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Article *in* Plant and Soil · October 2017

DOI: 10.1007/s11104-017-3442-3

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DOI 10.1007/s11104-017-3442-3

REGULAR ARTICLE

Biocrust morphology is linked to marked differences in microbial community composition

Angela M. Chilton · Brett A. Neilan · David J. Eldridge

Received: 1 April 2017 / Accepted: 26 September 2017

Abstract
Background and aims Biocrust morphology is often used to infer ecological function, but morphologies vary widely in pigmentation and thickness. Little is known about the links between biocrust morphology and the composition of constituent microbial community. This study aimed to examine these links using dryland crusts varying in stage and morphology.
Methods We compared the microbial composition of three biocrust developmental stages (Early, Mid, Late) with bare soil (Bare) using high Miseq Illumina sequencing. We used standard diversity measures and network analysis to explore how microbe-microbe associations changed with biocrust stage.
Results Biocrust richness and diversity increased with increasing stage, and there were marked differences in the microbial signatures among stages. Bare and Late stages were dominated by Alphaproteobacteria, but Cyanobacteria was the dominant phylum in Early and Mid stages. The greatest differences in microbial taxa were between Bare and Late stages. Network analysis indicated highly-connected hubs indicative of small networks.
Conclusions Our results indicate that readily discernible biocrust features may be good indicators of microbial composition and structure. These findings are important for land managers seeking to use biocrusts as indicators of ecosystem health and function. Treating biocrusts as a single unit without considering crust stage is likely to provide misleading information on their functional roles.

Responsible Editor: Sasha Reed

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-017-3442-3) contains supplementary material, which is available to authorized users.

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Keywords Cyanobacteria · Network analysis · Biological soil crust · Semi-arid, microbial ecology · Drylands · Soil function

Introduction

Biocrusts are complex associations of macroscopic, non-vascular organisms such as mosses, lichens and liverworts, and microscopic organisms such as cyanobacteria, fungi, bacteria and archaea, that form intimate associations with surface soils. Biocrusts are dominated by phototrophic organisms that require direct access to sunlight. Consequently, they are particularly

Published online: 04 October 2017

common in the interspaces between patches of perennial plants in areas such as drylands where vascular plant cover is sparse. Biocrusts are critically important for regulating carbon, nutrient and hydrological cycles, stabilising surface soils, and providing habitat for soil biota (Bowker et al. 2013; Zhao et al. 2016; Neher et al. 2009). In many landscapes, biocrusts are significant contributors to biomass, biodiversity and ecological functioning. Efficient functioning of biocrust communities relies heavily on the underlying microscopic components of the crust. Critical to the formation and stability of biocrusts is the stabilisation of soil particles resulting from the ecosystem engineering actions of bacteria, particularly large filamentous cyanobacteria (Garcia-Pichel and Wojciechowski 2009). This stabilisation is typically initiated by rain and is responsible for nutrient enrichment of the local soil profile encouraging subsequent colonisation by additional microorganisms, particularly more macroscopic mosses and liverworts. The important bioengineering role of cyanobacteria has been shown to be a precursor to the development of stable functional soil surfaces (Garcia-Pichel and Wojciechowski 2009; Rossi and De Philippis 2015; Zhang 2005).

Despite the large body of research carried out on the macroscopic, more visible components of biocrusts (e.g. Weber et al. 2016), comparatively little is known about the underlying microbial community structure. Profiling of the bacterial community of biocrusts using ribosomal gene sequencing is in its relative infancy, but has been performed for a number of locations worldwide. The majority of biocrust microbiome research has been carried out on soils from the western deserts of the United States, and more recently China (Li et al. 2014), Europe (Büdel et al. 2014) and Africa (Thomas and Dougill 2007). In Australia, however, the microbial signature of biocrusts has been poorly studied (Abed et al. 2012). Together these studies have revealed that biocrusts from drylands are dominated by cyanobacteria and are less bacterially diverse than those from more mesic areas (Zaady et al. 2010; Garcia-Pichel et al. 2003). The most widely reported cyanobacterium in the global literature is *Microcoleus*, particularly *Microcoleus vaginatus* (Starkenburg et al. 2011). More recent studies have shown that, although biocrusts are dominated by filamentous cyanobacteria, additional non-cyanobacterial phyla are also common (Nunes da Rocha et al. 2015). These include the ubiquitous Proteobacteria (primarily Alphaproteobacteria), Actinobacteria, Acidobacteria and Bacteroidetes. Less common phyla include Deinococcus-Thermus, Chloroflexi, Firmicutes, Verrucomicrobia, Planctomycetes and Gemmatimonadetes. Together these phyla represent a broad group of microbes that function as chemoorganoheterotrophs or ammonia-oxidizing chemoorganoautotrophs (Delgado-Baquerizo et al. 2016).

