Cyanobacterial community composition and their functional shifts associated with biocrust succession in the Gurbantunggut Desert

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Summary

Cyanobacteria, as key biocrust components, provide a variety of ecosystem functions in drylands. In this study, to identify whether a cyanobacterial community shift is involved in biocrust succession and whether this is linked to altered ecological functions, we investigated cyanobacterial composition, total carbon and nitrogen contents of biocrusts in the Gurbantunggut Desert. Our findings showed that the biocrust cyanobacteria in the Gurbantunggut desert were mostly filamentous, coexisting with abundant unicellular colonial Chrooococciopsis. Heterocystous Nostoc, Scytonema and Tolyphothrix always represented the majority of biocrust nitrogen-fixing organisms, comprising an average of 92% of the nifH gene reads. Community analysis showed a clear shift in prokaryotic community composition associated with biocrust succession from cyanobacteria- to lichen- and moss-dominated biocrusts, and filamentous non-nitrogen-fixing cyanobacteria-dominated communities were gradually replaced by nitrogen-fixing and unicellular colonial communities. Along the succession, there were concomitant reductions in cyanobacterial relative abundance, whereas Chl-a, total carbon and nitrogen contents increased. Concurrently, distinct carbon and nitrogen stores shifts occurred, implying that the main ecological contribution of cyanobacteria in biocrusts changes from carbon- to nitrogen-fixation along with the succession. Our results suggest that any activity that reverses biocrust succession will influence cyanobacterial community composition and eventually lead to large reductions in soil carbon and nitrogen stores.

Introduction

Drylands account for more than 40% of the global land area (Reynolds et al., 2007; Roncero-Ramos et al., 2019), where vascular plants are often sparsely distributed due to limited water and soil resources. However, the gaps between vascular plants are commonly covered with biological soil crusts or biocrusts, forming a mosaic of macroscopic vascular plants and microscopic biocrust patches across the landscapes (Elliott et al., 2014; Lan et al., 2019). Biocrusts, as a layer of top-soil assemblages composed of photoautotrophic and heterotrophic microorganisms, provide a variety of ecosystem services and functions in drylands, such as protecting soil from erosion (Eldridge and Leys, 2003; Rodríguez-Caballero et al., 2012), influencing hydrologic soil properties (Belnap, 2006; Felde et al., 2014) and enhancing soil fertility through photosynthesis and nitrogen fixation (Elbert et al., 2012; Thomas, 2012; Büdel et al., 2014; Rodriguez-Caballero et al., 2018; Thomas et al., 2018).

Cyanobacteria, especially filamentous cyanobacteria, such as Microcoleus, are often the main photosynthetic colonists involved with the initial establishment of biocrusts due to their unique physiological and ecological characteristics (García-Pichel and Pringault, 2001; Wu et al., 2013; Lan et al., 2014). Therefore, cyanobacterial soil crusts (cyanobacteria-dominated biocrusts with very limited lichens and mosses; Wu et al., 2014) are
generally regarded as the primary or early successional stages, which gradually improve the soil surface microenvironments and provide the opportunities for colonization of later successional lichens and/or mosses (Zaadý et al., 2000; Belnap, 2006). The photosynthetic dominants change through time during this process, resulting in biocrusts characterized by varying proportions of cyanobacteria, lichens and mosses (Housman et al., 2006; Zhang et al., 2007 Maier et al., 2018). Changes to the dominant species and community composition both stimulate and underpin biocrust ecological succession (Hagemann et al., 2014; Lan et al., 2014). In turn, different dominant photoautotrophic types associated with biocrust succession control microbial diversity, pigment composition and physiology (Lan et al., 2017; Maier et al., 2018).

Biocrust composition is sensitive to climate change and land disturbance, with a recent estimate suggesting that the global distribution of biocrusts could decrease by 25%–40% in the next 65 years (Rodriguez-Caballero et al., 2018). However, the extent to which biocrust are affected by climatic and land disturbance is still largely dependent upon their species composition. Eldridge and Delgado-Baquerizo (2019) reported that the decrease of relative abundance in biocrust Peitulaceae, Teloschistaceae and Bryaceae was related to the positive temperature legacies (temperature increased), while increase in relative abundances of Cladoniaceae, Lecideaceae, Thelotremataceae and Pottiaceae was related to the positive precipitation legacy (precipitation increased). Furthermore, a continental-scale biocrust survey found cyanobacterium Microcoleus steenstrupii to be more thermotolerant but less psychrotolerant than M. vaginatus (García-Pichel et al., 2013), with recent evidence from Fernandes et al. (2018) suggesting that Scytomena may be particularly sensitive to decreased precipitation, followed by M. steenstrupii and M. vaginatus. There is a growing evidence base and consensus that biocrust communities will be affected by future environmental changes, and these changes may convert large areas of later successional biocrusts to early successional stages (Housman et al., 2006). However, there is only limited understanding of how cyanobacteria, as the pioneer, important carbon and nitrogen fixers in biocrusts, are linked to biocrust ecological succession. The current simplified model (García-Pichel, 2003) derived from observation in North America suggests that bundle forming non-heterocystous cyanobacteria (M. vaginatus, members of the M. steenstrupii complex) are the pioneers that stabilize the soil (García-Pichel and Wojciechowski, 2009). Once the soil is stable, sessile heterocystous cyanobacteria can colonize (Couradeau et al., 2016). The two types occupy differentiated vertical micro-niches (García-Pichel and Belnap, 2001).

