Increasing relative abundance of non-cyanobacterial photosynthetic organisms drives ecosystem multifunctionality during the succession of biological soil crusts

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A B S T R A C T

Biological soil crusts (biocrusts) are essential for ecosystem functioning, especially in drylands. However, we lack the knowledge of how ecosystem multifunctionality (EMF) responds to the development of biocrusts and the key factors mediating EMF during biocrust succession. In this study, we tested a series of essential ecosystem functions of the biocrust system and calculated a weighted EMF index, a processes-based EMF index, and a resource storage and availability based EMF index. Sequencing of the 16S rRNA gene and ITS gene was used to test differences in the community compositions of 16S rRNA gene-based organisms and ITS gene-based fungi in different biocrust stages. Results showed that the changing patterns and driving factors of all the three multifunctionality indices were similar. Later developed biocrust stages exhibited higher values of all three EMF indices. The 16S rRNA gene-based diversity reduced with biocrust succession. Biodiversity-EMF relationships varied when considering different biocrust stages and organisms. Across all biocrust stages, significantly negative relationships existed between the EMF indices and 16S rRNA based α-diversity, whereas positive relationships occurred between the EMF indices and both 16S rRNA and ITS gene-based β-diversity. Further analyses indicated that the increasing relative abundance of non-cyanobacterial photosynthetic organisms (represented by chloroplast sequences and lichenized fungi) was the key predictor of all three EMF indices during biocrust succession. Specifically, these organisms were Streptophyta, Chlorophyta and Bacillariophyta and lichenized fungi, e.g., Verrucaria, Caloplaca and Aspicilia. This study provided a mechanistic understanding of how biological compositions and diversity drive EMF with biocrust development.

1. Introduction

Biological soil crusts (biocrusts, hereafter) consist of microscopic eukaryotic algae, fungi, cyanobacteria, associated heterotrophic bacteria and macroscopic lichens and mosses (Belnap et al., 2016). These organisms are usually poikilohydric and dominate in semi-arid and arid environments. Biocrusts are classified based on species composition, functional group (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 these traits (Colesie et al., 2016). Belnap and Eldridge (2003) suggest classifying biocrusts into bare soil, cyanobacterial crust, lichen crust and moss crust according to the dominant photoautotrophic organisms and their general successional sequence. General successional sequence commonly occurs in a wide variety of regions (Weber et al., 2016), including the Gurbantunggut Desert in China (Zhang et al., 2015, 2016, 2018).

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Filamentous and mobile cyanobacteria, e.g., *Microcoleus* spp., are pioneer colonizers in bare soil (Belnap and Eldridge, 2003). Next, soil-surface-bound *Nostoc* and *Scytonema* become the dominant species in cyanobacterial crusts (Belnap and Eldridge, 2003; Büdel et al., 2016; Weber et al., 2016). Afterwards, early lichen species (e.g., *Cocclem* spp.) emerge, followed by a series of early to late lichens (e.g., *Toniina* spp.) and bryophytes (e.g., *Syntrichia canadensis*) in the community, forming the later developed biocrusts (Belnap and Eldridge, 2003; Weber et al., 2016). The *Microcoleus* spp. can help to bind soil particles to form a crust in bare soil (Büdel et al., 2016). Then, the microalgae, lichens and mosses can enhance soil stability by increasing soil aggregation and decreasing erosion (Bowker et al., 2006; Lange and Belnap, 2016; Rosentreter et al., 2016). For example, the mycobionts (e.g., fungi) form the main structure of lichens, and the photobionts (e.g., green algae and cyanobacteria) provide nutrients via photosynthesis and N₂ fixation (Lange and Belnap, 2016; Rosentreter et al., 2016). The symbiotic lichens can retain soil moisture and increase soil fertility, hence promoting the ecosystem’s primary productivity (Billings et al., 2003; Houseman et al., 2006; Morillas et al., 2017; Gao et al., 2020) and mitigating the negative effects of climate warming on ecosystem productivity (Maestre et al., 2013).

