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

Following the destruction of the soil profile in a post-mining landscape there is the critical need to restore ecological integrity to the system. Mine rehabilitation is a complex process that involves many levels of understanding of difficult issues relating to ecosystem function. Harsh disturbance disrupts the spatial structure of soil microbial communities. Not the least is the removal or burial of bioactive soils that will have knock-on effects for rehabilitation efforts such as nutrient cycling and plant reestablishment (Jasper, 2007; Tongway and Ludwig, 1996). Microorganisms are critical components of soils that drive assorted micro-processes and impact soil ecosystem function on several levels. Successful ecological restoration of arid mining sites relies on a holistic approach where microbial recolonization can serve as an indicator of the integrity of the wider ecosystem (Tongway, 1990). In this sense, recent restoration approaches in arid landscapes includes the reestablishment of surficial crusts that develop as a protective skin across the bare soil or stony interspaces between plants. These encrusted surfaces can be physical, chemical or biological (microbial) in nature. Well established biological crusts (biocrusts) are intricately interwoven and structured, high-functioning communities, and variable in composition (incorporating cyanobacteria, algae, lichens, mosses, liverworts, micro-fauna and bacteria). 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).

However, biocrusts are very sensitive to disturbance and mining severely disrupts and compromises their structure and function. The recognition of the absolute importance of microbial communities in the topsoil has led to the accepted practice of stripping and storing in stockpiles as a form of "biobanking" for redistribution at the cessation of mining. During this process the crushing and burial of biocrusts results in the inability of crust organisms to photosynthesise due to the lack of light. Massive soil disturbance results in a loss of structure, resources and often has long lasting effects on energy transfers, soil stability, nutrient cycling and surface hydrology (Bowker, 2007; Tongway and Hindley, 2004). 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). Biocrust burial as a result of environmental stress and disturbance can result in serious impacts on biocrust microorganisms' viability and function. For example, grazing and drought caused significant declines in cyanobacterial richness and abundance which resulted in 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 generally over many years (Belnap and Eldridge, 2001; Belnap and Gillette, 1998). Should biocrust organisms remain inactive while they are wet, cell death and decomposition commonly occurs (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).

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).

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. 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). 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.

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 (Chamizo et al., 2012; Mager and Thomas, 2011). 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). As biocrusts develop in structural complexity, the diversity of organisms is regulated by water infiltration, temperature, light and additional disturbance (Belnap and Eldridge, 2001; Büdel et al., 2009; Elbert et al., 2012).

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 sequestering 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 surface between the grass plants and post-mining rehabilitation needs to take their role into account (Doudle et al., 2011). It follows that there is a real need for 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).

To our knowledge, the effects of topsoil stockpiling on biocrust organisms such as cyanobacteria, and their recovery time, following topsoil spreading, has not previously been investigated. In this research we focused on the cyanobacterial component of the biocrusts. This was in keeping with the mining framework in the ongoing development of informed rehabilitation plans that focuses on improved long-term outcomes. Overall, we sought to determine whether shallow biobanks of cyanobacterial-enriched topsoil would facilitate the recovery of essential soil microprocesses when re-spread following mine disturbance. The specific aims of the microbial biobanking research program were to: (a) define the cyanobacterial community structure applying a polyphasic approach with a special focus on species that drive early colonisation, nutrient cycling and soil stabilisation; and, (b) to examine the effects of stockpiling topsoil on cyanobacterial resilience to crushing and burial and their recovery following spreading of topsoil back across mined land. We hypothesised that cyanobacterial survival within a topsoil stockpile would reduce with both depth within the stockpile and elapsed time before topsoil re-spreading.

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 cover around 45% of

the landscape surfaces, equating to 2,000 hectares of the mining lease, and had been previously classified into three successional stages representative of the five biocrust types found growing across the landscape (Table S1, S2) (Doudle et al., 2011). Types 1–2 are light coloured, patchy, thin, and fragile cyanobacterial crusts 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). In this study the term biocrust covers whole crust samples that incorporated lichens cyanobacteria and mosses in varying proportions; however, the cyanobacterial component of this crust was the focus in terms of our polyphasic approach to community structure, succession and its biophysiochemical properties.

At J-A the landscape has been characterised into three distinct soil types that were associated with vegetation communities identified as soil management units (SMUs) (Doudle et al., 2011; Hou and Warland, 2005): SMU 1 – deep calcareous yellow sands associated with dune ridges and dominated by Red Mallee: *Eucalyptus oleosa* ssp. *oleosa* = open Mallee/Myall woodland; SMU 2 – shallow calcareous sandy loams dominated by Chenopod Shrubland: *Maireana sedifolia* and *Atriplex vesicaria*; SMU 3 – Western Myall: deep calcareous sandy loam dominated by *Acacia papyrocarpa Maireana sedifolia* = open Myall woodland. Site 1 occurs in a transition between SMU 2 and SMU 3 but was treated as most like SMU 2 (also see Table S1). In the first place, sample site locations (Fig. 1) were selected based on these soil management units (SMU) and a two-year old stockpile (Table S1). Secondarily, sites within these SMU were selected for the subsequent detailed studies of cyanobacterial succession and its resilience to longer-term stockpiling (Fig.2 and Section 2.4). On two occasions (July 2011 and March 2012) we sampled the biocrusts across the three main SMU sites (10 sites, see Fig. 1).

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 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 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 biocrust sampling from Sites 1–10 within J-A (Fig. 1). The sites selected encompassed the three SMU 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 a 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, about half the sample was removed from each Petri dish and fine sieved (1.70 mm). Duplicate sub-samples (~2 g) were analysed for total C and N and C:N ratio using a high temperature digestion in a vario MACRO Elemental Analyser (Elementar) (n=6 per SMU). Duplicate samples (10g) for the purpose of analysing soil pH and electrical conductivity (EC) 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. The remaining half of the Petri dish was used to determine Chlorophyll *a* concentration of the biocrusts. Following resurrection (by moistening) the crust was lightly homogenised and a 5 g sample used at 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.

Prior to the use of the samples for analysis 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.

At J-A we measured photosynthetic performance (recorded as yield, YII) of the biocrusts using a pulse-amplitude modulated (PAM) fluorometer (Pocket PAM; Gademann Instruments, Germany). The goal 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 adjacent to each sampled location (6 x 4 reps per site).

