Microbial Biobanking
Cyanobacteria-rich topsoil facilitates mine rehabilitation

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Abstract

Restoration of soils post-mining requires key solutions to complex issues where the disturbance of topsoil incorporating soil microbial communities can result in a modification to ecosystem function. This research was in collaboration with Iluka Resources at Jacinth-Ambrosia (J-A) mineral sand mine located in a semi-arid chenopod shrubland in southern Australia. At J-A, assemblages of microorganisms and microflora inhabit at least half of the soil surfaces and are collectively known as biocrusts. This research encompassed a polyphasic approach to soil microbial community profiling focused on 'biobanking' viable cyanobacteria in topsoil stockpiles to facilitate rehabilitation. We found that cyanobacterial communities were compositionally diverse topsoil microbiomes. There was no significant difference in cyanobacterial community structure across soil types. As hypothesised, cyanobacteria were central to soil micro-processes, strongly supported by species richness and diversity. Cyanobacteria were a significant component of all three successional stages with 21 species identified from ten sites. Known nitrogen-fixing cyanobacteria *Symploca, Scytonema*, *Porphyrosiphon, Brasilonema, Nostoc* and *Gloeocapsa* comprised more than 50% of the species richness at each site and 61% of the total community richness. In the first study of its kind, we have described the response of cyanobacteria to topsoil stockpiling at various depths and ages. Cyanobacteria are moderately resilient to stockpiling at depth and over time, with average species richness greatest in the top 10 cm of the stockpiles of all ages and more viable within the first six weeks, indicating potential for biocrust re-establishment. In general, the resilience of cyanobacteria to burial in topsoil stockpiles in both the short and long term was significant, however in an arid environment recolonization and community diversity could be impeded by drought. Biocrust re-establishment during mine rehabilitation relies on the role of cyanobacteria as a means of early soil stabilisation. At J-A mine operations do not threaten the survival of any of the organisms we studied. Increased cyanobacterial biomass is likely to be a good indicator and reliable metric for the reestablishment of soil micro-processes.

1.0 Introduction

Microorganisms are critical components of soils that drive micro-processes and impact soil ecosystem function on several levels. Biological soil crusts (biocrusts) are topsoil assemblages of bacteria, fungi, algae, mosses, and lichen which occur where vascular plant growth is restricted, namely arid lands. Highly structured, diverse biocrusts are a significant asset to arid soil ecosystems providing a protective, nutrient rich layer closely integrated into the soil surface (Delgado-Baquerizo et al., 2013; Maestre et al., 2012). As biocrusts develop in structural complexity, the diversity of organisms is regulated by water infiltration, temperature, light and disturbance (Belnap and Eldridge, 2001; Büdel et al., 2009; Elbert et al., 2012). Early successional biocrusts are mainly composed of bacteria, algae and cyanobacteria whereas late successional biocrusts can incorporate lichens, mosses, liverworts, algae, fungi and bacteria (Büdel et al., 2009).

Cyanobacteria in arid landscapes are exceptionally well-adapted to desiccation. Their polysaccharide sheaths and EPS production perform a vital role in maintaining cyanobacterial cell integrity, exchange of information and absorption of water during rehydration (Rossi et al., 2017). EPS has adhesive properties that binds non-aggregated soil particles into a protective encrusted surface that reduces the destructive impacts of wind and water (Eldridge and Leys, 2003; Rossi et al., 2017). Cyanobacterial biofilms provide stabilisation of initially disturbed surfaces that pave the way for diverse microbial communities, and form bioactive crust-like layers assimilated into the soil (e.g. Büdel et al., 2009; Rossi et al., 2017; Bowker et al., 2014). EPS forms the bulk of the biocrust structure. EPS more than doubles the biocrusts compressive strength and increases cohesiveness by up to six times (Hu et al., 2002).

Soil disturbance results in a loss of resources and often has long lasting effects on soil stability, nutrient cycling and surface hydrology (Bowker, 2007; Tongway and Hindley, 2004). Cyanobacterial re-establishment is a key indicator of early soil surface re-stabilisation, regulation of soil moisture and the balancing of soil carbon and nitrogen (Elbert et al., 2012). Restoration of ecosystem function post disturbance requires an appreciation of the dynamic functional status of the landscape prior to disturbance (Tongway and Ludwig, 1996), as well as an understanding of the net accumulative effects of disturbance on the components of the system. Consequently, it is necessary to appreciate the micro-processes that will assist in the restoration of soil function and to monitor recovery along the way. On the micro-scale, cyanobacterial species richness contributes to soil ecosystem function through micro-processes including carbon fixation through photosynthesis, atmospheric nitrogen fixation in a biological-available form, micro-nutrient breakdown and release, soil particle cohesion, regulation of moisture and soil surface structure (Delgado-Baquerizo et al., 2013; Elbert et al., 2012; Hu et al., 2002; Maestre et al., 2012 and others).

Physical disturbance can profoundly disrupt biocrust integrity, composition and physiological function where the impact is governed by site characteristics, severity, frequency and timing (Belnap and Eldridge, 2001). Mining disturbance alters the biophysical state of the biocrust community through excavation, crushing, mixing and burial. Biocrust burial within the natural environment as a result of environmental stress and disturbance can have severe negative impacts on biocrust integrity and function. For example, significant declines were recorded in cyanobacterial richness and abundance that was linked to a reduction in soil nutrient concentrations (Rao et al., 2012; Williams and Eldridge, 2011). Following disturbance, restoration and regrowth of biocrusts can take place unassisted, seasonally driven and may take many years (Belnap and Eldridge, 2001; Belnap and Gillette, 1998). Alternatively, assisted biocrust restoration places emphasis on the recovery of ecosystem function and necessarily addresses environmental constraints. This incorporates the knowledge of 'potential condition' based on experience with sites of ecological similarity that have undergone disturbance and recovery (Bowker, 2007). Biocrust recovery can be altered by dust deposition, fire and climatic conditions (Weber et al., 2016). When biocrusts recover naturally soil properties change. For example, in Southern African and Spanish rangelands an incremental accumulation of soil nutrients, organic matter and a build-up of silt and clay lead to the development of a resilient and multi-functional biocrust (Büdel et al., 2009; Maestre et al., 2012; Weber et al., 2016).

Mine rehabilitation is a complex process that involves many levels of understanding of difficult issues relating to ecosystem function where the removal or burial of the bioactive soils can have knock-on effects for rehabilitation efforts such as native seedling establishment (Jasper, 2007; Tongway and Ludwig, 1996). Successful ecological restoration of arid mining sites relies on a holistic approach where biocrust recovery to pre-disturbance levels is integral and can serve as an indicator of the integrity of the ecosystem (Tongway, 1990). Research into biocrust disturbance with a focus on recovery post-mining is rare. In the Namaqualand arid lands (Namibia, South Africa) low rainfall and high winds impact the rehabilitation of degraded lands following diamond mining and grazing (Carrick and Krüger, 2007). These researchers found that cyanobacteria and non-vascular plants that form a living and protective surface crust were crucial to surface stabilisation. Jasper, (2007) also recognised the importance of soil microbial communities including cyanobacteria in post-mine rehabilitation in the Jarrah forests of south-western Australia. In the Czech Republic and Germany chrono-sequential studies of old brown coal mine sites found in younger sites that green algal biofilms and a diverse range of cyanobacteria initiated the rehabilitation of the soils (Lukešová, 2001). In serpentinite mine tailings (New South Wales, Australia), McCutcheon et al., (2016) showed filamentous cyanobacteria accelerated carbonate mineral precipitation and stabilised the tailings. They demonstrated cyanobacteria had the capacity to adsorb magnesium while acting as a nucleation site and sequestered carbon. In our current study, preliminary research identified that in undisturbed chenopod shrublands at the edge of the Nullarbor Plain (South Australia) biocrusts cover more than 45% of the soil surfaces between the grass plants and post-mining rehabilitation needs to investigate their role (Doudle et al., 2011). It follows that there is a real need for a focus on practical approaches that contribute to the restoration of soil function and measure relevant aspects of success through soil microbial communities and

biocrust reestablishment, especially cyanobacteria (for example: Setyawan et al., 2016; Mazor et al., 1996; Fischer et al., 2014; Chiquoine et al., 2016; Doherty et al., 2015; Harris, 2003; Tongway and Hindley, 2004; Zhao et al., 2014).

In mining, the topsoil is often stripped and stored in stockpiles as a form of "biobanking" for redistribution at the cessation of mining. This process involves in the crushing and burial of biocrusts and results in the inability of crust organisms to photosynthesise due to the lack of light. To our knowledge, the effects of topsoil stockpiling on biocrust organisms such as cyanobacteria, and their recovery time, has not previously been investigated. Should biocrust organisms remain inactive while they are wet, cell death and decomposition may occur (Kidron et al., 2012; Rao et al., 2012). Nevertheless, in dry conditions, cyanobacteria and algae are known to remain desiccated and viable for millions of years (Vishnivetskaya et al., 2003).

Our study aimed to examine biocrusts, with a focus on cyanobacteria, within a mining framework to develop informed rehabilitation plans that achieve improved long-term outcomes. Specifically, we sought to determine whether shallow biobanks of cyanobacterial-enriched topsoil would facilitate biocrust recovery on mining disturbed lands. We identified key aspects of biocrust and cyanobacterial ecology that needed to be discerned which directed the establishment of this biocrust research project. The overall goals of the biocrust research program were to: (a) evaluate specific roles of natural, undisturbed biocrusts in ecosystem function at the mine site; (b) determine cyanobacterial community structure in terms of key species that drive early colonisation, biogeochemical cycling and soil stabilisation; and, (c) to investigate the effects of stockpiling topsoil on cyanobacterial survival after burial and subsequent recovery. We hypothesised that cyanobacteria would be essential as ecosystem engineers in soil habitat formation with a clear distinction in community structure between soil types and biocrust successional stages. It was also anticipated that in the topsoil stockpiles there would be a low survival rate especially at depth and over time.