Due to their vital functional roles in dryland ecosystems, biocrusts are good model systems for exploring the relationships between biodiversity and ecosystem functions (Bowker et al., 2010, 2011). With the resource accumulation during biocrust succession, the diversity, biomass and species compositions of cyanobacteria vary significantly (Zhang et al., 2009; Lan et al., 2012). Similarly, soil physiochemical properties, microbial activities and community compositions are distinct among biocrust stages (Zhang et al., 2015, 2016, 2018; Xu et al., 2020, 2021). The later biocrust stages show higher photosynthesis and N₂ fixation activities compared with the earlier stages (Housman et al., 2006; Lan et al., 2012). Also, nitrogenase activity is higher in cyanobacterial crusts than in lichen and moss crusts (Wu et al., 2009; Pushkareva et al., 2017). Biocrust development can shift heterotrophic communities and alter physiological properties with respect to respiration rates and NO and HONO emission patterns (Maier et al., 2018). However, it remains elusive how the ecosystem functions as a whole, known as ecosystem multifunctionality (EMF, hereafter), as well as how biodiversity-EMF relationships vary with biocrust succession.

Ecosystem multifunctionality is defined as the ability of an ecosystem to provide multiple functions and services (Hector and Bagchi, 2007). Generally, higher biodiversity leads to greater functions (Cardinale et al., 2012). For example, increasing plant richness is found to enhance EMF (Maestre et al., 2012). Plant species richness, soil bacterial richness, soil faunal richness and soil biodiversity are positively correlated with above and belowground EMF (Jing et al., 2015). Compared to a single ecosystem function, more biodiversity is required to sustain ecosystem multifunctionality in drylands (Bowker et al., 2013). EMF can be quantified by an EMF index (EMFI). Several approaches are available to calculate an EMFI: the single threshold (Gamfeldt et al., 2013), multiple threshold (Byrnes et al., 2014), orthologous (Miki et al., 2014) and multivariable model approaches (Dooley et al., 2015), as well as the most widely used averaging approach (Hooper and Vitousek, 1998; Maestre et al., 2012). Previous studies have focused on ecosystem functions which quantify ecosystem C storage and productivity (e.g., plant and microbial biomass, plant productivity), ecosystem nutrient pool buildup (e.g., plant N and P contents, soil organic matter and soil N and P contents) and nutrient cycling (e.g., extracellular enzyme activities) capabilities at the same time (Maestre et al., 2012; Jing et al., 2015; Delgado-Baquerizo et al., 2016). However, the processes-based ecosystem functions may have different drivers and responses to environmental changes compared to resource storage and availability-based functions. For example, soil organic matter may be accumulated through increasing productivity or low decomposition.

In this study, we sampled different stages of biocrusts at five sites in the Gurbantungut Desert (Xinjiang, China) and studied how biocrust development influenced ecosystem multifunctionality, processes-based multifunctionality (EMFprocesses) and resource storage and availability based multifunctionality (EMFpools). We aimed to test the following hypotheses: (1) the succession of biocrust will be associated with higher values of EMF indices, (2) biodiversity and the values of EMF indices are positively correlated, and (3) dominant organisms in biocrust systems are key drivers in determining EMF.

2. Materials and methods

2.1. Study sites and sampling descriptions

The Gurbantungut Desert is located between 44.18° to 46.33°N and 84.52° to 90.00°E. It is the largest fixed and semi-fixed desert in China, and 40–50% of the sand dunes are fixed and 15–25% of them are partially fixed by drought-tolerant shrubs and herbs. In June 2017, biocrust samples were collected in Beishawo, Caianan, Erzhan, Sanhaodian and Yizhan. The geographical locations of these sites are shown in Fig. S1. In each sampling site, soil samples from bare soil and cyanobacterial, lichen and moss crusts were randomly collected by a soil corer (2.5 cm diameter) at the depth of 0–2 cm. In total, we collected 100 samples, including five replicates for each biocrust stage at each site. The samples were separated into two parts. One part was air dried for soil property measurements, and the other part was stored at –20 °C for molecular analysis (Vestergaard et al., 2017). Detailed site description, experimental design and sample collection are available in the Supplementary material.

2.2. Measurements of chlorophyll-a, CO₂ flux, nitrogenase activity and soil physiochemical properties

Chlorophyll-a was extracted by acetone and determined by a spectrophotometer and quantified using trichromatic equations (Garcia-Pichel and Canestenholz, 1991). Net photosynthetic CO₂ was measured by a portable photosynthesis system (LI-6400XT, LI-COR, USA) in situ, as described by Su et al. (2012). Soil moisture and temperatures were determined by a moisture meter (HH2, Delta-T Devices Ltd., England) at a depth of 0–5 cm. Soil pH and salinity were measured in a soil water suspension (1:5, w/v) by a pH meter and an electrical conductivity meter, respectively. Soil texture was determined by the hydrometer method (Bouyoucos and John, 1962). Soil was extracted with 2 M KCl, and then the exchangeable NH₄-N and NO₃-N were measured by the indophenol blue and phenol disulfonic acid methods, respectively (Weatherburn, 1967; Doane and Horwáth, 2003). Nitrogenase activity was measured by the acetylene-ethylene reduction assay in the laboratory (Hardy et al., 1968). Soil organic matter was determined using the K₂Cr₂O₇-H₂SO₄ oxidation method (Nelson et al., 1996), total nitrogen (TN) by the Kjeldahl procedure (Bremner, 1965), total K by the molybdenum blue method (Murphy and Riley, 1962), available P by the Olsen’s method (Olsen, 1954), and available K by the 1 mol/L NH₄OAc leaching-flaming luminosity (Kolterman and Truong, 1953). Detailed descriptions for net photosynthetic CO₂ and potential nitrogenase activity measurements are available in the supplementary material.

