rates of seeds of *S. Setaria, S. glareosa* and *C. latens* on the petri dishes were 86% ± 4.2, 83% ± 3.6 and 79% ± 3.3, respectively.

To simulate animal burrowing, human trampling and other disturbances, 270 samples were mechanically destroyed. A spade was used to destroy the biocrusts so that half of the biocrust layer was broken or removed (MD: moderate disturbance), and the biocrust layer of some samples was removed completely to simulate mechanical damage or engineering construction disturbance (SD: strong disturbance). The remaining 135 intact biocrust samples were treated as those experiencing no disturbance (ND) (Fig. S4).

A factorial experiment was designed. Three types of biocrusts were selected for different succession stages. The three types of biocrusts were treated with the three disturbance levels (ND, MD and SD) and with three rainfall gradients (−50%, normal, +50%), resulting in 135 pots for each biocrust type. The control group contained sand without biocrusts, and each treatment had 5 replicates; thus, a total of 450 pots were established. In each pot, 100 seeds of *C. latens, S. glareosa* and *S. viridis* were sown. To simulate naturally seed fall onto the biocrusts, no external force was applied to bring seeds into contact with the soil beneath the biocrusts.

According to the interannual variation in multiyear rainfall in the Shapotou region, each rainfall is about 6 mm (180 mm/30 times) and is considered normal rainfall (Li et al., 2012). Therefore, a 50% increase and 50% decrease in rainfall were 3 mm and 9 mm, respectively. Totally, three rainfall gradients for -50%, normal and +50% were designed to study the effects of biocrusts on the tested plants during dry and wet years (Fig. S5) (Li et al., 2012; Song et al., 2017). Correspondingly, 200 mL, 400 mL or 600 mL distilled water (equivalent to 3 mm, 6 mm or 9 mm rainfall per time) was sprayed onto the pots for each rainfall gradient every 10 days during the experimental period, representing a 50% rainfall reduction (RR) and normal rainfall (NR) and a 50% rainfall increase (IR).

2.5. Soil nutrient content

Soil organic content (SOC) was determined by the dichromate oxidation method; total nitrogen (TN) was determined by the micro Kjeldahl method; total phosphorus (TP) was determined by the alkali diffusion method; available nitrogen (AN) was determined by the alkali diffusion method; available phosphorus (AP) was digested with NaHCO3 and determined by the Mo-sb colorimetry method (Bao, 2005).

2.6. Seed germination monitoring

The number of germinating seeds was observed and recorded every day starting at the beginning of the experiment. The emergence of a radicle marked seed germination. If no new seedlings emerged for 8 weeks, germination was no longer recorded. To prevent intraspecific competition in the limited pot space from interfering with the experimental results, we limited the number of the seedlings in each pot to a maximum of five, and any additional seedlings that emerged (more than five plants) were removed. Any new seedlings that germinated later in the experiment were removed immediately to prevent them from affecting the monitoring of seedling survival and growth.

In this experiment, we assumed that new seedlings could survive at a plant density of five plants per pot and monitored the mortality from the first seedling death until the end of the experiment. If none of the five seedlings died, this indicated that the mortality of the seedlings was zero; if any of the five plant seedlings died, the new seedlings remained, and the remaining number was equivalent to the number of dead seedlings. The mortality was calculated as the number of dead plants divided by the total number of germinated seedlings; the mortality of the treatments that had less than five plants was the number of dead plants divided by the number of germinated seedlings.

2.7. Data analysis

The differences in soil physical properties (including biocrust thickness) and nutrient contents (SOC, TN, TP, AN and AP) among the different types of biocrusts were tested by one-way ANOVA. The least significant difference (LSD) method was used for pairwise comparison. The test level was