We hypothesized that cyanobacterial community shift involved in biocrust ecological succession occurs at finer taxonomic resolution, and that the changes might affect the ecological functions of biocrusts. It is important to obtain a more detailed knowledge in the face of the known differential effects of climate change and soil disturbance on community composition and function. In this study, we investigated cyanobacterial community compositions, and associated ecological parameters (e.g. total carbon and nitrogen stores) in different successional types of biocrusts from the arid Gurbantunggut Desert, China (average annual precipitation 79.5 mm with potential evaporation more than 2600 mm; Zhang et al., 2007), to test the validity of current models of cyanobacterial community shift, increase our organismal resolution involved in biocrust succession, and lastly to assess if cyanobacterial community shift correlates with biocrust carbon and nitrogen stores.

Results
Cyanobacterial diversity and community structure

A total of 460 987 valid sequences (1121 OTUs) were obtained from four successional types of biocrusts (with four replicates of each giving a total of 16 samples) using 16S rRNA gene high-throughput sequencing (Table 1). The majority (87.82%) of sequences were assigned to 16 Phyla, and the remaining sequences were annotated as candidate divisions BJ-169, BRC1, FBP and unclassified (others in Fig. 1). The most abundant phyla were Cyanobacteria (26.05% of the total reads across all samples), Actinobacteria (23.29%) and Proteobacteria (21.16%); followed by Chloroflexi (5.20%), Acidobacteria (3.41%), Bacteroidetes (3.27%), Planctomycetes (2.13%) and Gemmatimonadetes (1.45%; Fig. 1A), although this varied depending on the successional types of biocrusts (Fig. 1B).

Table 2 shows the taxonomic assignments of cyanobacterial operational taxonomic units (OTUs) based on their positions within the phylogenetic trees constructed using 16S rRNA gene sequences (Fig. 2A and B). A total of 47 cyanobacterial OTUs were identified through the 16S rRNA gene sequences, which were divided into 20 Clades (Fig. 2; Table 2). Only two Clades including 10 OTUs were identified as unicellular colonial cyanobacteria belonging to genus Chroococcidiopsis; while 13 Clades (28 OTUs) were identified as non-nitrogen-fixing filamentous cyanobacteria, including Crinalium, Lyngbya, Microcoleus, Oculatella, Pseudosphormidium and Trichocoleus (Fig. 2A and B). The most abundant Clade 13 was assignable to M. vaginatus, which accounted for 43% of the total cyanobacterial sequences, followed by Clade16 (Nostoc spp.; 12.67%), Clade8
Cyanobacterial OTUs (nifH) 30 31
Cyanobacterial reads (nifH) 20 494
nifH gene reads 27 054
Cyanobacterial OTUs (16S rRNA) 38 46

was no significant difference between the different successional types (P > 0.05; Fig. 4A). Furthermore, the relative abundance of unicellular colonial cyanobacteria (Chroococcidiopsis) in the whole cyanobacterial community increased (P < 0.05), while the relative abundance of the filamentous non-nitrogen-fixing cyanobacteria decreased gradually along with biocrust succession (P < 0.05; Fig. 4B).

Except for Clades 2, 10 and 19, relative abundance for all the other cyanobacterial Clades was significantly different depending on biocrust successional types (P < 0.05; Table 2). Cyanobacterial community analysis revealed that the relative abundance of M. vaginatus decreased along with the succession, while members of the M. steenstrupii complex showed a significant increasing trend (Fig. 3A; Table 2). Similarly, with the succession of biocrusts, Chroococcidiopsis spp., Oculatella spp., Nostoc spp., S. hyalinum 861, S. hyalinum 275 and Tolypothrix distorta showed an increasing trend (Fig. 3A). Considering ESCs and DSCs as a whole (as well as lichen and moss soil crusts as a whole; LSCs and MSCs), a significantly increased relative abundance was observed for the Clades assigned as Chroococcidiopsis spp., Microcoleus sp. 1, Microcoleus spp., Nostoc spp., Oculatella spp., Pseudophormidium, S. hyalinum 275, S. hyalinum 861, Scytomena spp. and T. distorta in LSCs and MSCs compared with early cyanobacterial ESCs and DSCs; while the decrease was obtained for Crinalium sp. 1, Crinalium sp. 2, Crinalium sp. 3, Lyngbya sp., M. cf. chthonoplastes, Microcoleus sociatus and M. vaginatus (Fig. S2A and B).

At genus level, the relative abundance for every genus was significantly different among the different successional types (P < 0.05; Table S1). Overall, Microcoleus, Chroococcidiopsis, Nostoc and Scytomena are the main genera found in the biocrusts from the Gurbantunggut Desert, accounting for 92.70% of the entire cyanobacterial community. Compared with ESCs and DSCs, LSCs and MSCs had a higher relative abundance in the genera Chroococcidiopsis, Nostoc, Oculatella, Pseudophormidium, Scytomena and Tolypothrix; while early cyanobacterial and developed-cyanobacterial soil crusts (ESCs and DSCs) had a higher relative abundance of unicellular colonial cyanobacteria (Chroococcidiopsis) in the whole cyanobacterial community.