Significant differences in C and N and C:N between SMUs as well as differences in Chlorophyll *a* and YII between SMUs and the two-year old stockpile were tested by one-way ANOVA's and Tukey post hoc tests (Minitab 18).

2.3 Cyanobacterial community structure

2.3.1 Microscopy of biocrust cyanobacteria

Prior to the destruction of the samples for analysis, a portion of field samples collected (Section 2.2) subsections were removed from six of the eight Petri dishes, placed into six-cell plates and used to enumerate cyanobacterial richness and diversity and to classify colonies. These 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 (Crust Types 1–2, SMU 1) or Late (Crust Types 4–5, SMU 2, SMU 3) 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 (representative of SMUs 1–3) , three replicates were collected 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 SMU 3 areas with *Acacia papyrocarpa* (Western Myall) over-storey (Table S3). Three different age stockpiles (9 months, 20 months and 29 months) 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 age. 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. Sample moisture was maintained by the addition of sterile water within a laminar flow cabinet. To determine cyanobacterial growth and species richness, wet-mounts for each sample were examined under 16 x magnification. Identification was carried out as per methods detailed in Section 2.2.1. Cyanobacterial thalli and colony sizes were estimated using area of coverage of the field of view.

A two-way ANOVA was initially run with stockpile age and depth as independent variables and species richness as the effect. If there was no interaction between age and depth a one-way ANOVA with Tukey's post hoc test was run between the significant independent variable and species richness. Similarity analysis (hierarchical agglomerative clustering with the

SIMPROF test and non-metric multi-dimensional scaling [Primer 7]) was used to determine whether there was a significant difference in relative species abundance at each depth.

3.0 Results

3.1 Biophysical characteristics of biocrusts and cyanobacteria

Across the three soil management units (SMU) mean soil pH ranged from 8.5–8.6 while the two-year old topsoil stockpile was higher at pH 8.9 (Table 1). Electrical conductivity ranged from 92–140 μS cm^{-1}. 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 whereas the lowest values were found on SMU 2. There were no significant differences between SMU 1 and SMU 2 and SMU 3. Compressive strengths across Sites 4, 9 and 10 (SMU 1) were significantly lower than SMU 2, SMU 3 and the two-year old topsoil stockpile (Table 2).

Mean chlorophyll *a* concentration ranged between 10.34–13.64 μg g^{-1} soil for the intact undisturbed biocrusts. From the three different SMU the lowest values were found at SMU 1 (10.34 ± 2.13, p = 0.000) whereas SMU 2 and SMU 3 showed no significant differences between them (Fig. 3). Mean chlorophyll concentrations of biocrusts sourced from the two-year old topsoil stockpile (7.49 ± 1.01 μg g^{-1} soil) were almost half those of SMU 3 (13.53 ± 1.74 μg g^{-1} soil), which was the origin of the topsoil stockpile. The photosynthetic yields recorded ranged between 0.073 (stockpile) and 0.147 (SMU 2). SMU 3 and the two-year old stockpile showed lower values than SMU 1 and SMU 2 (p = 0.017) whereas SMU 1 and SMU 2 were not significantly different (Fig. 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%), *Porphyrosiphon* (16%) and *Scytonema* (16%). Secondary cyanobacteria present variously occupied <1 to 10%, and overall made up 34% of the community. *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). 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). When cyanobacterial community structure of different soil types was compared we found no significant difference in cyanobacterial community structure across soil types, however the results do suggest some spatial structuring exists across the three SMU (Fig. 5a). Moreover, 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. Individual sites generally displayed similar trends although there was some variability occurring between sites.

Of the 21 cyanobacteria species found at J-A, more than half (12 species) 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 (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 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 also highly visible (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 the two-year old topsoil stockpile that had originated from SMU 3 (deep 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 (*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. However, PERMANOVA analysis showed that there were significant differences in the composition and structure of the communities of each stage (Pseudo-*F*=4.9544, P(perm)=0.004, Unique perms=273). Post-hoc pair-wise comparisons showed Bare stage was significantly different to the crusted stages (Fig. 7, Table 4) while resolution between the Early and Late stages was less clear. The three-dimensional ordination of the samples showed a separation and grouping between Early and Late stages (Fig. 7).

3.4 Cyanobacterial tolerance to stockpiling

Cyanobacteria species richness in the stockpiles was affected by sampling depth (p=0.000), whereas stockpile age and its interaction with sampling depth did not exert any significant effect (p=0.378). As shown in Figure 8, species richness drastically decreased from 2 cm (5.11 ± 0.79) to 10 cm (2.22 ± 1.18). Then it fluctuated around 1.94 – 2.56 with no significant change between 10 and 50 centimetres (Fig. 8).

Five identifiable cyanobacterial genera were found in the stockpile soil samples: *Nostoc*, *Scytonema*, *Microcoleus*, *Porphyrosiphon* and *Leptolyngbya*. *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. *Nostoc sp.* (yellow) 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.

4.0 Discussion.

This research has demonstrated cyanobacteria to be a key component of soil microbial communities at J-A. These were compositionally diverse topsoil microbiomes that substantially contributed to the Myall-chenopod landscape. In this study cyanobacterial community richness and abundance were not related to soil and landform type and this was further supported through the sequenced samples where no significant differences were observed. At J-A any of the cyanobacteria could conceivably occur anywhere across the landscape. Their relative abundance was most probably determined by microenvironments and microhabitats such as light (sun and shade) and chemical gradients (Stal, 2003), as well as moisture availability (Büdel et al., 2018) and soil particle size (Büdel et al., 2009). We had hypothesised that cyanobacteria would be central to soil micro-processes, and this was strongly supported by extensive species richness and diversity values. The results have demonstrated how these micro-processes provide a strong foundation for the restoration of soil function. At J-A cyanobacteria contributed to soil structure and function during the early developmental stages of the biocrust. Photosynthesis

drove the productivity and growth of the biocrust that initiated carbon and nitrogen cycling and resulted in increases in soil nutrient concentrations right where vascular plants might use them.

