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Null-model analysis and changes in species interactions in biocrusts along a successional gradient in the Tengger Desert, northern China

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Abstract

Aims: In arid regions, the succession of biological soil crusts (biocrusts) usually accompanies substantial turnover of community composition. How interspecific interactions among biocrust-forming mosses, lichens, algae and cyanobacteria change along the successional gradient is largely unknown. According to the stress gradient hypothesis (SGH), the frequency or intensity of facilitative and competitive interactions within the community will vary along an environmental stress gradient, with an increasing role of competition relative to facilitation under more benign environmental conditions. In the process of biocrust succession, decreasing soil stress also occurs because of slow soil amelioration, which meets the assumption of the SGH. Therefore, we hypothesize that the changes in facilitative and competitive interactions within biocrust communities will follow the prediction of the SGH and that the nature of biocrust interactions will also change.

Location: Sand-binding vegetation belts for the Baotou–Lanzhou railway at the southeast fringe of the Tengger Desert in the Shapotou region of the Ningxia Hui Autonomous Region, western China.

Methods: We conducted a field evaluation at five adjacent sand-binding vegetation belts built in chronosequence to study the successional gradient. Small-scale patterns of co-occurrence and null models were used to evaluate the community-level interspecific interactions among the biocrust-forming mosses, lichens, algae and cyanobacteria. In addition, an accessory method of species-pair co-occurrence based on null models was also applied to potentially identify the nature of the interspecific interactions within the biocrusts and for the causal investigation of these patterns.

Results: A strong spatial segregation of co-occurrence patterns occurred between cyanobacteria and algae and species of mosses and lichens at early succession, and occurred among species of mosses and lichens at late succession, while the weak spatial aggregation between lichen species only occurred at middle succession.

Conclusions: Negative interspecific interactions were prevalent within biocrusts throughout succession. The co-occurrence patterns at both the community and species-pair levels are consistent, supporting the unimodal version of the SGH that species interactions shift toward competition under extremely stressed conditions.

Given the lack of research on interspecific interactions in biocrusts, our study is important for obtaining a deeper understanding of biocrust succession.

1 | INTRODUCTION

Species interactions, as key drivers of the structure and dynamics of plant communities, are a central topic in ecology (Holmgren et al., 1997; Valiente-Banuet & Verdú, 2008; Maestre et al., 2009a). During the process of succession, species interactions can determine species coexistence, and changes in species interactions have profound influences on species distributions and diversity (Michalet et al., 2006; Brooker et al., 2008; Bronstein, 2009).

Biological soil crust (biocrust) is a complex community consisting of various species of free-living cyanobacteria, algae, lichens, bryophytes, fungi and other organisms (Bowker et al., 2014, Garcia-Pichel et al. 2016). In water-limited arid and semi-arid regions, biocrust communities are especially notable and serve as important ecosystem engineers (Li et al., 2003; Bowker et al., 2013). Previous studies have found typical successional sequences of biocrusts on bare land or disturbed habitat, in which biocrust succession started with cyanobacteria and algae on a relatively stable physical crust, followed by lichens and ending with mosses (Li et al., 2003; Weber et al., 2016). In biocrust studies, abiotic factors, such as the climatic regime, soil properties, and microtopography, have long been considered as the driving force in shaping the community structure of biocrust, and the role of biotic interactions has been underestimated (Wilson et al., 1995; Maestre et al., 2008; Weber et al., 2016). Recent studies in Spain conducted at both local and regional scales have described the dominant role of competition in structuring the biocrust community (Maestre et al., 2010; Bowker et al., 2010). Studies at rock quarries have found that the association with moss facilitates the photosynthetic performance of the cyanobacterial lichen *Peltigera rufescens* (Weiss) Humb., which demonstrated a mechanistic basis for the facilitative effects between bryophytes and lichens (Colesie et al., 2012). Despite this important evidence, to date, few studies have investigated the change in species interactions of the biocrust community along a successional gradient (Bowker et al., 2014).

Null model analysis of species co-occurrence patterns has long been used to link spatial patterns and ecological processes (Gotelli, 2000; Gotelli & Declan, 2002). Null model analysis uses the Monte Carlo simulation approach to build null community matrices and then identifies non-random species co-occurrence from a comparison between real matrices and the null matrices (Gotelli, 2000; Gotelli & Declan, 2002; Maestre et al., 2008). Many studies have suggested that segregation and aggregation in the fine-scale spatial arrangement of species are closely related to competition and facilitation, respectively (Gotelli & Declan, 2002; Maestre et al., 2008; Bowker et al., 2010; Bowker & Maestre, 2012). In the frequently used method and software (EcoSim) developed by Gotelli (2000), Gotelli and Declan (2002), and Gotelli et al. (2013), the indices are designed to evaluate the co-occurrence pattern at the community level that can reflect the overall biotic interactions of the community. However, these community-level indices show no information about how species pairs co-occur within a community, which hinders the analysis of the shifting nature of interactions within biocrust communities. An EcoSim accessory method that was proposed by Sanderson (2000, 2004) and used the same null matrices generated from EcoSim provides a tool to identify the positive or negative co-occurrence between species pairs. In this study, we used the former analysis to evaluate the changes in community-level interactions among the biocrust community along a successional gradient, and we used the latter pair-wise method to potentially identify the nature of the interactions and for investigating the cause of these patterns.

A second question we address herein concerns how the biocrust interactions change along the successional gradient. According to a well-formalized conceptual model known as the stress gradient hypothesis (SGH), facilitation is common in stressful abiotic environments, whereas competition dominates interspecific interactions in benign abiotic environments, and the role of facilitation in communities monotonously increases across the gradient of environmental severity (Bertness & Callaway, 1994; Brooker & Callaghan, 1998; Brooker & Callaway, 2010; Michalet & Pugnaire, 2016). However, there is also some evidence that facilitation collapses to neutral interactions (Michalet et al., 2006) or switches to competition (Holmgren & Scheffer, 2010) at the extreme end of environmental gradients, showing a unimodal relationship between stress and species interactions (Callaway et al., 2002; Michalet et al., 2006; Holmgren & Scheffer, 2010; Butterfield et al., 2016). In our study, the successional gradient started from extremely stressed bare sand, in which most plants barely survive. Subsequently, the environmental stress was decreasing because establishing plants and biocrusts constantly changed the habitat and ameliorated the edaphic condition (Li et al., 2007b, 2010). Therefore, we present two working hypotheses: (a) the change in community-level biocrust interactions supports the prediction of the unimodal SGH because extreme stress is involved; (b) both facilitative and competitive interactions occur, and the nature and intensity of biocrust interactions change along the successional gradient.

