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RESEARCH ARTICLE

# Biocrust mediates the complexity and stability of bacterial networks in both biocrust and subsoil layers in the Tengger Desert

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**Abstract**

*Background and Aims* Biocrusts cover approximately 30% of the global dryland surface area, constituting a crucial atmosphere–soil interface. Bacteria living at this interface participate in almost all biogeochemical cycling processes that may profoundly alter soil and ecosystem multifunctionality and speed up ecosystem restoration. However, the successional dynamics of bacterial communities in both biocrusts and subsoil remain largely unclear.

*Methods* This study used α and β diversity assessments and molecular ecological networks to reveal the bacterial community succession in biocrusts and subsoil along a 65-year succession sequence (a succession of biocrust types ranging from cyanobacteria to lichens to mosses) on the southeastern edge of the Tengger Desert.

*Results* Our results showed that the bacterial α and β diversity and network complexity in the biocrusts and subsoil increased with succession. In the process of succession, there were distinct differences observed in bacterial community diversity and network complexity and stability between biocrusts and subsoil. In particular, the subsoil bacterial network properties, including nodes, links, average links per node, average clustering coefficient, connectance and relative modularity, were significantly higher than those of biocrust in late succession. Based on piecewise SEM, we also found that succession, soil physicochemical conditions, and biocrust bacterial community composition were the strongest direct drivers of subsoil bacterial community composition. The plant communities and biocrust bacterial community composition directly drove the network complexity and stability of the subsoil bacterial community.

*Conclusion* Our findings indicate that under the cover and protection of the biocrust layer, the diversity of the bacterial community in the subsoil layer increased more obviously, and the network was more complex and stable. This may emphasize the important roles of biocrusts as mediators of soil microbial communities.

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**Keywords** Biocrusts · Bacterial community composition · Cooccurrence network · Complexity and stability · Environmental driver

## Introduction

Drylands cover approximately 45% of the terrestrial land surface, representing the largest terrestrial biome on Earth (Schimel 2010). Vegetation in drylands is generally sparse, and biocrusts coexist alongside herbaceous and woody vegetation, creating landscape mosaics of vegetated, bare soil and biocrust-covered patches (Maestre et al. 2010). Biocrusts, including communities of cyanobacteria, algae, fungi, bacteria, lichens, and mosses, occur on or within the top few centimeters of the soil surface (Li et al. 2018). Biocrusts cover approximately 30% of the global dryland surface area, constituting a crucial atmosphere–soil interface (Rodriguez-Caballero et al. 2018). Acting as a selective force to mediate both the physical and biological environments of dryland ecosystems, biocrusts affect a range of ecosystem functions, such as the cycles of water, nutrients, and gases (Elbert et al. 2012; Chen et al. 2018b; Rodriguez-Caballero et al. 2018). For example, biocrusts can account for approximately 15% of global terrestrial carbon (C) and 40%–85% of nitrogen (N) fixation globally (Rodriguez-Caballero et al. 2018). Therefore, biocrusts play very important roles in drylands and are generally considered one of the organizing principles and critical zones in dryland ecosystems (Belnap et al. 2016). Studies have even further suggested that biocrusts may as one of multiple stable states rather than just influencing factor in dryland ecosystems, which is particularly important because biocrusts can decelerate heavy degradation of ecosystems (Kinast et al. 2013; Chen et al. 2020). More importantly, approximately 10–20% of drylands have already been degraded, and degradation is expected to increase in the future (Huang et al. 2016).

Accordingly, ecosystem restoration has become a global priority, especially in drylands (Strassburg et al. 2020). The natural succession of biocrusts often follows a general succession pattern, starting with cyanobacteria and algae at the initial colonization stage and then followed by lichens and bryophytes at a later stage (Weber et al. 2016), which generally takes decades to centuries (Pointing and Belnap 2012; Chen et al. 2020). During succession, the microbial community composition (Liu et al. 2017) and diversity (Mogul et al. 2017) in biocrusts are strongly transformed. For example, bacterial and eukaryotic communities have the maximum difference among successional stages and higher microbial mutual exclusion and more complex networks at later stages (Li and Hu 2021). Nevertheless, a study in a temperate desert in China found that microbial community diversity differed significantly between biocrust and subsoil but was minor among biocrust types (Liu et al. 2019). Importantly, microbial communities participate in almost all biogeochemical cycle processes and play critical roles in maintaining ecosystem function (Bardgett and van der Putten 2014; Zhou et al. 2020a, b), which may be an important indicator of biocrust recovery. In biocrusts, the phyla Proteobacteria and Actinobacteria were the major contributors to the nutrient cycles, and the intensities of the functional gene expression involved in the nutrient cycles increased significantly in later successional stages (Liu et al. 2018; Zhao et al. 2020; Qi et al. 2021). Unfortunately, most previous works on biocrust recovery focused on one of the successional phases or assessed biocrust recovery mostly based on morphological assessments (e.g., biocrust coverage) (Xiao et al. 2019). Several studies have focused on the mechanisms of microbial community assembly (Li and Hu 2021) and microbial interactions (Zhou et al. 2020a, b), but few have examined and assessed the entire succession of the microbial community in biocrust phases owing to the lack of information on accurate biocrust ages or long-term observations.

Previous studies assessing ecosystem succession in soil microbial communities focused on simple attributes such as taxonomic diversity and did not assess them together in most cases (Moreno-Mateos et al. 2017), the latter may provid pivotal knowledge to explore the microbial community and functions (Garcia-Garcia et al. 2019; Zhou et al. 2020a, b). However, ecosystem restoration requires moving beyond traditional biodiversity assessments to a broader perspective, which considers the interactions between species and the complexity and stability of ecosystems (Moreno-Mateos et al. 2017). In ecosystems, the complex interactions between different species constitute a complicated ecological network (Yuan et al. 2021). The network with species as nodes and their relationships as links is fundamental for

characterizing species interactions and the dynamics of ecosystems (Pržulj and Malod-Dognin 2016). Species richness, or the total number of interacting species (network size) in the network, has been used as the simplest descriptor of network complexity (Landi et al. 2018). Other network topology features, such as link, node degree and modularity, can be used to describe and characterize the complexity of species interactions in the network (Toju et al. 2017). Furthermore, network stability refers to the proportion of persisting species once equilibrium is reached and the speed at which the community returns to equilibrium after a perturbation (Thébault and Fontaine 2010). A long-term in situ warming experiment in Oklahoma showed that the microbial networks under warming and control conditions followed different successional trajectories over time and that warming significantly increased the complexity and stability of the networks more than the control (Yuan et al. 2021).

In a microbial network, species associate with each other directly or indirectly through competition, mutualism or predation, and their interactions are critical evolutionary pressures for natural selection during ecological evolution and could influence the response of communities to environmental change (Pilosof et al. 2017; Wagg et al. 2019), therefore affecting the community biodiversity, composition, complexity, and stability of microbial ecosystems (Ghoul and Mitri 2016; Ratzke et al. 2020). Furthermore, interactions have the potential to reorganize with the threats of global change and human activity (Yuan et al. 2021). However, knowledge of microbial interactions and their dynamics under long-term succession remains largely insufficient (Faust 2021), and these knowledge gaps lead to challenges in predicting the dynamics of the microbial community and its associated functions under future disturbances (Ratzke et al. 2020; Yuan et al. 2021). Considerable evidence has demonstrated that the complicated interactions between coexisting organisms could be represented by ecological cooccurrence networks, with species as nodes and their relationships as links (de Vries et al. 2018). Interaction networks can be used to assess the complexity and predict the stability of microbial communities (Banerjee et al. 2019; Yuan et al. 2021). Microbial cooccurrence networks have been increasingly used to explore the complex relationships among myriad microbial species (Faust 2021). However, how the interactions, complexity, and stability of microbial communities in biocrusts and subsoil change along with succession remains uncertain (Morrien et al. 2017; Guo et al. 2018).

In deserts, the surface of mobile dunes is fixed under restoration measures, cyanobacteria and algae settle on the physical crust first, and then the colonization and succession of biocrusts begins (Li et al. 2010). Researchers have found that the colonization and development of biocrusts either through natural or assisted processes can profoundly alter soil and ecosystem function and accelerate ecosystem recovery and succession (Miralles et al. 2013; Weber et al. 2016). As ecosystem engineers in high-abiotic-stress systems, the provision of nutrients by biocrusts could directly influence the bacterial community in the subsoil on a very fine scale (Liu et al. 2019). Biocrusts could also indirectly influence the microbial communities in subsoil due to changes in soil physicochemical conditions (Ghiloufi et al. 2019). In addition, the presence of different microbial communities in biocrusts and the leaching of certain secondary metabolites from biocrusts to the subsoil may influence the composition of the soil microbial communities (Ghiloufi et al. 2019). This presence in turn greatly influences the response of subsoil functioning to global environmental change. Nevertheless, very little is known about the succession dynamics of the community diversity of bacterial communities in biocrusts and in substrates colonized by biocrusts, and even less is known about bacterial community complexity and stability dynamics. How soil bacterial community diversity, complexity, and stability are affected by biocrust or the factors that determine changes in their subsoil bacterial communities remains largely understudied.

