

# Ravensworth Hunter Ironbark Complex Research Program Final Report



by

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GLENCORE



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*Cover photos. Top row: left to right: Seeding “hot spot” species (photo Carmen Castor), Wet seed mix in the laboratory before mixing with sand (photo Carmen Castor), Eucalyptus crebra seedling in the field (photo Yvonne Nussbaumer). Bottom row: Chrysocephalum apiculatum flowers, Swainsona galegifolia flowers and Einadia nutans fruit (photos Carmen Castor).*

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## 1. Executive Summary

In this report by the University of Newcastle to Ravensworth Operations on the Ravensworth Hunter Ironbark Complex Research Program the background rationale is given for the current experimental approach to rehabilitating towards Ecologically Endangered Communities (EECs) on the northern spoil dump area (Section 2). Current evidence suggests that over the long term, plant diversity on rehabilitation areas declines which could jeopardize meeting rehabilitation objectives. One possible reason for this is an inadequate seed bed preventing second generation seed germination and establishment, leading to the loss of species at the site once the first generation dies. In this program, seed bed and sustainability of plants will be investigated. The use of a variety of substrates is explored to ameliorate the seed bed (Section 3) and an explanation is given of how a statistically valid field trial is set up. Then a detailed explanation is given on how the target community species lists were determined (Section 4) and how they were used in the field experiment. Results on species establishment, cover and weediness are presented and discussed in Section 5. Experiment 1 refers to the establishment of the experiment and the seeding of the area with the EEC species, and is referred to as the matrix. Section 6 describes how the issue of herbaceous plant sustainability was experimentally investigated and what the outcomes were. Experiment 2 specifically refers to an experiment on the sustainability of herbaceous species which will be studied in detail to resolve the problem of the lack of persistence of many herbaceous species. Section 7 describes recommendations for rehabilitation based on information obtained to date. Subsoils and forest topsoil, even when highly degraded, still provide the best outcomes in terms of species diversity and sustainability of populations of EEC and other local species. When combined with mulch the effect is increased and in some cases the addition of OGM may further increase outcomes. The main usefulness of OGM is the high ground cover of selected native species it can produce. But a major problem is that it also encourages weedy species and invasive grasses which require intensive management and can ultimately threaten the outcome. Recommendations are formulated, and a revised site preparation plan, seeding plan and monitoring plan proposed.

## 2. Context and Problem

This research is undertaken by The University of Newcastle to satisfy Conditions 36 and 37 of Schedule 3 of the Ravensworth Operations Project Approval (PA 09\_0176). The conditions state:

### ***“Hunter Ironbark Research Program***

*36. The Proponent shall prepare and implement a Hunter Ironbark Research Program for the project to the satisfaction of the Director-General. This program must:*

- a. be prepared in consultation with DECCW, and be submitted to the Director-General for approval by the end of December 2011;*
- b. be directed at encouraging research into the mapping and recovery of EECs affected by the project, particularly:*
  - Central Hunter Grey Box – Ironbark Woodland EEC; and*
  - Central Hunter Ironbark – Spotted Gum – Grey Box Forest EEC.*

*37. The Proponent shall allocate at least \$200,000 towards the preparation and implementation of the Hunter Ironbark Research Program identified above and obtain the Director-General’s approval for allocation of funding under the program.”*

In response to this requirement we proposed a research program based on the current knowledge of long term difficulties experienced by rehabilitation sites in the Hunter Valley, NSW. Our experience for the work has been based on our research at the neighbouring Mount Owen mine where rehabilitation towards native ecosystems has been on-going since 1996.

While the establishment of most canopy and upper middle storey species seems to be successful, the lower storeys are constantly under threat by invasive grasses and weeds. Also in areas where native herbs and grasses have initially established, diversity tends to diminish over time. Experiments set up specifically to monitor the life cycling of these small plants have determined that while most set seed, seedlings frequently fail. This seems to indicate problems with the seed bed, either that overall conditions (humidity, nutrient availability, etc.) are not favourable to survival of seedlings, or the frequency of microsites (soil surface heterogeneity, nurse plants, etc.) is not sufficient for these species. For these reasons, at Ravensworth Operations northern rehabilitation area, we have chosen to address seed bed properties by setting up a variety of spoil ameliorations with different properties to test the life cycling capacity of a selection of herbaceous plants (Experiment 2). Not only will the soil itself influence herbaceous plant survival, but also the surrounding vegetation. For this reason, a background (matrix) vegetation community was also incorporated into the design based on the canopy, upper-middle storey and understorey species composition of the Central Hunter Grey Box-Ironbark Woodland and the Central Hunter Ironbark-Spotted Gum-Grey box Forest (Experiment 1). This has the advantage of becoming an independent level of study directly targeted at the effects of the experimental substrates on the main matrix of these vegetation types which is useful data for the continued success of the remaining rehabilitation areas.

### **3. Experimental Substrates**

#### **3.1 Substrates and Ameliorants**

With the objective of ameliorating spoil to support diverse native vegetation, a number of substrate combinations were trialled based on discussions between University and Mine Personnel, the availability of substrates and the suitability of substrates as indicated by previous research done in the Hunter Valley (Nussbaumer et al. 2012). For these reasons subsoil, Organic Growth Media (OGM), and wood mulch were chosen as ameliorants. Mine spoil and woodland topsoil were also included in the trial (Plate 3.1).

##### **3.1.1 Spoil**

Spoil is overburden and interburden from the mine pit. In this location, coal seams lie mainly in the Wittingham coal measures which are interspersed by sandstones and silt stones of marine origin. Analysis of the spoil from the shaped rehabilitation hill (WEA 1-3, SESL Australia 2013) shows that it is strongly alkaline, moderately saline and sodic, with limited nutrient value. Its sodicity means it can become highly dispersive. High levels of magnesium led to the recommendation (SESL Australia) to apply gypsum to enhance plant growth and to the recommendation (Umwelt 2010) to add organic amendments to overcome the dispersive properties and the lack of nutrients in spoil.

##### **3.1.2 Woodland Topsoil**

From the Environmental Assessment (Umwelt 2010), topsoils at the Ravensworth Operations site range from brown sand to brown loam. Topsoil analysis undertaken for Ravensworth Operations (BH 1 – 20, SESL Australia 2011) show that organic material is at low to very low levels, with few exceptions over the whole site. The advantage of this substrate is that it may contain some remnant soil microbes and some native seed bank. A major disadvantage is the presence of a potential weed seed bank.

##### **3.1.3 Subsoil**

Subsoils from the Liddell formation, which predominates on the mined area, range from sandy clay, light clay, sandy loam to loamy sand (Umwelt 2010). Salinity increases rapidly with depth and some of it is highly dispersive (BH 1-20, SESL Australia 2011). Nevertheless, being a more weathered material than spoil, the presence of soil microorganisms and a native seed



bank, albeit in low quantity, make this an interesting media to trial. Subsoil was used in experiments as a spoil ameliorant on Mount Owen mine in 2004 and 2005 (Nussbaumer et al. 2012, Cole et al. 2006) and has been the best media for establishing a biodiverse ecosystem which is not heavily invaded with weedy species.