### Table 1. Biocrust development status and basic characteristics derived from high-throughput sequencing.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>ESCs</th>
<th>DSCs</th>
<th>LSCs</th>
<th>MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successional type</td>
<td>Early cyanobacterial soil crusts</td>
<td>Developed-cyanobacterial soil crusts</td>
<td>Lichen soil crusts</td>
<td>Moss soil crusts</td>
</tr>
<tr>
<td>Colour</td>
<td>Light grey</td>
<td>Dark grey</td>
<td>Black</td>
<td>Brown</td>
</tr>
<tr>
<td>Dominants</td>
<td>Microcoleus spp.</td>
<td>Microcoleus spp.</td>
<td>Collema</td>
<td>Bryum</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.82–3.95</td>
<td>2.29–3.92</td>
<td>6.42–8.32</td>
<td>11.80–12.94</td>
</tr>
<tr>
<td>Development index</td>
<td>0.03–0.10</td>
<td>0.05–0.18</td>
<td>0.14–0.32</td>
<td>0.87–0.97</td>
</tr>
<tr>
<td>Cyanobacterial reads (16S rRNA)</td>
<td>7289–10 837</td>
<td>4591–11 752</td>
<td>7736–12 137</td>
<td>1892–5019</td>
</tr>
<tr>
<td>Cyanobacterial OTUs (16S rRNA)</td>
<td>38</td>
<td>48</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Cyanobacterial reads (nifH)</td>
<td>20 494–47 993</td>
<td>27 652–43 779</td>
<td>27 151–43 735</td>
<td>26 556–30 464</td>
</tr>
<tr>
<td>Cyanobacterial OTUs (nifH)</td>
<td>30</td>
<td>30</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td><strong>Note:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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abundance of sequences in the genera Crinalium, Lyngbya and Microcoleus (Fig. S2C and D).

Total carbon, total nitrogen and pigment content

From ESCs, DSCs, to LSCs and MSCs, biocrust chlorophyll-a (Chl-a) and carotenoid contents gradually increased, although the difference between any two successional types was not always significant at 0.05 level (Fig. 5A and B). Scytonemin content was highest in the LSCs, and all pigment contents were lowest in the ESCs (Fig. 5). In particular, the scytonemin content in ESCs was only 3.74% of that in the LSCs. In addition, there was a linear positive correlation between Chl-a and carotenoid content ($R^2 = 0.826; P < 0.001$), and the ratio of carotenoid to Chl-a content was maintained at 0.18 (Fig. S3A). By contrast, biocrust scytonemin content increased and then decreased with the increasing Chl-a content ($R^2 = 0.544; P = 0.002$; Fig. S3A).