2.0 Methods

2.1 Background and site description

Jacinth-Ambrosia (J-A) heavy mineral sand mine is located on the eastern edge of the Nullarbor Plain, South Australia, across the boundary of two Regional Reserves within the Eucla Basin region. The climate is semi-arid with a mean rainfall of 185 mm, mean maximum temperature of 27.6°C and minimum of 12.1°C (further detail provided in supplementary Figure S1, www.bom.gov.au). Tertiary sediments deposited in marine and terrestrial settings and the soil distribution of the area reflects the geological history, with at least five marine transgression and regression events depositing 40-50 m of sediments (Hou and Warland, 2005). The landscape is broadly undulating with low open woodlands that have a shrub understorey with chenopod shrub lands as well as dune fields that consist of parallel dunes and inter-dune swales (Doudle et al., 2011; Gillieson et al., 1996). Prior to mining disturbance, the landscape is superficially homogenous chenopod-dominated

vegetation but functionally patchy on the fine scale where the soil surfaces are extensively colonised by biocrusts. At J-A, biocrusts primarily consist of lichens, mosses, and cyanobacteria that cover around 45% of the landscape surfaces, equating to 2,000 hectares of the mining lease (Doudle et al., 2011).

5   The landscape has been characterised into three distinct soil types (Table 1) associated with vegetation communities called soil management units: SMU 1 – deep calcareous yellow sands associated with dune ridges; SMU 2 – shallow calcareous sandy loams; and SMU 3 – deep calcareous sandy loam (Doudle et al., 2011; Hou and Warland, 2005). The biocrusts at J-A had been previously classified into three successional stages representative of the five biocrust types found growing across the landscape (Table S2) (Doudle et al., 2011). Types 1–2 are light coloured, patchy, thin, and fragile cyanobacterial crusts
10   corresponding to early stages of development; Type 3 are well established cyanobacterial crusts with establishment of some mosses and lichens corresponding to intermediate stages of development; Types 4–5 biocrusts are well established with cyanolichens and/or green algal lichens and mosses corresponding with late stages of development (additional descriptions available in supplementary Table S1). Study locations were selected for: (a) vegetation communities, (b) soil types and underlying geology, (c) biocrust successional types, (d) areas identified for future mine operations and a dry salt lake (Lake
15   Ifould). The dry salt lake would potentially identify cyanobacterial species adapted to saline conditions similar in nature to ground water.

In the process of mining and preparation of the J-A site for future rehabilitation, topsoil (0–100 mm) is stored in low stockpiles, generally less than two metres in depth where stockpiles are comprised of topsoil sourced from only one
20   vegetation type. Later stockpiles will be returned to the surface of mined areas in the rehabilitation process, and the cyanobacterial activity therein must be quantified to enable educated planning and decision making regarding biocrust re-establishment. If low levels of biocrust organisms are detected below the top few centimetres, the addition of propagated biocrust organisms (e.g. cyanobacteria) to returned topsoil may be warranted. Activity and species richness within the stockpiled top soils may also vary with age and this may also influence the establishment of vegetation in the rehabilitation
25   process.

2.2 Biophysical characteristics of biocrusts and cyanobacteria

2.2.1 Field Sampling

Preliminary identification of biocrust types had been determined by Doudle et al., (2011) and provided the baseline data for
30   biocrust sampling from Sites 1–10 (Fig. 1, Table S1). The sites selected encompassed the three SMUs and five crust types (Table S1). All sites are of naturally occurring biocrusts except for Site 6 which is from a two-year-old topsoil stockpile regarded as an early (Type 1) biocrust representing recovery two years post disturbance (Fig. 2a-c). Within each site, eight 10 cm diameter samples were selected at random and removed to a depth of 1 cm using metal scraper (n=80), air dried

(>40°C), and stored in Petri dishes. Each Petri dish contained approximately 80 g of crust. The samples were packed to avoid crust disruption and transported to The University of Queensland's Central Analytical Laboratory at Gatton.

2.2.2 Biocrust biophysiochemical properties

For each site, duplicate sub-samples were taken from each Petri dish for each biophysiochemical analysis (n=16 per site). Soil was fine sieved (1.70 mm) and analysed for total C and N and C:N ratio using a high temperature digestion in a vario MACRO Elemental Analyser (Elementar). Soil pH and electrical conductivity (EC) samples were prepared using a 1:5 (soil to water) ratio and shaken for one hour. Following shaking, samples were left to stand for 30 min and EC measured using a Crison Conductivity Meter 525. The sample was mixed again, and pH was measured with a TPS pH meter MC-80 using an ionode IJ44C electrode. Chlorophyll *a* concentration of the biocrusts were determined following resurrection (by moistening) using a 1:5 ratio of (dry weight) biocrust to Dimethyl sulfoxide (DMSO) (Barnes et al., 1992) with samples placed in a warm bath (65°C) for a two-hour dark extraction, followed by centrifuging for five minutes (5000 g RCF). Chlorophyll *a* concentration was determined using Wellburn's, (1994) equations.

A pocket penetrometer (8 mm foot) was used to determine the compressive strength (kg cm^2) of the dry intact biocrusts samples. Overall the crust thickness was < 0.5 cm. Each sample (10 cm diameter x 2 cm depth) was placed on a solid surface and a total of twelve measurements (3 readings x 4 reps) were taken for each site. The measurement was taken at the point when the crust was broken, and the foot penetrated the crust surface.

Photosynthetic performance (recorded as yield, YII) was measured using pulse-amplitude modulated (PAM) fluorometer (Pocket PAM; Gademann Instruments, Germany). The aim was to demonstrate photosynthetic yield (YII) indicative of active growth of the biocrusts, using the detection of chlorophyll fluorescence from photosystem II (PSII). The sensor was placed onto the biocrust and once started, a series of short pulses of excitation light at high intensity that is amplified resulting in a brief closure of PSII and the measurement of fluorescence yield based on the Genty parameter which is the quantum yield (YII) of the charge separation of PSII (Genty et al., 1989) and recorded on a scale of 0–1 for all photosynthesis. In the field this process was completed at least six times for each sample (6 x 4 reps per site).

2.3 Cyanobacterial community structure

2.3.1 Microscopy of biocrust cyanobacteria

Field samples collected in section 2.2 were used to enumerate cyanobacterial richness and diversity and to classify colonies. The dried crust samples were resurrected in the glasshouse for three to five days. For each of the ten sites, twelve replicates

(subsampled from the eight samples) were analysed via light microscopy. For each replicate, a minimum of two wet-mount slides incorporating six representative portions of the cyanobacterial colonies were examined (n=144 colonies per site). For the dominant land type, Chenopod shrubland (Site 8), there were an additional 10 x 6-cell multi-well plates. These were treated similarly where two slides were examined from each of the 60 multi-wells (n=120). In total > 2,184 cyanobacterial colonies were examined. Initial inspection of the biocrust and the separation of individual species were made using an Olympus SZH10 microscope at 70 x magnification. Cyanobacterial filaments or colonies were carefully extracted with forceps to recover sufficient material that included important morphological features such as their colour, encasing sheaths as well as cellular structure. Live material was examined by Nomarski differential interference contrast (DIC) microscopy with a Jenaval (Jena Zeiss) and an Olympus BX51 compound microscope (magnifications 400–1000 x). Photomicrographs were taken using an Olympus SC100 digital microscope camera, and morphological measurements of vegetative cells were made from digital images of live material taken at 400 x magnification using Olympus cellSens® digital imaging software.

Identification was performed to a species level (wherever possible) in the laboratory using the following taxonomic references: Anagnostidis and Komarek, (2005, 2005); Sant'Anna et al., (2011); Skinner and Entwisle, (2002). It was often necessary to record the closest named species as attributes varied somewhat to temperate climate and aquatic specimens described in literature. Nitrogen fixing cyanobacteria were identified based on the three recognised types: (1) heterocystous species (those with specialised N-fixing cells); (2) non-heterocystous species that fix N aerobically and; (3) non-heterocystous species that fix N anaerobically (Bergman et al., 1997; Stal, 1995). Using a graticule, abundance was ranked on a scale of 1–8 where the main taxa are ranked in decreasing order of the relative percentage area occupied in a single view (Biggs and Kilroy, 2000). More than one species could be dominant, and all other taxa were ranked in relation to the dominant taxa as abundant, common, occasional and rare. To determine similarities between cyanobacterial communities, cluster analysis, SIMPROF, and non-metric multidimensional scaling (nMDS) were conducted using Primer v6 (Clarke & Gorley 2001).

2.3.2 16S rDNA profiling of native undisturbed biocrust microbiomes

For genomic profiling of naturally occurring successional biocrust communities, a location adjacent to Site 9 was visually determined to contain Bare, Early (Types 1-2) or Late (Types 4-5) stages of development (Table S2). Biocrust successional features were determined by morphological attributes of pigmentation, thickness and surface roughness as well as the presence/absence of lichens and mosses (Fig. 2d) (Chilton et al., 2017). Bare stage was characterised by loose soil particles with no visible biocrust structure. Samples were collected in July 2014. For each successional stage, three replicates were collected that were representative of SMUs 1–3 where a 10 cm^2 plot with 95% coverage of the desired biocrust stage was excised to the depth of the crust and non-aggregated soil discarded (Fig. 2e-g). Samples were processed at UNSW, Sydney.

