ILUKA

Jacinth Ambrosia

Research and Monitoring Summary

2014 - 2015

DOCUMENT CONTROL

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1 Introduction

Iluka Resource Ltd are pleased to present the progress report of the 2014 and 2015 Jacinth Ambrosia (J-A) research and monitoring programs. This report is supplemental to the J-A Annual Compliance Report (2015), which is submitted annually in March to the Department of State Development (DSD).

In their efforts to understand how the environment at J-A recovers from disturbance, the rehabilitation staff at J-A continue to work in collaboration with, among others, the University of Adelaide and the Adelaide Botanic Gardens. Knowledge gained from the research collaborations and on-site monitoring program feeds into the on-going rehabilitation activities at J-A enabling a continuous improvement process.

2 Site Characteristics

The J-A mineral sand mine is made up of two deposits within the Eucla Basin region of South Australia. The site is located approximately 200 km north-west of Ceduna and is located within the Yellabina Regional Reserve (Figure 1) which is currently managed for the conservation of wildlife and natural features while still permitting use of the natural resources of the land (mixed use reserve).

J-A is located within an arid environment and is dominated by chenopod shrubland plains and open myall woodlands interspersed with myall/mallee sand rises and creeks. The plant interspaces are well stabilised with thick and diverse biological soil crusts.

The long term annual mean rainfall from the nearby Tarcoola weather station is approximately 174 mm. Average monthly rainfalls are consistent across all months however more frequent and less intense rain events are experienced in winter months and less frequent, more intense rain events can occur in summer.

Mining at Jacinth commenced with pre-stripping of vegetation, topsoil and overburden in September 2009 with processing of ore commencing in November 2009. Mining at Jacinth is expected to continue into 2023 and will not commence at Ambrosia until approximately 2020.

As at December 2015, a total area of approximately 755 ha has been disturbed as part of mining operations at Jacinth. Progressive rehabilitation of mined areas commenced in 2013 and will progress with the life of the mine, with major infrastructure areas being rehabilitated at mine closure. To date approx. 22 ha has been rehabilitated.

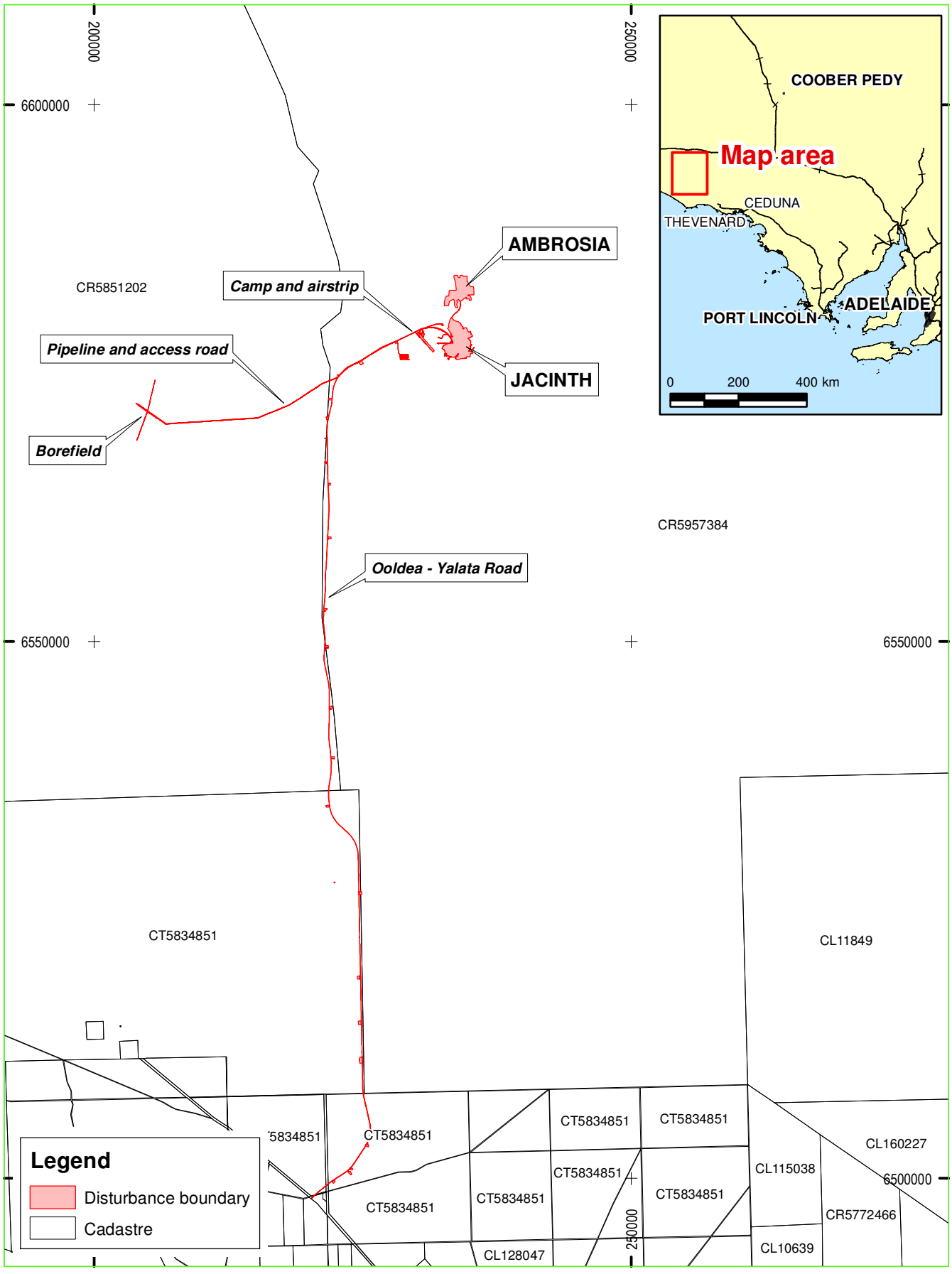
This progress report outlines some of the research and monitoring activities Iluka Resources Ltd are undertaking to enable interim closure criteria to be further developed and negotiated with the provision of relevant scientific data.

3 Alignment to Closure Criteria

The research and monitoring programs at J-A are designed to demonstrate compliance with the lease conditions and associated outcome and completion criteria approved by DSD.

The outcome and completion criteria, detailed in the J-A Mine Closure Plan, were developed prior to the commencement of mining, as such, the research and monitoring programs also serve to improve the relevance and empirical scientific basis for on-going review and refinement of the criteria where required.

The alignment of each project in the J-A research and monitoring program to the closure criteria is provided in Table 1.



JACINTH - AMBROSIA

LOCATION PLAN

ILUKA

ORIG: J.Williams DRAWN: D.G.S. SCALE: 1:500,000 (A4) DATE: 21 Jan. 2015 DWG No: 227256 ver.00

FIGURE: 1

Table 1 Research and monitoring program alignment with PEPR closure criteria

Outcome	Measurement Criteria	Relevant Section
Public safety		
No public injuries or deaths resulting from mine operations traffic or unauthorised access that could have been reasonably prevented.	All plant, equipment and mine related infrastructure has been removed from site. All retained infrastructure safe and stable (e.g. borefield pipeline)	Information related to on-going (operational) management of public safety is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of public safety will be developed 12 months prior to site closure.
Cultural heritage		
No disturbance to aboriginal artifacts or sites of significance unless prior approval under the relevant legislation is gained.	Demonstrate that no disturbance has occurred in areas for which heritage clearance approval has not been gained (output from GIS). Prior to lease handover, all heritage sites restored to pre-mining vegetation associations, and all artifacts restored to original position (or as agreed with FWCAC)	Information related to on-going (operational) management of cultural heritage is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of cultural heritage will be developed 12 months prior to site closure.
Waste management		
No demolition, industrial or solid wastes disposed of within rehabilitated site	Audit and inspection records demonstrate waste correctly stored and managed in accordance with Waste Management Plan Demolition or industrial wastes disposed of at appropriately licensed facility outside the project area	Information related to on-going (operational) management of waste materials is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of waste materials will be developed 12 months prior to site closure.

Outcome	Measurement Criteria	Relevant Section
Groundwater		
The extraction and use of groundwater does not adversely affect environmental processes or beneficial users that are reliant on that groundwater system.	Aquifer total drawdown is equal to or better than original model (per C37) predictions at mine closure. J-A regional groundwater model indicates that mine-site groundwater levels at mine closure are trending towards pre-mining baseline levels.	Information related to on-going (operational) management ground water is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of ground water will be developed 12 months prior to site closure.
Visual amenity		
The reconstructed landform is consistent with surrounding topography	No point in the rehabilitated landscape greater than 177 mAHD (+1 m of the highest designed mAHD) for domain 4A (Jacinth Pit). No point in the rehabilitated landscape less than 124 mAHD (the lowest designed mAHD) for domain 4A (Jacinth Pit). No point in the rehabilitated landscape greater than 160 mAHD (+1 m of the highest designed mAHD) for domain 4B (Ambrosia Pit). No point in the rehabilitated landscape less than 118 mAHD (the lowest designed mAHD) for domain 4B (Ambrosia Pit). No point in the rehabilitated landscape greater than 181 mAHD (+1 m of the highest designed mAHD) for domain 4C (Offpath TSF).	Information related to the rehabilitation soils profile is provided in the JA Annual Compliance Report. Final as-built designs of rehabilitated areas can be provided on request. Future JARMS will include reports and final as built designs for all rehabilitated areas for that period.

Outcome	Measurement Criteria	Relevant Section
Surface water		
Post mining ecosystem and landscape function is resilient, self-sustaining and indicating that the pre-mining ecosystem and landscape function will ultimately be achieved	Erosion rates of rehabilitated watercourses are comparable with upstream control sites Water quality in rehabilitated creeks comparable with upstream control sites	Appendix 15 Long-term watercourse monitoring activity report 2014 – 2015
Soil		
Soil profile and function is restored and capable of supporting agreed land use	Soil profile has been restored in accordance with Table 29 Indicative soil profile (3.10.4).	Appendix 7 Jacinth Ambrosia Stockpile Monitoring Report 2015 Appendix 9 Jacinth Ambrosia Dune and Creek Soil Characterisation Appendix 10 Jacinth Ambrosia Overburden Soil Balance 2015 Appendix 14 Changes to topsoil and subsoil stripping depth (2015) Additional information related to the rehabilitation soils profile is provided in the JA Annual Compliance Report
	No soil contamination in areas used for storage and handling of hazardous materials (as per NEPM).	No programs have been established relevant to this matter. Information related to on-going (operational) hazardous materials management is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of hazardous materials and potential contamination will be developed 12 months prior to site closure.

Outcome	Measurement Criteria	Relevant Section
	Surface radiation on the rehabilitated areas is consistent with pre-mining levels.	No programs have been established relevant to this matter. Information related to on-going (operational) radiation management is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of radiation will be developed 12 months prior to site closure.
	No salinisation of rehabilitated soil profile due to capillary rise	Information related to on-going (operational) management and monitoring of ground water mounding is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of ground water will be developed 12 months prior to site closure.
	Surface site contamination (salinity) does not exceed control site conditions.	No programs have been established relevant to this matter. Program to be developed 12 months prior to site closure. Closure programs for the management and monitoring of surface salinisation will be developed 12 months prior to site closure.
	Biological soil crust (minimum age class 1) as described in *Field guide for landscape function analysis for environmental monitoring and assessment, Minerals Regulatory Guidelines* (DMITRE, 2013)	Appendix 5 Landscape Function Analysis Appendix 10 Jacinth Ambrosia Overburden Soil Balance 2015 Appendix 11 Jacinth Ambrosia Topsoil Farm 2015
	Fugitive dust emissions from the rehabilitated landscape are consistent with control sites	Annual fugitive dust data is provided in Appendix 1 Jacinth Ambrosia Vegetation and Dust Monitoring Closure programs for the management and monitoring of ground water will be developed 12 months prior to site closure.

Outcome	Measurement Criteria	Relevant Section
Native vegetation		
Post mining ecosystem and landscape function is resilient, self-sustaining and indicating that the pre-mining ecosystem and landscape function will ultimately be achieved	Landscape Function Analysis indicates that rehabilitated systems are trending towards pre-disturbance landscape function based on comparison with control sites.	Appendix 1 Jacinth Ambrosia Vegetation and Dust Monitoring Appendix 2 Effects of groundwater mounding on native vegetation Appendix 3 Reduced Plant Establishment in Bay 1 of Cell 1 West – Investigation and Actions Appendix 4 Cell 1 rehabilitation trials 2014 – 2015 Appendix 6 Jacinth Ambrosia Photo Point Monitoring 2015 Appendix 8 Jacinth-Ambrosia Native Vegetation Seed Store Activity Report 2014 – 2015 Appendix 12 Progress report for soil sample analysis Appendix 13 Do plants grown in stockpiled soil for mine-site revegetation form arbuscular mycorrhizas? Appendix 16 Growth of tree seedlings in post mining reconstructed soils in an arid region of South Australia

Outcome	Measurement Criteria	Relevant Section
Pest species		
No introduction of new weeds or plant pathogens, nor increase in abundance of existing weed species in the lease area and adjacent areas caused by mining operations.	Weed species diversity and abundance at closure to be consistent with control sites.	Information related to on-going (operational) pest management is provided in the JA Annual Compliance Report. Closure programs for the management and monitoring of pest species will be developed 12 months prior to site closure.
No increase in abundance of pest animal species in the lease area and adjacent areas caused by mining operations.	Pest animal abundance at closure to be consistent with control sites.	

4 Research and Monitoring Summary

The vegetation monitoring results for 2015 generally reflect the warmer and drier climatic conditions in 2015. There was an increase in the proportion of plants in both the groundwater and dust monitoring transects showing signs of dieback close to mine operations. There was a decline in *M sedifolia* (pearl bluebush) health within 50 m of the mine footprint, potentially related to a reduction in the new growth of leaves for these plants (Appendix 1). Reduced growth may be a response to the low rainfall and monitoring of these plants will continue. Similarly, plants monitored for response to mounding groundwater showed a decline in health at impact and non-impact sites, although individuals at impact sites presented with higher rates of dieback than individuals at non-impact sites. It is possible that these individuals are responding to the additional stresses of being close to the mine site, i.e. groundwater mounding or the cumulative impacts of a range of environmental variables. Longer term monitoring will assist in identifying the cause of decline and the ability of individuals to recover (Appendix 2).

Rehabilitation sites also appeared to respond to the low and late winter rains in 2015. Sections of the latest rehabilitation area had very little germination in comparison to other rehabilitated areas and additional investigations were carried out to determine the likely cause (Appendix 3). The likely cause was late topsoil preparation and therefore a lack of rainfall exposure. Anecdotally this is further supported by the considerable increase in germination in 2016 following high summer rainfall events. All other rehabilitation sites showed an increase in vegetation density and diversity over time (Appendix 4) and Landscape Function Analysis showed rehabilitated landforms similar or trending towards analogue ecosystem function (Appendix 5) indicating closure criteria will be achieved. Rehabilitation photo point monitoring plates are provided in Appendix 6.

The results of the rehabilitation trials and the ongoing stockpile monitoring program (Appendix 7) have provided opportunities for identification of potential recalcitrant species. This information together with a gap analysis carried out on the JA seed lab have indicated a number of species to be the focus of future seed collection programs (Appendix 8), i.e. longer lived deep rooted species and some smaller annual species. Generally, species identified as absent from rehabilitation sites are the longer lived deep rooted shrub species and the seed viability for annual species degrades rapidly in the seed lab. Of particular interest is the long lived *M sedifolia*, the dominant species in the mallee and chenopod vegetation associations. Seed set in the *M sedifolia* is rare and unreliable, and much of the seed collected has been unviable. In 2016 opportunities to collect seed from the broader Eyre Peninsula area will be investigated in parallel to viability and germination trials.

A number of research programs have resulted in a change to the way in which clean overburden materials are stripped and returned. Previous to 2015 a 2 m sand layer was required in the rehabilitated soil profile. This commitment was based on limited pre-mine data and it was later identified that the volumes of material required to achieve that commitment was not available as the sand layer in the surrounding environment was not a uniform 2 m, present in a much more discrete spatial arrangement than originally mapped. The depth of sand in dune and creek systems was investigated in 2014 (Appendix 9) and it was determined that the depth of sand varied and for dune systems was limited to the dune

boundary. It was therefore determined that the depth of clean sand overburden in the rehabilitation soil profile should vary according to the volume of sand that could be excavated from the undisturbed soil during mining. Designs for rehabilitated creeks and dunes will be prepared in the rehabilitation planning phase.

The identification of a clean overburden deficit for rehabilitation resulted in investigations to manage and mitigate impacts to rehabilitation outcomes. The soil balance carried out in 2015 (Appendix 10) identified a deficit of red loam, brown loam and subsoil. The deficit of brown loam and red loam has been investigated and controls implemented, namely changes to the soil profile, rehabilitated vegetation associations and changes to the landform design (see JA Program for Environmental Protection and Rehabilitation (PEPR) for further discussion). Changes to the soil profile were approved by DSD in December 2015. It was identified that 57% of the subsoil required for rehabilitation was available at 31 December 2015. Topsoil was available in sufficient volumes, however historically a deficit has been identified. A number of options have been considered to reduce the deficit of subsoil and deficit of topsoil however studies investigating biological soil crusts (Appendix 11), soil seed bank (Appendix 12) and mycorrhiza (Appendix 13) with depth identified that an increase in stripping depth would be a suitable mitigating measure (Appendix 14).

The watercourses at JA are ephemeral in nature and only flow in response to heavy rainfall events. A long term water course monitoring program was established in 2015 (Appendix 15). The purpose of the monitoring is to provide baseline turbidity, erosion and weed data for comparison with rehabilitated watercourses and downstream water courses. Monitoring will be carried out after high rainfall events and on a biennial basis.

Studies carried out as part of the JA ARC Linkage program continued in 2014 and 2015. The program is a partnership between Iluka and the University of Adelaide, partially funded by the Australian Research Council. The purpose of the program is to determine how interactions between the roots of deep rooted vegetation species and the saline tails could impact on plant growth and therefore rehabilitation outcomes. In 2014 studies investigating the effect of tails on seedling emergence and growth indicated that for the deeper rooted trees, *Acacia papyrocarpa* (western myall), *Eucalyptus oleosa* and *E gracilis* germination and root development is not inhibited by tails as a growth media, however seedling growth can be overall reduced (Appendix 16). It is anticipated that the final program report will be provided to Iluka in 2016.

Appendix 1 Jacinth Ambrosia Vegetation and Dust Monitoring

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Jacinth Ambrosia Vegetation and Dust Monitoring

2015

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1 Introduction

Prior to mining disturbance, the topsoils of Jacinth – Ambrosia (J-A) mine were stabilised by a combination of vegetation and biological soil crusts (SWC 2008, Doudle 2010). As a result, wind erosion from undisturbed soils in the area was considered to be very low. Observed dust sources in high wind events in the undisturbed landscape include new fire scars, rabbit and wombat burrows and the surface of Lake Ifould. However, no baseline depositional dust data was collected prior to project operations.

The soil profile comprises five main soil types prior to ore exposure; topsoil, subsoil, sand (if in a dune landscape), brown loam and red loam. The first four are calcareous and alkaline whereas red loam is non-calcareous and has a lower pH (SWC 2008). All of these soil types are prone to wind erosion following disturbance events such as vegetation clearance, overburden removal, soil stockpiling and rehabilitation activities. The high grade heavy mineral (HM) ore mainly occurs in the Ooldea sand beneath the red loam, however economic quantities are also extracted from the red and brown loam. After removing the HM via processing with hypersaline water, the tailings mass is composed of highly saline red and brown loam and Ooldea sand. The tailings are currently returned directly to the previous mining pit cell, however for the first 2 years of the project the tailings reported to an off path Tailing Storage Facility (TSF).

The tailings drain rapidly and the resulting dry surface is highly prone to wind erosion. During high wind events, the finer fraction from the tailings has been observed to move from the TSF into the surrounding environment and the coarser fraction moves across the TSF surface (S Doudle, observations 2009-2011. JARMS 2011, The Effectiveness of Hypersaline 'Slimes' for Wind Erosion Management at the J-A Mine).

The impact of high loads of fugitive dust from the mine operations on plant species in the local area is not clear. Observations have identified particular plant species that are excellent visual indicators of dust presence. The leaves of each of these indicator species bear arrangements of fine hairs that sometimes extend to the stems which can trap dust. Build-up of dust on the leaves may result in reduced plant health, impacting on reproduction and potentially causing plant death. An additional pathway for plant death is due to burial by fugitive dust, particularly close to the mine footprint where higher dust loads are anticipated and for small forbs and ground covers.

It is a requirement of the Jacinth Ambrosia (J-A) Program for Environmental Protection and Rehabilitation (PEPR) that all project related vegetation clearance is approved by Department for State Development (DSD), as Native Vegetation Council (NVC) delegate, in accordance with the Native Vegetation Act 1991:

4. The licensee must, in constructing and operating the license, ensure that all clearance of native vegetation is authorised under appropriate legislation (Mining Lease Conditions, Second Schedule).

Where direct clearance of native vegetation is identified, offsets are identified in the form of a Significant Environmental Benefit (SEB) in accordance with the Guidelines for a Native Vegetation Significant Environmental Benefit Policy for the clearance of native vegetation associated with the minerals and petroleum industry (September 2005). The J-A Native Vegetation Management Plan (NVMP) outlines the SEB requirements for the J-A project related clearances. However the current SEB does not allow for any vegetation loss other than that resulting from direct clearances for project activities. To monitor and manage additional vegetation clearance outside of approved areas Iluka have committed to environmental outcomes (identified in the PEPR).

2 Methods

2.1 Pearl Bluebush Surveys

Pearl bluebush (*Maireana sedifolia*) has been identified as the most appropriate species to monitor as it is one of the most widely distributed species at J-A. The leaves and stems of this species are covered with a thick layer of fine hairs, referred to as trichomes (Figure 1). Dust is readily trapped in these in hairs. Whilst dust carried on light to medium winds appears to be deposited on top of these hairs and readily washed off during rainfall events, the dust from heavy wind events appears to penetrate the hairy layer and is captured and held by the plant (S Doudle, observations 2009-2011, Figure 1). The response of pearl bluebush to high dust loads is not known.

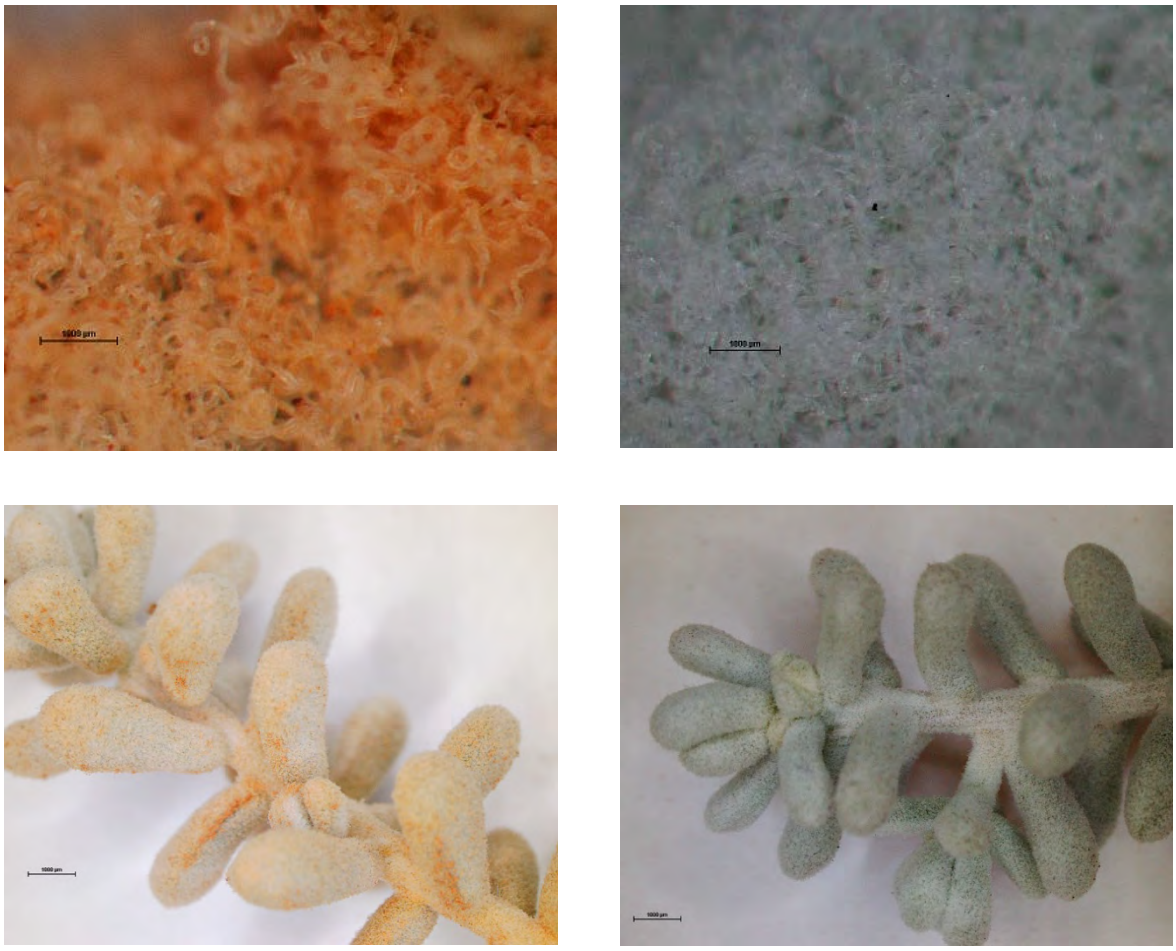


Figure 1 Hairs of Pearl Bluebush coated with dust on the left and without on the right at 80x magnification above and 8x magnification below

A set of six transects were established by the J-A rehabilitation team in June 2011 in locations adjacent to a range of different dust sources at the J-A mine. Transects extend north, south, east and west, away from the source of the dust. Only Pearl Bluebush was chosen for plant health measurements due to its ability to trap and hold dust, thereby clearly indicating the presence or absence of dust along these transects.

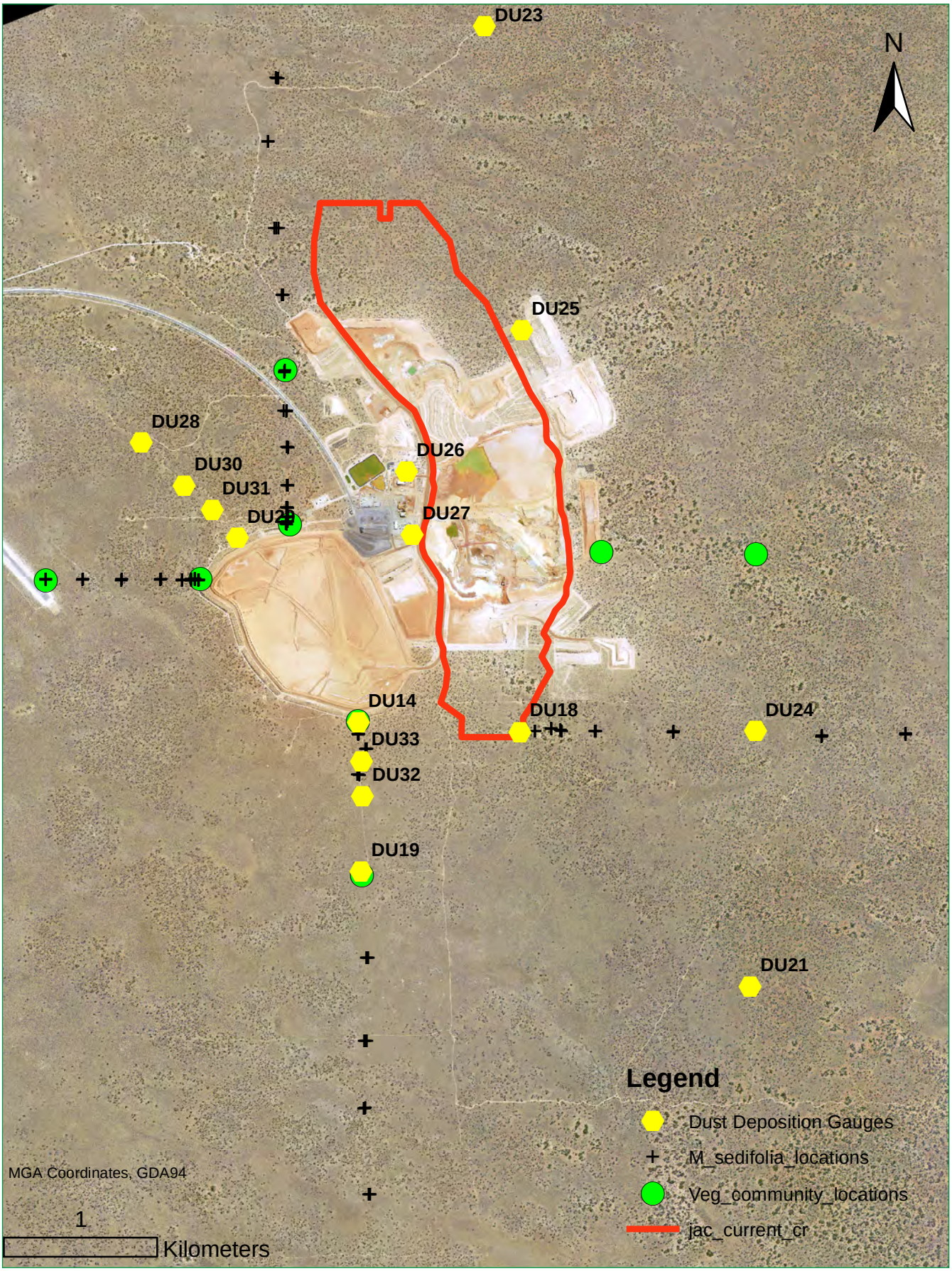
An additional two transects were added to the network in 2012 and all eight have been assessed for baseline plant health and dust covering levels. Four of these transects

(transect 6, 9, 11 and 12) have three replicated bushes at each monitoring location and these form the long term monitoring project. The remaining transects have had initial baseline measurement taken only and can be reactivated in the future if they are required or if additional monitoring resources become available. Only transects 6, 11 and 12 have been included in the analysis as transect 9 is not yet within the impact zone (but will become so once the Jacinth pit moves further south). The location of monitoring shrubs is provided in Figure 2.

Initially measurements recorded at each monitor shrub included old and new leaf health, presence or absence of flowers and fruit, the percentage of canopy cover looking from the top and from the side of the bush closest to the dust source, and the percentage of dust covering on the bush (Table 1). Based on the data analysis for previous years the monitoring program was streamlined to the presence and health of new and/or old leaves, flowering and fruiting and the proportion of the plant subject to dieback.

Table 1 Plant data recorded at each monitor shrub for each monitoring year

Feature	Characteristic	Score Options	2011 -2013	2014	2015
Health	Mature Leaf Health	Healthy, coating of dust, dying & dropping	✓	✓	✓
	New Leaf	Nil, healthy, dusty, unhealthy	✓	✓	✓
	Flowering	Mature, immature	✓	✓	✓
	Fruit	Mature, immature	✓	✓	✓
	Overall Plant Health	Score: 0 - 100 %	✓		
	Canopy down	Score: 0 - 100 %	✓		
	Canopy side	Score: 0 – 100%	✓		
	Dieback	Score: 0 – 100%		✓	✓
Dust	% of plant covered in dust	Score: 0 - 100 %	✓	✓	
	Dust direction (dust source)	N, E, S, W	✓		



J-A

Vegetation Condition (Dust) Monitoring locations

ILUKA

ORIG: JLEE DRAWN: JLEE SCALE: 1:34,994 (A4) DATE: Mar 2015 DWG No: Fig 2

FIGURE: **2**

2.2 Vegetation condition transects

In 2014 eight vegetation condition transects were established (Figure 2). Four transects were established at the edge of the mine footprint and four transects (controls) 1000 m from the mine footprint. Each transect is 50 m, divided into five 10 m by 2 m quadrats. For each quadrat the presence and abundance of each plant species is recorded, seedling or juveniles identified and cover estimates are recorded for bare ground, vegetation, biological surface crust and litter cover. Additional dieback information for closest trees to the transect is also recorded.

Data for all quadrats at the 1000 m transects will be pooled for data analysis where it can be shown there is no statistical difference.

3 Results and Discussion

3.1 Rainfall

Rainfall at JA for 2015 was the lowest recorded for the past three years, and slightly lower than the long term average for Tarcoola (JA – 150.4 mm, Tarcoola mean – 176.8 mm). The highest monthly rainfall was recorded in August, Figure 3.

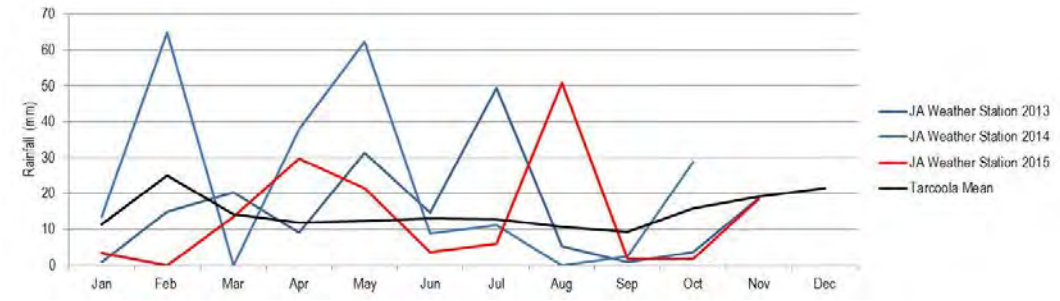


Figure 3 Monthly rainfall Jacinth weather station

3.2 Dust Deposition

Overall an increase in dust management awareness and practice has seen a reduction in the fugitive dust level produced by the mine in 2015. Particularly the off-path TSF which was previously responsible for much of the fugitive dust, including some off lease emissions historically.

The mean dust deposition recorded for 2015 (January to September) is slightly lower compared to all other years combined (Figure 4), however results are highly variable dependent on location. Generally gauges closer to the mine record higher dust deposition than those further away. Additionally there has been increased management of the off-path TSF in an attempt to minimise fugitive dust emissions. Access roads have been developed to increase the area of coverage by the water carts. This management has seen a reduction in dust recorded in the gauges located close to the off-path TSF.

Deposition gauges located south of the off-path TSF have declined in depositional dust for the gauge at the edge of the footprint in comparison to 2014 (October to December data has been excluded as records not available for 2015). Gauges located greater than 250 m from the off-path TSF show similar depositional levels. However, dust deposition rates to the

north of the off-path TSF are similar for both 2014 and 2015 (Figure 7), although dust deposition levels are half that of the gauges located to the south of the off-path TSF.

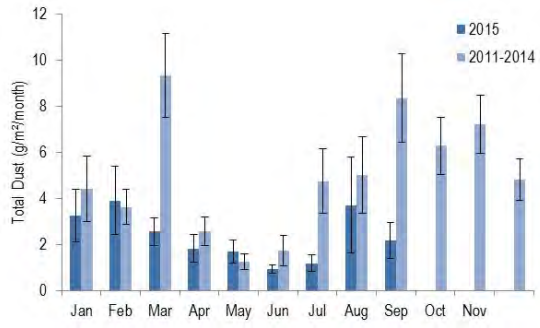


Figure 4 Mean dust deposition by month for all dust deposition gauge sites and all years.

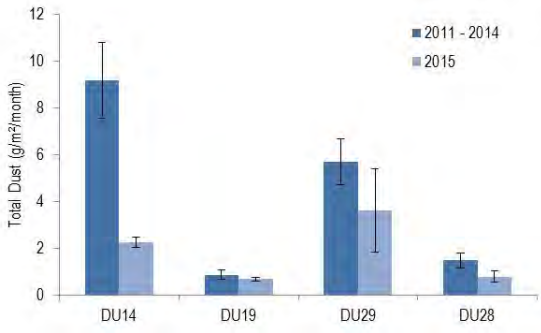


Figure 5 Mean dust deposition for DDG located at the off-path TSF (DU14 and DU29) and at 1000 m (DU19 and DU28)

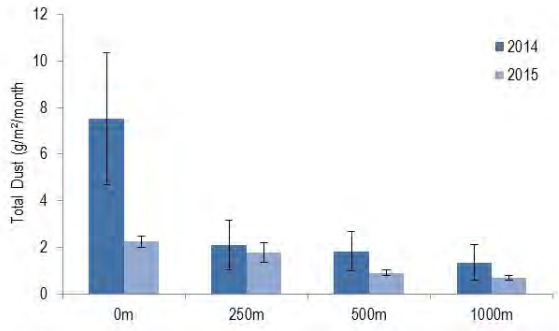


Figure 6 Mean dust deposition with distance south of the off-path TSF (2014 and 2015).

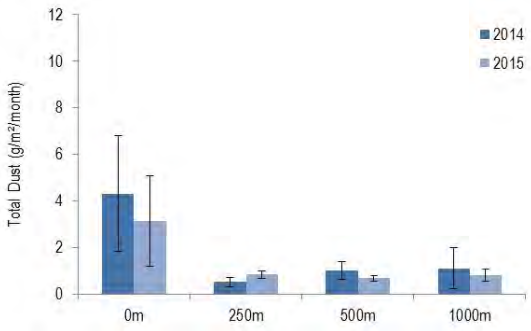


Figure 7 Mean dust deposition with distance north of the off-path TSF (2014 and 2015).

3.3 Maireana sedifolia

Maireana shrubs have been monitored since 2011. A total of 88 shrubs were monitored in March 2015 and 85 shrubs in November 2015 (Table 2). A difference in the numbers of shrubs between seasons was usually due to plants being difficult to find in the field. However sufficient plants are monitored for analysis. No plant deaths were recorded. Overall in 2015 dieback increased for plants within 50 m of the mine footprint when compared to 2014 dieback records. However, dieback for plants located greater than 250m for the footprint was similar for both 2014 and 2015. The increase in dieback recorded was likely due to the decrease in the proportion of shrubs that had healthy new leaves in 2015 compared to 2014. This response may be due to late and low rains for 2015 or may be a response to the dust deposition over time. The response of plants within 50 m of the mine footprint will need to be monitored carefully to identify any further degradation in health.

Table 2 Number of *M. sedifolia* shrubs monitored per monitoring event

Distance from Off-Path TSF	2011	2012		2013		2014		2015	
	Nov	Mar	Oct	Jan	Aug	Feb	Aug	Mar	Nov
0	1	5	2	12	9	3	8	9	12
25		1		6	6	3	6	6	6
50	1	2	3	9	9	3	12	9	6
75	1	1	3	3	3			3	3
100		2		6	6	3	6	6	4
200	1	1	3	3	3			2	3
250		2		6	6	3	6	6	6
300	1	1	3	3	3		3	3	3
500	1	2		6	6	3	6	6	5
750		2		6	6	3	6	6	6
1000	1	3	3	12	8	3	9	9	9
1500				6	6	3	3	6	6
2000				9	6	4	6	5	5
2500				6	6	3	6	6	6
3000				6	6	3	6	6	5
Total	**7**	**22**	**17**	**99**	**89**	**37**	**83**	**88**	**85**

The proportion of dieback recorded on plants ranged from a mean of 5% at plants greater than 500 m from the off-path TSF to 80% for plants located directly adjacent to the off-path TSF. Mean dieback has increased in 2015, when compared to 2014 surveys, for plants located within 50 m of the off-path TSF (Figure 8). Mean dieback was similar across both survey periods at all other distances.

The proportion of shrubs with healthy old leaves was highly variable with distance to the off-path TSF, ranging from nil to 100%, the majority of plants recorded new leaves, mean proportion ranging from 20% to 100% (Figure 9). No seasonal effect for leaf growth could be determined (Figure 10). No shrubs within 250 m of the off-path TSF were recorded with old leaves in 2014, this had increased to 27% of shrubs in 2015. However, there was a 10% decrease in plants with healthy new leaves from 2014 to 2015 (from 100% of shrubs in 2014 to 89% in 2015).

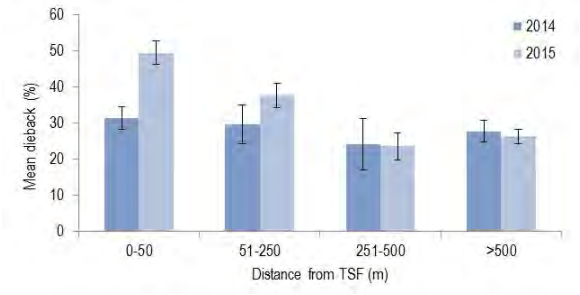


Figure 8 Mean proportion of dieback for monitoring shrubs with distance to off-path TSF

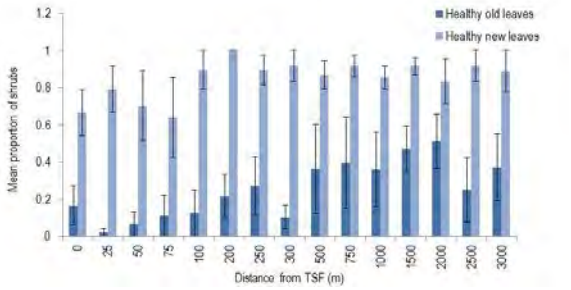


Figure 9 Mean proportion of shrubs with healthy leaves for all years monitoring

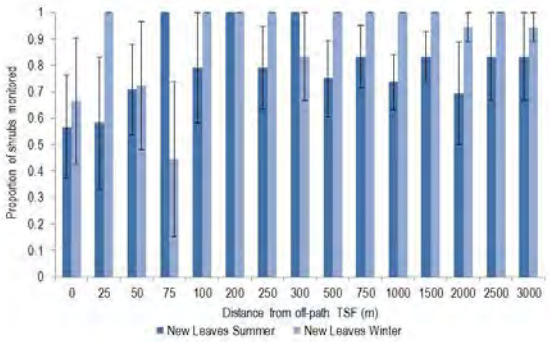


Figure 10 Mean proportion of shrubs with healthy new leaves during post summer and post winter monitoring events.

3.4 Vegetation condition

Overall species richness and diversity was similar for the impact and non-impact vegetation condition transects across 2014 and 2015. Vegetation and crust cover across the transects and years was similar, however the proportion of bare ground recorded at impact sites was greater than recorded at non-impact sites and for quadrats within 40 m of the mine footprint had increased since 2014. The bare ground recorded in the impact quadrats was loose surface sand (likely fugitive tails from the TSFs). Given the reduction in fugitive dust recorded it is possible that the increase in cover may be due to the movement of sand already present in quadrats rather than additional sand from TSFs. This will need further investigation and risk assessment to determine if and what course of action is required.

Species richness at transects ranged from 7 to 14 plants, however was similar for impact and non-impact sites for both 2014 and 2015 (Figure 11).

Although variable, mean plant abundances for the non-impact sites were similar across all quadrats for each year, data was therefore pooled for 2014 and 2015 (Figure 12). Plant abundance was lowest 10-20 m from the mine footprint in comparison to the non-impact sites, although had increased from 2014 to 2015 (Figure 13).

The proportion of transects that recorded the highest bare ground was within 10 m of the mine footprint and has increased since 2014 within 20 m of the impact (Figure 14). All transects within 50 m of the mine footprint had a higher proportion of bare ground compared to transects established 1000 m from the footprint.

Vegetation cover had reduced at all transects from 2014 to 2015, however the proportion of vegetation cover was similar across all impact transects and in comparison to the non-impact transects (Figure 15).

Biological crust cover was substantially less within 10 m of the mining footprint in comparison to the further transects (10-50 m) and the non-impact transects (Figure 16).

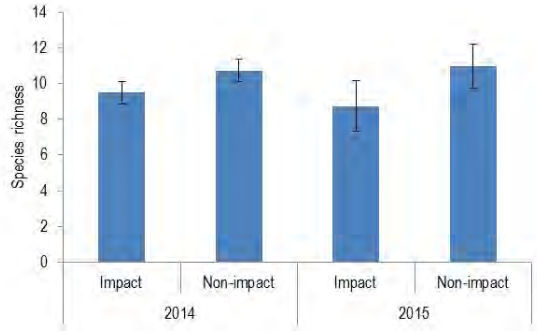


Figure 11 Mean species richness for impact and non-impact sites

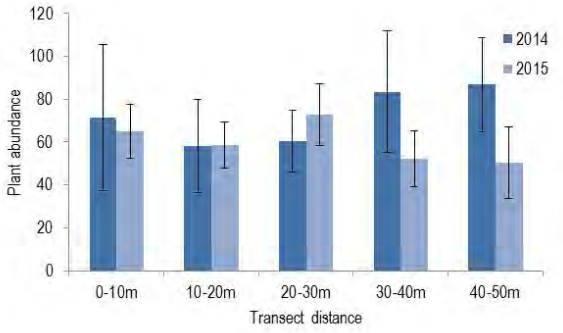


Figure 12 Plant abundance with time for non-impact sites

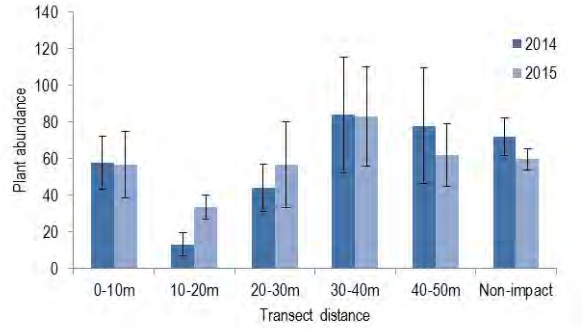


Figure 13 Mean plant abundance for impact transects with distance and mean non-impact data combined

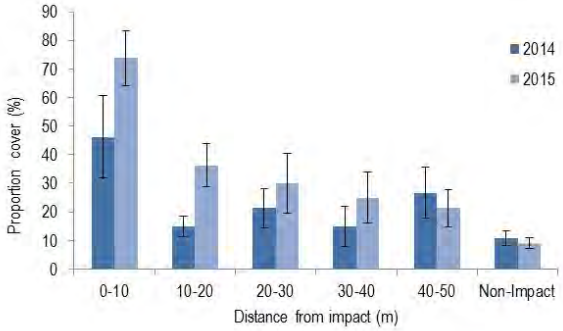


Figure 14 Proportion of bare ground recorded with distance from impact in comparison to non-impact sites

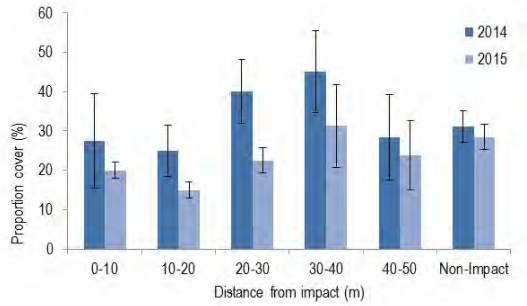


Figure 15 Proportion of vegetation cover recorded with distance from impact in comparison to non-impact sites

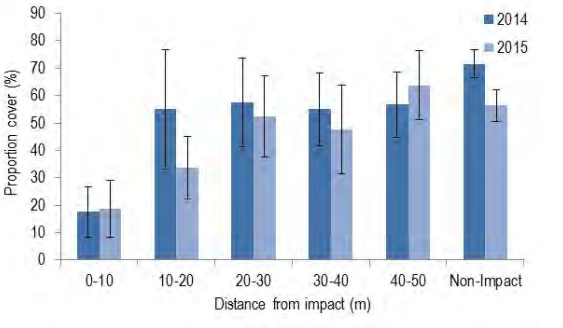


Figure 16 Proportion of BSC cover recorded with distance from impact in comparison to non-impact sites

4 Recommendations

Overall increased dust management on the off-path TSF has resulted in a decrease in the fugitive dust recorded in 2015. However, in contrast there has been an increase in the proportion of bare ground close to mine footprint. Additionally an increase in dieback on *M. sedifolia* was also recorded. It is not clear if the increase in bare ground and dieback is due to additional dust loads or climatic conditions, notably the drier than average rainfall for 2015. Based on the 2015 vegetation condition (dust) monitoring program the following recommendations are made:

- The health of *M. sedifolia* continue to be monitored to determine if recent increase in dieback due to climate conditions or ongoing pressure of high dust loads on plants.
- Continue to monitor the dust loads at the mine footprint to determine if increase in bare ground is due to additional fugitive dust or the movement of the material already present in the quadrats. Investigations to consider:
    - The installation of dust traps located at ground level at the impact vegetation condition transects to monitor sand movement.
    - The depth of sand across the impact vegetation condition transects.
- Dust management on the off-path TSF to continue. It is expected that rehabilitation of the off-path TSF will commence in 2016, therefore fugitive dust levels would be expected to decrease.
- Dust management of the in-pit TSF (particularly the sand tails walls) be investigated to minimise fugitive dust from these area. Investigations to consider:
    - Regular use of slimes on walls interior to the TSF.
    - Use of secondaries on the top of walls and the wall bunds to stabilise sands.

Appendix 2 Effects of groundwater mounding on native vegetation

ILUKA

Jacinth Ambrosia

Effects of saline groundwater mounding on native vegetation

2015

DOCUMENT CONTROL

Document Title:	Effects of saline groundwater mounding on native vegetation
Mine Status:	Operational
Revision:	Version 1.0
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Compiled by:	Joanne Lee
Owner:	JA Rehabilitation
Document No:	

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FIGURES

PLATES

1 Introduction

This report summarises the results of the vegetation health (groundwater) monitoring program to date.

The combined texture of the tailings produced at JA is coarse and saline water drains rapidly from the unlined tailings storage facilities and into the in-situ soil profile. Since mining and tailing began in October 2009, four tailings storage facilities have been used and groundwater mounds have progressively developed under each. A comprehensive summary of this groundwater mounding occurrence and subsequent response by Iluka is presented in J-A Groundwater Management Plan.

Some localised groundwater mounding was anticipated in the original mine planning and approvals. As part of the approvals process, Iluka have identified various groundwater levels (dependent on current surface land use) to minimise the impact of saline groundwater on the health vegetation and rehabilitation efforts. These groundwater levels are provided in the Program for Environmental Protection and Rehabilitation (PEPR).

1.1 Effects of rising groundwater on plants

Although salt can be detrimental to plant growth, there are species that tolerate relatively high salt levels (Barrett-Lennard & Malcolm 1999). A previous limited soil survey of JA prior to mining activity showed high soil salinity levels in two of the deeper soil types, brown loam (16-28 mS/m) and the deeper red loam (8-13 mS/m) (SWC, 2008). Tree roots were found to extend beyond 8 m in these soil pits, indicating the ability of some native species to survive under these conditions (SWC 2008).

Waterlogging is often associated with salinity caused by rising groundwater and this poses an additional set of stressors for plants. Plant tolerance to waterlogging and salinity is usually related to combinations of morphological and physiological adaptations (Bell 1999 and Aslam 2011). Pre-mining at JA the hypersaline groundwater levels were greater than 30 m below surface so vegetation would not be adapted to high or variable groundwater levels (WISH JA, 2011). Species capable of tolerating water logging are considered more likely to be able to tolerate the combination of salinity and waterlogging (Barrett-Lennard, 1986. van der Moezel, et. al 1988), however, these tolerances are not always linked. For example the growth of some Atriplex species, known for their salt tolerance, are constrained by waterlogging (Barrett-Lennard & Malcolm 1999).

Tolerance and plant response varies between species. A method to conduct rapid assessments of the effects of rising groundwater on adjacent vegetation has been developed, and the results of the monitoring presented here.

2 Methods

2.1 Soil salinity

In-fill drilling carried out in October 2014 offered an opportunity the measure soil salinity at depth in areas that had been impacted by groundwater mounding and had receded or was still present.

Soil salinity (1:5 soil/water method) was measured where groundwater was anticipated to be between 15 to 20 mBGL since 2011 using samples collected as part of the geological drilling program (impact measurements). Salinities were measured at sample depths 2m, 5m, 6m,

7m, 8m, 9m, 10m, 15m, 20m and 24m (or the end of drill hole). Salinity was also investigated at additional samples where groundwater was not expected to have been above 30 mBGL (according to modelling) as control measurements. Control salinities were measured at 2m, 5m, 10m, 15m, 20m and 24m.

2.2 Tree health

From 2011 to 2014 three branches were randomly chosen on each monitor tree and each was permanently labelled with coloured string and an aluminium tag identifying transect, tree, and branch numbers. In 2014 this was reduced to monitoring the tree overall, as recording separate branches is generally considered to be pseudo-replication and can skew results. A permanent photo point was established for each monitor tree and marked with a labelled wooden survey peg. Photos have been recorded 2011 to 2013, however this form of monitoring no longer comprises part of the program. Photo point monitoring is carried out separately and offers no useful information other than visual. Ad-hoc photos of trees will be taken as required or when of interest.

Initial health data captured (2011 – 2014) included the health of new and old leaves, symptoms of stress, evidence of flowering or fruiting and tree health scores (Table 1). In 2014 and 2015 the data collection was altered to provide clarification of tree response, as follows:

- The proportion of unhealthy leaves was recorded along with the symptoms to provide a clearer understanding of the tree health, a 'nil' entry indicates that the plant had no leaves showing stress symptoms.
- Recording tree health was removed from the program as data analysis in 2015 indicated the evaluation of tree health was ambiguous, different scores for the same tree on the same monitoring period was recorded and some trees had been identified as unhealthy but had high health scores. The proportion of dieback evident on each tree has been recorded since 2014 as a better, and more commonly used, health indicator.
- The recording of dropped leaves was not recorded in 2015 as this information is picked up in the dieback information.

The proposed monitoring schedule was previously quarterly to pick up seasonal differences on tree health, this has now been reduce to annually during quarter 4 of the year.

Trees have been identified as being located in an impact site, where the groundwater mound has been recorded within 30 m of the surface, or non-impact, all other locations. Note tree roots have been collected from the pit at a depth of 27.5 m, therefore 30 m was used as a conservative estimate of potential to impact.

Table 1 Summary of tree health monitoring data

Tree Feature	Characteristic	Score options pre-2014	Score options 2015	Score options 2015
Branch	Old Leaf Health	nil, healthy, unhealthy, dying, dead	nil, healthy, unhealthy, dying, dead	nil, healthy, unhealthy. Proportion of old unhealthy leaves
	Old Leaf Symptoms	nil, yellowing, burn, dead, dropped, combo (note), insect	nil, yellowing, burn, dead, dropped, combo (note), insect	Nil, yellowing, burn, dead, insect
	New Leaf	nil, healthy, unhealthy, dying, dead	nil, healthy, unhealthy, dying, dead	nil, healthy, unhealthy Proportion of unhealthy new leaves
	New Leaf Symptoms	nil, yellowing, burn, dead, dropped, combo (note), insect	nil, yellowing, burn, dead, dropped, combo (note), insect	Nil, yellowing, burn, dead, insect
	Flowering	nil, buds, flowering	nil, buds, flowering	nil, buds, flowering
	Fruit	nil, green, ripe non-viable, ripe viable, seed dropped	nil, green, ripe non-viable, ripe viable, seed dropped	nil, green, ripe non-viable, ripe viable, seed dropped
Whole Tree	Tree health	healthy, unhealthy, dying, dead	Proportion of dieback	Proportion of dieback
	Tree health score	0 – 10 (0 dead, 10 very healthy)		

2.3 Myall Locations

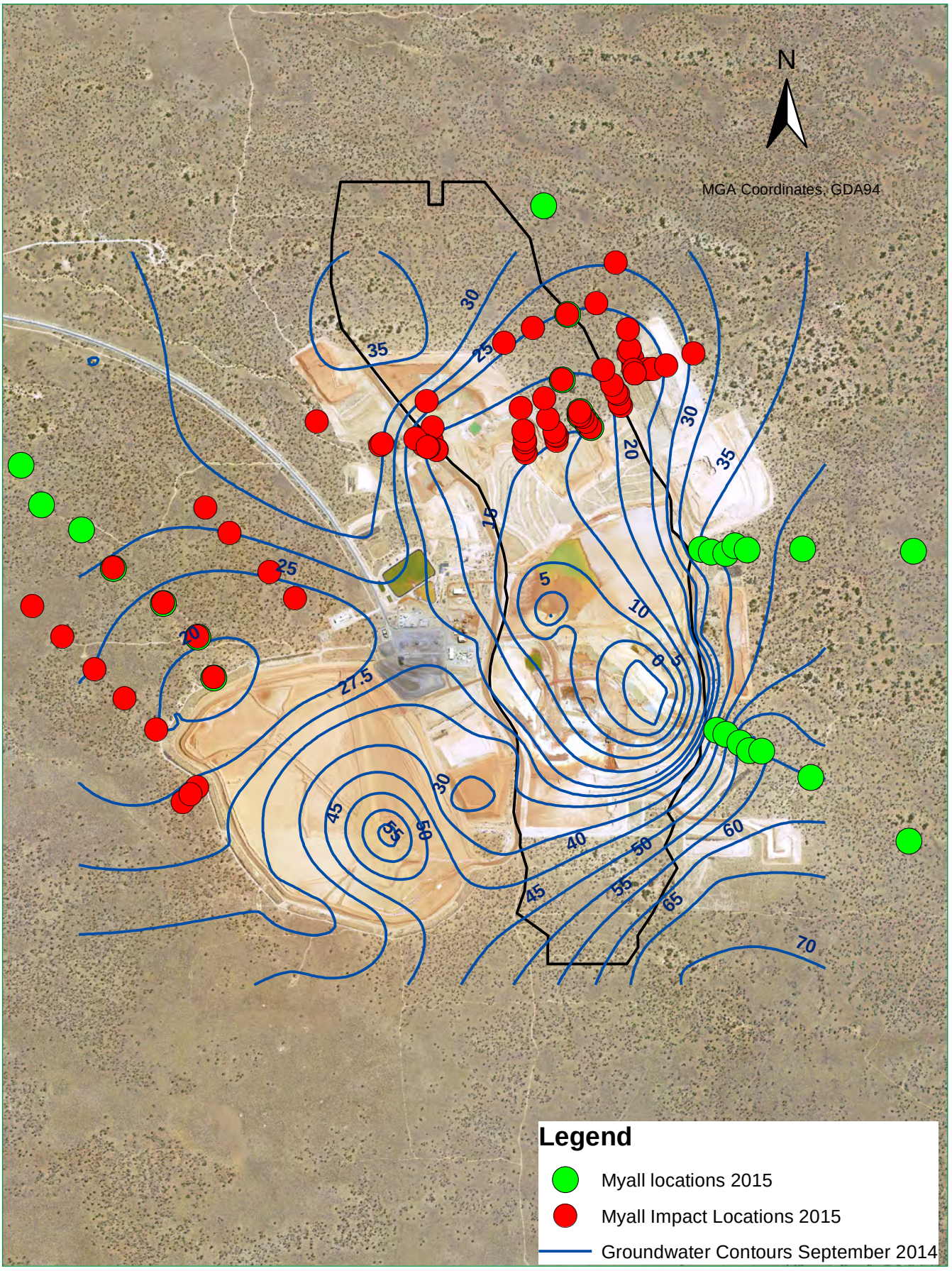
In 2011 transects to monitor the response of myall to groundwater mounding were installed (Figure 1). Monitoring transects were located as close as possible to an existing or proposed monitoring bore (MB), investigation hole (IH) or vibrating wire peizometer (VWP). All monitor trees along transects 1 to 4 (apart from distance 1,000 m) are in the future mine path (and some have now been cleared) and will be removed. Monitor trees along transects 5 to 12 are currently outside the mine path and expected to provide longer term data. Based on analysed data for previous years the number of transect to be monitored annually has been reduced to four only (transects 3,10,13 and 14), Figure 1. These transects provide enough of a sample size for both impact and non-impact sites.

2.4 Mallee Locations

In 2011 70 trees were identified to monitor response to groundwater mounding, additional trees have been added to the program to a total of 118 monitored trees (including those monitored opportunistically), Figure 2. However, 73 of these trees have now been cleared as part of the mine path, 45 trees remain in the monitoring program. Not all trees have been monitored for all periods, the number of trees monitored each period is provided in the results sections.

2.5 Mixed Cluster Locations

Mixed species monitoring cluster were also established in 2011. Initially all sites were located in the Cell 1 tailings facility: two in areas of concern for rising saline groundwater (MB07, transect 9, and adjacent Cell 1 Decant area – Cell 1 West, transect 10) and one control site (MB05, transect 11 – based on 30 impact site this site is now classified as an impact site), Figure 3. Both transect 10 and transect 11 have cleared as part of the mine path. In 2014 three control and two impact cluster sites were established to monitor any response in shallower rooted species to ground water mounding.



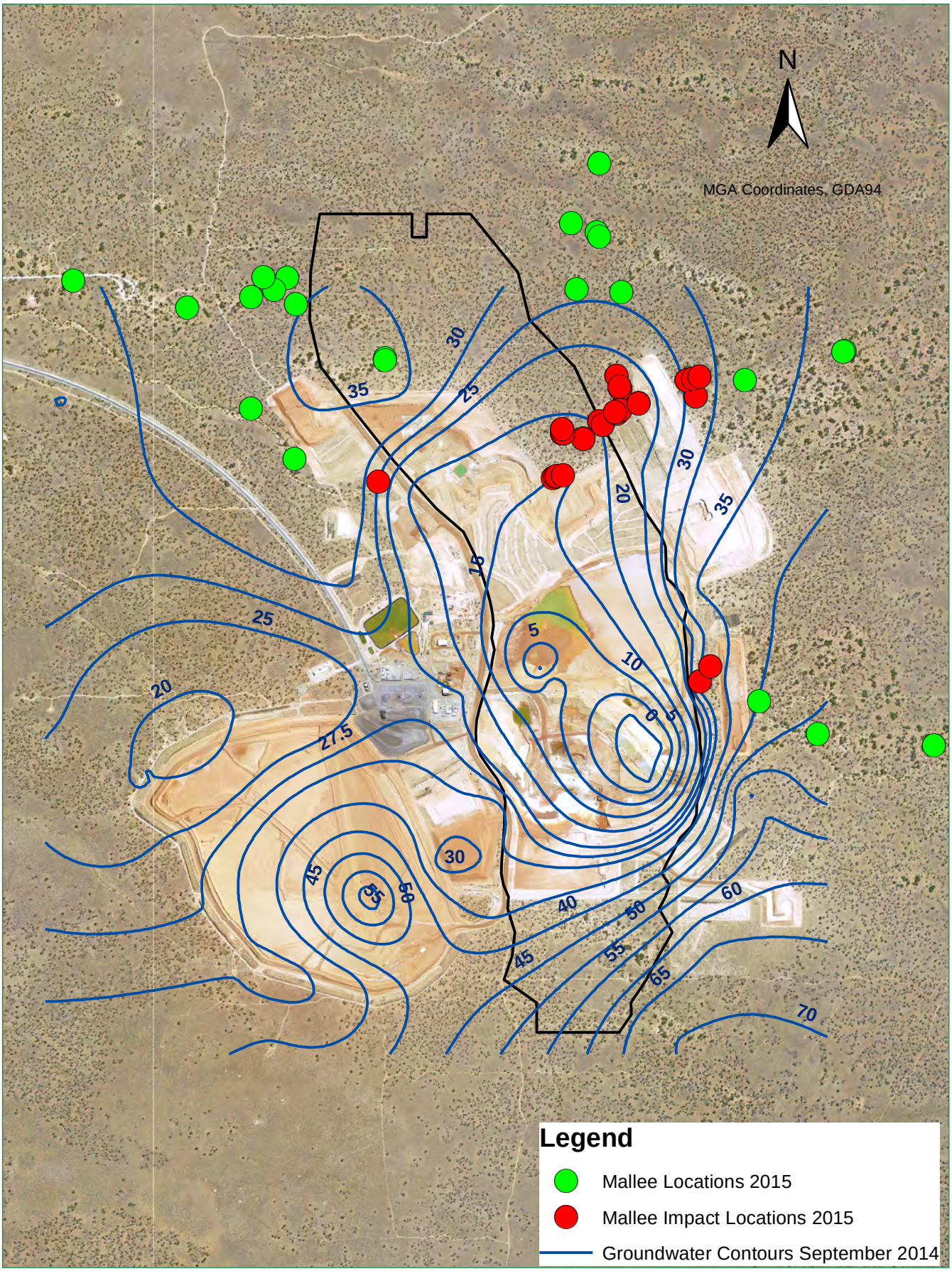
JA

Location of myall monitoring trees

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:23,881 (A4) DATE: Jan2016 DWG No: NA

FIGURE: **1**



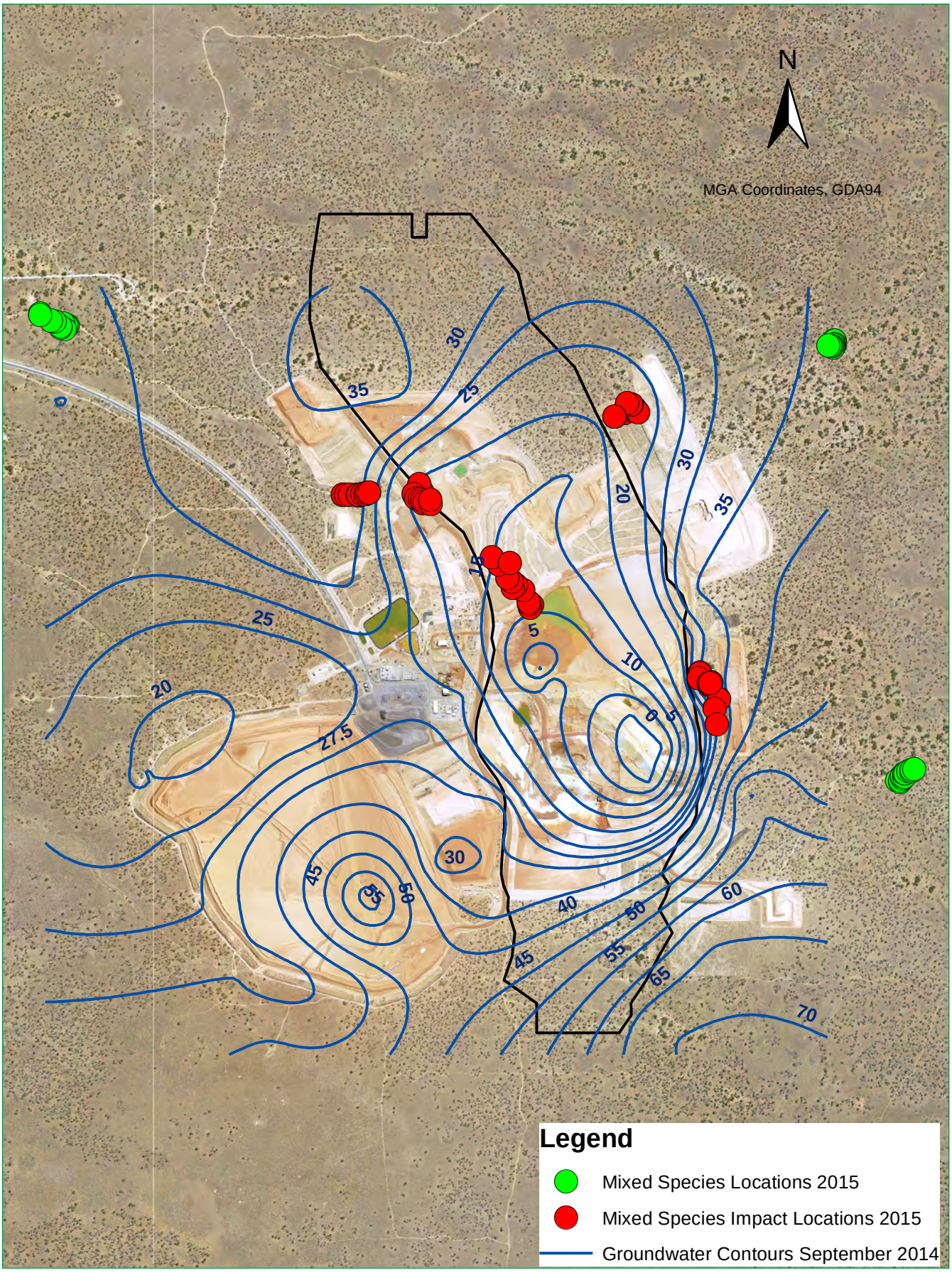
JA

Location of mallee monitoring trees

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:22,832 (A4) DATE: Jan2016 DWG No: NA

FIGURE: **2**



JA

Location of mixed species monitoring trees

ILUKA

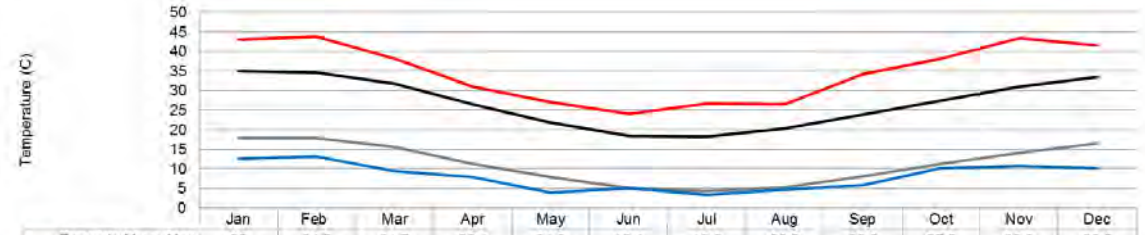
ORIG: JL DRAWN: JL SCALE: 1:22,832 (A4) DATE: Jan2016 DWG No: NA

FIGURE: 3

3 Results

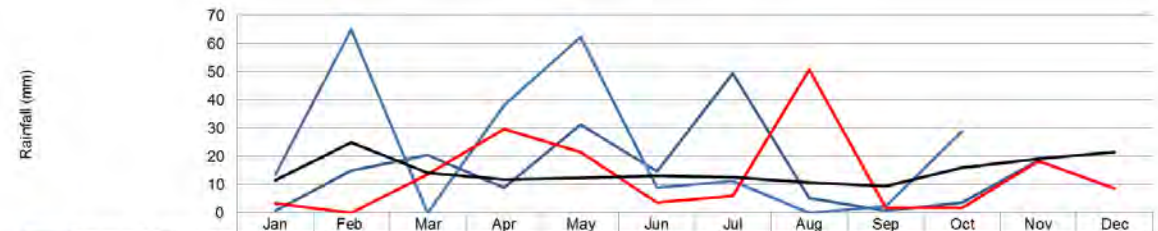
3.1 Climate

Overall the climate at JA was hotter and drier in 2015 than the long term average (Figure 4 and Figure 5). Temperatures were consistently warmer than the long term average (Figure 4). Rainfall at JA for 2015 was the lowest recorded for the past three years, and slightly than the long term average for Tarcoola (JA – 159 mm, Tarcoola mean – 176.8 mm). Winter rains arrived later than usual, the highest monthly rainfall was recorded in August, Figure 4.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Tarcoola Mean Max	35	34.5	31.7	26.4	21.8	18.4	18.2	20.2	23.9	27.5	30.9	33.5
Tarcoola Mean Min	17.9	17.9	15.5	11.2	7.9	5.1	4.2	5.3	8.1	11.2	14.1	16.5
JA 2015 Max	43.1	43.8	38.2	31	27.1	24.1	26.6	26.5	34.1	38.2	43.4	41.6
JA2015 Min	12.6	13.1	9.44	7.83	3.93	5.07	3.31	4.67	5.73	10.2	10.6	10.1

Figure 4 Monthly temperatures for JA weather station and Tarcoola monthly long term average



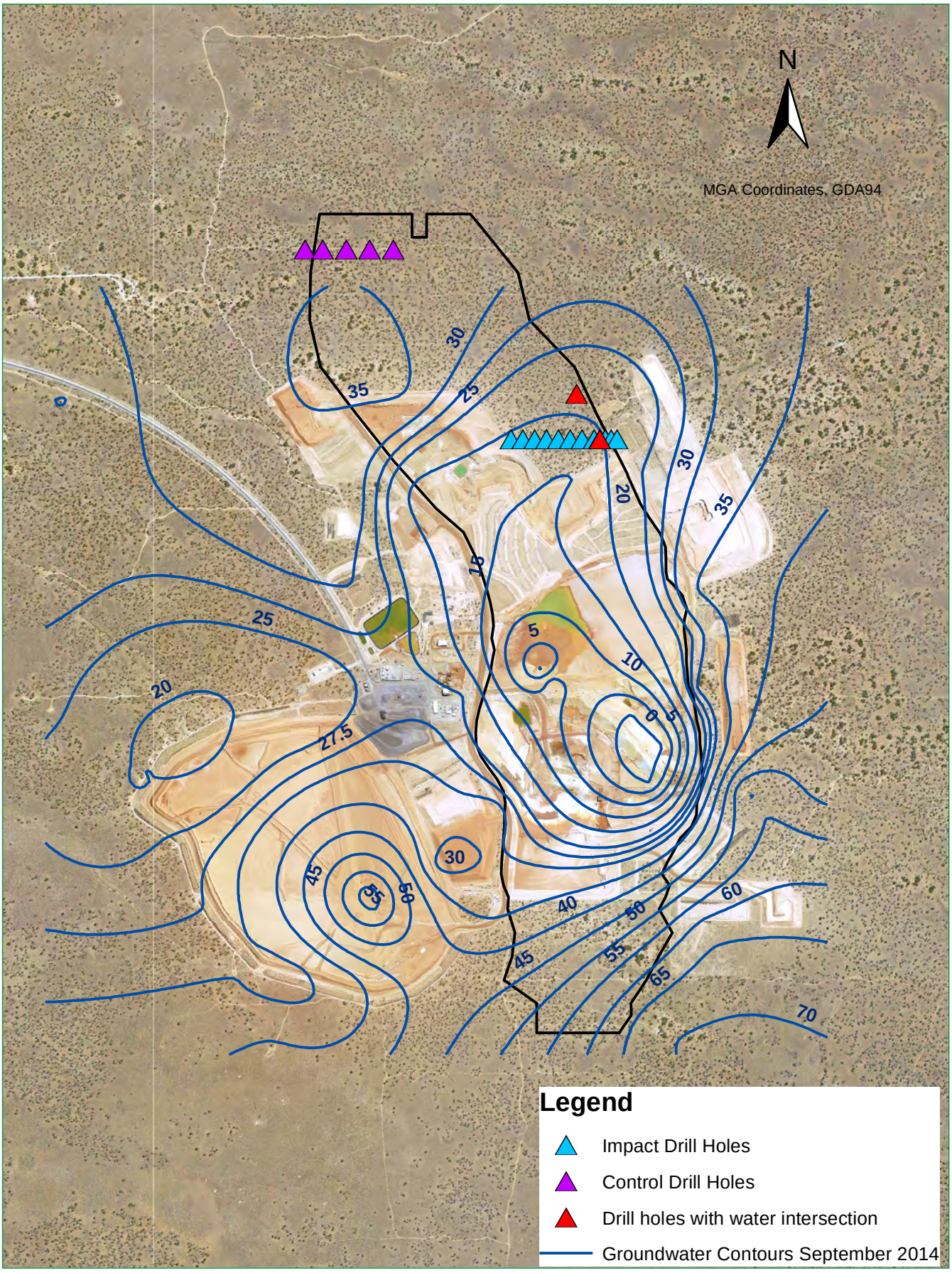
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
JA Weather Station 2013	0.8	15	20.4	9	31.4	14.6	49.4	5.2	0.8	3.6	18.6	
JA Weather Station 2014	13.6	64.9	0	38	62.35	8.88	11.24	0	2.4	28.8		28.8
JA Weather Station 2015	3.4	0	13.6	29.6	21.4	3.6	6	50.8	1.8	1.8	18.4	8.6
Tarcoola Mean	11.4	25	14.2	11.8	12.3	13	12.7	10.7	9.3	15.9	19.1	21.4

Figure 5 Monthly rainfall for JA weather station and Tarcoola monthly long term average

3.2 Soil salinity

Groundwater was intersected at two locations during a resource drilling program carried out in October 2014 (Figure 6) at 21 mBGL (JAN00124) and 29 mBGL (JAN00099). Both intersections were within the levels anticipated based on drill hole location and current modelling contours.

All salinities recorded were within the ranges currently recorded at JA (Figure 7). Salinities collected during other programs are given for comparison, the locations of the comparative measurements are outside the groundwater mounding area of influence.



JA

Soil salinity investigation locations

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:22,832 (A4) DATE: Jan2016 DWG No: NA

FIGURE: **6**

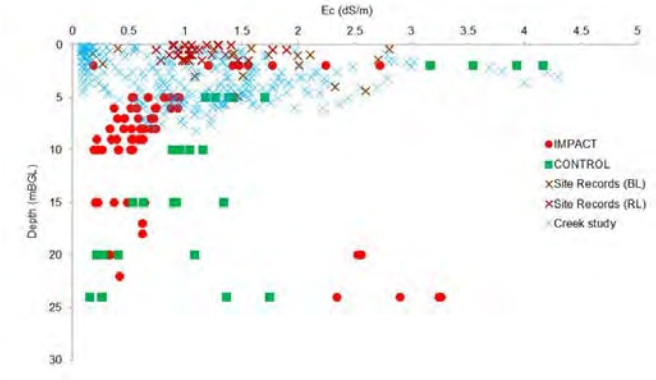


Figure 7 Soil salinity with depth recorded from samples collected as part of in-pit drilling. Impact salinities relate to area where groundwater has been recorded at a maximum depth of 20 m from surface. Control sites, site records and creek study records are given as a comparison with areas not impacted by groundwater mounding.

3.3 Mallee Health

A total of 37 mallee were monitored in September of 2014 (Table 2), with an additional 8 individuals that were missed in the monitoring surveyed in February 2015. The 2015 monitoring was delayed until 1 and 2 January 2016, a total of 39 individuals were surveyed. Trees were located at various distances from the mining pit (area of impact).

Table 2 Summary of mallee monitoring occasions

	Jul-11	Aug-11	Sep-11	Nov-11	Dec-11	Jan-12	Jun-12	Jul-12	Sep-14	Feb-15	Jan-16
Trees visited		28	52	26	97	14	51		37	8	39
Opportunistic monitoring	15			22				19			

Dieback was generally variable across 2014 to 2016, ranging from 80% to 5% across 2014 to 2016. Overall dieback was higher for individuals located within the impact site in comparison to those outside of the impact area (Figure 8) and there has been a slight increase in dieback from 2014 to 2016. However tree health scores for 2011 and 2012 were similar for both impact and non-impacted individuals (Figure 9). Tree health scores are not considered suitable to determine individual response to groundwater mounding and will no longer be considered in data analysis and reporting going forward.

The proportion of individuals that were identified with unhealthy old leaves was very low for 2011 and 2012, increasing in 2014 and 2015, and the majority of individuals in 2016 were recorded as having unhealthy old leaves (Figure 10). The increase in individuals with unhealthy old leaves in 2016 was due to a change in recording method where the proportion of leaves that were unhealthy was recorded, rather than the ambiguous healthy or unhealthy. Interestingly the mean proportion of unhealthy leaves on monitored trees was greater in the non-impact sites in comparison to impact sites (Figure 11).

For unhealthy old leaves insect damage was the most common symptom, accounting for 80% of old leaf symptoms (Figure 12). More individuals were identified with insect damage in non-impact area in comparison to impact sites. The proportion of individuals showing insect damage varied across all monitoring periods, Figure 10, with more individuals with insect damage in non-impact area across all years except the 2014-2015 period (if combined). For the 2014-2015 period 65% of individuals in the impact sites presented with some sign of

insect damage, in comparison to 24% presented signs of insect damage. Yellowing of leaves, suspected to be a response to saline stress was only recorded in 10% of individuals, similar for both impact and non-impact sites, Figure 12.

Very few individuals have been recorded with unhealthy new growth (new leaves), ranging from 7% in 2012 to 8% in 2016 (Figure 14). The majority of unhealthy in new leaves were recorded in non-impact sites; with the only records of unhealthy new leaves in the impact sites was 2016 (2.5%). For the three individuals that recorded unhealthy new leaf growth in 2016 the proportion of unhealthy leaves was quite high, 50% to 60%, two were located in the non-impact area and one inside the impact area. The proportion of individuals that were identified with no new leaf growth varied across monitoring periods (Figure 15), however there was generally more individuals without new leaves recorded in non- impact sites compared to impact sites (note that no non-impact individuals were monitored in 2015, and only 8 impact individuals monitored).

Particular observations of note include:

- Ma_0_49 in an impact site had high levels of insect damage to both old and new leaves, however all other trees in that area were observed to be healthy (Plate 1).
- Ma_5_13 and Ma_0_25 in an impact site showed epicormic growth (Plate 2 and Plate 3).

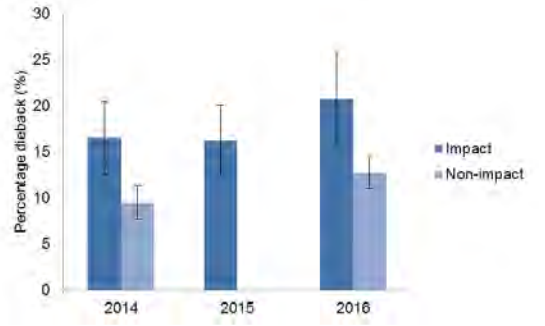


Figure 8 Mean proportion dieback for mallee during the monitoring periods 2014 – 2016.

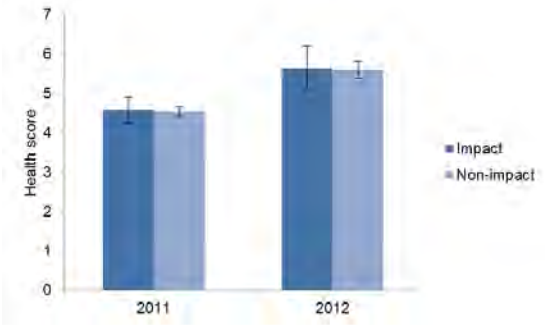


Figure 9 Mean health scores for mallee during the monitoring periods 2011 and 2012.

