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RESEARCH ARTICLE

Soil total organic carbon/total nitrogen ratio as a key driver deterministically shapes diazotrophic community assemblages during the succession of biological soil crusts

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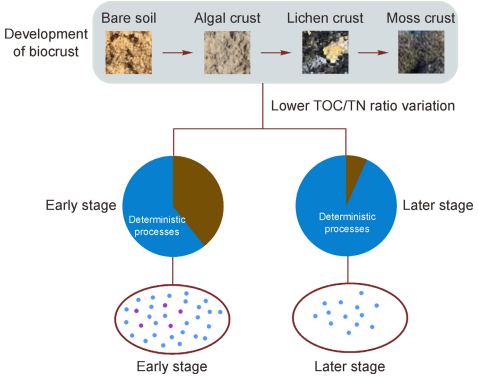
HIGHLIGHTS

- Biocrust succession alters diazotrophic diversity and community compositions.
- Deterministic processes govern diazotrophic community assemblages.
- The TOC/TN ratio is a key factor driving diazotrophic community succession.
- Diazotrophic networks become less complex with biocrust succession.

GRAPHICAL ABSTRACT



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ABSTRACT

The diazotrophic community in biological soil crusts (biocrusts) is the key supplier of nitrogen in dryland. To date, there is still limited information on how biocrust development influences the succession of diazotrophic community, and what are the most important factors mediating diazotrophic communities during biocrust succession. Using the high throughput *nifH* amplicon sequencing, the diazotrophs in soils at different developmental stages of biocrust were comparatively studied. The results evidenced the decreases of TOC/TN ratio and pH value with biocrust development. *Nostoc* and *Scytonema* were the most dominant diazotrophic genera at all biocrust stages, while *Azospirillum* and *Bradyrhizobium* were abundant only in bare soil. Diazotrophic co-occurrence networks tended to be less complex and less connected with biocrust succession. The soil TOC/TN ratio was the most dominant factor mediating diazotrophic diversity, community composition and assembly processes, while diazotrophic-diversity and NO_3^--N/NH_4^+-N ratio were positively correlated with the nitrogenase activity during biocrust succession. This study provided novel understandings of nitrogen fixation and succession patterns of diazotrophic community, by showing the effects of biocrust succession on diazotrophic diversity, community composition, community assembly and co-occurrence networks, and recognizing TOC/TN ratio as the most dominant factor mediating diazotrophs during biocrust succession.

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1 Introduction

Although dinitrogen (N_2) is abundant in atmosphere, nitrogen is still a constraint element in terrestrial and aquatic ecosystems (Vitousek et al., 2002), which limits the primary productivity (Vitousek et al., 2002; Gaby et al., 2018). Diazotrophic prokaryotes are able to fix (reduce) N_2 into ammonia and they are the main nitrogen suppliers throughout the Earth's ecosystems (Gaby et al., 2018), by imbibing the atmospheric N_2 into biosphere.

As the dominant vegetation in semiarid and arid environments, biocrusts (biological soil crusts) cover up to 70% of dryland area (Eldridge and Greene, 1994; Belnap et al., 2016), and play important roles in N cycling, especially for N-input by N_2-fixation (Belnap and Lange, 2003; Billings et al., 2003; Housman et al., 2006; Pepe-Ranney et al., 2016). The biocrusts are constituted by eukaryotic microalgae, prokaryotes (e.g., Cyanobacteria), lichens, fungi and mosses (Eldridge and Greene, 1994; Belnap et al., 2016). Biocrusts are classified based on species compositions, functional groups (e.g., cyanobacteria, green algae, lichens and mosses), surface appearance (light or dark), texture (smooth, rugose, rolling or pinnacled) and thickness, and a combination of them (Colesie et al., 2016). Belnap and Eldridge (2003) propose to classify biocrusts into bare soil, cyanobacterial crust, lichen crust and moss crust according to dominant photoautotrophic organisms and their general successional sequence (GSS). GSS commonly occurs in a wide variety of regions (Weber et al., 2016), including in Gurbantunggut desert, China (Zhang et al., 2015; Zhang et al., 2016; Zhang et al., 2018). Filamentous and mobile Cyanobacteria, e.g., *Microcoleus* spp., are the pioneer colonizers in bare soil (Belnap and Eldridge, 2003). Then, soil-surface-bound *Nostoc* and *Scytonema* turn to be the dominant species in cyanobacterial curst (Belnap and Eldridge, 2003; Büdel et al., 2016; Weber et al., 2016). Afterwards, early lichen species (e.g., *Collema* spp.) emerge, followed by a series of early- to later- lichens and bryophytes in community, forming later developed biocrusts (Belnap and Eldridge, 2003; Weber et al., 2016).

The biocrusts at later stages show higher activities of biological nitrogen fixation compared with those at early stages (Housman et al., 2006; Lan et al., 2012). For instance, the lichen crusts exert higher nitrogenase activity than the dark crusts (mixtures of cyanobacterial and lichen crusts) and cyanobacterial crusts (Belnap, 2002). However, the highest nitrogenase activity is also found in cyanobacterial crusts, rather than in lichen crusts or moss crusts (Wu et al., 2009; Pushkareva et al., 2017). These implicate that nitrogenase activities might be related to the compositions of diazotrophic communities and their interactions with environmental factors.

Similar to other microbes, diazotrophic community assembly is also shaped by both deterministic and stochastic processes (Stegen et al., 2013; Wang et al., 2017a; Wang et al., 2019), and the formers refer to the community assemblages under environmental filtering and biotic interactions, and the latter ones indicate dominant roles of random processes in community assembly, e.g., dispersal, birth-death events and ecological drifts (Vellend, 2010; Chase and Myers, 2011). Climate (e.g., temperature and precipitation), soil properties (e.g., pH and moisture) and plant diversity have been reported to influence diazotrophic diversity and community compositions in many habitats (Santos et al., 2014; Wang et al., 2017a; Yao et al., 2017).

Besides environmental factors, diazotrophic community assemblages in biocrusts are also determined by intraspecies and interspecies interactions. The negative interactions like predation (Bachelot et al., 2015) and competition (Cordero et al., 2012; Pande et al., 2014) implicate that the presence of a microbe harms the other members in the community; and the positive interactions like commensalism and mutualism indicate that the microbes benefit from the biochemical activities of others in the community (Sieber et al., 2012; Seth and Taga, 2014; Pande and Kost, 2017). Co-occurrence network analysis is an approach revealing how microorganisms occur together within a community that offers insights into the microbial interactions and assembly (Deng et al., 2012; Lupatini et al., 2014). In addition, it can reveal keystone taxa that have the largest influences in a community (Berry and Widder, 2014; Banerjee et al., 2016). For example, by conducting network analysis, a study reports that the climatic legacies have strong effects on the current distribution of ecological clusters of biocrust species across large spatial scale (Eldridge and Delgado-Baquerizo, 2019). Wang et al. (2017b) observe stronger negative than positive interactions of diazotrophs in desert and sandy grassland soils, and a simpler diazotrophic network structure in desert grassland soil. Our previous study also illustrates simpler prokaryotic co-occurrence networks at later biocrust stages (Xu et al., 2020).

To date, the shifts of diazotrophic diversity, community composition, community assembly and co-occurrence networks during the biocrust succession, as well as the key factors driving such variations are still not clear. Therefore, in the present study, we investigated the successional patterns of diazotrophic communities across the biocrust developmental stages in Gurbantunggut Desert, China. The objectives were (i) to explore the effects of biocrust succession on diazotrophic diversity and compositions; (ii) to identify the relative contributions of deterministic processes and stochastic processes to diazotrophic community assembly and the key drivers shaping diazotrophic community assembly; and (iii) to evaluate diazotrophic co-occurrence patterns during the biocrust succession.

2 Methods

2.1 Site and sampling descriptions

In June 2017, biocrust samples were collected at Beishawo (44.39° N, 87.92° E) in Gurbantunggut Desert, North-western China. Soil samples from bare soil, cyanobacterial crust, lichen crust and moss crust were randomly collected in five

replicates for each biocrust stage, with a soil corer (2.5 cm diameter) at the depth of 0–2 cm. In total, 20 biocrust soil samples were obtained and each of them was separated into 2 parts. One was air-dried for soil property measurements, and the other was stored at –20°C for molecular analysis (Vestergaard et al., 2017). Detailed descriptions for sites and samplings were reported previously (Xu et al., 2020).

2.2 Measuring environmental variables

The following variables of the biocrust soils were measured: chlorophyll-a content, net photosynthetic CO_2 flux, soil pH, total N (TN), total K (TK), total P (TP), exchangeable NH_4^+-N, NO_3^--N, total organic carbon (TOC), available P (AP) and available K (AK). Detailed descriptions for measuring these variables were described previously (Xu et al., 2020). The summary of these environmental variables in each biocrust stage was shown in the supplementary material (Table S1).