<table>
<thead>
<tr>
<th>Recovery year</th>
<th>Biocrust type</th>
<th>Thickness cm</th>
<th>Species composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Cyanobacterial crusts</td>
<td>0.22 ± 0.07a</td>
<td><em>Microcolea vaginatus</em>, <em>Hantzschia amphioxys</em>, <em>Phormidiaceae</em>, <em>Chlorella vulgaris</em>, <em>Division chlorophyta</em>, etc.</td>
</tr>
<tr>
<td>1973</td>
<td>Lichen crusts</td>
<td>0.46 ± 0.22b</td>
<td><em>Collema tenax</em>, <em>Lecidea decipiens</em>, <em>Xanthoparmelia desertorum</em>, <em>Diploschistes muscorum</em>, etc.</td>
</tr>
<tr>
<td>1956</td>
<td>Moss crust</td>
<td>1.04 ± 0.10c</td>
<td><em>Bryum argenteum</em>, <em>Barbula dirichoides</em>, <em>Didymodon nigrescens</em>, <em>Tortula bidentata</em>, <em>Barbula constricta</em>, etc.</td>
</tr>
</tbody>
</table>

Note: Different lowercase letters show significant differences at the level of 0.05, \( n = 5 \).
set to 0.05 ($P$ value). Independent sample t-tests were used to test the significant differences between the germination rates of three plants on the biocrusts and on the petri dishes.

The variation in seedling mortality among the different treatments was tested by one-way ANOVA. The germination rate data were collected using a repeated measures design, and a suitable mixed linear model was used for analysis (Littell et al., 1998), in which biocrusts, disturbance and rainfall were fixed factors. Tukey's test was used to test the difference between the treatments. The different species were analyzed by a mixed linear model. Before these analyses, the data were tested to determine if they conformed to the normality and variance homogeneity of the test hypothesis, and then a sine or logarithmic transformation was performed if necessary.

The interactive effects of biocrusts, disturbance and rainfall on the germination rate and mortality were tested by generalized linear models (GLMs) with logit connection functions and binomial error distributions (Venables and Ripley, 2013). We used the quasi likelihood method to overcome the uncertainty caused by the excessive discretization of data (Guisan et al., 2002). All statistical analyses were performed with SPSS 20 software (IBM SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Effects of biocrusts on plant seed germination and survival

Compared with the germination rate on petri dishes, the germination rates of the three species on all biocrusts were significantly lower ($P < 0.001$). The seed germination rate of $S. viridis$ was significantly different among the three biocrust types. The germination rate on lichen was the highest, followed by that on cyanobacteria, while the germination rate of $S. viridis$ seeds on moss was lower than that of the control group (Fig. 1a). The germination rates of $S. glareosa$ and $C. latens$ on the three types of biocrusts did not reach 15% and were significantly lower than those of the control group (Fig. 1b-c, $P < 0.05$). The germination rates of $S. glareosa$ seeds on lichen and cyanobacteria were higher than that on moss ($P < 0.001$). There was no significant difference in the $C. latens$ seed germination rate among the three types of biocrusts ($P < 0.05$).

The mortality of $S. viridis$ seedlings on the three types of biocrusts was significantly lower than that of the control group ($P < 0.001$). Fig. 1e shows that there was no death of $S. glareosa$ seedlings on moss, and there was no significant difference in the mortality rates between cyanobacteria and lichen. However, the mortality rates of these two biocrusts were higher than those of the control group ($P < 0.05$). The mortality of $C. latens$ on the three types of biocrusts from high to low was in the order cyanobacteria $>$ lichen $>$ moss, the difference between the three reached a significant level ($P < 0.05$), and the mortality on lichen and moss was lower than that of the control group.

3.2. Effects of disturbance on germination and survival

The germination rate of $S. viridis$ seeds on MD was significantly higher than those on ND, SD and the control ($P < 0.001$, Fig. 2a-c). The highest germination rate (17%) of $S. glareosa$ was found on the cyanobacteria (Fig. 2d). The germination rates on the MD and ND crusts were lower than those on the SD and control crusts (Fig. 2d-f, $P < 0.001$). $C. latens$ seeds were the first to germinate on the three SD biocrusts (Fig. 2g-i). The results showed that the germination rate under the SD treatment was significantly higher than that under MD ($P < 0.001$). However, the germination rate of $C. latens$ was very low on the ND treatment, which was significantly different from the other treatments ($P < 0.001$).