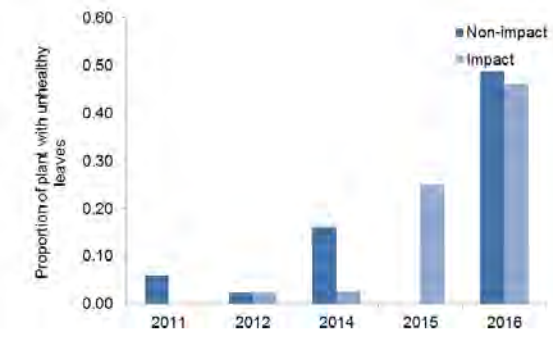


Figure 10 Proportion of mallee that were identified with unhealthy old leaves across all monitoring periods

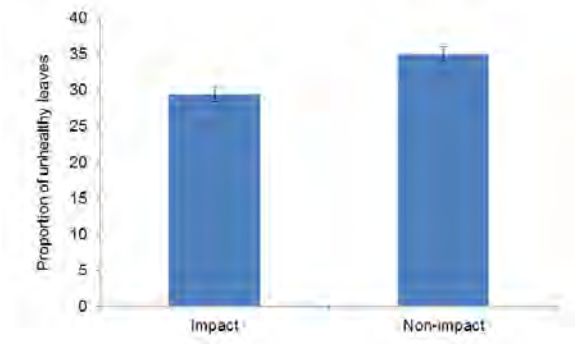


Figure 11 Mean proportion of unhealthy leaves per mallee for impact and non-impact sites

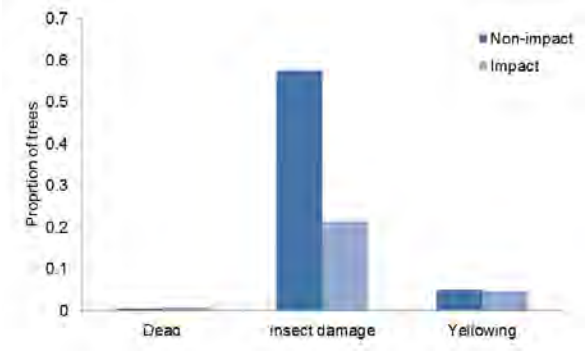


Figure 12 Proportion of mallee with various symptoms for all periods combined

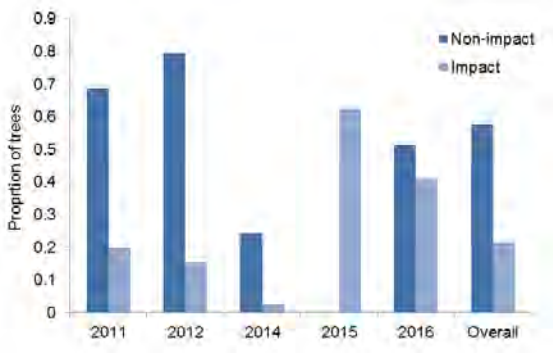


Figure 13 Proportion of mallee with insect damage across all monitoring periods

Figure 14 Proportion of mallee that were identified with unhealthy new leaves across all monitoring periods

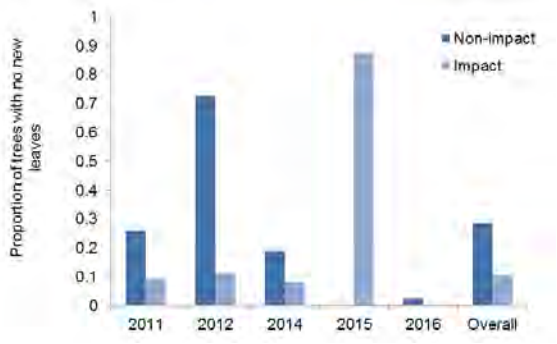


Figure 15 Proportion of mallee that did not have new leaf growth across all monitoring periods

Plate 1 Mallee (Ma_0_49) presenting high insect damage (galls present in both old and new leaves)

Plate 2 Mallee (Ma_0_25) presenting with epicormic growth

Plate 3 Mallee (Ma_5_13) presenting with epicormic growth

3.4 Myall Health

Monitoring of myall has been ongoing since 2011, with various numbers of individuals monitored each year (Table 3). A total of 28 myall were monitored January 2016 Trees were located at various distances from the mining pit (area of impact).

Table 3 Summary of myall monitoring occasions

	Jul-11	Nov-11	Mar-12	Jul-12	Dec-13	Sep-14	Jan-16
Number of trees monitored	27	51	19	77	63	63	28

Health scores of myall have generally increased over the 2011 to 2013 period (Figure 16), health scores were not recorded in 2014 and 2016. In 2011 the health score of individuals was higher for those within the impact area, however in 2012 and 2013 health scores in the impact sites were lower than those for the non-impact sites. Mean dieback has increased in 2016 in comparison to 2016, Figure 16. However dieback was similar for individuals in impact and non-impact sites. One particular myall (My_03_01) presented with a high proportion of dieback (Plate 4), likely due to mine pit disturbance (note this myall will be cleared for the mine path).

The proportion of myall with unhealthy old leaves was variable across years (Figure 18), with the numbers of individuals with unhealthy leaves greater in 2013 and 2016 in comparison to 2011 and 2012. Overall a greater proportion of individuals within the impact sites had unhealthy leaves in comparison to the individuals in the non-impact area (Figure 18). In 2015 the proportion of leaves showing symptoms was recorded. Myall within the impact area had a mean greater proportion of leaves with symptoms (26.3 SE ±11.8) in comparison to myall in the non-impact sites (10 SE±5), although results were highly variable. The most common old leaf symptom was dead leaves (remaining on individuals) and yellowing, both occurred more often in impact area, Figure 19.

Unhealthy new growth was only recorded in 2013, and the proportion of individuals presenting with unhealthy new growth was 1% in both the impact and non-impact sites. However the proportion of myall that has not shown any new growth has been consistently higher in the impact sites than non-impact sites (Figure 20).

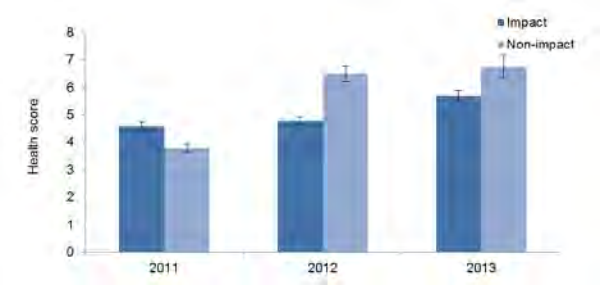


Figure 16 Mean health scores for myall across the 2011 to 2013 monitoring periods

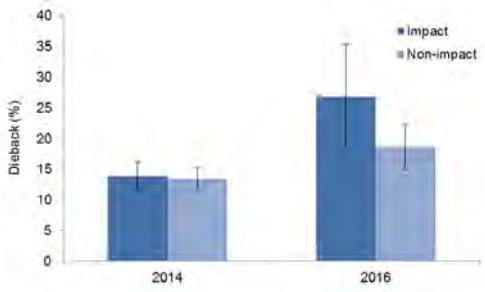


Figure 17 Mean dieback for myall across the 2014 and 2016 monitoring periods

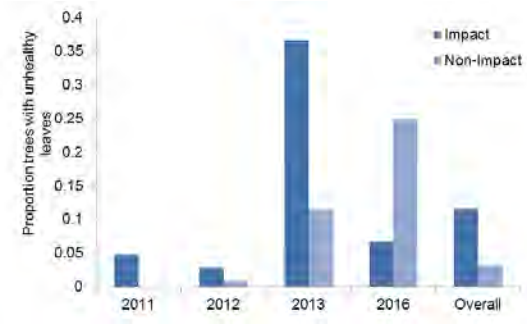


Figure 18 Proportion of myall with unhealthy old leaves across all monitoring periods

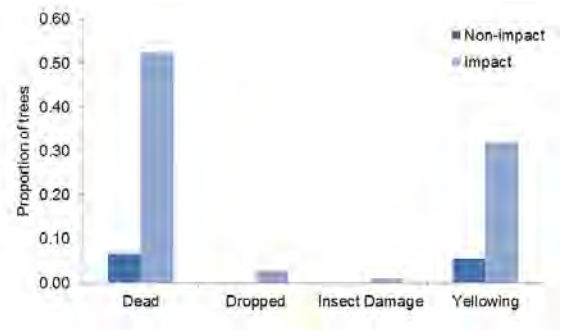


Figure 19 Proportion of symptoms for myall with unhealthy old leaves

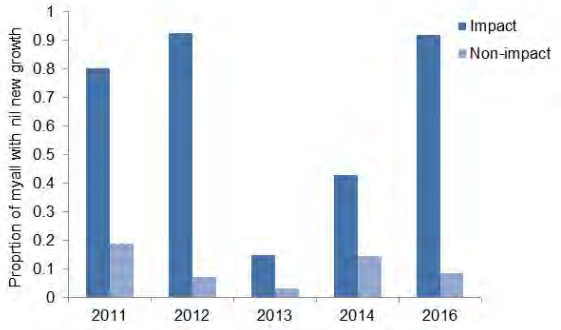


Figure 20 Proportion of myall that have nil new growth across all monitoring periods

Plate 4 Myall (My_03_01) showing dieback in response to disturbance

3.5 Mixed Species Health

Various numbers of different species have been monitored for a groundwater mounding stress response since 2011, Table 4. Initially 40 individuals were monitored, all within the area of impact (water at a maximum of 25 mBgl). One cluster was monitored in 2012, and then two clusters were removed for the mine path. The remaining cluster was monitored in 2013 and 5 additional transects were established in 2014 (2 impact and 3 non-impact).

Table 4 Summary of mixed species monitoring occasions

Species	Jul-11	Aug-11	Nov-11	May-12	Dec-13	Sep-14	Oct-14	Jan-16	Total
Acacia ligulata	1	1	1				6	3	12
Acacia papyrocarpa	5	5	5	5					20
Alectryon oleifolius	6	6	6	2			25	12	57
Dodenaea viscosa	4	4	4		4	3	6	6	31
Eremohphila latrobi							1	1	2
Eremophila alternifolia	4	4	4		4	1		3	20
Eremophila latrobi							2		2
Eremophila scoparia	1	1	1				10	6	19
Eucalyptus oleosa	6	6	6	4					22
Lycium australe	3	3	3	1	2	2	3	5	22
Maireana sedifolia	1	1	1			1	4	3	11
Myoporum platycarpum	2	2	2						6
Santalum acuminatum	4	4	4		3	1	14	8	38
Senna artemisiodes petiolaris							12	6	18
Senna artemisioides	3	3	3		3	2	5	6	25
Total	**40**	**40**	**40**	**12**	**16**	**10**	**88**	**59**	**305**

Plant health was recorded as healthy or unhealthy 2011 to 2013. Overall the majority of individuals in impact sites 2011 to 2012 were recorded to be healthy, however in 2013 the majority of individuals were recorded as unhealthy (Figure 21). Two individuals were

recorded as dead in 2012, an *E. oleosa* and a *L. australe*, these individuals were removed as part of the mine clearance program. The mean health score has decreased since 2011, ranging from 6.5 (±0.19) in 2011 to 4.7 (±0.31) in 2013, Figure 22. Dieback on individuals in both the impact and non-impact sites was recorded in 2014 and 2016. The percentage of dieback on individuals was similar for both impact and non-impact sites in 2014, however increased in 2016 with individuals at impact sites presenting with slightly higher dieback than non-impact sites, Figure 23. Plant deaths were recorded at impact and non-impact sites, three *L. australe* individuals in non-impact sites and two in impact sites were completely denuded.

The proportion of individuals that presented with unhealthy leaves was variable across monitoring periods (Figure 24). The highest recorded proportion was in the 2012 (67%) impact sites, however reduced below 2% in 2014 and 2016 for impact sites. Further only 12 individuals were monitored in 2012, with 7 presented with unhealthy, dead or dying leaves and 5 presenting with healthy leaves. In 2016 the proportion of individuals with unhealthy old growth was higher at non-impact sites.

No unhealthy new leaves were recorded at non-impact sites during 2014 and 2016, unhealthy leaves were only recorded at impact sites in 2011 (9%) and 2013 (13%). Further the proportion, of individuals that did not have any new growth was highest in 2011 (56%) and 2012 (92%), Figure 25. However, the proportion of individuals without new growth was similar for the 2013 (36%) and 2016 (42%) impact sites, and similar across impact and non-impact sites (46%) for 2016.

The dieback response across impact and non-impact sites for individual species varied, Figure 26. *E. scoparia* (49.3% SE±8.75) and *M. sedifolia* (60% SE±0%) had a higher mean dieback at impact sites in comparison to non-impact sites (20% SE±8% and 12.5% SE±1.11 respectively). Note that the dieback for *M. sedifolia* in impact sites was based on two individuals. In contrast *S acuminatum* (15% SE±3.32) and *S. artemisiodes petiolaris* (19.44% SE±5.4) had higher mean dieback at non-impact sites in comparison to non-impact sites (6.79% SE±1.79% and 8.89% SE±1.62 respectively). *E. alternifolia* presented with relatively high dieback in impact sites, however no plants are located in non-impact sites for comparison. All other species showed similar dieback for both impact and non-impact sites.

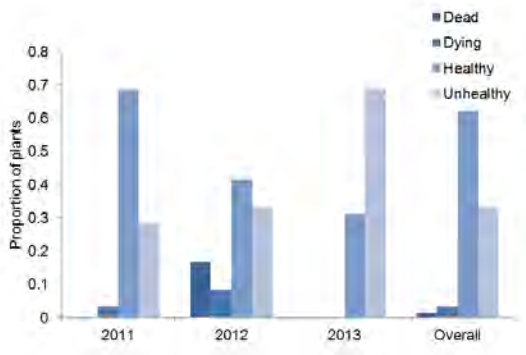


Figure 21 Health records for mixed species at impact sites 2011 to 2013

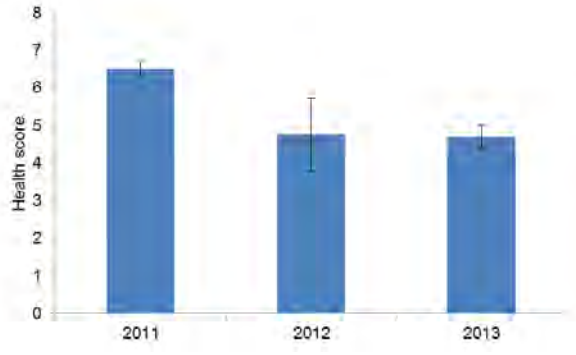


Figure 22 Mean health scores for individuals at impact sites 2011 to 2013

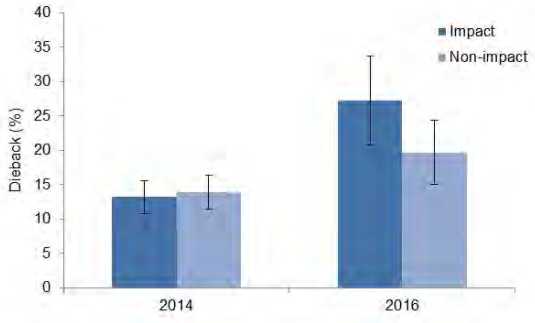


Figure 23 Mean dieback for mixed species at impact and non-impact sites, 2014 and 2016

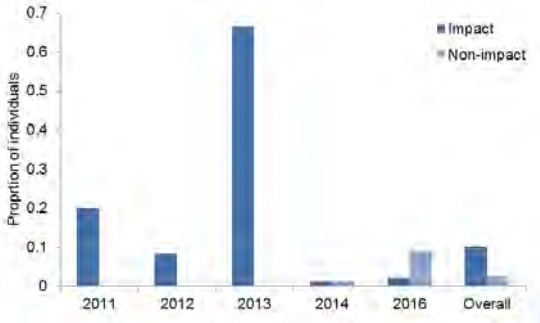


Figure 24 Proportion of individuals presenting with unhealthy old leaves across all monitoring periods

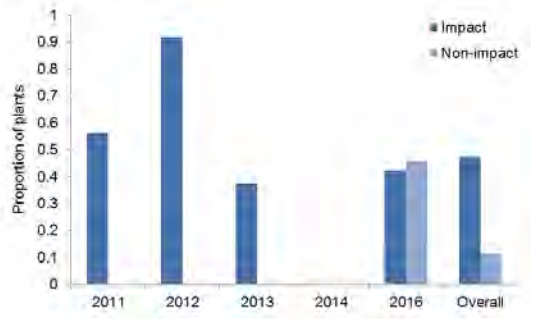


Figure 25 Proportion of mixed species individuals with no new leaf growth across all monitoring periods

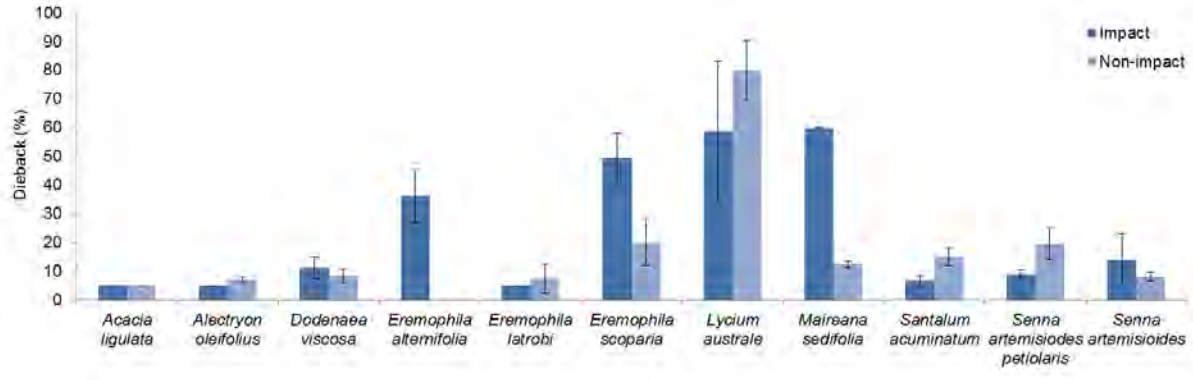


Figure 26 Mean dieback for individual species at impact and non-impact sites

4 Discussion

There has recently been a decline in plant health across both impact sites and non-impact sites with a general increase in dieback. In 2016 higher rates of dieback was recorded across all three monitoring programs, mallee and myall and mixed species. The general decline in plant health may be due to the drier and hotter than average conditions recorded at JA during the 2015 period, and all monitoring was carried out after a hot and dry summer. The *L. australe* individuals that dies over this period was most likely caused by age or dry conditions as individuals were lost at both impact and non-impact sites. These plants do have the ability to regenerate from roots, therefore they will continue in the monitoring program to determine if they respond to a change in climate conditions.

Individuals at impact sites presented with higher rates of dieback than individuals at non-impact sites. It is possible that these individuals are responding to the additional stresses of being close to the mine site, i.e. ground water mounding or the cumulative impacts of a range of environmental variables. Longer term monitoring will assist in identifying the cause of decline and the ability of individuals to recover.

Leaf response in individuals varied, myall had a greater proportion of individuals with unhealthy leaves in impact site, mallee had similar proportion of individuals with unhealthy leaves at impact and non-impact sites and the proportion of individuals at non-impact sites had more unhealthy leaves than non-impact sites. Further myall individuals were the only species to have a higher proportion of unhealthy leaves per individual in impact sites compared to non-impact sites. This may be due to the very slow decomposition rate for myall, and dead or dying leaves are more likely to remain on the individual for longer in comparison to other species. This would also indicate that the dieback for myall could be underestimated; longer term monitoring is required of these individuals.

Generally very few individuals were recorded with unhealthy growth across the monitoring periods, making data analysis and comparison difficult. However fewer myall individuals in impact areas had new growth in comparison to those in non-impact sites. Further fewer mixed species had new growth during the 2011 and 2012 period when the groundwater mound was highest, and considering the higher than average rainfall recorded for 2011 new growth would be expected for most species. The majority of these plants have now been removed, however it is possible that the lack of new growth maybe a plant stress response.

The majority of unhealthy responses recorded in mallee were caused by insect damage, at both impact and non-impact sites, however was recorded on a higher proportion of individuals at impact sites. Individuals under stress can be targeted and succumb more readily to insect attack. It is possible that the higher incidence of insect damage on individuals at impact sites is due to mine impacts stressing individuals.

Overall there appears to be some response by plant species to mining impacts, and these may have been influenced further by recent hot and dry climatic conditions. The majority of affected individuals are within the mine path and therefore anticipated to be cleared as part of mining activities. In addition, the attenuation of the groundwater mound over time and periods of higher rainfall may also encourage a positive response from affected plants. At this point in time no plant death can be attributed to the groundwater mound. However, ongoing monitoring is recommended to determine the long term extent of impacts on individuals to rising groundwater.

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Appendix 3 Reduced Plant Establishment in Bay 1 of Cell 1 West – Investigation and Actions

File Note

By:	Tina Law and Mark Dobrowolski	**CC:**	Jo Lee, Nick Travers
Date:	2 Sept 2015 Updated 10 Jan 2016	**Trim Ref:**	

Subject: Reduced Plant Establishment in Bay 1 of Cell 1 West – Investigation and Actions

Summary

The reduced plant establishment in Bay 1 of Cell 1 West rehabilitation is potentially caused by multiple factors, namely delayed ripping after topsoil placement, delayed seeding after topsoil placement missing critical winter rain, return of the topsoil in a single 0.2 m pass, and the physical or chemical characteristics of the topsoil used (from a soak area) used for Bay 1. Soil characterisation and seeding/watering trials have been implemented to determine the most influential factor(s).

Introduction

In Bay 1 of Cell 1 West, far fewer plants have established to date compared to other areas of rehabilitation that were completed in the first half of 2014 (Figure 1 shows a plan of the 2014 rehabilitation areas). This is evident from simple observation of the area, from photos (Plate A), and from survey data (Figures 2 and 3) that show the total plant abundance and species richness for the trial bays of Cell 1 West as measured in Jessop surveys conducted in the last quarter of 2014.

Along with various methods of soil return trialed in other bays of Cell 1 West, the topsoil/subsoil in Bay 1 was direct returned in a single pass (Figure 1). This involved the excavation of topsoil/subsoil within the clearance footprint to a depth of 0.2 m captured by a single pass with a dozer. The excavated soil was then trucked to Bay 1, pushed out to 0.2 m with a dozer, then ripped. The total area of Bay 1 is approximately 1 ha in size.

The reduced plant establishment in Bay 1 ***may*** be solved with application of additional seed and/or supplementary watering of the area, ***if*** a lack of seed, seed burial with single pass soil placement, or lack of rain following soil placement were the reasons for that poor establishment. However, as will be presented in the analysis below, there is no single reason that can unambiguously explain the reduced plant establishment in Bay 1. Without a clear understanding of the causal factors that reduced the plant establishment in Bay 1, remedial actions may be ineffective and waste resources, and the problem will likely be repeated.

In keeping with Iluka's commitment to high levels of environmental performance through best practice rehabilitation, an investigation into the causal factors of this problem has begun. This file note on the reduced plant establishment in Bay 1 of Cell 1 West therefore summarises the available evidence and an analysis of the factors that may have contributed to the reduced plant establishment and investigative actions currently in process.

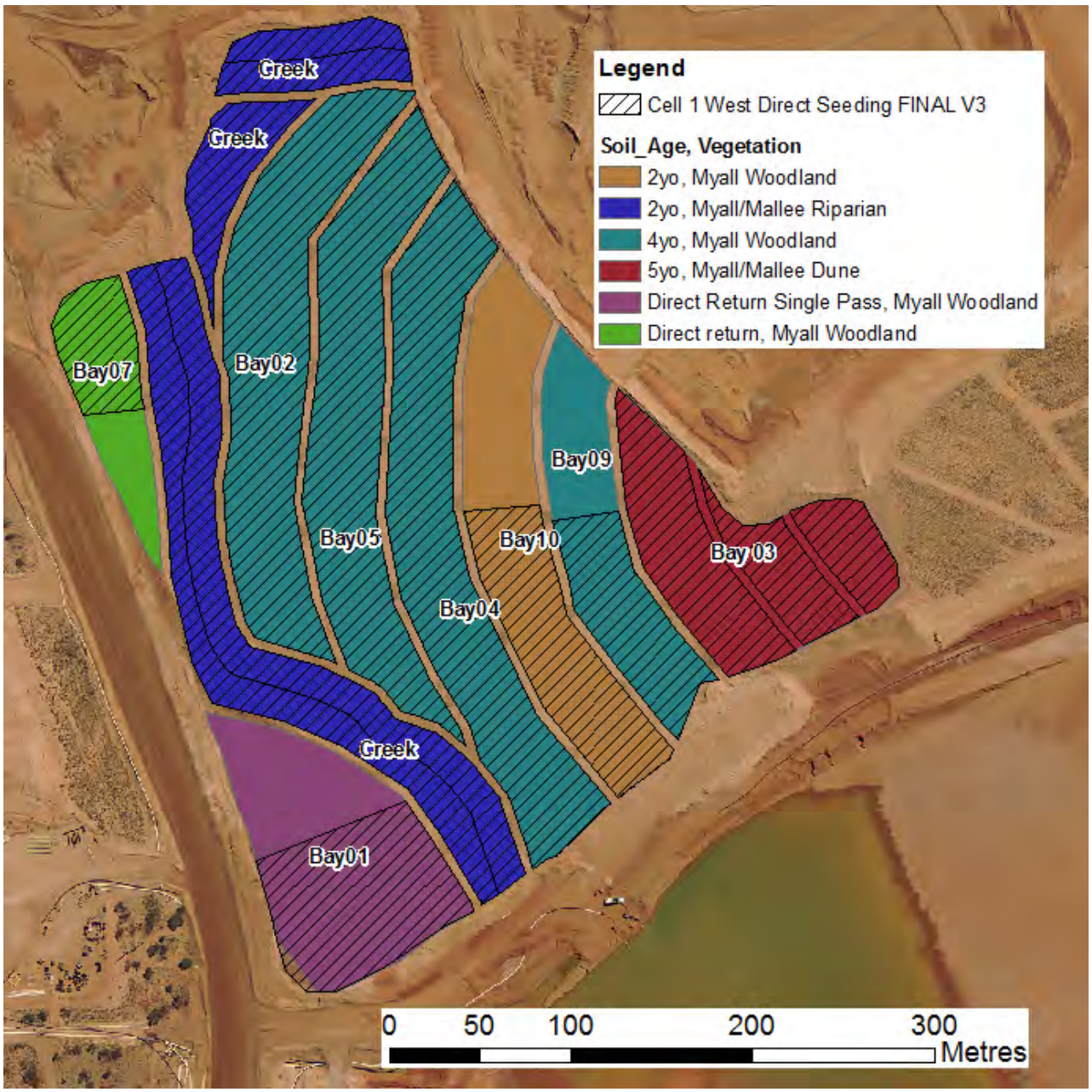

Figure 1 Plan view of the Cell 1 West bays (areas) of rehabilitation completed in 2014 showing the bay's soil age and vegetation type, bay's with topsoil direct returned and direct returned in a single pass, as well as the areas with direct seeding (seed broadcast by hand)

Plate A Photo of Cell 1 West taken 14/7/15 with Bay 1 in the background, Bay 10 in the foreground

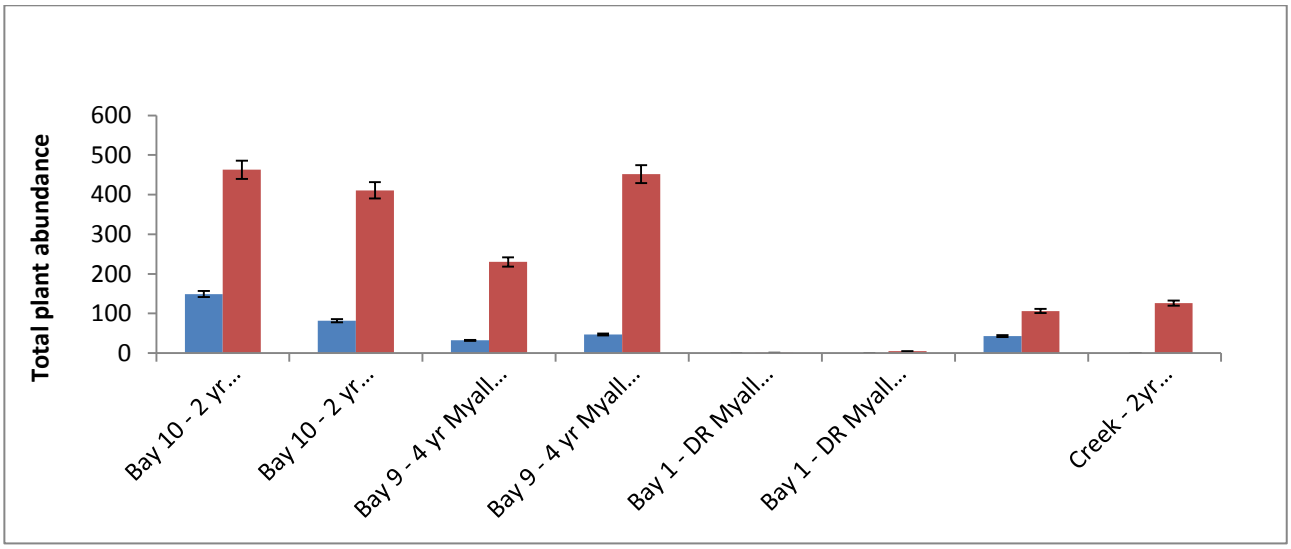

Figure 2 Total plant abundance of Cell 1 West trial bays measured in Jessop transects surveyed Nov 2014 & Nov 2015

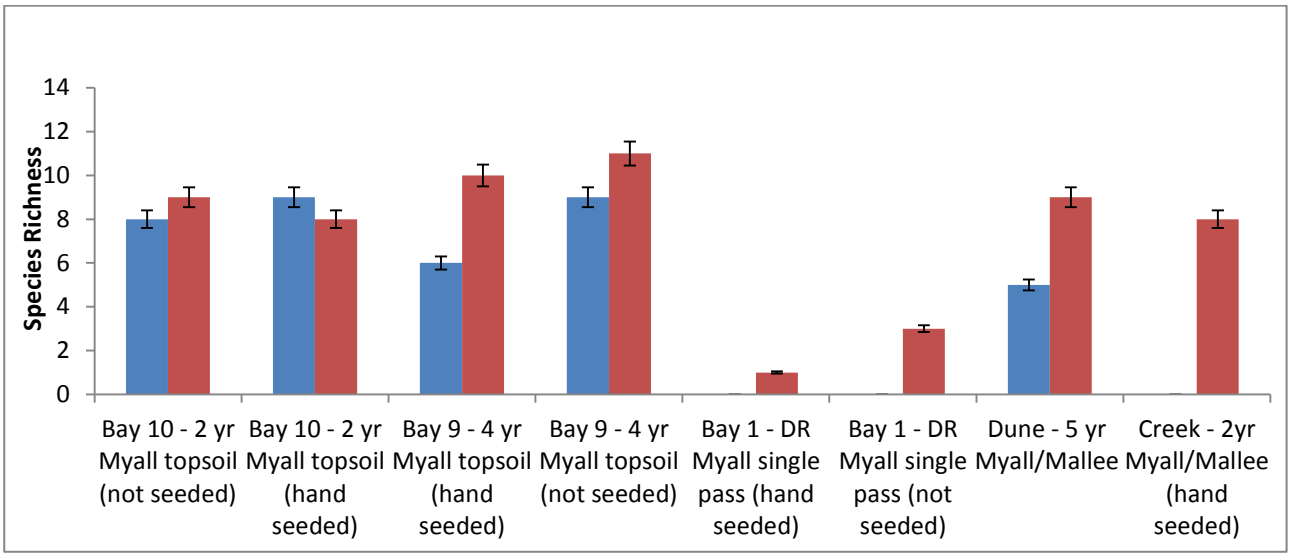

Figure 3 Total species richness of Cell 1 West trial bays measured in Jessop transects surveyed Nov 2014 & Nov 2015

Sequence of Rehabilitation

The sequence of events — topsoil/subsoil placement, ripping and significant rainfall — during the 2014 rehabilitation of the Cell 1 West bays could be a critical factor in explaining the reduced plant establishment in Bay 1. That sequence has been carefully reconstructed from notes, diary entries and photographs and is illustrated in Figure 4.

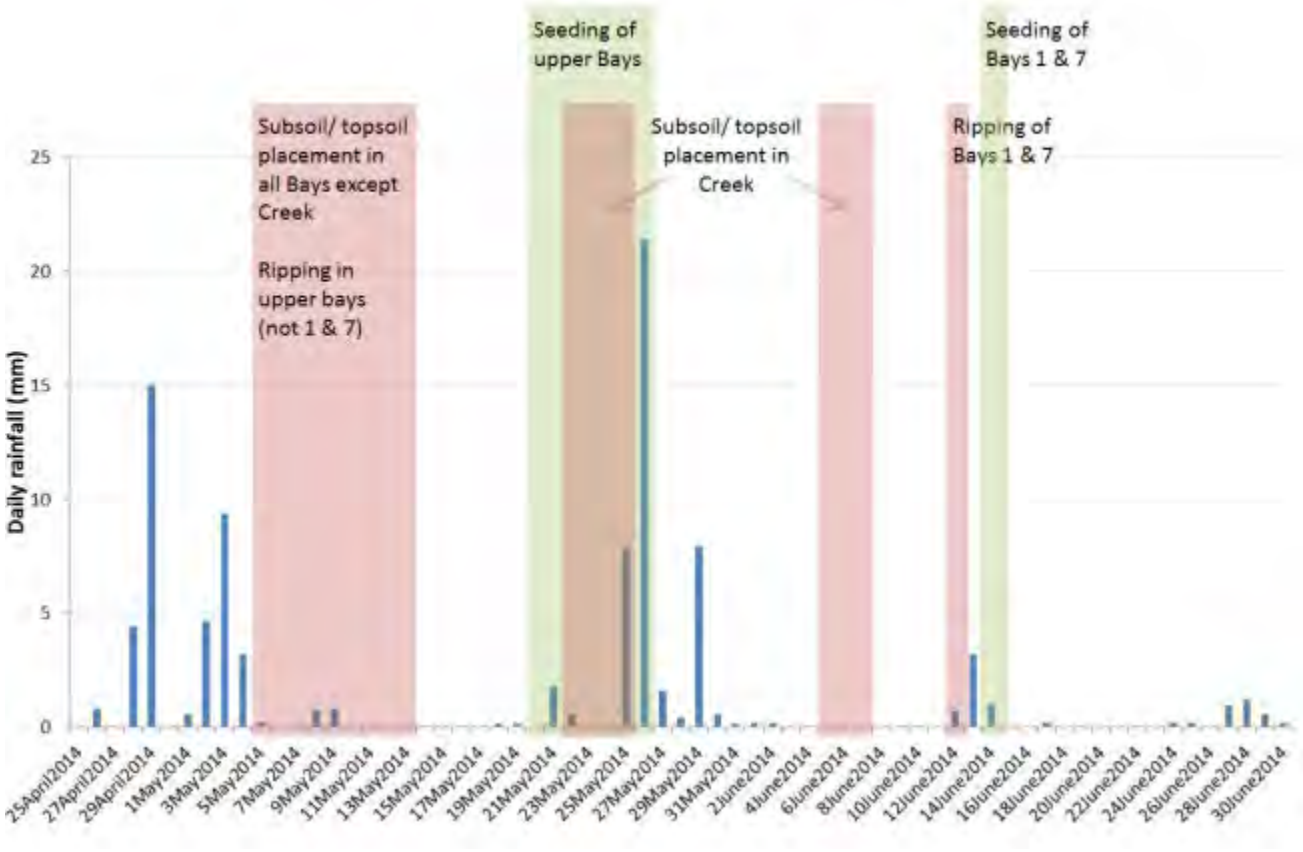

Figure 4 The timeline of 2014 rehabilitation events in Cell 1 West overlaying the daily rainfall

This sequence shows that although all subsoil and topsoil placement had occurred prior to the 25–29 May rainfall event, ripping and seeding of Bays 1 and 7 did not occur until ~12–14 June, well after that event. The 25-29 May rainfall event was the last significant rainfall that occurred in the cool season of 2014; June to September of 2014 were particularly dry and the ~30 mm of October rainfall fell too far into the warm season to be effective for plant germination and establishment (Figure 5).

It is worth appreciating the pronounced affect that rainfall timing and total rainfall have on overall rehabilitation success at J-A (apart from Bay 1). Cell 1 East rehabilitation established in April–May 2013 received good winter rains (~100 mm in May–August 2013) allowing excellent establishment and since then has received 331 mm over 2.25 years growth (to end July 2015). In comparison Cell 1 West rehabilitation established in May 2014 received only one good winter rainfall event (~40 mm in the 25–29 May event, 63 mm in total May–August 2014) allowing only sparse establishment and since then has received 109 mm over 1.25 years growth (to end July 2015).

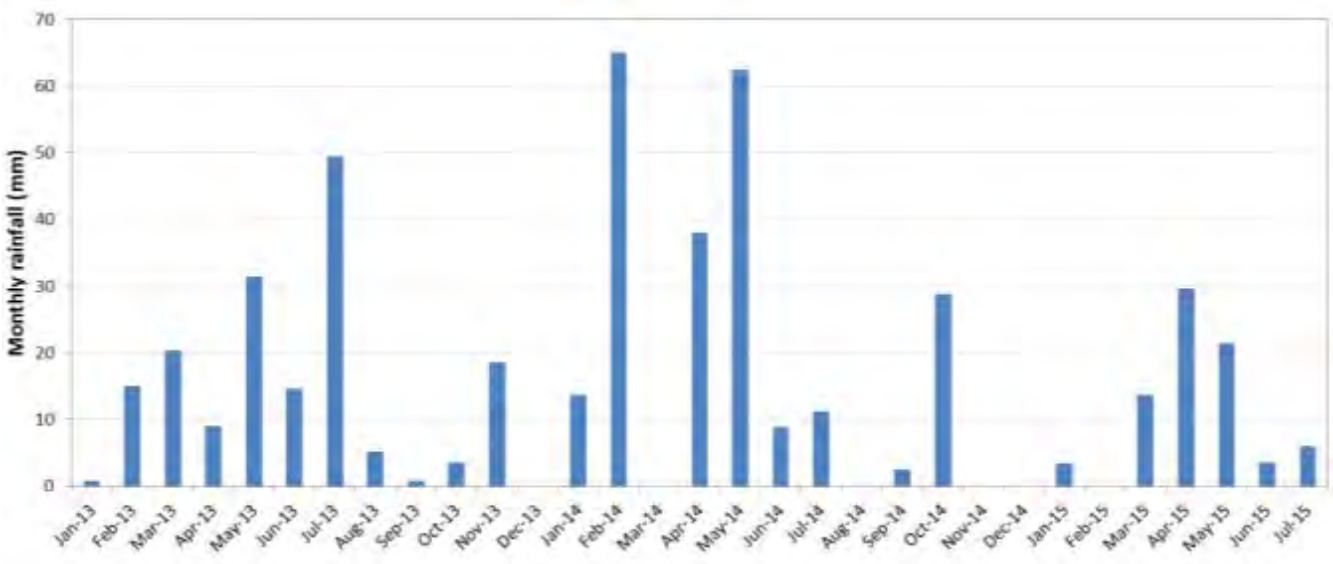

Figure 5 Monthly rainfall 2013–2015 at the J-A weather station

General Observations

General observations, made on 22 August 2015, of plant establishment and soil surface characteristics are presented below.

- Bay 1 had few to nil plants established, with the exception being seedlings around wood debris that appear to have germinated in soil brought in with that debris. The surface crust was a deeper red colour than other bays (e.g. Bay 4 and 10) and the soil strength when dry was ‘strong’ compared to only being ‘firm’ in other bays.
- Bay 7 had few plants established, although notably more than Bay 1 and they were not all associated with wood debris.
- The Creek areas had few plants established, similar to Bay 1, although these areas were not ripped extensively to reduce water erosion and down the slope of the waterway.
- Other bays (e.g. Bay 2, 4, 5, 10) had considerable plant establishment and a surface crust more brown in colour than Bay 1, and a soil strength when dry that was ‘firm’.

Since these observations were made there has recently been a germination response recorded in the trial plots due to recent winter rainfall. The number of seedlings recorded in the plots on 4 September 2015 (prior to additional watering) ranged from nil to 78, indicating that a lack of rainfall may have been an important limiting factor. However this does not explain the differences between Bay 01 and Bay 07 and investigations will continue to determine causal factors.

Analysis of Potential Factors Affecting Plant Establishment

Although the timing of- and total rainfall can explain the pronounced difference in plant establishment between Cell 1 East and Cell 1 West rehabilitation, it does not explain differences between bays within Cell 1 West. All bays (except the creek) had both topsoil and subsoil placed 5–13 May prior to the most significant rainfall event in winter of 2014, 25–29 May (40 mm), so difference among the bays are not due to missing that rainfall, at least for topsoil stored seed.

Missing that 25–29 May 2014 rainfall event may have had some effect on the germination of broadcast seed, given areas of Bays 1 and 7 were direct seeded after than last significant rainfall event in winter of 2014 (Figure 4). There was no notable difference in areas that were direct seeded in Bays 1 and 7 (Figure 1) compared to those areas that received no additional seed. This seed is therefore likely awaiting rain to germinate and the good August 2015 rainfall (~35 mm on 23 August) has resulted in plant establishment.

Ripping of Bays 1 and 7 five weeks after their placement, rather than immediately after like all other bays, may some of the reduced plant establishment. The dozer may have disturbed or crushed germinating seedlings and/or resulted in greater compaction of the soil in these bays due their being wet following the 25–29 May rainfall event. This could have depleted the seed resource of the topsoil of these areas. However, this does not fully explain the observed plant establishment, which is comparatively good in Bay 7 compared to Bay 1 (plants virtually absent), although no survey data are available for Bay 7 (a Jessop transect will be established in Bay 7 in 2015 for comparison). Also, compaction would only affect later plant survival rather than initial germination and establishment, so the almost complete lack of even small plants establishing then dying (a likely lack of germination) seen in Bay 1 is not consistent with this scenario. A survey using a penetrometer across Bays 1, Creek, 4, and 10 on 22 August 2015 (essentially in a line from background to foreground through the photograph in Plate A) showed that the subsurface compaction pattern was similar across all bays: little resistance in rip lines and compaction at 0.2–0.3 m along dozer tracks. The surface crust was stronger in Bay 1 than other areas as noted in general observations.

The return of topsoil in Bay 1 in a single pass may have buried the soil seedbank and led to the reduced plant establishment. This could explain the difference in plant establishment between Bay 1 and Bay 7, given topsoil for Bay 7 was from the same source but direct returned in two separate soil profiles, thus not likely to have buried the soil seedbank. There would be an expectation, however, of some seed germinating and plants establishing in Bay 1 if topsoil return in a single pass was the only factor of influence, rather than the almost complete absence of plants in Bay 1. This suggests the topsoil physical and/or chemical factors may also have reduced the plant establishment in Bay 1.

The topsoil of Bay 1 and Bay 7 was direct returned from the same area, most likely a soak area located between dunes (Figure 6). Vegetation in this area was sparse and dominated by grass and herbaceous species. It may naturally have had fewer seed in the soil seedbank because of this vegetation. However, seed was broadcast in Bays 1 and 7, so recent August 2015 rainfall indicate the soil seedbank was not a limitation. Soaks can also have soil conditions that limit seed germination and plant establishment, including containing more clay-sized particles, being sodic, and containing higher amounts of boron. A higher clay fraction could result in hard-setting soil that inhibits seedling emergence, higher sodicity can also cause excessively strong soil crust, and high boron content in soils can inhibit germination. The observed soil strength in Bay 1 compared to Bays 4 and 10 is consistent with the physical limitations to seed germination/emergence however

recent germination make this unlikely. However, soil sampling and analysis is required to answer if these limitations are reducing plant establishment.

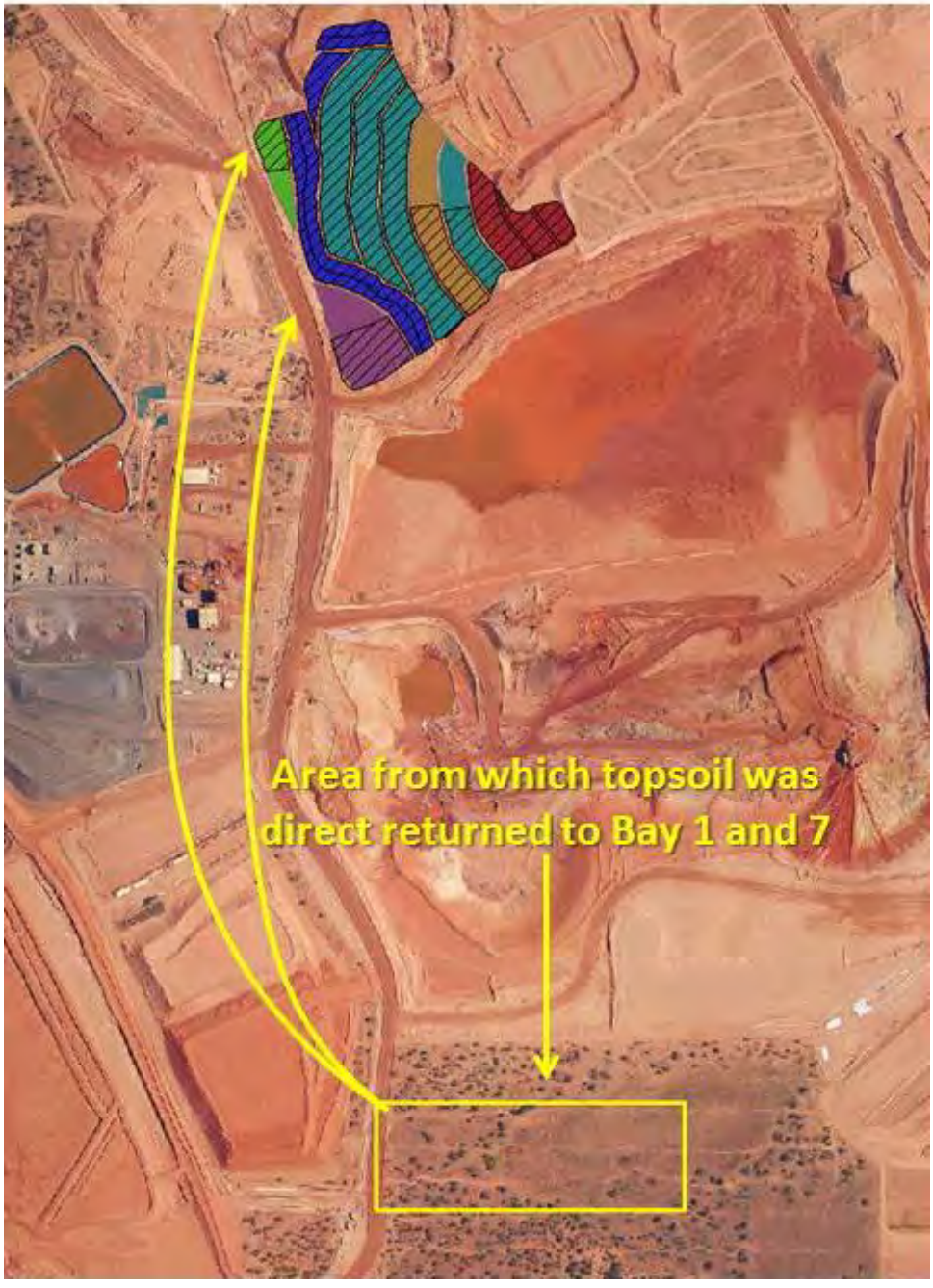

Figure 6 2013 aerial photo of J-A showing soak located between dunes from which topsoil was direct returned to Bays 1 and 7 of Cell 1 West rehabilitation in 2014

In summary, there are a multiple factors that potentially have resulted in the reduced plant establishment in Bay 1. These factors are listed in Table 1 below, with their possible effect or explanatory power, the further information required to assess their influence, and actions that will rectify them.

Table 1 Potential factors reducing plant establishment in Bay 1 of Cell 1 West rehabilitation

Potential factor	Possible effect/ explanation	Additional information required	Action to rectify
Rehabilitation in Cell 1 West completed behind schedule missing early May rains	Does not explain Bay 1 result Explains poorer plant establishment compared to Cell 1 East	nil	Ensure future rehabilitation earthworks are completed on schedule to ensure maximum exposure to seasonal rains
Bays 1 and 7 were ripped and seeded behind schedule missing late May rains (for broadcast seed)	Partially explains result in Bays 1 and 7	A trial of seeding/watering will determine the influence of this factor	Await good rainfall (which fell in August 2015) for broadcast seed to germinate Broadcast seed over remaining Bay 1 & 7 areas in 2016
Dozer crushed germinating seedlings in Bays 1 and 7 depleting soil seedbank	May explain Bay 1 and 7 performance compared to other bays. Does not explain difference between Bay 1 and 7	A trial of seeding/watering will determine if additional seeding will rectify this problem	Await good rainfall (which fell in August 2015) for broadcast seed to germinate Broadcast seed over remaining Bay 1 & 7 areas
Dozer spread of topsoil led to soil compaction	Subsoil compaction is unlikely to explain Bay 1 result	nil	
Single pass return of topsoil in Bay 1 buried seed	May explain some of Bay 1 result but cannot explain areas broadcast with seed	Establish Jessop transect in Bay 7 to assess single pass return of topsoil	
Soil physical/ chemical characteristics limit plant establishment	Unknown if these explain result in Bay 1 or 7	Soil analysis to determine hardsetting potential, sodicity and boron content	Dependent on soil analysis results
Soak topsoil has low soil seedbank	May explain some of Bay 1 and 7 result	Sifting of topsoil to see if seed is present	Broadcast seed over remaining Bay 1 and 7 areas

Actions Completed to Date

To date the following actions have been implemented:

- Soil sampling and analysis
- Establishment of a seeding/watering trial to investigate seed and water limitations in Bay 1.

All actions have been implemented in consultation with Principal Rehabilitation Scientist, Mark Dobrowolski.

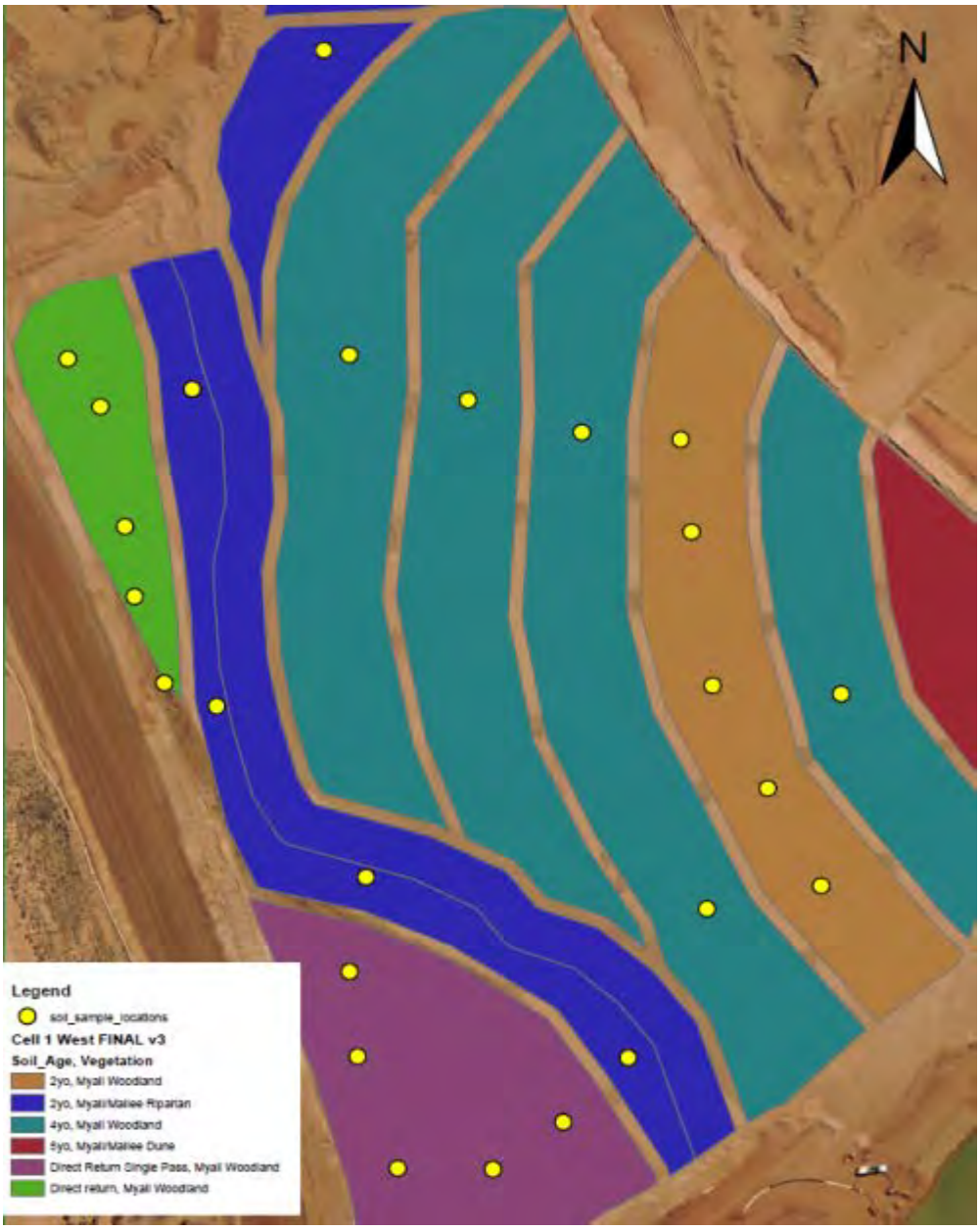

Figure 7 Soil sampling locations in the bays of Cell 1 West rehabilitation sampled 22 August 2015

Soil sampling and analysis

To determine if the soil characteristics of Bay 1 are limiting germination and plant establishment, particularly hard-setting potential, sodicity and boron content, along with other nutritional and chemical characteristics, a soil sampling strategy has been implemented.

Soils samples were collected on 22 August 2015 and forwarded to EP Analysis for analysis. Samples were collected from five treatment groups based on the source of topsoil and application method. For each treatment group, 5 composite samples were collected. Table 2 below outlines the source and method of topsoil/subsoil placement and Figure 7 shows the location of the sample points.

The results are expected to be provided in the coming fortnight.

Table 2 Sample collection areas and their soil source for the sample analysis program

Collection Area	Source of soil	Analyses to be performed
Bay 1	Direct return myall woodland Single pass (subsoil and topsoil) using a dozer to excavate, trucked to area then spread using a dozer	pH EC (1:5) & ECe organic carbon total nitrogen ammonium nitrogen nitrate nitrogen phosphorus sulfur Exchangeable calcium, potassium, magnesium, and sodium boron $CaCO_3$ particle size (sand%, silt%, clay%)
Bay 7	Direct return myall woodland Subsoil truck and dozer, topsoil truck and spread using a tractor scoop	
Bay 10	2-year old myall woodland Subsoil and topsoil trucked to area then spread using a tractor scoop (TS stockpile 22 and SS stockpile 22)	
Creek	2-year old myall/mallee riparian Subsoil and topsoil trucked to area then spread using a dozer (Ck TS stockpile 01 and Cl SS stockpiles 01 and 04)	
Bay 2 (sample 1) Bay 5 (sample 2) Bay 4 (samples 3, 4) Bay 9 (sample 5)	All 4-year old myall woodland Subsoil and topsoil trucked to area then spread using a tractor scoop (TS stockpile 20 and SS stockpile 20)	

Seeding/watering trial

Trial plots have been established in Bay 1 to determine if lack of rainfall and/or loss/exhaustion of soil seedbank has influenced lack of plant establishment. On the 24 August 2015 a series of eight 10 m × 10 m trial plots (four treatments × two replicates) were established within Bay 1 (see Figure 8).

The treatments applied were:

- Hand seeding
  A seed mix incorporating species previously identified as having rapid germination responses (within 5- 10 days) was applied by hand on 25/8/15. The seed mix applied to each trial plot contained *Austrostipa nitida* (7.2 g); *Atriplex vesicaria* (10 g); *Maiereana turbinata* (0.6 g) and *Zygophyllum ovatum* (5 g).

- Hand watering
  Trial plots are to be artificially watered on a weekly basis with potable water. One thousand litres of water per trial bay is an approximate volume to simulate a 10 mm rain event, which allows for approximately ~50 mm of water penetration into the soil profile (based on recent field observations).

- Hand seeding and hand watering
  The combination of the two treatments above.

- Control
  No treatments applied.

At the commencement of the trial the number of visible germinates in each trial plot was recorded by walking three transects within each plot. In addition a photo point has been established at each plot. A record of visible germinates and a photo record will be captured every week for four weeks.

Upon conclusion of the four week period a comparison will be made to determine if there is a notable variation in germination for each of the treatments applied.

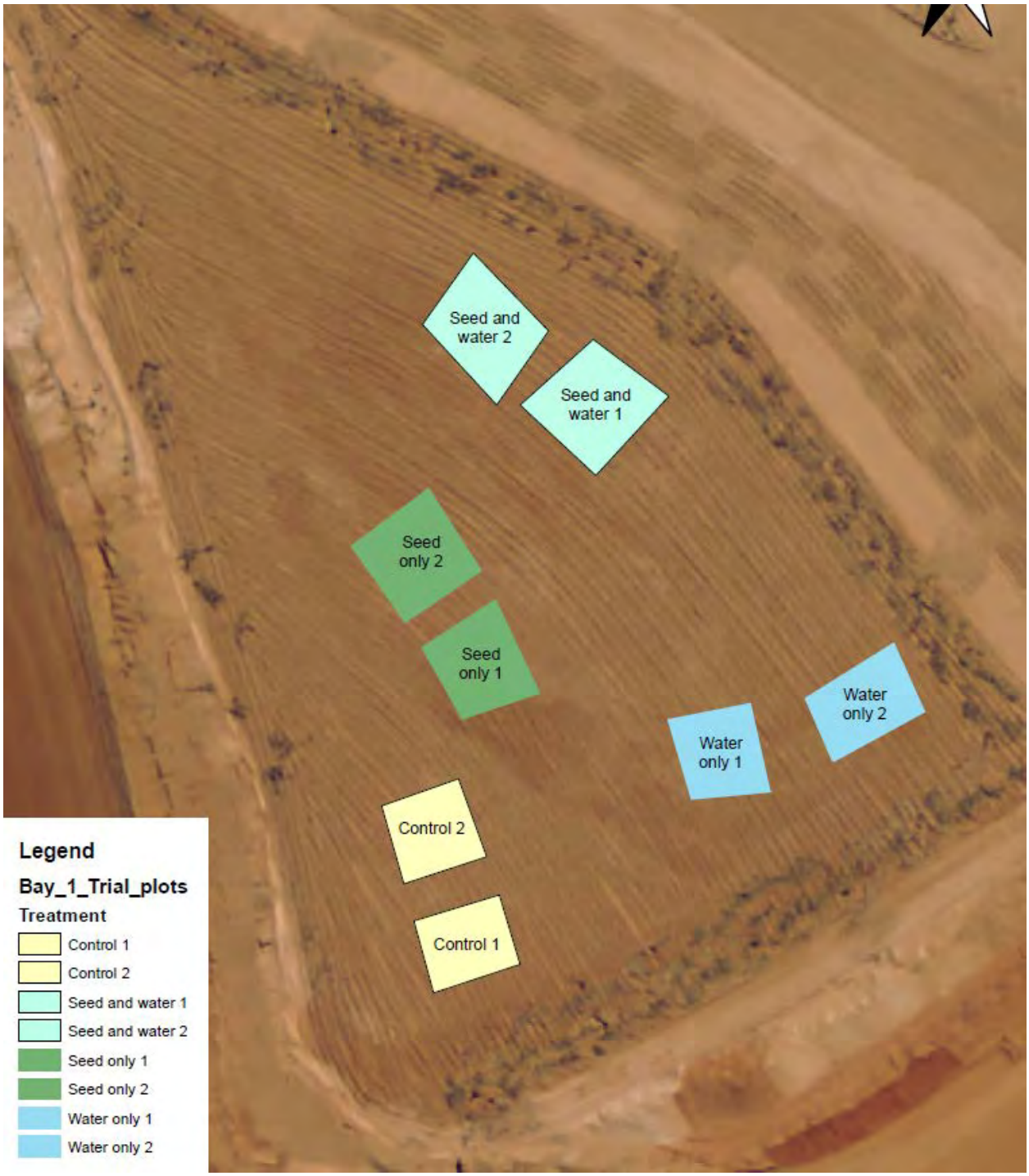

Figure 8 Hand seeding/watering trial plots in Bay 1

Results

Trial was established on 28.8.15 and number of germinates present within treatment plots measured as a baseline. The total number of germinates in each plot was measured again on 4.10.15.

Plots were watered weekly between commencement of trial and final measurement and 3mm of rainfall was recorded during the five week period (max. 1.2mm in 24 hr).

Figure 9 shows the results of the trial. The greatest increase in germinates was recorded in the Water A plot, Seed A also showed a considerable increase in germinates. No germinates were recorded in Control B for the whole trial.

It is not possible to make assumptions on such a limited trial however the addition of water and/or seed was shown to improve plant densities in the plots that received a treatment.

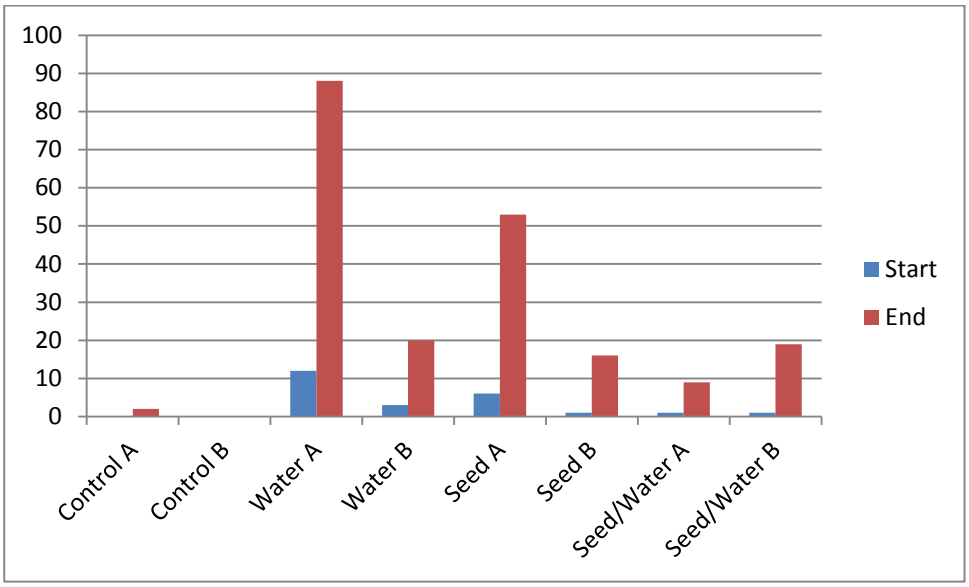

Figure 9 Results of seeding/watering trial

Update

Soil analysis results were received on 25.9.15 and were reviewed by Principal Rehab Scientist Mark Dobrowolski. Results can be found at: I:\JA\B - ERCR\08 - Rehabilitation & Closure\File Notes\2015\Cell 1 West\EP Analysis soil results.xslx

A comparison between Bay 1 and four other Bays showed no significant variance in soil properties that would affect plant growth. Variation in properties was shown across all samples.

The receipt of low rainfall, and potentially a low seed bank are likely factors influencing the poor germination in Bay 1, as hypothetically demonstrated in the results of the trial. The current plan of action is to hand seed Bay 1 extensively in February 2016, prior to the commencement of the rain season. The seed mix will consist of species that are known to have a rapid germination response and have been observed to set seed readily within the other rehabilitation areas. This action is hoped to assist in increasing the density of plant species in this Bay.

Two Jessop transects and vegetation density quadrats are established in the Bay. These will continue to be monitored on an annual basis.

Appendix 4 Cell 1 rehabilitation trials 2014 – 2015

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JA Cell 1 Rehabilitation Trials

2014 - 2015

DOCUMENT CONTROL

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1 Introduction

In 2013 the first of the in-pit rehabilitation areas was completed with the reshaping of the landform, replacement of overburden, ripping and seeding at Cell 1 East. As part of the replacement of the topsoil the opportunity was taken to trial different rehabilitation techniques. This included using different aged topsoils to determine if the viability of the soil seed bank varied with age and also a comparison between direct seeding methods.

In 2014, Cell 1 West was established in a similar manner to that of Cell 1 East to allow confirmation and comparison of results received in the previous trials. It should be noted that the source of the majority of the soil in Cell1 West is from myall woodland vegetation association (myall), with all of Cell 1 East comprising of myall/mallee woodland (mallee).

The trials comprise:

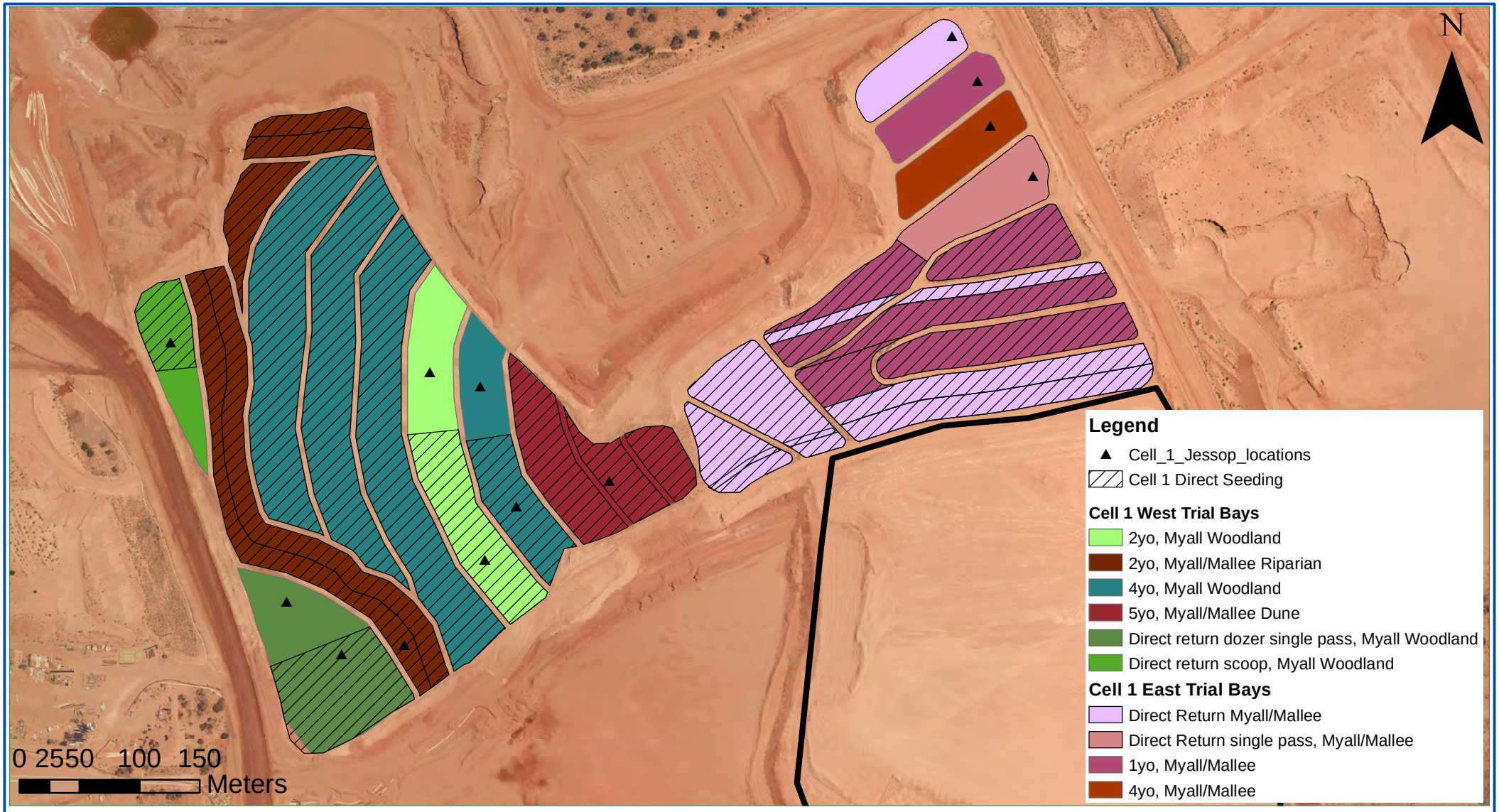
- Comparison of species diversity and abundance for subsoil and topsoil that has been stockpiled for various time periods and subsoil and topsoil directly returned from the mine path clearance i.e. not stockpiled.
- Comparison of species diversity and abundance when topsoil and subsoil is cleared and applied as a single unit, as opposed to two distinct layers.
- Comparison of species diversity and abundance when direct seeding is carried out in comparison to areas where germination is from the soil seed bank only.
- Comparison of soil replacement techniques (carry grader or paddock dumping/dozer push), this is in Cell 1 West only.

2 Trial Design and Monitoring Method

Cell 1 East contains four trial bays (Figure 1). Two trial bays were prepared using different aged topsoil and subsoil i.e. one year old and four year old and two were prepared using different methods of direct returned soils i.e. single pass (topsoil and subsoil collected together) and separation of subsoil and topsoil profiles.

Cell 1 West replicates the four trial bays, using two year old and four year old topsoils and two related to different methods of direct returned soils as in Cell 1 East. However the single pass in this Cell was returned using a dozer rather than the scoop that was used in Cell 1 East. Two additional areas are monitored in the myall/mallee in the riparian and dune areas.

A summary of treatments is provided in Table 1.



Jacinth - Ambrosia
Cell 1 Rehabiltation Trials

ILUKA

FIGURE: 1

ORIG: T Law DRAWN: T Law SCALE: 1:4,468 DATE: March 2016

Table 1 Summary of rehabilitation trial treatments

Site ID	Location	Vegetation Type	Soil age	Seeded	Soil application method
C1E_1YO	Cell 1 East	Mallee	1	No	Tractor scoop
C1E_4YO	Cell 1 East	Mallee	4	No	Tractor scoop
C1E_DR	Cell 1 East	Mallee	Direct return	No	Tractor scoop
C1E_DR1PAS	Cell 1 East	Mallee	Direct return single pass	No	Tractor scoop
C1W_2YO	Cell 1 West	Myall	2	Yes / no	Tractor scoop
C1W_4YO	Cell 1 West	Myall	4	Yes / no	Tractor scoop
C1W_DR	Cell 1 West	Myall	Direct return	Yes / no	Tractor scoop
C1W_DRPas	Cell 1 West	Myall	Direct return single pass	Yes / no	Dozer
C1W_2YO_MalRip	Cell 1 West	Mallee (dune)	2	No	Tractor scoop
C1W_5YO_MalDun	Cell 1 West	Mallee (riparian)	5	No	Tractor scoop

Monitoring is completed in each cell on an annual basis and includes the following:

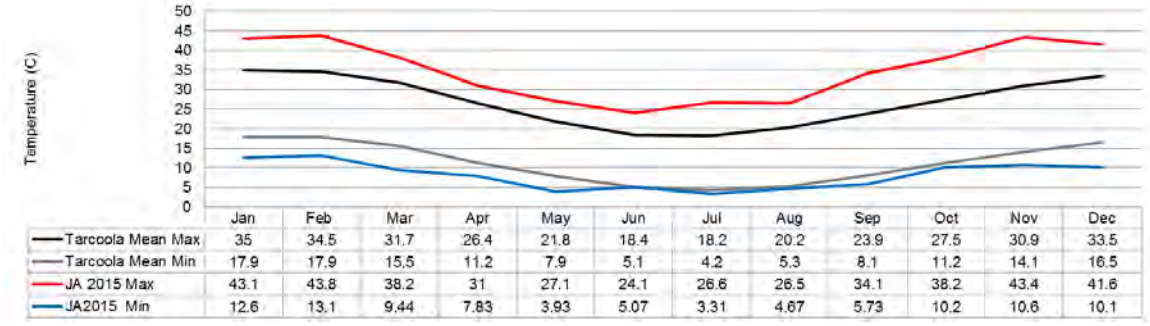
- Quadrats: Based on the Biological Survey of South Australia method (Heard and Channon, 1997) this survey enables an assessment of the composition and cover of flora species within the rehabilitated vegetation.
- Jessop Transects: Installed along the middle axis of the monitoring quadrats. All species that occur within a 1m of the centre line are recorded for each metre of the transect line.
- Photographic Monitoring Points: Photograph frames are aligned along the Jessup transects (provided in Appendix 1).

Figure 1 Layout of Cell 1 Rehabilitation Trials

3 Results

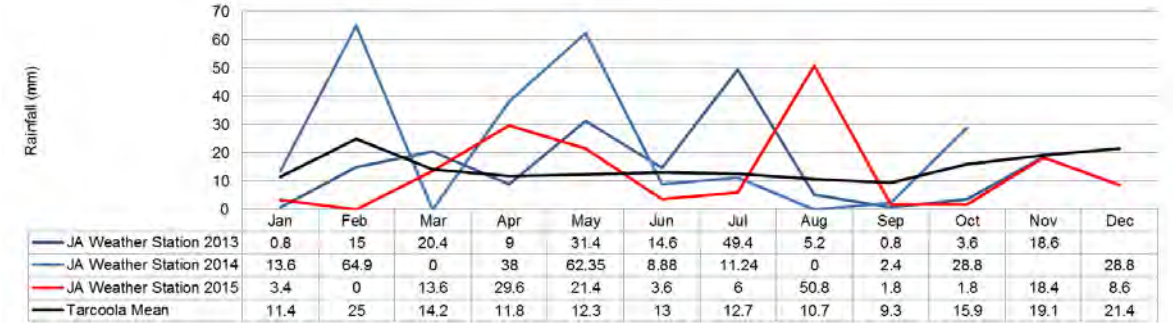
3.1 Climate

Overall the climate at JA was hotter and drier in 2015 than the long term average (Figure 4 and Figure 5). Temperatures were consistently warmer than the long term average (Figure 4). Rainfall at JA for 2015 was the lowest recorded for the past three years, and slightly than the long term average for Tarcoola (JA – 159 mm, Tarcoola mean – 176.8 mm). Winter rains arrived later than usual, the highest monthly rainfall was recorded in August, Figure 4.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Tarcoola Mean Max	35	34.5	31.7	26.4	21.8	18.4	18.2	20.2	23.9	27.5	30.9	33.5
Tarcoola Mean Min	17.9	17.9	15.5	11.2	7.9	5.1	4.2	5.3	8.1	11.2	14.1	16.5
JA 2015 Max	43.1	43.8	38.2	31	27.1	24.1	26.6	26.5	34.1	38.2	43.4	41.6
JA2015 Min	12.6	13.1	9.44	7.83	3.93	5.07	3.31	4.67	5.73	10.2	10.6	10.1

Figure 2 Monthly temperatures for JA weather station and Tarcoola monthly long term average



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
JA Weather Station 2013	0.8	15	20.4	9	31.4	14.6	49.4	5.2	0.8	3.6	18.6	
JA Weather Station 2014	13.6	64.9	0	38	62.35	8.88	11.24	0	2.4	28.8		28.8
JA Weather Station 2015	3.4	0	13.6	29.6	21.4	3.6	6	50.8	1.8	1.8	18.4	8.6
Tarcoola Mean	11.4	25	14.2	11.8	12.3	13	12.7	10.7	9.3	15.9	19.1	21.4

Figure 3 Monthly rainfall for JA weather station and Tarcoola monthly long term average

3.2 Species Richness

A total of 34 native vegetation species have been recorded in the mallee vegetation rehabilitation monitoring sites, Table 1, in comparison to 36 species recorded in the analogue sites. For the myall vegetation association 26 species have been recorded in the rehabilitation monitoring sites, Table 1, compared to 38 species in the myall analogue sites. A complete table of species presence absence in rehabilitation and analogue sites is provided in Appendix 2.

Generally species richness increased with time across all rehabilitation treatments, Figure 3 and Figure 4. In Cell 1 East (mallee vegetation association) the direct return topsoil treatment had consistently greater species richness than all other treatments. The direct return single pass had the lowest recorded species richness across all treatments. Similarly the direct return single pass treatment had the lowest species richness for all myall treatments. However, the direct return topsoil treatment in Cell 1 West (myall vegetation association) had the second lowest species richness. It is important to note that the direct return and direct return single pass treatments for Cell 1 West were subject to much less rainfall than other Cell 1 West treatments, further discussion is provided in *Reduced Plant Establishment in Bay 1 of Cell 1 West – Investigation and Actions* (JARMS, 2015).

The aged topsoil treatments all recorded similar species richness regardless of vegetation association type.

Table 2 The number of rehabilitation monitoring sites native vegetation species were recorded (2013 – 2015).

	Mallee			Myall	
Species	**2013**	**2014**	**2015**	**2014**	**2015**
Acacia papyrocarpa	1	1			
Atriplex vesicaria	4	6	6	5	6
Rytidosperma caespitosum		2	1		1
Austrostipa nitida	4	5	5	4	6
Calotis hispidula	2			3	
Cephalipterum drummondii	1	1	2	3	
Chenopodium curvispicatum	4	4	6	4	5
Enchylaena tomentosa		4	2	2	3
Eremophila sp.		1			
Eriochiton sclerolaenoides	4	4	5	4	6
Eucalyptus oleosa					4
Euphorbia drummondii			2		
Euphorbia tannensis			1		
Lepidium phlebopetalum	3	1	2	2	3
Maireana erioclada		3	2	1	1
Maireana pentatropis	4	2	2	3	
Maireana radiata	4	4	5	2	4
Maireana sedfolia		1	1		
Maireana trichoptera			2		
Rhagodia parabolica		2			
Rhodanthe floribunda					5
Rhodanthe stuartiana	2	3	3		1
Salsola australis	4	6	6	5	6
Sclerolaena brevifolia			2		4
Sclerolaena diacantha		3	3		
Sclerolaena obliquicuspis		1	2		6
Sclerolaena sp.	4	3	1	5	
Sida ammophila			1		
Stenopetalum lineare			5	1	4
Tetragonia eremaea		1	1	4	5
Tetragonia implexicoma			1		
Vittadinia cervicularis			1		1
Zygophyllum apiculatum	2	1	1		
Zygophyllum aurantiacum	4	3	3	3	2
Zygophyllum erantiacum			1		
Zygophyllum eremaeum	4		1		1
Zygophyllum ovatum	4	6	6	4	5

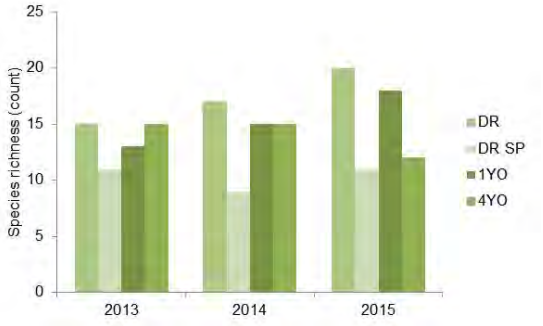


Figure 4 Species richness recorded in quadrats per year for topsoil treatments for Cell 1 East (mallee vegetation association)

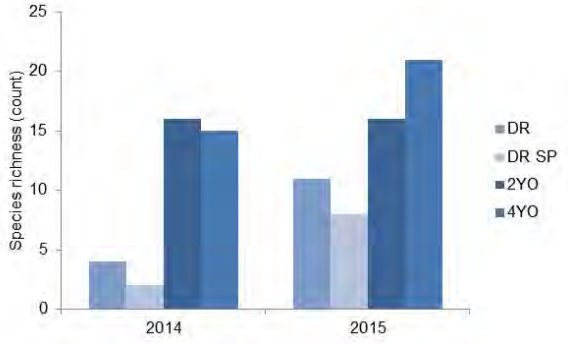


Figure 5 Species richness recorded in quadrats per year for topsoil treatments for Cell 1 West (myall vegetation association)

3.3 Vegetation Abundance

Overall vegetation abundance increased with time across all treatments, Figure 5 and Figure 6. Vegetation abundances were generally similar across all mallee treatments in 2013 and 2014. In 2015 abundances increased considerably across all treatments, however the direct return and 4 year old soil treatments recorded the highest abundances. The direct return and aged topsoil material (2 year old and 4 year old) recorded similar abundances to the mallee aged treatments in the first year of rehabilitation (2014). Abundances recorded increased considerably for the aged topsoil treatments but remain similar for both direct return treatments in 2015. Very little vegetation has been recorded in the direct return single pass treatments; see *Reduced Plant Establishment in Bay 1 of Cell 1 West – Investigation and Actions* (JARMS, 2015).