Each biocrust replicate for Bare, Early and Late stages of development were homogenised and genomic DNA extraction performed using the FASTDNA Spin Kit for Soil (MP Bio Laboratories, USA) according to the manufacturer's instructions. Molecular libraries of the 16S rDNA V123 hypervariable region generated via PCR as per Chilton et al., (2017) and submitted to the Ramaciotti Centre for Genomics (UNSW, Australia) for a 2x300 bp sequencing run on an Illumina MiSeq instrument. Sequencing data was processed using Mothur version 1.34.0 (Schloss et al 2009) and described in detail in Chilton et al., (2017). Singleton and doubleton OTUs were removed and samples rarefied to 8598 sequences each across 3785 OTUS. The curated Greengenes database (McDonald et al 2012) was used to assign taxonomy to OTUs. Diversity values were derived using the DIVERSE function within the Primer package (Anderson et al 2008) upon standardized OTU values. ANOVA with post hoc Tukey's tests was used to test for significant differences between stages. Multivariate analyses were performed in Primer upon a Bray-Curtis dissimilarity matrix generated from square-root transformed abundance data. Samples were represented in two and three-dimensional space within a nMDS plot. Pair-wise, a posteriori comparisons of factor Stage were performed using the PERMANOVA function with 9999 Monte Carlo permutations. Homogeneity of dispersion for each stage was tested using PERMDISP.

2.4 Cyanobacterial tolerance to stockpiling

Stockpile sampling was carried out in March 2012 with samples sourced from topsoil stockpiled from areas with *Acacia papyrocarpa* (Western Myall) over-storey (Table S3). Stockpiles established at three different time points (9 months, 20 months and 29 months) as well as corresponding undisturbed sites were sampled in triplicate at six depths (0–2, 2–4, 4–6, 10, 25 and 50 cm). This resulted in 18 replicates for each soil depth for each stockpile time point. Holes were dug to >50 cm depth with a shovel and the exposed profile removed with an ethanol-wiped spatula before taking a soil sample. A second sample at 50 cm was autoclaved to serve as a culturing control. Samples were stored in paper bags and processed at The University of Queensland. For each sample, 20 g of soil was set up in petri dishes with 9 ml of water and sealed with Parafilm®. Petri dishes were incubated for six weeks at 26°C under a 12-hour photoperiod regime and rotated weekly to prevent site specific effects. Samples were maintained as wet by the addition of sterile water within a laminar flow cabinet. To determine cyanobacterial growth and richness, wet-mounts for each sample were examined under 16 x magnification for cyanobacterial thalli and colony size was estimated via area of coverage of the field of view. Where multiple colonies were present in a slide, the relative abundance of each was used to divide the cover between the colonies accordingly. Where no growth was observed, five soil samples were taken randomly from the sample, mounted and examined under 400 x magnification for the presence of cyanobacterial cells. ANOVAs were performed with post hoc Tukey's tests performed to determine any differences between treatments.

3.0 Results

3.1 Biophysical characteristics of biocrusts and cyanobacteria

Soil pH across the three soil management units (SMUs) ranged from pH 8.4–8.6 while the two-year old topsoil stockpile was higher at pH 8.9 (Table 1). Electrical conductivity ranged from 92–140 µS cm^{-1}. There were no significant differences in pH

5 and EC between field samples and intact samples (data not shown). Total nitrogen was typically <0.1% across all sites and total carbon ranged between 1–2% with higher percentages generally found across SMU 2 and 3 (Table 1). The ratio between carbon and nitrogen was the greatest across SMU 3 and the two-year old topsoil stockpile, also originating from SMU 3.

For the intact undisturbed biocrusts mean chlorophyll *a* concentrations (10.33–13.64 µg g^{-1} soil) differed across all sites and

10 SMU 1 biocrusts were significantly lower (p = 0.05) compared to SMU 2 and 3 (Fig. 3). The mean chlorophyll concentrations of biocrusts sourced from T2, the two-year old topsoil stockpile (7.49 ± 1.01 µg g^{-1} soil) were almost half the concentration of SMU 3 (13.53 ± 1.74 µg g^{-1} soil), which was the origin of the topsoil.

The photosynthetic yield measurements showed there was variability between sites (Fig. 3). The yields recorded all fell

15 within the expected range and when data was separated into their respective SMU it provided a clearer picture of the variability. SMU 3 had the lowest mean overall while there were no differences between the others.

The penetrometer index of crust compressive strengths across the ten sites showed that Sites 4, 9 and 10 in SMU 1 were significantly different from each other as well as significantly lower than SMU 2, SMU 3 and T2, two-year old topsoil

20 stockpile sites (Table 2). The means for SMU 1–3 were also significantly different (p < 0.0001), and it was confirmed by a student's t-test that the difference was between SMU 1 and SMU 2 and 3.

3.2 Cyanobacterial community structure

A total of 21 cyanobacterial species were identified across the ten sites using microscopy (Table 5; Figs. S2–S9). The majority of species richness and abundance was comprised of four filamentous genera: *Symploca* (18%), *Schizothrix* (16%),

25 *Porphyrosiphon* (16%) and *Scytonema* (16%). Secondary cyanobacteria present variously occupied <1 to 10%, and overall made up 34% of the community. The known nitrogen-fixing cyanobacteria *Symploca, Scytonema*, *Porphyrosiphon, Brasilonema, Nostoc* and *Gloeocapsa* comprised more than 50% of the species richness at each site and formed 61% of the total community richness (Fig. 4). In this study cyanobacterial community structure was tested against the three different soil types to determine whether soil type was influential in determining community structure. There was no significant difference

30 in cyanobacterial community structure across soil types, however the results do suggest some spatial structuring exists across the three SMU (Fig. 5a). The structural relationship between all samples shows greater similarities rather than dissimilarities,

of which there are only a small number of samples that are significantly different (Fig. 5b). Soil type did not explain these differences. This suggests that most of the species could potentially be found anywhere across the three zones and their richness and abundance is controlled by other factors. *Symploca* occurred more frequently and was more abundant in the majority of the samples examined therefore was the most significant contributor to the community (data not shown).

5 Individual sites generally displayed similar trends although there was some variability occurring between sites.

Of the 21 species more than half (12 species) of cyanobacteria were identified in SMU 1 where four primary genera made up 75% of the community: *Symploca*, *Schizothrix*, *Scytonema* and *Symplocastrum* (for more detail see Fig. S10). Cyanobacterial crusts from the dune regions on SMU 1 (deep calcareous yellow sands) were representative of crust types 1–3; patchy, brittle

10 (when dry) early-successional crusts as well as formed dark crusts that were mid to late-successional and included cyanolichens (also see Doudle et al., 2011).

Cyanobacterial crusts from the chenopod shrublands and open woodlands in SMU 2 (shallow calcareous sandy loam) represented a broad range of crust types (2–5) but overall could be described as late-successional. Lichens and mosses were

15 highly visible (also see Doudle et al., 2011). There were 21 cyanobacteria recorded: four were primary genera that made up 63% of the community including: *Schizothrix*; *Porphyrosiphon*; *Scytonema* and *Symploca* (for more detail see Fig. S11).

Cyanobacterial crusts from the open woodlands in SMU 3 (deep calcareous sandy loam, Fig. 2c) represented a broad range of crust types (2–5) but like SMU 2 could also be described as late-successional. Lichens and mosses were highly visible

20 (see Doudle et al., 2011). There were nine cyanobacteria recorded of which four were primary genera that made up 85% of the community: *Symploca*, *Porphyrosiphon*, *Scytonema* and *Schizothrix* (for more detail see Fig S12). Cyanobacteria with the capacity to fix nitrogen contributed to 77% of the community structure.

Cyanobacterial crusts from Site 6 were from the two-year old topsoil stockpile (T2) that had originated from SMU 3 (deep

25 calcareous sandy loam) would be described as early successional crusts with some seasonal mosses. There were eight cyanobacteria recorded of which four were primary genera that made up 84% of the community: *Symploca*; *Symplocastrum*; *Porphyrosiphon*; and *Scytonema* (also see Fig. S13). It was interesting to note that *Symplocastrum* was co-dominant with *Symploca* whereas in the other communities it ranged between 8-13%. Sub-surface species *Schizothrix* (found in top 5 mm) only contributed to 4% of the richness compared to 10-20% elsewhere. Cyanobacteria with the capacity to fix nitrogen

30 (*Symploca*, *Porphyrosiphon*, *Scytonema* and *Brasilonema*) contributed to 61% of the community (Fig. 4).

3.3 16S rDNA profiling of native undisturbed biocrust microbiomes

Microbial community profiling using high through-put sequencing revealed cyanobacteria comprised a significant component of all three stages forming the majority of sequences in Early and Late stages (Fig. 6a). There was a diversity of morphotypes observed including simple filamentous, heterocystous and unicellular types (Fig. 6b). The most abundant genera identified were *Leptolyngbya, Phormidium, Tolypothrix, Nostoc, Brasilonema, Chroococcidiopsis* and *Acaryochloris*. Unclassified Nostocaceae were dominant within Bare soils while Early stages observed a relative even increase in *Phormidium*, *Brasilonema* and the unicellular genera (e.g. *Chroococcidiopsis, Acaryochloris, Xenococcaceae*). Late stage biocrusts showed a slight resurgence of Nostocaceae. There was no significant difference in the richness, evenness or diversity between the three stages (Table 3). However, there were significant differences in the composition and structure of the communities of each stage. The Bare stage was significantly different to the crusted stages (Fig. 7, Table 4) while resolution between the Early and Late stages was less clear. Ordination of the samples within three dimensions showed Early and Late stages grouped separately however, PERMANOVA failed to identify a significant difference (Table 3).