## 2 | METHODS

2.1 | Study area

This study was conducted in Shapotou in the Ningxia Hui Autonomous Region, located at the southeastern fringe of the

Tengger Desert (37°32′–37°26′ N, 105°02′–104°30′ E, 1,300–1,350 m above mean sea level [a.m.s.l.]), which is a typical ecotone between a desertified steppe and sandy desert (Li et al., 2003). According to the data of a local meteorological station (SDRES, CAS) from 1955 to 2016, the annual mean temperature is 10.0°C, with a −6.9°C mean temperature in January and a 24.3°C mean temperature in July. The annual mean precipitation is 186 mm, with 80% of this falling between May and September. The annual mean wind velocity is 2.9 m/s. The annual potential evaporation is approximately 2,900 mm. The soil type is wind-borne sand, or Eutric Arenosols in the World Reference Base (FAO-ISRIC-ISSS, 1998) To protect the Baotou–Lanzhou railway, a series of sand-binding artificial vegetation belts was built directly on sand dunes with the shrubs *Caragana korshinskii* Kom. and *Artemisia ordosica* Krasch. and straw checkerboards. These artificial vegetation belts were designed to sustain without irrigation and fertilization and built along the railway with a width of 500 m each and were revegetated in a chronosequence (1956, 1964, 1981, 1987, and 1990; Li et al., 2003, 2007a). We did not choose the belt revegetated in 1990 because the outermost one has been subjected to artificial disturbance from tourism in recent years. Instead, the nearby (1.3 km) sand-binding belt built in 2000 was used, which was built to extend the successional sequences for research purposes and is located at the Soil Water Balance Experimental Field of the Shapotou Desert Experimental Research Station, Chinese Academy of Science. All five selected belts (1956, 1964, 1981, 1987, and 2000), having the same climatic condition and being built with an identical sand-binding treatment (Li et al., 2007b), are representative of the different successional times for biocrusts

(62, 54, 37, 31 and 18 years; Figure 1). After revegetation, biocrust succession spontaneously began at the stabilized sand dunes.

2.2 | Biocrust community sampling

Within each revegetation belt, we set ten 1.5-m line intercepts along the soil surface to evaluate the community composition of the biocrust in late August 2018. Taking into account spatial representativeness, transects were randomly set in the direction of the belts and at least 5 m apart. To avoid the influence of plants, litter and micro-geomorphology, the transects were intentionally placed on flat ground at least 30 cm away from the nearest shrub or tussock and at least 2 m from the nearest mound (Choler et al., 2001; Li et al., 2010). Along each line transect, we sprayed distilled water to to make the biocrust more visually identifiable, and then we recorded the presence of mosses, lichens, cyanobacteria, algae, rocks, and bare soil with a 1-mm resolution. Mosses and lichens were identified to the species level in the field, while cyanobacteria and algae were classified as one "species" (CA) because they cannot be identified in the field and their combination rather than the single species is more functionally compatible to species of mosses and lichens (Li et al., 2010). Therefore, one transect data set consisted of 1,500 records in sequences similar to, e.g., "MMMMLLLMMMM…". The total number of a given letter (species) was counted as its abundance, and each continuum of a letter in the sequence was called a patch. This resolution was sufficient for our study because the smallest patches were ≥1 mm. The moss species included *Bryum argenteum* Hedw. (BA), *Didymodon vinealis* (Brid.) Zander (DV), and *Syntrichia caninervis* Mitt.
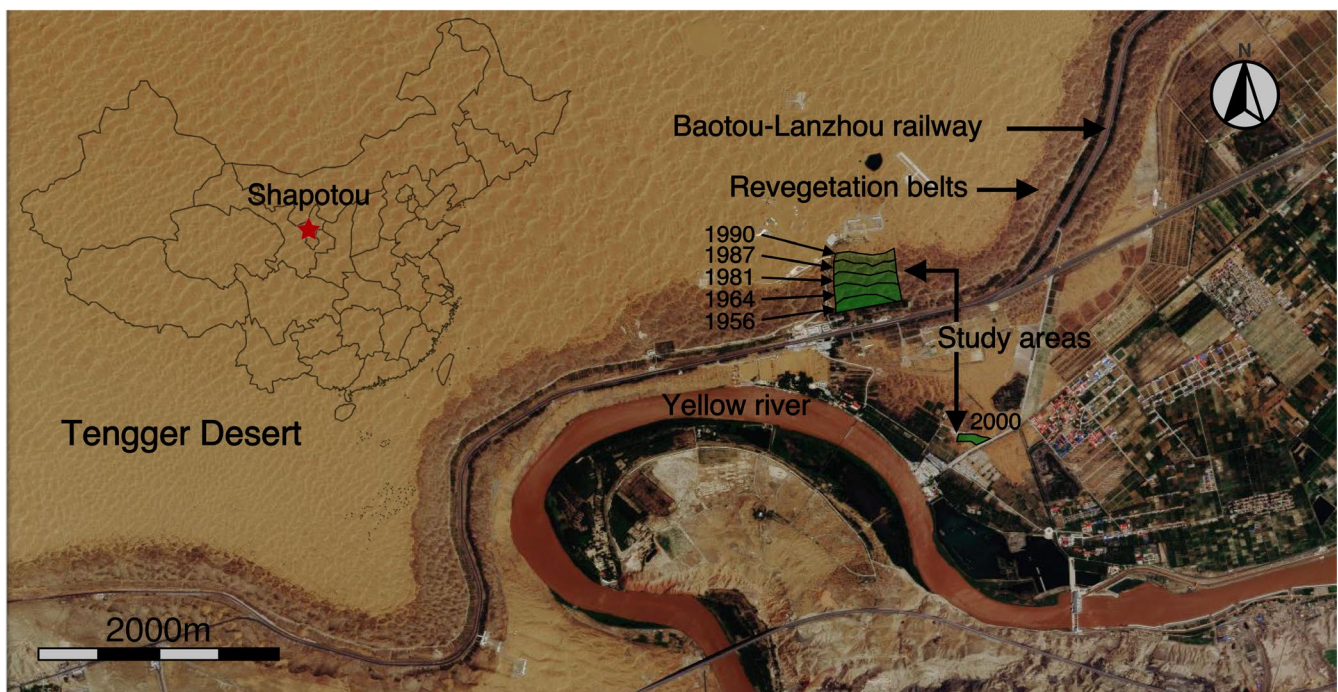
FIGURE 1 Aerial map of our study region. The green color marks the location of our sampling areas that are along the Baotou–Lanzhou railway in the Shapotou region of the Tengger Desert, China