2.3. Formulating ecosystem multifunctionality indices

Considering that there may have different drivers and responses of the processes-based and nutrient-pool-based ecosystem functions, we calculated three EMF indices, namely EMF, EMFprocesses and EMFpools, respectively to represent all functions together, processes-only and nutrient-pool only functions.

To calculate an EMFI of the biocrust system, we considered (1) ecosystem C storage and primary productivity, which can be represented by chlorophyll-a contents (green/photosynthetic biomass) and net photosynthetic CO₂ and (2) resource storage and availability, which can
be represented by soil organic matter, exchangeable NH$_4^+$-N and NO$_3^-$-N, total N and P contents and available P and K contents and (3) nutrient cycling capability which can be represented by potential nitrogenase activity. These parameters indicated the functional potential of a biocrust stage (Table S1). Firstly, cyanobacteria, eukaryotic algae and moss contain chlorophyll-a, which can represent the biomass to fix C in the biocrust. In addition, net photosynthetic CO$_2$ indicates the potential primary productivity. Moreover, soil nutrient contents are essential in maintaining ecosystem functions, such as primary productivity, and they are mediated by biocrust developmental stages. Organic matter content is an indicator of soil fertility and C cycling. Total soil N represents the N storage in a nutrient pool of each biocrust stage. Exchangeable NH$_4^+$-N and NO$_3^-$-N and available P and K are important available nutrients for mosses, lichens and microorganisms (Schlesinger and Bernhardt, 2013; Delgado-Baquerizo et al., 2016b). Nitrogenase activity represents the potential N input in a dry ecosystem.

Since it is not recommended using highly correlated ecosystem functions to calculate an EMFI (Manning et al., 2018), using the above functional parameters, a weighted EMFI was calculated to down-weight highly correlated functions following three main procedures. Firstly, all functions were standardized by dividing their maximum observed values. Then, following the procedures proposed by Manning et al. (2018), a clustering analysis was conducted to obtain the weight coefficient of each function. In total, five clusters of ecosystem functions were obtained (Fig. S2a). Each cluster was assigned 1 as the weight coefficient, and the parameter weight coefficient was 1, divided by the number of functions in each cluster. Finally, the weighted EMFI was calculated with the following formula (Maestre et al., 2012; Manning et al., 2018):

$$\text{weighted EMFI} = \frac{1}{m} \times \sum_{i=1}^{n} a_i \times f_i$$

where $m$ is the number of function clusters; $n$ represents the number of functions selected; and $a_i$ and $f_i$ represent the weight coefficient and standardized value of function $i$, respectively. We also calculated the unweighted EMFI to verify the significance of the weighted EMFI using the averaging methods (Maestre et al., 2012).

Net photosynthetic CO$_2$ and potential nitrogenase activity can represent important processes related to ecosystem functions. Thus, the processes-based EMF$\text{processes}$ was calculated by averaging the standardized values of net photosynthetic CO$_2$ and potential nitrogenase activity. In addition, the remaining ecosystem functions were clustered (Fig. S2b) and weighted to calculate the nutrient-pool based EMF$\text{pool}$, which indicates the capability for resource storage and nutrient provisions in a biocrust system.

2.4. Collections of climate data

Using spatial analysis tools of ArcGIS10.2 (ESRI, USA), climate data, including mean annual temperature (MAT) and mean annual precipitation (MAP), were obtained according to the geological coordinates of sampling sites using the WorldClim database (http://www.worldclim.org/ version2) with a resolution of 2.5 min (Fick and Hijmans, 2017).