Yet we found various cyanobacteria responded differently to high-level disturbance. In the first study of its kind we have shown there was no time-depth interactions over the different ages of the stockpiles. The greatest determination of species richness occurred at quite shallow depths with highly significant decreases in richness beyond 6 cm depth, even in the nine-month old stockpile. Nonetheless, the resilience of the individual cyanobacterial species to burial in topsoil stockpiles appeared good, however in an arid environment recolonization and community diversity could be impeded by drought (Williams and Büdel, 2012).

4.1 Cyanobacterial community structure

Overall, at J-A there was a rich cyanobacterial community comprised of twenty-one species 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). Surprisingly though, *Microcoleus* species that were recorded at J-A 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 necessarily play a dominant role in early stabilisation and colonisation of the soil. At J-A, *Symploca* and *Scytonema* appeared to be important as early colonising cyanobacteria in the biocrusts. These species have also been recorded as playing a key role in carbon sequestration in northern Australian cyanobacterial crusts (Büdel et al., 2018).

Elsewhere, multiple studies have demonstrated the high value of biocrust attributes as drivers of soil micro-processes that restore soil function (e.g. (Barger et al., 2016; Belnap and Eldridge, 2001; Bowker et al., 2014; Büdel et al., 2009; Chilton et al., 2017; Chiquoine et al., 2016; Weber et al., 2016). Similarly, in southwestern Queensland and northern Australia cyanobacterial species richness was strongly linked to increased bioavailable nitrogen and carbon uptake (Büdel et al., 2018; Williams et al., 2018; Williams and Eldridge, 2011). Environmentally induced strategies, which have developed over a long evolutionary history reflect their habitat. Cyanobacterial richness at J-A was determined according to their morphological features (e.g. outer protective sheaths, UV protection, EPS production) which in many cases provided the basis of attributes that pertained to fundamental survival strategies.

Filamentous cyanobacteria 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). 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). Important crust-building cyanobacteria in this study also appeared to be *Symploca* that was associated with EPS production, a principal feature of early colonising crust formation (Hu et al., 2002). 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) but share critical morphological features enabling biocrust formation and maintenance. Other key cyanobacteria indicative of biocrust formation and development were *Leptolyngbya*, *Phormidium*, *Tolypothrix*, *Nostoc*, *Brasilonema*, *Chroococcidiopsis* and *Acaryochloris*. Notably, the identification of *Brasilonema*, not been previously recorded in Australian soils, was supported with sequencing data. 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). 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*).

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). Nevertheless, it has now been documented that both *Nostoc commune* and *N. flagelliforme* are not separate species, rather the spaghetti-like tubes are unique to the ecotype and 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 stockpiling of topsoil. This type of mechanical disturbance results in burial and translocation of the biocrust. The survival of cyanobacteria following burial has rarely been studied and never been analysed in topsoil stockpiles. 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). In this study we have demonstrated that without further disturbance a range of cyanobacterial species survived stockpiling for over two years, an important feature

underpinning the concept of biobanking soil microbial communities. Yet we also discovered that species richness was significantly greater only in the shallow surfaces of the stockpiles (i.e. <6 cm) and that there were significant losses between 0–10 cm. The depletion appeared to be rapid in that it had already taken effect in the youngest stockpile (9 months) with were no further age effects.

In previous studies, cyanobacteria had been cultivated 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). *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 for a prolonged period of time albeit under optimum conditions. It is likely 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).

Taking into account the length of time it took for buried cyanobacteria to resurrect suggested they had regrown from vegetative material that had been inactive since the stockpiling process, which in turn resulted in long lag times for growth (Bristol, 1919; Lipman, 1941; Shaw et al., 2003). In addition, there may be the potential for photo-damage to occur as many sub-surface cyanobacteria would now be exposed in the topsoil removal and stockpiling process. This could also disrupt and slow down the recovery process once re-spread; this was observed in the laboratory following an out of season heat wave where many sub-surface species were trapped on the surface and died (W. Williams, unpublished data). In the context of rehabilitation, it is not practical to store stockpiles at very shallow depths due to the land area they would occupy. We have shown that under optimum laboratory conditions a diverse range of cyanobacteria recovered from a range of stockpile depths. It would be important to monitor the recovery of cyanobacteria from stockpiled soil to ascertain whether there is adequate inoculum to support the regrowth of biocrusts.

4.3 Limitations

As this was the first study of its kind, further investigation would be needed to determine what factors are influential in the decline of species richness following burial. We do not yet understand if there may be a critical time frame for holding the topsoil or if rainfall penetration into the stockpile results in the demise of cyanobacteria (possibly consumed by bacteria). On the other hand, in the longer term provided conditions are favourable, there may be adequate cyanobacterial survival, at least down to 50 cm, to recolonise the soil surfaces. Additionally, dust containing cyanobacteria from nearby locations might also be blown across the rehabilitated site, thereby facilitating recovery (e.g. Burrows et al. 2009; McKenna Neuman et al. 1996).

In this study the sampled buried cyanobacteria in the topsoil were provided optimum conditions of moisture, light and warmth in the laboratory. Under field conditions, rainfall deficiencies, especially drought, could be a limiting factor following the spreading of the topsoil during rehabilitation. This leads to the need for additional studies on both increased depth, younger stockpiles and areas of regeneration under natural weather conditions as to what time frame the biocrust recolonises. The role of bacteria in biocrust reestablishment should also be investigated further as they clearly exist as a poorly understood component of these microbial communities (Chilton et al. 2017; Zhang et al. 2016).

5.0 Conclusions

The Eucla Basin, in which J-A is located, is situated on the eastern edge of the Nullarbor Plain. Naturally occurring biocrusts occupy at least 45% of the landscape with a diverse community structure that contributes to landscape resilience and function. Cyanobacteria naturally occur within the first few millimetres of the surface of the biocrust where access to resources such as light and moisture are essential. Biocrusts contain cyanobacterial species such as *Phormidium* and *Leptolyngbya* that are regarded as early colonisers, and other species such as *Scytonema* and *Nostoc* that contribute bioavailable nitrogen to the nutrient cycle.