Fig. 3a-c shows that the mortality of $S. viridis$ seedlings on the MD cyanobacterial crust was significantly lower than that on the ND cyanobacterial crust ($P < 0.001$), and the mortality on SD cyanobacterial crust was significantly higher than that on ND cyanobacterial crust ($P < 0.001$). The order of mortality on cyanobacteria was SD $>$ ND $>$ MD. In particular, $S. viridis$ seedlings had mortality as low as 0% on MD moss crust. In contrast with that $S. viridis$ seedlings, the mortality rates for $S. glareosa$ on the ND and SD cyanobacterial crusts were not significantly different ($P < 0.05$), but they were significantly higher than those on MD cyanobacterial crust and the control group (Fig. 3a, $P < 0.05$). The mortality rate of $C. latens$ on ND cyanobacteria was as high as 100%, which was higher.

![Fig. 1. Cumulative germination and mortality rates of $S. viridis$ (a, d), $S. glareosa$ (b, e), and $C. latens$ (c, f) on different types of biocrusts. Error bar indicate standard error.](image-url)
than that of other treatments \((F_{i g. ~3g, ~P < 0.001})\). The mortality rate of \(C. \text{latens}\) on the SD cyanobacterial crust was the lowest (18 %), which was significantly lower than that on the MD cyanobacterial crust, and the mortality rates on the SD and MD cyanobacterial crusts were significantly lower than that of the control group \((P < 0.05)\). The mortality of \(C. \text{latens}\) on the ND lichen crusts was lower than that on the MD lichen crusts \((P < 0.05)\). The mortality of \(C. \text{latens}\) seedlings on moss crusts was lower than that of the control group \((P < 0.05)\), and the \(C. \text{latens}\) seedling mortality from high to low was ND > MD > SD. As shown in Fig. 3i, its mortality was lower than that on the ND moss crust and higher than that on the SD moss crust \((P < 0.05)\).

### 3.3 Effects of rainfall on germination and survival

In cyanobacterial and lichen crusts (Fig. 4a-b), the germination rate of \(S. \text{viridis}\) seeds under IR was significantly higher than those under NR and RR \((P < 0.001)\), and the germination rate in NR was also significantly higher than that in the control group \((P < 0.05)\). When the rainfall was reduced by 50 %, the germination rate of \(S. \text{viridis}\) decreased significantly. In contrast to cyanobacteria and lichens, the germination rate of \(S. \text{viridis}\) seeds on moss crusts was not significantly different from that of the control group in the IR treatment \((P < 0.05)\) but was higher than that under NR and RR.

Generally, the germination rate of \(S. \text{glareosa}\) seeds on the three biocrusts was significantly lower than that of the control group \((P < 0.001)\), even though it increased with the 50 % rainfall (Fig. 4d-f). Compared with NR, IR did not increase the germination rate of \(S. \text{glareosa}\) seeds on cyanobacterial crusts \((P < 0.05)\), but RR significantly reduced the germination rate \((P < 0.05)\). On lichen crusts, the seed germination rate of \(S. \text{glareosa}\) increased in the order RR < NR < IR (Fig. 4e). On moss crusts, the seed germination rate of \(S. \text{glareosa}\) was not significantly different \((P > 0.05)\) under IR and RR, but both were higher than that under NR (Fig. 4f, \(P < 0.05)\).

Similar to the trends in \(S. \text{glareosa}\), the seed germination rate of \(C. \text{latens}\) on the three biocrust types was significantly lower than that of the control group (Fig. 4g-i, \(P < 0.001)\). For cyanobacterial crusts, the \(C. \text{latens}\) seeds presented a germination rate from high to low in the order IR > RR > NR, and the germination rate under IR was significantly higher than those under NR and RR \((P < 0.05)\), but there was no significant difference between NR and RR \((P > 0.05)\). The germination rates of \(C. \text{latens}\) on lichen and cyanobacterial crusts were very low under NR and RR. On moss crusts, \(C. \text{latens}\) did not germinate under any of the rainfall treatments (Fig. 4i).

For the cyanobacterial crusts, there was a significant difference in \(S. \text{viridis}\) seedling mortality among the three rainfall treatments \((P < 0.05)\). The lowest mortality rates were 1.9 % in the IR group and 100 % in the RR group (Fig. 5a). Similar to the trends on the cyanobacterial crusts, the mortality of \(S. \text{viridis}\) on RR lichen crusts also reached 100 %, but its mortality rates under IR and NR were 0.59 % and 2.78 %, respectively (Fig. 5b). There was a significant difference in the mortality of \(S. \text{viridis}\) under the three rainfall conditions \((P < 0.001)\). For moss crusts, the mortality of \(S. \text{viridis}\) seedlings was only 22.5 %, which was significantly lower than that of the control group, even in the RR treatment, and there was no mortality of \(S. \text{viridis}\) seedlings under the IR treatment (Fig. 5c).