(SC) (Li et al., 2003). The lichen species included *Collema coccophorum* Tuck. (CC), *Endocarpon pusillum* Hedw. (EP), *Fulgensia bracteata* (Hoffm.) Räsänen (FB), *Xanthoparmelia camtschadalis* (Ach.) Hale (XC), *Endocarpon simplicatum* (Nyl.) Nyl. (ES), and *Fulgensia desertorum* (Tomin) Poelt (FD) (Li et al., 2010). In particular, the dominant species BA and CC were ubiquitous among all transects.

2.3 | Soil sampling

In this study, because all belts were under the same climatic conditions, the soil properties were considered to be the main abiotic differences in each belt (Li et al., 2017). We took soil cores to sample the 0–3 cm of soil under the biotic layer of biocrusts in late August 2018. To fully evaluate the soil conditions, four equal-sized soil cores spread evenly along each 1.5-m transect were mixed together as one soil sample. A total of 50 soil samples were air-dried at ambient temperature to a constant weight (3–5 days), crushed and then sieved with a 2-mm mesh. The following parameters were measured: the soil pH and electrical conductivity (EC) were determined by a portable multimeter (HQ30d); soil soluble salt (SS) and $CaCO_3$ accumulation were analyzed using the methods described by the Nanjing Institute of Soil Research (1980); the soil particle sizes, i.e., clay of 0.1–0.25 mm (Clay1), clay of 0.05–0.1 mm (Clay2), silt of 0.002–0.05-mm (Silt) and dust < 0.002 mm (Dust), were analyzed using the pipette method (Hopmans & Bristow, 2001); soil organic matter (SOM) was determined with the dichromate oxidation method described by Nelson et al. (1982); total carbon (TC) and total nitrogen (TN) were measured by elemental analysis (vario MACRO cube); and total phosphorus (TP) was measured by Mo–Sb colorimetry.

2.4 | Co-occurrence patterns and null-model analysis

Co-occurrence patterns and null-model analyses have been used to explore biotic interactions in recent years (Gotelli & Declan, 2002; Maestre et al., 2008; Bowker et al., 2010; Bowker & Maestre, 2012). Null-model analysis of species co-occurrence uses the swap algorithm to build random community matrices and then identifies non-random species associations from a comparison of real matrices and null matrices (Gotelli & Declan, 2002; Maestre et al., 2008). This use of null models can help unveil the linking patterns and processes (Gotelli & Declan, 2002). In this study, we divided each 1.5-m transect into fifty 3-cm segments and organized them into a presence/absence matrix, where each row represented a different species and each column represented a segment. We chose 3 cm as the segment size to effectively capture the species co-occurrence because this value is close to the mean patch size. This matrix was arranged as raw data for co-occurrence pattern evaluation at both the community and species-pair levels. Then, we stochastically built null matrices to reflect communities that

are not structured by any deterministic ecological process. There are many algorithms to produce null matrices that apply equiprobable, proportional or sums-fixed constraints on rows or columns (Gotelli, 2000). In our research, the fixed/equiprobable algorithm (row sums fixed and columns equiprobable) was used to randomize each observed matrix with 10,000 iterations because its assumptions are close to those in this study. First, this algorithm fixes the row sums (species incidence rate is fixed); that is, the null matrices preserve the differences in the commonness and rarity of species. Second, the column equiprobable constraint guarantees that each species is assumed to be equally probable to occur in all segments. This assumption simulates the situation in which all homogeneous 3-cm segments in a line transect have an equal chance to be occupied. In addition, the fixed/equiprobable algorithm has low probability of Type 1 errors and good power to detect non-randomness (Gotelli, 2000).

For the community-level analysis, we used the C score described in Stone and Roberts (1990) to quantify the co-occurrence patterns of the biocrust community structure. The C score evaluates the average segregation between all possible species pairs in the checkerboard distribution; thus, it is robust to noise in the data (Gotelli, 2000; Bowker et al., 2010). The C score is calculated for each pair of species as $(R_i − S) (R_j − S)$, where R_i and R_j are the total occurrences of species i and j, respectively, and S is the number of co-occurrences for both of them; this score is then averaged over all possible species pairs. Then, we evaluated the C score on both the observed matrices and simulated null matrices to obtain the standardized effect size for the C score (SES_C; Gotelli, 2000). SES_C was calculated as $(I_{obs} − I_{sim})/SD_{sim}$, where I_{obs} is the C score for a given matrix, and I_{sim} and SD_{sim} are the mean and standard deviation for the simulated matrices. A higher C score indicates less co-occurrence between all species pairs in a community, which is logically related to differential habitat utilization, different dispersal modes or competitive exclusion (Gotelli, 2000). However, our research method can minimize the influences of habitat selection and dispersal differences, and the co-occurrence patterns are able to potentially address the importance of species interactions because (a) the short line transects were intentionally placed on flat ground, which provided spatial homogeneity, and (b) the abundant dispersal source nearby made species unlikely to be dispersal-limited at such a small sampling scale. To indicate the significance, we used an SES value of 1.96 to represent the confidence level of 95%. That is, if |SES| > 1.96, the observed co-occurrence indices fall beyond the ±1.96 standard deviation from the mean of the null matrix space. Therefore, if the observed C score is significantly greater than that from the null matrices ($SES_C > 1.96$), these species co-occur less often than expected by chance and are thus likely to be structured by competitive interactions; otherwise, $SES_C < −1.96$ suggests significant facilitative interactions (Maestre et al., 2008).