2.3 Measuring potential nitrogenase activity

The nitrogenase activity was measured by the acetylene-ethylene reduction assay in laboratory (Hardy et al., 1968). Pre-experiment was conducted to examine linear relationship between the incubation time and the ethylene production. Fifteen grams of air-dried biocrust soil was weighted into a 220 mL serum bottle, equally mixed and adjusted to 40% water-holding capacity with distilled water, and pre-incubated at 25°C for 72 h. Then, the bottle was sealed with a thick butyl rubber stopper and aluminum cap, followed by injecting 11 mL (5%) of C_2H_2 into each single bottle. A headspace gas sample (2 mL) was collected by a syringe after 4 h of incubation at 25°C. The C_2H_4 in each sample was quantified with a gas chromatograph (SP-2100A, Beijing-Fenrui, China). Nitrogenase activity was expressed as nmol C_2H_4 g^{-1} dry soil h^{-1}.

2.4 DNA extraction, molecular analysis and amplicon sequencing

Metagenomic DNA was extracted from 1 g biocrust soil sample using the PowerSoil® DNA Isolation kit (Qiagen, Hilden, Germany). The quantitative PCR (qPCR) was conducted to quantify the absolute abundances of diazotrophs, bacteria and fungi, targeting *nifH* gene, 16S rRNA gene and ITS gene, respectively. The ratio of ITS to 16S rRNA copy numbers was defined as F/B ratio. Primer set of nifH2 and nifH1 was used for qPCR and PCR of *nifH* gene. It was chosen due to its high coverage (Zehr and McReynolds, 1989), and the ability capturing high diversity of diazotrophs similar to primer set of IGK-DVV (Gaby et al., 2018). However, the sequences of both IGK and DVV contain base inosines, which bring high cost in barcode-linking primer synthesis. Negative controls were performed to verify contamination. Amplicons were sequenced by an Illumina Hiseq platform with 2 × 250 bp kits. Detailed descriptions of qPCR and PCR procedures were shown in supplementary material. Original sequencing data were deposited at National Center for Biotechnology Information, Sequence Read Archive (NCBI, SRA) with the accession number of PRJNA596827. Sequencing data files and associated abiotic properties were also available at Microbiome Database (http://egcloud.cib.cn) with project number PRJ-AMPLI-d3faedeec6056b45a63436a817bbe177.

2.5 Bioinformatic analyses

QIIME pipeline (Caporaso et al., 2010) was used to process raw sequences. After sequence quality checking, low quality sequences were removed. Two samples were discarded due to their low sequence reads. Chimera checking was conducted by Usearch8 in *de novo* mode (Edgar et al., 2011). RDP FrameBot was used to correct frameshift mutation sequences (Wang et al., 2013). Totally 550 595 sequences from 18 samples with successful corrections were clustered into Operational Taxonomic Units (OTUs) at an identity level of 96% using UCLUST algorithm (Farnelid et al., 2013). In total 7338 OTUs were obtained. Taxonomic annotations for *nifH* OTUs were conducted by BLASTn algorithm at an 80% cutoff with a reference database (Wang et al., 2019). MEGAN program was used to process taxonomical information of BLASTn results (Huson et al., 2007). All samples were re-sampled to 407 sequences per sample for downstream analyses. This number of rarefied sequences is reasonable because rarefaction curves showed a sequencing depth around 250 sequences that was enough to examine the diazotrophic α-diversity with biocrust succession (Fig. S1).

2.6 Construction of co-occurrence networks

We inferred a meta-community co-occurrence network for diazotrophs across the biocrust stages and sub-networks in early (including bare soil and cyanobacterial crusts) and later (including lichen and moss crusts) stages to check the overall microbial interactions and their variations during the biocrust development. The networks were analyzed using the molecular ecological network analyses pipeline (MENAP) at https://ieg2.ou.edu/MENA, with the default settings (only keeping those OTUs occurring in more than half of the total samples) (Deng et al., 2012). Network modules were identified by fast greedy modularity optimization method. Network graphs were visualized by Cytoscape 3.7.2. Keystone species were identified by the within-module connectivity (Zi) and among-module connectivity (Pi). Nodes with Zi less than 2.5 and Pi less than 0.62 were considered as peripheral species, those with $Zi < 2.5$ and $Pi > 0.62$ as connector species, those with $Zi > 2.5$ and $Pi < 0.62$ as module hubs, and those with $Zi > 2.5$ and $Pi > 0.62$ as network hubs. Connector species, module hubs and network hubs were considered as putative keystone species (Deng et al., 2012).

2.7 Evaluating habitat generalists and specialists

Habitat generalists and specialists were identified by "niche breadth" method proposed by Levins (1968), using *"niche.width"* function in *"spa"* package, with the following formula:

$$B_j = 1 \div \sum_{i=1}^{N} P_{ij}^2$$

where B_j signifies niche breadth and P_{ij} is the proportion of individual belonging to species *j* presenting in a given habitat *i*. Species with higher B-values are habitat generalists as they can probably distribute in a wider range of habitats, while species with lower values of niche breadth are specialists (Pandit et al., 2009; Logares et al., 2013). Generalists OTUs were designed to those with B-values greater than 3.237 that corresponded to the OTUs associated with a wide range of developmental stages; specialists were those with B-values equal to 1 that corresponded to the OTUs associated with some specific biocrust stages; remaining OTUs were regarded as intermediate taxa (Logares et al., 2013).

2.8 Statistical analyses

All statistical analyses were conducted using R 3.5.3 (R-Core-Development-Team, 2010). Mean nearest taxon index (MNTD) and nearest taxon index (NTI), β mean nearest taxon index (βMNTD) and βNTI were calculated by the "picante" package, using the "*mntd* " "*ses.mntd*" and "*comdistnt"* functions (Webb et al., 2008; Stegen et al., 2012; Stegen et al., 2013). MNTD is the phylogenetic distance between each OTU in one community, while NTI quantifies the standard deviation between observed MNTD and its random distribution of the null models. Similarly, βMNTD quantifies the closest phylogenetic distance between communities, and βNTI is the difference between observed βMNTD and its null distributions. A NTI value higher than + 2 (or mean NTI greater than 0) indicates significant phylogenetic clustering, whereas a NTI value less than −2 (mean NTI less than 0) indicates phylogenetic over-dispersion; a βNTI value < −2 or > + 2 indicates less or greater than expected phylogenetic turnover, meaning that deterministic processes dominate microbial community assembly (Fine and Kembel, 2011; Stegen et al., 2012; Stegen et al., 2013). A βNTI value > −2 and < + 2 indicates that stochastic processes dominate microbial community assembly. Differences in diazotrophic α-diversity, *nifH* abundances and community similarities among biocrust stages were measured by non-parametric Wilcoxon test using the "*wilcox.test*" function. Analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) were used interactively to explore the differences of diazotrophic community compositions among biocrust stages using the "*cmdscale*," "*anosim*," "*adonis*" functions respectively, in the "vegan" package. Distance-based redundancy analysis (dbRDA) was used to check the relationships between diazotrophic β-diversity (differences in community compositions) and environmental variables. Relative importance of environmental variables on diazotrophic community compositions was assessed by the permutation test based on dbRDA scores, using the "*envfit*" function. Indicator analysis (Dufrene and Legendre, 1997) was used to screen indicative diazotrophic OTUs that could be distinguishable among the biocrust stages, using the "*multipatt*" function in the "*indicspecies*" package. Mantel test was used to explore the relationships between diazotrophic β-diversity indices, βNTI and the differences in environmental variables using the "*mantel*" function.

3 Results

3.1 Diazotrophic diversities and their driving factors with biocrust succession

The *nifH* abundance was higher in cyanobacterial crust than those in other stages and it was lowest in bare soil and lichen crust (Fig. 1A). The taxonomic diversity (represented by Shannon index) and the phylogenetic diversity (NTI) of diazotrophs were significantly ($P < 0.05$, Fig. 1A) higher in cyanobacterial crust than those in other stages, and they were lowest in bare soil, demonstrating that the biocrust development significantly altered the diazotrophic α-diversity. The NTI values were significantly higher than 0 in all the biocrust stages and were more than + 2 in most of biocrust stages (except the bare soil, Table S3), indicating that during biocrust succession, diazotrophic community shifted to be more phylogenetically clustered than expected by chance. Both taxonomic ($P = 0.036$) and phylogenetic ($P = 0.050$) diversities showed positive relationships with the potential nitrogenase activity (Fig. 1B). No significant relationship was observed between the *nifH* abundance and potential nitrogenase activity ($P = 0.233$, Fig. 1B).

The distance base-redundancy analysis showed different clusters of biocrust samples, indicating obvious differences of diazotrophic β-diversity (changes in community compositions) among distinct biocrust stages (Fig. 2A). Further ANOSIM analysis revealed that biocrust development significantly influenced diazotrophic β-diversity (Table 1). The diazotrophic community in bare soil was significantly different from those in other biocrust stages ($P < 0.05$); in addition, diazotrophs in cyanobacterial crust were also different from those in lichen crust and moss crust ($P < 0.05$), whereas the samples of lichen crust overlapped with those of moss crust samples ($P > 0.05$, Table 1 and Fig. 2A). PERMANOVA analyses revealed similar patterns (Table 1), suggesting the successional changes of diazotrophic community during biocrust development.