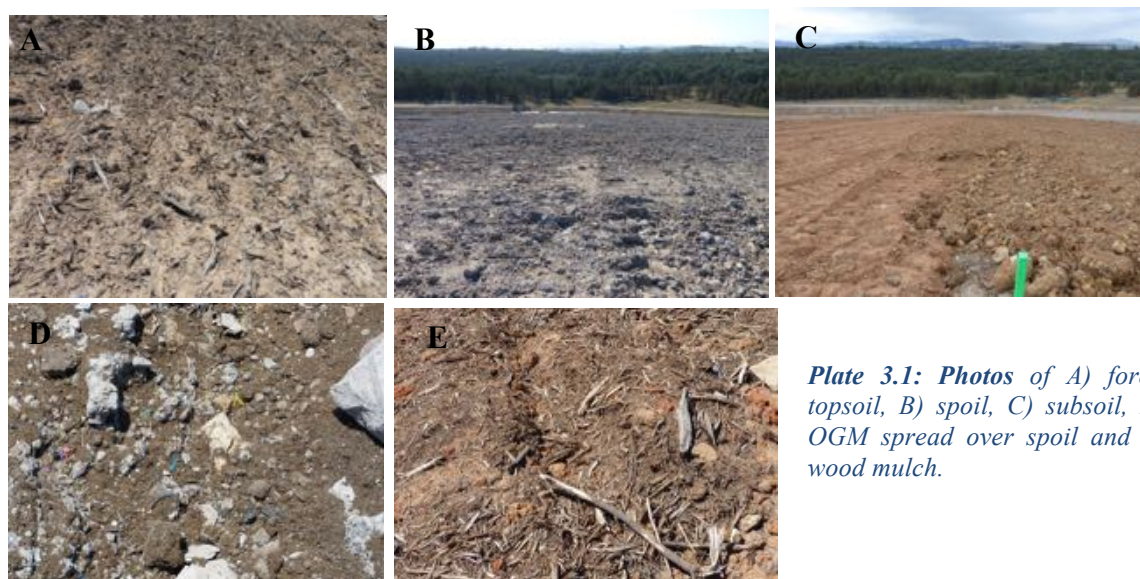
### 3.1.4 Organic Growth Media (OGM)

Organic Growth Media was made available to Ravensworth Operations by Global Renewables. OGM is made from composted mixed waste defined as “a) residual household waste that contains putrescible organics and/or b) waste from litter bins that are collected by or on behalf of local councils” (EPA mixed waste exemption 2011). It may be mixed with certain other wastes of organic origin. It contains nutrients, organic material and microbes beneficial for plants. Chemical and physical contaminants in the form of plastic, glass, metal and weed seeds are at an acceptably low level for mine rehabilitation at approved application rates. Analysis of the substrate onto which the OGM will be applied is required to ensure that the levels of contaminants do not exceed EPA regulations. Appendix 1 shows pre-application substrate testing for 4 samples containing 6 cores each.

OGM has been trialled on several other mines in the Hunter Valley (Kelly 2008, Spargo 2012, pers. com. Bill Baxter, Colin Davies) but most are only based on one-plot trials. The overall conclusion is that OGM increases establishment and growth of woodland species. Thorough incorporation of the OGM into the underlying substrate is recommended as it can burn young seedlings. An issue with OGM that will become apparent only in the long term is whether the plastic content will affect the fertility of animals through bioaccumulation in the food chain. Research into adverse effects of plastics on human and animal health has a long history, but, due to the great utilitarian value of these products, has largely been ignored (see: Halden, 2010).

### 3.1.5 Wood Mulch

Wood mulch was sourced from the vicinity of the Northern Remnant at Ravensworth Operations where authorised clearing works were in progress and is mainly from *Allocasuarina luehmannii* trees. Wood mulch has also been tested at Mount Owen Mine (Read 2002) and has been routinely incorporated into topsoil for application over spoil (Nussbaumer et al. 2012). It provides organic input, resistance to surface erosion and microsites for microorganisms and seedlings.



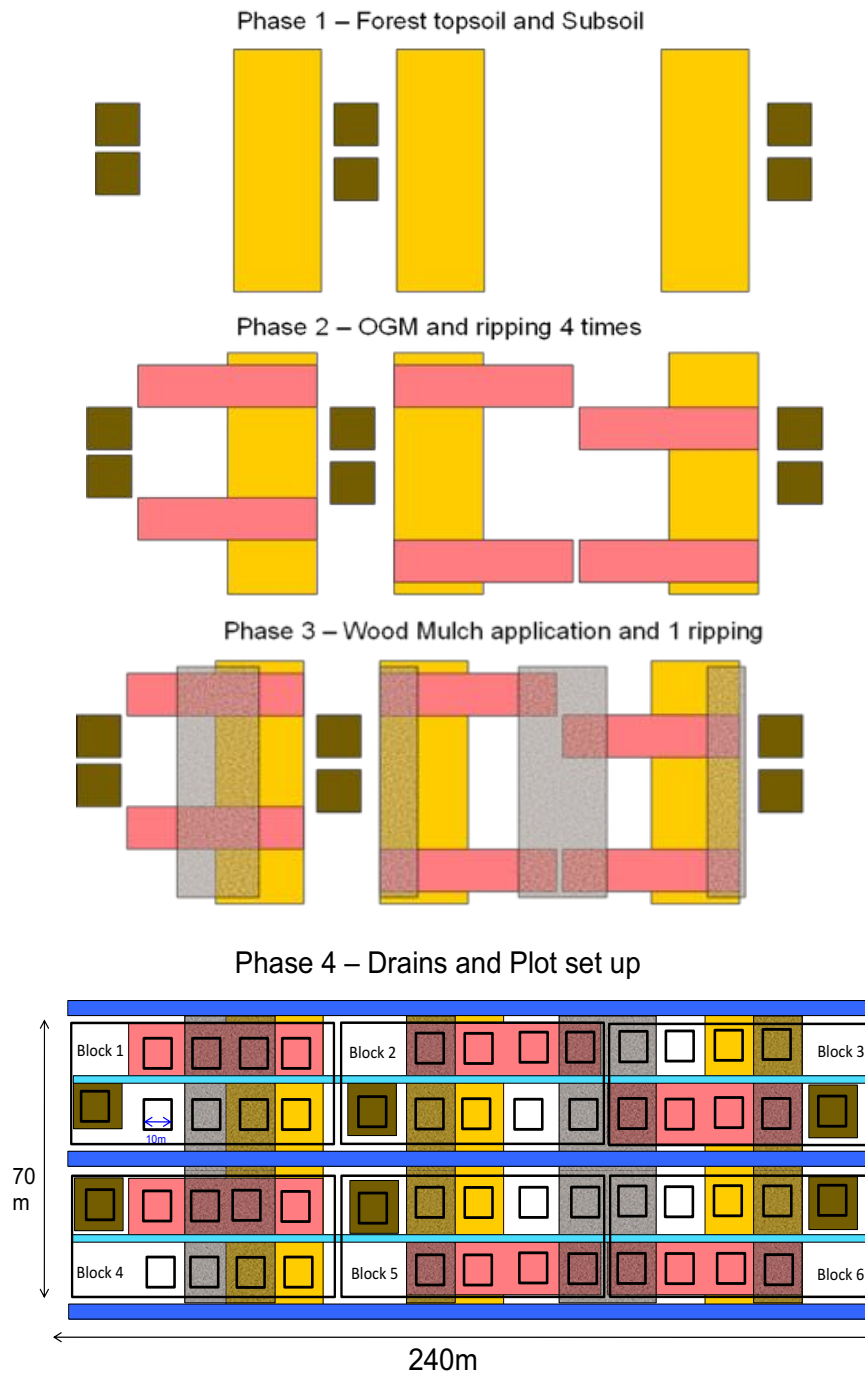
*Plate 3.1: Photos of A) forest topsoil, B) spoil, C) subsoil, D) OGM spread over spoil and E) wood mulch.*