Biocrust communities can be characterised in many ways including: level of development (Belnap et al. 2008), biocrust morphology type (Thomas and Dougill 2007; Pócs 2009), level of pigmentation (Couradeau et al. 2016) or constituent organisms (e.g. cyanobacterial vs chlorolichen; (Budel et al. 2009). These characteristics are inherently descriptive and subjective, but are often associated with a particular stage of biocrust maturity.

Generally, biocrusts shift from thin, lightly-coloured cyanobacterial-dominated crusts to thicker, darker, more complex assemblages. Little is known, however, about whether the outward appearance or morphology of biocrusts, often based on crust type, pigmentation and level of development, is a useful proxy of the underlying microbial community structure. Linking the microbial community structure to the outward appearance of biocrusts is critically important if we are to use biocrusts as indicators of ecosystem health and functioning (Castillo-Monroy et al. 2011) or as model systems to examine effects of different stresses such as overgrazing or climate change (Bowker et al. 2014; Garcia-Pichel et al. 2003).

Previous research has revealed that the composition of microbial communities within biocrusts is complex and dynamic, and changes under different environmental settings. For example, in the Kalahari Desert, bacterial community structure varied markedly with vegetation type, and was distinct from the subsoil microbiome (Thomas and Dougill 2007). In the Negev Desert in Israel, precipitation was found to be the strongest driver of cyanobacterial diversity and abundance (Hagemann et al. 2015). In the deserts of the western United States, studies of biocrusts along a developmental gradient from thin cryptic species to dark mature biocrusts showed that changes in biocrust composition can have a direct effect on the soil microbial community. Warming was associated with the replacement of the keystone heat-sensitive *Microcoleus vaginatus* by the more heat-tolerant *Microcoleus steenstrupii* (Couradeau et al. 2016). Although both morphology and bacterial

community composition have been used to better understand the functional role of biocrusts, relatively little is known about how these relate to each other.

Here we examine the pattern of community composition of the microbial community associated with biocrusts ranging in development from bare surfaces to highly developed, floristically rich and deeply pigmented biocrusts containing lichens and mosses. We hypothesised that differences in biocrust development would be reflected in substantial differences in microbial community structure. We tested this hypothesis using indicator species analysis and co-occurrence networks. Our aim was to deepen our understanding of the links between biocrust form and function and improve our understanding of the use of biocrusts as model systems and ecosystem indicators.

Methods

Study area and field sampling

The Kalgooleguy Regeneration Reserve is an area of crown land north-west of Cobar, New South Wales Australia (−31.49° S, 145.84° E) covering an area of 4777 ha (Electronic Appendix S1). The climate is characterized by low and variable rainfall (mean annual rainfall 390 mm), high rates of evaporation (~ 2200 mm $yr.^{-1}$), hot dry summers (maximum 28–39 °C, minimum 14–24 °C) and cool to mild winters (maximum 13–20 °C, minimum 2–8 °C). The reserve is located on the Cobar Peneplain, a low undulating plain punctuated by stony ridges and ranges, and characterized by well-drained red and red-brown clay loams and loams, with increasing clay content with depth (Typic Haplargids or Red Earths), with variable amount of stones in the profile. The reserve is dominated by eucalypt woodlands and Acacia shrublands dominated by *Eucalyptus populnea*, *Callitris glaucophylla*, *Acacia aneura* and dense patches of shrubs (*Dodonaea viscosa*, *Eremophila longifolia*, *Senna artemisioides*, *Acacia* spp.) varying in cover from <5% to about 50%. The soil surface is dominated by a variable cover of biocrusts ranging from cyanobacterial films to well-developed lichen crusts.