In the co-occurrence analysis of species pairs, we followed the principle of Sanderson (2000, 2004) to count the number of co-occurrences S (similar to the C score) as a "natural co-occurrence" index for all species pairs in the matrix. We evaluated S from both

the real matrices and null matrices, and we summed S from 10 line transects in each revegetation belt (S_{sum}). Then, we calculated the standardized effect size for S_{sum} (SES_S) as ($I_{obs} − I_{sim}$)/SD_{sim}, where I_{obs} is the observed S_{sum} and I_{sim} and SD_{sim} are the mean and standard deviation of 10,000 simulated S_{sum}, respectively. For SES_S evaluation, we used the criterion of 1.96 as well. Therefore, $SES_C > 1.96$ or $SES_C < −1.96$ indicates a significantly positive or negative association between a species pair. Co-occurrence pattern and null-model analyses were conducted using R software (version 3.5.2) with the *EcoSimR* package (Gotelli et al., 2013).

2.5 | Statistical analyses

The soil parameters among different years of revegetation were compared by one-way analysis of variance (ANOVA). Multiple comparisons were performed with least-significant-difference tests. Principal components analysis (PCA) was used to integrate soil parameters and identify the main factor. The correlation matrix for PCA was built using the Pearson coefficient covariance. Principal component selection was performed according to the broken-stick model, which was first proposed by Macarthur (1957). These calculations were carried out using the *Vegan* package (Dixon, 2003) in R software (version 3.5.2). The SGH was tested by modeling interactions as a function of the physical stress using least-squares regression analysis. Specifically, we regressed SES_C against the corresponding PC1. The classic SGH would be supported by a negative linear relationship between SES_C and PC1, while the unimodal SGH would be supported by a concave curve. Given this, we employed first-, second-, and third-order polynomial regressions to distinguish linear and non-linear relations. The best-fitting model was selected according to the result of an F-test and the lowest Akaike information criterion (AIC). The data that support the findings of this study are openly available in Github at https://github.com/Agrostis-Sun/DataArchive. All regressions were performed in R software (version 3.5.2).

3 | RESULTS

3.1 | Soil properties

After revegetation, the concentrations of TC, TN, TP and SOM in the soil accumulated over time ($p < 0.001$). EC, SS and $CaCO_3$ also significantly increased ($p < 0.001$), but not pH ($p = 0.21$). The soil particle distribution significantly changed as well, increasing for Dust, Silt and Clay2 ($p < 0.001$) but decreasing for Clay1 ($p < 0.001$; Table 1). According to the correlation biplot, most variables were closely correlated, having the same change tendency with a small angle relative to the first PCA axis, except for pH and SS. The broken-stick model chooses the first components that explain more variance than is possible by randomly dividing the variance into parts. The proportion explained by PC1 was 0.7259,

TABLE 1 Physical and chemical soil properties (at a depth of 0–3 cm under biocrust) of belts with different numbers of years since revegetation ($n = 10$, mean ± SE), and the result of the ANOVA

Parameter	Unit	Year of revegetation					MS	F
		1956	1964	1981	1987	2000		
pH		7.77 ± 0.050[a]	7.64 ± 0.040[a]	7.72 ± 0.028[a]	7.69 ± 0.039[a]	7.70 ± 0.022[a]	0.02062	1.476
Electrical conductivity (EC)	µs/cm	464 ± 43[a]	366 ± 18[ab]	397 ± 25[ab]	324 ± 39[b]	321 ± 23[b]	35,594	3.60[*]
Soil soluble salt (SS)	g/kg	0.50 ± 0.082[a]	0.34 ± 0.058[ab]	0.36 ± 0.047[ab]	0.12 ± 0.052[b]	0.22 ± 0.050[b]	0.211	6.04[***]
$CaCO_3$	g/kg	53.17 ± 2.28[a]	40.91 ± 2.89[b]	37.23 ± 1.87[b]	17.80 ± 3.32[c]	6.45 ± 0.88[d]	3,520	59.52[***]
Soil organic matter (SOM)	g/kg	5.36 ± 0.25[a]	5.00 ± 0.24[a]	3.96 ± 0.11[b]	2.79 ± 0.43[c]	1.59 ± 0.13[d]	24.48	36.85[***]
Total carbon (TC)	g/kg	9.49 ± 0.39[a]	7.81 ± 0.44[ab]	6.76 ± 0.24[b]	3.76 ± 0.64[c]	1.70 ± 0.17[d]	99.05	57.54[***]
Total nitrogen (TN)	g/kg	0.60 ± 0.016[a]	0.43 ± 0.025[b]	0.43 ± 0.018[b]	0.22 ± 0.027[c]	0.16 ± 0.012[c]	0.33	77.89[***]
Total phosphorus (TP)	g/kg	0.68 ± 0.036[a]	0.69 ± 0.028[a]	0.69 ± 0.014[a]	0.54 ± 0.044[b]	0.36 ± 0.014[c]	0.21	23.66[***]
Clay (0.1–0.25 mm, Clay1)	%	54.24 ± 2.00[a]	59.04 ± 2.28[b]	60.44 ± 1.49[c]	76.89 ± 2.33[c]	89.32 ± 0.98[c]	2,162.9	59.43[***]
Clay (0.05–0.1 mm, Clay2)	%	17.19 ± 0.89[a]	14.38 ± 1.07[a]	14.22 ± 0.63[a]	10.46 ± 1.01[b]	4.74 ± 0.40[c]	235.08	34.22[***]
Silt (0.002–0.05 mm)	%	22.89 ± 1.27[a]	21.18 ± 1.33[a]	20.64 ± 0.97[a]	9.79 ± 1.18[b]	4.59 ± 0.56[c]	658.4	52.98[***]
Dust (<0.002 mm)	%	5.67 ± 0.23[a]	5.40 ± 0.31[a]	4.70 ± 0.18[a]	2.87 ± 0.30[b]	1.36 ± 0.14[c]	34.12	59.12[***]

MS indicates mean squared error. The significance for superscript letter is .05.

*$p < .05$; **$p < .01$; ***$p < .001$.

and the percent eigenvalue bar for PC1 was higher than would be expected under the broken-stick model (Figure 2). Therefore, it is reasonable to use PC1 as a measurement of abiotic stress. The proportion of abiotic variables explained by PC1 was as follows: TC, 96.69%; $CaCO_3$, 95.78%; Clay1, 95.63%; Silt, 92.02%; Dust, 90.2%; SOM, 89.46%; Clay2, 86.54%; TN, 83.34%; TP, 82.41%; EC, 33.54%; SS, 25.5%; and pH, 2.1%.