A series of variables were significantly ($P < 0.05$, Table 2) correlated to the changes of diazotrophic community compositions during biocrust succession, including the ratios of soil TOC/TN (Fig. 2B) and TOC/TP (Fig. 2C), soil texture (clay, silt and sand contents), photosynthetic CO_2 flux and chlorophyll-a content, available K and F/B ratio. Soil pH influenced diazotrophic community (Fig. 2D) at a marginally significant value ($P < 0.1$, Table 2) (Pritschet et al., 2016). Diazotrophic communities in bare soil were positively correlated to TOC/TN ratio, pH and soil sand content, whereas photosynthetic CO_2 flux, chlorophyll-a content, TOC/TP ratio, F/B ratio, soil clay and silt contents showed significant and positive correlations with the diazotrophs in lichen and moss crusts (Fig. 2).

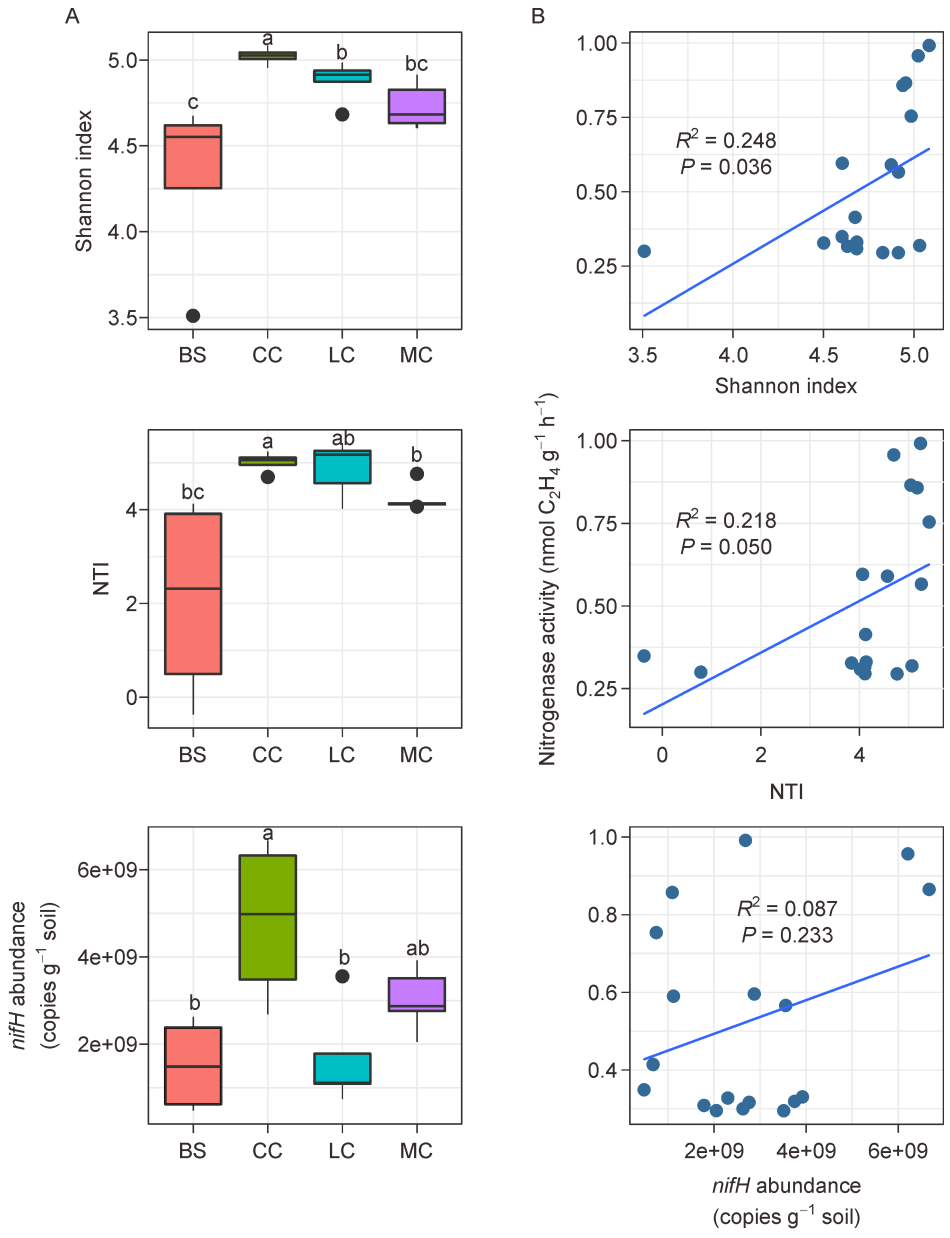

Fig. 1 (A) Diazotrophic taxonomic diversity (Shannon index), phylogenetic diversity (Nearest taxon index, NTI) and *nifH* abundances at different biocrust stages; (B) relationships between potential nitrogenase activity and diazotrophic taxonomic diversity, phylogenetic diversity and *nifH* abundance. BS, Bare soil; CC, Cyanobacterial crust; LC, Lichen crust; MC, Moss crust. Different lower-case letters indicate significant differences at $P < 0.05$

Soil TOC/TN ratio constantly showed significant ($P < 0.05$) correlations with both α- and β-diversity indices of diazotrophs (Table S2). Meanwhile, the ratio of exchangeable NO_3^--N/NH_4^+-N was significantly and positively correlated with both diazotrophic taxonomic and phylogenetic diversities (Fig. S2a and b), while the ratio of TOC/TP, soil clay, sand, chlorophyll-a content and photosynthetic CO_2 flux showed significant ($P < 0.05$) or marginal ($P < 0.1$) correlations with diazotrophic β-diversity (Table S2). These results suggested that the TOC/TN ratio was the most important factor influencing diazotrophic diversity during the biocrust succession. In addition, the ratio of NO_3^--N/NH_4^+-N showed a significant and positive relationship with potential nitrogenase activity (Fig. S2c). This probably implicated that a relatively low NH_4^+-N content (e.g., in cyanobacterial and lichen biocrusts, Table S1) could stimulate more diverse diazotrophs and maintain higher nitrogenase activity.

3.2 Diazotrophic community compositions and assembly in biocrust succession

Diazotrophic communities at different biocrust stages mainly consisted of Proteobacteria and Cyanobacteria, which accounted for more than 99% of the total reads in all samples.