Given the accelerating rate of dryland degradation under global change (Huang et al. 2016), with the threats of soil degradation and erosion, studies on the long-term dynamics of biocrust and soil bacterial communities and driving factors during succession are urgently needed to inform the conservation and restoration of dryland ecosystem functioning and services. To understand the succession dynamics of bacterial communities in both biocrusts and subsoil and explore the factors that determine the changes in subsoil bacterial communities, a 65-year long-term restoration succession sequence on the southeastern edge of the Tengger Desert was selected as a case study. Here, we hypothesized that 1) the succession

of soil bacterial diversity and network complexity and stability in the biocrust and subsoil does not linearly increase with time but rather is a highly dynamic process; 2) the bacterial community diversity, network complexity, and stability in the biocrust and subsoil are inconsistent with the succession process; and 3) biocrusts can mediate not only bacterial community composition but also network complexity (node number, link number, average links per node, etc.) and stability (vulnerability and robustness). An insight into microbial community dynamics in biocrusts and subsoil has high application significance for restoration management to maintain ecosystem stability and enhance the ecological function of artificial ecosystems (Guo et al. 2018; Moreno-Mateos et al. 2017).

## Materials and methods

### Experimental sites and field sampling

The experimental sites were located in the Shapotou revegetated area (37°32′N, 105°02′E; 1330 m above sea level) on the southeastern edge of the Tengger Desert in northern China (Fig. 1). The region has a typical continental monsoon and desert climate, characterized by a long winter with strong winds and a short summer with drought. The annual average temperature is 9.6 °C, the annual average precipitation is 180 mm, and the annual average pan evaporation is approximately 2900 mm. In addition, precipitation in this region is concentrated from July to September (mostly heavy rains). These data were obtained from long-term observations of the Shapotou Desert Research and Experiment Station, which is located in the Shapotou revegetated area and 100 m away from the collection sites. The annual average wind velocity is 2.9 m/s, and the annual number of dust storm days per year is 59 (Li et al. 2014). The soil is wind-borne sand (according to Eutric Arenosols in the World Reference Base, FAO-ISRIC-ISSS, 1998) with a pH of 7.5–11.2. The dominant plants in this area were Psammophytes, including *Hedysarum scoparium* Fisch., *Artemisia ordosica* Krasch., *Caragana korshinskii* Kom., *Setaria viridis* (L.) Beauv., *Eragrostis minor* Host, and *Bassia dasyphylla* (Fisch. et al. et Mey.) O. Kuntze, et al.

To prevent the Baotou–Lanzhou railway from possibly being damaged by wind erosion and sand burial, a 16 km-long artificial vegetation project was built along the railway by utilizing the coupled methods of checkerboards and shrub plantations to stabilize blowing dunes in 1956 (Li et al. 2010). Straw checkerboards (1 m × 1 m) were constructed, within which 2-year-old xerophytic shrubs were planted (*A. ordosica*, *C. korshinskii*, and *H. scoparium*). The artificial ecosystem was shielded from further anthropogenic disturbances such as fires and grazing since plant establishment. To improve the regional environment, this nonirrigated revegetation system has been duplicated several times for the expansion of protective achievements. Following the stabilization of sand dunes, the colonization and development of biocrusts are considered important indicators of desertification reversal. The revegetation system has provided a unique and long successional chronosequence for investigating and revealing the evolutionary dynamics of biocrust-related ecosystem processes. In the present study, we used a "space-for-time" approach (Li et al. 2007); biocrusts and subsoil were sampled in July 2021 in the revegetation enclosures, constructed in 1956 (65 years of revegetation, 65Y, moss-dominated biocrust), 1964 (57 years of revegetation, 57Y, moss-dominated biocrust), 1973 (48 years of revegetation, 48Y, moss-dominated biocrust), 1981 (40 years of revegetation, 40Y, lichen-moss mixed biocrust), 1991 (30 years of revegetation, 30Y, lichen-dominated biocrust), and 2000 (21 years of revegetation, 21Y, cyanobacteria-dominated biocrust). In addition, mobile sand (MS) nearby was used as a representation of the initial status. Before ecological restoration, the basic conditions of the sites in the study area were consistent.

Five 10 × 10 m sampling plots in each revegetation area were delineated with at least 20-m buffers between plots. Five 1 × 1 m subplots were established from a diagonal intersection and four vertices of each plot. The plant diversity (Shannon index) was investigated; shrub cover, herb cover, and biocrust cover were estimated; herbaceous biomass and litter biomass were investigated; and the thickness of the biocrusts was also measured. Five biocrusts and subsoil cores (3.5 cm diameter, 5 cm depth) from the subplots of each plot were sampled, and equal amounts of each of the five cores were pooled and thoroughly mixed to form one composite biocrust or subsoil sample. All samples were obtained with a sterile trowel to prevent contamination. Biocrust or subsoil samples were

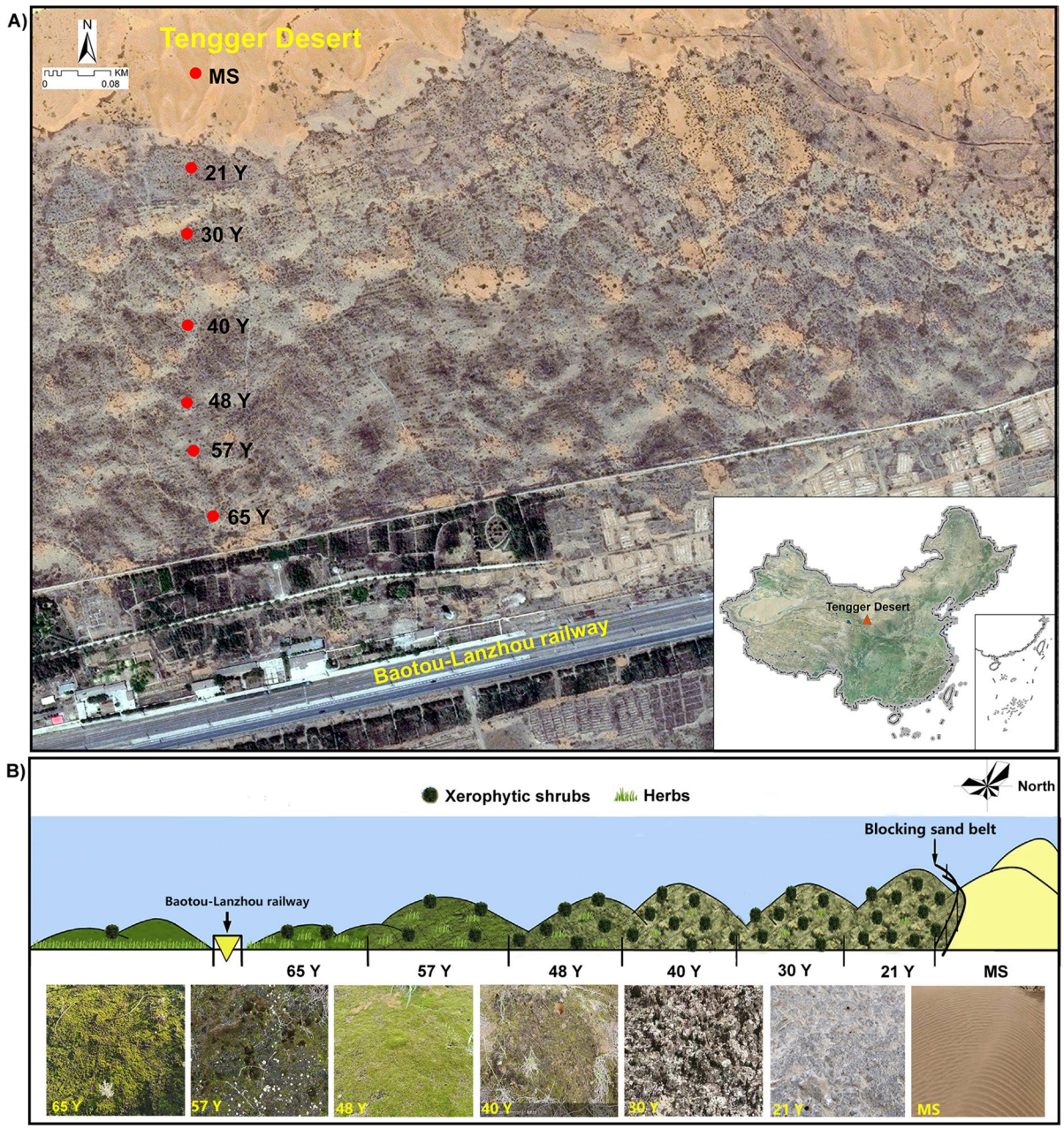



**Fig. 1** Study area and sampling sites on the southeast edge of the Tengger Desert. (**A**) Study area and sampling sites, (**B**) schematic description of the artificial ecosystem and biocrusts in different succession stages

preserved in a car refrigerator, taken back to the laboratory, passed through a 1-mm sieve to remove stones and plant debris immediately, and homogenized thoroughly. Then, biocrust and subsoil samples were stored at − 80 °C for nucleic acid-based molecular analysis. Finally, 35 biocrust samples (5 cores × 7 plots) and 35 subsoil samples (5 cores × 7 plots) were obtained. In addition, three other parts of the subsoil samples were collected for the determination of soil physicochemical properties. In the first part, the soil samples in the cutting ring sampler and aluminum box were dried and weighed to determine the soil bulk density (BD, g/cm$^3$)

and soil water content (SW, %); the second part was stored at 4 °C after being passed through a 2-mm mesh sieve and removing plant roots for determination of the soil enzyme activities and soil available nutrients; and the third part was air-dried to measure other soil properties.