Total carbon and total nitrogen contents of the biocrusts ranged from 4.22 to 23.50 g kg$^{-1}$ and 0.51 to 1.49 g kg$^{-1}$ respectively, and there were significant differences between biocrust types ($P < 0.05$; Fig. 4C). Both total carbon and total nitrogen contents gradually increased with biocrust succession (from ESCs to MSCs), and the ratio of total carbon to total nitrogen was consistent at around 13.5, ($R^2 = 0.604; P < 0.001$, Fig. S3B).
<table>
<thead>
<tr>
<th>Clade number</th>
<th>Taxa</th>
<th>OTUs</th>
<th>Species from NCBI (accession no.)</th>
<th>X²</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clade 1</td>
<td><em>Pseudophormidium</em> sp. a</td>
<td>OTU347</td>
<td><em>Pseudophormidium</em> sp. WJT61-NPBG13 (KJ939055)</td>
<td>11.805</td>
<td>3</td>
<td>0.008</td>
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<tr>
<td>Clade 2</td>
<td><em>Chroococcidiopsis</em> sp.</td>
<td>OTU891</td>
<td><em>Chroococcidiopsis</em> sp. MKC.28 (MF67540)</td>
<td>4.848</td>
<td>3</td>
<td>0.183</td>
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<tr>
<td>Clade 3</td>
<td><em>Crinalium</em> sp. 1</td>
<td>OTU11</td>
<td><em>Crinalium epipsammum</em> SAG 22.89 (NR 112218)</td>
<td>8.365</td>
<td>3</td>
<td>0.039</td>
</tr>
<tr>
<td>Clade 4</td>
<td><em>Trichocoleus desertorum</em></td>
<td>OTU276</td>
<td><em>Trichocoleus desertorum</em> 64et (KX759133); Trichocoleus desertorum Mon69 D (MK478718)</td>
<td>8.568</td>
<td>3</td>
<td>0.036</td>
</tr>
<tr>
<td>Clade 5</td>
<td><em>Crinalium</em> sp. 2</td>
<td>OTU172</td>
<td><em>Crinalium</em> sp. UMPCCC 1112 (KM218881)</td>
<td>13.792</td>
<td>3</td>
<td>0.003</td>
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<tr>
<td>Clade 6</td>
<td><em>Crinalium</em> sp. 3</td>
<td>OTU445</td>
<td><em>Crinalium</em> sp. UMPCCC 1112 (KM218881)</td>
<td>9.253</td>
<td>3</td>
<td>0.026</td>
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<tr>
<td>Clade 7</td>
<td><em>Lyngbya</em> sp.</td>
<td>OTU20; OTU305; OTU493; OTU530; OTU483; OTU583; OTU264; OTU485; OTU1053</td>
<td><em>Lyngbya</em> aestuarii ISB2 (KJ499108)</td>
<td>12.295</td>
<td>3</td>
<td>0.006</td>
</tr>
<tr>
<td>Clade 8</td>
<td><em>Chroococcidiopsis</em> spp.</td>
<td>OTU394; OTU494; OTU1075</td>
<td><em>Chroococcidiopsis</em> thermals CHAB1690 (JX494785); <em>Chroococcidiopsis</em> sp. BB79.2 SAG 2023 (AJ344552); <em>Chroococcidiopsis</em> sp. CMEE 246 (JF810073); <em>Chroococcidiopsis</em> sp. A789-2 (JF810071); <em>Chroococcidiopsis</em> sp. CC1 (DQ914863.2); <em>Chroococcidiopsis</em> sp. UFS-A4UI-NPMV4-B4 (KC525099); <em>Chroococcidiopsis</em> sp. CXA029 4 (MH463398)</td>
<td>10.522</td>
<td>3</td>
<td>0.015</td>
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<tr>
<td>Clade 9</td>
<td><em>Oculatella</em> ssp.</td>
<td>OTU75; OTU512</td>
<td><em>M. sociatus</em> SAG 26.92 (AF284808); <em>M. sociatus</em> MPI 96MS.KID (AF284809)</td>
<td>5.051</td>
<td>3</td>
<td>0.168</td>
</tr>
<tr>
<td>Clade 10</td>
<td><em>Microcoleus sociatus</em></td>
<td>OTU21</td>
<td>*Microcoleus cf. chthonioplastes Yyy1100 (KC463182)</td>
<td>11.471</td>
<td>3</td>
<td>0.009</td>
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<tr>
<td>Clade 11</td>
<td><em>Microcoleus</em> cf. chthonioplastes</td>
<td>OTU284; OTU348</td>
<td><em>Microcoleus</em> cf. chthonioplastes Yyy1100 (KC463182)</td>
<td>9.375</td>
<td>3</td>
<td>0.025</td>
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<tr>
<td>Clade 12</td>
<td><em>Microcoleus</em> sp.</td>
<td>OTU74; OTU571</td>
<td><em>M. vaginatus</em> KZ-23-1 (MK211228); <em>M. vaginatus</em> USPCI-1 (EU586737); <em>M. vaginatus</em> 127–1 (AJ871222); <em>M. vaginatus</em> Pasv-RS27 (KU175689)</td>
<td>11.669</td>
<td>3</td>
<td>0.009</td>
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<tr>
<td>Clade 13</td>
<td><em>Microcoleus</em> sp.</td>
<td>OTU505</td>
<td><em>M. steenstrupii</em> 177-4B (AF355392); <em>M. steenstrupii</em> 177-7A (AF355395); <em>M. steenstrupii</em> ATAD-2-DP02 (KX311865); <em>M. steenstrupii</em> 187–2 (AJ871986); <em>M. steenstrupii</em> COL109 (KX996383); <em>M. steenstrupii</em> 141–2 (AJ871981); <em>M. steenstrupii</em> 129–1 (AJ871979)</td>
<td>10.318</td>
<td>3</td>
<td>0.016</td>
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<tr>
<td>Clade 14</td>
<td><em>Nostoc</em> sp.</td>
<td>OTU96; OTU482; OTU555</td>
<td><em>Nostoc cf. indistinguendum</em> F15-VF4 (AY577540); <em>Nostoc</em> sp. Lukesova 5/96 (AM711546); <em>Nostoc</em> punctiforme KZ-2-2-2 (MK211227); <em>Nostoc</em> commune Mon62 A (MK478701); <em>Nostoc</em> flagelliforme str. Sunlezuqugi (GU10186)</td>
<td>8.647</td>
<td>3</td>
<td>0.034</td>
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<tr>
<td>Clade 15</td>
<td><em>Toxopthrix distorta</em></td>
<td>OTU310</td>
<td><em>Toxopthrix</em> sp. Ep Yyy1400 (KC463234); T. distorta CAU13 (MG641916)</td>
<td>9.088</td>
<td>3</td>
<td>0.028</td>
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<td>Clade 16</td>
<td><em>S. hyalinum</em> 275</td>
<td>OTU275</td>
<td><em>S. hyalinum</em> Mon66 C (MK478708)</td>
<td>12.463</td>
<td>3</td>
<td>0.006</td>
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<tr>
<td>Clade 17</td>
<td><em>Scytonema</em> spp.</td>
<td>OTU78; OTU298; OTU406</td>
<td><em>Scytonema</em> sp. CMT-2BRIN- HLNPC8 CGSFID17 (KF934149)</td>
<td>7.699</td>
<td>3</td>
<td>0.053</td>
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<td>Clade 18</td>
<td><em>S. hyalinum</em> 861</td>
<td>OTU861</td>
<td><em>S. hyalinum</em> mm 1 (MF574180); <em>S. hyalinum</em> AMS54C (MG641906)</td>
<td>11.206</td>
<td>3</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Clades 1 and 4 include the species in genus *Leptolyngbya*, thus the OTU347 and OTU276 may also be *Leptolyngbya* spp. In this table, we just classified the two OTUs based on the minimum genetic distances with *Pseudophormidium* sp. WJT61-NPBG13 (KJ939055) and *Trichocoleus desertorum* 64et (KX759133).*
Discussion

In this study, we investigated cyanobacterial communities of biocrusts in the extreme and Gurbantunggut desert. The cyanobacterial community in this region may represent the changing trend of many biocrust cyanobacterial communities from drylands, where aridity has been predicted to increase (Lin et al., 2015). Furthermore, we reveal a clear cyanobacterial community shift associated with biocrust succession, and an allied shift in biocrust carbon and nitrogen content.