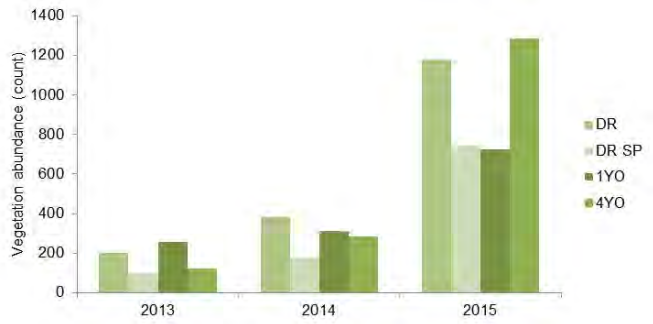


Figure 6 Total vegetation abundance recorded in Jessops per year for topsoil treatments for Cell 1 East (mallee vegetation association)

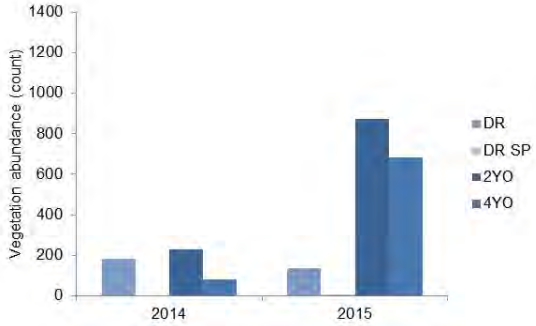


Figure 7 Total vegetation abundance recorded in Jessops per year for topsoil treatments for Cell 1 West (myall vegetation association)

3.4 Species Abundance

Higher abundances of some species were recorded at rehabilitation locations in comparison to analogue sites, Table 2:

- The annual *S australis*[1] was recorded in high numbers across both vegetation associations. Higher abundance was recorded in the mallee vegetation association, and with abundances increasing over time in both vegetation associations.
- The grass *A nitida* was recorded in high numbers across both vegetation associations. Higher abundance was recorded in the mallee vegetation association, and with abundances increasing over time in both vegetation associations. There is only a single record of *A nitida* at an analogue site (chenopod) in 2009.
- The perennial shrub *M. radiata* was recorded in high numbers across both vegetation associations. Higher abundance was recorded in the mallee vegetation association, with abundance increasing over time in both vegetation associations. *M radiata* has been recorded at analogue vegetation associations consistently across all monitoring periods in low to moderate numbers.
- The annual *Z. ovatum* has been recorded at consistently high numbers in both rehabilitated vegetation associations in comparison to analogue sites. *Z ovatum* has been recorded at all analogue locations however was only recorded in 2010.

A number of species were recorded in lower abundances (or absent) from Jessops in the rehabilitation monitoring sites in comparison to analogue sites, Table 3:

- The long lived perennial shrub *Maireana sedifolia* has not been recorded in any rehabilitation locations. This species is generally in high densities in all undisturbed vegetation associations.
- The perennial shrub *Atriplex vesicaria* has been recorded in moderate numbers in all rehabilitated vegetation associations; however it is one of the most common species in the myall and chenopod vegetation associations.
- The low spreading shrub *Zygophyllum aurantiacum* has been recorded in low numbers in both the myall and mallee rehabilitation areas. This species is relatively common in the analogue mallee sites.
- *Maireana trichoptera*, a perennial herb, is found in moderate numbers in the myall vegetation association, however has only been recorded at the mallee rehabilitation sites in similar numbers to the mallee analogue sites.
- *Maireana erioclada* is found in moderate numbers in the myall vegetation association, however has only been recorded at the mallee rehabilitation sites in similar numbers to the mallee analogue sites.

Notably a number of the larger, longer lived shrubs have not been recorded in the rehabilitation trials.

[1] Previous name *S. kali* was misapplied to Australian material and *S. tragus* has under recent molecular studies been identified as *S. australis* R.Br. Ref: Chinnock, R.J. (2010).

Table 3 Mean count of native species abundance in Jessops for the mallee and myall vegetation associations

Species	Mallee			Myall	
	2013	**2014**	**2015**	**2014**	**2015**
Atriplex vesicaria	10.25	14.5	12.75	11.25	11
Rytidosperma caespitosum		5.25			
Austrostipa nitida	23.5	139.5	148.75	53.75	237
Calotis hispidula			0.75	0.25	
Cephalipterum drummondii	1.75	1	13.5	0.25	
Chenopodium curvispicatum	5.75	5.5	3.25	5	3
Enchylaena tomentosa		1.25	1.25		0.5
Eriochiton sclerolaenoides	2	3.75	4	2.25	5.25
Erodium sp					0.25
Eucalyptus oleosa					0.25
Euphorbia drummondii			0.5		
Lepidium phlebopetalum	0.5		17.5		0.25
Maireana erioclada		2.5	2		
Maireana pentatropis	3.75			0.25	
Maireana radiata	6.75	7.5	70.5		2.5
Maireana trichoptera	1.25		5.25		
Rhagodia parabolica		0.25			
Rhodanthe stuartiana		0.5	9.75		
Salsola australis	44	36	462.25	12.25	70.75
Sclerolaena brevifolia			1		0.25
Sclerolaena diacantha		0.25			
Sclerolaena obliquicuspis			3.75		15
Sclerolaena sp.	12.5	15.75	101	0.5	
Sida ammophila			0.5		
Stenopetalum lineare			1.5		
Tetragonia eremaea			0.5	1.5	12.75
Tetragonia implexicoma			0.5		
Vittadinia cervicularis			0.5		0.25
Zygophyllum apiculatum	0.5		0.25		
Zygophyllum aurantiacum	16	4.5	0.5	0.25	
Zygophyllum eremaeum	0.5		1.5		0.25
Zygophyllum ovatum	31.75	59.5	88.75	35.25	30

Table 4 Standardised average density (per Jessop) of species in analogue vegetation associations

Species	Chenopod	Mallee	Myall
Atriplex vesicaria	28.07	7.55	22.13
caespitose Rytidosperma caespitosum	3.40		
Chamaesyce drummondii			0.02
Chenopodium curvispicatum		0.12	1.05
Enchylaena tomentosa		0.22	0.63
Enneapogon avenaceus	0.63		
Eremophila scoparia		0.38	
Eriochiton sclerolaenoides			1.13
Frankenia serpyllifolia			0.01
Lycium australe			0.05
Maireana erioclada	0.02	0.58	2.74
Maireana pentatropis		0.40	0.02
Maireana radiata		0.32	0.29
Maireana sedifolia	5.33	2.16	2.13
Maireana trichoptera	0.09	0.43	3.82
Maireana turbinata	0.57	0.25	0.85
Minuria cunninghamii	0.06		0.07
Ptilotus obovatus		0.08	
Rhagodia candolleana argentea		0.79	0.27
Rhagodia spinescens		0.27	
Rhagodia ulicina			0.24
Salsola australis		2.83	0.83
Santalum acuminatum		0.30	
Scaevola spinescens		0.18	
Sclerolaena diacantha	7.63	0.31	0.16
Sclerolaena obliquicuspis	32.77	0.52	2.79
Senna artemisioides coriacea			0.02
Senna artemisioides petiolaris		0.06	
Senna cardiosperma gawlerensis		0.01	
Zygophyllum apiculatum		0.62	
Zygophyllum aurantiacum		1.85	0.07
Zygophyllum eremaeum		0.03	

3.5 Direct seeding

For myall vegetation association treatments in Cell 1 West monitoring was carried out in areas where additional seed had been applied by hand to compare with germination from the natural seedbank (no seed added). Different seed species were applied to the different vegetation associations in the cell, Table 4.

Species that were applied that have not yet been recorded in the monitoring sites include:

- *Acacia papyrocarpa*
- *Dodonea viscosa ssp. augustissima*
- *Acacia oswaldii*
- *Senna artemisioides ssp. coriacea*
- *Senna artemisioides ssp. petiolaris*

For species that did germinate there was little difference in abundances at seeded treatments compared to unseeded treatments for both perennial (Figure 7) and annual species (Figure 8). Both *E tomentosa* and *E oleosa* were recorded in un-seeded myall vegetation treatments, these species were not recorded in the seeded treatments.

Table 5 Additional seed applied to seeded rehabilitation treatments

Species	Myall	Mallee dune	Mallee riparian
Austrostipa nitida	✓	✓	✓
Atriplex vesicaria	✓	✓	✓
Maireana trichoptera	✓	✓	✓
Acacia papyrocarpa	✓	✓	✓
Salsola tragus	✓	✓	✓
Maireana radiata	✓	✓	✓
Eriochiton schlerolaenoides	✓	✓	✓
Eucalyptus oleosa ssp ampliata		✓	✓
Dodonea viscosa ssp. augustissima			✓
Acacia oswaldii			✓
Senna artemisioides ssp. coriacea			✓
Enchylaena tomentosa var. tomentosa		✓	
Senna artemisioides ssp. petiolaris		✓	

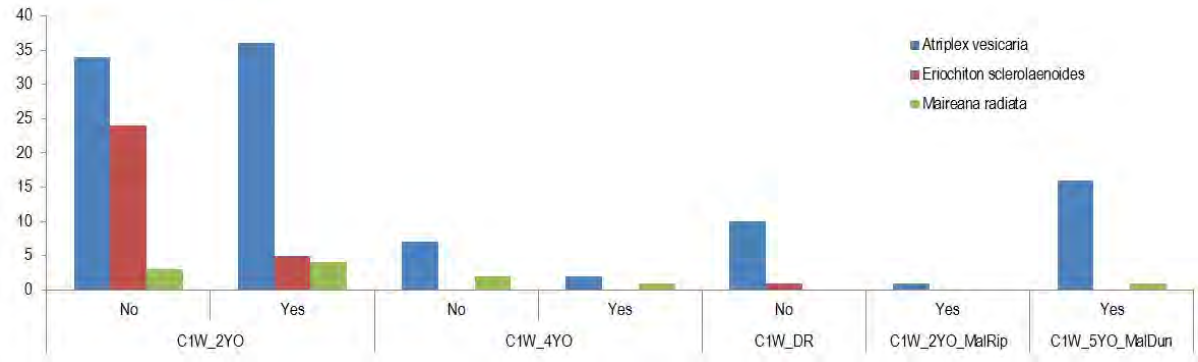


Figure 8 Perennial species abundance in Jessop transects for seeded (yes) and un-seeded (no) treatments

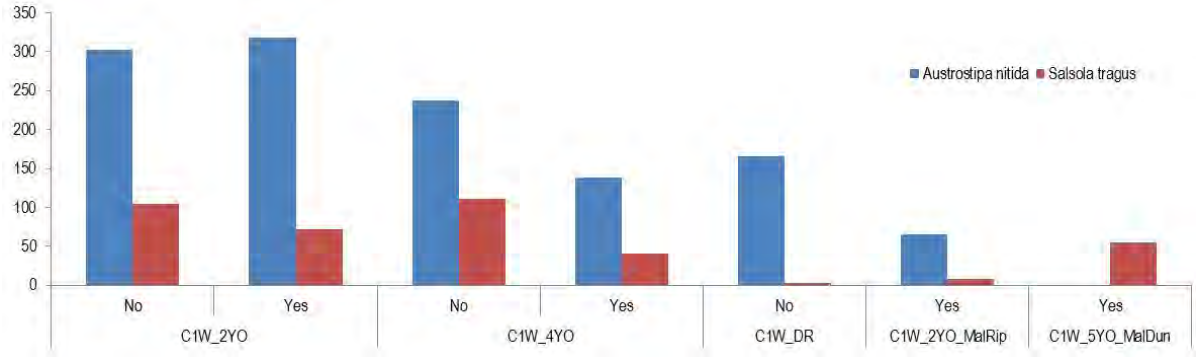


Figure 9 Annual species abundance in Jessop transects for seeded (yes) and un-seeded (no) treatments

3.6 Soil replacement methods

Monitoring locations have not yet been established in the treatments where overburden material was returned using different equipment (i.e. dozer in comparison to tractor scoop). Additional sites to will be established in 2016.

4 Discussion and Recommendations

Overall the numbers of species recorded in rehabilitation locations was less than recorded at the analogue sites. Fewer of the larger longer lived vegetation species were present in the rehabilitation sites, including Santalum, Senna, Acacia, Alectryon and notably the long lived *Maireana sedifolia* (pearl bluebush). *M sedifolia* is generally found in high densities in both the chenopod and myall vegetation associations. The absence of *M sedifolia* in the soil seedbank is likely due to a lack of viable seed set. *M sedifolia* seeding at JA has been recorded infrequently and the seed collected is often unviable. Many of the harder coat species (Santalum, Senna and Acacia) require some form of break in the seed coat to germinate, i.e. through scarifying or soaking. Although applied seed is pre-treated before planting seeds in the natural seedbank may require a large rain fall event to prompt germination. This has been noted anecdotally in Cell 1 East where *A papyrocarpa* have germinated after the large summer rainfall in 2014. For other species germination triggers may not be known, and some species are known to be particularly difficult to germinate (i.e. Alectryon and some Santalum species). Given that lack of response by some longer lived species, germination investigations (field and nursery) should be carried out to determine the efficacy of applying additional seed to rehabilitation areas and for more difficult to germinate species for nursery grown seedlings. The results of this work would form the basis of the JA Seed Management Procedure.

Importantly the richness and abundance of vegetation in the rehabilitation areas have increased with time. Although the community composition in the rehabilitation areas is currently dominated by annuals and short to moderate lived perennials it is anticipated that this will alter as the areas age. Continued monitoring will provide information about species that have the potential to be recalcitrant and measures can put in place to ameliorate impacts (i.e. additional seeding or planting of seedlings). Currently the densities of all species in undisturbed areas have not been calculated, these will be estimated to track rehabilitation performance.

The direct return of topsoil material is considered to be best practice for mine site rehabilitation as soil biology is maintained resulting in better vegetation outcomes. This has been supported here in Cell 1 East with higher diversity and abundance in the direct return treatment. The poor response in Cell 1 West is likely a reflection of the lack of rainfall received for that treatment in comparison to the aged topsoil treatments (further studies have shown increased germination with additional watering). Although direct return was the best performer in term of vegetation richness and diversity the aged topsoil treatments have also performed well and stockpiling material for up to 5 years has shown no significant negative impact on richness or diversity. However this result should be treated with caution as recent studies have shown very low seed viability for material stockpiled for 6 years (*Progress report for soil sample analysis*, JARMS 2015). Additional investigations into how the seedbank viability of stored material reflects on field germination will need to be carried out.

Additional monitoring sites will need to be established to determine any response to the different soils application techniques (i.e. dozer in comparison to tractor scoop).

Appendix 1 – Rehabilitation monitoring plates

Plate 1 C1E_DRSP 2014


Plate 2 C1E_DRSP 2015

Plate 3 C1E_4YO 2014

Plate 4 C1E_4YO 2015


Plate 5 C1E_1YO 2014


Plate 6 C1E_1YO 2015

Plate 7 C1E_DR 2014


Plate 8 C1E_DR 2015

Plate 9 C1W_DRSP 2015

Plate 10 C1W_4YO 2015

Plate 11 C1W_1YO 2015

Plate 12 C1W_DR 2015

Appendix 2 – Presence / absence data for myall and mallee analogue and rehabilitation sites.

Scientific Name	Mallee		Myall	
	Analogue	Rehab	Analogue	Rehab
Acacia papyrocarpa	+	+	+	
Alectryon oleifolius ssp. canescens			+	
Amyema quandang var. quandang			+	
Atriplex vesicaria	+	+	+	+
Rytidosperma caespitosum		+		
Austrostipa eremophila				
Austrostipa nitida	+	+	+	+
Brachyscome sp.		+	+	
Calotis hispidula		+		+
Cephalipterum drummondii		+		+
Chenopodium curvispicatum	+	+	+	+
Enchylaena tomentosa var.	+	+	+	+
Eremophila scoparia	+			
Eriochiton sclerolaenoides	+	+	+	+
Eucalyptus oleosa				+
Euphorbia drummondii	+	+	+	
Euphorbia tannensis ssp. eremophila			+	
Frankenia serpyllifolia			+	
Lycium australe			+	
Maireana appressa			+	
Maireana erioclada	+	+	+	+
Maireana georgei	+			
Maireana integra				
Maireana pentatropis	+	+	+	+
Maireana radiata	+	+	+	+
Maireana sedifolia	+	+	+	
Maireana trichoptera	+	+	+	
Maireana turbinata	+		+	
Minuria cunninghamii	+		+	
Ptilotus incanus/obovatus	+			
Rhagodia candolleana ssp.	+			
Rhagodia candolleana ssp. argentea	+		+	
Rhagodia parabolica		+		
Rhagodia spinescens	+		+	
Rhagodia ulicina			+	
Rhodanthe floribunda				+
Rhodanthe stuartiana		+		+
Salsola australis	+	+	+	+
Santalum acuminatum	+		+	
Scaevola spinescens	+			
Scaevola brevifolia		+		+
Sclerolaena diacantha	+	+	+	
Sclerolaena obliquicuspis	+	+	+	+
Sclerolaena patenticuspis	+		+	
Senna artemisioides ssp.	+			
Senna artemisioides ssp. petiolaris	+			

Scientific Name	Mallee		Myall	
	Analogue	Rehab	Analogue	Rehab
Senna artemisioides ssp. coriacea	+		+	
Senna cardiosperma ssp. gawlerensis	+		+	
Sida ammophila		+		
Sida cunninghamii			+	
Sida fibulifera			+	
Sida trichopoda	+			
Stenopetalum lineare		+		+
Tetragonia eremaea		+	+	+
Vittadinia sp.		+	+	+
Zygophyllum apiculatum	+	+		
Zygophyllum aurantiacum ssp.	+	+	+	+
Zygophyllum eremaeum	+	+	+	
Zygophyllum ovatum	+	+	+	+

Appendix 5 Landscape Function Analysis

ILUKA

Jacinth Ambrosia

Landscape Function Analysis

2015

DOCUMENT CONTROL

Document Title:	Landscape Function Analysis
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1 Introduction

The key rehabilitation monitoring tool at JA is Landscape Functions Analysis (LFA). Originally developed for rangeland monitoring, LFA is ideally suited to monitor the JA environment. After rehabilitation has occurred LFA surveys are conducted at various intervals over time on the rehabilitated areas and compared against relevant analogue sites. LFA is a recognised system which monitors structural development of ecosystems and the re-development of ecosystems process such as stability, nutrient cycling and infiltration.

2 Methods

Permanent LFA transects have been established in rehabilitation areas and corresponding analogue sites. A total of 13 Rehabilitation LFA sites have been established and 9 analogue sites (3 per vegetation association) since 2010 (Table 1).

The lengths of transects vary in order to capture at least five replications of each patch type. Monitoring at each site follows the methods of Tongway & Hindley, 2004. Indices are generated for stability, infiltration/runoff and nutrient cycling status using the scoring techniques and spread sheets provided.

Definitions for each of the indices are as follows:

- Stability –the ability of the soil to withstand erosive forces, and to reform after disturbance.
- Infiltration/Runoff – how the soil partitions rainfall into soil-water (water available for plants to use) and runoff water which is lost from the local system, or may also transport materials (soil, nutrients and seed) away.
- Nutrient cycling status – how efficiently organic matter is cycled back into the soil.

In addition to the traditional LFA measurements, an assessment of the successional phase of the biological soil crust (BSC) on the LFA transects was made using a method adapted from Budel et al. 2009 (Figure 1 - Figure 6). An additional category has been added, Type 0.5, where filaments are visible to the eye when the soil surface is excavated (Figure 2). Monitoring the successional recovery of biological soil crusts on rehabilitation areas adds to the understanding of how long it could take for highly disturbed BSC's to begin contributing ecosystem services such as soil stabilisation, nitrogen fixation, carbon sequestration and development of niches to capture resources.

LFA surveys are carried out at the end of the year when annual species have reached the end of their lifespan (senesced).

Figure 1 Class 0, no visible signs on surface or subsurface.

Figure 2 Class 0.5, no visible signs on surface, filaments visible subsurface

Figure 3 Class 1, light coloured, visible traces.

Figure 4 Class 2, light, thin, not diverse, irregular covering.

Figure 5 Class 3, dark, medium thickness, medium diversity, complete covering.

Figure 6 Class 4, dark, very thick, very diverse, complete covering. Lichen dominant, including light coloured lichens.

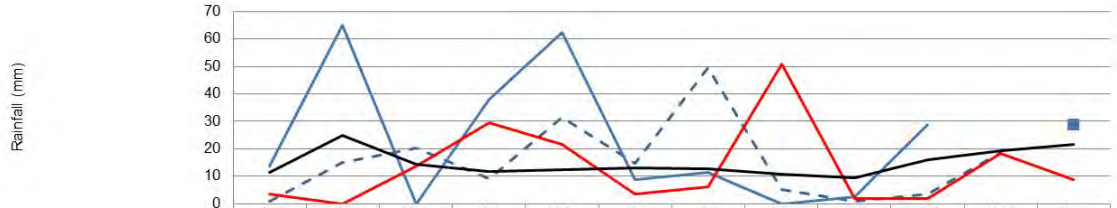
Table 1 Summary of LFA monitoring events per site

Site	Site	Year Rehabilitated	Year monitored		
			Year 1	Year 2	Year 3
LFA_AN_001	ANALOGUE - Chenopod	NA	2015		
LFA_AN_002	ANALOGUE - Chenopod	NA	2015		
LFA_AN_003	ANALOGUE - Chenopod	NA	2011	2012	2015
LFA_AN_004	ANALOGUE - Mallee	NA	2015		
LFA_AN_005	ANALOGUE - Mallee	NA	2015		
LFA_AN_006	ANALOGUE - Mallee	NA	2011	2012	2015
LFA_AN_007	ANALOGUE - Myall	NA	2015		
LFA_AN_008	ANALOGUE - Myall	NA	2015		
LFA_AN_009	ANALOGUE - Myall	NA	2015		
LFA_JA_001_A	Canberra Camp	2009	2011	2012	2015
LFA_JA_002	Tank Farm 1	2009	2011	2012	2015
LFA_JA_004_A	PWD N Wall west spillway	2010	2011	2012	
LFA_JA_004_B	PWD N Wall east spillway	2010	2011	2012	
LFA_JA_005	Dam 11	2010	2011	-	
LFA_JA_007	Borefields TN4	2012		2014	
LFA_JA_007	Borefields TN4	2012	-	2014	
LFA_JA_008	Borefields TN5	2012		2014	
LFA_JA_008	Borefields TN5	2012	-	2014	
LFA_JA_009_A	Cell 1 East	2013	2014	2015	
LFA_JA_009_B	Cell 1 East	2013	2014	2015	
LFA_JA_010_A	Cell 1 West	2014	2015		
LFA_JA_010_B	Cell 1 West	2014	2015		

3 Results

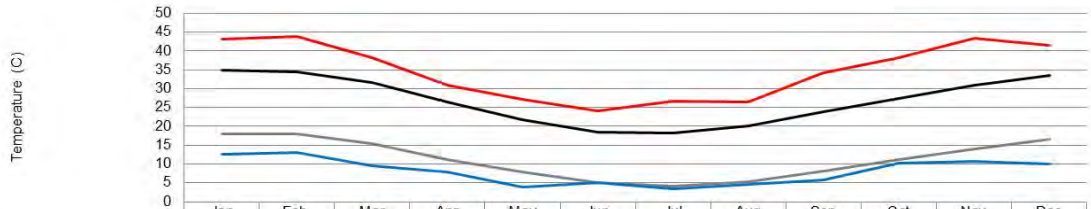
3.1 Climate

Overall the climate at JA was hotter and drier in 2015 than the long term average (Figure 7 and Figure 8). Temperatures were consistently warmer than the long term average (Figure 7). Rainfall at JA for 2015 was the lowest recorded for the past three years, and slightly than the long term average for Tarcoola (JA – 159 mm, Tarcoola mean – 176.8 mm). Winter rains arrived later than usual, the highest monthly rainfall was recorded in August, Figure 8.



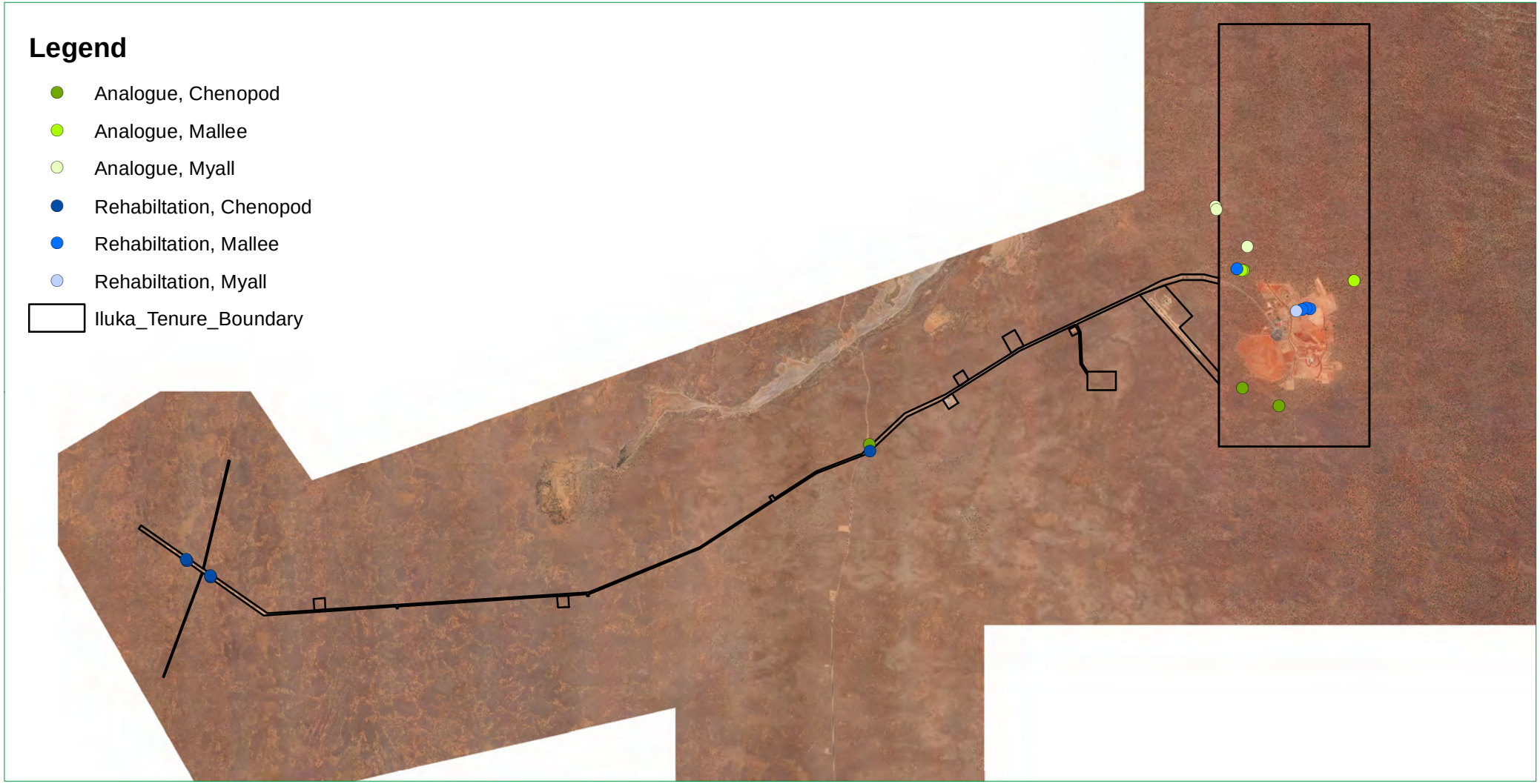
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
JA Weather Station 2013	0.8	15	20.4	9	31.4	14.6	49.4	5.2	0.8	3.6	18.6	
JA Weather Station 2014	13.6	64.9	0	38	62.35	8.88	11.24	0	2.4	28.8		28.8
JA Weather Station 2015	3.4	0	13.6	29.6	21.4	3.6	6	50.8	1.8	1.8	18.4	8.6
Tarcoola Mean	11.4	25	14.2	11.8	12.3	13	12.7	10.7	9.3	15.9	19.1	21.4

Figure 7 Site monthly rainfall data



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Tarcoola Mean Max	35	34.5	31.7	26.4	21.8	18.4	18.2	20.2	23.9	27.5	30.9	33.5
Tarcoola Mean Min	17.9	17.9	15.5	11.2	7.9	5.1	4.2	5.3	8.1	11.2	14.1	16.5
JA 2015 Max	43.1	43.8	38.2	31	27.1	24.1	26.6	26.5	34.1	38.2	43.4	41.6
JA2015 Min	12.6	13.1	9.44	7.83	3.93	5.07	3.31	4.67	5.73	10.2	10.6	10.1

Figure 8 Site monthly temperatures



JA

LFA Monitoring Locations

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:149,114 (A4) DATE: March16 DWG No: NA

FIGURE: **9**

3.2 Ecological Function Indices

3.2.1 Indices with time

Thirteen sites were monitored in 2015. Seven additional analogue sites were included in program which now comprises three sites for each vegetation association (chenopod, myall and mallee). Two new sites were established in the 2014 rehabilitation area (Cell 1 West). All other sites have been monitored previously (Table 1).

For sites that had been monitored since 2011 soil stability was generally similar to analogue sites (Figure 10). Although the Tank Farm had a slight decline in soil stability in 2012, the index is now greater than the initial year one measure and similar to the rehabilitation sites. The soil stability index at Canberra Camp is slightly higher than found at the analogue site.

Infiltration indices are also similar across rehabilitation sites and analogue sites (Figure 11). A similar infiltration index has been recorded at Canberra Camp and the mallee analogue site since 2012. The mallee analogue site has shown a consistent decline from 2011, mirrored by the Canberra site from 2012 to 2015. The Tank Farm and the chenopod analogue sites have generally remained similar and consistent across years.

Nutrient cycling has steadily increased for the Canberra Camp and Tank Farm rehabilitation site since 2011 (Figure 12). However the mallee analogue site has shown a decrease since 2011 and the chenopod site since 2012. The nutrient cycling index at the mallee analogue site is now less than that of the rehabilitation site.

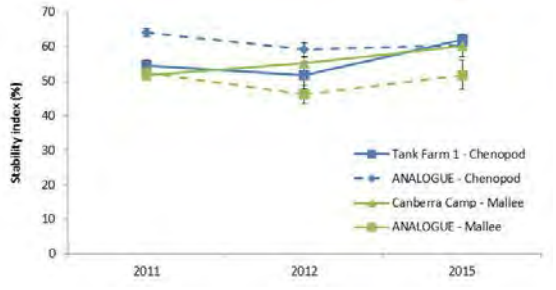


Figure 10 Stability index for sites with multiple years monitoring

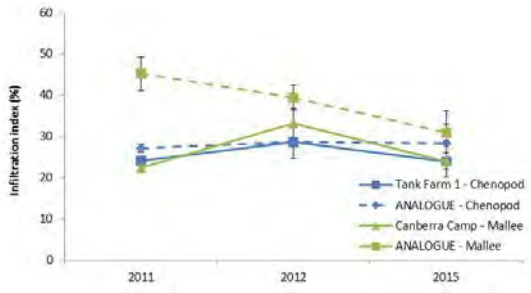


Figure 11 Infiltration index for sites with multiple years monitoring

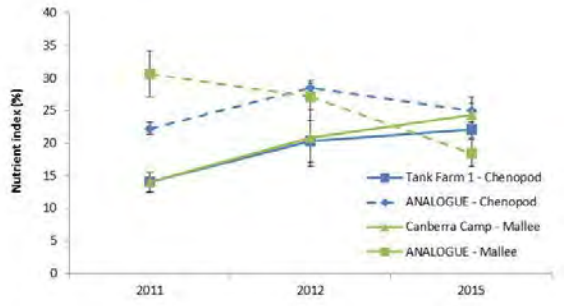


Figure 12 Nutrient cycling index for sites with multiple years monitoring

3.2.2 Indices for vegetation types

Mallee

The performance of the mallee rehabilitation sites in 2015 was similar to that of the analogue sites. Stability at the mallee sites in 2015 was generally similar (Figure 13). Stability at the Canberra Camp rehabilitation site (60.2%) was greater than both all analogue sites (ranging from 48.8% to 57.1%). Cell 1 sites also showed similar stability ranging from 36.2% at Cell 1 West to 44.2% at Cell 1 East. Infiltration for mallee sites ranged from 10.1% to 40.6% (Figure 14). Generally infiltration was slightly lower at rehabilitation sites, ranging from 18.1% to 24%, compared to analogue sites, ranging from 29.6% to 40.6%.Nutrient cycling was variable across rehabilitation and analogue sites (Figure 15). Analogue site 06 recorded a lower nutrient cycling index (18.5%) compared to other analogue sites (23.8% and 29%), but was similar to the Canberra Camp site (24.4%) and one of the Cell 1 East sites (20.7%). The lowest nutrient cycling indices were recorded in Cell 1 East (13.8%) and Cell 1 West (13.4%).

Chenopod

The performance of chenopod rehabilitation was variable in 2015 when compared to analogue sites. The stability for the chenopod rehabilitation site and analogue sites was similar in 2015 (Figure 16). Stability at the analogue sites ranged from 60.5% to 72.2%, stability at Tank Farm 1 was 62%. There was very little variability within sites. Infiltration at the rehabilitation site (Tank Farm) was slightly lower (24%) than the analogue sites (ranging from 28.2% to 29.6%), Figure 17. Nutrient cycling indices was variable across analogue sites (ranging from 25% to 35.6%), the rehabilitation site (Tank farm 1) was slightly lower again (22%), Figure 18.

Myall

Cell 1 West rehabilitation site performed consistently lower than myall analogue sites across all three indices (Figure 19 to Figure 21). Stability at myall analogues ranged from 59.4% to 64.1%, and was greater than stability recorded at the myall rehabilitation site (44.2%), Figure 19. Infiltration at analogue sites ranged from 28.6% to 33%, compared to 19.7% at the rehabilitation site, Figure 20. Nutrient cycling indices at analogue sites raged from 25% to 27% compared to 15% at the rehabilitation site, Figure 21.

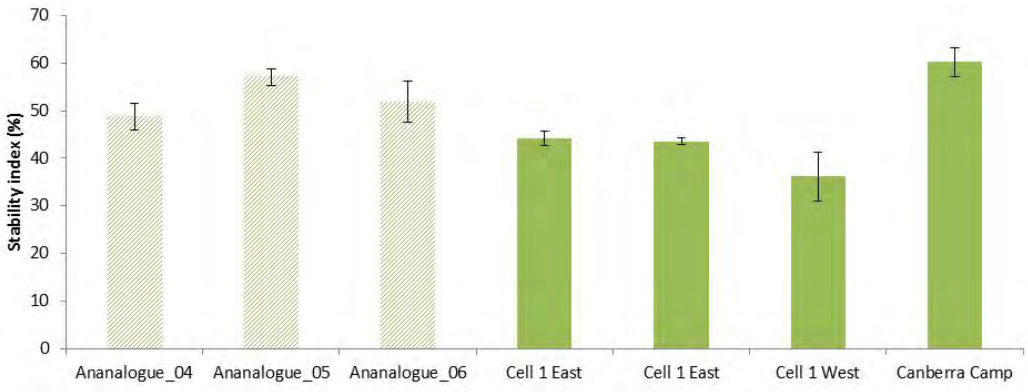


Figure 13 Stability indices for all mallee sites monitored in 2015

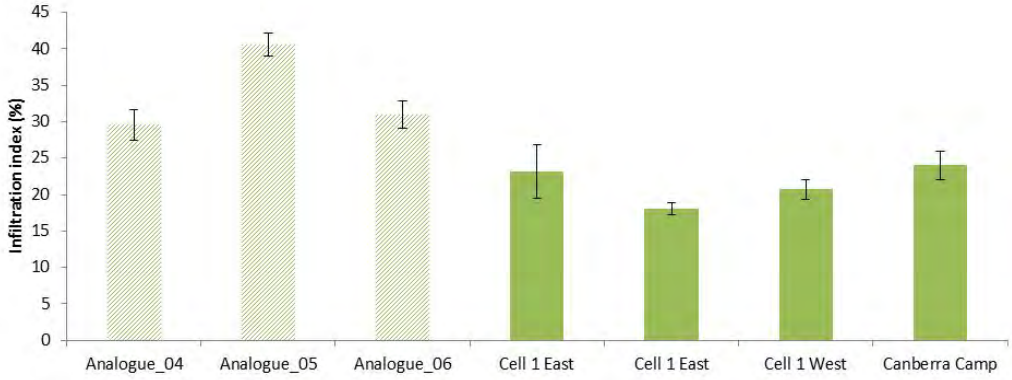


Figure 14 Infiltration indices for all mallee sites monitored in 2015

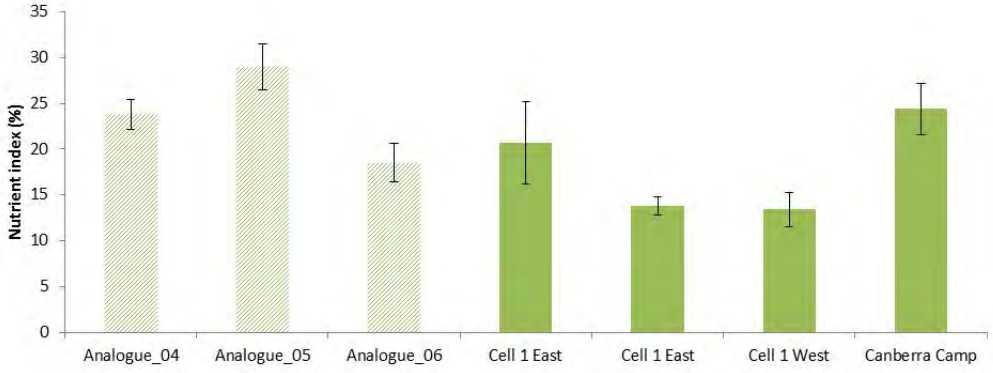


Figure 15 Nutrient cycling indices for all mallee sites monitored in 2015

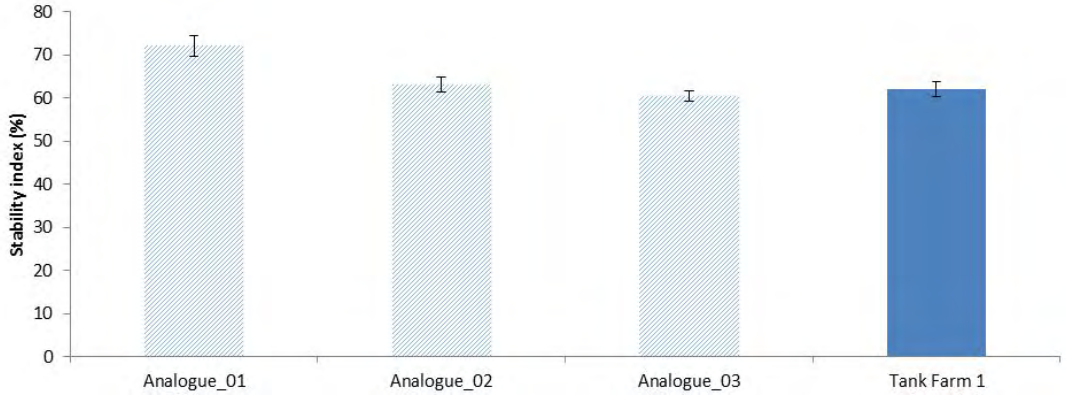


Figure 16 Stability indices for all chenopod sites monitored in 2015

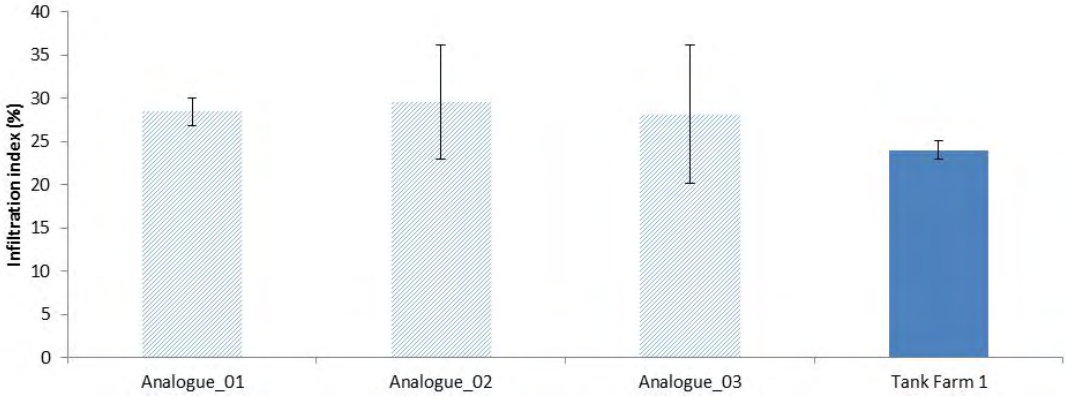


Figure 17 Infiltration indices for all chenopod sites monitored in 2015

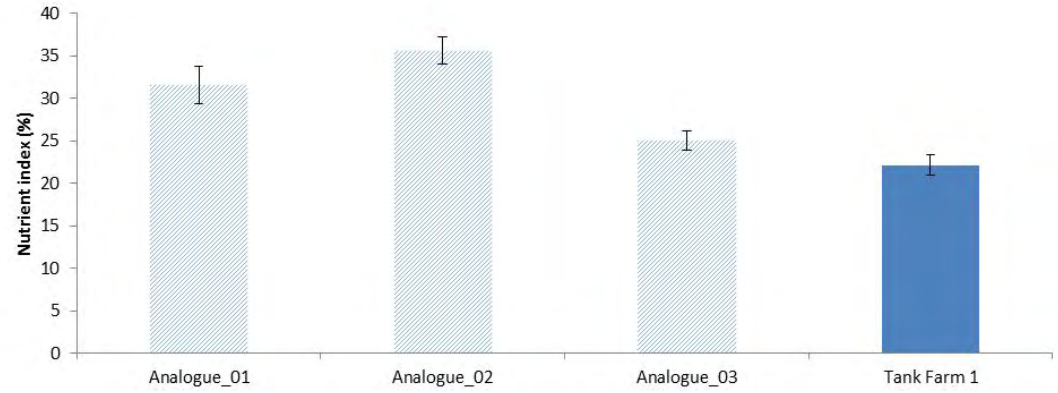


Figure 18 Nutrient cycling indices for all chenopod sites monitored in 2015

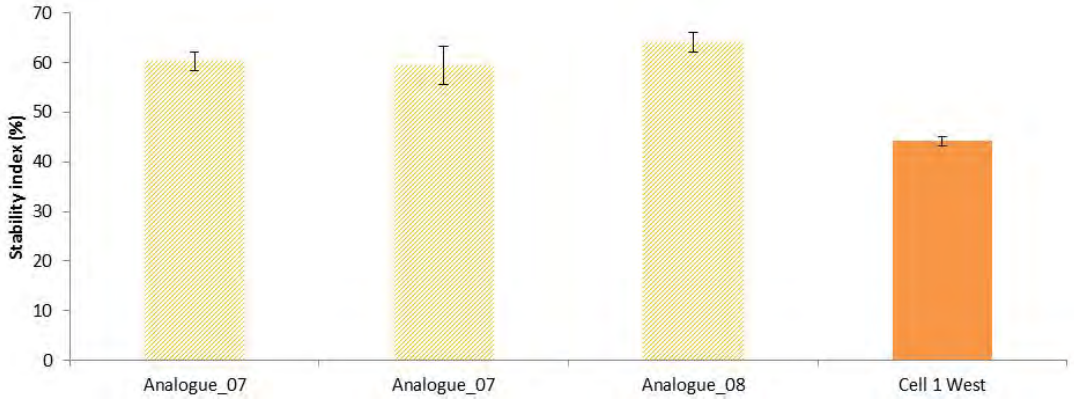


Figure 19 Stability indices for all myall sites monitored in 2015

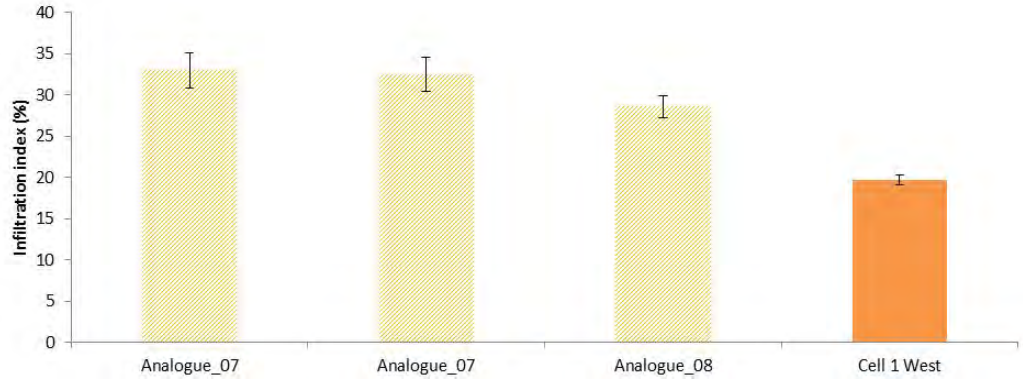


Figure 20 Infiltration indices for all myall sites monitored in 2015

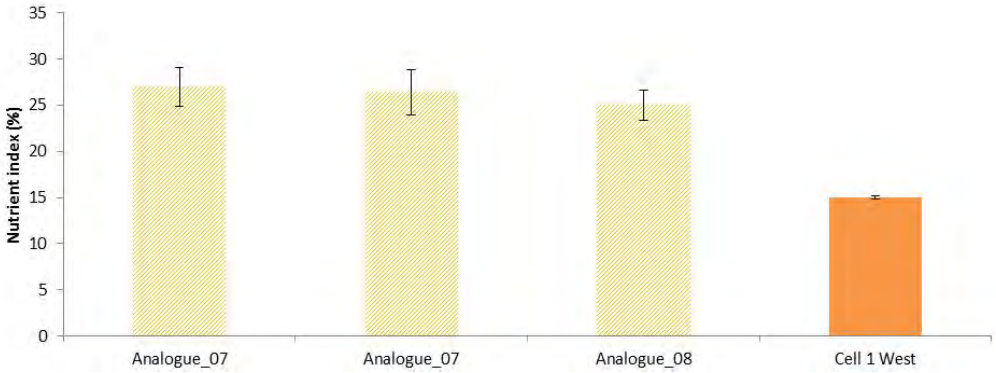


Figure 21 Nutrient cycling indices for all myall sites monitored in 2015

3.3 Ground Cover

The proportion of bare ground at rehabilitation sites was generally greater than that at analogue sites across all vegetation types (Figure 22 to Figure 24). Note that bare ground here only refers to a lack of vegetation, biological soil crust cover is considered in the function indices, stability and nutrient cycling indices.

The proportion of bare ground recorded at mallee rehabilitation sites ranged from 80.7% to 87.6%, in comparison analogue sites ranged from 53.7% to 65.7%, Figure 22. The highest recorded bare ground was at the chenopod rehabilitation site Tank Farm, where bare ground accounted for 97.1% of the LFA transect, Figure 23. In comparison the chenopod analogue sites ranged from 70.1% to 86.5%. The proportion of bare round recorded at the myall rehabilitation site was 86%, compared to analogue which ranged from 26% to 53%, Figure 24.

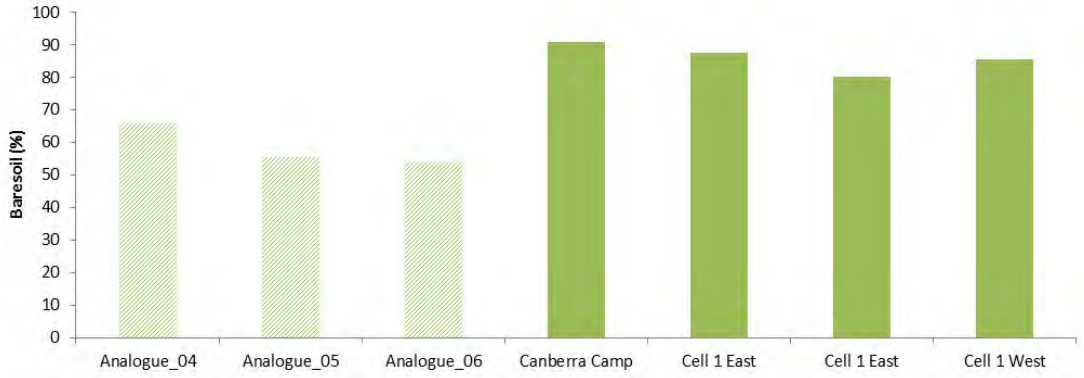


Figure 22 Proportion of bare ground at mallee sites in 2015

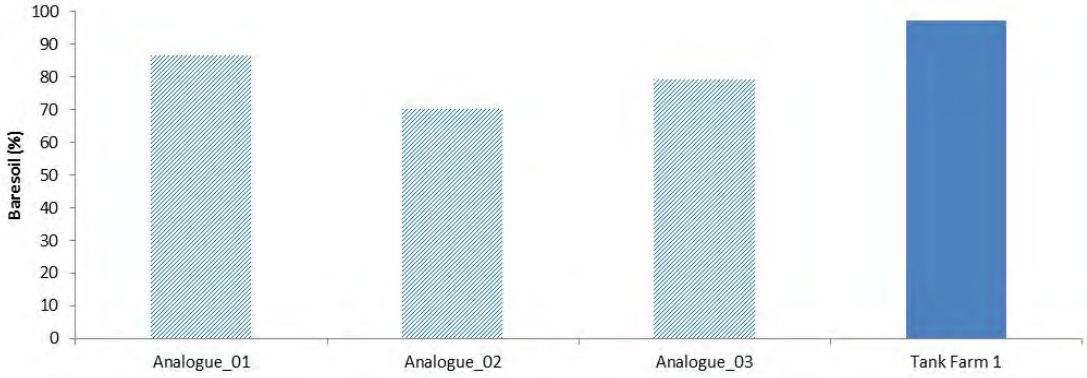


Figure 23 Proportion of bare ground at chenopod sites in 2015

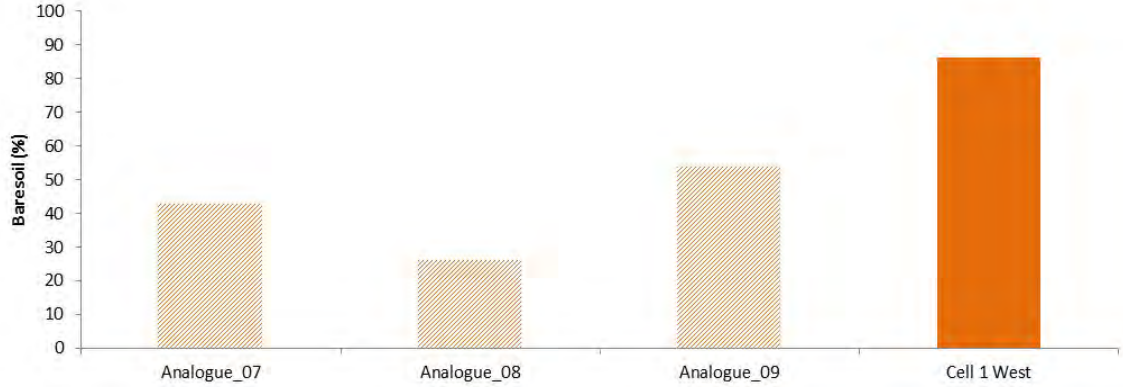


Figure 24 Proportion of bare ground at myall sites in 2015

3.4 Cryptogram cover

Biological soil crust (BSC) was recorded on all transects, however for rehabilitation sites contribution was not considered to be significant (Class 1) compared to the significant cover (Class 4 - greater than 50%) observed in analogue sites.

4 Discussion

The soil stability index provides information on the ability of the soil to withstand erosive forces, and to reform after a disturbance event. The response over time in mallee and chenopod vegetation associations has been similar for both rehabilitation and analogue sites. Further in 2015 the stability indices recorded for rehabilitation and analogue sites was also similar. In contrast the soil stability at the myall vegetation associations was lower at the rehabilitation site in comparison to the analogues sites. However the myall rehabilitation site monitored was only 1 year old, and had been subject to hotter and drier climate conditions in the first year of rehabilitation compared to all other rehabilitation sites. Additionally monitoring will be required to determine if the slow response by the myall soils is due to the soils characteristics or the poor climate conditions. Additional LFA sites will be established in the newly rehabilitated (2016) Cell 2 East myall areas.

Infiltration provides information on how water moves through the system, rainfall can either infiltrate soil surfaces and be available for plants or move through the system and potentially take with it nutrient and seed. Overall infiltration indexes showed similar responses over time across mallee and chenopod vegetation associations. Generally a reduced BSC cover would increase infiltration rates for disturbed areas, and increased surface roughness at rehabilitation sites (due to ripping) would also encourage rainfall infiltration. However the infiltration index here also considers litter and vegetation cover, which was higher at analogue sites than rehabilitation sites likely accounting for the similar responses. Further a decline in infiltration for mallee sites between 2011 and 2015 may be due to poor climate conditions resulting in reduced canopy and litter cover (due to less annual growth).

The nutrient cycling index describes how efficiently organic matter is cycled back into the soil. Nutrient cycling indexes varied across sites regardless of vegetation association or disturbance. Generally nutrient cycling potential of rehabilitation sites increased with time, however one analogue site declined. The increase surface roughness at rehabilitation sites would increase the nutrient cycling potential, and will increase with time with increased vegetation and BSC cover.

As anticipated ground cover at rehabilitation sites was less than that of analogue sites however this is expected to increase with time as perennial plants establish. BSC was recorded at all rehabilitation sites and the contribution of the crust to soil stability will improve with time. Further it has been observed at JA the often BSC develop and contribute to soil stability well before it is visible on the surface. Therefore the contribution to soil stability is likely underestimated in the LFA process.

Overall there has been good vegetation growth and soil stabilisation in the JA rehabilitation areas. The stability of soils has generally increased with time or shown similar climatic response as analogue sites.

5 References

Tongway DJ, Hindley NL (2004). Landscape Function Analysis: procedures for monitoring and assessing landscapes with special reference to minesites and rangelands. CSIRO Sustainable Ecosystems

Rehabilitation Sites

Plate 1 LFA JA 001 - 2011

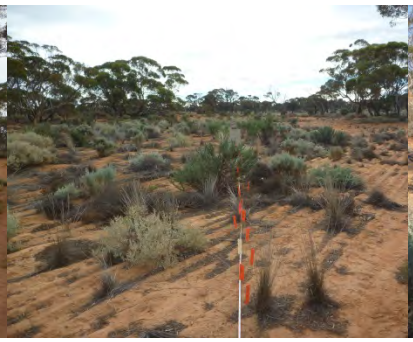


Plate 2 LFA JA 001 - 2012

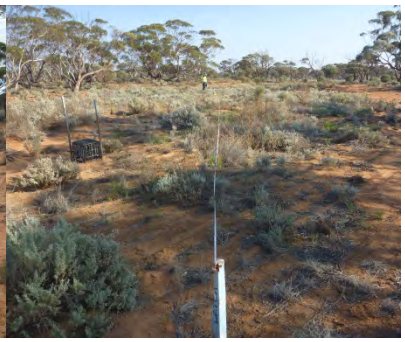


Plate 3 LFA JA 001 - 2015

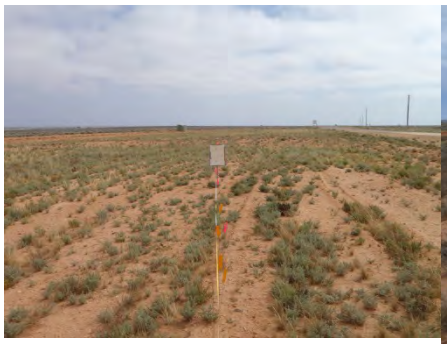


Plate 4 LFA JA 002 - 2011

Plate 5 LFA JA 002 - 2012

Plate 6 LFA JA 002 - 2015

Plate 7 LFA JA 007A – 2014

Plate 8 LFA JA 008B – 2014

Plate 9 LFA JA 009 – 2014

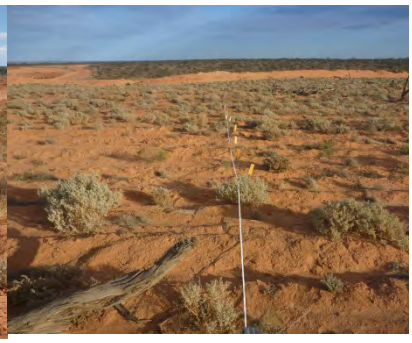


Plate 10 LFA JA 009 – 2015

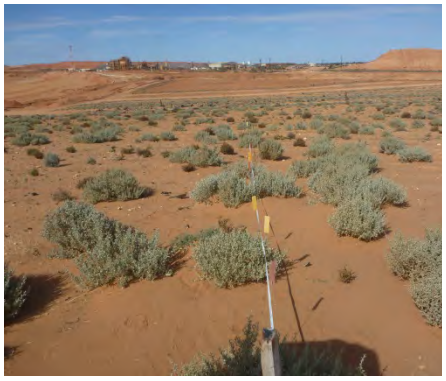


Plate 11 LFA JA 010 – 2015

Analogue Sites

Plate 12 LFA AN 002 - 2015

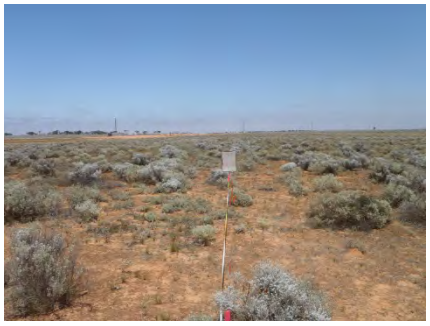


Plate 13 LFA AN 003 - 2011

Plate 14 LFA AN 003 - 2012

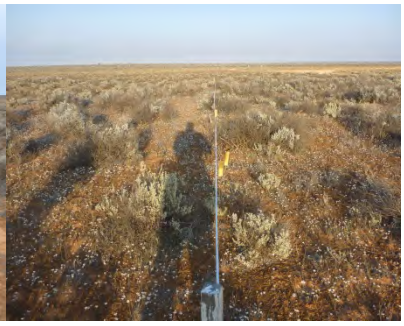


Plate 15 LFA AN 003 - 2015

Plate 16 LFA AN 004 - 2015

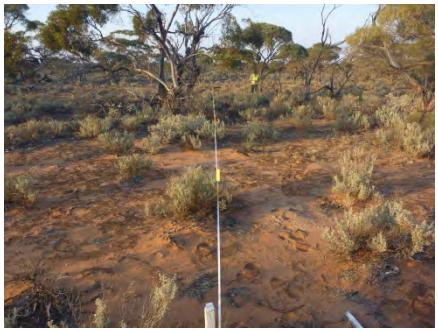


Plate 17 LFA AN 005 - 2015

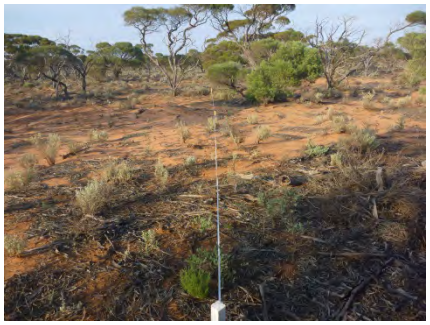


Plate 18 LFA AN 006 - 2015

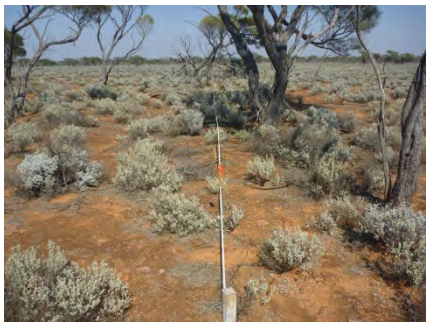


Plate 19 LFA AN 007 - 2015

Plate 20 LFA AN 008 - 2015

Plate 21 LFA AN 009 - 2015

Appendix 6 Jacinth Ambrosia Photo Point Monitoring 2015

ILUKA

Jacinth Ambrosia Photo Point Monitoring

2015

DOCUMENT CONTROL

Document Title:	Jacinth Ambrosia Photo Point Monitoring
Mine Status:	Operational
Revision:	Version 1.0
Date Issued:	12 January 2016
Review Frequency:	-
Compiled by:	Joanne Lee
Owner:	JA Rehabilitation
Document No:	

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FIGURES

PLATES

1 Introduction

Photo monitoring provides a visual representation of vegetation change over time, and is considered to be a simple form of monitoring. This technique for monitoring is based on the establishment of permanent photo locations or photo points, which can be revisited at regular intervals. Planning and thought is therefore needed prior to the establishment of a photo point to determine the exact nature of the photos and what they will reflect over time. For J-A photo point monitoring will be used to identify:

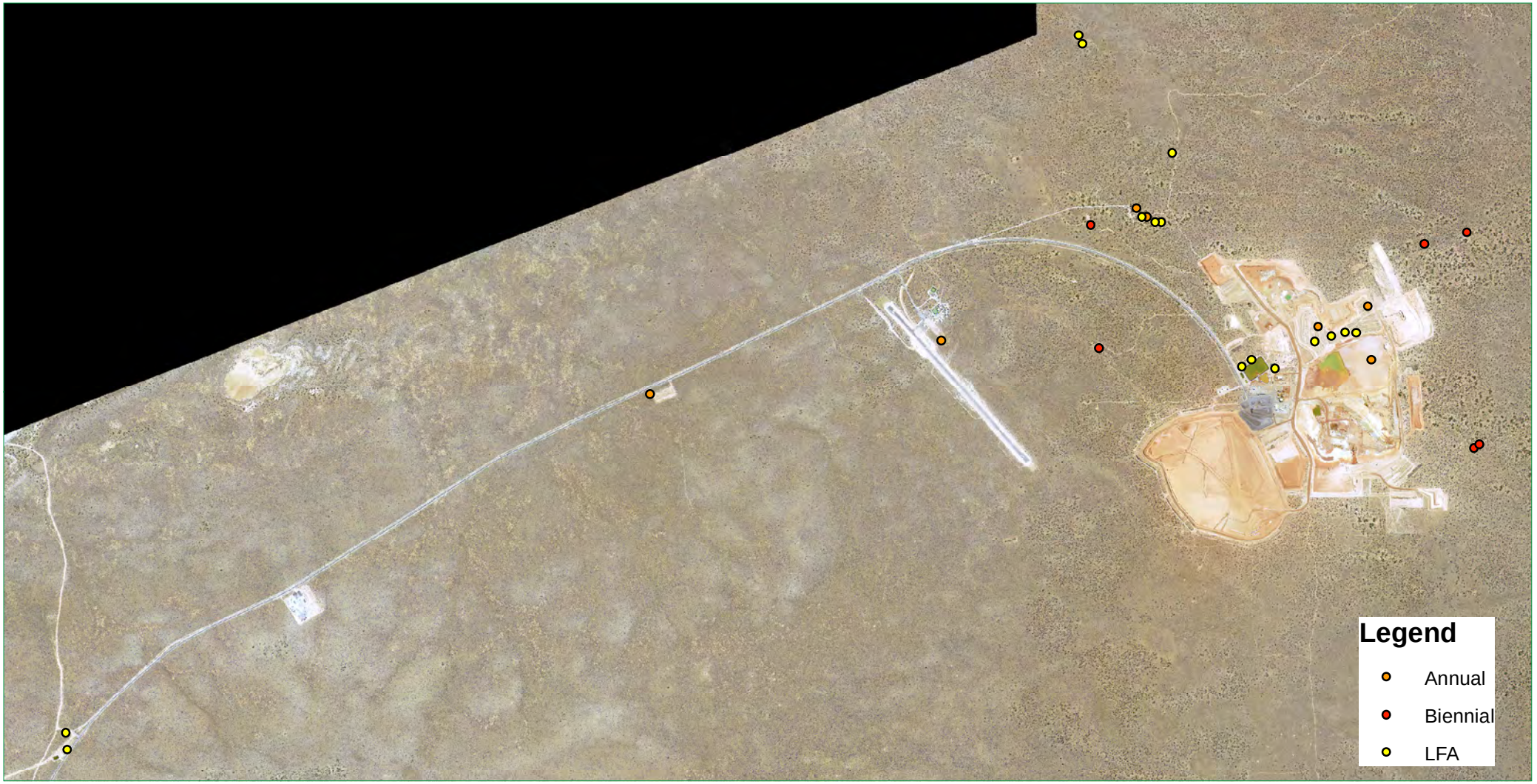
- The success of rehabilitation efforts through comparison of vegetation before disturbance with images over time after rehabilitation of those areas.
- Changes in landform or vegetation over time due to indirect mine impacts.
- Changes in landform and vegetation with climatic changes in areas not impacted by the mine activities for comparison with rehabilitated areas or areas indirectly impacted by mine activities.

2 Methods

A total of 319 photo points have been established since 2009 at JA (Figure 1 and Figure 2). Photo points have been monitored on an ad-hoc basis from 2009. All recorded photo points were visited from the 8 to 15 December 2015 to determine suitability for ongoing photo point monitoring. Photo points were then identified as one of four types:

- Active, comprising:
    - Annual – Photo points currently monitored annually.
    - Biennial - Photo points currently monitored biennially (water course monitoring).
    - LFA – Photo points monitored in accordance with LFA monitoring (1,2,5 and 10 years post rehabilitation)
- Rehab – Photo points to be monitored at rehabilitation of the location.
- Inactive – Photo point determined not suitable for monitoring at this time, but may be activated at some time in the future if required.

Results of photo point monitoring related to LFA and water course monitoring are described in the relevant reports. However, the locations are recorded here for completeness.



N

MGA Coordinates, GDA94

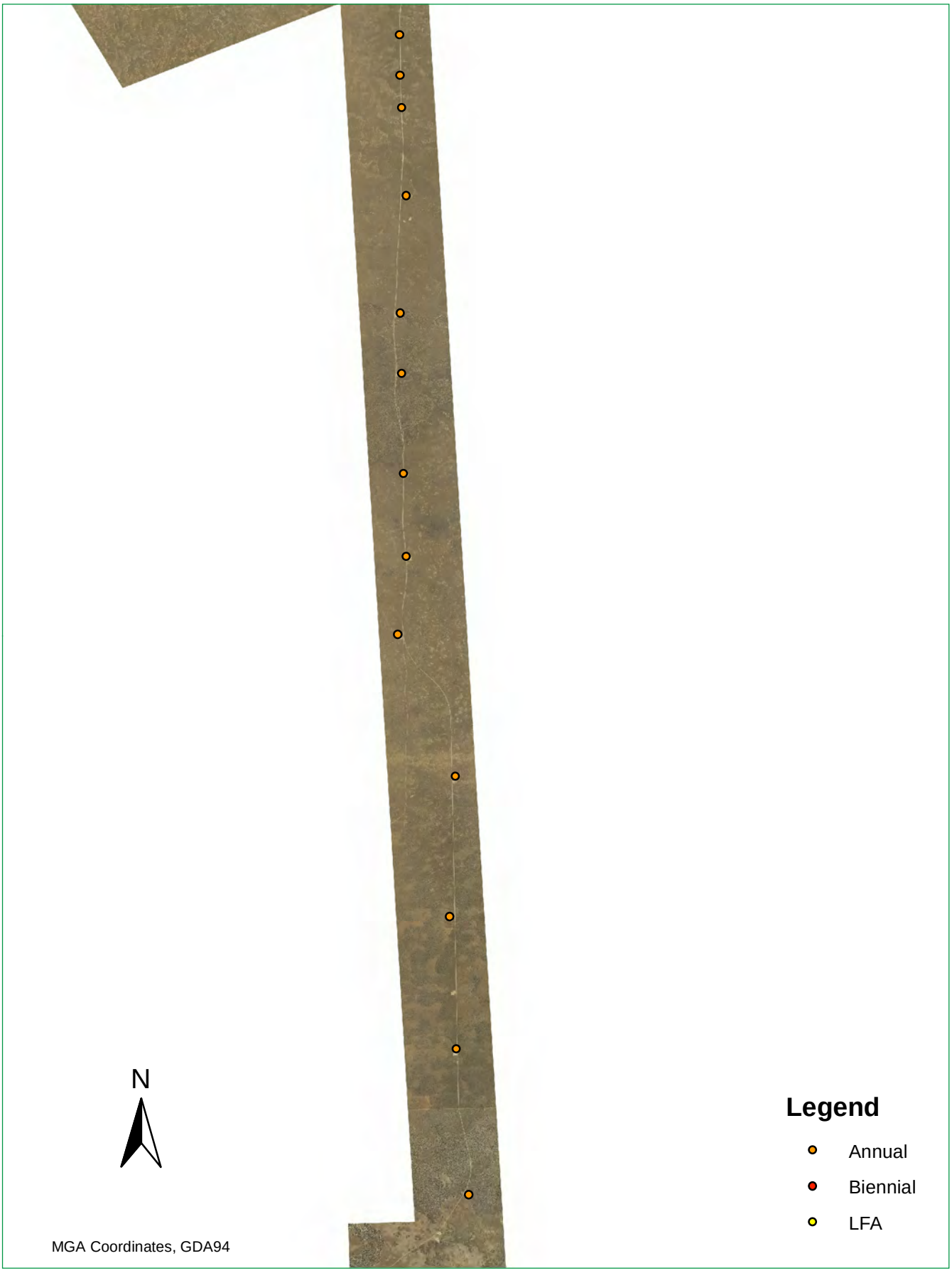
JA

Photo point monitoring locations ML and MPL

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:50,919 (A4) DATE: January 16 DWG No: NA

FIGURE: 1



JA

Photo point monitoring locations
Ooldea Road

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:306,088 (A4) DATE: December 2015 DWG No: NA

FIGURE: **2**

3 Results

Overall 207 of the 319 (65%) of photo point were identified to be unsuitable for photo point monitoring and therefore determined inactive. Sixty three photo points were identified as potentially suitable to monitor rehabilitation at J-A. Twenty one photo points are captured in the current annual photo point monitoring program. Six water course monitoring and 22 LFA photo points were also recorded in the photo point database. A summary of the photo points is provided in Table 1.

A brief description of the review of the 2015 photo point monitoring provided below:

Access tracks – The majority of the photo points labeled as access tracks were located on the Ooldea Road. As the Ooldea Road is not to be rehabilitated those photo points have been determined to be inactive. One photo point was located at the location of the wind mast, currently rehabilitated, therefore is to be monitored annually commencing 2015. Two photo points were located on currently used unsealed access tracks and therefore annual monitoring will occur once rehabilitation of those areas is complete.

Airstrip – A total of nine photo points were located on the airstrip, seven were determined to be unsuitable (therefore inactive). Two photo points were identified as suitable for annual monitoring, which will occur once rehabilitation of the areas is complete.

Borrow pits – A total of 82 photo points were located at borrow pits, of those 65 were determined as unsuitable (therefore inactive). Fourteen photo point were located on currently rehabilitated borrow pits and identified as suitable for annual monitoring. A further three photo points were identified as suitable for monitoring upon completion of rehabilitation in those areas.

Creeks – Three photo points were located at creeks, all were considered to be unrequired as all creeks are monitored in accordance with the watercourse monitoring program, which includes photo point monitoring biennially. A total of six photo points are currently monitored as part of the water course monitoring program.

Cultural track – The cultural track is not be rehabilitated therefore no photo points are required.

Dust monitor – The dust monitoring photo points were located along the Ooldea Road to determine dust impacts on vegetation, however the road is now sealed. The dust monitor photo points are no longer required.

Monitor bore – Of the eight photo points located at monitoring bores five were identified as suitable, monitoring of these points will commence once the bores are decommissioned and rehabilitated.

Permanent camp – A total of 17 photo points have been established at the current camp of which two were determined as suitable. Monitoring of these points will commence once the camp is decommissioned and rehabilitated.

Pipeline – Two photo points were located along the bore field pipeline. One was identified as not suitable (therefore inactive). Monitoring of the other photo point will commence once the bore field and pipeline are decommissioned and rehabilitated.

Pit – A total of 18 photo points have been established in the Jacinth pit. Two points are located in rehabilitation areas (Cell 1 East and Cell 1 West) and one point in the anticipated 2016 rehabilitation area (Cell 2 East). These photo points will be monitored annually. The suitability of the remaining 15 photo points will be determined as those areas are rehabilitated. One additional photo point will be established in the pit clearance area each year. Four LFA monitoring points are located in the rehabilitated Cell 1 East and Cell 1 West.

Process plant – Twenty four photo points are located at the process plant and surrounds. The suitability of the photo points will be determined as those areas are rehabilitated.

Tank farm – All three tank farm photo points were identified as unsuitable. The tank farm currently is monitored with LFA transects, therefore the photo points no longer required (inactive).

Temp camp – Three photo points are located at the construction camps, now rehabilitated. These points are monitored annually. The temporary camp is also monitored with a LFA transect.

TSF – Six photo points are located at the off-path TSF. The suitability of photo points will be determined as areas of the off-path TSF are rehabilitated.

Turkeys nest – One photo point is located at a turkeys nest at the bore field, monitoring of this point will commence once the bores are decommissioned and the areas rehabilitated. A further seven LFA monitoring points are located at turkeys nests at the bore field. Note additional photo points will be added in 2016 for the Ooldea Road turkeys nests rehabilitated in 2015.

Other - A number of other photo point locations were identified in the photo point register related to vegetation growth, however were identified as not suitable (therefore made inactive).

Analogue – Nine LFA analogue sites are established in the three vegetation association and photo point monitoring occurs in line with the frequency of LFA monitoring.

Table 1 Summary of JA photo points

Location	Annual	Biennial	LFA	Inactive	Rehab	Total
Access Roads	1			66	2	69
Airstrip				7	2	9
Borrow Pit	14			65	3	82
Creeks		6		3		9
Cultural Track				17		17
Dust monitor				25		25
Monitoring Bore				5	4	9
Perm Camp				15	2	17
Pipeline				1	1	2
Pit	3		4		15	22
Process Plant			2		24	26
Tank Farm			1		3	4
Temp Camp	3		1			4
TSF					6	6
Other				3		3
Turkeys Nest			5		1	6
Analogue			9			9
Total	**21**	**6**	**22**	**207**	**63**	**319**

An example of the currently active photo points is provided in Plates 1 to Plate 19. Locations of the plates is provided in Figure 3.

4 Recommendations

- Sighting posts to be installed for currently active photo points. The compass point bearing of the photo point to be recorded. Technical Works Instruction to be updated accordingly.
- Active photo points to be monitored annually (or biennially as required) in winter or spring.
- Additional pre-disturbance photo points to be established in the pit areas and stockpile areas each year, minimum one per domain to be disturbed.

Plate 1 Pit 05 - Cell 1 West 2009

Plate 2 Pit 05 - Cell 1 West 2015

Plate 3 Pit09 mb15 - Cell 1 East 2013

Plate 4 Pit09 mb15 - Cell 1 East 2015

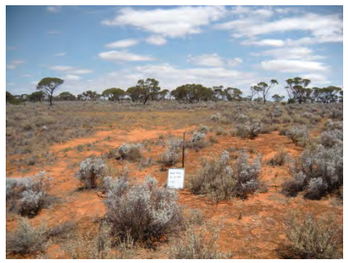


Plate 5 Wind Mast 2015

Plate 6 BP05NE - Borrow Pit 05 2009

Plate 7 BP05NE - Borrow Pit 05 2010

Plate 8 BP05NE - Borrow Pit 05 2011

Plate 9 BP05NE - Borrow Pit 05 2013

Plate 10 BP05NE - Borrow Pit 05 2015

Plate 11 BP18NE - Borrow Pit 18 2009

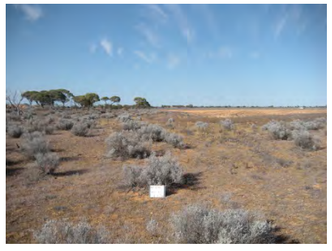


Plate 12 BP18NE - Borrow Pit 18 2015

Plate 13 19 - Temporary Camp 2009

Plate 14 C19 - Temporary Camp 2011

Plate 15 C19 - Temporary Camp 2015

Plate 16 C20 - Temporary Camp 2009

Plate 17 C20 - Temporary Camp 2010

Plate 18 C20 - Temporary Camp (C20) 2013

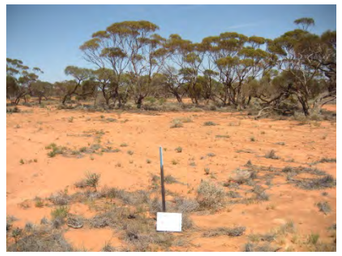
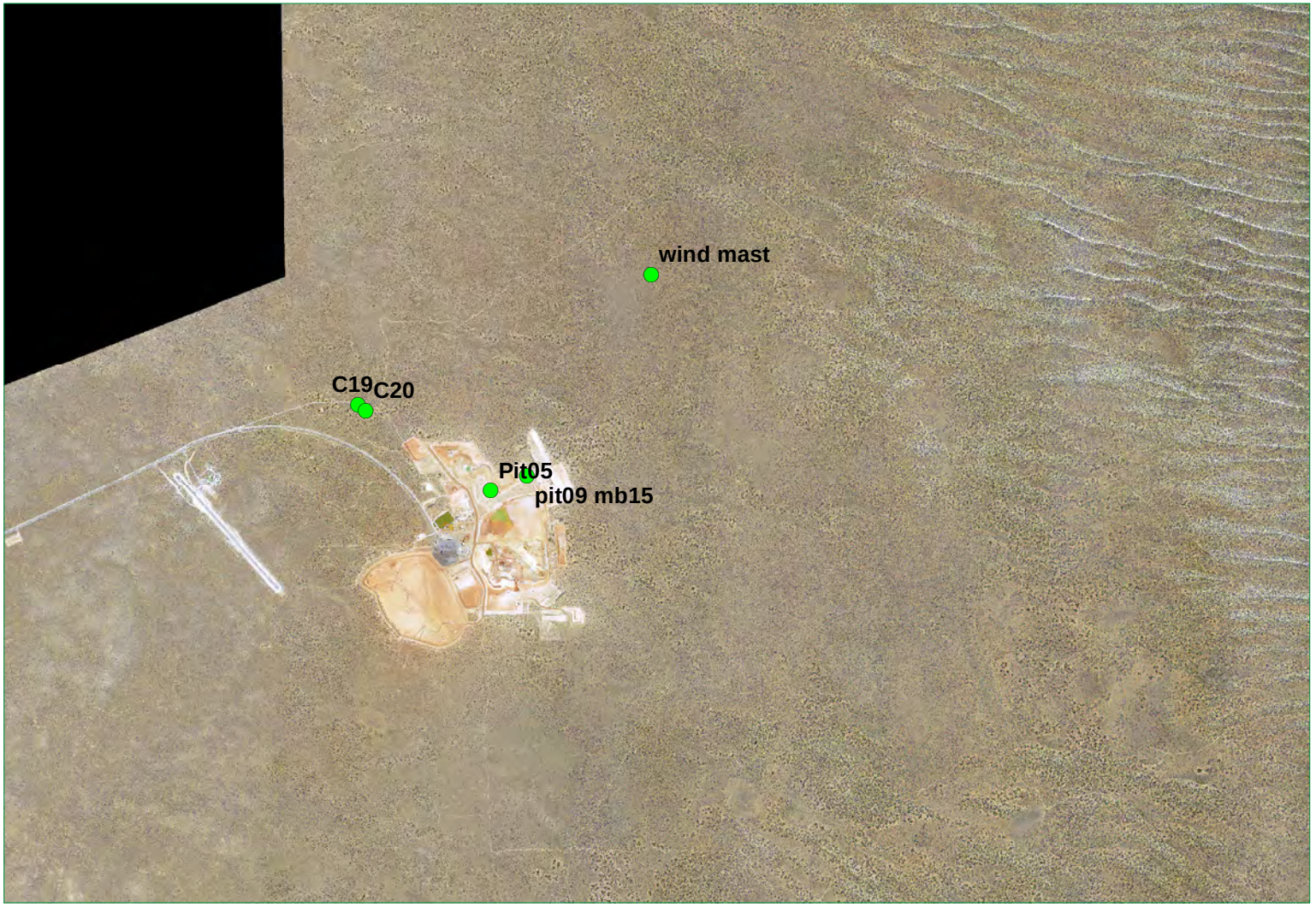


Plate 19 C20 - Temporary Camp (C20) 2015



N

MGA Coordinates, GDA94

JA

Photo point monitoring locations Plates

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:295,407 (A4) DATE: January 16 DWG No: NA

FIGURE: 3

Appendix 7 Jacinth Ambrosia Stockpile Monitoring Report 2015

ILUKA

Jacinth Ambrosia

Stockpile Monitoring Report

2015

DOCUMENT CONTROL

Document Title:	Jacinth Ambrosia Stockpile Monitoring report
Mine Status:	Operational
Revision:	-
Date Issued:	July 2015
Review Frequency:	-
Compiled by:	J Lee
Owner:	JA Rehabilitation
Document No:	

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TABLES

FIGURES

1 INTRODUCTION

At the Jacinth Ambrosia (JA) mine both vegetation and overburden are cleared in advance of the mine path. Clearing is performed in a specific sequence: Woody vegetation is cleared and collected; the topmost 0.05 m layer of soil ('topsoil') is stripped; and a 0.15 m layer of soil ('subsoil') is stripped. Topsoil and subsoil are stockpiled separately for rehabilitation purposes. The soils from the chenopod shrubland and myall woodland vegetation associations (stockpiled together) are also stockpiled separately from the soils from mallee woodlands.

Newly created stockpiles are stabilised with potable water to form a structural crust. If created correctly, and if further traffic is excluded, a structural crust is strong enough to protect the stockpile from wind erosion. The structural crust is further strengthened over time by rainfall and the development of a biological soil crust (BSC).