## Microbial community characterization

Biocrusts and subsoil microbial DNA were extracted from 1 g of each well-mixed sample by grinding and purified with the PowerSoil DNA isolation kit (Mobio Laboratories, Carlsbad, CA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop 2000c (Thermo-Fisher Scientific), and DNA quality was checked by 1% agarose gel electrophoresis. The genetic polymorphism within the nuclear rDNA operon was assessed by adopting a nested polymerase chain reaction (PCR) strategy with a thermocycler PCR system (GeneAmp 9700, ABI, USA). Then, the 16S rRNA gene was PCR-amplified using the specific primer set 338F (5'- ACTCCTACGGGA GGCAGCA-3') and 806R (5'- GGACTACHVGGG TWTCTAAT-3'), targeting the V3–V4 regions. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, and a final extension at 72 °C for 7 min. The PCRs were performed in triplicate, and the barcode sequences of each sample were unique. The resulting PCR products were extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's instructions. Sequencing libraries were generated using equimolar and paired-end sequencing on an Illumina NovaSeq 6000 platform (BMK Technology Co., Ltd., Peking) according to standard protocols.

After sequencing, the original data were sorted into valid reads. Quality filtering was performed on the raw fastq files to obtain high-quality clean reads by Trimmomatic (v. 0.33) (Bolger et al. 2014). Clean paired-end reads were merged using FLASH (v. 1.2.11) (Magoč and Salzberg 2011) according to their overlap. Finally, sequences of each sample were assigned based on barcodes and primers, reads containing ambiguous bases were discarded, and reads of less than 200 bp in length or with a quality score < 25 were eliminated by UCHIME version 8.1 (Edgar et al. 2011). The minimum, mean, and maximum numbers of reads were 48971, 76580.09 and 107776, respectively. Sequences with greater than or equal to 97% similarity were clustered to the same operational taxonomic unit (OTU) by UPARSE (version 7.1 http://drive5.com/uparse/). Taxonomic identification was performed using the Naive Bayes classifier in QIIME2 against the SILVA database (Release 132, http://www.arb-silva.de; Pruesse et al. 2007) with a confidence threshold of 70%. To avoid large differences in sequencing depth across samples, singleton OTUs were removed, and sequences were resampled to the same sequence depth (30,000 sequences per sample) across all samples (Yuan et al. 2021). Then, the relative abundance of an operational taxonomic unit (OTU) in each sample was calculated. The taxonomic composition of the networked communities under the biocrusts and subsoil at the phylum, family, genus, and OTU levels were analyzed, and Mann–Whitney U tests were used to evaluate the abundance change of each taxon. Alpha diversity (Shannon index) analysis was used to reflect the diversity and richness of the bacterial community, and beta diversity was calculated using nonmetric multidimensional scaling (NMDS) and the permutational multivariate analysis of variance (PERMANOVA) method with R (http://www.r-project.org/) based on the Bray–Curtis distance to analyze the overall differences in bacterial community composition.

## Network construction and characterization

Cooccurrence networks were constructed based on Pearson correlations of log-transformed OTU abundances, and the correlation cutoff threshold was determined in an automatic fashion by a random matrix theory (RMT)-based approach (Zhou et al. 2010; Yuan et al. 2021). The RMT-based network approach could minimize the uncertainty in network construction and comparison by its mathematically defined nonarbitrary correlation cutoff. Although no method is perfect, we believe that this approach is the most appropriate choice for this study. Network construction was performed by the Molecular Ecological Network Analysis Pipeline (MENAP, http://ieg4.rccc. ou.edu/MENA/). Only OTUs appearing

in three replicates (a total of five replicates for each developmental stage) were used for network construction. The relative abundances of OTUs were log-transformed, Pearson's correlation coefficient was calculated for each pair of OTUs, and the missing data were filled with a small value (0.01) if paired valid values were available. This correlation matrix was then transformed into an adjacency matrix by taking the absolute values. An appropriate threshold was identified from a series of thresholds from 0.30 to 1.00 until significant nonrandom patterns were detected in the network, and only correlations above the optimal threshold were used for defining the adjacency matrix.

Various network topological indices were calculated in the MENAP interface, including $n$ (total number of nodes, which is used to indicate network size), $L$ (total number of links), average K (average links per node), average CC (average clustering coefficient, the extent to which nodes are clustered), Con (connectance, the proportion of realized links in all possible links) and modularity (the degree to which a network is compartmentalized into different modules), which were calculated as per the method of Deng et al. (2012). To test the significance of the constructed empirical networks, 100 random networks were generated by rewiring the links among the nodes while constraining $n$ and $L$ randomly. The same topological properties of the 100 random networks were calculated, and then the means and standard deviations were calculated and compared with those from the corresponding empirical networks. All networks in this study were constructed and analyzed using the MENAP. Modularity measures the degree to which a network is compartmentalized into different modules. As the network size and connectivity varied substantially among the different networks, relative modularity (RM) is more meaningful for comparing modular structures across different networks. In this study, RM was calculated using the following formula (Yuan et al. 2021):

$$\mathrm{RM} = \frac{M - \overline{M_r}}{\overline{M_r}}$$

where $M$ is the modularity of the empirical network and $\overline{M_r}$ is the mean modularity of the random networks.

Robustness and vulnerability were used to characterize the stability of the networks. The stability of the networks was indicated based on both simulations of extinction and empirical observations (Montesinos-Navarro et al. 2017). To test the effects of species removal on the remaining species, the abundance-weighted mean interaction strength (wMIS) of node $i$ was calculated as follows:

$$\mathrm{wMIS_i} = \frac{\sum_{j \neq i} b_j s_{ij}}{\sum_{j \neq i} b_j}$$

where $b_j$ is the relative abundance of species $j$ and $s_{ij}$ is the association strength between species $i$ and $j$, measured by Pearson's correlation coefficient. After removing the selected nodes, if wMISi = 0, all of the links to species $i$ were removed; if wMISi < 0, then t mutualistic association between species $i$ and other species is insufficient for its survival; thus, node $i$ was considered isolated and thus removed from the network. This deletion was repeated until all species had positive wMISs. Robustness is defined as the proportion of the remaining species in this network after random or targeted node removal. In this study, random removal robustness was measured when 50% of random nodes were removed, and targeted removal robustness was measured when five module hubs were removed (Yuan et al. 2021).

The vulnerability of the networks was assessed by the maximal vulnerability of nodes in the network. The vulnerability of each node was measured by the relative contribution of the node to the global network efficiency. The vulnerability of the networks was calculated as follows:

$$\mathit{Vulnerability} = max\left(\frac{E - E_i}{E}\right)$$

where $E$ is the global efficiency and $E_i$ is the global efficiency after removing node $i$ and its entire links (Deng et al. 2012). The global efficiency of a network was calculated as the mean of the efficiencies over all pairs of nodes as follows:

$$E = \frac{1}{n(n-1)} \sum_{i \neq j} \frac{1}{d(i,j)}$$

where $d(i,j)$ is the number of edges in the shortest path between nodes $i$ and $j$.

The detailed calculation method of robustness and vulnerability was described by Yuan et al. (2021). All calculations were performed in R.

## Determination of soil physicochemical properties and biocrust attributes

Soil nitrate ($NO_3^-N$) and ammonium ($NH_4^-N$) were determined using the phenol disulfonic acid colorimetric method and the indophenol blue colorimetric method, and soil available nitrogen (AN, mg/kg) for each sample was defined as the sum of soil nitrate nitrogen and ammonium nitrogen. Soil available phosphorus (AP, mg/kg) was extracted with sodium bicarbonate and then determined by the molybdenum blue method. Soil pH was measured using an electronic pH meter. Soil organic matter (SOM, g/kg) was determined by the potassium dichromate external heating method. The soil total carbon (TC, g/kg) and total nitrogen (TN, g/kg) contents were tested by the dry combustion method (Vario MACRO cube, Elementar, Germany). The soil total phosphorous (TP, g/kg) concentration was measured using the colorimetric method with ammonium molybdate-vanadate as the coloring reagent. The soil particle size distribution was determined by a laser particle size meter (Mastersizer 2000; Malvern), and the soil particle size fraction was graded according to the standards of the United States Department of Agriculture. Soil enzyme activities were assessed in light of peroxidase, dehydrogenase, urease, alkaline protease, and sucrase activities and measured according to Dick (2020). Biocrust attributes were determined by morphological features, including biocrust thickness and cover.