### 3.2 Setting up the Experimental Site

The experimental design was based on statistically valid blocking of treatment replicates and the practicality of spreading substrates with machinery. The implementation of the design was performed by Mine Personnel (Sean Pigott, Daracon, EAMS) in August to September 2013. Subsoil and woodland topsoil were spread first to a depth of 20-30cm in predetermined positions (Plate 3.2 & Figure 3.1). OGM was spread next at a rate of 6m<sup>3</sup>/plot, equivalent to 100dst/ha, with a dozer and was incorporated into the underlying substrate by 4 passes of a ripper to a depth of 10-20cm (Plate 3.2). The ripping was done on all plots starting at the top of the slope and moving down the site to reduce the amount of OGM cross-contamination. The ripper moved very slowly and lifted the tynes between subsoil and spoil plots to reduce the amount of material being pulled into adjoining plots. The mulch was applied at 6m<sup>3</sup> per plot and then ripped in once, following the same procedure described above. Some manual mulch application was necessary to homogenise the cover as the mulch layer was quite uneven both within and between plots (Plate 3.1). Drains were then excavated parallel to the contour to avoid contamination between OGM and non-OGM plots by runoff during rainfall. Ten by ten meter plots were then set up in the middle of each treatment combination, producing a total of 54 plots. On closer inspection of the plots, some cross contamination of treatments had occurred despite the care taken, and two additional plots in blocks 2 and 3 were set up on spoil areas for compensation. These were included in the experimental design and were seeded like the other plots.



**Plate 3.2. Setting up the experimental site.** A) Subsoil (orange) and OGM (brown) have been layered over the spoil (grey). Piles of mulch are visible at the top of the site waiting to be spread after several ripping passes shown in B). In C) The plots have been set up and some additional mulch is being spread by the small excavator which also fixed the drains which had been put in previously with a dozer.



**Figure 3.1: Implementation sequence of experimental set up and the final experimental design.** Phase 1 consisted of laying down the forest topsoil (brown) and the subsoil (orange). Phase 2 consisted of layering the OGM (pink) and carefully ripping 4 times to incorporate the OGM without dragging it into neighbouring areas. Phase 3 consisted of spreading the wood mulch (mottled brown) and one ripping pass followed by manual spreading. Phase 4 consisted of drains (blue) being put in place along the contours to stop OGM being washed into adjacent plots by rain runoff. Plots (10x10m) were then set up over the whole site (black squares), and divided into blocks (larger black squares). In all, 6 blocks were set up, each containing nine plot types: Spoil, spoil & mulch, spoil & OGM, spoil & mulch & OGM, subsoil, subsoil & mulch, subsoil & OGM, subsoil & mulch & OGM, and forest topsoil. Spare spoil plots were placed in the empty space in blocks 2 and 3.



### 3.3 Soil evaluation of the Experimental Site

#### 3.3.1 Soil Sampling

After site set-up, samples were taken from every experimental plot as a zero baseline against which the future rehabilitation area may be compared to in terms of nutrient content and limiting factors to plant establishment and growth. Four samples were taken on the 6<sup>th</sup> of January and the 14<sup>th</sup> of February 2014 (5 and 6 months after site substrate setup) with a 9cm diameter and 10cm deep corer at 2x2m in from each corner post of the 10x10m plots: in total 216 samples. These samples were dried at 40°C for 2-5 days and stored in plastic jars for future analysis. In January 2015 samples from blocks 1, 2 and 5 (the least affected by erosion and the representative for best outcomes on site) were processed for analysis by SWEP Analytical Laboratories. The four cores from each plot were homogenised and a subsample of soil fines extracted (as per SWEP guidelines) so that 24 samples, the nine treatments and 3 replicates corresponding to the three blocks, were analysed.

A second set of four subsamples from each plot were taken on the 9<sup>th</sup> of December 2014 using a hand auger with a radius of 5cm to a depth of 10cm at 1x1m in from each corner. Core samples were taken from blocks 1, 2 and 5 as above. The four subsamples were then homogenised and processed in the university soil laboratories to determine physical properties such as water holding ability, texture, organic content and bulk density, as part of an honours project. These factors effect plant growth, survival and germination of second generation plants. Detailed information of methods and results can be found in Scanlon (2015).

Five analogue areas were also sampled using the above methods for the Honours project (Table 3.1) and were processed in a similar way for chemical analysis and physical properties. More details can also be found in Scanlon (2015).

**Table 3.1: Analogue site codes and descriptions.**

Site code	Identifier in Appendix C	Site description
R1	Safe Site 1	Ravensworth State Forest (RSF) – <i>Swainsona galegifolia</i> area under Eucalyptus forest
R2	Safe Site 2	Forest East Offset Mt Owen Complex on the Edge of RSF – <i>Calotis lappulacea</i> and <i>Chrysocephalum apiculatum</i> area in wooded grassland
R3	Ref site NW offset	North West Offset Mt Owen Complex – <i>Hypericum gramineum</i> , <i>Swainsona galegifolia</i> and <i>Desmodium brachypodum</i> area in wooded grassland
R4	Ref site Rav Einadia	Northern Offset Ravensworth Operations – <i>Einadia nutans</i> area near <i>Allocasuarina luehmannii</i> woodland
R5	Ref site S1R1	RSF monitoring plot – <i>Desmodium brachypodum</i> area under Eucalyptus forest

#### 3.3.2 Results of Soil Chemical and Physical Characteristics

The chemical properties of the treatments at site set-up were analysed to obtain a baseline against which the evolution of the site can be evaluated later on (full data in Appendix 3).

**Forest** topsoil treatments typically had physiologically optimal pH, low nutritional values (N, P), carbon content similar to Analogue sites and acceptable levels of salt and ion exchange capacity (Table 3.2). Bulk density and available water were similar to analogue sites, but gravimetric water is the lowest of all substrates which may cause it to select for drought tolerant species and slow growth. While low nutrients are normal in analogue sites (where these elements are held in the vegetation) on this recently established site if new inputs through nitrogen fixing plant-rhizobia symbiosis fail, or mycorrhizal interactions fail, it could cause a stall in new vegetation growth.

As is typical for **spoils** in the Central Hunter, the spoil treatments had high pH, sodicity and sulfur levels. Though the sulfur levels were not high enough to be a problem, the high pH may inhibit the growth of some plants due to decreased uptake of iron and zinc. The high sodicity (exchangeable sodium percentage) also explains both the crust developed on the spoil plots and the gully erosion that has formed on site. From a soil chemistry point of view this treatment would likely be very poor for plant growth and establishment, particularly from seed, as radical penetration would be difficult and the soil environment moderately hostile despite good water holding capacity. Additionally, it has the highest bulk density of all substrates indicating that compaction and rock content can become an issue.