3.4 Cyanobacterial tolerance to stockpiling

Examination of stockpile soil samples via microscopy revealed five cyanobacterial genera: *Nostoc, Scytonema, Microcoleus, Porphyrosiphon* and *Leptolyngbya*. Average genera richness was highest in stockpiled samples at and above 10 cm depth for all stockpile ages (Fig. 8 a, b). These differences were significant between 50 cm and 0–2 cm in all stockpiles (29-month $F_{(1,5)}$ = 7.00, p= 0.024; 20-month $F_{(1,5)}$ + 8.37, p = 0.016; 9-month $F_{(1,5)}$ = 18.00, p = 0.002). Genera richness at the intermediate depths was not significantly different. Differences were significant between 25 cm and 0–2 cm in the twenty- and nine-month old stockpiles ($F_{(1,5)}$ = 32.73, p = 0.000; F = 16.20, p =0.000) respectively. Counterintuitively, after nine and twenty months of stockpiling, average genera richness was higher in stockpiled samples when compared with undisturbed samples except for 20-month old 4–6 cm samples (Fig. 8 a, b) but this difference was only significant in the nine-month old stockpile at 10 cm depths ($F_{(1,5)}$ = 8.27, p = 0.017). High variability between replicates accounted for the lack of significance in the rest.

When comparing average genera richness at different depths between stockpiles, richness was greater in material stockpiled for the least amount of time above 10 cm depth but not at or below this level (Fig. 8b). Average genera richness was variable within adjacent undisturbed areas (Fig. 8a). The variability in genera richness within replicates was high (refer to Fig. S14 a–c). The cover of genera was relatively constant between sites in the undisturbed samples, but in stockpiled samples *Nostoc* cf *commune* was more prevalent ($F_{(1,5)}$ = 5.97, p = 0.012).

Nostoc cf. *commune, Nostoc* yellow, *Microcoleus* and *Leptolyngbya* were present in more stockpiled samples than undisturbed samples. Conversely, *Scytonema* and the black form of *Leptolyngbya* were more prevalent in undisturbed

samples. When all samples were combined, *Nostoc* cf *commune* had the greatest cover in both stockpiled and undisturbed samples followed by yellow *Nostoc* in stockpiles only. The coverage by the remaining genera was similar for most other genera although *Microcoleus* and *Leptolyngbya* had greater cover in stockpiled areas and *Porphyrosiphon* had slightly higher coverage in undisturbed samples. The cover of *Scytonema* was almost the same in stockpiled and undisturbed areas ($F_{(1,17)} = $ 0.00, $p = 0.969$) (also see Fig. S15).

Nostoc cf. *commune* exhibited the best survival response in stockpiling as it had significant coverage in samples from all depths in all stockpile ages. *Nostoc* had the greatest coverage in the oldest stockpiles, followed up by the most recently created stockpiles. These differences were significant from depths of 6 cm to the surface (4–6 cm $F_{(1,5)} = 28.83$, $p = 0.000$; 2-4 cm $F_{(1,5)} = 4.89$, $p = 0.023$; 0-2 cm $F_{(1,5)} = 3.72$, $p = 0.049$). These patterns were not reflected in the total coverage by *N.* cf. *commune* in corresponding undisturbed areas. The *Leptolyngbya* black morphotype only occurred between 20 and 40 cm depth and were found in only one stockpile samples but were present in six samples from three adjacent areas. *Stigonema* genus was found in six stockpile samples spanning all ages and in only one adjacent sample in all cases in the upper 10 cm of the soil profile.

Nostoc cf. *commune*, *Porphyrosiphon*, *Microcoleus* and *Scytonema* were the first genera to develop to an identifiable stage. Filaments of the *Stigonema* genus were found in low numbers and appeared to be recently formed. It was only present in samples examined in the latter stages of the identification process. The yellow form of *Nostoc* exhibited a much slower rate of development than *Nostoc* cf *commune* and could only be definitively determined as a form of *Nostoc* when examined after 13 weeks of incubation. Samples from undisturbed areas sourced at depths from between 10 cm and 50 cm initially showed no visible signs of growth when examined after six weeks of incubation. Six weeks later cyanobacterial growth was evident yet in many cases had not advanced to the point where morphotypes could be distinguished.

4.0 Discussion

In terms of rehabilitation the natural capital in topsoil that has been removed in the mining process is often not recognised or poorly understood. Soils properties are crucial in the re-establishment of a raft of ecosystem services that include fertility, structure, climate regulation and biodiversity (Dominati et al., 2010). Cyanobacteria are ecosystem engineers in that they have the capacity to provide many of these crucial ecosystem services (Jones et al., 1994). This research demonstrated that the cyanobacterial communities in the J-A biocrusts were compositionally diverse topsoil microbiomes that substantially contributed to the Myall-chenopod biomes. We had hypothesised that cyanobacteria would be central to soil micro-processes and this was strongly supported by extensive species richness and diversity. In the first study of its kind we have shown the response of cyanobacteria to topsoil stockpiling at various depths and ages. In this study we have shown cyanobacteria are moderately resilient to stockpiling at depth and over time, providing they are manipulated dry (W. Williams, unpublished

data). Cyanobacteria from the top 10 cm were found to be more viable within the first six weeks and showed potential for biocrust re-establishment. We found greater cyanobacterial richness in the nine and 20-month stockpiles compared to undisturbed samples adjacent to the stockpiles. In general, the resilience of cyanobacteria to burial in topsoil stockpiles in the longer term appeared good, however in an arid environment recolonization and community diversity could be impeded by

5   drought (Williams and Büdel, 2012)

In this study cyanobacterial community richness and abundance were not related to soil and landform type and this was further supported though the sequenced samples where no significant differences were observed. At J-A any of the cyanobacteria could conceivably occur anywhere across the landscape, with their relative abundance likely determined by microenvironments and microhabitats such as light (sun and shade) and chemical gradients (Stal, 2003), as well as moisture

10   availability (Büdel et al., 2018) and soil particle size (Büdel et al., 2009).

4.1 Cyanobacterial community structure

Key cyanobacteria indicating biocrust formation and development were *Leptolyngbya, Phormidium, Tolypothrix, Nostoc, Brasilonema, Chroococcidiopsis* and *Acaryochloris*. These genera have consistent morphological traits with those observed via microscopy. Notably, the identification of *Brasilonema* was supported with sequencing data and had not been previously

15   recorded in Australian soils. Simple filamentous types are often attributed with the primary crust building role, able to span inter-particle gaps within the soil via supra-cellular structures (e.g. *Microcoleus*, see Garcia-Pichel and Wojciechowski 2009). Sequencing data showed *Phormidium* was the dominant cyanobacterium for this role and it is likely that *Symploca* identified though microscopy was the principal *Phormidium* present. *Microcoleus* sp. and *Porphyrosiphon* were also identified as early colonisers however these genera are currently poorly resolved phylogenetically (Garcia-Pichel et al 2013)

20   but share critical morphological features enabling biocrust formation and maintenance. Notwithstanding, the important crust-building cyanobacteria in this study appeared to be *Symploca* that was associated with EPS production, and principal feature of early colonising crust formation (Hu et al., 2002).

The cyanobacterial richness at J-A was determined according to their morphological features. In many cases these features

25   (e.g. outer protective sheaths, UV protection, EPS production) provided the basis of attributes that pertained to fundamental survival strategies. Environmentally induced strategies of arid land cyanobacteria reflect their habitat, these survival traits have developed over a long evolutionary history. Many primary (common to abundant) and secondary (uncommon) cyanobacteria recorded at J-A exhibited thick gelatinous sheaths (*Porphyrosiphon, Schizothrix, Microcoleus, Nostoc*) or were associated with the production of EPS (*Symploca, Nostoc, Schizothrix, Leptolyngbya*). Filamentous cyanobacteria

30   formed the major part of the J-A crust structure with tufts, webs or creeping masses closely intertwined (e.g. *Porphyrosiphon*, *Symploca*, *Scytonema*, *Schizothrix*, *Microcoleus*). These are often assimilated with unicellular forms (e.g.

Gloeocapsa, *Chroococcus*, *Chroococcidiopsis*) or gelatinous colonies of *Nostoc* (see supplementary material Fig. S16 for images of growth habits).

Twenty-one cyanobacteria were recorded from 13 genera. Four species were unicellular and the remaining seventeen were filamentous. Some cyanobacteria found at J-A (*Microcoleus paludosus, Nostoc* sp.*, Gloeocapsa*) had also been recorded at Lake Gilles (SA) about 400 km southeast of J-A (Ullmann and Büdel, 2001). Although *Microcoleus* species were recorded at J-A they did not dominate the biocrust compared with many reports from the United States, Asia and elsewhere (e.g. see Belnap and Eldridge, 2001). This infers that the early colonisers such as *Microcoleus* would not play a dominant role in early stabilisation and colonisation of the soil. At J-A *Symploca* and *Scytonema* appeared to be an important colonising cyanobacterium in the biocrusts and have been recorded as playing a key role in carbon sequestration in northern Australian cyanobacterial crusts (Büdel et al., 2018).

The taxonomic status of *Brasilonema* remained uncertain and may be a variety of *Scytonema*, however, genomic data supported morphological identification and the type has also been recorded in other terrestrial habitats globally. Due to its similar morphological attributes and genomic data, in this study we called this cyanobacterium *Brasilonema* (Fiore et al., 2007; Vaccarino and Johansen, 2012). *Nostoc commune* var. *flagelliforme* had been recorded at J-A along with *Nostoc commune* across the shallow and deep sandy loams. Although *N. flagelliforme* appeared rarely, it had been previously documented from sites in south-western South Australia, Western Australia, Northern Territory (Skinner and Entwisle, 2002) and Victoria (W. Williams, unpublished data). A joint Spanish-Australian study has now shown that both *Nostoc commune* and *N. flagelliforme* contain the same genomic markers and cannot be separated, rather the spaghetti-like tubes that are unique ecotype likely associated with aridity (Aboal et al., 2016). This is supported by the semi-arid environment at J-A and it may be more widespread in Australia than previously recorded as it is often only clearly visible following rains.