Cyanobacterial composition and diversity of biocrusts

Studying the composition and diversity of cyanobacteria in biocrusts is an important step towards understanding their ecological functions in drylands. In total,
47 cyanobacterial OTUs were identified based on 16S rRNA gene sequences; a similar number to that previously reported by Dojani et al. (2014) in the succulent Karoo and Nama Karoo of Southern Africa by Steven et al. (2012) in the Nevada Desert of North America and by Muñoz-Martín et al. (2019) in the eastern Iberian...
Fig. 4. Cyanobacterial relative abundance, total carbon and total nitrogen content in the different successional types of biocrusts. A. Relative abundance changes of cyanobacterial 16S rRNA genes and nitrogen-fixing cyanobacterial nifH genes along with biocrust succession. B. Relative abundances of the different morphological groups of cyanobacteria (16S rRNA) in the different successional types of biocrusts. C. Total carbon and total nitrogen content in the different successional types of biocrusts. Single asterisk means the relative abundance of nitrogen-fixing cyanobacteria in the whole nitrogen-fixing communities; double asterisk means the cyanobacterial relative abundance in bacterial communities. For each parameter, the different lowercase letters indicate that the values are significantly different at the 0.05 level ($P < 0.05$).
Peninsula of Spain, although there are some differences in composition. Many cyanobacterial species in biocrusts are cosmopolitans, such as *M. vaginatus* and *M. steenstrupii* (Garcia-Pichel et al., 2013); however, there are some specifics that may be regional. For example, *Crinalium* was identified in the present study but was not found in previously mentioned studies.

Cyanobacteria in the biocrusts of the Gurbantunggut desert are mostly filamentous. In particular, filamentous non-nitrogen-fixing cyanobacterial species were the most abundant taxa, which are consistent with reports from many other drylands (e.g. Hagemann et al., 2014; Fernandes et al., 2018; Muñoz-Martín et al., 2019). This reflects the important role of filamentous cyanobacteria in the formation and maintenance of biocrusts, especially the bundle-forming filamentous cyanobacteria, such as the species of the genus *Microcoleus*. The filaments entangle soil particles, and secrete polysaccharides, cementing soil grains to form a fine crust structure (Lan et al., 2014; Mugnai et al., 2018). The most common heterocystous nitrogen-fixing filamentous cyanobacteria in our study were in the genera of *Nostoc*, *Scytonema* and *Tolypothrix*, although some other low abundance of nitrogen-fixing genera, such as *Calothrix*, were also found with Cydrasil analysis (Fig. S1), while this genus is typical of North American biocrusts (Yeager et al., 2007).

In addition to the filamentous cyanobacteria, there are abundant unicellular colonial cyanobacteria in the Gurbantunggut desert biocrusts. Most are in the genus *Chroococcidiopsis*, which is the major unicellular biocrust cyanobacterial genus (Hagemann et al., 2014; Williams et al., 2016; Muñoz-Martín et al., 2019). However, compared with other biocrust cyanobacterial communities (Steven et al., 2012; Dojani et al., 2014; Fernandes et al., 2018), the relative abundance of *Chroococcidiopsis* in the Gurbantunggut biocrusts is higher, sometimes exceeding 30% of total cyanobacterial abundance (Fig. 3), which may likely do with the extreme arid environment of this setting (Billi et al., 2000). Globally, *Chroococcidiopsis* variants have been regarded as common primary producers occurring in either hot or cold extreme arid deserts (Bahli et al., 2011).

The most dominant OTU across all biocrusts was *M. vaginatus* in the Gurbantunggut desert. This species has also been found to be dominant in other soil biocrusts worldwide (e.g. Starkenburg et al., 2011; Couradeau et al., 2019), and has been considered the most abundant soil cyanobacterium globally (Garcia-Pichel, 2003). The mean relative abundance of *M. vaginatus* in the present study was 43% of all cyanobacterial reads, which is likely to be caused by the low sensitivity of *M. vaginatus* to aridity than other biocrust cyanobacteria, such as the nitrogen-fixing cyanobacteria *Scytonema* and filamentous non-nitrogen-fixing cyanobacteria *M. steenstrupii* (Fernandes et al., 2018). Therefore, with the decline of precipitation in some areas under the global climate change, our results suggest that biocrust cyanobacteria community may develop towards the community structures with more abundance of *M. vaginatus* and *Chroococcidiopsis* spp.