The JA soil stockpiles are a critical rehabilitation resource and as such a program was established in 2009 to regularly assess their condition, record vegetation and monitor for signs of biological soil crust growth. Monitoring included:

- assessment of soil dump stability including identification of threats and management options, e.g. feral animals, unauthorised trafficking or saline water use;
- identification of any plant species that germinate and grow on the different soil types;
- recording the lifespan, growth rates and contribution to the seed bank of species that grow on the stockpiles; and
- monitoring the soil surface for signs of BSC growth.

The stockpile monitoring program also provides valuable information on plant and BSC community succession for rehabilitated areas. This information will be used to assist the development of final completion criteria for the JA mine.

2 METHODS

Monitoring points were located and permanently pegged and the location surveyed for future reference.

At each stockpile, vegetation and crust was recorded in a 10 m long Jessop transect. Overall, twenty 1 m^2 quadrats were monitored for each stockpile. The first 10 quadrats were used to describe the class and proportion of cover for BSC. The final 10 quadrats were

monitored for the number of individuals of all plants species present and their proportional cover. Previous monitoring events (2009–2012) described cover in classes, 1 < 5%, 2 = 5–25%, 3 = 25–50%, 4 = 50–75%, 5 > 75%. For the current monitoring period (2015) cover was recorded as a proportion of the quadrat and then assigned a class (as per previous surveys) during data entry.

In addition to the Jessop transect quadrats, a larger radial quadrat was established (20 m diameter) and plant abundance, cover and life stage was recorded.

A photograph of each Jessop transect was taken.

A detailed method for the stockpile monitoring program is supplied in TWI-022 Stockpile Monitoring.

3 RESULTS

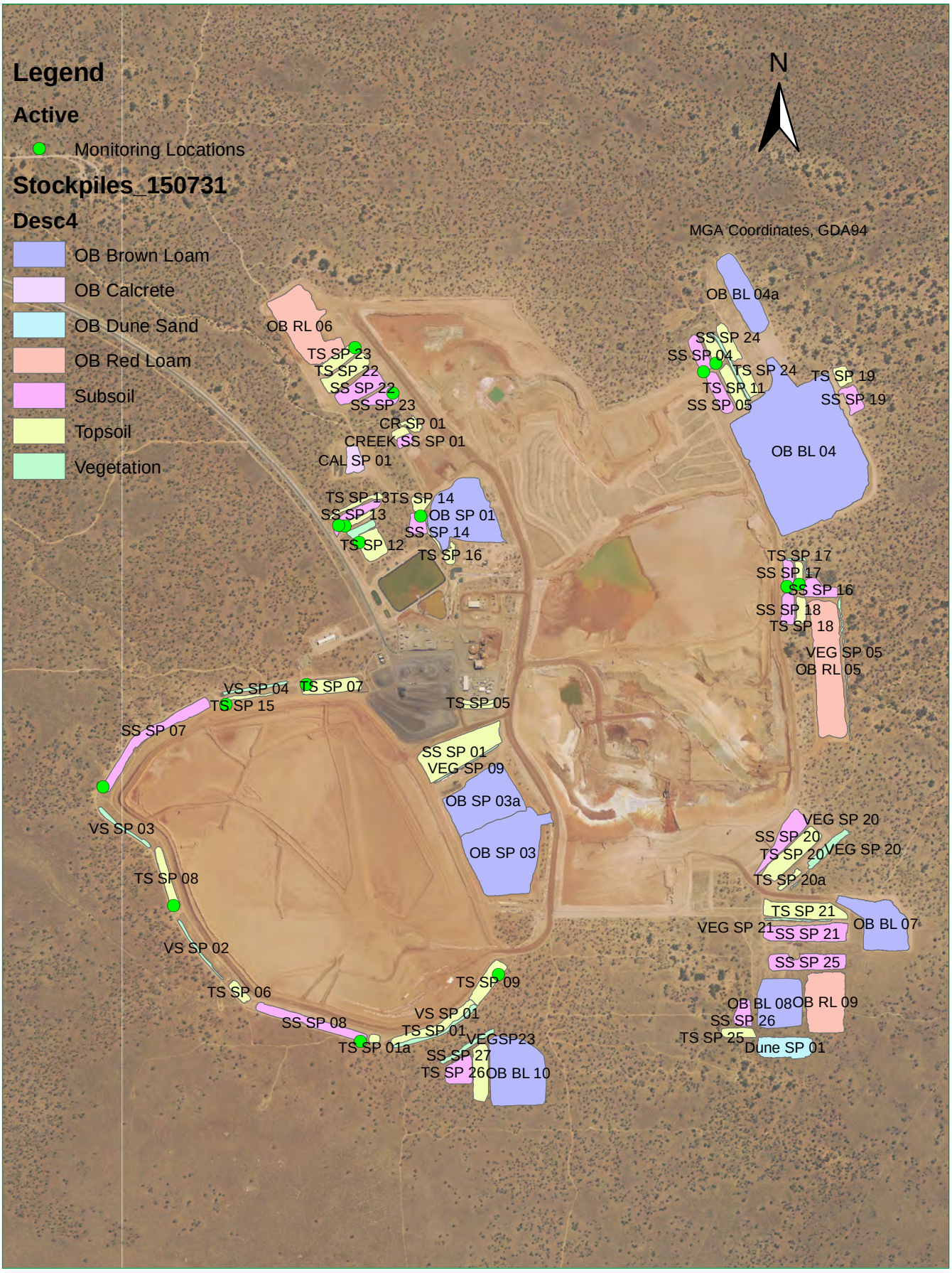
Monitoring of stockpiles has been carried out periodically between 2009 and 2015 (Table 1). Not all stockpiles have been monitored each year due to use of material for rehabilitation or disturbance of stockpiles due to clearance activities. In 2015 16 stockpiles (8 subsoil and 8 topsoil) were identified for long term monitoring, these will be monitored annually in future programs.

Table 1 Summary of stockpiles monitored each year

Stockpile ID	Age	Vegetation association	2009	2010	2012	2015	Long term program
Topsoil							
TS SP 05	2009	Myall	✓	✓	✓		
TS SP 06	2009	Myall/Chenopod	✓	✓	✓	✓	✓
TS SP 07	2009	Myall/Chenopod	✓	✓	✓	✓	✓
TS SP 08	2009	Myall/Chenopod	✓	✓	✓	✓	✓
TS SP 09	2009	Myall/Chenopod	✓	✓	✓	✓	✓
TS SP 10	2009	Myall	✓	✓	✓	✓	✓
TS SP 11	2009	Myall/Mallee		✓	✓		
TS SP 12	2009	Myall	✓	✓	✓	✓	✓
TS SP 13	2009	Myall	✓	✓	✓	✓	✓
TS SP 15	2009	Myall/Chenopod	✓	✓	✓		
TS SP 16	2009	Myall	✓	✓	✓		
TS SP 17	2009	Mallee	✓	✓	✓	✓	✓
TS SP 18	2010	Myall		✓	✓		
TS SP 19	2010	Myall		✓	✓		
TS SP 23	2009	Mallee		✓		✓	✓
Subsoil							
SS SP 01	2009		✓				
SS SP 04	2009	Myall	✓		✓	✓	✓
SS SP 05	2009	Myall/Mallee	✓		✓		
SS SP 07	2009	Myall/Chenopod	✓		✓	✓	✓
SS SP 08	2009	Myall/Chenopod	✓		✓	✓	✓
SS SP 13	2009	Myall			✓	✓	✓
SS SP 14	2009	Myall	✓		✓	✓	✓
SS SP 16					✓	✓	
SS SP 17	2009	Mallee	✓		✓	✓	✓
SS SP 18	2010	Myall			✓		
SS SP 19	2010	Myall			✓		
SS SP 23	2009	Mallee				✓	✓

The location of monitoring stockpiles is provided in Figure 1 Stockpile monitoring locations.

Images of representative stockpiles are given in Plate 1 to Plate 18.



Jacinth Ambrosia

Stockpile Monitoring Locations 2015

ILUKA

ORIG: JLEE DRAWN: JLEE SCALE: 1:18,721 (A4) DATE: August 2015 DWG No: NA

FIGURE: 1

3.1 Rainfall

Total annual rainfall over the monitoring program is provided in Figure 2. Complete local rainfall data from the JA weather station is only available from 2013 onwards (note that data is missing for December 2013 and November and December 2014). Generally rainfall was initially similar to the long term annual average. Higher than average rainfall was recorded in 2011 at Tarcoola and at JA in 2014 (both due to large single events in February). Total annual rainfall since 2011 has been consistently lower than average.

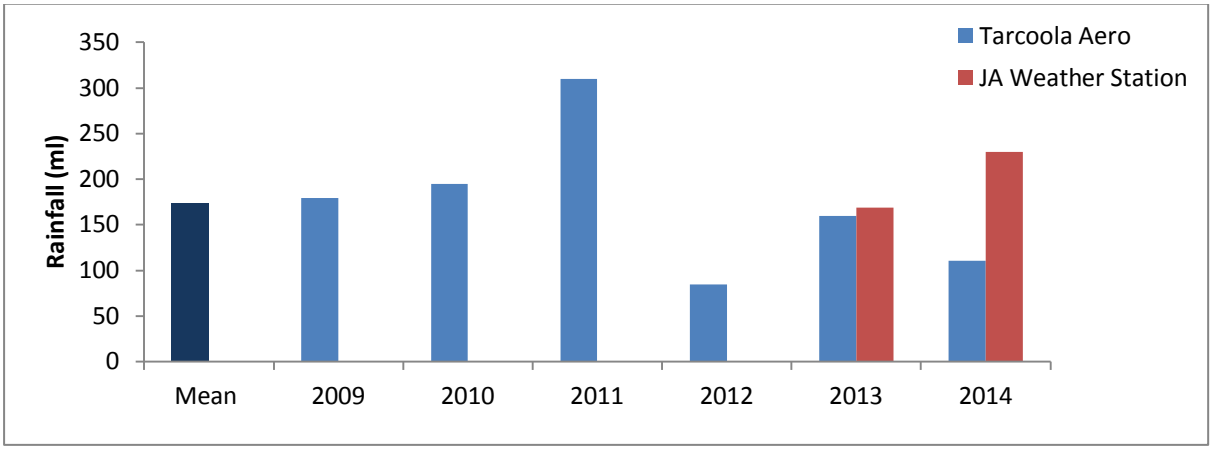


Figure 2 Total annual rainfall (Tarcoola Aero)

3.2 Vegetation

Species richness across stockpiles ranged overall from one species recorded to 12 species, Figure 3 and Figure 4. The only instance of one plant recorded was a subsoil stockpile monitored in 2009. Mean species richness for subsoil (6.17 species) and topsoil (5.5 species) was similar when records for all periods combined, however topsoil had on average a greater species richness for 2009 (Figure 5). No subsoil stockpiles were monitored in 2010. In 2012 and 2015 species richness for subsoil and topsoil stockpiles was similar.

The mean numbers of species recorded in the larger radial quadrats was greater than recorded in the Jessop quadrats (Figure 6). The highest mean species richness was recorded in 2012, 10 species for topsoil stockpiles and 11 species for subsoil stockpiles. Similar to the Jessop data the radial data indicates that species richness is similar in subsoil and topsoil stockpiles. The mean increase in species for 2012 to 2015 is 3.7 species for subsoil and 3.5 species for topsoil stockpiles.

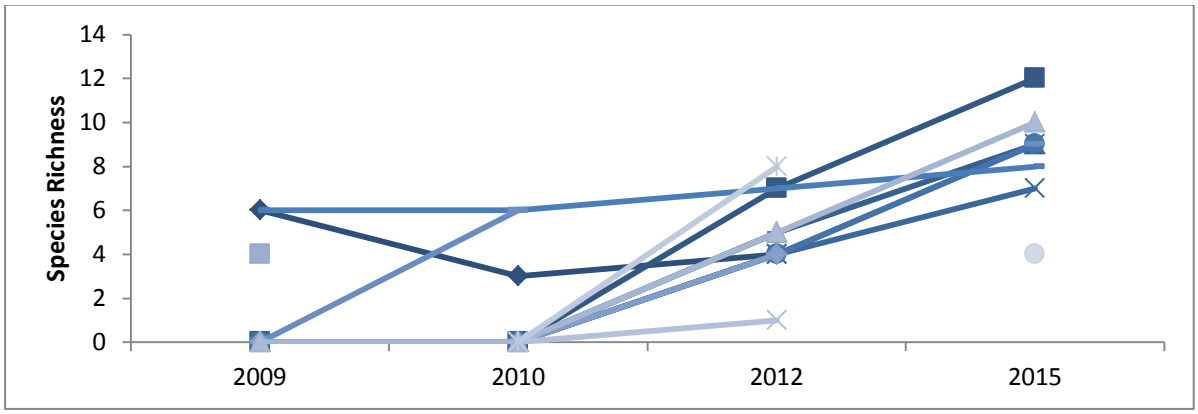


Figure 3 Species richness for topsoil stockpiles across survey periods 2009 – 2015

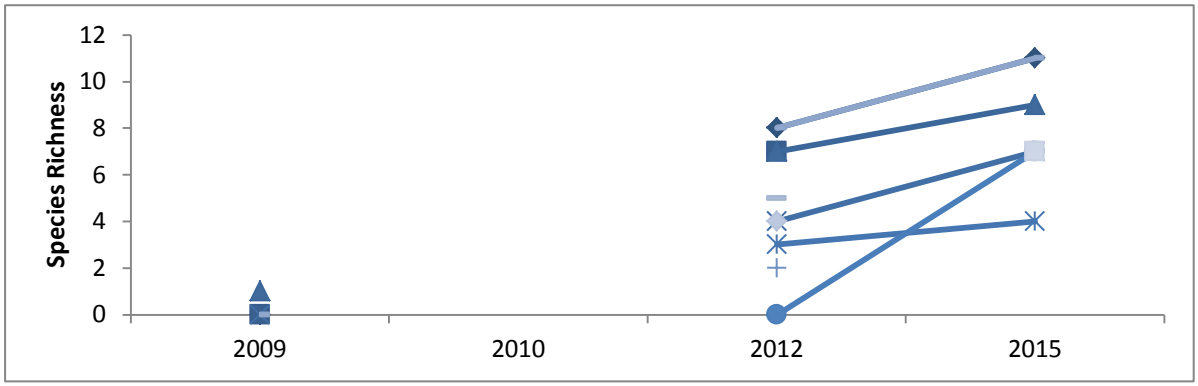


Figure 4 Species richness for subsoil stockpiles across survey periods 2009 – 2015

Species richness for all stockpiles increased with time, Figure 7. Species richness did not increase from 2009 to 2010, however an additional 8 species were recorded in 2012, and a further additional 3 species recorded in 2015.

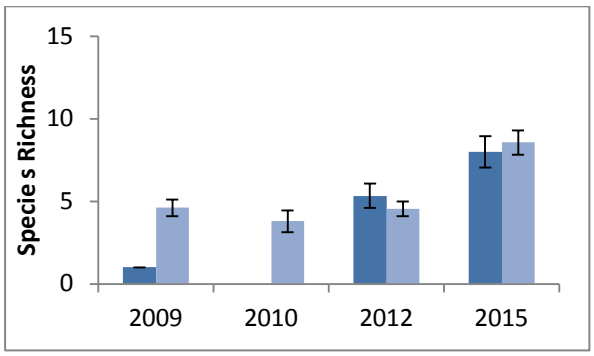


Figure 5 Jessop data - Mean species richness for stockpiles across monitoring periods. Error bars indicate standard error.

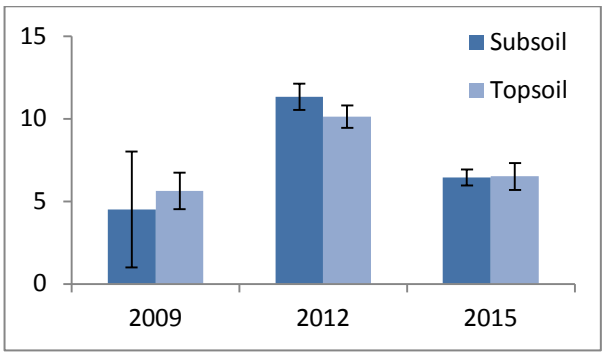


Figure 6 Radial data - Mean species richness for stockpiles across monitoring periods. Error bars indicate standard error

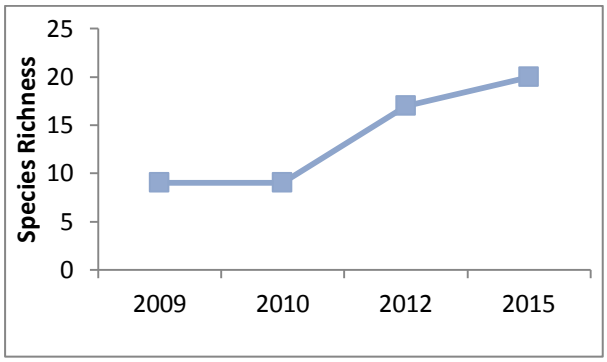


Figure 7 Species richness for all stockpiles with time.

The number of plants per stockpile ranged from one individual (the subsoil stockpile monitored in 2009) to 94 individuals (recorded on a subsoil stockpile in 2015), Figure 8 and Figure 9. Overall mean plant abundance across for all monitoring periods was similar for subsoil (28.41 individuals) and topsoil (26.47 individuals). Similar to species richness plant abundance was significantly higher on topsoil stockpiles in 2009 in comparison to the subsoil stockpiles monitored (Figure 10). However in 2012 and 2015 plant abundances were similar for both subsoil and topsoil stockpiles (Figure 10).

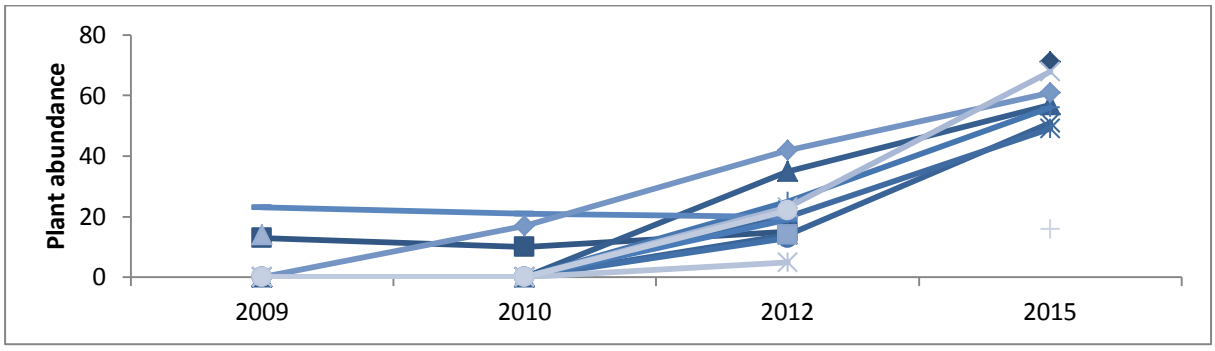


Figure 8 Plant abundance for topsoil stockpiles across survey periods 2009 – 2015

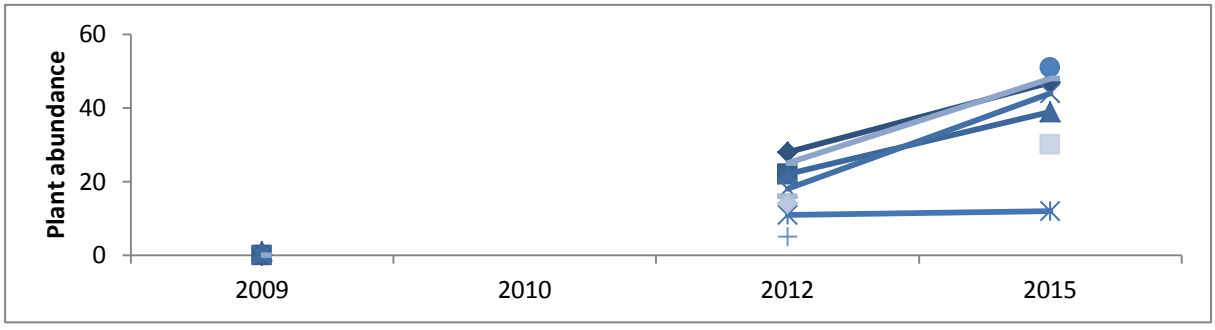


Figure 9 Plant abundance for subsoil stockpiles across survey periods 2009 – 2015

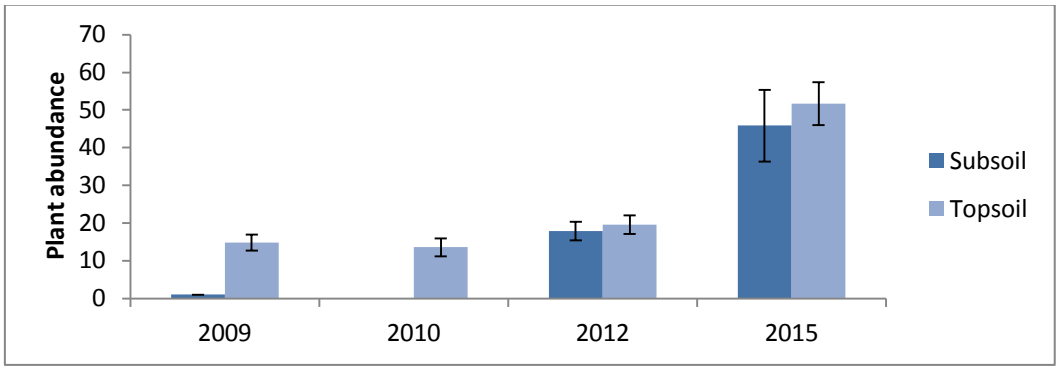


Figure 10 Mean plant abundance for stockpiles across monitoring periods. Error bars indicate standard error.

A total of 25 species have been recorded during the stockpile monitoring program. Species recorded for the first time in 2015 include *Maireana pentatropis*, *M. suaedifolia*, *Zygophyllum aurantiacum* and *Z. eremaeum* (Table 2). *Sclerolaena obliquicuspis* was first identified in 2015, however this species is the most common of *Sclerolaena* species and it is likely unidentified *Sclerolaena* species previously recorded are *S. obliquicuspis*.

Generally abundances of the more common species increased over time (Figure 11).

3.3 Biological Soil Crusts

Crust cover and class information is only available from the 2015 monitoring program.

Overall crust was recorded in 83% of stockpile quadrats. For subsoil stockpiles crust was recorded in 67% of quadrats. In comparison, topsoil quadrats where crust was recorded in 98% of quadrats. No crust was identified on one subsoil stockpile (SSSP13); all other stockpiles had crust recorded in at least 3 quadrats. All crust recorded was identified as Class 1.

Overall crust cover for all stockpile quadrats was 23.9% (±1.67). Mean crust cover for quadrats was greater on topsoil stockpiles (35%±2.5) in comparison to subsoil stockpiles (13.1%±1.4), Figure 12.

Table 2 Species recorded each year of the monitoring program

Species	2009	2010	2012	2015
Annuals				
Cephalipterum drummondii	✓			
Lepidium phlebopetalum	✓		✓	
Rhodanthe floribunda	✓	✓	✓	✓
Salsola australis[1]	✓		✓	✓
Tetragonia eremaea	✓	✓		
Zygophyllum ovatum	✓	✓	✓	✓
Grasses				
Austrostipa nitida	✓	✓	✓	✓
Shrubs				
Atriplex vesicaria	✓	✓	✓	✓
Chenopodium sp	✓		✓	✓
Enchylaena tomentosa var. tomentosa	✓		✓	✓
Eremophila scoparia	✓		✓	✓
Eriochiton sclerolaenoides	✓		✓	
Maireana erioclada	✓		✓	
Maireana pentatropis				✓
Maireana radiata	✓	✓	✓	✓
Maireana sedifolia	✓	✓	✓	
Maireana sp.				✓
Maireana suaedifolia				✓
Maireana trichoptera			✓	✓
Rhagodia sp		✓	✓	✓
Sclerolaena obliquicuspis				✓
Sclerolaena sp	✓	✓	✓	
Zygophyllum apiculatum		✓		
Zygophyllum aurantiacum ssp. aurantiacum				✓
Zygophyllum eremaeum				✓
Weeds				
*Brassica tournefortii**			✓	
*Sonchus oleraceus**				✓

[1] Previously described as *S. tragus* and *S. kali*

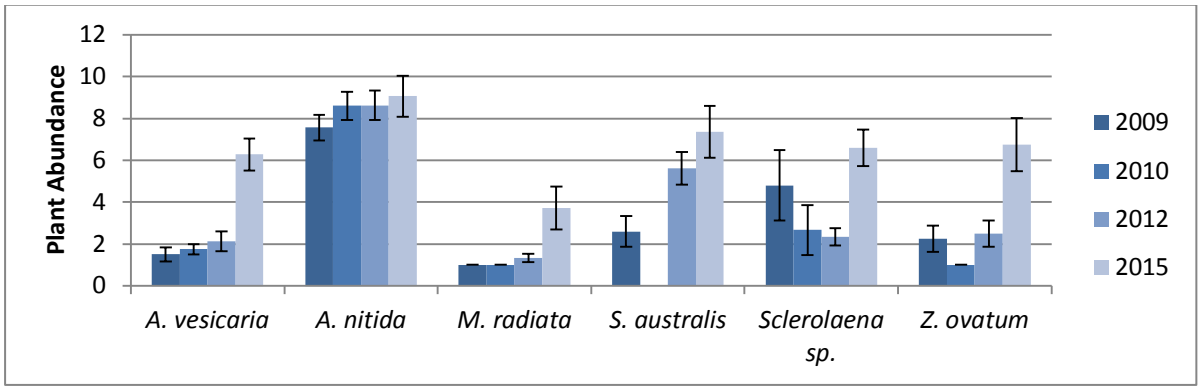


Figure 11 Mean species abundances across all stockpiles

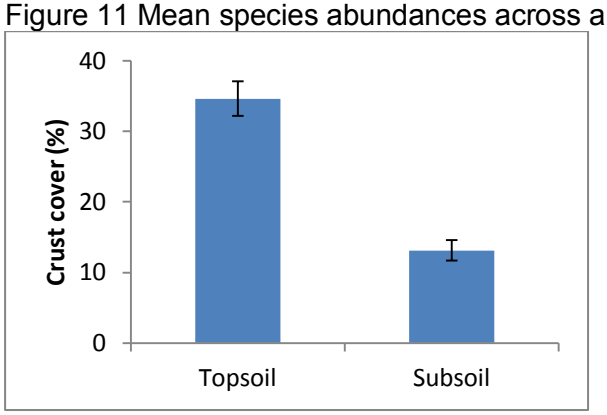


Figure 12 Mean crust cover for subsoil and topsoil stockpiles. Error bars indicate standard error.

4 DISCUSSION

The establishment of vegetation generally occurred faster on topsoil stockpiles than on subsoil stockpiles with nearly 50% of species richness occurring in the first two years. In 2012 (three years after stockpile were established) species richness in both subsoil and topsoil stockpiles was similar. Plant abundance showed similar response as species richness. The numbers of plants that established on stockpiles was much greater for topsoil stockpiles than subsoil stockpiles. However plant abundance was similar for both subsoil and topsoil stockpiles by 2012. Species richness and abundance of subsoil stockpile was not monitored in 2010 (two years after establishment). Topsoil stockpiles showed little increase in the one year period (2009 to 2010) so it is difficult to determine the response on subsoil stockpiles during this time.

The total annual rainfall for 2011 was above average and the increase in species richness and abundance on all stockpiles may be a reflection of the increased rainfall.

Similar species richness and abundance would indicate that the subsoil stockpiles contain some seedbank. Low germination rates initially may be due to low seedbank stocks and increase from 2010 onwards due to seed set of the initial species. There is some potential for replacement of the seed bank following seeding of species present on the stockpiles, particularly for species lacking seed dormancy and that readily set seed. Any replacement would be limited to the surface of the stockpile only.

Alternatively the delay in vegetation growth on subsoil stockpiles may be due to seed dormancy. For Australian plant species physical and physiological are considered to be the most common dormancy mechanisms. Studies of 16 common species recorded at JA (1) indicate three forms of dormancy, namely physiological, physical and a combinational dormancy (both physical and physiological dormancy). Of the plants studied eight have been recorded during the stockpile monitoring program:

1. *Atriplex vesicara* - no dormancy identified, however temperature dependent germination and light dependence also recorded, viability reduces rapidly with time (in the soil seedbank). First identified on stockpile in 2009.
2. *Eriochiton sclerolaenoides* - temperature dependent germination and viability of seed rapidly reduces with time. First identified on stockpile in 2009.
3. *Lepidium phlebopetalum* - physiological dormancy identified. First identified on stockpiles in 2009.
4. *M. erioclada* - no dormancy identified when seed removed from fruit, however physiological dormancy identify when seed remained in fruit. Some temperature dependence for germination. First identified on stockpiles in 2009.
5. *M. radiata* – no dormancy identified when seed removed from fruit, however cyclic and light and temperature dependent physiological dormancy identify when seed remained in fruit. First identified on stockpiles in 2009.
6. *M. trichoptera* – no seed dormancy identified, however first identified on stockpiles in 2012.
7. *Rhodanthe floribunda* – cyclic physiological dormancy identified (light dependence also recorded). First identified on stockpiles in 2009.
8. *Zygophyllum aurantiacum* – cyclic physiological dormancy identified. First identified on stockpiles in 2012.

Any seed dormancy would be similar for seeds in both topsoil and subsoil stockpiles (unless dormancy was also related to depth of burial, i.e. dormancy is more difficult to break when buried deeper).

No plant species with hard seed coats were recorded during the stockpile monitoring program. This indicates that conditions did not allow water imbibition (i.e. soaking or scarification) or alternatively these species are not present in the seedbank.

Differences between the 2009 radial data results and the 2009 Jessop data results indicate that the Jessop are not as representative of the stockpile abundance and richness as they could be (this was also noted anecdotally during the monitoring program). Radial data indicates that species richness for subsoil and topsoil was similar for all monitoring periods, although much more variable for subsoil in 2009. The reduced richness may be due to the lower densities, i.e. less likely for a particular species to fall within the Jessop if at low densities.

Biological soil crust cover was consistently lower on subsoil stockpiles compared to topsoil stockpiles. The presence of BSC is limited to the soil surface and for subsurface communities at the crust - soil surface interface. Soil crust has been recorded up to 15 cm in depth in other arid environments (2), however at JA Cyanobacteria were also found to exist naturally in the undisturbed soils of J-A at depths of 50 cm (3). Results here indicate that the presence of visible BSC in the subsoil profile is significantly less than found in the topsoil, however it is likely that much of the BSC cover will be beneath the soil surface.

5 RECOMMENDATIONS

Based on the stockpile monitoring the flowing recommendations are provided:

- Monitoring program:
    - Compare stockpile monitoring data with rehabilitation data, and increase the size of the Jessop to a minimum of 20 m.
    - Record all crust and vegetation data in each quadrat (quadruple the sample size).
    - Include crust information in rehabilitation trial monitoring (not currently recorded).
    - Move Jessop transects to central locations on the stockpiles.
- Additional investigation required:
    - Investigate the actual depth of the seed bank in undisturbed soils. This information will be important for the investigations into the suitable depth for topsoil stripping.

- Investigate the biological viability response (BSC and seedbank) with depth of storage and time for subsoil and topsoil stockpiles for stockpile greater than 2 years old and at depth greater than 0.5 m.
- Continue investigating seed dormancy and viability for JA species.

- Planning:
  - Develop and implement a seed collection plan that strategically targets seed species based on dormancy and viability analysis and minimises any impact through reductions in the soil seedbank outside of the mine footprint.

6 REFERENCES

1. Pound L, Facelli JM, Steggles E, Ainsley P. Investigating seed ecology dynamics of plant species native to Jacinth Ambrosia mineral sands deposit - final research report. Botanic Gardens of Adelaide, South Australia, 2009.

2. Smith RJ, Benavides JC, Jovan S, Amacher M, McCune B. A rapid method for landscape assessment of carbon storage and ecosystem function in moss and lichen ground layers. Bryologist. 2015 Spr;118(1):32-45. PubMed PMID: WOS:000354224700004. English.

3. Schneemilch M. Activity of cyanobacteria in topsoil stockpiles of varying ages at the Jacinth-Ambrosia mine site. Inversity of Queensland, 2013.

7 PLATES

Plate 1 TS SP 13 2009

Plate 2 TS SP 13 2010

Plate 3 TS SP 13 2011

Plate 4 TS SP 13 2012

Plate 5 TS SP 13 2015

Plate 6 SS SP 17 2009

Plate 7 SS SP 17 2011

Plate 8 SS SP 17 2012

Plate 9 SS SP 17 2015

Plate 10 TS SP 08 2009

Plate 11 TS SP 08 2010

Plate 12 TS SP 08 2011

Plate 13 TS SP 08 2012

Plate 14 TS SP 08 2015

Plate 15 SS SP 08 2009

Plate 16 SS SP 08 2011

Plate 17 SS SP 08 2012

Plate 18 SS SP 08 2015

Plate 19 TS SP 08 2009

Plate 20 TS SP 08 2010

Plate 21 TS SP 08 2011

Plate 22 TS SP 08 2012

Plate 23 TS SP 08 2015

Appendix 8 Jacinth-Ambrosia Native Vegetation Seed Store Activity Report 2014 – 2015

ILUKA

Jacinth-Ambrosia
Native Vegetation Seed Store
Activity Report
2014 - 2015

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TABLES

PLATES

1 Introduction

At the commencement of mining, a native vegetation seed store was established at J-A to assist with rehabilitation activities. The seed store is a purpose built, climate controlled storage container that is managed by the rehabilitation team. Seed is collected and maintained in line with the *Florabank Guidelines and Model Code of Practice for community-based collectors and suppliers of native plant seed* (2000).

When seasonally available, seed is collected from vegetation within the mining lease. Subject to plant production of seed at the time of clearance, seed collection also occurs from all native vegetation strata (trees, shrubs and grasses) prior to clearance activities taking place.

A database is maintained that records the quantity of seed for each species and seed quality parameters such as viability and germination rates. The seed stock is regularly assessed to determine the potential regenerative capacity and ensure viability is sustained.

The J-A Native Seed Store currently contains a collection of 63 species from 20 families (Appendix A, Table 1).

2 Seed Collection 2014 – 2015

Seed collection activities are generally associated with rainfall events that are sufficient to encourage plant production of seed. Rainfall at J-A for 2014 and 2015 was 258 mm and 159 mm respectively. This is compared to the annual mean rainfall for the area of 177 mm (Tarcoola BOM site). As a consequence of low rainfall in 2015, seed collection for this period was less than that collected in 2014.

Table 1 below provides a summary of the seed collected for the 2014 – 2015 period.

Table 1 Summary of seed collected at J-A in 2014 and 2015

Year	Scientific Name	Common Name	Quantity (grams)
2014	*Acacia ligulata*	umbrella bush	38
	Austrostipa nitida	Balcarra grass	1919
	Atriplex vesicaria	bladder saltbush	880
	Dodonaea viscosa	narrow-leaf hopbush	257
	Eriochiton sclerolaenoides	woolly-fruit bluebush	186
	Ptilotus obovatus	pink mulla mulla	117
	Rhagodia parabolica	fragrant saltbush	10
	Rhodanthe floribunda	common white sunray	180
	Santalum spicatum	quandong	491
	Senna artemisioides ssp petiolaris	flat-stalk senna	253
	Vittadinia cervicularis	New Holland daisy	6
2015	*Alectryon oleifolius*	bullock bush	293
	Eucalyptus socialis	red mallee	2
	Maireana sedifolia	pearl bluebush	1.3
	Santalum acuminatum	quandong	992
	Santalum spicatum	sandalwood	131

3 Seed Viability Tests

Viability tests are conducted on a regular basis on native seed stored in the J-A seed collection. Testing is required to determine the quality of the seed and to estimate the germination potential for each seed lot. Testing also assists in detecting any loss in quality over time (Plate 1).

With the exception of batches that contained less than 100 seeds, a viability test of the seeds within the collection was tested in 2015 by performing a cut test of a sample of 25 – 100 seeds, the quantity varied depending on the amount of seed available from each collection. It was found that 27% of the samples tested had a viability rate <50%. Much of the collection did not have the viability recorded when initially collected, so it is unknown if there has been deterioration in viability or, the seeds were collected with low viability rates.

During the testing process four of the seed lots within the store were found to be infested with insects and were disposed of these included batches from the following species *Atriplex vesicaria*, *Maireana pentatropis*, *Senna cardiosperma ssp. gawlerensis*, *Vittadinia cervicularus*. It was also found that some seed lots were not cleaned sufficiently when initially placed in the store resulting in a misleading estimated seed volume for those species. The seed weight for these batches has been amended.

Viability tests will be conducted again in 2016 and a comparison will then be made to determine any deterioration in germination potential.

Plate 1 Examples of viable and nonviable seeds

4 Seed Collection Strategy for 2016

4.1 Seed Collection

A gap analysis of species not currently found in the collection, or only present in low quantities, has been conducted and as a result a number of species have been identified as targets for collection in 2016 these include:

- *Alectryon oleifolius ssp. canescens*
- *Dodonea viscosa ssp. angustissima*
- *Eremophila latrobei ssp. glabra*
- *Eremophila scoparia*
- *Lycium australe*
- *Maierana sedifolia*
- *Myoporum playtcarpum ssp. playtcarpum*
- *Santalum spicatum*
- *Scleroleana obliquicuspis*
- *Senna artemisioides ssp. coriacea*
- *Senna artemisioides ssp. petiolaris*
- *Senna cardiosperma ssp. gawlerensis*

Other species already present will also be collected opportunistically dependent on availability.

4.2 Germination Tests

Germination tests are an additional tool to determine the viability of seed in storage and are a reliable way to determine what results may be achieved in the field, especially for seeds that are known to have dormancy mechanisms.

In 2016 germination tests will be conducted on a selection of species. For the species with no or simple dormancy mechanisms the tests will be carried out in the on-site greenhouse. Species with more complex dormancy mechanisms will be tested by the Seed Centre at the Botanic Gardens of Adelaide.

Austrostipa nitida seeds will also be tested by students completing the Restoration Ecology course at the University of South Australia, Mawson Lakes Campus. To determine variability in germination rates of different aged seeds.

4.3 Pearl blue bush investigations

Also planned for 2016 are projects related to one of the primary species present in the chenopod vegetation association i.e. pearl blue bush (*Maireana sedifolia*). Pearl blue bush is a slow growing species, living more than 150 year. Very few seeding events have occurred during the life of the mine and therefore little seed collected. In addition to this the seed that has been collected was identified as non-viable.

The two projects proposed to address the seed deficit are:

Pearl blue bush reproduction

Pear bluebush reproduces infrequently, and at J-A little viable seed has been available for collection.

Although past studies have looked at various aspects of blue bush reproduction, it is not clear what triggers flower production in this species nor the conditions necessary to produce viable seeds. The aim of this honours project will be to investigate environmental cues that facilitate flowering in this species through a series of field and glasshouse experiments.

Pearl blue bush genetics

Given the lack of viable seed available for collection at J-A it would be beneficial to determine if it would be possible to use seed collected from plant sources outside of the Yellabinna region. An investigation of the genetic variability of pearl blue bush across the State would enable Iluka to determine if they can source seed from other regions without compromising the genetic integrity of the pearl blue bush in the Yellabinna.

Both the University of Adelaide and the University of South Australia have been approached to assign Honours students to complete the above projects with financial and field based assistance from Iluka.

Appendix A

Scientific Name	Common Name	Quantity (grams)
Acacia ligulata	umbrella bush	959
Acacia oswaldii	umbrella wattle	1647.2
Acacia papyrocarpa	western myall	10373
Acacia ramulosa	sand dune mulga	5
Alectryon oleifolius	bullock bush	80
Atriplex vesicaria	bladder saltbush	32391
Austrostipa eremophila	desert spear grass	2001
Austrostipa nitida	Balcarra grass	13640
Austrostipa platychaeta	feather spear grass	1088
Brachyscome ciliaris var. ciliaris	variable daisy	3
Casuarina pauper	Belah	1
Cephalipterum drummondii	pom pom heads	278
Cratystylis conocephala	bluebush daisy	98
Dodonaea viscosa	narrow-leaf hopbush	417
Duboisia hopwoodii	pituri	6
Enchylaena tomentosa	ruby saltbush	407
Enneapogan nigricans	black nineawn	1874
Eragrostis falcate	sickle love-grass	1
Eremophila alternifolia	scented emubush	3
Eremophila latrobei ssp glabra	Latrobe's emubush	1
Eriochiton sclerolaenoides	woolly-fruit bluebush	7094
Eucalyptus gracilis	white mallee	11
Eucalyptus oleosa	red mallee	848
Eucalyptus socialis	inland red mallee	19
Frankenia sp.	sea-heath	653
Glycine sp.	glycine	4
Goodenia pinnatifida	scrambled eggs	79
Hakea francisiana	grass-leaf hakea	1
Lepidium phlebopetalum	veined peppercress	276
Maireana erioclada	rosy bluebush	1656
Maireana integra	entire-wing bluebush	52
Maireana pentatropis	erect mallee bluebush	1968
Maireana radiata	radiate bluebush	1184
Maireana sedifolia	pearl bluebush	268
Maireana tricophtera	hairy-fruit bluebush	258
Maireana turbinata	top-fruit bluebush	1043
Marsdenia australis	bush banana	5
Melaleuca interioris	broom honey-myrtle	3
Minuria cunninghamii	bush minuria	3
Myoporum platycarpum	false sandalwood	15
Ptilotus nobilis ssp. nobilis	pink mulla mulla	16
Ptilotus obovatus	silver mulla mulla	4769
Pychnosorus pleiocephale	soft billy buttons	186
Radyera farragei	bush hibiscus	92
Rhagodia parabolica	fragrant saltbush	98

Scientific Name	Common Name	Quantity (grams)
Rhagodia spinescens	spiny saltbush	547
Rhodanthe floribunda	common white sunray	970
Rytidosperma caespitosum	white-top	1135
Salsola tragus	buckbush	3671
Santalum acuminatum	quandong	23209
Santalum spicatum	sandalwood	6729
Sarcocornia sp.	glasswort	5109
Scleroleana sp.	-	1732
Scleroleana obliquicuspis	limestone coppurburr	66
Scleroleana patenticuspis	spear-fruit bindyi	665
Senna artemisioides ssp petiolaris	flat-stalk senna	1377
Senna cardiosperma ssp. gawlerensis	Gawler Ranges senna	387
Tetragonia eremaea	native spinach	1723
Vittadinia cervicularis	New Holland daisy	1069
Zygophyllum apiculatum	gallweed	375
Zygophyllum aurantiacum	shrubby twinleaf	2110
Zygophyllum eremaeum	pale-flower twinleaf	1337
Zygophyllum ovatum	dwarf twinleaf	374

Appendix 9 Jacinth Ambrosia Dune and Creek Soil Characterisation

ILUKA

Jacinth Ambrosia Dune and Creek Soil Characterisation

2014

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1 INTRODUCTION

This report presents the results of a drilling program carried out in August 2014 to determine the depth of sand in association with myall/mallee woodlands and the creek systems of J-A.

1.1 Dune Characterisation

The J-A rehabilitation management plan currently specifies the return of 2.4 m of dune sand to areas identified as myall/mallee woodland. It was identified that mallee would only grow in sand of this depth and that myall/mallee vegetation in proposed project area all had this depth of sand. The depth of sand information was based on a very limited sampling data set. Further, the vegetation mapping identified large areas of myall/mallee habitat. This resulted in a large amount of sand required to rehabilitate these areas.

It became apparent in 2012/2013 that dunes did not have 2.4 m depth of sand uniformly across the proposed J-A pit area. Depth of sand varied and mallee were found to grow in areas where there was no or limited sand. In addition areas identified as myall/mallee vegetation was over estimated and where mallee was associated with dune features these area were a discrete portion of the area.

Subsequent discussion with DSD regarding this issue resulted in the following outcomes:

- The current myall/mallee woodland boundary for topsoil and subsoil removal would be maintained. Species associated with sand dunes occur both on the sand dune and in proximity to the sand dune.
- Detailed soil surveys are to be conducted for each dune feature prior to overburden removal. This will assist in identifying the location and depth and enable the removal of the sand as a discrete unit.
- The dune sand from Cell 1 East was removed in 2009, prior to the more detailed dune understandings developed in 2012. Subsequently the amount of dune sand from Cell 1 East was not originally measured. To progress with rehabilitation of the area in 2012, an estimation method was developed using principals derived from the dune study and using original aerial photography (Sand Dune Study).
- Original material type ratios to be determined for each dune prior to overburden removal via detailed dune soil surveys.
- Reapply approximately the same quantity of dune sand during rehabilitation as was stripped during overburden removal. The design should be a consolidated shape, not trying to mimic the original random sand dune. To maximise the area of sand, the features should be 1 m deep.

1.2 Creek Characterisation

A total of six river types have been identified in the JA catchment area (Alluvium, 2013), comprising (Figure 1):

1. Chain of pans
2. Interdunal bank confined channel, sand
3. Interdunal bank confined gully, sand
4. Interdunal wandering, sand
5. Terminal lunette chain of pans
6. Valley fill, sand

Descriptions of these river types can be found in the Alluvium Report (Jacinth Ambrosia Watercourse Rehabilitation, October 2013).

Of these six river types, four types will be disturbed due to project activities and subsequently require restoration:

1. Interdunal bank confined channel, sand
2. Interdunal bank confined gully, sand
3. Interdunal wandering, sand
4. Valley fill, sand

Three of these river types fall (interdunal bank confined channel, interdunal bank confined gully, and interdunal wandering) within both myall woodland and myall/mallee woodland vegetation association in the impact area. Valley fill sand is only found in myall in the impact area.

It was identified that the river styles to be restored require a sand base as part of the soil profile, however the depth of sand varies for river style, vegetation type and location.

1.3 Aims

The aims of the dune and creek characterisation study are to:

- Determine depth of sand at the dune and creek features;
- Verify accuracy of using historical drill hole logs to determine depth of sand; and
- Verify accuracy of excavation techniques to determine sand depth.

2 METHODS

2.1 Drilling Program

Drilling was carried out at various locations using a hollow stem auger. Samples were collected at 0.5 m increments.

2.2 Location of Dune Drilling Program

The Jacinth Dune South is approximately 2.4 ha. The dune drilling program comprised 29 holes across three transects running NW-SE 50 m apart. Drill holes were at 50 m intervals (Figure 1).

All drilling was carried out to a depth where the final depth of sand could be determined, based on in-field analysis of samples.

2.3 Location of Creek Drilling Program

The proposed soil survey comprised three drill locations for each river type and vegetation type to be impacted by project activities (i.e. 21 drill holes across 7 locations), Table 1. Holes were 20 m apart at each location straddling the creek centre (Figure 2).

All holes were planned to be drilled to 9 m deep, unless refusal is reached or final depth of sand determined (based on in-field analysis of samples).

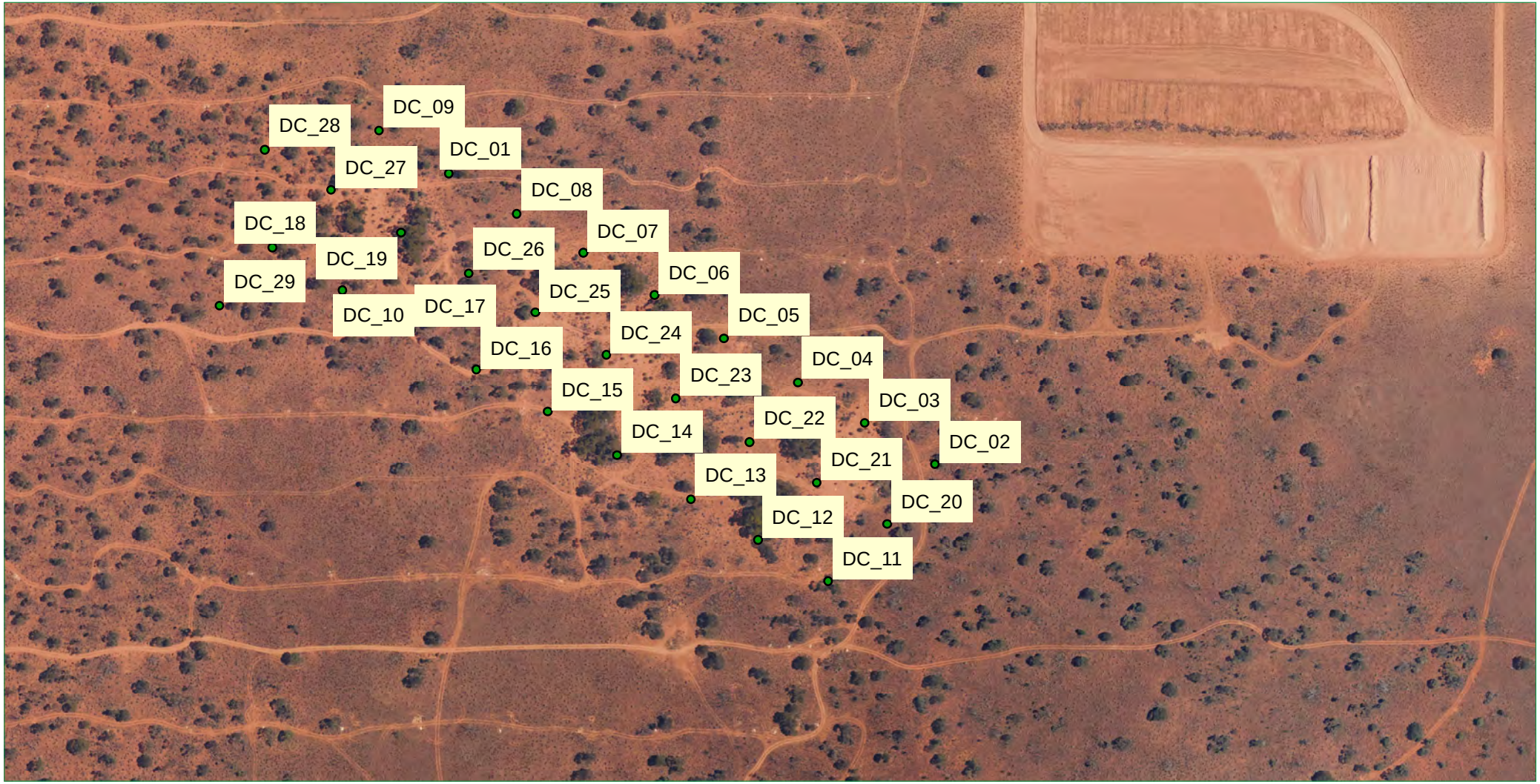
Table 1 Creek drill holes by river type and habitat

Hole ID	River Type	Habitat
CC 1	Interdunal bank confined gully	Myall Woodland
CC 2		
CC 3		
CC 4	Interdunal bank confined channel	Myall Woodland
CC 5		
CC 6		
CC 7	Interdunal bank confined gully	Myall/Mallee Woodland
CC 8		
CC 9		
CC 10	Interdunal bank confined channel	Myall/Mallee Woodland
CC 11		
CC 12		
CC 16	Valley fill	Myall Woodland
CC 17		
CC 18		
CC 19	Interdunal wandering	Myall/Mallee Woodland
CC 20		
CC 21		
CC 22	Interdunal wandering	Myall Woodland
CC 23		
CC 24		

2.4 Sample Analysis

All soil samples were collected at 0.5 m increments and analysed by EP Analysis on site (during the drilling program) and at the EP Analysis Laboratory. Sample analysis comprised:

- Texture
- Conductivity EC 1:5 weight
- pH 1:5 (water)
- $CaCO_3$
- Water repellent (Y or N)
- Smoke (Y or N)
- % rock



0.2

Kilometers

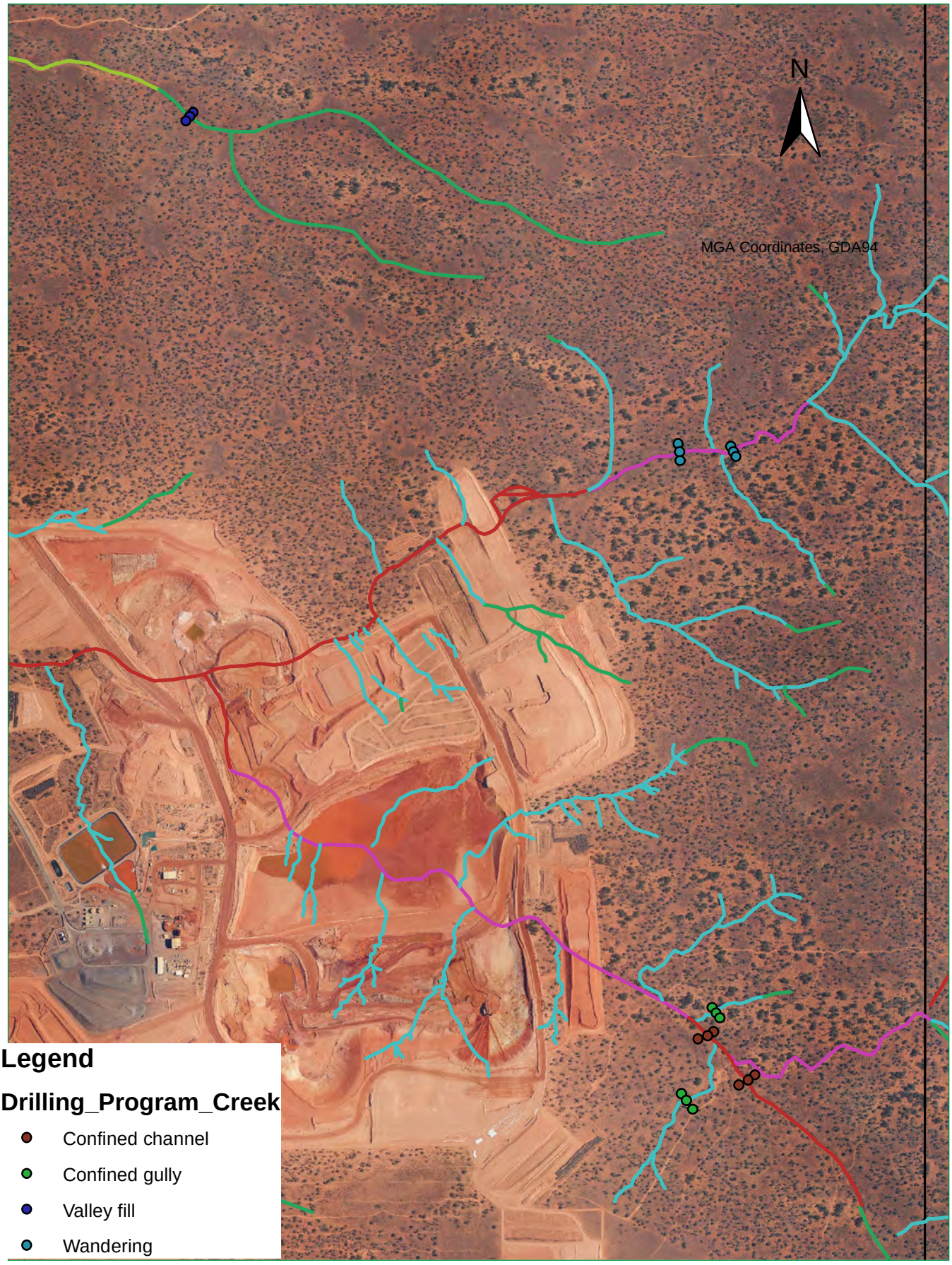
J-A

Layout of dune characterisation drilling program

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:3,585 (A4) DATE: January 2015 DWG No: Fig. 1

FIGURE: **1**



J-A

Layout of creek characterisation drilling program

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:16,134 (A4) DATE: January 2015 DWG No: Fig. 2

FIGURE: 2

3 RESULTS

3.1 Dune Drilling Program

Generally the sands (sand, loamy sand and clayey sand) were located to the uppermost portions of the drill holes (Figure 3); however occasional lenses of sand were identified at depth. The formation of the dune is likely to remnants of sand deposition of sand against the windward side (north-western) of a natural rise.

Depth of sand was variable across the dune. The deepest record of sand on the dunes was at 9.8 mbgl, however this record did not form part of a continuous layer of sand. The deepest continuous record of sand was at DC24, in the central section of the dune at 5.8 mbgl. The shallowest record of sands was recorded at DC02 and only continued for 0.8 mbgl. The uppermost sand layer generally gave way to loamy soils; very little clay materials were recorded.

All samples were very highly calcareous.

The pH ranged from moderately alkaline (pH 7.9 – a single record) to very strongly alkaline (pH 9.9 – a single record). This is similar to previously recorded on site where SWC reported the pH of sands to range from 9.2 to 9.85, and brown loams at J-A (as recorded in the soils database) have a recorded pH between 7.8 and 9.54.

Salinity ranged from 0.7 mS/cm to 4.2 mS/cm. Generally salinity increased with depth, however then started decreasing below approximately 2 m to 3 m (Figure 3). Overall salinities are similar to those previously recorded at J-A; the SWC report recorded sands with EC ranging from 0.6 mS/cm to 0.26 mS/cm, and EC for brown loams have been recorded ranging from 0.149 mS/cm to 4.14 mS/cm.

Past soil studies have shown the the salinity of brown loam is generally greater than that of the underlying red loam, further supported by this drilling program.

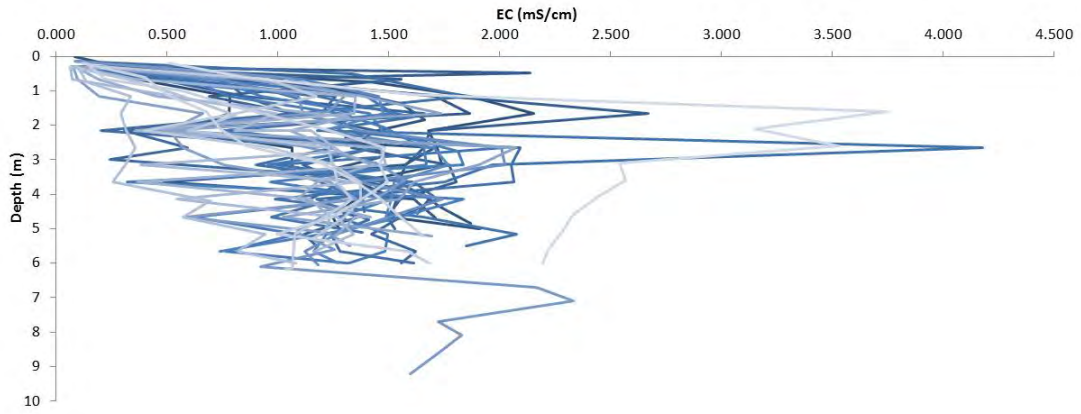


Figure 3 EC 1:5 of drill holes samples with depth for dune characterisation drilling program

mRL	DC09	DC01	DC08	DC07	DC06	DC05	DC04	DC03	DC02
172									
171.5									
171									
170.5									
170								LS	LS
169.5							LS	LS	CS
169							LS	CS	SCL
168.5				LS	LS	CS	CS	SCL	SCL
168				LS	LS	LC	LSCL	LSCL	SL
167.5				LS	LS	LS	LS	LSCL	SL
167			LS	CS	LS	L	CS	LSCL	SL
166.5			LS	CS	SL	SCL	SL	SCL	SCL
166			LS	SL	CS	SL	CS	SCL	LSCL
165.5		S	LS	SL	SCL	SCL	SC	CL	SL
165		LS	LS	SC	SCL	LSCL	SCL	CL	LSCL
164.5		LS	LS	SCL	SCL	SCL			
164	LS	LS	LS	LSCL	CL	SCL			
163.5	LS	LS	LSCL	SC	SC	SCL			
163	LS	CS	SL	SC					
162.5	CS	CS	SCL						
162	LS	SC	CL						
161.5	LSCL	LSCL	CL						
161	SL	CL	CL						
160.5	CL	SCL							
160	CL								
159.5	LC								

mRL	DC28	DC27	DC19	DC26	DC25	DC24	DC23	DC22	DC21	DC20
172										
171.5									LS	
171									LS	LS
170.5						LS		LS	LS	LS
170						LS	LS	LS	SCL	LS
169.5						LS	LS	SCL	LSCL	CS
169					LS	LS	LS	SCL	SCL	CS
168.5					LS	LS	CS	LS	SCL	SCL
168				LS	LS	LS	SL	SCL	LSCL	SCL
167.5				LS	LS/CS	LS	SL	SCL	SL	LC
167				LS	LS/CS	LS	LS	CS	LSCL	CL
166.5			LS	LS	CS	LS	SCL	LSCL	CL	LSCL
166			LS	LS	CS	LS	CS	CL		SC
165.5			CS	LS	CS	CS	SC	LS		
165		S	LS	LS	CS	CS	SCL			
164.5		S	LS	CS	SCL	SCL	SC			
164		LS	LS	SL	LSCL					
163.5	LS	LS	CS	LSCL						
163	LS	CS	SL	SCL						
162.5	LS	CS	LSCL	CS						
162	CS	CS	SCL	SCL						
161.5	CS	LSCL	CL							
161	CS	SCL	SC							
160.5	SL	SC								
160	SCL	SCL								
159.5	CL	SL								
159	LSCL	LC								
158.5	LMC									

mRL	DC29	DC18	DC10	DC17	DC16	DC015	DC014	DC013	DC012	DC011
172										LS
171.5								LS	LS	LS
171							LS	LS	LS	LS
170.5							LS	LS/CS	LS	SCL
170						CS	LS	SCL	SCL	LSCL
169.5					LS	LS	CS	CS	SCL	LSCL
169				LS	CS	LS	CS	LSCL	CL	LSCL
168.5				LS	CS	CS	SL	SCL	CL	CL
168				LS	CS	CS	LSCL	LC	CL	SC
167.5			LS	LS	SL	SL	SL	CL	CL	LC
167	CS		LS	LS	SCL	SCL	S	SCL	SCL	SCL
166.5	CS		LS	CS	SCL	SCL	LMC	SL	SCL	CL
166	CS	LS	LS	CS	SCL	SCL	LMC	SCL		
165.5	CS	LS	LS	CS	SC	CL	SL	CL		
165	SL	LS	CS	SL	SCL	SC	SCL			
164.5	LSCL	LS	CS	SL	SCL	SL				
164	LSCL	LS	SCL	SL	LSCL					
163.5	SC	LS	SL	SL	SCL					
163	CL	CS	SL	SL						
162.5	CL	SCL	SL	CS						
162	LC	LSCL	SL							
161.5		LSCL	SCL							
161		SL								
160.5		SCL								
160		LS								
159.5		SCL								
159		SCL								
158.5		SCL								
158		SL								
157.5		LC								
157		SCL								

Figure 4 –Texture of drill hole samples by depth in the northern, central and southern transects of dune characterisation drilling program

3.2 Creek Drilling Program

Sand was recorded at all locations; however depth varied (Figure 7). Further, sand at depth was recorded for all myall/mallee vegetation locations. Not all central creek sites (active creek zone) had sand in the upper soil profile 0 m to 0.3 m. Further descriptions of the soils profiles are given below.

Salinity ranged from 0.08 mS/cm to 4.3 mS/cm. Generally salinity increased with depth, then decreasing from approximately 2 m (Figure 5). Overall salinities are similar to those previously recorded at J-A, the SWC report recorded sands with EC ranging from 0.6 mS/cm to 0.26 mS/cm, and EC for brown loams have been recorded ranging from 0.149 mS/cm to 4.14 mS/cm.

Calcareous material found in the samples ranged from nil to very high. Generally the very highly calcareous records were found in the upper profiles to 5 m.

The proportion of rock per sample was largely below 30%, increasing from surface to approximately 2 m to 3 m before decreasing with depth (Figure 6).

The pH ranged from mildly alkaline (pH 6.7) to very strongly alkaline (pH 9.8). This is similar to previously recorded on site where SWC reported the pH of sands to range from 9.2 to 9.85, and brown loams at J-A (as recorded in the soils database) have pH recorded between 7.8 and 9.54.

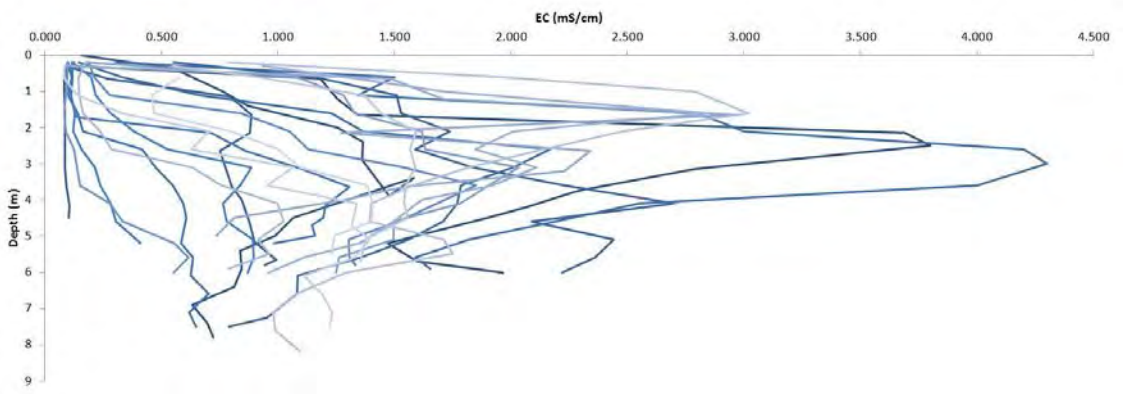


Figure 5 EC 1:5 of drill hole samples with depth for creek characterisation drilling program

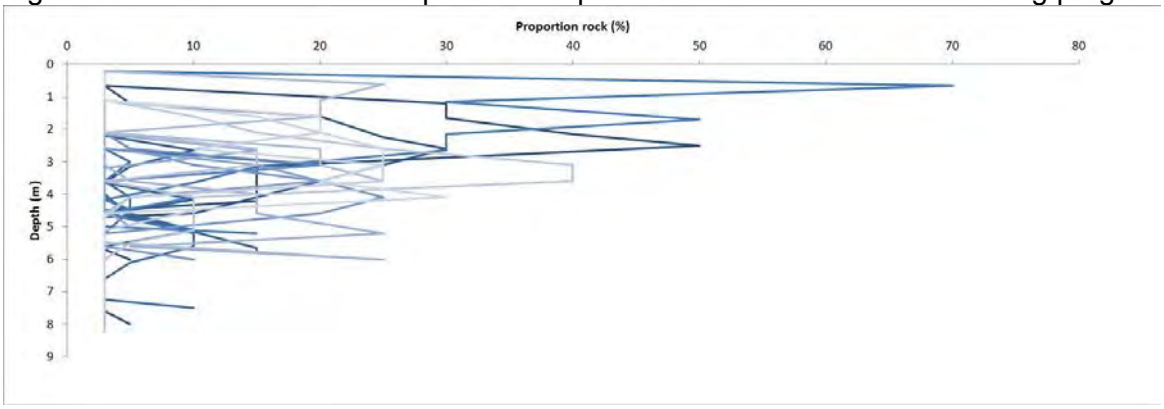


Figure 6 Proportion of rock in drill hole samples with depth for the creek characterisation program

3.2.1 Creek Soil Profiles

Interdunal bank confined gully

Myall woodland – sand to a depth of 1.8 m was recorded on the eastern side of the creek, clayey sands recorded to 0.3 mbgl at the central drill hole (active creek zone). No sand was recorded to the west of the creek. Loam soils were recorded in upper sections of the profile, below sand where present across all bore holes. Clays predominated below the loams.

Myall/mallee woodland – sand was recorded at surface across all three boreholes. The deepest sand was recorded on the eastern side of the creek to 2.8 mbgl, only surficial sand (0.3 mbgl) as recorded in the active creek zone and the on the western side. Underlying the sand profile was a mix of loam and clay layers.

Interdunal bank confined channel

Myall woodland – the only sand recorded at was to 0.3 mbgl in the active channel zone. The remaining soil profile across all bore holes was a mix of loams and clays.

Myall/mallee woodland – sand was recorded at all bore holes aross the creek. The active creek zone recorded the deepest sand, to 4.8 mbgl, underlying a discrete layer of sandy loam to 0.3 mbgl. Both the western and eastern bore holes had sand at surface to 1.8 mbgl and 0.8 m respectively. The remaining profiles were a mix of loams and clays.

Interdunal wandering

Myall woodland – a small amount of sand was recorded at the northern and active channel ones, at a depth of 2.8 mbgl and 2.3 mbgl respectively. Generally the surface profile comprised loam soils overlying clays.

Myall/mallee woodland – sand was recorded at all bore holes from surface to a depth of 2.8 mbgl. Generally loam soils were recorded below the sand with some discrete layers of clays.

Valley fill

Myall woodland – sand was recorded at surface at the southern borehole to a depth of 1.3 mbgl. A discrete layer of sand was recorded in the active channel at a depth of 1.3 mbgl. all other record of sand were located at depth from 3.8 mbgl.

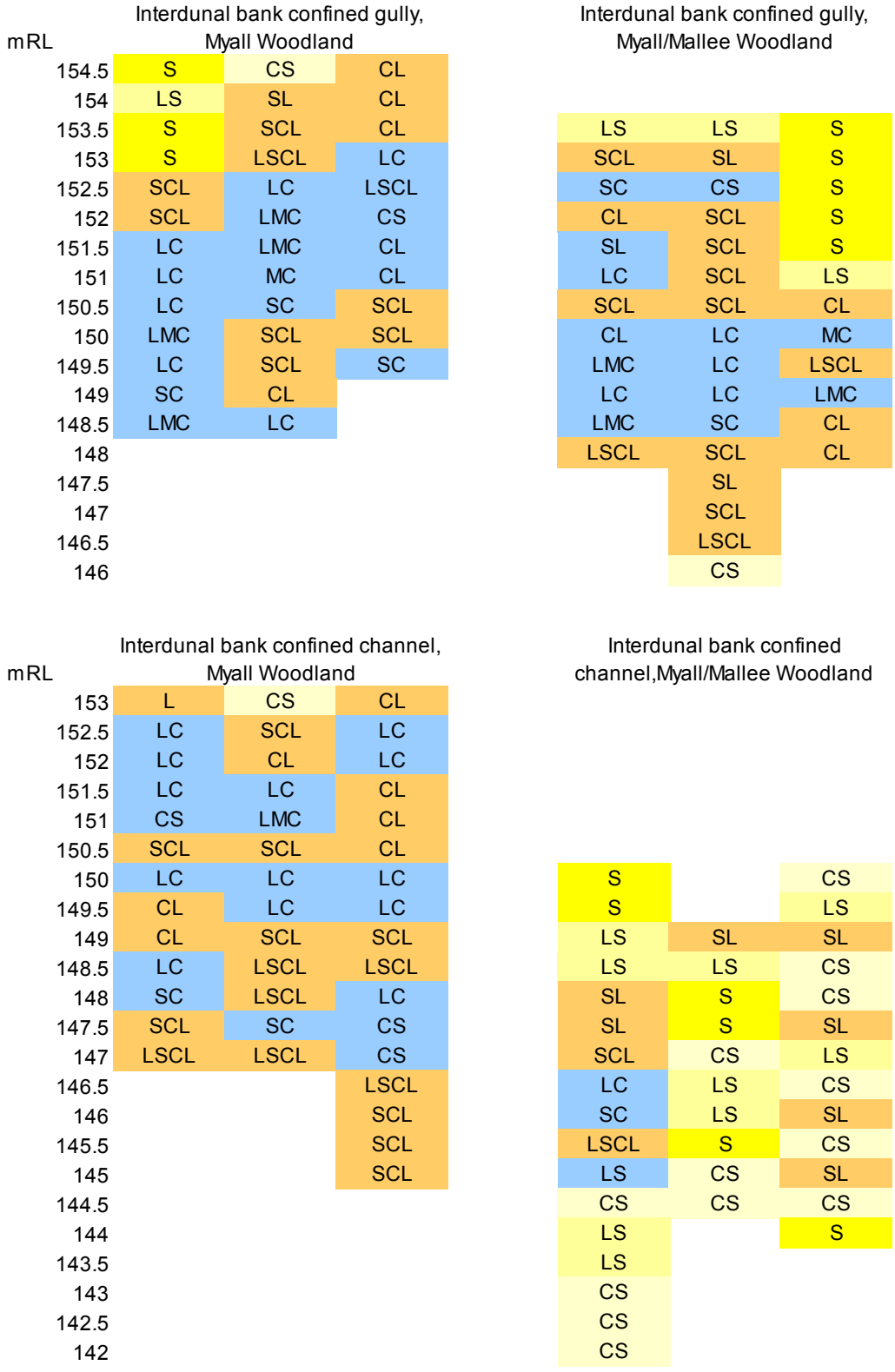


Figure 7 Texture of drill hole samples by depth from creek characterisation drilling program

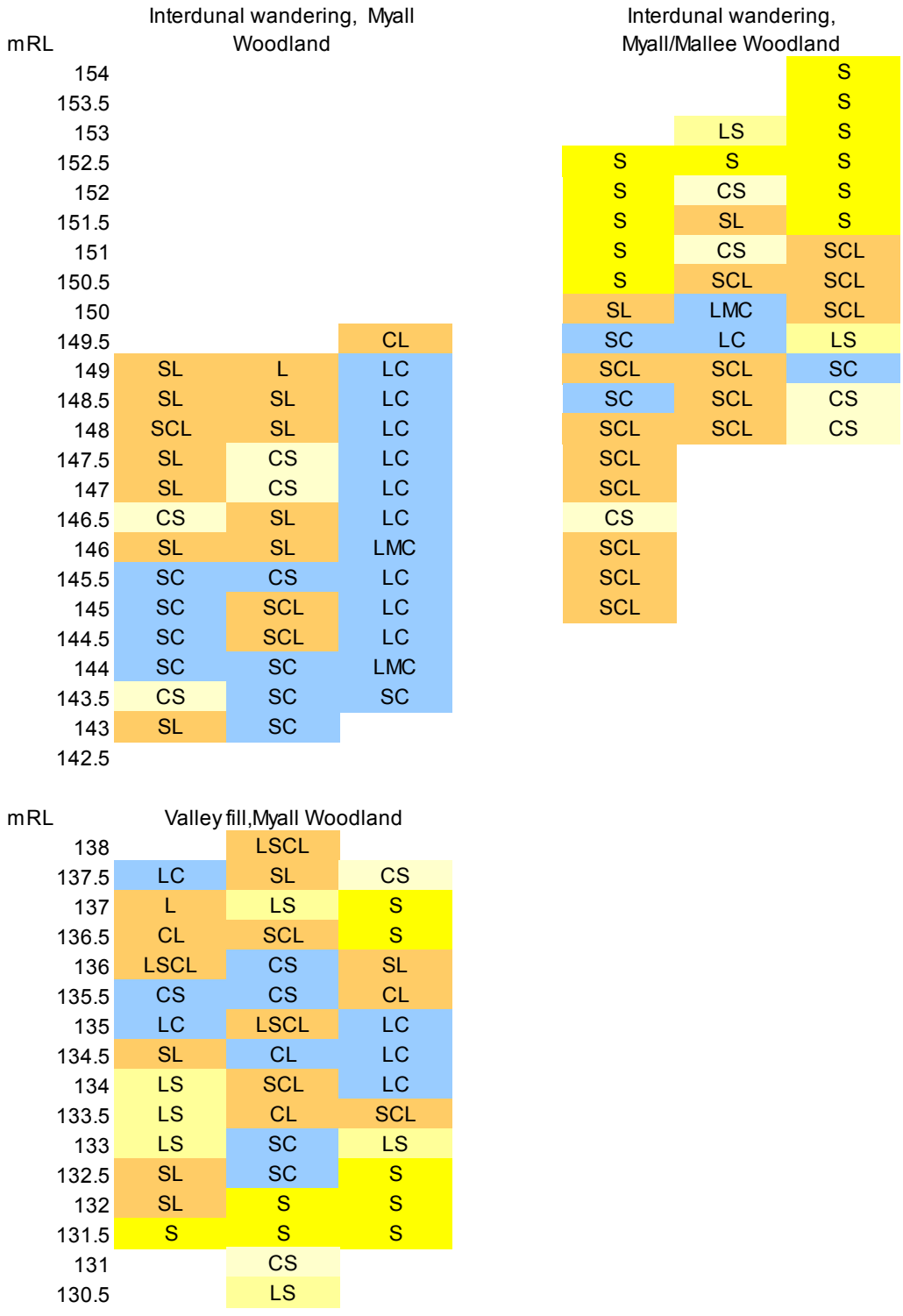


Figure 7 Texture of drill hole samples by depth from creek characterisation drilling program

4 Summary and Recommendations

Sand was present in all myall/mallee woodland locations associated with creeks and dunes. However, for each location depth varied. For the dune depth of sand ranged from 0.8 mbgl at the edges to 5.8 mbgl in the center of the dune. Similarly for creeks the depth of sand continuous from surface varied from 0.3 mbgl to 5.8 mbgl.

Sand was not present at surface for all active creek zones. The interdunal wandering (myall) and valley fill (myall) creeks only had sand lenses at depth, 1.8 m and 1.3 m from surface respectively.

The proportion of rock found in creek bore holes generally increases to 2 m to 3 m before decreasing with depth.

Based on this study it is recommended that:

- Small discrete sand dune features to be developed in rehabilitation areas designated myall/mallee woodlands. The depth of sand should vary, and be returned in a lens shape similar to natural (i.e. shallower at the edges, deeper on the center).
- Dunes and creeks should be designed on a case by case basis for each rehabilitation site (cell). The final design will describe:
    - Estimated amount of sand and/or calcrete collected from the area as part of overburden removal.
    - Availability of sand and/or calcrete for each feature.
    - The type of creek to be restored and/or size of dune to restored.
    - The depth and thickness of sand and/or calcrete for each feature.
    - Results of modelling carried out to assess erosion likelihood.

The proposed changes to the soil profile includes sand at various depth and thickness for both dune and creek zones and the addition of a calcrete layer, of various depths and thickness, for creeks (Table 2).

Table 2 Proposed soil profile reconstruction

Soil Materials	Landscape Vegetation Unit					
	Myall/Mallee Woodland		Myall Woodland		Chenopod Shrubland	
	Depth (m)	Thickness (m)	Depth (m)	Thickness (m)	Depth (m)	Thickness (m)
Topsoil	0.05	0.05	0.05	0.05	0.05	0.05
Subsoil	0.20	0.15	0.20	0.15	0.20	0.15
Sand[1]	Various	Various	Various	Various	n/a	n/a
Calcrete Layer[2]	Various	Various	Various	Various	n/a	n/a
OB2 Brown loam	4.90	3.20	2.50	3.20	0.50	0.30
OB3 Red sandy loam	8.10	2.30	5.75	2.30	1.50	1.00
Tailings	to pit floor	variable	to pit floor	variable	to pit floor	variable

[1] Yellow sand associated with dune and creek features
[2] Calcrete associated with creek features only

5 REFERENCES

Alluvium, 2013. Jacinth Ambrosia Watercourse Rehabilitation. Richmond, Victoria

SWC, 2009. Unsaturated zone modeling of water and salt movement in modcod and overlying overburden materials, Eucla Basin. Perth Western Australia

Appendix 10 Jacinth Ambrosia Overburden Soil Balance 2015

ILUKA

Jacinth Ambrosia

Overburden Soil Balance

2015

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TABLES

FIGURES

1 Introduction

Overburden resources at the Jacinth Ambrosia (JA) mine site are important for successful rehabilitation of the mining impact areas. All clean overburden compromising red loam, brown loam, sand, subsoil and topsoil are collected and stockpiled for this purpose. It was identified in 2014 that for some overburden materials there would be a deficit of material required for rehabilitation. This was investigated and the rehabilitation design approved in the current (at that time) Mining and Rehabilitation Program (MARP) was reviewed. A new final landform design was developed where the final height for portions of the rehabilitated in-pit TSF was reduced (Figure 1), the depth of clean overburden was reduced for red loam (Table 1 and Table 2) and the area of the chenopod vegetation association was increased (Figure 1). The new design was approved in the Program for Environmental Protection and Rehabilitation (PEPR) in 2016. As part of the new approved design a commitment was made to monitor the available clean overburden for the life of the mine.

The JA Rehabilitation Management Plan states "*The topsoil and subsoil balances are regularly reviewed to ensure appropriate final topsoil and subsoil replacement depths*". Further the annual stockpile balance is a commitment in the PEPR (PEPR Section 5.9), Table 1.

This document reports on the soil balance at JA for the 2014 to 2015 period.