## Statistical analyses

Networks were visualized using the interactive platform Gephi version 0.9.2 (https://gephi.org/). All statistical analyses were conducted in R 2021 version 4.0.5 software (https://www.r-project.org/). One-way analysis of variance (ANOVA) and post hoc LSD analysis were performed to determine the differences in the bacterial community diversity and composition, the relative proportion of the classifications, the network complexity, and stability in biocrusts and subsoil at different stages. We conducted regression analysis on the network complexity attribute and stability attribute. To make the models more accurate, we compared and selected the regression models by combining $R^2$ and AIC (akachi information criteria) and finally chose the trinomial regression (Supplementary Material 3).

An intriguing question is whether and how environmental factors affect microbial community diversity, composition, and network properties in biocrusts and subsoil. We thus address this question using the partial Mantel test to decipher drivers for these significant relationships. Therefore, Pearson's correlation analyses and Mantel tests (with the Mantel function) were performed to examine how soil physicochemical properties, plant characteristics, and biocrust characteristics were correlated with bacterial community diversity, composition, and network properties in biocrusts and subsoil, which were conducted in the vegan (version 1.4–268) package in R (Oksanen et al. 2019). The normality and equal variance of datasets were tested, and the data were standardized by log transformation. A principal component analysis (PCA, R package *FactorMineR* 1.29) was used to reduce the dimension of multidimensional variables and facilitate indicator selection by categorically grouping the plant (shrub cover, herb biomass, herb cover, litter biomass and plant diversity), soil (SOM, TC, TN, AN, TP, AP, pH, SW, BD, sand, silt, clay, peroxidase, dehydrogenase, urease, alkaline protease and sucrase), biocrust (biocrust cover and thickness), biocrust bacterial community and subsoil bacterial community network (*n*, *L*, average K, average CC, Con, RM, target robustness, random robustness, vulnerability, alpha and beta diversity) variables into principal components while minimizing the loss of information (Marsboom et al. 2018). Principal components with high eigenvalues ($>1$) were considered the best representatives of explaining ($>53.37\%$) the variability, and the data used for PCA were pretransformed to avoid the horseshoe effect (Marsboom et al. 2018).

Finally, we fitted a piecewise SEM (Lefcheck 2015) to assess the relative importance of the plant community, soil physicochemical conditions, and biocrust in determining subsoil bacterial community composition and network properties. Piecewise SEM is conceptually similar to classical path analysis; it solves each component model separately different from using global estimation from a single variance–covariance matrix and thus can operate

with smaller sample sizes and allow for models with their own sampling distributions (Lefcheck 2015). Compared with traditional variance covariance-based SEM, piecewise SEM can piece multiple separate linear models together into a single causal network. Therefore, Shipley's test of *d*-separation was used to assess the overall fit of the piecewise SEM (Shipley 2013), and Fisher's C test statistic was generated and Akaike's information criterion (AIC) was used to compare the models with some paths included or removed by piecewise SEM (http://jonlefcheck.net/2014/07/06/piecewise-structural-equation-modeling-in-ecological-research/). The best model using the lowest AIC values was adopted to present the multivariate effects of the variables. Piecewise SEMs were conducted in the piecewiseSEM package of R 2021 version 4.0.5 software.

## Results

### Bacterial diversity and community composition

The bacterial community diversity (Fig. 2A) and OTU numbers (Fig. 2C) in the biocrust layer increased significantly with succession in the first 30 years and then tended to be relatively stable. Comparatively, the bacterial community alpha diversity (Fig. 2B) in the subsoil increased significantly with succession. However, the OTU numbers in subsoil trends could be divided into two stages (Fig. 2D), increasing with succession during the first 40 years and then suddenly transitioning to another increasing state from 40 to 65 years. As shown in the nonmetric multidimensional scaling ordination plots based on Bray–Curtis dissimilarity matrices, the bacterial community composition in biocrusts was clustered into four distinguishable groups, in which MS, 21 Y and 30 Y were separated from all groups, while 40 Y, 48 Y, 57 Y and 65 Y were not clearly separated (Fig. 2E). Similarly, the bacterial community composition was separated in the subsoil at all successional stages (Fig. 2F). The proportions of unique genera and OTUs in both biocrust and subsoil bacterial communities were particularly low (Fig. S1).

The dominant phyla and genera of the bacterial communities in the biocrusts and subsoil were different (Fig. S2). Before biocrust development, Actinobacteria (32.60%) accounted for the highest proportion in MS. After crust development, the relative abundance of Actinobacteria in the biocrust layer decreased sharply (21Y: 9.53%, 56Y: 4.66%), and Proteobacteria and Acidobacteria were the top 2 bacterial phyla in all biocrust types. For the subsoil, Actinomyces accounted for the highest relative abundance in the subsoil layer of cyanobacteria crust (21Y: 38.44%) and lichen crust (30Y: 31.48%), then decreased sharply to less than 20% after succession to moss crust (48Y) (Fig. S2), and Acidobacteria and Proteobacteria exceeded Actinobacteria in the late successional stage. Notably, the proportion of Cyanobacteria in 21Y was as high as 12.79%, much higher than in other samples, including the biocrust and its subsoil.

At the genus level, uncultured_bacterium_c_Acidimicrobiia (6.41%, Actinobacteria) had the highest relative abundance in MS, while it decreased rapidly after biocrust development, with a relative abundance of 0.5% (21Y) in cyanobacteria crust and 1.19% (30Y) in lichen crust; the highest value in the moss crust was 1.17% (40Y), and the lowest value was 0.77% (56Y). The genus uncultured_bacterium_f_Sphingomonadaceae (Proteobacteria) had the highest relative abundance among all the other biocrust types, and it increased gradually with succession. In the subsoil, RB41 (Acidobacteria) had the highest relative abundance in the subsoil layer of lichen and moss crust, while uncultured_bacterium_c_Acidimicrobiia (9.28%) had the highest relative abundance in the subsoil of cyanobacteria crust. The genus uncultured_bacterium_c_Subgroup_6 (Acidobacteria) was the second most abundant bacterial genus in all subsoils, and the genera after third place were different in all subsoils (Fig. S2).

### Bacterial cooccurrence network complexity

To determine how the bacterial network complexity in biocrusts and subsoil changes with succession, thirteen networks were constructed (Fig. 3A), and six network topological parameters were regressed against time (Fig. 3B–G). For bacterial network complexity in the biocrusts, the nodes, links, average K, average CC, connectance and relative modularity all suggest "temporal partitioning". Bacterial network complexity in the biocrusts increased with succession during the first 40 years, and the complexity in biocrusts reached the approximate largest level in the