4.2 Cyanobacterial tolerance to stockpiling

Physical disturbance of biocrusts occurs on a large scale at the J-A mine site with the removal and temporary stockpile storage of topsoil. This type of mechanical disturbance results in the burial and translocation of the biocrust. The impacts of burial within the natural environment are rarely studied. In China, artificial sand burial at shallow depths showed there were significant reductions in chlorophyll concentration, UV synthesis, total carbohydrates (EPS) and damage to photosynthetic activity (Rao et al., 2012). In a semi-arid grassland in Australia, wind-borne sand burial of cyanobacterial crusts during a severe drought resulted in a significant reduction in surface dwelling cyanobacteria and significant reductions in biological-available nitrogen (Williams and Eldridge, 2011).

Here we have shown that without further disturbance, a proportion of cyanobacteria can survive stockpiling for over two years. This is not surprising due to the recognised ability of cyanobacteria to survive in extreme environments. In previous

studies, cyanobacteria have been grown from samples sourced at 18 cm depths in Japanese rice paddy soils (Fujita and Nakahara, 2006), 50 cm in the UK (Esmarch, 1914), and 70 cm depths in the USA (Moore and Karrer, 1919). Yet, the cyanobacteria from the surface depths of the stockpiles (i.e. <10 cm) appeared more resilient in the short-term.

The species sampled at J-A have a proven track record of survival under extreme conditions. *Microcoleus* and *Leptolyngbya* have survived and remained viable after up to three million years frozen in lake sediments in permafrost (Vishnivetskaya et al., 2003). Vegetative *Nostoc commune* material retains viability following several decades of storage in desiccated form (Bristol, 1919; Lipman, 1941). Reactivation of vegetative material after decades of storage was successful but several months (Lipman, 1941) to a year (Bristol, 1919) of incubation can be necessary for growth to take place. These results were reflected in the current study where growth was not observed in the undisturbed areas below 10 cm depth for several months. It may be that the longer the period of inactivity, the longer time taken for reactivation to occur (Billi and Potts, 2002; Williams et al., 2014), or less material is viable therefore it takes longer to rebuild colonies (Agrawal and Singh, 2002).

Akinetes are desiccation resistant cells produced by certain filamentous cyanobacteria that can survive for long periods. *Nostoc* and *Scytonema* produce akinetes (Kaplan-Levy et al., 2010; Tomaselli and Giovannetti, 1993) but many of the other species sampled in this study cannot, therefore alternative survival methods are in action. Heterotrophic growth is also possible for some cyanobacteria (Flores and Herrero, 2010). Cyanobacteria can survive in darkness through utilisation of alternate carbon sources in drinking water systems (Codony et al., 2003) and this may also be true for soil cyanobacteria (Reisser, 2007). *Nostoc* have the potential to grow at low light in caves and under ice (Dodds et al., 1995) and even in darkness (Huang et al., 1988). Belnap and Gardner, (1993) reported *Microcoleus vaginatus* sheaths at depths to 10 cm and considered the sheaths to be remnant from a time when the surface was lower than the current day due to a lack of chlorophyll. It is possible that heterotrophic growth was still occurring at these depths for which chlorophyll is unnecessary.

The species richness in taxa at depths in undisturbed areas was like that of surface samples yet with much slower growth. The fact that these organisms took much longer to grow than those sampled from upper layers would suggest that they have grown from vegetative material that has been photosynthetically inactive for long periods. Long term inactivity of vegetative material can result in long lag times for growth following re-activation (Bristol, 1919; Lipman, 1941; Shaw et al., 2003) and this was observed in species sourced from depths that are incapable of akinete production.

In the context of rehabilitation, it is not practical to store stockpiles at very shallow depths, however in the longer term under optimum conditions a diverse range of cyanobacteria did recover, indicating the depth of burial is an important feature of recovery over time. Gradual removal of the topsoil from the stockpiles at shallow depths may facilitate and provide a greater opportunity for microbial recovery. The timing of topsoil amendments would be crucial in terms of moisture availability for the initial cyanobacterial resurrection and to facilitate enough growth to stabilise the newly laid soil surfaces.

5.0 Conclusions

This study investigated the nature and role of biocrusts, with particular attention paid to cyanobacteria, in the natural J-A landscape with the aim of assessing the role of these crusts in post-mining soil rehabilitation. Prior to mining, top soil is stripped and stockpiled for re-distribution following mining. The persistence of cyanobacteria at depth in soil stockpiles was examined and it was found that this diminished substantially below the top few centimetres and over time. Thus, to maximise the ability of new biocrusts to form over rehabilitated soil surfaces, stockpiles should be as shallow as space allows and the soil spread back over mined land as soon as practicable. Diversity indices determined through sequencing of the whole bacterial community did not provide any advantages over morphological identification of cyanobacteria as a measure of soil fertility.

At J-A microbial biobanks created through shallow scraping of topsoil and low-profile topsoil stockpiles are clearly valuable eco-resources. As re-establishment of biocrusts would be dependent on rainfall, future research should be focused on the timing of rehabilitation and how soon cyanobacteria recolonise to form a protective crust. Ongoing monitoring biocrust recovery will provide an effective means of measuring important soil function processes.

Table 1: Intact biocrust soil physicochemical descriptions for all sites EC = electrical conductivity in μS cm⁻¹; total percentage of nitrogen present (N%), total percentage of carbon present (C%) and carbon to nitrogen ratios (C:N) for all sites. *SMU 3 is the origin of the topsoil stockpile aged two years.

Vegetation	Soil	SMU	Site	pH	EC	N%	C%	C:N
Mallee	Deep calcareous yellow sand		4	8.6	92	0.07	1.02	15.1
Mallee		1	9	8.5-8.6	107	0.07	0.93	13.7
Mallee			10	8.5-8.6	118	0.09	1.37	15.8
Myall	Shallow calcareous sandy loam		1	8.4-8.5	122	0.10	1.20	11.7
Mallee		2	5	8.4	133	0.13	1.84	13.8
Chenopod			8	8.6	124	0.10	1.32	12.8
Myall	Deep calcareous sandy loam		2	8.5	113	0.09	1.09	12.1
Myall		3	3	8.6	114	0.09	1.99	21.4
Myall			7	8.5-8.6	140	0.10	1.76	18.3
Myall	Topsoil stockpile	SMU 3*	6	8.9	119	0.08	1.57	19.6

Table 2: Biocrust compressive strengths measured with a penetrometer, means and standard deviations (SD) for SMU 1–3 (kg cm⁻²) tests with p-values, values in bold that are different from 0 with a significance level alpha=0.05, NS = not significantly different.

SMU	Means ± SD	SMU 1	SMU 2	SMU 3
SMU 1	2.79 ± 1.41	**0**	**0.001**	**0.005**
SMU 2	3.75 ± 0.79	**0.001**	**0**	NS
SMU 3	3.97 ± 0.70	**0.005**	NS	**0**

Table 3: Cyanobacterial mean (± Standard Error) of richness (Margalef's index), evenness (Pielou's index) and diversity (Shannon index) across successional stages.

	Bare		Early		Late	
	Mean	SE	Mean	SE	Mean	SE
Richness (d)	167.4	5.344	146.8	5.818	142.8	15.69
Evenness (J')	0.816	0.011	0.791	0.005	0.788	0.027
Diversity (H')	5.977	0.103	5.692	0.064	5.642	0.281

Table 4: Permutational analysis of variance (PERMANOVA) of pair-wise comparisons of Bray-Curtis dissimilarity between biocrust stages and bare soil. P(MC) = probaility values obtained using 9999 Monte Carlo permutations. Permdisp showed no significant differences in variation of spread (pseudo F=3.8068, P(perm) = 0.068).

Groups	t	P(perm)	Unique permutations	P(MC)
Bare, Early	2.6216	0.0979	10	0.0107
Bare, Late	2.5742	0.0959	10	0.0120
Early, Late	1.2793	0.0953	10	0.1993

Table 5: Diversity across sites on a presence absence basis for all seasons and Lake Ifould (salt lake). Different species attributed to a genus (i.e. sp. 1,2,3) have all been separated based on their morphological features and size but could not be positively identified.