Within the reserve we identified three stages of crusts based on thickness, pigmentation and composition (sensu Belnap et al. 2008; Eldridge and Rosentreter 1999). Early stage crusts (hereafter 'Early-stage') were defined as thin, lightly-coloured smooth crusts dominated by cyanobacteria and with little evidence of colonisation by mosses or lichens. Mid-stage crusts (hereafter 'Mid-stage') were thicker and more pigmented (darker) and showed evidence of colonisation by mosses and lichens. Late stage crusts (hereafter 'Late-stage') had the greatest pigmentation and thickness and were dominated by lichens and mosses (Electronic Appendix S2). These three crust types were compared with uncrusted, bare surfaces (hereafter 'Bare-stage').

In April 2013 we collected samples of the four different stages from three large sites within the reserve. Sites were separated by distances of about 1.5 km. At each site we collected four samples of each stage within large plots of about 50 by 50 m resulting in 16 samples per site. The distance between samples within a plot ensured that all samples were spatially independent at the scale of the organism studied. For each sample, 10 cm^2 plots were collected to the depth of the biocrust and stored in paper bags and transported to the laboratory and at the University of New South Wales and stored at 4 °C.

Molecular analysis

Environmental genomic DNA was isolated from 500 mg of homogenised soil using the FASTDNA Spin Kit for Soil (MP Bio Laboratories, USA) according to the manufacturer's instructions. The hypervariable regions V1-V3 of the 16S rRNA gene were amplified using unique combinations of barcoded 27F/519R primers. The pooled DNA libraries were submitted to the Ramaciotti Center for Genomics (UNSW, Australia). Sequencing was performed on an Illumina MiSeq using a MiSeq Reagent Kit v3 with a 2x300bp run format. Sequencing data were received de-multiplexed via the Illumina cloud-computer BaseSpace and are available on the NCBI Small Read Archive (SRA) under project PRJNA396825. For this study, only amplicons generated from the forward primers (27F) were used in order to avoid artificial inflation of diversity measures due to poor confidence in contig formation (Kozich et al. 2013; Nielsen et al. 2016). Sequence reads were processed and analysed using Mothur version 1.34.0 (Schloss et al. 2009) according to the standard operating procedure developed by Kozich et al. 2013. Briefly, sequences were checked for quality using a threshold average quality score of 30 over 50-base increments. Sequences less than 200 bases, with greater than 8

homopolymers or containing ambiguous bases were removed. Amplicons were aligned and trimmed to a consensus region using a customised V1–3 version of the SILVA alignment database (Quast et al. 2013). Preclustering was performed where by rare sequences with ≤1 per 100 bp difference to abundant sequences were merged. Chimeras were detected and removed using the in-built application UCHIME (Edgar et al. 2011). Sequences were classified using the GreenGenes database (version gg_13_8_99, August 2013) (DeSantis et al. 2006) with an 80% pseudobootstrap confidence score. Sequences not classified at kingdom level or classified as Mitochondria, Archaea or Eukaryota were removed. Samples were rarefied to 14,434 sequences resulting in a curated dataset of 678,398 sequences across 47 samples with an average length of 259 bases. Operational taxonomic units (OTUs) were generated at a 0.03 distance threshold via a distance-based matrix with average neighbour clustering performed at Order level (Schloss and Westcott 2011). OTUs were then assigned taxonomy using the same GreenGenes database.