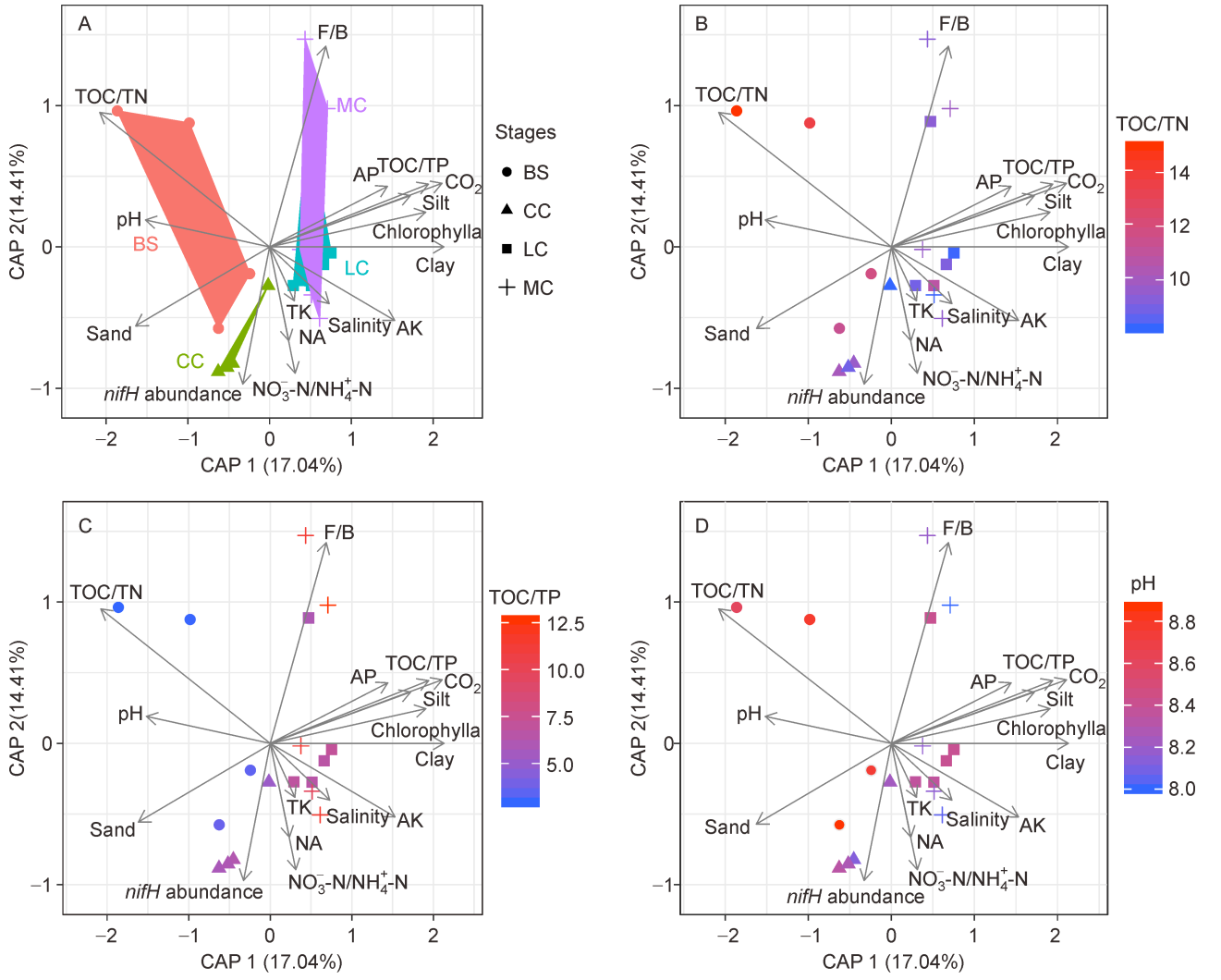

Fig. 2 Distance-based redundancy analysis (dbRDA) of diazotrophic communities based on (A) different biocrust stages, (B) TOC/TN ratio, (C) TOC/TP ratio and (D) pH. AK, available potassium; AP, available phosphorus; NA, potential nitrogenase activity; CO_2, net photosynthetic CO_2; TK, total potassium; F/B, the ratio of ITS to 16S rRNA gene abundances.

Table 1 Analyses of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) of diazotrophic communities among biocrust developmental stages based on the relative abundances considering Bray–Curtis dissimilarity matrices.

Pairwise comparisons [a]	ANOSIM		PERMANOVA	
	r [b]	P	R^2 [b]	P
BS vs. CC	0.302*	0.026	0.195*	0.025
BS vs. LC	0.462**	0.006	0.222**	0.008
BS vs. MC	0.316*	0.016	0.190**	0.009
CC vs. LC	0.668*	0.014	0.235*	0.015
CC vs. MC	0.503*	0.011	0.222**	0.006
LC vs. MC	0.032	0.552	0.095	0.770

[a] BS: Bare soil, CC: Cyanobacterial crust, LC: Lichen crust, MC: Moss crust;

[b] Significance codes: *$P<0.05$, **$P<0.01$.

At the order level, Rhizobiales, Rhodospirillales and Nostocales dominated in bare soil, while Nostocales accounted for more than 98% of the relative abundances of diazotrophs in the later three biocrust stages (Fig. S3a). At genus level, *Azospirillum* (averagely 20.52% of total reads in bare soil, but statistically not significant from other stages, $P>0.05$) and *Bradyrhizobium* (2.64%, $P<0.05$) only showed high relative abundances in bare soil, while *Nostoc* (averagely 46.52% in all samples) and *Scytonema* (19.15%) were abundant in bare soil and the other biocrust stages (Table S3, Fig. S3b). The relative abundances of representative genera *Nostoc* and *Scytonema* showed non-significantly positive relationships with potential nitrogenase activity (Fig. S4).

In total, 208, 354, and 748 OTUs were identified as habitat generalists, intermediate and specialists, respectively, according to their B-distributions (Fig. S5). Positive relationships were observed between the relative abundances of the OTUs and their B-values ($P<0.001$, Fig. 3A), indicating that abundant species might have broader environmental tolerances and distribute in a wider range of biocrust stages. Because niche B value only provided possible niche breadth of each OTU, indicator analysis was further used to identify the taxa strictly associated with each biocrust stage. Nine OTUs strictly associated with bare soil were mainly from the Alphaproteobacteria (*Azospirillum,* 6/9), Betaproteobacteria (*Burkholderiales,* 1/9) and Nostocales (2/9). Nine OTUs were found to be strictly associated with cyanobacterial crust, including 7 from *Nostoc punctiforme* and 2 from *Scytonema*. The lichen crust harbored 3 indicator OTUs, including a *Scytonema hofmannii*, a *Nostoc punctiforme* and a Nostocales. Four OTUs were indicators of moss crust stage in which one OTU was affiliated to *Nostoc punctiforme* and 3 OTUs were from the order Nostocales (Fig. 3B).

Table 2 Permutation analysis of distance-based redundancy analysis (dbRDA) scores and environmental variables showing their relative importance on diazotrophic community compositions.

Environmental variables [a]	R^2	*P*	Significance [b]
NO_3^--N/NH_4^+-N	0.123	0.365	
TOC/TN	**0.716**	**0.002**	**
TOC/TP	**0.545**	**0.002**	**
TK	0.032	0.776	
AP	0.31	0.064	
AK	0.358	0.044	*
pH	0.321	0.060	
Salinity	0.095	0.453	
Clay	**0.623**	**0.002**	**
Silt	0.425	0.017	*
Sand	0.498	0.005	**
Chlorophyll-a	0.508	0.005	**
CO_2 flux	**0.638**	**0.002**	**
NA	0.068	0.627	
F/B	0.341	0.038	*
nifH abundance	0.144	0.330	

[a] AK, available potassium; AP, available phosphorus; NA, potential nitrogenase activity; CO_2 flux, net photosynthetic CO_2; TK, total potassium. [b] Significance codes: $^{*}P<0.05$, $^{**}P<0.01$.

Deterministic processes dominated diazotrophic community assemblages in both early and later biocrust stages (Fig. 4), and the contributions of stochastic processes decreased from early to later biocrust stages (Fig. 4). Variations in the soil TOC/TN ratio and pH during the biocrust succession had significantly positive influence on diazotrophic βNTI (Mantel test, $P<0.01$, Fig. 5). Meanwhile, the differences in TOC/TN and pH were lower among the cyanobacterial, lichen and moss stages of the biocrust ($P<0.05$, Table S1) than those compared to bare soil. These results indicated that, by decreasing the soil TOC/TN ratio and pH value, the dominance of deterministic processes increased in shaping diazotrophic community assembly with biocrust succession.

3.3 Diazotrophic co-occurrence networks in early and later biocrust stages

The dominant co-occurred diazotrophic taxa were Nostocaceae and Scytonemataceae (Fig. 6A). In general, more negative interactions were observed in both meta-network and sub-networks, and positive interactions decreased from early stages to later stages (Table 3 and Fig. 6A). In addition, 23, 11 and 6 OTUs were identified as keystone species in the meta-network and two sub-networks for early and later stages, respectively. These OTUs were mainly affiliated to *Nostoc punctiforme* and *Scytonema hofmannii* (Tables S5, S6 and S7). With similar total nodes in two sub-networks, the later stage sub-network exhibited lower total links (Table3). In addition, the lower *avgK* in later stage sub-network indicated the connection was weaker in later stage than in early stage (Fig. 6B). Meanwhile, the higher average path distance in later stage sub-network indicated that node connections were relatively weak in later stage compared to those of early stage. These results indicated diazotrophic networks became less complex and less connected with biocrust succession (Fig. 6).

4 Discussion

4.1 Soil TOC/TN ratio was the dominant factor influencing diazotrophic communities

The α-diversity of diazotrophs at bare soil was largely lower than those in the other biocrust stages (Fig. 1A). Such diversity pattern for diazotrophs was different from those of total bacteria and fungi in Gurbantunggut Desert, which showed no significant differences among biocrust developmental stages (Zhang et al., 2015; Zhang et al., 2018). The β-diversity revealed successional changes of diazotrophic community compositions with biocrust development