**Subsoils** were an intermediate between forest and spoil treatments with no chemical or physical characteristic exceptionally high or exceptionally low. Subsoil would be a suitable alternative to spoil where forest topsoil is unavailable. The slightly higher nitrogen content of subsoil and subsoil with mulch compared to the analogue is interesting and could be due to subsoil under *Allocasuarina* trees (also nitrogen fixers with *Frankia* bacteria) containing higher amounts released on the cutting down of trees before extraction of the subsoil.

For the characteristics measured, adding **mulch** made very little difference to most chemical or physical characteristics of the soil at this early stage. However, there has been a significant increase in survival of plants on mulch plots. Mulch reduced nitrogen levels when compared to the non-mulched substrate pair which is probably due to increased microbial activity. Mulch did not contribute much to organic carbon content values reported here as it was removed from the soil during preparation of samples for chemical and physical analysis (issue with standard soil methods). Nevertheless, as mulch contributes to maintaining microsites for seed germination, invertebrate habitat and fungal growth, and its decay, we would expect that in several years' time it would contribute to an overall increase in soil carbon on this plot treatment and contribute to the modification of physical properties. Mulch was responsible for a significant increase in percent gravimetric water content.

**OGM** had a very large effect on the chemistry and physical characteristics of the soil increasing gravimetric water, organic matter, cation exchange capacity, electrical conductivity, calcium to magnesium ratio as well as many macro and micro nutrients. It should also be noted that, depending on the standards used for evaluation, salinity and phosphorus may be at excessively high levels. How this affects species growth and species diversity will be answered by analysing the final plant survey data.

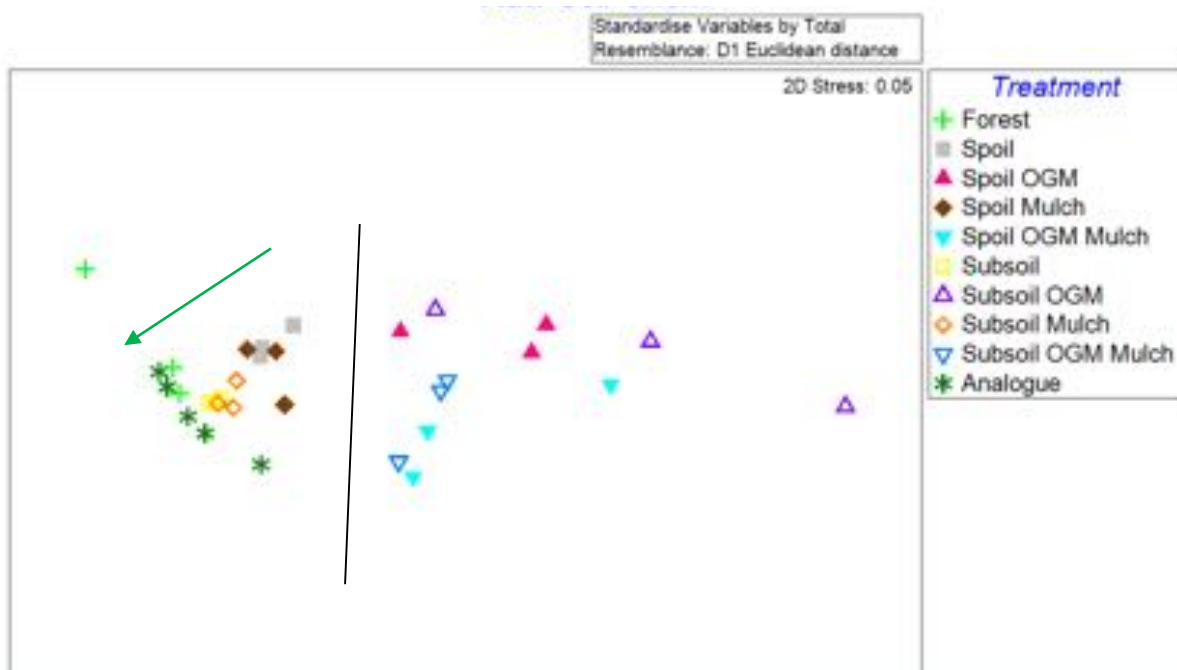
**Analogue sites**, which were established for the second part of this project concerning the niche conditions of certain herbaceous species, showed values expected for the region and provided a baseline to compare the experimental sites with. pH is acidic as expected in a forest ecosystem; nitrogen and phosphorus are appropriate for a self-sustaining ecosystem where nutrient cycling is ongoing; organic matter and organic carbon are high enough for soil structure to be in place; electrical conductivity is down and the Ca/Mg ratio is good. Bulk density shows a good intermediate value indicating the soil is structured and not compacted and gravimetric water is indicating good water holding capacity. Adding mulch and/or OGM to spoil or subsoil brings the gravimetric water up to levels found on analogue sites.

Multi-Dimensional Scaling (Primer 6) was used to illustrate the relation of treatments to one another (Figure 3.2). Treatments that include OGM, alone or in combination with other substrates, group together illustrating the large effect of OGM on soil chemistry. Non-ameliorated substrates (spoil, subsoil and forest) and in combination with mulch, align on a gradient from spoil to subsoil to forest topsoil which is probably produced by the effects of weathering of the substrates. It clearly shows that if forest topsoil is not available for

rehabilitation, then subsoil is the next closest in chemical properties, especially if mulch is also used. Forest topsoil is very similar to most analogue sites.

**Table 3.2: Main chemical and physical characteristics of the treatments used in the experimental site (n=3) and the sites used as undisturbed analogue (n=5).**

	Forest topsoil	Spoil	Spoil Mulch	Spoil OGM	Spoil OGM Mulch	Subs.	Subs. Mulch	Subs. OGM	Subs. OGM Mulch	Analogue
pH(1:5 Water) <sup>†</sup>	5.9	9.0	8.6	8.4	8.0	6.7	6.9	7.4	7.4	5.5
Available Nitrogen (ppm)	0.3	11.3	3.6	116.4	81.7	7.8	4.5	132.0	77.6	3.1
Available Phosphorus (ppm)	0.1	0.1	0.1	36.6	43.6	0.1	0.1	91.8	43.7	1.2
Total Organic Matter (%)	5.3	6.1	9.7	12.7	14.8	4.4	6.8	14.9	12.3	6.7
Total Organic Carbon (%)	2.6	3.1	4.9	6.3	7.4	2.2	3.4	7.4	6.1	3.3
Electrical Conductivity (1:5 Water) ( $\mu\text{S}/\text{cm}$ )	161.7	704.7	508.3	1320.0	796.3	270.0	316.3	1386.7	669.0	85.4
Exchangeable Sodium Percentage	5.1	19.5	17.4	19.1	11.3	6.7	6.5	12.8	7.8	1.86
Ca/Mg Ratio	0.95	0.47	0.45	1.44	1.75	0.83	1.09	3.53	2.68	1.58
Bulk Density ( $\text{g}/\text{cm}^3$ )	1.03	1.39	1.16	1.20	1.08	1.14	1.03	0.93	1.02	0.99
Gravimetric Water (%)	4.68	8.36	9.11	7.77	12.03	5.37	9.09	13.53	13.11	10.69



**Figure 3.2: Multi-Dimensional Scaling to illustrate the similarity between treatments (using 21 chemical variables).** Colour coding is in the legend. For each substrate three blocks (1, 2 and 5) are shown. Black line divides OGM treatments from those which don't have OGM; green arrow illustrates progression from spoil to subsoil to forest soil and analogue sites.