Statistical and network analyses

We calculated measures of richness, diversity and evenness using the Diverse function in the Primer/PERMANOVA package (Anderson et al. 2008). Differences in richness, diversity and evenness were determined using mixed-models ANOVA. Our model structure accounted for differences among the three sites, the four stages and their interactions. Tukey's Least Significant Difference (LSD) tests were used to determine differences among the four stages. We use the same model structure to examine differences in the composition of the 16S rRNA OTUs in relation to stage. With PERMANOVA, pair-wise, a posteriori comparisons were made, where necessary, using a multivariate analogue of the t statistic, the probability levels being obtained by permutation. Homogeneity of spread for factor Stage was confirmed using PERMDISP with 999 permutations (pseudo $F = 2.12$. P(perm) = 0.414). We then used non-metric multidimensional scaling ordination (nMDS) to derive the first two dimensions of the nMDS biplot on log-transformed OTU abundance data to represent the compositional differences among the four stages. The 2D solution provided a suitable representation of the bacterial data (stress = 0.16). Indicator Species Analysis (De Cáceres and Legendre 2009) was used to determine the degree of association between OTUs and stage type. Operational Taxonomic Units were randomized among the stages and a Monte Carlo procedure performed with 999 iterations to determine the statistical significance of the indicator values generated. Co-occurrence analysis of OTUs for network analysis was measured using the SparCC command within mothur with 100 iterations and 10,000 permutations (Friedman and Alm 2012). Only OTUs contributing greater than 0.25% to a stage and occurring across 3 or more sites were included to avoid spurious associations. False discovery rates were kept below 5% using Benjamini-Hochberg corrected P-values ($q < 0.0016$) (Benjamini and Hochberg 1995). Significant OTU-OTU correlations for each crust type were visualised as scale-free networks using the Cytoscape package version 3.2.1 (freely available at: http://www.cytoscape.org/). Non-random co-occurrence patterns for the network OTUs were checked with the checkerboard score (C-score) with the R package EcoSimR under a null model preserving row and column sums with default settings (Gotelli and Ellison 2013). For each network, overall topological parameters of connectivity, centrality and density were calculated (Assenov et al. 2008).

Results

Richness and diversity of microbial taxa

Across all samples we recorded a total of 44,005 OTUs at a 0.03 distance threshold. Less than 3% of all OTUs accounted for 75% of total abundance and 60% of OTUs occurred as singletons. For the total dataset, i.e. considering all OTUs, there were no significant differences in richness ($F_{3,6} = 4.15$, $P = 0.065$), diversity ($P = 0.064$) or evenness ($P = 0.59$) among the four stages. However, when we excluded singletons from the analyses, both richness ($F_{3,6} = 5.60$, $P = 0.036$) and diversity ($F_{3,6} = 5.63$, $P = 0.035$), but not evenness ($P = 0.63$) increased significantly with increasing biocrust stage (Table 1).

Community composition

We found a significant difference in the composition of OTUs among the four stages (Pseudo-$F_{3,6} = 3.37$, P(perm) = 0.002). Multiple comparison tests revealed that the composition of Bare was significantly different to

Table 1 Mean (± SE) richness, diversity (Margalef's index) and evenness (Pileau's index) of microbial OTUs across the four stages for samples excluding singletons

Attribute	Bare		Early		Mid		Late	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Richness	2134[a]	90.2	2408[ac]	82.8	2581[bc]	98.0	2720[b]	51.0
Diversity	223[a]	9.5	252[ac]	8.7	271[bc]	10.4	286[b]	5.4
Evenness	0.80[a]	0.01	0.79[a]	0.01	0.81[a]	0.01	0.82[a]	0.01

Different letters within an attribute indicate a significant difference among the four stages at $P < 0.05$

the other stages ($t > 1.87$, $P < 0.016$), and that Early was different from Late ($t = 1.72$, $P = 0.017$; Fig. 1). Phylotyping of all OTUs identified 16 abundant bacterial classes across seven phyla and all biocrust stages (Fig. 2). Alphaproteobacteria was the dominant phylum within the Bare and Late stages, but Cyanobacteria was the dominant phylum within Early and Mid stages.

Microbial indicators of biocrust stage

Indicator species analysis revealed 18 OTUs comprising 13 genera that characterised the Bare stage. *Herbiconiux* (Actinobacteria) was the strongest indicator and *Flavisolibacter* (Bacteroidetes) the most abundant (Table 2). The Late stage was the only biocrusted stage with a unique OTU indicator species, the terrestrial green-algae *Chloroidium*. The three biocrusted stages were characterized by eight OTUs from six genera, all of the phylum Cyanobacteria. An unclassified Oscillatoriophycideae OTU was the strongest indicator of biocrusted soils and *Phormidium* the most abundant genus. There were no indicators of Early or Mid stages. All biocrust indicator OTUs were most similar to other biocrust submissions whereas the Bare stage indicator OTUs had greatest similarity to non-biocrust environments.