Table 1 Rehabilitation soil profiles 2009 to 2015 (MARP approved)

Soil Materials	Landscape Vegetation Unit		
	Myall/Mallee Woodland	Myall Woodland	Chenopod Shrubland
	Thickness of layer (m)	Thickness of layer (m)	Thickness of layer (m)
Topsoil	0.05	0.05	0.05
Subsoil	0.15	0.15	0.15
OB1 – Yellowsand	2.40	0.00	0.00
OB2 – Brown Loam	2.3	2.3	0.30
OB3 – Red Sandy Loam	3.20	3.20	1.00
Capillary Break –	0.35 - 0.5	0.35 - 0.5	0.35 - 0.5

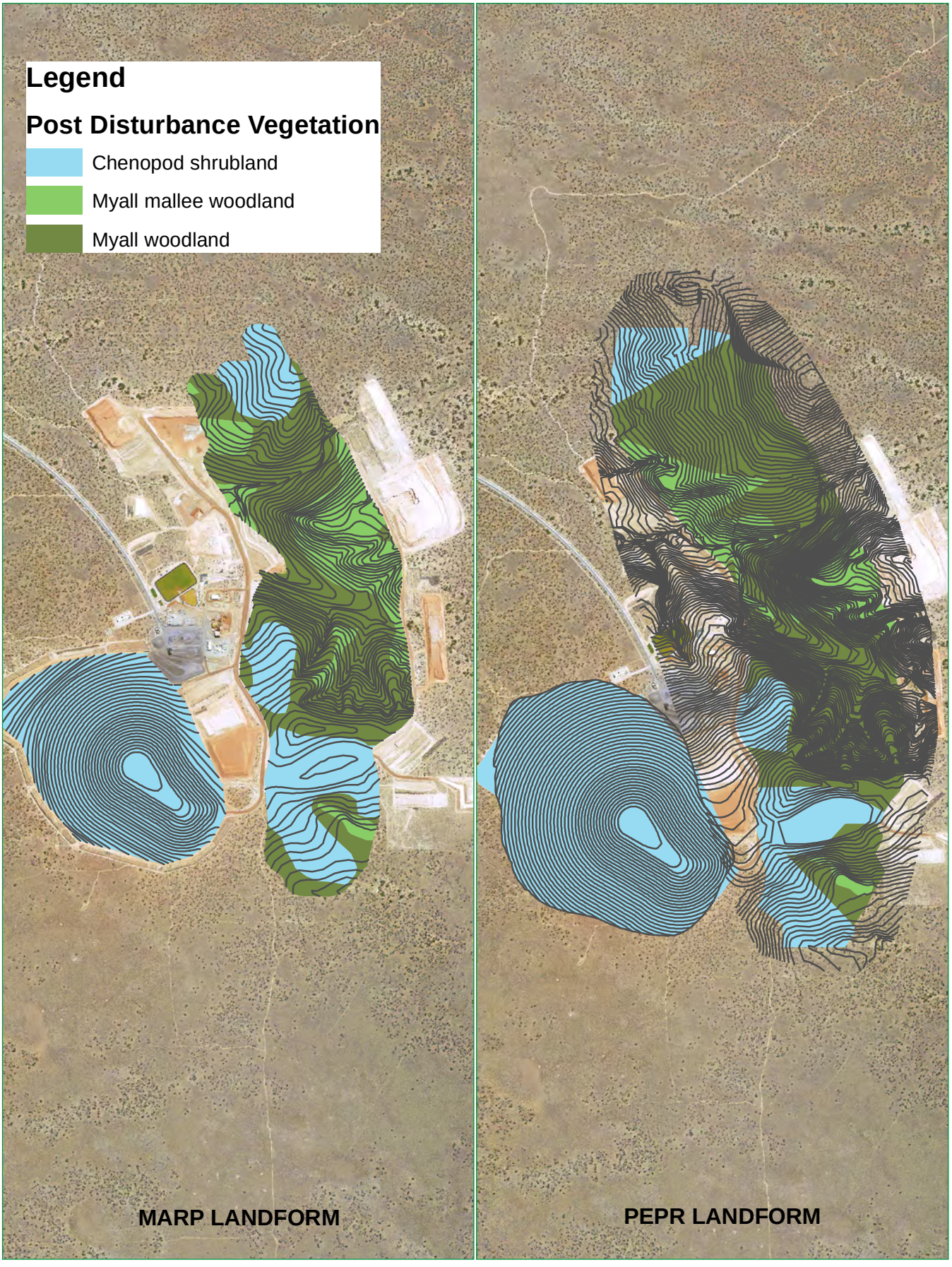
Table 2 Rehabilitation soil profiles 2015 to current (PEPR approved)

Soil Materials	Landscape Vegetation Unit		
	Myall/Mallee Woodland	Myall Woodland	Chenopod Shrubland
	Thickness of layer (m)	Thickness of layer (m)	Thickness of layer (m)
Topsoil	0.05	0.05	0.05
Subsoil	0.15	0.15	0.15
Sand[1]	Various	Various	n/a
Calcrete Layer[2]	Various	Various	n/a
Brown loam	Minimum of 2.30[3]	2.30–3.20[3]	0.30
Red loam	Minimum of 2.30[3]	2.30–3.20[3]	1.00
Tailings	variable	variable	variable

Table 3 Measurement criteria and monitoring requirements for outcomes in relation to soils (Impact S1 only)

Impact ID	Potential Impact	Controls and management strategies	Uncertainties	Commitments to Address Uncertainties	Outcome
S1	Significant loss of topsoil and subsoil resources due to erosion	• Implementation of the Native Vegetation Management Plan • Implementation of the Rehabilitation Management Plan • Implementation of the Dust and Air Quality Management Plan • Implementation of the Surface Water Management Plan • Progressive rehabilitation of disturbed area, commencing within first few years of operations • Annual stockpile monitoring • Restricting access to stockpiles • Prohibiting topsoil and subsoil stripping when winds exceed 15 km/h	Stability of topsoil and subsoil stockpiles Stability of rehabilitated soil surface	Stockpile monitoring program **Annual stockpile balance** Dust deposition monitoring	Soil profile and function is restored and capable of supporting agreed land use

[1] Yellow sand associated with dune and creek features.
[2] Calcrete layer associate with creek features only.
[3] Brown and red loam layers together with sum to 5.5m thickness, but the thickness for each layer individually can vary as indicated.



JA

In-pit landform design and post disturbance vegetation units

ILUKA

ORIG: JL DRAWN: JL SCALE: 1:30,000 (A4) DATE: Mar16 DWG No: Fig. 1

FIGURE: 1

2 Methods

Annual materials movements for all clean overburden are collated monthly from survey certificates and recorded in the materials movement register. Movements include addition to stockpiles from clearing of new areas, addition to stockpiles through movements from other stockpiles and reductions in stockpile volumes through use of material for rehabilitation. All clean overburden movement are recorded. Reconciliation of the stockpiles is then carried out, i.e. stockpile volume year start, recorded additions and use of materials from stockpiles and stockpile volume at year end.

The volumes of overburden material required to rehabilitate all open areas as at the end of 2015 is calculated and compared with the total volumes of overburden material available in stockpiles. Any shortfall of material is then to be investigated and plans put in place to ameliorate. Inactive stockpiles are not scanned annually to determine changes in volume and unexpected volume losses recorded. Spot height checks are captured of a random selection of subsoil and topsoil stockpiles for comparison with survey data to determine any loss due to erosion events for stockpiles not active during the 2015 period.

3 Results

Overall more overburden reported to stockpiles than was used for rehabilitation purposes. A total of approximately 755,000 m^3 reported to stockpile, 360,000 m^3 reported from stockpile to rehabilitation and 385,000 m^3 was direct returned from the mining pit to rehabilitation. A summary of the stockpiled overburden is provided in Table 2. This was generally reflected in the stockpile volumes, where red loam, brown loam, dune sand and topsoil recorded a difference between what was recorded in the survey certificates and actual stockpile volumes of 1% or less of the total material stockpiled. Subsoil recorded a 4% difference (more material was recorded in stockpile volumes than was moved during the year). All of these differences are considered minor and likely due to the accuracy of GPS data, material settling when stockpiled, and some fugitive losses.

The required overburden for rehabilitation of all open areas as at 31 December 2015 is consistently greater than available overburden, except for topsoil, Table 3, overall there is a deficit of approximately 2,400,00 m^3 of clean overburden material. There is a significant deficit of red loam (75% of required), brown loam (76% of required) and subsoil (57% of required). Topsoil is available in sufficient volumes, however 7,200 m^3 of this material will report to the 2016 rehabilitation area (Cell 2 East).

Table 4 Summary of overburden movements at in 2015

2015 material movements	Red Loam	Brown Loam	Dune Sand	Subsoil	Topsoil	Total
To stockpile	230,601	373,289	46,553	81,802	22,536	754,781
From stockpile	327,460	4,401		27,019		358,880
Direct return to cell 2	48,659	336,348				385,007
Difference m^3	-96,859	368,888	46,553	54,783	22,536	

Table 5 Summary of stockpiled material volumes at 31 December 2014 and 31 December 2015

Year	Red Loam	Brown Loam	Dune Sand	Subsoil	Topsoil
2014 m^3	1,675,000	2,936,081		263,000	201,665
2015 m^3	1,556,382	3,329,552	46,553	331,967	222,911
Difference m^3	-118,618	393,471	46,553	68,967	21,246

Table 6 Summary of overburden required for rehabilitation of open areas as at 31 December 2015

Location	area ha	area m^2	Red Loam m^3	Brown Loam m^3	Subsoil m^3	Topsoil m^3
Jacinth Pit	186	1,868,156	3,369,298	3,841,972	280,223	93,407
Off-Path TSF	108	1,087,000	1,087,000	326,100	163,050	55,756
Stockpiles[4]	707	7,070,000			58,358	27,176
Infrastructure	56	566,000			84,900	28,300
Total	**1057**	**10,570,000**	**4,456,298**	**4,168,072**	**586,531**	**204,639**
Available			3,329,552	3,155,474	331,967	222,911

4 Discussion

Some differences between recorded material movements and stockpile volumes is to be expected, potential reasons for differences comprise:

- DGPS equipment accuracy – material movements (monthly survey certificate) are recorded at the location the material was collected from or reported to, stockpiles volumes are based on scans of the stockpiles.
- Stockpile subsidence - settling of material will account for some of the difference over time, particularly for the finer materials (i.e. brown loam, subsoil and topsoil) which can collapse with disturbance (often observed as 'bulldust'). These materials then settle when wet and are compacted with equipment, resulting in a reduced recorded volume. Further, DGPS records in the collection or reporting point may also be inaccurate as material collapses, i.e. overestimation of material to stockpile and under estimation of material reporting to rehabilitation locations. A combination of these factors would account for a relatively significant difference when moving large volumes of material.
- Fugitive dust - finer materials are more likely to be subject to fugitive dust movements during handling, particularly during peak windy periods (September to November).

[4] Note that area under topsoil stockpiles will not require topsoil or subsoil application and areas under subsoil stockpiles will not require topsoil application.

The results of the 2015 soil balance indicate that similar volumes of material are reporting to the stockpiles as is being collected, therefore loss due to recording accuracy and fugitive dust can be considered minimal. Differences in volumes are likely to be due to materials settling in the stockpiles over time.

The deficit in overburden material is not unexpected and changes to soil profile depths and landform height for rehabilitation have been approved in the JA Program for Environmental Protection and Rehabilitation (PEPR). The approved landform design reduces the depth of clean overburden required through an increase in the area of chenopod vegetation association, which has a 1.3 depth of clean overburden material compared to 5 m depth for the deeper rooted vegetation associations (myall and mallee). Further areas of the final landform a have been lowered to reduce overburden requirements. All changes to landform and soil profile have been risk assessed and approved in the JA PEPR. Monitoring of the overburden soil balances will continue for the life of the mine. It is anticipated with these changes the deficit in brown loam and red loam would be reduced over time.

Appendix 11 Jacinth Ambrosia Topsoil Farm 2015

ILUKA

Jacinth Ambrosia Topsoil Farm

2015

DOCUMENT CONTROL

Document Title:	Jacinth Ambrosia Topsoil Farm
Mine Status:	Operational
Revision:	Version 1.0
Date Issued:	18 November 2015
Review Frequency:	-
Compiled by:	Joanne Lee
Owner:	JA Rehabilitation
Document No:	

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FIGURES

PLATES

1 Introduction

The importance of topsoil for the rehabilitation of the J-A landscape is recognised on-site and consequently topsoil is treated as a valuable resource. However, despite the ongoing management of the topsoil stockpiles there is currently a topsoil deficit to achieve the required soil depth. The topsoil deficit may have been caused by a number of factors including change in surface area i.e. off-path TSF; and incidental losses of topsoil during overburden removal and stockpiling.

A project was implemented to investigate if additional topsoil could be manufactured by spreading diluted topsoil over an underlying substrate (subsoil) and allowing the biological crust and vegetation seedbank to develop over time. This farmed topsoil could then be used to cover the recognised topsoil deficit.

The Topsoil Farm was established mid-2013 and commenced with the stripping and placement of topsoil on subsoil.

2 Method

An undisturbed area of vegetation within the mine footprint was selected as the topsoil source to avoid any additional vegetation clearance (Figure 1). Care was taken to locate the Topsoil Farm in area that was to remain undisturbed for a minimum of five years to allow the topsoil to develop.

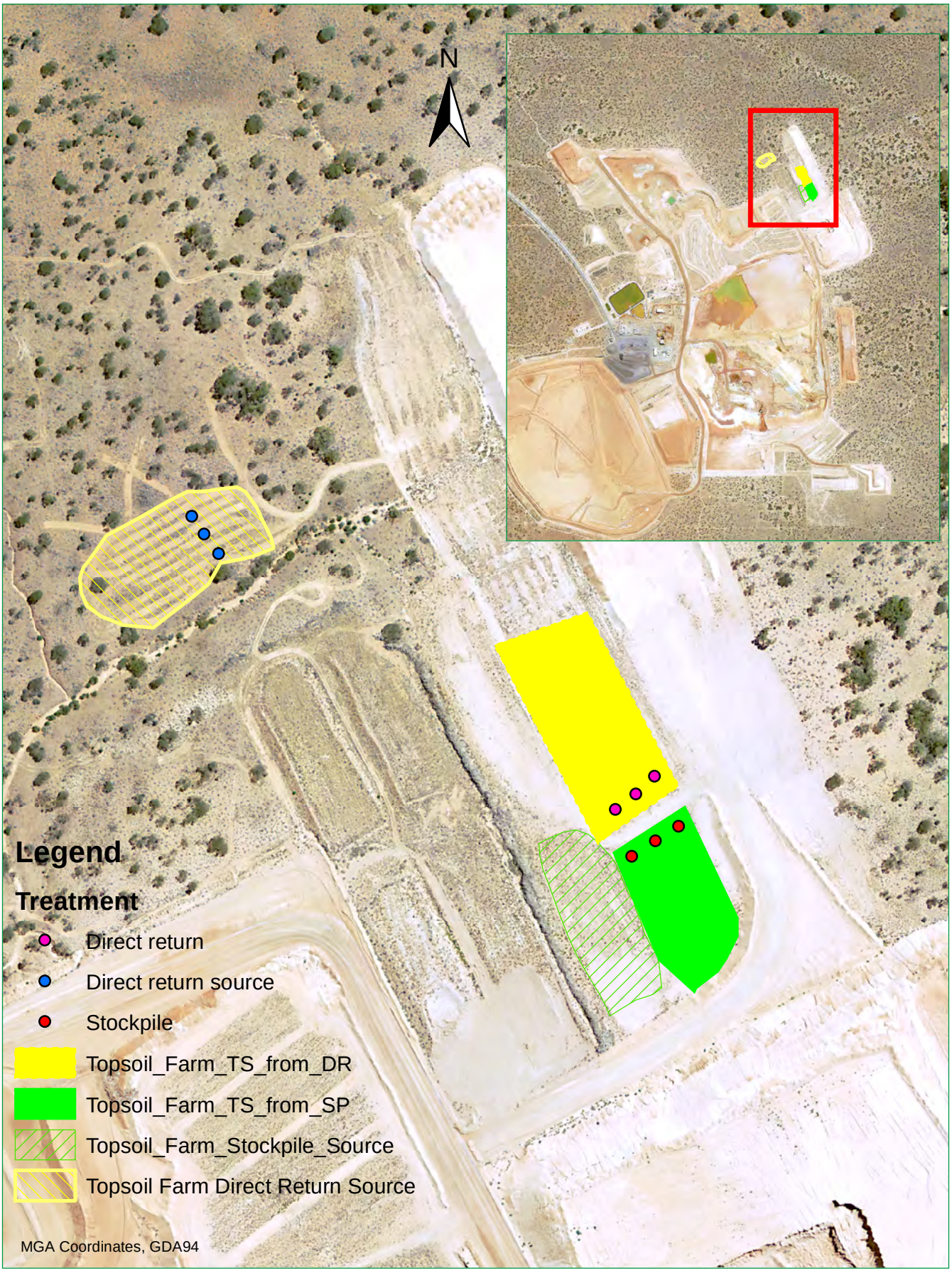
The Topsoil Farm currently comprises three treatments:

1. Stockpiled topsoil applied to subsoil at a diluted rate.
2. Direct return topsoil from an undisturbed area applied to subsoil at a diluted rate.
3. The source area for the direct return topsoil material.

Topsoil was collected from the source area with a carry grader and then applied at a diluted rate across the top of an area that was stripped down to subsoil. Where the topsoil was collected from the undisturbed area the topsoil was scalped in strips with vegetation left in between the strip to encourage an edge effect (Figure 1, Plate 4 to Plate 6).

The regeneration of the biological crust and vegetation will be monitored over time. Modified Jessop transects were established in topsoil application areas and the undisturbed topsoil source area. Three transects were located in each of the treatments. The proportion of BSC cover and total number of plant species were counted in each Jessop quadrat.

All treatments have been monitored in December 2013 and October 2015.



100 Meters

Jacinth Ambrosia

Topsoil Farm location and layout

ILUKA

ORIG: JLEE DRAWN: JLEE SCALE: 1:3,205 (A4) DATE: Nov15 DWG No: NA

FIGURE: 1

3 Results and Discussion

Vegetation and BSC was recorded in all Jessop transects (Plate 1 to Plate 9).

Species richness was similar across all treatments and years, ranging from 9 in 2015 at the stockpile treatment to 12 at both the direct return treatment area and the direct return source area in 2015. Species richness in 2013 ranged from 10 to 11 species.

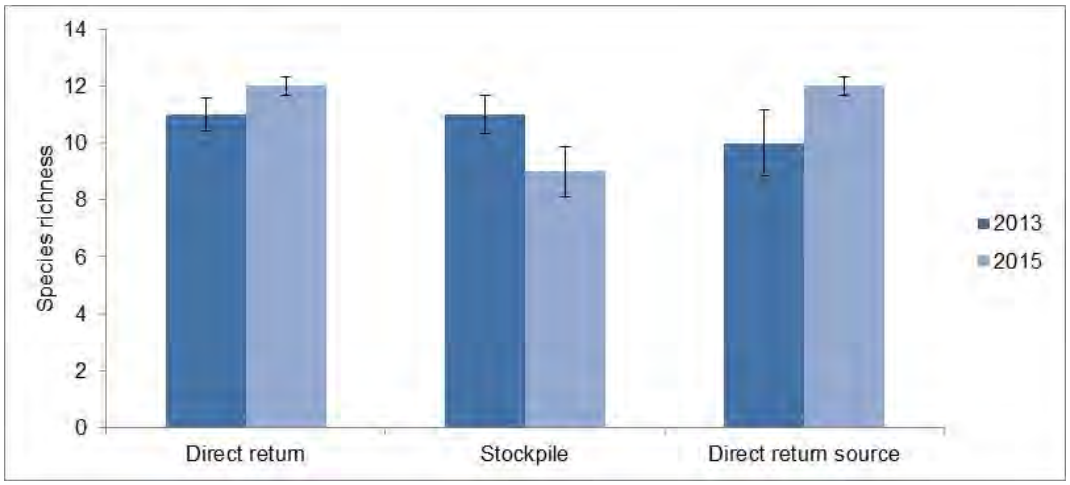


Figure 2 Mean species richness for Topsoil Farm Jessop transects, for each treatment. Error bars indicate standard error.

Overall total plant abundance has increased across all treatments in comparison to the 2013 survey (Figure 2). The high abundances recorded were due to the annual *Cephalipterum drummondii* and presence of *Austrostipa* spp (grasses), the total recorded abundances for each species per treatment and year is provided in Table 1. In 2015 the proportion of annuals and grasses comprised 79% of plant abundance in the direct return treatment, 76% in the stockpile treatment and 95% of the direct return source area.

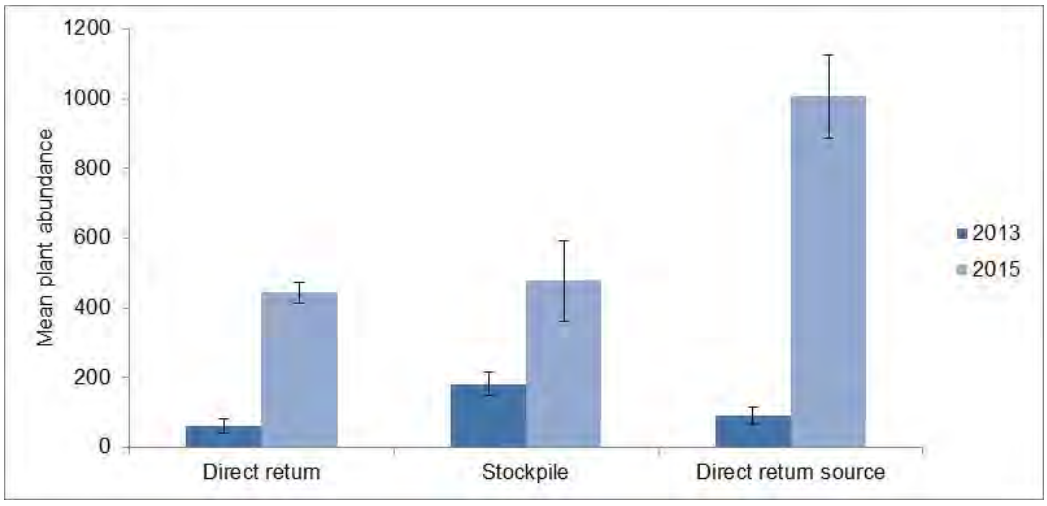


Figure 3 Mean plant abundance for Topsoil Farm Jessop transects, for each treatment. Error bars indicate standard error.

The considerable increase in the numbers of grasses (*Austrostipa* spp) recorded is likely due to the above average summer rains in 2014, increases in grass abundances was anecdotally noted across the entire site.

Table 1 Total numbers of individuals in Jessop transects per year

	Direct return		Direct return source		Stockpile	
Species	**Dec-13**	**Oct-15**	**Dec-13**	**Oct-15**	**Dec-13**	**Oct-15**
Annuals						
Brassica tournefortii[1]		3				16
Cephalipterum drummondii	8	2	94	1482		1
Calotis hispidula	3					
Erodium spp			2			
Rhodanthe stuartiana		2				2
Salsola australis[2]	52	259	28	32	117	160
Sonchus spp[1]		6		2		11
Tetragonia eremaea			8	1		
Zygophyllum eremaeum	3		1		4	39
Zygophyllum ovatum	7	88	1	96	44	176
Grasses						
Austrostipa nitida		13	2	1	3	6
Austrostipa spp	57	821	20	1264	94	743
Trees and shrubs						
Acacia papyrocarpa					1	
Acacia sp.					1	
Atriplex vesicaria	45	53	5	4	121	114
Brachyscome ciliaris var. ciliaris			6			
Chenopodium curvispicatum	2	1			17	2
Enchylaena tomentosa						3
Euphorbia drummondii				10		
Lepidium phlebopetalum	4	1		5		
Lycium australe			12	13		
Maireana integra		5		9		3
Maireana pentatropis	2				2	
Maireana radiata	2	1			11	9
Maireana sclerolaenoides[3]	31	9	9	35	48	15
Maireana sedifolia	2	3				
Maireana spp	4			26	1	
Maireana trichoptera	1	3		6		9
Sida spodochroma				2		
Sclerolaena obliquicuspis	60	39	26	35	81	74
Zygophyllum aurantiacum		27	17		2	50

[1] Introduced species
[2] Previously *Salsola tragus*
[3] Previously *Eriochiton sclerolaenoides*

High numbers of the annual *Cephalipterum drummondi* were recorded in the direct return treatments in comparison to the stockpile treatment. However higher numbers of the perennial *Atriplex vesicaria* were recorded in the stockpile treatment in comparison to the direct return treatments. The stockpiled material had been stored for less than a year before being used in the program. Therefore any difference in community composition for the treatments is likely to be related to the species present in initial vegetation association (or the underlying seedbank) rather than any loss of viability of annual seeds due to extended storage.

Lycium australe recorded in the direct return source area was due to the reshooting of root material. No *L. australe* seedlings in any treatment were recorded.

BSC was recorded in all treatments and identified as Class 1, slight discoloration visible at surface, early successional stage (Plate 10 to Plate 12). Generally there was reasonable BSC coverage in all Jessop transects (Figure 4), and where lower proportions were observed this was due to burial by mobile sands. Mean proportion cover per Jessop ranged from 41% in the stockpiled treatment to 65% cover in the direct return source area. There was little difference in the proportion cover for the treatments where the topsoil was applied (direct return and stockpile).

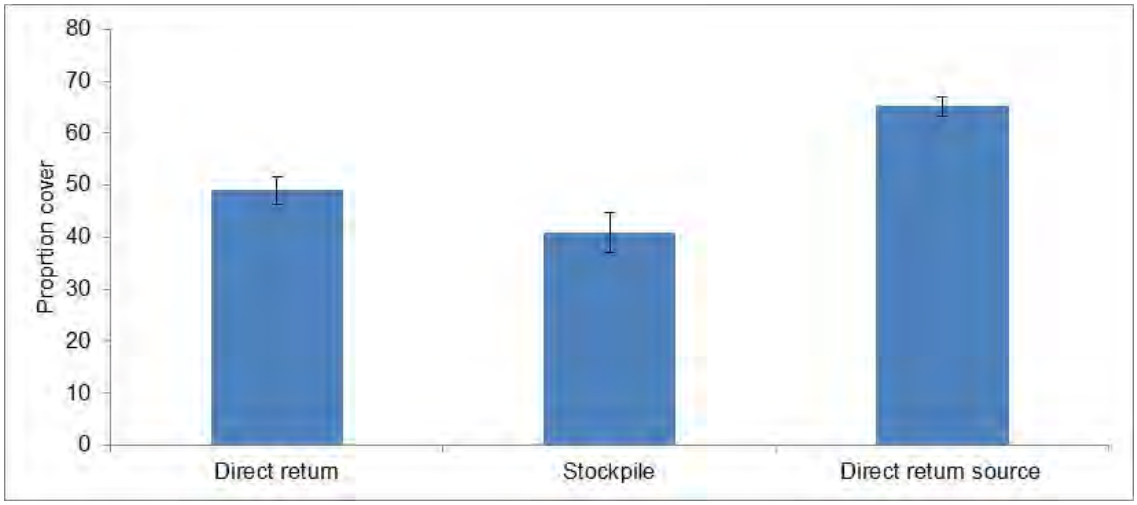


Figure 4 Mean proportion cover of BSC for Jessop's across all treatments and years. Error bars indicate standard error.

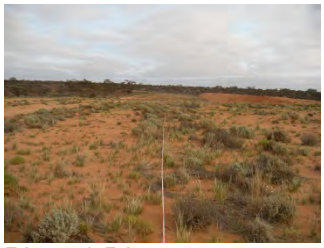


Plate 1 Direct return treatment, transect A

Plate 2 Direct return treatment, transect B

Plate 3 Direct return treatment, transect C

Plate 4 Direct return source, transect A

Plate 5 Direct return source, transect B

Plate 6 Direct return source, transect C

Plate 7 Stockpile topsoil treatment, transect A

Plate 8 Stockpile topsoil treatment, transect B

Plate 9 Stockpile topsoil treatment, transect C

Plate 10 Direct return BSC

Plate 11 Direct return source BSC

Plate 12 Stockpile BSC

4 Recommendations

Given the high proportion of annuals recorded there is potential that the resulting seedbank would contain much greater proportions of annual seeds in comparison to undisturbed seed banks. There is also potential to reduce longer lived species in the seedbank if they germinate and do not have sufficient time (or the right conditions) to set seed before soil stripping. Further, if the material from the Topsoil Farm is stored annual seeds would lose viability sooner than longer lived species[4]. This would likely result in an overall loss in seed viability (for both annual and longer lived species) for the farmed soils.

In addition, topsoil farming is highly intensive, requiring heavy machinery, and large areas available for clearing to develop enough topsoil to make any practical contribution to the current JA topsoil deficit. It is recommended that no further topsoil farming be carried out, however the monitoring program will continue as valuable information regarding vegetation and BSC succession can be obtained through the program.

The importance of BSC for the stability of rehabilitated soils is well known. The BSC at JA responds well to disturbance and can recolonise soils rapidly, however there is little known how disturbance impacts on the BSC communities. It is recommended that samples of the BSC from the Topsoil Farm are further investigated to determine community composition and abundances for the different disturbance types.

[4] South Australian Seed Conservation Centre, 2011. Restoration Technology Project, a report to Iluka Resources Ltd

Appendix 12 Progress report for soil sample analysis

REPORT TO ILUKA RESOURCES

PROGRESS REPORT FOR SOIL SAMPLE ANLAYSIS

MARCH 2016

South Australian Seed Conservation Centre

Botanic Gardens of SOUTH AUSTRALIA

Summary

Soil samples were analysed to determine the number of viable seeds per sample. The analysis was approached using two methods. Firstly to spread out the soil in the glass house under moist conditions and score the number of seedlings that emerged. Secondly, to sieve the soil samples and separate the seeds from soil particles under a microscope and determine the viability of the seeds using x-ray imaging and viability tests. The results show that few viable seeds were present in the soil samples. In total 276 seeds were sieved from the soil samples and 38 were filled as determined by x-ray and cut testing. A total of 24 seedlings were observed to emerge from soil samples that were placed under irrigation. The results suggest that the majority of viable seeds may not persist in the soil or that the seeds may have been nonviable upon dispersal.

Methods

Soil Samples

Soil samples were collected from the Iluka mine site, Yellabinna Regional Reserve. Thirty three samples were delivered to the Seed Conservation Centre on 2nd October 2015.

Germination of seedlings in the glass house

The seedling experiment started on the 6th November 2015. A subsample of soil (450 g) was weighed from each sample for analysis. The subsamples were split into three replicates of 150 g (± 1g) each.

Seedling trays were prepared with a lining of shade cloth to prevent losing soil through the drainage holes. The cloth was covered with ~ 1 cm of seed raising mix which followed by ~ 1 cm of sterilised (autoclaved at 120°C, 120 kPa for 20 min) washed sand. This matrix was chosen so that the trays did not dry out too quickly but allowed free drainage away from the seedling roots. Sand was sterilised to kill any weed seed present. The trays were lightly sprayed with water then the sample replicates were spread evenly over the surface (Figure 1).

The trays were placed in a glass house with overhead mist irrigation for 15 min per day and an air conditioner set to cool when the temperature rose above 28°C. The number of seedlings emerging was scored weekly. The trays were scored for 12 weeks, the results are summarised in Table 1.

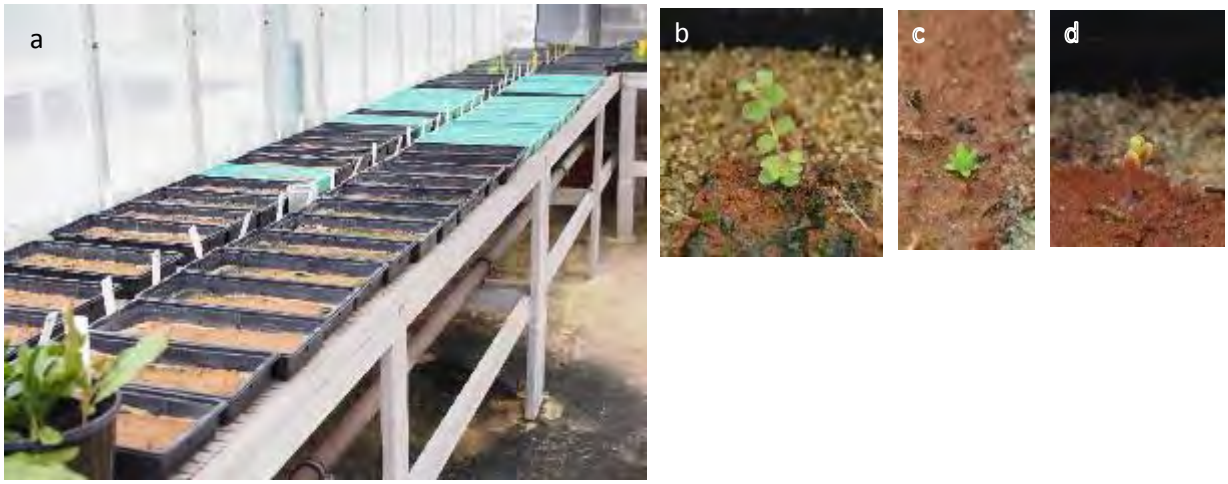


Figure 1. Seedling emergence experiment showing (a) trays with soil samples under irrigation in the glass house; (b) *Euphorbia* seedling; (c) *Crassula* seedling; (d) *Zygophyllum* seedling.

Soil Sieving

The amount of soil was weighed before sieving and analysis under a microscope. Soil was fractioned through the following series of sieve sizes (µm): 1400, 1000, 850, 500, 250. The seeds were sorted from the soil particles and other debris under a microscope for each of the fractions. The majority of seeds were greater than 850 µm diameter. Fine particles (less than 250 µm) were not analysed for all samples as no seeds were detected from the first 2 samples examined, and the final fraction was time consuming to analyse. Few species of plants recorded at Yellabinna Regional reserve have seeds with a diameter less than 250 µm. One exception is species of *Crassula* and those seeds would be likely to germinate in the seedling study.

Thirty-three samples were sifted, counted and imaged. Seeds that were recovered from soil samples were imaged under a dissecting microscope. The seeds were also imaged using an x-ray machine to determine the number of filled seeds per sample. All of the seeds were cut test after x-ray and cut filled seeds were imaged under the dissecting microscope (Appendix 2).

Results and Discussion

Seed Germination

The results from the seedling emergence experiment and soil sieving are summarised in Table 1. The complete data, including replicates, are shown in Appendix 1. The seedlings that have been identified to genus level were species from *Salsola*, *Crassula*, *Cullen*, *Eucalyptus*, *Zygophyllum* and *Euphorbia*.

Table 1 Soil sample analysis data using two methods to estimate the number of viable seeds per sample; Soil sieving experiment with microscopic analysis and seedling emergence experiment.

Sample Code	Weight (g)	# Seeds counted from soil sieving experiment	# Filled Seeds	Total # seedlings (450 g soil) Seedling emergence experiment
SP01A	240	17	1	0
SP01B	434	6	0	0
SP01C	458	14	0	0
SP01D	373	11	0	0
SP01E	423	21	0	0
SP01F	466	21	1	0
SP10A	327	1	0	0
SP26A	474	10	4	0
SP26B	434	10	3	2
SP26C	416	9	4	4
SP26D	371	9	3	1
SP26E	375	7	0	1
SP26F	332	2	0	2
SP7A	474	3	0	5
SP7B	456	5	0	2
SP7C	470	6	1	0
SP7D	393	10	0	0
SP7E	349	8	0	1
SP7F	392	23	0	1
SP8A	409	21	9	1
SP8B	420	1	0	1
SP8C	390	4	1	1
SP8D	326	7	1	1
SP8E	345	7	0	0
SP8F	422	8	0	0
UD1A	439	4	1	0
UD1B	369	4	3	0
UD2A	443	6	1	0

UD2B	395	5	0	0
UD2C	392	2	0	1
UD3A	487	9	4	0
UD3B	454	4	1	0
UD3C	421	1	0	0

The results from the seed sieving experiment show that there were more nonviable than viable seeds present in all of the soil samples. Seeds may have been nonviable upon dispersal or have subsequently been degraded in the soil through various processes of seed ageing or degradation resulting from predation or fungal attack. X-ray and seed images are shown in Appendix 2.

The sample with the highest number of seedlings (5) was SP7A, however the no viable seeds were sieved from the other half of the sample. It may be difficult to correlate the results from the two experiments due to the low seed numbers. Of the samples that were analysed using sieving the highest number of filled seeds was found in sample SP8A (7 grass and 2 *Tetragonia* sp seeds). Only one seed germinated from this sample in the seedling emergence experiment and was an unidentified dicotyledon seedling. The other samples all had low numbers of seedlings that emerged (2 or less) and low numbers (4 or less) of filled seeds observed after sieving.

Only one monocotyledon seedling emerged in the experiment, from sample SP26F. This was surprising as the largest number of filled seeds found after sieving were grass seeds. Physiological dormancy can occur in grass seeds and may have supressed germination, or the seeds may have appeared filled but were not viable.

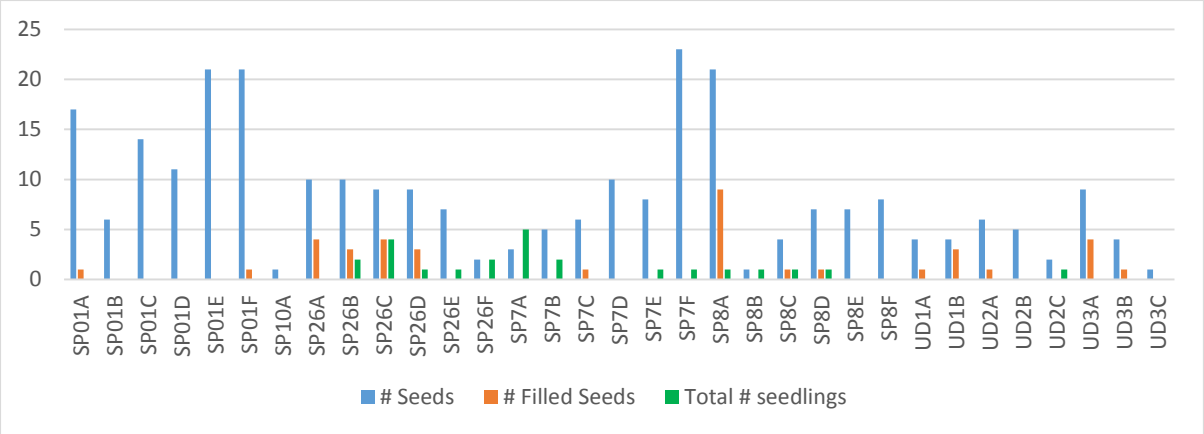


Figure 2 Graph of seed counts showing: ■ Number of seeds extracted from soil samples; ■ number of filled seeds extracted from soil samples; ■ Number of seedlings observed.

The overall results (Table 1 and Figure 2) show that the viable seed numbers are low (< 10) per kilogram of soil sample. The results from both methods show that the seed quantity is low. Using the two methods has provided more information than using only one technique. Seeds are not necessarily evenly distributed throughout the soil seed bank and there may be patches with higher concentrations near parent plants and or through deposition by animals or insects. Therefore it can

be difficult to take representative samples. For future work it may be necessary to take larger soil samples and concentrate the seed fraction by sieving before analysis.

Recommendations

One method to increase the number of seeds to be analysed would be to increase the soil sample size and sift through 250 µm mesh to enrich the seed fraction before analysis.

Appendix 1 Data from the seedling emergence experiment

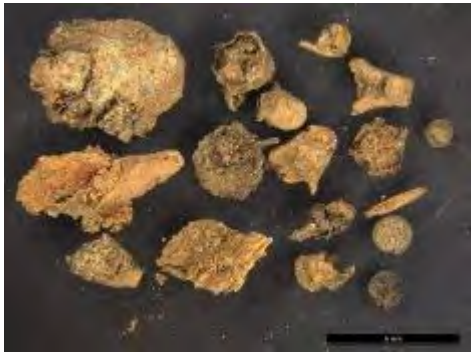
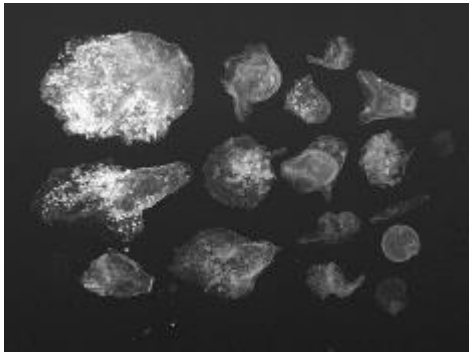
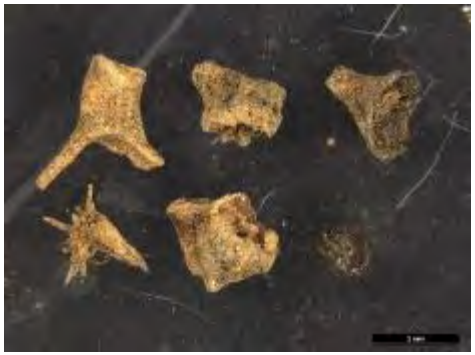
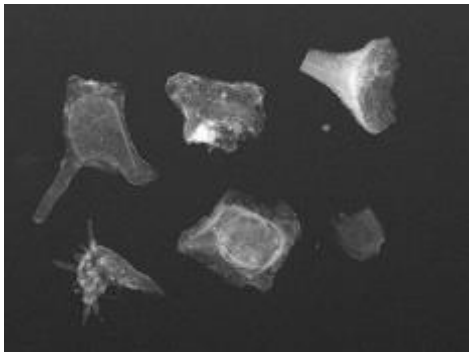
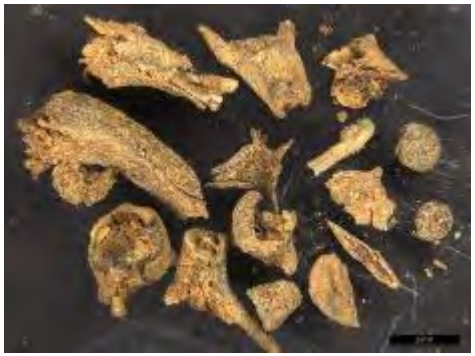
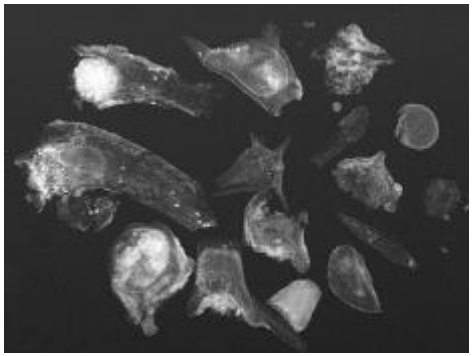
			Wk1		Wk2		Wk3		Wk4		Wk5		Wk6		Wk7		Wk8		Wk9		Wk10		Wk11		Wk12		
Tray Number	**Sample Code**	**Weight (g)**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	
1.1	UD3B	150																									
1.2	UD3B	150																									
1.3	UD3B	150																									
2.1	SP26F	150			1		1		1		1		1		1		1		1		1		1		1		unidentified
2.2	SP26F	150						1		1		1		1		1		1		1		1		1		1	Crassula
2.3	SP26F	150																									
3.1	UD2C	150																									
3.2	UD2C	150																									
3.3	UD2C	149											1		1		1		1		1		1		1		Eucalyptus
4.1	UD3C	150																									
4.2	UD3C	150																									
4.3	UD3C	149																									
5.1	SP26C	150				1		1		1		1		1		1		1		1		1		1		1	Zygophyllum
5.2	SP26C	150																									
5.3	SP26C	149				1		1		1		3		3		3		3		3		3		3		3	2 Crassula; 1 Salsola
6.1	UD2B	150																									
6.2	UD2B	150																									
6.3	UD2B	149.5																									
7.1	SP26B	150																									
7.2	SP26B	150										1		1		2		2		2		2		2		2	1 Crassula; 1 Eucalyptus
7.3	SP26B	149																									
8.1	SP01F	150																									
8.2	SP01F	150																									
8.3	SP01F	149																									
9.1	SP01E	150																									
9.2	SP01E	150																									
9.3	SP01E	149																									
10.1	SP26E	150																									

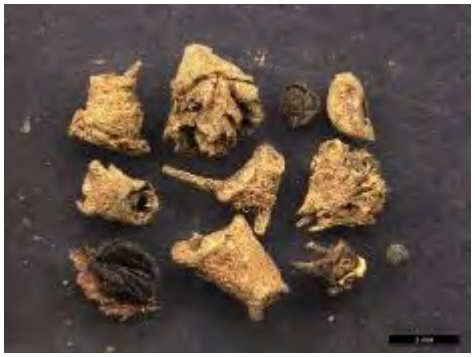
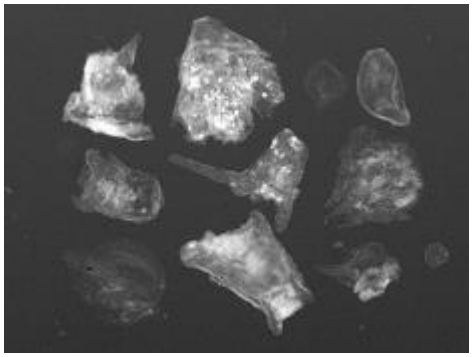
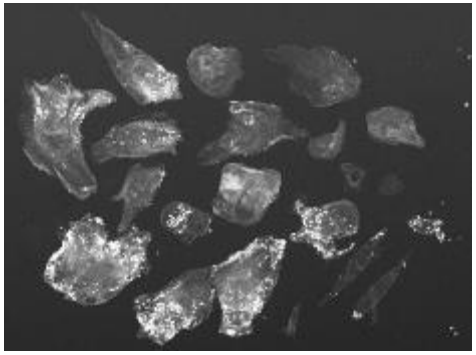
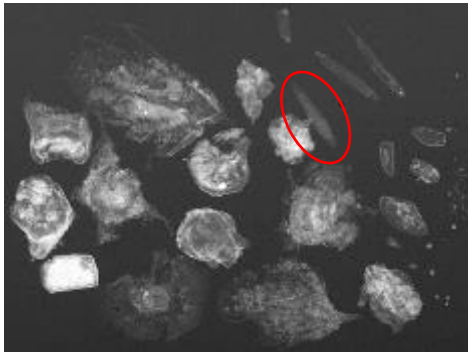
			Wk1		Wk2		Wk3		Wk4		Wk5		Wk6		Wk7		Wk8		Wk9		Wk10		Wk11		Wk12		
Tray Number	**Sample Code**	**Weight (g)**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	
10.2	SP26E	150														1		1		1		1		1		1	unidentified
10.3	SP26E	149																									
11.1	UD1B	150																									
11.2	UD1B	150																									
11.3	UD1B	150																									
12.1	UD2A	150																									
12.2	UD2A	150																									
12.3	UD2A	149																									
13.1	UD1A	150																									
13.2	UD1A	150																									
13.3	UD1A	149																									
14.1	SP26A	150																									
14.2	SP26A	150																									
14.3	SP26A	149																									
15.1	UD3A	150																									
15.2	UD3A	150																									
15.3	UD3A	149																									
16.1	SP01D	150																									
16.2	SP01D	150																									
16.3	SP01D	149.5																									
17.1	SP26D	150																									
17.2	SP26D	150																									
17.3	SP26D	149.5						1		1		1		1		1		1		1		1		1		1	unidentified
18.1	SP7A	150				1		1		1		1		1		1		1		1		1		1		1	unidentified
18.2	SP7A	150				1		1		1		1		1		1		1		1		1		1		1	Zygophyllum
18.3	SP7A	149				3		3		3		3		3		3		3		3		3		3		3	unidentified
19.1	SP7B	150				1		1		1		1		1		1		1		1		1		1		1	Cullen
19.2	SP7B	150						1		1		1		1		1		1		1		1		1		1	Zygophyllum
19.3	SP7B	149																									
20.1	SP7D	150																									

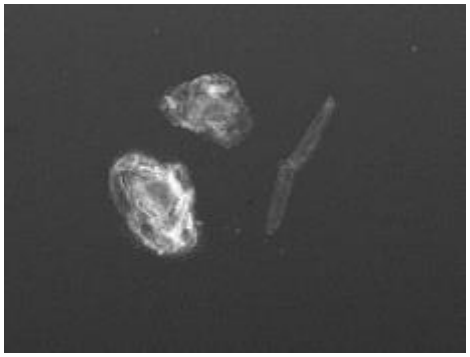
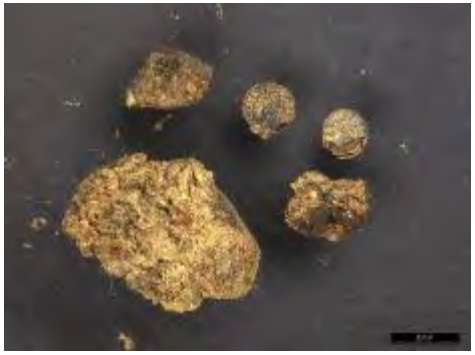
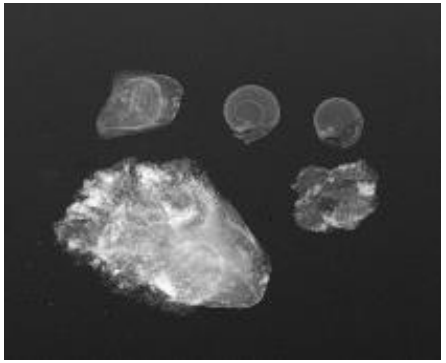
			Wk1		Wk2		Wk3		Wk4		Wk5		Wk6		Wk7		Wk8		Wk9		Wk10		Wk11		Wk12		
Tray Number	**Sample Code**	**Weight (g)**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	
20.2	SP7D	150																									
20.3	SP7D	149																									
21.1	SP7C	150																									
21.2	SP7C	150																									
21.3	SP7C	149																									
22.1	SP8F	150																									
22.2	SP8F	150																									
22.3	SP8F	149																									
23.1	SP01B	150																									
23.2	SP01B	150																									
23.3	SP01B	149.5																									
24.1	SP8D	150																									
24.2	SP8D	150										1		1		1		1		1		1		1		1	Euphorbia
24.3	SP8D	149.5																									
25.1	SP8E	150																									
25.2	SP8E	150																									
25.3	SP8E	149																									
26.1	SP10A	150																									
26.2	SP10A	150																									
26.3	SP10A	149																									
27.1	SP8C	150																									
27.2	SP8C	150																									
27.3	SP8C	149										1		1		1		1		1		1		1		1	Euphorbia
28.1	SP8B	150				1		1		1		1		1		1		1		1		1		1		1	unidentified
28.2	SP8B	150																									
28.3	SP8B	149.5																									
29.1	SP01C	150																									
29.2	SP01C	150																									
29.3	SP01C	149																									
30.1	SP7F	150																									

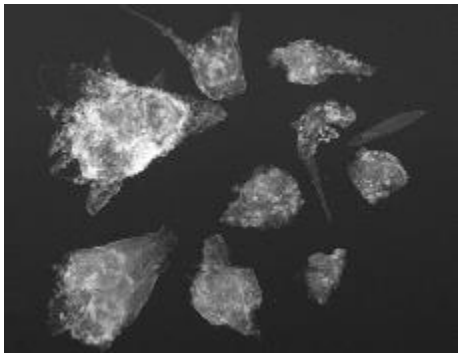
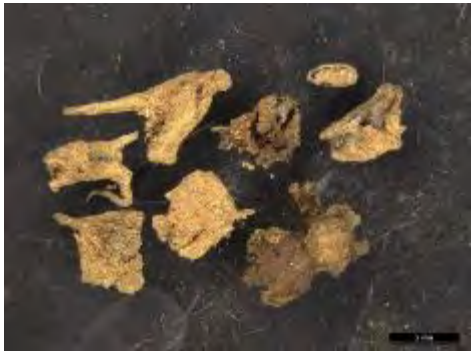
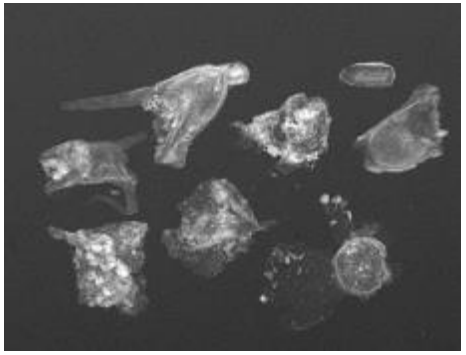
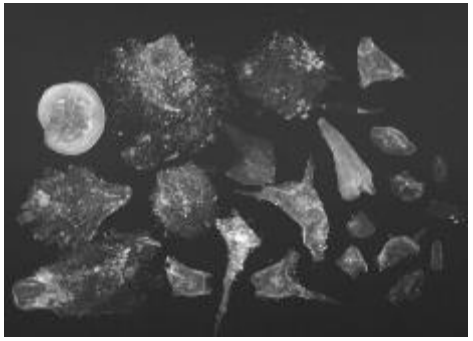
			Wk1		Wk2		Wk3		Wk4		Wk5		Wk6		Wk7		Wk8		Wk9		Wk10		Wk11		Wk12		
Tray Number	**Sample Code**	**Weight (g)**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	**M**	**D**	
30.2	SP7F	150																									
30.3	SP7F	149				1		1		1		1		1		1		1		1		1		1		1	Cullen
31.1	SP7E	150												1		1		1		1		1		1		1	Cullen
31.2	SP7E	150																									
31.3	SP7E	149																									
32.1	SP8A	150				1		1		1		1		1		1		1		1		1		1		1	unidentified
32.2	SP8A	150																									
32.3	SP8A	149																									
33.1	SP01A	150																									
33.2	SP01A	150																									
33.3	SP01A	149.5																									

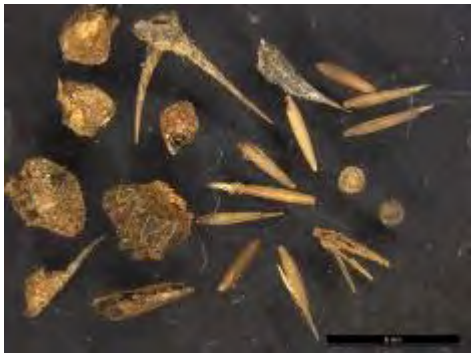
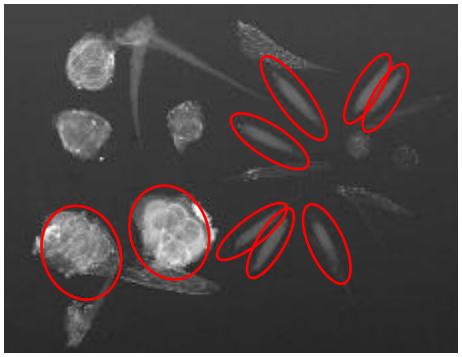
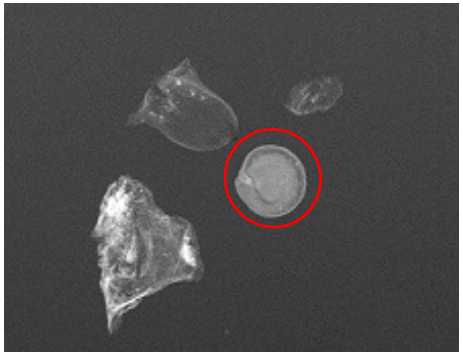
Appendix 2. X-ray and microscope images of seeds isolated from the soil sieving experiment. Filled seeds circled in red on the x-ray image. Filled seeds were cut and imaged.

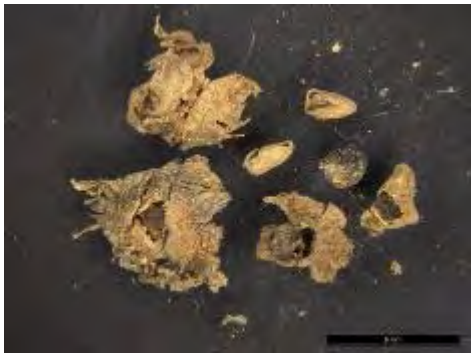
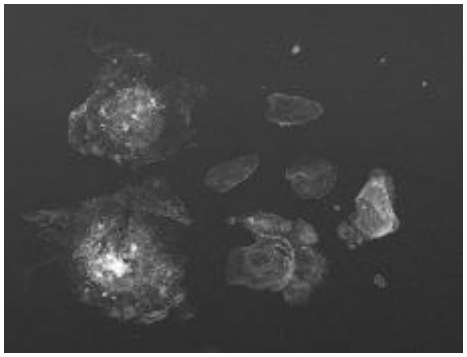
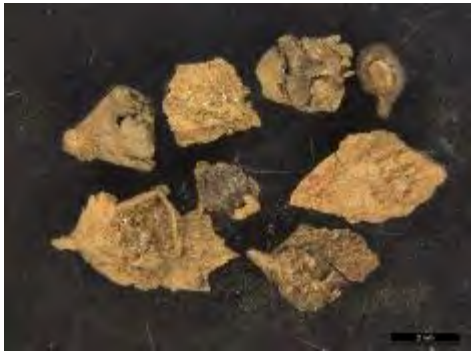
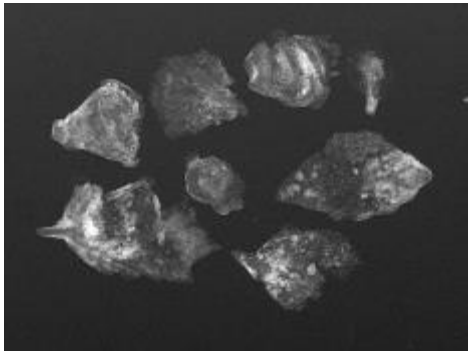
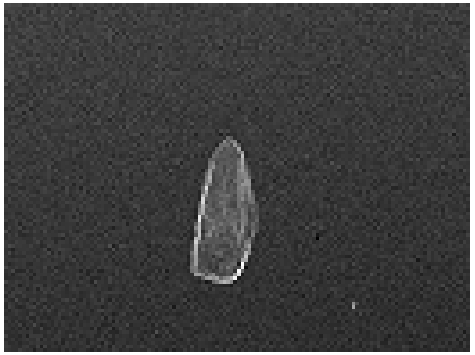
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP01A 17 seeds total 0 filled seeds			No filled seeds
SP01B 6 seeds total 0 filled seeds			No filled seeds
SP01C 14 seeds total 0 filled seeds			No filled seeds

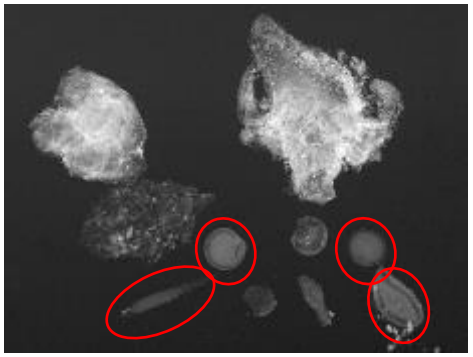
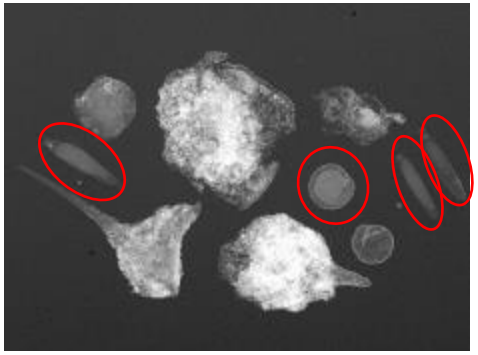
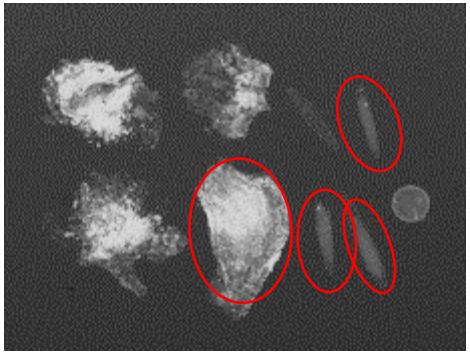
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP01D 11 seeds total 0 filled seeds			No filled seeds
SP01E 21 seeds total 0 filled seeds			No filled seeds
SP01F 21 seeds total 1 filled seed grass			

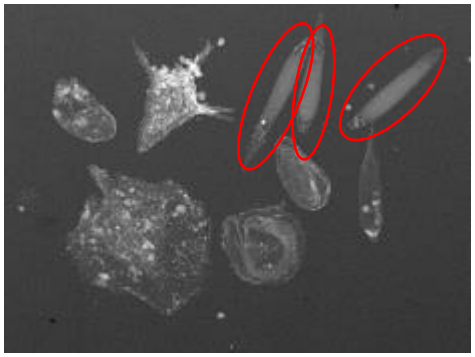
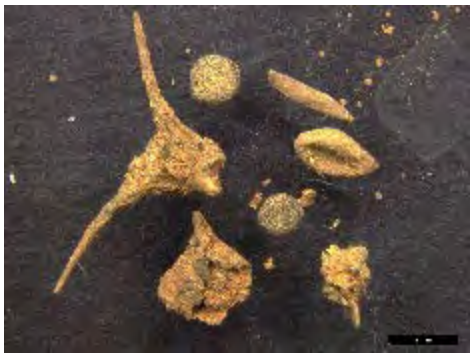
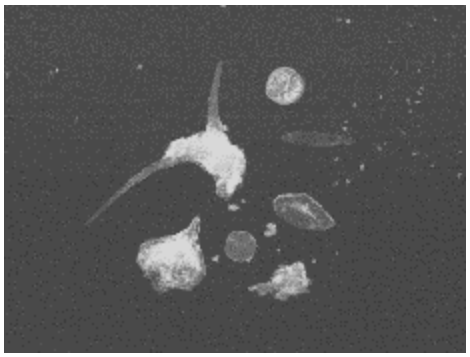
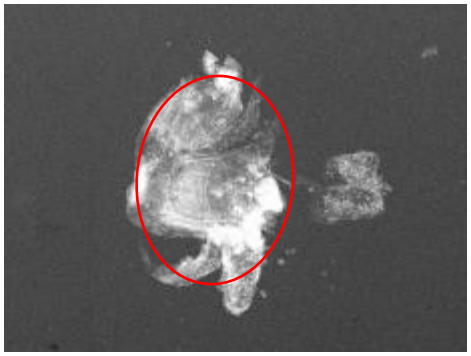
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP7A 3 seeds total 0 filled seeds			No filled seeds
SP7B 5 seeds total 0 filled seeds			No filled seeds
SP7C 5 seeds total 1 filled grass seed			

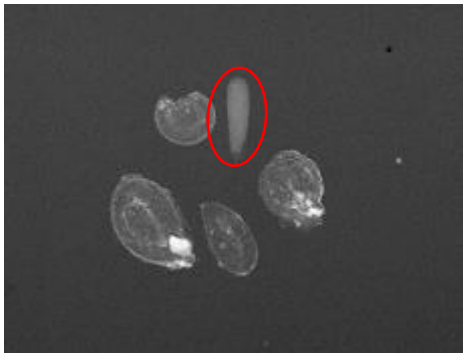
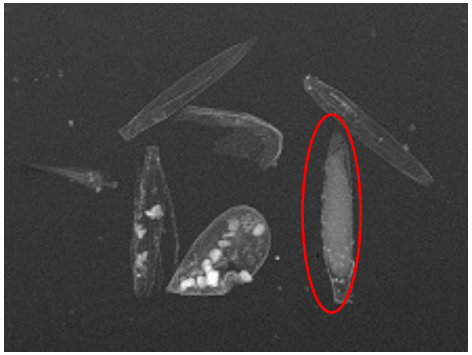
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP7D 10 seeds total 0 filled seeds			No filled seeds
SP7E 8 seeds total 0 filled seeds			No filled seeds
SP7F 23 seeds total 0 filled seeds			No filled seeds

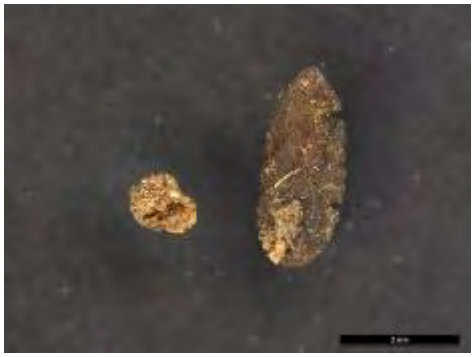
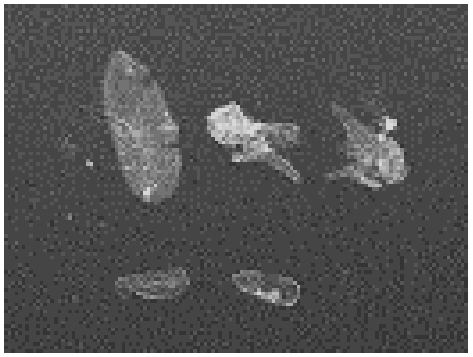
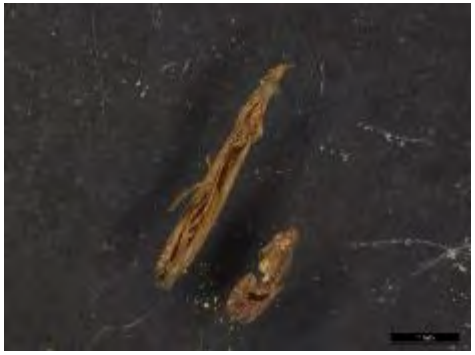
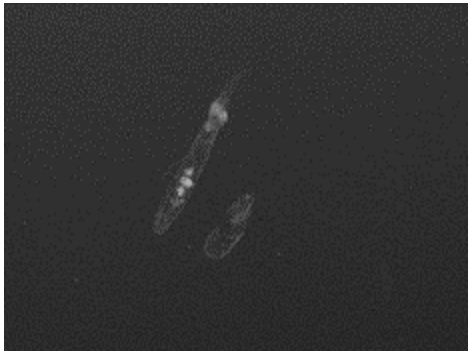
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP8A 21 seeds total 8 filled seeds - 7 grasses - 2 Azioaceae (Tetragona sp)			
SP8C 4 seeds total 1 filled Chenopodium seed			
SP8D 7 seeds total 1 filled seed			

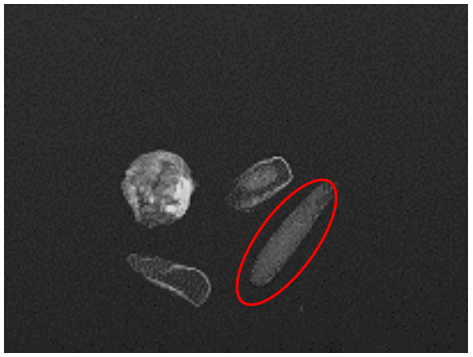
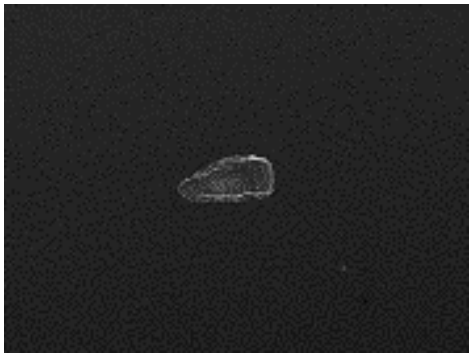
Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP8E 7 seeds total 0 filled seeds			No filled seeds
SP8F 8 seeds total 0 filled seeds			No filled seeds
SP10A 1 seed total 0 filled seeds			No filled seeds

Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP26A 10 seeds total 4 filled seeds -1 grass seed -2 Chenopodium seeds -1 Myoporum platycarpum			
SP26B 10 seeds total 3 filled seeds - 1 Chenopodium - 3 grass seeds			
SP26C 9 seeds total 3 filled grass seeds 1 filled Salsola seed			

Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
SP26D 9 seeds total 3 filled grass seeds			
SP26E 7 seeds total 0 filled seeds			No filled seeds
SP26F 2 seeds total 1 filled Salsola seed			

Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
UD1A 5 seeds total 1 filled seed			
UD1B 4 seeds total 3 filled seed			
UD2A 6 seeds total 1 filled seed (grass)			

Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
UD2B 5 seeds total 0 filled seed			No filled seeds
UD2C 2 seeds total 0 filled seed			No filled seeds
UD3A 9 seeds total 4 filled seeds (grass)			

Number of seeds identified per sample	Seed image	X-ray Image	Image of filled seeds after cutting
UD3B 4 seeds total 1 filled grass seed			
UD3C 1 seed total 0 filled seed			No filled seeds

Appendix 13 Do plants grown in stockpiled soil for mine-site revegetation form arbuscular mycorrhizas?

Do plants grown in stockpiled soil for mine-site revegetation form arbuscular mycorrhizas?

A report submitted to Iluka Resources, by the University of Adelaide, December, 2015.

Report prepared by Associate Professor Timothy Cavagnaro and Ms Rebecca Stonor.

Executive summary

Arbuscular mycorrhizas (AM) are associations formed between the roots of most (~80%) terrestrial plant species, and a specialised group of beneficial soil fungi. The formation of AM can improve plant nutrient acquisition, plant drought tolerance, resistance to plant pathogens, and can help improve soil structure. As such, they have an important role to play in the growth of individual plants, as well as the establishment of plant communities. They are also recognised as valuable indicators of soil health and restoration of mine sites.

A study investigating the formation of AM by test plants (*Medicago truncatula*) in soils collected and supplied to the University of Adelaide by Iluka Resources was undertaken. These soils were collected from various sites including soil stockpiles associated with mining activities and from undisturbed control sites. The soils were sampled from a range of sampling depths at each site.

The majority of soils collected contained arbuscular mycorrhizal fungi (AMF) that readily colonized the roots of the test plants. Soils from both the undisturbed and stockpile sites were colonized by AMF to a good extent in a large number of samples tested. Almost two thirds of samples were colonized by AMF. Of the 12 samples not colonized by AMF, seven of those came from two stockpiles (SP01 and SP7). Mycorrhizal colonization of roots of plants grown in soil collected from stockpiles SP8 and SP26 were generally high, and in many cases were higher than in samples from the undisturbed sites. Interestingly, soil collected from the surface layer of the undisturbed sites had no AM colonization. Although plants were small, there were clear differences in biomass between the various soil samples tested. There was some evidence of a threshold for AM colonization above which plant growth was generally good.

This study did not provide an opportunity to identify the factors underlying variation in levels of AM colonization of roots. This variation may be associated with soil physicochemical properties, management of the soil, or the time the soils have been stockpiled. These factors are potentially worthy of further investigation so that AM colonization can be maximized in restoration activities. The variation in levels of AM colonization between stockpiles is intriguing, and potentially provides an opportunity to manage AMF in the soil for example by blending soil from stock piles.

Background

Arbuscular mycorrhizas (AM) are associations formed between the roots of most (~80%) terrestrial plant species, and a specialised group of beneficial soil fungi (Smith and Read, 2008). The formation of AM can improve plant nutrient acquisition. Hyphae of arbuscular mycorrhizal fungi (AMF) can extend beyond the root surface by more than 10 centimetres (Jakobsen et al., 1992; Li et al., 1991), with common hyphal densities of >10 m of hyphae per gram of soil (Cavagnaro et al., 2005; Drew et al., 2003; Jakobsen et al., 1992). This extensive absorbing network, which extends beyond the rhizosphere nutrient depletion zones that form around roots, allows AM to access a larger volume of soil than roots not colonized by AMF.

There is clear evidence that AMF can help plants acquire nutrients including phosphorus (P), zinc (Zn), ammonium (NH_4^+), nitrate (NO_3^-), copper (Cu), potassium (K), and others (Cavagnaro, 2008; Lehmann et al., 2014; Marschner and Dell, 1994); for example, up to 90% of plant P and 20% of plant Zinc (Zn) can be provided by AMF, although estimates vary among studies and study systems. Arbuscular mycorrhizas can also improve plant drought tolerance, resistance to plant pathogens, and can help improve soil structure. As such, they have an important role to plant in the growth of individual plants, as well as the establishment of plant communities (van der Heijden et al., 1998). They are also recognised as valuable indicators of soil health (Damsma et al., 2015) and as being important in the establishment of plant communities, including those on restored mine sites (Reddell and Milnes, 1992).

While AMF are near ubiquitous in soil, their presence in soil, and hence their ability to from a symbiosis with plant roots, can be affected be a range of factors. These factors can include soil disturbance (e.g. removal and stockpiling of soil during mining operations), compaction and nutrient addition in excessive amounts. Thus, the extent to which plants form AM can be impacted by past and current soil use and management. Given that AM play an important role in supporting plant growth and nutrition, and community establishment, and that stockpiling of soil may adversely affect these beneficial fungi, an important question in mine site restoration is: Do plants grown in stockpiled soil for mine-site revegetation form AM?

In this report we present results of a study investigating the formation of AM by test plants (*Medicago truncatula*) in soils collected and supplied to the University of Adelaide by Iluka Resources (Project A157946). These soils were collected from various sites including soil stockpiles associated with mining activities and from undisturbed control sites. The soils were sampled from a range of sampling depths at each site.

The most commonly used method for quantifying the formation of AM by plants is measurement of “mycorrhizal colonization”. Mycorrhizal colonization, which is assessed microscopically, is simply the percentage (or proportion) of the root length that contains the fungus. Thus, the greater the precent root length colonized, the greater extent to which the association has been formed. Where roots contain no

mycorrhizal colonization, the percentage of root length colonized is zero. Although not part of the project brief, the study also permitted assessment of the growth of the plants, and so these data are also included in this report as they provide some addition interesting insights.

Methods

Soils were supplied to the University of Adelaide by Iluka Resources on the 30th of Sept, 2015. Samples were returned to the laboratory, weighed and sieved to remove rocks and coarse woody debris (Figure 1a). In order to grow test plants in the soil, it is first necessary to determine a suitable moisture content at which to water the plants. This is routinely set at 75% of water holding capacity (WHC). Ideally WHC is determined for each soil sample separately, but due to small sample sizes (~800g/sample), it was necessary to determine WHC on a composite soil sample made up of a sub-sample collected from all soil samples. Fortunately all soils were of a similar texture, and consequently WHC would be similar for all of the soils. The WHC was determined using a sintered glass funnel connected to a 100 cm water column (Ψ_m = −10 kPa) (Figure 1b). Soil was packed in the glass funnel to a bulk density of 1.4 g/cm, saturated with water and allowed to drain for 48 hrs and weighed. The soil was then dried at 105 °C for 48 hr and gravimetric moisture content calculated. The gravimetric moisture content at field capacity was 0.14 g water g^{-1} dry soil. Plants were watered during the growth phase of the study to 75% of WHC (i.e. 0.11 g water g^{-1} dry soil).

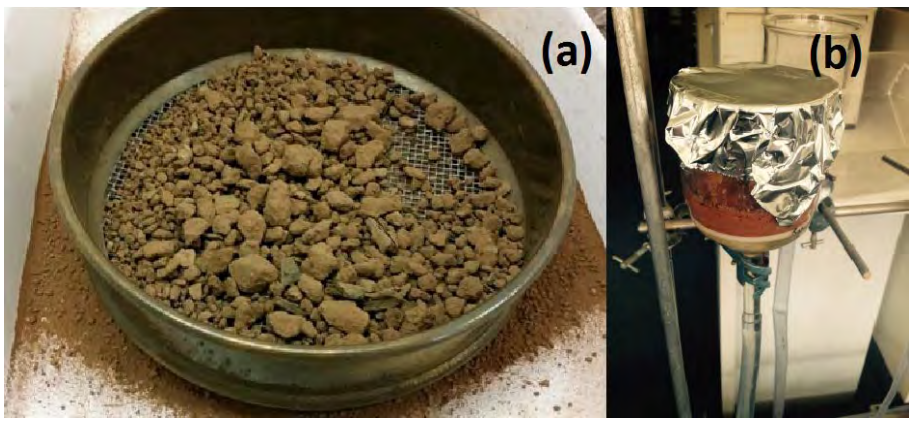


Figure 1. Soil (a) sieving and (b) water potential determination.

To each of 33 pots, 500 g of air dry soil was added. The pots were then watered up to 75% of WHC and into each pot two seeds of *Medicago truncatula* (cv Jemmalong) were sown. This species was selected as it readily forms mycorrhizal associations and grows well in a very wide range of soils. Two pregerminated seeds were sown into each pot to ensure there was sufficient biomass for assessment of mycorrhizal colonization; in all pots there were two plants at the time of harvest, with the exception of pots 20 and 26 where only one plant established. The pots were then placed in SunBags which help to minimize water loss from the pots, and minimize the risk of cross contamination of pots between samples by airborne spores of mycorrhizal fungi. The pots were then placed in a greenhouse on the Waite Campus

of the University of Adelaide where conditions were set to 21.6^{o}C and a relative humidity of 55.5%.

All pots, with the exception of one (No. 15), were destructively harvested 49 days after planting. Pot 15 was harvested one week before the other pots (42 days after planting) as it was dying, and was not expected to survive until the planned harvest date. This one plant still yielded roots for assessment of mycorrhizal colonization and so is in included in our analysis. Plants were harvested by washing them from the soil with water (Figure 2). All the shoots and a sub-sample of the roots were oven-dried (50 ^{o}C) until a constant mass was achieved, and dry weights determined. The second root sample was used for assessment of mycorrhizal colonisation (reported as percentage root length colonized) using the gridline intersect method (Giovannetti and Mosse, 1980), after roots were cleared with KOH (10% W/V) (Phillips and Hayman, 1970) and stained with ink and vinegar (Vierheilig et al., 1998).

Figure 2. Roots washed from soil.

As part of our routine laboratory procedures, we also grew two *Medicago* seedlings in a standard laboratory soil that is known to have a high level of AMF inoculum potential. Percentage colonization data (see below) from this control pot are included to provide a benchmark against which the test samples can be compared.

Results

All data are presented in table format in Appendix 1. The main findings, including some simple data exploration, are presented in the following section as Figures.

Plant growth.

The shoot dry weight (SDW) and root dry weight (RDW) of the plants (Figure 3) varied considerably between the samples analysed. Whereas plants grown in the soil from the undisturbed reference sites were generally consistent across sampling depths (with the exception of sample UD2B), those from the stockpile sites were much more variable, and no consistent patterns with sampling depth were observed. As noted in the methods, biomass data are for two plants per pot, unless otherwise indicated. Root and shoot fresh weights followed similar patterns as dry weights (see Appendix 1).

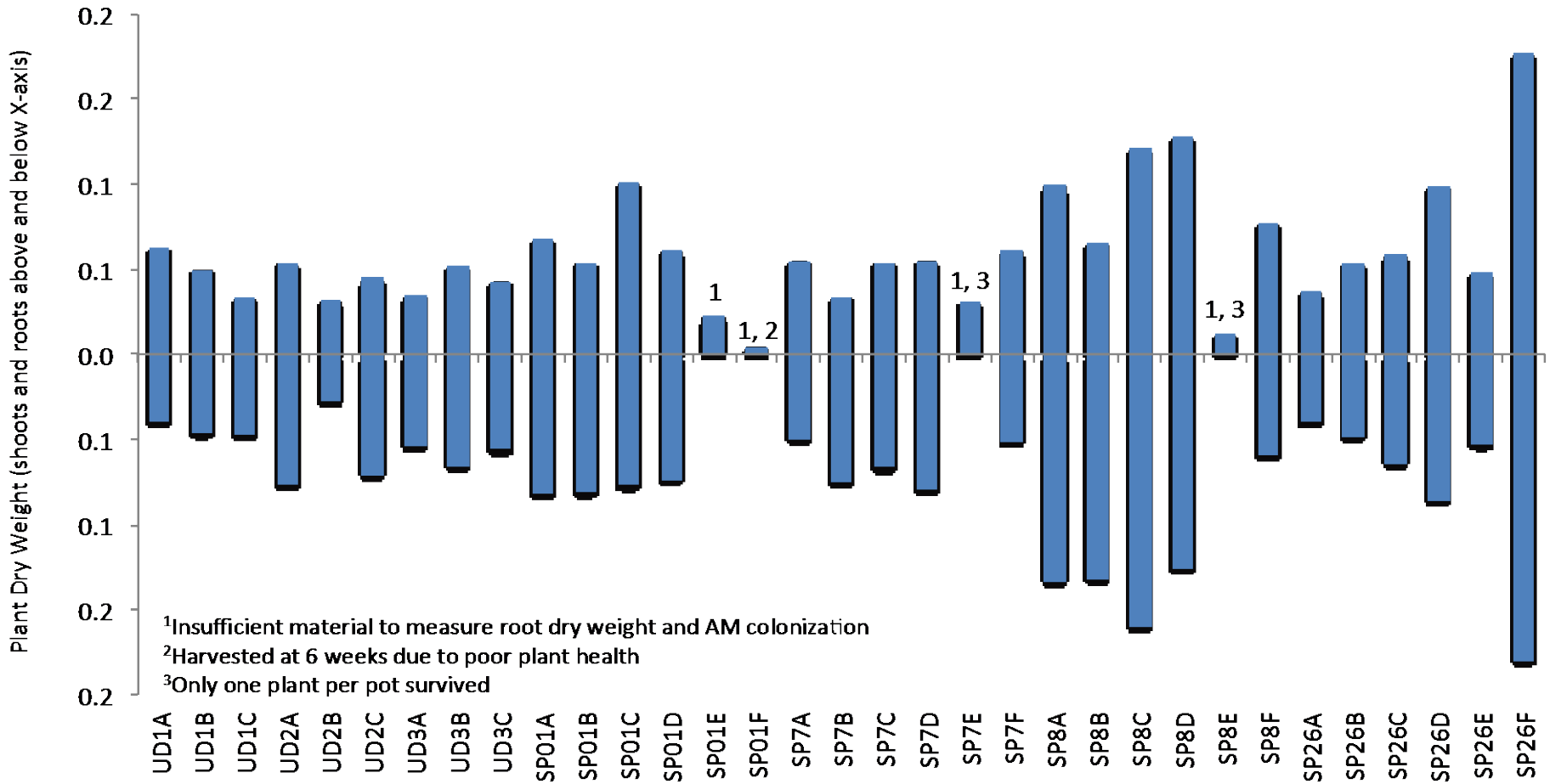


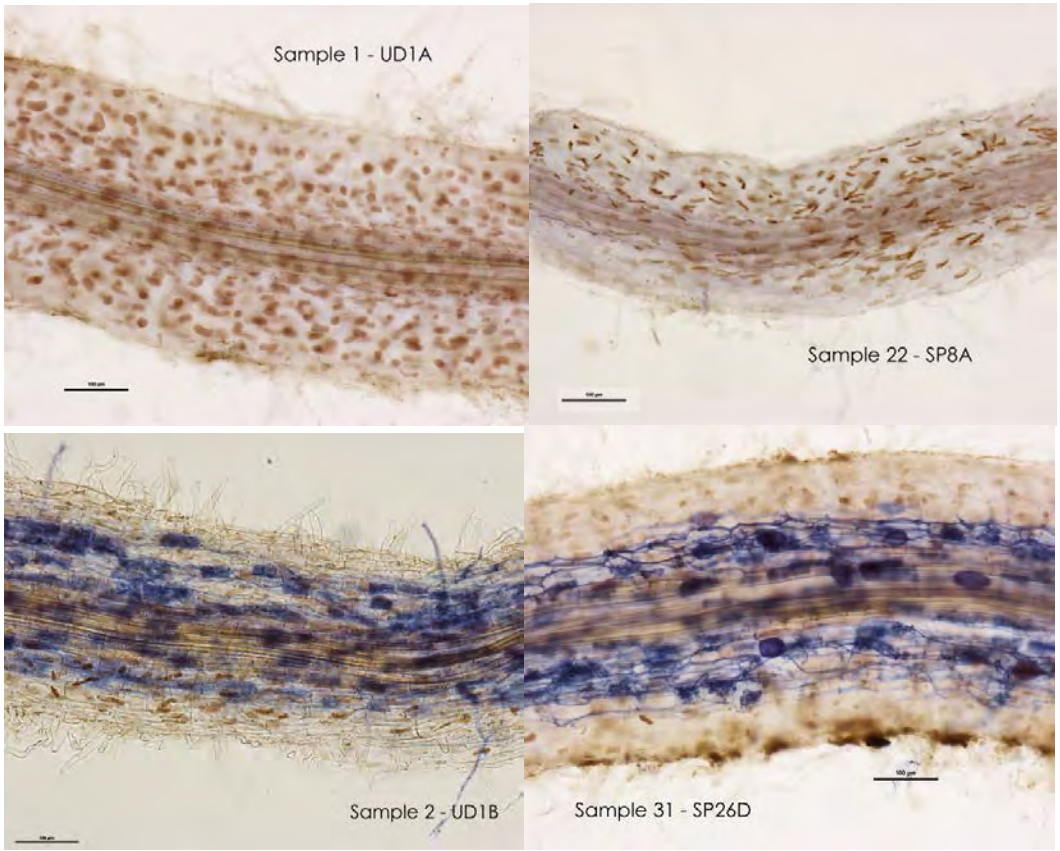
Figure 3. Biomass of test plants at time of harvest. N.B. shoot and root dry weights appear above and below X-axis respectively.