The mortality of \(S. \text{glareosa}\) on the three biocrusts was 100 % under RR (Fig. 5d-f). On the lichen crusts, \(S. \text{glareosa}\) mortality was similar to that on the cyanobacteria crusts (Fig. 5d-e). For moss crusts, however, the mortality of \(S. \text{glareosa}\) was 0 % under IR and NR and 100 % under RR. The difference between the three treatments was very significant \((P < 0.001)\). Compared with \(S. \text{viridis}\), \(S. \text{glareosa}\) seedlings died at a later timepoint under all rainfall treatments.

In general, the mortality of \(C. \text{latens}\) seedlings was higher than that of \(S. \text{viridis}\) and \(S. \text{glareosa}\) seedlings. For cyanobacterial crusts, the mortality
of *C. latens* reached 100 % under NR and RR. However, the mortality under IR was 61.5 %, which was not significantly different from that of the control group (**Fig. 5g**, *P* > 0.05). The mortality of *C. latens* on lichen crusts was slightly lower than that on cyanobacterial crusts (**Fig. 5h**). There was no significant difference between the mortality of *C. latens* under the IR and NR treatments (*P* > 0.05), but both were significantly lower than that of the control group (*P* < 0.05). It should be noted that the seedling mortality of *C. latens* on moss was 0 % under IR and RR, while mortality under NR (33.3 %) was significantly lower than that of the control group (**Fig. 5i**, 65.1 %, *P* < 0.05).

### 3.4. Interactions among the effects of biocrusts, disturbance and rainfall

In this study, GLMs were used to analyze the germination rate of the three test plants. The results in **Table 2** show that the likelihood ratio was significantly <0.001, which means that compared with a model that does not contain any independent variables, the current model could explain the seed germination rate and mortality data of *S. viridis*, *S. glareosa* and *C. latens* more rigorously.

**Table 3** shows that, compared with disturbance and rainfall, the effect of biocrusts on *S. viridis* seed germination was the least significant, but it was also statistically significant (*P* < 0.05). The interactions among biocrusts × disturbance, biocrusts × rainfall and between disturbance × rainfall also had significant effects on the seed germination of *S. viridis* (*P* < 0.001). However, the three-dimensional interaction effect of biocrusts × disturbance × rainfall was not significant (*P* > 0.05). For *S. glareosa*, other than the two-dimensional interactions of biocrust × disturbance and the three-dimensional interactions of biocrust × disturbance × rainfall, the main effects and two-dimensional interactions had significant effects on seed germination (*P* < 0.05). The two-dimensional interactions of biocrust × rainfall (*P* > 0.05) and three-dimensional interactions of biocrust × disturbance × rainfall (*P* > 0.05) had no significant effects on the seed germination of *C. latens*, while the other main effects and two-dimensional interactions had significant effects on the seed germination of *C. latens* (*P* < 0.05). Table 3 shows that the main effects, two-dimensional effects and three-dimensional effects on the seedling mortality of *S. viridis* reached a significant level (*P* < 0.05), except for the interaction of biocrusts × rainfall. For *S. glareosa*, only biocrust × disturbance had no significant effects on seed germination, and the other main effects and two-dimensional interactions had significant effects on the seed germination of *S. glareosa* (*P* < 0.05). It should be noted that the rainfall gradient was the only variable that had no significant effect on the mortality of *C. latens* (*P* > 0.05).

### 4. Discussion

#### 4.1. Biocrust succession stages affect seed germination and survival

The results showed that the effects of different succession stages of biocrusts on the seed germination of the three plants were consistent: cyanobacteria, lichen and moss crusts inhibited the seed germination of *S. viridis*, *S. glareosa* and *C. latens* more rigorously. Even on rough lichen crusts, low-lying microhabitats suitable for seed germination are difficult to find; as a result, seeds remain on the surface of biocrusts and are unable to absorb sufficient amounts of water for germination (Zhang and Belnap,
2015; Havrilla and Barger, 2018). However, the seed size of *S. glareosa* is smaller than that of *C. latens*, so *S. glareosa* seeds may penetrate cracks in the biocrusts or find sheltered locations on the rough lichen. The seeds of *S. viridis* are relatively small and a small portion of them can enter the low-lying microenvironments on the surfaces of rough lichen crusts (Fig. S3), which can increase the contact area between seeds and the soil for absorbing water and promote germination. Most of the *S. viridis* seeds remain in an environment where they can receive light, and intact biocrusts can also inhibit the germination of seeds (Hernandez and Sandquist, 2011; Song et al., 2017).