2.5. DNA extraction and amplicon sequencing

Soil DNA was extracted from 1 g of a biocrust soil sample using the Power Soil DNA Isolation kit (Qiagen, Hilden, Germany). Primers 515F and 909R were used to amplify the 16S rRNA gene, whereas gT57 and ITS4 (Bodor and Urban, 2007) were used to amplify the fungal ITS gene. Both primer sets were also used to determine the absolute abundances of 16S rRNA and ITS genes by quantitative PCR (qPCR). Detailed qPCR and PCR procedures are shown in the Supplementary material. Negative controls were performed to verify contamination. Libraries were constructed with equimolar mixed amplicons using a TruSeq DNA kit and sequenced using an illumina Hiseq platform with 2 x 250 bp V2 Kits.

The original sequence data were deposited at the National Center for Biotechnology Information, Sequence Read Archive (NCBI, SRA) with the following accession numbers:PRJNA451397 (16S) and PRJNA451416 (ITS). Both sequencing data files and associated abiotic properties were also available at the Microbiome Database (http://ecogud.cib.cn) with project numbers PRJ-AMPLI-0a583055711755db4f36e0e17bdc11ae (16S) and PRJ-AMPLI-229248be4e5981549703f4470ca951d (ITS).

2.6. Bioinformatics analysis

Paired-end reads were merged using the FLASH software (Magoc and Salzberg, 2011). Low-quality sequences were removed, namely, those sequences with a length < 300 bp (250 bp for ITS sequences), >2 ambiguous base ‘N’ and an average base quality score < 30. Twenty-seven fungal samples were discarded for their low sequence reads. Chimeras were removed using the UCHIME algorithm (Edgar et al., 2011). Afterward, the QIIME Pipeline (V1.9.7) (Caporaso et al., 2010) was used to identify the operational taxonomic units (OTUs) at the cutoff of 97% identity threshold. For the better prediction of functional groups, the Greengene database (McDonald et al., 2012) and the UNITE database (Kõljalg et al., 2013) were used to assign the taxonomy of 16S rRNA sequences and ITS sequences, respectively. All data were rarefied to 11,714 and 11,389 sequences per sample, respectively, for 16S rRNA datasets (including prokaryotes and chloroplasts sequences) and fungi ITS datasets for the downstream analyses. The putative functional profiles for prokaryote and chloroplast sequences were predicted using the FAPROTAX database (Louca et al., 2016), and the ecological guilds of fungi (functional groups) were predicted using the FUNGuild database (Nguyen et al., 2016).

2.7. Statistical analysis

All statistical analyses were conducted using R 3.4.3 (R-Core-Development-Team, 2010). The taxonomic Shannon diversity and community Bray-Curtis distance matrix were calculated using the “vegan” package (Oksanen et al., 2007). Phylogenetic diversity (mean nearest taxon distance, MNTD) was calculated using the “picante” package (Stegen et al., 2013; Wang et al., 2017). Comparisons of ecosystem functions among biocrust stages or sampling locations were analyzed by the non-parametric Kruskal-Wallis test. Relationships between the EMF indices and β-diversity indices based on 16S rRNA and ITS sequence data were assessed by the multiple regression on distance matrices (MRM) analyses, using the “ecodist” package (Goslee and Urban, 2007). Considering the functional profiles (groups) of prokaryotes and fungi together, Random Forest analysis (Liaw and Wiener, 2002) was used to identify the biomarkers governing EMF using the “randomForest” package. The significance of each biomarker’s importance on all three EMF indices was assessed using the “rfPermute” package (Archer, 2016).

Model selection procedures based on the corrected Akaike information criterion (AICc) were applied to evaluate the best predictors of the EMF indices using the “stepAICc” function. Then, with the resulting models, three model averaging procedures were used to select the best predictors using the “MuMln” package (Barton, 2016). We considered climate variables (including MAT and MAP), soil pH, soil salinity, soil texture (clay, silt and sand contents), the ratio of ITS to 16S rRNA gene abundance, 16S rRNA gene and fungal diversity indices (including MNTD and Shannon diversity) and functional groups with random forest $P$ values less than 0.01 as potential predictors. Before modeling, all the above variables were standardized by dividing their maximum observed values to transform parameter estimates to a comparable scale.
3. Results

3.1. Variations of various ecosystem functions with biocrust development

Soil available K, soil organic matter, chlorophyll-a content, photosynthetic CO$_2$ and total N and total P contents increased significantly with biocrust development (Fig. 1). The NO$_3$-N content was lower in bare soil than in other biocrust stages and it did not change significantly from cyanobacterial crust to moss crust. Soil NH$_4$-N contents in bare soil and cyanobacterial crust were lower than that in moss crust. In addition, soil-available P contents were lower in bare soil and lichen crust, and higher in cyanobacterial and moss crusts. Potential nitrogenase activity was higher in cyanobacterial and lichen crusts and decreased from cyanobacterial to moss crusts (Fig. 1).