Mycorrhizal colonization of roots.

Roots were colonized by AMF. Examples of colonized roots are shown in Figure 4. Of the 33 soil samples tested, 21 resulted in AM colonization of roots (Figure 5). Where roots were colonized, levels ranged from 0.5 to 66% of root length colonized. In almost half of the samples where roots were colonized, levels of colonization were greater than 25% of the root length. Given the early growth stage of the plants, these levels of colonization indicate the presence of active AMF communities in the soil.

The most striking result was that AM colonization was almost absent from roots grown in soil from stockpiles SP01 and SP7, and present to good levels in most soil layers from stockpiles SP8 and SP26. There are many potential explanations for these differences, including soil physicochemical properties of the stockpiles, the presence of vegetation on the stockpiles (more cover typically results in a build up of AMF), or age of the stockpile. Determination of these drivers is worthy of further investigation. Further, there may be potential to blend soils from different stockpiles to maximize the likelihood of plants forming AM following restoration. Again, this requires further investigation.

Figure 4. Photos of roots colonized by AMF (bottom images with fungi stained blue) and roots not colonized (top images).



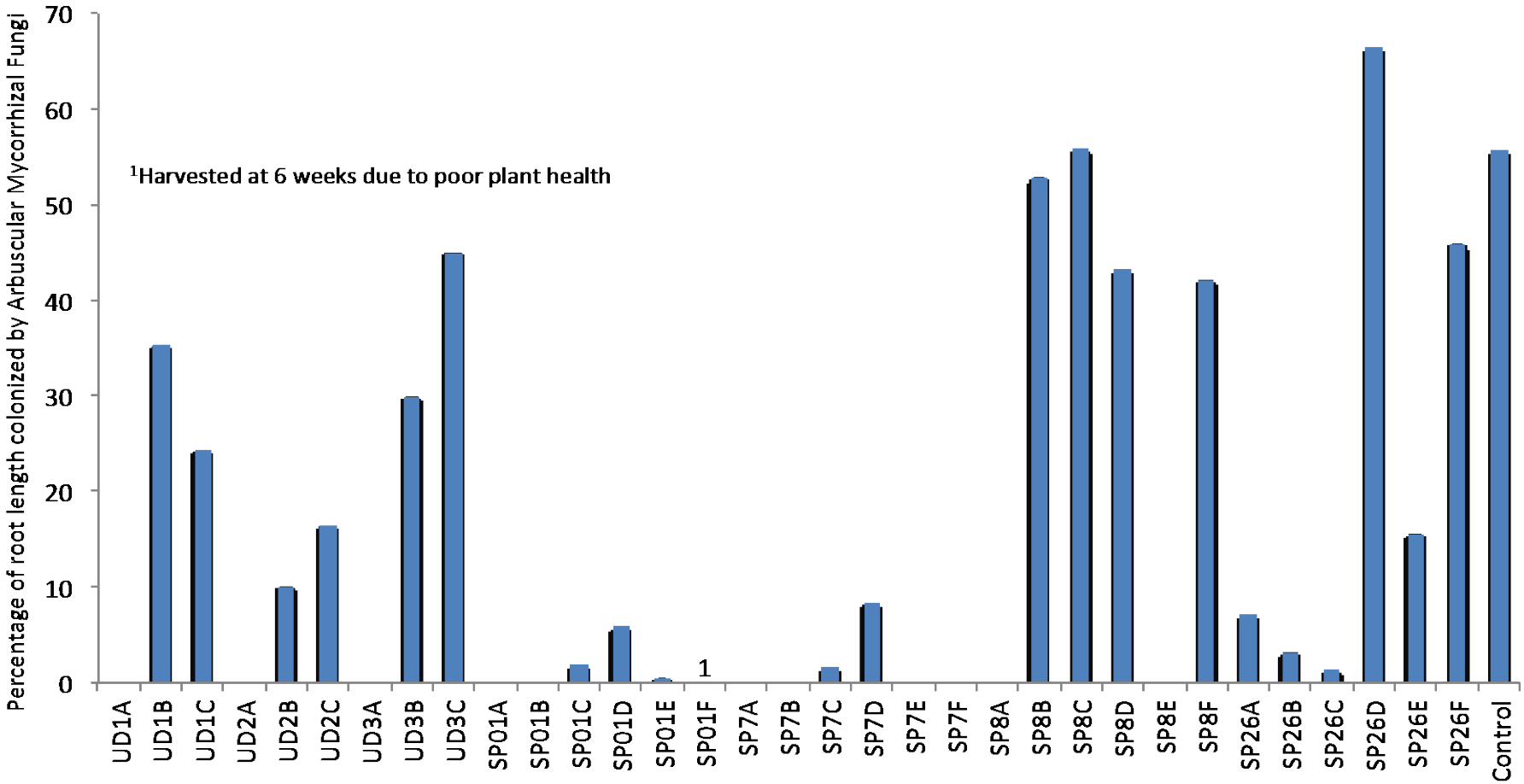


Figure 5. Mycorrhizal colonization of test roots.

In an attempt to further explore the data, AM colonization versus soil depth was plotted (Figure 6a). Values are presented as average level of colonization for a given soil layer within each sample type (i.e. undisturbed or stockpile). Interestingly, these data showed that while levels of AM colonization were good in soils from the undisturbed sites, this was only true for the lower soils layers, with no AM colonization of roots grown in soil collected from the 0-2cm soil layer. While the reasons for the remain unknown, it may be related to physical disturbance of this soil layer (e.g. due to wind), high soil temperatures at the soil surface, or physicochemical properties in this later.

Although variable, levels of AM colonization of roots grown in soil from the stockpile sites provide an interesting result. It is clear that AM colonization increased sharply with soil depth (Figure 6b), but that it was, for reasons unknown, greatly depressed from the 50 cm soil layer. Whilst speculation only, this may be due to soil compaction in the layer during stockpile preparation.

Finally, AM colonization was plotted against shoot dry weight (Figure 7) to explore a potential relationship between plant biomass and AM colonization. In soil from the undisturbed reference sites (Figure 7a), there was no clear pattern. However, in soil from the stockpiles (Figure 7b), there was evidence of a potential threshold of approximately 40% of root length colonized where plant biomass was increased. While not a direct demonstration of a mycorrhizal benefit, these data point to a potential relationship worthy of further investigation.

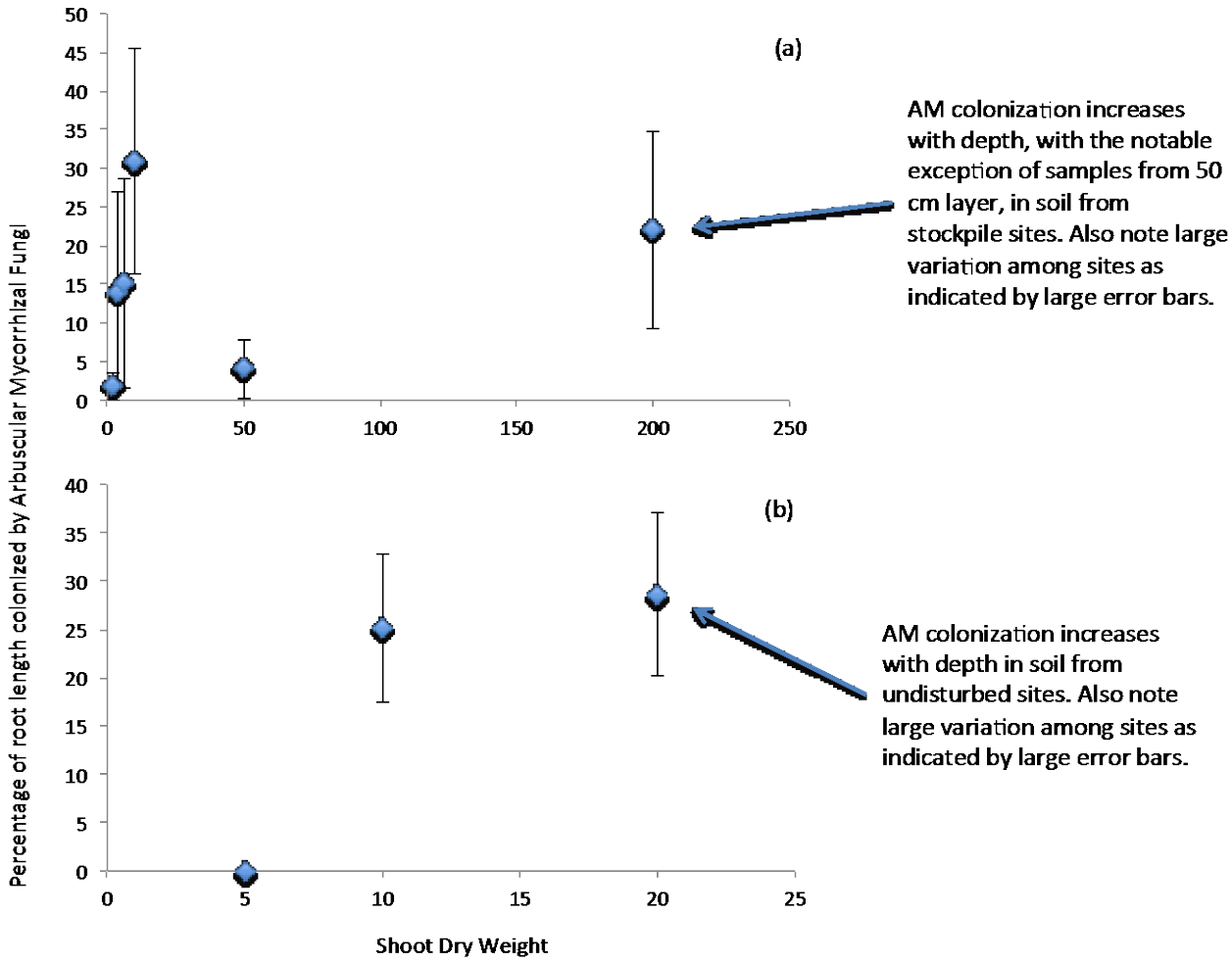


Figure 6. Relationship between AM colonization and root soil sampling depth for (a) stockpile and (b) undisturbed, sites. N.B. vales are mean and S.E of all samples for a given depth within a given soil type (see text).

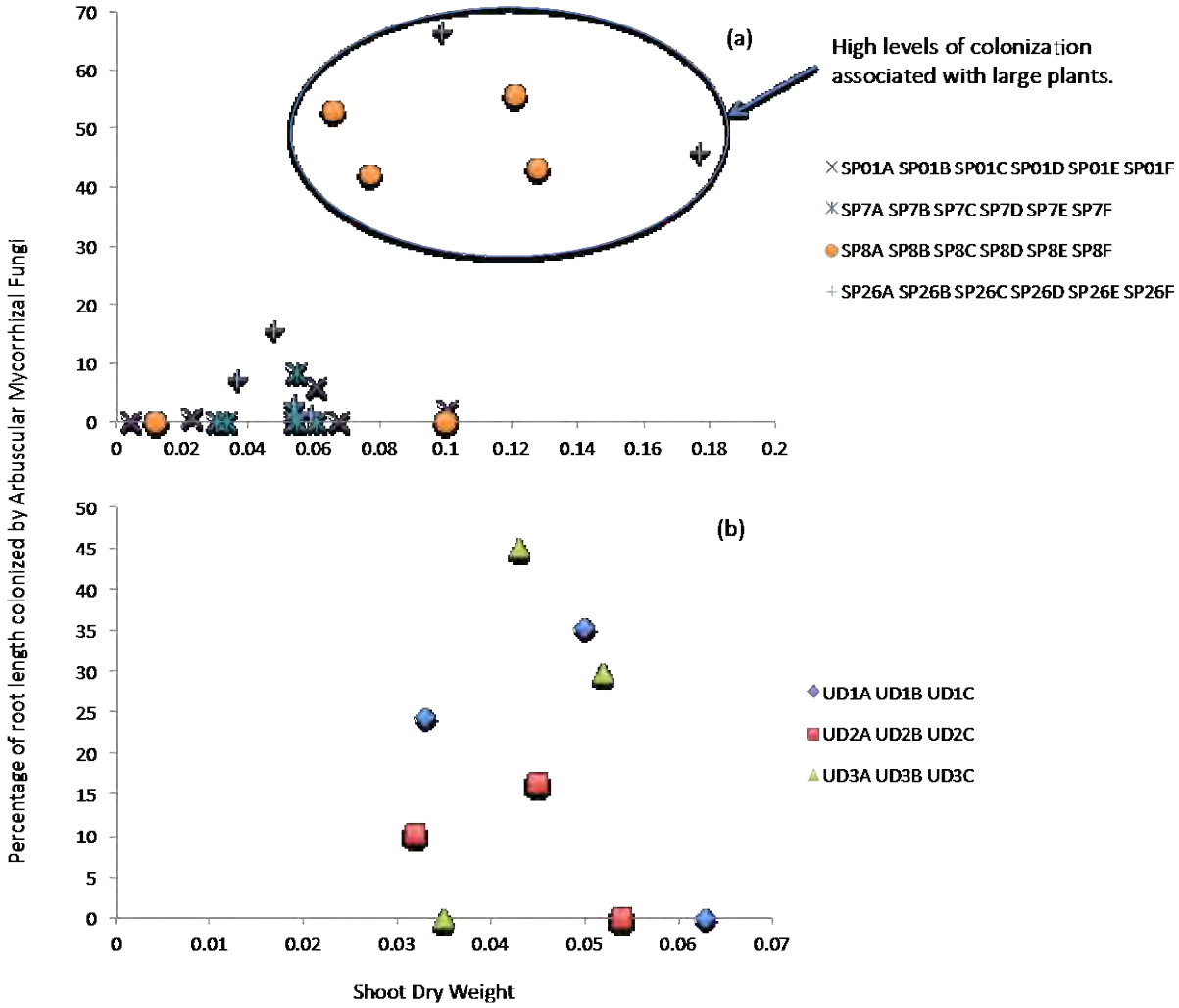


Figure 7. Relationship between shoot dry weight and mycorrhizal colonization in samples from (a) stockpile and (b) undisturbed sites.

Conclusions and summary of major findings

The majority of soils collected contained AMF that readily colonized the roots of the test plants. Soils from both the undisturbed and stockpile sites were colonized by AMF to a good extent in a large number of samples tested. Almost two thirds of samples were colonized by AMF. Of the 12 samples not colonized by AMF, seven of those came from two stockpiles (SP01 and SP7). Mycorrhizal colonization of roots of plants grown in soil collected from stockpiles SP8 and SP26 were generally high, and in many cases were higher than in samples from the undisturbed sites. Interestingly, soil collected from the surface layer of the undisturbed sites had no AM colonization. Although plants were small, there were clear differences in biomass between the various soil samples tested. There was some evidence of a threshold for AM colonization above which plant growth was generally good.

This study did not provide an opportunity to identify the factors underlying variation in levels of AM colonization of roots. This variation may be associated with soil physicochemical properties, management of the soil, or the time the soils have been stockpiled. These factors are potentially worthy of further investigation so that AM colonization can be maximized in restoration activities. The variation in levels of AM colonization between stockpiles is intriguing, and potentially provides an opportunity to manage AMF in the soil for example by blending soil from stock piles.

Appendix 1

Sample ID	Crust	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Percent colonisation	Observations
UD1A	A	0.255	0.063	0.486	0.039	0	
UD1B	N	0.234	0.050	0.481	0.047	35	
UD1C	N	0.137	0.033	0.419	0.047	24	
UD2A	A	0.236	0.054	0.724	0.076	0	
UD2B	W	0.135	0.032	0.359	0.027	10	
UD2C	N	0.148	0.045	0.473	0.071	16	
UD3A	A	0.165	0.035	0.385	0.054	0	1 weed germinated
UD3B	A	0.172	0.052	0.515	0.066	30	
UD3C	N	0.211	0.043	0.544	0.056	45	
SP01A	W	0.320	0.068	0.680	0.082	0	
SP01B	N	0.235	0.054	0.689	0.081	0	
SP01C	N	0.210	0.101	0.576	0.077	2	
SP01D	N	0.228	0.061	0.564	0.073	6	
SP01E	N	0.098	0.023	0.154		0.5	
SP01F	N	0.043	0.005	0.035		0	Dead at 6 weeks
SP7A	N	0.225	0.055	0.486	0.050	0	
SP7B	A	0.171	0.034	0.609	0.075	0	1 weed germinated
SP7C	A	0.233	0.054	0.702	0.067	2	
SP7D	A, W	0.306	0.055	0.661	0.079	8	
SP7E	N	0.111	0.031	0.174		0	Only one plant
SP7F	Y	0.240	0.061	0.382	0.052	0	
SP8A	A, W	0.504	0.100	1.030	0.133	0	
SP8B	A, W	0.385	0.066	0.899	0.132	53	
SP8C	N	0.788	0.121	0.790	0.160	56	
SP8D	Y	0.834	0.128	0.947	0.126	43	
SP8E	N	0.029	0.012	0.060		0	Only one plant
SP8F	N	0.584	0.077	0.530	0.060	42	
SP26A	N	0.152	0.037	0.345	0.040	7	
SP26B	A	0.257	0.054	0.502	0.048	3	
SP26C	A, W	0.211	0.059	0.516	0.064	1	
SP26D	A, W	0.526	0.099	0.728	0.086	66	
SP26E	W	0.203	0.048	0.427	0.053	16	
SP26F	N	1.051	0.177	1.021	0.180	46	Flowers
Control	N	2.460	0.434	1.463	0.240	56	Nodules

Table A1. Complete summary data. Evidence of surface crusts was noted where A= algae, W = white crust, N = no crust.

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Appendix 14 Changes to topsoil and subsoil stripping depth (2015)

File Note

By:	Joanne Lee	CC:	Nick Travers, Tina Law
Date:	9 April 2016	Trim Ref:	
Subject:	Changes to topsoil and subsoil stripping depth (2015)		

Introduction

Currently at Jacinth Ambrosia (JA) clean overburden is stockpiled during the mining process for rehabilitation purposes. The reconstructed soil profile comprises loams, sand, subsoil and topsoil at various depths for each vegetation association located in the project area, Table 1. Soil balance works at JA have indicated a deficit of the majority of soils required for rehabilitation, Table 2 (JA Soil Balance 2015). The deficit of brown loam and red loam has been investigated and controls implemented, namely changes to the soil profile, rehabilitated vegetation associations and changes to the landform design (see JA Program for Environmental Protection and Rehabilitation (PEPR) for further discussion). Changes to the soil profile were approved December 2015. A commitment was made to carry out a stockpile balance annually to determine quantities of overburden available at the end of year in comparison to the quantity of clean overburden required for rehabilitation at that point in time.

The 2015 JA soil balance identified a deficit of most overburden materials, and for subsoil 57% of required was available at 31 December 2015. Topsoil was available in sufficient volumes, however historically a deficit has been identified. The increase in topsoil stripping depth commenced in October 2015, and is likely to account for the change from topsoil deficit to slight surplus. Due to the small quantities of topsoil collected as part of the stripping process topsoil material is at a greater risk to wind and water erosion and quantities will continue to be monitored. This document provides supporting information for the decision to increase soil stripping depth.

A number of options have been considered to reduce the deficit of subsoil and deficit of topsoil, comprising:

1. Topsoil farming - the farming of topsoil by adding small quantities of topsoil to subsoil and brown loam to increase seed store and BSC activity. This has been identified as unsuitable due to the area of area to be disturbed to farm, and the likelihood of changing the soil biology to disturbance specialist species, losing later successional communities (of both BSC and vegetation). Additional discussion is provided in the JA Topsoil Farm Report 2015.
2. Changing soil profile - reducing the rehabilitation soil profile of subsoil and topsoil was considered initially however it was identified that there would be a higher risk of losing the topsoil layer (and associated biology) due to wind or surface water events if it was made shallower than 0.05 m.
3. Changing stripping depths - increasing the material collected during ground disturbance was identified as a potential source of additional material however the depth of the soil biota in undisturbed soils needed to be considered, particularly that of BSC due to its importance for soil stability. Previous works indicated that BSC was present to greater than 0.20 m depth in undisturbed soils (Attachment 1), however it was not clear if the soil seedbank and densities of mycorrhiza decreased with depth (both important for vegetation growth). Additional studies were carried out to determine the depth of soil seed banks and mycorrhiza in undisturbed soils. Data and recommendations for changes to soil stripping depths are presented here.

Table 1 Rehabilitation soil profiles 2015 to current (PEPR approved)

Soil Materials	Landscape Vegetation Unit		
	Myall/Mallee Woodland	Myall Woodland	Chenopod Shrubland
	Thickness of layer (m)	Thickness of layer (m)	Thickness of layer (m)
Topsoil	0.05	0.05	0.05
Subsoil	0.15	0.15	0.15
Sand[1]	Various	Various	n/a
Calcrete Layer[2]	Various	Various	n/a
Brown loam	Minimum of 2.30[3]	2.30–3.20[3]	0.30
Red loam	Minimum of 2.30[3]	2.30–3.20[3]	1.00
Tailings	variable	variable	variable

Table 2 Summary of overburden required for rehabilitation of open areas as at 31 December 2015

Location	area ha	area m^2	Red Loam m^3	Brown Loam m^3	Subsoil m^3	Topsoil m^3
Jacinth Pit	186	1,868,156	3,369,298	3,841,972	280,223	93,407
Off-Path TSF	108	1,087,000	1,087,000	326,100	163,050	55,756
Stockpiles[4]	707	7,070,000			58,358	27,176
Infrastructure	56	566,000			84,900	28,300
Total required	**1057**	**10,570,000**	**4,456,298**	**4,168,072**	**586,531**	**204,639**
Available			3,329,552	3,155,474	331,967	222,911

Methods

The predominant vegetation types at Jacinth are the Myall and Chenopod vegetation associations. Due to similarities between the species present within these associations the topsoil collected as part of the clearance process is stored together in stockpiles. The depth of the seedbank and mycorrhiza in undisturbed soils was investigated for the myall woodlands.

Previously topsoil was stripped to a depth of 0.05 m and subsoil 0.02 m (0.15 m thickness). It was proposed that topsoil stripping depth be increased to 0.10 m and subsoil increased to 0.30 m (0.2 m thickness). Given that topsoil contains the majority of the soil biota important for

[1] Yellow sand associated with dune and creek features.
[2] Calcrete layer associate with creek features only.
[3] Brown and red loam layers together with sum to 5.5m thickness, but the thickness for each layer individually can vary as indicated.
[4] Note that area under topsoil stockpiles will not require topsoil or subsoil application and areas under subsoil stockpiles will not require topsoil application.

rehabilitation soil samples were taken from undisturbed soil profiles in the myall vegetation association at a depth of 0-5 cm 5-10cm and at 20cm (total of 9 samples).

Samples were sent to the SA Seed Conservation Centre at the Adelaide Botanic Gardens where the numbers of viable seed in each sample was identified (for further details see - *Progress Report for Soil Sample Analysis*, South Australian Seed Conservation Centre, February 2016) and the University of Adelaide's Waite Campus to determine the presence of mycorrhiza (for further details see - *Do plants grown in stockpiled soil for mine-site revegetation form arbuscular mycorrhizas*?, University of Adelaide, December 2015).

Results and Discussion

Overall results indicated that soil biology was at a depth of 0.1 m was similar to that of 0.05 m. and seed and mycorrhiza was also present at a depth 0.2 m.

Mycorhizza colonisation of plants was recorded for all soils samples, regardless of depth in undisturbed soils (Attachment 3). It is not anticipated that stripping topsoil to a depth of 0.10 m would impact negatively on the restoration of mycorrhiza in rehabilitated soil profiles.

The densities and viability of seeds present in the undisturbed samples was consistently low across all soil depths, Figure 1 and Figure 2. Low numbers of viable seeds were also recorded in the stockpiled material; it is therefore suspected that the seedbank in the myall woodland is naturally low. It is not anticipated that stripping to 0.1 m would impact additionally on rehabilitation efforts, however the low seedbank will need to investigated and remediation considered if required (i.e. additional seeding or seedlings).

Based on studies carried out to date any deleterious impacts to rehabilitation efforts due to increased stripping depth could be ameliorated by the addition of seed to topsoil. The greater risk is the soil deficit, therefore stripping topsoil to a depth of 0.10 m and subsoil to a depth 0.3 m is recommended. Soil balances will continue annually and stripping depth can be reviewed as required.

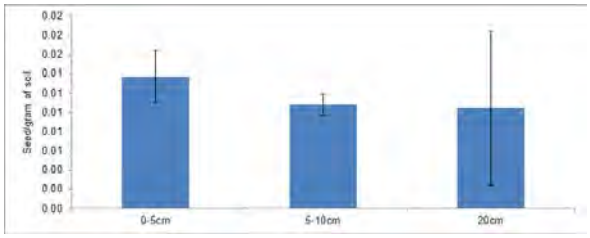

Figure 1 Numbers of seeds per gram of soil recorded in undisturbed myall woodlands. Error bars indicate standard error.

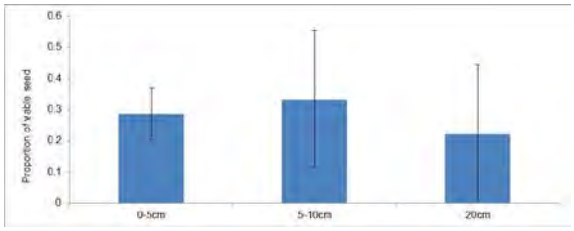

Figure 2 Proportion of viable seeds recorded in undisturbed myall woodlands. Error bars indicate standard error.

ATTACHMENT 1

Prepared for

Iluka Resources Limited

Subject

Activity of cyanobacteria in topsoil stockpiles of varying ages at the Jacinth-Ambrosia mine site

Author

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22 February 2013

UniQuest Project No: C00468

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Activity of cyanobacteria in topsoil stockpiles of varying ages at the Jacinth-Ambrosia mine site

Declaration

This report has been prepared in accordance with UniQuest's Quality Management System, which is compliant with AS/NZS ISO 9000:2000.

Project Group	Project Report No.	Date
Biocrust - BSC	BSC-004	7 Feb 2013

REPORT BY

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ABSTRACT

The Jacinth-Ambrosia (J-A) mineral sands mine is situated in semi-arid South Australia. Biological soil crusts (BSCs) are dominated by cyanobacteria however incorporate lichens and mosses and cover a large proportion of the soil surface in this area.

BSCs perform a number of important ecological functions that alter soil properties including modifying soil moisture and nutrients and stabilisation of soils. Soil cyanobacteria in particular, perform many important ecosystem functions that include carbon and nitrogen fixation, nutrient entrapment and cycling as well as soil moisture and temperature regulation, all of which can influence plant germination and growth. Vegetation growing in BSC rich soils is therefore also influenced by BSCs. For these reasons, it is important to consider restoring BSC organisms in rehabilitation of the J-A site.

A large proportion of BSC organisms, including cyanobacteria, rely on photosynthesis for energy and for this reason, it is assumed that BSC activity is generally concentrated in the top few centimetres of the soil. A second assumption follows that cyanobacterial viability will be lost in the absence of light where moisture is available, but this hypothesis has not been widely tested, and specifically has not been examined in the J-A context.

To this end, topsoil at the site was removed and placed in low stockpiles when mining commenced. It was expected that the cyanobacteria in these topsoils would be killed through the stockpiling process due to lack of light. The current study was undertaken to determine the effects of stockpiling on the crust forming cyanobacteria native to the J-A site.

It was found that cyanobacteria did survive stockpiling for up to 29 months without loss of cyanobacterial diversity. However, different taxa exhibited different responses to stockpiling. Cyanobacteria were also found to inhabit undisturbed areas at lower depths. The mechanisms by which survival occurs are unknown but growth rates suggest it may be through reactivation of vegetative material.

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1. INTRODUCTION

Biological soil crusts (BSCs) consisting of cyanobacteria, algae, lichens, mosses, liverworts, micro-fungi and bacteria perform a wide array of ecosystem functions in semi-arid areas (Eldridge, 1993, Bowker *et al.*, 2008, Dunne, 1989, Eldridge & Greene, 1994, Graetz & Tongway, 1986, Mager & Thomas, 2011) including the Jacinth-Ambrosia (J-A) mineral sands mine site in north-western South Australia. Soil cyanobacteria thrive in alkaline soils (Reisser, 2007a) such as those of the J-A area (Doudle, 2010). Post mining restoration of BSCs may assist in overcoming a range of problems that present following vegetation clearance. For example, erosion, nutrient loss, altered water infiltration and storage in the soil as well as having associated effects on vegetation establishment and growth (Hawkes, 2004, Hernandez & Sandquist, 2011, Langhans *et al.*, 2009, Li *et al.*, 2005, Nebeker & St. Clair, 1980).

Soil cyanobacteria in particular, perform many important ecosystem functions (Belnap *et al.*, 2001). Cyanobacteria can fix nitrogen and carbon, (Büdel *et al.*, 2008, Eldridge & Greene, 1994, Belnap *et al.*, 2001) create other nutrients, (Belnap & Harper, 1995, Harper & Belnap, 2001, Beyschlag *et al.*, 2008, Harper & Pendleton, 1993, Tongway & Smith, 1989) alter soil moisture holding capabilities (Obana *et al.*, 2007) and influence soil temperature (George *et al.*, 2003), each of which can in turn influence plant germination (Hawkes, 2004, Rivera-Aguilar *et al.*, 2005, Godínez-Alvarez *et al.*, 2011, Langhans *et al.*, 2009, Eckert *et al.*, 1986, Bliss & Gold, 1999, Nebeker & St. Clair, 1980), and subsequent growth (Belnap & Harper, 1995, McIlvanie, 1942, Pendleton *et al.*, 2003, Belnap, 1995, Pendleton & Warren, 1995, Dadhich *et al.*, 1969, Nebeker & St. Clair, 1980, Watanabe *et al.*, 1951). Soil cyanobacteria comprise a major component of the BSCs at J-A (Doudle, 2010) and were therefore targeted for this study.

A large proportion of BSC organisms, including cyanobacteria, rely on photosynthesis for energy and for this reason, it is assumed that BSC activity is generally concentrated in the top few centimetres of the soil (Büdel *et al.*, 2008, Eldridge & Greene, 1994). A second assumption follows that cyanobacterial viability will be lost in the absence of light where moisture is available, but this hypothesis has not been widely tested, and specifically has not been examined in the J-A context.

In the process of mining and preparation of the J-A site for future rehabilitation, topsoil is stored in low stockpiles, commonly of less than two metres in height. Soil types correspond with vegetation types in the J-A area. Consequently, stockpiles are comprised of topsoil sourced from only one vegetation type. 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 in regard to BSC re-establishment. If low levels of BSC organisms are detected below the top few centimetres, the addition of propagated BSC organisms to returned topsoil may be warranted. Activity and diversity of the organisms within the stockpiled topsoils may vary with age and this may also influence the establishment of vegetation in the rehabilitation process.

The effects of topsoil stockpiling on BSC organisms has not been investigated to date. More broadly, few studies have investigated the presence of cyanobacteria below the top few centimetres of soil (Reisser, 2007b). The aim of this research was to investigate the activity of soil cyanobacteria in topsoil that had been stockpiled for three different periods. Activity of soil cyanobacteria within stockpiles was compared with activity of undisturbed BSCs in areas adjacent to stockpiles through mirrored replication of soil sampling methods in these areas. The information gained through this study will be integral in the planning and implementation of rehabilitation of the BSCs at J-A mine site.

2. MATERIAL AND METHODS

2.1 Stockpile soil sampling

Samples of soil were sourced from varying depths (Table 1) in stockpiles of topsoil taken from areas with a *Acacia papyrocarpa* (Western Myall) overstorey that is associated with a particular soil type. Corresponding samples were taken from the same depths in surrounding areas with similar soil types that were unaffected by clearance or soil disturbance to determine BSC activity at depth in undisturbed areas. Soil sampling was undertaken on 8-9 March 2012.

A total of six stockpiles that were established in three different years were sampled to determine any changes in cyanobacterial activity that may occur with stockpile age. Holes were created to enable access to the soil profile for sampling of two stockpiles for each of the three different periods of stockpiling. Three replicate holes were dug in each of the six stockpiles and corresponding samples were obtained from an equal number of holes in adjacent undisturbed areas. This amounts to 36 holes in total when taking into account the corresponding holes in undisturbed areas. The locations of the sampling sites are shown in Appendix I with GPS coordinates recorded in Table 1. Holes were dug to a depth of greater than 50 cm and a total of seven soil samples were taken from each of the hole at six different series of depths throughout the soil profile using a geologist's hammer and a spatula. A second sample from taken from the lowest depth of 50cm was autoclaved to act as a control. The samples were transferred to labelled paper bags then transported to the laboratory. Care was taken to ensure the samples were taken from the nominated depth and not contaminated by soil from the profile above. The surfaces of the exposed soil profile were brushed with a paintbrush prior to sampling to remove any excess dust that may have fallen into the hole during excavation.

2.2 Sample preparation

Samples were weighed into individual plastic Petri dishes with between 20.000 and 20.020 grams per samples. Great care was taken to reduce the likelihood of cross contamination of samples. Profile samples were weighed from 50 cm backwards to finish with the 0-2 cm sample as this is considered the most likely layer to contain the highest concentration of micro-organisms. Implements that were used to add or remove material from the samples were sterilised after use using methylated spirits and a Bunsen burner. The balance and surrounding area were wiped regularly with 70% methylated spirits and hands were washed between profiles.

After weighing, approximately 9 ml of sterile distilled water was added to each sample using a 20 ml sterile syringe. The syringe was dipped into methylated spirits between samples to remove any dust particles that may have been disturbed. Samples were then sealed with parafilm and labelled. Control samples taken from 50cm were autoclaved at 100^0C and 121 psi for 20 minutes in glass Petri dishes that were wrapped in alfoil. These samples were then transferred to plastic Petri dishes and watered and sealed as per the other samples.

2.3 Incubation

The Petri dishes containing samples were then transferred to shelves in a temperature controlled room that were illuminated with banks of grow lux tubes for a 12 hour photoperiod. Temperatures on the shelves ranged from 26-27^0C and Petri dishes were rotated weekly to prevent site specific effects. When samples appeared to be drying, they were individually opened in a laminar flow cabinet and watered with sterile distilled water. Records of the dates of watering for individual samples were kept. Great care was taken to ensure cross contamination of samples did not occur. Only one Petri dish was ever opened at one time and surfaces were wiped with 70% methanol after each dish was opened. These steps were also repeated during the identification process.

Table 1 Topsoil stockpile ages, locations and sampling depths

Stockpile number	Date of topsoil stockpiling	Stockpile Replicate	Latitude	Longitude	Adjacent replicate	Latitude	Longitude	Soil sample depths (cm)	Date sampled
10	31/10/2009	1	0234527	6578956	1	0234433	6578944	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
10	31/10/2009	2	0234542	6578921	2	0234422	6578933	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
10	31/10/2009	3	0234557	6578881	3	0234436	6578913	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
12	6/11/2009	1	0233396	2578200	1	0233414	6578180	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
12	6/11/2009	2	0233377	6578206	2	0233437	6578227	0-2, 2-4, 4-6, 10, 25, 50, 5 (sterilised control)	8/3/12
12	6/11/2009	3	0233362	6578218	3	0233431	6578263	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
18	13/07/2010	1	0234870	6578033	1	0234878	6577787	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
18	13/07/2010	2	0234852	6578023	2	0234873	6577776	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
18	13/07/2010	3	0234860	6578007	3	0234853	6577782	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	1	0234999	6578800	1	0234922	6578851	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	2	0235000	6578820	2	0234918	6578856	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	3	0235003	6578834	3	0234913	6578849	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
20	2011	1	023457	6577150	1	0234938	6577286	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
20	2011	2	0234881	6577174	2	0234936	6577311	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
20	2011	3	0234900	6577191	3	0234965	6577284	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
21	2011	1	0234832	6576977	1	0234894	6577031	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
21	2011	2	0234861	6576974	2	0234893	6577043	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
21	2011	3	0234882	6576975	3	0234882	6577046	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12

2.4 Identification of cyanobacteria

Cyanobacteria are difficult to taxonomically assign for a number of reasons. Firstly, comprehensive keys are only available for taxa of middle European environments and these do not cover all of the cyanobacterial groups that have been described. Of the groups that are described, few organisms have been investigated using molecular analyses. Furthermore, taxonomic morphological traits (both micro and macro) vary with nutrition, climate, substrate types (Komárková *et al.*, 2010) and phase of growth leading to inconsistent and unreliable identification. In comparison, molecular methods use highly conserved gene regions to determine the relatedness of unknown cyanobacteria to those characterised in nucleotide databases. A high level of correlation is required between sequences in order to definitively identify cyanobacteria. Generally >95% sequence similarity is required for identification of genera (Komárková *et al.*, 2010) and >98% sequence correlation is necessary for identification of species. Variations in the method by which the DNA is extracted and the portion of the sequence that is read can affect the outcomes of gene sequencing. Also, the paucity of environmental cyanobacteria sequences in nucleotide databases can still result in poor taxonomic resolution, especially for cyanobacteria from novel niches (Garcia-Pichel *et al.*, 2001). Molecular investigations have not yet been undertaken in relation the species present at the J-A site and such undertakings are highly time and resource consuming. It was therefore considered prudent to divide the cyanobacteria present in the samples into morphotypes based on similar cellular, filamentous and thallic features. This approach corresponds with that of many other cyanobacterial researchers

After six weeks of incubation, the samples were examined microscopically to identify the cyanobacterial morphotypes present and measure their relative cover. The filamentous morphotypes were keyed out using Komárek and Anagnostidis (2005a) to genus level where possible. Future molecular investigation is required to validate or refute these identifications with any certainty. Each sample was examined at 16 times magnification in order to locate cyanobacterial thalli. The area of each colony was estimated by the relative amount of space taken up in the microscope field of view under the magnification in which the entire thallus fit. Where the thallus would not fit in the field of view at six times magnification, the relative area taken up in the entire Petri dish was estimated. Samples of thalli were mounted on slides in sterile distilled water under a coverslip and examined using a compound microscope to assist with identification. Where multiple morphotypes were present in a colony, the microscopic relative abundance of each was used to divide the cover between the morphotypes accordingly. Where no growth was observed, five soil samples were taken randomly from the

sample, mounted and examined under 400 times magnification for the presence of cyanobacterial cells.

When filamentous cyanobacteria begin to grow, they are colourless and lack the visible cellular structure necessary for differentiation into morphotypes. Where developing cyanobacteria were encountered in the samples, the area of coverage was recorded but no additions to the number of morphotypes were included as identification was not possible.

The identification process was time consuming particularly initially when familiarisation and identification of the morphotypes was undertaken. All controls were examined first followed by undisturbed samples starting with the deepest samples then sequentially through the remaining samples from deepest to surface layers. The stockpile samples were then examined in the same order. Identification of cyanobacteria in the undisturbed samples was carried out over a 50 day period but it took only two weeks to examine all stockpile samples. When all samples had been examined, the undisturbed samples were inspected again for the presence of slow growing species.

2.5 Statistical analyses

ANOVAs were performed with post hoc Tukey's tests performed to determine any differences between treatments.

3. RESULTS

3.1 Morphotype differentiation

A total of ten cyanobacterial morphotypes were identified from the samples. The majority were filamentous with the exception of one morphotype. It was red in colour and had changed morphologically with time. Initially, no green colouration was evident thus the categorisation of this organism as cyanobacterial was not clear cut. With time, areas of chlorophyll appeared but further observation through ensuing growth phases, sampling and potential DNA analysis of this morphotype is necessary.

The remaining eight morphotypes could be clearly identified as filamentous cyanobacteria. Of these seven, five were widespread throughout the samples. These included *Nostoc, Scytonema, Microcoleus, Porphyrosiphon* and *Leptolyngbya.* A second *Leptolyngbya* morphotype was black in colour and this did not appear to be sheath colouration. Morphological analyses could not definitively determine whether this was a different form of the green and yellow colour *Leptolyngbya* morphotype that had a greater distribution throughout the samples. A similar situation existed with *Porphyrosiphon.* A yellow form was present in a small number of samples and may be either another morphotype or the same form with different colouration. The least frequently sampled morphotype was *Stigonema*.

A second morphologically distinct *Nostoc* species also occurred in the samples. It was initially yellow in colour and appeared to be non-filamentous. During the latter stages the solitary cells developed into another form of *Nostoc.*

3.2 Cyanobacterial diversity

Average morphotype diversity was highest in stockpiled samples at and below 10 cm depth for all stockpile ages (Figure 1, Figure 2 and Figure 3). These differences were significant at 50 cm in all stockpiles (29 month DF 5, F 7.00, P 0.024 20 month DF 5, F 8.37, P 0.016 9 month DF 5, F 18.00, P 0.002) and 25 cm in 20 and 9 month old stockpiles (DF 5, F 32.73, P 0.000 DF 5, F 16.20 respectively). Following 29 months of stockpiling, average morphotype diversity was highest in adjacent undisturbed areas above 6 cm depth (Figure 1) although this difference was not significant. Conversely, after nine and 20 months of stockpiling, average morphotype diversity was higher in stockpiled samples when compared with undisturbed samples with the exception of 20 month old 4-6 cm samples (Figure 2 Figure 3) but this difference was only significant in the nine month old stockpile at 10 cm depths (DF 5, F 8.27, P 0.017). High variability between replicates accounted for the lack of significance.

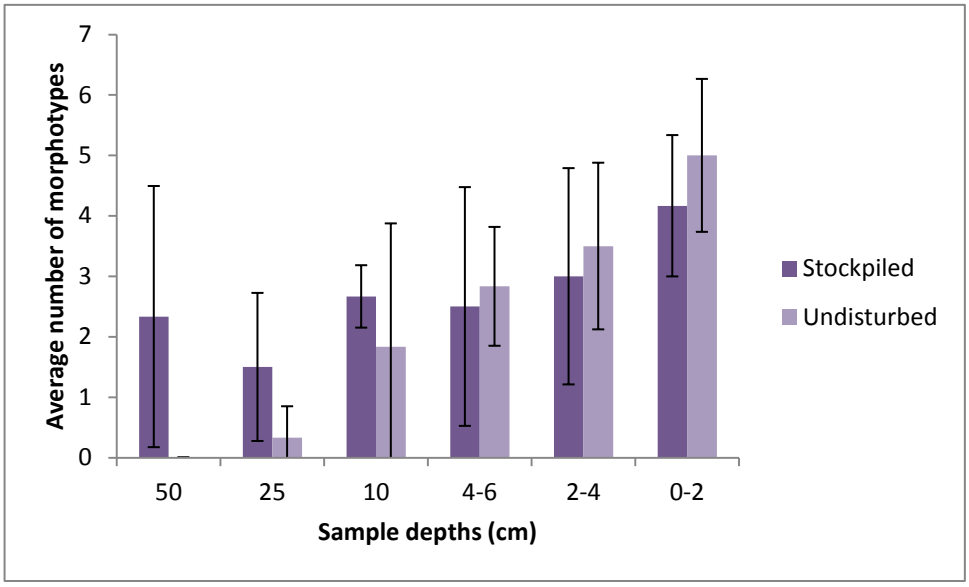

Figure 1 Cyanobacterial diversity following 29 months of stockpiling

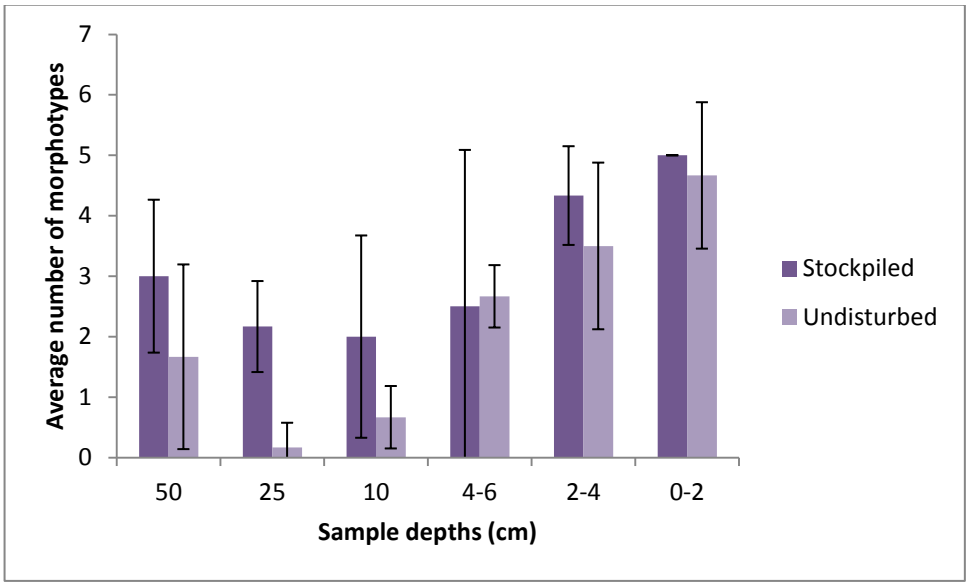

Figure 2 Cyanobacterial diversity following 20 months of stockpiling

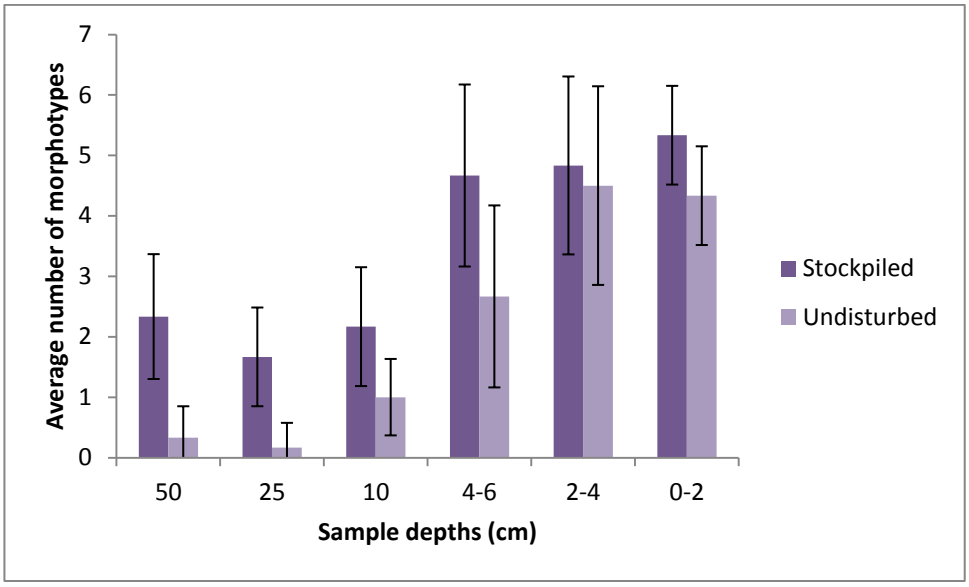

Figure 3 Cyanobacterial diversity following 5 months of stockpiling

When comparing average morphotype diversity at different depths between stockpiles, diversity was greater in material stockpiled for the least amount of time above 10 cm depth but not at or below this point (Figure 4). Average morphotype diversity was variable within adjacent undisturbed areas (Figure 5). The variability in morphotype diversity within replicates was high as shown in the error bars of Figure 1, Figure 2 and Figure 3. This variability was evident in the lack of significant difference in species diversity at any depth between stockpiles of different ages.

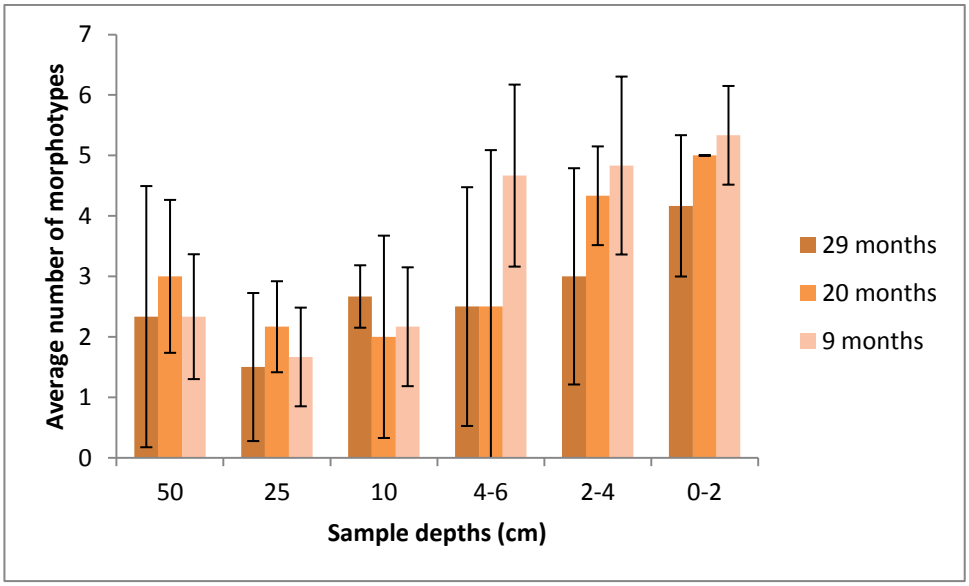

Figure 4 Cyanobacterial diversity in stockpiles of different ages

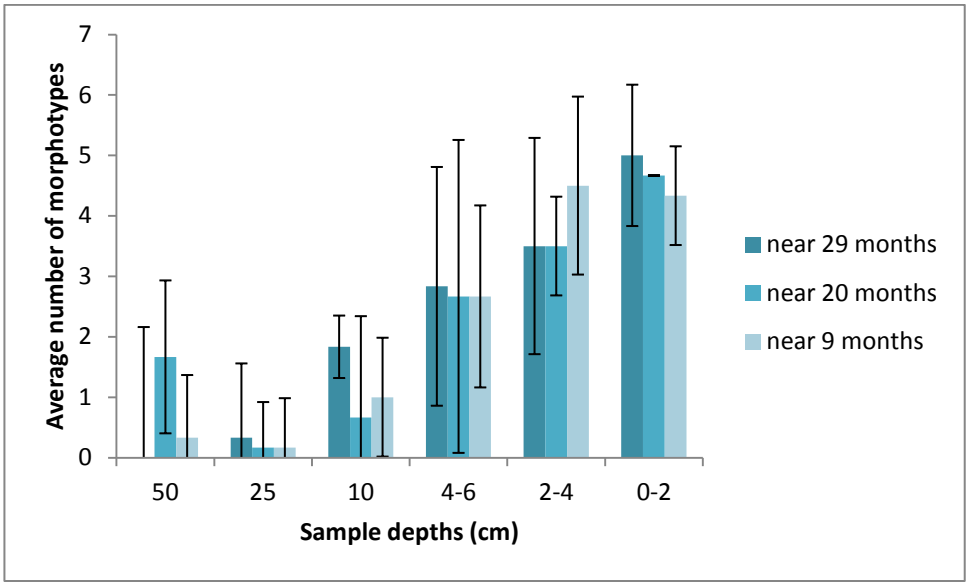

Figure 5 Cyanobacterial diversity in undisturbed areas

3.3 Cyanobacterial distribution

Nostoc cf *commune* exhibited the best survival response in stockpiling as it covered a significant area of samples from all depths in all stockpile ages. This morphotype covered the largest area of the samples in the oldest stockpiles followed by the most recently created stockpiles. These differences were significant from depths of 6 cm to the surface (4-6 cm DF 5, F 28.83, P 0.000 2-4 cm DF 5, F 4.89, P 0.023 0-2 cm DF 5, F 3.72, P 0.049). These patterns were not reflected in the total area covered by *N.* cf *commune* in corresponding adjacent areas.

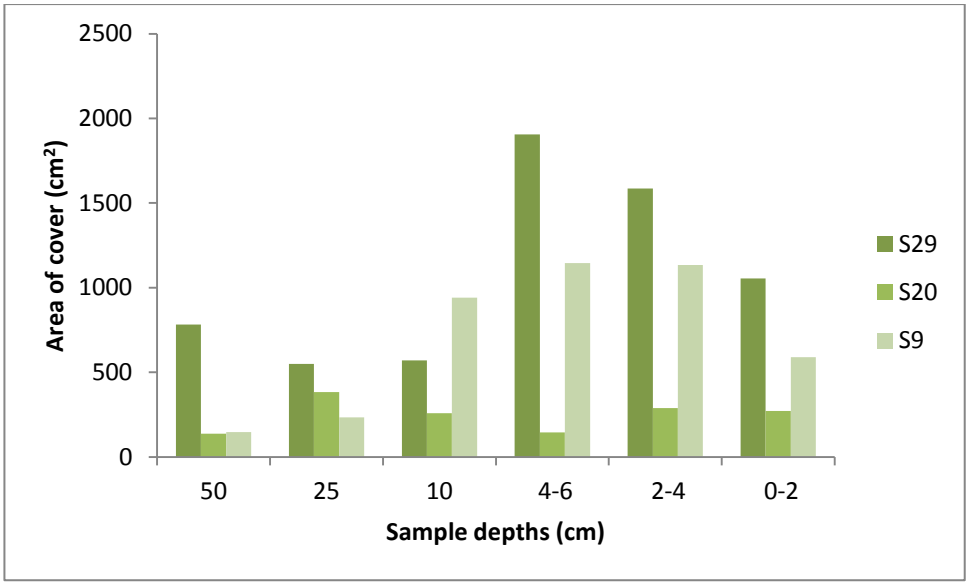

Figure 6 Total area covered by *Nostoc* cf *commune* in stockpile samples (legend shows stockpile age)

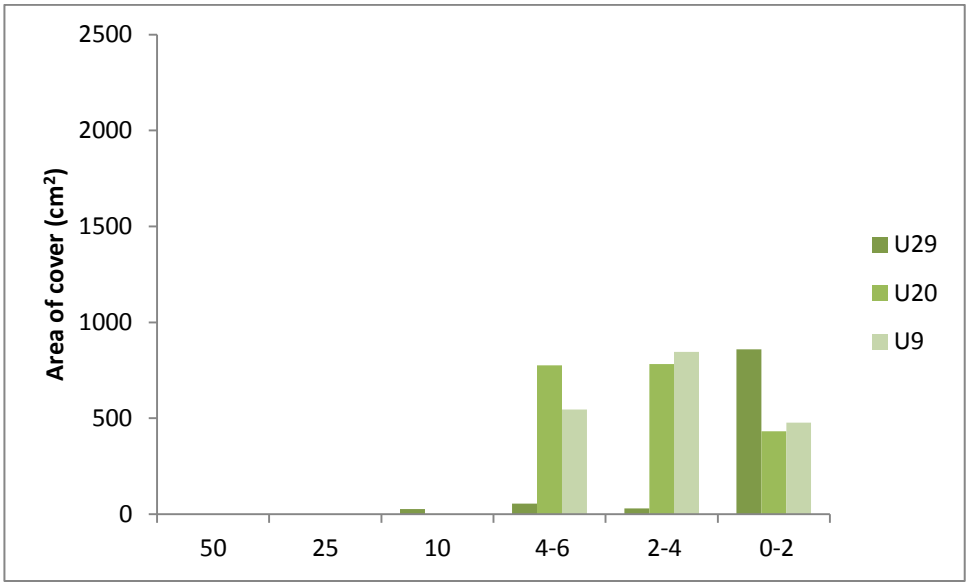

Figure 7 Total area covered by *Nostoc* cf *commune* in undisturbed samples (legend shows age of nearest stockpile)

The distribution of yellow *Nostoc* was similar in that it was present in only one surface sample from an adjacent area (Figure 9) yet was found in all stockpiles (Figure 6). This morphotype was more prevalent at lower depths as with the stockpile samples.

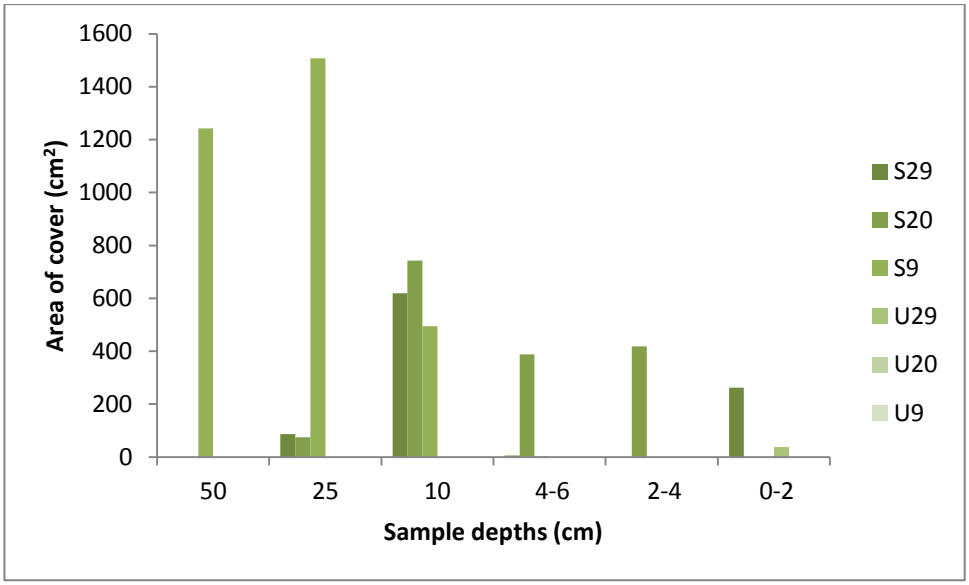

Figure 8 Total area covered by yellow *Nostoc* in stockpiles (S) and undisturbed (U) samples

The *Leptolyngbya* black morphotype only occurred between 20 and 40 mm depth and were found in only one stockpile samples but were present in six samples from three adjacent areas.

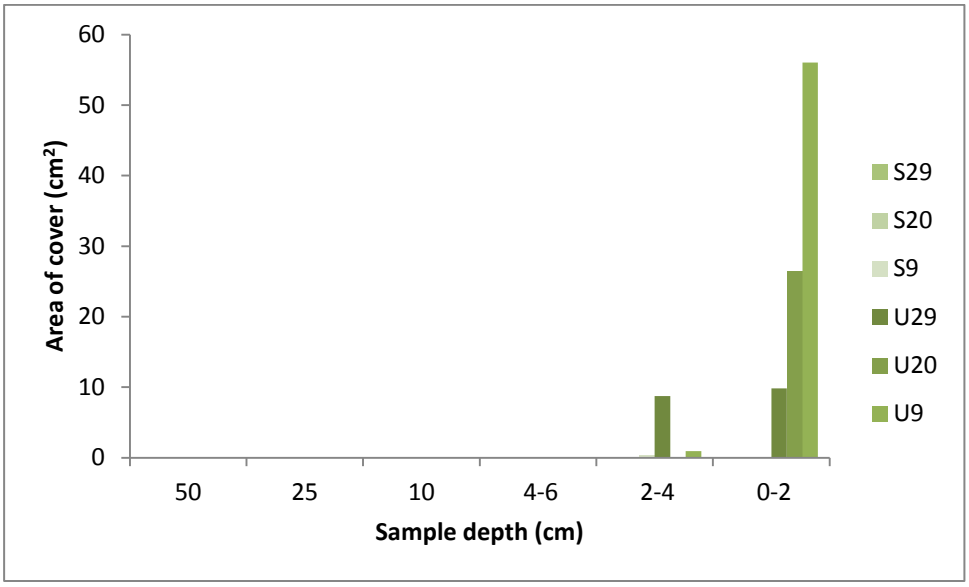

Figure 9 Total area covered by *Leptolyngbya* in stockpiles (S) and undisturbed (U) samples

The *Stigonema* morphotype was found in six stockpile samples spanning all ages and in only one adjacent sample in all cases in the upper 10 cm of the soil profile.

The red non-filamentous organism was present in only one of the undisturbed samples but observed in stockpiles at various depths. This morphotype was found in all stockpiles except those created five months prior to sampling (Figure 10).

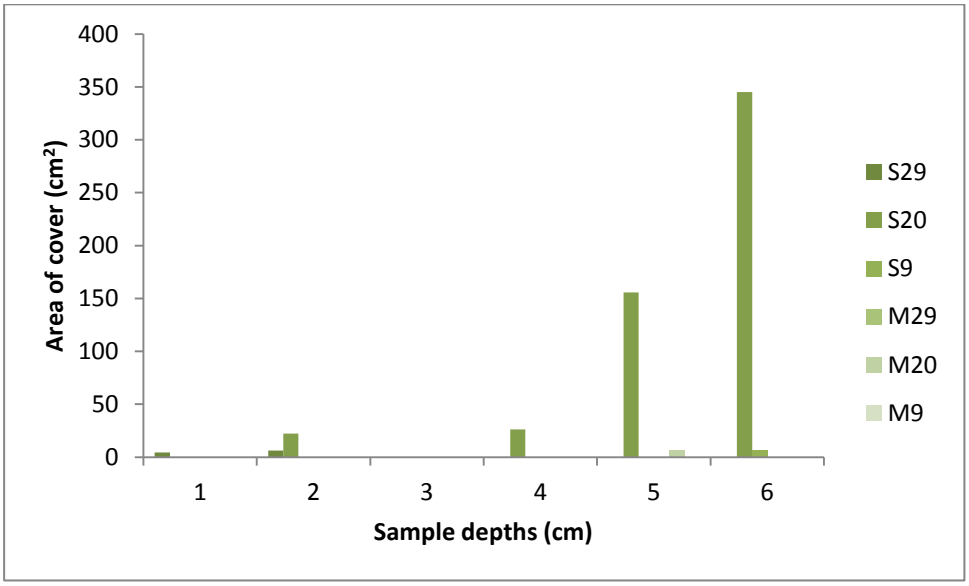

Figure 10 Red non-filamentous distribution in stockpiles (S) and undisturbed (U) samples

The total area covered by *Porphyrosiphon, Scytonema* and *Leptolyngbya* appeared to decrease with stockpile age (Figure 11) but differences were not significant and variation was

again evident in adjacent samples (Figure 12). The area covered by each of the morphotypes was relatively constant between sites in the undisturbed samples. The same was not true for the stockpiled samples where variation in morphotype cover was evident between stockpiles. This difference was statistically significant only for *Nostoc* cf *commune* (DF 5, F 5.97, P 0.012).

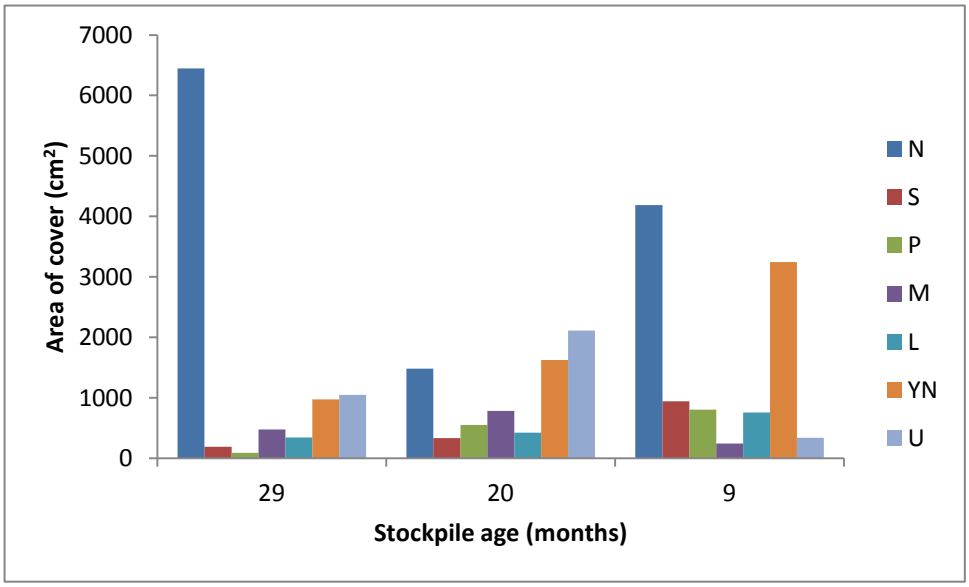

Figure 11 Total area of cover for all species in all stockpiled samples.

(N *Nostoc* cf *commune,* S *Scytonema,* P *Porphyrosiphon,* M *Microcoleus,* L *Leptolyngbya,* YN yellow *Nostoc,* U underdeveloped cyanobacteria)

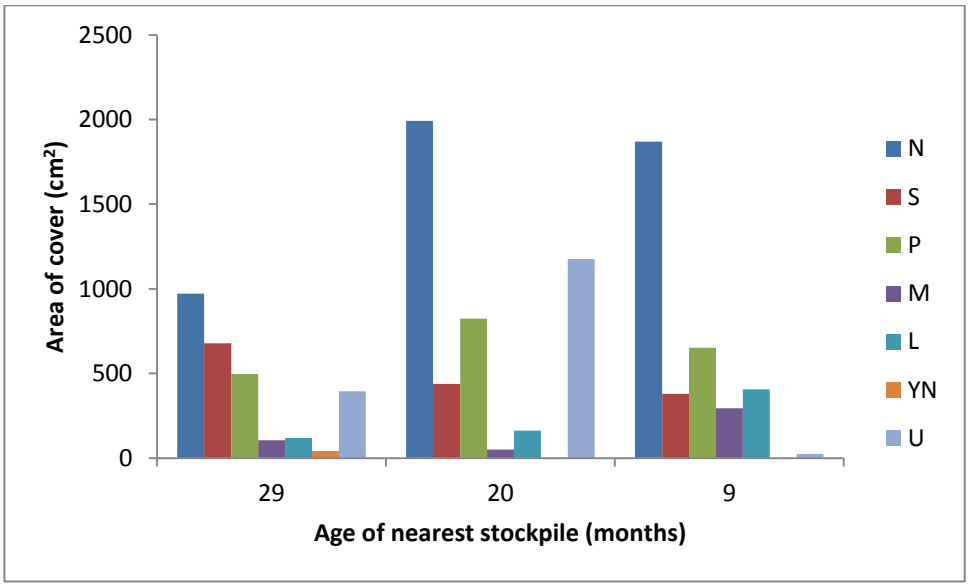

Figure 12 Total area of cover for all species in all undisturbed samples.

(N *Nostoc* cf *commune,* S *Scytonema,* P *Porphyrosiphon,* M *Microcoleus,* L *Leptolyngbya,* YN yellow *Nostoc,* U underdeveloped cyanobacteria)

Nostoc cf *commune, Nostoc* yellow, *Microcoleus* and *Leptolyngbya* were present in more stockpiled samples than undisturbed samples (Figure 13). Conversely, *Scytonema* and the black form of *Leptolyngbya* were more prevalent in undisturbed samples (Figure 14).

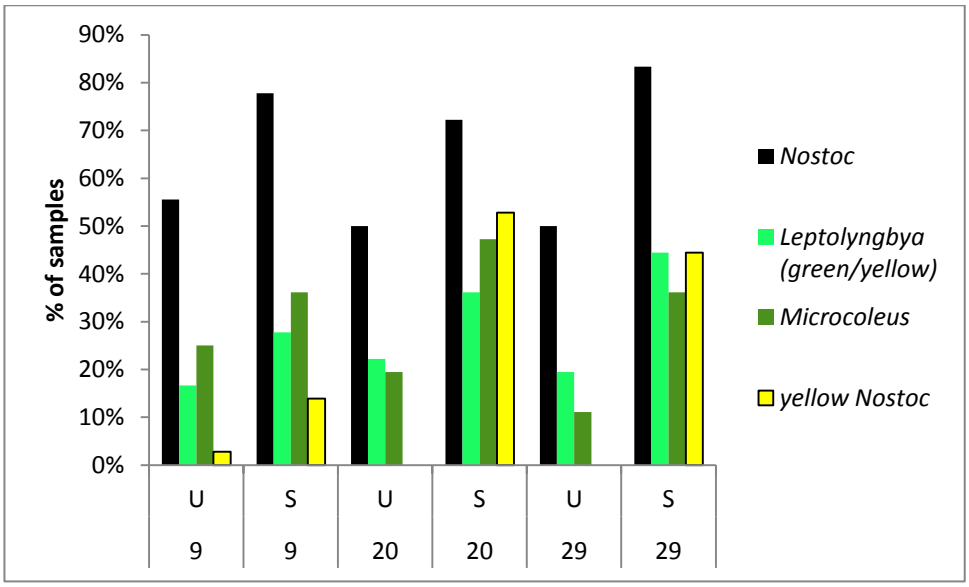

Figure 13 Morphotypes identified in a higher percentage of stockpiled samples (S) when compare with undisturbed samples (U) of varying ages (months)

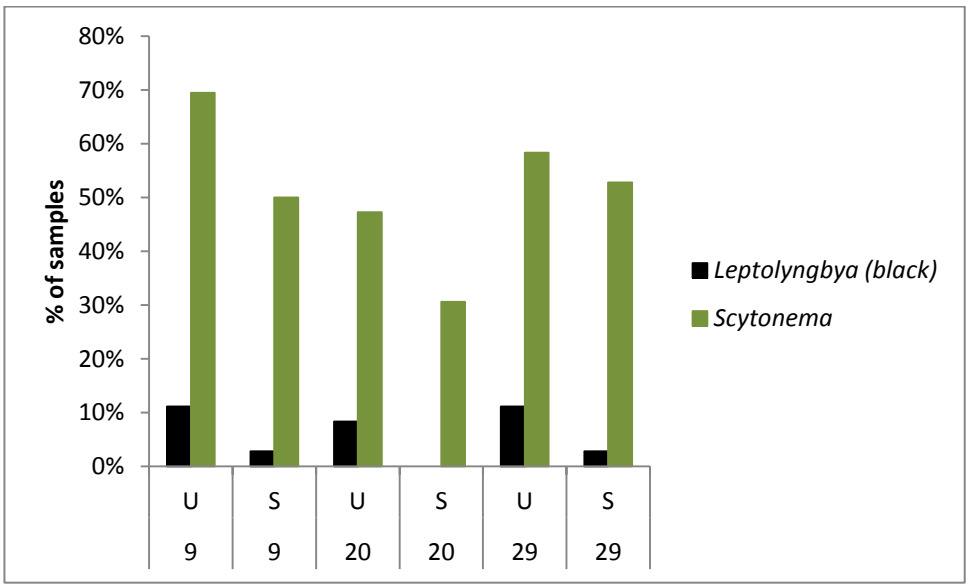

Figure 14 Morphotypes identified in a higher percentage of undisturbed samples (U) when compared to stockpiled samples (S) of varying ages (months)

When all samples were combined, *Nostoc* cf *commune* covered the greatest area in both stockpiled and undisturbed samples followed by yellow *Nostoc* in stockpiles only (Figure 15). The area covered by the remaining morphotypes was similar for most other morphotypes although *Microcoleus* and *Leptolyngbya* covered a greater area in stockpiled areas and *Porphyrosiphon* covered slightly higher area in undisturbed samples. The cover of *Scytonema* was almost identical in stockpiled and undisturbed areas and this lack of difference was supported statistically (DF17, F 0.00 P 0.969).

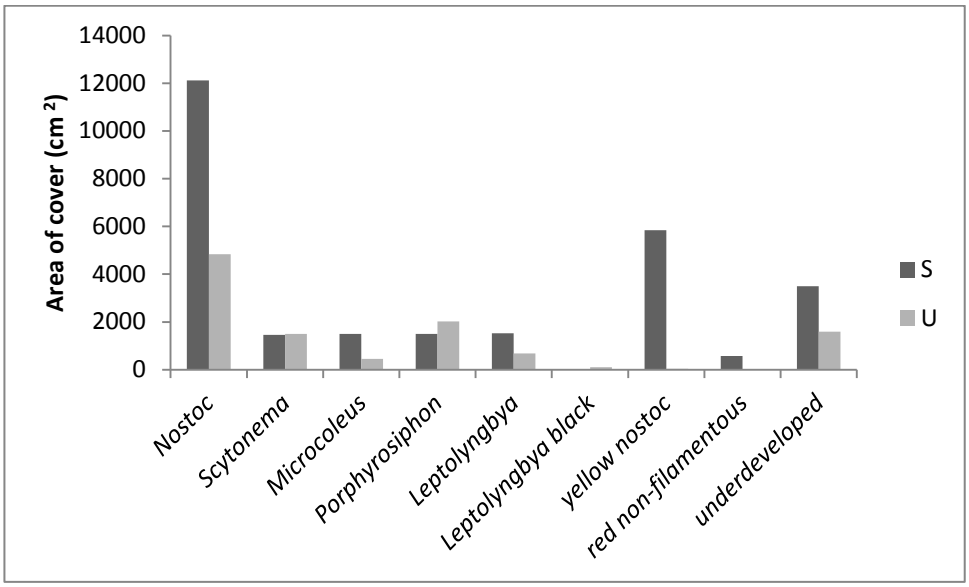

Figure 15 Total area of cover for each cyanobacterial morphotype in all stockpiled (S) and all undisturbed (U) samples

Contamination occurred in seven of the autoclaved controls. Only one morphologically distinct *Nostoc* morphotype grew in the control samples. The contamination source could be traced back to a single watering event. Fungal growth was only noted on control samples.

3.4 Growth rates

Nostoc cf *commune, Porphyrosiphon Microcoleus* and *Scytonema* were the first morphotypes to develop to an identifiable stage. Filaments of the *Stigonema* morphotype were found in low numbers and appeared to be recently formed. It was only present in samples examined in the latter stages of the identification process. The yellow form of *Nostoc* exhibited a much slower rate of development than *Nostoc* cf *commune* and could only be definitively determined as a form of *Nostoc* when examined after 13 weeks of incubation.

Samples from undisturbed areas sourced at depths from between 10 cm and 50 cm initially showed no visible signs of growth when examined after six weeks of incubation. When re-examined six weeks later cyanobacterial growth was evident but in many cases had not advanced to the point that most of the morphotypes could be distinguished. However, the few colonies that had grown to the point that morphotypes could be distinguished included each of the morphotypes that were present in the samples from the surface layers with the exception of the red non-filamentous organism, the black form of *Leptolyngbya* and the *Stigonema* morphotype. The results of the initial examinations were included in analyses to avoid skewing the data by including growth from a much later date.

4. DISCUSSION

4.1 Cyanobacterial diversity

Large scale sampling over a broad area of desert in China revealed what is considered a high diversity of cyanobacterial morphotypes from 17 genera (Zhang *et al.*, 2011). The small scale sampling in this study from a relatively small area performed selectively on only one soil type yielded morphotypes from at least seven genera, but this number may increase with time and molecular analyses. Diversity in cyanobacterial communities tends to be underestimated when using microscopy for identification (Schlesinger *et al.*, 2003, Taton *et al.*, 2003, Garcia-Pichel *et al.*, 2001), new taxa are yet to be described (Hu *et al.*, 2012) and revision of existing taxa is underway (Komárek & Anagnostidis, 2005b).

It is likely that the different coloured morphotypes should be grouped together. Colour variation occurs within cyanobacterial species and this can be influenced by age, nutrition (Sanmartín *et al.*, 2010) and even the other cyanobacteria growing nearby (de Chazal *et al.*, 1992). Some morphological diversity was noted in *Scytonema* but it was not pronounced enough to distinguish taxa. Either there is morphological diversity within one *Scytonema* morphotype or two morphotypes exist. Determination of taxa may be possible after longer periods of incubation. Considering the limited time of incubation, the small sample area of a single soil type and non-molecular identification process, cyanobacterial diversity at J-A is high. Further sampling across a range of soil types and the analysis of molecular analyses is needed to definitively determine the number of taxa present.

4.2 Survival at depths

Despite the recognised ability of cyanobacteria to migrate through the soil and survive long periods of desiccation, investigations of soil cyanobacteria to date have focussed on only the top few millimetres of soil (Hu *et al.*, 2003, Zhang *et al.*, 2011, Garcia-Pichel *et al.*, 2003). Similarly, few studies have been undertaken to determine the effect of burial on cyanobacterial activity. Effectively, burial occurs in stockpiling of topsoil material at J-A.

One study investigated burial of cyanobacterial crusts with sand to depths of 5 cm (Rao *et al.*, 2012). Chlorophyll α, chlorophyll fluorescence, carbohydrate content and crust thickness were measured over a 35 day period and reductions in each were interpreted as detrimental to BSC persistence (Rao *et al.*, 2012) but all of these features would naturally decline with reductions in photosynthesis. Thomas and Dougill (2007) conversely suggested buried cyanobacterial

crusts would regenerate and grow with greater thickness through the covering sand when adequate moisture and amenable conditions arose. Belnap and Eldridge (2003) reported that smaller cyanobacteria were killed with burial resulting in reduced crustal community diversity but this was not reflected in the current study.

The findings of this research show that cyanobacteria can survive burial through stockpiling. Responses to stockpiling varied between morphotypes with some showing greater survival than others but all cyanobacteria native to undisturbed areas were represented in the stockpiles. Survival extends to depths of half a metre (the extent of sampling in this study) and may well extend beyond this. Cyanobacteria were also found to exist naturally in the undisturbed soils of J-A at depths of 50 cm. Although this result is unusual, this may be due to a research focus on surface layers. Where researchers have sampled at greater depths, similar results to that of this study have been found.

4.3 Survival mechanisms

The majority of cyanobacteria identified from both the stockpiled and undisturbed samples were filamentous. Filamentous cyanobacteria can survive in extreme conditions due in part to the ability to produce four different cell types: vegetative trichomes; smaller hormogonia for short range movement and colonisation; nitrogen fixing heterocytes; akinetes that can survive conditions unsuitable for growth (Singh & Montgomery, 2011, Flores & Herrero, 2010). The trichomes of motile species (Belnap & Gardner, 1993, Garcia-Pichel & Pringault, 2001) and hormogonia of non-motile species (Singh & Montgomery, 2011, Flores & Herrero, 2010) are capable of movement through the soil in response to stimuli.

4.3.1. Akinetes

Akinetes are thick walled cells similar to spores that do not contain chlorophyll (Kaplan-Levy *et al.*, 2010) and exhibit reduced rates of metabolism when compared with vegetative material (Thiel & Wolk, 1983) . Akinetes can develop in response to decreasing light intensity (Kaplan-Levy *et al.*, 2010) as would be experienced in burial. In some *Nostoc* strains, entire filaments can develop into akinetes thereby creating large volumes of the resting cells (Kaplan-Levy *et al.*, 2010) that could act as a reservoir of growth material within the soil. Akinetes do not contain chlorophyll are desiccation resistant and can survive much longer periods than vegetative material but do not survive high temperatures (Kaplan-Levy *et al.*, 2010). They have been found in sediment cores and may survive at greater depths (Kaplan-Levy *et al.*, 2010).

Germination of akinetes in requires oxygen in some *Nostoc* but triggers such as light and temperature are necessary for other genera (Kaplan-Levy *et al.*, 2010). Where akinetes are buried at depths, conditions for growth would therefore only occur when they are uncovered as burial reduces infiltration of light, oxygen and soil has insulator properties that would keep temperatures low.

The genetic pathways to akinete and heterocyte formation are similar (Kaplan-Levy *et al.*, 2010) thus non-heterocyte forming species cannot produce akinetes. Only two morphotypes sampled at J-A were observed to produce heterocytes; *Scytonema* and *Nostoc* cf *commune.* The ability of *Scytonema* and *Nostoc* to withstand desiccation has been experimentally investigated with both maintaining high cell density following 12 months of drought and readily re-activating when watered (Tomaselli & Giovannetti, 1993).
Nostoc is one of the most widely distributed cyanobacteria on Earth (Dodds *et al.*, 1995). The *Nostoc* cf *commune* morphotype was spread across all depths in the stockpiled material in evidence of the ability of these cyanobacteria to survive stockpiling for periods of more than two years. The akinetes of *Nostoc* have the potential for aerial dispersal (Dodds *et al.*, 1995) and this ability to colonise by air may explain the greater relative distribution of the *Nostoc* cf *commune* morphotype in both stockpiled and undisturbed samples.

