Jacinth Ambrosia Research and Monitoring Summary (JARMS)

2012 – 2013

ILUKA

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1 INTRODUCTION

Iluka Resource Ltd are pleased to present the progress report of the 2012 and 2013 Jacinth Ambrosia (J-A) research and monitoring programs. This report is supplemental to the J-A Annual Compliance Report, which is submitted annually in March to the Department of Manufacturing, Innovation, Trade, Resources and Energy (DMITRE).

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 ongoing rehabilitation activities at J-A enabling a continuous improvement process.

The projects discussed within this report cover a range of topics, to assist the reader, the report has been assembled so that projects are grouped according to a common theme.

2 SITE CHARACTERISTICS

The Jacinth-Ambrosia (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 2.1) 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).

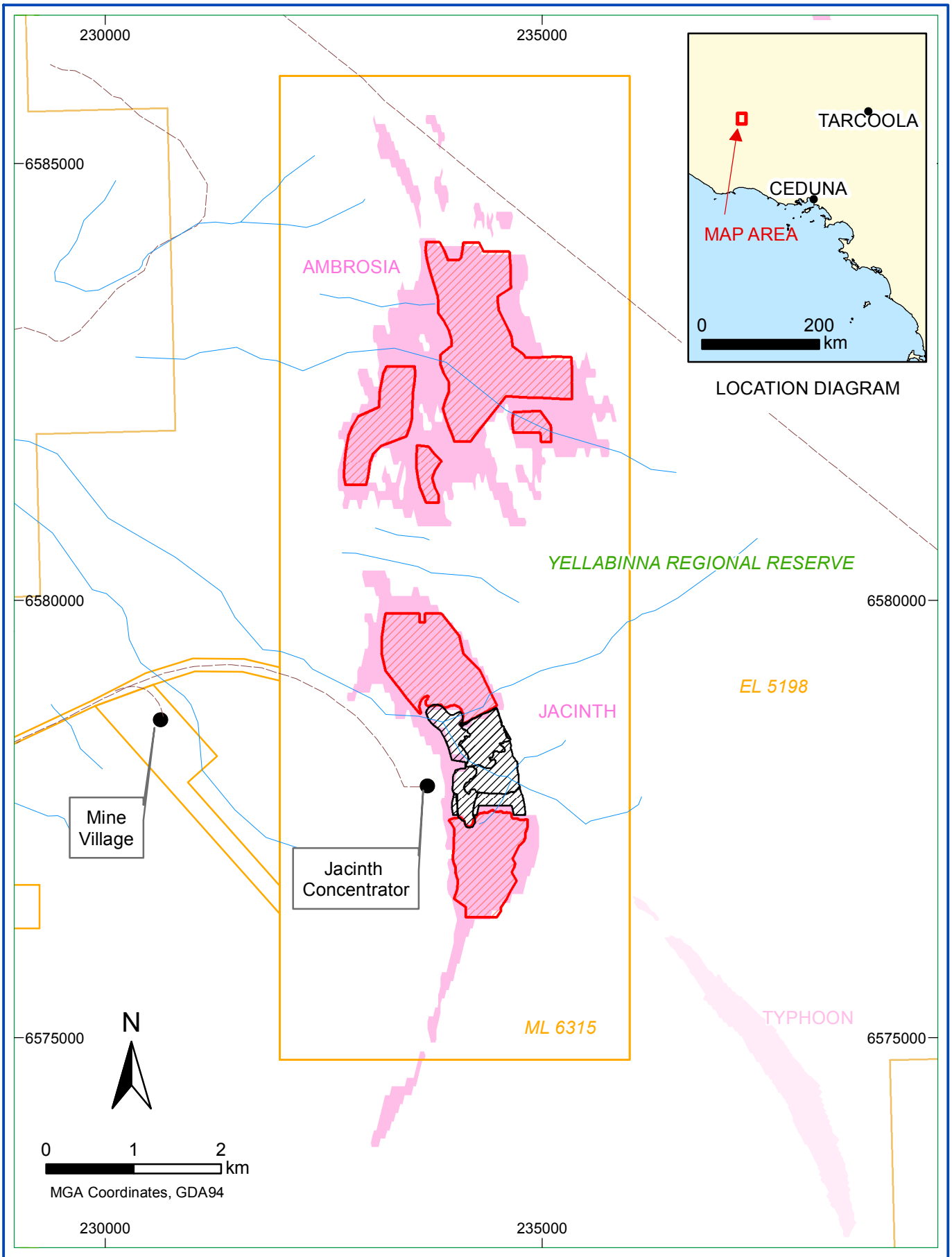
J-A is located within an arid environment and is dominated by chenopod shrubland plains and open myall woodlands interspersed with myall/mallee sand rises and creeks. The plant interspaces are well stabilised with thick and diverse biological soil crusts.

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 2013, a total area of approximately 800 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 7.5 ha has been rehabilitated with another 15 ha planned for rehabilitation in 2014.

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.



Legend

- Iluka Tenements
- Previous Mining
- HM Reserves
- HM Resource

JACINTH & AMBROSIA RESOURCE OVERVIEW PLAN SA OPERATIONS

ILUKA

CHK'D: R Gibson DRAWN: D.Boyd 8/02/2014 SCALE: (A4) DATE: DWG No: 216671v02 1:60,000

FIGURE:2-1

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 DMITRE.

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 3-1.

It should be noted that several other projects are currently in planning or progress at J-A that are not included in this current JARMS. Progress reports on these projects will be included in future JARMS reports.

Table 3-1 Research and monitoring program alignment with MARP closure criteria

Objective	Criteria	Relevant Section
Public Safety		
Ensure that land is physically safe for people to access.	Mine related hazards have been removed from the landscape.	No programs have been established relevant to this matter.
Cultural Heritage		
Ensure that cultural heritage is protected.	Heritage sites and artefacts are not damaged, and any necessary disturbance is in accordance with agreed management plans.	No programs have been established relevant to this matter.
Ecosystem reestablishment		
Pre-existing soil profile and function are reinstated.	Post mining soil profile established in accordance with Table 4.1 and Figure 4.1 of the J-A Rehabilitation Plan.	4, 5, 7
	Rainfall infiltration and soil moisture comparable to analogue sites.	4, 5
	Salt migration into clean soils is restricted	4.4, 5.1, 6.1, 9
	Post mining surface soil function is re-established and is comparable with analogue sites.	4, 5, 7
Habitat re-establishment The post mining ecosystem and landscape function is resilient, self-sustaining and indicating that the pre-mining ecosystem and landscape function will ultimately be achieved.	Placement of leaf litter and logs comparable to analogue sites	5.3

Objective	Criteria	Relevant Section
Flora species diversity The post mining ecosystem and landscape function is resilient, self-sustaining and indicating that the pre-mining ecosystem and landscape function will ultimately be achieved.	An agreed percentage[#] of over story species listed in Appendix A of the J-A Mine Closure Plan (and with consideration for analogue sites) are recorded within rehabilitation areas not subject to tailings backfill, for each landscape vegetation unit.	4, 5, 8,10
	An agreed percentage[#] of over story species listed in Appendix A of the J-A Mine Closure Plan (and with consideration for analogue sites) are recorded within rehabilitation areas subject to tailings backfill, for each landscape vegetation unit.	4, 5, 6, 9
	An agreed percentage[#] of perennial under story species listed in Appendix A of the J-A Mine Closure Plan (and with consideration for analogue sites) are recorded within rehabilitation areas, for each landscape vegetation unit.	4, 5, 8, 10
	An agreed percentage[#] of annual under story species listed in Appendix A of the J-A Mine Closure Plan (and with consideration for analogue sites) are recorded within rehabilitation areas, for each landscape vegetation unit.	4, 5, 8, 10
Flora species abundance The post mining ecosystem and landscape function is resilient, self-sustaining and indicating that the pre-mining ecosystem and landscape function will ultimately be achieved.	Within rehabilitation areas not subject to tailings backfill, projected foliage cover of rehabilitated over story species reaches an agreed percentage compared to analogue sites occurring within the same landscape vegetation unit.	4, 5, 8, 10
	Within rehabilitated areas, projected foliage cover of rehabilitated under story species reaches an agreed percentage compared to analogue sites occurring within the same landscape vegetation unit.	4, 5, 8, 10
No net adverse impacts from site operations on native fauna in lease area and adjacent areas.	Vegetation strata identified at analogue sites occur within the rehabilitation area. Fauna recovery at closure (at control sites) to be consistent with baseline data and indicating positive trends (as per the Fauna Management Plan)	4, 5, 8, 10

Objective	Criteria	Relevant Section
Landform Stability		
Ensure that landforms are compatible with surrounding topography	Landform shape and slopes are compatible with surrounding topography.	5
	No slope > 10% variance from agreed landform design.	5
	No landform elevations > 200m AHD.	5
	Drainage channels re-established and pre-mining catchment flows are restored.	5.1
	Erosion rate comparable with pre-mining landscape as surface soils and vegetation re-establishes.	5

To be determined in consultation with the regulatory authorities, and based upon (1) known keystone species, and (2) propagation difficulty of species in place based on observations (pre-mining and analogue sites).

4 AUSTRALIAN RESEARCH COUNCIL LINKAGE PROJECT

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4.1 Introduction and Research Aims

A partnership was formed in September 2011 between Iluka Resources Ltd. and the University of Adelaide with the purpose of researching plant-soil-water relations at the Jacinth-Ambrosia (J-A) mine site. The primary objective is to provide information to assist rehabilitation managers at J-A to return ecosystem and landscape function to mine-affected areas that will be both resilient and sustainable. Initially the partnership focussed on conducting a series of pilot studies to trial different methods and technologies in preparation for a larger Australian Research Council Linkage Project (ARCLP). In June 2012, the Australian Research Council announced successful projects including the University of Adelaide application titled: "*Root distribution and salinity and soil water dynamics in a chenopod shrubland: implications for restoration ecology*". This was a considerable achievement when considering a total of 504 applications were submitted from around Australia with a range of research focuses, including medicine, arts and engineering. Of these applications, 185 projects (36.7%) were successful, with only 15 of these based in South Australia.

The ARCLP commenced in October 2012 and is due to complete in October 2015. The University of Adelaide research team includes Associate Professor José Facelli who specialises in arid zone ecology, Associate Professor Jennifer Watling whose area of expertise is plant eco-physiology and Professor David Chittleborough who specialises in soil science. The primary investigator is Dr Emma Steggles who recently completed her PhD which focussed on soil seed bank dynamics and seed germination in Western myall open woodland at the J-A mine site. Dr Kate Holland, an EcoHydrologist in the Hydrology Group at CSIRO Land and Water has also recently joined the group. The Iluka research team includes Samantha Doudle (Rehabilitation Specialist 2009-2013) and more recently Tina Law and Joanne Lee (Rehabilitation Specialists). Iluka Resources also provides technical support for the project and acknowledgements of their contributions to the project are provided in each research area.

The goal of the ARCLP is to study the root distributions and plant-soil-water relations of key plant species at the J-A mine site. The mine site is located in the far west of South Australia, and the plant community consists of *Acacia papyrocarpa* (Western Myall) open woodland with chenopod understory. The research has three main aims:

1. To investigate the spatial distribution of roots and how this relates to soil characteristics for key plant species at J-A.
2. To examine soil-water relations for key plants species at JA.
3. To determine the responses of key species to water availability and increased salinity in natural and reconstructed soil profiles.

The first research area offers a unique opportunity to gather critical information about root structure and soil water dynamics in an ecological context, at depths difficult to reach in a regular research project. Progressive mapping and soil sampling of plant roots to match overburden removal schedules will allow roots, soil and water to be sampled to a degree of detail unequalled in any previous research. A combination of techniques are being employed, including classical root mapping, air spading, molecular fingerprinting of roots,

characterisation of water in plants, root and soil using stable isotope analyses, and eco-physiological measurements of plant performance. This will provide a complete model of water usage in the system to determine how water utilisation relates to plant performance and the nature, distribution and significance of nutrients in plant growth.

From the rehabilitation point of view, this project will provide urgently needed information. Many new mining projects are being developed in the arid lands of southern Australia. As mine lease conditions and approvals require the restoration of vegetation to enable a stable landscape and a sustainable ecosystem, it is necessary to understand how the various species will perform in different reconstructed soil profiles. Early decision and planning will ensure effective and efficient mine closure. By determining which species rely primarily on surface or shallow stores of water, we can identify those less likely to be affected by hypersaline tailings (or tailings affected by other stress factors) buried within the reconstructed soil profile.

The second research area focuses on sources of water used by key plants species and their plant-soil-water relations. Roots of deep-rooted species will need to penetrate through the hypersaline zone to access ground water for their long-term survival. Deep rooted species, such as western myall (*Acacia papyrocarpa*) and red mallee (*Eucalyptus oleosa*), may employ hydraulic redistribution (HR) to store water deep within the soil profile for use during dry seasons and severe droughts. This process plays an important ecological role because it enables roots in near-surface horizons to subsist through dry periods and also makes water available to associated understory vegetation. Identifying HR in these two dominant species is critical for rehabilitation because modified soils and tailings may not have the same properties to enable water storage.

The third component of the project will provide important information on how reconstructed soil profiles and hyper-saline tailings will potentially affect the establishment, growth and long-term survival of key plant species. Results from salinity and water potential measurements will help to identify those species or genotypes that may be more salt tolerant and therefore more likely to prosper under higher salinity levels.

The following sections provide a summary of project results to date.

4.2 Spatial distribution of roots and soil characteristics

The underground component of ecosystems can be considered the last frontier in terrestrial ecology and despite repeated calls to intensify the research of the underground environment, our understanding of below-ground processes remains shallow. Most studies seldom investigate depths beyond 2 m or if they do then they are schematic at best. Indeed, the detailed study of root distribution and deep soil water dynamics is often considered intractable due to logistic considerations; however, open-cut mining offers exciting possibilities to study deep-rooted systems. Previous root mapping work at J-A has shown there is a discrepancy between the pre-disturbance soil profile depth utilised by western myall and the proposed post mining soil profile depth, raising questions about whether western myall and other deep-rooted species can subsist in the much shallower overburden profiles. The ARCLP aims to assess the vertical and horizontal root distribution of key species and correlate root density and distribution with soil profile and soil water properties. During 2012 and 2013, the focus was on developing research techniques in three main areas to help investigate plant root distributions at JA:

1. Mapping root architecture.
2. Identifying roots at depth;
   - Anatomical and morphological characterisation of roots through microscopy;
   - Root identification and root presence in mixed soil samples through molecular techniques.

3. Characterising soil chemical and water properties.

4.2.1 Mapping root architecture

A step system was trialled in November 2011 (Figure 4.1) with the purpose of providing access to root samples at depths greater than 2 m below surface. The motivation for trialling a step system was based on previous steps established in the corner of the mine pit (Cell 1) in 2010, which enabled safe access to root samples at incremental depths down to 30 m below surface. Unfortunately the trial steps were unsuccessful because they lacked stability and they had to be filled in to a depth of 3.5 m. They differed from the steps in Cell 1 which were stable due to their position in the corner of the pit wall and the heavily battered slope of the wall. The trial steps did however provide a good opportunity to sample a range of roots where parent plants were known, and these were used to develop methods for using molecular and microscopy techniques for identification purposes. Root cross-sections and external characteristics were also photographed, measured and recorded in a data base.


(a)

(b)

Figure 4.1 (a) Excavation of steps during 2011 (b) exposed vertical roots

A new approach to access root material at depth was developed whereby roots are progressively mapped and soil sampled to match the mine overburden removal schedule. An opportunity to start testing this new approach arose in May 2012 ahead of clearance in an area referred to as Cell 1 West (Figure 4.2). At this site, a mature Western myall tree growing along a section of creek line had exposed roots along its southern side (Figure 4.3). A differential GPS (Trimble 5800 and TSC3 controller) was used to pinpoint x, y and z coordinates of the roots, with particular emphasis placed on capturing vertical root positions to assist future relocation during over-burden removal. Root samples, photographs and information on root diameters and bark descriptions were also collected to assist future relocations. A small excavator was used to trace one of the primary lateral roots out from the base of the tree in a north-easterly direction. Unfortunately due to time constraints prior to clearance the root had to be abandoned before knowing its full lateral extent. We can however, confirm that primary lateral roots of western myall can extend greater than 20 m from the base of the tree (Figure 4.4).

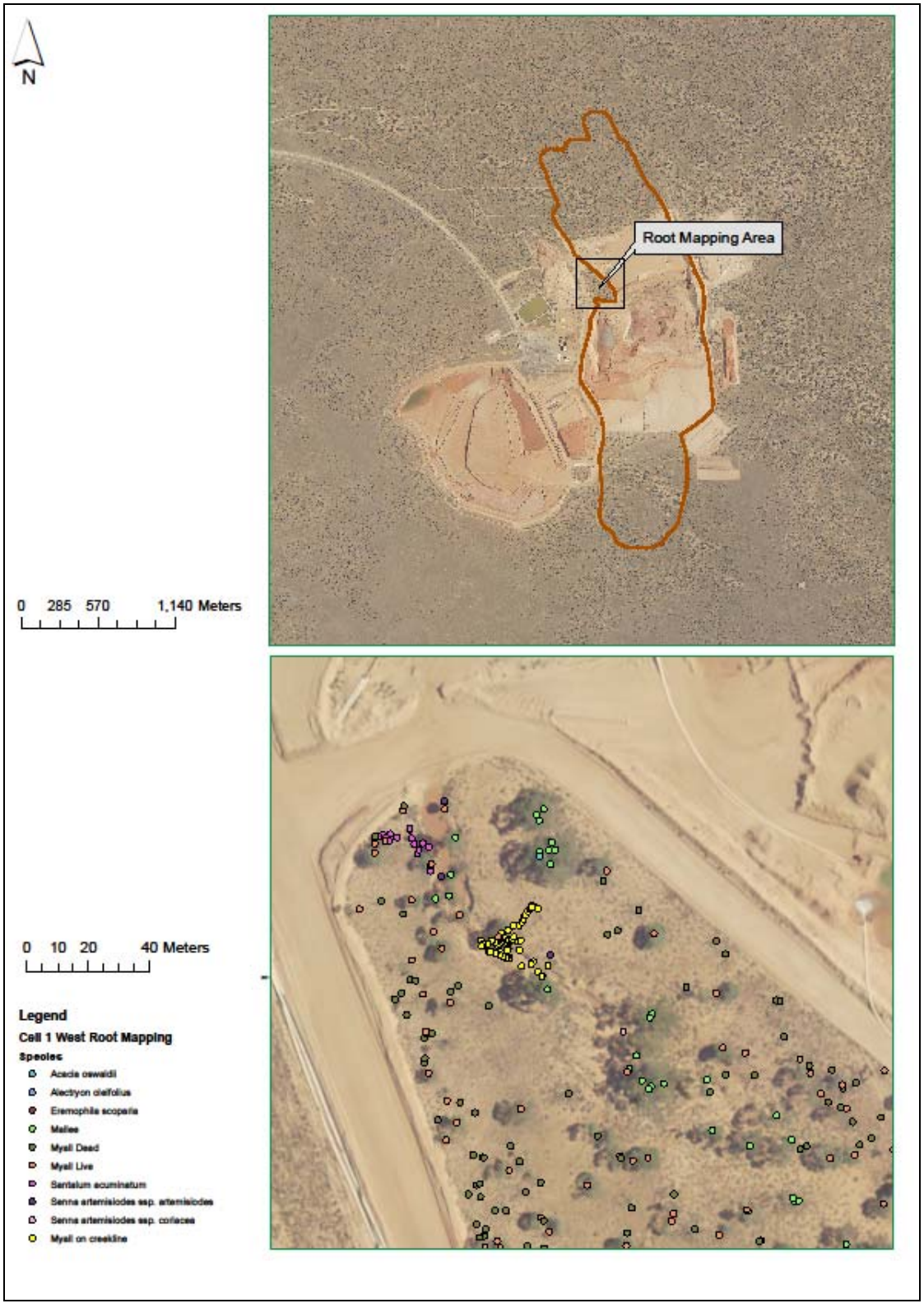

Figure 4.2 Location of root mapping area in Cell 1 West (top image) and GPS positions of all trees and large shrubs prior to clearance. A western myall primary lateral root, represented by yellow dots, extended more than 20m NE from the base of the tree (bottom image).


(a)


(b)


(c)

Figure 4.3 (a) Western myall roots exposed in a section of creek line in Cell 1 West (b & c) surface lateral roots turning vertical into the soil


(a)


(b)


(c)

Figure 4.4 Excavating and tracing western myall surface lateral roots

In addition to tracing the roots of each western myall tree, a GPS location and species identification was recorded for every tree in the Cell 1 West area (Figure 4.2). The survey was taken at the base of each tree trunk with the hope that tap roots may be located progressively as over-burden was removed, or roots could be assigned to the nearest likely tree. Some success was had with relocating roots during July and August 2012, however unforeseen changes to the overburden removal method for this particular area meant that we were unable to continue relocating roots. Although there has been no further progress made in mapping roots during overburden removal, opportunistic root and soil samples are continually being collected from the mine pit to build a data base of information which includes root physical characteristics, rooting depths and their positions as well the soil properties associated with each root specimen such as water and salt content. This database provides valuable information for the Iluka rehabilitation team.

In 2012 an air-spade was trialled at J-A to determine whether it could be used to expose root systems without damaging them and therefore assist with the relocation of roots for root mapping (Figure 4.5). The air-spade is produced by Air-Spade Technology, Verona, PA, USA and distributed in Australia through Drillers World, NSW. It is powered by an air compressor supplying air at 0.8 m3 s±1 and a pressure of 0.6 MPa (air stream with a speed of Mach 2). The tool uses a supersonic air stream that passes over smooth objects (such as a stone or root) but when it comes in contact with soil pores, air is compressed in it and the pore splits open (Nadezhdina and Cermak 2003). Soil is therefore blown away and the roots and other smooth objects remain undamaged, and this includes fine roots. This method was found to be suitable for root mapping work at J-A because it worked extremely quickly in the light soils and was much faster than hand-excavation. The technique has been previously used to excavate *Eucalyptus* spp. roots at Lake Chillinup in the South Stirlings region in Western Australia (Pate and Verboom 2009). An air-spade was purchased for the project and has been useful for a range of activities such as exposing western myall roots to enable the installation of sap flow meters as part of hydraulic redistribution studies.

(a)

(b)

Figure 4.5 (a) Use of an air-spade at JA (b) exposed surface roots of a western myall

Acknowledgements

Shane Doudle (Iluka Technical Officer) excavated and assisted with the mapping of Western myall roots near the Cell 1 West creek line and also collected GPS positions of trees in Cell 1 West. Shane, Con Miller and Kerry Saunders (Iluka Technical Officers) helped to relocate roots in July and August 2012.

4.2.2 Plant root identification by DNA sequencing and determining species root presence from soil samples using molecular techniques

Phase 2 Summary Report: January – August 2012

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Introduction

Plant investments belowground are generally more than half of total plant biomass (Caldwell and Richards 1986) and this makes understanding root structure and function critical to managing and restoring native ecosystems. This research formed part of a series of pilot studies to investigate methods for mapping root architecture and soil properties associated with key species at the Jacinth-Ambrosia (J-A) mine site. Root mapping involves the gradual excavation and retrieval of roots at various depths and where possible, the tracing of roots back to the above ground portion of the plant for positive identification. As this is not generally feasible for layers deeper than 2 m, molecular techniques were investigated as a potential means to identify and quantify roots in mixed samples.

The mine site is located in the far west of South Australia and the plant community consists of *Acacia papyrocarpa* (western myall) open woodland with chenopod understory. The dominant plant taxa of particular interest at the site include: *Acacia* and *Senna* spp. (Leguminosae); *Eucalyptus oleosa* (Myrtaceae); *Alectryon oleifolius* (Sapindaceae); *Myoporum platycarpum* (Myoporaceae); *Santalum* spp. (Santalaceae); and *Maireana*, *Atriplex* and *Chenopodium* spp. (Chenopodiaceae). Outcomes from this research will be used to aid the characterisation of J-A perennial vegetation into deep (5 m+), medium (1-5 m) and shallow rooted (< 1 m) species and help determine which species play important ecosystem roles in the undisturbed environment.

Although plant roots have been formally studied for over 250 years, the primary focus has been on relatively shallow-rooted crop species (Jackson et al. 1996). Very little is known about the distribution of roots in natural ecosystems due to the difficulty of distinguishing between species at depth (Mommer et al. 2011). Morphological identification has been used in some studies but is relatively limited, only differentiating between two (Genney et al. 2002; Mommer et al. 2012) or three species (Janecek et al. 2004). Biochemical techniques such as near infrared reflectance spectroscopy and plant wax markers (Dawson et al. 2000; Roumet et al. 2006) have been used successfully to identity and quantity species in mixed root samples; however, these methods are best suited to controlled experiments with low variability in soil chemistry, plant age and plant species richness (Mommer et al. 2011).

DNA-based techniques are revolutionising the way roots are being studied, particularly in the area of identification and quantification from mixed root samples. The first research to be published using DNA based techniques for plant root identification was Jackson et al. (1999) and Linder et al. (2000). DNA was extracted from tree root fragments and species identified using species-specific regions of the DNA (internal transcribed spacers) which they compared to a reference database. Quantitative real-time PCR on species-specific markers has also been used to identify species and quantify species abundances in mixed root samples, with relative abundance translatable to root biomass (Mommer et al. 2008; Haling et al. 2011). These techniques have been applied to root studies in diverse plant communities (Mommer et al. 2010; Kesanakurti et al. 2011) and have also been used to discriminate among roots of different individuals of the same species (Saari et al. 2005; Lang et al. 2010). Mommer et al. (2011) provides a good discussion on the current state of

molecular techniques used for plant species identification and quantification from mixed root samples including work done on ancient plant DNA. This work has led to the development of protocols and commercially available kits that have been specifically designed for degraded and/or old woody root tissues (Parducci and Petit 2004; Gugerli et al. 2005; Finkeldey et al. 2010).

This report summarises the molecular work undertaken during Phase 2 of the Iluka Resources Ltd. – University of Adelaide Rehabilitation Partnership between January and August 2012. Research aims were to (1) identify plant roots through DNA sequencing and (2) determine species root presence from soil samples using molecular techniques.

Methods

Plant root identification by DNA sequencing

In November 2011, a series of steps were excavated adjacent to mature *Acacia papyrocarpa* and *Eucalyptus oleosa* trees at the Jacinth-Ambrosia mine site. These steps enabled roots to be sampled to an approximate depth of 3.5 m and traced back to individual plants. A range of different root specimens were collected at this time and transported to the University of Adelaide where they were stored on silica gel prior to DNA extraction. A separate selection of air-dried roots collected from the floor of the mine pit in 2009/2010 (S1-36, R36, R39, R46, S58 and S62) were also sent for identification.

The outside of roots were cleaned with 100% ethanol (EtOH) to remove soil and contaminating DNA. Roots greater than 5 mm in diameter were dissected to extract DNA from the cambium tissue. DNA was extracted using the DNeasy Plant Mini kit (QIAGEN) using the manufacturer's protocol, with the exception that tissue was not frozen with liquid nitrogen prior to bead-beating in a Retsch mill (2 x 1 min at 25 Hz), as this did not affect the potential to PCR-amplify the resulting extracts. DNA was extracted from leaf tissue from plant voucher specimens using the same method. In order to reduce costs, tungsten carbide beads were recycled for DNA extractions. We found that washing with 5% decon and 100% EtOH was insufficient to remove contaminating DNA from beads, leading to contamination of subsequent DNA extracts (e.g. PCR products amplified from extraction blanks, multiple bases at a single position in the electropherogram). Washing beads for 1 minute with 0.4 M HCl led to no visible products in extraction blanks and clean sequences. DNA extractions were performed in a dedicated pre-PCR lab.

The internal transcribed spacer 2 (ITS2) was PCR-amplified using a plant-specific forward primer (ITS2P, Hugh Cross, unpublished data) and ITS2 S3R (Chen et al. 2010). PCR amplification was performed in a reaction mix containing: 1.5 mM $MgCl_2$, 1 mM dNTPs, 5 pmol each of forward and reverse primer, 0.5 U AmpliTaq Gold DNA polymerase in 1 x reaction buffer (Applied Biosystems, Melbourne, Australia), and 1 µL DNA extract in a total reaction volume of 10 µL. Reactions were PCR-amplified on a DNA Engine Tetrad 2 (Bio-Rad Laboratories, Gladesville, Australia) using a thermal cycle of 10 min at 94 °C, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min.

PCR products were separated by electrophoresis and visualised on 2% agarose gel, then cleaned using ExoSAP-IT (USB Corporation, USA). Cycle sequencing was performed using a program of 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Extension products were purified on a MultiScreen SEQ_{384} filter plate (Millipore, Kilsyth, Australia) using a standard vacuum clean-up protocol, and sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Individual sequences were assembled into contigs, edited and a consensus sequence produced using Geneious Pro 5.4.6 (Drummond et al. 2011). Putative identifications for each consensus sequence were generated by

performing a local BLAST search against a reference DNA sequence database generated from plant voucher specimens from the Jacinth-Ambrosia site.

Next generation sequencing of soil DNA to identify roots present

Due to the difficulty of distinguishing roots of different plant species, especially fine roots, it is often unclear how many different roots from a soil sample should be analysed to determine the species present. An alternative to Sanger sequencing DNA extracts from individual root samples is to use next-generation sequencing (NGS) to characterise the DNA present in a bulk sample. For example, extracting and sequencing the DNA from a soil sample with roots of 10 different plant species present could reveal the identity of those 10 species.

To test this approach, we collected soil samples from beneath an *Acacia papyrocarpa* tree in a well-characterised plant community at the Jacinth-Ambrosia mine site. A plant list was compiled of the species of sub-shrubs and forbs growing beneath the canopy of the tree, including those plants that could be identified by remnant material. Plant species were also included up to 5 m outside the canopy edge. Ten replicate sets of soil samples were collected from around the tree at the canopy edge. Each replicate consisted of four depths: 3 – 11 cm, 20 – 28 cm, 50 – 58 cm and 100 – 108 cm (i.e. allowing for the diameter of the rings). Sampling commenced 3 cm below the surface to avoid the inclusion of seeds, as the majority of the soil seed bank is known to occur within the top 3 cm of the soil profile (Pake and Venable 1996; Guo et al. 1998) and to avoid visible litter.

Before sampling, a fresh profile was obtained by scraping the soil with a trowel. Bulk density rings (8 cm diameter) were then pushed into the side of the soil profile at a pre-measured depth and extracted with the trowel. Decon 90™ (5% v/v) followed by ethanol (EtOH 95% v/v) was used to decontaminate cores and equipment between each sample collection. Soil samples were stored inside two plastic zip- lock bags with orange indicating silica gel prior to processing.

Samples were then sieved and macroscopic roots were separated and identified by sequencing the ITS2 locus as described above. We extracted DNA from ca. 0.25 g sieved soil from each sample using a MoBio PowerSoil kit following the manufacturer's protocol. We PCR-amplified the *trn*L P6 loop using the primers *trn*L c and h (Taberlet et al. 1991; Taberlet et al. 2007) modified to include the Ion Torrent sequencing adaptors and a seven base pair molecular identification (MID) tag specific to each sample. The *trn*L P6 loop was used in preference to ITS2 for the soil samples as it is shorter (ca. 150 base pairs versus 400-500 base pairs), and more likely to amplify degraded DNA present in a soil sample.

PCR amplification was performed in a reaction mix containing: 1.5 mM $MgCl_2$, 1 mM dNTPs, 5 pmol each of forward and reverse primer, 0.5 U AmpliTaq Gold DNA polymerase in 1 x reaction buffer (Applied Biosystems, Melbourne, Australia), and 1 µL DNA extract in a total reaction volume of 10 µL. Reactions were PCR-amplified on a DNA Engine Tetrad 2 (Bio-Rad Laboratories, Gladesville, Australia) using a thermal cycle of 10 min at 94 °C, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Triplicate PCR products for each sample were pooled and cleaned using the Agencourt AMPure XP PCR Purification kit (Beckman Coulter Genomics, NSW), and quantified using the NanoDrop 2000 (ThermoScientific). Products were pooled in equimolar concentrations. Amplicon libraries were prepared for sequencing using the Ion OneTouch system (Life Technologies) at the Australian Genome Research Facility (AGRF). Enriched Ion Sphere particles were loaded on a 314 Ion semiconductor chip and sequenced on the Ion PGM at AGRF.

Bioinformatics processing

Ion PGM reads were processed using the USEARCH clustering algorithm (Edgar 2010) and Geneious 5.6.3 (Drummond et al. 2011) for all other steps. DNA sequences were sorted by MID tag, retaining only sequences perfectly matching the MID and primer, as errors in the barcode and primer increase the likelihood of errors in the rest of the sequence. Primer sequences and regions with more than a 5% chance of an error per base were trimmed from the 5' and 3' ends of the sequence reads. Sequences were sorted by length and reads less than 100 bp were discarded. The USEARCH program was used to cluster sequences sharing 98% identity, and a consensus sequence produced for each cluster. Clusters containing only one read ('singletons') were discarded.

Putative identifications for each consensus sequence were generated by a local BLAST search against a *trn*L reference DNA sequence library of 44 species. Seven sequences were generated *de novo* for taxa whose roots were known to be present in the soil samples (Table 4.3). To reduce the computational load, one BLAST hit was retained per cluster, and only when the E-value was less than 1×10^{-50} and the pairwise identity was equal to or greater than 97%.

Results

Plant root identification by DNA sequencing

The ITS2 reference DNA sequence database created for the site contained 68 sequences representing 57 species, including the two subspecies of *Eucalyptus oleosa* (ssp. *ampliata* and *oleosa*) and two subspecies of *Senna artemisioides* (ssp. *coriacea* and *petiolaris*). Reference sequences were generated for 37 taxa *de novo*, the remainder were sourced from GenBank. Each taxon possessed a unique ITS2 sequence, with the exception of *Maireana georgei* and *M. trichoptera*. It was not possible to definitively distinguish the two *S. artemisioides* subspecies [potentially two substitutions, resequence to confirm. N.B: A single substitution (A>G) between *E. oleosa* subspecies, a single substitution (T>C) between the two *Sclerolaena* species.]. Several species represented by more than one sequence in the database showed intra-specific variation in ITS2 (*Alectryon oleifolius*, *Ptilotus obovatus*, *Salsola kali*, *Santalum spicatum* and *S. acuminatum*).

The 27 root samples processed resulted in 25 putative identifications (Table 4.1). One sample (27c) failed to PCR-amplify. This can occur due to PCR inhibitors in the DNA extract (often apparent from coloured extracts) and is typically due to excess plant material overloading the DNA extraction columns. For this reason, we recommend processing no more than 20-30 mg root tissue in each DNA extraction. Another sample (23) produced a very low quality sequence due to a degraded PCR clean-up reagent (ExoSAP-IT) and could not be identified. The sample was not repeated as the roots had been traced back to the plant to confirm the species identification (*Acacia papyrocarpa*).

Table 4-1 Species identifications based on internal transcriber spacer 2 (ITS2) DNA sequences for root samples from J-A mine site

Sample	ACAD no.	Details	PCR	Seq	Putative ID	E-value	Pairwise ID (%)
5	12062	10 mm woody roots, was 12009	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	99.5
23	12064	2-3 mm roots, 12011?	y	poor	-		
27a	12066	2-3 mm roots	y	y	*Chenopodium curvispicatum*	0	100
27b	12067	2-3 mm roots	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	100
27c	12068	Fine (1 mm) roots	n	n	-		
27d	12069	Fine (1 mm) roots	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	100
27e	12070	5 mm woody roots	y	y	*Rhagodia spinescens*	0	99.8
27f	12071	5 mm woody roots	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	100
27g	12072	10 mm woody roots	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	100
32	12073	5 mm woody roots	y	avg	*Eucalyptus oleosa* ssp. *oleosa*	0	100
34	12074	2-3 mm roots	y	avg	*Chenopodium curvispicatum*	6.49E-159	100
35	12075	5 mm woody roots	y	avg	*Alectryon oleifolius*	5.92E-180	98.8
S1-36	12158	2-3 mm roots	y	poor	*Acacia papyrocarpa*	2.73E-147	94.7
R36	12358	thick roots, >5 m deep	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	100
R39	12359	thick roots, >5 m deep	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	99.5
R46	12360	thick roots, >5 m deep	y	y	*Eucalyptus oleosa* ssp. *oleosa*	0	99.5
S58	12361	thick roots, >5 m deep	y	y	*Acacia papyrocarpa*	0	99.8
S62	12362	thick roots, >5 m deep	y	poor	*Eucalyptus oleosa* ssp. *oleosa*	7.68E-166	100

Next generation sequencing of soil DNA to identify roots present

From the vegetation survey, nine plant species were recorded beneath and adjacent (within 5 m) the Western myall canopy: (1) *Atriplex vesicaria* ssp. *variabilis*; (2) *Maireana erioclada*; (3) *M. integra*; (4) *M. sedifolia*; (5) *M. trichoptera;* (6) *Chenopodium curvispicatum*; (7) *Salsola australis*; (8) *Sclerolaena obliquicuspis*; and (9) *Austrostipa nitida*. Sanger sequencing of nine roots separated from the soil samples identified the presence of seven

taxa (Table 4.2), including four species in the 3-11 cm sample (My1R1-A), two taxa in the 20-28 cm sample (My1R1-B) and only *Acacia papyrocarpa* at 50-58 cm depth (My1R1-C). No macroscopic roots were present in the 100-108 cm soil sample (My1R1-D). Visualising PCR products of the four soil DNA extracts on agarose gels gave similar results, with strong products for My1R1-A and B, weak products for C and no visible products for D.

Table 4-2 Species identifications based on Sanger sequencing of sieved root samples from J-A mine

Sample	ACAD no.	Details	PCR	Seq.	Putative ID	E-value	Pairwise ID (%)
My1R1-A1	12194	3-11 cm deep, Coarse root/twig	y	y	*Maireana radiata*	0	99.9
My1R1-A2	12195	3-11 cm deep, dark "curvy" root	y	avg	*Atriplex vesicaria*	0	99.8
My1R1-A3	12196	3-11 cm deep, pale root	y	y	*Acacia papyrocarpa*	0	99.5
My1R1-A4	12197	3-11 cm deep, long straight twig/root	y	y	*Acacia papyrocarpa*	5.49E-179	99.5
My1R1-A5	12198	3-11 cm deep, fine roots	y	y	*Sclerolaena uniflora*	0	98.6
My1R1-B1	12199	20-28 cm deep, thick roots, pale (Myall?)	y	y	*Maireana georgei* or *M. trichoptera*	0	100
My1R1-B2	12200	20-28 cm deep, fine roots	y	y	*Atriplex vesicaria*	0	99.5
My1R1-C2	12206	50-58 cm deep, fine roots	y	y	*Acacia papyrocarpa*	0	99.5
My1R1-C3	12206	50-58 cm deep	y	y	*Acacia papyrocarpa*	1.46E-179	99.5

Next generation sequencing on the Ion PGM produced 40 223 sequences with one of the three sample-specific MID tags; 33 684 of these contained the full correct *trn*L h primer. 26 523 sequences remained after quality trimming and removing reads less than 100 bp. Clustering yielded 881 consensus sequences (My1R1-A: 408, My1R1-B: 312, My1R1-C: 161). BLAST searching against the reference *trn*L database identified 11 taxa in My1R1-A, six taxa in My1R1-B and four in My1R1-C (Table 4.3).

Table 4-3 Species identifications based on next generation sequencing of trnL P6 loop amplified from soil DNA extracts at the J-A mine

Sample (depth)	My1R1-A (3-11 cm)	My1R1-B (20-28 cm)	My1R1-C (50-58 cm)
Taxa present	*M. radiata*	*M. radiata*	*M. radiata*
	Sclerolaena uniflora	*Sclerolaena uniflora*	*Sclerolaena uniflora*
	Acacia papyrocarpa	*Acacia papyrocarpa*	*Acacia papyrocarpa*
	Austrostipa spp.	*Austrostipa* spp.	*Austrostipa* spp.
	Atriplex spp.	*Atriplex* spp.	-

Sample (depth)	My1R1-A (3-11 cm)	My1R1-B (20-28 cm)	My1R1-C (50-58 cm)
	Rhodanthe spp.	-	-
	Acacia oswaldii	-	-
	Cratystylis conocephala	-	-
	Roepera apiculata (Zygophyllum apiculatum)	-	-
	Amyema spp.	-	-
	Vittadinia spp.	-	-
	-	*M. trichoptera*	-
No. taxa	11	6	4

Discussion

Performing a BLAST search against the ITS2 reference database gave E-values (the number of hits expected to be observed by chance when searching a database of a particular size) of zero or close to zero (<1 x 10^{-145}) for each of the 25 sequences, with the percentage pairwise identity greater than 94% in all cases and 99.5-100% for 22 sequences (Table 4.1). Roots were assigned to nine different taxa, with all roots collected from 50 cm below the surface or deeper identified as *A. papyrocarpa* or *E. oleosa* ssp. *oleosa*.

The weakest BLAST hit (lowest E-value and % pairwise identity) was for S1-36, which yielded a very poor quality DNA sequence, most likely due to a contaminated DNA extraction bead. In spite of the poor quality sequence, the E-value was much lower for *A. papyrocarpa* than any other sequence in the reference database, providing some confidence in the identification. My1R1-A5 had a 98.6% pairwise identity to *Sclerolaena uniflora*, but may represent one of the other four congeneric species present at the site but currently not in the reference database. Sample 35/12075 had a 98.8% pairwise identity to *Alectryon oleifolius*. The lower pairwise identity for this sample may reflect the use of a GenBank sequence for *A. oleifolius* in the reference database, and potentially represents intraspecific variation in ITS2 for this species. The low E-value and 100% pairwise identity for S62 demonstrates that in some cases a confident assignment can be made with even a poor quality sequence. However, such assignments should be interpreted with caution as the assigned species could represent contaminating DNA.