## 4. Plant Species

### 4.1 Species List Development

Several sources were used to create a species list that covers both the Central Hunter Grey Box-Ironbark Woodland EEC (CHBIW) and the Central Hunter Ironbark-Spotted Gum-Grey Box Forest EEC (CHISGGBF) for Ravensworth Operations. Lists were available from Ravensworth Operation and Bulga Mine for both vegetation types (per. com. Stephen Cox – Umwelt) reflecting the local composition of these EECs. Lists were downloaded from the OEH web page in 2013 (1: <http://www.environment.nsw.gov.au/determinations/centralhuntergreyboxFD.htm> 2: <http://www.environment.nsw.gov.au/determinations/centralhunterironbarkFD.htm>) and species lists from Peake (2006) covering the Central and Upper Hunter were also used to determine the species composition at a larger scale for these two vegetation types. This procedure was adopted because there is always a large variability between plot based species lists. The use of only one site for a species list may reduce the possible biodiversity that can be achieved over a larger area like a rehabilitation site. Lastly, the species list for the Northern Offset, adjacent to the rehabilitation area, was also included. It contains several vegetation types but is mainly CHBIW (Plate 4.1) and the species contained therein have the possibility to interact with the rehabilitation area and may benefit by the increase in their overall distribution (mainly through cross pollination, reduction of herbivore intensity, etc...). The final list contained 312 species (Appendix 3).

### 4.2 Seeding List Development

To determine what a suitable seeding list would be, the complete species list was categorized using a variety of criteria. Firstly, species which are crucial identifiers of the EECs. For example, *Corymbia maculate* (spotted gum) was identified and highlighted. These species would absolutely need to be incorporated into the seeding mix. Next, the species which appeared in a number of the lists (i.e. widespread), were identified. Species that appeared in several locations in both EECs or consistently in one EEC were also identified and highlighted. Then ecological attributes like flowering period were considered for upper strata to identify which species would achieve a spread of flowering covering the whole year thus providing food for resident and transitory nectar and pollen feeders (in particular the regent honeyeater, the swift parrot and the grey headed flying fox, but also sugar gliders and others). Finally, commercial seed availability was considered. This process produced a final seeding list for the vegetation of 50 species.

### 4.3 Matrix Seeding (Experiment 1)

#### 4.3.1 Seed List

Seeds were obtained through two main providers: Diversity Native Seeds (Geoff Williams) and Royston Petri Pty Ltd. Some additional seeds were contributed by CSER Research from stock collected at Mount Owen by Future Harvest (Greg Major). The seed was divided into 4 groups depending on the amount available, the need for a pre-treatment of seed to promote germination and their usefulness as a study subject:

A = seed not requiring any treatment;

B = herbaceous species not requiring any treatment which are of interest or which have low quantities of seed available;

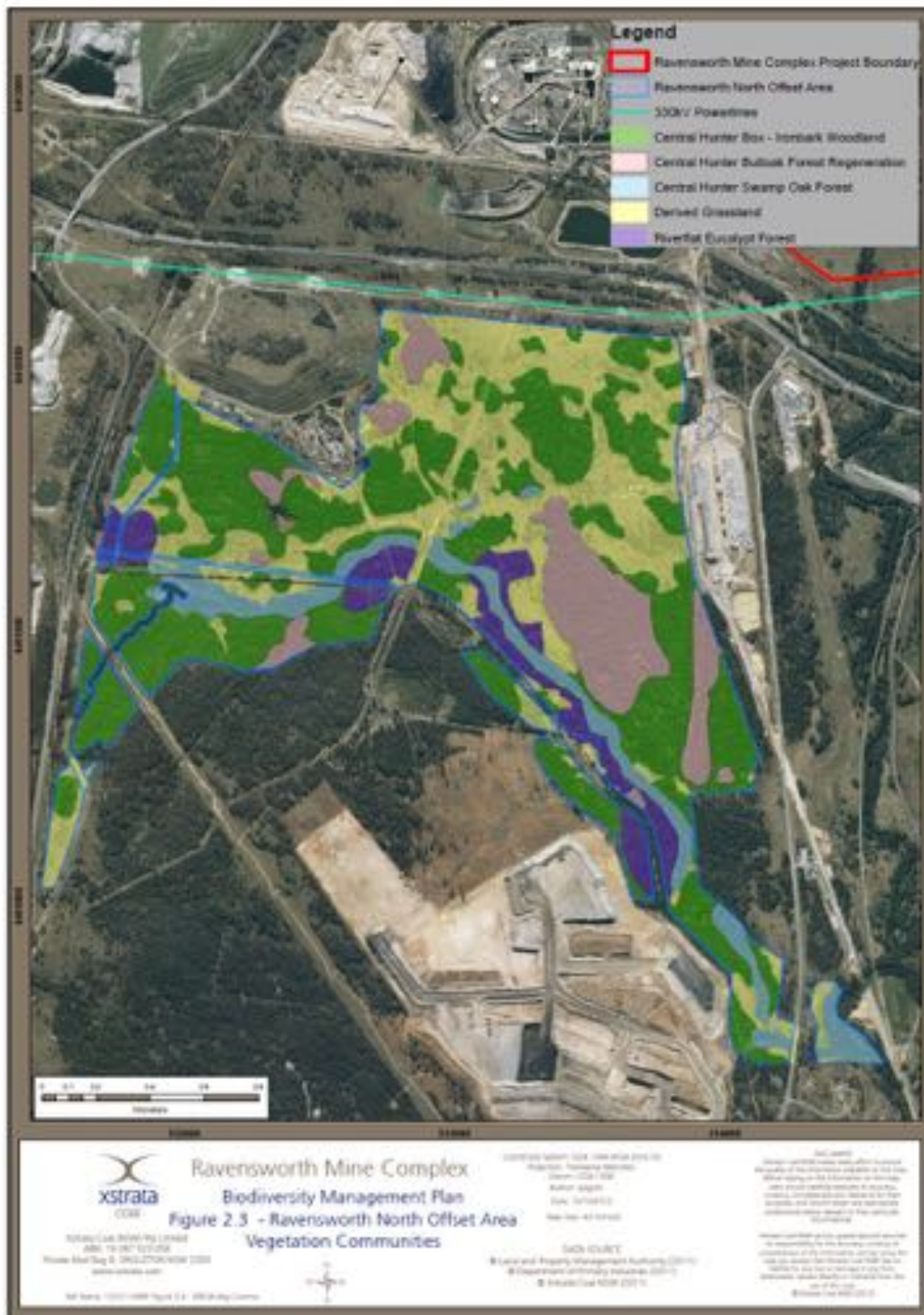
C = seed which need to be heat treated to promote germination;

D = herbaceous species which will be grown from seed in the shade house and planted into the field in autumn.