Cyanobacterium	SMU 1	SMU 2	SMU 3	T2 Stockpile	Lake 5 Ifould
Aphanothece					x
Brasilonema	x	x	x	x	x
Chroococcidiopsis		x		x	
Chroococcus sp. 1	x	x	x	x	
Chroococcus sp. 2					x
Gloeocapsa	x	x			x
Leptolyngbya	x	x			x
Microcoleus cthonoplastes					x
Microcoleus paludosus	x	x	x	x	x
Microcoleus sociatus					x
Microcoleus vaginatus	x	x			x
Nostoc commune		x	x	x	x
Nostoc flagelliforme		x			
Nostoc pruniforme		x			
Nostoc sp.	x	x			
Porphyrosiphon sp. 1	x	x	x	x	
Porphyrosiphon sp. 2		x			x
Schizothrix sp. 1		x			x
Schizothrix sp. 2	x	x	x	x	
Schizothrix sp. 3					x
Scytonema sp. 1	x	x	x	x	
Scytonema sp. 2		x			x
Scytonema sp. 3		x			
Scytonema sp. 4					x
Symploca sp. 1	x	x	x	x	x
Symploca sp. 2		x			
Symplocastrum sp. 1	x	x	x	x	x
Symplocastrum sp. 2					x
Species richness	**12**	**21**	**9**	**10**	**18**
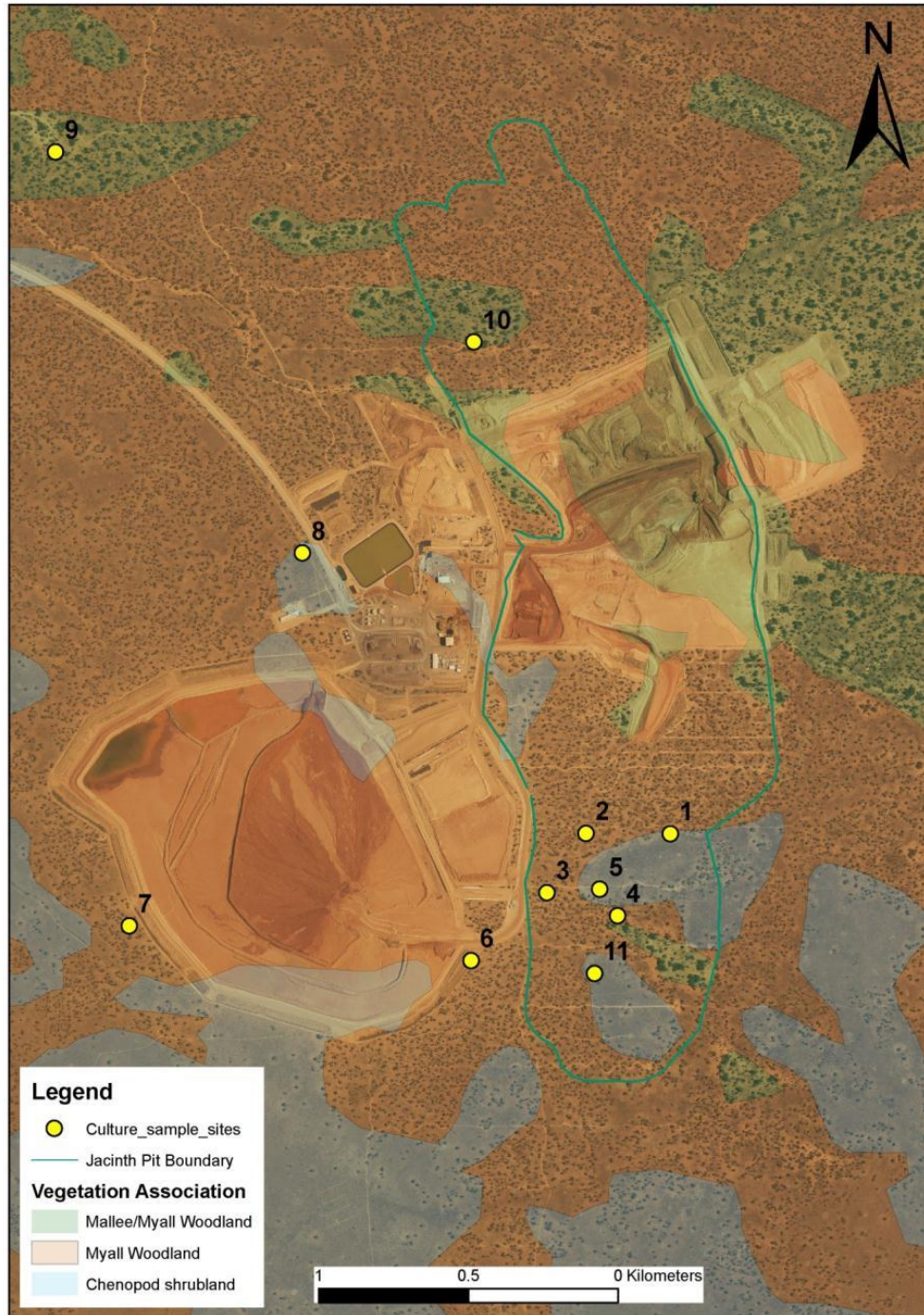
Figure 1: Image of biocrust sample sites located within the vegetation associations described in Table S1 (supplied, S. Doudle). Site 11 was initially investigated but later discarded as it was a fourth replicate of the Chenopod Shrubland.
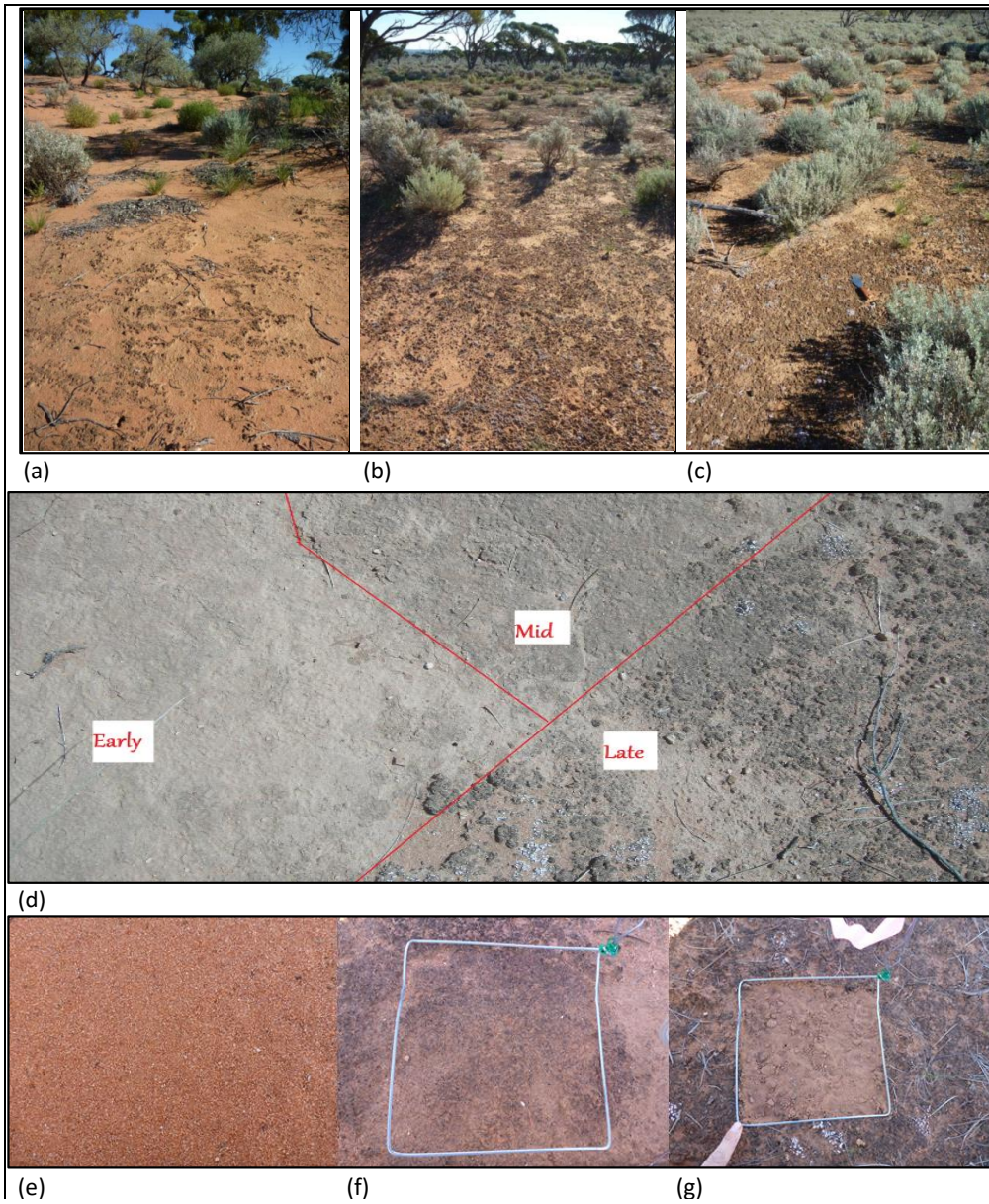
Figure 2: (a) SMU 1 (Sites 4, 9 and 10): Type 1–3 Biocrusts on deep calcareous yellow sands (dunes); (b) SMU 2 (Sites 1, 5 and 8): Primarily types 4 and 5 biocrusts on shallow calcareous sandy loam; (c) SMU 3 (Sites 2, 3 and 7): Types 1-5