Scytonema responded less well to stockpiling as evident by reductions in cover with stockpile age. Although some species of *Scytonema* can produce akinetes (Tomaselli & Giovannetti, 1993), this does not necessarily translate to the ability of the morphotype found at J-A. *Scytonema* was noted as growing mainly at the surface by Campbell (1979) and this distribution was reflected in the current study however, this morphotype was observed growing at depths in undisturbed areas following longer periods of incubation.

4.3.2. Survival of vegetative material

It is not only akinetes that can survive desiccation (Billi & Potts, 2002). Due to the inability of the remaining morphotypes to produce akinetes, alternative methods of survival must have occurred in stockpiles and at depths in undisturbed areas. Although the majority of soil micro-organisms are concentrated at the surface of the soil, they do exist much deeper in the soil profile in lower numbers (Torsvik & Øvreås, 2008, Fujita & Nakahara, 2006, Reisser, 2007b, Esmarch, 1914). Viable cyanobacteria have been grown from samples of undisturbed soil to depths of 70 cm in the USA (Moore & Karrer, 1919) at depths of 18 cm in rice paddys in Japan (Fujita & Nakahara, 2006) and to depths of 50 cm in the UK (Esmarch, 1914). Cyanobacteria

have also been found to be associated with the root zone of rice crops in India, well below the soil surface (Prasanna *et al.*, 2009). Viable *Microcoleus,* and *Leptolyngbya* morphotypes survived and remained viable after up to three million years frozen in lake sediments in permafrost (Vishnivetskaya, 2009).

Vegetative *Nostoc commune* material remained viable after several decades of storage in desiccated form (Lipman, 1941, Bristol, 1919). Reactivation of vegetative material following 86 to 87 years of herbarium storage was successful but interestingly took between five and 10 months of incubation in culture before growth was observed (Lipman, 1941). Similarly, *Nostoc* in soil stored dry for up to 70 years took more than a year to develop (Bristol, 1919). These results suggest that the longer the period of inactivity, the longer time taken for reactivation to occur. Extended periods of desiccation in vegetative *Nostoc* material resulted in greater lengths of time to rehydration and resumption of metabolic activity but this time was reduced with subsequent drying for shorter periods followed by rewetting (Shaw *et al.*, 2003).

4.3.3. Heterotrophic growth

Heterotrophic growth is also possible for some cyanobacteria (Flores & 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, 2007b). *Nostoc* have the potential to grow at low light in caves and under ice (Dodds *et al.*, 1995) and even in darkness (Huang & Chow, 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.

4.4 Growth rates as an indicator of cyanobacterial survival mechanisms at J-A

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

The akinete producing morphotypes may have survived in this form, and germination of akinetes is known to occur more slowly than re-activated vegetative material (Kaplan-Levy *et al.*, 2010), but the rates of growth for each form have not been comparatively examined after long term inactivity. Similarly, no research is available on the ability, timing and effects of switching from heterotrophic to autotrophic growth. Further research on the ability of the J-A cyanobacterial morphotypes to grow heterotrophically and produce akinetes may shed further light on the survival mechanisms employed for each of the morphotypes when buried.

Cyanobacteria are known to be spread by wind and water. It is therefore possible that this spread could occur on a vertical basis at J-A as has been recorded in other areas (Reisser, 2007b). Smaller particles are more easily washed into the ground and can be spread by air on dust particles (Reisser, 2007b). The extent of this vertical spread by this manner at J-A would seemingly be restricted to depths of less than 10 cm as this is where cyanobacterial diversity was greatest in both stockpiled and undisturbed areas. Growth rates at these depths were similar to those at the surface in undisturbed areas suggesting recent photosynthetic activity of these cyanobacteria.

As the J-A site is in a semi-arid area with mobile sand dunes, it is possible that the cyanobacteria present in the samples sourced from the greatest depths in undisturbed areas are relicts from a time when the surface was at a different level. Sand deposition may have covered and rendered these organisms inactive over long periods, hence the longer period of incubation required for visible growth in comparison with samples sourced from closer to surface level.

4.5 Comparative growth rates of morphotypes

Numerous other researchers reported *Microcoleus* (Hu *et al.*, 2012, Belnap, 2006) was one of the first cyanobacteria to grow along with *Scytonema* and *Nostoc* (Pandey *et al.*, 2005). This was true for this study with the addition of *Porphyrosiphon* as an early appearing morphotype. In the case of *Microcoleus, t*his may be due to motility. On agar plates, *Microcoleus* trichomes can travel up to 500 times their own length in one day (Campbell, 1979) and in soil are capable of vertical upward movement at a rate of 5 mm every 24 hours where adequate moisture is available (Belnap & Eldridge, 2003). Water and light trigger motility in *Microcoleus* but movement in darkness also occurs (Campbell, 1979). This species leaves the sheath to migrate towards the light then, as drying occurs travels below the surface and excretes a new sheath for protection from desiccation (Hu *et al.*, 2012). In calcareous soils such as those of J-A, calcium carbonate can be incorporated into sheaths giving a white appearance when dry (Campbell, 1979).

A significant area of samples was covered with underdeveloped cyanobacteria that showed little cellular differentiation or colouration. This is likely evidence of slow forming cyanobacteria. Other researchers have commented on the need to follow the growth phases of developing cyanobacteria for some months before definitive identification can be reached as few distinguishing features could be gleaned from the early stages of development (Bristol, 1919).

A number of studies of undisturbed areas have shown that different cyanobacteria have different growth times with some species taking more than four months to develop (Pandey *et al.*, 2005). Bristol (1919) also found *Nostoc* morphotypes that took much longer to grow to a point at which they could be identified. Similar findings came from this study where a yellow form of *Nostoc* could only be identified as such in the latter stages of the study. The *Stigonema* morphotype was also found in low numbers due to a much slower growth rate. For this reason, further examination of samples is warranted over time to assess diversity between undisturbed and stockpiled samples in slower growing species. Samples will therefore be maintained and monitored, with photographic records kept to enable assessment of any changes in morphotype composition and diversity that occur over time.

Fungal growth was observed only on autoclaved control samples. Cyanobacteria can secrete compounds that repel fungi (Reisser, 2007b) and it appears a similar action occurred in this case. This repulsion can be due to competition for carbon that can occur when cyanobacteria are growing heterotrophically (Reisser, 2007b). Further investigation into the antifungal excretions of the cyanobacteria native to J-A could yield medically significant compounds and those with benefits for the food production industry.

4.6 BSC recovery

Generally, of all biological crust forming species, cyanobacteria are the most capable of recovery following disturbance (West, 1990, Johansen, 1993, Eldridge & Greene, 1994). Due to their motility, cyanobacteria can re-establish crusts even when completely removed, but this process can take decades (Belnap & Eldridge, 2003). Recovery of BSCs are fastest when material is disturbed but maintained and the timing of disturbance can effect survival; biocrusts recover more readily when disturbed in wet seasons when compared to recovery following disturbance when dry (Belnap & Harper, 1995). Motility, desiccation resistance and rapid reactivation are the characteristics that enable colonisation and survival of cyanobacteria in arid and semi-arid areas (Campbell, 1979) and these traits are present in J-A soil cyanobacteria.

Recovery of crusts is dependent on water availability and is reduced with high temperatures (Belnap & Eldridge, 2003). For this reason, the growth observed under incubation conditions in this study cannot be expected under field conditions in the semi-arid J-A environment without the addition of water. However, if restoration incudes some initial watering and is timed to coincide with seasonal availability of water, either through precipitation or fog, cyanobacterial growth may establish to the point that soil stabilisation occurs.

Further field based, larger scale experimentation is necessary to determine to what point stabilisation of soil can occur with the minimal application of water. Any further water application following the return of stockpiled stockpiles should increase the rate of BSC recovery. The preferential growth of cyanobacteria on fine substrates (Harper & Marble, 1988) is perhaps due to the ease of movement and growth under these conditions. The soils of J-A are fine in texture, and for this reason, rapid colonisation with cyanobacteria following soil return in the restoration process could reduce air pollution that can be harmful both to mine workers and the vegetation at the site.

4.7 Study limitations

The conditions provided during incubation were constant and may favour the growth of certain species. Different cyanobacterial species also have different growth rates with some taking more than four months to develop (Pandey *et al.*, 2005; Bristol, 1919). The timing at which the samples were examined in this study therefore gives only a snapshot of the growth at that instance under the conditions provided.

Although great care was taken to reduce the likelihood of cross contamination of samples, it is difficult to maintain sterility under field conditions particularly when handling soil. When considering that cyanobacteria can be transported by wind, there is the possibility that contamination of samples occurred. This was not expressed however, in the rates of observed growth that have occurred in each of the samples.

Based on the growth rates reported by previous researchers (Bristol, 1919, Lipman, 1941, Shaw *et al.*, 2003) a fragment of cyanobacteria from the surface would grow more rapidly than one that has been buried for several months or years. The observed time taken for the initial visible signs of growth to occur in the deepest samples would suggest no contamination has occurred here. When initially examined, growth was visible at depths of 50 cm in stockpiled samples but none was observed in samples from this depth in adjacent undisturbed areas. At the time that thalli, protective pigments, branching and other mature phase morphological

indicators were present in all stockpile samples, the initial phases of growth were just emerging in the deepest samples of the undisturbed areas. Similarly, the comparatively high numbers of organisms in samples from depths in stockpiles can only be explained by survival of these organisms when buried

5. FUTURE RESEARCH

The use of a sonic drill to source samples could greatly reduce sampling time and the likelihood of cross contamination during sampling. The University of Adelaide plan to utilise a sonic drill at the J-A site in the future and arrangements could be made to sample stockpiles at this time. Potential problems with this technique could arise in accessing stockpiles as the drill is truck mounted.

Cyanobacteria survived stockpiling to depths of 50 cm but topsoil stockpiles at J-A can reach heights of 2 m. Investigation of survival of cyanobacteria at the deepest part of each stockpile should be carried out as any differences in community composition there may influence rehabilitation efforts. Similarly, determining the effect of stockpiling on alternative soil types is necessary to test for any differences with substrate types and corresponding cyanobacteria community assemblages. Installation of the necessary meters could assess any penetration of light, heat and water into stockpiles.

In future sampling studies, material should be examined prior to incubation for the presence of vegetative material and akinetes. This will assist in identifying the survival strategies employed by each of the taxa. Experiments could also be conducted to determine the ability of organisms to grow heterotrophically when supplied with carbon and water in the presence of darkness.

Isolation and molecular characterisation of cyanobacteria with different morphologies could assist in defining generic boundaries and potentially identifying species that may be present in sequence libraries. This will give a more complete picture of the cyanobacterial community diversity at the J-A site and enable the production of identification keys for the use of staff and researchers.

6. CONCLUSIONS

The diversity of soil cyanobacteria at the J-A site appears to be relatively high. The results of this study show that all of the soil cyanobacteria from the J-A site can survive stockpiling for up to 29 months. Rates of coverage suggest that some morphotypes survive stockpiling better than others.

All morphotypes were also found at depths to 50 cm in undisturbed areas. Growth rates and knowledge of species survival mechanisms suggests that survival at depths occurs through desiccated vegetative material that may have been inactive for long periods but further work is required to determine this experimentally. It is possible that some species are capable of heterotrophic growth.

Growth rates also vary between morphotypes and it is likely that more morphotypes will develop with time. Samples will be maintained in incubation and monitored for further growth and any changes in species diversity and cover. Molecular examination of taxa could assist in definition of generic and specific boundaries.

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Appendix 15 Long-term watercourse monitoring activity report 2014 – 2015

ILUKA

Jacinth-Ambrosia

Long-term watercourse monitoring

Activity Report

2014 - 2015

TABLE OF CONTENTS

FIGURES

1 Introduction

The watercourses at Jacinth Ambrosia (J-A) are ephemeral in nature and only flow in response to heavy rainfall events. The development of these ephemeral channels is generally controlled by high magnitude, low frequency floods and to a lesser degree modified by smaller flow events. The highly localized nature of rainfall in the J-A region means that only a section of watercourse may experience flow following rainfall, with runoff being absorbed in the system before reaching downstream areas, sediment movements are therefore also patchy (Alluvium, 2013).

According to Interim Completion Criteria in the J-A Rehabilitation Management Plan, erosion rates of the rehabilitated land form must be comparable with upstream control sites. Given the irregularity of erosion and sedimentation in the J-A region, monitoring sites have been established (see Figure 1) to determine erosion patterns within pre-mining creeks systems. This information will enable an informed comparison of erosion rates within rehabilitation areas.

2 Method

Previous surface water studies completed at J-A (Alluvium, 2013, 2013) identified six River Styles© within the J-A catchment. Four of these styles occur within the pit; interdunal bank confined channel; interdunal bank confined gully; interdunal wandering and, valley fill. A monitoring site has been established for each of the styles to be rehabilitated, and within each of the vegetation associations i.e. myall/mallee woodland or myall woodland to ensure that erosion patterns consistent with each River Styles and vegetation type are investigated. The valley fill style is not monitored as it has no distinct creek bed and is not suitable for the monitoring methodology used. Monitoring sites for rehabilitated areas will be established upon reinstatement of the relevant pit areas.

The monitoring method used has been developed in line with recommendations from Alluvium Consulting (Alluvium, 2013). At each monitoring site the following activities are to be completed:

- Annual comparison of aerial imagery of study areas using a consistent resolution;
- On ground photo monitoring, with photos taken every two years or immediately after a flood event;
- A cross section survey to capture measurement of creek bed dimensions. Top and toes of each bank; channel invert and, any points where the gradient of the bank changes are recorded using GIS survey equipment;
- A 50m longitudinal survey profile of the centre line channel of the watercourse is established and GIS survey equipment used to record the topography every 5m or where there are obvious changes in topography of the channel centre line;
- A record of the location and height of the flood debris line / high water mark is taken;
- Erosion pins, with the exposed length painted, are installed on the bank to changes in surface;
- A cross-section survey measuring biological soil crust and vegetation growth on the bank and creek bed and,

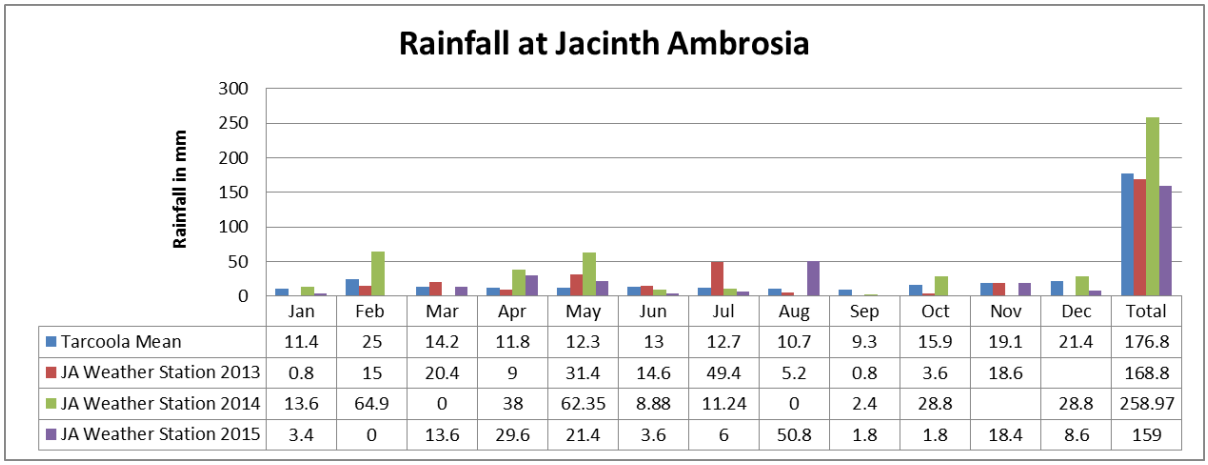
- Record of weed presence within the creek bed.

3 Preliminary Results

To date only one survey record has been captured for each of the six monitoring sites, aerial imagery is available for the last five years. The information for each monitoring site is provided below. A comparison of the aerial photographs from 2010 to 2014 does not reveal a great variation in each of the sites with the exception of LWM004 that appears to have had sediment build up over time.

Upon collection of more survey data it will be possible to determine topographic changes of the creek bed.

Rainfall from January 2013 to December 2015 is provided below:



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Tarcoola Mean	11.4	25	14.2	11.8	12.3	13	12.7	10.7	9.3	15.9	19.1	21.4	176.8
JA Weather Station 2013	0.8	15	20.4	9	31.4	14.6	49.4	5.2	0.8	3.6	18.6		168.8
JA Weather Station 2014	13.6	64.9	0	38	62.35	8.88	11.24	0	2.4	28.8		28.8	258.97
JA Weather Station 2015	3.4	0	13.6	29.6	21.4	3.6	6	50.8	1.8	1.8	18.4	8.6	159

Figure 1 J-A weather station rainfall (mm) from January 2013 to December 2015

3.1 LWM001

River Style: Interdunal bank confined gully
Vegetation Association: mallee woodland

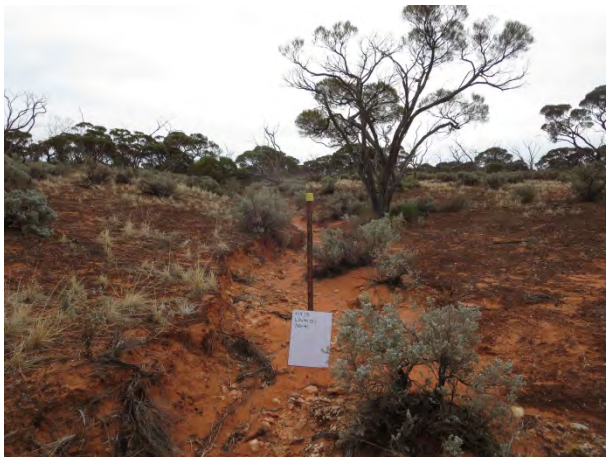


Figure 2 LWM001 photopoint facing north 2/5/2015

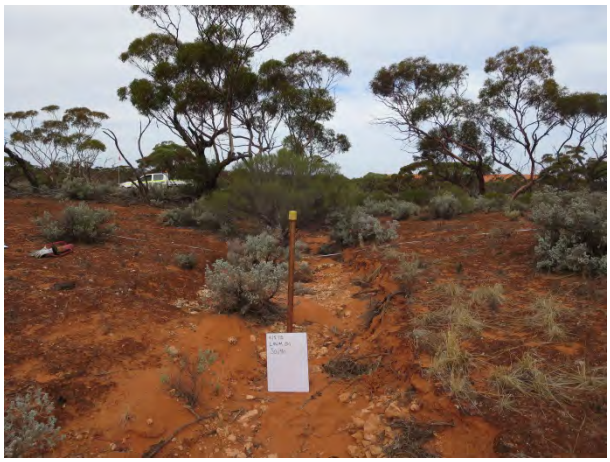
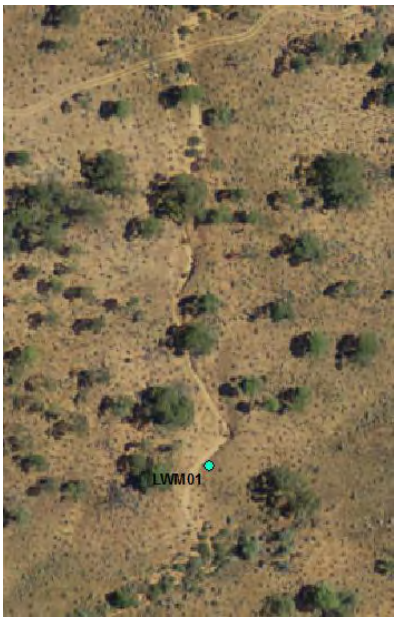


Figure 3 LWM001 photopoint facing south 2/5/2015

3.1.1 Aerial imagery comparison

2010

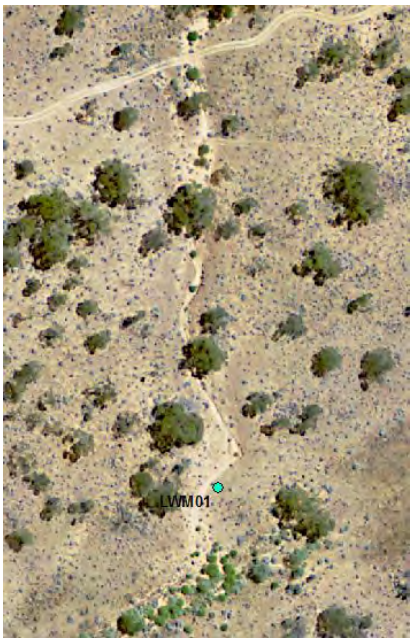
2011



2012

2013

2014



3.1.2 Cross-section profile

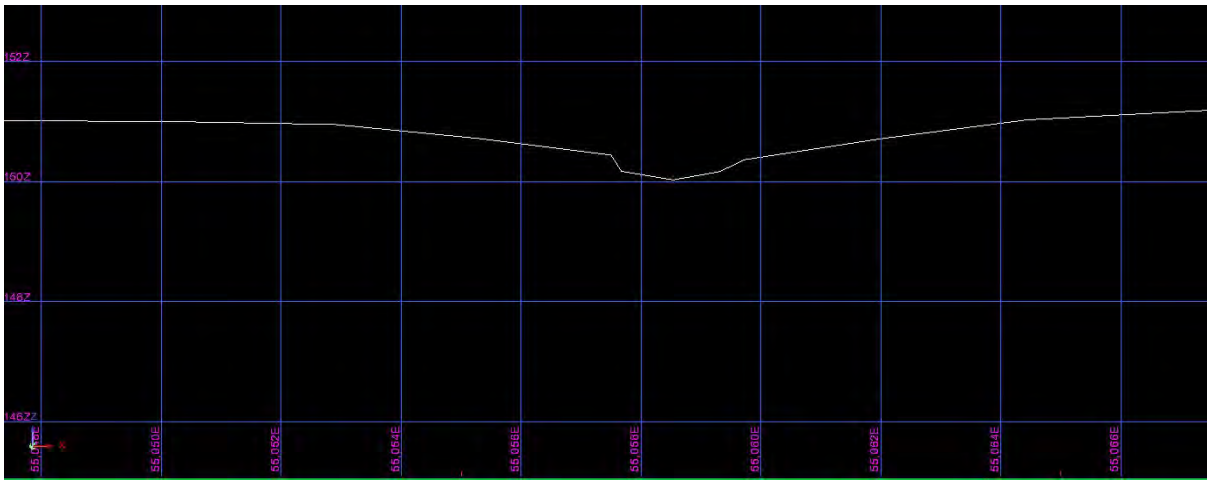


Figure 4 Cross section profile - site LWM001

3.1.3 Longitudinal profile

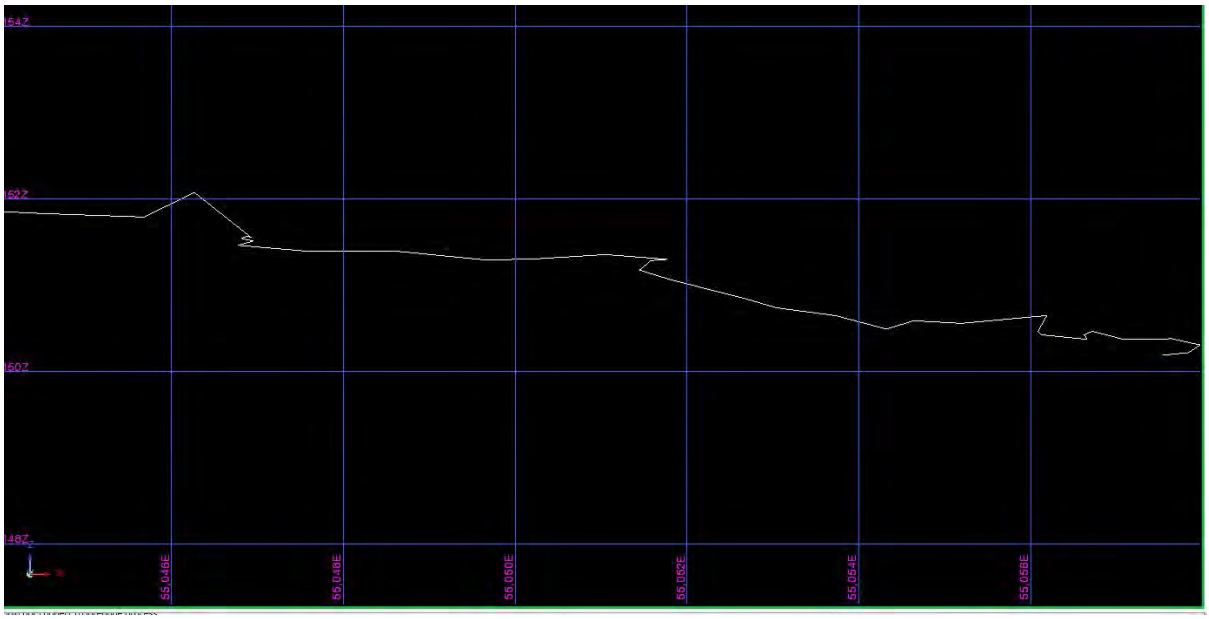


Figure 5 Longitudinal profile - site LWM001

3.1.4 Erosion pins

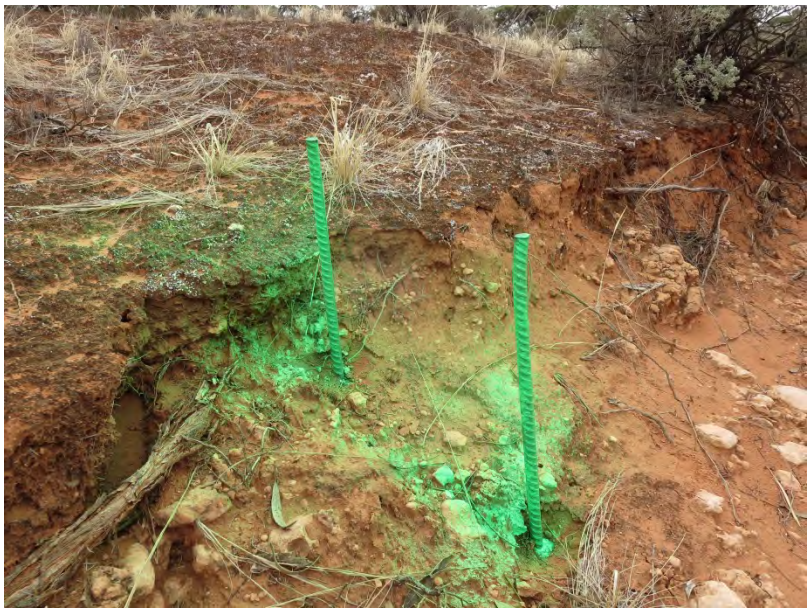


Figure 6 Erosion pins 2/5/2015

3.2 LWM002

River Style: Interdunal bank confined gully
Vegetation Association: myall/mallee woodland

Figure 7 LWM002 Photopoint facing west 25/11/2015

Figure 8 LWM002 Photopoint facing east 25/11/2015

3.2.1 Aerial imagery

2010

2011

2012

2013

2014

3.2.2 Cross-section profile

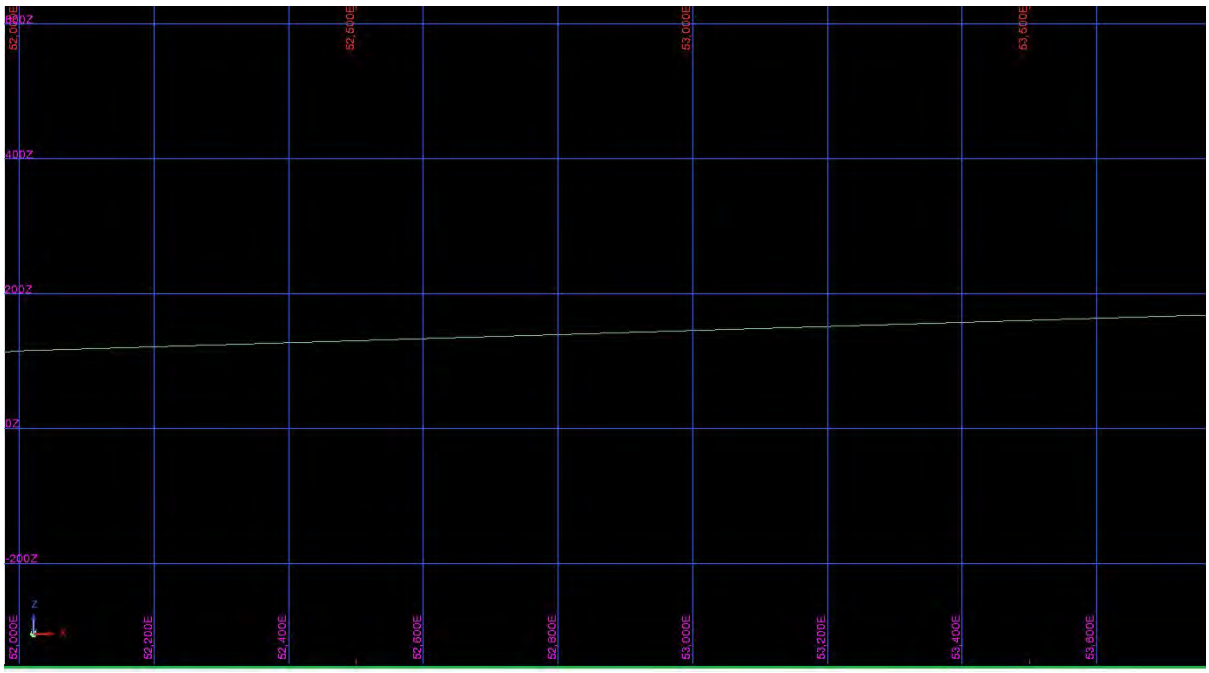


Figure 9 Cross-section profile - site LWM002

3.2.3 Longitudinal profile

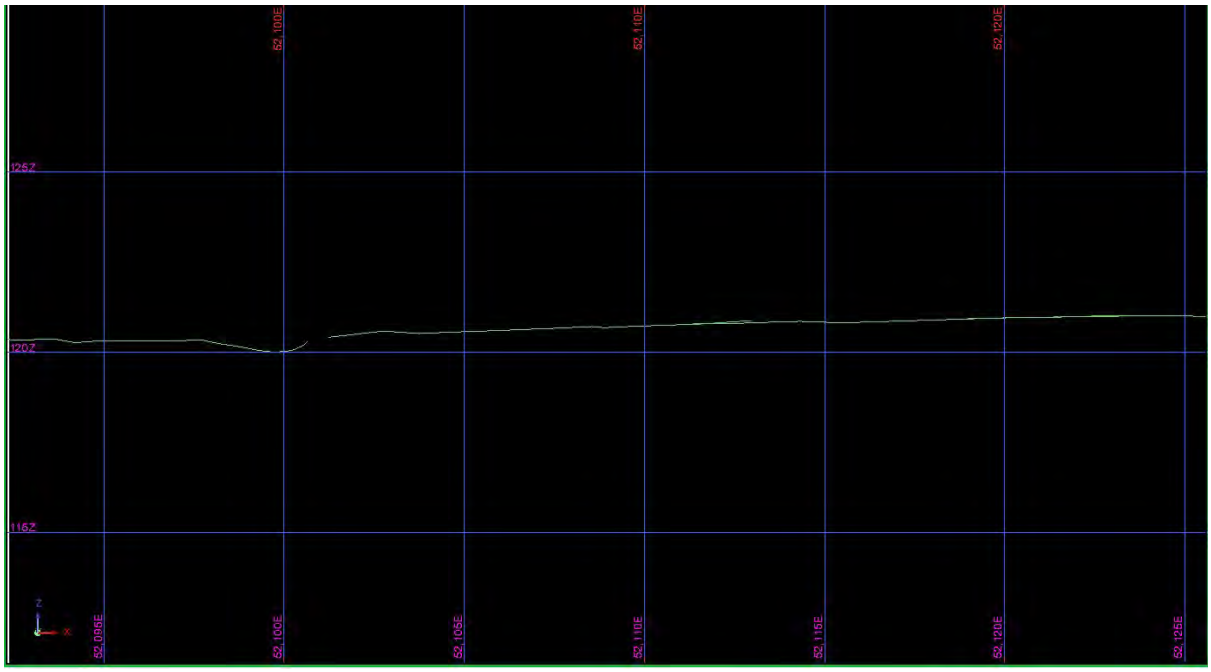


Figure 10 Longitudinal profile site - LWM002 25/11/2015

3.2.4 Erosion pins

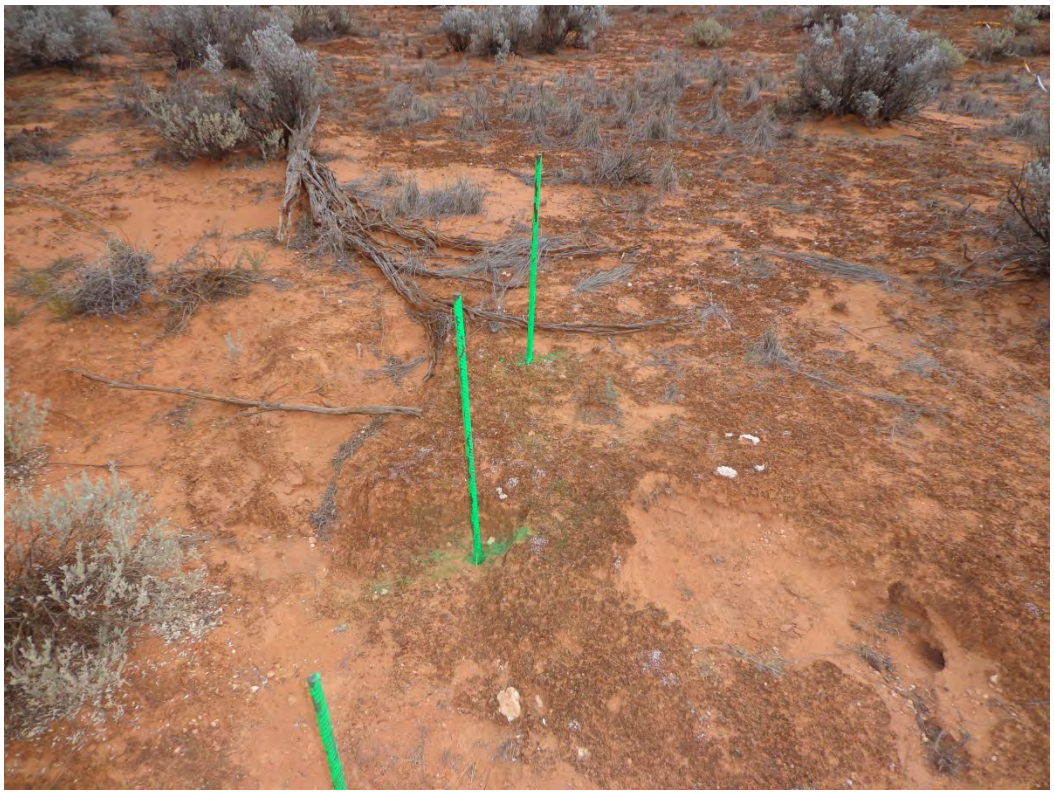


Figure 11 Erosion pins 25/11/2015

3.3 LWM003

River Style: Interdunal bank confined channel
Vegetation Association: mallee woodland

Figure 12 LWM003 photopoing facing west 26/11/2015

Figure 13 LWM003 photopoint facing east 26/11/2015

3.3.1 Aerial imagery

2010

2011

2012

2013

2014

3.3.2 Cross-section profile

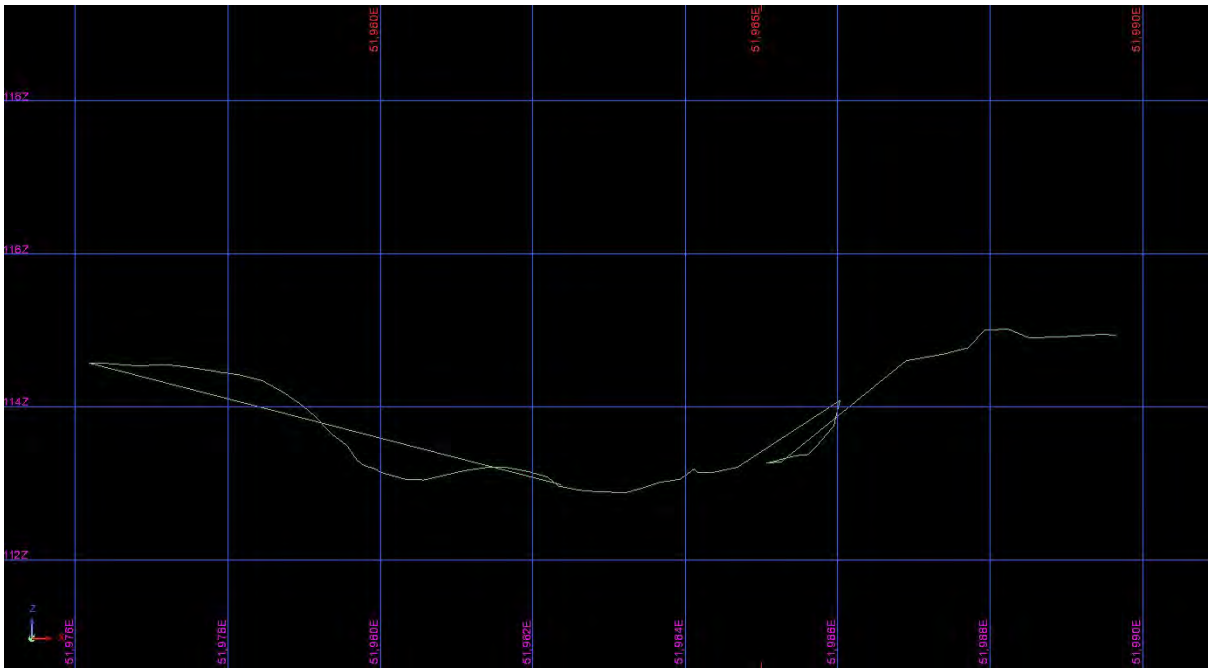


Figure 14 Cross-section profile - site LWM003 26/11/2015

3.3.3 Longitudinal profile

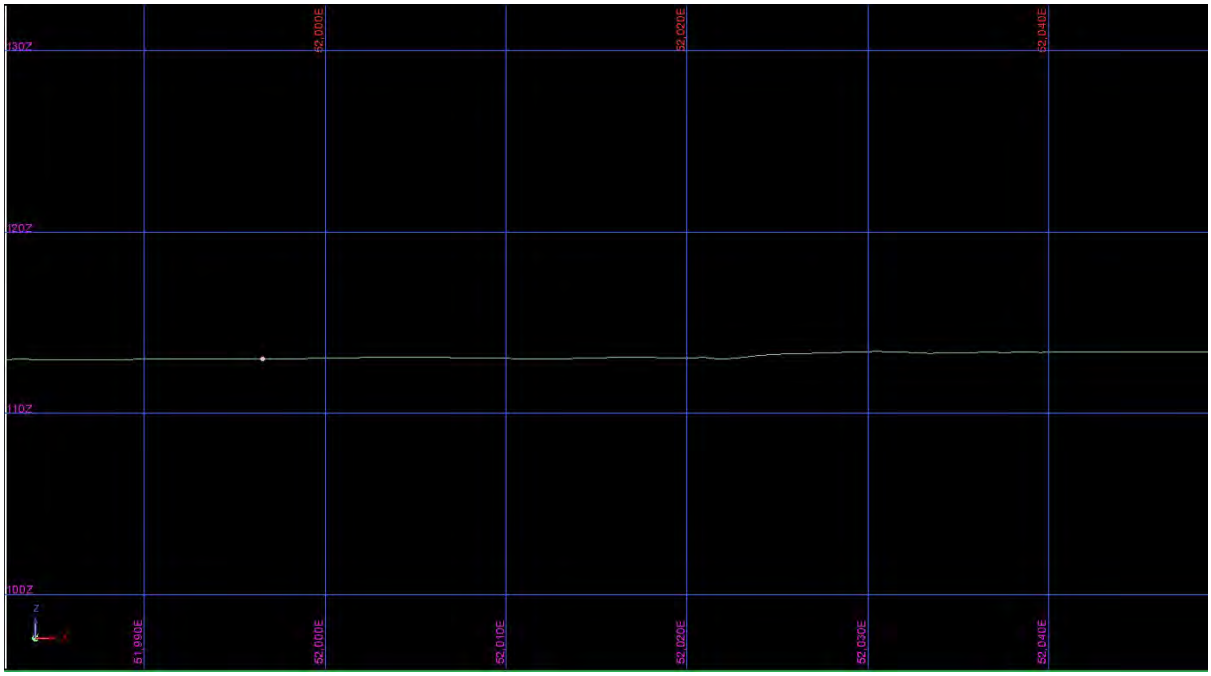


Figure 15 Longitudinal profile - site LWM003 26/11/2015

3.3.4 Erosion pins

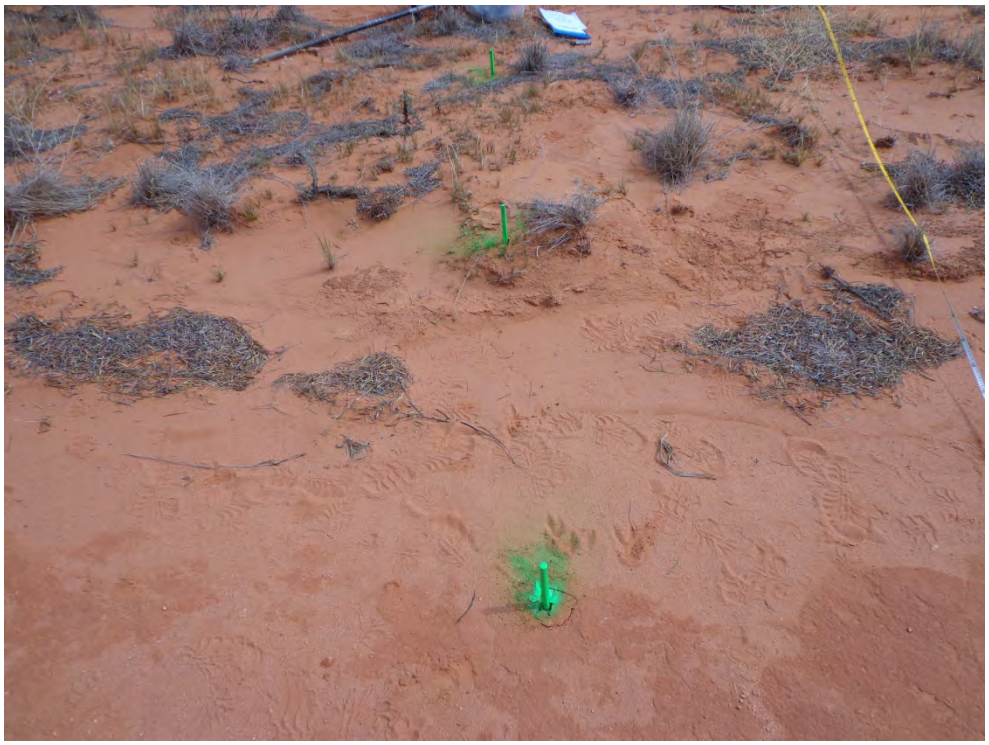


Figure 16 Erosion pins site LWM003 26/11/2015

3.4 LWM004

River Style: Interdunal wandering
Vegetation Association: mallee woodland

Figure 17 LWM004 photopoint facing east 26/11/2015

Figure 18 LWM004 photopoint facing west 26/11/2015

3.4.1 Aerial imagery

2010

2011

2012

2013

2014

3.4.2 Cross-section profile

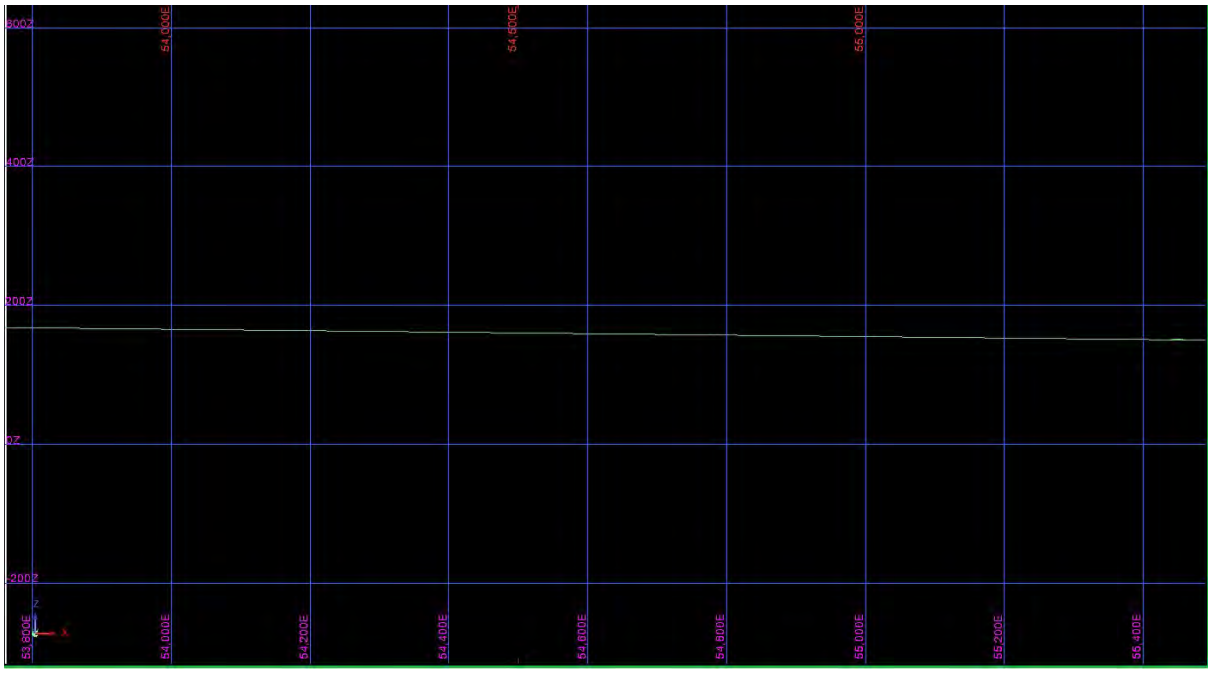


Figure 19 Cross-section profile - site LWM004 26/11/2015

3.4.3 Longitudinal profile

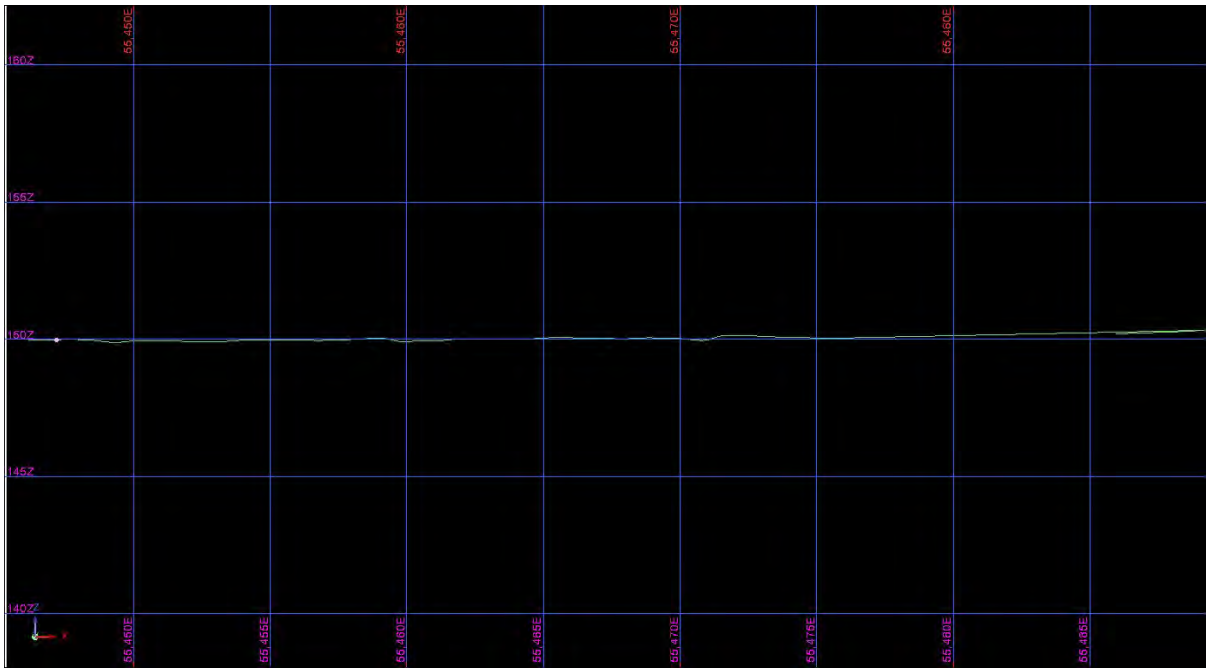


Figure 20 Longitudinal profile - site LWM004 26/11/2015

3.4.4 Erosion pins

Figure 21 Erosion pins site LWM004 26/11/2015

3.5 LWM005

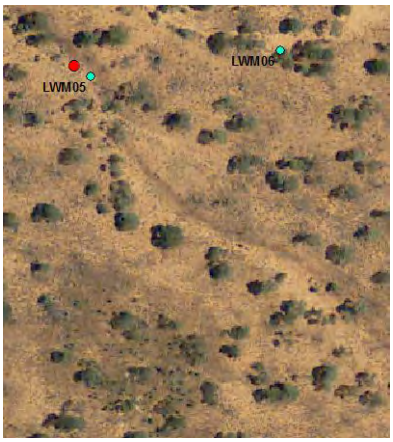
River Style: Interdunal bank confined channel
Vegetation Association: myall/mallee woodland

Figure 22 LWM005 photopoint facing east 27/11/2015

Figure 23 LWM005 photopoint facing west 27/11/2015

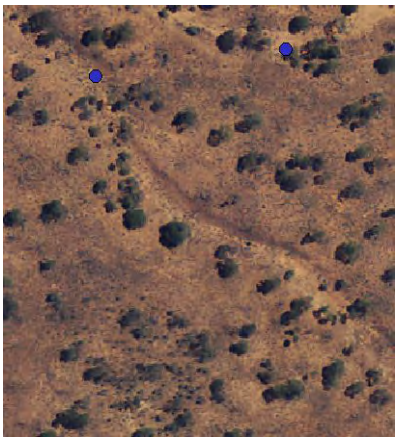
3.5.1 Aerial imagery

2010

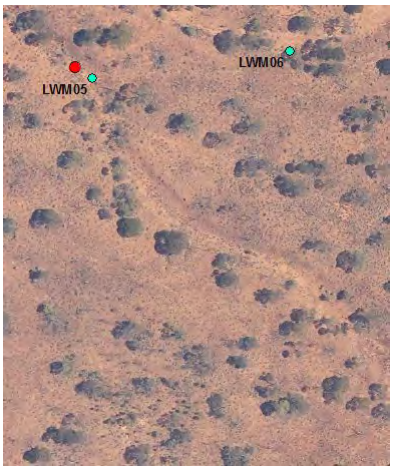


2011

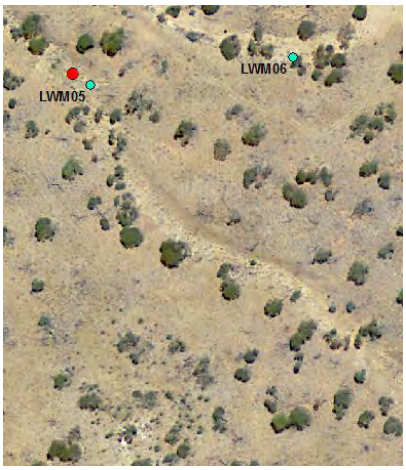
2012



2013



2014



3.5.2 Cross-section profile

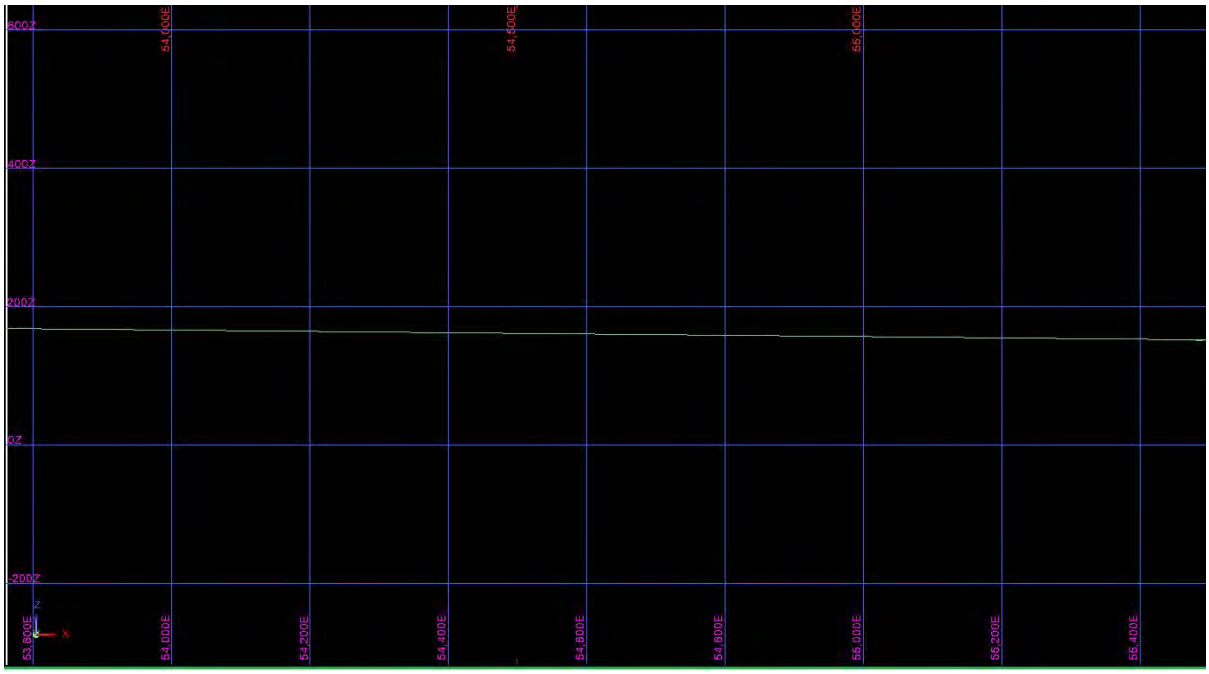


Figure 24 Cross-section profile - site LWM005 27/11/2015

3.5.3 Longitudinal profile

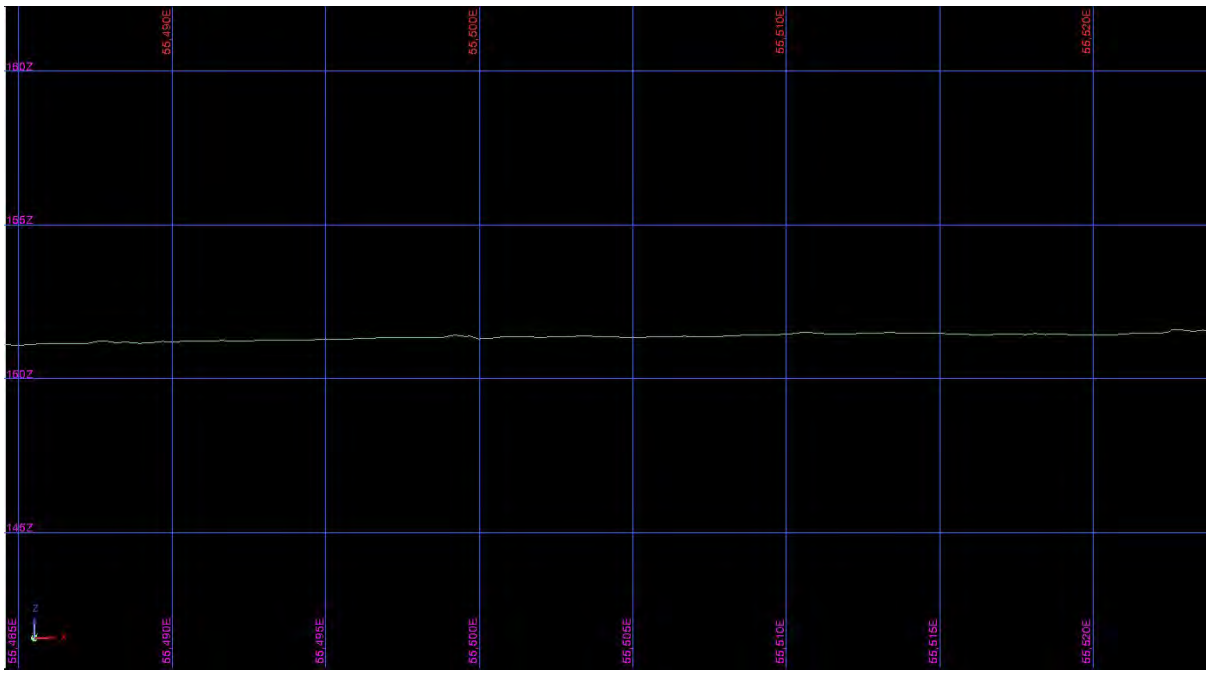


Figure 25 Longitudinal profile - site LWM005 27/11/2015

3.5.4 Erosion pins

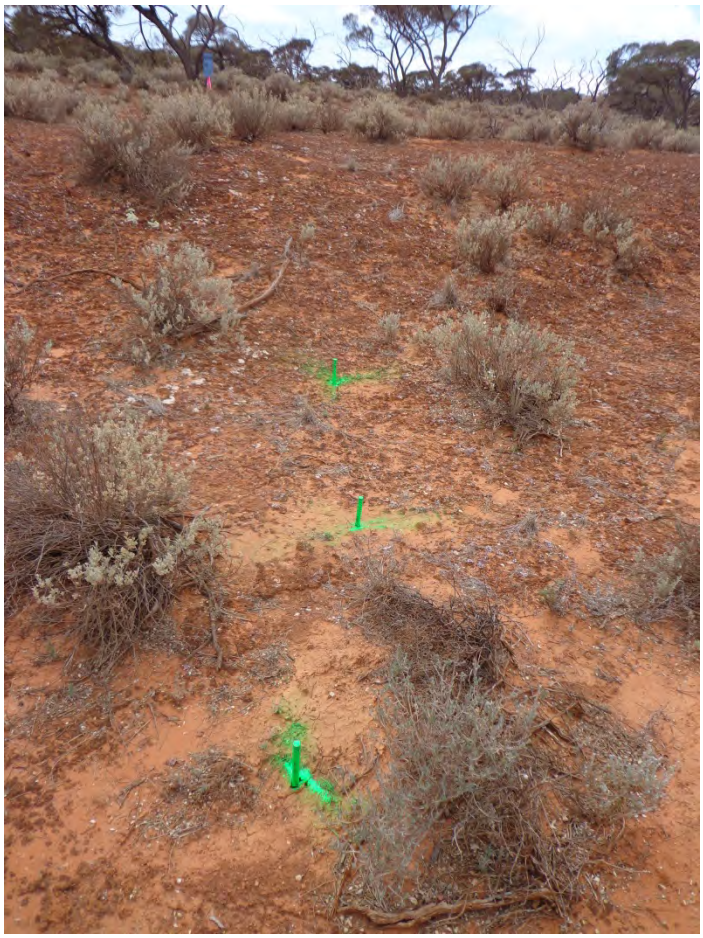


Figure 26 Erosion pins site LWM005 27/11/2015

3.6 LWM006

River Style: Interdunal wandering
Vegetation Association: myall/mallee woodland

Figure 27 LWM006 photopoint facing east 30/11/2015

Figure 28 LWM006 photopoint facing west 30/11/2015

3.6.1 Aerial imagery

2010

2011

2012

2013

2014

3.6.2 Cross-section profile

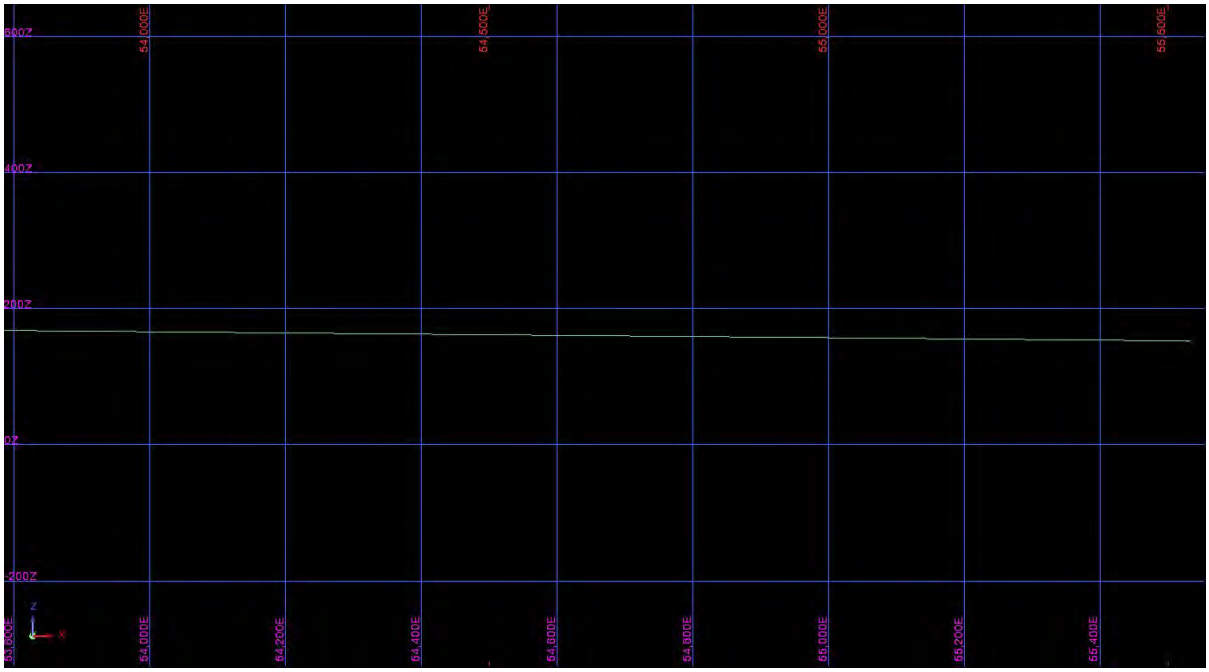


Figure 29 Cross-section profile - site LWM006 30/11/2015

3.6.3 Longitudinal profile

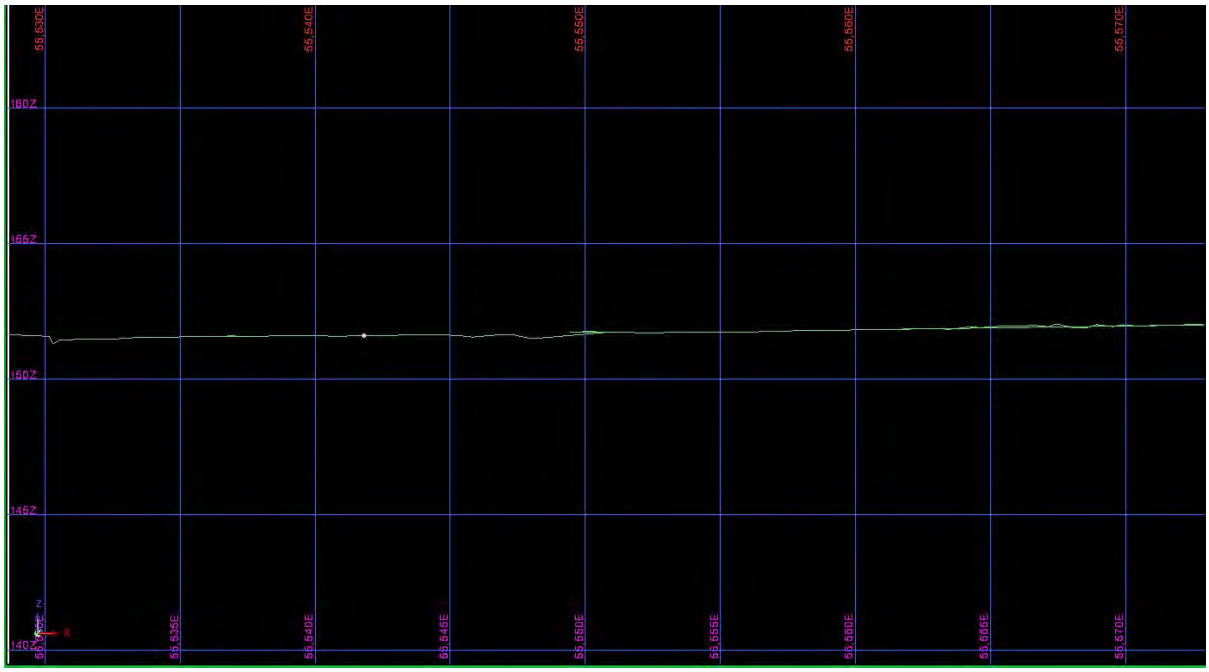


Figure 30 Longitudinal profile - site LWM006 30/11/2015

3.6.4 Erosion pins

Figure 31 Erosion pins site LWM006 30/11/2015

Appendix 16 Growth of tree seedlings in post mining reconstructed soils in an arid region of South Australia

Growth of tree seedlings in post-mining reconstructed soils in an arid region of South Australia.

Lucy Cunningham

Supervisors: A/Prof José M. Facelli and Dr Emma Steggles

A thesis submitted as a requirement for the Bachelor of Science (Honours)

The University of Adelaide

November 2014

SCHOOL OF EARTH AND ENVIRONMENTAL SCIENCES

THE UNIVERSITY of ADELAIDE

Declaration

The work presented in this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this thesis being made available for photocopying and loan.

……..

Lucy Cunningham

November 2014.

Acknowledgements

I would like to thank everyone that I worked with at the Iluka Jacinth Ambrosia Mine who accommodated me and welcomed me into their community. In particular, Kerry Saunders for assisting with the setup and watering of my seedlings and the rehabilitation specialists Joanne Lee and Tina Law for offering their expertise and support. I would like to thank my field assistants, Renate Faast and Lindy Scott who assisted me with my projects and shared their insights with me.

I especially thank my supervisors José Facelli and Emma Steggles for their complete support and advice throughout the year. José's knowledge of statistics and literature structuring has significantly helped me and guided me throughout the year. Emma's incredible knowledge, guidance and support were second to none, and she made the project a great experience and a lot of fun for me.

I am highly thankful to the Ranson Mortlock Trust for the scholarship which financially assisted me throughout the year.

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Lucy Cunningham

Honours Student, the University of Adelaide.

Table of Contents

Abstract

Expansions of the mining industry and public concerns about the associated environmental impacts have increased the need for ecological restoration. Mining causes long lasting detrimental impacts on the environment which can remain in the landscape for decades and centuries. Mining rehabilitation is highly challenging, particularly in arid regions where water and nutrients are limiting and species are slow growing. One of the ways of addressing these challenges is to obtain a greater understanding of ecosystem function through scientific research. Open cut mining can provide researchers with unique opportunities to better understand ecological processes through large-scale field trials and destructive sampling. Mining can severely modify soils by altering soil structure, causing a loss of nutrients and increasing salinity. For the rehabilitation process soils are stockpiled and returned to the mining pit overlying the waste tailings in reconstructed soil profiles. This study focuses on whether reconstructed soil profiles and tailings provide suitable substrates to support the emergence, growth and root development of three woody, deep rooted native species. The species at the focus of this study are *Acacia papyrocarpa* (Fabaceae), *Eucalyptus oleosa* (Myrtaceae) and *Eucalyptus gracilis* (Myrtaceae).

To assess the suitability of three post mining soil substrates (topsoil, subsoil and tailings) to support the emergence of the three study species a glasshouse experiment was undertaken. The two *Eucalyptus* species showed the same response with higher emergence in subsoil and tailings than topsoil. *Acacia papyrocarpa* differed in its response, with emergence highest in topsoil. A field trial was used to examine seedling establishment and growth response to different reconstructed soil profiles. The physiological variables measured (midday and pre-dawn water potentials and photosynthetic yields) did not distinguish any differences in plant performance between treatments. However, biomass for the two *Eucalyptus* species was lower in tailings than the reconstructed soil profiles. Leaf area showed an interactive effect of species and substrate on the eucalypt seedlings and this also showed lower biomass in the tailings for both species. *Acacia papyrocarpa* did not show any differences across the soil profiles for any of the measured variables in the field trial, likely caused by the short duration of the experiment (4 months) and the slow growing nature of the species. Thirdly, to investigate whether tailings were conductive of root growth and expansion, a glasshouse experiment was established. Three different soil treatments were constructed in 0.5 m deep profiles (two reconstructed profiles of topsoil, subsoil and brown sandy loam, topsoil subsoil and tailings and a profile of entirely tailings) and root biomass was quantified after four

months of growth. In the top 20 cm depths, roots for all species were highest in profiles which contained topsoil and subsoil and significantly lower in the tailings. Roots in the 20-50 cm depths did not differ in the varying profiles for *E. gracilis* and *A. papyrocarpa.* However, *E. oleosa* had lower root biomass in the 20-50 cm depth of the tailings profile compared to the reconstructed profiles. These findings show that whilst all analysed soil substrates are conductive to emergence, early stage growth and root development of the three species, seedling performance was overall reduced in tailings with the exception of eucalypt emergence.

Introduction

Mining is expanding in the arid and semi-arid regions of South Australia. Most studies on arid lands in the past focused on the impact of stock grazing, but little information exists on the impact of mineral exploration and extraction in these areas. The recent construction of a mine in a regional reserve in the far west of South Australia has increased the need for understanding how native plant species respond to severe landscape disturbance. Open cut mining activities require the removal of vegetation and soil from the landscape, resulting in erosion, dust pollution, soil compaction, land subsidence, and the production of high quantities of waste products (Miller 2005). This causes severe disruption of ecological processes such as the flow of nutrients and water through the landscape (Wassenaar *et al.* 2013). Increasing public pressures and government regulations now require mines to generate mine closure plans and rehabilitate areas, which often require the native ecosystem to be reinstated. This provides a great challenge as many ecosystem processes in these environments are poorly understood. These ecosystems are fragile, easily degraded by disturbance and often require long periods of time to recover. Arid and semiarid areas are characterised by high seasonal summer temperatures, soils with low nutrient levels that are often saline and low precipitation which is highly variable and unpredictable, making water the dominant controlling factor for biological processes (Noy-Meir 1973; Ludwig and Marsden 1995). Rehabilitation in arid and semi-arid areas is further challenged due to the slow growth of many of the ecologically important native plant species (Cortina *et al.* 2013).

The first challenge for restoration is the establishment of seedlings, which is the plant life cycle stage most susceptible to stress because seedlings have different and often more strict environmental thresholds than mature plants (Facelli 2008; Meloni *et al.* 2008). Ecological research can identify the fundamental requirements and limitations affecting an ecosystem

which can then direct management and lead to successful ecological rehabilitation outcomes. Understanding the physiological adaptations and tolerances at the seedling stage for a species can be used to identify what environmental limitations might prevent the establishment of the native species. The ability of a species to tolerate salinity, water limitation and nutrient limitation can help identify their suitability for rehabilitation projects.

Salinity can negatively affect plants by lowering soil water potential, making water less available to plants and by creating inorganic imbalances within cells forming nutritional deficiencies (Vitousek *et al.* 1982; Rhodes *et al.* 1986; Hu and Schmidhalter 2005; Facelli 2008; Hu *et al.* 2009; Asensio *et al.* 2013). In arid and semi-arid regions, salinity levels naturally change within the soil profile with seasonal moisture fluctuations relative to soil water content (Bui 2013). Evaporation of water from the soil surface in dry periods can generate an upward movement of salts through the soil profile through capillary movement, producing an accumulation of salts in the top layers of soil (Endo *et al.* 2012; Bui 2013). However, rainfall can flush salts down the profile through leaching as water infiltrates (Qi *et al.* 2002; Meloni *et al.* 2008). These processes are influenced by the compaction and structure of the soils. The use of heavy machinery in mine rehabilitation processes can detrimentally alter soil properties by destroying soil structure causing decreased porosity, increased compaction and increased soil impedance, which alters nutrient and water movement through the soil profile (Chong and Cowsert 1997; Salazar *et al.* 2009; DeLong *et al.* 2012). These are some of the major factors challenging rehabilitation of post mining areas.

The use of native vegetation species in mine rehabilitation is preferred as these species have survival mechanisms and tolerances suited to the prevailing climatic conditions (Maier and Mendez 2008). The overstorey vegetation species in arid regions contribute significantly to biodiversity and ecosystem function by creating different microclimates beneath their canopies. This occurs through processes such as decreasing the evaporative demand by shading and altering water, nutrient and soluble salt abundances in the soil through their root systems which may facilitate the establishment of other plant species (Facelli and Brock 2000; Jeddi *et al.* 2009). These species also provide a suite of resources and habitats for parasitic plants, birds and diverse invertebrate assemblages (Reid and Lange 1988; Reid 1989; Ireland and Andrew 1995; Dew and Schwartz 2013). Woody species further improve ecosystem processes through soil modification by building up the litter layer which increases soil organic carbon, soil nutrients, water infiltration and reduces water runoff (Jeddi *et al.* 2009; Cortina *et al.* 2013 and references therein). To use woody species in rehabilitation,

species must be capable of tolerating the prevailing conditions in the post mining environment which differ from their natural habitat (Grigg *et al.* 2010).

This study investigates whether three key native deep rooted species in an arid region of South Australia can establish and grow under the new ecosystem processes present in a post mining environment. This information will improve current understandings of future recruitment of seedlings in these environments. The Jacinth Ambrosia mine is an open cut mineral sands mine which uses hypersaline water sourced from a palaeochannel in the ore beneficiation process, making the waste product hypersaline (Goode and Doudle 2009). In the rehabilitation process, the mine pit is filled with a waste by-product referred to as ModCod (modified co-disposed sandy and clayey tailings) to a design level, followed by the placement of previously stockpiled substrates (including red sandy loam, brown sandy loam, a sandy capillary break material, subsoil and topsoil) returned in the order which they naturally occur. However, soil depths, soil structure, compaction and porosity are changed in reconstructed substrates compared to natural areas and consequently ecological processes may differ. For the rehabilitation at the site, soils are always reconstructed over ModCod. This research includes experiments which analyse seedling growth and response in treatments consisting entirely of ModCod without overlying soils to observe whether the altered soil characteristics and salinity impact on the plant-soil water relations of deep rooted species. This could provide an early indication of whether the altered characteristics may be problematic in the future when root development reaches these layers in the rehabilitation sites.

The objective of this project was to identify whether reconstructed soil profiles provide a suitable substrate to support the emergence, growth and root development of three species: Western Myall *Acacia. papyrocarpa* (Benth.), Red Mallee *Eucalyptus oleosa (*F. Muell) and Yorrell, *Eucalyptus gracilis* (F. Muell). This study aims to answer three research questions: Firstly, are topsoil, subsoil and ModCod suitable substrates to support seedling emergence for the three species? Secondly, can reconstructed soil profiles and ModCod support early seedling growth? Thirdly, are the modified soil substrates able to support root growth? To determine whether substrates were conductive to seedling emergence a glasshouse experiment was established. Treatments were chosen to best reflect reconstructed soil profiles at the mine and to test the effects of ModCod on plant emergence. To identify whether post mining reconstructed soils can support early seedling growth of the three species, seedlings were planted in an existing field trial consisting of multiple soil profiles reconstructed to

various depths. To gain a deeper understanding of the root development in the soil substrates, a glasshouse experiment was established using 0.5 m deep reconstructed soil profiles.

Methods

Site Description

The study site was located at the Jacinth Ambrosia (JA) mine site within Yellabinna Regional Reserve in the southwest of South Australia. This reserve which spans an area of 20, 000 km allows land management for conservation and permits the use of natural resources (Figure 1) (DEWNR 2013).

Figure 1: Map of Yellabinna Regional Reserve (yellow shaded area) and the Jacinth-Ambrosia mine (red rectangle). Image modified from DEWNR (2013).

Climate

The climate is considered semi-arid to arid with hot summers and mild to cool winters (Copley and Kemper 1992). Long term weather data can be inferred from the closest Bureau of Meteorology site located at Tarcoola, 220km east of the site (Figure 2). The mean monthly maximum and minimum temperatures from 1997-2014 were 37.0°C and 19.9°C in January

and 18.8°C and 4.7°C in July (BOM 2014). Large rainfall events occur in summer during La Niña years (reflected in the high average February rainfall) and can cause flooding (Figure 2) (Puckridge *et al.* 2000). An onsite weather station provided daily maximum rainfall and minimum and maximum temperatures for the four month period which the experiment was undertaken (Figure 3).

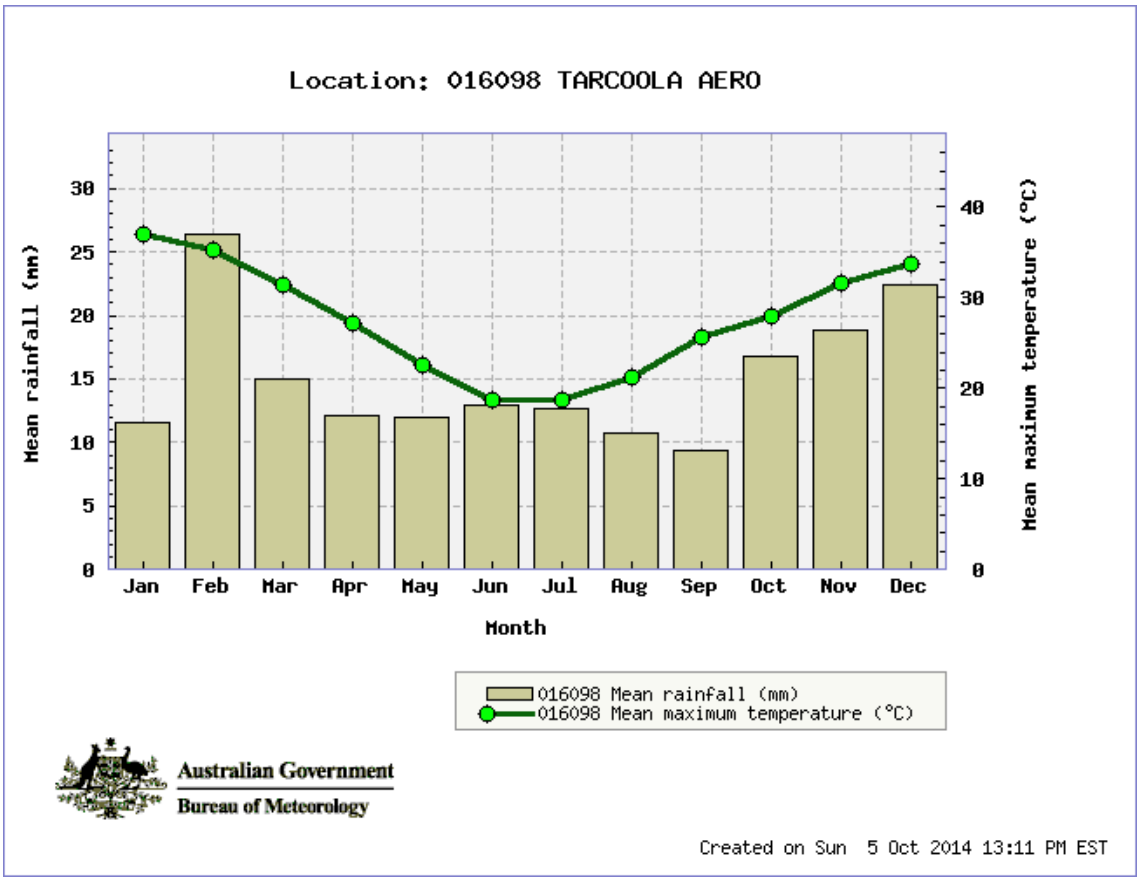

Figure 2: Mean monthly rainfall and maximum temperature data at Tarcoola weather station (BOM 2014).