Network analysis

Co-occurrence networks derived from abundant OTUs showed non-random assembly patterns (C-scores, Table 3), with highly-connected hubs (a group of nodes exceedingly more highly connected than the average) indicative of small world networks (Electronic Appendix S3). The number of nodes (OTUs) remained relatively consistent across each stage (Table 3). Network densities and clustering coefficients (measures of node connectivity) were also stable, with the exception of the

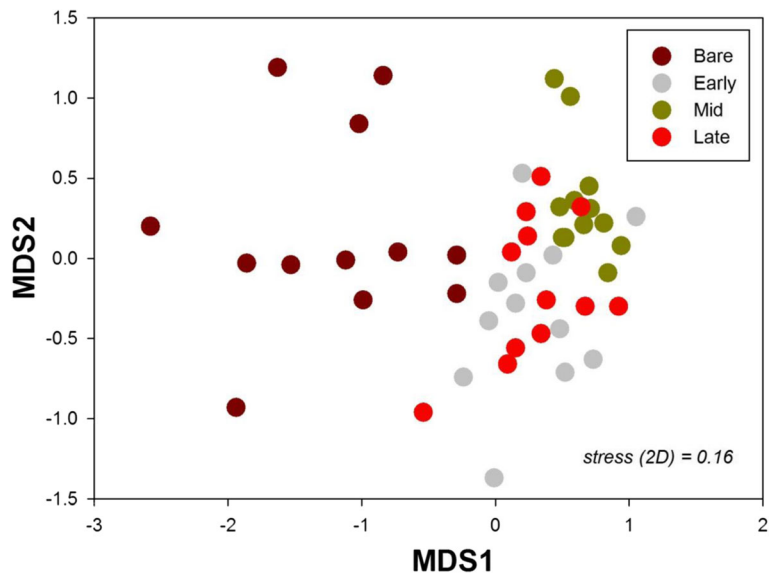

Fig. 1 Non-metric multidimensional biplot of the 48 sites from the four stages based on composition of OTU with an abundance >1

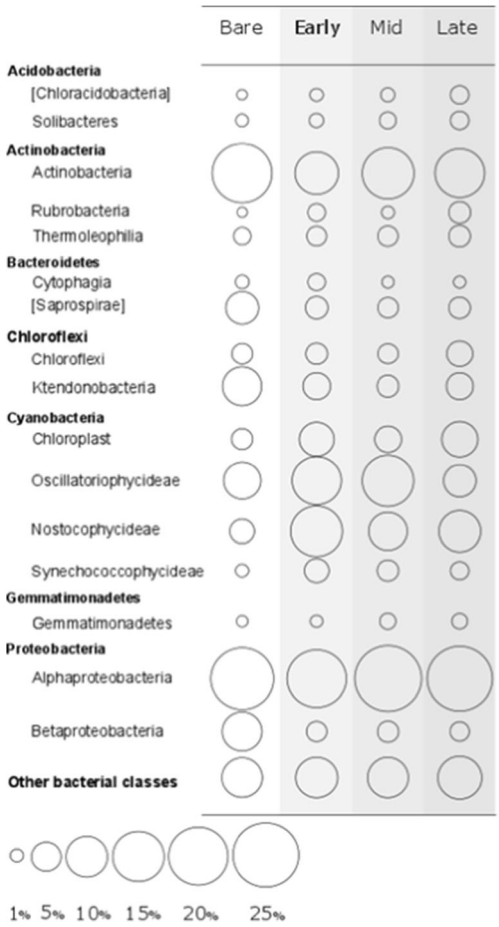

Fig. 2 Relative abundance (%) of the major bacterial classes grouped by phylum for Bare, Early, Mid and Late stage biocrusts

Early stage which exhibited a large drop in connectivity that resulted in an increased network diameter. Degree (number of connections per node) followed a power-law distribution with a few highly connected nodes forming edge-dense hubs resulting in modular network topologies. High clustering coefficient and density scores indicated the Bare stage network had the greatest modularity (Eldridge et al. 2015). Proteobacteria was the greatest contributor to nodes and correlations across all stages with the exception of the Early stage, where cyanobacteria comprised the greatest number of nodes. Phylum level patterns of node correlations were observed. Cyanobacterial nodes shifted from within-phylum to cross-phyla correlations from the Bare to Early stages whereas all non-cyanobacterial phyla consistently formed more cross-phyla than within-phylum associations. The number of negative interactions increased as biocrust stage advanced (Table 3).