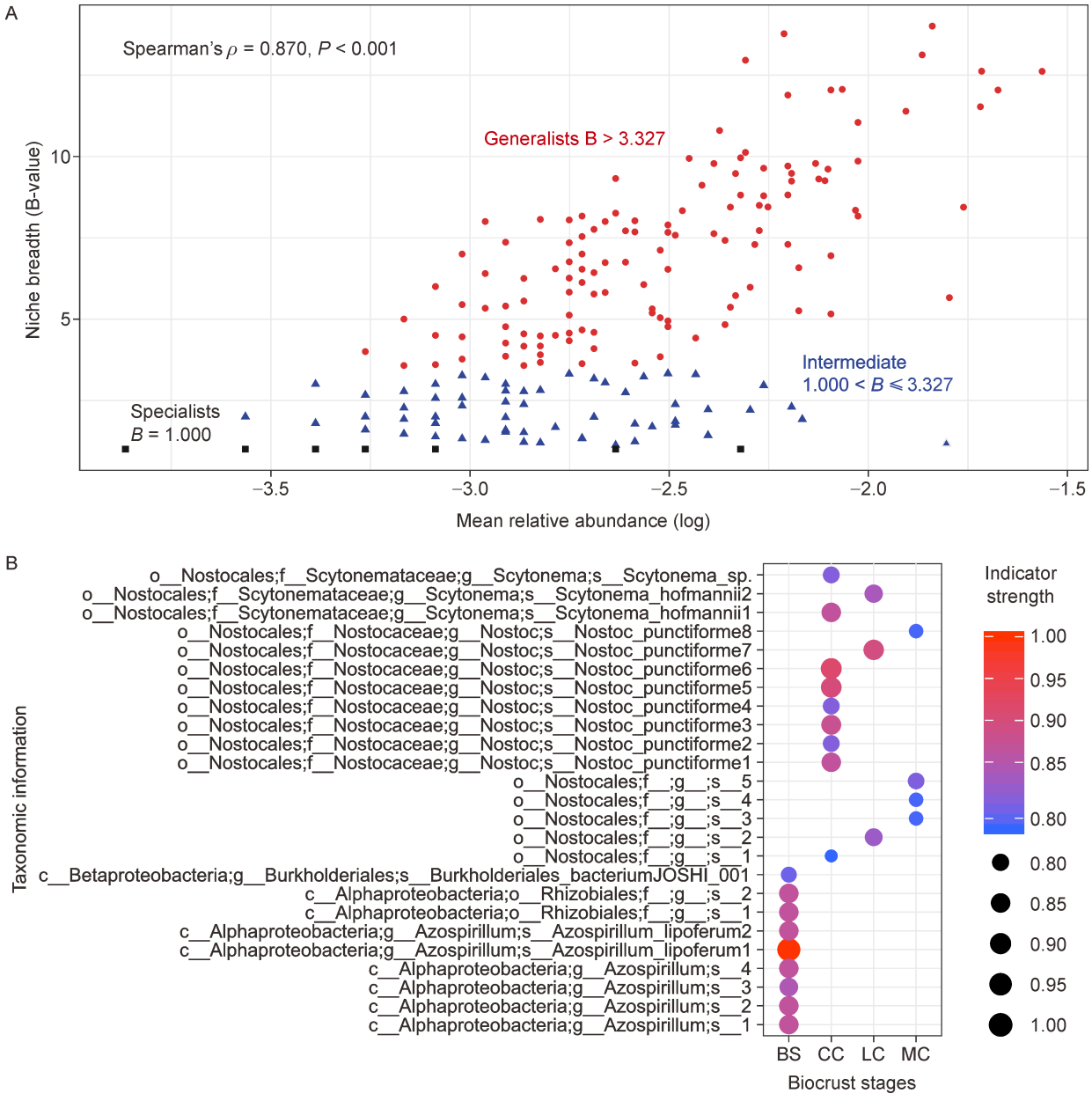

Fig. 3 (A) Habitat specialists and generalists of diazotrophs among biocrust developmental stages. Each dot represents an OTU. Generalists (circles), intermediate (triangles) and specialists (squares) OTUs are indicated. (B) OTUs strictly associated with specific biocrust stages. Point sizes show the strengths of indicator OTUs (Indval). only those OTUs with p values less than 0.05 are shown.

(Table 1, Fig. 2), which were similar to the variations of total bacteria and fungi reported previously (Zhang et al., 2015; Zhang et al., 2018). Thus, both the α- and β-diversity indices demonstrated that the diazotrophic community changed in relative abundances and community compositions with biocrust succession.

The environmental gradients created by biocrust development were proved to transit diazotrophic community assembly from stochastic to deterministic processes (Fig. 4 and Fig. 5). This was in accordance with previous studies that the importance of community assembly processes may vary with environmental gradients (Rominger et al., 2009; Vellend et al., 2014; Zhang et al., 2016). Such environmental gradients can be represented by the differences in soil TOC/TN and pH with biocrust development (Fig. 5). Soil pH in alpine meadow is reported to influence diazotrophic community assembly deterministically accompanied by phylogenetically clustering (Wang et al., 2017a). Similar pH-driven pattern is observed with respect to diazotrophs in the rhizosphere and bulk soil of wheat (Fan et al., 2018). The TOC/TN ratio of a given biocrust system may serve as the indicator of N limitation. Photosynthetic CO_2 flux was positively associated with the diazotrophic β-diversity, indicating that higher amount of C input altered diazotrophic community compositions

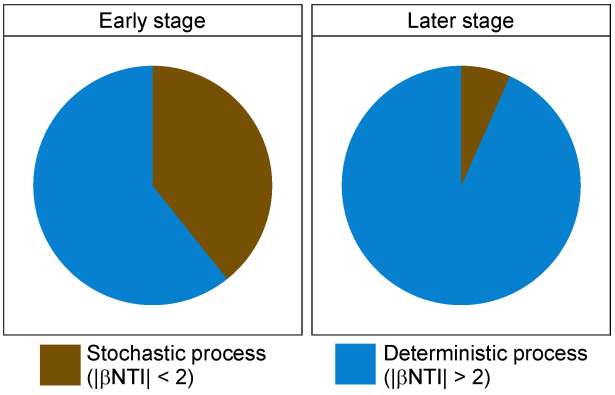

Fig. 4 Relative contributions of deterministic and stochastic processes to diazotrophic community assembly in early and later biocrust stages.

during biocrust development. In biocrust system, the fixed N by cyanobacteria could accumulate at higher rate than C, therefore TOC/TN ratio declined (Table S1), which in turn brought environmental selection pressure shifting diazotrophic community assembly with more dominance of deterministic processes (Fig. 5). In addition, the soil C/N ratio is reported to be a main factor to determine the community compositions of natural arbuscular mycorrhizal fungi (Dumbrell et al., 2010), as well as the bacterial community in wildfire burned and unburned fields (Ferrenberg et al., 2013). Therefore, we suggested the soil TOC/TN ratio to be the most powerful controlling factor of diazotrophic communities in biocrust systems. This observation is reasonable considering the dominant roles of photoautotrophic *Nostoc* and *Scytonema*, which can mediate TOC/TN ratio by photosynthesis and nitrogen fixation processes.

4.2 Abiotic and biotic factors jointly regulate nitrogenase activity

Abiotic (e.g., soil TOC/TN, pH, NO_3^--N and NH_4^+-N contents) and biotic (*nifH* gene abundances, diversity and community composition) and factors might jointly affect the processes of nitrogen fixation. The fixed N compounds in biocrust are mostly released in terms of NO_3^--N to the surrounding organisms and then leached into the deep soil layer or lost as N_2O or N_2 into atmosphere via denitrification (Belnap, 2002; Abed et al., 2013). In addition, evidence shows that global change is accelerating soil N_2O emission, which constitutes nearly two-thirds of global N_2O emission (Dai et al., 2020). In the present study, the higher NO_3^--N/NH_4^+-N ratio in the cyanobacterial crust and lichen crust stages might be related to the previously reported facts that higher nitrification potential (Rosentreter et al., 2016) and higher relative abundance of nitrifying bacteria presented in lichen crust stage (Maier et al., 2018). Therefore, it is reasonable that NO_3^--N/NH_4^+-N rate was positively correlated with potential nitrogenase activity.

Positive relationship between diazotrophic diversity and potential nitrogenase activity corroborated the positive biodiversity-ecosystem function relationships (Cardinale et al., 2012; Maestre et al., 2012). However, we did not find significant relationship between the nitrogenase activity and *nifH* gene abundances (Fig. 1B). The measurement of *nifH* gene abundance based on DNA quantifies both dead and active cells, and the *nifH* genes in some species of diazotrophs may not be expressed (Schöler et al., 2017). For example, the symbiotic diazotrophs in Rhizobiales (Fig. S3) do not fix nitrogen in free-living state. Also, only the heterocysts presents the nitrogenase activities in many cyanobacteria, which might be the principle diazotrophs in the

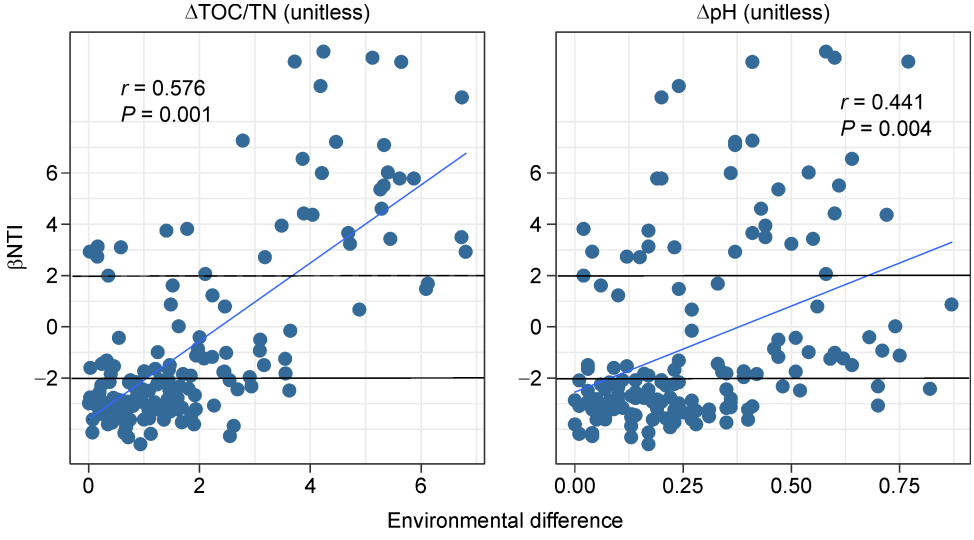

Fig. 5 Relationships between βNTI and the differences in TOC/TN ratio and pH. Horizontal dashed black lines indicate upper and lower significance thresholds at βNTI = + 2 and –2, respectively. *r* and *P* indicate mantel test correlation coefficient and significance based on Spearman's *rho*.