In conclusion, restricting the amount of root material in the DNA extraction, the use of adequate bead-cleaning procedures, and an adequate DNA sequence reference database can produce reliable species identifications by sequencing ITS2 of root tissue from the J-A mine site.

All genera identified by direct sequencing of root DNA extracts from the soil samples (Table 4.2) were also identified using NGS (Table 4.3). However, 2.75 to 4-fold more taxa were identified in each sample by NGS than direct sequencing. This could represent an inability to identify and separate roots of distinct species for Sanger sequencing. However, all roots in My1R1-C were morphologically similar, and ITS2 sequences from two separate root DNA extracts from My1R1-C yielded the same species identification (*A. papyrocarpa*).

Given soil DNA extractions were performed on a small amount of material (0.25 g), it seems unlikely that root material from 4-11 species was present in each sample. This is clearly illustrated by the presence of *Amyema* spp., a rootless branch parasite. It is likely that the soil DNA amplified represents not only live roots, but also DNA from leaf litter, seeds, pollen and roots that have leached through the soil profile or from degraded plant material within

the sample that is no longer visible to the naked eye. Indeed, crops cultivated 40-50 years ago in abandoned fields can still be detected in soil DNA by amplifying the *trn*L P6 loop (Yoccoz et al. 2012). Amplification of the *trn*L P6 loop from soil DNA cannot robustly identify live plant roots present within a soil sample. Amplifying a larger locus such as ITS2 may be less prone to amplifying degraded DNA and give a better indication of taxa with live roots in a soil sample. Next generation sequencing could be used to identify species in a bulk root sample containing multiple taxa, although this still requires the relatively labour-intensive step of sieving soil samples.

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4.2.3 Microscopy of root material as a method for species identification

Phase 2 Summary Report: January – August 2012

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Introduction

This report provides a summary of the progress made towards investigating root microscopy as a diagnostic tool to identify plant species. The work was undertaken as part of Phase 2 of the Iluka Resources Ltd. – University of Adelaide Rehabilitation Partnership between January and August 2012. One of the primary aims of this ongoing partnership is to map root architecture and soil properties associated with key species in Western myall open woodland at the Jacinth-Ambrosia mine site in South Australia. This will involve the gradual excavation and retrieval of roots at various depths. Where possible, roots will be traced back to the above ground portion of the plant for positive identification; however, this is generally not feasible for layers deeper than 2 m. Microscopy was therefore investigated as a potential method to characterise anatomical features of the various root specimens for identification purposes. A second approach was to use molecular techniques to distinguish between species, the progress of which forms a separate report.

In a search of the literature, only one paper was found to use microscopic diagnostic features (i.e. starch grains) for identification purposes (Cortella and Pochettino 1994). Often information on the anatomical and histological features in roots is used to assess the stage of a root's development, which influences its activity and moisture and nutrient uptake (Cortella and Pochettino 1994; de Neergaard et al. 2000). Root microscopy is also used to examine external properties such as root hairs, bacteria, polymers and mycorrhiza (Dart 1971; Mendelssohn and Postek 1982; Mast et al. 2009). Methods for studying root anatomy do not generally differ from methods used for plant stems and leaves (de Neergaard et al. 2000) and past research includes both light microscopy and scanning electron microscopy.

Methods – Fixing, embedding and sectioning root samples

In November 2011, a series of steps were excavated adjacent to mature *Acacia papyrocarpa* and *Eucalyptus oleosa* trees at the Jacinth-Ambrosia mine site. These steps enabled roots to be sampled to approximately 3.5 m depth and traced back to individual plants. A range of different root specimens collected at this time were stored in plastic bags and transported to Adelaide Microscopy at the University of Adelaide for processing.

As the penetration of fixative and resin through large tissues is problematic (Waterhouse 2012), only the smaller specimens (< 2 mm diameter) were chosen for microscopy. The sectioning of larger specimens would also have required specialised equipment (i.e. sledge microtome) not currently available through Adelaide Microscopy. Root segments were cut into small lengths approximately 3 mm long. As segments were considered quite large, root lengths were cut longitudinally to assist the penetration of fixative and subsequent chemicals into the samples. Root samples were then fixed in 4% paraformaldehyde / 0.25% glutaraldehyde in phosphate buffered saline prior to embedding.

Fixed root samples were washed with phosphate buffered saline prior to being dehydrated through a series of ethanol solutions (70%, 90% and 100% v/v) with three changes at each concentration every 30 minutes. Root samples were then infiltrated overnight in a 1:1 ratio of ethanol (100% v/v) and LR White (resin). Then samples were infiltrated by 100% LR White, with the resin changed every 2 days for a total of 4 days.

Root samples were embedded in LR White inside individual gelatine capsules. To assist the orientation of each sample, a small piece of paper bent into a 'v' shape was placed inside each gelatine capsule prior to inserting the sample. This enabled root samples to be

orientated such that the cut end was facing the end of the capsule. Polymerisation was achieved by placing gelatine capsules containing samples and resin into an oven at 50°C for 24 hours. The embedded root samples were then ready for sectioning.

To prepare each capsule (block) for sectioning, the block was 'faced' by cutting away the outer gelatine capsule around the embedded root sample using a gem blade. The excess resin at the end of the block was removed until the root sample was reached. Resin around the sides of the root and the paper that was used to assist with sample orientation were also removed to reduce the size of the block face. The block face therefore comprised the root sample with only a small amount of excess resin.

Blocks were then sectioned in a Leica EM UC6 Ultramicrotome using glass knives (8 mm wide glass). The right hand edge of the knife (the least sharp end) was used to smooth off the block face such that a glass-like appearance was achieved, with the block brought forward 0.2 µm at each rotation. Once smooth, the block was positioned to the sharp, left hand side of the block. A drop of water was placed on the knife face within which sections were collected. Sections of 1 µm were collected (transverse sections through the root sample) into the water drop. These sections were then transferred into a water drop on a microscope slide. Once several sections had been collected on one slide, it was then placed onto a heating element to dry the slide and adhere the sections to the slide.

Stained and unstained slides were prepared for each type of root. For the stained slides, toluidine Blue O was applied for approximately 5 seconds and then rinsed off using reverse osmosis (RO) water. These slides were then dried again on a heating element. Cover slips were permanently mounted to both stained and unstained slides.

A total of seven different root samples were processed in this manner. Image analysis was undertaken using bright field and fluorescent light microscopy with an Olympus BX51 microscope and Analysis™ software. Between 2 and 4 capsules were sectioned for each of the seven types of root. The best images were used to analyse cellular and anatomical structures.

Results

Early analysis of images showed that penetration of fixative and resin through small root samples was successful. Samples were cut longitudinally to assist with penetration; however, making these cuts resulted in the 'shredding' of some samples such that cellular integrity was lost, with some cell layers splitting apart. Root samples assessed were approximately 2 mm in diameter or less. It appears that this longitudinal cutting was not required for roots of this size. Hence future processing of similar samples should include cutting some samples longitudinally but leaving some samples whole.

From images obtained it appears that the roots sampled were well developed roots with regard to anatomy; however, it is likely that the age of root samples varied. Hence, differences in anatomy at first-glance may reflect differences in the age of roots rather than true anatomical differences that may exist between species. In order to make comparisons between species, it may be necessary to view samples of similar age and development.

That said, our understanding of root anatomy is still developing and some assistance from experts would assist in interpreting the images that have been collected. The labelling of images below, reflects our best attempts to interpret the anatomy of roots in each image. A glossary of terms relating to root cellular structures is provided below.

Acacia papyrocarpa

The primary xylem consists of protoxylem (smaller cells) and metaxylem (larger cells) and is present in this particular specimen in a pentarch shape (Figure 4.6). This particular root has

undergone secondary growth with secondary xylem forming a ring around the primary xylem. Vascular cambium is present between the secondary xylem and phloem. The phloem includes primary phloem (thin-walled cells), phloem fibres (thickened cell walls, light blue in colour), degenerating cells and pink staining cells. External to the phloem is the endodermis which appears as a very dark blue staining band (2-3 cell layers) of unorganised cells. The cortex appears to be collapsing.

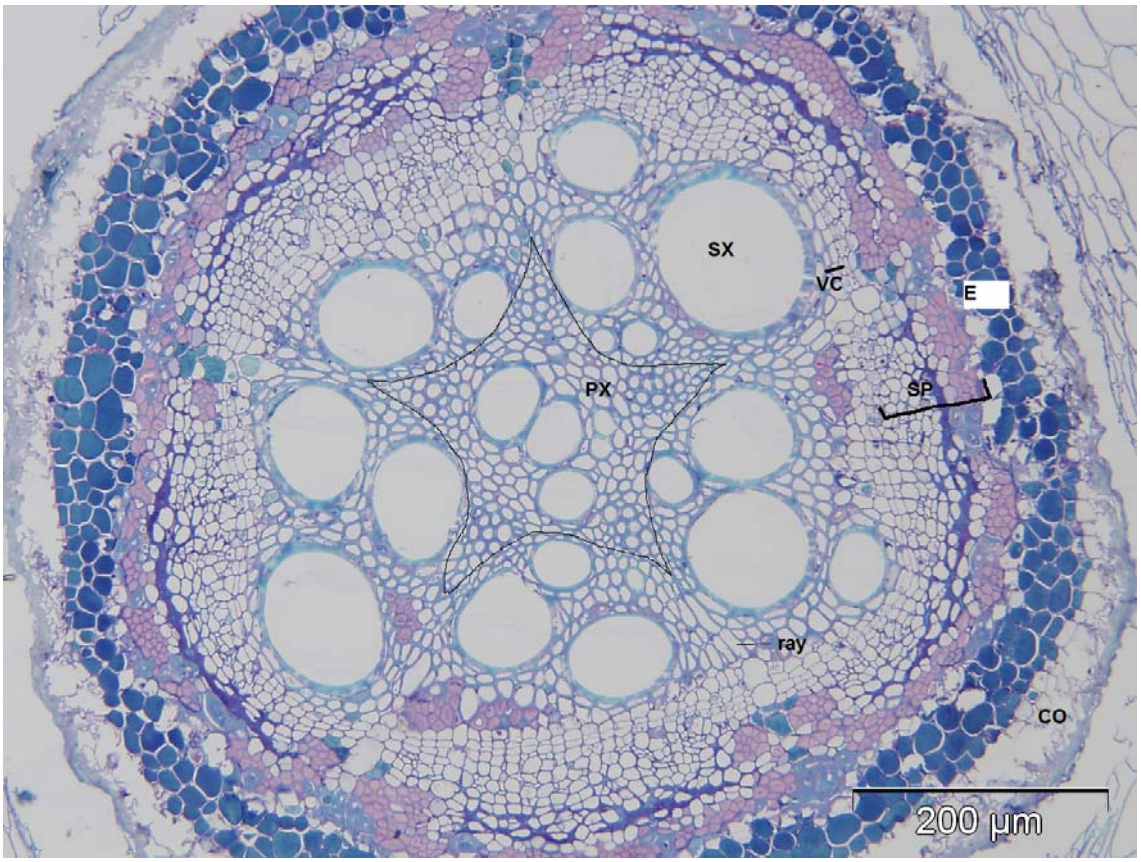

Figure 4.6 *Acacia papyrocarpa* root cross-section. PX - primary xylem, SX - secondary xylem, VC - vascular cambium, SP - secondary phloem, E - endodermis, CO – cortex

Eucalyptus oleosa

The primary xylem consists of protoxylem (smaller cells) and metaxylem (larger cells) and is present in this particular specimen in a tetrarch shape (Figure 4.7). This particular root has undergone secondary growth with secondary xylem present around the primary xylem in an undulating ring. This undulating pattern to the secondary xylem was consistent across three root samples. Vascular cambium is present between the secondary xylem and phloem and appears to be quite a thick layer of cells organised in a linear radial pattern. The band of phloem has very distinct clumps of phloem fibres present. These have thickened cell walls and have stained very dark purple in colour. This observation was also consistent across three root samples. The endodermis appears as a lightly staining band (2-3 cell layers) of cells. The cortex appears to be collapsing.

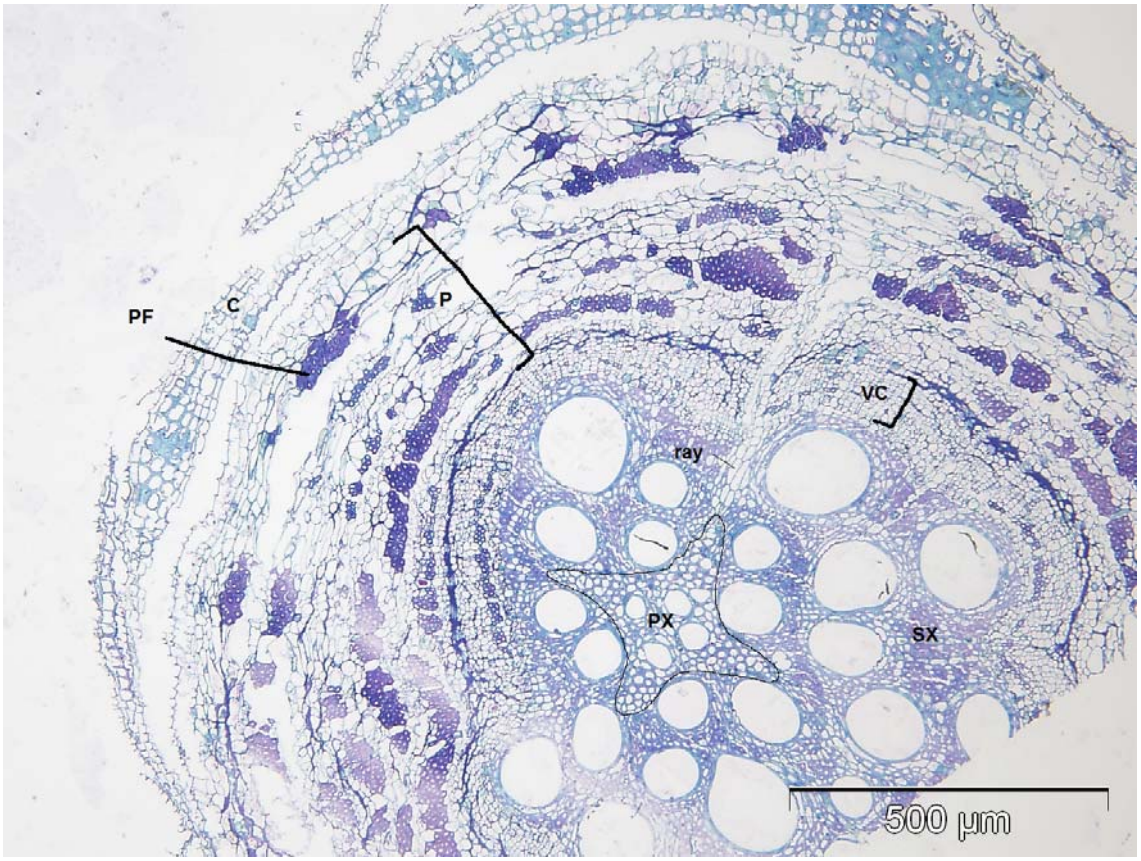

Figure 4.7 *Eucalyptus oleosa* root cross-section. PX - primary xylem, SX - secondary xylem, VC - vascular cambium, P - phloem, PF - phloem fibres, E - endodermis, C – cortex

Chenopodium curvispicatum

The vascular network is present in this species as discrete bundles with the xylem interior to the phloem (Figure 4.8). This network of vascular bundles is present throughout the majority of the root interior. The phloem consists of cells without secondary thickening and also degenerating cells that stain dark purple throughout. There are no obvious phloem fibres and a vascular cambium is not distinguishable. No vascular rays are observable. The vascular bundles are surrounded by a very dark staining, lignified ground tissue (possibly sclerenchyma?). The overall pattern of cellular structure was consistent across two root samples.

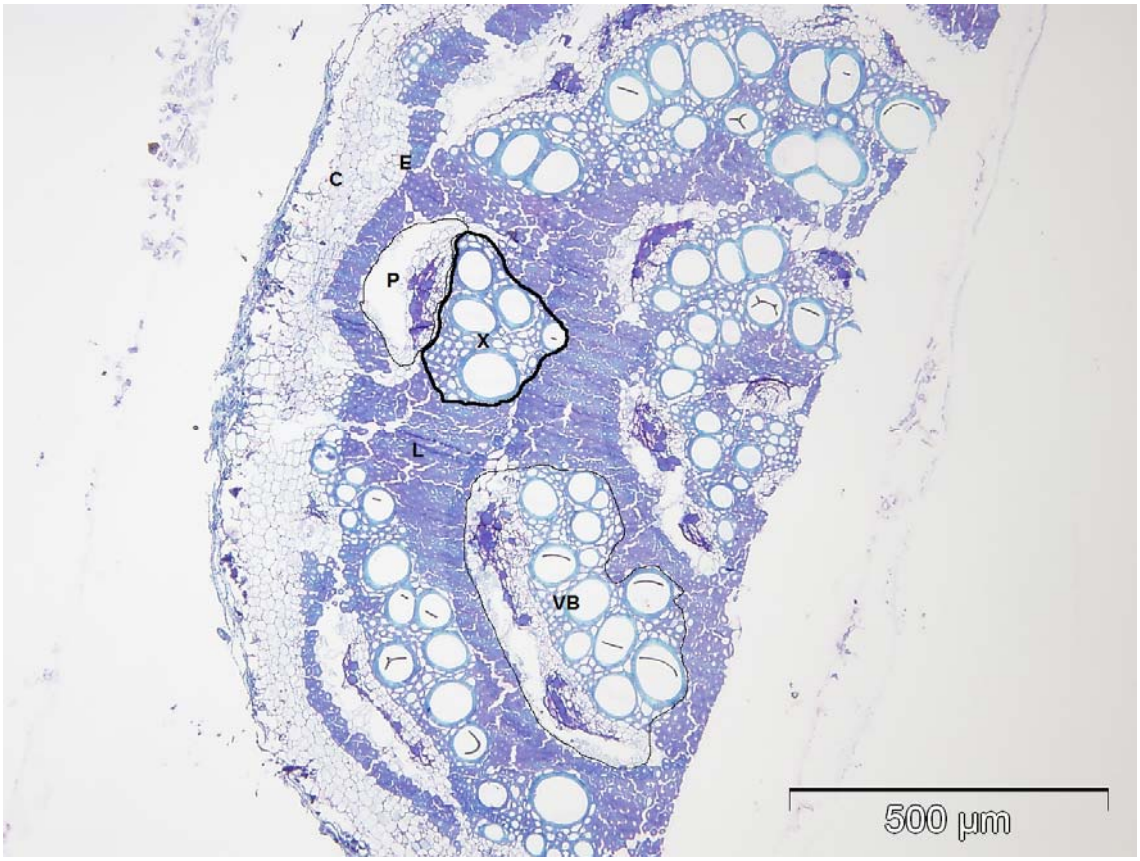

Figure 4.8 *Chenopodium curvispicatum* root cross-section. X - xylem, P - phloem, VB - vascular bundle, E - endodermis, c - cortex

Discussion

This research was undertaken to determine whether microscopy techniques could be used to identify plant roots to species based on anatomy. Patterns in root anatomy were observed between three species and these may potentially be used to differentiate between them; however, a greater sample size would be required to confirm uniformity in observed patterns. It must also be noted that the cellular anatomy of plant roots varies considerably depending on the age of roots, reflecting growth from early development through to secondary growth (Bowes 1996). In order to fully understand patterns present at the species level samples of roots at varying ages need to be assessed. That noted, the main differences between the samples of the three species observed that may be consistent were:

- *Acacia papyrocarpa* - dark blue, solid staining of the endodermis;
- *Eucalyptus oleosa* - secondary xylem present in 'undulating' shape and distinct clumps of dark staining phloem fibres;
- *Chenopodium curvispicatum* - vascular tissue present in discrete vascular bundles surrounded by a solid mass of cells with thickened cell walls that stain darkly.

Fluorescence was found to be useful to help distinguish features not easily detected using bright field light microscopy and is a recommended technique for any further microscopy work. Consideration however, needs to be given to the efficacy of using microscopy as a diagnostic tool, considering the total time and costs required to process seven root specimens in this study (Table 4-4).

Table 4-4 Time and costs involved in processing seven root samples for microscopy

Microscopy Step	Time (hours)	Cost
Sample processing	6.5	
Sample embedding	3.0	
Training for sectioning	2.5	
Block preparation/sectioning/staining	30.0	
Cover slip mounting/bright field microscopy/image capture	5.0	
Image analysis	7.5 (but ongoing)	
Total	**54.5** +	**$3764***

**Total cost consists of salary for one casual technician ($3,075) and a service fee from Adelaide Microscopy ($689).*

The total cost equates to $537 per sample. Most of the cost was for a casual salary for one technician and the remainder covered the service fee from Adelaide Microscopy for the use of laboratory equipment and supplies as well as technical support. This service fee was kept relatively low because Adelaide Microscopy is a research Centre of the University of Adelaide.

Overall, this research study has shown that root anatomy has the potential to be diagnostic; however, the time and costs involved with undertaking microscopy may be inhibitive, especially considering that DNA techniques are increasingly cost-effective (refer to Phase 2 summary report on molecular research). Microscopy cannot produce a practical or quick reference guide for identifying roots in the field and requires skilled technicians with access to specialised equipment.

Nevertheless, the methods and techniques developed in this study may be used to further examine anatomical differences in roots, which may provide insight into the roles of different root types as well as hydraulic conductivity between species. Growing roots undergo anatomical and morphological changes that influence their activity and nutrient uptake (de Neergaard et al. 2000). In particular, hydraulic conductivity and minimum water potential gradients are two parameters that influence the hydraulic properties of a root system. Research done by Rieger and Litvin (1999) found that thinner roots or roots with a thin cortex had higher hydraulic conductivity levels than larger/thinner cortex roots. Also, that the minimum water potential gradients required to induce flow were higher in species with an exodermis in the root radial path. Microscopy may therefore be a useful tool to examine plant-soil-water relationships as part of the continuing University of Adelaide-Iluka Resources Ltd. Australian Research Council Linkage Grant.

Acknowledgements

We would like to acknowledge the advice and technical support provided by Lyn Waterhouse, Agatha Labrinidis and Ruth Williams from Adelaide Microscopy. Thanks also to Gwen Mayo from the Australian Centre for Plant Functional Genomics (ACPFG) for providing initial advice on embedding and sectioning specimens as well assistance with the identification of cellular structures.

Glossary of Root Anatomy Terms

Term	Definition	Reference
Apical meristem	A meristem that is located at the apex or most distal part of an organ.	(Dictionary.com 2012)
Atactostele	The stele of monocots, consisting of a complex, three-dimensional network of collateral vascular bundles.	(Mauseth 2009)
Cork cambium	A sheet-like fundamental type of meristem. The cork cambium (phellogen) produces the cork tissue of the bark (secondary tissue).	(Mauseth 2009)
Cortex	The portion of a shoot (root) between the epidermis and the vascular tissue.	(Dictionary.com 2012)
Endodermis	A specialised tissue in the roots and stems of vascular plants, composed of a single layer of modified parenchyma cells forming the inner boundary of the cortex.	(Dictionary.com 2012)
Epidermis	A thin layer of cells forming the outer integument of seed plants and ferns.	(Dictionary.com 2012)
Eustele	A type of ectophloic siphonostele in which the stele is made up of one ring of vascular bundles that surround a pith.	(Mauseth 2009)
Exodermis	A temporary, protective layer of cells in some roots, as in certain orchids.	(Dictionary.com 2012)
Exodermis	A layer of cells located immediately interior to the epidermis of some roots and having the characteristics of an endodermis .	(Mauseth 2009)
Fibre	An elongated and usually tapered sclerencyma cell with thick, usually lignified, second walls. It is usually dead at maturity.	(Bowes 1996)
Meristem	A cell or group of cells whose principal function is to divide in an organized manner.	(Dictionary.com 2012)
Metaphloem	The primary phloem that forms in a vascular bundle after that portion of the bundle has stopped elongating. The sieve elements are larger than those in the protophloem, and there will be either companion cells or albuminous cells present.	(Mauseth 2009)
Metaxylem	The part of the primary xylem that is the last to be formed, usually having weblike or pitted surfaces.	(Dictionary.com 2012)
Parenchyma cell	An unspecialised, highly vacuolated cell with typically only a primary wall of uniform thickness; it occurs as extensive regions of tissue in the pith, cortex and mesophyll of the plant body.	(Bowes 1996)

Term	Definition	Reference
Pericycle	The outermost cell layer of the stele in a plant, frequently becoming a multilayered zone.	(Dictionary.com 2012)
Phloem	The part of a vascular bundle consisting of sieve tubes, companion cells, parenchyma, and fibres and forming the food-conducting tissue of a plant.	(Dictionary.com 2012)
Primary phloem	Phloem derived directly from the growth of an apical meristem.	(Dictionary.com 2012)
Primary xylem	Xylem derived directly from the growth of an apical meristem.	(Dictionary.com 2012)
Procambium	The meristem from which vascular bundles are developed.	(Dictionary.com 2012)
Procambium	A portion of the sub-apical meristem that produces the cells that mature into the primary xylem and primary phloem. In woody plants, a portion of the procambium is converted into the fascicular cambium.	(Mauseth 2009)
Protophloem	The part of the primary phloem that develops first, consisting of narrow, thin-walled cells.	(Dictionary.com 2012)
Protostele	A vascular cylinder in which the xylem is located at the centre as a solid mass and there is no pith.	(Mauseth 2009)
Protoxylem	The part of the primary xylem that develops first, consisting of narrow, thin-walled cells.	(Dictionary.com 2012)
Protoxylem	The first primary xylem to differentiate within a vascular bundle at any particular level of that bundle. The tracheary elements may be either tracheids or vessels, but they are always relatively small and have only extensible types of secondary wall (annular or helical).	(Mauseth 2009)
Ray	A panel of parenchyma extending radially across the secondary vascular tissues; a ray is formed from an initial in the vascular cambium and is of variable width and height.	(Bowes 1996)
Sclerenchyma	A supporting tissue whose cells are commonly dead at maturity and possess thick, lignified secondary walls, as in fibres and sclereids.	(Bowes 1996)
Secondary phloem	Phloem derived from the cambium during secondary growth.	(Dictionary.com 2012)
Secondary xylem	Xylem derived from the cambium during secondary growth.	(Dictionary.com 2012)
Stele	The central vascular network of the shoot and roots. In the roots, the pericycle and all tissues interior to it are the stele.	(Mauseth 2009)

Term	Definition	Reference
Vascular bundle	A strand of tissue composed of primary xylem and phloem (and in dicotyledons cambium) running lengthwise in the shoot.	(Bowes 1996)
Vascular cambium	A sheet-like fundamental type of meristem. The vascular cambium produces secondary xylem and phloem.	(Mauseth 2009)
Vascular tissue	A general term referring to both the xylem and phloem.	(Bowes 1996)
Vascular tissues	Plant tissue consisting of ducts or vessels, that, in the higher plants, forms the system (vascular system) by which sap is conveyed through the plant.	(Dictionary.com 2012)
Xylem	A compound tissue in vascular plants that helps provide support and that conducts water and nutrients upward from the roots, consisting of tracheids, vessels, parenchyma cells, and woody fibres.	(Dictionary.com 2012)

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4.3 Water sources and plant-soil-water relationships

4.3.1 Preliminary investigation of methods to determine plant water sources at the Jacinth-Ambrosia mine site, South Australia

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Introduction

A pilot study was undertaken at the Jacinth-Ambrosia (J-A) mine site in June 2012 to investigate whether stable water isotopes and plant-soil water potentials could be used to gain insights into water use by *Acacia papyrocarpa* (western myall).The mine site is located in the far west of South Australia and the primary vegetation type impacted by the mine is western myall open woodland with chenopod shrub understorey. This type of open woodland extends along a narrow 200-250 mm rainfall belt starting northwest of Port Augusta and Whyalla in South Australia and fringing the Nullarbor Plain into Western Australia (Johnson and Burrows 2001) (Whibley and Symon 1992) (Figure 2.1). This study has relevance for other key tree species at the site including red mallee (*Eucalyptus oleosa*), bullock bush (*Alectryon oleifolius*), false sandalwood (*Myoporum platycarpum*), umbrella wattle (*Acacia oswaldii*), quandong (*Santalum acuminatum*) and sandalwood (*S. spicatum*).

The Mining and Rehabilitation Program (MARP) developed between Iluka Resources Ltd and the South Australian Government has specific rehabilitation commitments to restore western myall open woodland in areas affected by mining. Western myall is a long-lived tree to 10 m high, often with multi-stems and a rounded canopy that spreads outwards with age (Maslin 2001; Kutsche and Lay 2003). Growth is very slow, taking approximately 75 years for individuals to reach maturity and their lifespan exceeds 250 years (Lange and Purdie 1976). Very little is known about water use patterns in Western myall and although generally identified as a deep-rooted species, it was not until recent mining activity at J-A led to Western myall roots being intercepted at 22 m below surface that we had evidence of how deep their root systems extended. This discovery identified a discrepancy between the root-zone depth in undisturbed areas and the shallower overburden depth in post mining areas, which raised concerns about how altered plant-soil-water relations may affect plant survival in the long-term.

One of the key purposes of the pilot study was to investigate and trial methods documented in other studies to determine whether they could be similarly applied to vegetation and soils at J-A. Past research has successfully used oxygen-18 ($\delta^{18}O$) and deuterium ($\delta^{2}H$) isotope signatures to assist their investigations of plant water use in a variety of tree species (Thorburn et al. 1993a; Mensforth et al. 1994; Brunel et al. 1997; Lamontagne et al. 2005; Holland et al. 2006; Swaffer et al. 2013). Provided there is no further fractionation of isotopes as water is taken up by plants, the isotopic composition of xylem water should match that of the water source (Mensforth et al. 1994). Fractionation is the change in proportion of relative abundances of molecules with different atomic isotopes and occurs in a variety of processes that affect differentially molecules with different weights. For example, evaporation increases the relative abundances of water molecules with heavier oxygen or hydrogen isotopes. Soil at varying depths will therefore have different fractions of the various molecular weights and this can provide distinctive isotopic signatures. It is well known that fractionation occurs

within leaves due to transpiration but the degree to which this affects the isotopic composition elsewhere within the plant varies (Brunel et al. 1995). Some researchers have in the past assumed constant fractionation rates and have ignored this uncertainty (Moore and Semmens 2008), yet the assumption that fractionation does not occur during the uptake of water by plants is not universal (Thorburn et al. 1993b) and is likely to be species-specific.

Thorburn et al. (1993b) ruled out isotope fractionation in four *Eucalyptus* species using a seedling experiment and this meant that subsequent sampling of xylem tissue in mature trees could be done using a single position on the tree, in this case twigs (Thorburn et al. 1993a; Mensforth et al. 1994). For this pilot study, very little was known about fractionation in western myall. To gain some indication, we sampled from different positions on each tree i.e. twigs, trunk, different lateral roots and the tap root. Deep-rooted species with bimodal root architecture are likely to have roots accessing water from a variety of sources with different signatures (Cramer et al. 1999) and consequently the multiple sampling approach was also critical to gaining a holistic impression of plant water use patterns in western myall. Brunel et al. (1995) validated the use of stable water isotopes in a semi-arid field situation and determined randomly distributed errors for δ^2H (5%) and $\delta^{18}O$ (1%), which are considered in our results. The errors are based on potential inaccuracies caused by water extraction methods, spatial heterogeneity in soil profiles and within plant canopies, the time taken for water to move through a plant and assumptions about fractionation of isotopes during water uptake.

Potential water sources at J-A include surface water, soil water, drainage systems such as creek lines and lakes as well as groundwater aquifers. Isolating the exact source of water is not always feasible as the isotopic composition of xylem water may be a mixture of more than one source (Brunel et al. 1995). Isotope mixing models are therefore generally used to assess the feasible proportional contribution of each of the possible tree water sources, and in this pilot study we used IsoSource™ (United States Environmental Protection Agency). This program examines all possible combinations of each source contribution (0–100%) in small increments (e.g. 1%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g. <0.1‰) are considered to be feasible solutions. The frequency and range of potential source contributions are determined, providing a range of potential source water solutions that could result in the end product signature (Phillips and Gregg 2003).

In addition to stable water isotope signatures, water potential (Ψ) gradients are commonly used to determine the accessibility of water to plants. Measuring soil water potentials collected at different depths can highlight layers in the soil profile from which roots can extract water. Soil water potential, measured in Mega Pascals (MPa), represents the sum of water potentials based on soil dryness (matric) and soil saltiness (osmotic), where a water potential of 0 MPa is equivalent to free water and more negative values are drier and/or more saline (Holland et al. 2006). As plant soil-water availability is dependent on soil pore size distribution and the decoupling of water flowing through macropores (Brooks et al. 2010), only soil regions with higher soil water potentials than twig water potentials are available to a tree at any given time (Holland et al. 2006). Twig water potential can be used as an indication of overall plant water potential, as water flow from roots to leaves is proportionate to a root–leaf potential difference and to a root–leaf hydraulic conductance term (Cook and O'Grady 2006). Water potentials from free flowing waters such as groundwater aquifers can also be compared using osmotic potentials calculated from the chloride concentration of the water (Holland et al. 2006).

The depth at which plants are able to access water from the soil profile has important implications for the hydrological balance of an ecosystem, as well as for carbon and nutrient cycling (Canadell et al. 1996). The clarification of rooting depths and plant-soil-water relations for key species at J-A will assist mine rehabilitation managers to devise specific

strategies to achieve long-term survival of deep-rooted species in reconstructed soil profiles, and subsequently this pilot study had two key aims:

1. To investigate the feasibility of using stable water isotope signatures and water potentials to determine water sources for plants at J-A.
2. To examine spatial variability in the isotope composition within western myall that may guide future sampling methods.

Methods

Study Site

The study site is situated at the Jacinth Ambrosia mine site (J-A) in the far west of South Australia (Figure 2.1). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (BOM 2012). Rainfall is generally consistent during winter months, however, large summer rainfall events that often produce floods occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). Mean annual rainfall between 1904 and 1999 was approximately 174 mm (BOM 2012). All meteorological data has been obtained from the nearest weather station 220 km east of the study site at Tarcoola.

Soils at the study site are deep calcareous sandy loam soils consisting of a thick layer of brown sandy loam (average 4 m) overlying calcrete (Specht 1972; Pratt 2008; Doudle 2010). Beneath the calcrete layer, red sandy loam extends to a depth of approximately 10 m overlying white sand (Pratt 2008). The physio-chemical characteristics of the brown and red loam can vary and areas of higher pH are generally associated with the presence of calcium carbonate (Bean et al. 2012).

Groundwater at the mine is restricted to fractured rock aquifers, which are heterogenous and may have dual-porosity characteristics where groundwater is stored in preferential pathways and/or the rock matrix (Bean et al. 2012). Natural groundwater depth is generally between 40 and 50 m below surface level and salinity levels are extreme, around 10,000 mg/L (Bean et al. 2012).

Tree selection and sampling for stable water isotope analysis

Three trees were selected south of the tailing storage facility at J-A (Figure 4.9). The site was chosen due to the close proximity of the trees to monitoring bores, providing access to both deep and shallower groundwater sources. Soil profile characteristics were also available for the site. Trees were of similar life form and one tree was sampled per day over a period of three consecutive days in mid-June (Figure 4.10). Two primary opposing lateral roots (i.e. north and south facing) were located at the base of each trunk and exposed using shovels and trowels. The primary lateral roots are large (approximately 20 cm diameter) and woody, often with a secondary lateral root attached, which is thinner (approximatley 5 cm diameter) and relatively smooth. Opposing secondary laterals were located on two out of three trees (except Myall 3). All roots were sampled within 50 cm of the trunk and between a depth of 20 cm and 50 cm beneath the soil surface. Taproots were exposed by using shovels, but a small excavator was needed to access the taproot of Myall 2. The taproot of Myall 3 was not accessible.

An increment borer was initially used to obtain cores from large primary roots, taproots and trunks; however this proved to be impractical due to the wood density. A modified wad punch (Part no. 07645400, Blackwoods, Regency Park, SA) and hammer was therefore trialled and found to be an efficient way to extract cores from both roots and trunks (Figure 4.10). Between 20 and 25 cores were collected per sample and immediately placed in kerosene

inside 150 ml glass jars sealed with metal lids and secured with electrical tape to avoid evaporation. Similarly, tree trunks were sampled using a wad punch on opposite sides of the tree, matching the direction of the lateral roots.

Twigs (approximately 15 mm diameter) were collected from each tree canopy on opposite sides of the tree, matching north and south aspects. The bark was removed from twigs as per Brunel, Walker et al. (1997) and twig lengths (an approximate total length of 200 mm) were cut into 15 mm sections and placed in kerosene as per CSIRO protocols (Figure 4.10). Small secondary roots were also processed in this manner. Refer to Figure 4.11 for more information.

Potential water sources – groundwater, rainwater and soil water

Five potential water sources were examined in this study (refer to Figure 4.9 for distances and positions of bores):

1. Closest accessible groundwater source (MBN01D: -40 m below surface level).
2. Closest accessible perched groundwater source (MBN01S: -35 m below surface level).
3. Next closest accessible groundwater source (IH18: -24 m below surface level).
4. Rainwater collected during sampling period.
5. Soil water ≤ 1m below surface level.

Water samples were obtained from monitoring bores MBN01D, MBN01S and IH18 after scheduled purging and recharging of aquifers by OTEK Practical Environmental Solutions (Adelaide, South Australia), two weeks after tree and soil sampling. Rain fell at the site (< 5 mm) on the night prior to the first day of sampling and this was also collected and analysed for $\delta\ ^{18}O$. Groundwater and rainwater samples were collected in triplicate and stored upside down in glass McKartney bottles as per CSIRO protocols.

To collect soil samples, a 1.2 m-deep trench was excavated approximately 5 m to the west of each tree (i.e. outside the canopy edge). Soil bulk density rings (258 cm^3) were used to collect samples from the freshly exposed face of each trench at -0.1, -0.3, -0.5 and -1.0 m intervals (Figure 4.10). Each sample was packed into a 500 ml glass jar with a metal lid and sealed with electrical tape to minimise evaporation.

Isotope analysis

Azeotropic distillation (Revesz and Woods 1990) was used to extract water from plant xylem tissue and soils. Analysis of $\delta\ ^{18}O$ was done using mass spectrometry as per Thorburn et al. (1993b) and Brunel et al. (1997). All isotope extractions and analyses were carried out by Isotope Analysis Service, CSIRO Land and Water (Waite, South Australia). Prior to commencing, a set of each sample type (excluding source waters) was collected and sent to CSIRO to ensure that the quantities of plant and soil material collected were sufficient to provide at least 2 ml of water for the isotope analysis. Deuterium analysis of plant xylem and soil water extracts was not available at the time of this study and therefore only $\delta^{18}O$ is examined here. IsoSource™ (US EPA) was used to determine bounds for the contributions of each source as per Phillips and Gregg (2003).

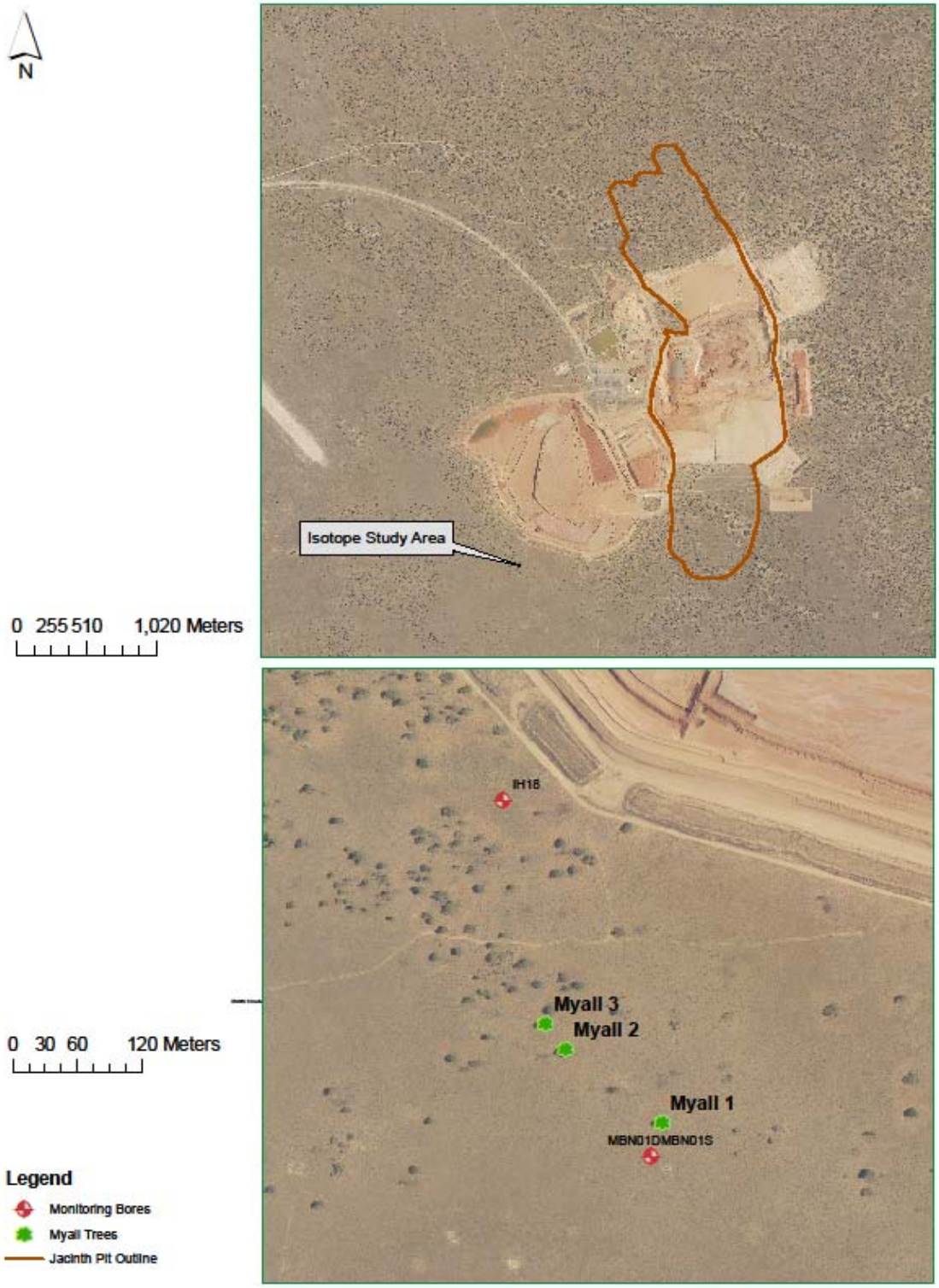

Figure 4.9 Location of the study area relative to the J-A mine site (top) and relative position of the western myall trees to ground-water monitoring bores


(a)


(b)


(c)


(d)

Figure 4.10 Western myall 1 (a); using a wad punch to collect xylem tissue from roots (b) preparing twigs (c); and collecting soil samples for water potential measurements (d)

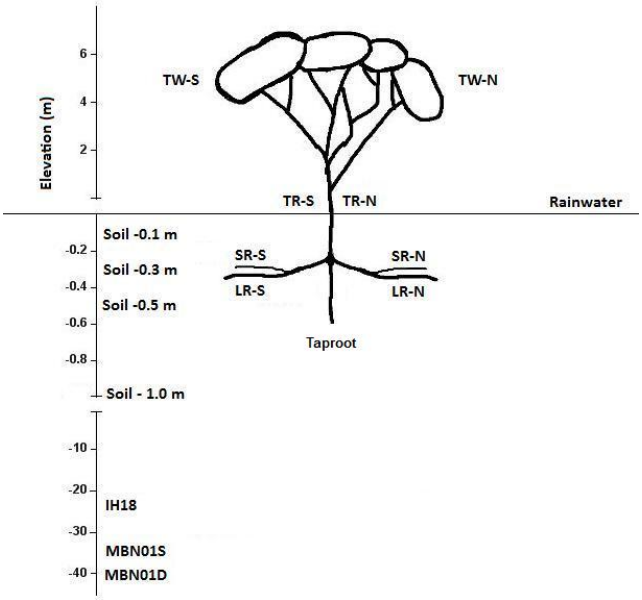

Figure 4.11 Diagram showing the position of samples. TW = twig; TR = trunk; SR = small root; LR = large root; Tap = taproot; N = north; S = south; IH18, MBNO1S & MBNO1D = groundwater monitoring bores

EC and pH measurements

Additional groundwater, rainwater and soil samples were collected concurrently with other samples, to measure electrical conductivity (EC) and pH. All measurements were done onsite, except groundwater samples, which were undertaken by OTEK Practical Environmental Solutions (Adelaide, South Australia). Onsite, EC and pH were measured using a Myron L Company 6Psi ultrameter II and soil EC was determined using the 1:5 soil/water method and converted to ECe with a texture conversion factor as per Wetherby (2003).