5 biocrusts on deep calcareous sandy loam (Photographs by S. Doudle, 2011); (d) different biocrust stages (top) north of Stockpile 19, adjacent to Site 9; (e) Bare, (f) Early and (g) Late stages, also showing biocrust sample already removed from Late stage (Photographs A. Chilton).
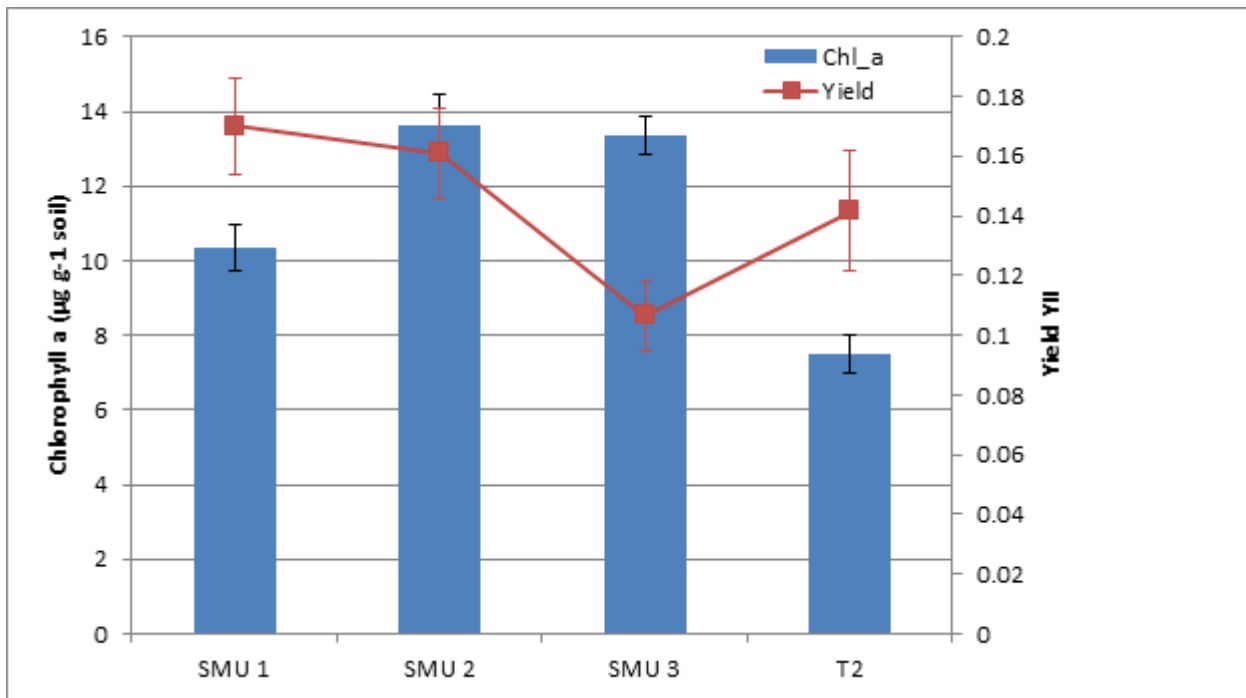
Figure 3: Chlorophyll *a* concentration (µg g^{-1} soil) following resurrection (2 weeks) after a desiccation (dry) period of 6 months. Soil Management Unit 1 (SMU 1) was significantly lower than SMU 2–3 (p = 0.05). Mean values and standard error of the mean (SEM) displayed where SMU 1 = Sites 4, 9, 10; SMU 2 = Sites 1, 5, 8; SMU 3 = Sites 2, 3, 7; T2 = two-year old Topsoil stockpile (originating from SMU 3). Photosynthetic yield (YII) of photosystem II (PSII) for SMU 1–3 and T2 (Site 6 two-year old topsoil stockpile), displaying mean values and standard error of the mean (SEM).
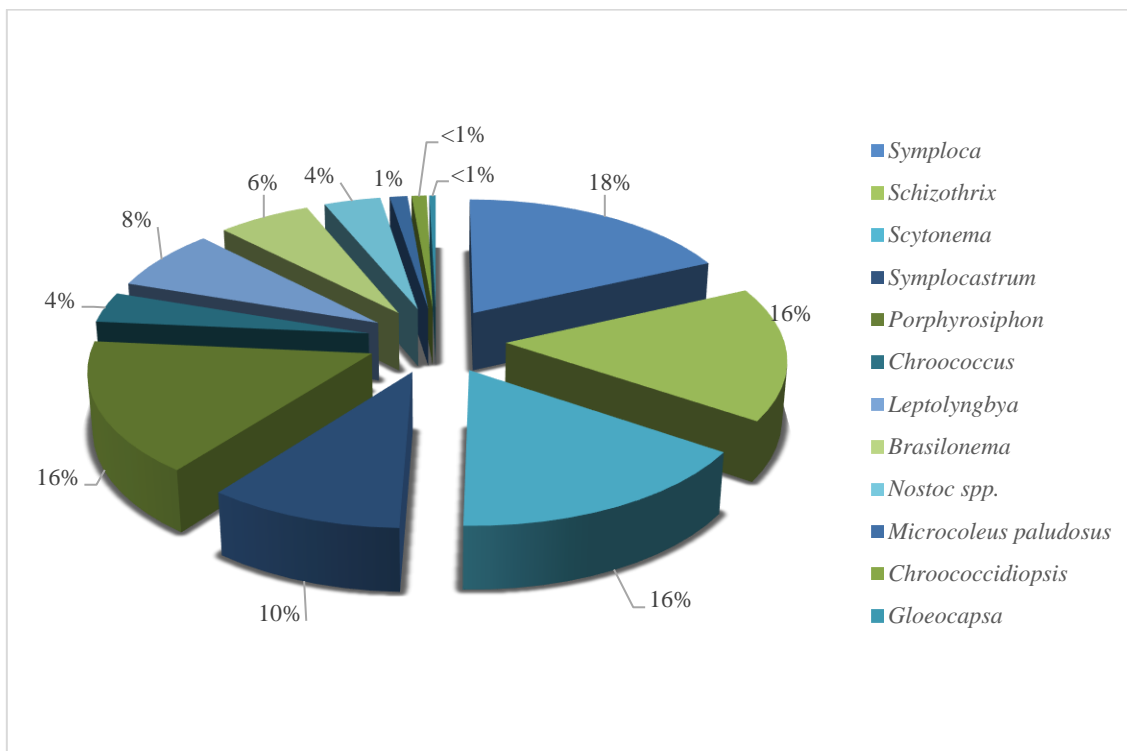
Figure 4: Cyanobacterial community structure across all sites expressed as a percentage of the total community based on mean richness and abundance scores. N-fixing cyanobacteria contributed to 61% of the community structure.
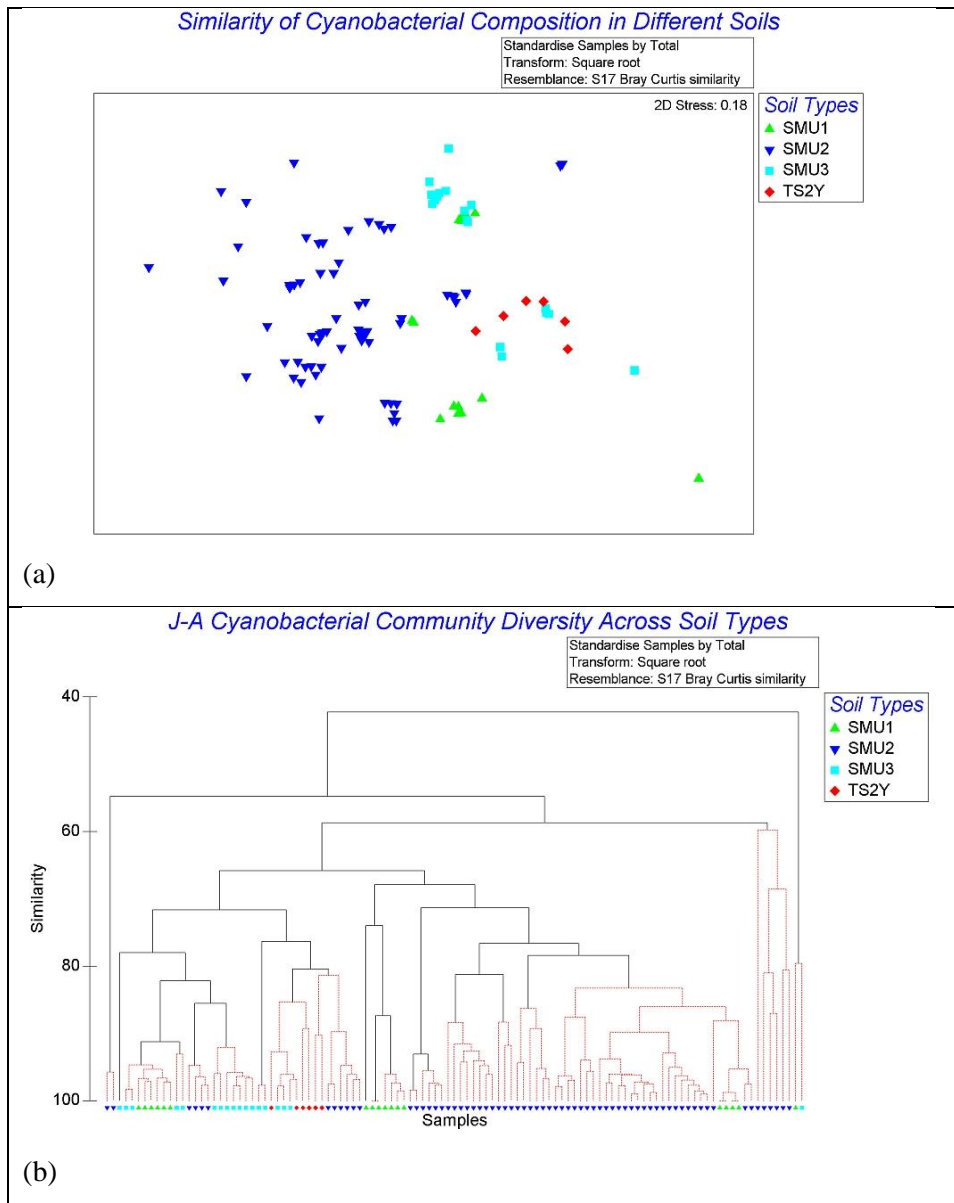
Figure 5(a): Cyanobacterial community structure based on indexed abundance and diversity across all sites displayed in an nMDS plot (Bray Curtis similarity). SMU 1-3 refer to key soil management units; SMU 2 appeared to have more range in the clusters compared to the closer groupings of SMU 1 and 3. T2 (TS2Y) is the two-year old topsoil stockpile; note these are clustered more closely. Figure 5(b): Similarities between samples within their SMU's are displayed in a Bray Curtis dendrogram. Black continuous lines show significant differences between samples ($p = 0.05$) and lighter lines indicate that most samples were nor significantly different to each other.
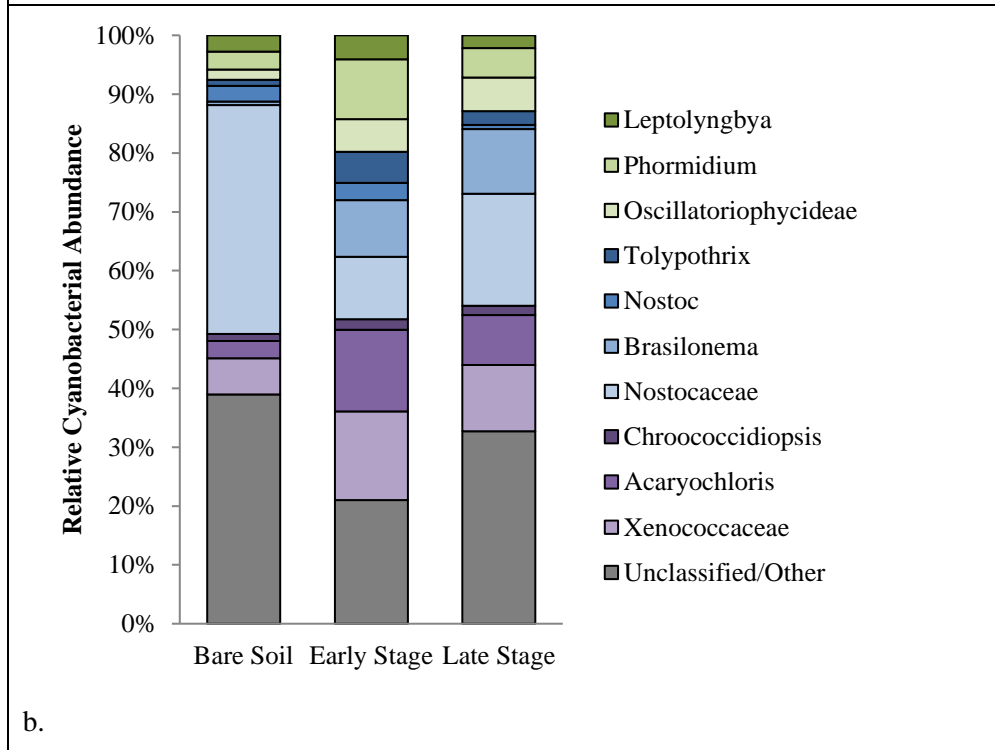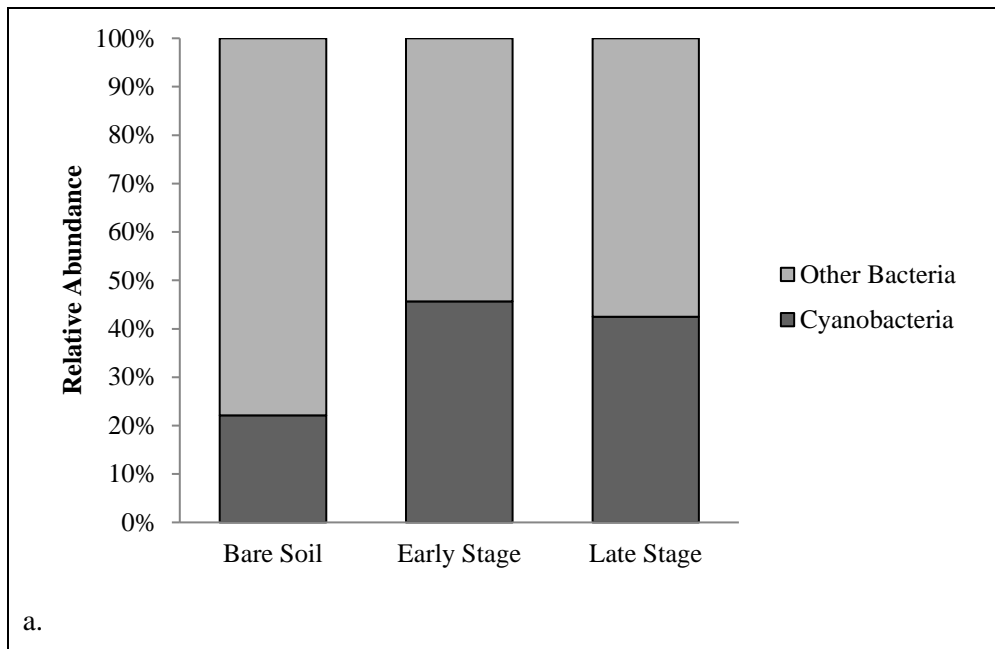
Figure 6(a): Relative abundance of cyanobacteria to other bacteria within Bare soil and Early and Late stage biocrusts.

