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Interactive comment on "Microbial Biobanking Cyanobacteria-rich topsoil facilitates mine rehabilitation" *by* Wendy Williams et al.

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Responses to Reviewer 1 We would like to thank Reviewer 1 for their recognition of the importance of this research. The suggestions provided by this thorough review has helped us improve the manuscript considerably. One of the difficulties in the preparation of the ms was the compilation of the work and the transformation of a mine report to a scientific paper. This explains a lot of generalisations especially in the discussion. I believe we have now addressed all your concerns in full. Comments and questions below: R1: Abstract needs more specificity; alter first sentence with focus on degradation; remove sentence from lines 21, 22 as it is not relevant to this study. Response: Abstract fully revised to include specific results and sentence removed. "Abstract Degradation from mining activities requires key solutions to complex issues where the removal or disturbance of topsoil incorporating soil microbial communities can result in a shift in ecosystem function. The 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 diverse biocrusts included a significant representation of cyanobacteria, lichens and mosses that inhabited nearly half of all soil surfaces. Cyanobacteria often dominate dryland soils and work as ecosystem engineers, in that they are in sufficiently large quantities to initiate biocrust establishment and facilitate soil surface stabilisation. This research encompassed soil microbial community profiling (using a polyphasic approach) with a focus on 'biobanking' topsoil for rehabilitation purposes. Biocrust successional stages were linked to soil types and formed the basis of the experimental design. Sequencing showed cyanobacteria were a significant component of all three successional stages. Microscopically, 21 cyanobacterial species were identified across the ten sites. Known nitrogen-fixing cyanobacteria Symploca, Scytonema, Porphyrosiphon, Brasilonema, Nostoc and Gloeocapsa comprised more than 50% of the diversity at each site and formed 61% of the total community diversity. There was no significant difference in cyanobacterial community structure across soil types which suggests that diversity and abundance is not controlled by soil type. Chlorophyll a concentrations sourced from the 2-year old topsoil stockpile was 7.49 μg g-1 soil, almost half the concentration of its source soil (13.53 μg g-1 soil). A total of nine cyanobacterial morphotypes were identified from the samples from nine stockpiles. Average morphotype richness was highest in stockpiled samples at and above 10 cm depth for all stockpile ages. Biocrust re-establishment during mining rehabilitation relies on the role of cyanobacteria as a means of early soil stabilisation. Ongoing monitoring of biocrust recovery is important as it provides an effective means of measuring important soil restoration processes." R1: Additional up-to-date references required in introduction, in particular p2, L10, include additional cyanobacterial restoration references Response: More recent references have been added to introduction (see edited ms below) and additional cyanobacterial restoration references have been added throughout. Paragraph incorporating P2, L10 now reads: "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 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 the chenopod shrublands at the edge of the Nullarbor Plain (South Australia) biocrusts cover 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)." R1: Use of word biofilm Response: I would partially disagree with R1 regarding the use of biofilm being more a less restricted to aquatic and marine environments. For example: Rossi and De Philippis (2015) specifically refer to the role of EPS in the creation of a biofilm in arid environments as the first step in biocrust establishment. I have revised its use (and context) and referenced it appropriately. Sentence incorporating biofilm in introduction now reads: "Cyanobacterial biofilms provide initial stabilisation of disturbed surfaces that pave the way for diverse microbial communities, and form bioactive crust-like layers integrated into the soil surface (e.g. Büdel et al., 2009; Rossi et al., 2017; Bowker et al., 2014). R1: define chlorophyll (type) measured (p2, L27) also in M&M section Response: Sentence now reads "EPS more than doubles the biocrusts compressive strength and increases cohesiveness by up to six times with a ratio of at least 2:1, EPS to chlorophyll a (Hu et al., 2002)." Response: see M&M revisions – chlorophyll a has been defined in all areas. In M&M main sentence now reads: "In order to define the cyanobacterial component of the biocrust, chlorophyll a pigmentation (unique to cyanobacteria) was measured following resurrection." R1: p2, L32 change 'was to were' Response: completed R1: "Research into. . .is rare" be more specific. Response: Refer to paragraph revised paragraph above that has included additional references and a more complete description of known research to date. R1: p3, Line 22 change plant-available to biological-available Response: completed R1: Clarify focus of work Response: We have removed site descriptions and background to start of methods and clarified the lead in paragraph to hypotheses to read as follows: "This project is based on designing mining rehabilitation plans that will achieve improved long-term outcomes. The restoration of landscape function and the accompanying need for the restoration of the soil ecosystem that included biocrusts after high-levels of disturbance directed the development of this biocrust research project. As cyanobacterial communities often develop their species richness, abundance and structure in response to their environment (e.g. Aboal et al., 2016; Büdel et al., 2009; Williams et al., 2014; Williams and Büdel, 2012), it was important to examine the biocrust community structure and survival. Within this design a polyphasic approach considers the essential ecosystem services provided through the