The overall seeding plan was to create a background matrix containing the main Hunter Ironbark Complex species (Experiment 1: Groups A, B & C = 50 species, Table 4.1), especially



the trees and grasses, and then add the herbaceous study species which are the primary focus of this project at a later date (Experiment 2: Group D, 6 species, see Section 6.).



*Plate 4.1: Vegetation community map of the Ravensworth North Offset area from the Biodiversity Management Plan March 2013 (Umwelt 2013).*

### 4.3.2 Seed Viability

Most of the seed was supplied with seed viability (Table 4.1). Those that weren't, or for which there is no reliable information on the longevity of seed in storage, were tested. For each species three petri dishes were set up with sterile fine sand, sown with a known weight of seed and, where practical, a known number of seed. These were then watered and sealed with parafilm and placed in a germination cabinet at 25°C day temperature for 14 hrs and 15°C night temperature for 10 hrs. Germination was evaluated every week for the first 3 months and every two weeks after that till 6 months. A seed is considered germinated when the radicle emerges 1mm from the seed coat. The method by which the seed providers evaluated the viability of seed is unknown. The method we have used is germinability combined with an evaluation of viability at the end of 6 months using the cut test (cutting the seed and determining whether the endosperm and/or embryo is white and undamaged). Both methods have disadvantages: germination tends to overestimate field germinability due to abundant water availability and takes a long time and appropriate facilities to do; the cut test can be difficult to do in hard or small seeds and requires some knowledge of seed morphology to evaluate correctly. The results on germinability were complemented by a cut test after 6 months to determine how many seeds were still viable and what this would mean for seed bank formation in the field.

### 4.3.3 Seeding Method

As the experimental design in the field contained 6 blocks, the seed was weighed and divided into 6 lots. Each of these lots was further subdivided to allow each experimental plot and each surrounding area to be sown with two seed batches each (Figure 4.1). Plots were subdivided in this way to make the seeding more even across the area. The more a plot is subdivided and sown with a known amount of seed, the more even the seeding and the expected emergence over a plot will be. As the objective of this seeding was to create a background matrix for a Hunter Ironbark Complex Woodland, it was decided that one subdivision of plots was the most cost-effective. With four batches of seed per treatment, 9 treatments per block, 6 blocks in the experiment and an additional two spoil plots, 224 batches of seed were measured out for both Group A and Group C species. Each batch of seed for group A species was added to a 1L jar (Plate 4.2) and had 0.5L of sand added to mix and dilute the seed in preparation for seeding.

Because the species in Group C germinate better after heat treatment, once all species were assembled and placed in 1L jars, 0.25L of just off boiling water was added to each jar and left to soak overnight. The following day the water was removed and 0.5L sand added to each batch and mixed. Seed was sown the following day.

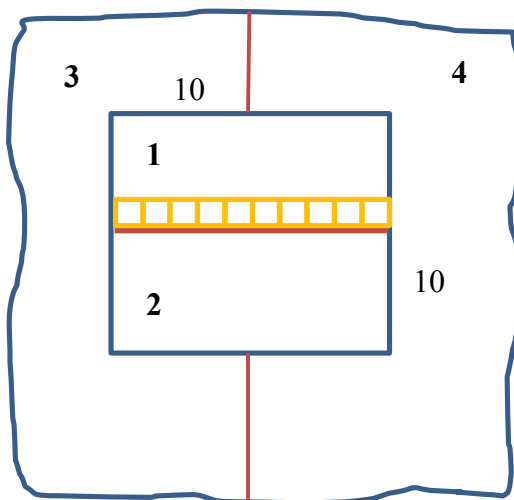
The ten species in group B did not need any treatment but were in insufficient quantities to add to the initial matrix seeding. Many of these species had been generously donated to the research by Diversity Native Seed. These seed were still divided into 6 batches, one for each block, but were then divided into 9 smaller batches and sown only into the experimental plots following a pre-determined seeding pattern to be able to track their emergence in the future. The 10x10m experimental plots were divided in half and 1x1m plots were laid out along this dividing line (Figure 4.1). Each species was given a number which was randomly assigned to each plot and the seed were sown according to the pattern produced. An additional factor was incorporated into the design with Blocks 1,3 and 5 being modified by raking and loosening the soil surface to create a better seed bed, because in trials at Mount Owen mine surface seeding of herbaceous species was not successful, possibly due to soil surface crusting. Raking has been identified in the literature as being a possible treatment for increasing emergence for herbaceous species when seeding into existing vegetation.

**Table 4.1: Final seeding list for the matrix of the Hunter Ironbark Vegetation Complex Experiment October 2013, the origin of seed and viability.** Suppliers were GW: Geoff Williams from Diversity Native Seeds, R: Royston Petri Pty. Ltd., MtO: from stock collected at Mount Owen by Greg Major from Future Harvest. Some seed had viability information (expressed in number viable seed per gram) shown in the column marked Viability, and others were evaluated or re-evaluated over a 6 month period by The University of Newcastle and shown in the column marked Germinability and expressed as number of germinants per gram of seed (see methods). In this last column + indicates that there were still more than 5% viable seed at the termination of the germinability period and <sup>n/e</sup> were not evaluated.

Species	Life form	Amount delivered in grams	Supplier	Viability # V/g	Germinability # G/g
<b>GROUP A - Dry</b>					
<i>Allocasuarina luehmannii</i>	tree	130	GW		105
<i>Angophora floribunda</i>	tree	260	GW	46	
<i>Aristida ramosa</i>	grass	130	GW	115	
<i>Atriplex semibractata</i>	herb	65	GW		122+
<i>Austrostipa scabra</i>	grass	650	GW	201	
<i>Austrostipa verticillata</i>	grass	130	GW		32 <sup>n/e</sup>
<i>Bursaria spinosa</i> var. <i>spinosa</i>	shrub	140	GW	136	312
<i>Callitris endlicheri</i>	tree	390	GW	31	
<i>Cassinia quinquefaria</i>	shrub	25	GW	43	117
<i>Chloris truncata</i>	grass	400	GW	1370	
<i>Corymbia maculata</i>	tree	130	GW	185	
<i>Dichondra repens</i>	herb	140	R	94%	
<i>Dodonaea viscosa</i>	shrub	80	GW		144
<i>Eragrostis brownii</i>	grass	130	GW		1851
<i>Eucalyptus crebra</i>	tree	350	R		229
<i>Eucalyptus fibrosa</i>	tree	470	R		140
<i>Eucalyptus moluccana</i>	tree	520	GW	1100	
<i>Eucalyptus tereticornis</i>	tree	260	GW	516	
<i>Kunzea ambigua</i>	shrub	25	R	6000	14567
<i>Microlaena stipoides</i> var. <i>stipoides</i>	grass	780	GW	104	
<i>Olearia elliptica</i> var. <i>elliptica</i>	shrub	260	GW	134	373
<i>Ozothamnus diosmifolius</i>	shrub	130	GW	190	353
<i>Panicum effusum</i>	grass	560	GW	903	
<i>Rytidosperma fulvum</i>	grass	130	GW		97 <sup>n/e</sup>
<i>Themeda australis</i>	grass	2600	GW	19	
<i>Vittadinia pterochaeta</i>	herb	65	GW	1460	567+
<b>GROUP B - Hot spots</b>					
<i>Cymbopogon refractus</i>	grass	60	GW	96	
<i>Einadia trigonos</i> subsp. <i>leiocarpa</i>	herb	65	GW	706	
<i>Enchylaena tomentosa</i>	herb	13	GW	77	
<i>Eragrostis leptostachya</i>	grass	15	GW		1044
<i>Eremophila debilis</i>	herb	33	GW	12	1
<i>Glycine clandestina</i>	herb	33	GW	129	
<i>Glycine latifolia</i>	herb	33	GW		30+
<i>Glycine tabacina</i>	herb	33	GW		102+
<i>Solanum cinereum</i>	herb	40	GW	190	
<i>Wahlenbergia</i> spp.	herb	35	GW		8536