Discussion

Biocrusts comprise a wide range of physical types or stages, ranging from thin cyanobacterial layers to thick, highly developed crusts dominated by a rich community of lichens, mosses and liverworts. The extent to which these different biocrust forms reflect differences in their underlying microbial signatures is, however, poorly known. In this study we show that increases in biocrust morphology and complexity corresponded with increased richness and diversity of biocrust-inhabiting microbes. Bare surfaces had a different complement of bacterial taxa to biocrusted surfaces, irrespective of their complexity. Network arrangement also differed among the four stages, with greater heterogeneity and more negative interactions with increasing biocrust development. Our results indicate that recognisable features of biocrust surfaces such as differences in thickness, cover and development are associated with marked differences in microbial communities. Thus different biocrust surface types are likely to reflect differences in biocrust capacity to moderate critical soil and ecological processes.

Biocrust stages as a proxy for microbial community structure

Gross morphological attributes such as colour, shape and thickness have been used widely to differentiate biocrusts into discrete community types, by developmental stage (Belnap et al. 2008) or morphological group (Eldridge and Rosentreter 1999; Read et al. 2014; Mallen-Cooper and Eldridge 2016). Some of this differentiation is based on the notion that form reflects function (e.g. Eldridge and Rosentreter 1999) and therefore that different forms should be indicative of different species assemblages with unique functions (Kidron et al. 2015). In our study we found that biocrust richness increased with increases in developmental stage, from Bare to Late stages, but there were no differences in composition among the three biocrusted (Early, Mid, Late) stages, which were largely dominated by cyanobacteria (Fig. 2). We would expect to detect substantial differences between bare and biocrusted stages for a number of reasons. The Bare stage showed little evidence of surface differentiation, a generally hardened surface with few cracks, and little incorporation of litter. Compared with Bare, the biocrusted surfaces had relatively

Table 2 Bacterial taxa significantly associated ($P < 0.01$) with different biocrust stages and biocrust-stage combinations using Indicator Species Analysis

Stage and phylum	Class	Genus	IV	RA (%)	BLAST	
					Accession	Similarity (%)
Bare						
Actinobacteria	Actinobacteria	*Herbiconiux*	0.98	17	KT773540	96
Proteobacteria	Betaproteobacteria	*Pseudoburkholderia*	0.98	28	KX508248	100
Proteobacteria	Betaproteobacteria	*Limnobacter*	0.98	46	JF809120	97
Bacteroidetes	[Saprospirae]	*Segetibacter*	0.97	17	AB696124	99
Actinobacteria	Actinobacteria	*Friedmanniella*	0.90	12	AF409005	98
Proteobacteria	Alphaproteobacteria	*Acidisphaera*	0.89	12		<95
Proteobacteria	Betaproteobacteria	*Pseudogulbenkiania*	0.88	11		<95
Firmicutes	Bacilli	*Bacillus*	0.88	14	KJ600919	99
[Thermi]	Deinococci	*Deinococcus*	0.87	15		<95
Bacteroidetes	[Saprospirae]	*Flavisolibacter*	0.85	71	JX797411	96
Chloroflexi	Ktedonobacteria	unclassified	0.83	49		<95
Actinobacteria	Actinobacteria	*Nocardioides*	0.82	1	KT772263	99
Cyanobacteria	Chloroplast	unclassified	0.80	12	HM725590	100
Late						
Cyanobacteria	Chloroplast	*Chloroidium*	0.80	30	HM731584	99
Crusted						
Cyanobacteria	Oscillatoriophycideae	unclassified	0.98	21		<95
Cyanobacteria	Nostocophycideae	*Toxopsis*	0.97	18	GU362214*	100
Cyanobacteria	Nostocophycideae	*Cylindrospermum*	0.97	11		<95
Cyanobacteria	Nostocophycideae	*Aphanizomenon*	0.94	13	JQ383870#	100
Cyanobacteria	Nostocophycideae	*Tolypothrix*	0.91	36	GU362210*	100
Cyanobacteria	Oscillatoriophycideae	*Phormidium*	0.87	50	JQ383804#	98

IV Indicator Value; *RA* relative abundance

*sequence from Oman biocrust

sequence from Nevada, USA, biocrust

high surface microtopography, up to 10 mm, with variable cracks and greater evidence of biological activity e.g. spider holes and small ant holes. A wide range of surface characteristics would likely provide more refugia for microbial communities in dryland ecosystems than bare soils, which are essentially homogeneous and hostile.