3.2. Variations of ecosystem multifunctionality with biocrust development

The EMF, EMF$_{processes}$ and EMF$_{pools}$ all increased significantly with the development of biocrust at all sites ($P < 0.0001$, Figs. 2 and S3), but none of these indices was significantly different among sampling sites ($P > 0.05$). The weighted and unweighted EMFs were significantly and positively correlated ($R^2 = 0.951$, $P < 0.001$, Fig. S4), suggesting that our results were robust based on the selected functions and multifunctionality index. The EMF$_{processes}$ and EMF$_{pools}$ showed significant and positive relationship ($R^2 = 0.434$, $P < 0.001$, Fig S5), indicating that with biocrust succession, process-based ecosystem functions were closely related to resource storage and availability. In addition, the EMF$_{processes}$ and EMF$_{pools}$ explained 70.40% and 86.30%, respectively, of the weighted EMF variations (Fig S5). Soil nutrient proxies, including organic matter (Spearman’s rho = 0.874, $P < 0.001$, Table S2) and total nitrogen (rho = 0.863, $P < 0.001$), and primary productivity proxies, including chlorophyll-a contents (rho = 0.832, $P < 0.001$) and net photosynthetic CO$_2$ (rho = 0.836, $P < 0.001$), were highly correlated with the weighted EMF, suggesting that these functions mostly drove the pattern of EMF. Soil NH$_4$-N, NO$_3$-N and potential nitrogenase activities were not significantly correlated with the weighted EMF. Similarly, the EMF$_{processes}$ variation pattern was also mainly driven by net photosynthetic CO$_2$ (rho = 0.931, $P < 0.001$), while total N (rho = 0.827, $P < 0.001$) and soil organic matter (rho = 0.856, $P < 0.001$) mostly drove the EMF$_{pools}$ variations (Table S2).

3.3. Variations of 16S rRNA genes and fungal diversity and their relationships with EMF

Both taxonomic (Shannon) and phylogenetic (MNTD) α-diversity indices based on 16S rRNA gene sequences were higher in bare soil than in other biocrust stages ($P < 0.05$, Fig. S6). Fungal phylogenetic diversity did not show a significant difference among the biocrust stages ($P > 0.05$), while fungal taxonomic diversity ($P < 0.05$) was lower in the lichen crust than in other stages. Although the explained variances were low, the phylogenetic diversity ($R^2 = 0.103$, $P = 0.001$, Fig. 3a) and taxonomic diversity ($R^2 = 0.044$, $P = 0.035$) of the 16S rRNA genes showed significantly negative relationships with EMF across all biocrust stages. However, the 16S rRNA gene-based taxonomic diversity showed a significant and positive relationship ($R^2 = 0.159$, $P = 0.049$, Fig. S7, Table S3) with EMF in the moss crust stage, similar to the relationships between EMF and fungal phylogenetic diversity in bare soil, although this was only supported by a statistically marginal significance ($R^2 = 0.115$, $P < 0.1$). In addition, a marginally significant and negative relationship was observed between EMF and fungal phylogenetic diversity in the cyanobacterial crust ($R^2 = 0.216$, $P < 0.1$). Biodiversity-EMF relationships were likely domain- and biocrust-stage-dependent. Similarly, at all biocrust stages, significantly negative relationships were observed between 16S rRNA gene diversity and both EMF$_{processes}$ and EMF$_{pools}$ (Figs. S8–S9). Meanwhile, domain- and biocrust-stage-dependent biodiversity-EMF$_{processes}$ biodiversity-EMF$_{pools}$ relationships were also observed (Figs. S8–S9, Tables S4–S5).

However, significantly positive correlations were observed between all three EMF indices and the 16S rRNA gene-based β-diversity, whether in terms of taxonomy or phylogeny (Fig. 3b and S10). Fungal α-diversity did not affect all three EMF indices significantly ($P > 0.1$, Fig. 3a, S8–S9). Moreover, fungal taxonomic and phylogenetic β-diversity showed significant correlations with EMF and EMF$_{pools}$, though $R^2$ values were lower than those of the 16S rRNA gene-based diversity indices (Fig. 3b and S10).