Measuring twig, groundwater and soil water potentials

Pre-dawn twig water potential measurements were collected from the myall tree to be sampled that day using a Scholander pressure chamber (PMS Instrument Company, USA, info@pmsinstrument.com) as per Scholander et al. (1965). Three replicate twig samples (approximately 15 mm diameter) were obtained from north and south-facing aspects of the canopy and each twig was placed directly into the pressure chamber and measured, thus avoiding the need for storage and potential water loss.

To measure soil water potentials at four depths -0.1, -0.3, -0.5 and -1.0 m, additional soil collections were made from each trench at the time of sampling for $\delta^{18}O$, EC and pH. Soil bulk density rings were again used and soil was placed into a 300 ml glass jars with metal lids and sealed with electrical tape. Total soil water potential was calculated by adding together matric (P_m), osmotic (P_o) and gravitational (P_g) pressure potentials. Matric potential was determined by the 'filter paper' technique (Greacen et al. 1989). Although the accuracy of this method may be compromised when applied to soil with gravimetric water contents below 0.278 g/g, results were still considered feasible estimates of soil P_m. Water retention curves are better suited for calculating P_m in drier soils and although not undertaken in this study, these will be carried out as part of future ARCLP research. The relation P_o = O.36 x EC x 10^3 was used for estimating the osmotic pressure of soil solutions from EC measurements as per Allison et al. (1954). Gravimetric water content (g g^{-1}) was calculated from oven dried soil (120°C for 24 h). Groundwater osmotic potentials were also calculated using EC measurements as per Allison et al. (1954). Gravitational pressure (0.098 MPa m^{-1}) was added to both soil and groundwater water potentials as per Taiz and Zeiger (2010).

Results

Results are presented in Figure 4.12 and Figure 4.13 and Table 4-5 and Table 4-6**Error! Reference source not found.** below.

$\delta^{18}O$ signatures

Figure 4.12 provides a comparison of δ18O signatures between western myalls and potential water sources. The blue line represents the line of best fit in relation to twig signatures and the blue circle highlights the signature of Myall 1 small north root which is more similar to that of surface soil-water.

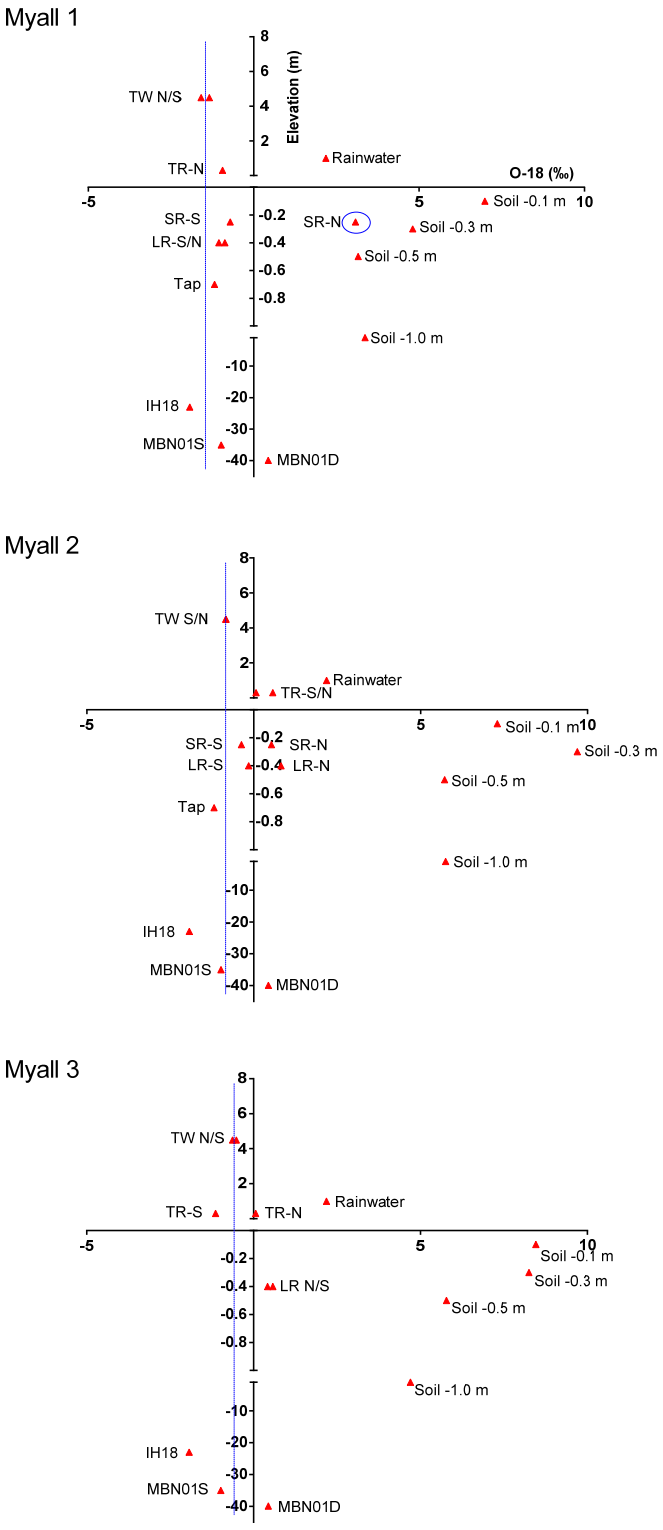

Figure 4.12 A comparison of δ18O signatures between western myalls and potential water sources. TW = twig; TR = trunk; SR = small root; LR = large root; Tap = taproot; N = north aspect; S = south aspect; IH18, MBNO1S & MBNO1D = groundwater monitoring bores.

IsoSource

Table 4-5 IsoSourceTM results showing source proportions in Myall 1 twigs, taproot and small north root; Myall 2 twigs and taproot and Myall 3 twigs.

Mixture	Source	Increment %	Tolerance	Source Proportion %
Myall 1 TWIG	Soil 0.1	1.5	0.01	0 - 5
Myall 1 TWIG	Soil 0.3	1.5	0.01	0 - 6
Myall 1 TWIG	Soil 0.5	1.5	0.01	0 - 9
Myall 1 TWIG	Soil 1.0	1.5	0.01	0 - 8
Myall 1 TWIG	MBN01D	1.5	0.01	0 - 20
Myall 1 TWIG	MBN01S	1.5	0.01	0 - 41
Myall 1 TWIG	IH18	1.5	0.01	51 - 93
Myall 1 TAP	Soil 0.1	1.5	0.01	0 - 8
Myall 1 TAP	Soil 0.3	1.5	0.01	0 - 11
Myall 1 TAP	Soil 0.5	1.5	0.01	0 - 14
Myall 1 TAP	Soil 1.0	1.5	0.01	0 - 14
Myall 1 TAP	MBN01D	1.5	0.01	0 - 32
Myall 1 TAP	MBN01S	1.5	0.01	0 - 80
Myall 1 TAP	IH18	1.5	0.01	20 - 90
Myall 2 TWIG	Soil 0.1	1.5	0.01	0 - 11
Myall 2 TWIG	Soil 0.3	1.5	0.01	0 - 10
Myall 2 TWIG	Soil 0.5	1.5	0.01	0 - 14
Myall 2 TWIG	Soil 1.0	1.5	0.01	0 - 14
Myall 2 TWIG	MBN01D	1.5	0.01	0 - 45
Myall 2 TWIG	MBN01S	1.5	0.01	0 - 96
Myall 2 TWIG	IH18	1.5	0.01	0 - 89
Myall 2 TAP	Soil 0.1	1.5	0.01	0 - 9
Myall 2 TAP	Soil 0.3	1.5	0.01	0 - 8
Myall 2 TAP	Soil 0.5	1.5	0.01	0 - 9
Myall 2 TAP	Soil 1.0	1.5	0.01	0 - 9
Myall 2 TAP	MBN01D	1.5	0.01	0 - 32
Myall 2 TAP	MBN01S	1.5	0.01	0 - 78
Myall 2 TAP	IH18	1.5	0.01	21 - 92
Myall 3 TWIG	Soil 0.1	1.5	0.01	0 - 12
Myall 3 TWIG	Soil 0.3	1.5	0.01	0 - 12
Myall 3 TWIG	Soil 0.5	1.5	0.01	0 - 17
Myall 3 TWIG	Soil 1.0	1.5	0.01	0 - 20
Myall 3 TWIG	MBN01D	1.5	0.01	0 - 53
Myall 3 TWIG	MBN01S	1.5	0.01	0 - 93
Myall 3 TWIG	IH18	1.5	0.01	0 - 86
Myall 1 SMALL NORTH	Soil 0.1	1.5	0.01	0 - 53
Myall 1 SMALL NORTH	Soil 0.3	1.5	0.01	0 - 71
Myall 1 SMALL NORTH	Soil 0.5	1.5	0.01	0 - 86
Myall 1 SMALL NORTH	Soil 1.0	1.5	0.01	0 - 88
Myall 1 SMALL NORTH	MBN01D	1.5	0.01	0 - 53
Myall 1 SMALL NORTH	MBN01S	1.5	0.01	0 - 44
Myall 1 SMALL NORTH	IH18	1.5	0.01	0 - 39

Water Potentials

Figure 4.13 shows a comparison of water potentials between myall twigs and potential water sources. The blue line represents the line of best fit in relation to twig pre-dawn water potential.

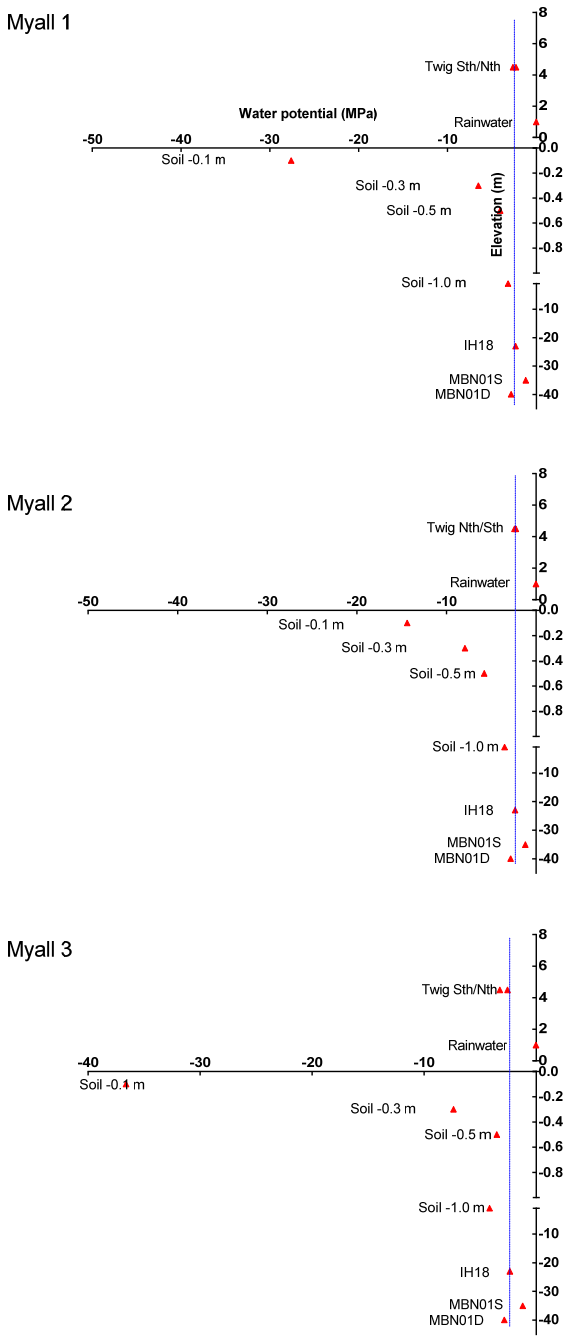

Figure 4.13 A comparison of water potentials between myall twigs and potential water sources. TW = twig; N = north aspect; S = south aspect; IH18, MBNO1S & MBNO1D = groundwater monitoring bores.

Salinity, pH and Soil Gravimetric Water Content Measurements

Table 4-6 Soil, groundwater and rainwater properties including salinity and pH measurements. Soil EC was converted to ECe using a texure conversion factor as per Wetherby (2003). SCL = sandy clay loam; LSCL = light sandy clay loam; CL = clay loam

Sample Location	Sample Depth Below Surface (m)	Salinity ECe (dS/m)	pH	Gravimetric Water Content (%)	Texture
Myall 1	0.1	2.87	8.65	2.6731	SCL
	0.3	14.63	9.70	5.6438	LSCL
	0.5	20.09	9.81	7.0511	CL
	1.0	45.11	9.69	5.5534	SCL
Myall 2	0.1	4.79	8.33	2.8720	SCL
	0.3	9.46	9.66	4.2649	SCL
	0.5	12.09	9.87	5.0153	SCL
	1.0	5.59	10.09	2.0917	LSCL
Myall 3	0.1	6.02	8.72	3.5616	SCL
	0.3	14.05	9.55	6.2133	SCL
	0.5	24.19	9.87	9.8580	CL
	1.0	32.50	9.74	5.9711	SCL
MBN01D	39.9	68.2	6.1	-	-
MBN01S	35	23.9	3.3	-	-
IH18	23	59.0	3.3	-	-
Rain Water	0	0.7	6.6	-	-

Discussion

Using stable water isotope signatures and water potentials to determine plant water sources at J-A

The pilot study results verify the suitability of stable water isotopes, in this case $\delta^{18}O$, to help identify patterns of water-use in Western myall trees at J-A. Oxygen-18 signatures were distinctly different between groundwater sources (IH18 -1.93‰, MBN01S -0.98‰ and MBN01D +0.44‰) as well as between surface soil-waters (ranging between +3.16‰ and +9.7‰), thus making it possible to distinguish between them. Conceivably, xylem water $\delta^{18}O$ signatures from other deep-rooted species at J-A could be compared to those of potential water sources in a similar manner to Western myall.

A limitation of this pilot study is that no profiling of soil water $\delta^{18}O$ signatures was undertaken below 1 m deep. Without this information we cannot be certain that Western myalls are accessing soil-water deeper within the soil profile with similar isotope signatures to groundwater. This deficit will be addressed in future research through the use of a drill rig, which will be used to access intact soil samples for $\delta^{18}O$ analysis incrementally to depths of around 14 m^{+}. Regardless, there have been some very important results obtained from this preliminary research.

To begin with, groundwater cannot be discounted as part of the mix of source waters for western myalls. Twig and taproot signatures from Myall 1 and Myall 2 closely align with those of either MBN01S or IH18 groundwater sources (Figure 4.12). This relationship is also reflected in IsoSource™ results (graphs available upon request), where IH18 groundwater was proportionally high in Myall 1 twig (51 – 93%), Myall 1 taproot (20 – 90%) and Myall 2 taproot (21 – 92%).The results also indicate potential contributions by MBN01D and MBN01S. The range of feasible source contributions in Myall 3 was not well constrained and therefore not very informative regarding tree water sources. Water potentials reveal that all three western myalls were likely capable of extracting water from MBN01S and IH18, but may not have been able to access water from MBN01D (Figure 4.13).

With what we currently know about western myalls, it is both feasible that their roots could reach groundwater aquifers and that they may be able to use hyper-saline water. Recently, DNA techniques have been used to identify western myall roots 22 m below the surface in the floor of the J-A mine pit. These roots were observed to continue vertically through the soil profile and may therefore reach the depths needed to access groundwater (i.e. IH18: -24 m, MBN01S: -35 m and MBN01D: -40 m). In terms of salt tolerance, anecdotal evidence based on soil chemistry data collected at J-A suggests that western myalls utilise water with high levels of salinity (Doudle 2012). A number of *Eucalyptus* species occurring on a floodplain of the River Murray in South Australia have been shown to regularly utilise groundwater despite very high EC levels of around 33 dS/m (Thorburn et al. 1993a) and extreme salt tolerance up to 20 dS/m is documented in a number of *Acacia* species (WADAF 2013).

It has been difficult to find published research on deep groundwater (i.e. -20^{+} m) use by plants in a similar type of geology and environment to J-A. In this research, the groundwater $\delta^{18}O$ signatures seem isotopically enriched (i.e. IH18: -1.93‰, MBN01S: -0.98‰ and MBN01D: +0.44‰) in comparison with other studies of shallower groundwater sources (i.e. < 5 m below surface). For example, Mensforth et al. (1994) documented groundwater $\delta^{18}O$ levels between -2.0 and -4.5‰, Holland et al. (2006) between -2.26 and -4.61‰ and Swaffer et al. (2013) between -4.5 and -5.0‰. We are currently exploring the possibility that the tailings storage facility (TSF) has influenced $\delta^{18}O$ signatures in nearby groundwater aquifers, perhaps reflected in the relatively high pH value (6.1) recorded in MBN01D water samples. The pH values for IH18 and MBN01S were both 3.3, which are more akin to natural groundwater values (Doudle 2013). Additional isotope analyses will also be done on water samples collected from groundwater aquifers approximately 1 km east of the isotope study site for comparison.

Overall, IsoSource™ proportions showed that surface soil water made very little contribution to twig and taproot mixtures in all three western myalls (< 20%), most likely reflecting the very dry surface soil conditions at the site leading up to sampling (refer to GW content measurements in **Error! Reference source not found.**Table 4.6. Although the range of feasible source contributions was not well constrained in Myall 1 small north root, surface soil water did seem to play a more important role in the mixture (Table 4-5). Soil water potential estimates indicate that it was unlikely for Myall 1 to be able to extract water at any of the four soil depths tested (≤ 1m) and the tree was therefore sourcing soil water at greater depths. In future ARCLP research, water retention curves which are better suited for

calculating matric potentials in dry soils, will be used in preference to the filter paper method. This will improve the accuracy of total water potential values and enable greater precision for pinpointing positions in the soil from which trees are capable of accessing soil water.

Another important consideration here is the potential role of vertical and/or lateral redistribution of water within the root system. This process is critical for our understanding of plant-soil-water relations, as xylem water within a root may contain isotope signatures from sources it is not physically in contact with. Hydraulic redistribution (HR) is being investigated in Western myall as part of the ARCLP project, using heat ratio method (HRM) sap flow meters (ICT International, Armidale, NSW). The process has been documented in other arid systems and rangelands (Richards and Caldwell 1987; Ryel et al. 2002; Hultine et al. 2003) and has important implications for other plant species as water is raised from deep stores for use by roots in near-surface horizons, thus making it available to associated understory vegetation. Evidence of facilitation through HR has been documented in other ecosystems (Caldwell and Richards 1989; Dawson 1993; Brooks et al. 2002; Ludwig et al. 2003; Zou et al. 2005) including a species of *Acacia* (Ludwig et al. 2003).

Spatial variability in the isotope composition within Western myall to guide future sampling

Western myalls evidently access water from a range of sources as shown by the variability in $\delta^{18}O$ signatures found in the small number of trees and root types sampled here. Our results highlight the importance of sampling xylem tissue from twigs, taproots and as many lateral roots as possible, to gain an overall impression of water use in western myall. There was very little variation in $\delta^{18}O$ signatures between twigs on the north and south sides of trees.

Conclusions

In future isotope research at J-A, groundwater and soil samples will be collected to greater depths using a drill rig and the analysis of other micro-elements will be considered to help “fingerprint” different soil layers. In addition to IsoSource™, Bayesian models will be used for analysing isotope data e.g. MixSIR (Moore and Semmens 2008). Planning is currently underway to incorporate all the information gained from this pilot study into the experimental design for the next stage of this research.

Acknowledgements

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4.3.2 Hydraulic redistribution of western myall

Introduction

Hydraulic redistribution (HR) is a process that some plants are able to employ to redistribute water via their root systems into deeper soil layers during seasons of high rainfall or during large summer rainfall events. In dry seasons or periods of drought, the water stored deep in the soil then moves upwards through the root system via hydraulic lift, and can therefore be used by the plant to sustain its surface roots. This is thought to be a survival strategy of deep-rooted species to survive extended drought periods in arid environments (Bleby et al. 2010). The process also has important implications for landscape hydrology and potentially the spatial distribution of understory plant species that may rely on the redistribution of water towards the surface (Burgess et al. 2001).

A research component of the ARCLP is to investigate whether deep-rooted species at JA utilise HR, which may have implications for the long-term success of rehabilitation at the site. Identifying HR in the two most abundant deep-rooted species at JA, western myall and red mallee, is critical because modified soils and tailings may have different water storage capacities when compared with those of undisturbed soil. This has the potential to reduce plant rooting depths and restrict roots from accessing deeper water sources previously used to maintain surface roots through dry periods and droughts.

Methods

Hydraulic redistribution can be identified through the detection of reverse sap flow in root systems using the heat ratio method. Sap flow meters (SFMs - ICT International, Armidale, NSW) are strategically installed in a number of position on the tree, such as the trunk, primary lateral and vertical roots and the tap root, in order to capture the movement of water towards the tree (positive flow) or away from the tree (negative flow). A SFM has two temperature probes, each with two thermocouple junctions, and one heater probe (Figure 4.14). The sensor measures heat pulse velocity (HPV) by obtaining the ratio of downstream sapwood temperature to upstream sapwood temperature (at points equidistant from the heat source) following the release of a heat pulse into the sap stream and its subsequent movement via conduction and convection (ICT 2008).

An initial pilot study was carried out between May 2012 and February 2013 to investigate HR in western myall, with the installation of six SFMs on a tree situated within the mine lease (Figure 4.15). Two opposing main lateral roots were excavated with shovels and trowels on the East and West side of the tree, close to the base of the trunk. Instruments were installed on the two laterals and the trunk and upper branches on both sides of the tree. Two stem psychrometers (ICT International) were also installed to measure fluctuations in stem water potential and five soil sensors (Decagon 5TE, ICT International, Armidale, NSW) were installed to measure changes in soil water content (i.e. east at 20 cm and 60 cm depths and west at 5 cm, 20 cm and 60 cm depths).

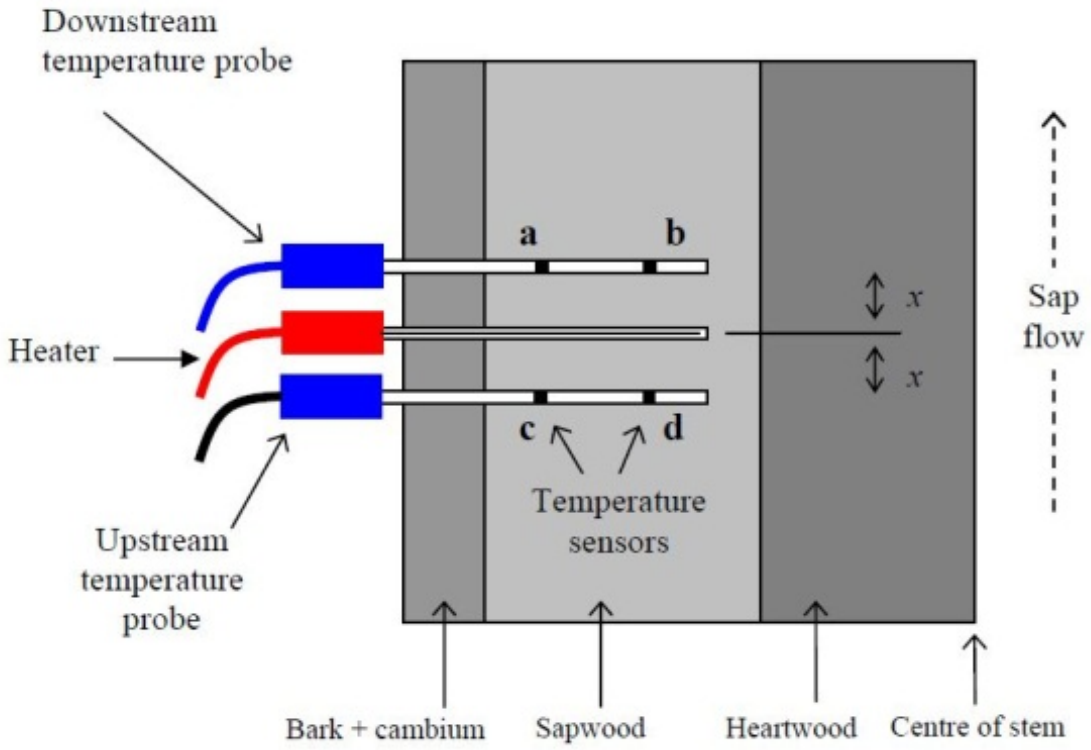
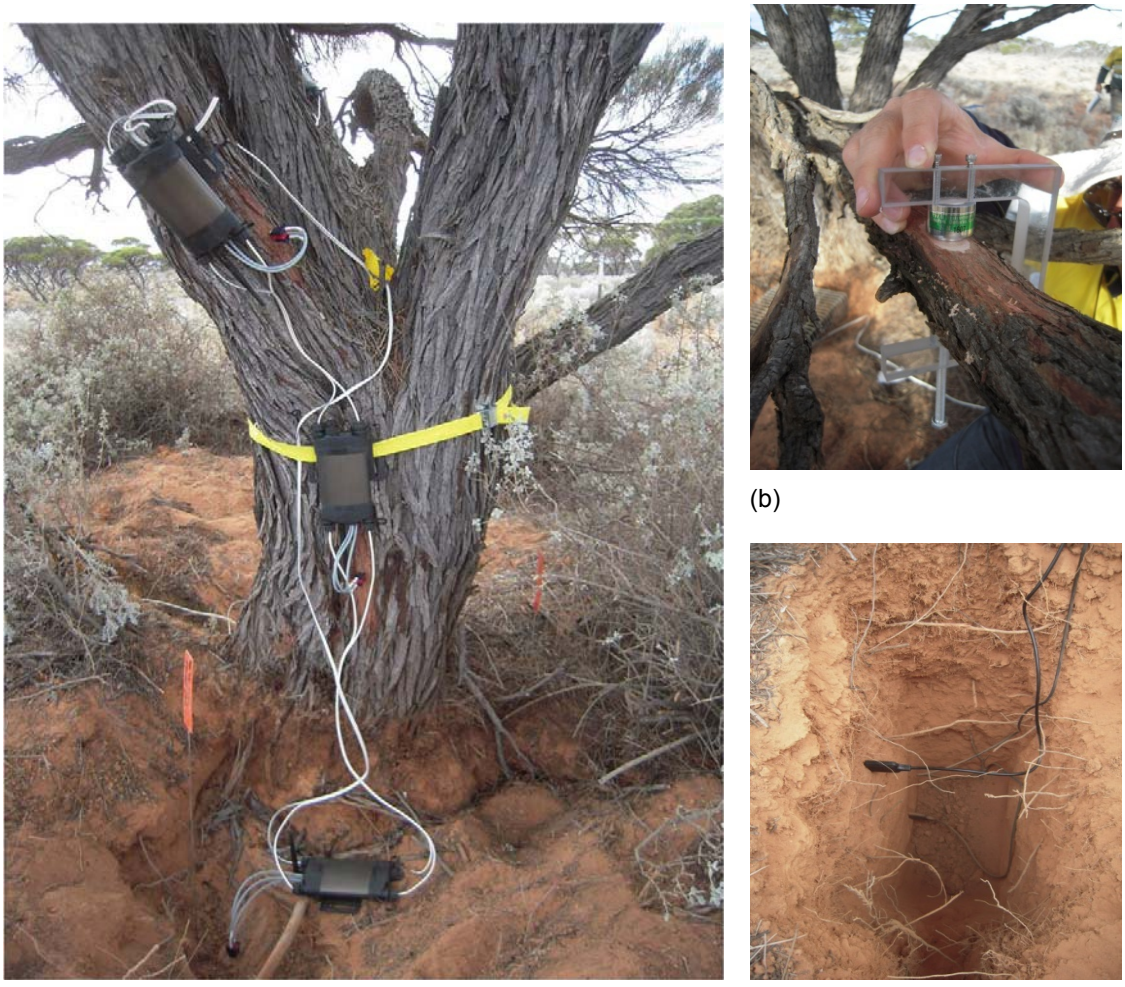


Figure 4.14 A schematic diagram showing a sap flow sensor installed into a stem. Temperature sensing needles are located upstream and downstream from the heater needle (x). Source: ICT (2008)



(a) (b) (c)

Figure 4.15 (a) HRM sap flow instruments installed on a western myall tree; (b) installation of a stem psychrometer; (c) 5TE soil moisture-temperature-EC sensors.

The period between May 2012 and February 2013 proved to be very dry at JA with only 37.4 mm of rain falling in scattered events between June 2012 and February 2013. This meant that no atypical sap flow response to rainfall was observed during the pilot study period. Once the ARCLP commenced officially, more instruments were purchased and the SFMs were uninstalled from the pilot study tree and incorporated into a more extensive installation on two western myall trees in May 2013 (HR Myall 1) and July 2013 (HR Myall 2). An airspade was used to excavate primary lateral roots and the tap root down to a depth of approximately 70 cm (Figure 4.16). A total of 16 SFMs and two stem psychrometers were fitted to each tree and a set of Decagon soil sensors were installed adjacent to each tree at 5, 10, 20, 40 and 60 cm depths.

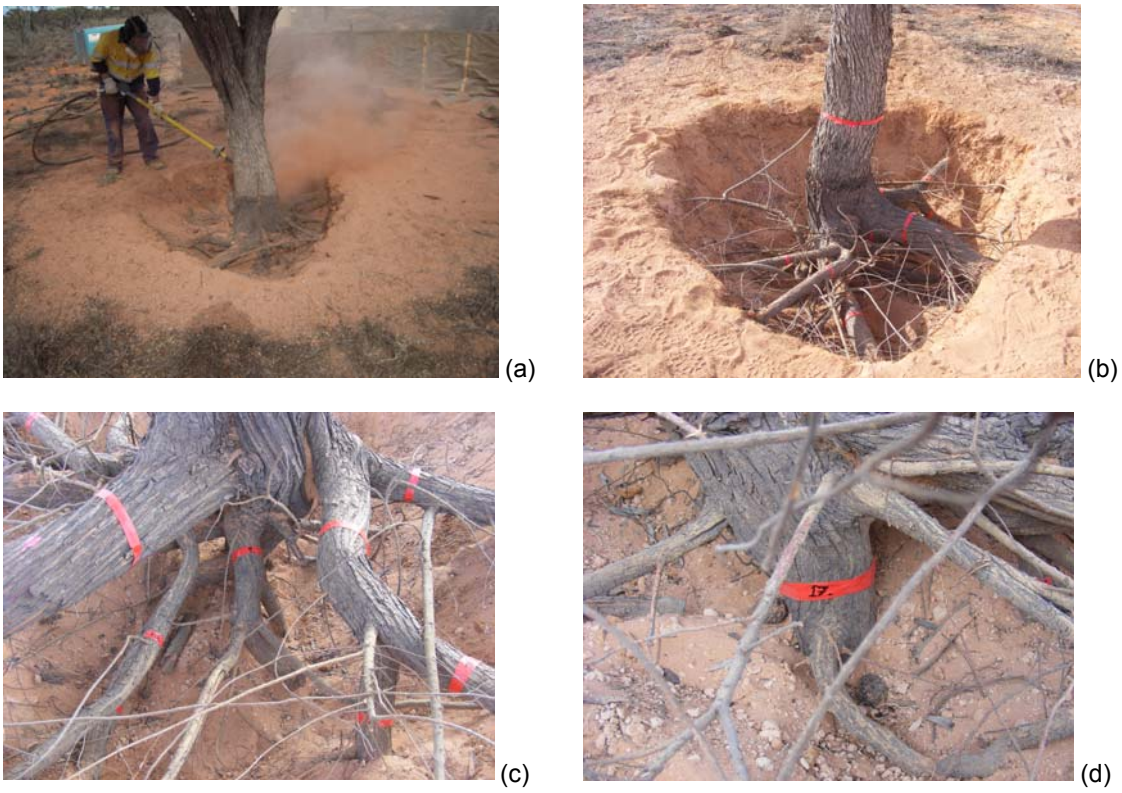


Figure 4.16 (a) Air-spading to expose roots around base of the first of two western myall trees, referred to as HR Myall 1; (b) red electrical tape indicates the positions of the SFMs; (c) root 9 (centre of imate) branches into two verticals; (d) the tap root

Preliminary Results

In July 2013, approximately two months after installing instruments on the first western myall tree and during installation of the second tree, JA experienced a rainfall event with 49 mm of rain falling between 11th and 23rd July (Figure 4.17). Soils were extremely dry at JA, as very little rain had fallen in the preceding year leading up to this event, and consequently the sap flow velocity patterns in both trees responded almost immediately (Figure 4.18 & Figure 4.19). A range of SFV responses were observed between the various positions, with sensors measuring positive or negative changes to sap flow to varying degrees. The most exciting responses were observed in the tap roots and some of the primary 'vertical' roots (i.e. those roots observed to enter the soil at an angle >75 degrees) of both trees. Examples are provided below (Figure 4.18 & Figure 4.19).

A similar-sized rainfall event occurred in February this year and sap flow velocities in both trees responded in a similar manner to that of the previous July rain event, thus providing two seasonal responses. A thorough examination of the data is currently underway, the outcome of which will include a comprehensive report for the Iluka rehabilitation team. Plans are to uninstall instruments from the two Myall trees in July 2014 and to reinstall them on two red mallee trees in order to investigate HR in this species.

Acknowledgements: Alec Downey (ICT International) and A/Prof Steve Burgess (University of Western Australia) provided advice on the design for the pilot study; Alec Downey and Shane Doudle assisted with installing instruments in the pilot study. Thanks goes to Tom Hands, Lucy Cunningham and Jamie Kohler for their assistance with installing instruments on HR Myall 1 and HR Myall 2.

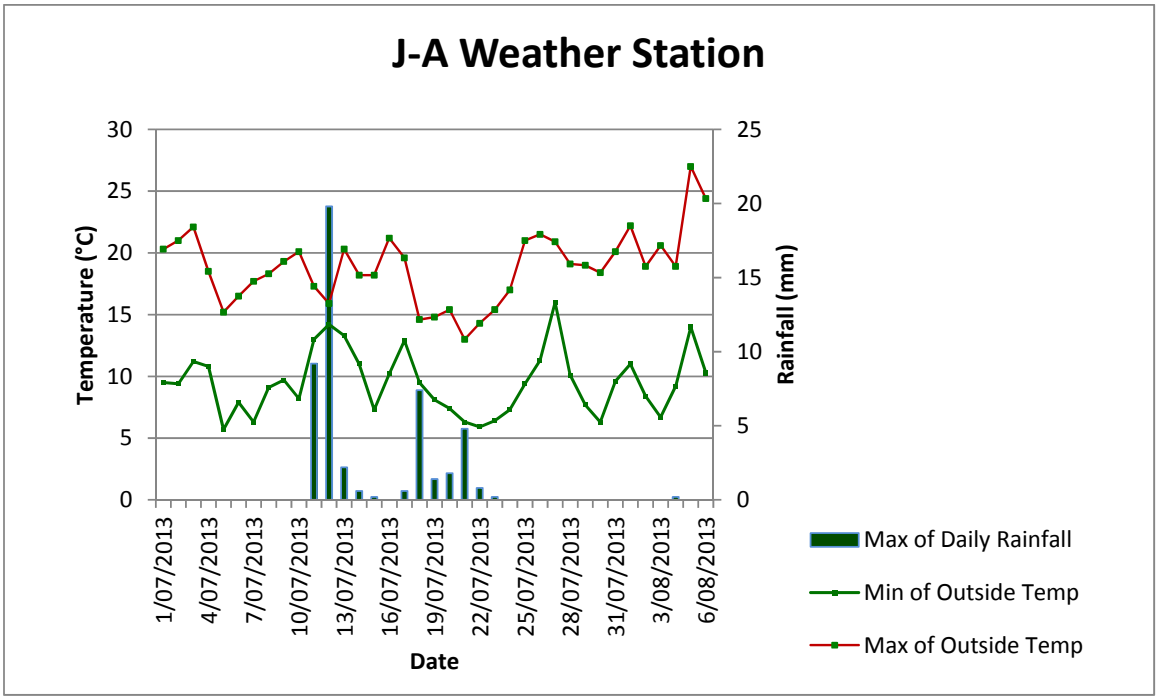


Figure 4.17 JA rainfall event, with 49mm of rain falling between 11 and 23 July 2013

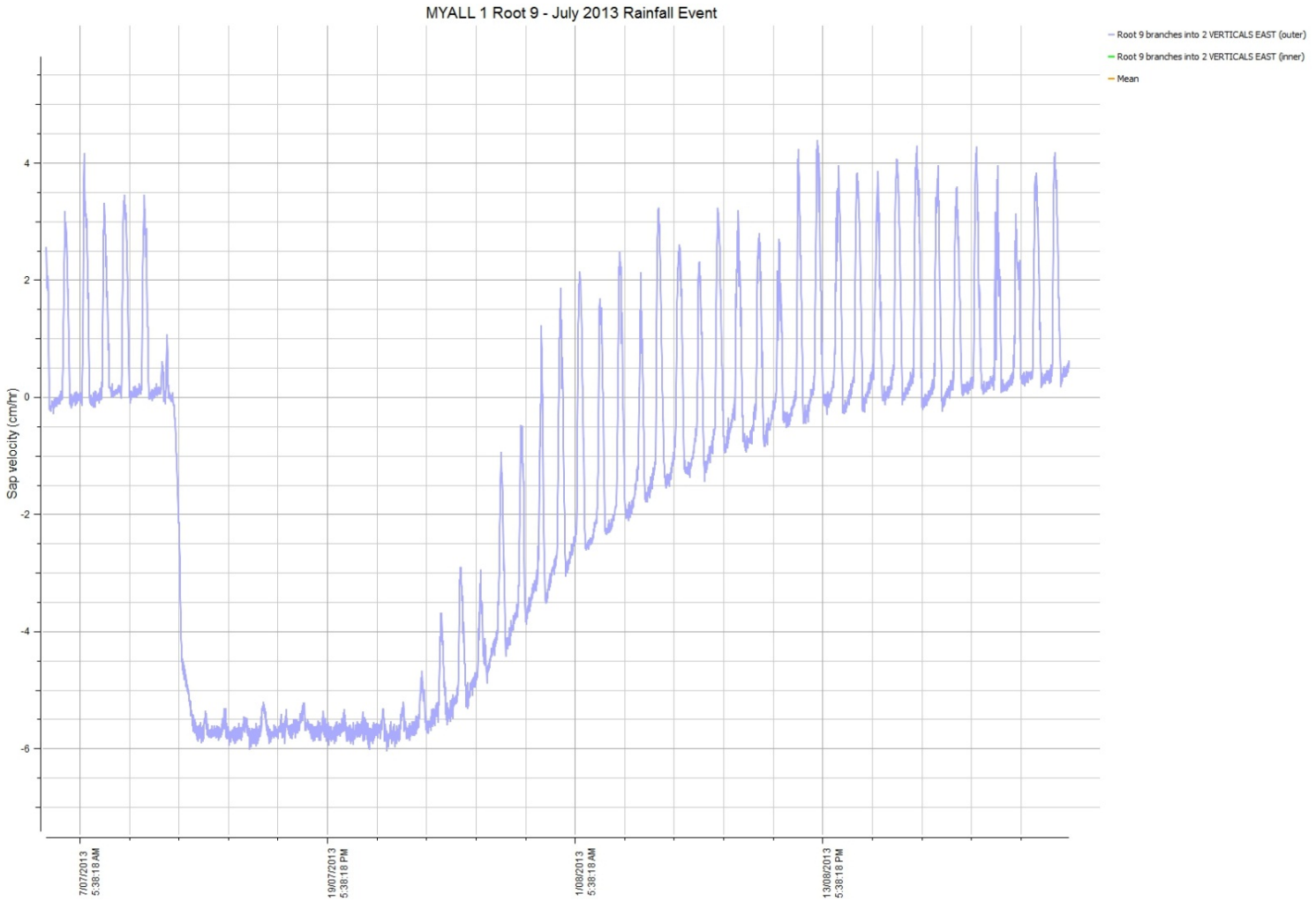


Figure 4.18 Reverse sap flow in HR Myall 1, vertical root 9, indicative of hydraulic redistribution

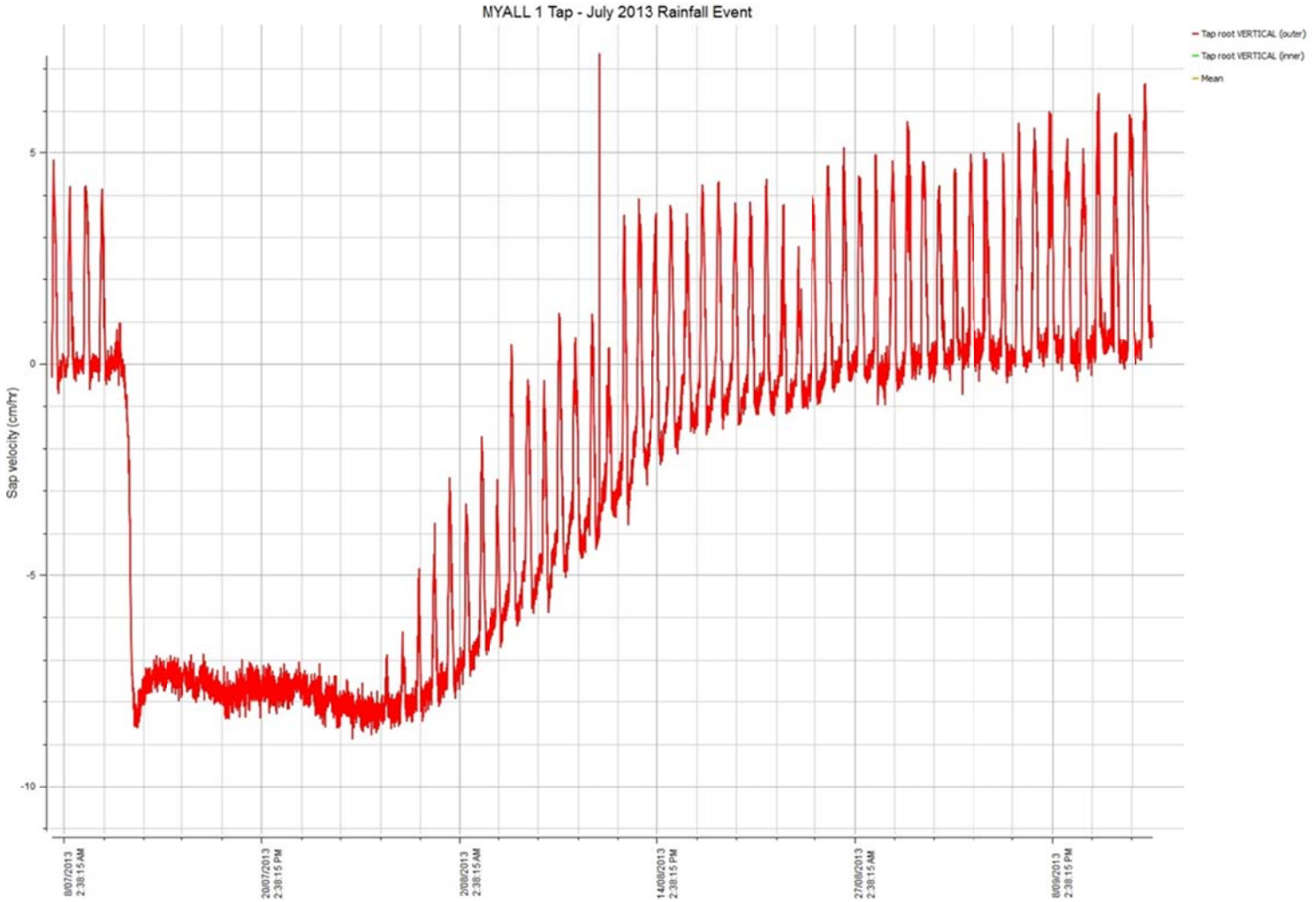


Figure 4.19 Reverse sap flow in HR myall 1 tap root indicative of hydraulic redistribution

4.4 Plant growth response to salinity migration in natural and reconstructed soil profiles

Initial root mapping studies at J-A have intercepted Western myall roots growing at 22 m depth below surface, with the total depth of root exploitation currently unknown. There is a clear mismatch between the depth of soil profile the trees exploit pre□disturbance and the depth of soil profile proposed for its growth post mining (Doudle and Schneemilch 2012).