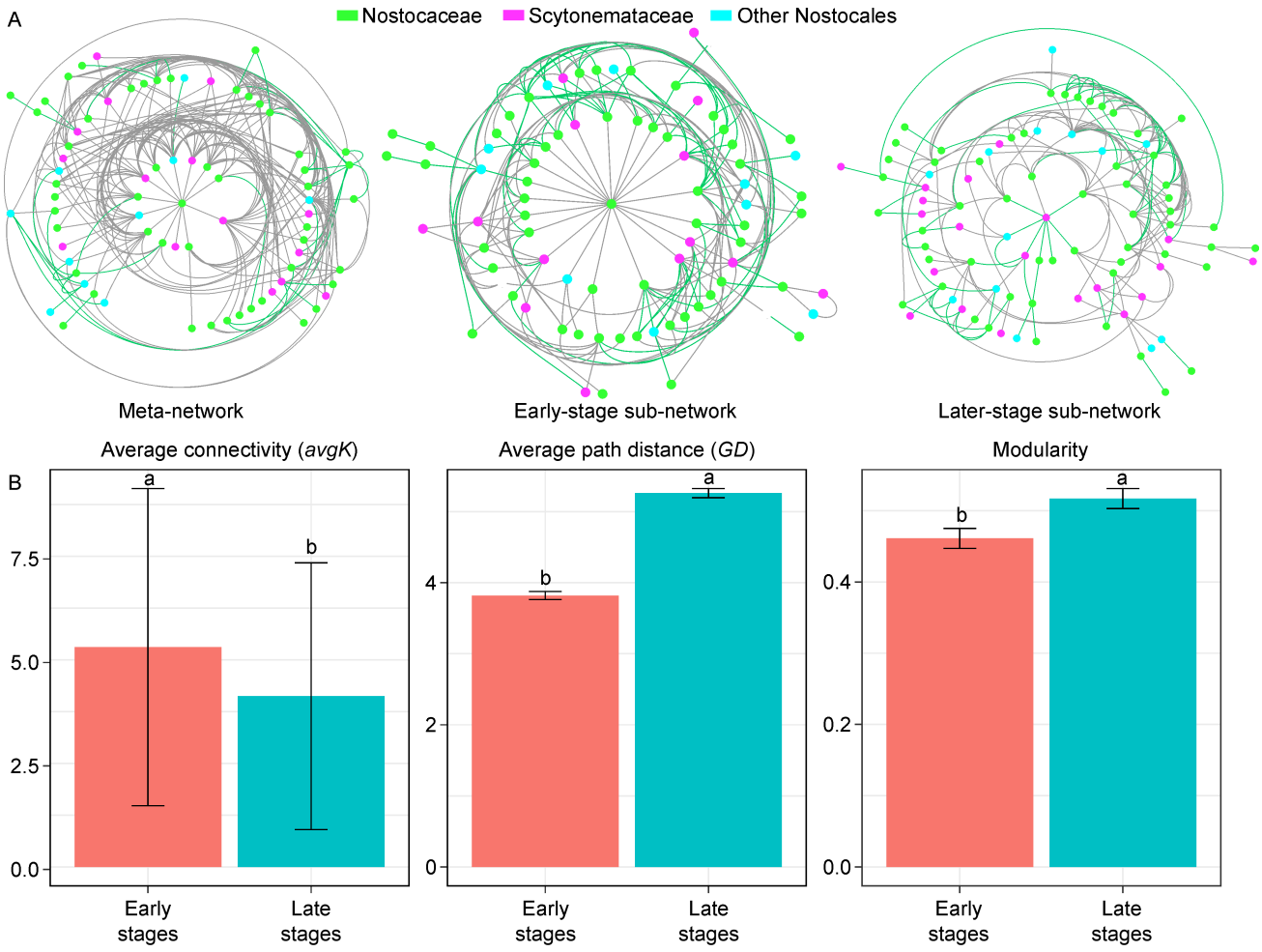

Fig. 6 (A) Diazotrophic co-occurrence networks in whole biocrust succession stages (meta-) and early- and later-stages. Each node signifies an OTU. Positive interactions are marked with green lines and negative by gray lines. (B) Network topological indices in early and later biocrust stages. Different letters indicate significant difference of *avgK* at $P < 0.05$, and *GD* and Modularity at $P < 0.001$. Topological indices illustrate specific properties of networks. Higher average path distance or geodesic distance (*GD*) indicates that all the nodes in the network are less connected. High modularity means that a network is well separated into modules, and a lower average connectivity (*avgK*) signifies a less complex network (Deng et al., 2012).

Table 3 Topological properties of co-occurrence molecular ecological networks of diazotrophic communities for early and later biocrust stages (sub-network) and all biocrust stages as a whole (meta-network)

Network types	Similarity threshold	Total nodes	Total links	Positive edge (%)	R^2 of power-law
Meta-network	0.510	75	251	13.94	0.614
Sub-network-early stage	0.710	81	204	40.69	0.774
Sub-network-later stage	0.710	89	183	33.33	0.755

cyanobacterial stage and in the lichen stage (Wirtz et al., 2003; Rosentreter et al., 2016). Furthermore, the abundance of N_2 fixers and the nitrogenase activity in the biocrusts are regulated by many factors, including anthropogenic activities across temporal and spatial scales (Büdel et al., 2016). Stronger effects of abiotic factors would overwhelm the roles of diazotrophic communities on nitrogenase activities. Significant relationships between nitrogenase activity and *nifH* gene abundances may be shown when biotic factors play main roles in controlling nitrogenase activity.

4.3 *Nostoc* and *Scytonema* are key diazotrophs in biocrust systems

Using low resolution T-RFLP method, a dominant role of

Nostoc species in poor developed and mature biocrusts is reported (Yeager et al., 2004), which suggests that the N_2-fixing species do not vary significantly between the poorly developed and mature crusts. However, our findings proved significant differences in diazotrophic community with biocrust succession using high throughput *nifH* amplicon sequencing method (Table 2 and Fig. 2). In bare soil, *Azospirillum* and *Bradyrhizobium* from Alphaproteobacteria accounted for considerable amount of diazotrophs, which is partially in accordance with Pepe-Ranney et al. (2016), where Clostridiaceae and Proteobacteria are the most common taxa assimilating atmospheric N_2 in early biocrust stage. *Azospirillum* is sensitive to temperature and precipitation (Santos et al., 2014; Yao et al., 2017), and it is stress tolerant (Bashan et al., 2004). *Bradyrhizobium* are able to take advantages of alternating cycles of dry and wet (Koponen et al., 2003) and some strains in this genus could fix nitrogen in free-living condition (Hara et al., 2019). Such traits may enable these two genera to survive and fix N_2 in the early biocrust stages. As biocrust succession, the proportions of *Azospirillum* and *Bradyrhizobium* decreased and the *Nostoc* and *Scytonema* were found to be the most dominant type of N_2 fixers, which accounted for 80% of diazotrophic community (Fig. S3). This may be explained by the fact that diazotrophs in bare soil suffer higher stress than those in other biocrust stages, thus, they must invest more energy for survival rather than for competition (Fierer et al., 2012). Competition may be stronger in nutrient-rich biocrust stages, where diazotrophs may invest more energy in C and N sequestrations using high yield and acquisition strategies (e.g., *Nostoc* and *Scytonema)*. The *Nostoc* and *Scytonema* are heterocystic cyanobacteria which are common in biocrusts detected in various locations on earth (Büdel et al., 2016), and they can serve as the photobionts of lichens in later biocrust stage. In addition, most of the indicative OTUs were identified as *Nostoc punctiforme*, which is reported to be desiccation-resistant, with their filaments holding together desert soil particles (Fleming and Castenholz, 2007). *Nostoc* can release extracellular polysaccharide materials for binding the extracellular carbohydrates into polymeric matrix (Kim and Or, 2017), which may contribute to crust formation by glue trichomes to soil particles (Colesie et al., 2016; Mugnai et al., 2018), and gather organic and inorganic materials to prevent them from erosion (Colesie et al., 2016).