3.4. Predictors of EMF during biocrust succession

The most important biomarkers of EMF were C-fixing organisms, including non-cyanobacterial photosynthetic organisms (represented by chloroplast sequences and lichenized fungi, same hereafter), followed by anoxygenic phototrophic, anoxygenic phototrophic S oxidizing and methylo trophic prokaryotes (Fig. S11a). Meanwhile, chloroplast sequences and lichenized fungi were also key biomarkers of the EMF$_{processes}$ and EMF$_{pools}$ (Fig. S11 b and c). In addition, phototrophic prokaryotes were also important drivers of EMF$_{processes}$ (Figs. 4 and S11). Further analysis indicated that these processes were also supported by ordi nary least squares regression (Fig. S12), which showed logarithmic relationships between the multifunctionality indices and the relative abundance of chloroplast sequences, and positive linear relationships between the EMF indices and the relative abundance of lichenized fungi.

3.5. Photosynthetic organisms in different biocrust stages

The 16S rRNA gene sequences related to chloroplasts were from Streptophyta (including sequence DQ514105.1), Chlorophyta (AJ292689.1) and Bacillariophyta (FJ595580.1). Relative abundances of Bacillariophyta and Chlorophyta chloroplast sequences were extremely low compared to those of Streptophyta (Fig. 5a). The relative abundances of Bacillariophyta chloroplast sequences were higher in cyanobacterial and lichen crusts and lower in the bare soil and mosses stages, while relative abundances of Chlorophyta and Streptophyta were higher in the later biocrust stages. The relative abundances of chloroplast sequences showed significantly positive relationships with chlorophyll-a content and net photosynthetic CO$_2$ flux (Fig. S13), indicating that chlorophyll-a content and primary productivity contributed to the C fixation potential (C sequestration) in biocrust systems. In addition, the average relative abundance of cyanobacteria was high (9.69%) in all samples; however, it was higher in cyanobacterial and lichen crusts than in moss crust, and it was lowest in the bare soil (Fig S14). In addition, the relative abundance of cyanobacteria was significantly ($R^2 = 0.09$, $P < 0.001$) and positively correlated to potential nitrogenase activity (Fig. S15).

The most abundant lichenized fungi were from the genus Verrucaria (average relative abundance of 28.62%), followed by the genera Caloplaca (0.80%), Aspicilia (0.22%), Phaeophyscia (0.16%) and Circinaria (0.05%) and the family Corticiaceae (0.0015%). Most of these taxa were more abundant in later biocrust stages, except the family Corticiaceae (Fig. 5b). In addition, the relative abundances of both chloroplast sequences and lichenized fungi were negatively correlated to biodiversity (Fig. S16). We summarized the successional changes in ecosystem functions, biodiversity, environmental factors and microbial properties along the developmental stages of biocrust in Fig. S17.

4. Discussion

To our knowledge, this is the first effort to investigate the variations of ecosystem multifunctionality, processes-based EMF$_{processes}$ and
Fig. 1. Selected ecosystem functions at different biocrust stages. Dots out of box represent outliers. Different letters indicate non-parametric Kruskal-Wallis statistical significance at $P < 0.05$. AK, available potassium; SOM, soil organic matter; PNA, potential nitrogenase activity; TN, total N; TP, total P. Extreme outliers are discarded for NH$_4^+$-N and PNA for better visualization. $n$ indicates the numbers of samples used in each panel.
nutrient-pool-based EMF pools with biocrust succession. Our results showed that the development of biological soil crusts was associated with enhanced EMF, EMF processes and EMF pools, and the most important predictors related to all three EMF indices were non-cyanobacterial photosynthetic organisms. The similar variation patterns and driving factors for all three EMF indices suggested the process-based ecosystem functions and resource storage and availability functions were highly coupled with the development of biocrust.

4.1. Biocrust development is key to enhancing ecosystem functions

This study demonstrated that soil nutrient proxies (e.g., SOM and TN, Table S2) contributed mostly to EMF, indicating that EMF was mainly driven by nutrient accumulation during biocrust succession. In addition, N resources in biocrust systems of the Gurbantunggut Desert are likely more limited (around 0.25 g kg $^{-1}$) compared to crust soils in the Tengger Desert (Li et al., 2011), alpine meadow of the Qinghai-Tibet plateau (Wang et al., 2017) and desert and typical steppes in neighboring regions (Li et al., 2008; Xu and Wan, 2008). Increasing total N in later biocrust stages may increase the biomass of biocrust communities (Vitousek et al., 2002). This was further supported by our data that TN and photosynthetic biomass were significantly and positively correlated (Spearman’s rho = 0.759, P < 0.001).