In these studies, we found that at J-A cyanobacteria were a diverse community that had a proven capacity as ecosystem engineers. Many of these cyanobacteria were early colonisers and were represented by a high proportion of filamentous and N-fixing species. Biocrust functionality is aided by a diverse cyanobacterial population and its connective properties via EPS where nutrients and resources can be exchanged. The destruction of structural integrity and fragmentation presents hurdles, however biocrust resilience, especially cyanobacteria, can over time consolidate and recolonise.

At J-A, rare earth mining processes require the removal of the topsoil, this soil is mechanically scraped off and relocated to topsoil stockpiles. These stockpiles act as a biobank in that they contain the biocrust microorganisms and are valuable eco resources. A major component of these biocrusts is cyanobacteria known for their capacity to survive extreme environments with the ability to remain in a desiccated state for long periods of time. Nevertheless, the stockpiling process destroys the macrostructure of the soil profile thus fragmenting and translocating the biocrusts throughout the stockpiles. In the process the burial of cyanobacteria at depth in soil stockpiles showed that species richness diminished substantially below the top few centimetres across all ages. Limited access to light for photosynthesis while buried can cause cyanobacterial death. Here, our results support the early return of topsoil stockpiles to facilitate the reestablishment of biocrusts and soil microbial community function. A direct-return process planned at J-A would improve outcomes by reducing the associated impacts of topsoil storage on key microbial communities such as cyanobacteria. Further research should focus on the (1) establishment of an optimum time for topsoil storage and, (2) the relationship of N-fixing cyanobacteria to seedling recruitment and plant establishment.

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. *SMU3 is the origin of the topsoil stockpile aged two years which was not included in the data analysis with SMU1–3 as there were only two samples. Different letters indicate significant differences in columns.

Vegetation	Soil	SMU	Site	pH	EC	N%	C%	C:N
Mallee	Deep calcareous yellow sand	1	4,9,10	8.6^{a}	92^{a}	0.07^{c}	1.11^{b}	$14.9^{a,b}$
Myall, Mallee, Chenopod	Shallow calcareous sandy loam	2	1,5,8	8.5^{a}	122^{a}	0.11^{a}	$1.40^{a,b}$	13.0^{b}
Myall	Deep calcareous sandy loam	3	2,3,7	8.5^{a}	113^{a}	0.09^{b}	1.61^{a}	17.3^{a}
Myall	Topsoil stockpile	Origin *SMU3	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, letters indicate significant differences

SMU	Means ± SD	SMU 1	SMU 2	SMU 3
SMU1	$^{b}2.79 \pm 1.41$	**0**	**0.001**	**0.005**
SMU2	$^{a}3.75 \pm 0.79$	**0.001**	**0**	NS
SMU3	$^{a}3.97 \pm 0.70$	**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. No significant difference in diversity measures was found between 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. A test for homogeneity of multivariate dispersions (PERMDISP) showed no significant differences in variation of spread of samples (pseudo *F*=3.8068, *P*(perm) = 0.068). Significant pair-wise differences are in bold.

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 the two-year-old stockpile (T2). 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 5
Aphanothece				
Brasilonema	x	x	x	x
Chroococcidiopsis		x		x
Chroococcus sp. 1	x	x	x	x
Gloeocapsa	x	x		
Leptolyngbya	x	x		
Microcoleus paludosus	x	x	x	x
Microcoleus vaginatus	x	x		
Nostoc commune		x	x	x
Nostoc flagelliforme		x		
Nostoc pruniforme		x		
Nostoc sp.	x	x		
Porphyrosiphon sp. 1	x	x	x	x
Porphyrosiphon sp. 2		x		
Schizothrix sp. 1		x		
Schizothrix sp. 2	x	x	x	x
Scytonema sp. 1	x	x	x	x
Scytonema sp. 2		x		
Scytonema sp. 3		x		
Symploca sp. 1	x	x	x	x
Symploca sp. 2		x		
Symplocastrum sp. 1	x	x	x	x
Species richness	**12**	**21**	**9**	**10**