**Cyanobacterial community shift associated with biocrust succession**

Our results reveal a clear cyanobacterial community shift associated with biocrust succession. ESCs and DSCs were mainly dominated by *M. vaginatus*, while...
nitrogen-fixing and unicellular colonial cyanobacteria, such as *Chroococcidiopsis* spp. and *Nostoc* spp., occurred profusely in LSCs and MSCs (Fig. 3A). The relative abundance of different species in the genus *Microcoleus* also changed with biocrust succession (Fig. S1A). Garcia-Pichel et al. (2013) first observed biocrust cyanobacterial substitution between different species in the genus *Microcoleus* and deduced this was largely driven by temperature. They proposed that *M. vaginatus* was more psychrotolerant, and *M. steenstrupii* was thermotolerant; therefore, climatic warming could lead to the replacement of *M. vaginatus* to *M. steenstrupii* in many dryland biocrusts. To some extent, our data also support this hypothesis. With biocrust succession, large quantities of scytonemin and carotenoids pigments develop (Fig. 5), giving later successional biocrusts, such as LSCs and MSCs, the ability to absorb more solar radiation energy, reducing biocrust albedo, thereby increasing biocrust temperature (Redfield et al., 2002; Couradeau et al., 2016). The resulting elevated temperatures may be unfavourable for the growth of *M. vaginatus*, which is further supported by the decreased relative abundance of *M. vaginatus* in LSCs and MSCs (Fig. 3A). Overall, the results of our study are in agreement with current models of biocrust cyanobacterial community shift derived from North American biocrusts (Garcia-Pichel and Wojciechowski, 2009; Garcia-Pichel et al., 2013), but we could define important novel aspects for some forms that are not the main, dominant types, and we could clearly demonstrate that biocrust succession actually involves significant increases with development in nitrogen-fixing cyanobacterial relative abundance, even as cyanobacterial populations become less dominant as lichens and mosses appear.

Functionally, community shift associated with increasing photosynthetic biomass (Chl-a content) suggests that the carbon-fixing role of cyanobacteria was gradually replaced with more cyanobacteria contributing to nitrogen fixation as biocrust succession progresses. This community shift may also relate to the differences in biocrust water absorption/retention between succession types, as well the sensitivity of biocrust cyanobacteria to moisture conditions. Nitrogen-fixing cyanobacteria *Scytonema* spp. has been regarded as the most sensitive biocrust taxa to drought (compared with *M. vaginatus* and *M. steenstrupii*; Fernandes et al., 2018), and biocrust water absorption/retention ability increases with their ecological succession (Redfield et al., 2002; Lan et al., 2015). As a result, a large proportion of nitrogen-fixing cyanobacteria are found in the later successional biocrusts (Housman et al., 2006; Couradeau et al., 2016). However, with decreasing moisture availability in the future (Huang et al., 2016), cyanobacterial community substitution associated with biocrust succession thus may be slowed down or even prevented.

A combination of climate change and increased soil disturbance is predicted to reduce biocrust coverage (Rodriguez-Caballero et al., 2018) and favour succession back to cyanobacterial soil crusts in some areas (Housman et al., 2006). This reversal of biocrust succession may result in a community shift to *M. vaginatus* dominated biocrust communities. At the same time, increasing aridity may cause the replacement of the nitrogen-fixing cyanobacteria (such as *Scytonema* spp.) by non-nitrogen-fixing and drought-tolerant filamentous cyanobacteria, such as *M. vaginatus* (e.g. Fernandes et al., 2018). However, there is some uncertainty because *M. vaginatus* is very sensitive to warming (Garcia-Pichel et al., 2013; Couradeau et al., 2016). In addition, nitrogen-fixing *Scytonema* also prefer warmer biocrust habitats (Muñoz-Martín et al., 2019). Therefore, with increasing aridity, warming and disturbance occurring in conjunction, it is still difficult to draw a unified conclusion on how the biocrust cyanobacterial community will develop and change in the future, but it will depend on the relative strengths of each factor. In a given region, several factors may work together to form a specific biocrust distribution and community compositions. Nevertheless, the sensitivity of biocrust cyanobacteria to aridity, warming and disturbances makes them potentially very good indicators of environmental change.

**Effects of cyanobacteria on biocrust carbon and nitrogen storage**

Biocrusts are widely distributed in dryland regions and are estimated to account for approximately 12% of the global land area (Rodriguez-Caballero et al., 2018); therefore, they are of great significance in carbon and nitrogen contribution in drylands and global biogeochemical cycle (Elbert et al., 2012). Our results regarding biocrust total carbon and total nitrogen contents increased with biocrust succession (Fig. 4C), are in agreement with the results of Garcia-Pichel and Belnap (1996), and those of Couradeau et al. (2016). The higher total carbon in the LSCs and MSCs could be explained by the increase of photosynthetic biomass (Fig. 5A) and carbon fixation ability (Lan et al., 2017) in the later successional types; while the higher total nitrogen in the later successional biocrusts was accompanied by the increasing relative abundance of nitrogen-fixing cyanobacteria (Fig. 4B), such as *Nostoc* and *Scytonema*.

The previous work in the Tengger Desert found that cyanobacterial and microalgal biomass decreased along with biocrust development and succession (Wu et al., 2014). Together, these previous and present results indicate that the contribution of cyanobacteria to biocrust carbon is gradually reduced with succession. An increasing number of lichens and mosses occupy a greater number
of biocrust niches, competing for light and water resources, and leaving the cyanobacteria with diminishing living space and resources. Although cyanobacterial relative abundance decreased significantly along with the succession, our nifH gene data revealed cyanobacteria always represented the vast majority of biocrust nitrogen-fixing organisms (approximately 92% of the nifH genes on average), and this is in agreement with the results from North American biocrusts (Yeager et al., 2007). In addition, non-cyanobacterial nitrogen fixers, however, may be also important in biocrust nitrogen fixation, particularly during the earliest phases of biocrust succession (Pepe-Ranney et al., 2016), in mutualistic association with M. vaginatus (Couradeau et al., 2019; Nelson et al., 2021).