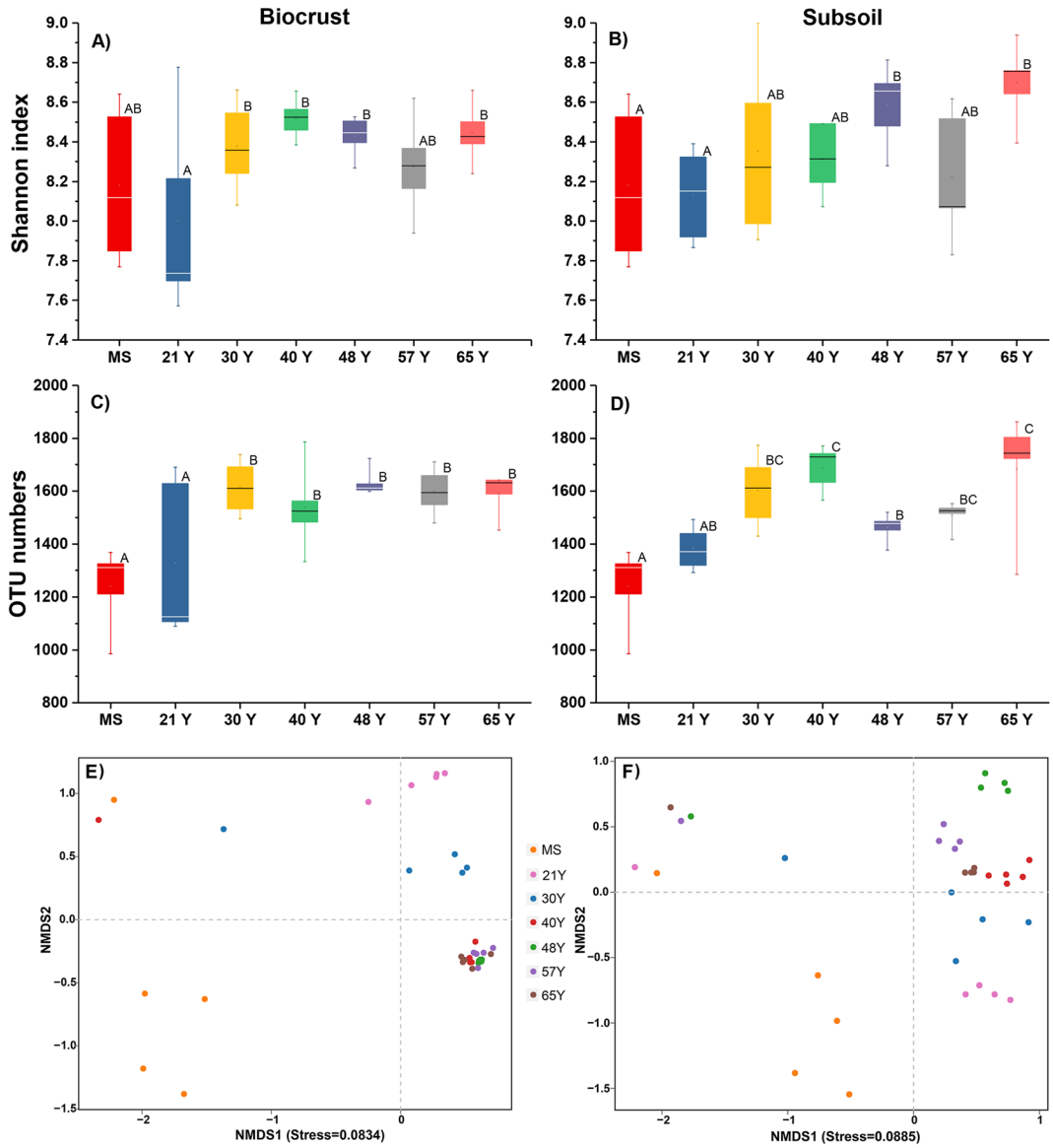



**Fig. 2** The bacterial community diversity is indicated by the Shannon index (at the OTU level (**A**, **B**)) and OTU numbers (**C**, **D**) in biocrusts and subsoil of different succession stages. Different uppercase letters indicate significant differences at $P < 0.05$. The similarity of bacterial community composition (**E**, **F**) is indicated by the nonmetric multidimensional scaling (NMDS) method based on Bray–Curtis distances in biocrusts and subsoil

40 years and then experienced a sudden decrease to a lower state at approximately 48 years, with a slow increase in the later stages with biocrust development (Fig. 3B–D). The average CC experienced a sharp decrease, reached the approximate lowest level at 57 years, and increased from 57 to 65 years (Fig. 3E). However, bacterial network complexity in the subsoil increased during the initial 21 years. Then, although the network size ($n$) of the bacterial community in the subsoil increased with time from 21 to 40 years, the network connectivity, average connectivity, average clustering coefficient, connectance, and relative modularity all experienced a decrease; the network connectivity and average connectivity decreased to the level of MS; and the average clustering coefficient, connectance, and relative modularity

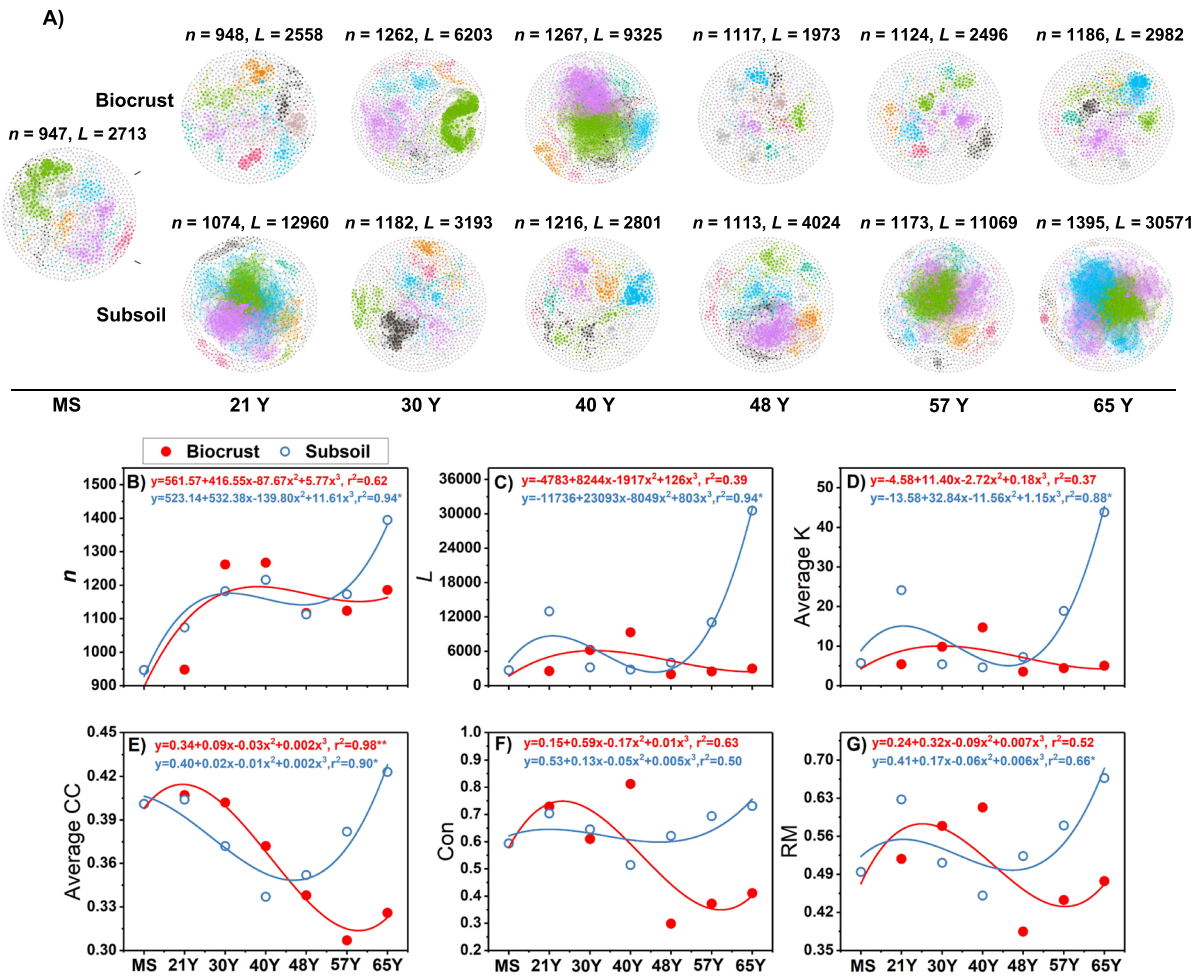



**Fig. 3** Succession of bacterial community networks over time in biocrust and subsoil (**A**). Large modules with ≥ 5 nodes are shown in different colors, and smaller modules are shown in gray; temporal changes in bacterial network topology, including *n* (**B**), *L* (**C**), average K (**D**), average CC (**E**), Con (**F**), and RM (**G**). In each panel, filled red symbols represent networks in the biocrust bacterial community, and open blue symbols represent networks in the subsoil bacterial community. The fitting formula and adjusted $r^2$ and *P* values from polynomial fitting are shown. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$

were much lower than those in MS. In addition, once succession exceeded 48 years and the growth rate of network complexity in the subsoil increased dramatically, the complexity increased linearly with time. In addition, the number of positive and negative links in both biocrust and subsoil increased first and then decreased in the early and middle stages of succession, and they increased more significantly in the soil layer than in the biocrust layer in late succession. However, the proportion of positive and negative links in both biocrust and subsoil showed opposite trends with the progress of succession (Fig. S3).