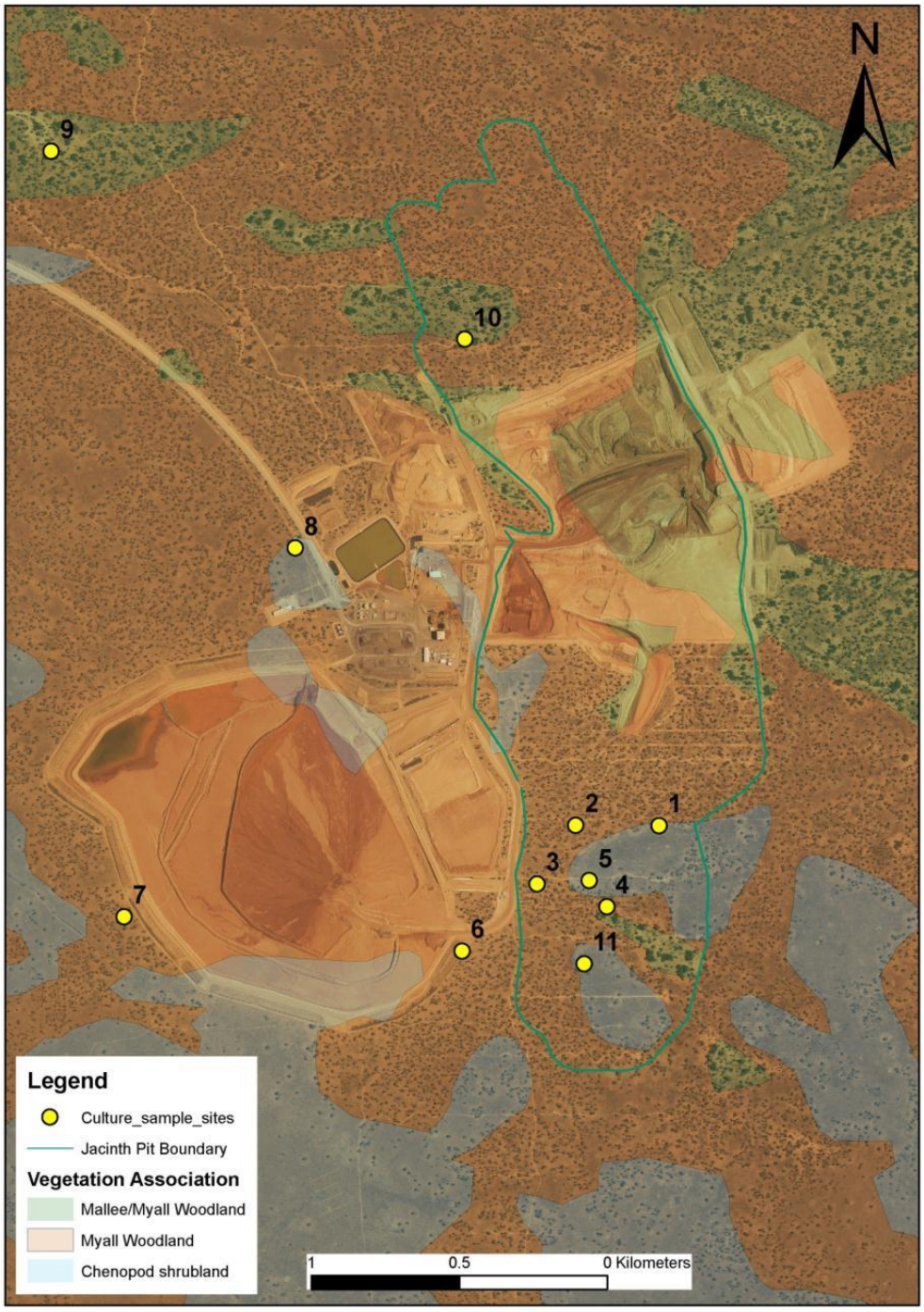


Figure 1: Image of biocrust sample sites located within the vegetation associations described in Table S1 (supplied, S. Doudle). Site 11 was initially investigated but later discarded as it was a fourth replicate of the Chenopod Shrubland.

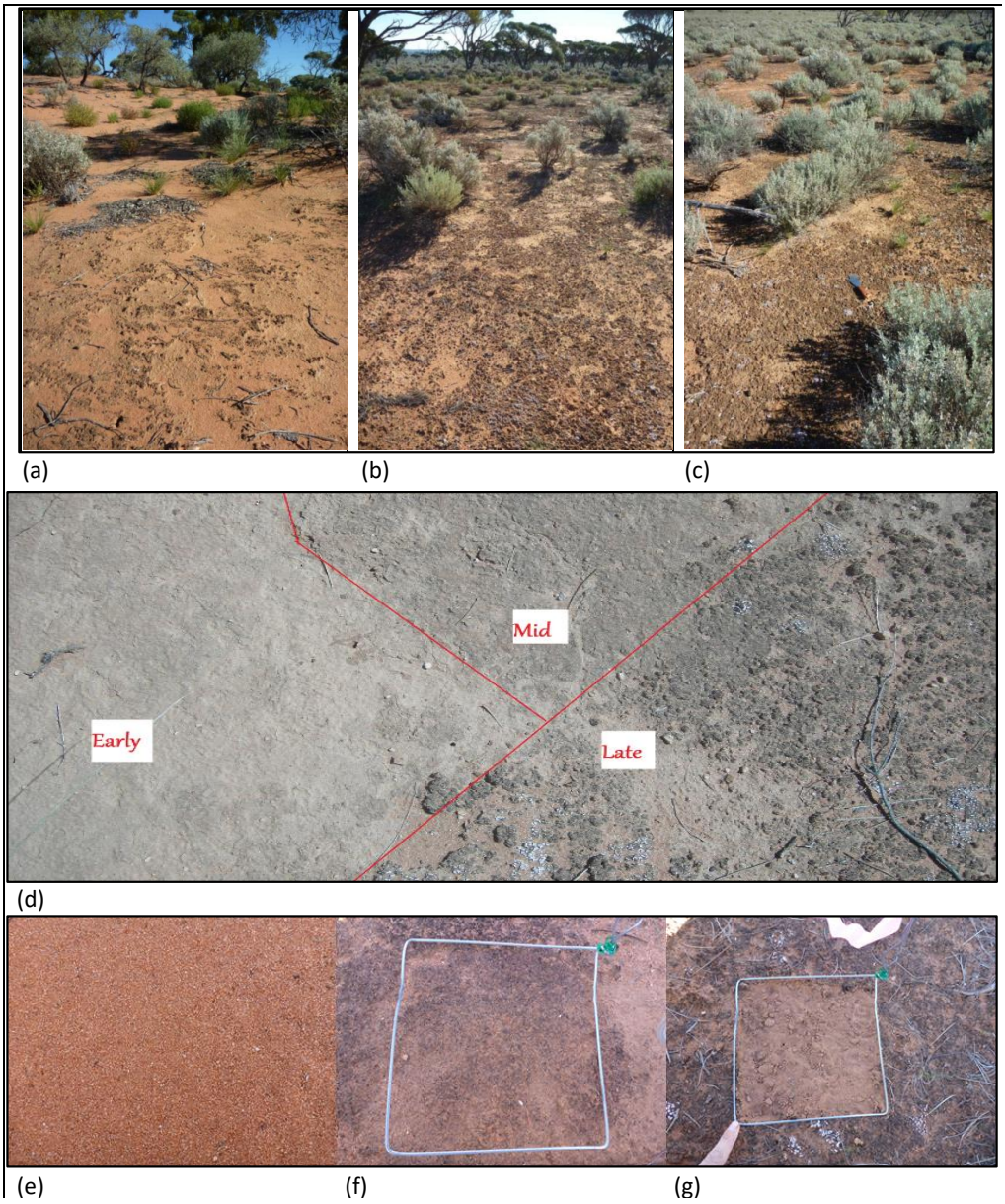


Figure 2: (a) SMU 1 (Sites 4, 9 and 10): Type 1–3 Biocrusts on deep calcareous yellow sands (dunes); (b) SMU 2 (Sites 1, 5 and 8): Primarily types 4 and 5 biocrusts on shallow calcareous sandy loam; (c) SMU 3 (Sites 2, 3 and 7): Types 1-5 biocrusts on deep calcareous sandy loam (Photographs by S. Doudle, 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).