Our work across regional eastern Australia has shown that biocrusts at a later stage of development support a richer community of mosses, lichens and liverworts, and richer vascular plant associates (Eldridge and Tozer 1997; Eldridge 1998a, b). This would likely provide opportunities for a larger range of bacterial taxa. A richer plant community should support a greater range of plant root types, a wider spectrum of root exudates (Berg and Smalla 2009; Bezemer et al. 2006) and therefore a greater range of microhabitats for bacteria (Lamb et al. 2011). Evidence from Australian drylands indicates that microsite differentiation can modify the abundance of soil ammonia oxidizing bacteria, with reductions on bare soils, but increases in areas of biological activity around structures such as ant nests that are often found in well-developed biocrusts (Delgado-Baquerizo et al. 2016). Further, as increasing development is associated with a thicker surface biocrust, we would expect greater levels of soil carbon and nitrogen, given that carbon and nitrogen are concentrated in the uppermost biocrust layers (Steven et al. 2013; Mueller et al. 2015).

Table 3 Topology metrics and C-score measures derived from scale-free co-occurrence networks of abundant OTUs (greater than 0.25% for each stage) for Bare, Early, Mid and Late stage biocrust microbial communities

	Stage			
Network metric	Bare	Early	Mid	Late
Number of Nodes (OTUs)	286	252	275	242
Number of Edges	1311	648	1064	947
Clustering coefficient	0.29	0.179	0.247	0.224
Density	0.032	0.02	0.028	0.032
Network centralisation	0.084	0.068	0.74	0.101
Network heterogeneity	0.712	0.794	0.698	0.839
Network Diameter	8	11	9	8
Average number of neighbours	9.2	5.1	7.7	7.8
Connected components	4	2	3	5
Negative interations (%)	23	36	40	41
C-Score measures				
Observed Index	1.07	0.86	0.97	0.65
Mean of Simulated Index	1.05	0.83	0.94	0.06
Standard Effect Size (SES)	1.91	9.96	9.70	20.76

Edges represent significant ($p < 0.05$ after Benjamini-Hochberg procedure) positive and negative correlations

Cyanobacterial bloom promotes biocrust formation

The primary microbial drivers of biocrust formation are filamentous cyanobacteria. These stabilise loose soil particles by producing exometabolites (Baran et al. 2015; Garcia-Pichel and Wojciechowski 2009), modifying soil physical properties and enriching the metabolic potential of the soil. We found a persistent population of diverse filamentous cyanobacteria across all stages, including the Bare stage, that changed in relative abundance as the crust developed. We propose that biocrust-forming cyanobacteria are prevalent within arid top-soils and that, given favourable conditions, undergo a bloom in population akin to those within aquatic systems (Fuhrman 2009). Increases in the relative abundance of cyanobacteria between Bare and Early stages combined with the high production of exopolymeric substances by cyanobacteria likely lead to increases in microbial biomass necessary to form a cohesive biocrust layer. These early biocrusts likely originate from raindrop impacted physical crusts, which are often precursors of biocrusts in highly erodible Australian loams (Eldridge 2001). Interestingly, the greatest increase in cyanobacterial abundance was due to Nostocophycideae types. Nostocophycideae are typically documented as later additions to biocrust communities, and are attributed with major roles in nitrogen fixation and darkening of the biocrust (Belnap et al. 2008; Yeager et al. 2004). Here, they were detected within bare soil and are an abundant component as soon as the soil is stabilised in thin, light-coloured crusts. This is an important consideration for future work determining the nutrient-cycling and ecological roles of these biocrusts.

A clear difference between Bare and crusted stages detected with Indicator Species Analysis shows that the Bare stage is characterised by non-cyanobacterial OTUs (excluding chloroplasts) whereas the crusted stages are characterised by cyanobacterial OTUs. The lack of cyanobacterial indicators within the Bare stage, despite abundant and diverse representation, indicates that cyanobacterial OTUs inhabiting the Bare stage are not an independent population but likely originate from the surrounding biocrusts (Shade et al. 2012). These may represent residual populations following physical disturbance (Kuske et al. 2012) or inundation by sediment (Williams and Eldridge 2011). However, we found no evidence of a remnant biocrust matrix on the Bare stages during our sampling. All cyanobacterial indicator OTUs were most similar to other biocrust submissions whereas the Bare stage indicator OTUs had greatest similarity to non-biocrust environments. This supports the finding that biocrusts are a niche populated by specialised organisms able to form and sustain biocrusts (Elliott et al. 2014).