Species	Life form	Amount delivered in grams	Supplier	Viability # V/g	Germinability # G/g
<b>GROUP C - Heat treated</b>					
<i>Acacia amblygona</i>	shrub	175	GW		99
<i>Acacia decora</i>	shrub	450	GW	72	
<i>Acacia falcata</i>	shrub	370	GW	72	
<i>Acacia implexa</i>	shrub	130	GW	35	
<i>Acacia implexa</i> - Batch 2	shrub	178	Mt O		46
<i>Acacia parvipinnula</i>	shrub	200	R		23
<i>Acacia salicina</i>	small tree	420	R		20
<i>Brachychiton populneus</i>	tree	1700	GW	9	
<i>Daviesia genistifolia</i>	shrub	75	GW		164+
<i>Daviesia ulicifolia</i>	shrub	75	GW		141
<i>Hardenbergia violacea</i>	shrub	25	GW	37	
<i>Hardenbergia violacea</i> - Batch 2	shrub	130	Mt O		29
<i>Indigofera australis</i>	shrub	130	GW	312	
<i>Kennedia rubicunda</i>	shrub	70	R		30+
<i>Pultenaea microphylla</i>	shrub	65	GW	426	
<i>Senna artemisioides</i> subsp. <i>zygophylla</i>	shrub	30	GW		14+



**Figure 4.1: Treatment area zones.** The large irregular blue outline delineates a treatment. The 10x10m blue square delineates the experimental plot. Matrix seeding was conducted over both the experimental plot area (1 & 2) and the surrounding treatment area (3 & 4). Both areas were divided into two as shown by the red lines (horizontal in the experimental plot and vertical in the surrounding area), and each half was seeded with a batch of seed from Groups A and C. By seeding in this manner a more even seed spread was obtained than if just one batch were spread over the whole area. Yellow squares are areas where additional hot spot species (Group B) were individually sown. Areas 1 & 2 were monitored; areas 3 & 4 serve as a buffer zone.



**Plate 4.2: Photos of seed preparation.** A) Distributing seed lots into jars. B) Hot water treated seed (group C). C) Jars containing seed mixed with sand. Some *Vittadinia* sp. seed can be seen on top of the sand prior to mixing. D) Spreading "Hot Spot" seed (Group B) in the field on a raked plot.



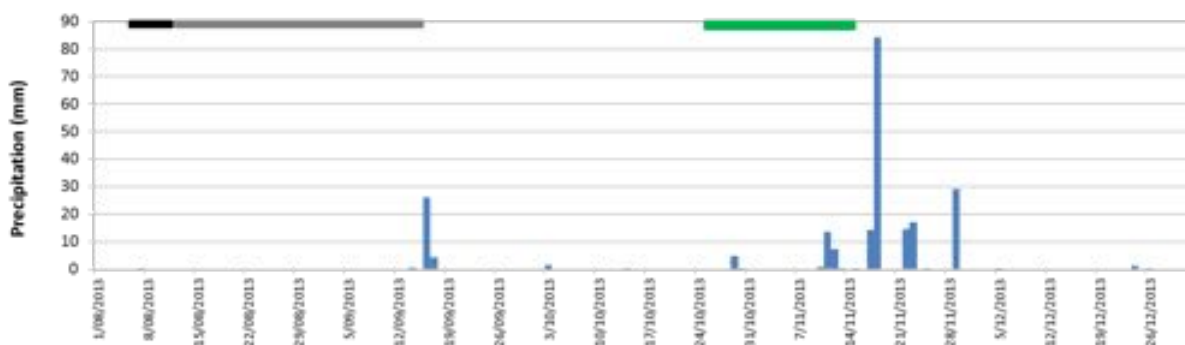
#### 4.4 Rainfall

Rainfall in this area is characterised by storm events with high rainfall which can occur in any month, interspersed with dry periods. Overall the main weather pattern predicts high rainfall in the summer months with lowest rainfall in winter and early spring (Table 4.2). Rainfall recorded at Singleton was 772, 650.3 and 899.3 mm per year respectively for 2013, 2014 and 2015.

**Table 4.2: Average rainfall at Singleton STP meteorological station.** This station (number 61397 Bureau of Meteorology) has only been in operation for 14 years.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Average	68.4	91.9	59.5	62.6	29.6	69.0	27.1	32.0	37.8	42.6	83.9	73.9	671.3

Daily rainfall from the Ravensworth Open Cut weather station, located near the Narama West Mine approximately 4.5km to the South, has been aligned with major setting up events of the experiments described in this report. Figure 4.2 shows rainfall (precipitation in mm) between the 1<sup>st</sup> of August 2013 and the 31<sup>st</sup> of December 2013. Overall, rainfall was below average for this period. The timing of earth works for the experimental rehabilitation site is shown by the black bar at the top of the graph in the second week of August. Sporadic interventions at a minor scale, like redistribution of mulch and setting up of experimental plot limits, were conducted in the period following that until mid-September and is shown by the grey bar. Seeding of the different batches was done between the 25<sup>th</sup> of October and the 14<sup>th</sup> of November and is indicated by the green bar on the graph. Timing of seeding was very opportune as a larger rain event happened about this time. On the 18<sup>th</sup> November 84mm fell which, in addition to giving the system a good water input, also caused erosion in and around the experimental site. During January 2015 many seedlings were observed, especially Eucalypts demonstrating that rain had not severely damaged the site. Monitoring of the site was done at the end of two years and the results are presented below (see: Section 5).



**Figure 4.2: Precipitation in mm per day between the 1<sup>st</sup> of August 2013 and the 31<sup>st</sup> of December 2013.** The black and grey horizontal bars represent the time over which the site earthworks and the set-up of the experimental area occurred. The green bar represents the seeding period. Data from the Narama West mine weather station.