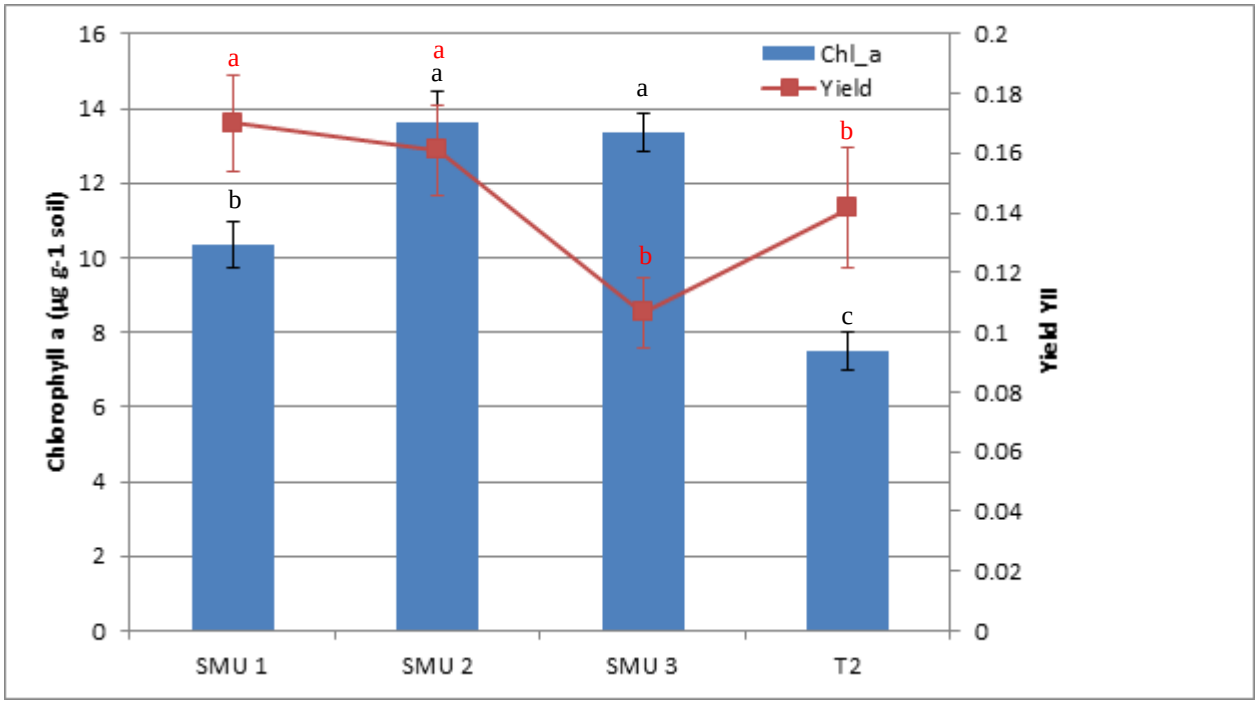


Figure 3: Chlorophyll *a* concentration (µg g^{-1} soil) following resurrection (2 weeks) after a natural desiccation (dry) period of 6 months. Soil Management Units: SMU 1–3 and T2 (two-year old topsoil stockpile) and Photosynthetic yield (YII) of photosystem II (PSII), with mean values and standard error of the mean (SEM). Different letters indicate significant differences between SMU.

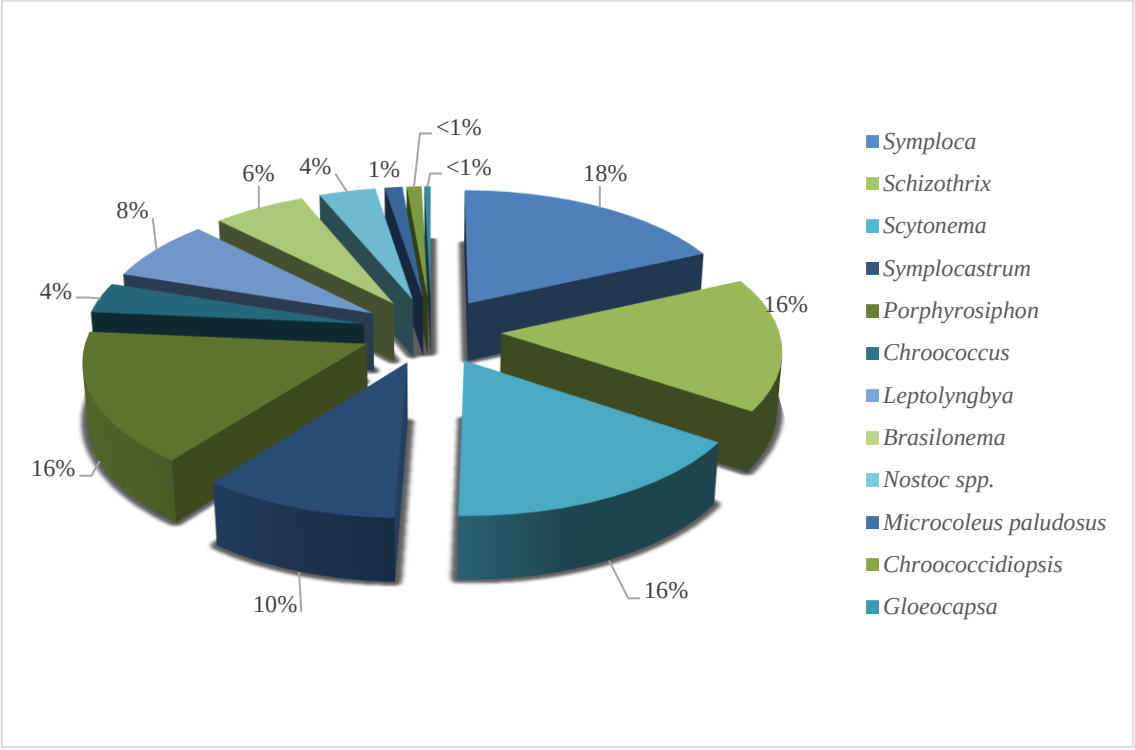


Figure 4: Cyanobacterial community structure across all sites expressed as a percentage of the total community based on mean richness and abundance scores. N-fixing cyanobacteria contributed to 61% of the community structure.