Further research was therefore required to determine whether western myall trees have the ability to:

- Survive long-term in a much shallower than normal soil profile.
- Be able to grow roots into saline tailings beneath the rehabilitated soil profile and use the soil moisture stored within.

Initial investigations involved a large pot trial that was established at JA in 2011, referred to as the Blue Drum Trial. The pots contained varying depths of soil over saline tailings and were planted with Western myall seedlings. An initial progress summary was included in the first JARMS report (Doudle and Schneemilch 2012) and an update is provided below. A large field trial was also established at JA in 2013, referred to as the Cell 1 Trial, which aims to investigate this issue further by examining plant-soil-water interactions of three deep-rooted species growing in tailings, various depth overburden profiles as well as undisturbed soils.

4.4.1 Blue Drum Trial

Methods

A large pot experiment was designed to investigate the effects on Western myall growth and root development when saline tailings were encountered in the root zone. Six different soil treatments, each with an increasing depth of brown and red sandy loam, were placed over saline tailings (ModCod) and replicated four times (Figure 4.20). Soil was placed into empty 200 litre triple-rinsed sodium hypochlorite drums. To prepare the drums, the tops were cut off with a reciprocal saw and drainage holes were drilled in the bottom. Drum placement in the trial was randomised and four drums each were placed on six wooden pallets and positioned next to the rehabilitation facilities at JA.

The amount of material required for each soil type was calculated and brought to the trial location with a front end loader. Soil samples were taken from each soil type and later tested in the JA Rehabilitation Facility for $ECe_{1:5}$, texture and pH_{WATER}. A bobcat and a telehandler with bucket attachments were used to manoeuvre the soil over the correct drum. Soil was scraped out of the bucket and packed into each of the drums to the required depth. This process was repeated with each soil type until all of the required soil profiles were created in each of the drums. Drums were shaken regularly to settle the contents as the soil was being applied. All drums were covered with the sawn off lids and tarpaulins and allowed to settle for one month.

A single one year old Western myall seedling was planted in each drum and lightly watered to settle the soil around the seedling root zone. Drums were exposed to natural weather conditions of rainfall, temperatures, etc. In addition, a mist dripper system was set up for each drum and plants received 2 minutes of watering every 4 days over summer. This watering strategy was designed to minimise the possibility of salt leaching through the drainage holes in the base of the drums.

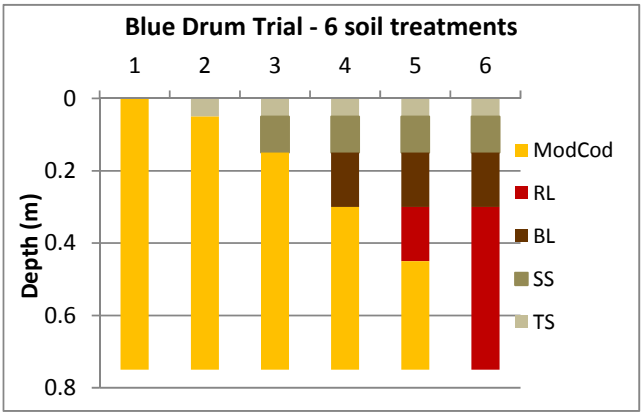


Figure 4.20 Left: blue drum trial; Right: six soil profile treatments examined in the blue drum trial, each with four replicates. ModCod = saline tailings; RL = red sandy loam; BL = brown sandy loam; SS = subsoil; TS = topsoil

Regular plant growth, plant health and photosynthesis efficiency measurements were recorded in 2011, 2012 and 2013. Growth measurements included plant height, plant width, total number of leaves, number of new leaves, leaf dieback and leaf death. A pulse amplitude modulated photosynthesis yield analyser (Mini-PAM: Heinz Walz GmbH, Effeltrich, Germany) was used intermittently to measure photosynthesis in the plants, as reduced photosynthesis efficiency can be indicative of plant stress.

The trial was initially designed to run over four years, with one replicate to be sacrificed each year for root mapping. As considerable variation in plant growth was observed within each treatment replicate, it was decided that the final processing of drums should be done altogether, within as close a timeframe to each other as possible, which would allow for statistical analysis of results. Consequently final growth measurements, plant harvesting, soil sampling and root washing was undertaken for the first two replicates of six drums each during November 2013, the third replicate was processed in December 2013 and the fourth replicate was processed in January 2014.

For each drum, plant measurements (as referred to previously) were recorded and the plant was harvested for shoot biomass and leaf surface area measurements (Figure 4.21a). Three auger ‘spears’ were inserted down to the base of each drum and soil was sampled at incremental depths in keeping with each soil type (i.e. 0-1, 1-5, 5-15, 20-30, 30-40, 40-50 & 50-60 cm). One spear was positioned towards the centre of a drum and two spears were positioned on opposite sides of a drum. These soil samples were collected for soil moisture, $EC_{1:5}$ and pH measurements (Figure 4.21b). As root nodules were observed on many of the root systems, a fourth spear was inserted into each drum and soil was sampled in increments as described above for total nitrogen analysis (CSBP, Bibra Lake, Western Australia).

After plant harvest and soil sampling, each drum was repositioned in a suitable area for root washing. An opening was cut in the side of each drum approximately 40 cm wide and down to the base of the drum. Two sets of metal rods were inserted through pre- drilled holes to criss-cross the drum at 10 cm below and 20 cm below the soil surface. The purpose of the rods was to provide support for the root system as it was being washed. A piece of wire was used to fasten the top of the root system so it remained in its original position.

A garden hose with an adjustable nozzle was used to wash a stable flat soil face against which a metal frame could be placed and used to score root density per 10 cm^2, at 10 cm increments down the profile (Figure 4.21c). Once this was done, the garden hose with

adjustable nozzle was used to carefully wash the soil from the roots. The soil generally loosened and washed away with relative ease (Figure 4.21d). Broken roots were retrieved by washing the slurry through sieves and using forceps to pick out any roots present (Figure 4.21e). Each set of roots were cleaned carefully, photographed, placed into paper bags and stored in a drying room for biomass measurements. According to the depth at which they occurred (i.e. 10 cm increments), root nodules were carefully detached from the root system and transferred to vials containing reverse osmosis (RO) water to keep them hydrated (Figure 4.21f). Nodules were later photographed and scored as viable (i.e. white/pink colouration) or non-viable (brown/black colouration), placed into paper bags and stored in a drying room for biomass measurements (i.e. viable and non-viable kept separate).

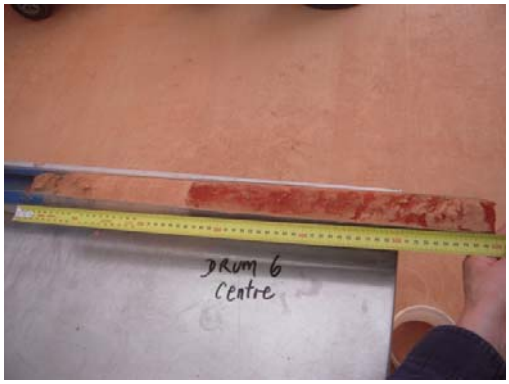
Preliminary Results

Preliminary results reported in JARMS 2011 showed that soil salinity in the top three layers was slight (< 4 $ECe_{1:5}$ dS/m), the red sandy loam was high (~ 8 $ECe_{1:5}$ dS/m) and the ModCod was extremely high (~ 22 $ECe_{1:5}$ dS/m). The pH_{WATER} readings were all alkaline (Doudle and Schneemilch 2012). The Western myall seedlings survived and grew in all treatments, including saline tailings. Only two deaths were recorded in the juvenile plant stage from Treatment 1 and Treatment 4. Unfortunately we cannot present final results from the trial in this JARMS report as plant, soil and root samples are still being processed and the data is yet to be analysed. A report will be made available to the rehabilitation team as soon as possible.

Acknowledgements

Shane Doudle, Con Miller, Keli Payne and Richard Mills assisted with the establishment of this trial. Shane Doudle, Kerry Saunders, Tim Jury, Lucy Cunningham, Brandon Willis and Tina Law assisted with the final collection of plant, soil and root data.

(a)



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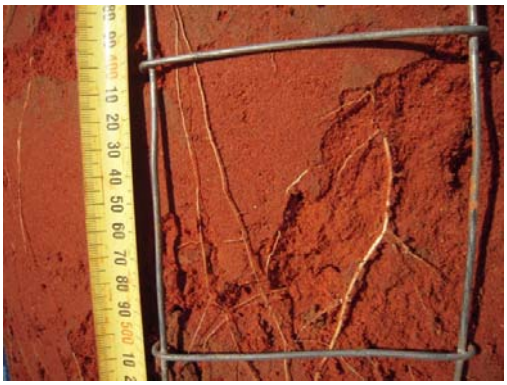

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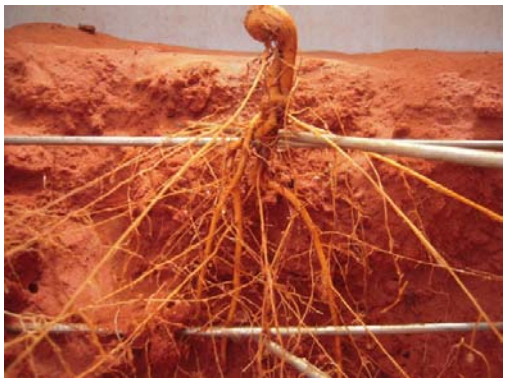

(d)

(e)

(f)

Figure 4.21 (a) Western myall plant harvested for shoot biomass and leaf surface area measurements; (b) soil sampling with an auger spear; (c) scoring root density within a 10 cm^2 area at 10cm depth increments; (d) surface roots exposed by root washing; (e) sieving soil to retrieve broken roots; (f) viable root nodules.

4.4.2 Cell 1 Trial

Introduction

The Cell 1 Trial is a large-scale field trial initiated by Samantha Doudle (Iluka Rehabilitation Specialist, 2009-2013) and now a large component of the ARCLP. Progressing on from the blue drum trial, it aims to examine to a greater extent the growth performance and survival of three deep-rooted species occurring at J-A in different reconstructed soil profiles over saline tailings. The focus species are western myall (*A. papyrocarpa*), red mallee (*Eucalyptus oleosa*) which occurs on sandy rises and along creek lines at JA, and yorrell (*E. gracilis*) which occurs around the margins of a nearby salt lake and is thought to be a salt tolerant mallee species. The trial has been designed as a long-term study with plant structural, morphological and physiological measurements being collected alongside soil chemistry and soil-water properties, to achieve the following aims:

1. To examine whether western myall, red mallee and yorrell are able to produce sustainable populations on shallow soil profiles over saline tailings.
2. To determine effects of shallow soil profiles on their plant water use.
3. To examine whether each species is capable of extracting and using moisture from saline tailings and if so, determine where the salt accumulates, i.e. exclusion at root zone or uptake, tissue accumulation and loss with subsequent salt accumulation in surface soil.
4. To monitor the movement of soil water and salinity through the various soil profile treatments.
5. To provide field verification of the capillary break modelling outcomes.
6. To compare changes in soil chemical and physical characteristics as a result of disturbance.

Knowledge gained from the Cell 1 Trial will be used to inform rehabilitation managers at JA and directly relates to achieving MARP objectives to ensure: 1) pre-existing soil profile and function are reinstated and 2) the post mining ecosystem and landscape function is resilient and self-sustaining.

Methods

In April 2013, a large-scale field experiment referred to as the Cell 1 Trial was installed adjacent to the first rehabilitated area at the JA mine. Carry graders were used to dig trenches to various depths in saline tailings and then overlay the tailings with different soil profile treatments (Figure 4.22). A differential GPS was used to ensure the soil was applied with precision i.e. within 2 mm accuracy. Five different soil profiles were constructed with different arrangements of topsoil, subsoil, brown sandy loam and red sandy loam over saline tailings (Figure 4.23).

The experiment is a split plot design with species nested across five reconstructed profile treatments replicated twice (i.e. 10 strips in total). Each strip is partitioned into seven plots (9 x 12 m), with two plots in each strip planted with a single western myall, red mallee or yorrell seedling and referred to as the ‘main plants’ (Figure 4.24). The seventh plot in each strip will be planted with multiple seedlings of all three species to be harvested after 6, 12 or 18 month intervals to assess early root growth and development. These are referred to as ‘sacrifice plants’ and their initial harvest will form part of a university Honours project in 2014.

Soil-water and EC sensors were installed during construction to monitor water dynamics and salinity changes in the soil profile. A total of 42 soil sensors (Decagon EM50, ICT International) were installed at various depths and these log data hourly. The same plant and soil measurements are being collected in undisturbed vegetation for comparison with those in the trial. To obtain baseline soil data, soil samples were collected from the different

soil types at various depths during trial construction, and analysed for Colwell Phosphorous, Colwell Potassium, Sulphur (KCl 40), Organic Carbon (Walkley-Black), Nitrate Nitrogen, Ammonium Nitrogen, Electrical Conductivity, pH (water), pH (CaCl2), Boron, Trace elements (DTPA: Copper, Zinc, Manganese, Iron), Exchangeable Cations (Calcium, Magnesium, Sodium, Potassium, Aluminium), Calcium Carbonate, Chloride, Particle Size (MIR Method) and Total Nitrogen (CSBP, Perth, Western Australia). Samples were also collected to assess field capacity, wilting point and bulk density (Soil Water Solutions, Naracoorte, South Australia).


(a)


(b)

Figure 4.22 (a) Carry-grader excavating one of the trenches; (b) overlooking the eastern half of the trial. The blue and red crates visible in the photograph were initially used to protect newly planted seedlings prior to the installation of a rabbit and kangaroo proof fence around the trial.

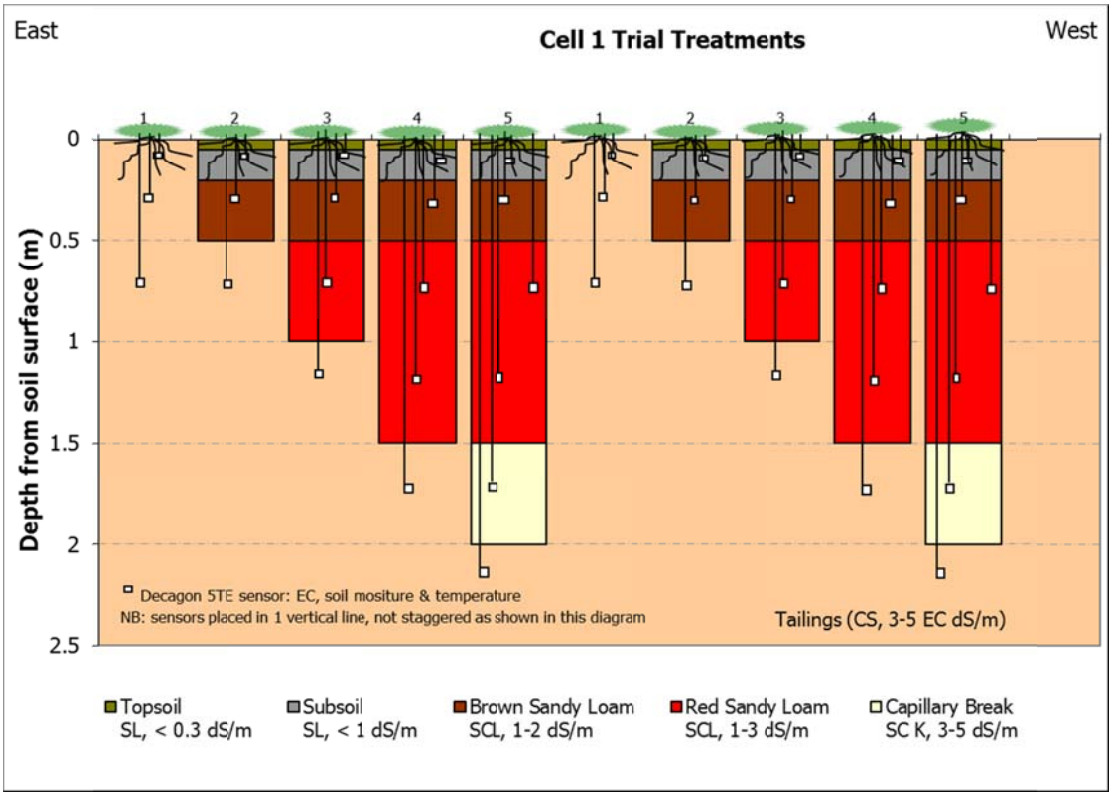

Figure 4.23 Side profile showing soil depths and placement of soil sensors (EC, temperature & soil moisture)

		W- myall	Red mallee	Sacrifice plants	Red mallee	W-myall	Yorrell	Yorrell
STRIP 1 ModCod	T1 R1 & R2	1	2	3	4	5	6	7
STRIP 2 TS, SS BL	T2 R1 & R2	8	9	10	11	12	13	14
STRIP 3 TS, SS, BL, <RL	T3 R1 & R2	15	16	17	18	19	20	21
STRIP 4 TS, SS, BL, >RL	T4 R1 & R2	22	23	24	25	26	27	28
STRIP 5 TS, SS, BL, RL, CB	T5 R1 & R2	29	30	31	32	33	34	35
STRIP 6 ModCod	T1 R3 & R4	36	37	38	39	40	41	42
STRIP 7 TS, SS BL	T2 R3 & R4	43	44	45	46	47	48	49
STRIP 8 TS, SS, BL, <RL	T3 R3 & R4	50	51	52	53	54	55	56
STRIP 9 TS, SS, BL, >RL	T4 R3 & R4	57	58	59	60	61	62	63
STRIP 10 TS, SS, BL, RL, CB	T5 R3 & R4	64	65	66	67	68	69	70

Figure 4.24 Each strip is partitioned into seven plots (9x12m), with two plots in each strip planted with a single western myall, red mallee or yorrell seedling. The seventh plot in each strip will be planted with seedlings of all three species for destructive sampling at 6,12 or 18 month intervals.

To avoid plant losses during dry months (November 2013 – March 2014), watering cans were used to apply 9 litres of water to each plant generally every fortnight or more/less often in response to weather conditions. Watering times and dates were recorded. Water wells (Greenwell Water Savers) were dug into the soil around each plant to ensure the application of water was as consistent as possible between trees. Ongoing erosion issues led to concrete having to be used to secure the water wells (Figure 4.25).

Figure 4.25 (a) Main plants protected by crates prior to rabbit and kangaroo-proof fence being installed. The natural regeneration from respreads topsoil has been removed since this photograph was taken to exclude competition effects. (b) examples of health plant growth in Treatment 2 measured in March 2014.

The trial is composed of a series of experiments which have been designed to achieve multiple aims (Table 4-7).

Table 4-7 Summary of Cell 1 Trial experiments, aims and methods

Experiment Name	Aims	Significance and application for rehabilitation	Summary of methods
Plant Sustainability (main plants)	To examine western myall, red mallee and yorrell are able to produce sustainable populations on shallow soil profiles over saline tailings.	Investigations post MARP discovered tree roots commonly at 22 m and continuing deeper. There is a mismatch between the depth of soil these species exist in normally and the shallower rehabilitation profile depths. If the outcome is: Yes - JA will have sustainable populations of these species. No - 2 options a) smaller pockets of deeper soil? b) deep rooted species return to pit rehab areas?	Measure plant growth and health parameters on Cell 1 trial and in situ plants. Measure: * **Plant growth** - stem diameter, height, # leaves, height x width plant and leaf index, habit * **Plant health** - Health score, Mini-PAM (photosynthetic efficiency), IRGA (gas exchange), reproduction (flowering, fruiting, viability)
Water Balance (main plants)	To determine the effect of shallow soil profiles on plant water use.	The mismatch between undisturbed and rehabilitated soil profiles may have reduced PAW to such an extent that deep rooted plants are less resilient to harsh summers and drought. If the outcome is: Yes - more confidence in current rehabilitation plan, post root mapping discoveries. No - 2 options a) smaller pockets of deeper soil? b) deep rooted species return to pit rehab areas?	Determine **plant and soil water balance** for each species in Cell 1 trial treatments & in situ. Measure * Rainfall * Evapotranspiration * Runoff * Change in soil water storage * Recharge
Plant Water Use from Mod Cod (sacrificial plants)	To determine whether western myall, red mallee and yorrell are able to extract and use moisture from saline tailings and if so, determine where the salt accumulates e.g. exclusion at root zone, or uptake, tissue accumulation and loss	Assuming deep rooted species are able to establish roots in saline tailings, if they are able to use the soil moisture from this layer it will substantially increase their effective rooting depth and therefore PAW. If they are able to use the water we need to establish where the salt from the tailings is accumulating to identify potential toxicity zones, which may impact their own root growth or that of other species. **Plant water source**	Using sacrificial plants, determine plant water sources under different seasonal conditions. Measure: * PAW in ModCod, RL, BL, SS, TS. * Tissue testing (Na+, Cl-) * Shallow probe samples, 1:5 ECe * Root depth - sacrificial seedlings * Stem water potential (pressure bomb on sacrificial plant) and soil water potential.

	with subsequent salt accumulation in surface soil.	If the outcome is: Yes - potentially increases PAW, and therefore increases plant resilience. No - plants will only have the rehab profile to extract PAW. **Salt toxicity** If the outcome is: Yes - monitor fate of salt and impact on plants over time, i.e. wetting front No - maintain status quo.	
Soil Water and Salinity Movement (main plants)	To monitor the movement of soil water and salinity through the various soil profile treatments, including wetting front identification.	According to the MARP we need to re-establish rainfall infiltration and soil moisture characteristics and therefore we need to measure what these are in undisturbed and rehabilitated soil profiles. If ModCod is exposed to rainfall some flushing of salt does occur (blue drum trial, EC measurements from Cell 1). We need to understand the net flux of leaching and evaporation over time to see if the effective rooting depth of the rehab profile can be increased by a period of tailing pre-exposure to leaching. **Soil Water Characteristics** If the outcome is: Yes - maintain status quo. No - revisit the BL vs. RL ratio if one or the other has greater water holding capacity and PAW. **ModCod Leaching** If the outcome is: Yes - leave tails exposed for however long it takes to flush. No - rehab can occur when tailing is finished.	Monitor soil moisture and EC movement through the soil profile. Monitoring * Soil moisture: 5TE soil sensors * Soil ECe: 5TE soil sensors Salt movement *EP Analysis hollow auger rig in sacrificial plots after 2 years (i.e. April 2015)
Capillary Break (main plants)	To provide field verification of the capillary break modelling outcomes i.e.	Modelling outcomes suggested a capillary break was not required and this needs field verification.	Monitor soil moisture and EC movement through the soil profile. Measure: * Soil moisture: gravimetrics.

	no capillary break required.	If the outcome is: Yes - keep installing capillary break No - don't include capillary break in future rehab profiles.	* Soil ECe: Decagon 5TE in year 1, gravimetrics.
Disturbed vs Undisturbed Soil Characteristics	To compare changes in soil chemical and physical characteristics as a result of disturbance.	Bulk handling of soil may change soil physical or chemical characteristics such as water holding capacity, nutrient status, toxicity levels, root penetration, PAW, soil porosity & gas exchange. Changes to these parameters could negatively impact on plant growth therefore early identification of problems will improve rehab outcomes. If the outcome is: Yes - detrimental to long term plant growth & survival - may need to revisit the BL vs. RL ratio, deep ripping, fertiliser, increasing direct return. No - status quo	Replicated samples of in-situ and disturbed trial material for each soil type sent to CSBP. Measure: Texture, EC, CEC, Cl, boron, pH, N, P, micronutrients, analysis. Replicated samples of in-situ and disturbed trial material for each soil type sent to Cliff Hignett (Soil Water Solutions) for theoretical PAW.

Preliminary Results

Aspects of the trial are still being established and unfortunately we are unable to present any initial data at this stage. There were no plant deaths over the summer period and many of the plants are flourishing. Erosion caused by windborne sand from nearby mining activities has impacted on the trial, resulting in a total loss of topsoil (i.e. approximately 5 cm). Currently there are plans to reapply the topsoil layer in May 2014, after adjacent areas in the mine pit become stable. Despite some delays in the final installation and issues with erosion, the trial will prove to be a valuable research tool for the Iluka rehabilitation team. Given the large scale of the experiment, it is expected that measurements will be continued well after the conclusion of the ARCLP to assess how the eco□physiology of these species in these environments change through their life phases.

Acknowledgements

Samantha Doudle designed the trial and with Shane Doudle orchestrated the construction of the trial and the installation of soil sensors. Thanks to Exact Mining Group for undertaking the earthworks. Kerry Saunders has assisted with many aspects of the trial and his help has been much appreciated. Brandon Willis, Lucy Cunningham and Lindy Scott have assisted with watering and collecting plant growth and health measurements.

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5 LANDSCAPE DESIGN

The aim of the landscape design research is to investigate key components of in-situ landscape arrangements from landscape to micro-topography level, to create sustainable rehabilitation landforms.

5.1 Categorising Jacinth and Ambrosia catchments and designing surface water management structures (consultancy)

Alluvium Consulting and Iluka Resources Ltd

5.1.1 Introduction

A watercourse rehabilitation study was carried out in 2013 (Alluvium, 2013) with the following project objectives:

- Review of environmental baseline information relevant to the project.
- Hydrologic modelling to determine flow rates for a range of ARI events.
- Hydraulic modelling to identify the pattern and distribution of flow for each ARI event.
- Geomorphological categorisation and delineation of parameters of watercourses.
- Development of functional designs for rehabilitation and construction of watercourses
- Recommendations for field studies to examine erodibility of the landscape and the efficacy of erosion controls.
- Training of J-A staff in the design principles for waterway rehabilitation.

The project report documents these outcomes and provides recommendations for the catchment reconnections which could also be applied to other Iluka owned sites.

A summary of the report is provided below with the full report available upon request.

5.1.2 Methods

The method that has been developed for this project has been developed to provide guidance and design parameters for watercourse rehabilitation and catchment reconnection at J-A mine. The method has also been developed within the wider context of achieving the following long term outcomes for Iluka:

1. A resilient and self-sustaining rehabilitated creek system indicating that the pre-mining ecosystem and landscape function will ultimately be achieved.
2. Adoption of best practice sustainable creek rehabilitation design to provide high confidence of a successful and timely planning approval process.
3. No significant and demonstrable impact on rehabilitated mining landscape including the rehabilitated mining landscape.

The attainment of these outcomes requires a thorough understanding of the watercourse characteristics, the mine rehabilitation plans and level of acceptable risk in terms of watercourse erosion or sedimentation and the identified criteria for mine closure. The method adopted is described in Table 5.1 and consists of five phases.

Table 5-1 Method outline and list of data used

Phase	Description	Data list
Phase 1	Review and synthesise all available background information. Refine project method based on the findings.	• Previous reports and GIS layers • 1 ,2 and 10 m contour data • Topo grid for pre mining landscape • Rainfall records (Tarcoola, Jacinth Ambrosia) • 2005/10/11/12 aerial photos • Vegetation and soil surveys • Arid zone literature
Phase 2	Undertake desktop assessment to characterise watercourses (using River Styles® framework) in terms of geomorphology and develop hydrologic and hydraulic model to develop an understanding of baseline conditions.	• As above • New hydrologic model built using RORB • New baseline hydraulic model built using HecRAS
Phase 3	Undertake fieldwork to refine River Styles® characterisation and hydraulic model. Using this information, define river parameter criteria (i.e. width, depth, slope) based on River Style categorisation. Conduct training workshop for Iluka staff about river processes and principles for river rehabilitation. Engage staff through a workshop to gain understanding of mine rehabilitation objectives and planning and identify acceptable levels of risk.	• Fieldwork conducted 29 – 31 May 2013 • Detailed topographical survey of selected reaches was undertaken during this period • Refined River Styles® characterisation based on fieldwork and refined hydraulic model based on topographic survey. • Identify key parameters that define different River Styles®. • Training and risk workshop was conducted on May 30 2013
Phase 4	Develop framework and functional designs for the rehabilitation of River Styles® - incorporate functional design into proposed mine rehabilitation landscape to identify and mitigate erosion risk.	• MARP • HecRas model • Proposed rehabilitation levels DEM • DEM of rehabilitated Cell 1
Phase 5	Advise on the future strategy for disconnected catchments and develop framework for monitoring and evaluation.	• MARP • Mine plan

5.1.3 Summary of baseline findings

Watercourses exist in a system with an upper catchment functioning as a sediment source zone, a middle catchment functioning as a sediment transport zone and a lower catchment where sediment is deposited. Over the long term, watercourses adjust their form to achieve a state of equilibrium where rates of erosion are similar to rates of deposition. An abrupt change in a system variable can disturb this balance by initiating a period of excess stream erosion or deposition. This can have significant implications for the ecological, economic and amenity values of the watercourse.

The J-A watercourses are ephemeral and are shaped by rainfall and flow events that are highly variable both spatially and temporally. When the watercourses do flow, they experience significant transmission losses for smaller and medium-sized flows. These significant transmission losses can result in ongoing cycles of incision and deposition along

the watercourse. Vegetation, and BSC, plays a crucial role in slope and watercourse bed and bank stability in the J-A catchment.

The J-A watercourse system is complex and vulnerable to accelerated erosion. The fluvial processes and physical form can vary substantially along the length of a watercourse. A geomorphic categorisation of the J-A watercourses is required to develop an understanding of stream form and function and to identify key parameters that can be used as a basis for the rehabilitation design of the disturbed watercourses.

5.1.4 River Styles® Categorisation

Overview

A geomorphic classification of watercourses in the Jacinth-Ambrosia catchment has been undertaken using the River Styles® framework. This approach enables consistent categorisation of watercourses in the region based on common geomorphic forms and processes. This allows us to identify common parameters that can be used as the basis for the rehabilitation design of watercourses disturbed by mining operations at the two mine sites.

The River Styles® framework was developed by Gary Brierley and Kirstie Fryirs (2000) to provide a rigorous geomorphic approach for examining river character, behaviour, condition and recovery potential. It is a four stage process, of which stages one and two will be applied in this assessment:

1. Catchment-wide baseline survey of river character and behaviour
2. Catchment-framed assessment of river evolution and geomorphic river condition

River Styles® Categories

A River Style® classification was completed for 100 km of watercourse network within the Jacinth Ambrosia catchment. A total of six River Styles® were identified, five of which are new styles within the recognised River Style® nomenclature. This is a reflection of the few known instances when this framework has been applied to an arid region. The distribution of River Styles® is shown in Figure 5.1 and the River Style® classification tree for these watercourses is provided in Figure 5.2.

All styles are within a laterally unconfined valley setting (Table 5-2), with 60% of watercourses having a discontinuous channel and the remaining 40% having a continuous channel.

Table 5-2 River Styles in the Jacinth Ambrosia region

Valley setting	River Style®
Laterally unconfined valley setting - continuous channel (LUV CC)	Interdunal bank confined gully, sand Interdunal bank confined channel, sand Interdunal wandering, sand
Laterally unconfined valley setting - discontinuous channel (LUV DC)	Valley fill, sand Chain of pans Terminal lunette chain of pans

The planform and potential adjustment of River Styles® in the laterally unconfined valley setting is not considered to be controlled by the valley margin. Watercourses in the Jacinth Ambrosia region have developed within extensive interdunal floodplains. Bed and bank materials are susceptible to erosion during flows and significant channel change typically

occurs in response to large, rare flow events while minor modifications can occur during smaller flow events or through aeolian processes. There are two groups of unconfined systems; those with a continuous channel and those with a discontinuous channel. The distribution of these channel types is shown in Figure 5.3.