## Bacterial cooccurrence network stability

The network vulnerability (the maximum decrease in network efficiency when a single node was deleted from the network) in biocrusts and subsoil showed opposite trends. In biocrusts, the network vulnerability first decreased and then increased over time and reached the approximate largest level in the 48 years (Fig. 4A). The network vulnerability of the bacterial community in the subsoil first increased and then decreased over time, reached the approximate lowest level at 48 years and remained stable after 48 years

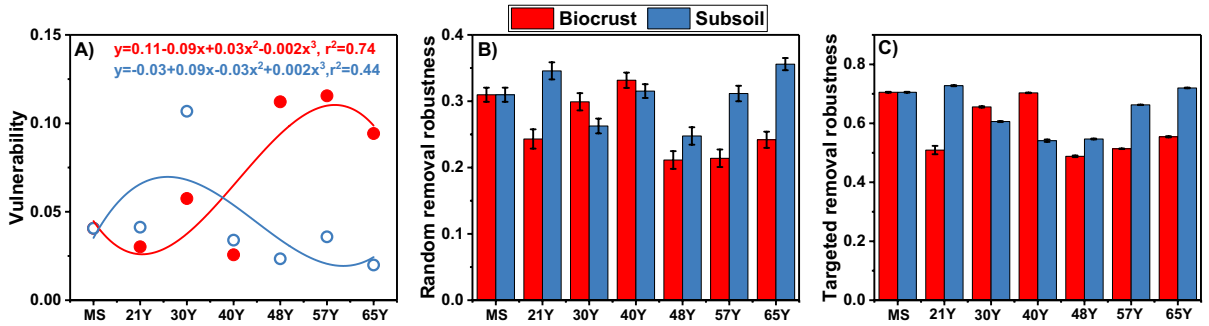



**Fig. 4** Temporal changes in network vulnerability, network robustness based on random species loss, and targeted removal of module hubs. **A** Filled red symbols represent networks in the biocrust bacterial community, and open blue symbols represent networks in the subsoil bacterial community. The fitting formula and adjusted $r^2$ and $P$ values from polynomial fitting are shown. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$. **B** Robustness measured as the proportion of taxa remaining, with 50% of the taxa randomly removed from each of the empirical networks. **C** Robustness measured as the proportion of taxa remaining with five module hubs removed from each of the empirical networks. In B and C, each error bar corresponds to the standard deviation of 100 repetitions of the simulation

of succession. We simulated species extinction and calculated the robustness (resistance to node loss) of the networks. For bacterial networks in the biocrusts, based on either random species loss or targeted removal of module hubs, the robustness of the network decreased in the initial 21 years, then increased at a high rate, and finally reached the approximate largest level in 40 years. A dramatic decline in stability occurred from 40 to 48 years, and then stability increased slowly over time after 48 years of biocrust attributes (Fig. 4B–C). For bacterial networks in the subsoil, both based on random species loss and targeted removal of module hubs, the network robustness increased in the initial 21 years and decreased in the next 20 years; meanwhile, after 48 years of biocrust attributes, the network robustness of the subsoil increased dramatically over time, similar to network complexity. In the later stage of succession, the network robustness in the subsoil was higher than that in the biocrusts, and the increase rate was also higher than that in the biocrusts.

Correlations between bacterial community diversity and environmental factors.

We identified correlations between microbial diversity and environmental factors (Fig. 5). The alpha diversity in biocrust was significantly positively correlated with biocrust cover and plant diversity, and the beta diversity was significantly correlated with biocrust cover and peroxidase. However, there were no significant relationships between network properties and environmental factors (Fig. 5A). Comparatively, the beta diversity in the subsoil was extremely significantly correlated with pH and peroxidase and significantly correlated with several environmental factors. In addition, the network properties in the subsoil were significantly correlated with herb biomass, litter biomass, SW, and dehydrogenase. However, there were no significant relationships between community diversity in the subsoil and environmental factors (Fig. 5B). Altogether, these results indicated that more environmental factors have stronger effects on bacterial communities in the subsoil than in biocrusts.

## Potential drivers of the subsoil bacterial community composition and network properties

Two piecewise SEMs were used to assess the relative importance of succession, plant community, subsoil physicochemical conditions, biocrust development, and bacterial community in determining subsoil bacterial community composition (Fig. 6A) and subsoil bacterial community network properties (Fig. 6B). The piecewise SEMs revealed that the predictors explained 52% ($R^2$) of the variation in subsoil bacterial community composition and 80% ($R^2$) of the variation in subsoil bacterial community network properties (Fig. 6). Succession had negative effects on subsoil bacterial community composition ($P < 0.05$), but its effects on subsoil bacterial community network properties were not significant. Furthermore, succession had positive effects on the

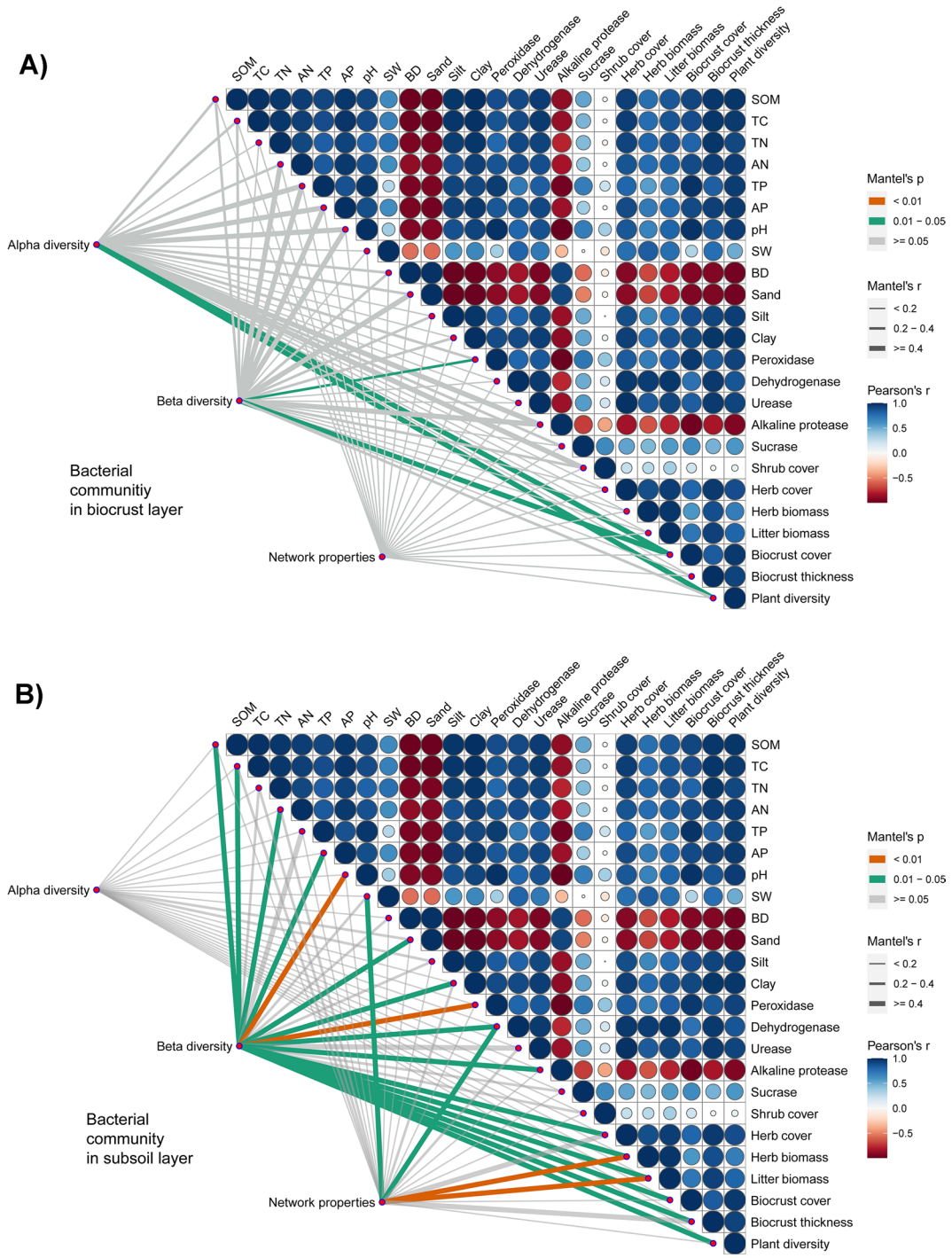
plant community ($P < 0.05$), subsoil physicochemical conditions, and biocrust development ($P < 0.001$) but no significant link with biocrust bacterial community composition (Fig. 6A). The model also revealed that, of all variables assessed, changes in succession and soil physicochemical conditions occurred. The composition of the biocrust bacterial communities constituted the strongest direct driver of changes in the composition of subsoil bacterial communities. The surrounding plant communities had an additional indirect effect on the subsoil bacterial community composition due to their influence on the soil physicochemical conditions. In contrast, no link between the composition of subsoil bacterial communities and biocrust development could be detected. The changes in the surrounding plant communities and composition of the biocrust bacterial communities constituted the strongest direct driver of changes in the network complexity and stability of the subsoil bacterial community (Fig. 6B).