3.2 | Species composition

After revegetation, the composition of the biocrust community gradually changed (Figure 3). The abundance of cyanobacteria, algae, and lichens and the amount of bare soil decreased with succession, while that of mosses increased. In the later stages of succession (belts 1981, 1964, and 1956), the relative composition remained stable. In addition, a high proportion of bare soil was also found at early succession, which accompanied small and fragmented species patches.

3.3 | Community-level analysis of co-occurrence patterns

Second-order polynomial regressions presented the best-fitting results based on the lowest AIC and highest significance ($p = 0.0076$, AIC $= 177.32$), which indicated a unimodal relationship

between SES_C and PC1 (Table 2). High SES_C values were reached at early and late succession, which indicated that these communities were highly structured (Figure 4). Moreover, the SES_C values in belts 1981 and 1987 were close to 0, which indicated that their net interaction intensities were close to what would be expected by chance (Figure 4).

3.4 | Species-pair analysis of co-occurrence patterns

From the results of SES_S (Figure 5), the negative associations between CA and other species were significant in belts 2000 and 1987, i.e., early succession, suggesting a strong competitive replacement for CA. In addition to CA, a negative association was widespread between mosses and lichens, such as BH ↔ CC (−4.72), BH ↔ EP (−1.97) and BH ↔ XC (−2.38) in belt 1987 and BH ↔ CC (−2.72) and BH ↔ XC (−2.58) in belt 1981. Subsequently, negative associations were stronger and more widespread in belts 1964 and 1956, and were among moss species, such as BH ↔ SC (−2.35) and BH ↔ DV (−3.65); among lichen species, such as CC ↔ EP (belt 1964: −5.60, belt 1956: −3.26); and between mosses and lichens, such as BH ↔ CC (−2.90) and BH ↔ EP (belt 1964: −4.58, belt 1956: −5.60). Notably, only the association CC ↔ XC was significantly positive (belt 1981: 2.09, belt 1964: 1.99), and it appeared during middle succession. In general, the results of the pair-wise and community-level co-occurrence patterns were consistent, i.e., species segregation decreased and then increased with succession proceeding.
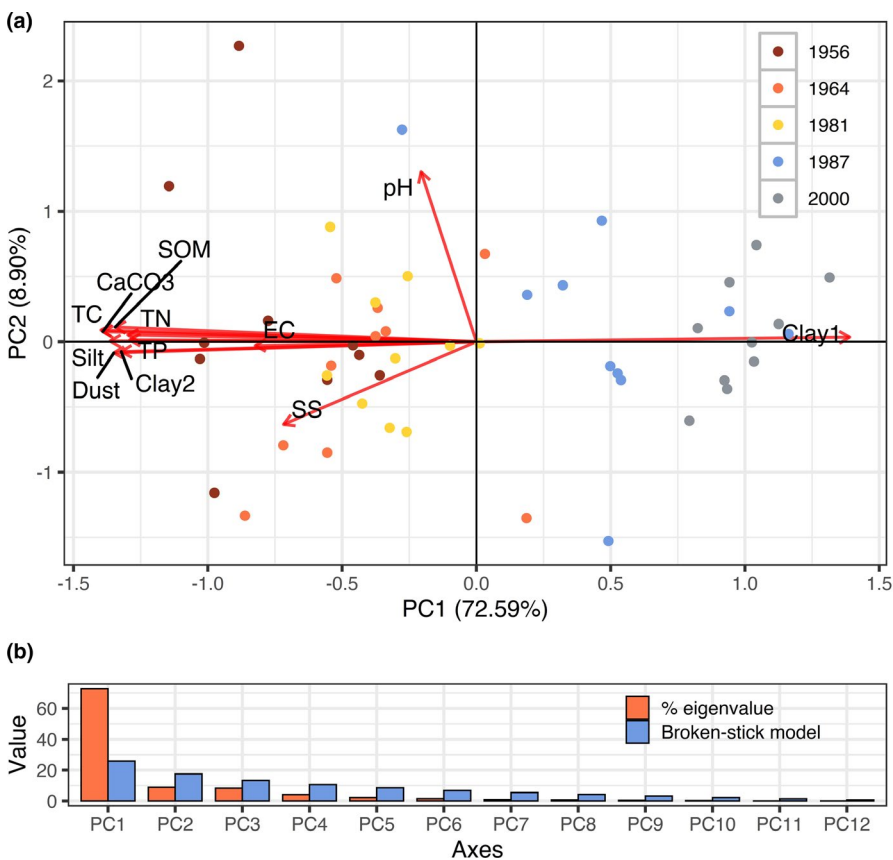
FIGURE 2 Principal components analysis (PCA) of physical and chemical soil properties and PC selection. (a) PCA shown as a correlation biplot. Abbreviation list: EC, electrical conductivity; SS, soil soluble salt; SOM, soil organic matter; TC, total carbon; TN, total nitrogen; TP, total phosphorus; Claqy1, 0.1–0.25-mm clay; Clay2, 0.05–0.1-mm clay; Silt, 0.002–0.05-mm silt; Duat, <0.002-mm dust. (b) PC selection with the broken-stick model. The broken-stick distribution reflects the ranked expected proportions of the broken pieces. The eigenvalue bars that are higher than the broken-stick bars are the PCs that are selected. According to this selection criterion, only PC1 is a PC of the soil abiotic environment