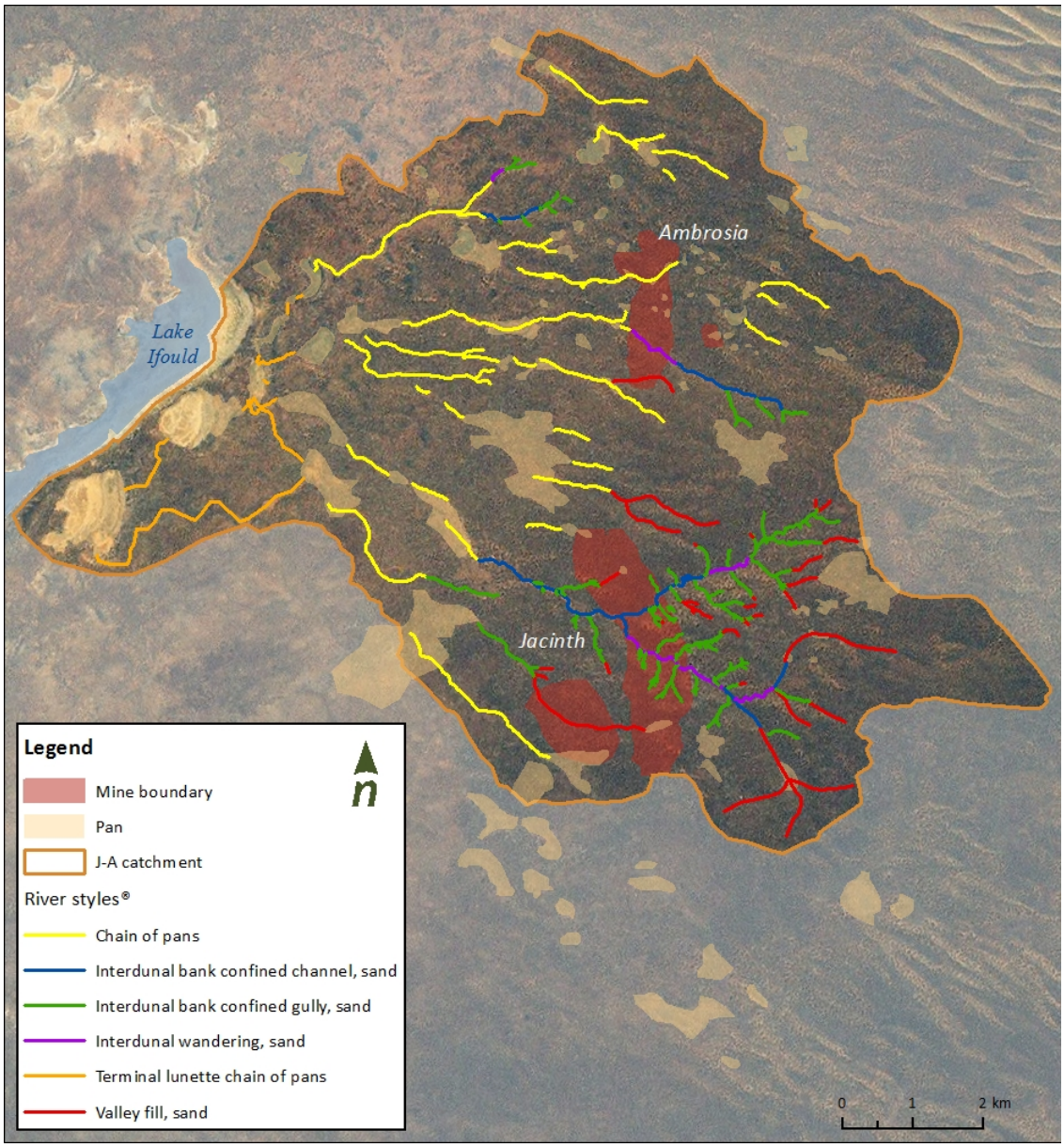


Figure 5.1 Distribution of River Styles in the Jacinth Ambrosia catchment

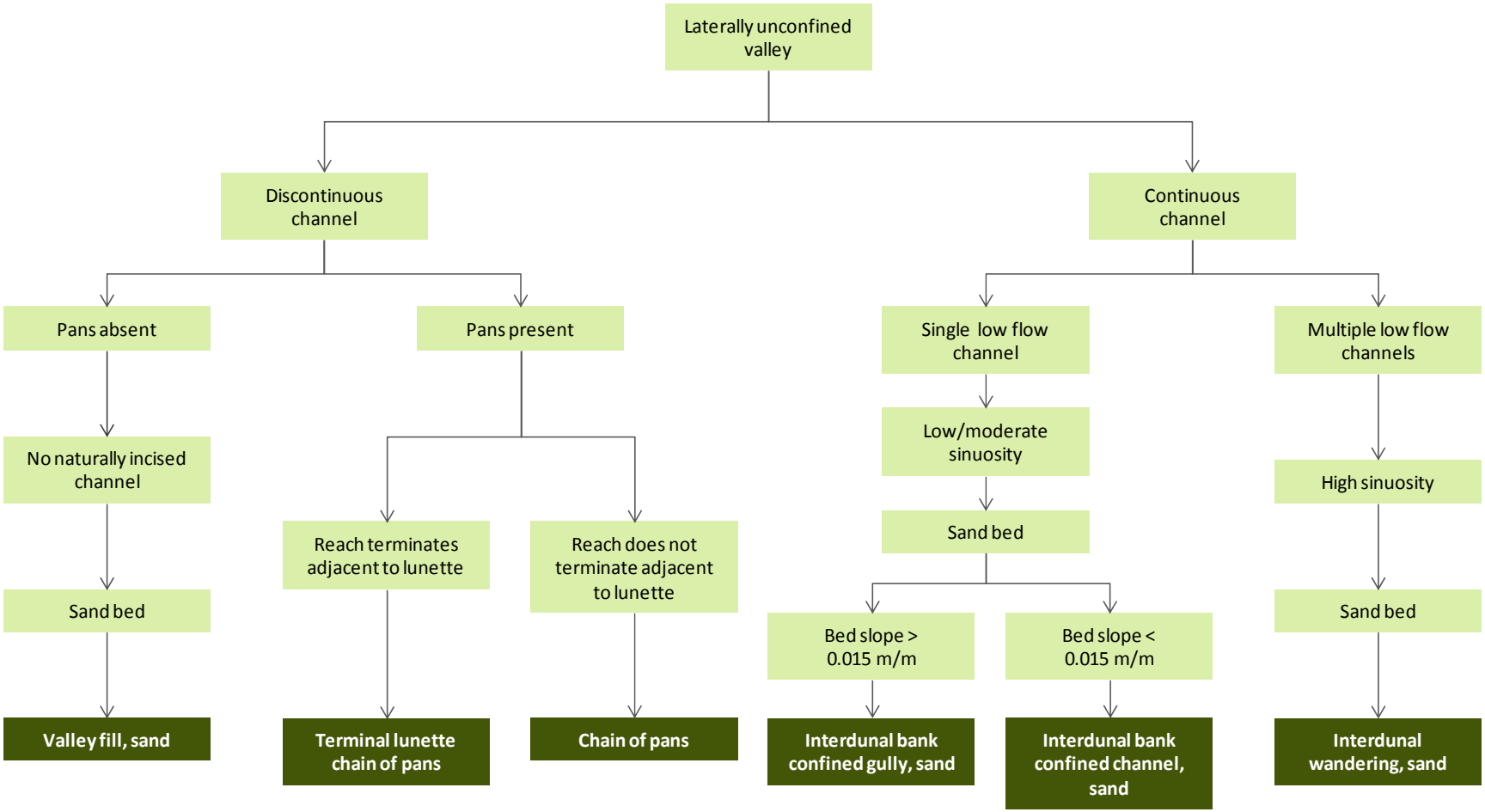


Figure 5.2 River Style tree developed for the assessment of watercourses in the Jacinth-Ambrosia mine area

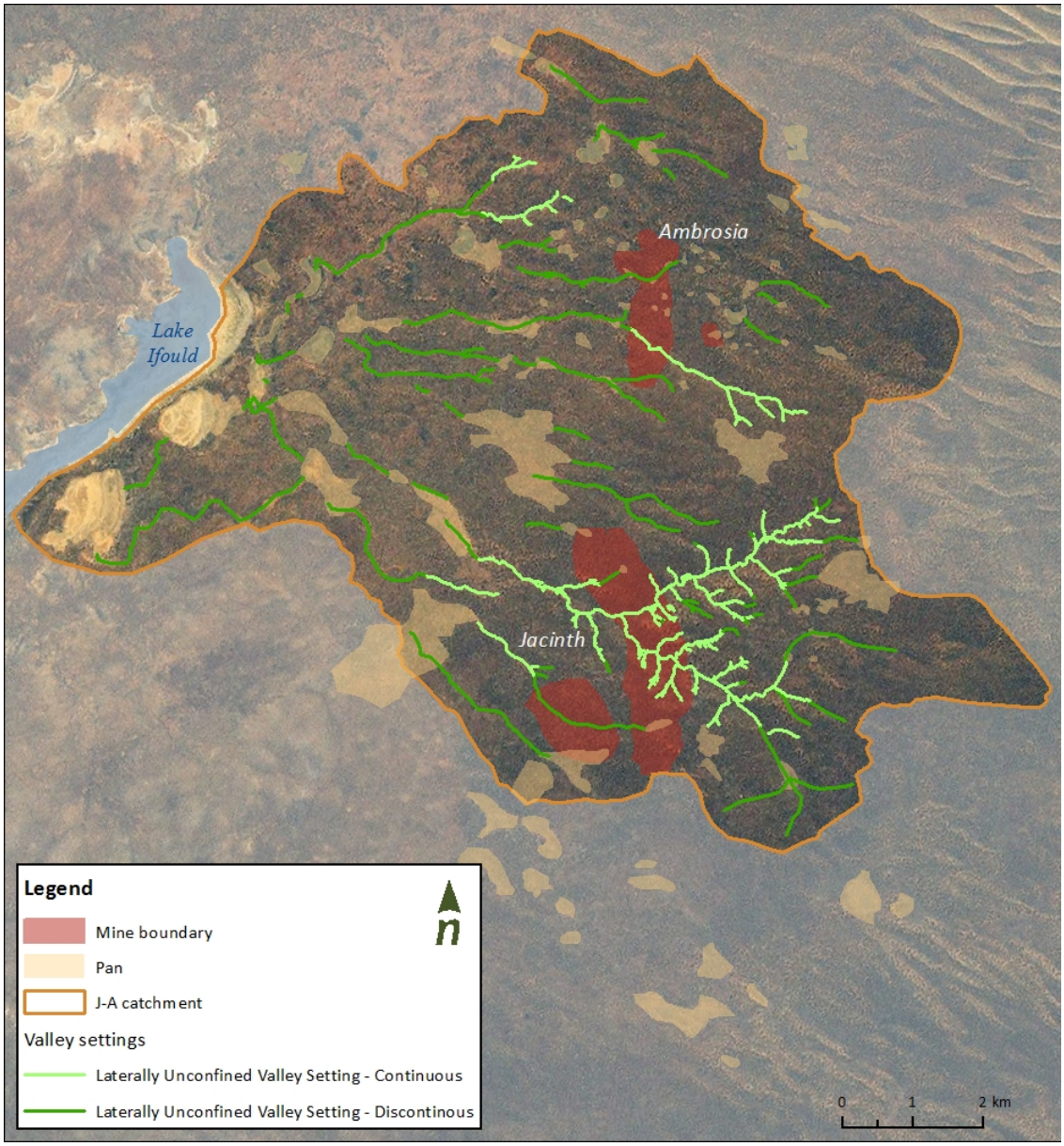


Figure 5.3 Continuous and discontinuous watercourse in the Jacinth-Ambrosia catchment

Continuous channel watercourses

Unconfined continuous watercourses include interdunal bank confined channel, interdunal bank confined gully and interdunal wandering River Styles®. These typically lie in the mid and upper catchment, including within the mine boundaries. Watercourses in each of these River Styles® have incised or are experiencing ongoing incision. The two interdunal bank confined styles tend to exist in sub-catchments with a more classical dendritic stream network. The two styles can be distinguished based on active deepening: the interdunal bank confined channel reaches tend to be relatively stable, while the interdunal bank confined gully reaches tend to be smaller and actively incising.

Discontinuous channel watercourses

Unconfined discontinuous watercourses in the Jacinth Ambrosia region include chain of pans, terminal lunette chain of pans and valley fill River Styles®. Terminal lunette chain of pans reaches are found only near the downstream extent of the catchment, adjacent to the lunette the fringes Lake Ifould. Other chain of pans watercourses are generally found through the lower catchment area and lie predominantly downstream of the two mine boundaries, although there are some reaches upstream of the Ambrosia mine lease. Valley fill reaches lie in the upper catchment and, in all cases, are the most upstream reaches of their watercourses.

The Valley Fill and Chain of Pans River Styles® are vulnerable to incision, which is typically triggered by a flow event and is related to biological soil crust (BSC) cover and/or presence of incision heads in downstream reaches. Incision events in these reaches have the potential to liberate large volumes of sediment for transport to downstream reaches.

The length of watercourse with each River Style® across the Jacinth-Ambrosia mine area is shown below (Table 5-3). Chain of pans is the most common River Style® across the area, reflecting the abundance of salt and sand pans throughout the middle catchment.

Table 5-3 Length of each River Style® found across the Jacinth Ambrosia region

Valley setting	River Style®	Length (km)	% of valley setting	% of all watercourses
LUV CC	Interdunal bank confined gully, sand	26.44	67%	26%
LUV CC	Interdunal bank confined channel, sand	8.44	21%	8%
LUV CC	Interdunal wandering, sand	4.46	11%	4%
LUV DC	Valley fill, sand	17.19	28%	17%
LUV DC	Chain of pans	35.51	58%	35%
LUV DC	Terminal lunette chain of pans	8.07	13%	8%

The defining attributes and parameters of each River Style ® are explained in detail within the Alluvium Report (2013) which is available upon request. A summary of the parameter values are provided in Table 5-4.

Table 5-4 Summary of parameter values for each River Style

River Style®	Channel width (m)	Depth (m)	Width-depth ratio	Channel slope (m/m)	Catchment area (ha)	2 year stream power (N/m s)	50 year stream power (N/m s)
LUV CC - Interdunal bank confined channel, sand	17 - 40	1.2 – 2.5	13 - 19	0.009 – 0.01	550 – 1200	16 - 34	25 – 43
LUV CC - Interdunal bank confined gully, sand	9 – 17	0.9 – 1.5	7 - 15	0.025 – 0.03	12 – 30	15 - 47	25 - 40
LUV CC - Interdunal wandering, sand	35 – 90	1 – 2.5	20 – 40	0.01	350 – 700	7 - 16	21 – 36
LUV DC - Chain of pans	-	-	-	0.009 – 0.012	-	3 - 21	10 – 26
LUV DC - Terminal lunette chain of pans	-	-	-	0.0008 – 0.022	-	-	-
LUV DC - Valley fill, sand	19 - 28	0.2 – 0.5	60 - 148	0.016 – 0.026	11 - 55	4 - 7	8 – 13

5.1.5 Design Principles and Framework

The design principles and framework are underpinned by an understanding of the regional hydrology and geomorphology and the parameters for each River Style as described in the previous sections. They have been developed within the context of J-A MARP criteria and achieving the following long term outcomes for Iluka:

- A resilient and self-sustaining rehabilitated watercourse system indicating that the pre-mining ecosystem and landscape function will ultimately be achieved.
- Adoption of best practice sustainable watercourse rehabilitation design to provide high confidence of a successful and timely sign-off process.
- No significant and demonstrable impact on the rehabilitated mine landscape or offsite.

Design Principles

The design principles have been divided into two areas: *technical* and *operational.* Technical design principles relate to ensuring the MARP criteria are met in terms of creating sustainable watercourse landscape units. Operational design principles relate to management objectives and levels of acceptable risk during operation and post rehabilitation.

Based on the above, the following *technical* design principles for Jacinth Ambrosia mine have been identified:

1. All disturbed watercourses will be rehabilitated and reconnected to the wider catchment.
2. Geomorphic and hydrologic concepts will underpin watercourse design to ensure that the post mine landscape function and shape of watercourses is achieved.
3. Design parameters will be based on the regional empirical relationships developed for the River Styles identified from the J-A mine catchment area.
4. Sediment dynamics of rehabilitated watercourses will be provided for in design.
5. Some adjustment of the watercourse form and shape through erosion and deposition will be anticipated in recognition of the rehabilitated watercourse operating as a natural watercourse.
6. Additional design measures will be adopted in locations where erosion risk into underlying tailings is considered high.
7. Vegetation is an integral component of watercourse design and future ecological function.

The operation requirements for watercourse rehabilitation planning and design were discussed with Iluka management staff during a workshop facilitated by Alluvium in May 2013. The following key risks related to surface water to the wider mine rehabilitation areas were identified:

- Capillary rise of salt into overburden - if surface water is allowed to regularly pool on areas rehabilitated on top of ModCod the seepage could trigger capillary rise of the salt from ModCod into the overlying overburden and into the plant root zone.
- Exposure of ModCod - uncontrolled gully erosion on top of ModCod areas could have the potential to expose saline tailings and release saline water into the rehabilitated riparian system, particularly on the shallow Chenopod shrubland profiles.
- Loss of topsoil - the topsoil layer containing plant seed and BSC is very thin and is the key to providing rapid landscape stability and kick starting ecosystem functions. A suite of measures need to be undertaken to minimise topsoil loss from water and wind

erosion. These measures need to be robust enough to protect the rehabilitated areas during sequential drought years.

Based on these risks and maximising operational and economic efficiency during rehabilitation works, the following operational design principles were identified during the discussion:

1. Any erosion into tailings is unacceptable.
2. Design and rehabilitation approach must meet the MARP criteria.
3. Watercourse rehabilitation approach needs to be accepted by regulator.
4. Design needs to be economic and allow for low level of construction precision.
5. Future mine liability needs to be minimised including minimal impacts offsite during and post rehabilitation

The technical and operational design principles provide the basis for the Jacinth Ambrosia watercourse rehabilitation design framework described in the following section.

Design Framework

The proposed design framework is focused on providing a flexible approach to watercourse rehabilitation that Iluka can use and refine over time. This contrasts with the development of a once-off, prescriptive watercourse rehabilitation design that doesn't accommodate future change. The design framework is underpinned by an understanding of the natural fluvial processes operating in the J-A watercourses. It provides two different approaches to watercourse rehabilitation (template and empirical) based on the River Styles identified at Jacinth Ambrosia mine (Figure 5.4).

Template design approach

The template approach is to be applied for the River Styles Interdunal Bank Confined and Interdunal Wandering to replicate and re-establish the original watercourses that have been disturbed. These River Styles relate to the main watercourses including Jacinth North and South Creeks and Ambrosia South Creek. The design approach for these watercourses has been developed to ensure that:

- the catchment is reconnected.
- natural, sustainable watercourse function is returned.
- ensure flow capacity is retained.
- minimise erosion risk.

While there is a requirement to 'put back' the original planform and channel gradient of the main watercourses, there is scope to modify some parameters such as channel width and bank slopes within a range of defined values (Table 5-5 and Table 5-6). This allows some flexibility in rehabilitation planning and construction whilst recognising that the main watercourses have the greatest potential to initiate erosion and contribute to offsite impacts if a significant deviation from the defined parameters is made. The design parameters for the minor creeks within the disturbed area are described in Table 5-7.

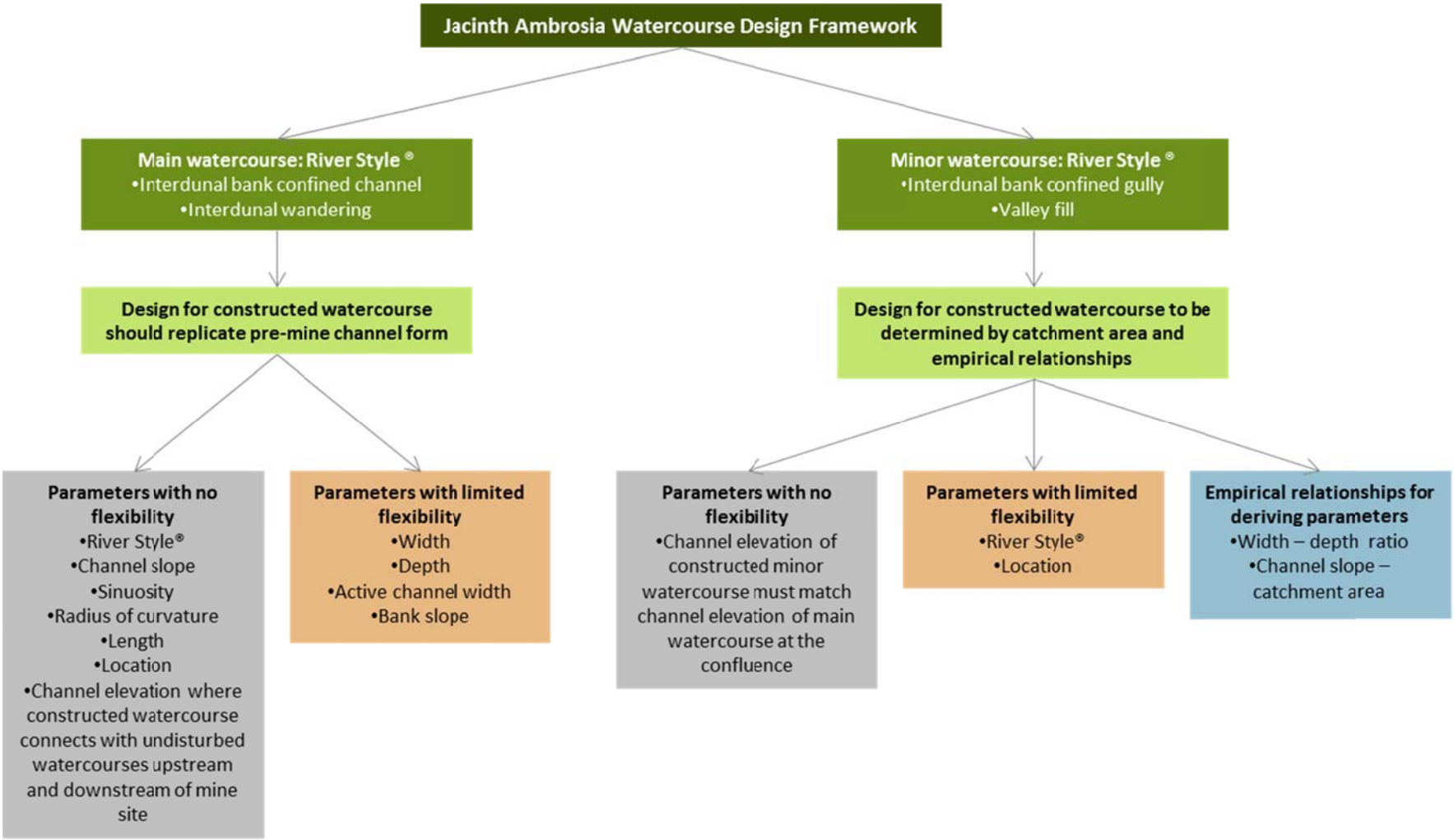


Figure 5.4 Jacinth Ambrosia watercourse design framework

Table 5-5 Design parameters for Jacinth North Creek and South Creek within the disturbed area

Parameter	Unit	Jacinth North Creek (Interdunal Bank Confined)	Jacinth South Creek Interdunal Wandering
Channel gradient	(m/m)	0.0091	0.0093
Width	(m)	17 – 55 (mean value 35)	55 - 90 (mean value 60)
Width–depth ratio	-	14 – 18	46 - 60
Active channel width	(m)	5 - 10 (mean value 8)	10 - 30 (mean value 18)
Inside bank slope	(V:H)	1:10 – 1:20	1:20 – 1:30
Outside bank slope	(V:H)	1:3 – 1:5	1:5 – 1:8
Straight bank slope	(V:H)	1:5 – 1:10	1:10 – 1:15
Sinuosity	-	1.07	1.13
Radius of curvature	(m)	100	80
Stream Power (2 yr ARI)	(N/m s)	15- 20	5 – 15
Stream Power (50 yr ARI)	(N/m s)	35 - 60	20 - 40

Table 5-6 Design parameters for Ambrosia South Creek within future disturbed area

Parameter	Unit	Ambrosia South Creek (Interdunal Wandering)
Channel gradient	(m/m)	0.0121
Width	(m)	40 – 65 (mean value 52)
Width–depth ratio	-	20 – 28
Active channel width	(m)	10 -25 (mean value 16)
Sinuosity	-	1.11
Radius of curvature	(m)	70
Stream Power (2 yr ARI)	(N/m s)	5 – 10
Stream Power (50 yr ARI)	(N/ m s)	20 - 35

Table 5-7 Indicative design parameters for minor creeks within the disturbed area

Parameter	Unit	Bank confined gully	Valley fill
Width	(m)	10 - 17	19 - 27
Width–depth ratio	-	10 - 16	-
Active channel width	(m)	1.3 - 4	No active channel
Bank slope	(V:H)	1:6	Gentle slope to centreline of catchment

Additional design elements

In additional to the design framework for the different River Styles, several design elements need to considered as part of the construction of watercourses. These include:

- Depth of overburden versus River Style
- Transition between River Styles
- Catchment connections
- Watercourse confluence
- Provision of sediment supply
- Vegetation establishment

These design elements are discussed in detail within the Alluvium report.

5.1.6 Future strategy for catchment and watercourse disconnection

A draft plan outlining the mine rehabilitation measures has been developed to demonstrate the sequence of events that are scheduled to occur (Doudle and Eckert, 2013). A summary of these measures is presented in Figure 5.5.

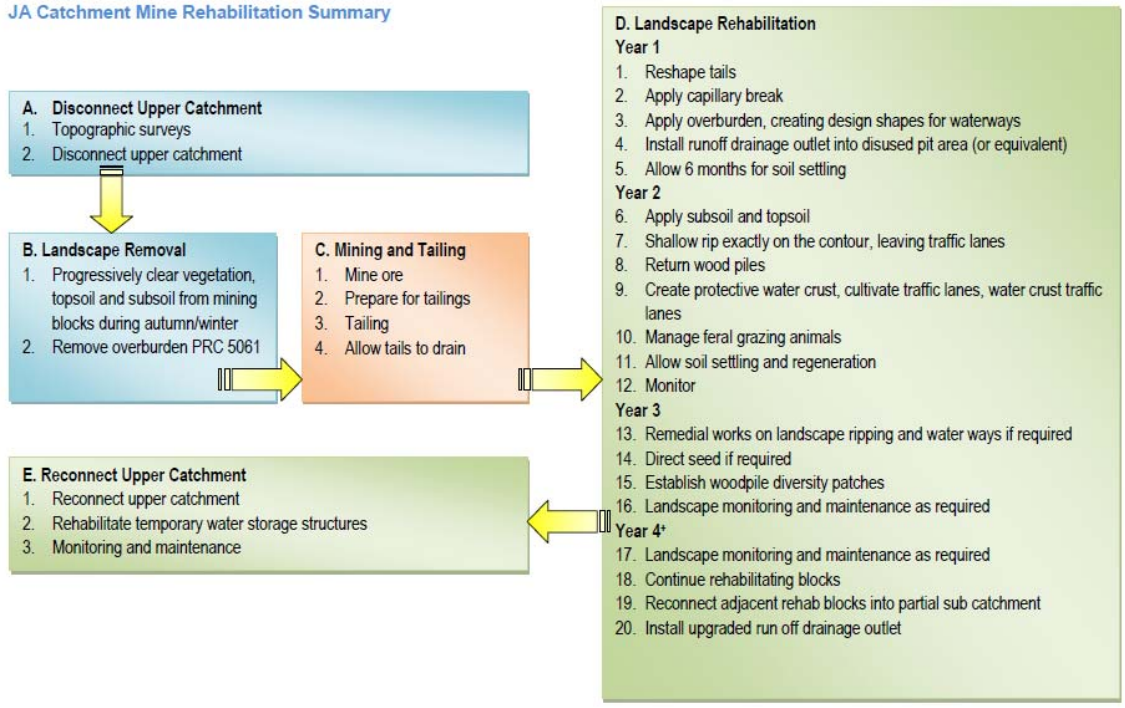


Figure 5.5 Summary of JA catchment mine rehabilitation measures

With the exception of meeting pre-mining topography targets, the current rehabilitation schedule does not take into account requirements for reconstructing the watercourses. Additional measures will be incorporated into future rehabilitation and consideration will be given to the recommendations provided by Alluvium in their report.

5.2 Categorising sand dune soils and vegetation to improve rehabilitation outcomes for this landscape type

Iluka Resources

5.2.1 Introduction

In the J-A baseline soil surveys conducted in 2009, three major soil management units were identified, comprising:

SMU 1 – deep calcareous sandy loam associated with 2 – 7 m deep dunal ridges.

SMU 2 – shallow calcareous sandy loam associated with localised topographic depressions and the Nullarbor Plain landscape to the west. The soils consist of a thin layer (<1 m) of brown sandy loam overlying a consolidated calcrete layer.

SMU 3 – deep calcareous sandy loam, which is the dominant soil unit, which consists of a thick layer of brown sandy loam (average 4 m) overlying calcrete.

Subsequent vegetation surveys then identified and linked a dominant vegetation association to each SMU:

SMU 1 – mallee/ myall woodland

SMU 2 – chenopod shrubland

SMU 3 – myall woodland

As part of the soil and vegetation association mapping, an assumption had been made that mallee only grew on deep dunal sands and therefore areas with this vegetation associated were prescribed a rehabilitation sand profile of 2.4 m (Table 4.1 of the J-A Rehabilitation Management Plan, 2009).

The pre-mining mapping and planned post-mining extent of the mallee/myall woodland landscape unit is shown in Figure 5.6.

Since mining commenced at J-A in 2009 field observations have shown that the soil prescription for this landscape unit is not realistic. This is discussed in more detail in Section 5.2.2.

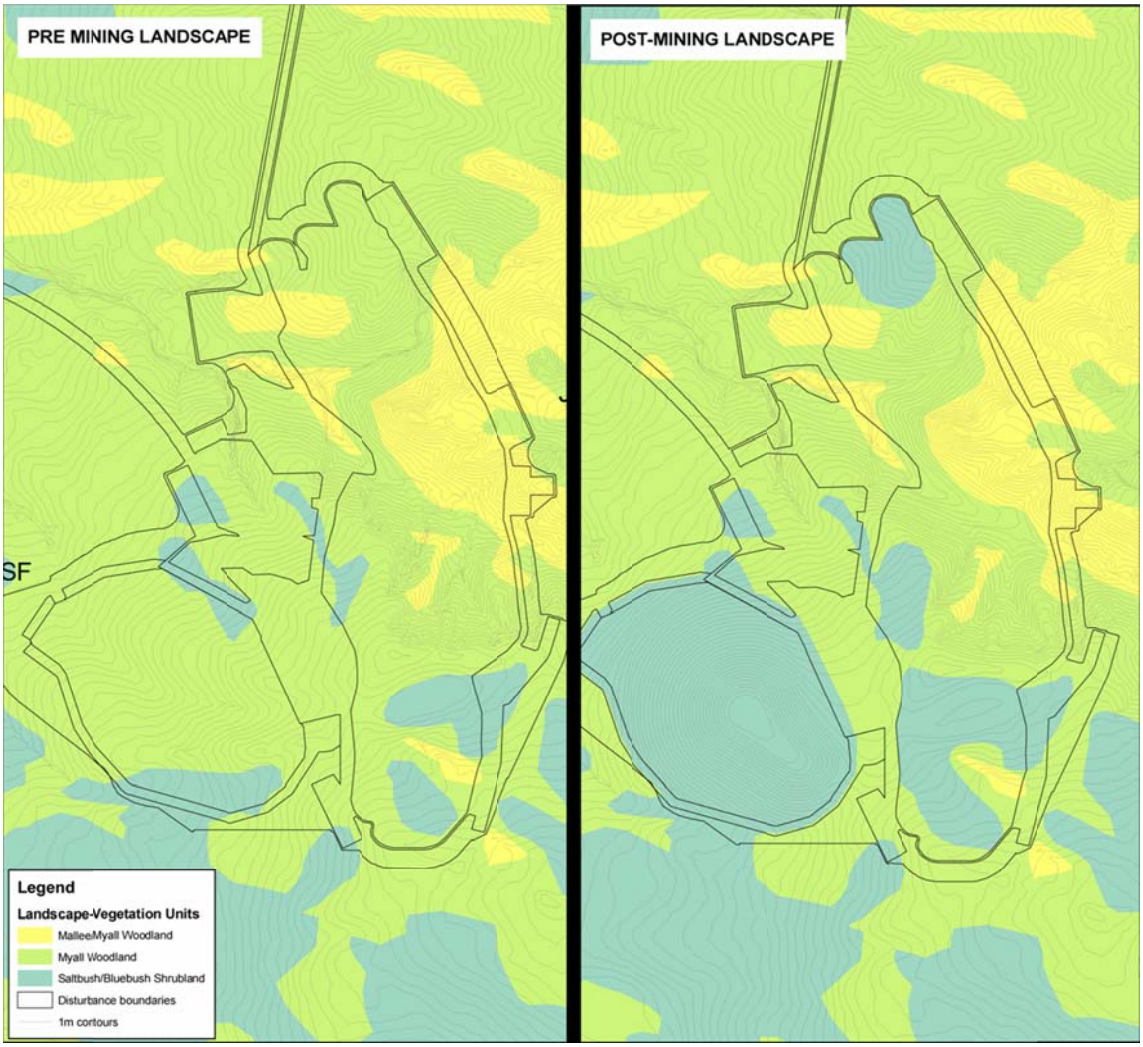


Figure 5.6 Comparison of pre-mining and post-mining vegetation landscape units

5.2.2 Observations

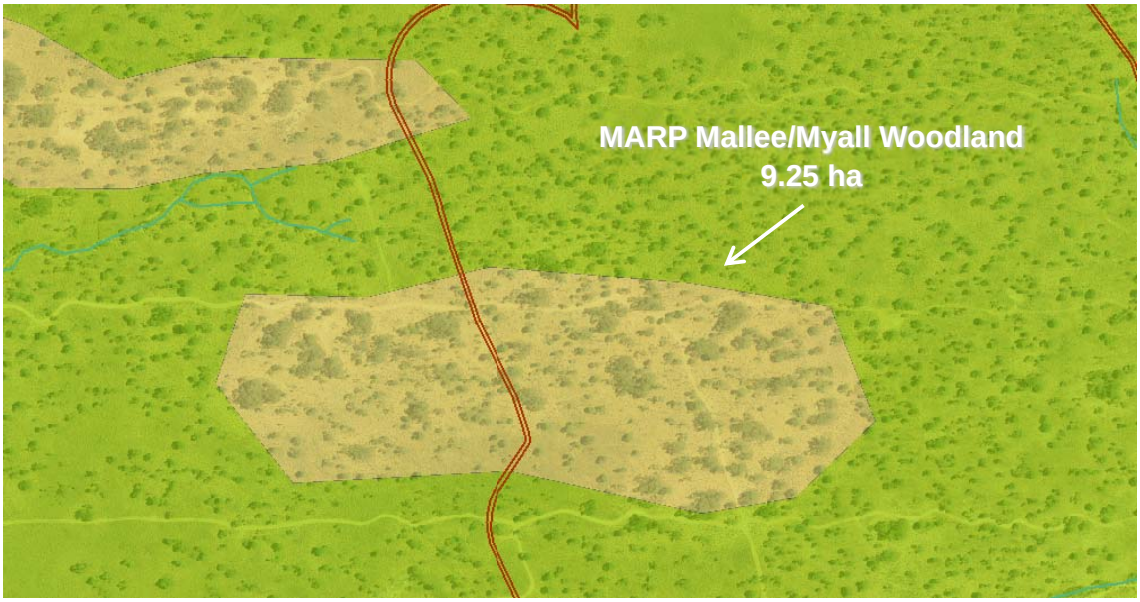
Field observations and detailed vegetation surveys have demonstrated that the original vegetation surveys were an over-simplification of the extent of myall/mallee woodland occurrence at J-A. In 2012, a focus sand dune was chosen on the north-western side of the Jacinth pit. This dune was chosen as it was in the immediate mine path and scheduled for overburden removal. The area was mapped in the MARP as 9.25 ha of myall/mallee woodland, which according to MARP Table 4.1 would require approximately 222,000 BCM of

sand to recreate a 2.4 m sand depth across the entire area (

Figure 5.7).

A detailed vegetation survey was conducted across the area using a GPS to identify exact locations of mallee, myall and other key sand indicator species i.e. *Senna artemisiodes* ssp. *Coreacea* and *Eremophila scoparia*. Myall was widespread over the entire area. Mallee was less common and occurred in patches throughout the area. The other sand indicator species were also distributed across the area in patches.

The mallee was visually mapped showing there are large areas within the zone area that don't contain any mallee. Approximately 4.8 ha or 51% of the area defined by the MARP mapping as myall/mallee woodland actually contains mallee (Figure 5.7).



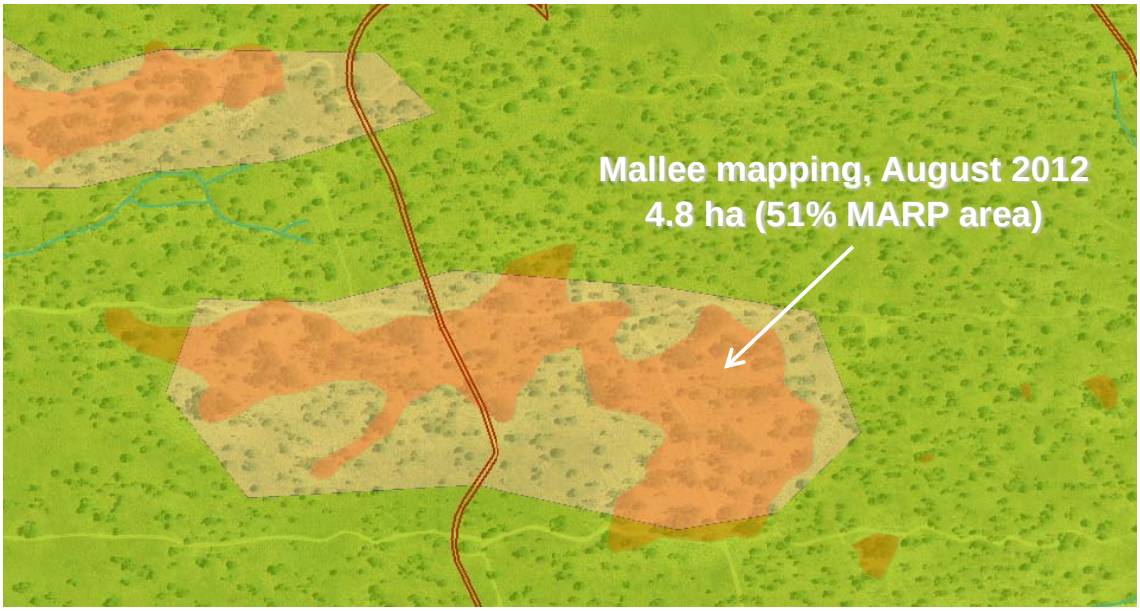


Figure 5.7 Top figure showing extent of mallee/myall woodland as outlined in the MARP. Bottom figure showing the extent of mallee based on a detailed vegetation survey

A detailed soil survey was subsequently conducted across the area to determine sand location and depth. Mini soil pits (1.5 m deep) and auger holes were sampled at 20 cm increments and analysed for texture, pH, ECe, $CaCO_3$ and water repellence. Sand dune material was distinguished from underlying brown loam using the same parameters as the original vegetation and soil surveys, namely sand to loamy sand texture, relatively low salinity compared to underlying materials and often above a minor unconsolidated calcrete layer at the texture change boundary (SWC 2009). Nine out of the twenty four holes contained dune sand and of these only two contained a depth of sand greater than 2 m.

5.2.3 Outcomes and Recommendations

Based on the above observations, discussions were had with government regulators in October 2012 and it was agreed that the following actions would occur moving forward:

- 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 (Figure 5.6).
- 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.

5.3 Landscape Function Analysis (LFA)

Iluka Resources

5.3.1 Progress

The key rehabilitation monitoring tool at J-A is Landscape Functions Analysis (LFA). Originally developed for rangeland monitoring, LFA is ideally suited to monitor the J-A 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.

Information generated from the initial years of LFA monitoring and vegetation surveys will be used to negotiate specific closure criteria for J-A, including targets for vegetation diversity and abundance for each soil unit and vegetation association.

LFA surveys are conducted on a 1, 2, 5 and 10 year schedule per monitoring site and none of the LFA survey sites that had been monitored and reported on in the previous JARMS period were scheduled for monitoring during 2012 and 2013.

No additional LFA sites were established in 2012 and 2013. However it is expected that new sites will be established in 2014 and these will be reported on in the next JARMS.

6 SOIL MANAGEMENT

The aim of this theme of projects is to assess the quantity and quality of soil material available for rehabilitation to thereby maximise its contribution to the creation of sustainable landscapes.

6.1 Determine effectiveness of using diluted topsoil for rehabilitation

Iluka Resources Ltd

6.1.1 Introduction

The importance of the role of topsoil in 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 likely to be insufficient topsoil to enable soil cover compliant with the rehabilitation soil profile outlined in the Mining and Rehabilitation Plan (MARP) and Table 4.1 of the J-A Rehabilitation Management Plan. 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 (brown loam or 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 brown loam and subsoil. A preliminary vegetation survey to measure regrowth was conducted in December 2013.

6.1.2 Method

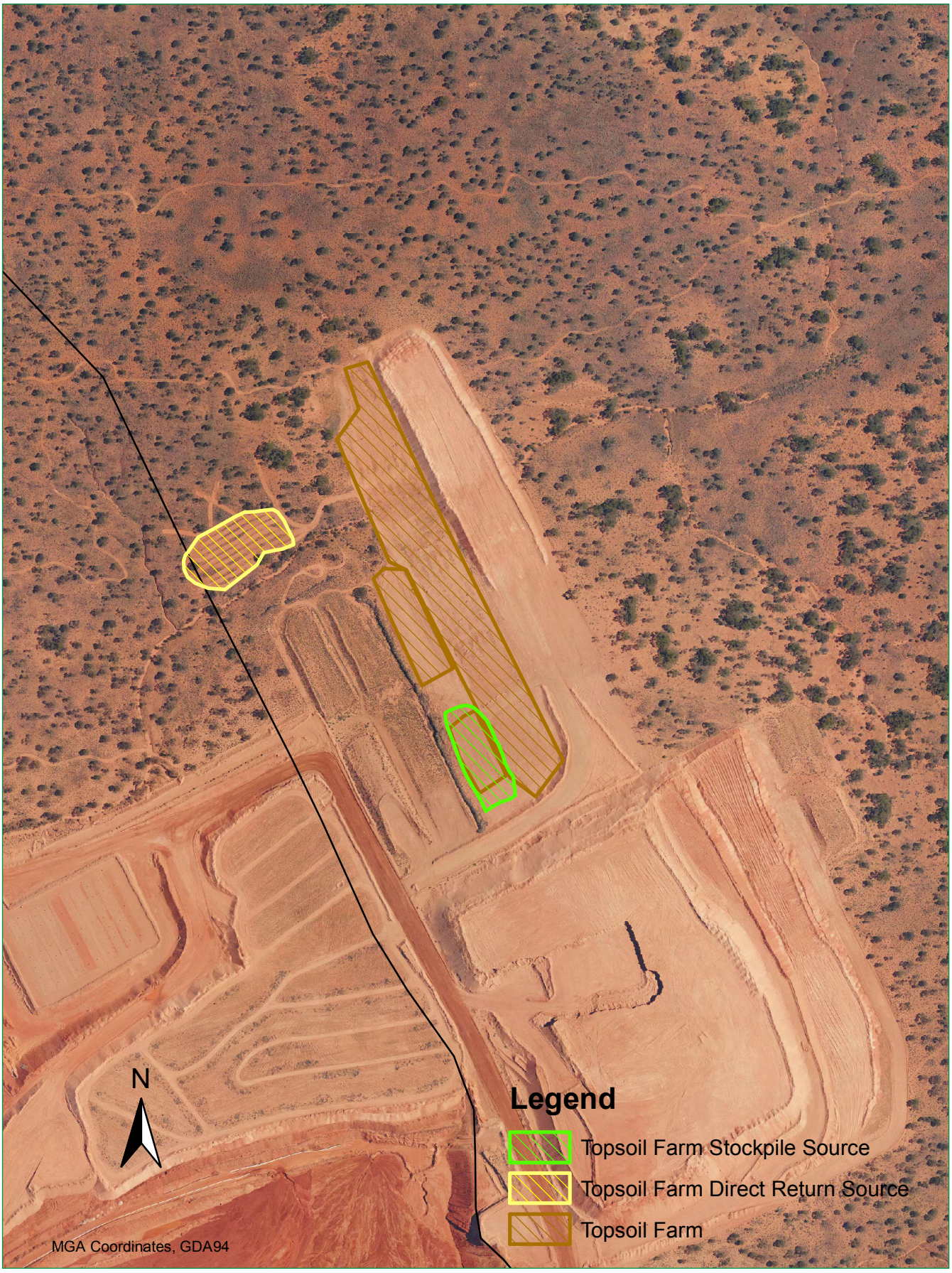
An undisturbed area of vegetation within the mine lease footprint was selected as the topsoil source to avoid any additional vegetation clearance (Figure 6.1). Care was taken to locate the Topsoil Farm in area that was to remain undisturbed for a minimum of five years to allow the topsoil to develop.

The Topsoil Farm currently comprises three treatments:

1. Stockpiled topsoil applied to brown loam at a diluted rate.
2. Direct return topsoil from an undisturbed area applied to brown loam at a diluted rate.
3. Direct return topsoil from an undisturbed area applied to subsoil at a diluted rate.

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 brown loam or 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 (Plate 6-1).

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. Six transects of 20 m were located in the application areas for treatment one and treatment two. Three transects were located in the undisturbed source area to monitor biological crust and vegetation growth in areas where topsoil was removed. Monitoring was not carried out on treatment three; direct return topsoil from source area applied to subsoil, however monitoring will commence in 2014 for this treatment. Monitoring of all treatments will continue annually.