## Discussion

The bacterial communities in biocrust and subsoil differed significantly with ecosystem succession over time on the basis of bacterial diversity indices. Previous studies assessing ecosystem succession in soil bacterial communities focused on simple attributes such as taxonomic diversity and did not combine them in most cases (Moreno-Mateos et al. 2017). We found that bacterial Shannon diversity and bacterial dissimilarities in both biocrusts and subsoil increased with vegetation restoration over time. Generally, an increase in soil bacterial diversity will likely increase soil multifunctionality, positively impacting soil quality and functions (Delgado-Baquerizo et al. 2016). Thus, bacterial community diversity is generally used to identify the successional stages of biocrusts, and a higher community diversity of bacteria is associated with more stable and developed successional biocrust stages (Maier et al. 2018). Twenty-one years might be a turning point in biocrust bacterial community diversity, which transforms to another state after 30 years. In the subsoil, the turning point was approximately 40 years, which was later than that of the biocrust bacterial community. This can also be confirmed by changes in the relative abundance of the bacterial community. For example, the relative abundance of Actinobacteria in MS was the highest, while from 21Y, the highest relative abundance changed to Proteobacteria. Furthermore, the relative abundance of Actinobacteria decreased rapidly after 21Y and continuously with succession. In the subsoil, the relative abundance of Actinobacteria was the highest in the first three successive stages and decreased continuously after 40Y, while Acidobacteria increased continuously after 40Y (Fig. S2). This could be because more Proteobacteria may contribute to the production of more exopolysaccharides to facilitate soil stabilization and biocrust formation (Abed et al. 2019).

This finding furthers past work by showing that biocrust bacterial community diversity is more sensitive to environmental changes in the early stage of succession and that reaching a relatively stable state takes only approximately 21 years; meanwhile, it takes a longer time in subsoil bacterial communities. A previous study in this area found that there were two turning points in herb coverage, which occurred at 19 years (17–26 years) and 38 years (32–40 years), and the turning point in shrub coverage occurred at 37 years (13–37 years), which corresponded to the changes in bacterial community diversity in the biocrust and subsoil, respectively, in this study (Chen et al. 2019). This may be related to the natural succession of revegetation. The revegetation project reduced wind erosion; then, biocrusts colonized, and their cover and thickness increased over time, further increasing the water holding capacity of the biocrust layer and eventually decreasing infiltration to subsoil layers (Chen et al. 2018b). Shrub cover probably increased initially due to high deep soil water availability but decreased from then onward as biocrust increased and deep soil water decreased (Li et al. 2014). Eventually, biocrust exceeded a threshold where grasses could expand, resulting in an abrupt shift in community structure, and the associated bacterial community also changed correspondingly (Chen et al. 2020). This result is also consistent with the estimated succession of moss-dominated biocrusts in the Chinese Loess Plateau, which is approximately 15–20 years (Xiao et al. 2019), and similar to the estimated rates from the Negev Desert of approximately 20 years based on biodiversity assessments (Kidron et al. 2008).

Some studies demonstrate that judging the successional stage and effectiveness only from the perspective of diversity leads to bias (Moreno-Mateos




et al. 2017). Moving beyond traditional diversity assessments to a broader perspective, the interactions between species and the complexity and stability of ecosystems were quantified in this study. Our results showed that the trends of bacterial network complexity and stability in the subsoil could be divided into three stages, and the turning points were 21 years and 40 years. According to these three periods, we can divide the successional stages into early (MS-21 years), middle (21–40 years), and

◂**Fig. 5** Relationships among the community diversity, composition, network structures, and environmental variables in biocrusts (**A**) and subsoil (**B**). The edge width corresponds to Mantel's *r* value, and the edge color denotes the statistical significance. Pairwise correlations of these variables are shown with a color gradient denoting Pearson's correlation coefficient. Soil variables included soil organic matter (SOM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), and soil available nutrients, including soil available nitrogen (AN) and soil available phosphorus (AP); pH; soil water content (SW); bulk density; soil texture, including sand, silt, and clay proportions; enzyme activity, consisting of peroxidase, dehydrogenase, urease, alkaline protease, and sucrase; plant variables, including shrub cover, herb cover, herb biomass, litter biomass, and plant diversity; and biocrust variables, including biocrust cover and thickness

later successional stages (48–65 years). The network complexity increased and stability decreased in the biocrust bacterial community in the early stage, and both the complexity and stability increased in the middle stage. Then, a sudden decrease in complexity occurred from 40 to 48 years and increased slowly in the later stage. Although subsoil is the source of bacteria of the biocrust (Steven et al. 2013; Liu et al. 2019), once biocrusts begin to develop on the sand surface, they dominate the shift of the subsoil bacterial community during the transition process of biocrust types, such as cyanobacteria crust changing to lichen crust after 21Y, and lichen-moss mixed crust at 40Y is a transitional biocrust type. Several studies have reported Acidobacteria to be avid rhizosphere colonizers, and their preponderance in the biocrust and subsoil indicate their active roles in plant−soil ecosystems (da Rocha et al. 2010). In addition, the relative abundance of the Cyanobacteria phylum in the cyanobacterial crust was higher than that in the other biocrusts and decreased gradually with succession in the biocrust layer. This indicates that the fixation of carbon and nitrogen mainly depended on Cyanobacteria at the early succession stage. As the composition of the bacterial community changed, the increase in RB41 (Acidobacteria) and uncultured_bacterium_f_Longimicrobiaceae (Gemmatimonadetes) replaced some cyanobacteria in fixing carbon and nitrogen. In this process, the shift of dominance or key bacteria (Proteobacteria or Cyanobacteria) will change the competition and cooperation between species in the bacterial community, which will affect the complexity and stability of the bacterial network (de Vries et al. 2018; Bastida et al. 2014).

Previous studies in this area suggested that the artificially restored ecosystem had undergone a "regime shift" from a nearly bare state to a moderate grass cover state after more than 30 years (Chen et al. 2018a). Although biodiversity in biocrusts recovered to a relatively high level before the ecosystem state transition approximately 21 years ago, the community network complexity and stability will take much longer to recover. This may be because bacterial species are sensitive to the improvement of harsh external environments, so the diversity and complexity of biocrust bacterial communities increased in the early stages. It has been confirmed that microbes living in harsh environments have narrow ecological niches and are sensitive to environmental changes, which may contribute to their instability (Wu et al. 2021). Pearson's correlation analyses showed that biocrust bacterial community diversity is related to biocrust development and plant diversity, while community composition is related to biocrust development and peroxidase activity. No significant correlations were found between abiotic or biological factors and biocrust bacterial network properties, indicating that although soil and other environmental factors were improved during the succession process, they had more effects on biocrust bacterial community diversity and composition and few effects on network complexity and stability (Chilton et al. 2018; Li et al. 2019; Zhang et al. 2022).

However, during the early and middle stages, the succession processes in the subsoil and biocrust were different, and the network complexity and stability in the subsoil increased in the early stage and then decreased in the middle stage. In the early and middle stages of succession, the proportion of positive links decreased sharply (Fig. S3), suggesting that although the subsoil environment improved in the early stage and the soil bacterial community diversity increased rapidly, the environment was still very harsh and had limited resources, resulting in the relationship between bacterial species still being dominated by competition (de Vries et al. 2018). In the later stages of succession, both the number of positive and negative links in the subsoil bacterial network increased sharply, and the proportion of positive links increased, but the proportion of negative links decreased sharply. This may be attributed to the gradual enrichment of resources in the subsoil with succession and the rapid colonization