FIGURE 3 Change in species composition in different belts after revegetation. The summed unidimensional interception length is used to indicate the abundance of each component. The length of the transect is 1.5 m, with 10 replications for each treatment. Different letters over the bars indicate significant differences at the 0.05 level (Bonferroni-adjusted, $p < .05$)
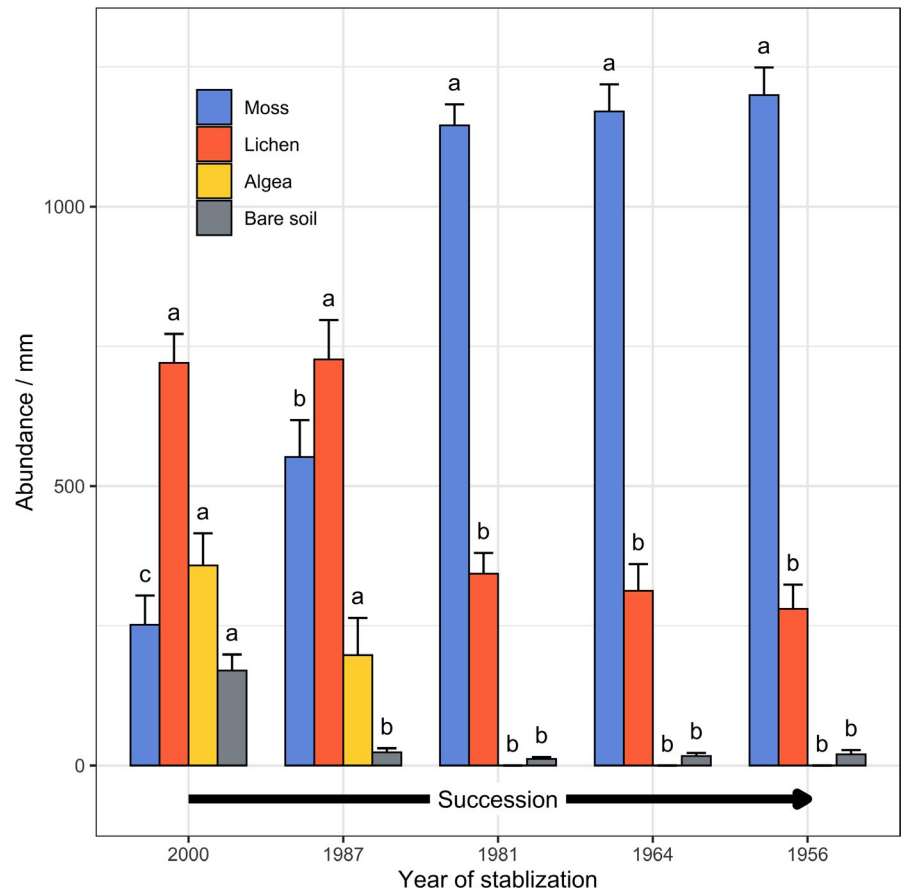
4 | DISCUSSION

4.1 | Soil properties and abiotic stress

The results of soil analysis along a successional gradient are in agreement with the already well-recognized fact that plants and biocrust ameliorate abiotic environments in railway protective belts after revegetation (Li et al., 2003, 2007b). Therefore, the process of succession was accompanied by a decrease in local abiotic stress. In the Shapotou region, three aspects of soil physicochemical properties are related to the performance of biocrust: topsoil moisture, stability and fertility (Li et al., 2007b, 2010). Water availability is the primary limiting factor in arid ecosystems, and soil stability is one of the most important prerequisites for biocrust formation because biocrusts rarely establish on topsoil subjected to extensive wind and water erosion and even die under severe sand burial (Belnap & Lange, 2003). Several studies have demonstrated the important influence of soil texture, especially the accumulation of fine particles, on soil development and soil water storage (Li et al., 2007b; Zaady et al., 2014). Therefore, the increase in Clay2, Silt and Dust suggested increased soil stability and soil water-holding capability under the given precipitation regime (Table 1). Fine particles, together with aeolian dustfall from wind erosion captured by established plants and biocrust communities, represent the most important sources of nutrients in desert ecosystems (Reynolds et al., 2001). In addition, the SOM also increases the water-retention capacity of soil, thereby

ameliorating the water stress (Celik et al., 2010). The results showed that TN, TP, TC, and SOM increased with time (Table 1) but were negatively associated with PC1 (Figure 2), which indicated an overall increase in soil fertility. Admittedly, most soil parameters except for SS and pH were correlated with each other (Figure 2); thus, we could not distinguish their relative contributions to soil conditions, although we could encapsulate them into PC1. Therefore, PC1 is representative of the total change in soil conditions along the succession, including topsoil moisture, stability and fertility, that bridges the extremely stressed condition and benign condition for a biocrust community. In sum, we fully believe that the first PC axis is a sufficient representative of the relevant stressors to evaluate the SGH.

4.2 | Biocrust interactions support the unimodal SGH

In the successional gradient of this study, species interactions showed a non-linear change in which competitive interactions dominated the community at both early and late succession, while neutral interactions dominated at middle succession (Figure 4). Contrary to the prediction of the classic SGH (Bertness & Callaway, 1994; Brooker & Callaghan, 1998), our results showed that the relation between interactions and abiotic stress followed a unimodal curve. One important reason for the unimodal change is probably that our successional gradient included the extremely stressed

environment because the succession of biocrusts began with the extremely arid unstable and barren soil condition, where most higher plants barely survive. To date, only a few studies concerning interspecific interactions in biocrust have been conducted. Our results differ from the results of those studies in several ways, mainly with regard to the extreme stress. A geographical-scale study of a lichen community was conducted by Maestre et al. (2010) along the long coastal desert of Chile to evaluate the relationship between co-occurrence patterns and water and nutrient availability. Their study, using multiple surrogates of abiotic stress and nested spatial scales, generally supported the prediction of the SGH that co-occurrence will shift from prevailing interspecific segregation to aggregation as abiotic stress increases. However, the exclusive focus on lichens, one important component of biocrusts, may have truncated the stress gradient, leading to a different conclusion than that of this study. A test of SGH with a biocrust community was also conducted by Bowker et al. (2010). Their study sites covered 112,400 km^2 of central, southern and eastern Spain, and they found that aridity was positively related to the balance between facilitation and competition. In that study, no

apparent or observable cyanobacteria and algae crust appeared on the soil surface because mosses and lichens usually replace cyanobacteria and algae during the climax of biocrust succession, which we believe is the major difference between that study and ours. Given that cyanobacteria and algae crusts are closely associated with extremely harsh or disturbed environments, we strongly suggest that more attention be paid to the role of cyanobacteria and algae to draw a complete picture of species interactions in biocrust communities.