Our results showed significant differences in the EMF indices among biocrust stages while no significant differences among sampling sites. In addition, the changing patterns of all three EMF indices with biocrust stages were similar in all sites (Fig. S3). Moreover, spatial-dependent variables such as MAP did not show significant effects on EMF (Fig. 4), suggesting that geographical heterogeneity in our sampling area could not explain the EMF variations. All of these lines of evidence supported our first hypothesis that more developed biocrusts would exhibit higher EMF.

4.2. Biodiversity-EMF relationships are biocrust-stage-dependent

Many studies have reported positive relationships between biodiversity and EMF (Maestre et al., 2012; Jing et al., 2015; Delgado-Baquerizo et al., 2016b). However, we observed significant and negative relationships between all three EMF indices and 16S rRNA gene-based diversity (Fig. 3a, S8a and S9a) across biocrust stages. This is not surprising because with increasing productivity, competitive exclusion may alter biodiversity-multifunctionality relationships from positive to negative, whether based on biomass- or non-biomass-related ecosystem functions (Thompson et al., 2005; Jiang et al., 2008). Another study also reports that bacterial phylogenetic diversity is negatively related to ecosystem functions in soils along a fertility gradient (Perez-Valera et al., 2015). Likewise, bacterial diversity in biocrusts decreases with the increase of N availability (Wang et al., 2015). Therefore, the negative biodiversity-EMF relationships across biocrust stages were probably caused by competitive exclusion with increasing nutrient resources in later biocrust stages. This is reasonable considering that, besides mosses, microbes (including eukaryotic microalgae, prokaryotes and fungi) are the main organisms in the Gurbantunggut biocrusts (Zhang et al., 2015, 2016, 2018). Microbes in bare soil is under higher stress than those in other biocrust stages; thus, they must invest more energy for survival rather than for competition (Fierer et al., 2012; Song et al., 2017). Competitive exclusion may be stronger in nutrient-rich biocrust stages, which can increase the relative abundances of those species closely related in phylogeny, and therefore reduce phylogenetic diversity (Figs. S6 and S16). Because species with closer phylogenetic
distances are usually functionally more related (Purschke et al., 2013), we speculated that, during biocrust succession, such negative biodiversity-EMF relationship implicated high functional redundancy (e.g., those organisms related to C sequestration) within 16S rRNA gene-based communities.

According to Alsterberg et al. (2017), the relative importance of biodiversity on EMF is known to change along a temporal scale. Therefore, it is greatly important to monitor the temporal relationship between biodiversity and EMF (Nannipieri et al., 2019). These points can partially explain the observed biocrust-stage-dependent relationships between biodiversity and the EMF indices.

Cyanobacteria and nitrogenase activity were positively related (Fig. S15), which could be an example of a positive relationship between the species abundance of a specific group of organisms and their associated ecosystem functions. Meanwhile, our previous study also shows significantly positive relationships between diazotrophic diversity and

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**Fig. 3.** Ordinary least squares (OLS) analyses showing relationships between EMF and phylogenetic α-diversity (represented by mean nearest taxon distance, MNTD) and taxonomic α-diversity (represented by Shannon index) (a). Shade areas indicate 95% confidence interval of fit. Multiple regression on distance matrices (MRM) analyses showing correlation coefficients between EMF and phylogenetic (represented by βMNTD distance matrix) and taxonomic (represented by Bray-Curtis distance matrix) β diversity (b). *** P < 0.001, * P < 0.05.

**Fig. 4.** Model averaging procedures (ΔAICc < 2) showing standardized regression coefficients of model predictors and associated 95% confidence intervals for EMF (a), EMF_processes (b) and EMF_pools (c). Confidence intervals that do not cross the zero line indicate that the predictors (e.g., chloroplast sequences and lichenized fungi in all three panels) under consideration are significantly (P < 0.05) associated with the EMF indices.
nitrogenase activity (Xu et al., 2021). Thus, we concluded that, during the succession of biocrust, diverse (negative, neutral or positive) relationships between biodiversity-ecosystem functions are possible because of different changing patterns of functions and organisms, as well as biocrust stages and sampling time. Further, positive relationships between the EMF indices and 16S rRNA gene-based and fungal β-diversity indicated that changes in community compositions among habitats might drive the EMF variations. These changes could be represented by increasing the relative abundances of non-photosynthetic organisms (mosses, eukaryotic microalgae and lichenized fungi, Figs. 3–5).