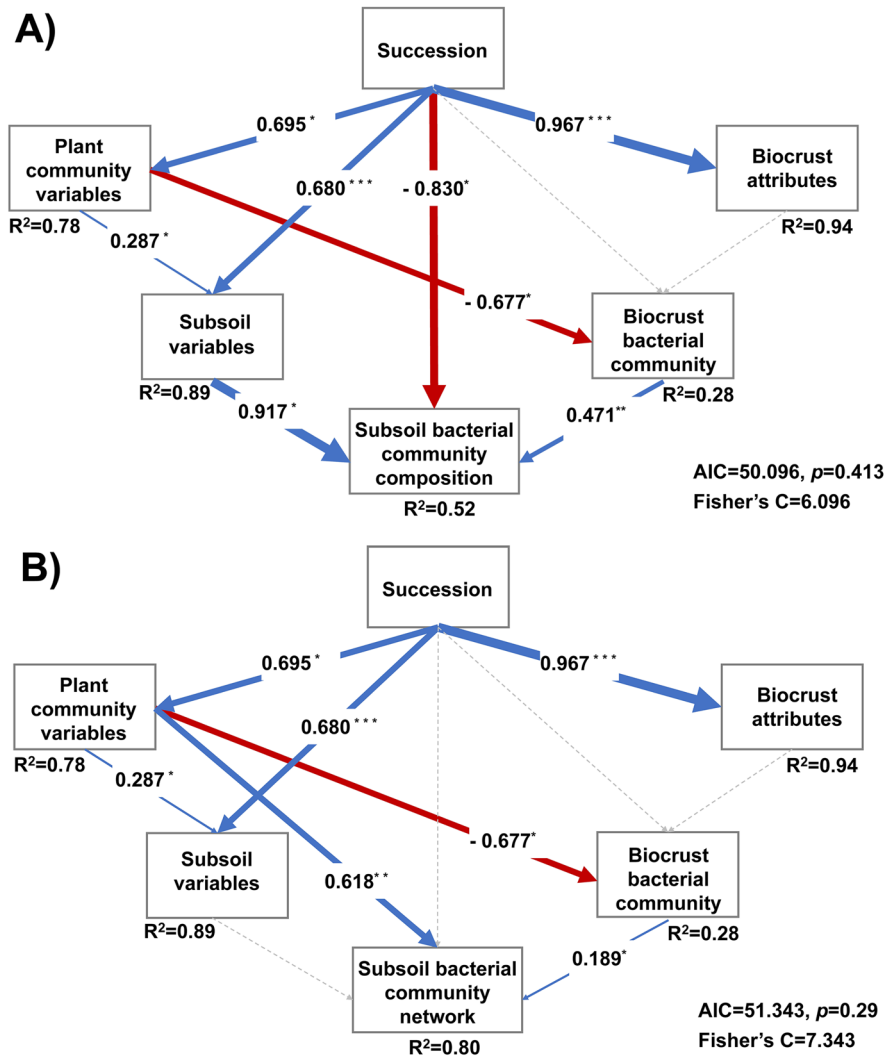



**Fig. 6** Results of the piecewise SEMs to assess the relative importance of succession, plant community, subsoil physicochemical conditions, biocrust development, and bacterial community in determining subsoil bacterial community composition (**A**) and subsoil bacterial community network properties (**B**). Blue lines indicate positive effects, whereas red lines indicate negative effects; paths with nonsignificant coefficients are presented as gray dotted lines. Numbers near arrows are standardized path coefficients, and the width of arrows indicates the strength of significant standardized path coefficients ($P < 0.05$). Significant values are indicated by * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$), and numbers near the boxes indicate the variance explained by the model ($R^2$). Parameters for estimating the model fit, i.e., Akaike information criterion (AIC) and Fisher's exact test (Fisher's *C* and *P*), are also given

of bacterial communities. The increased proportion of positive links, which may represent positive biological interactions, may indicate cooperative behaviors (e.g., cross-feeding, commensalism and mutualistic interactions) between bacterial species and shared environmental requirements rather than competition for limited resources or distinctive environmental niches (de Vries et al. 2018). In addition, the turning points of diversity, complexity, and stability of the bacterial community in the subsoil were all 40 years, lagging behind the transition of the ecosystem state. Our research also showed that the impacts of environmental factors on bacterial communities in biocrusts and subsoil were different. Soil pH is regarded as the most important determinant for soil bacterial communities (Rousk et al. 2010), and our study confirms this point. Peroxidases are capable of degrading complex carbon compounds and are thought to be expressed for the acquisition of not only carbon but also of nitrogen in the soil ecosystem, which affects bacterial composition in both biocrusts and subsoil (Liu et al. 2020). The network properties in the subsoil were significantly correlated with herb biomass, litter biomass, SW, and dehydrogenase. Litter of plant origin is the main source of soil organic matter (Castellano et al. 2015), and dehydrogenase activity can reflect soil bacterial redox metabolism (Tan et al. 2017), which is involved in soil nutrient cycling of N and P, increasing the soil nutrient availability (Wu et al.

2022) and thereby affecting the bacterial community complexity and stability in the subsoil.

Specific soil physicochemical conditions may directly select for particular bacterial species in the soil (Vieira et al. 2020). Alternatively, environmental conditions may affect the bacterial community in the subsoil indirectly by determining the developmental stage and physiological status of the biocrusts (Liu et al. 2019). In addition, biocrusts and bacterial communities undergo different development depending not only on the soil physicochemical conditions but also on the surrounding plants (Zhang et al. 2022). We assessed the potential drivers of soil bacterial communities in the subsoil in more detail by employing an SEM approach. The results revealed that succession, soil physicochemical conditions, and biocrust bacterial community composition constituted the strongest direct drivers of changes in the subsoil bacterial community composition. While soil physicochemical conditions have a stronger effect than other factors on subsoil bacterial community composition, our results indicate that they have no significant effects on subsoil bacterial community network complexity and stability. The plant community and biocrust bacterial community composition constituted the strongest direct driver of changes in the network complexity and stability of the subsoil bacterial community. Previous research has shown that changes in plant communities have long-lasting associations with bacterial networks and communities and strongly govern their recovery (de Vries et al. 2018).

Some studies have suggested that the larger the biocrust cover is, the stronger its effects on the chemical properties of the subsoil and the greater its effects on other soil properties and soil microorganisms (Chilton et al. 2018). There were no significant effects of biocrust on either the biocrust or subsoil bacterial communities in this study. However, the morphological indices of biocrust, such as biocrust coverage and thickness, had relatively rapid growth in the early stage of succession and reached a high and stable level (approximately 80% and 12 mm) at 40 years; thus, their influence may be similar during the later stage. This confirmed our hypothesis that biocrust bacterial community composition had positive effects on both subsoil bacterial community composition and network complexity and stability. These results provide new evidence that biocrust profoundly influences the sustainability of the structure and function of desert ecosystems, particularly their bacterial communities, which drive functions that include but are not limited to the decomposition of organic matter and nutrient acquisition and then greatly influence the response of subsoil bacterial communities and soil function to global environmental change (Belnap et al. 2016; Li et al. 2018; Su et al. 2020). Notably, plant community variables determined the composition of the biocrust bacterial community and were the strongest direct drivers of the network complexity and stability of the subsoil bacterial community. In addition, plant community variables had an additional indirect effect on the subsoil bacterial community composition due to their influence on the soil physicochemical conditions and biocrust bacterial community. These results confirmed earlier findings that on a local scale or for similar soils, plant community variables, including composition and biomass, are predictors of soil bacterial communities. This is possibly due to differences in the quality, quantity, and diversity of the litter produced by different plant communities and used as a major growth substrate of bacteria in soil (Su et al. 2020). In addition, some compounds exuded by particular plant species can cause distinct changes in soil bacterial communities (Vieira et al. 2020). Advancing earlier findings, we further found that in the presence of biocrusts, plants had different effects on bacterial communities in the biocrusts and the subsoil layers. The plant communities had direct effects on biocrust bacterial community composition, but no direct link could be detected with subsoil bacterial community composition.

Our work confirms an interesting point for the restoration of ecosystems: biocrust and subsoil recovery do not linearly increase with time but rather are highly dynamic processes, and fluctuations are unavoidable features of ecosystems, particularly semiarid ecosystems (Weber et al. 2016). Some results showed that bacterial diversity was parallel to the successional stage, gradually increased with succession, and may partially stem from the following reasons: a short study period, nonconsecutive measurements, and an analysis of limited variables that cannot reflect the bacterial community comprehensively (Miralles et al. 2020; Moreno-Mateos et al. 2017). This study can increase our understanding of the succession process of the bacterial community in biocrust and subsoil, shed further light on the regulatory mechanism of the soil bacterial community, and be particularly

valuable for verifying predictions about the effects of biocrusts on the soil bacterial community derived from knowledge of long-term succession sequence data. Biocrusts could mediate the soil bacterial community, not only community composition but also network complexity and stability, and this finding would be particularly important but underappreciated. In addition, more consideration should be given to the interactions between plants, biocrusts, and soil when exploring soil processes and functions. This information would provide more empirical data for ecosystem succession model simulation and prediction.

## Conclusions

In this study, we used a 65-year long-term restoration succession sequence to investigate the variation in the bacterial community in both biocrusts and subsoil and the contribution of the plant community, soil physicochemical conditions, and biocrust to the subsoil bacterial community on the southeastern edge of the Tengger Desert, which has a very fragile and harsh environment. The results revealed that the recovery of soil bacterial α and β diversity and network complexity in biocrust and subsoil did not increase linearly with time but rather was a highly dynamic process. The bacterial community diversity and network complexity and stability in the biocrusts and subsoil were not consistent with the succession process. Our study also identified that the changes in succession, soil physicochemical conditions, and biocrust bacterial community composition were the strongest direct drivers of changes in subsoil bacterial community composition. The plant communities and biocrust bacterial community composition were the strongest direct drivers of changes in the network complexity and stability of the subsoil bacterial community. Biocrusts could mediate the soil bacterial community not only community composition but also network complexity and stability. Collectively, these findings may help us to understand the temporal dynamics of bacterial communities in a long-term restoration succession sequence in an arid artificial ecosystem, and the results provide useful insights into the linkage between plants, soil, biocrusts (morphological development and bacterial community), and subsoil bacterial communities in restored ecosystems. These temporal dynamics are of great importance for predicting the development process in ecosystems by biocrusts, and the regulatory mechanism has great application potential for restoration management to maintain ecosystem stability and enhance the ecological function of artificial ecosystems.

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**Author contributions** G.S. and X.L. designed the research. G.S. and Z.Y. conducted the experiment and analyzed the data. G.S. and Y.H. wrote the manuscript. G.S., Z.Y., Y.H. and X.L. interpreted the results and revised the manuscript.

**Data availability** All data generated or analyzed during this study are included in this published article and its supplementary information files.

**Declarations**

**Ethics approval** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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