## 4.3 | Change in biocrust interactions in succession

Having confirmed the change in community-level interactions within biocrusts along the succession, we turn to the question of why the biocrust interactions change in a unimodal manner. This question has been of considerable interest over the last two decades, but attempts to answer it have not been very fruitful because researchers have historically treated biocrusts more as a component of the ecosystem than as multispecies communities (Weber et al., 2016). In fact, vascular plants serve a more widely recognized model system to evaluate the SGH. Due to the substantial differences in life forms and life history, biocrusts provide new angles to verify the SGH and its generality (Bowker et al., 2014). In the process of succession, the species composition underwent substantial turnover, in which the proportions of cyanobacteria, algae and lichens decreased and that of mosses increased, which is basically consistent with the well-recognized successional trajectory in arid and semi-arid ecosystems, where cyanobacteria and algae colonize the bare soil first, followed by lichens and mosses (Li et al., 2010; Lan et al., 2012; Weber et al., 2016). Therefore,

TABLE 2 Polynomial regressions between the standardized effect size based on the C score (SES_C) and the first axis from the principal components analysis (PC1)

Order	R^2	F	df	p-value	AIC
1st	0.0078	0.38	1 and 48	0.54	185.32
2nd	**0.19**	**5.42**	**2 and 47**	**0.0076**[**]	**177.32**
3rd	0.22	4.22	3 and 46	0.010[*]	177.55

Note: Best-fitting models are marked in boldface.
*p < .05; **p < .01; ***p < .001.

$$SES = 1.61 - 0.42 \cdot PC1 + 1.57 \cdot PC1^2$$
$$(R^2 = 0.19, P = 0.0076)$$
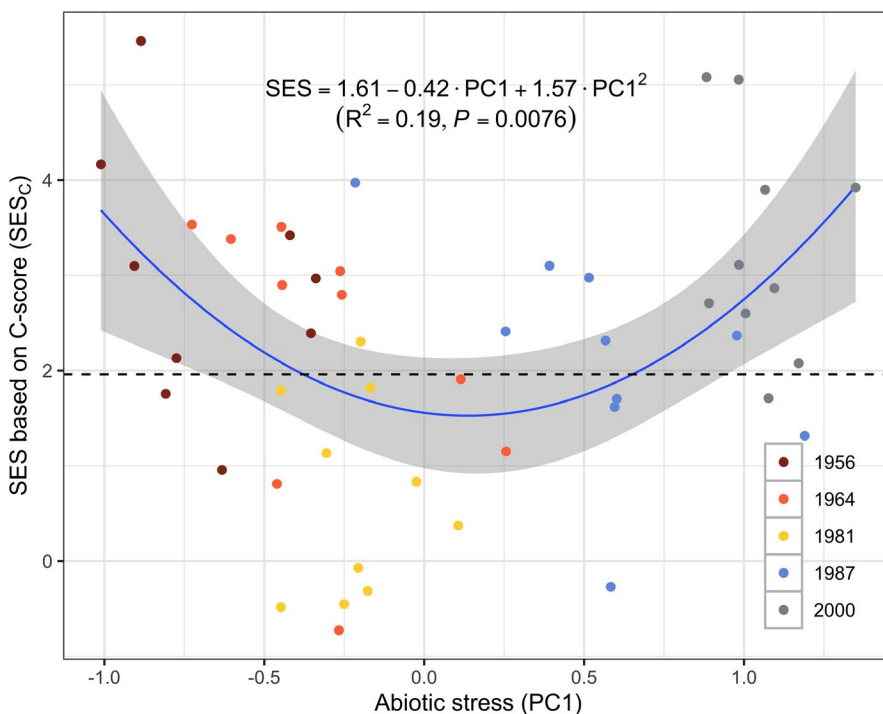
FIGURE 4 Standardized effect size based on the C score (SES_C) for each transect as a function of the abiotic stress. Each point represents one transect with different revegetation years. Second-order polynomial regressions are fitted with 95% confidence intervals

the change in community-level interactions within biocrusts can be interpreted in terms of the shifting nature of species interactions (species turnover).

At the beginning of biocrust succession, once the soil has stabilized, the development and succession of biocrust spontaneously begin, in which cyanobacteria and algae first colonize the physical crust (Li et al., 2003). Li et al. (2017) investigated six desert regions of northern China across a precipitation gradient and introduced a diagrammatic replacement triangle to explain the relationship between variations in the composition of biocrust and variations in environmental parameters, in which improved soil stability and moisture promote the replacement of cyanobacteria and algae by mosses and lichens. Therefore, strong competitive displacement was likely to occur. Cyanobacteria and algae, as colonizers of the biocrust community, are competitively inferior to mosses and lichens, forming segregated co-occurrence patterns and a strong negative association of cyanobacteria and algae with lichens and mosses (Figure 5a).

In our study, except for belt 2000, species competitions were widespread among species of lichens and mosses (Figure 5b–e).