4.3. Non-cyanobacterial photosynthetic organisms enhance EMF through mediating C sequestration

The chloroplast sequences were assigned to Streptophyta, Chlorophyta and Bacillariophyta (Fig. 5a), indicating that chloroplast sequences were from mosses, and/or eukaryotic microalgae (Büdel et al., 2016). The relative abundances of diatoms (Bacillariophyta) and green algae (Chlorophyta) were extremely low compared to the relative abundances of Streptophyta (mostly mosses, Fig. 5a). The relative abundance of Bacillariophyta showed a decreasing pattern from lichen crust to the moss crust stage. The structural complexity of moss (presence of non-vascular plant tissue with lots of chloroplasts in contrast to single-cell diatoms and green algae) resulted in high abundances of chloroplast sequences (Fig. 5a), chlorophyll-a contents and high carbon fixation potential (Figs. 1 and S13) in later biocrust stages, as well as the logarithmic relationship between the relative abundances of chloroplast sequences and the EMF (Fig. S12). Considering that the dominant functional biomarkers were C- rather than N-fixing organisms (Fig. S11), and the non-significant relationship occurred between EMF and potential nitrogenase activity (Table S2), we speculated that C sequestration with biocrust succession was the key process promoting EMF.

Lichens are also important organisms enhancing C sequestration (Fig. S13a). The high relative abundances of lichenized fungi and their positive relationships with the EMF (Figs. 4 and S12) indicated their important roles in enhancing ecosystem multifunctionality. The Verrucaria were the most dominant lichenized fungi (Fig. 5b), which can engage in symbiosis with green algae and heterokonts, e.g., Heterococcus and Petroderma (Lakatos et al., 2004). The members of order

Fig. 5. Relative abundances of chloroplast sequences (a) and lichenized fungi (b) in different biocrust stages. Dots out of box represent outliers. Different letters indicate non-parametric Kruskal-Wallis statistical significance at P < 0.05.
Verrucariales are major components in the biocrust systems of Tengger and Gurbantunggut deserts (Wang et al., 2015; Zhang et al., 2018). In addition, subdominant lichenized fungi can also exert essential ecosystem functions, e.g., Caloplaca and Aspicilia, that can bind detritus and stabilize soil (Rosentreter et al., 2016). Lichenized fungi can absorb water and nutrients and protect photobionts by filamentation, and photobionts can provide organic compounds (e.g., extracellular saccharides) to lichenized fungi (Lange and Belnap, 2016; Rosentreter et al., 2016). The increases in extracellular polysaccharides, biomass and soil organic matter with biocrust development enhance the water-holding capacity (Chamizo et al., 2018). The input water in such dry scenery can activate bacterial metabolisms (Garcia-Pichel and Frangiul, 2001), decompose organic matter and further promote lichen growth (Swenson et al., 2018). The symbiotic lichens together with mosses became dominant with biocrust development, increasing the competition exclusion ability and hence decreasing biodiversity (Fig. S16). On the other hand, the increasing abundances of non-cyanobacterial photosynthetic organisms enhanced the EMF. Finally, negative biodiversity-EMF relationships may occur when considering all biocrust stages. These findings supported our third hypothesis. 5. Conclusions This study advanced our understanding of ecosystem multifunctionality in the biocrust system, at least on three points: (1) biocrust development was associated with enhanced EMF, EMF_Functionality in the biocrust system, at least on three points: (1) biocrust development was associated with enhanced EMF, EMF processes and EMF_Hierarchies (2) 16S rRNA gene-based diversity indices were negatively correlated to all the EMF indices across biocrust stages; and (3) non-cyanobacterial photosynthetic organisms, including those lichenized organisms, play important roles in accumulating resources and subsequently enhancing ecosystem multifunctionality. Recognizing how multiple factors interactively mediate ecosystem functions with biocrust development is of great importance in future research. Therefore, an EMF index containing more ecosystem functions (e.g., soil respiration and decomposition rates, soil faunal biomass and microbiota) is necessary. A synthesis of more data from various biocrust systems would broaden our understandings of the structure and functions of biocrust systems at regional and global scales. Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Acknowledgements This work was supported by the National Natural Science Foundation of China (U20A2008, 320271548, 42077206), the National Key Research and Development Program of China (2018YFE0107000), the National Science Fund for Distinguished Young Scholars (41925028) and China Biodiversity Observation Networks (Sino BON). We thank the colleagues in Xinjiang Institute of Ecology and Geography, CAS for their help in field sampling and in providing environmental data. We appreciate the excellent edit work by Lisa Sheppard. Opinions expressed in this paper are those of the authors and not necessarily of the Illinois State Water Survey, the Prairie Research Institute, or the University of Illinois. No conflicts of interest have been declared. The authors gratefully acknowledge the support for this research from the Illinois State Water Survey at the University of Illinois at Urbana-Champaign. Appendix A. Supplementary data Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115052.

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