Despite the high abundance and diversity of cyanobacteria in our samples, and the putative worldwide distribution of the genus, no OTUs were assigned to *Microcoleus*. Rather, *Phormidium* was the most abundant cyanobacterial genus and was found consistently throughout all the stages, particularly the Early stage. *Microcoleus vaginatus* is often identified as the primary cyanobacterium of biocrusts, particularly in the early stages and more often in North American samples (Garcia-Pichel et al. 2003). *Microcoleus* and *Phormidium* are poorly resolved phylogenetically within the Phormidiaceae family, however, beyond taxonomic discrepancies, these types share important biocrust-forming attributes such as the formation of long, sheathed filaments with large cells, features which likely support the ability to form supra-cellular ropes to stabilise soil grains. Cyanobacteria with these features

are thought to be able to travel large distances in bare soils.

Biocrust stages defined by microbe-microbe associations

Our network analyses indicated that there were major differences in connectivity among the four biocrust stages, indicating differences in their capacity to 1) recover from disturbance, 2) deviate from equilibrium, and 3) perform multiple functions (Allison and Martiny 2008; Bissett et al. 2013). The Bare stage network had the greatest modularity (formation of hubs), suggesting high reactivity and low resilience (Ruiz-Moreno et al. 2006). This community structure may be an important trait for bacteria within oligotrophic arid and semi-arid soils for the prompt uptake of nutrients and response to infrequent wetting events. High reactivity and low resilience may also explain how cyanobacteria can colonise and dominate bare surfaces and initiate biocrust formation. An essential factor in the formation and growth of biocrusts is the presence of biocrust-forming bacteria, primarily cyanobacteria. We observed a cyanobacterial hub within the Bare stage network that likely indicates a niche where members respond to environmental stimuli in the same way (Fuhrman 2009). *Phormidium* was the main genus in this hub, but several other cyanobacterial genera such as *Brasilonema*, *Leptolyngbya* and *Cylindrospermum* were also present. This may indicate a degree of functional redundancy within bare soil, and suggests that these genera are also implicated in biocrust formation.

A sharp decline in the number of edges from Bare to Early stages resulted in strong de-centralisation of the Early stage network. We theorise that the Early stage microbial community has yet to effectively adapt to the modified conditions induced by cyanobacteria colonisation and biocrust formation. A shift from within-phylum to among-phyla correlations, which was unique for Cyanobacteria (Electronic Appendix S4), may be an ecological strategy that promotes biocrust formation. By Mid and Late stages, node connectivity appeared restored, but many of these were negative correlations. We suggest that this is a reflection of resource partitioning (Fuhrman and Steele 2008), likely due to the substrate preferences of heterotrophic bacteria (Baran et al. 2015) and indicates biocrust maturity. Overall, a sequence of high to low network connectivity was followed by a trend towards recovery of network complexity, a pattern observed in salt marsh chronosequences, where loss of network complexity could be due to loss of taxonomic diversity (Dini-Andreote et al. 2014).

Concluding remarks

Overall we found that measures of microbial diversity and richness increased with biocrust development. We showed that changes in cyanobacterial abundance corresponds with biocrust formation and disrupts community structure of the top-soil. Our results indicate that readily discernible biocrust features may be good indicators of microbial composition and community structure. These findings are important for land managers seeking to employ biocrusts as indicators of ecosystem health and functioning. Specifically, the developmental stages of biocrusts should be taken into consideration when evaluating their contribution to arid landscapes. Our work informs on the dynamic nature of the microbial community of biocrusts, providing further understanding for their use as models systems.

Acknowledgements We thank Angela E. Chilton for assistance with sample collection, Jason Woodhouse for assistance with the network analysis and Samantha Travers for comments on the manuscript. Angela M. Chilton was supported by an Australian Post-Graduate Award.

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