The mortality of *S. glareosa* on cyanobacterial and lichen crusts was significantly higher than that of the control group. This is not consistent with previous observations that biocrusts promote plant survival and growth (Su et al., 2009). This may be because the seeds of *S. glareosa* are large and are completely exposed to the air after germination, and its young roots have difficulty penetrating the biocrust layer and entering the soil, resulting in water deficiency and death. Serpe et al. (2008) studied the colonization of two herbaceous plants on lichen (*Diploclothes muscorum*), and their results are consistent with that of this study. They postulated that even if seeds germinate on biocrusts, the success rate of their colonization can be ignored because young roots have difficulty penetrating biocrusts, resulting in necrosis of the root tips. Although the mortality rates of *C. latens* on lichen and moss crusts were lower than that of the control group, the standard error of the mortality histogram (Fig. 1F) showed that the data for *C. latens* on the two biocrust types were more discrete, while the mortality on the cyanobacterial crusts had no standard error. This is because the germination rate of *C. latens* on intact crusts is very low. To a certain extent, the germination and survival of *C. latens* in a certain pot could be considered a small probability event.

In summary, the effect of biocrusts on the mortality of the three tested plants was species-specific (Zhang and Belnap, 2015; Havrilla et al., 2019). For *S. viridis*, the existence of biocrusts significantly reduced seedling mortality. However, some fraction of the *S. glareosa* seedlings were able to survive on the cyanobacterial and lichen crusts. Moss crusts represent the highest stage of succession in the revegetated areas, and they have strong inhibitory effects on the germination of *C. latens* seeds. Although the mortality of *C. latens* seedlings gradually decreased with succession, in view of its extremely low germination rate on intact biocrusts, we concluded that *C. latens* is not suitable for establishment in a revegetated area during the present stage of succession.

4.2. Disturbance and rainfall promote seed germination and survival

Intact biocrusts can prevent seeds from entering the soil and keep the seeds exposed at the soil surface; as a result, these seeds are unable to maintain a moist environment, and this delays the germination of the plant seeds (Deines et al., 2007). Therefore, the germination rate of seeds on intact crusts was significantly lower than that on disturbed crusts. The results showed that seed germination was significantly affected by the disturbance of biocrusts. For example, the germination rate of *S. viridis* seeds on MD biocrusts was the highest, while *S. glareosa* and *C. latens* seeds had the highest germination rates on the SD biocrusts (Fig. 2). Based on previous research, these differences may be due to the different seed sizes of the three plants. *S. viridis* seeds are smaller (Fig. S2), and they can enter the soil below disturbed biocrusts, which increases the contact area between the seeds and the soil and further promotes the germination of the seeds (Zhang and Belnap, 2015; Song et al., 2017). However, the seeds of *S. glareosa* and *C. latens* are larger, particularly *C. latens* seeds, which have
dense hairs. Even on the MD biocrusts, only a few C. latens seeds entered the soil and germinated normally. Only the SD biocrusts were able to provide a consistently large soil contact area for C. latens seeds. A large number of studies have shown that disturbance can improve seed germination on biocrusts (Belnap et al., 2003b; Li et al., 2010; Hernandez and Sandquist, 2011). Briggs and Morgan (2011) found that the germination rate of large subshrub seeds on disturbed biocrusts was significantly higher than that on intact crusts. This finding is consistent with the results of this study.

In this study, both MD and SD biocrusts significantly reduced S. viridis mortality compared with the control. This is because even if the biocrusts are disturbed or removed, the structured soil (instead of sand) below the biocrusts (especially moss crusts) can reduce rainfall infiltration and increase the moisture content of the surface soil relative to that of the control group (Hernandez and Sandquist, 2011; Havrilla and Barger, 2018). However, the seeds of S. glareosa are larger, and even on MD and SD biocrusts, they cannot be buried in the soil. Even if some seeds succeed in germinating by utilizing with limited water available on the surface of biocrusts, the young roots are exposed to air and die from water deficiency.