Similar to most plant species, lichens and mosses also compete for space, nutrients and light, and their competitive interactions lead to the stable coexistence of multiple species (Armstrong & Welch, 2007; Bowker et al., 2010). The growth rate is an important attribute in species interactions because rootless biocrust constituents require direct contact with the substrate to take up water and nutrients (St. Clair et al., 2009). Because of their horizontal distribution (some mosses have 3D structures and multilayers) and spatially precluding morphology, species with high colonization rates are more likely to reach exclusive pre-emption of space and success in competition (Armstrong, 2010). As in plant species, the life-history strategy associated with morphology also influences the outcome of competition (Armstrong & Welch, 2007). For example, when lichens gradually colonize the substrate and come into contact with each other, the growth form and morphology determine the relative position of overlapping thalli. Foliose lichens, with flat leaf-like thalli, are more likely to shade and overgrow lower-position thalli, such as those of gelatinous and crustose lichens (Pentecost, 1980). In contrast, the lower-position thalli are able to undermine their competitors by elevating the upper thalli from the substratum
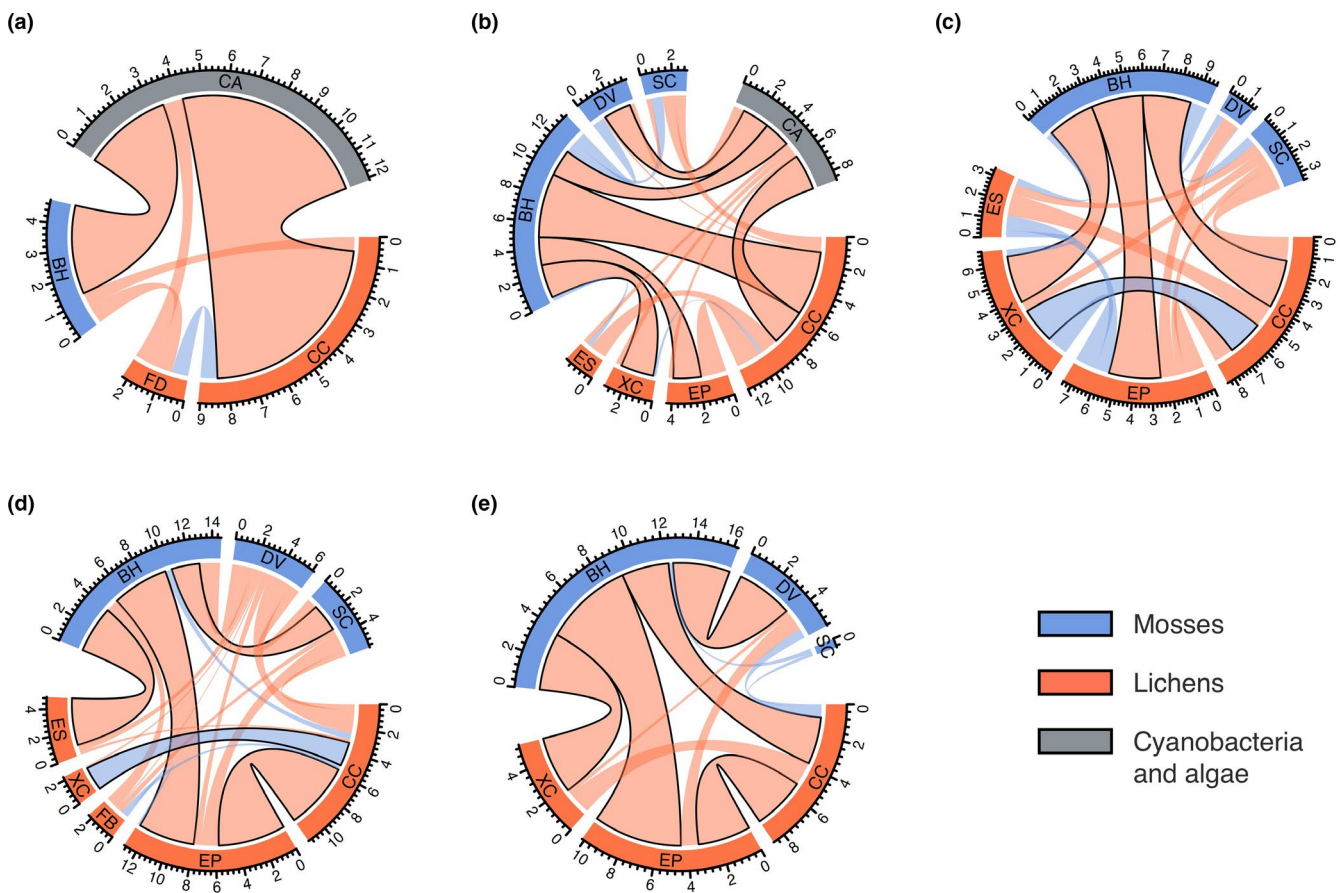
FIGURE 5 Changes in the standardized effect size based on the co-occurrence number (SES$_S$) between biocrust species pairs at different revegetation belts. (a–e) Belts revegetated in 2000, 1987, 1981, 1964 and 1956, respectively. The widths of links are proportional to the absolute value of SES$_S$. Positive and negative links are colored in blue and red, respectively, and significant links (|SES$_S$| > 1.96) are bordered. Species codes: BA, *Bryum argenteum* Hedw.; DV, *Didymodon vinealis* (Brid.) R.H. Zander; SC, *Syntrichia caninervis* Mitt.; CC, *Collema coccophorum* Tuck.; EP, *Endocarpon pusillum* Hedw.; FB, *Fulgensia bracteata* (Hoffm.) Räsänen; XC, *Xanthoparmelia camtschadalis* (Ach.) Hale; ES, *Endocarpon simplicatum* (Nyl.) Nyl.; CA, cyanobacteria and algae

(Armstrong & Welch, 2007). However, our experimental design does not consider biological mechanisms nor verify any mechanisms; these speculations provide important insights for further consideration.

In the intermediate part of the successional gradient, the community-level interaction changed from negative to neutral, which may arise from either the decreasing intensity of interactions or the offset by facilitation. First, the competitive hierarchy in biocrust is not fixed, and the position in the resource niche of species-specific optima affects the competitive outcome (Bowker et al., 2010). For example, in the middle succession, conditions of both the soil moisture and stability are ameliorated, which are also vital factors for mosses and lichens respectively. Therefore, mosses and lichens are prone to co-occur because of two relatively sufficient key resources. Moreover, this process also explains the replacement of lichens by mosses under the moisture soil condition of late biocrust succession because mosses have a more developed hydraulic conductivity system and greater water consumption than lichens (Michel et al., 2015; Li et al., 2017). Second, the evidence of positive association between CC and XC may support facilitative interactions, which provides a clue for the offset explanation (Figure 5c, d). To our knowledge, studies on biocrust facilitation are very few. The first evidence of facilitative effects of biocrusts was reported by Colesie et al. (2012), in which the lichen *Peltigera rufescens* benefited from its associated mosses via CO_2 enhancement and microclimate amelioration. Secondary chemicals of biocrusts are also important mechanisms of facilitation, some of which can promote the uptake of essential micronutrients, reduce intracellular chemical toxicity, and inhibit pathogenic bacteria and fungi (Hauck et al., 2009; Kowalski et al., 2011). Therefore, species facilitating nutrient uptake and species with a chemical defense mechanism can cooperate with each other and mutually benefit from this association. However, these interpretations are only speculative, and more physiological experiments are needed in the future.

5 | CONCLUSIONS

In summary, in response to our working hypothesis, we found that competition dominated the biocrust community throughout succession, while the community-level interactions were neutral in the intermediate part of the stress gradient. The co-occurrence patterns at both the community and species-pair levels supported the unimodal version of the SGH because a long stress gradient bridged by biocrust succession in this study included the extremely stressed environment. In addition, in view of the lack of research on interspecific interactions of biocrusts, our study is of pioneering value. To the best of our knowledge, this attempt is the first to evaluate the facilitative and competitive interactions during the process of biocrust succession. We suggest that future work investigate the detailed mechanisms of these interactions via physio-ecological experiments to obtain a deeper understanding of biocrust succession.

AUTHOR CONTRIBUTIONS

Jingyao Sun: conceptualization, methodology, software, field work, manuscript drafting. Xinrong Li: conceptualization, interpretation of data, funding acquisition. Rong Liang Jia: species identification, field work. Ning Chen: visualization, validation. Tian Zhang: software, field work.

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