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reestablishment of biocrusts. The conceptual design is based on the net ecosystem benefits that must be achieved through biocrust regeneration. It follows that cyanobacterial inoculum in the topsoil stockpiles would be central to early stabilisation of mobile surfaces subjected to the potential impacts of wind and rain splash erosion. We sought to determine whether shallow 'biobanks' of cyanobacterial-enriched top soil would facilitate biocrust recovery of this mine site." R1: Methods need revision for clarity, specific points to address are addressed in responses below Responses: The methods have been completely revised into a clear sequence under revised headings as follows: 2.0 Methods 2.1 Background and site description 2.2 Field Methods 2.3 Ecophysiological properties of biocrust cyanobacteria 2.4 Cyanobacterial community structure 2.5 16S rDNA profiling of native undisturbed biocrust microbiomes 2.6 Cyanobacterial tolerance to stockpiling Specific points addressed below: R1: 'Fig 1 (11 sites) vs. 10 sites sampled' Response: Figure description now states that Site 11 was not used. R1: "natural samples vs. stockpile samples" Response: In the last paragraph of the introduction we had already clearly stated they were natural undisturbed samples versus stockpile samples that had been crushed and buried. "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." R1: 'Explain crust types' Response: We have added a description into the first section 'Site description and background' "The biocrusts at J-A had been previously classified into three primary successional stages representative of the five biocrust types found growing across the landscape (Doudle et al., 2011); Types 1–2: light coloured, thin cyanobacterial crust in early stages of development; Type 3: cyanobacterial crust, well established, intermediate stages of development; Types 4–5: biocrust, well established with cyanolichens and/or green algal lichens and mosses, late successional stage of development (additional descriptions available in supplementary Table S1). Study locations were selected

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from the main vegetation associations across the three soil types and Lake Ifould (a dry salt lake)." R1: 'p5, line 2 what type of tool?' Response: Inserted "Within each area 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." R1: 'p7, line 3 what type of chlorophyll and improve description' Response: Chlorophyll a defined throughout. Description of methods improved: "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 calculated using Wellburn's (1994) equations." R1: 'p6, lines 10-11 clearly explain use of penetrometer' Response: Description revised to read "A pocket penetrometer (8 mm foot) was used to determine the compressive strength (kg cm-2) of the dry intact biocrusts. Four measurements were taken from each sample location, providing 12 replicates per site. The measurement was taken at the point when the crust was broken, and the foot penetrated the crust surface." R1: 'Explain measurements of photosynthetic activity from what you did' Response: This has been revised to read "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. Allowing a short space of time between readings, this process was completed several times for each sample." R1: 'More detail in sequencing methods and was sequencing done for stockpile samples?' Response: Please note that sequencing was not used in stockpiles as unfortunately there was insufficient budget to cover

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this. Now the methods are rewritten this is clearer. Page 5, Line 11: Biocrusts were collected using a paint scraper and spatula which were wiped between each sample using 70% ethanol. Figure 3 shows how biocrusts were selected. More information on DNA library generation: Sentence updated to - 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. Clearer statistical analyses: Methods section has been re-structured. Each approach now has the specific statistical analyses used under that section. This section in the methods has been rewritten as follows: "2.3 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 3-5) 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. 3), (Chilton et al., 2017). Bare stage was defined by loose soil particles with no 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 cm2 plot with 95% coverage of the desired biocrust stage was excised to the depth of the crust and non-aggregated soil discarded (Fig. 4). 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

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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 non-metric multidimensional scaling plot (nMDS). 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." R1: 'Please provide the type of statistical analysis for each specific section' Response: see above and further descriptions added throughout revised methods (refer to main ms). Results R1: Please present your results step by step Response: The results section has been revised to reflect the methods section, main headings as follows: 3.0 Results 3.1 Ecophysiological properties of biocrust cyanobacteria 3.2 Cyanobacterial community structure 3.3 16S rDNA profiling of native undisturbed biocrust microbiomes 3.4 Cyanobacterial tolerance to stockpiling