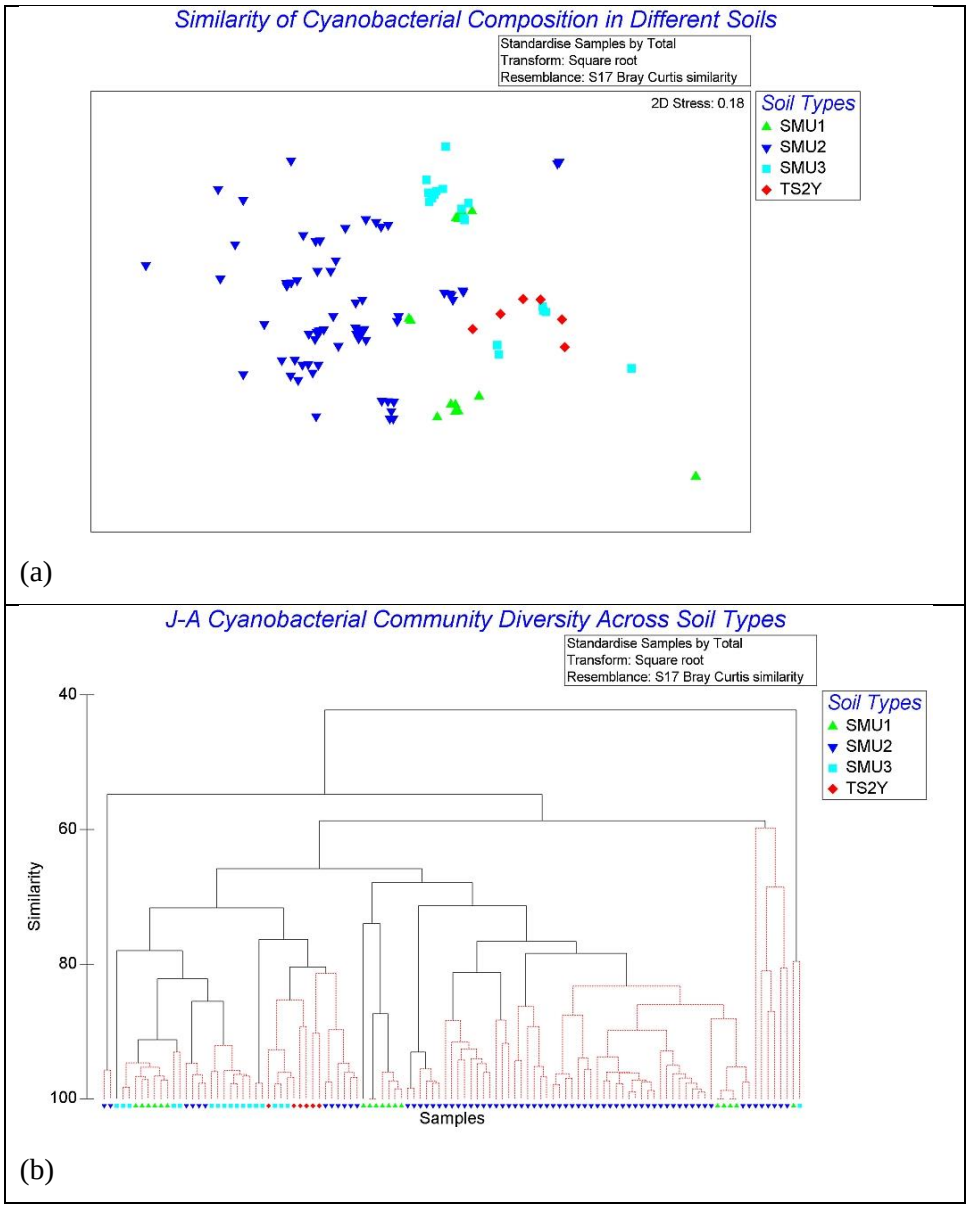
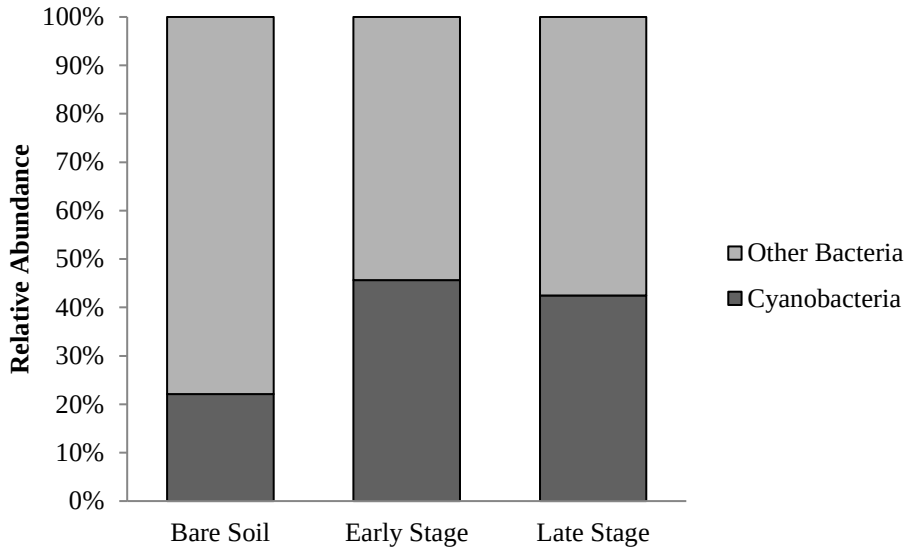
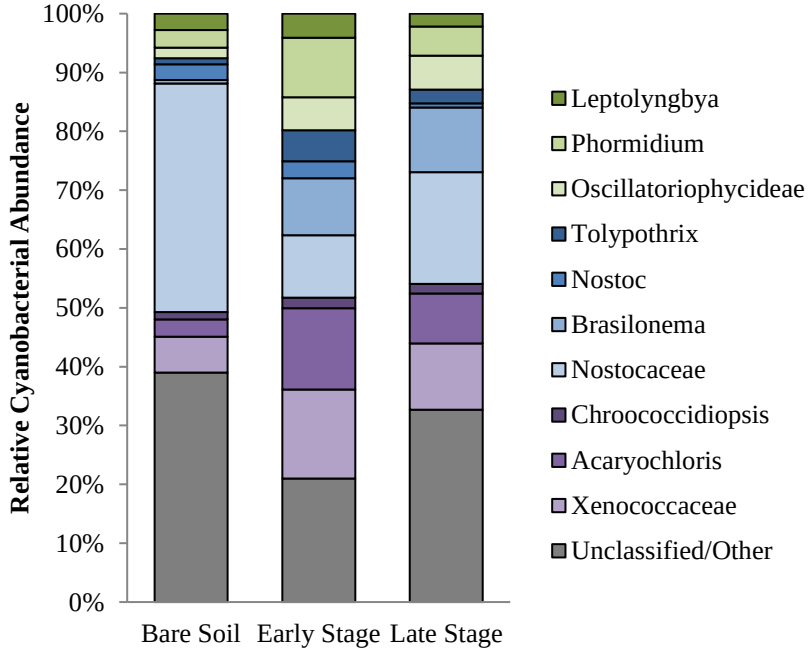