Figure 6(b): Abundance of cyanobacterial genera and groups. Green = Simple filamentous types, Blue = Heterocystic types, Purple = Unicellular. Grey = Unclassified/Other includes chloroplasts.
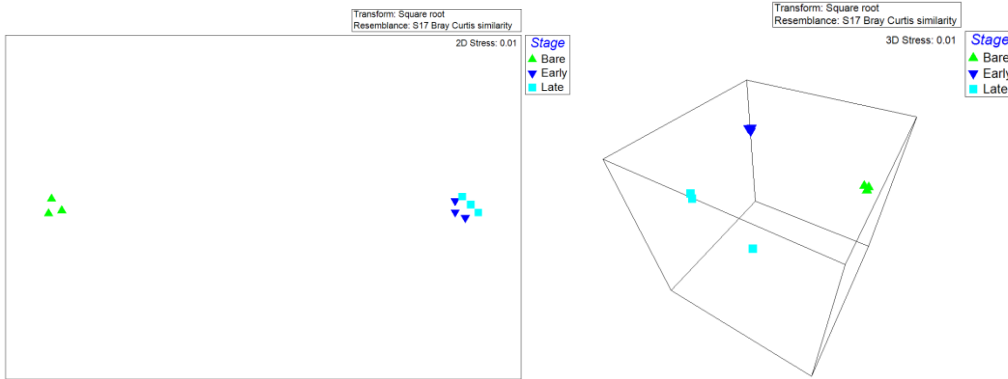
Figure 7: Non-metric Multidimensional Scaling of Bare soil and Early and Late Stage biocrusts within two dimensions (left) and three dimensions (right).
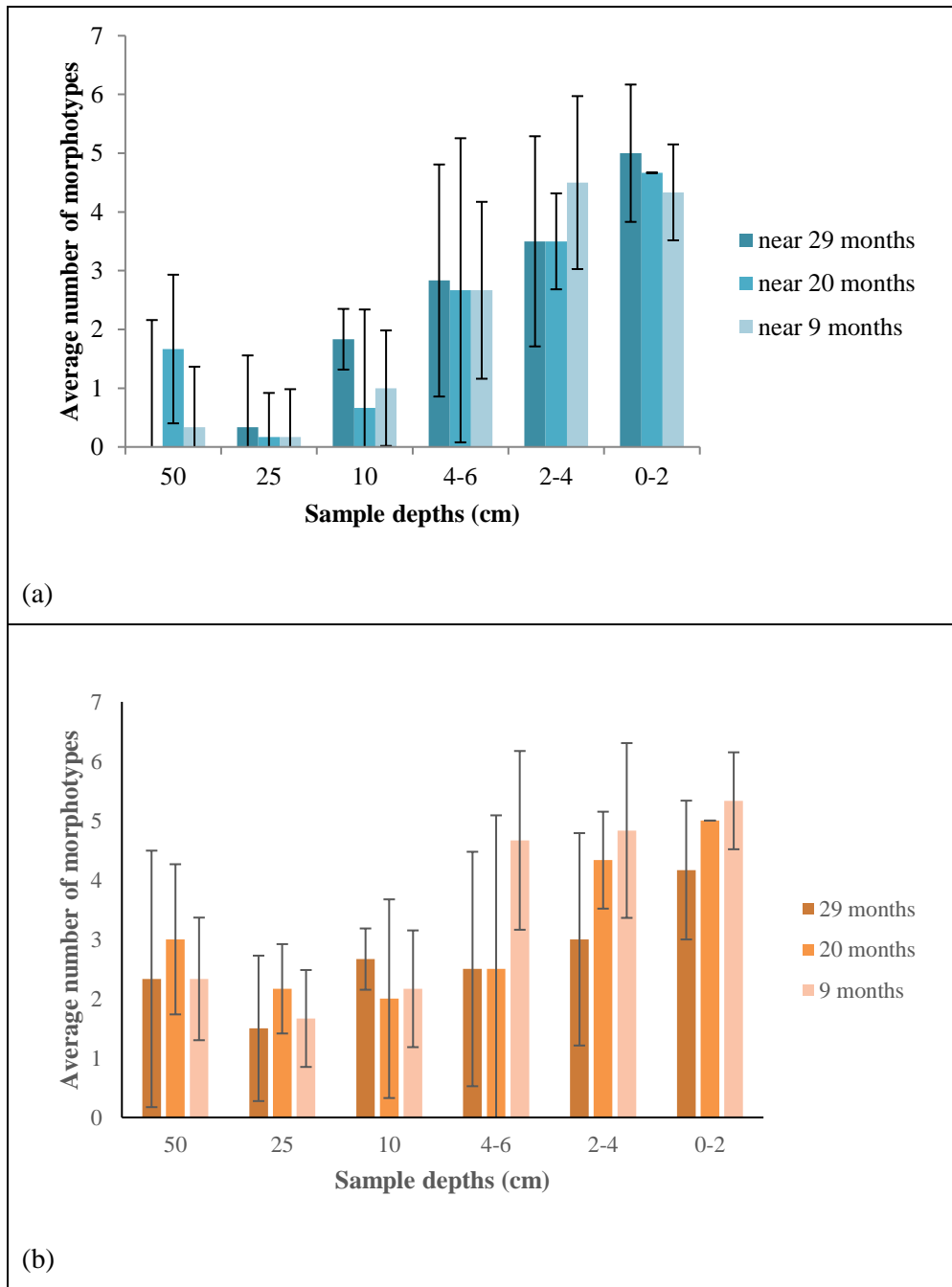
Figure 8: Cyanobacterial richness: (a) undisturbed were significant between 50 cm and 0–2 cm in all stockpiles (p= 0.024; 20-month p = 0.016; 9-month p = 0.002) and, (b) stockpiles of different ages were significant between 25 cm and 0–2 cm in the twenty- and nine-month old stockpiles (p = 0.000; p =0.000).

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