R1: p8, line 24 recast sentence "in Table 1..." Response: this sentence has been removed as it was unnecessary. R1: p8, line 25 'what do you mean by ecologically significant...' Response: the word "ecologically" has been removed R1: p9 define chlorophyll type, add mean concentration (missing), surface area reporting preferred Response: chlorophyll a inserted, mean concentration added. In this case we reported in μg g-1 soil as we needed to compare with disturbed topsoil and topsoil stock piles. It was later used (ms in preparation) to define the concentrations per g soil to add in restoration trials. I understand that globally comparisons by surface area are easier however we were constrained by the requirements of the mine project. We do however have some earlier biomass area data done as part of an honours project that was carried out as a preliminary study that I will add to final ms revisions. R1: p9, line 6 – had T2 been introduced previously? Response: T2 had been defined in two figure descriptions "T2 = 2YO Topsoil stockpile originating from SMU 3" however in the script

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two-year has also been described as 2YO stockpile (see below) and further down has had (T2) added for clarity of reference between results and figures. R1: clarify sites in this section Response: The first sentence now reads "Soil pH across the three soil management units (SMUs) ranged from 8.4–8.6 while the two-year old (2YO) topsoil stockpile was higher at 8.9 (Table 1)." R1: p9, line 7 incorrect use of Levene's test, explain analysis Response: this was removed and correctly identified as not relevant, analysis described in revised methods R1: please state clearly what methods were used for each section i.e. community structure, stockpiles, were polyphasic approach used across all of these? Response: This has been clarified in revised methods and explained in previous section (sequencing was not used in stockpiles as unfortunately there was insufficient budget). R1: p9, line 19 'unicellular. . .' what genera does this refer to? Response: inserted "(e.g. Chroococcidiopsis, Acaryochloris, Xenococcaceae)." These are also identified in Figure 7. R1 p9, line 20 – explain differences in richness, evenness and diversity according to. . .. The methods section has been re-structured to better reflect which statistical analyses were used for which set of data. Please find the relevant sentence: "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." R1: p9, line 24-25 also 26-30 remove sentences, not relevant to results or stating what we know. Response: sentences removed R1: p10, lines 7-8 soil texture can influence community structure. . .not in results? Response: The results relate to soil types rather than textures and written as soil types (SMU 1-3, soil management units) which were previously determined by a soil consultant and described in detail in methods section 2.1 (see below) R1: p10, lines 8-9 ideas are opposite? Response: this sentence clarified R1: p10, line 16 'introduce soil textures, please do it before. . .' Response: Soil management units are now fully described in Methods Section 2.1 "The landscape has been characterised into three distinct soil types associated with vegetation communities (Table 1) called soil management units: SMU1 – deep calcareous yellow sands associated with dune ridges; SMU2 – shallow calcareous sandy loams and, SMU3 –