Our results suggest the cyanobacterial community structure shift reflects the metabolism characteristics of the different successional biocrusts. This is in turn also closely related to biocrust ecological functions, such as carbon and nitrogen fixation and storage. With increasing aridity, warming and disturbance, biocrust cyanobacterial community structure associated with ecological succession could also be affected. Any reverse of biocrusts to early successional stages, such as cyanobacterial soil crusts, not only means dominant photosynthetic organisms such as lichens and mosses in the biocrusts being replaced by cyanobacteria but also the relative abundance of nitrogen-fixing cyanobacteria will be at risk of decline, which eventually leads to a decline in carbon and nitrogen storages in the dryland biocrusts.

Conclusion

This study of 16S rRNA and nifH genes in different successional biocrusts from the Gurbantunggut desert in China shows that cyanobacterial community shift accompanies biocrust succession, with cyanobacterial relative abundance becoming lower as the succession progresses. However, heterocystous Nostoc, Scyttonema and Tolypothrix always represent the majority of biocrust nitrogen-fixing organisms, contributing an average of 92% of the nifH genes. The specific cyanobacterial community in the extreme arid Gurbantunggut desert is mainly characterized by filamentous M. vaginatus with abundant unicellular colonial Chroococcidiopsis spp., suggesting that with the likely decline of water availability, biocrust cyanobacteria communities may develop community structures with greater abundances of M. vaginatus and Chroococcidiopsis spp. Moreover, cyanobacterial community analysis demonstrates that filamentous non-nitrogen-fixing cyanobacterial communities are gradually replaced by nitrogen-fixing and unicellular colonial communities along with the biocrust succession. Combined with biocrust carbon and nitrogen analysis, our results suggest the carbon-fixing role of biocrust cyanobacteria is gradually replaced with more cyanobacteria contributing to nitrogen fixation as succession progresses. The cyanobacteria present in each of the successional biocrusts have different sensitivities to moisture, temperature and disturbances, which makes them potentially very good indicators of environmental change, and require us to consider biocrust types when analysing cyanobacterial community structure. These results also suggest any activity that reverses or resets biocrust succession will influence the cyanobacterial community structure, affecting ecological functions, such as carbon and nitrogen fixation and store in drylands.

Experimental procedures

Experimental region

The study sites are located in the Fukang region of Xinjiang Uygur Autonomous Region, located on the southern edge of the Gurbantunggut Desert (44°29′-44°44′N, 88°19′-88°39′E). It has a temperate continental climate and an altitude of 520–550 m above sea level. The annual average air temperature is 7.3°C with the extreme minimum temperature lower than −30°C, and the average annual precipitation is approximately 79.5 mm, falling mainly from April to July, while the annual potential evaporation is more than 2600 mm (Zhang et al., 2007; Zhou et al., 2020). The region is covered by extensive fixed and semi-fixed sand dunes. Soils are sandy, composed of 73%–83% sand, which support a sparse natural vegetation cover dominated by Haloxylon ammodendron and H. persicum (Zhang et al., 2007; Zhou et al., 2012, 2020). In between vegetation patches, most of the surface is covered by biocrusts, including cyanobacterial, lichen and moss soil crusts (Zhang et al., 2007; Li et al., 2016).

Sample collection

Biocrusts were collected from two sites. Site 1 (44°44′ N; 88°39′E) was characterized by a low cover (c. 10%) and light grey of ESCs. In contrast, biocrust cover at site 2 (44°29′ N; 88°19′E) exceeded 50% of the soil surface in places, and there was a broader range of well-developed biocrust types, with dark grey DSCs, black LSCs and brown MSCs. In this study, the above-mentioned four biocrust types were collected to represent the different stages of ecological succession (Table 1). Each biocrust type was randomly collected at four different locations away from shrubs (at least 0.5 m), and all the sampling locations were separated from each other over 20 m. Biocrust samples were carefully scooped from the soil surface with a small trowel to ensure the natural
thickness of biocrusts was collected. One set of samples were placed in small tubes and stored in a -80°C fridge for subsequent DNA extraction; the other set was directly placed in Petri dishes and kept in a desiccator for subsequent physicochemical analysis.

Biocrust development index calculation

Major biocrust photosynthetic types were observed and identified with a binocular microscope/stereomicroscope according to the reference guides of Hu et al. (1980) and Rosentreter et al. (2007). Biocrust thickness (TH) was determined using a Vernier calliper, and an index of biocrust development (DI) was calculated as follows:

\[
\text{DI}_i = \frac{\text{TH}_i \times \text{Chl}_i}{(\text{TH}_{\text{max}} \times \text{Chl}_{\text{max}})}
\]

where DI\(_i\) indicates the biocrust development index in a given sample \(i\). TH\(_i\) and Chl\(_i\) are biocrust thickness and Chl-a content (analysing details are in the next section) in the sample \(i\); while TH\(_{\text{max}}\) and Chl\(_{\text{max}}\) are respectively the maximum biocrust thickness and Chl-a content across all the samples.