The seeds of subshrub C. latens are larger and were expected to have the same mortality rates as those of S. glareosa, but the results of the study showed that their mortality rates on MD and SD biocrusts was significantly

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Source of difference</th>
<th>Germination Type III Wald chi square</th>
<th>DF</th>
<th>Sig.</th>
<th>Mortality Type III Wald chi square</th>
<th>DF</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. viridis</td>
<td>(intercept)</td>
<td>2129.284 1 0.000 131.004 1 0.000</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Biocrusts</td>
<td>25.553 2 0.042 3.400 2 0.182</td>
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<td></td>
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<tr>
<td></td>
<td>Disturbance</td>
<td>916.264 2 0.000 41.543 2 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Rainfall</td>
<td>354.578 2 0.000 141.571 2 0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biocrusts × disturbance</td>
<td>40.014 4 0.000 12.664 4 0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Biocrusts × rainfall</td>
<td>45.001 4 0.000 2.520 4 0.640</td>
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<td></td>
<td>Disturbance × rainfall</td>
<td>139.468 4 0.000 92.157 4 0.000</td>
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<td></td>
<td>Biocrusts × disturbance × rainfall</td>
<td>13.907 8 0.084 30.156 8 0.000</td>
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<tr>
<td>S. glareosa</td>
<td>(intercept)</td>
<td>872.776 1 0.000 253.908 1 0.000</td>
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<tr>
<td></td>
<td>Biocrusts</td>
<td>6.934 2 0.031 11.743 2 0.002</td>
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<tr>
<td></td>
<td>Disturbance</td>
<td>225.474 2 0.000 75.708 2 0.002</td>
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<td></td>
<td>Rainfall</td>
<td>170.506 2 0.000 30.019 2 0.000</td>
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<td></td>
<td>Biocrusts × disturbance</td>
<td>4.130 4 0.388 8.582 4 0.072</td>
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<td>Biocrusts × rainfall</td>
<td>23.522 4 0.000 12.720 4 0.012</td>
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<td>Disturbance × rainfall</td>
<td>69.203 4 0.000 23.869 4 0.000</td>
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<td></td>
<td>Biocrusts × disturbance × rainfall</td>
<td>15.260 8 0.054 20 8 0.010</td>
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<tr>
<td>C. latens</td>
<td>(intercept)</td>
<td>740.432 1 0.000 201.996 1 0.000</td>
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<tr>
<td></td>
<td>Biocrusts</td>
<td>1.312 2 0.518 15.689 2 0.000</td>
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<tr>
<td></td>
<td>Disturbance</td>
<td>304.812 2 0.000 57.659 2 0.000</td>
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<tr>
<td></td>
<td>Rainfall</td>
<td>138.308 2 0.000 10.539 2 0.463</td>
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<td></td>
<td>Biocrusts × disturbance</td>
<td>10.457 4 0.033 11.099 4 0.026</td>
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<td></td>
<td>Biocrusts × rainfall</td>
<td>7.838 4 0.097 14.332 4 0.006</td>
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<tr>
<td></td>
<td>Disturbance × rainfall</td>
<td>59.502 4 0.000 16.956 4 0.001</td>
<td></td>
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<tr>
<td></td>
<td>Biocrusts × disturbance × rainfall</td>
<td>4.248 8 0.833 19.063 8 0.014</td>
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</table>

A fitting model was compared with an intercept-only model.
lower than that of the control group. This may be due to the high water demand of *C. latens*; it is possible that after the simulated rainfall, some of the surface water evaporated rapidly and rest of the water infiltrated downward (Li et al., 2010; Chamizo et al., 2016). Especially during the later stages of the experiment, the poor water holding capacity of sand led to the death of *C. latens* seedlings due to water deficiencies. Eckert et al. (1986) and Zamfir (2000) suggested that the effect of biocrusts on plant seedling survival was related to seed size and quality and to plant life form.