200 Meters

Jacinth Ambrosia

Topsoil Farm location and layout

ILUKA

ORIG: JLEE DRAWN: JLEE SCALE: 1:5,617 (A4) DATE: April 2014 DWG No: JARMS Fig. 6.1

FIGURE: 6.1

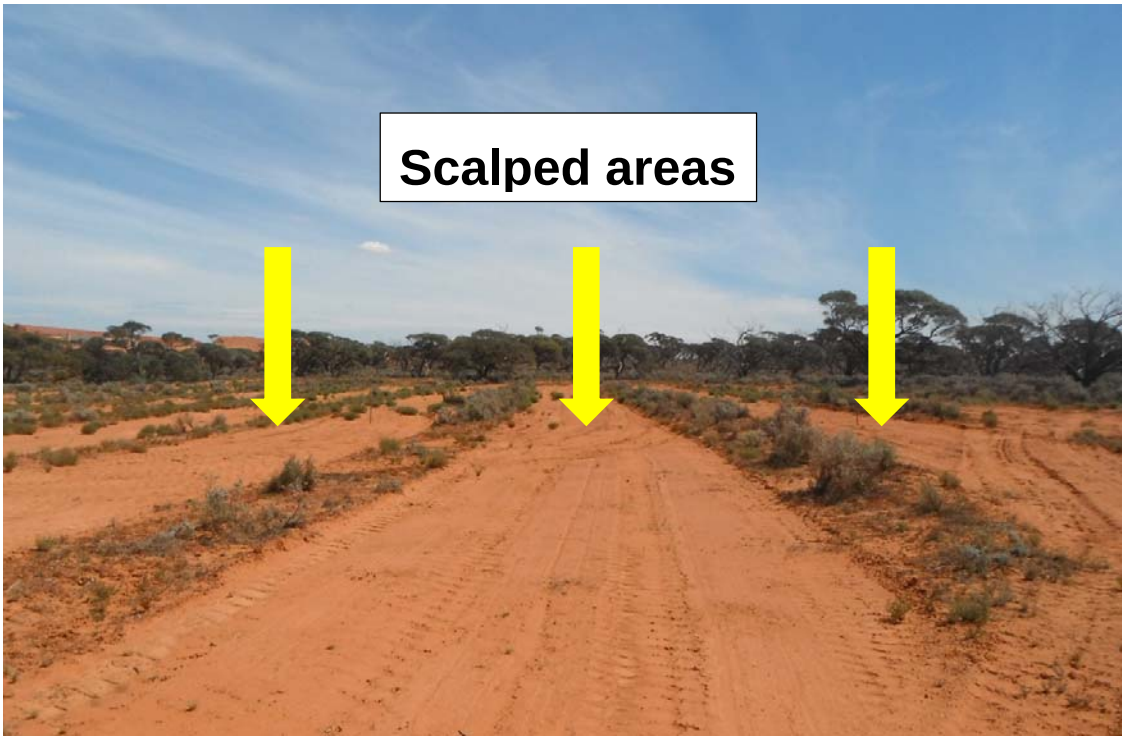


Plate 6-1 Previously undisturbed area with scalped strips (topsoil source)

6.1.3 Results and Discussion

The mean number of species recorded across the monitored treatments was similar, ranging from 11 at the direct return on brown loam and 10 species on the stockpiled topsoil on brown loam treatment (Table 6-1). The mean number of species on the topsoil source area was also similar (11 species). This may be due to the requirements of the plant species, the species germinating may be disturbance specialists and the community may therefore change with time. Additional analysis of the community composition and the species research will clarify how similar the communities are across the treatments and identify possible causes for any differences.

The mean number of individual plants recorded in the transects ranged from 182 (stockpiled topsoil on brown loam) to 61 (direct return topsoil on brown loam). The mean number of plants recorded was much higher where the stockpiled soil had been applied to the brown loam (Table 6-1). This result must be interpreted with caution as the result was from a single monitoring event, although the count of plants was consistently higher across all transects in the stockpiled topsoil treatment. The count of plants on the area where the topsoil was sourced from was similar to that of the direct return soil on brown loam.

Table 6-1 Results of topsoil farming monitoring for 2013

	Direct return topsoil on brown loam	Stockpiled topsoil on brown loam	Topsoil source area	Direct return topsoil on subsoil
Mean abundance	61	182	92	Not monitored
Mean species richness	11	10	11	Not monitored

No measurement of biological crust was taken during the 2013 period, however a monitoring program will be developed for 2014 to measure the regeneration of the biological crust in the Topsoil Farm.

6.1.4 Recommendations

Based on the current results the following recommendations are made:

- Monitoring will continue in all three treatments annually and will include a program to monitor the response of the biological crust to the different treatments.
- Include a subsoil stockpile and brown loam stockpile in the monitoring program as a baseline against which to compare results.
- Given that the characteristics of topsoil, subsoil and brown loam are very similar additional investigations will be carried out to determine the potential to manufacture topsoil from brown loam or subsoil without the addition of a topsoil inoculant. These studies will determine if with the addition of artificially grown biological crust inoculants and collected seed, topsoil of suitable quality to ensure good rehabilitation outcomes can be created.

6.2 Promoting the re-establishment of biocrusts for the long term control of invasive species

Angela Chilton - University of New South Wales

Iluka Resources

6.2.1 Introduction

A survey of the Iluka Resource Ltd Jacinth-Ambrosia (J-A) mine site pre-operations recorded the presence of *Carrichtera annua* which has since increased in number. Since operations have begun, other species including *Brassica tournefortii* have been identified. These are notorious invasive species of arid lands worldwide to which they are well adapted, notably to disturbed sites. Current mitigation strategies include manual pulling and herbicides. However, these are labour intensive, impractical on large scales and curative rather than preventative. Promoting the re-establishment of biocrusts presents an alternative method for the long term control of these invasive species. However, the effect of biocrusts on vascular plants has been found to be highly site and species dependent. It is therefore important to consider the biocrust/plant relationship in the context of the elected environment. To this end, an examination of the effect of six different biocrust coverage types on the germination of the two invasive plant species *Carrichtera annua* and *Brassica tournefortii* was undertaken.

6.2.2 Methods

Six different biocrust cover types were selected (Table 6-2). These included Bare Soil as a control, three different re-establishment treatments and two natural biocrusts. Each biocrust cover type had two different seed depths, surface (top) and subsurface (buried). Each treatment was conducted with four replicates of 25 seeds over 14 days. All sample material was sourced from the mine site and the experiment conducted at the UNSW, Sydney, Glasshouse. Data was analysed in Excel and Primer 6.

Table 6-2 Types of biocrust cover types used

Treatment	Treatment Category	Description
Bare Soil	Control	Soil free of any biocrust
Growth Inoculum	Artificial	Biomass enriched biocrust soil grown for 21 days
Fresh Inoculum		Biomass enriched biocrust soil not grown
Crush		Crumbled mixed biocrust
Early	Natural	Core of early stage biocrust (Cyanobacteria)
Late		Core of late stage biocrust (moss)

6.2.3 Key results

The findings demonstrated the species dependent nature of biocrust-vascular plant interactions. While *C.annua* was overall inhibited by burial, *B.tournefortii* was inhibited by placement on the surface. *C.annua* seeds had an overall high rate of germination (max = 94%). Independent of seed depth, Early stage natural biocrusts showed significant inhibition of germination compared to the Bare soil control while Late stage biocrust showed inhibition for surface placed seeds (Figure 6.2 and Table 6-3). Artificial biocrust coverage types showed no significant inhibition upon germination compared to the Bare soil control for either seed depth ($p>0.05$). For surface placed *B.tournefortii* seeds, both artificial and natural biocrust cover types demonstrated significant inhibition of germination compared to the Bare soil control, with the Early biocrust treatment observing zero germination (Table 6-3). Conversely, there was no significant inhibition of germination observed for buried *B. tournefortii* seeds ($p>0.05$). However, germination rates for *B. tournefortii* were low overall for both seed depths (max = 16.5%).

Table 6-3 Significant p values for treatments compared to Bare soil. Only p values less than 0.05 shown.

Seed Species	Treatment Type	Seed Location	P value
Carrichtera annua	Early	Surface (Top)	0.030
	Late		0.030
	Early	Subsurface (Bottom)	0.035
Brassica Tournefortii	Early	Surface (Top)	0.022
	Late		0.037
	Crush		0.042
	Growth		0.026
	Fresh		0.026

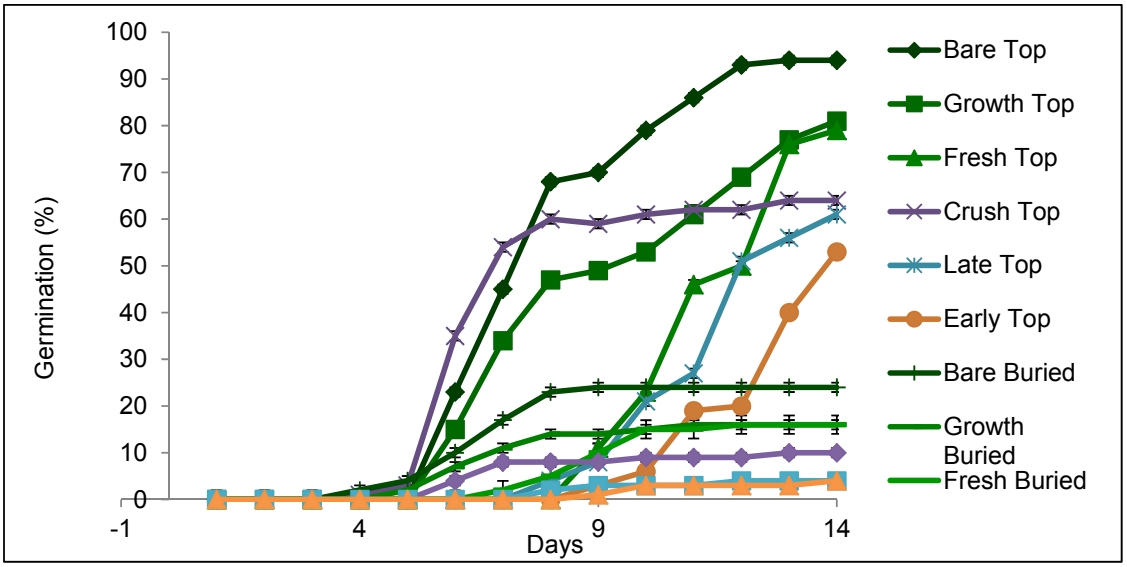


Figure 6.2 Germination of *C.annua*. Error bars shows standard error of the mean.

6.2.4 Conclusions

The differing responses of the two seed types demonstrates the complexity of the biocrust-vascular plant relationship. Overall, *C.annua* was inhibited by naturally occurring biocrusts while *B.tournefortii* was only significantly inhibited by surface placement upon both artificial and natural biocrust cover types. The re-establishing of biocrusts is a crucial aspect of arid land restoration. While this study used artificial biocrust types representative of potential remediation strategies, these treatments would likely need considerably longer time in order to demonstrate similar physically inhibitory effects as naturally occurring, established biocrusts. In addition, several factors which may also explain the observation of greater occurrence of these invasive weeds at disturbed sites have not been accounted for here. These include allelopathic effects and surface water run-off from biocrusts carrying seeds to disturbed sites where they are deposited.

6.3 Cell 1 East Rehabilitation Trials

Iluka Resources

6.3.1 Introduction

In mid-2013, the first of the Jacinth pit areas planned for rehabilitation was reinstated with land forming, ripping and seeding. Rehabilitation of the first of the in-pit TSF (Cell 1 East) presented an opportunity to trial different rehabilitation techniques. The topsoil layer of Cell 1 East was established in a manner that allows a comparison of the persistence of the seed bank in various aged topsoils as well as determining the efficacy of direct seeding techniques. More specifically the trials comprise:

- Comparison of species richness and abundance for subsoil and topsoil that has been stockpiled for various time periods and subsoil and topsoil returned directly from mine path clearance (i.e. not stockpiled).
- Comparison of species richness and abundance when topsoil and subsoil is cleared and applied as a single unit in comparison to the current soil profile required in the MARP.
- Comparison of species richness and abundance when direct seeding is carried out in comparison to areas where vegetation germination is via the soil seed bank only.

- Vegetation growth response to ripping compared with areas that have not been ripped.
- Survivability of vegetation planted as seedlings over time.

6.3.2 Trial Design

Cell 1 East was divided into a number of trial bays, each relating to specific treatment (Figure 6.7). Three trial bays were prepared using different aged topsoils and suboils, soils that had been stockpiled for one year, soils that had been stockpiled for four years and soils that were cleared and immediately replaced in the trial bay (direct return, i.e. not stockpiled). The application of the subsoil and topsoil were carried out according to the MARP profile for these treatments (ie subsoil 0.15 m deep and topsoil 0.05 m deep). In addition another bay was prepared using a combination of topsoil and subsoil collected together (direct return single pass) and applied directly from a cleared area (i.e. not stockpiled). The soil age trial bays were not seeded, all vegetation growth is from the soil seed bank. The remaining bays in Cell 1 East were then prepared using a combination of direct return and one year old soils.

All other trial bays (outside of the soil age trial bays) were direct seeded, additional seed was broadcast by hand. Additionally seedlings of various species were planted in the direct seeded bays with the intention of monitoring survivability over time.

Transects were established to test the response of vegetation growth to ripping and seeding, comprising four treatments, additional seeding +/- ripping and seedbank only +/- ripping. Each treatment transect was 20 m long.

6.3.3 Monitoring

Photopoints were established for each of the Cell 1 East trials and photopoint monitoring was carried out monthly for 2013. Future photopoint monitoring will be carried annually.

Modified Jessop transects were established to record vegetation species richness and abundance in the soil age trial bays and the direct return single pass trial. A 20 m transect was established from the photopoint markers and the number of each species present in a 1 m^2 quadrat directly adjacent the transect was recorded. In addition a 0.4 ha quadrat was established in each trial bay and each species was recorded and given an abundance estimate.

To compare the response by vegetation to seeding +/- ripping all vegetation transecting a single 20 m transect was recorded.

Vegetation response to direct seeding for the various topsoil ages available was not monitored, however additional monitoring points will be established for future programs.

6.3.4 Preliminary Results

Monitoring of the trial bays commenced December 2013, results shown here are preliminary only.

Soil Age Trials

Overall species richness for the different aged soils was similar, ranging from 14 species to 9 species (Figure 6.3). The most number of species was recorded in the trial bay with soils that had been stockpiled for four years (14 species), the least number of species was recorded in the trial bay where soil was direct returned (9 species). Where the topsoil and subsoil was collected and returned as a single unit (direct return single pass) 12 species were recorded (Figure 6.3).

The total number of plants recorded (abundance) in the Jessop transects ranged from 255 plants to 98 plants (Figure 6.4). The highest number of plants was recorded in the trial bay with soils that had been stockpiled for one year (255 plants). The lowest number of plants recorded was in the four year old soils (122 plants); this was similar to the direct return single pass trial bay (98 plants).

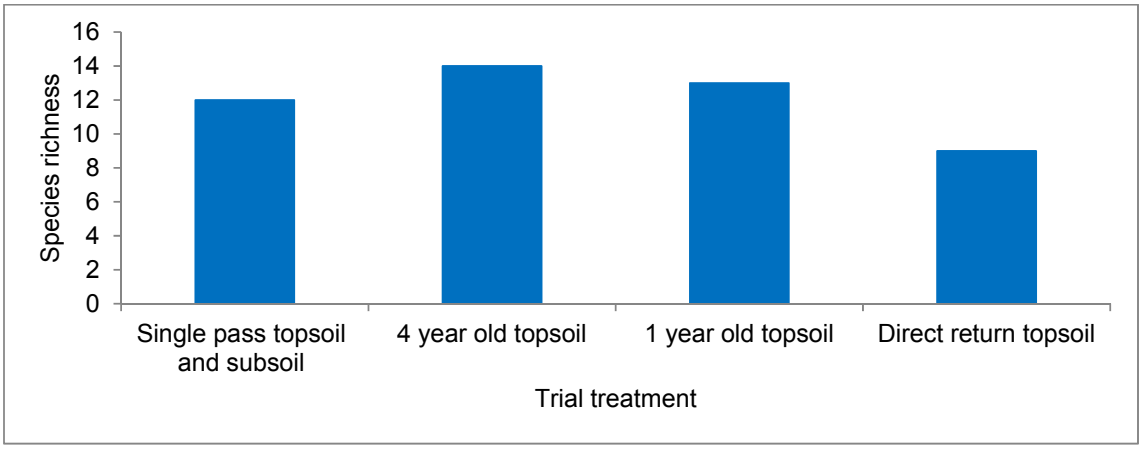


Figure 6.3 Species richness for different topsoil ages and treatments

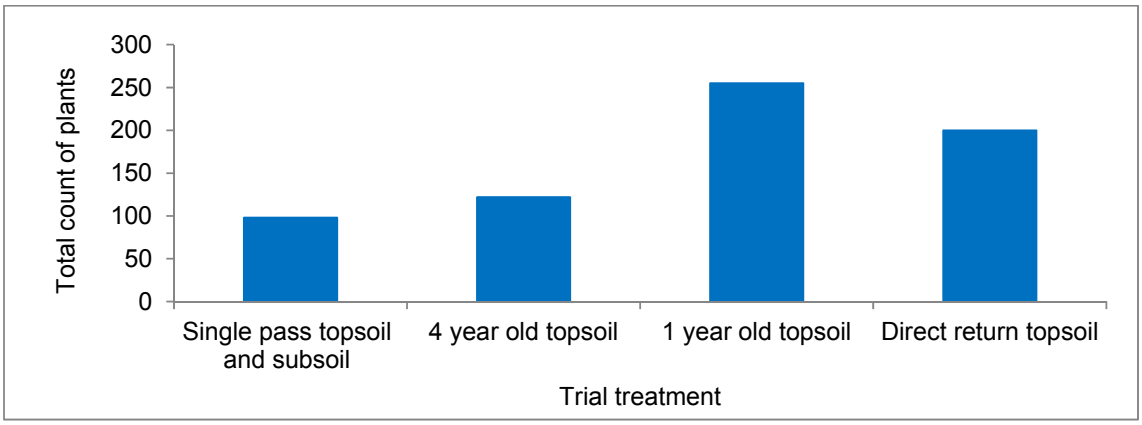


Figure 6.4 Abundance of plants for different topsoil ages and treatments

Seeding and Ripping

The numbers of species recorded in the seeding and ripping treatments was similar across all treatments, ranging from 11 species in the direct seeded and ripped transect to 9 species in the not seeded or ripped transect (Figure 6.5).

The total counts of plants on the transects ranged from 64 plants to 34 plants (Figure 6.6). The numbers of plants on the ripped treatments was the same and the highest (64 plants). The lowest number of plants recorded was on the transect that was not seeded or ripped (34 plants) and was approximately half that of the other three treatments.

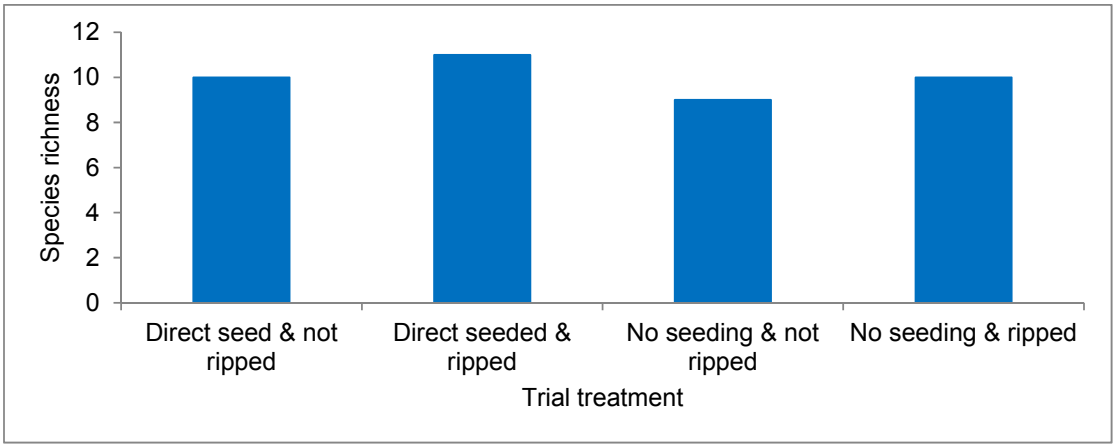


Figure 6.5 Species richness for different seeding and ripping treatments

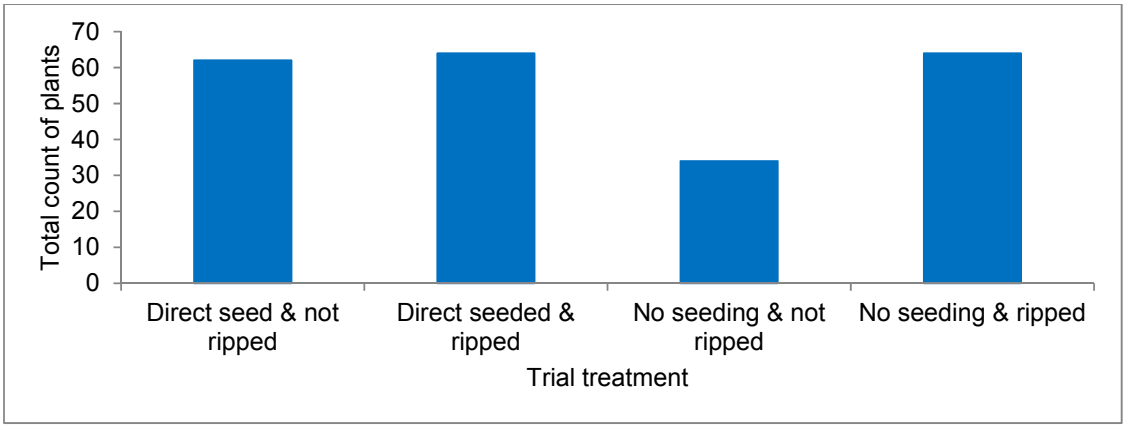


Figure 6.6 Plant abundance for different seeding and ripping treatments

Seedling persistence

Of the 67 seedlings that were planted only nine *Eucalyptus Oleosa* seedlings could be relocated in 2013. Eight plants were recorded as still alive. The height and number of the branches on the seedlings was recorded for future monitoring programs.

6.3.5 Conclusion

The results here must be interpreted with caution as they are only from a single trial season. However overall the number of species recorded in the soil age trials was similar and further the species recorded were similar across the trials. Any differences in community composition across the soil age trials will be investigated further and the resultant information will provide a preliminary recalcitrant species list, i.e. species that do not easily germinate from the soil seedbank. Recalcitrant species will then be the focus of germination and direct seeding trials and seedling survivability trials.

The direct return single pass and four year old soil trials performed the poorest for vegetation abundance, however again these results must be interpreted with caution as only the first year of growth is presented here. The performance of the trial bays over the longer term will provide further detail on the suitability of these rehabilitation methods.

Species richness was again similar across the direct seeding and ripping methodology trial bays. The similarity of species across the entire rehabilitation area is more likely due to the requirements of the species present; it is likely that some of the species present are

disturbance specialist taking advantage of the open areas. It may also be possible that the absence of a mature biological crust which may usually inhibit seed germination of many plant species (as it has been show to do for some weed species) may no longer be an inhibiting factor. Additional studies of the soils biological crust life stages and effects on germination would be required to understand this interaction.

Abundance of plants on the different seeding and ripping methods were similar except for the trial where there was no additional seeding was carried out and the transect was not ripped. Although this may indicate the importance of ripping for successful revegetation outcomes the experiment was too small and discrete to draw any conclusions. Regardless, ripping of rehabilitation areas is generally considered to be beneficial for infiltration and germination and will be continue to be part of the J-A rehabilitation program as a best practice standard.

Rehabilitation to be carried out in 2014 (Cell 1 West) will replicate these trials as closely as possible and monitoring of both trial areas will be carried out and reported on annually in the Mining and Rehabilitation Compliance Report. More detailed analysis of results and reporting will be carried out in the 2015 JARMS.

100

Meters

Janith Ambrosia

Cell 1 East Rehabilitation Trial

ILUKA

ORIG: JLEE DRAWN: Drawn SCALE: 1:2,873 (A4) DATE: April 2014 DWG No: JARMS Fig. 6.6

FIGURE: 6.7

7 VEGETATION SALT TOLERANCE

The following studies aim to investigate the tolerance of J-A vegetation species to soil salinity at various life stages.

7.1 Potential effect of saline water seepage on deep rooted vegetation (J-A field monitoring)

Iluka Resources Ltd

7.1.1 Introduction

This progress report summarises the methods used to proactively and reactively monitor the vegetation health in response to unexpectedly rapid rising of saline groundwater at J-A over the past two years (Table 7-1). The studies are still in progress and a limited number of results have been analysed and presented. It is expected that the progress of all three programs will continued to be reported as they become available.

This study has shown that red mallee is a potentially useful vegetation species to assist with predictions of rising saline groundwater. The potential now exists to expand the red mallee monitoring network to obtain baseline data for areas in advance of the mine path. Methods of rapid monitoring of plant health for numerous other keystone species have also been developed. Verification of the symptoms used for monitoring plant health can be achieved through using multiple data sources at vegetation health monitoring sites, including groundwater levels and electromagnetics.

The outcomes of this work will provide information of operational use to Iluka and of scientific value to the wider community. Rising groundwater and salinity issues are of great importance to land managers throughout Australia and the world. This importance is reflected in the provision of governmental funding (via an Australian Research Council Grant through the University of Adelaide) to continue investigations of plant water use and root mapping arising directly from the findings of this study.

The data from this study to date supports the Iluka proposed interim depth beyond which groundwater should not be allowed to rise of 20 m below the soil surface. The accuracy of this prediction will be developed over the next three years. Contributions will come from the continuation and expansion of the monitoring network and the information generated in the new University of Adelaide/Iluka Resources Ltd, Australian Research Council Partnership Project.

Further work is required to improve understanding of the effects of rising groundwater on soil salinity levels as the saline groundwater recedes via pumping or mound dispersion. This has important implications for overburden removal and storage in advance of the mine path. Saline groundwater must not be allowed to rise into overburden that will later be required for rehabilitation.

Groundwater Mounding

The combined texture of the tailings produced at JA is coarse and this has allowed the saline water to rapidly drain from the unlined tailings storage facilities and into the soil profile. Since mining and tailing began in October 2009, two 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 the JA Groundwater Management Plan.

Some localised groundwater mounding was anticipated in the original mine planning and approvals. As part of the approvals process, Iluka were required to eventually negotiate a

level beyond which groundwater should not rise to minimise the impact of saline groundwater on the health of local and downstream vegetation and ecosystems. The background information required to set a useful level did not exist. To address this and other rehabilitation related knowledge gaps, Iluka and the University of Adelaide have developed research and monitoring programs to better understand the unique local environment, identify the impacts that may occur during mining operations and develop mitigations strategies to manage those impacts.

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 pit 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). At JA the hypersaline groundwater levels were greater than 30 m below surface pre-mining so waterlogging was not something that the vegetation was naturally adapted to (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 varies between species as do plant responses to stress. In light of the groundwater mounding issues, a method by which to conduct rapid assessments of the effects of rising groundwater on adjacent vegetation was needed. It was therefore necessary to identify visual indicators of stress response for plants native to the J-A area and identify useful indicator species for saline groundwater rise.

Diagnostic plant responses to salinity and waterlogging

Only 2% of the world's plants are considered salt tolerant and the Chenopodiaceae family contain the largest number (Aslam, 2011). This family is represented by more species than any other plant family at JA and members constitute a significant proportion of the shallow rooted vegetation cover (Badman, 2006). Shallow rooted species, including saltbush (*Atriplex* sp.) and bluebush (*Maireana* sp.) should be the last to be affected by rising groundwater.

As saline groundwater rises it progressively interacts with deeper rooted plant species. The dominant deep rooted tree species at JA is the myall (*Acacia papyrocarpa*), a very long lived and widely distributed species (Facelli & Brock 2000). The JA Rehabilitation program has been conducting myall research with the aim of learning as much as possible about their biology to increase the likelihood of successful restoration of the trees post mining. As part of this program myall monitoring transects were established in 2011, initially to begin developing a longer term understanding of myall growth and health. At the same time a new tailings storage facility (Cell 1) was established and shortly afterwards a nearby groundwater monitoring bore (MB07) began to rapidly rise. This provided circumstances under which to monitor responses to salinity and waterlogging.

The rising tailings-fed groundwater mound at Cell 1 was located directly adjacent to two ephemeral creek lines and several small sand rises, all of which had red mallee populations

(*Eucalyptus oleosa ssp. ampliata*). A South Australian study (Litchfield 1956) noted that red mallee (*Eucalyptus oleosa*) were only found on freely draining, non-saline soils and this implied that their tolerance to both water logging and salinity was low. At JA red mallee similarly occurs exclusively in the less saline landscape types; sandy rises, dunes and creek lines (Badman 2006, EBS 2008). Red mallee near Cell 1 have begun to show symptoms of disease.

Symptoms of salt stress in Eucalyptus species vary. When seedlings of *E. oleosa*, *E. diversifolia* and *E. incrassata* were exposed to high salt concentrations, the damage resembled that caused by drought (Parsons 1967). These symptoms included leaf in-rolling and death leaves with older leaves most severely affected (Parsons, 1967).

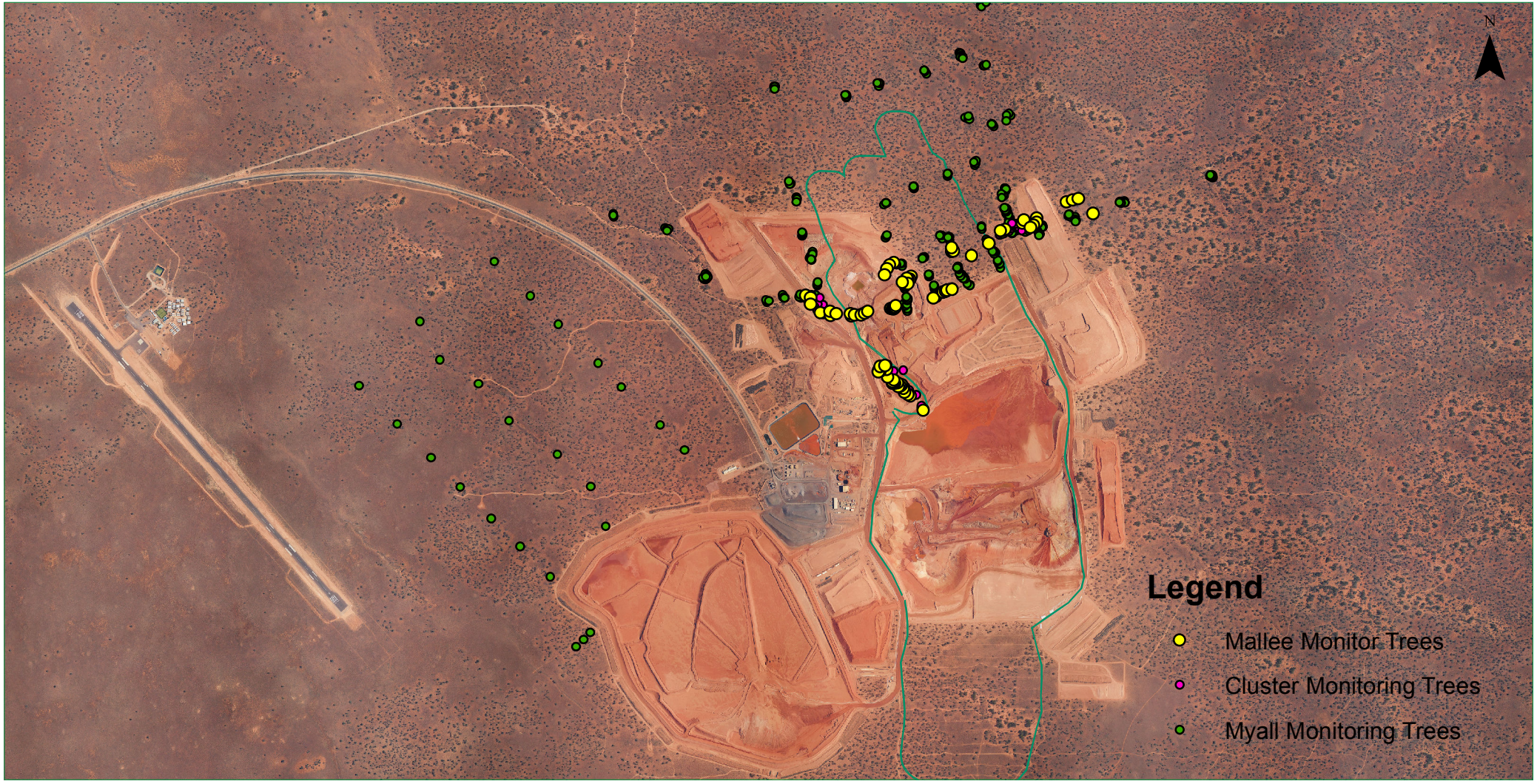
Eucalyptus oleosa has more recently been identified as comprising seven different subspecies occurring across southern Australia (Johnson & Hill 1999). The study by Parsons (1967) was conducted in south-eastern SA and was therefore likely to have been a different subspecies to that occurring at JA. In addition, subsequent studies have shown that tolerance of water logging and salinity in seedlings does not necessarily reflect subsequent tolerance of the same stressors in adult plants (Bell 1999). Therefore the responses observed in seedlings of a different subspecies by Parsons (1967) may not reflect the response of red mallee individuals to elevated salt concentrations at JA.

Symptomatic responses to increased salinity and waterlogging stress have been recorded in other Eucalyptus species. *Eucalyptus globulus* and *E. camaldulensis* symptoms began with yellowing (chlorosis) of leaf edges in mature leaves that progressed to those leaves dropping from the plant (Meddings et al 2001). Other waterlogging and salt sensitive Eucalyptus species seedlings exhibited healthy tissue death (necrosis) first at the growing tips followed by leaf patches and rapid aging of mature leaves (van der Moezel et. al, 1998). Conversely, the pattern of leaf damage in more tolerant species seedlings appeared first in older leaves then spread to the younger leaves (van der Moezel et. al, 1998).

This progress report summarises the three methods used to proactively and reactively monitor the vegetation health in response to rising saline groundwater rise at JA over the past 2 years (Table 7-1).

Table 7-1: Timeline of triggers and responses for J-A vegetation health monitoring activities.

Start Date	Trigger	Response	Response Status
October 2010	Rehabilitation requirement to better understand plant root depth requirements	J-A Rooting Mapping Project. University of Adelaide ARC project.	Proactive
March 2011	Rehabilitation requirement to better understand myall	Method 1: Myall Monitoring Transects (Figure 7.1).	Proactive
	Requirement for vegetation health monitoring network		
July 2011	Symptoms of disease in pockets of vegetation	Method 2: Mixed Species Clusters Monitoring (Figure 7.1).	Reactive
August 2011	Identification of red mallee as a potential indicator species of saline groundwater rise	Method 3: Mallee Monitoring Transects (Figure 7.1).	Reactive
July 2012 – December 2013	Analysis of data from Red Mallee Monitoring (this report)	Continue Myall Monitoring Transects	Proactive
		Continue and expand Mallee Monitoring Transects	Proactive



2

Kilometers

Jacinth Ambrosia

Groundwater Vegetation Monitoring Locations

ILUKA

ORIG: JL DRAWN: SCALE: 1:25,816(A4) DATE: April 2014 DWG No

FIGURE: 7.1

7.1.2 Myall Monitoring Study

Aims

Monitor the long term health and growth characteristics of the most widely spread, deep rooted species in the J-A area - Myall (*Acacia papyrocarpa*).

Determine if Myall is a useful indicator species of saline groundwater rise.

Methods

Transects were established to achieve both aims by choosing two areas that could experience potential impacts from rising saline groundwater. These areas, Cell 1 N and TSF NW, were identified from the JA groundwater monitoring network data (Figure 7.1).

Transects

The Cell 1 N Myall transects were established on March 1 2011. On each of the eight transects (chosen from a consistent compass heading) eight Myall monitor trees were selected based on their location correlating most closely with distances from the tailings facility: 0, 10, 20, 50, 100, 200, 500 and 1,000 m. Six transects extended north from the tailings area and one each extended east and west (Figure 7.1).

The TSF NW transects were established on 6 May 2011. On each of the three transects, a target of seven Myall trees monitor trees were selected based on their location correlating most closely with equidistant intervals from the tailings area: 0, 20, 40, 60, 80, 100 and 1200 m. Each transect extended from the TSF in a north westerly direction. Three monitor trees were also established adjacent MB02.

Monitoring

In order to provide relevant groundwater data, the transects began as close as possible to an existing or proposed monitoring bore (MB), investigation hole (IH) or vibrating wire peizometer (VWP) (Table 7-2). All monitor trees along transects 1 to 4 (apart from distance 1,000 m) are in the future mine path and will be removed within 2 to 4 years. During clearance and overburden removal these monitor trees will become part of an ARC funded project to map plant roots and understand plant water use. Monitor trees along transects 5 to 12 are currently outside the mine path and expected to provide longer term data.

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. A permanent photo point was established for each monitor tree and marked with a labelled wooden survey peg.

Health monitoring data capture forms were developed in ArcPad 8.0 Studio (ESRI®) (Table 7-3). Field data capture was conducted with either a Trimble Nomad or Trimble Yuma using ArcPad 8 software, a mobile mapping and geographic information system. At each monitor tree each of the three branches was visually assessed and photographed. Based on the branch assessments an overall tree score was determined and an overall tree photo taken from the photopoint. Upon return to base the data was downloaded, edited and stored in a desktop GIS system (ArcMap 9.3.1, ESRI®).

The proposed monitoring schedule is quarterly to pick up seasonal differences on tree health.

Table 7-2: Cell 1 Myall monitoring transects and associated monitoring bores.

Location	Transect #	MB start transect	MB end transect
Cell 1	T1	MB16	Nil
Cell 1	T2	MB13	Nil
Cell 1	T3	MB14	Nil
Cell 1	T4	IH48	Nil
Cell 1	T5	MB07	Nil
Cell 1	T6	MB05	MB06
Cell 1	T7	MB07	Nil
Cell 1	T8	MB05	Nil
TSF	T9	Nil	Nil
TSF	T10	MB03, VWP14	MB08
TSF	T11	IH36	MBN07
TSF	T12	MB02	Nil

Table 7-3: Myall transect health assessment categories.

Tree Feature	Characteristic	Score Options
Branch	Old Leaf Health	nil, healthy, unhealthy, dying, dead
	Old Leaf Symptoms	nil, yellowing, burn, dead, dropped, combo (note), insect
	Old Leaf Notes	
	New Leaf	nil, healthy, unhealthy, dying, dead
	New Leaf Symptoms	nil, yellowing, burn, dead, dropped, combo (note), insect
	New Leaf Notes	
	Flowering	nil, buds, flowering
	Fruit	nil, green, ripe non-viable, ripe viable, seed dropped
Whole Tree	Tree health	healthy, unhealthy, dying, dead
	Tree health score	0 – 10 (0 dead, 10 very healthy)

Results

The data presented here corresponds to the monitoring of the myall transects carried out on four occasions, June 2011, November 2011, March 2012 and July 2012 (Table 7-1). The Cell 1 myall transects are located to the north of the mining pit (Figure 7.1) and were monitored on three occasions. The TSF myall transects were included in the monitoring program in 2012 and are located adjacent to the off-path tailings storage facility (TSF) (Figure 7.1), these transects were monitored on two occasions.

Table 7-4 Summary of trees sampled for each sampling period

	Jun-11	Nov-11	Mar-12	Jul-12	Total
Cell 1	27	51		63	141
TSF			19	14	33
Total	27	51	19	77	174

Cell 1 West

Overall tree health

A total of 127 trees were given health ratings as part of the Cell 1 program, of which all were given an overall health description as healthy or unhealthy (Table 7-5). A total of 100 trees were given a health score rating between 0 and 10 (Table 7-5). Dead trees (recorded as zero) were not monitored therefore not included the overall tree health analysis.

The majority of trees monitored were located directly adjacent to the pit (0 m), 50 m from the pit, 100 m from the pit, 500 m from the pit and 1000 m from the pit (Table 7-5) accounting for 62% of all the trees monitored.

Table 7-5 Total numbers of trees monitored for health with distance from pit.

Distance from pit (m)	Number of trees given a health description (Healthy / Unhealthy)	Number of trees given a health score (1 – 10)
0	16	13
10	2	2
20	3	3
30	4	3
40	3	2
50	12	9
60	5	4
75	6	5
80	2	1
100	18	14
120	4	4
130	2	1
200	8	7
250	7	5
500	18	14
750	2	1
1000	15	12
Total	**127**	**100**

Overall the majority of the trees surveyed as part of the Cell 1 program were identified to be healthy (Figure 7.2); only eight of the 127 trees (6%) were identified to be unhealthy overall.

The health score of trees shows a relatively normal distribution, with the majority of scores falling between a score rating of three and six (88%), only one tree was recorded with a health score of two and one tree with a health score of nine (Figure 7.3).

Only one percent of myalls were given a health score less than two (none were given a rating of one), and 13% of myalls were given a health score of less than three, indicating that unhealthy trees are scored somewhere between two and three. There does not appear to be a relationship between the health descriptions and health rating scores.

Although health scores were well spread across all transects, generally higher proportions of lower health scores were observed within 80 m of the pit (Figure 7.4). From 100 m to 1000 m the tree scores were variable, with a higher representation of higher health scores. When the data for health scores was pooled for 0 m to 80 m, and 100 m to 1000 m the trees closer to the pit are skewed to lower health scores (Figure 7.5). Trees greater than 100 m from the pit show a normal response for health scores (Figure 7.5).