Pigment, total carbon and nitrogen analysis

For biocrust pigment, total carbon and total nitrogen analysis, samples were first ground with mortar and pestle and then passed through a 50-mesh sieve (300 μm). The pigment extraction was carried out in darkness over 12 h using 100% acetone at 4°C. The extract solutions were centrifuged at 8000 rpm for 10 min, and the absorption values at 384, 490 and 663 nm of the supernatant solution were determined using an UV-spectrophotometer (Beijing Persee General Instruments, China). The contents of Chl-a, carotenoids and sctycytonin were calculated according to the trichromatic equations of Garcia-Pichel and Castenholz (1991). To determine the total nitrogen content of the biocrusts, the ground samples were digested at 121°C for 30 min with alkaline potassium persulfate, and spectrophotometry was used to determine the concentrations using methods described by He et al. (2019). Total carbon content for the ground biocrust samples was measured using a Vario TOC analyser without any acid treatment (Elementar, Germany).

DNA extraction and PCR amplification

DNA extraction for each biocrust sample was carried out according to the instructions of the manufacturer of the soil DNA extraction kit (Omega, USA). DNA concentration and purity were determined by a NanoDrop2000 spectrophotometer (Thermo Scientific, USA), and DNA extraction quality was detected using 1% agarose gel electrophoresis.

Thereafter, the 16S rRNA gene was amplified with primers 338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’; Couradeau et al., 2016); while nifH gene was amplified with primers nifH-F (5’-AAGGYYGAWCGYAARTGCCACCAC-3’) and nifH-R (5’-TGTTSGCRTACATSGCCCAT-3’; Fiorentino et al., 2017). Both of the amplification systems (20 μl) contained 4 μl of 5× FastPfu buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM each), 0.4 μl of FastPfu polymerase, 0.2 μl of bovine serum albumin and 10 ng of the template DNA. The PCR amplification procedures were as follows: initial denaturation at 95°C for 3 min, followed by 27/35 cycles (27 cycles for 16S rRNA gene and 35 cycles for nifH gene) of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, and a final extension at 72°C for 10 min. All the PCR amplifications were carried out using a GeneAmp 9700 PCR System (ABI, USA).

High-throughput sequencing

The 16S rRNA and nifH gene amplification products were extracted using 2% agarose gel and purified by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). All the purified gene products were quantified using a QuantFlou™-ST quantitative system (Promega, USA) and then sequenced on the Miseq PE300 according to the Illumina MiSeq platform standard protocols (Illumina, USA). Sequencing was performed by the Major BioTech (Shanghai, China). All the sequence data were deposited to NCBI Short Read Archive database under BioProjects PRJNA723170 and PRJNA723272.

16S rRNA/nifH gene sequence analysis

All the 16S rRNA/nifH gene raw sequences were demultiplexed and quality-controlled using Trimmomatic trimmer and merged using Fast Length Adjustment of SHort reads (FLASH). The chimeric sequences were identified and removed using the UCHIME, and then each sample obtained 23 912–32 432 16S rRNA gene sequences and 27 054–54 404 nifH gene sequences (Table 1). The effective sequences were clustered into different OTUs with a 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/), and the representative sequence of each OTU was given. The RDP classifier algorithm (http://rdp.cme.msu.edu/) was then used to identify the OTUs against the Silva/ FunGene database (http://www.arb-silva.de/ and http://fungene.cme.msu.edu/; Silva database for 16S rRNA gene and FunGene database for nifH gene) with the confidence threshold of 70%. The Good’s coverage ranged from 99.24% to 99.98%, indicating that most of the
biocrust microorganisms containing the 16s rRNA/nifH gene had been revealed (Table 1).

Cyanobacterial sequence analysis

Regardless of the 16S rRNA or nifH genes, the non-cyanobacterial and chloroplast sequences were eliminated from our dataset, and all of the OTU representative sequences annotated as cyanobacteria were manually blasted against the GenBank database to match with the cultivated cyanobacterial sequences. Those sequences with more than 97% similarity and derived from specific cyanobacteria-research articles/labs were retrieved. The cyanobacterial OTU representative sequences were then aligned along with the sequences retrieved from the GenBank database to ultimately determine the phylogenetic (taxonomic) position of each OTU (see Statistical analysis for the details). In addition, the DADA2 (Callahan et al., 2016) and Cydrasil (Roush et al., 2021) were also used to check our cyanobacterial taxa (see Supplementary Material for the details). Cyanobacterial relative abundance in this study was defined as the percentage of cyanobacterial 16S rRNA gene sequences in all 16S rRNA gene sequences, and the relative abundance of nitrogen-fixing cyanobacteria in nitrogen-fixing microorganisms was defined as the percentage of cyanobacterial nifH gene sequences in all nifH gene sequences. To further analyse the cyanobacterial community structure, the relative abundance of each cyanobacterial taxon (such as clade, genus) was defined as the percentage of the corresponding 16S rRNA gene sequences in cyanobacterial 16S rRNA gene sequences.

Statistical analysis

The variation of each parameter was analysed by one-way ANOVA or non-parametric test according to their normality, and the variation analysis was performed on Statistical Product and Service Solutions (SPSS v.20). Bacterial communities at phylum level were clustered by Hierarchical Clustering using a Multi Experiment Viewer. The phylogenies of 16S rRNA and nifH gene sequences (including the OTU representative sequences and the retrieved sequences from GenBank) were analysed using Maximum Likelihood Tree with 1000 bootstrap replications, and the gaps/missing data were completely deleted. The Maximum Likelihood Tree was performed on a Mega 6.0 software. Adjusted $R^2$ values were evaluated by Curve Estimation to fit curves using SigmaPlot 12.5 software.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting Information.