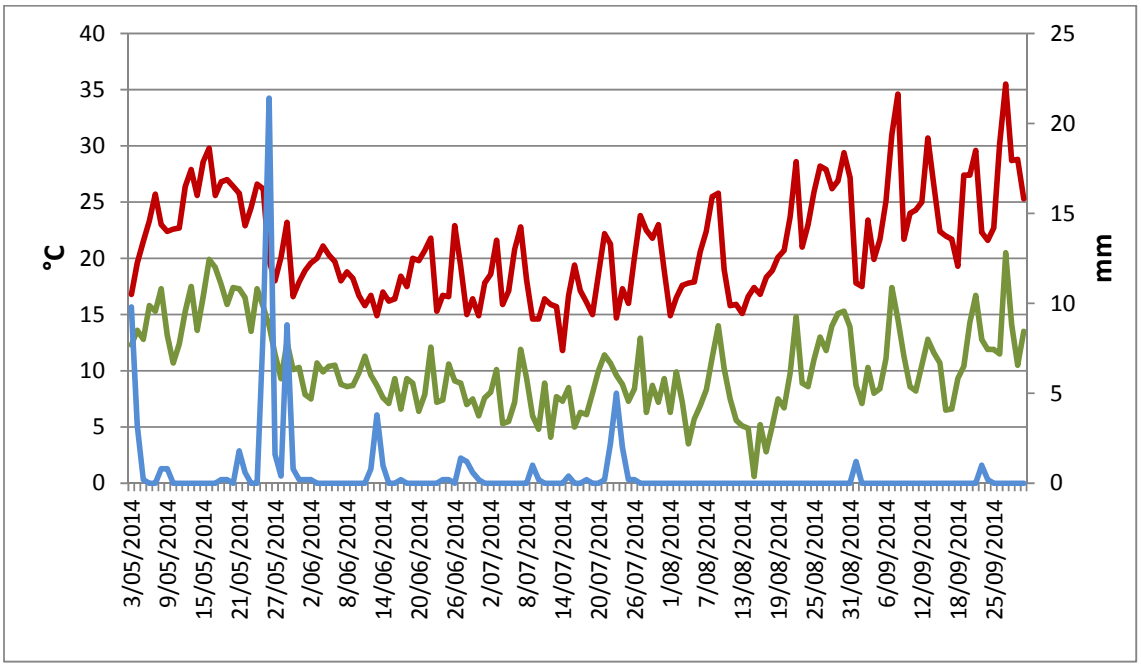

Figure 3: Daily maximum temperatures (red), daily minimum temperatures (green) and daily maximum rainfall (blue) over the four month period from March to September 2014.

Soils

During pit construction, soils are collected in categorical types and stockpiled separately. The ModCod tailings are stored in a designated tailings storage facility or directly returned to the pit floor. For the rehabilitation process, soil layers are returned to the pit overlying the ModCod to recreate the original topography and soil profile layers in the sequence which they naturally occurred (Appendix A). Soil horizons in undisturbed areas vary in depth, but generally the term topsoil refers to the soil between 0 and 5 cm depths and subsoil refers to the soil between 5 and 15 cm depths. Topsoil and subsoil are the layers which seeds are naturally stored in, and support initial seedling germination, emergence and establishment. Brown sandy loam spans depths 15 cm to 3 m. This study focuses on the top 0.5 m depths due to time limitations and therefore these are the soils at the focus of this study. Their properties are summarised in Table 1.

Table 1: Soil properties of the target soils occurring at JA. Sourced from Goode and Doudle (2009).

Soil types	Properties		
	Physical	Chemical	Hydrological
Topsoil	Poorly developed Non dispersive	Low organic matter Low nitrogen and phosphorus. Alkaline Low salinity	High water holding content
Subsoil	Poorly developed Non dispersive	Low organic matter Low nitrogen and phosphorus. Alkaline Low salinity	High water holding content
Brown Sandy Loam	Weak structure Low-moderate bulk density, low soil strength High fine sand and coarse silt content Poor macro-structural stability Very low hard setting potential Non dispersive	Low nutrient retention capacity Strongly alkaline Very highly saline	Considerable macro and meso-porosity (> 30 µm pores) High saturated hydraulic conductivity High water holding capacity
ModCod	Very weak structure Moderate-high bulk density	Low organic matter Low nitrogen and phosphorus Alkaline saline	Free draining

Vegetation

The vegetation consists of a chenopod shrubland with an open overstorey of slow growing, long lived species. The area has no recent history of stock grazing and is relatively undisturbed with the exception of grazing from introduced species such as rabbits. The dominant overstorey species in the area is *A. papyrocarpa* which has a scattered distribution forming an open woodland (Figure 4a). A second overstorey species, *E. oleosa*, has a range restricted to sandy rises and creeklines in the area (Figure 4b). Both species contribute greatly to the regions biodiversity and the heterogeneity of the system. The canopies of *A. papyrocarpa* have been found to alter the micro-environmental conditions of the soil beneath, distinctly effecting plant community structure (Facelli and Brock 2000). Furthermore, vegetation assemblages associated beneath *E. oleosa* have been found to contain the highest species richness within the area (Goode and Doudle 2009). Several shrub and grass species including *Enchylaena tomentosa* and *Rhagodia* species have been observed to have distributions exclusively in below canopy environments (Facelli and Brock 2000).

One of the adaptive strataegies used by species to survive in this environment is hydraulic redistribution, which reffers to the movement of water through the root systems, not only upwards through hydraulic lift for plant transpiration, but also downwards and laterally where it can be stored and utilised during dry periods (Yu *et al.* 2013). Recent studies have shown this strategy is used by *A. papyrocarpa* which suggests the establishment of deep root systems may be highly important in the survival of this species during dry periods (Steggles *et al*. unpublished data). Root specimens of both species have been recovered in the floor of the mine pit and consequently they are known to extend to below 20 m^{+} deep in the area (pers. Com. E. Steggles 2014).

The third species, *E. gracilis* naturally occurs at the margins of a saline lake (Lake Ifould) located 6 km from the mine (Goode and Doudle 2009). This species is capable of surviving and growing in saline, alkaline, and bicarbonate conditions, and has been identified as ideal for the reclamation and rehabilitation of alkaline landscapes (Bell *et al.* 1993b; James *et al.* 2002). *Eucalyptus gracilis* is included in the study to identify whether it can tolerate growth in post mining conditions, and if necessary, act as an alternative species to *E. oleosa* which is known to be less salt tolerant (Doudle and Schneemilch 2012; Guerin and Ainsley 2013)

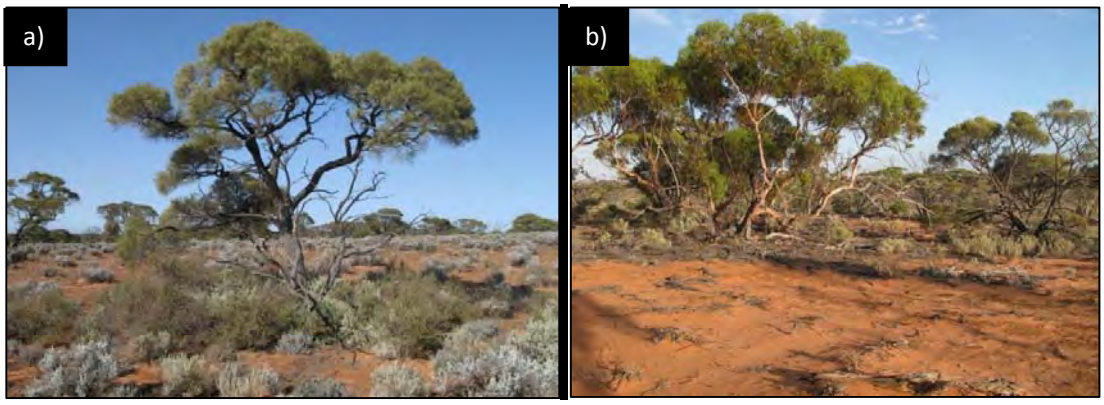

Figure 4: The vegetation types within the study area. a) A chenopod shrubland with an open overstorey of *A. papyrocarpa*. b) A sandy rise with *E. oleosa* and *A. papyrocarpa* overstorey and chenopod understorey.

Seedling emergence in post mining substrates – Glasshouse experiment

A glasshouse experiment was established to gain information on the germination and emergence success of the three species in the post mining soil substrates. Seeds used in this experiment were collected from JA in 2010 and had been stored in a dark, controlled environment room at a temperature of 15 ± 2°C and a relative humidity of 15% (as per South Australian Seed Centre seed storage protocols). Soils were collected from topsoil and subsoil stockpiles and the tailings storage facility at the mine and transported to a research glasshouse in Adelaide. Three sub-samples were collected for each soil type for baseline pH and electrical conductivity ($EC_{1:5}$) measurements. Eighteen trays (10 cm (l) x 5 cm (w) x 4 cm (d)) were filled with topsoil, subsoil or ModCod (Figure 5). Six trays of each soil type were then allocated to each of the three species and ten seeds were planted per tray. Seeds of *A. papyrocarpa* were pre-treated prior to sowing by nicking the seed coat with a scalpel to break physical dormancy. *E. oleosa* and *E. gracilis* seeds were sown directly into the soil with no prior treatment. Trays were watered twice daily with 15 minutes of mist through an automated watering system and moved around regularly to avoid position effects. Seedling emergence was recorded every two-to-three days between June and August, for a total period of 58 days.

Data was analysed using GraphPad PRISM V6 software. As the general patterns over time were similar for all combinations of soils and species, only final emergence was analysed. The data was transformed by the square root of the number of emerged seedlings +1, then analysed using ANOVA. The two eucalypt species were analysed together in a two way

ANOVA as they had similar and comparable biology, whilst the physiology of *A. papyrocarpa* differs and was analysed individually in one way ANOVA. Tukeys post hoc tests were used as required to identify differences. Significance was set at $P<0.05$.

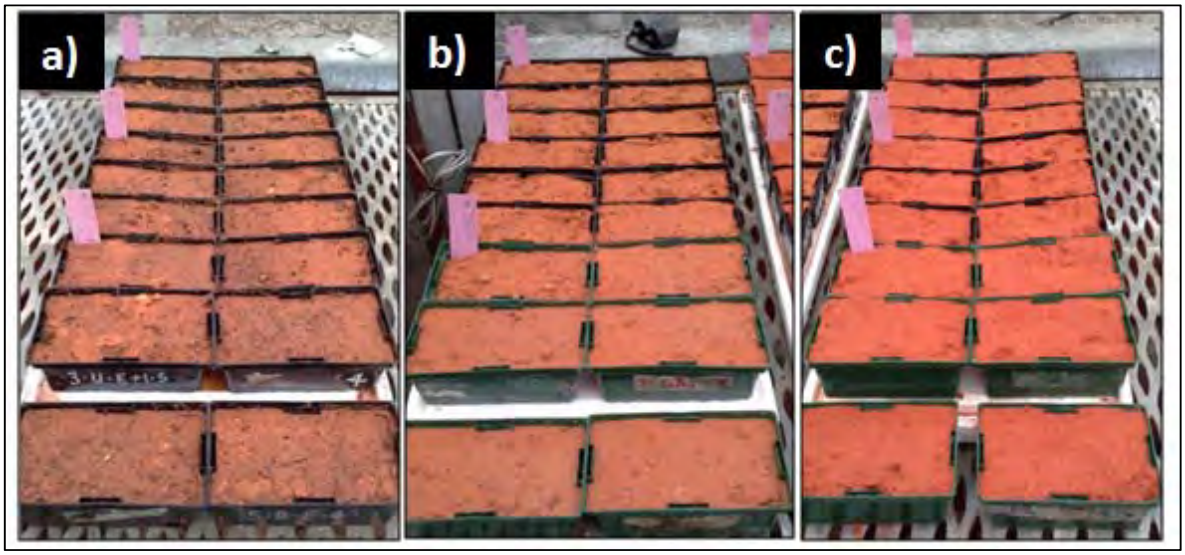

Figure 5: The seedling emergence experiment undertaken in the Adelaide Botanical Gardens glasshouse. Trays filled with a) topsoil, b) subsoil, and c) ModCod.

Seedling establishment in reconstructed soil substrates – Field trial

A previously established field trial was used to gain information on the growth and establishment of seedlings in reconstructed soil substrates. The trial was constructed with trenches (84 m long x 9 m wide) dug in an area of the mine pit that had been backfilled with ModCod tailings, then trenches were filled with different soil treatments. Soils were retrieved from stockpiles and reassembled in sequential layers which reflect pre-disturbance. These layers have reduced artificial depths and soil thicknesses which allow plant roots come into contact with different soil layers in a shorter timeframe, allowing plant growth responses to be observed earlier. Five different substrate treatments were constructed using varying depths of topsoil, subsoil, brown sandy loam, red sandy loam, a capillary break coarse sand material and ModCod (Figure 6). Each of the reconstructed profile strips was divided into seven 9 x 12 m plots and one plot across each was allocated for this study. Each profile was constructed twice, creating a complete block design consisting of two blocks with nested replicates. A profile consisting entirely of ModCod was included to identify whether the different soil properties alter the plant-soil-water relations of establishing seedlings and whether this impacted on their growth. This was expected to provide results on the effect of this substrate

on the seedlings in a short timeframe as roots could immediately come in contact with ModCod. Due to the short term nature of an Honours project, this study focused on early stage seedling growth and soils within the first 0.5 m depths. The trial is completely fenced with rabbit and kangaroo-proof wire to exclude grazing.

A research glasshouse in Adelaide was used to propagate 170 seedlings of each of the three species in nursery bags filled with topsoil collected from a stockpile at JA. Seeds had been collected and stored as per glasshouse experiment methods. Seeds for *E. oleosa* and *E. gracilis* were directly sown into nursery bags, whilst *A. papyrocarpa* seeds were pre-treated prior to sowing by nicking the seed coat with a scalpel to break the physical dormancy. However, using this method, the emergence success of *A. papyrocarpa* was low (< 20%). To increase success rates, additional seeds were nicked using a scalpel and placed into 70 mm Petri dishes with two Whatman™ filter papers and 30ml sterile water. Petri dishes containing 20-50 seeds were sealed with plastic wrap and placed in a growth cabinet with a 12 h light period and maintained at temperatures of 15 degrees for 5-7 days prior to planting into nursery bags. This significantly improved the success rate and the method was repeated multiple times until the required numbers of seedlings were established. All nursery bags received watering for 15 minutes twice a day delivered via an automated mist watering system. Seedlings were thinned to one plant per nursery bag after 5 weeks of growth to reduce competition. Each nursery bag was fertilised after 6 weeks with 50mL of diluted Seasol® (30 ml in 9 L) and four to six granules of Osmocote® native gardens slow release fertiliser. The application of fertiliser was consistent across all seedlings. After 13 weeks of growth, seedlings were transported to JA for planting in the field trial.

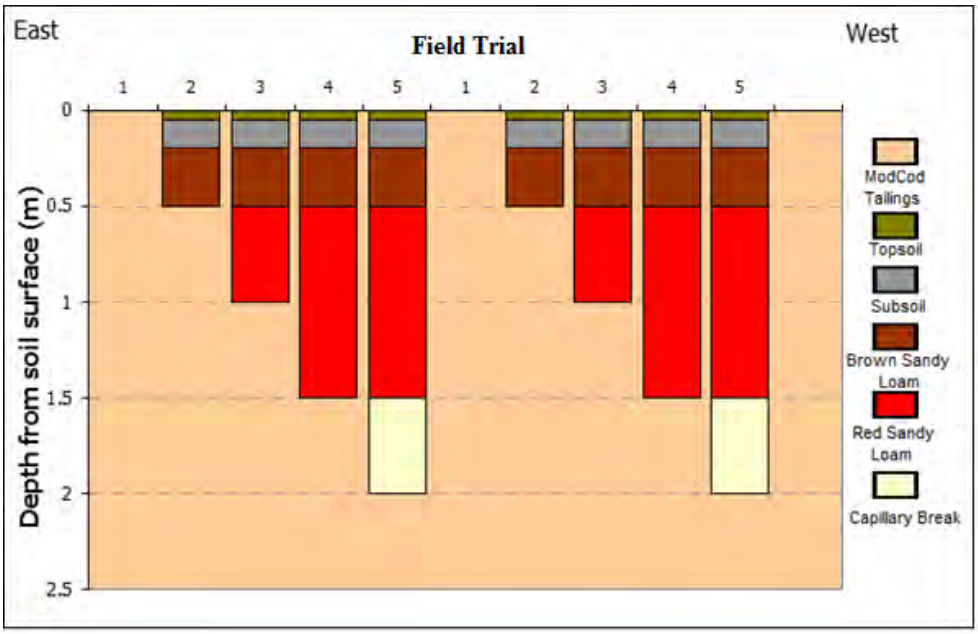

Figure 6: Field experiment trial design. One of the treatments consists entirely of ModCod tailings. The other four treatments are reconstructed soil profiles made with soils that had been stockpiled during the mine construction phase and replaced in the order they naturally occurred. Artificial depths are shallower than proposed rehabilitation depths.

Within the allocated 9 x 12 m plots on the field trial, eighteen positions were created. This allowed for three harvest times i.e. four month harvest (this project) and two subsequent harvests at 12 and 18 months from the planting date (Figure 7). Six of these positions were allocated for this Honours project and their position within the plots was chosen to allow maximum space for root growth of later harvest plants. Three seedlings (one of each species) were plated at each of the six positions making a total of six replicates per block. The seedlings were planted in a north orientated triangular arrangement to maximise the space between them (27 cm apart). A 600 mm diameter watering well (GreenWell™) was placed around each seedling cluster for protection against abrasive windblown particles generated from mining activities. Seedlings were randomly allocated to a treatment and plot using a random number generator. To account for shading effects caused by the height of the wells, *A. papyrocarpa* was always planted in the north position, while *E. oleosa* and *E. gracilis* were randomly allocated to the east and west positions. This allowed comparisons to be made between the *Eucalyptus* species as these positions received relatively consistent light conditions and the biology of these species is similar and comparable. Photographs

illustrating the field trial are shown in Figure 8a, b. Two additional plots were established in an undisturbed area away from the mining area using the same experimental design to allow comparisons.

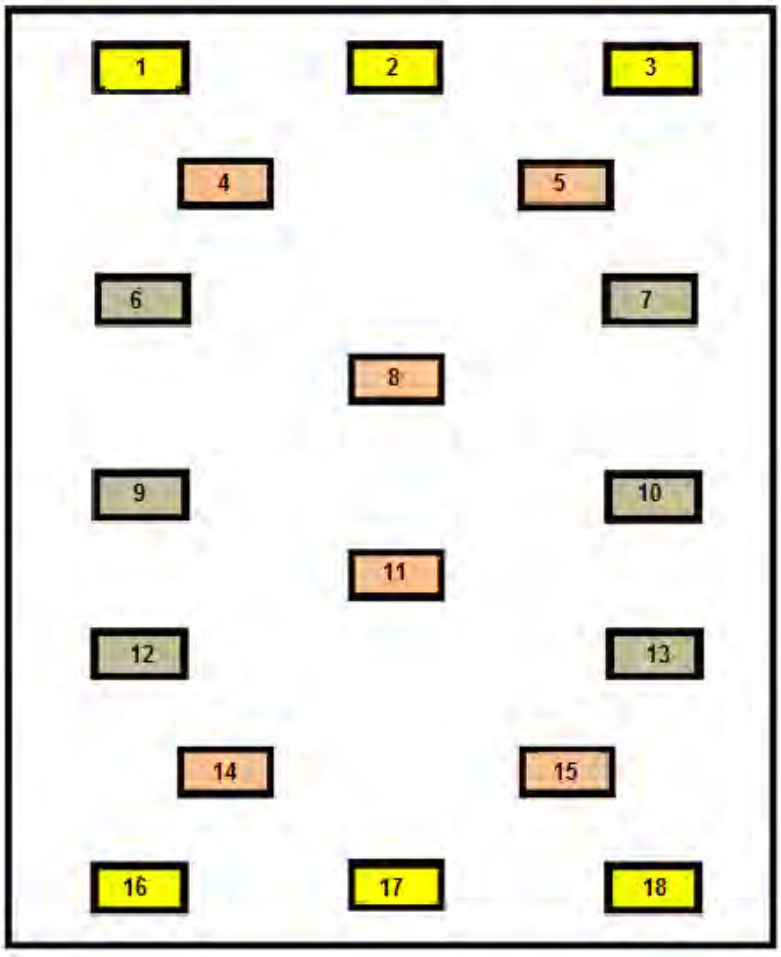

Figure 7: Planting layout in each 9 x 12 m plot. The 4 month harvest (yellow) positioned towards the edges of the plot is the focus of this project. Later harvests (grey and orange) are positioned to allow for maximum root expansion.

Figure 8: a) Facing north across the Cell 1 Trial. The reconstructed soil profiles are seen as strips running north-south. b) Facing west across the trial. The wells on the left and right edges contain the four month harvest seedlings.

Seedlings were planted between the 9th and 13th of May 2014. Nursery bags were removed from the plants with scissors to minimise shock and the visible root morphology of each seedling was photographed before planting. Each well was watered with nine litres of potable water after planting. Watering wells helped to maintain consistency between treatments when watering, as each plant received four additional litres of potable water every fortnight. This was necessary to ensure seedling survival due to the extremely low rainfall received at the site post planting and throughout the duration of this experiment.

At time of planting, seedlings were photographed and measured for height, widest canopy dimension and a measurement perpendicular, number of new and mature leaves and health notes were recorded. Midday photosynthesis yields were obtained from 20 plants of each species prior to being planted using a miniaturized pulse-amplitude-modulated photosynthesis yield analyser (MiniPAM) of H. Walz (Effeltrich, Germany) at a constant

light intensity of approximately 1400 PAR. Twelve additional seedlings of each species were harvested and dried in an oven at 70 ˚C for 48 h and stored for subsequent leaf area measurements. These measurements formed the plant baseline data for this study. Subsequent plant growth and MiniPAM measurements were repeated after two months. After four months of growth at the site, the seedlings were harvested (9-16th of September 2014). Seedling final heights and canopy dimensions were measured. Wells within each treatment were randomly assigned to midday or predawn harvest times using a random number generator. Pre-dawn harvests occurred between 05:00-06:30 h, and midday 11:00-14:00 h over a period of 6 consecutive days. During these time periods photosynthetic yield was measured at 1400 PAR at midday and 0 PAR at pre-dawn. Seedling stems were then cut at ground level and water potentials were obtained using whole seedlings or a branch when the stem diameter was too large for the grommet, using a Scholander pressure chamber (Scholander *et al.* 1965) (PMS Instruments, Corvallis, OR, USA). Seedlings were photographed then stored in plastic bags and kept cool. Upon return to the laboratory, seedlings were rinsed with RO water, blotted dry and stored in paper bags for transport back to Adelaide.

Leaves were removed from stems and branches before drying in an oven at 70˚C for 48 hours, then weighed and counted. Leaf area was calculated using a leaf area meter (ΔT Area Meter, Delta T Devices, Cambridge, England). Due to the high number of leaves on the eucalypt seedlings the total leaf areas were calculated as estimates. To achieve this, leaf samples from four replicate seedlings across each treatment were measured for leaf area and weight. Subsamples were removed and then re-weighed until a total sample weight was gained. GraphPad PRISM software was used to plot a linear regression for the cumulative weights and corresponding light meter values. Seedling leaf total biomass was obtained and substituted into the linear regression to estimate total leaf area per seedling. The small number of phyllodes harvested for *A. papyrocarpa* allowed the ‘leaf’ area to be directly obtained for each seedling.

Soil samples were collected in 10 cm increments, down to a depth of 40 cm across the ModCod profiles (treatment 1) and the shallowest reconstructed profiles (treatment 2). Bulk densities were collected in three replicates using the methods described in White *et al.* (2012) Soil weights were recorded then samples were oven dried at 70 °C for 48 h. Samples were then sent to CSBP soil analysis laboratory (Bibra Lake, Western Australia) for standard nutrient analyses (Ammonium N, Nitrate N, P, K, S, Organic C, pH_{H2O}, pH_{CaCl2},

$EC_{1:5}$,EC_{CaCl2}, Cu, Fe, Mn, Zn, Al, Ca, Mg, Na, B). Two replicates of additional soil samples of surface soil and 10 cm intervals were collected using a spear auger to depths of 40 cm were kept cool and sent to CSBP for analysis of total nitrogen.

Final harvest data is presented in this thesis. Normality and dispersion of the data was analysed using IBM SPSS Statistics 20 software. The seedling biomass needed to be Log_{10} transformed and total leaf areas were square root transformed to reduce dispersion. The two eucalypt species were analysed together in a two way ANOVA as they had similar and comparable biologies, whilst the physiology of *A. papyrocarpa* differs and was therefore analysed independently with one way ANOVA. Tukeys post hoc tests were used as required to identify differences between treatments. Significance was set at $P<0.05$.

Root development in post mining substrates – Glasshouse experiment

A glasshouse experiment was established to gain more detailed information on the root growth of seedlings of the three species in reconstructed soil substrates. PVC pipes of 12 mm diameter were cut to 0.5 m lengths and capped on one end, with drainage holes. The pipes were lined with plastic bags which also permitted drainage. Three different treatments were constructed by adding different substrates in layers (Figure 9). Treatment 1 consisted of, from top to bottom, 5 cm of topsoil, 15 cm of subsoil and 30 cm of brown sandy loam. Treatment 2 consisted of 5 cm of topsoil, 15 cm of subsoil and 30 cm of ModCod. Treatment 3 consisted of 50 cm of ModCod. Eight replicates per treatment were established for each species. Baseline pH and $EC_{1:5}$ measurements were done for each soil type prior to commencing the experiment.

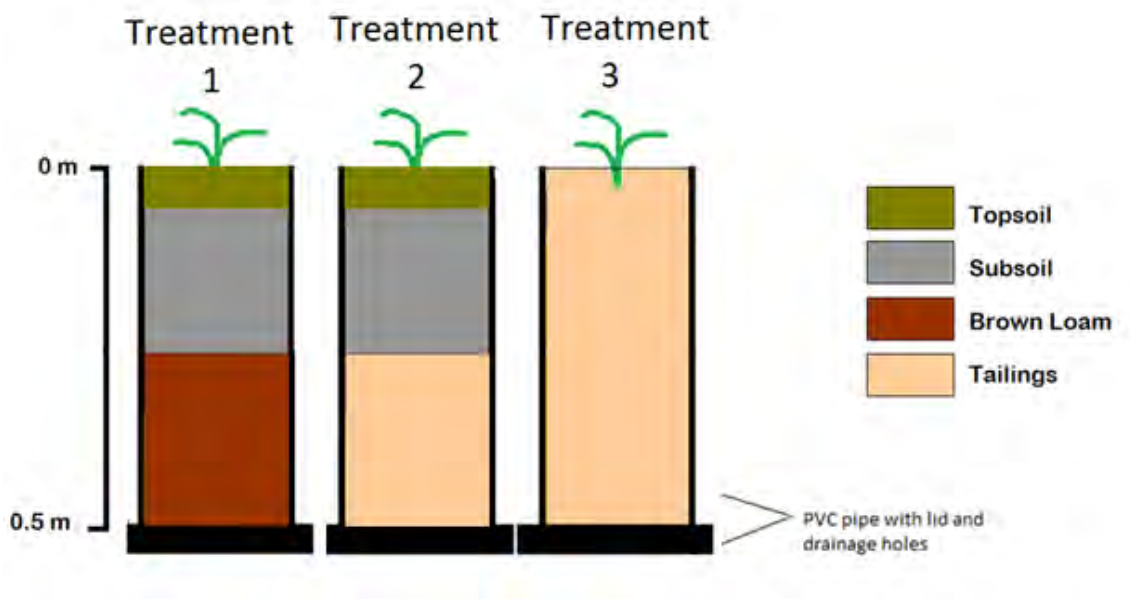

Figure 9: The design of the root observation experiment. Treatment 1: 5 cm topsoil, 15 cm subsoil and 30 cm brown sandy loam. Treatment 2: 5 cm topsoil, 15 cm subsoil and 30 cm ModCod. Treatment 3 consisted of 50 cm of ModCod.

Seeds collected and stored as per emergence glasshouse experiment were used in this experiment. Seeds were germinated in a growth cabinet with 12 h light periods and temperature of 15°C for 7 days. Germinants were transferred to the pipes on the 29/5/14 and watered with 250 ml of rainwater. Pipes subsequently received 50 ml of water every 2-3 days. After eleven weeks mould was observed growing on the phyllodes of *A. papyrocarpa* and watering was reduced to 50 ml twice a week for the final six weeks of the experiment. Seventeen weeks after planting (29/9/14), the seedlings were harvested. The above ground biomass was oven dried at 70°C for 48h and weighed to obtain dry biomass. Soils were removed from the pipes within their plastic linings and sectioned into samples of 0-5 cm, 5-20 cm and 20-50 cm depths to correspond with the different soil types in the reconstructed profiles. Soils were wet sieved through two stacked 2 mm sieves and root biomass was collected. Roots were subsequently oven dried at 70 °C for 48h and weighed to obtain total dry root biomass.

Normality and dispersion of the biomass was analysed using SPSS software. Above ground biomass and root biomass needed to be $\log_{10}$ transformed to reduce dispersion and then analysed through ANOVAs and graphed using GraphPad PRISM software. The above ground biomass for the two eucalypt species were analysed together in a two way ANOVA as

they had similar and comparable biologies, whilst *A. papyrocarpa* was analysed independently with one way ANOVA. The root biomass was analysed for each species individually in a two way ANOVA analysis comparing biomass across substrate and depth. Where applicable, Tukeys multiple comparisons post-hoc tests were used and significance was set to $P<0.05$.

Results

Seedling emergence in post mining substrates - Glasshouse Experiment

Soil Properties

The substrates used in the emergence experiment showed varied EC and pH (ANOVA, $p \leq 0.01$, Figure 10, Table 2). ModCod had a higher EC than the other substrates (Tukey $p < 0.05$). The EC of topsoil and subsoil were not different (Tukey, $p > 0.05$). The pH of subsoil was higher (Tukey $p < 0.05$) than those of topsoil and ModCod tailings, which were not different from each other (Tukey, $p > 0.05$, Figure 10). The pH range observed (8.3 to 8.9) is within the tolerance ranges of the species of interest (James *et al.* 2002; Bui *et al.* 2014)

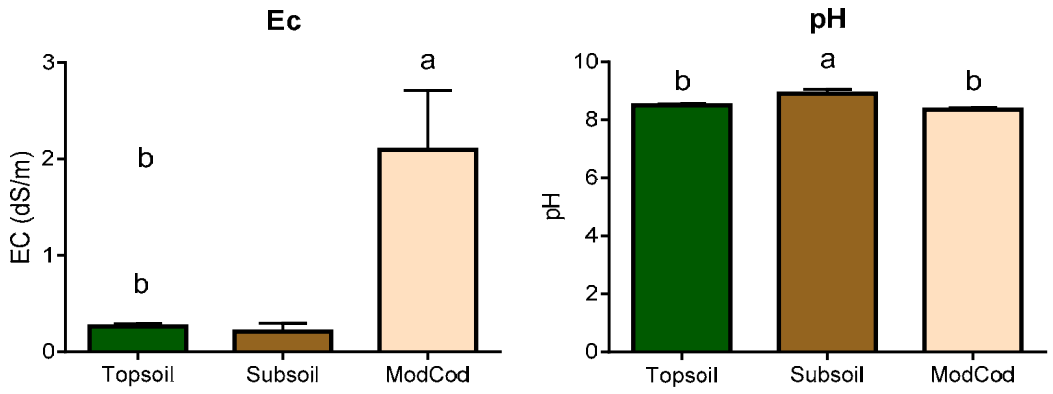

Figure 10: Chemical analyses of the topsoil, subsoil and ModCod substrates used in the seedling emergence experiment, a) EC and b) pH.

Table 2: Summary table of the one way ANOVA analyses for EC and pH of the substrates used in the emergence experiment.

EC	SS	DF	F	P
Substrate	6.913e+006	2	26.80	0.0010
Residual	773941	6		
pH ANOVA				
Substrate	0.4834	2	24.92	0.0012
Residual	0.05820	6		

Emergence

The final emergence of *E. oleosa* and *E. gracilis* showed no interaction or effect of species after 58 days (ANOVA, $p > 0.05$, Figure 11a, Table 3). Emergence varied between the substrates (ANOVA $p \leq 0.01$) and was higher in the subsoil and ModCod than in the topsoil (Tukey, $p < 0.05$). Substrate also had an effect on the emergence of *A. papyrocarpa* (ANOVA, $p > 0.05$ Figure 11b, Table 3) but contrarily, was highest in the topsoil and lowest in ModCod (Tukey, $p < 0.05$).

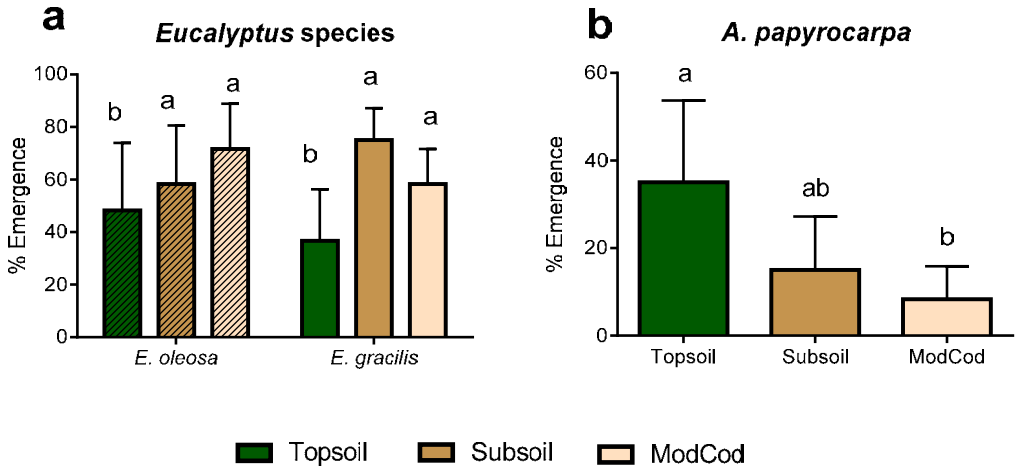

Figure 11: Final emergence results in topsoil (green), subsoil (brown) and ModCod (cream) substrates for a) *E. oleosa* (patterned) and *E. gracilis* (plain), and b) *A. papyrocarpa*

Table 3: Summary table of the two way ANOVA analysis of *E. oleosa* and *E. gracilis* emergence and the one way ANOVA of *A. papyrocarpa* emergence in post mining substrates.

E. oleosa and *E. gracilis* emergence	SS	DF	F	P
Interaction	0.7412	2	1.995	0.1536
Species	0.02513	1	0.1353	0.7156
Substrate	2.443	2	6.576	0.0043
A. papyrocarpa emergence				
Substrate	3.710	2	5.741	0.0141
Residual	4.847	15		

Seedling establishment in reconstructed soil substrates – Field trial

Seedlings at the undisturbed site showed poor health and significantly altered growth and physiological responses. This is expected to be caused by effects such as competition from surrounding plants, increased herbivory from insects and other factors which were not controlled for. Due to these confounding factors, data from the undisturbed area was omitted from the results and only comparisons among the five treatments on the trial are presented.

Soil Properties

Soil samples collected in the shallow reconstructed soil profile (treatment two) and the Modod profile (treatment one) in the field trial indicated no interaction for EC but effects of depth and substrate ($p \leq 0.01$ Figure 12a, Table 4). EC increased with depth, and was higher at the 30-40 cm depth than the 0-10 cm and 10-20 cm depths (Tukey $p < 0.05$). The reconstructed soil profile had higher EC than the Modcod (Tukey $p < 0.05$). No interaction or depth effects were found for pH but there was a significant effect of substrate (ANOVA $p \leq 0.01$, Figure 12b, Table 4). pH was higher in the ModCod than the reconstructed profile (Tukey, $p < 0.05$). Bulk density indicated no interaction but an effect of depth and substrate (ANOVA $p \leq 0.05$, Figure 12c, Table 4). Bulk density increased with depth and was lower at the 0-10 cm depth than the 20-30 cm depth (Tukey, $p < 0.05$). The ModCod substrate had higher bulk density than the shallow reconstructed soil profile (Tukey, $p < 0.05$). Volumetric water content did not detect an interaction but depth and substrate had significant effects

(ANOVA $p \leq 0.01$, Figure 12d, Table 4). Volumetric water content was higher in the shallow reconstructed profile than the ModCod (Tukey, $p < 0.05$). Water content was lowest in the 0-10 cm depth and consistent across the subsequent depths (Tukey, $p < 0.05$). Nutrient analyses revealed total nitrogen had a significant interaction between depth and substrate (ANOVA, $p < 0.01$, Figure 12e, Table 4). Total nitrogen was higher in the shallow reconstructed profile at the soil surface and down to 20cm depths (Tukey, $p < 0.05$). At depths beyond 20 cm, nitrogen decreased and was statistically similar to the ModCod across all depths (Tukey, $p < 0.05$). Exchangeable aluminium showed no interaction or depth effect, but a significant substrate effect (ANOVA, $p < 0.01$, Figure 12f, Table 4). Aluminium was higher in the ModCod than the shallow reconstructed profile. Exchangeable sodium also showed no interaction or depth effect but a significant substrate effect (ANOVA, $p < 0.05$, Figure 12g, Table 4) Sodium was higher in the reconstructed profile than the ModCod.

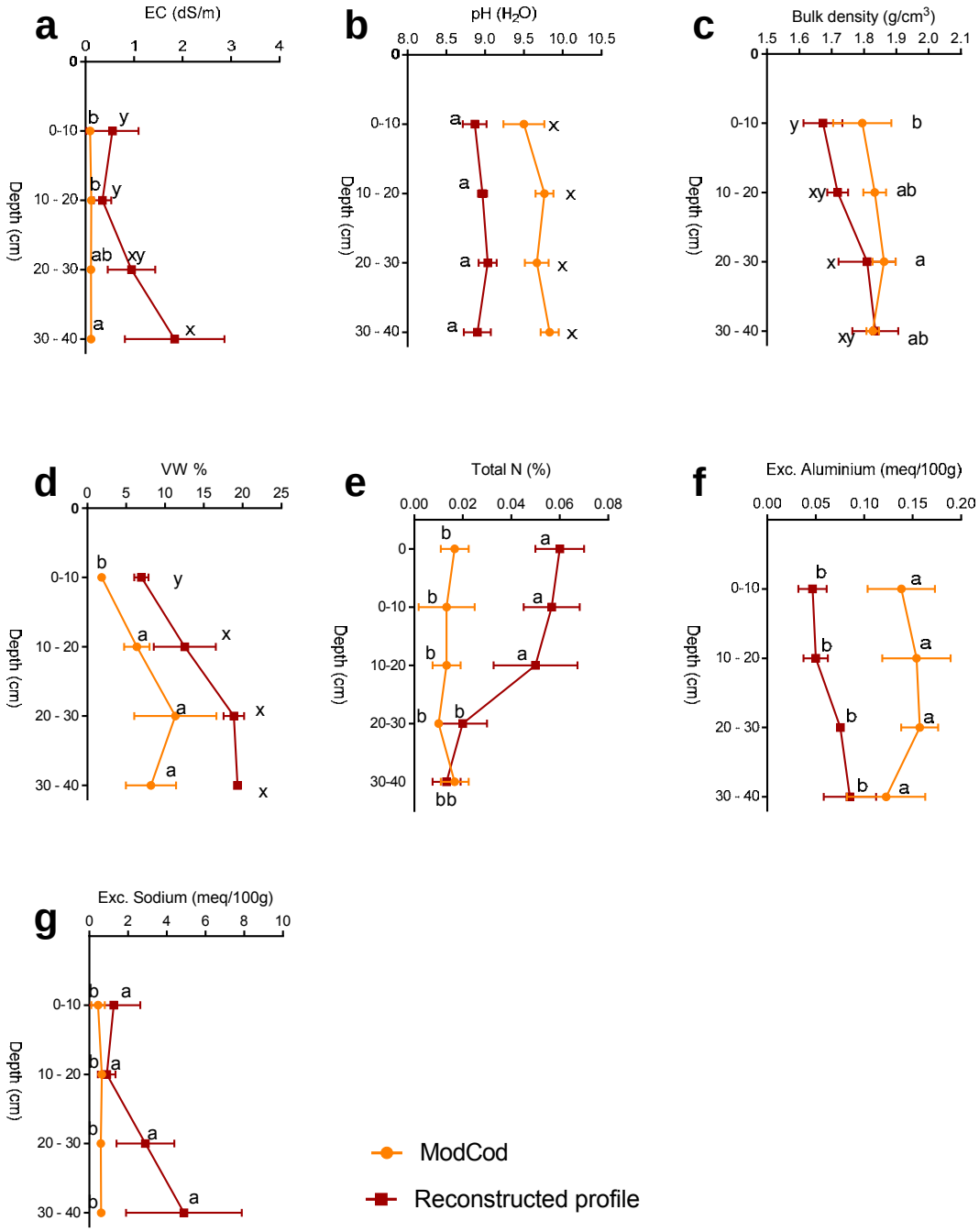

Figure 12 :The chemical and physical soil analysis results for the ModCod (orange) and shallow reconstructed soil profile (red) substrates down to depths of 40 cm. a) EC, b) pH, c) bulk density, d) volumetric water content, e) total nitrogen, f) exchangeable aluminium g) exchangeable sodium.

Table 4: Two way ANOVA results comparing the soil properties between the ModCod and shallow reconstructed profile(treatment two) in the field trial.

EC	SS	DF	F	P
Interaction	1.984	6	2.368	0.0613
Depth	3.226	3	7.702	0.0009
Substrate	4.094	2	14.66	< 0.0001
Residual	3.351	24		
pH				
Interaction	0.09500	3	1.333	0.2985
Depth	0.1433	3	2.012	0.1529
Substrate	3.375	1	142.1	< 0.00011
Residual	0.3800	16		
Bulk density				
Interaction	0.04850	6	2.942	0.0269
Depth	0.02941	3	3.568	0.0289
Substrate	0.3853	2	70.11	< 0.0001
Residual	0.06595	24		
Volumetric water content				
Interaction	57.37	6	1.804	0.1408
Depth	480.9	3	30.25	< 0.0001
Substrate	609.1	2	57.47	< 0.0001
Residual	127.2	24		
Total N				
Interaction	0.002747	4	7.630	0.0007
Depth	0.003013	4	8.370	0.0004
Substrate	0.00507	1	56.33	< 0.0001
Residual	0.0018	20		
Exc. Aluminium				
Interaction	0.003795	3	1.824	0.1834
Depth	0.001728	3	0.8305	0.4964
Substrate	0.03721	1	53.64	< 0.0001

Residual	0.01110	16		
Exc. Sodium				
Interaction	14.57	3	2.885	0.0682
Depth	15.36	3	3.040	0.0594
Substrate	21.87	1	12.99	0.0024
Residual	26.94	16		

Physiology

Midday measurements of photosynthetic yield detected no interaction or species effects but a significant effect of substrate (ANOVA, $p \leq 0.01$, Figure 13a Table 5) Midday photosynthetic yield was higher in seedlings growing on the reconstructed profiles in treatments three and four (with 0.5 m and 1.0 m of red loam in the profile) than ModCod (treatment one) (Tukey $p < 0.05$). Pre-dawn photosynthetic yields displayed no interaction or substrate effects but a significant species effect (ANOVA, $p < 0.05$, Figure 13b, Table 5). Yield was higher in *E. oleosa* seedlings than *E. gracilis* seedlings (Tukey $p < 0.05$). Midday water potential measurements indicated no interaction or effect on soil treatment but a significant species effect (ANOVA $p <0.01$, Figure 13c Table 5**Error! Reference source not found.**). *Eucalyptus gracilis* had lower midday water potentials than *E. oleosa* (Tukey $p < 0.05$). There was no significant interaction effect for pre-dawn water potential but there were significant species and soil treatment effects (ANOVA $p < 0.05$, Figure 13d, Table 5). *Eucalyptus gracilis* seedlings had lower pre-dawn water potentials than *E. oleosa* seedlings (Tukey $p < 0.05$). Pre-dawn water potential was significantly lower for seedlings growing in treatment three (reconstructed profile including 0.5 m of red loam) than seedlings in the ModCod substrate (Tukey $p < 0.05$). Substrate did not have an effect on any of the physiological parameters measured for seedlings of *A. papyrocarpa* (ANOVA, $p > 0.05$,Figure 14, Table 6).

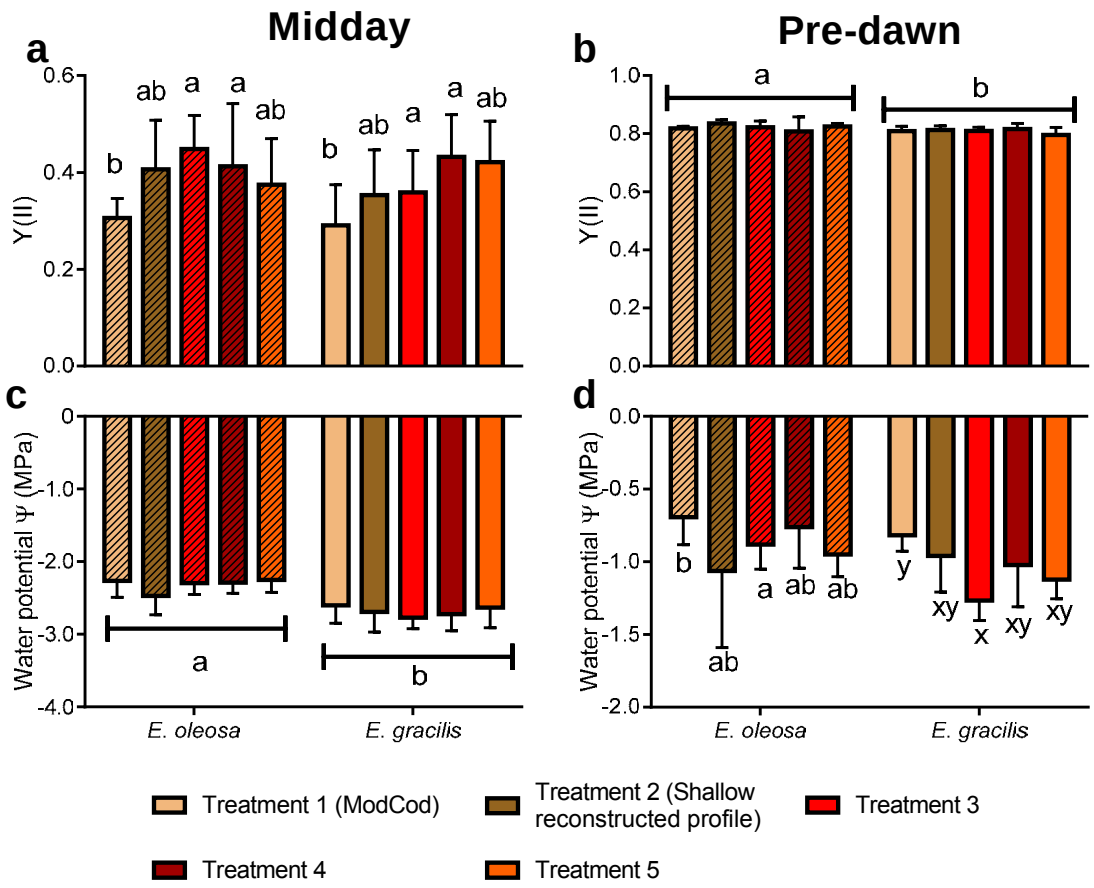

Figure 13: Midday and pre-dawn physiological data comparisons of *E. oleosa* (patterned) and *E. gracilis* (plain) seedlings grown in the field trial. a) Midday photosynthetic yield, b) pre-dawn photosynthetic yield, c) midday water potential, d) pre-dawn water potential.

Table 5: Two way ANOVA results comparing the physiological measurements of photosynthetic yield and water potential between *E. oleosa* and *E. gracilis* seedlings grown in the five reconstructed soil substrates on the field trial.

Midday photosynthetic yield	SS	DF	F	P
Interaction	0.03584	4	1.114	0.3605
Species	0.005005	1	0.6223	0.4339
Substrate	0.1128	4	3.506	0.0134
Residual	0.4022	50		
Pre-dawn photosynthetic yield				
Interaction	0.002493	4	1.231	0.3097
Species	0.002522	1	4.982	0.0301
Substrate	0.001427	4	0.7047	0.5925
Residual	0.02531	50		
Midday water potential				
Interaction	0.1177	4	0.6067	0.6596
Species	2.033	1	41.93	< 0.0001
Substrate	0.1873	4	0.9657	0.4346
Residual	2.424	50		
Pre-dawn water potential				
Interaction	0.3959	4	1.551	0.2021
Species	0.4261	1	6.674	0.0128
Substrate	0.8104	4	3.174	0.0212
Residual	3.192	50		

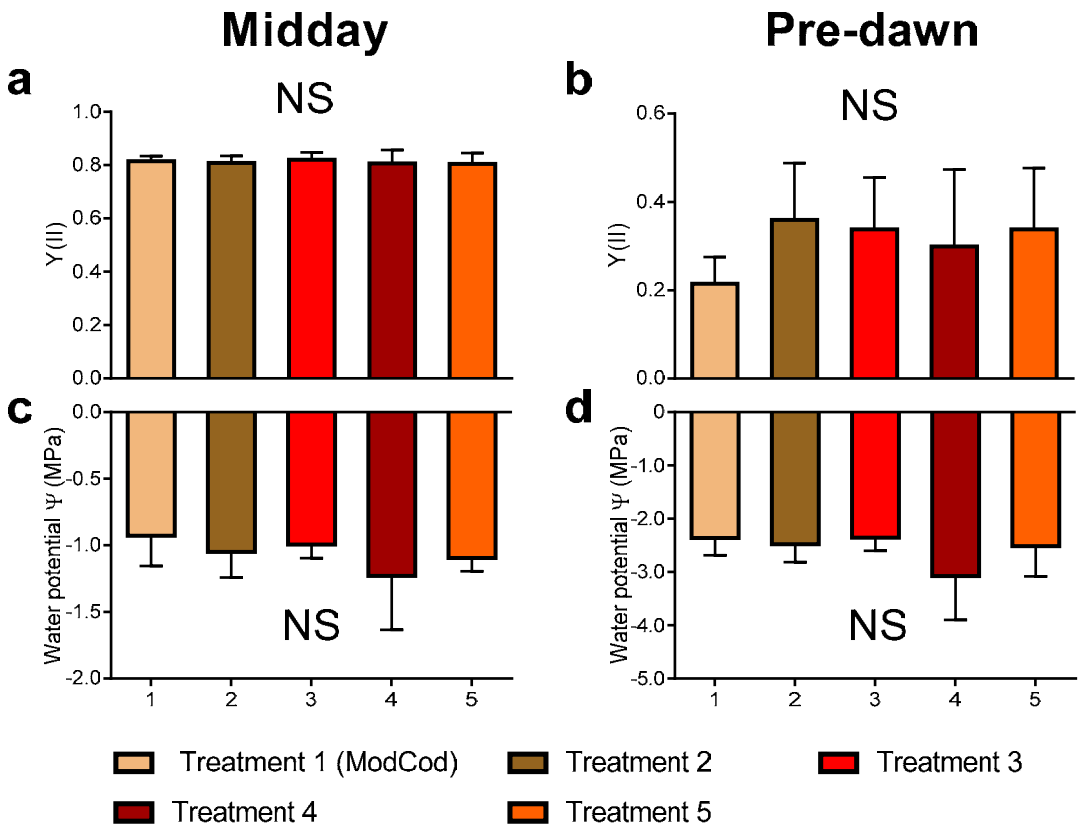

Figure 14: The midday and pre-dawn physiological data of *A. papyrocarpa* seedlings grown in the field trial. No significant results were detected. a) Midday photosynthetic yield, b) Pre-dawn photosynthetic yield, c) midday water potential, (d) pre-dawn water potential.

Table 6: One way ANOVA summaries of physiological measurements undertaken on *A. papyrocarpa* seedlings growing in the five soil substrates of the field trial.

Pre-dawn photosynthetic yield	SS	DF	F	P
Substrate	0.07527	4	1.116	P = 0.3732
Residual	0.3878	23		
Midday photosynthetic yield				
Substrate	0.001044	4	0.2091	0.9308
Residual	0.02995	24		
Pre-dawn water potential				
Substrate	0.2218	4	1.058	0.4041

Residual	0.9954	19		
Midday photosynthetic yield				
Substrate	0.001044	4	0.2091	0.9308
Residual	0.02995	24		

Growth Measurements

Comparisons of the leaf biomass of seedlings for the two eucalypt species found no interaction but independent species and substrate effects (ANOVA $p < 0.01$, Figure 15a, Table 7). *Eucalyptus gracilis* had higher leaf biomass than *E. oleosa* (Tukey $p < 0.05$). Seedlings grown in the reconstructed soil profiles had higher leaf biomass than seedlings grown in the ModCod profile (Tukey $p < 0.05$). Leaf area estimates for the eucalypt species identified an interaction between species and treatment indicating simultaneous effects of these two factors (ANOVA, $p < 0.01$, Figure 15b, Table 7). Leaf area was lower in seedlings grown in ModCod (Tukey, $p < 0.05$). *Eucalyptus gracilis* seedlings had the highest leaf area in the reconstructed substrate in treatment three which was higher than the leaf area measured for *E. oleosa* in the same treatment and *E. oleosa* growing in the shallowest reconstructed profile (treatment two) (Tukey, $p \leq 0.05$). Substrate did not have any effect on the leaf biomass or leaf area of *A. papyrocarpa* (ANOVA, $p > 0.05$, Table 8, Figure 16).

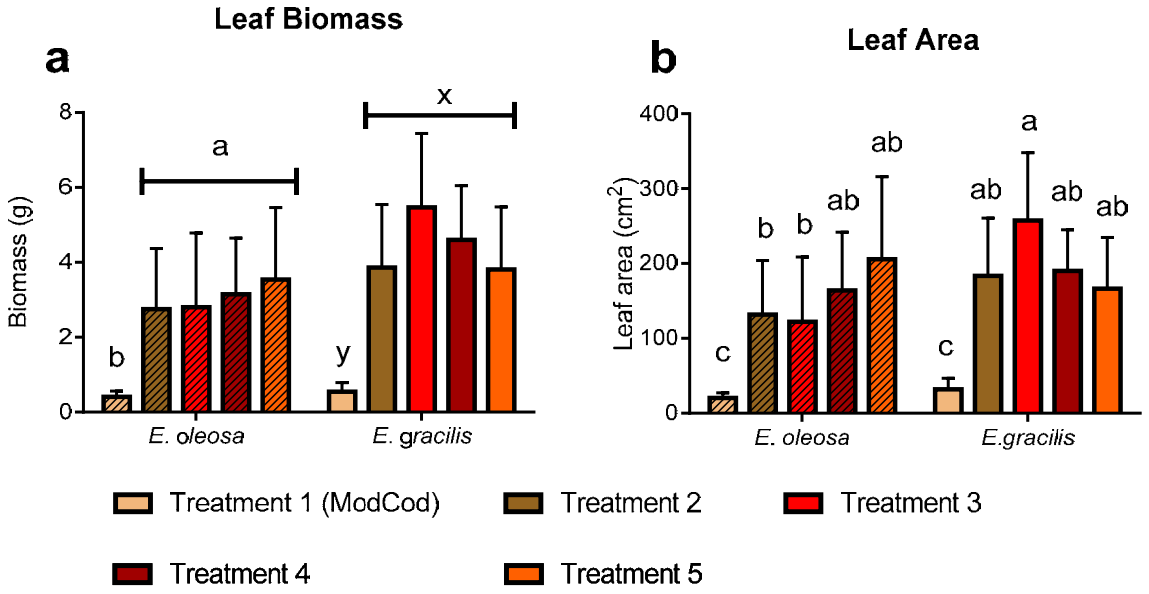

Figure 15: Comparisons between *E. oleosa* (patterned) and *E. gracilis* (plain) seedlings of a) total leaf biomass, b) estimated leaf area.

Table 7: The two way ANOVA summary table of the leaf biomass and total leaf area comparisons of *E. oleosa* and *E. gracilis* seedlings grown in the field trial.

Leaf biomass	SS	DF	F	P
Interaction	0.3027	4	1.346	0.2574
Species	1.047	1	18.64	< 0.0001
Substrate	14.65	4	65.15	< 0.0001
Residual	6.182	110		
Leaf area				
Interaction	131.6	4	4.124	0.0038
Species	89.89	1	11.27	0.0011
Substrate	1218	4	38.18	< 0.0001
Residual	877.4	110		

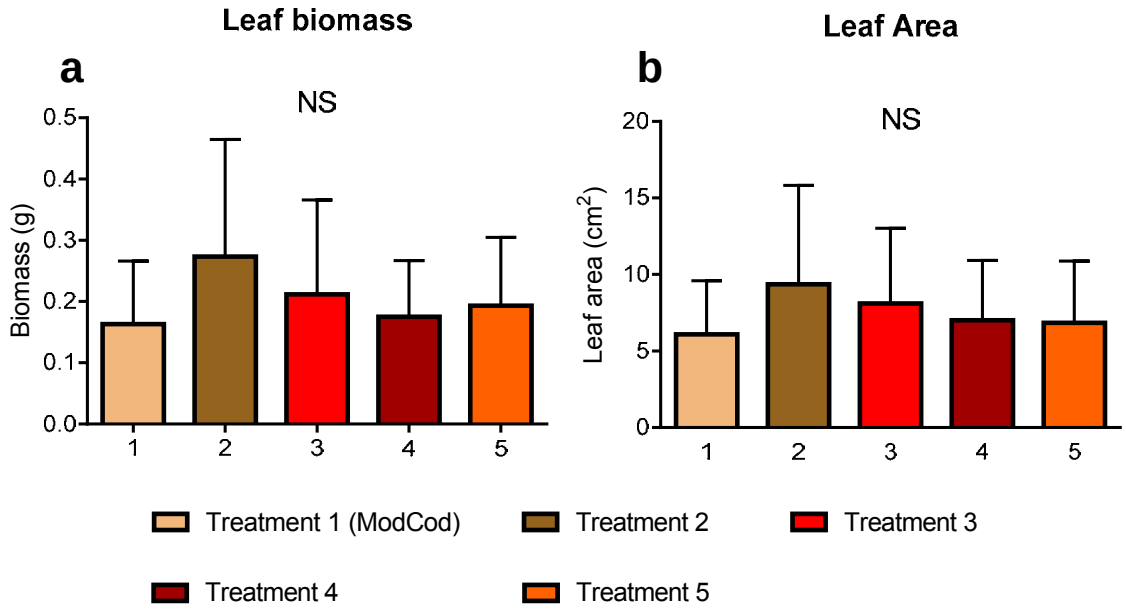

Figure 16: ***Acacia papyrocarpa*** **a) seedling leaf biomass and b) leaf (phyllode) area across the soil treatments on the Cell 1 Trial.**

Table 8: Summary table of the one way ANOVAs for the total leaf biomass and leaf area for seedlings of *A. papyrocarpa* grown in the field trial.

Leaf biomass	SS	DF	F	P
Substrate	0.1941	4	0.5020	0.7343
Residual	4.928	51		
Leaf area				
Substrate	1.582	4	0.4875	0.7448
Residual	41.38	51		

Root development in post mining substrates – Glasshouse experiment

Soil Properties

The substrates used in the root development experiment showed varied EC and pH values (ANOVA, $p \leq 0.01$, Figure 17, Table 9). ModCod and brown sandy loam had higher conductivities than topsoil and subsoil (Tukey $p < 0.05$). pH was highest in ModCod (Tukey $p < 0.05$).

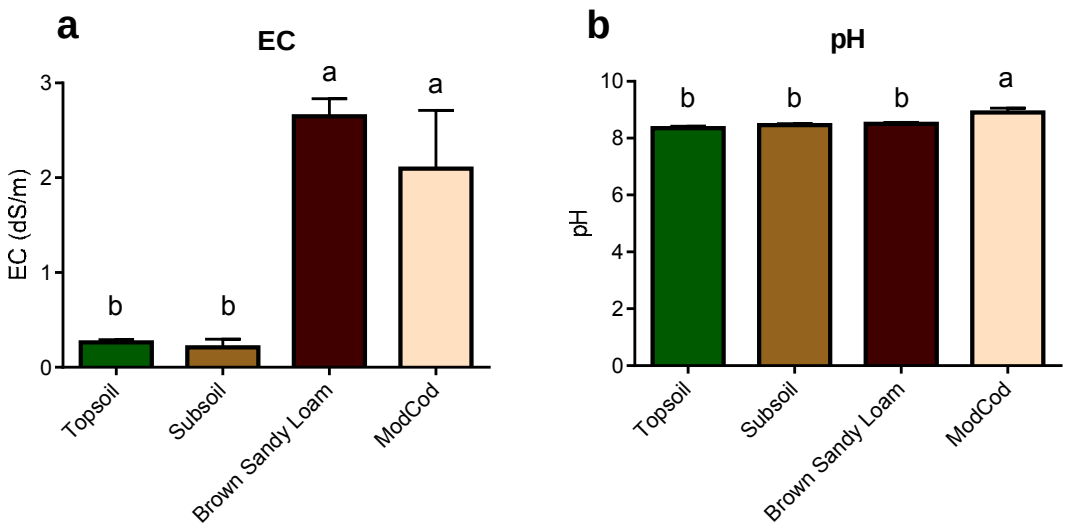

Figure 17: Chemical analyses of the topsoil, subsoil and ModCod substrates used in the root development experiment, a) EC and b) pH.

Table 9: One way ANOVA results of the EC and pH of the substrates used in the root development experiment.

EC	SS	DF	F	P
Treatment	1.413e+007	3	44.75	< 0.0001
Residual	842139	8		
pH				
Treatment	0.5182	3	21.77	0.0003
Residual	0.06347	8		

Above Ground Biomass

After four months of growth, the above ground biomass of *E. oleosa* and *E. gracilis* seedlings had no interactive effects but significant species and soil effects (ANOVA $p < 0.01$, Figure 18a, Table 10). All treatments significantly differed, with the highest above ground seedling biomass recorded in the topsoil/subsoil/brown sandy loam substrate, and the lowest seedling biomass recorded in the ModCod sequence (Tukey $p < 0.05$). The total biomass data collected for *A papyrocarpa* did not detect any significant differences between the treatments (ANOVA, $p > 0.05$, Figure 18b, Table 10).

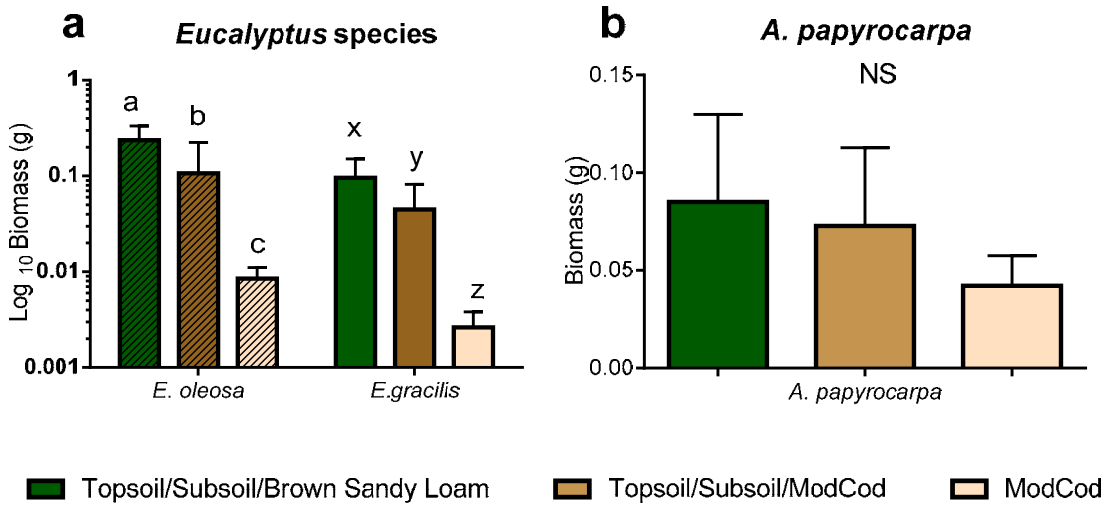

Figure 18: Above ground biomass collected for each species in the root development experiment. a) *E. oleosa* (patterned) and *E. gracilis* (plain), b) *Acacia papyrocarpa*.

Table 10 ANOVA summaries of the two way analysis for *E. oleosa* and *E. gracilis* above ground biomass and the one way analysis *A. papyrocarpa* above ground biomass in the root development experiment.

E. gracilis and *E. oleosa* above ground biomass	SS	DF	F	P
Interaction	0.2136	2	0.6908	0.5068
Species	1.984	1	12.83	0.0009
Substrate	17.30	2	55.94	< 0.0001
Residual	6.493	42		
A. papyrocarpa above ground biomass				
Substrate	0.2593	2	1.662	0.2137
Residual	1.638	21		

Root Biomass

Root biomass data collected for *E. oleosa* showed a significant interaction between substrate and depth (ANOVA $p < 0.01$, Figure 19a,Root biomass collected in 0-5 cm, 5-20 cm and 20-50 cm intervals for a) *E. oleosa*, b) *E. gracilis* and c) *A. papyrocarpa* in the root observation glasshouse experiment. Substrates consisted of: 0-5 cm topsoil, 5-15 cm subsoil and 20-50 cm brown sandy loam (green) Table 11). Root biomass was lowest in the ModCod substrate across all depths (Tukey, $p < 0.05$). The 0-5 cm root biomass in the ModCod was only comparable with the other depths in the ModCod and the bottom 20-50 cm of the topsoil/subsoil/ModCod profile. Biomass was highest in the 0-5 cm and 5-20 cm depths in the substrates where topsoil and subsoil were present (Tukey, $p < 0.05$). *Eucalyptus gracilis* root biomass showed a significant interaction effect between depth and soil substrate (ANOVA $p < 0.05$, Figure 19b, Table 11**Error! Reference source not found.**). Root biomass was lowest in ModCod at the 0-5 cm and 5-20 cm depths (Tukey, $p < 0.05$). Root biomass was highest in the top 20 cm of soil in the two treatments where topsoil and subsoil were present in these depths (Tukey, $p < 0.05$). The biomass at the 20-50 cm depths did not vary across the treatments for *E. gracilis. Acacia papyrocarpa* root biomass also had a significant interaction between soil substrate and depth (ANOVA $p < 0.01$, Figure 19c, Table 11). Root abundance was highest in the top 20 cm of the soil in the two substrates which contained topsoil and subsoil in these depths (Tukey, $p < 0.05$). The root biomass in the 20-50 cm range was not different to the biomass in this depth range of the other soil substrates (Tukey, $p > 0.05$).

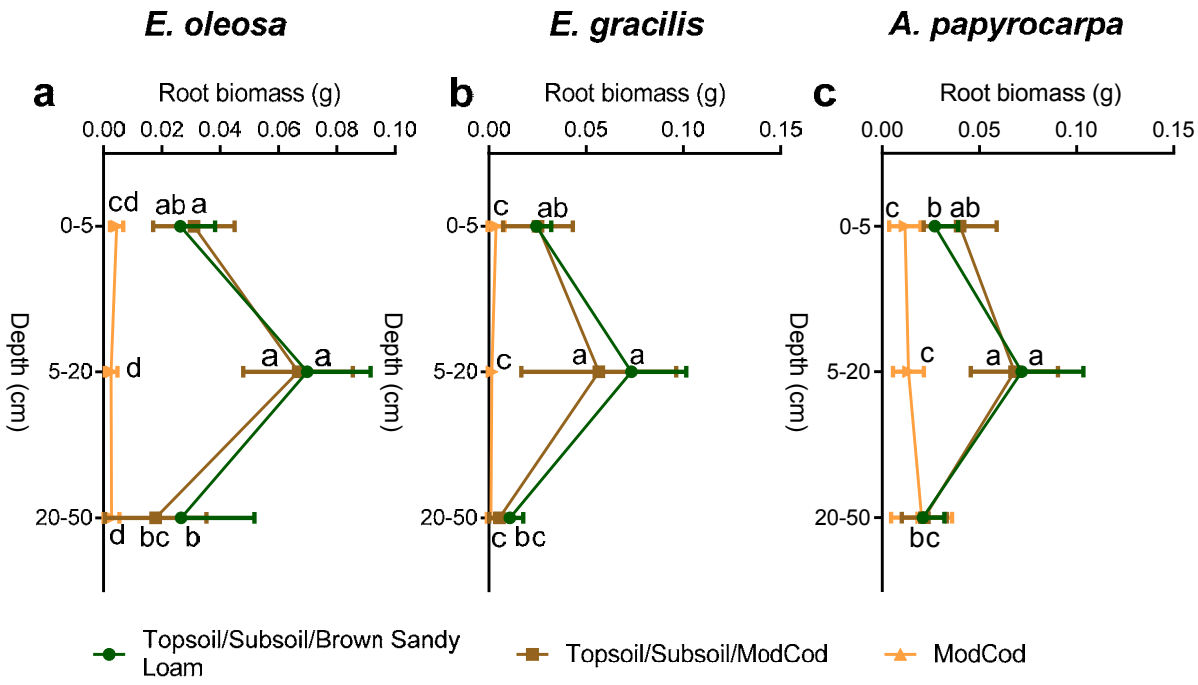

Figure 19: Root biomass collected in 0-5 cm, 5-20 cm and 20-50 cm intervals for a) *E. oleosa,* b) *E. gracilis* and c) *A. papyrocarpa* in the root observation glasshouse experiment. Substrates consisted of: 0-5 cm topsoil, 5-15 cm subsoil and 20-50 cm brown sandy loam (green), 0-5 cm topsoil, 5-15 cm subsoil and 20-50 cm ModCod (brown) and 50 cm ModCod (orange)

Table 11: Summary table of the two way ANOVAs for the root biomass of each species collected in 0-5 cm, 5-20 cm and 20-50 cm depths in the three soil substrates.

E. oleosa root biomass	SS	DF	F	P
Interaction	1.539	4	3.776	0.0084
Depth	1.814	2	8.899	0.0004
Treatment	14.19	2	69.60	< 0.0001
Residual	6.013	59		
E. gracilis root biomass				
Interaction	1.242	4	2.583	0.0473
Depth	4.296	2	17.87	< 0.0001
Treatment	10.59	2	44.05	< 0.0001
Residual	6.490	54		

A. papyrocarpa root biomass				
Interaction	1.189	4	4.460	0.0031
Depth	1.290	2	9.674	0.0002
Treatment	2.897	2	21.73	< 0.0001
Residual	4.132	62		

Discussion:

This study found that there were only moderate differences in the responses of seedlings of the three species in the different substrates and reconstructed soils, despite their contrasting properties. The ability of the three species to emerge and grow in these soils indicates that seedling establishment is possible in the reconstructed soils under favourable conditions. The results can partially, but not completely, be explained by the substantial changes in the chemistry of the substrates which, under field conditions quickly become more favourable for plant growth.

This study has shown that topsoil, subsoil and ModCod were suitable substrates to support seedling emergence for each of the three species. These results show that topsoil provides resources and conditions that enhance seedling emergence of *A. papyrocarpa*. Generally, topsoil contains a suite of microbial organisms as well as high abundances of nutrients and organic matter which are beneficial in supporting plant growth (Grant *et al.* 1996; Frouz *et al.* 2001; Graham and Haynes 2004; Cortina *et al.* 2013). Conversely, the two eucalypt species have shown the ability to emerge more successfully in the subsoil and ModCod substrates. The chemical and physical properties of topsoil and subsoil are similar, and it is unclear why these species would favour subsoil over topsoil with the current data. The stockpiling process may have altered the chemical or microbial properties of the subsoil in a manner which benefits these species. Increased contact between the soil particles and the seeds may have reduced water loss around the seeds leading to more successful emergence (Liu *et al.* 2008). Further analyses of the physical and microbial characteristics of these substrates may provide insights into why this was found. Increased emergence in ModCod was unexpected. Greater establishment success in mining wastes compared to topsoil have been found in another published study, however their results were due to a lack of competition from other species and competition was controlled in this experiment (Huxtable *et al.* 2005). Contrarily, a similar study analysing different eucalypt species germinating in saline mining wastes

showed decreased emergence success (Madsen and Mulligan 2006). *Eucalyptus gracilis* naturally inhabits salty areas and therefore the species has adaptations which allow it to successfully and competitively establish in the salty substrates which may have contributed to this result (Bell *et al.* 1993a). However, *Eucalyptus oleosa* naturally inhabits relatively low salinity soils and is less salt tolerant, further suggesting that chemical or microbial differences influenced the emergence result(Doudle and Schneemilch 2012; Payne 2012; Guerin and Ainsley 2013). Regular watering may have masked the effects of soil type or salts may have been flushed from the soil over time. Seedling establishment may be very different in the rehabilitation areas in the absence of regular watering.

This study has also shown that the reconstructed soil profiles and ModCod are capable of supporting early seedling growth. All soil substrates were capable of supporting seedling growth for the three species in both the field and glasshouse experiments. Under well watered conditions the type of substrate had very little effect on the above ground growth of *A. papyrocarpa*. This indicates the chemical and physical properties of all substrates are suitable for supporting seedling growth when water availability is high. Similarly, all substrates were capable of supporting growth of the two eucalypt species; however biomass was decreased in the ModCod substrate in the field and glasshouse experiments. Due to the use of saline water in the mining process, ModCod was expected to be saline. Whilst this was the case in the glasshouse experiments; soil analyses at the end of the field study found lower electronic conductivity and exchangeable sodium in the ModCod. The ModCod soils used in the glasshouse experiments were collected from deep within the tailings storage facility stockpile and protected from the influence of rainfall and therefore the salts had not been flushed. This allowed observations to be made under the saline conditions. However, the trial had been constructed for over twelve months before this experiment was undertaken and the free draining nature of the ModCod suggests the salts at the surface of the trial had flushed down to deeper depths beyond 40 cm. This is evidenced by much higher EC measurements recorded for the ModCod during the construction of the trial (data not shown, Pers. Com. E. Steggles, 2014). It is not known at this stage how far down the profile the salts have moved through flushing or whether they will accumulate at the most frequent depth of the wetting front after rains (Terkeltoub and Babcock 1971; Park *et al.* 2013). Another possibility is the salts may rise back to the surface through capillary forces when the profile is wet again in future rains (Endo *et al.* 2012; Bui 2013). These processes may develop such harsh conditions that root penetration becomes inhibited which may ultimately compromise the establishment

and survival of these species in the long term. This issue requires further investigation in the future.

The above ground and total leaf biomass data collected in the field and glasshouse experiments showed similar patterns of decreased growth in profiles consisting entirely of ModCod, despite the differences in ECs. Therefore, these differences cannot be explained by salts alone, and an alternative cause is likely a lack of available nutrients. Nutrient limitation is commonly found in post mining soils (Bradshaw 1997; van Etten *et al.* 2012; Asensio *et al.* 2013). Soil analyses undertaken on the field trial revealed that the elements more often limiting plant growth (N, P, K, Ca, Mg, S, Fe, and Mn) were lower in the ModCod compared to the shallow reconstructed profile (Appendix B)(Han *et al.* 2011). Generally, mine waste products often have low microbial and biological activity which may have also contributed to the decreased plant growth (Frouz *et al.* 2001; Helingerová *et al.* 2010). Another possible factor affecting growth in ModCod could be aluminium toxicity. High aluminium contents can negatively affect yield of PSII and root growth (Fonseca *et al.* 2014). Aluminium exposure primarily affects plant roots, where it can cause reduced growth, oxidative stress and nutrient imbalances (Silva 2012). However, the toxic trivalent form of aluminium Al^{3+} associated with aluminium toxicity is found in acidic conditions (pH<5) while the pH at this site is alkaline, (between 8 and 9) a range where aluminium is often in the forms of $Al(OH)_4^-$ and aluminate, which are not likely to cause toxicities (Dalhaize and Rya 1995).

Seedlings received sufficient watering to ensure survival due to scarce rainfall over the study period. This minimised seedling deaths, avoided reducing sample sizes and allowed sufficient growth to occur within the relatively short timeframe available. Water is often the factor most limiting in arid and semiarid habitats (Caldwell *et al.* 1977; Donovan and Ehleringer 1992; Rhizopoulou and Davies 1993). Rainfall was low throughout the period of this study and high mortality would have been obtained if watering was not undertaken. Despite consistent watering, the volumetric water content differed across the substrates in the field experiment at time of harvest. The soil chemical and physical properties in the substrates differed which is the likely cause of this variation. Bulk densities in the ModCod and reconstructed substrates were found to be in the range of 1.6-1.9 g/cm ^{3} which is higher than the site soil pre-mining bulk densities of 1.27- 1.67 g/ cm^3 (SWC 2007). This increase is likely caused by the use of heavy machinery in the removal and replacement processes and loss of soil structure caused by the disturbance (Chong and Cowsert 1997; DeLong *et al.* 2012). Increased bulk density causes a reduction in pore space decreasing water penetration, increasing runoff and leading

to lower soil water content which can limit water availability to vegetation (Thompson *et al.* 1987; Ballard 2000). The free draining characteristics and lack of structure of the ModCod substrate may have resulted in different water holding capabilities, evaporative and infiltrative rates and capillary continuity within the soil. Furthermore, organic matter contents in the soil has been found to greatly influence soil moisture content, and the lack of organic carbon in the ModCod may have contributed to the lower water content (Sauwa *et al.* 2013).

The physiological parameters recorded for seedlings on the trial indicated that they were not stressed in relation to the different substrates. *Acacaia papyrocarpa* showed no difference for any measured parameters, and this is likely due to regular watering reducing water stress and shade from the watering well reducing light stress. Future studies where shading effects are omitted and water is not supplied may yield a greater influence of substrate. The physiological measurements of the two eucalypt species were inconclusive. Whilst some statistical differences were found, they were not consistent across the substrates. Photosynthetic yield and water potential measurements were undertaken in the field experiment as they are effective tools for detecting stress and physiological differences within plants. However, biomass and leaf area appear to be better indicators in this study under the given circumstances.

Each species was able to successfully develop roots in the different substrates. A significant resource allocation to the root development was observed in the glasshouse experiment for all three species. The top 20 cm depths subsoil and topsoil substrates had higher biomass across all species compared to ModCod in this depth range. Root development in the top 20 cm of soil is important for seedlings to access soil nutrients which are unavailable in the deeper soil layers (Dougill *et al.* 1998). However, surface soils in the arid zone dry out during the hot, dry summer months and roots close to the soil surface are prone to desiccation (Enright and Lamont 1992; Rokich *et al.* 2001). To avoid desiccation, these species need to establish a tap root to allow them to access soil water over dry periods. Tap root development needs to occur early in the plants growth stage in order to extend deeply into the soil at a rate exceeding of the water evaporation rates drying the soil (Enright and Lamont 1992). In arid climates, the upper 5- 10 cm depths are mostly dry within 5-25 days after rain (Noy-Meir 1973). Drying of subsequent depths is slower (dependant on the amount of rainfall and soil type) and can take a number of weeks to dry the following 10-30 cm depths (Noy-Meir 1973).

The development of deep root systems is highly beneficial to improving soil structure by generating channels that can increase water flow deeper into the profile which can reduce the effects of compaction over time (Thorne *et al.* 2013). Root expansion into deeper soil layers can also reduce competition for water from shallow rooted species (Kambatuku *et al.* 2013). Furthermore, recent studies (Steggles *et al.* unpublished data) on mature *A. papyrocarpa* have indicated that this species effect hydraulic redistribution and reverse sap flow, suggesting they rely on storing water deep down in the soil to redraw upon in periods of low rainfall and drought survive (Steggles unpub. manuscript). Hydraulic distribution has also been measured in a range of eucalypt species, and is likely a process used by *E. oleosa and E. gracilis* (Burgess *et al.* 1998; Forster 2014) This suggests the successful establishment of deep root systems is crucial in the long term survival of these species.

The glasshouse emergence and root development experiments provided constrained environmental conditions and examine the intrinsic responses of the seedlings growing and emerging in the different substrates whilst controlling for other factors which might have masked the fundamental differences. The next step for this would to be to investigate if these results are similar under the more unpredictable conditions present at the mine site where climatic variables such as temperature, water and light are more variable.

The first hurdle of any restoration project is the establishment of seedlings, either through emergence from seeds or as planted individuals. This study demonstrated that the substrates used for back filling the open mine are suitable for seedlings to emerge and establish. However this is just the first stage required for establishing a self-sustained population. After establishment, several different challenges can arise. These include the ability of plants to effectively source and store water over dry periods and droughts, and the ability to find sufficient nutrients to continue growth and reach maturity. Long term monitoring and continued research in this area is required to in order to identify whether self-sustained populations of these species are viable, however this research does provide a small but significant initial indication of success.

Importantly, one of the findings of this study is that exposure to elements can remove several compounds deleterious to plants from the substrates (particularly ModCod). Information about the dynamics of leached substances in the reconstructed profile is critical for the future survival and suitability of these species in rehabilitated areas. Understanding whether the salts will leach and accumulate in a particular soil zone or redistribute and surface through

capillary forces may drastically influence the overall success of the site rehabilitation. A final statement on the viability of the revegetation effort will clearly require longer term data.

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Appendices

Appendix A: Specifications for the reconstructed soil depths in the rehabilitation undertaken at JA. Sourced from Goode and Doudle (2009)

Table 4.1 Proposed soil profile prescriptions by Landscape Unit for the Jacinth Mine

Soil materials	Landscape Unit					
	Myall/Mallee Woodland		Myall Woodland		Chenopod Shrubland	
	Depth (m)	Thickness (m)	Depth (m)	Thickness (m)	Depth (m)	Thickness (m)
Topsoil	0.05	0.05	0.05	0.05	0.05	0.05
Subsoil	0.20	0.15	0.20	0.15	0.20	0.15
OB1 – Yellowsand	2.60	2.40	n/a	0.00	n/a	0.00
OB2 – Brown Loam	4.90	2.3	2.5	2.3	0.50	0.30
OB3 – Red Sandy Loam	8.10	3.20	5.75	3.20	1.50	1.00
Capillary Break – Ooldea Sand / suitably graded material*	8.60	0.35 - 0.5	6.25	0.35 - 0.5	2.0	0.35 - 0.5
Co-disposed tailings	to pit floor	Variable	to pit floor	Variable	to pit floor	Variable

* Or alternative demonstrated equivalent technology. The objective of the capillary break is to slow salt migration to 0.5 m over 500 years.

Appendix B: Results of the soil nutrient analyses comparing the ModCod ,shallow reconstructed soil profile and undisturbed area from the field experiment