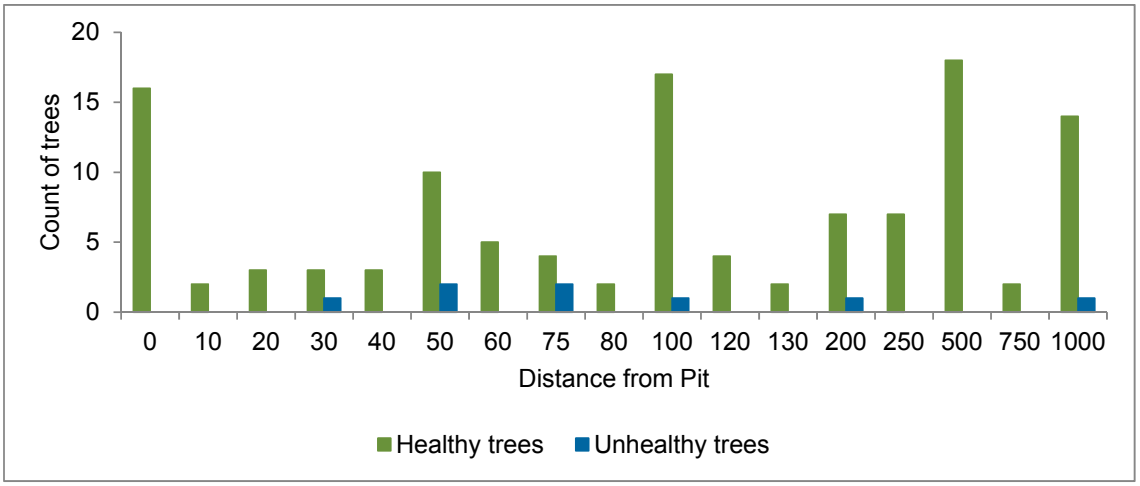


Figure 7.2 Cell 1 transect myall health with distance from pit

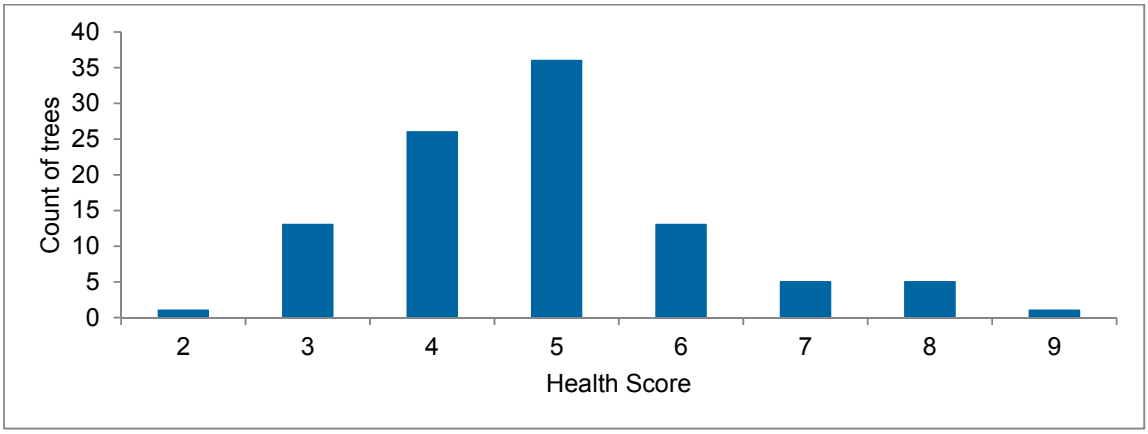


Figure 7.3 Health scores of myall on Cell 1 transects

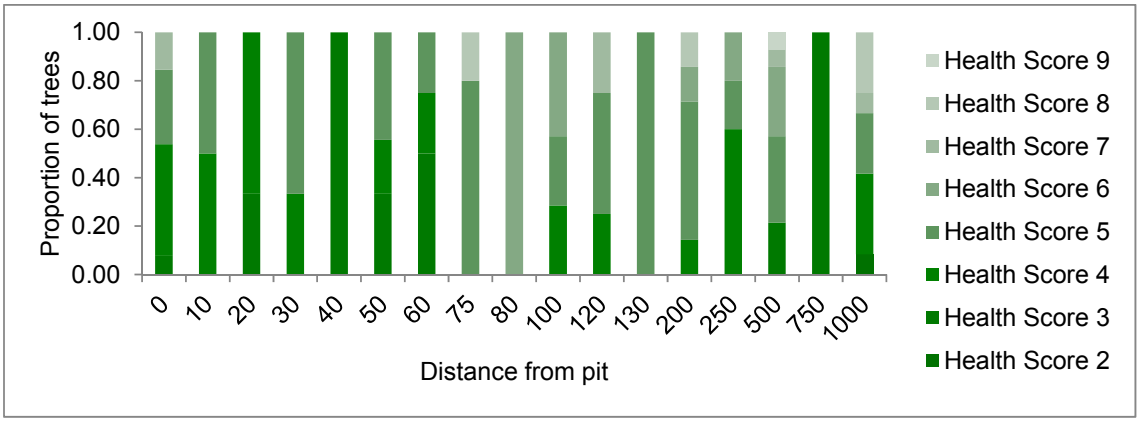


Figure 7.4 Proportional Cell 1 myall tree health scores with distance from pit

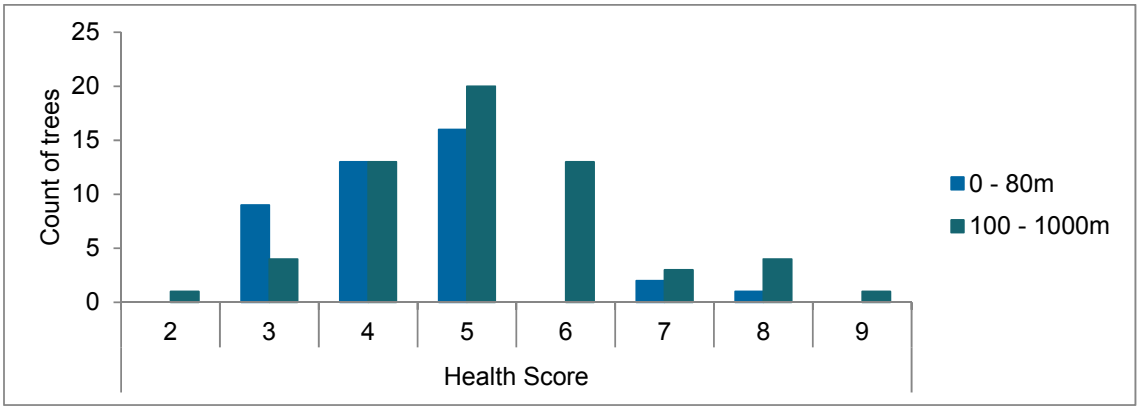


Figure 7.5 Combined health scores for 0 m - 80 m from pit and 100 m - 1000 m from pit

Reproductive output

Flower and seed production was generally variable across all distances from the pit (Figure 7.6). Flowers were produced on approximately 34% of branches, however seed production was only recorded on 11% of branches.

When data is combined reproductive output decreases with distance from pit, with trees located greater than 750 m from the pit having the lowest flowering, fruiting and seeds recorded (Figure 7.7). Trees located less than 500 m from the pit show similar records of fruiting and seeding.

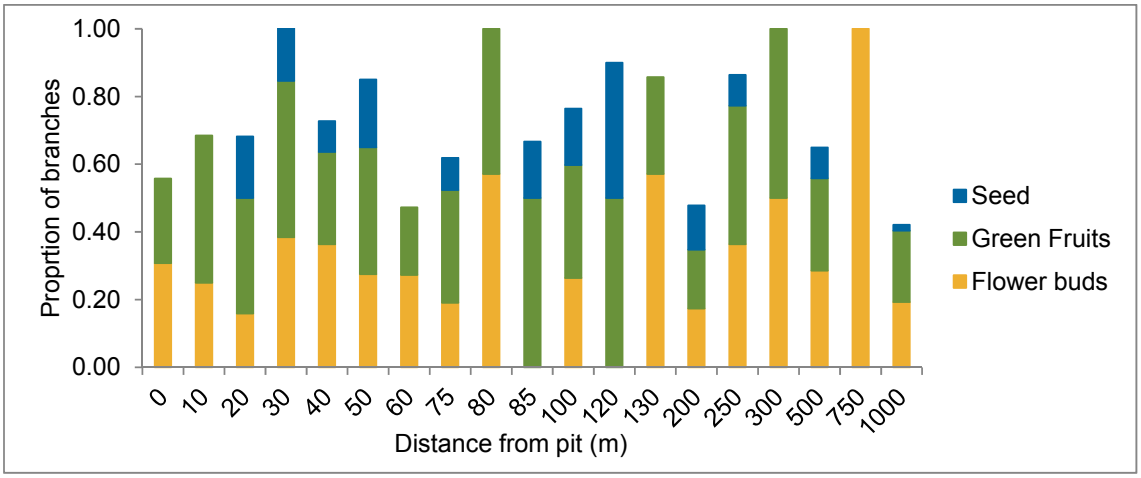


Figure 7.6 Proportional flowering and seed production on Cell 1 myall tree branches with distance from pit.

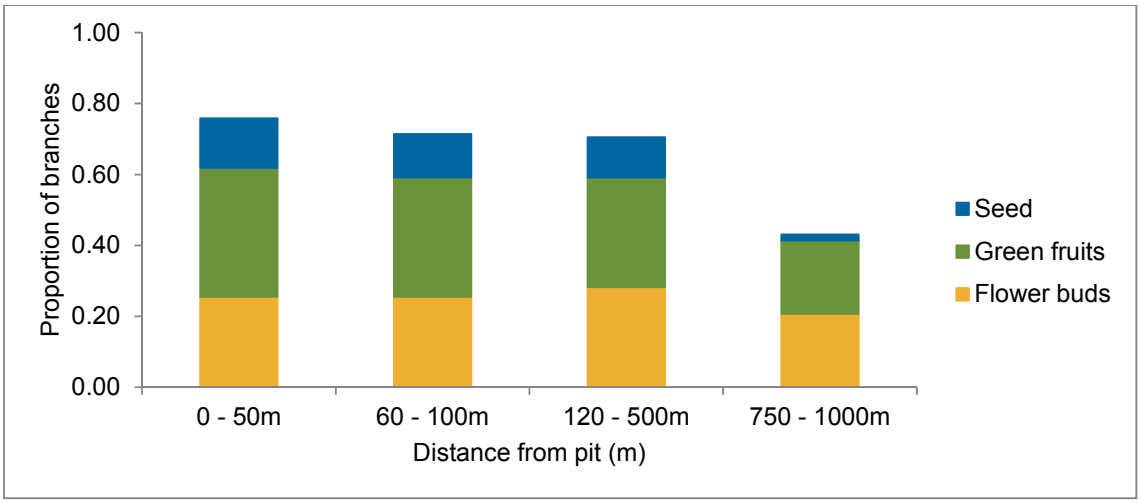


Figure 7.7 Proportion of flowering and seed production at combined distances from pit.

Leaf health

New leaf growth was only recorded on five occasions, on three branches located 10 m from the pit and on two branches located 100 m from the pit.

Overall the majority of old leaves on the trees were healthy, with only a few leaves recorded as being unhealthy or dead (Figure 7.8). More branches with unhealthy leaves were recorded on trees located within 100 m of the pit, accounting for approximately 76% of all unhealthy leave records.

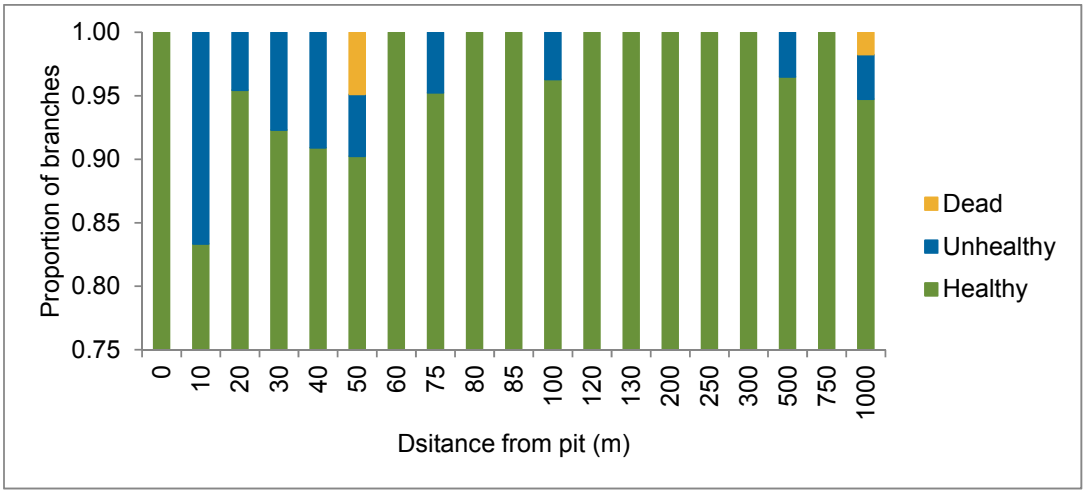


Figure 7.8 Old leaf of Cell 1 myall trees with distance to pit

Tailings Storage Facility

No data was available for distance from pit for the tailings storage facility (TSF) monitoring program at the time of the analysis, however all trees were labelled starting from one at the TSF. Therefore tree numbers have been used here to determine any impacts from TSF groundwater.

All trees surveyed as part of the TSF myall monitoring program were identified as healthy, with tree scores between three and seven (Table 7-6). The lowest health scored trees were located closer to the TSF than the highest scored trees.

Table 7-6 Summary TSF myall health scores

Tree number (distance from TSF)	Health score			
	3	4	5	7
1	2		2	
2	2	2	2	
3		2	6	
4		4	2	
5			4	
6			2	2
7			4	2
Total	4	8	22	4

Reproductive output

No TSF monitored trees flowered during the survey programs; however seeds were recorded on branches of trees closest to the TSF (Figure 7.9).

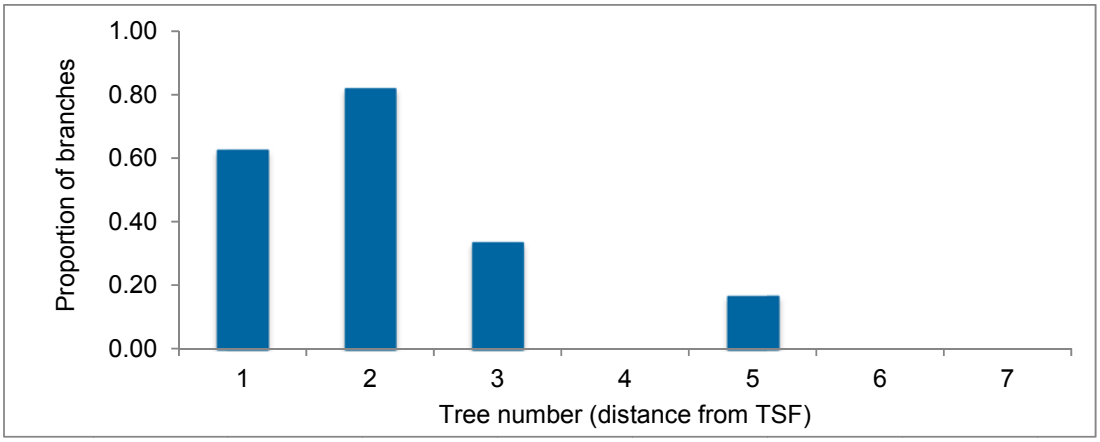


Figure 7.9 Proportion of branches with seed on the TSF myall transects

Leaf health

No new growth was recorded on any of the TSF myall monitoring trees.

The majority of old leaves were identified as being healthy with only two occasions of unhealthy leaves record, at tree number three and tree number four. Unhealthy leaves comprised 3% of all TSF records.

Discussion

The response by myall to rising groundwater at this point in time is ambiguous. The majority of trees have been described as healthy overall, however health scores are lower for those trees located closer to the groundwater impact area (cell 1 and TSF). Cell 1 data indicates that there is potential for impact on trees within 80 m of groundwater mounds, and the TSF trees closer to the impact area recorded lower health scores also. The response in tree health cannot clearly be attributed to groundwater as there are a number of other impacts that may be a causal (for example dust) and these have not been measured here. Further analysis of the tree health in conjunction with analysis of the groundwater response is required. Modelling of the groundwater at Cell 1 and the off-path TSF has recently been carried out for JA, this data will be collated and included in future analysis.

Interestingly reproductive output appears to have increased closer to the impact zones. Trees at Cell 1 showed consistently higher flowering, and seeding with 500 m of the impact zone, similarly the first three trees on the TSF transects were identified as seeding, only a single tree was recorded as seeding for trees four to seven. Seeding may be a response to stress, however it is unlikely that the impacts from rising groundwater would be observed 500 m from the impacts area. Further analysis considering the location of the groundwater mound needs to be carried out here.

There was no apparent change in leaf health in response to proximity to the impact zone for both Cell 1 and TSF trees. It may be that when stressed trees drop leaves quickly, however branches with dead leaves have been observed to remain on trees across survey occasions indicating that this not likely. The usefulness of this health measurement will be reconsidered during the analysis of the 2014 data.

Recommendations

- The tree health descriptors, healthy and unhealthy, are not useful indicators of trees health, the majority of trees were recorded as healthy, although this was not reflected

in the health scores which were variable. Less subjective health measurements will be considered for future monitoring (for example proportion of canopy cover).

- Tree health responses may be due to a number of environmental factors, other than mounding groundwater. Future analysis of the data in will be carried out conjunction with the modelling of the groundwater mound movement to more clearly determine contributing factors.

- Tree health will require monitoring as the pit moves across the landscape. Additional transects will set up in front of (but outside of) the mining pit. These transects will act as baseline for current data and for monitoring of trees as the as pit moves and may potentially impact myalls.

- Health of trees may be a response to age rather than other environmental factors. The age of the trees in this study has not been considered. It is recommended that the trees included in the monitoring program are age scored.

7.1.3 Mixed Species Cluster Monitoring

Aims

Monitor the short term health of vegetation estimated to have a range of root depths extending from less than five to beyond 10 m in search of species that could provide an early visual indication of rising saline groundwater.

Method

Three sites were chosen close to the Cell 1 tailings facility: two in areas of concern for rising saline groundwater (MB07 and adjacent Cell 1 Decant area – Cell 1 West) and one control site (MB05) (Figure 7.1). The eastern monitoring bore (MB07) was chosen because it was the first bore in the area to register rapidly rising groundwater. Following commencement of tailing in the Cell 1 area in January 2011 the groundwater was first registered in this shallow bore in mid June 2011 (28.93 m below surface). Cell 1 West was chosen as the second location due the possibility of root interaction with seepage from the nearby Decant pond which was the collection point for all saline tailings run-off water at the time. The western monitoring bore (MB05) was chosen as the control as groundwater levels had been deep and stable at 35.5 m.

Mixed species monitoring clusters were established 2 July 2011 at each location, encompassing different species with an estimated range of root depths. At the time when the mixed species cluster monitoring was establishment only myall (*A. papyrocarpa*) and red mallee (*E. oleosa ssp ampliata*) were suspected to have roots exceeding 10 m depth below surface (from initial J-A root mapping work, Oct 2010 to Feb 2011) and pearl bluebush (*M. sedifolia*) was suspected to have roots not exceeding 5 m depth (from mature plant relocation experiment, late 2010). At the same time a plant water use and root mapping research project was being developed with the University of Adelaide (U of A). In the absence of other data the initial estimations of the root depths of different species came from the developing U of A proposal (Table 7-1).

Plant species were chosen based on their proximity to each of the bores and pond. Representatives from six plant species were chosen for monitoring within 58 m of the monitoring bore MB07, seven species where chosen within 63 m of the monitoring bore MB05 and three species where chosen within 180 m of the Decant Pond at Cell 1 West.

Each monitor plant in the cluster was marked and labelled using wooden survey pegs and individual permanent photo points were established. The plant health monitoring data capture forms originally developed for the Myall transects were also used to monitor the clusters (Table 7-3). Due to the variation in plant size within the clusters, visual assessments were conducted after consideration of the entire plant, with no branch replication as per the myall transect technique.

Field data capture was conducted with either a Trimble Nomad or Trimble Yuma using ArcPad 8 software, a mobile mapping and geographic information system. Upon return to base the data was downloaded, edited and stored in a desktop GIS system (ArcMap 9.3.1, ESRI®).

Monitoring was proposed to be conducted on a monthly basis until potential early warning target species were identified. The monitoring intensity reduced to a target of twice a year after this goal was achieved.

Table 7-7: Proposed Root Depth Categories (source, draft U of A research proposal)

Root Depth Category	Scientific Name	Common Name	Number Monitored
Deep (>10 m)	*Acacia papyrocarpa*	Myall	MB07 n = 5, MB05 n = 0, Decant n= 0
	Eucalyptus oleosa ssp ampliata	Red Mallee	MB07 n = 0, MB05 n = 2, Decant n= 4
	Myoporum platycarpum	False Sandalwood	MB07 n = 0, MB05 n = 2, Decant n= 0
Medium (5-10 m)	*Acacia ligulata*	Umbrella bush	MB07 n = 0, MB05 n = 1, Decant n= 0
	Alectryon oleifolius	Bullock Bush	MB07 n = 0, MB05 n = 4, Decant n= 2
	Dodonea viscosa	Sticky Hop Bush	MB07 n = 4, MB05 n = 0, Decant n= 0
	Eremophila alternifolia	Narrow Leaf Emu Bush	MB07 n = 3, MB05 n = 1, Decant n= 0
	Eremophila scoparia	Broom Emu Bush	MB07 n = 0, MB05 n = 1, Decant n= 0
	Lycium australe	Australian Boxthorn	MB07 n = 2, MB05 n = 0, Decant n= 1
	Santalum acuminatum	Quandong	MB07 n = 3, MB05 n = 1, Decant n= 0
	Senna artemisioides ssp. artemisiodes	Silver Senna	MB07 n = 3, MB05 n = 0, Decant n= 0
Shallow (<5 m)	*Maireana sedifolia*	Pearl Bluebush	MB07 n = 1, MB05 n = 0, Decant n= 0
Total			MB07 n = 16, MB05 n = 12, Cell 1 decant n = 12, Total n = 40

Results

Monitoring was carried out on four occasions; all trees were visited July, August and November 2011 (Table 7-8). However the majority of trees were cleared for mining early 2012 and the remaining 12 trees were visited again in May 2013.

The majority (66%) of trees were considered to be healthy, 29% unhealthy and 6% were dead or dying (Figure 7.10), however given that no baseline trees were monitored the factors relating to health of the trees in the monitoring program cannot investigated.

All species were recorded either flowering or fruiting during the monitoring program except *Maireana sedifolia* (Figure 7.11). Reproductive response to mounding groundwater cannot be determined as baseline monitoring was not carried out.

Table 7-8 Summary of visits to cell 1 mixed species monitoring trees

Species	Jul-11	Aug-11	Nov-11	May-12	Total
Acacia ligulata	1	1	1		3
Acacia papyrocarpa	5	5	5	5	20
Alectryon oleifolius	6	6	6	2	20
Dodonea viscosa	4	4	4		12
Eremophila alternifolia	4	4	4		12
Eremophila scoparia	1	1	1		3
Eucalyptus oleosa	6	6	6	4	22
Lycium australe	3	3	3	1	10
Maireana sedifolia	1	1	1		3
Myoporum platycarpum	2	2	2		6
Santalum acuminatum	4	4	4		12
Senna artemisioides	3	3	3		9
Total	40	40	40	12	132

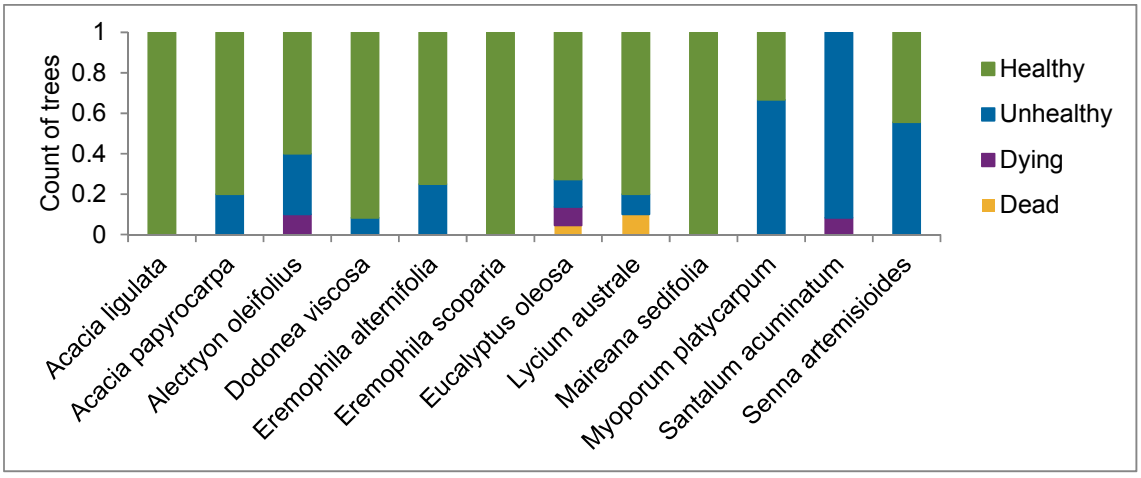


Figure 7.10 Tree health for cluster cell species

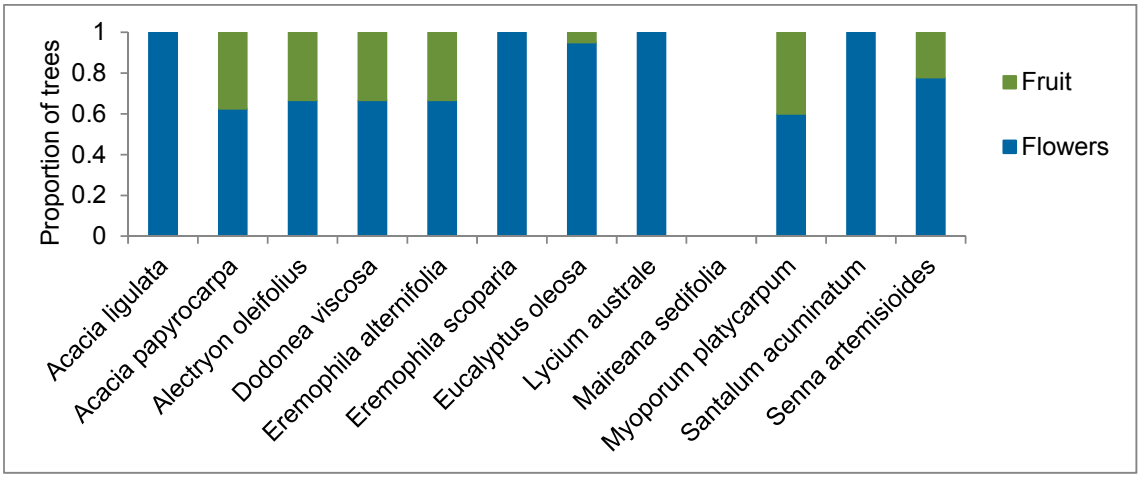


Figure 7.11 Reproductive output of cell 1 mixed species clusters

Discussion and Recommendations

Species response to mounding groundwater cannot be determined as baseline data was not available. Further the health of the various vegetation species will require monitoring as the pit moves across the landscape. Additional transect will be set up in front of (but outside of) the mining pit path. These transects will act as baseline for current data and for monitoring of trees as the as pit moves and may potentially impact vegetation.

The tree health descriptors, healthy and unhealthy, are not useful indicators of trees health, the majority of trees were recorded as healthy. Less subjective health measurements will be considered for future monitoring (for example proportion of canopy cover or health indicators).

7.1.4 Red Mallee Monitoring

Aims

Define, catalogue and map the symptoms of health stress in red mallee (*Eucalyptus oleosa ssp ampliata*) in the JA area and monitor these over time. Focus on this species is particularly important since it is the deepest rooted species on site that exhibits suspected salt stress.

Methods:

In August 2011 every mallee along the creeks surrounding the northern and western edge of the Cell 1 tailings facility was established as an individual monitoring point – a combined distance of 1.9 km and 70 red mallee (Table 7-9).

These 70 red mallees were divided into five monitoring zones; two close to the Cell 1 tailings facility where the first symptoms of ill health were observed on 15 August 2011 (N creek and Cell 1 west), one zone each to the east and west of N creek and one zone up-slope from the Cell 1 creek. Within each zone the red mallee occurred in distinct patches, providing focus groups. These groups were used determine any correlations between red mallee health and rising saline groundwater through assessment of multiple data sources, e.g. groundwater levels, soil conductance (electromagnetics) (Figure 7.12).

The monitoring method developed for the myall transects and mixed species clusters was not used. A less detailed and faster monitoring method was established to ensure rapid ground coverage and data capture. The monitor trees were not tagged or labelled and no

permanent photo points were established. Instead trees were re-identified at each monitoring event using ArcPad 8 software, the mobile mapping and geographic information system on the Trimble Nomad or Yuma.

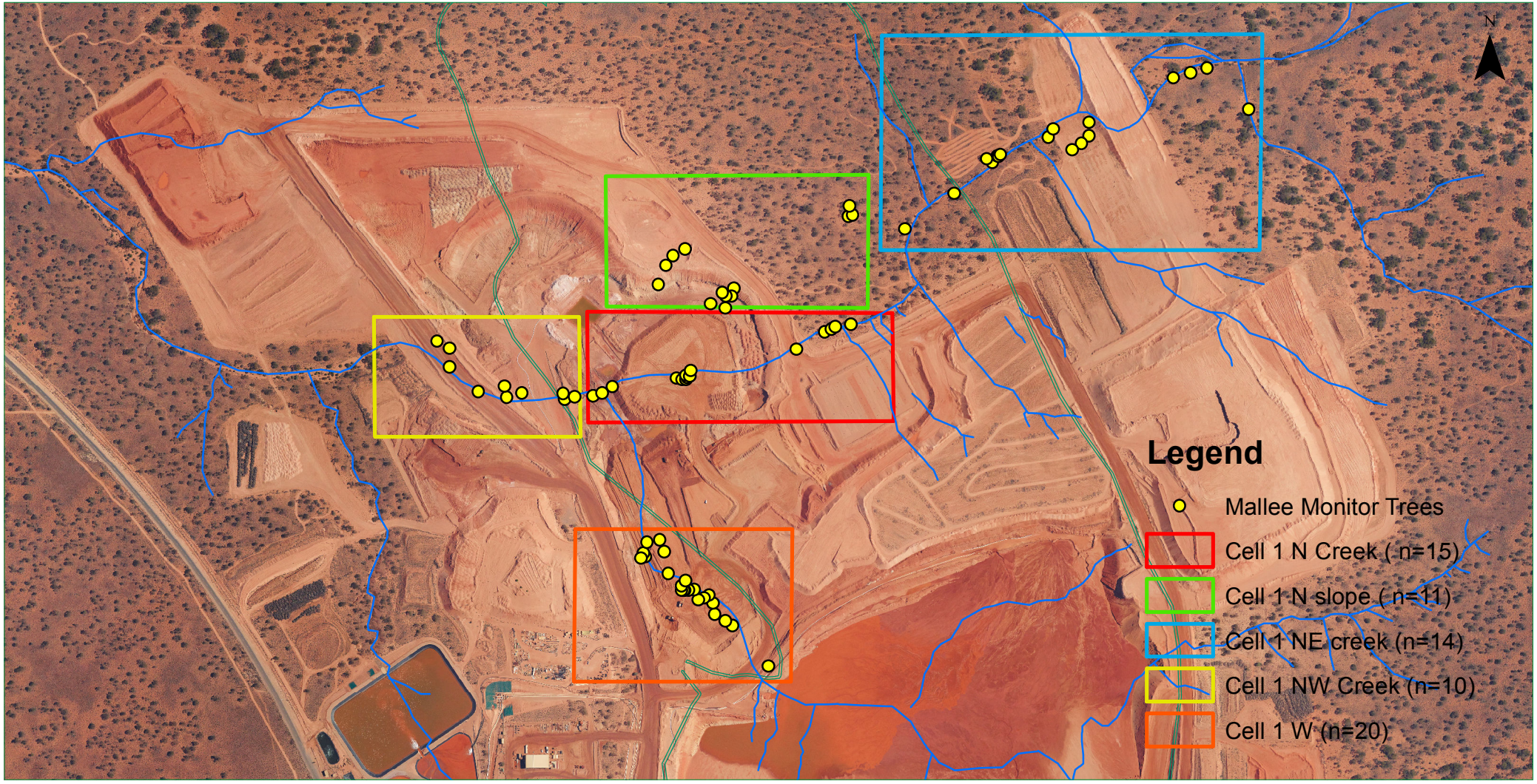
A simpler health monitoring data capture form was developed in ArcPad 8.0 Studio (ESRI ®) Table 7-10). Health categories were determined using the data and observations collected during the mixed species cluster monitoring program and from closer observations made during preparation of the red mallee monitoring. Based on this background, red mallee leaf symptoms were divided into two types:

1. Leaf discolouration symptoms – tipping (necrosis), total leaf or inter-veinal yellowing (chlorosis), purple hue, total leaf death (senescence).
2. Insect damage symptoms – 'leaf cutters', 'leaf stickers', 'leaf miners', 'branch borers'.

These symptoms have not yet been investigated in detail to identify the actual mechanism or organism responsible for the damage but insects are known to preferentially target sick and weakened individuals.

Visual observations from myall transect monitoring and mixed species cluster monitoring suggested that insect damage symptoms on red mallee leaves were wide spread, whereas leaf discolouration appeared closer to the Cell 1 tailings dam. On this basis and the background provided from the published information an assumption was made that leaf discolouration symptoms should form the basis of the simplified tree health categories for the red mallee monitoring. Five monitoring categories were established. Leaf insect symptoms were only included in the scoring if they were abundant and were an obvious symptom of tree health (Table 7.10).

Health assessments were made after walking around each tree and visually inspecting the tree at the base and in the canopy from the ground. Red mallee are tall trees with the leaf canopy generally at the top, hence fine details like small leaf tipping may not have been noticed in poor light conditions. A health category was assigned to each tree and any health related notes were also captured. To ensure consistency the same operator conducted each monitoring event. Upon return to base the data was downloaded, edited and stored in a desktop GIS system (ArcMap 9.3.1, ESRI®).



Jacinth Ambrosia

Mallee Monitoring Locations

ILUKA

ORIG: JL DRAWN: SCALE: 1:9,122 (A4) DATE: April 2014 DWG No

FIGURE: 7.12

Table 7-9: Mallee monitoring zones, focus groups and associated monitoring bores or investigation holes.

Cell 1 Mallee monitoring zones	Total # Mallees in monitoring zone	Focus groups of Mallee within monitoring zones	Mallee ID #
N creek	15	IH 53 Group (n=3)	11, 12, 17
		IH 52 Group (n=3)	8 - 10
		MB13 Group (n=5)	18 - 22
Cell 1 W	20	Sth Group (n=4)	71 - 74
		Nth Group (n=7)	62 - 68
NW creek	10	MB 05 Group (n=3)	1 - 3
NE creek	14	MB07 Group (n=2)	25, 27
N slope	11	Nil	Nil
Total	70	16	

Table 7-10: Mallee monitoring health assessment categories.

Health Score	Health Category	Definition
5	Healthy	No signs of any stress
4	Health?	Some signs of stress in localised yellowing, not necessarily related to salt stress (symptoms itemised in notes)
3	Tipping	Obvious tipping of leaves, often only on some branches, most likely salt stress
2	Unhealthy	Definite tipping and progressive death of leaves, often only on some branches, most likely salt stress. Sometimes epicormic growth after leaf loss.
1	Dead	Total leaf loss, no recovery.
	Notes	Description of symptoms

Opportunistic monitoring of mallee was also carried out on a number of occasions on the myall transects, the health assessment of these trees was the same as that for the myall (Table 7-3).

Results

Monitoring of mallee trees was carried out on eight occasions across 2011 and 2012 (Table 7-11). No additional monitoring of the mallee transects was carried out since the June 2012 and all results are presented in the 2011 JARMS. Opportunistic monitoring of mallee was carried out on the myall transects on three occasions, July and November 2011 and July 2012 (Table 7-11).

Table 7-11 Summary of mallee monitoring occasions

	Jul 2011	Aug 2011	Sep 2011	Nov 2011	Dec 2011	Jan 2012	Jun 2012	Jul 2012	Total
Number of trees visited		28	52	26	97	14	51		268
Opportunistic monitoring	15			22				19	56

For the monitoring carried out on the mallee transects the two categories with the most robust tree health, 'Healthy' and 'Health?' occurred in all monitoring zones. 'Healthy' trees ranged from 65% in NW creek zone to 9% in N creek zone. Trees in the 'Health?' category showed no clear trend for any zone.

The two categories with concerning tree health, 'Tipping' and 'Unhealthy', were more restricted in their occurrence. 'Tipping' occurred in N creek and Cell 1 W zones, with a few observations also in NW creek and N slope. 'Unhealthy' trees occurred in the N creek (29%), Cell 1 West (15%) and NE creek (4%) and no 'Unhealthy' trees were observed in the NW creek or N slope zones.

Monitoring of the closest bores indicate that saline groundwater levels of approximately 5 m to 10 m below surface has impacted on red mallee health. Some minor impacts were recorded in locations where saline groundwater was recorded at approximately 20 m below surface. However no health impacts were recorded where saline groundwater was recorded at approximately 35 m below surface level.

Soil sampling and analysis in areas affected by saline groundwater rise identified that all levels of soil sampled were defined as saline, having an ECe value > 4 dS/m (Hazelton and Murphy, 2007). Almost every sample from the top 5 m was highly saline, possibly reflecting the capillary rise recorded only 7 m away in the creek bank. The topsoil was extensively disturbed during drilling set up so this sample cannot be considered as representative. Soil salinity reaches extremely saline levels (>16 ECe dS/m) at approximately 8 m and from 12 to 18 m those levels nearly double in magnitude up to 42 dS/m.

Opportunistic sampling of mallee was carried out on three occasions on the myall transects, July and November 2011 and July 2012 (Table 7-11) on 31 trees. Only two trees were recorded as being unhealthy overall. Similarly, the majority of branches were identified as healthy, with only 11 (6%) branches recorded as unhealthy. Of the 11 unhealthy branches, eight were within 200 m of the tailings facility, three unhealthy branches were recorded 450 m from the tailings facility.

The majority of leaves on the opportunistic mallee were identified as healthy (92%), however of the branches bearing unhealthy leaves 94% were within 200 m of the pit (Figure 7.13). New leaf growth was recorded on over half of all branches (54%), and the majority were identified as being healthy (47%). No trend for new leaf health and distance from tailings facility was observed.

Fruiting was recorded at all distances from the tailings facility during the opportunistic mallee monitoring (Figure 7.15). No trend with distance from tailings facility was observed.

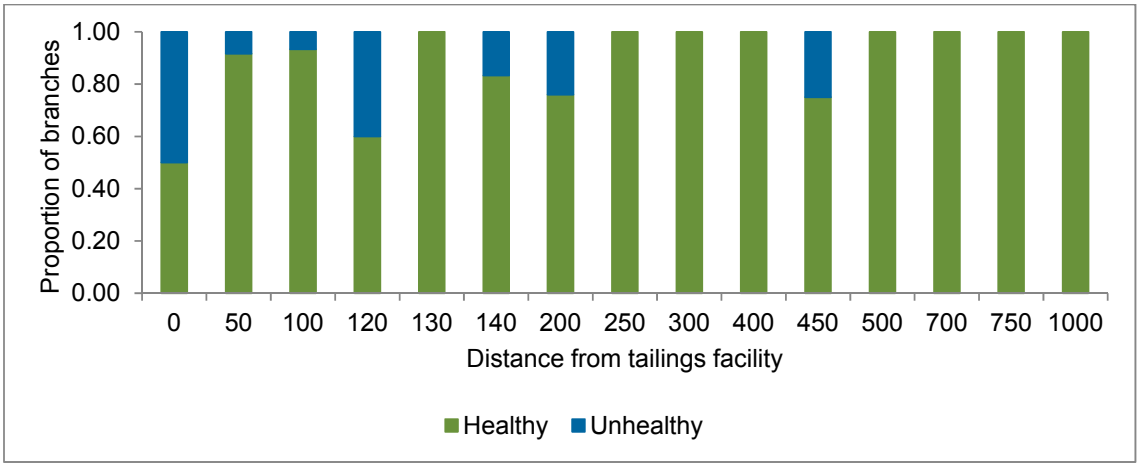


Figure 7.13 Proportion of health of old leaves on mallee branches

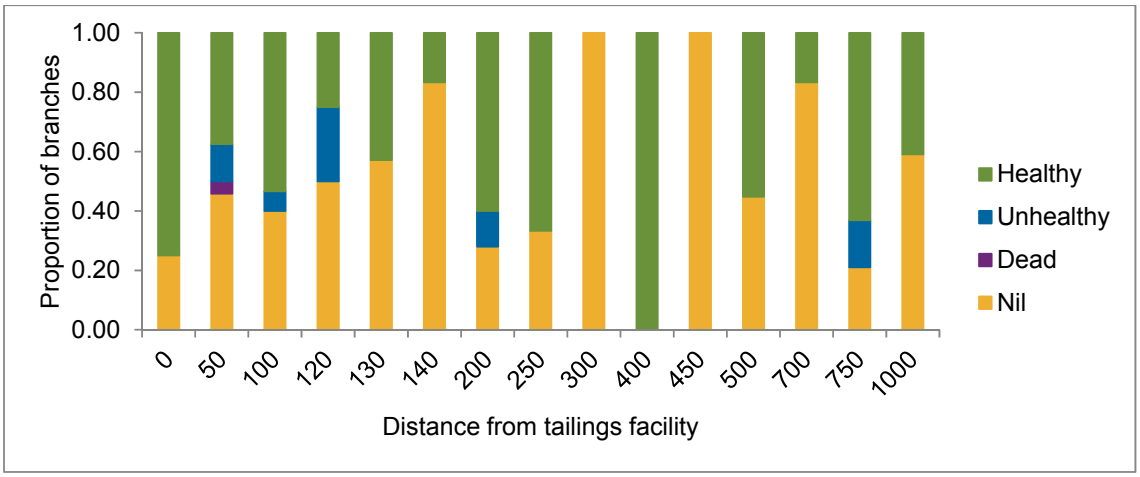


Figure 7.14 Proportion of health of new leaves on mallee branches

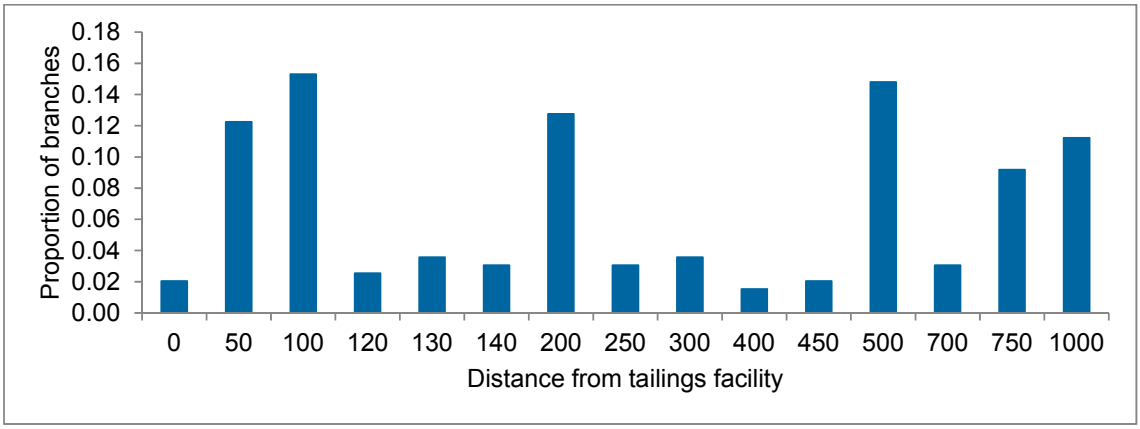


Figure 7.15 Proportion of branches flowering with distance from tailings facility

Discussion

Overall impacts to mallee as a result of groundwater rise was detected where groundwater was recorded up to 20 m below surface level. Symptoms recorded included yellowing of leaves, browning of leaf tips and epicormic growth. Monitoring has not been carried out since June 2012 therefore the recovery of the trees has not yet been determined.