The adequacy of moisture largely determined the seed germination of the three tested plant species. For example, the seed germination rates of the three plants under 50% increased rainfall conditions were significantly improved (P < 0.05), while *S. glareosa* and *C. latens* had almost no seed germination on cyanobacterial and lichen/moss crusts, respectively, with 50% reduced rainfall. This indicates that in years with abundant rainfall in desert areas, the presence of sufficient water can promote the invasion and establishment of native vegetation species of the adjacent grassland ecosystems into the revegetated area (Belnap et al., 2006; Eskelinen and Harrison, 2014; Song et al., 2020). Nevertheless, for the shrub *C. latens*, the seed germination rate was still very low even under 50% increased rainfall (Fig. 4). This indicates that biocrusts play a dominant role in the ecological filtering role of new species in the revegetated area during the current stage of succession. Therefore, even in the presence of sufficient amounts of water, *C. latens* cannot become established in the revegetated ecosystem.

### 4.3. Biocrusts mediate plant introduction and establishment

The results of the GLMs showed that the effect of biocrusts on the seed germination of *S. viridis* was reduced when disturbance and rainfall gradients occurred at the same time (Table 3), and disturbances affected seed germination by increasing the contact area between the seeds and the soil, which was conducive to water absorption by the seeds from the soil to promote germination (Hernandez and Sandquist, 2011; Havrilla and Barger, 2018). This effect was more noticeable in *S. glareosa* and *C. latens* seeds. Li et al. (2005) studied the relation of biocrusts on seed germination could be masked by the increase in water content. Therefore, from this point of view, water is indicated to be the main factor that determines the introduction of natural plants into the revegetated area. However, our results show that biocrusts are the main biological factors affecting the introduction of natural plant species. It has been suggested that biocrusts plays “sieving” roles during the seed-spreading stage of natural plant establishment, which has been confirmed in a large number of studies (Hernandez and Sandquist, 2011; Zhang and Belnap, 2015; Song et al., 2017; Havrilla et al., 2019; Slate et al., 2019). For example, Zhang and Belnap (2015) found that biocrusts had no significant effects on the germination of smooth plant seeds but could inhibit the germination of plant seeds with subsidiary appendages in the Gurbantunggut desert. Hernandez and Sandquist (2011) suggested that the presence of biocrusts acted as a biological barrier to the introduction of foreign plants, but the biocrusts played a certain role in promoting invasions after they were disturbed.

In our study, biocrusts had no significant effect on the mortality of *S. viridis* seedlings but significantly impacted the mortality of *S. glareosa* and *C. latens* (Table 3). The results showed that the seedlings of *S. viridis* could survive during different succession stages in the revegetated area. However, the spatial niche of *S. glareosa* and *C. latens* during the current succession stage of the revegetated area was limited by the existence of the biocrusts. This limiting effect was more noticeable for *C. latens*. The settlement process of *C. latens* in the revegetated area was greatly inhibited during the seed germination stage. However, the effect of biocrusts on *C. latens* was greatly weakened by increasing water content and disturbance (Hernandez and Sandquist, 2011; Havrilla and Barger, 2018; Slate et al., 2019; Song et al., 2020). Langhans et al. (2009) studied the effect of biocrusts on vascular plants and found that with artificial increases in water content, biocrusts not only promoted seed germination but also significantly promoted the survival and establishment of seedlings.

### 5. Summary

In conclusion, during the process of introduction and establishment of plant species in revegetated systems, as a key biological factor, biocrusts play an ecological filtering role in plant species introduction into the revegetation ecosystem and promote the establishment and survival of plant species adapted to the current revegetated community. The plant species that are currently unsuitable are filtered out by the biocrusts. Therefore, species that enter the artificially vegetated area and are not filtering out by the biocrusts can find suitable niches in the revegetated community and can coexist well with the other species in the community. This discovery clarifies the scientific significance of biocrusts in maintaining the health and stability of revegetated areas and provides a scientific basis for the management of artificially vegetated ecosystems. Our research encourages scientists to further study the important characteristics of these ecosystems and informs ecosystem managers to pay attention to the protection and rational utilization of biological soil crusts.

**CRediT authorship contribution statement**

**Guang Song:** Data curation, Methodology, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing. **Rong Hui:** Data curation, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Hao-tian Yang:** Data curation, Funding acquisition, Methodology, Formal analysis. **Bing-yao Wang:** Funding acquisition, Project administration, Investigation. **Xin-rong Li:** Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157135.

**References**