Figure 5(a): Cyanobacterial community structure based on indexed abundance and diversity across all sites displayed in an nMDS plot (Bray Curtis similarity). Soil management units represented by SMU 1–3 and two-year old topsoil stockpile (TS2Y) Figure 5(b): Similarities between samples within their SMU 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.



(a)



(b)

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.

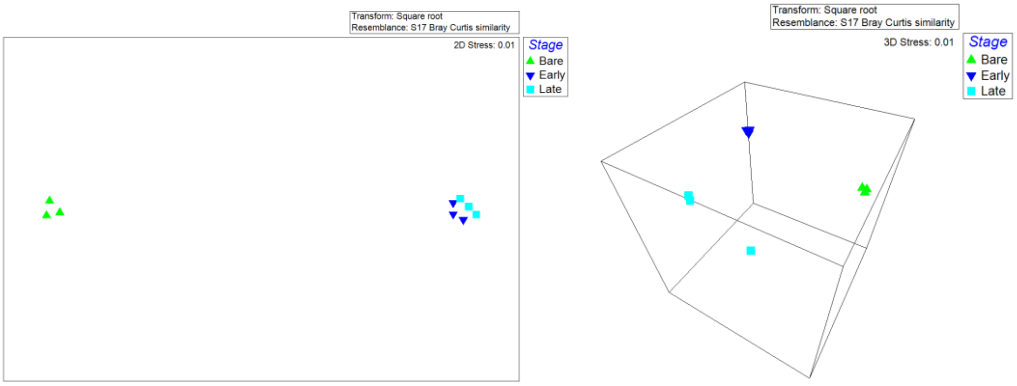


Figure 7: Non-metric Multidimensional Scaling of Bare soil and Early and Late Stage biocrusts within two dimensions (left) and three dimensions (right).

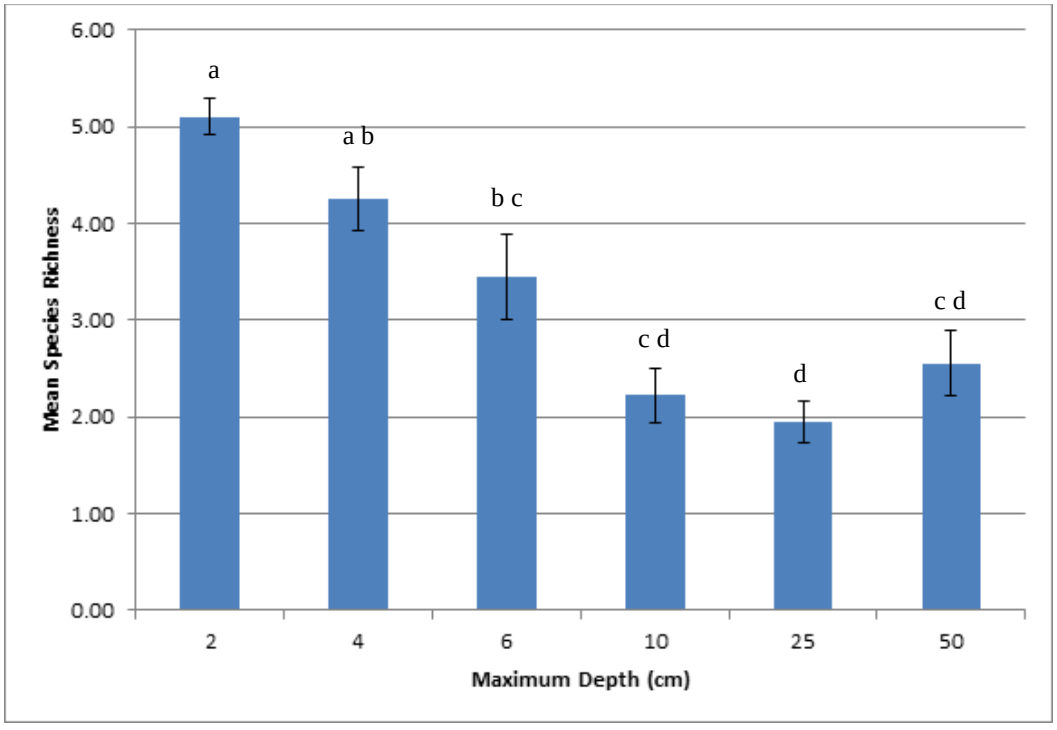


Figure 8: Mean number of cyanobacterial morphotypes with standard errors in topsoil stock piles. Different letters indicate significant differences between depths.

b

Acknowledgements

This project was funded and supported by Iluka Resources Ltd. and the Jacinth-Ambrosia Rehabilitation team. Thanks to Sam Doudle, Iluka Rehabilitation Specialist for her recognition of the importance of biocrusts at Jacinth-Ambrosia and for initiating this research program. This was a UniQuest project that was carried out at The University of Queensland's School of Agriculture and Food Sciences, Gatton Campus and the University of New South Wales. Especially appreciated was the strong support, project advice and laboratory resources provided by Assoc. Prof Vic Galea and Katherine Raymont. Special thanks to Dr Glenn McGregor for technical advice, assistance with cyanobacterial identification and the use of specialist microscopes and facilities at the Ecosciences Precinct (Qld), as well as report editing. Special thanks to David Tongway who assisted in Landscape Function Analysis field work, and two anonymous reviewers who provided valuable insights and editing to greatly improve this manuscript.

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