The results of the mallee creek surveys were supported by the results of the opportunistic monitoring. Mallee health was appeared to be impacted within 200 m of the pit, indicating a potential response to the rising groundwater. However it is important to note that it is unclear how far from the pit the rising groundwater has impacted.

Where saline groundwater rise was recorded within 5 m of surface level the entire soil profile was saline to extremely saline. Further sampling and monitoring is required to verify the level of soil salinity left behind by receding saline water tables in the different soil types.

Recommendations

The health categories used in this study do not distinguish between the different levels of decline noted. Expansion of the categories is required to distinguish between the extent of the tipping, leaf loss, and epicormic growth or branch death.

The health of the mallee will require monitoring as the pit moves across the landscape. Additional transects will be set up in front of (but outside of) the mining pit path. These transects will act as baseline for current data and for monitoring of trees as the as pit moves and may potentially impact vegetation.

Additional sampling and monitoring of the soil profile will be carried to determine the residual salinity from the saline groundwater rise. Particular attention will be paid to areas where groundwater levels of up to 20 m below surface level were recorded. Baseline sampling will be carried out in areas unaffected by groundwater rise.

7.1.5 References

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8 VEGETATION CHARACTERISTICS – OTHER

This section provides information on miscellaneous projects that were established to address issues of importance to future J-A rehabilitation outcomes.

8.1 Effect of dust smothering on *Maireana sedifolia*

Progress Report 2013

Iluka Resources Ltd

8.1.1 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. Hairy leaf and stem coverings provide protection against numerous biotic and abiotic stresses (Amme, 2005) but clogging of leaf stomata with dust can act to reduce photosynthetic activity and therefore growth (Turner, 2011). Respiration and transpiration can also be effected and may result in increases in water loss (Farmer, 1993) and changes in leaf temperature (Pradjapati, 2011). This ultimately may result in reduced plant health, impacting on reproduction and potentially causing plant death.

It is a requirement of the Jacinth Ambrosia (J-A) Mining and Rehabilitation Plan (MARP) that all project related vegetation clearance is approved by Department for Manufacturing, Innovation, Trade, Resources and Energy (DMITRE), 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 MARP). The outcomes and assessment criteria relating to dust impacts on vegetation are outlined below in Table 8.1.

Table 8-1 Aspect, impact, outcome assessment criteria and monitoring details - flora (as per MARP operations pp. 92)

ID	Aspect and Impact	Outcome	Assessment Criteria	Monitoring Details		
				Type	Frequency	Parameters Monitored
CJA_0002	Vegetation death to 'smothering' of vegetation with dust from operational activities (vegetation on the fringe of cleared areas and regenerating vegetation)	All clearance of native vegetation is authorised under appropriate legislation	Demonstrate that actual clearance boundaries are within authorised clearance boundaries (output from GIS)	Biological survey mapping	Annually	Changes in abundance, composition or condition against control site or background data to identify changes outside approved clearance boundaries. Monitoring includes: plant mortality; plant health as measured by vigour of new growth, flowering and fruiting; extent of smothering (monitored by visual observations)

8.1.2 Methods

Survey Species

Pearl bluebush (*Maireana sedifolia*) has been identified as the most appropriate species to monitor on the ML 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 (Plate 8-1). 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 Plate 8-1) The response of pearl bluebush to high dust loads is not known.

Dust Deposition Gauges

Dust deposition has been monitored monthly on site since August 2008, and dust deposition gauges (DDG) are located at various distances from dust sources. The dust gauges were moved at some point between 2009 and 2013 therefore for the purposes of this report dust depositional rates for 2008 are considered to be baseline, and the 2012 and 2013 depositional data was considered to show impact levels.

For the purposes of this report the 2012 and 2013 DDG locations were grouped according to how far they were from the closest dust source (Table 8-2).

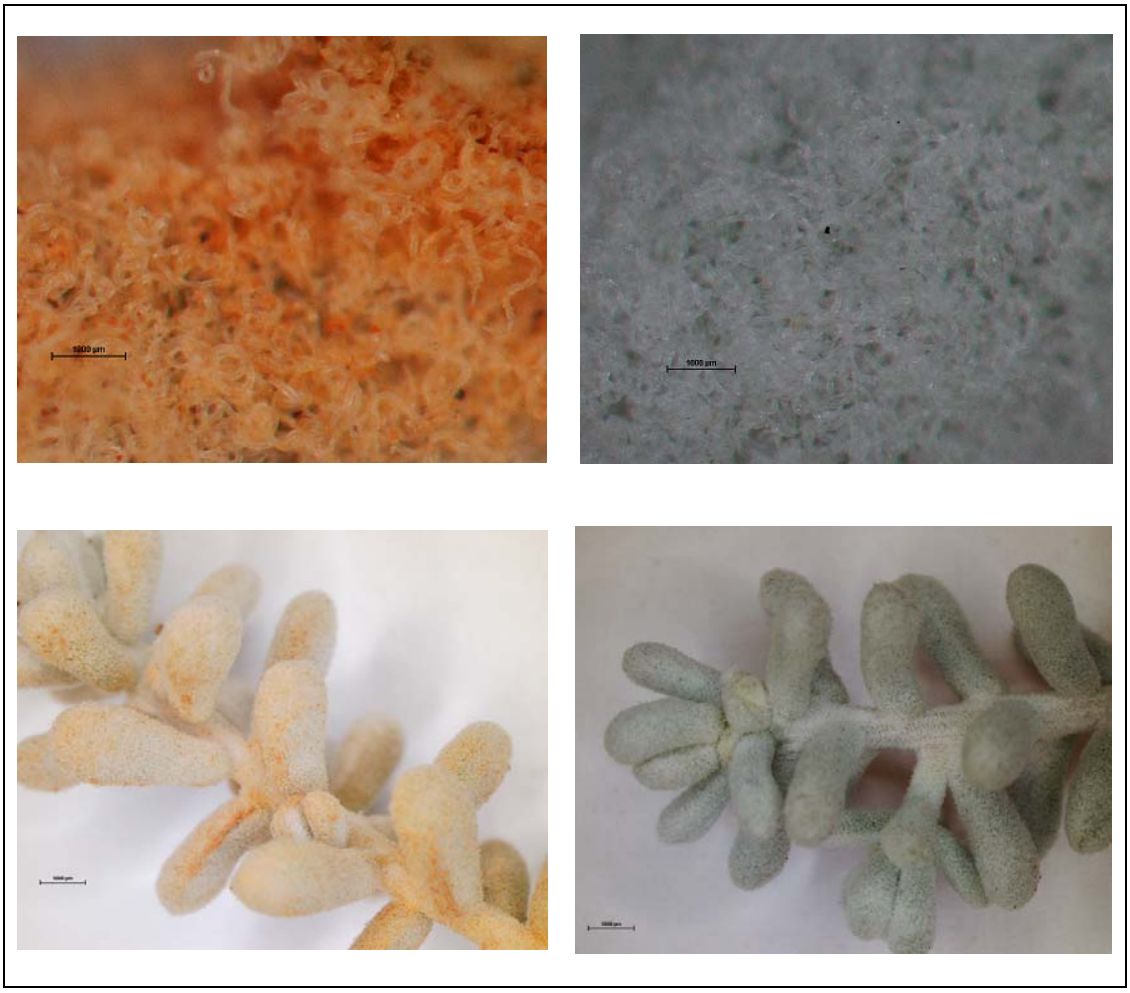


Plate 8-1 Close magnification of trichomes present on leaves (top left & right) and leaves of pearl blue bush coated with dust (bottom left) and without (bottom right)

Table 8-2 Description of DDG and analysis groups

Analysis group	DDG	Closest dust source	Distance from source
0 m	DU14	TSF	Directly adjacent
	DU25	Pit, stockpiles	200 m
	DU29	TSF	Directly adjacent
	DU26	Pit	Within source
	DU27	HMC	Within source
1 km	DU18	TSF, stockpiles	800 m
	DU28	TSF	900 m
	DU24	Pit, stockpiles	1200 m
	DU19	TSF	1000 m
2.5 km	DU17	TSF, stockpiles	2500 m
	DU23	Pit, stockpiles	2200 m
	DU21	Pit, stockpiles	2500 m

NB: Both DU14 and DU19 are the average of three gauges positioned next to each other for quality control purposes.

Pearl Bluebush Surveys

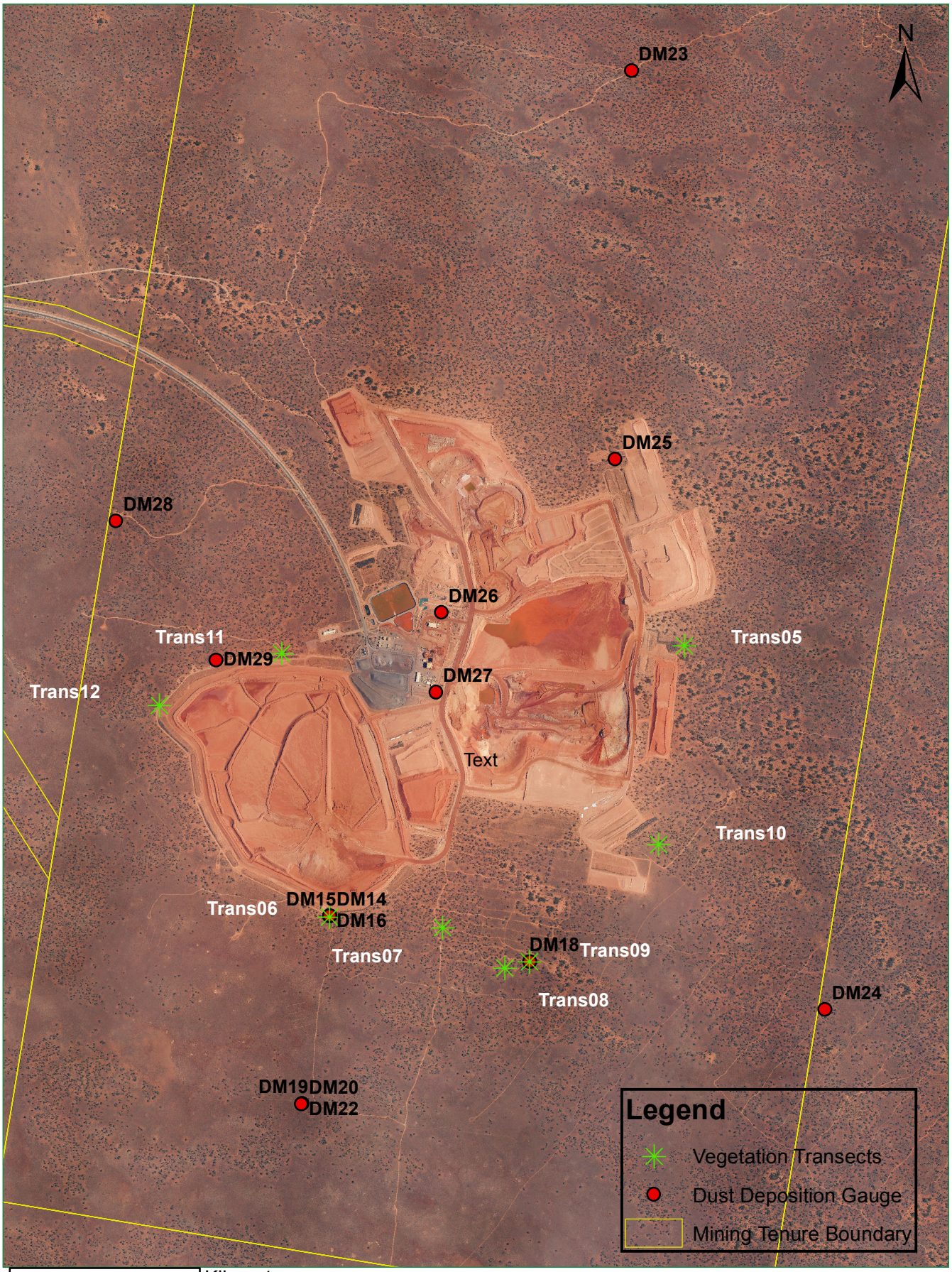
A set of six transects were established by the J-A rehabilitation team in June 2011 in locations adjacent to a range of different dust sources at the J-A mine. Transects extend north, south, east and west, away from the source of the dust. Only pearl bluebush was chosen for plant health measurements 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.

At each monitor shrub an assessment was made of 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 8.3). Photos were taken of canopy cover percentage from the top and side. Upon return to base the data was downloaded, edited and stored in a desktop GIS system (ArcMap 9.3.1, ESRI®).

Table 8-3 Plant data recorded at each monitor shrub

Feature	Characteristic	Score Options
Health	Mature Leaf Health	Healthy, coating of dust, dying & dropping
	New Leaf	Nil, healthy, dusty, unhealthy
	Flowering	Yes or No
	Fruit	Yes or No
	Overall Plant Health	Score: 0 - 100 %
	Canopy down	Score: 0 - 100 %
	Canopy side	Score: 0 – 100%
Dust	% of plant covered in dust	Score: 0 - 100 %
	Dust direction (dust source)	N, E, S, W



JACINTH AMBROSIA

Location of dust deposition gauges and pearl bluebush monitoring transects

ILUKA

MGA Coordinates, GDA 94, Zone 53

ORIG: JLEE DRAWN: JLEE SCALE: 1:28,277 (A4) DATE: April 2014 DWG No: JARMS Fig. 8.1

FIGURE: 8.1

8.1.3 Results

Dust Deposition Monitoring

Baseline dust deposition monitoring results averaged from 2.15 g/m2/month to 0.42 g/m2/month (Figure 8.2). These levels are similar to those recorded in similar arid and semi-arid environments, Uno station located north of Kimba has recorded base line levels of 1.7 g/m2/month, however rates of up to 4.4 g/m2/month have been recorded.

As anticipated, higher levels of depositional dust were recorded during the known windier periods (October and November).

Figure 8.2 Baseline depositional dust (g/m^3/month) recorded in 2008 at J-A

	August	September	October	November	Uno Station
Average	0.42	1.09	2.15	1.33	1.77
Minimum	0.02	0.4	0.45	0.42	0.2
Maximum	4.92	4.21	5.11	4.23	4.4

Uno Station annual baseline results are given for comparison of deposition rates in a similar environment.

Overall dust deposition for the 2012 and 2013 period ranged from to 0.11 g/m^3/month to 32.97 g/m^2/month (Figure 8.3). As anticipated high dust deposition was recorded at dust deposition gauges (DDGs) closest to the mine footprint (0 m) ranging from a maximum of 32.97 g/m^2/month to a minimum 0.45 g/m^2/month and the highest deposition rates were recorded at the gauges located south of the TSF (and Figure 8.4). Dust levels recorded at DDGs at greater than 1 km from the source were similar to baseline levels.

Figure 8.3 Mean depositional dust (g/m2/month) recorded 2012 and 2013 at J-A

Distance from source	0m					~1km				~2.5km		
Depositional Dust (g/m^2/month)	DU 14	DU 25	DU 29	DU 26	DU 27	DU 18	DU 28	DU 24	DU 19	DU 17	DU 23	DU 21
January	5.19	7.16	5.99	18.60	23.91	1.33	0.56	0.70	0.64	1.25	1.53	0.76
February	1.28	6.99	4.94	13.47	14.44	0.47	0.25	0.85	0.45	0.89	0.98	0.79
March	12.74	3.59	9.16	11.21	13.39	1.23	2.40	0.77	0.81	0.81	1.52	1.20
April	4.17	1.75	1.05	1.95	4.36	0.48	0.30	0.28	0.18	0.38	0.50	0.38
May	2.52	0.45	1.47	1.41	2.86	0.26	0.62	0.15	0.20	0.29	0.13	0.11
June	2.09	0.54	0.54	1.23	5.74	0.41	1.20	0.19	0.35	0.39	0.60	0.48
July	8.61	0.58	0.50	1.85	7.23	1.74	5.17	0.47	0.35	0.50	0.26	0.35
August	22.13	0.99	0.66	4.97	24.67	2.19	4.43	0.56	0.63	0.44	0.24	0.52
September	1.76	0.97	0.63	2.59	14.00	0.47	0.19	0.29	0.10	0.24	0.41	0.41
October	32.97	9.42	8.32	15.19	28.11	3.21	1.01	1.10	1.89	1.51	3.05	1.27
November	21.72	5.17	6.99	7.25	3.11	1.51	0.62	0.77	1.66	1.24	1.71	0.92
December	22.03	9.47	8.02	19.63	25.26	1.78	1.33	0.86	1.04	1.31	1.88	0.85
Average	11.43	3.92	4.02	8.28	13.92	1.25	1.50	0.58	0.69	0.77	1.06	0.67

Green text indicates minimum recorded levels per gauge and red text indicates maximum dust levels recorded per gauge

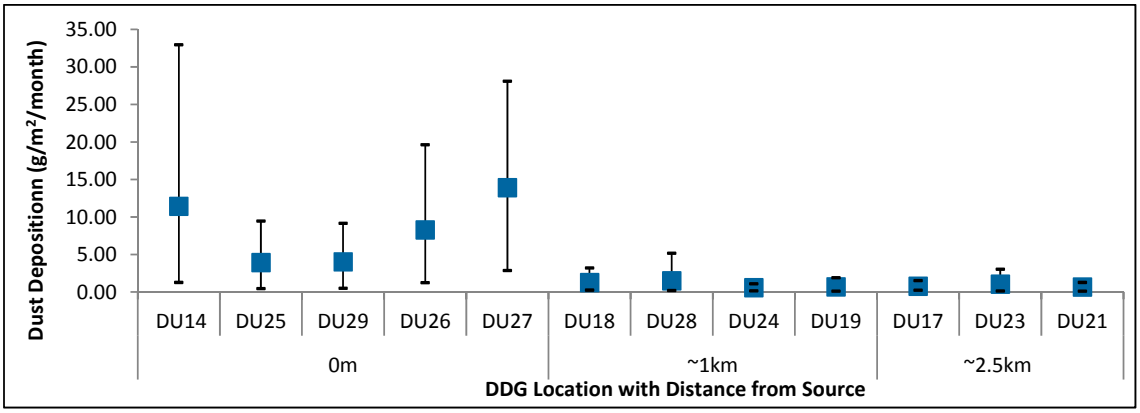


Figure 8.4 Mean dust deposition with distance from dust source 2012 and 2013. Error bars indicate maximum and minimum recorded levels

Pearl Bluebush Surveys

The results from surveys of transect 6, 9, 11, 12 are presented here and compared with EBS Baseline survey data collected in 2008 and 2009.

A total of 114 shrubs were sampled at various times across seven sampling occasions between November 2011 and August 2013 (Figure 8.5). All 114 shrubs were monitored in January and August 2013, forming the basis of the long term sampling program.

Figure 8.5 Number of shrubs monitored at J-A from November 2011 to August 2013

	Survey Date							
Transect Number	**Nov-11**	**Mar-12**	**Jul-12**	**Oct-12**	**Jan-13**	**Feb-13**	**Aug-13**	**Total**
6	6	6		17	30	9	30	98
9	7	7	4		24	8	25	75
11		8			36		36	80
12		8			24		23	55
Total	**13**	**29**	**4**	**17**	**114**	**17**	**114**	**308**

Dust Cover and Distance from Source

Dust cover on monitoring shrubs ranged from 100% to 0%. As anticipated there is an effect of distance from dust source on the proportion of dust covering the monitoring shrubs (Figure 8.6). The proportion of dust cover decreases rapidly with distance from the source, before levelling off at 500 m.

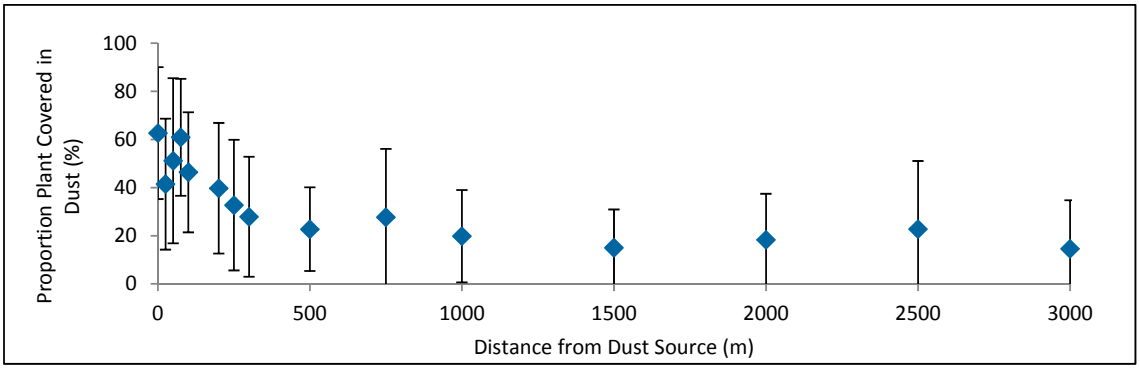


Figure 8.6 Mean proportion of monitoring shrubs covered by dust across distances from dust source. (Chart is based on all J-A survey data collected between November 2011 and August 2013. Error bars indicate standard deviation)

Dust Cover and Plant Health

Health scoring of all shrubs ranged from 90% health to 10% health. Overall plant health decreases with increasing proportion of monitoring shrub covered in dust (Figure 8-7), as dust cover increases plant health decreases. Plant health is a cumulative scoring estimate of all measures considered for the survey; canopy cover, flowering and the health of leaves.

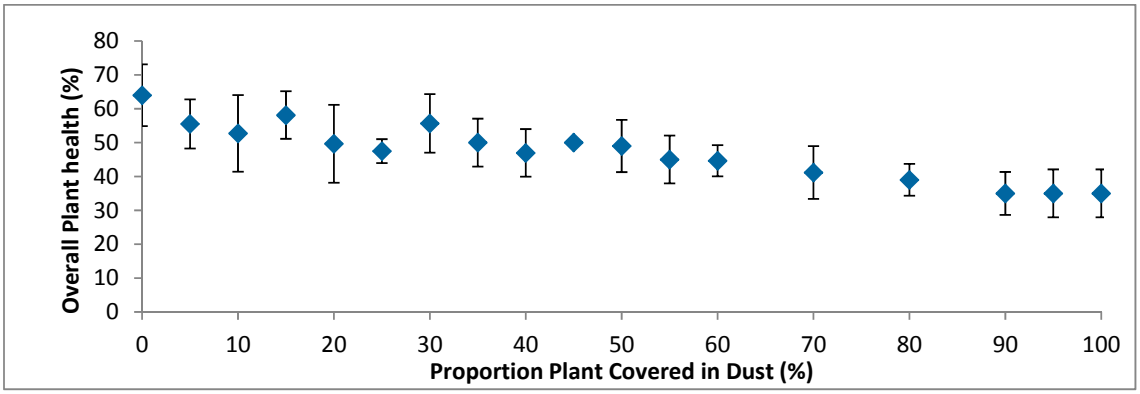


Figure 8.7 Mean overall plant health of monitoring shrubs in relation to the proportion of the shrub covered in dust. (Chart is based on all J-A survey data collected between November 2011 and August 2013. Error bars indicate standard deviation)

Dust Cover and Canopy Cover

Overall canopy cover (looking from above) of monitoring shrubs ranged from 10% to 90% across all monitoring periods. Side canopy cover (canopy cover on side facing dust source) ranged from 5% to 90% overall across all monitoring periods. Mean canopy cover is similar for both top and side cover (Figure 8.8). Based on current collected data there does not appear to be any relationship between canopy cover and the proportion of the shrub covered in dust. Canopy cover was highly variable although consistently lower than the baseline levels recorded in 2008 and 2009 by EBS Ecology. Overall canopy cover was lower when compared to baseline, however this is for all dust cover levels and therefore may not be related to dust on plants.

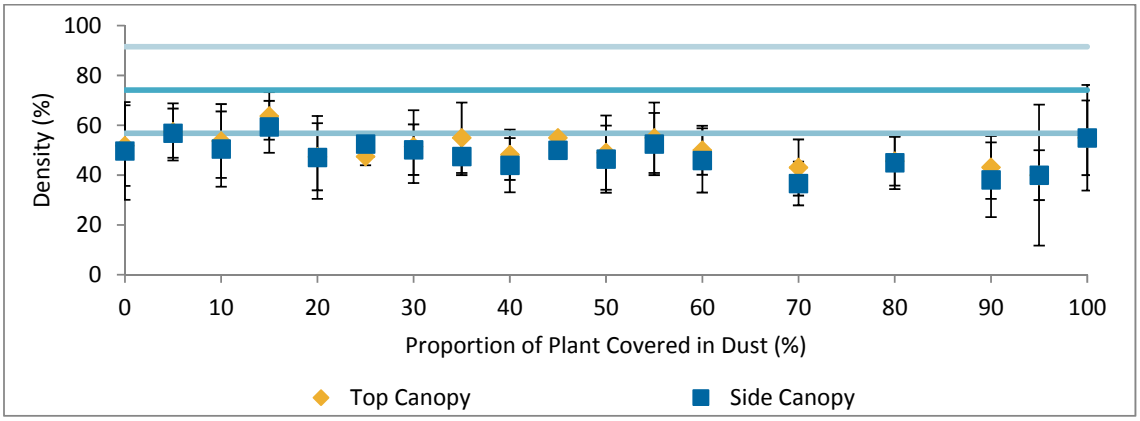


Figure 8.8 Comparison of canopy densities (looking from top of canopy and side of canopy from the dust source) collected at J-A across all surveys with baseline data collected by EBS Ecology. Green solid lines indicate the mean baseline canopy densities collected

Dust Cover and Flowering

Pearl bluebush viable seed production has been low and unreliable at J-A. The shrubs within the mine lease set seed on a large scale during March 2009, however no viable seed was found from hundreds of viability checks made in many locations. Of the monitoring shrubs flowering was recorded on 56 occasions (out of a possible 359 occasions) between 2011 and 2013. There does not appear to be a relationship between flowering and the proportion of the plant covered in dust, although no plants flowered when greater than 90% of the plant was covered in dust (Figure 8.9). The data should be treated with caution as the data set is limited and any trends may not be identified until additional surveys have been carried out.

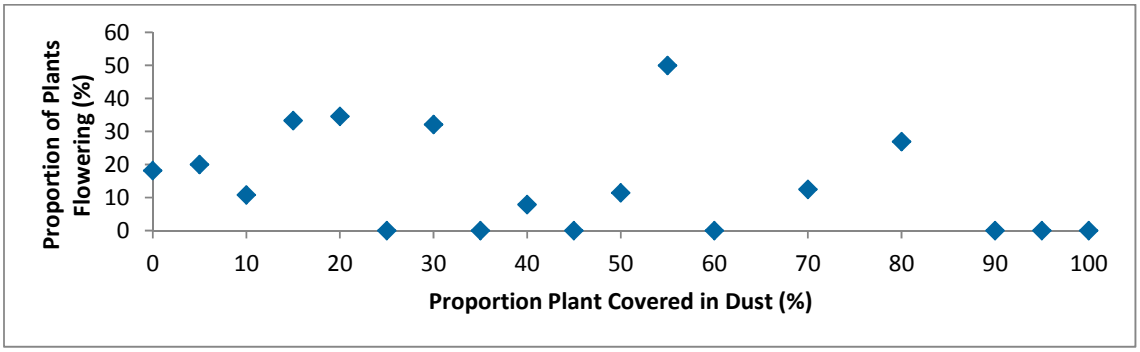


Figure 8.9 Proportion of monitoring shrubs found to be flowering in relation to the proportion of shrub covered in dust. This chart is based on all J-A survey data collected between November 2011 and August 2013

Dust Cover and Leaf Health

No shrubs with healthy older leaves were found on plants with greater than 40% dust cover, with the number of shrubs having healthy old leaves decreasing with dust cover (Figure 8.10). Although interestingly there is no apparent relationship between dying and dropping leaves and unhealthy leaves and the level of dust cover on the shrub. Importantly there does

appear to be a decline in in the number of shrubs with healthy new leaves with increasing dust cover of the shrub. The growth of healthy new leaves may be an indicator a plants ability to recover from stress. Few shrubs showed unhealthy new leaves and further the numbers of shrubs with unhealthy new leaves did not show any effect for the proportion of the plant covered in dust.

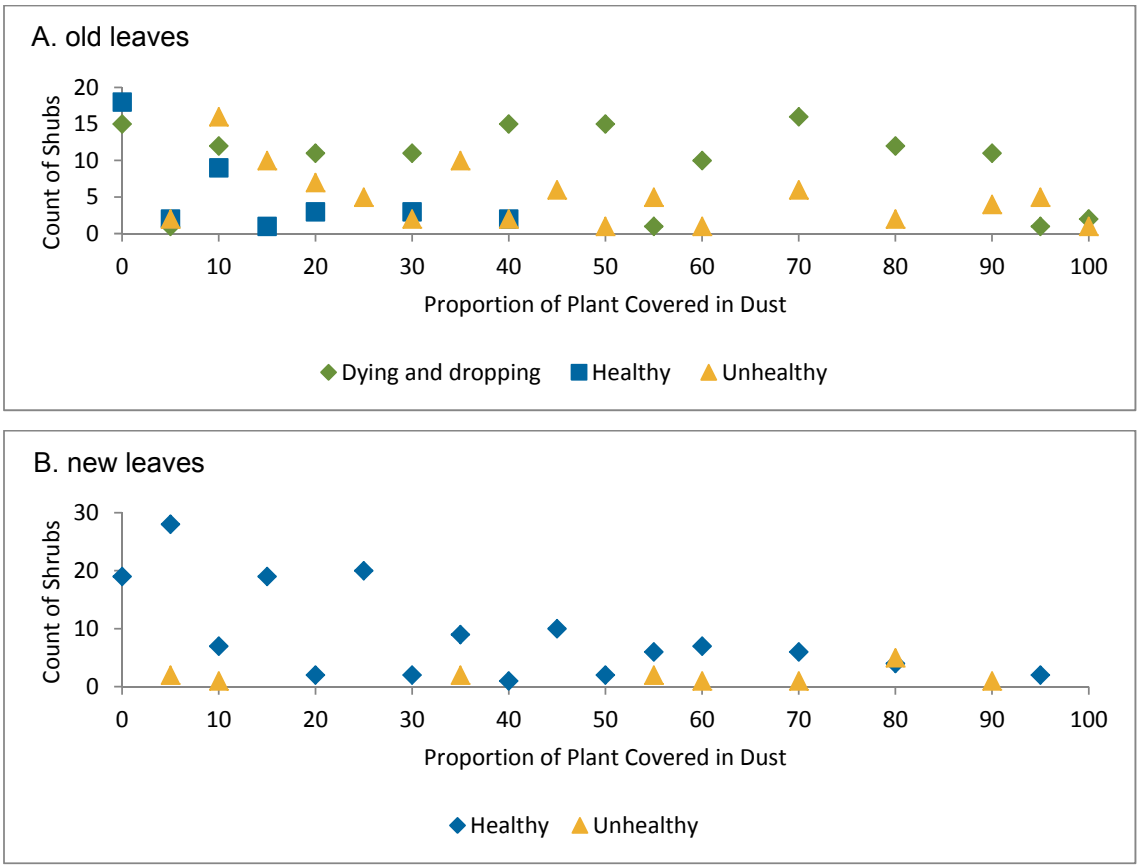


Figure 8.10 Leaf health in relation to the proportion of the plant covered in dust. (A) shows results for old leaves on plants and (B) shows results for new leaves on plants. This chart is based on all J-A survey data collected between November 2011 and August 2013

Dust Cover and Rainfall

Survey data collected in January and August 2013 was analysed separately to determine any seasonal effects on shrub dust cover (Figure 8.11). Notably the dust cover on shrubs in August 2013 was less than the dust cover on shrubs recorded in January 2013. This is likely due to the wash off effect of an above average rainfall in 2013 (Figure 8.12) where 54.4 mm was recorded in July, well above the long term average of 12.9 mm.

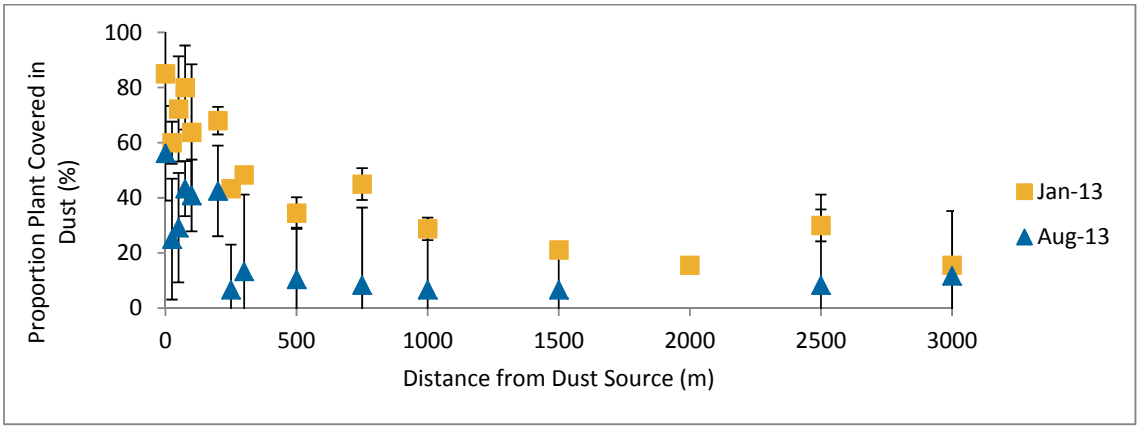


Figure 8.11 Mean proportion of monitoring shrubs covered in dust for January and August 2013 with distance from dust source. This chart is based on the January 2013 and August 2013 survey data, error bars indicate standard deviation

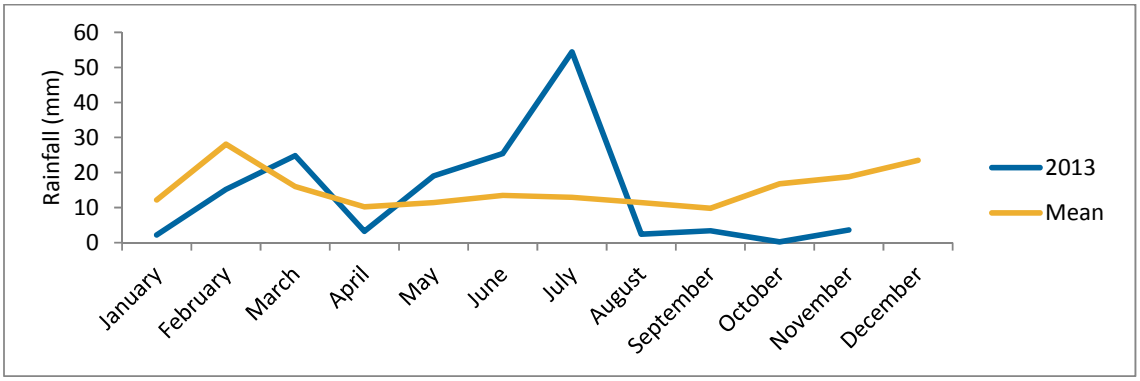


Figure 8.12 Mean rainfall for Tarcoola and rainfall recorded at Tarcoola in 2013

8.1.4 Conclusion

DDG data shows that mine operations are having an impact on the local fugitive dust levels. As anticipated dust deposition rates were higher closer to the source, gauges located in the 0 m group showing very high deposition rates and gauges located at 1 km and 2.5 km showing lower levels similar to baseline. Monitoring shrubs also showed an effect for dust cover with distance from source, shrubs closer to the source had a higher proportion of dust cover than those further away. Dust cover reduced steadily with distance from the source levelling at shrubs located 500 m to 3000 m. No dust depositional data is available between 0 m and 1000 m, therefore the area of influence is unclear at this point at time. Additional gauges located between the 0 m and 1000 m would provide useful information about the area of impact.

At this stage is it difficult to determine the long term impact of the additional dust loads on the pearl bluebush. The additional dust loading on plants appears to have a negative impact on the overall health of plants and the health of leaf growth, however monitoring shrubs do not show any effects for flowering and canopy cover. Anecdotally many of the shrubs observed with zero dust cover (outside the fugitive dust emission area) also showed leaf and canopy cover loss. It is possible that canopy cover is related to a different factor (i.e. age or

climate conditions), and another measure may be more suitable to determine changes to plant health in the short time. Additional monitor sites outside the ML could provide information on the health of plants regionally.

The numbers of shrubs with healthy leaves appears to be influenced by the level of dust cover. No healthy older leaves were found on shrubs with a greater than 40% dust cover, and further the number of shrubs with healthy new leaves decreased with increasing dust cover. Monitoring the leaf response of shrubs may be a more efficient way to determine rapid changes in plant health than canopy cover. The response of leaf growth to dust cover has the potential as a leading indicator for canopy cover, i.e. a lack of new healthy leaf growth would indicate a reduction in canopy cover prior to the reduction in canopy cover being observed.

There is potential for dust loads to be washed off plants by rain, however for plants such as pearl bluebush with trichomes the wash off effect may be reduced due to the 'stickiness' of the leaves and stems. There was a rainfall wash off effect of the dust cover across all monitored shrubs, however this response may be due to the higher than average rainfall recorded in 2013. Washing off of dust cover during average or lower than average years may be reduced. Further information will need to be collected at various rainfalls before the impact of the washing off effect can be determined and how this impacts on the long term shrubs survivability and reproduction.

Pearl bluebush are a long lived species and may be slow to respond to changes in the environment. The response of the smaller annual species and overall community composition has not yet investigated. Community composition and abundances are an important part of the environment and all vegetation is considered native vegetation under the Native Vegetation Act. The loss of smaller annuals and less long lived species may impact cumulatively on the longer lived species, for example reduced plant cover could potentially lead to an increase in erosion and decrease in nutrient and water availability for other longer lived species. Additional monitoring of plant communities overall will be required to determine overall vegetation impacts.

There is still too little data collection to determine changes to the survivability of the pearl bluebush as a function of dust deposition, and at this stage it does not appear the impacts could be considered clearance under the Native Vegetation Act. However, it is not clear if high loads continue how the shrubs will respond. Monitoring should continue for early warning of any rapid decline in plant health to the point of mortality. Further surveys and investigation into measures of dust deposition rates, pearl bluebush health (and other species) are required to determine the total area of influence and the ability of plants to recover from the high dust loads.

Recommendations

Based on the information provided in this report the following recommendations are made:

- The current monitoring program should continue in January and August of each year to look at the ability of rain events to wash dust off plants and to determine if there is any ability of the plant to recover from any effect the dust loads may have on growth or reproductive ability. Additional baseline monitoring should also be carried out well outside the fugitive dust emission zone (likely outside of the Mining Leases) to test for regional responses to climate. This will require a scientific permit issued by DEWNR.
- Monitoring a single species does not give an indication of how other species of plants are being affected by the additional dust loads. Unless this is known then the usefulness

of the indicator species is limited. Further losses of other species from the local environment may further exacerbate stresses already placed on plants due to higher dust loads. The loss of annuals and other shrubs has the potential to increase erosion or change the soil seed bank. It is recommended that additional plant species and community composition changes are monitored for impacts from dust smothering.

- It is not clear if canopy cover is related to plant health (i.e. it potentially may be an indicator of plant age), therefore canopy cover may not be a suitable indicator. It is recommended that investigations into other measures of suitable plant health continue, however the current survey methods should continue as there is little information or data available for pearl bluebush health. The long lived pearl bluebush may be slow to respond to increasing dust loads and other species may be detrimentally impacted whilst we monitor this species. The impacts to other plant species should also be investigated.
- Additional DDGs should be located at 250 m and 500 m from the dust source on the transects with the highest dust loads (transect 6 and transect 12) and at 500 m from the dust source on transects with lower dust loads (transect 11 and Transect 9). This will allow the area of influence to be determined more accurately. The monthly dust deposition monitoring program should continue and be reviewed and analysed annually with the pearl bluebush survey data.
- Dust deposition has been observed around the edges of the mine footprint and can be seen to be piling up against vegetation. The sand is newly deposited (i.e. overlays intact crust). Measurement of the depth of sand over crust should be measured at recorded at various sites around the mine (these can be incorporated in the community composition monitoring program). The levels may need monitoring over time to determine the efficacy of dust management measures and the impacts on local vegetation identified.
- A precautionary approach should be taken, and dust management measures (as per the Dust Management Plan and Native Vegetation Plan) should be implemented on the TSF and other open areas.
- Given that the TSF may no longer be required for tailings storage the final rehabilitation of the TSF should be investigated. This will require additional landform studies and trials into the ability of the chenopod communities to regenerate.