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deep calcareous sandy loam (Goode, 2009; Doudle et al., 2011)." "The landscape has been characterised into three distinct soil types associated with vegetation communities (Table 1) called soil management units: SMU1 – deep calcareous yellow sands associated with dune ridges; SMU2 – shallow calcareous sandy loams and, SMU3 – deep calcareous sandy loam (Goode, 2009; Doudle et al., 2011)." R1: Section 3.5 'too many numbers in community structure data' Response: This section has been refined as follows: 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. S4). 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. S5). 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 (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 S6). Cyanobacteria with the capacity to fix nitrogen contributed to 77% of the community structure. Cyanobacterial crusts from Site 6 were from the 2YO 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. S7). 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. R1: p11, line 1 2YO topsoil acronym query Response: this has been addressed early in results (see above) R1: p11, line 13 'you did not sample Stigonema. . .' Response: This paragraph has been recast to read: "Examination of stockpile soil samples via microscopy revealed five cyanobacterial morphotypes corresponding with the genera: Nostoc, Scytonema, Microcoleus, Porphyrosiphon and Leptolyngbya (Fig. 17). Average morphotype richness was highest in stockpiled samples at and above 10 cm depth for all stockpile ages (Figs. 13, 14)." R1: p12, line 1 'area mentioned. . ..where has it been introduced previously..' Response: In methods area has been described as 'area of coverage' – "To determine cyanobacterial growth rates 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." Subsequently, the references to the word area in results were changed to cover or coverage to better reflect the description in the methods. R1: p12, lines 19-20 remove sentence Response: Sentence removed R1: p12, lines 21-22 use of the term growth rates Response: above mentioned sentence highlighted refers to growth that was measured over time (via area of coverage). Discussion R1: Please rewrite Response: The discussion has been rewritten with the useful comments by R1 incorporated. The full discussion is added below: 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 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 known as 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 extensive biocrusts present. We had hypothesised that cyanobacteria would be central to soil micro-processes and this was strongly supported by high species diversity. In this study cyanobacterial community diversity and abundance was not related to soil type. 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, 2000), as well as moisture availability and soil particle size (Büdel et al. 2009). Cyanobacterial community richness and abundance were not affected by soil type as shown by the similarity between early stage (SMU 1) and late stage (SMU 3), further supported though the sequenced samples where there were no significant differences. Contrary to our hypothesis there was not always a clear distinction in community structure between soil types and biocrust successional stages, notably in the early and late stages. 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. 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 drought (Williams and Büdel, 2012) 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 recorded in Australian soils. Simple filamentous types are often attributed with the primary crust building role, able

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to span inter-particle gaps within the soil via supra-cellular structures (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) but share critical morphological features enabling biocrust formation and maintenance.

The cyanobacterial richness at J-A was determined according to their morphological features. In many cases these features (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 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. 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 has been recorded as playing

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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, and due to its similar morphological attributes was called Brasilonema in this study (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 and the Northern Territory (Skinner and Entwisle, 2002) and Victoria (W. Williams, unpublished data). Due to its scarcity N. flagelliforme is of high commercial value in Asia where it is considered a delicacy (Gao, 1998). 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). 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. Only a few studies have recorded the impacts of burial within the natural environment because of drought as well as under artificially reconstructed burial trials. A study based in China showed that 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, 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). The findings of this study show that 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). The species sampled at J-A have a well 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 10cm depth for several months. It may be that the longer the period of inactivity, the longer time taken for reactivation to occur. Akinetes are desiccation resistant cells produced by certain filamentous cyanobacteria that can survive for long periods. Nostoc and Scytonema can 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 growth 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 veg-

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etative 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.

5.0 Conclusions Biocrusts and cyanobacteria are a major component of the J-A landscape that protect and enhance soil function. These studies focused on the cyanobacterial community structure at J-A and its recovery following topsoil stockpiling. It was apparent that the top few centimetres of the stockpiles were the most reactive to cyanobacterial regeneration. As even low-profile stockpiles are in the vicinity of 2 m additional time would be needed for re-establishment of biocrusts which would likely be dependent on rainfall. It follows that the timing of rehabilitation would be important so as to take advantage of favourable climatic conditions. Cyanobacteria are well adapted to long periods without water, the optimisation of short growing seasons, wet-dry cycles, low water potentials, tolerance of high UV and low light intensities, fluctuating temperatures and in some cases high salinity. Cyanobacterial strategies central to survival include EPS production, spectral adaptation, nitrogen fixation and motility. Biocrust re-establishment during mining rehabilitation relies on the role of cyanobacteria as a means of early soil stabilisation. Provided there is adequate cyanobacterial inoculum in the topsoil stockpiles their growth and the subsequent crust formation should take place largely unassisted. Ongoing monitoring of biocrust recovery is important as it provides an effective means of measuring important soil restoration processes. Detecting increases in key species and shifts of community structure will likely provide more informative and robust verification of desired rehabilitation outcomes. Cyanobacterial species richness is an important measure of biocrusts that incorporate micro-processes central to a healthy and functional soil ecosystem. Increased cyanobacterial biomass is likely to also be a good indicator and reliable metric. Diversity indices derived from sequencing data of the whole bacterial community are poor measures of biocrust formation and development.

Tables and Figures: All corections have been made including: Table 4: Table heading

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updated to include dependent variable “Table 4: Permutational analysis of variance (PERMANOVA) of pair-wise comparisons of Bray-Curtis dissimilarity between biocrust stages and bare soil” Figure 6: Axis added Figure 7: Graph transformed to relative abundance

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