4.4 Biocrust development simplifies co-occurrence network of diazotrophic community

In early stage networks, higher proportion of positive associations was observed among the diazotrophic communities than those in later stage networks (Table 3 and Fig. 6). This suggested that compared to the later biocrust stages, diazotrophs were much more coherent to the environmental changes in early stages, whereas competitions or differential niche adaptations might play more roles in shaping diazotrophic community interactions with biocrust succession.

The smaller number of keystone species in later biocrust stages indicated the possible loss of key species with biocrust development. Those lost key species were important in shaping network structure because of their high within or between module connectivity (Fig. S6). Therefore, with their loss, the later stage sub-network broke into more modules and some links (related to the key species) lost, resulting in less links and average connectivity, as well as the longer path distance in the later stage sub-network (Fig. 6B). Modularity of a network indicates the ability of a network to compartmentalize modules (Newman, 2006). Taxa within a module are usually functionally related (Stephen et al., 2017). Therefore, the modules can represent potential ability of specific ecological functions (Delgado-Baquerizo et al., 2018). The increase of modularity with biocrust succession indicated that diazotrophic community might differentiate into more specific functions with more resource input in later biocrust stages.

5 Conclusions

This study firstly reported the successional pattern of diazotrophic community with biocrust development. The results revealed significant differences in diazotrophic community diversity and compositions among biocrust developmental stages and further identified soil TOC/TN ratio as the most dominant factor explaining the variations of diazotrophic community with biocrust development in Gurbantunggut Desert. These findings provided important implications for managing biocrusts in desert ecosystems.

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References

Abed, R.M., Lam, P., de Beer, D., Stief, P., 2013. High rates of denitrification and nitrous oxide emission in arid biological soil crusts from the Sultanate of Oman. ISME Journal 7, 1862–1875.

Bachelot, B., Uriarte, M., Mcguire, K., 2015. Interactions among mutualism, competition, and predation foster species coexistence in diverse communities. Theoretical Ecology 8, 297–312.

Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., Richardson, A.E., 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable

soil. Soil Biology & Biochemistry 97, 188–198.

Bashan, Y., Holguin, G., de-Bashan, L.E., 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Canadian Journal of Microbiology 50, 521–577.

Belnap, J., 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils 35, 128–135.

Belnap, J., Eldridge, D., 2003. Disturbance and Recovery of Biological Soil Crusts. In: Belnap, J., Lange, O.L., eds. Biological Soil Crusts: Structure, Function, and Management. Springer, Berlin, Heidelberg. 363–383

Belnap, J., Lange, D., 2003. Biological Soil Crusts: Structure, Function, and Management. Springer Berlin Heidelberg.

Belnap, J., Weber, B., Büdel, B., 2016. Biological Soil Crusts as an Organizing Principle in Drylands. In: Weber, B., Büdel, B., Belnap, J., eds. Biological Soil Crusts: An Organizing Principle in Drylands. Ecological Studies (Analysis and Synthesis). Springer, Cham, 3–13.

Berry, D., Widder, S., 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Frontiers in Microbiology 5, 219.

Billings, S.A., Schaeffer, S.M., Evans, R.D., 2003. Nitrogen fixation by biological soil crusts and heterotrophic bacteria in an intact Mojave Desert ecosystem with elevated CO_2 and added soil carbon. Soil Biology & Biochemistry 35, 643–649.

Büdel, B., Dulić, T., Darienko, T., Rybalka, N., Friedl, T., 2016. Cyanobacteria and Algae of Biological Soil Crusts, In: Weber, B., Büdel, B., Belnap, J., eds. Biological Soil Crusts: An Organizing Principle in Drylands. Ecological Studies (Analysis and Synthesis). Springer, Cham, 55–80.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67.

Chase, J.M., Myers, J.A., 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 366, 2351–2363.

Colesie, C., Felde, V.J.M.N.L., Büdel, B., 2016. Composition and Macrostructure of Biological Soil Crusts. In: Weber, B., Büdel, B., Belnap, J., eds. Biological Soil Crusts: An Organizing Principle in Drylands. Ecological Studies (Analysis and Synthesis). Springer, Cham, Berlin, 159–172.

Cordero, O.X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain, F., Le Roux, F., Mincer, T., Polz, M.F., 2012. Ecological populations of bacteria act as socially cohesive units of antibiotic production and resistance. Science 337, 1228–1231.

Dai, W., Bai, E., Li, W., Jiang, P., Dai, G., Zheng, X., 2020. Predicting plant–soil N cycling and soil N_2O emissions in a Chinese old-growth temperate forest under global changes: uncertainty and implications. Soil Ecology Letters 2, 73–82.

Delgado-Baquerizo, M., Reith, F., Dennis, P.G., Hamonts, K., Powell, J.R., Young, A., Singh, B.K., Bissett, A., 2018. Ecological drivers of soil microbial diversity and soil biological networks in the Southern Hemisphere. Ecology 99, 583–596.

Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. BMC Bioinformatics 13, 113.

Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67, 345–366.

Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H., 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME Journal 4, 337–345.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics (Oxford, England) 27, 2194–2200.

Eldridge, D.J., Delgado-Baquerizo, M., 2019. The influence of climatic legacies on the distribution of dryland biocrust communities. Global Change Biology 25, 327–336.

Eldridge, D.J., Greene, R., 1994. Microbiotic soil crusts- a review of their roles in soil and ecological processes in the rangelands of Australia. Soil Research (Collingwood, Vic.) 32, 389–415.

Fan, K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biology & Biochemistry 121, 185–192.

Farnelid, H., Bentzon-Tilia, M., Andersson, A.F., Bertilsson, S., Jost, G., Labrenz, M., Jürgens, K., Riemann, L., 2013. Active nitrogen-fixing heterotrophic bacteria at and below the chemocline of the central Baltic Sea. ISME Journal 7, 1413–1423.

Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Melbourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. ISME Journal 7, 1102–1111.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., Caporaso, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences of the United States of America 109, 21390–21395.

Fine, P.V., Kembel, S.W., 2011. Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. Ecography 34, 552–565.

Fleming, E.D., Castenholz, R.W., 2007. Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. Environmental Microbiology 9, 1448–1455.

Gaby, J.C., Rishishwar, L., Valderrama-Aguirre, L.C., Green, S.J., Valderrama-Aguirre, A., Jordan, I.K., Kostka, J.E., 2018. Diazotroph community characterization via a high-throughput nifH

amplicon sequencing and analysis pipeline. Applied and Environmental Microbiology 84, e01512–e01517.

Hara, S., Morikawa, T., Wasai, S., Kasahara, Y., Koshiba, T., Yamazaki, K., Fujiwara, T., Tokunaga, T., Minamisawa, K., 2019. Identification of nitrogen-fixing Bradyrhizobium associated with roots of field-grown sorghum by metagenome and proteome analyses. Frontiers in Microbiology 10, 407.

Hardy, R.W., Holsten, R.D., Jackson, E.K., Burns, R.C., 1968. The acetylene-ethylene assay for n(2) fixation: laboratory and field evaluation. Plant Physiology 43, 1185–1207.

Housman, D.C., Powers, H.H., Collins, A.D., Belnap, J., 2006. Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments 66, 620–634.

Huson, D.H., Auch, A.F., Qi, J., Schuster, S.C., 2007. MEGAN analysis of metagenomic data. Genome Research 17, 377–386.

Kim, M., Or, D., 2017. Hydration status and diurnal trophic interactions shape microbial community function in desert biocrusts. Biogeosciences 14, 5403–5424.

Koponen, P., Nygren, P., Domenach, A.M., Le Roux, C., Saur, E., Roggy, J.C., 2003. Nodulation and dinitrogen fixation of legume trees in a tropical freshwater swamp forest in French Guiana. Journal of Tropical Ecology 19, 655–666.

Lan, S., Li, W., Zhang, D., Hu, C., 2012. Composition of photosynthetic organisms and diurnal changes of photosynthetic efficiency in algae and moss crusts. Plant and Soil 351, 325–336.

Levins, R., 1968. Evolution in Changing Environments: Some Theoretical Explorations. Princeton University Press.

Logares, R., Lindström, E.S., Langenheder, S., Logue, J.B., Paterson, H., Laybourn-Parry, J., Rengefors, K., Tranvik, L., Bertilsson, S., 2013. Biogeography of bacterial communities exposed to progressive long-term environmental change. ISME Journal 7, 937–948.

Lupatini, M., Suleiman, A.K.A., Jacques, R.J.S., Antoniolli, Z.I., de Siqueira Ferreira, A., Kuramae, E.E., Roesch, L.F.W., 2014. Network topology reveals high connectance levels and few key microbial genera within soils. Frontiers in Environmental Science 2, 10.

Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., García-Gómez, M., Bowker, M.A., Soliveres, S., Escolar, C., García-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., Bran, D., Conceição, A.A., Cabrera, O., Chaieb, M., Derak, M., Eldridge, D.J., Espinosa, C.I., Florentino, A., Gaitán, J., Gatica, M.G., Ghiloufi, W., Gómez-González, S., Gutiérrez, J.R., Hernández, R.M., Huang, X., Huber-Sannwald, E., Jankju, M., Miriti, M., Monerris, J., Mau, R.L., Morici, E., Naseri, K., Ospina, A., Polo, V., Prina, A., Pucheta, E., Ramírez-Collantes, D.A., Romão, R., Tighe, M., Torres-Díaz, C., Val, J., Veiga, J.P., Wang, D., Zaady, E., 2012. Plant species richness and ecosystem multifunctionality in global drylands. Science 335, 214–218.

Maier, S., Tamm, A., Wu, D., Caesar, J., Grube, M., Weber, B., 2018. Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. ISME Journal 12, 1032–1046.

Mugnai, G., Rossi, F., Felde, V.J.M.N.L., Colesie, C., Büdel, B., Peth, S., Kaplan, A., De Philippis, R., 2018. Development of the polysaccharidic matrix in biocrusts induced by a cyanobacterium inoculated in sand microcosms. Biology and Fertility of Soils 54, 27–40.

Newman, M.E., 2006. Modularity and community structure in networks. Proceedings of the National Academy of Sciences of the United States of America 103, 8577–8582.

Pande, S., Kost, C., 2017. Bacterial unculturability and the formation of intercellular metabolic networks. Trends in Microbiology 25, 349–361.

Pande, S., Merker, H., Bohl, K., Reichelt, M., Schuster, S., de Figueiredo, L.F., Kaleta, C., Kost, C., 2014. Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. ISME Journal 8, 953–962.

Pandit, S.N., Kolasa, J., Cottenie, K., 2009. Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. Ecology 90, 2253–2262.

Pepe-Ranney, C., Koechli, C., Potrafka, R., Andam, C., Eggleston, E., Garcia-Pichel, F., Buckley, D.H., 2016. Non-cyanobacterial diazotrophs mediate dinitrogen fixation in biological soil crusts during early crust formation. ISME Journal 10, 287–298.

Pritschet, L., Powell, D., Horne, Z., 2016. Marginally significant effects as evidence for hypotheses: Changing attitudes over four decades. Psychological Science 27, 1036–1042.

Pushkareva, E., Kvíderová, J., Šimek, M., Elster, J., 2017. Nitrogen fixation and diurnal changes of photosynthetic activity in Arctic soil crusts at different development stage. European Journal of Soil Biology 79, 21–30.

R-Core-Development-Team, 2010. R: A language and environment for statistical computing. Computing 14, 12–21.

Rominger, A.J., Miller, T.E., Collins, S.L., 2009. Relative contributions of neutral and niche-based processes to the structure of a desert grassland grasshopper community. Oecologia 161, 791–800.

Rosentreter, R., Eldridge, D.J., Westberg, M., Williams, L., Grube, M., 2016. Structure, Composition, and Function of Biocrust Lichen Communities. In: Weber, B., Büdel, B., Belnap, J., eds. Biological Soil Crusts: An Organizing Principle in Drylands. Ecological Studies (Analysis and Synthesis). Springer, Cham, 121–138.

Santos, H.F., Carmo, F.L., Duarte, G., Dini-Andreote, F., Castro, C.B., Rosado, A.S., van Elsas, J.D., Peixoto, R.S., 2014. Climate change affects key nitrogen-fixing bacterial populations on coral reefs. ISME Journal 8, 2272–2279.

Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S., Schloter, M., 2017. Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biology and Fertility of Soils 53, 485–489.

Seth, E.C., Taga, M.E., 2014. Nutrient cross-feeding in the microbial world. Frontiers in Microbiology 5, 350.

Sieber, J.R., McInerney, M.J., Gunsalus, R.P., 2012. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Annual Review of Microbiology 66, 429–452.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. ISME Journal 7, 2069–2079.

Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012.

Stochastic and deterministic assembly processes in subsurface microbial communities. ISME Journal 6, 1653–1664.

Stephen, A., Jack, A., Jonathan, W., Seren, M., Caitlyn, M., Mary, R., Benjamin, L., Francis, Q., Krista, L., 2017. Consequences of tropical forest conversion to oil palm on soil bacterial community and network structure. Soil Biology & Biochemistry 112, 258–268.

Vellend, M., 2010. Conceptual synthesis in community ecology. Quarterly Review of Biology 85, 183–206.

Vellend, M., Srivastava, D.S., Anderson, K.M., Brown, C.D., Jankowski, J.E., Kleynhans, E.J., Kraft, N.J., Letaw, A.D., Macdonald, A.A.M., Maclean, J.E., Myers-Smith, I.H., Norris, A. R., Xue, X., 2014. Assessing the relative importance of neutral stochasticity in ecological communities. Oikos 123, 1420–1430.

Vestergaard, G., Schulz, S., Schöler, A., Schloter, M., 2017. Making big data smart—how to use metagenomics to understand soil quality. Biology and Fertility of Soils 53, 479–484.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E. B., 2002. Towards an Ecological Understanding of Biological Nitrogen Fixation, In: Boyer, E.W., Howarth, R.W. (eds.). The Nitrogen Cycle at Regional to Global Scales. Springer, Dordrecht. 1–45.

Wang, Q., Quensen, J.F. 3rd, Fish, J.A., Lee, T.K., Sun, Y., Tiedje, J. M., Cole, J.R., 2013. Ecological patterns of *nifH* genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. mBio 4, e00592–e13.

Wang, Y., Li, C., Kou, Y., Wang, J., Tu, B., Li, H., Li, X., Wang, C., Yao, M., 2017a. Soil pH is a major driver of soil diazotrophic community assembly in Qinghai-Tibet alpine meadows. Soil Biology & Biochemistry 115, 547–555.

Wang, Y., Li, C., Shen, Z., Rui, J., Jin, D., Li, J., Li, X., 2019. Community assemblage of free-living diazotrophs along the elevational gradient of Mount Gongga. Soil Ecology Letters 1, 136–146.

Wang, Y., Li, H., Li, J., Li, X., 2017b. The diversity and co-occurrence patterns of diazotrophs in the steppes of Inner Mongolia. Catena 157, 130–138.

Webb, C.O., Ackerly, D.D., Kembel, S.W., 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics (Oxford, England) 24, 2098–2100.

Weber, B., Bowker, M., Zhang, Y., Belnap, J., 2016. Natural Recovery of Biological Soil Crusts After Disturbance. In: Weber, B., Büdel, B., Belnap, J., eds. Biological Soil Crusts: An Organizing Principle in Drylands. Ecological Studies (Analysis and Synthesis). Springer, Cham, 479–498.

Wirtz, N., Lumbsch, H.T., Green, T.A., Türk, R., Pintado, A., Sancho, L., Schroeter, B., 2003. Lichen fungi have low cyanobiont selectivity in maritime Antarctica. New Phytologist 160, 177–183.

Wu, N., Zhang, Y.M., Downing, A., 2009. Comparative study of nitrogenase activity in different types of biological soil crusts in the Gurbantunggut Desert, Northwestern China. Journal of Arid Environments 73, 828–833.

Xu, L., Zhu, B., Li, C., Yao, M., Zhang, B., Li, X., 2020. Development of biological soil crust prompts convergent succession of prokaryotic communities. Catena 187, 104360.

Yao, M., Rui, J., Niu, H., Heděnec, P., Li, J., He, Z., Wang, J., Cao, W., Li, X., 2017. The differentiation of soil bacterial communities along a precipitation and temperature gradient in the eastern Inner Mongolia steppe. Catena 152, 47–56.

Yeager, C.M., Kornosky, J.L., Housman, D.C., Grote, E.E., Belnap, J., Kuske, C.R., 2004. Diazotrophic community structure and function in two successional stages of biological soil crusts from the Colorado Plateau and Chihuahuan Desert. Applied and Environmental Microbiology 70, 973–983.

Zehr, J.P., McReynolds, L.A., 1989. Use of degenerate oligonucleotides for amplification of the nifH gene from the marine cyanobacterium Trichodesmium thiebautii. Applied and Environmental Microbiology 55, 2522–2526.

Zhang, B., Kong, W., Wu, N., Zhang, Y., 2016. Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. Journal of Basic Microbiology 56, 670–679.

Zhang, B., Zhang, Y., Li, X., Zhang, Y., 2018. Successional changes of fungal communities along the biocrust development stages. Biology and Fertility of Soils 54, 285–294.

Zhang, B., Zhou, X., Zhang, Y., 2015. Responses of microbial activities and soil physical-chemical properties to the successional process of biological soil crusts in the Gurbantunggut Desert, Xinjiang. Journal of Arid Land 7, 101–109.