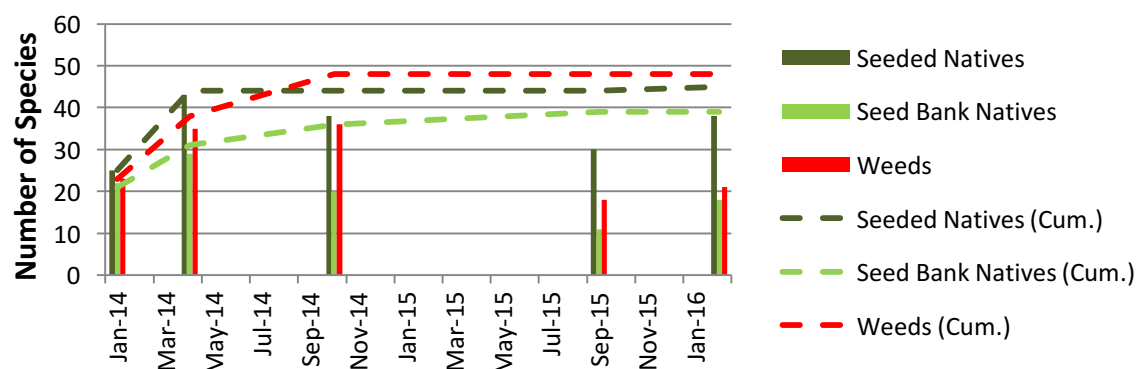
## 5. Results and Development of Vegetation (Experiment 1)

### 5.1 Species Richness

#### 5.1.1 Species richness development over time

On five dates in January, April and October 2014, September 2015 as well in February 2016 a general species list was compiled to gauge the evolution of the site in terms of emergence of sown species, native species contributed by seed bank from the forest topsoil and the subsoil and the invasion or contamination by native and weed species (Plate 5.1 and 5.2). Species lists were compiled based on observations (visual inspection) across the whole site (Appendix 2) and were meant to give a quick evaluation of progress and problems.

Early in the site development, seeded species, other native species mainly emerging from the seed bank and weedy exotic species were observed (Figure 5.1). All three categories emerged rapidly and number of species visible at one time peaked at 103 in April 2014. By the end of this reporting period, February 2016, 132 species had been observed on site at one time or another, but only 77 were still visible in the ecosystem, and the increase in species for all three categories had plateaued (Figure 5.1, cumulative curves). This means that without further input by seeding or planting, natural colonisation or contamination by weeds, the possible total number of species at this site is stagnant at 132. Most of the decline seen in standing vegetation (species visible at any one time) was due to some exotics disappearing from the above ground vegetation. More detailed analysis of the seeded natives will be done in the final plot survey below (see: 5.1.2).



**Figure 5.1** Bar graph illustrating number of species observed at survey time points and the cumulative curves for species number over time on the experimental site. Species were analysed in terms of being seeded, natives emerging from the seed bank, or exotic weeds.



**Plate 5.1:** Native plants on the experimental site in January 2013, 3 months after seeding. A) shows a spotted gum (*Corymbia maculata*) and some windmill grass (*Chloris truncata*). The stylus, for scale, is the length of a normal pen. B) shows a collection of different acacias which have come up in this little depression. C) shows some *Sida corrugata*, a species which has come out of the subsoil seed bank.



**Plate 5.2 Native plants on the experimental site. In Aug 2015:** A) *Eucalyptus*, *Acacia falcata*, *Acacia decora* (flowering), *Dodonea viscosa*. B) *Hardenbergia violacea* and *Acacia amblygona* flowering. C) *Indigofera australis* with *Eucalyptus* and *Acacia falcata* in the background.

Most native species had emerged by April 2014, 6 months after seeding. Forty-four of the 50 seeded species were observed by April 2014. But some of the seeded native species were not easy to detect in a visual inspection, for example, *Austrostipa scabra* and *Eragrostis leptostachya* were not detected during the last two surveys and *Callitris endlicheri* was not seen until the full survey was conducted. Seeded species will be analysed further in the following section (Section 5.1.2.) which will report on diversity and cover of experimental plots. This data is shown to illustrate the usefulness and limitations of rapid assessments.

Seed bank species on the other hand continued to appear until September 2015, although at a decreasing rate. Many of the seed bank species only emerged in small numbers and self-sustaining populations did not form. This does not make the subsoil an undesirable substrate from a seed contribution point of view. Many of the seeded species may have had contributions from individuals from the seed bank. The only species for which this is undoubtedly clear is *Acacia amblygona*. In forest topsoil, large numbers of this species grew and cannot be attributed solely to seeding. Their importance resides in their contribution to the gene pool of this species. Collected seed can have a variety of origins and sometimes locally less suitable genotypes may be introduced. By retaining the potential for contribution of local genotypes through the soil seed bank, these are not lost and may be important for climate adaptation and the future evolution of the species.

Weeds and exotic species were observed on the site from the very start (Plate 5.3). The most notable was Japanese millet which grew profusely in OGM treated plots in January 2014. This was not deliberately sown and its origin is unknown as the OGM supplier doesn't use cover crops and the spreader on site had not been in contact with the seed. The other larger infestation was of *Galenia pubescens* in plots containing mulch in blocks 1, 3, 4 and 6. This seed must have been in the mulched vegetation and illustrates the care that must be taken when sourcing products for rehabilitation sites. These plants were initially manually removed by CSER to avoid losing the experimental value of the site, but continued in the system. Other noxious weeds that were found are *Opuntia stricta*, *Carthamus lanatus* (saffron thistle) and one plant each of *Ricinus communis* (castor oil plant) and *Xanthium spinosum* (Bathurst burr). The last three were manually removed and taken off site. See Appendix 3 for full list of species.

As time progressed it became apparent that *Chloris gayana* (Rhodes grass) and *Pennisetum clandestinum* (Kikuyu) were also present on site (Plate 5.4). These species have the potential to exclude other species, including small trees if left uncontrolled. It was initially planned to apply herbicide to these two species to allow the site to be evaluated in terms of best possible conditions for native ecosystem development but weather conditions (wind) and seasonal factors (arrested growth in winter) impeded the application of herbicide. The native grass

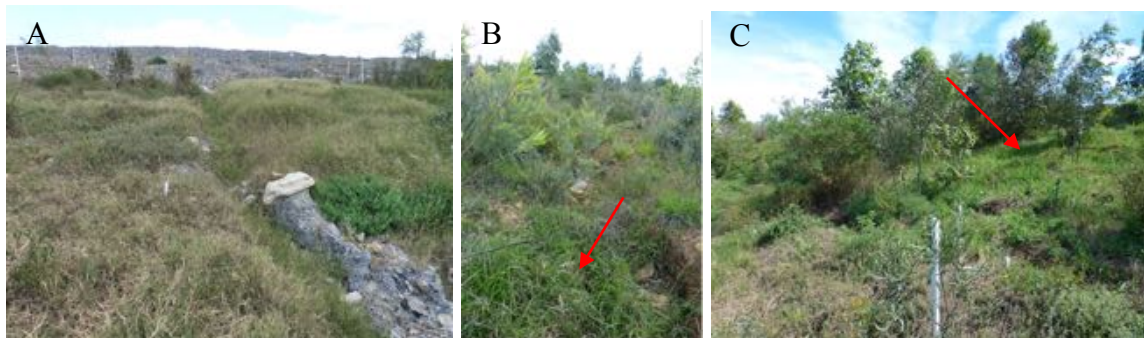


*Cynodon dactylon* (Couch) is also cause for concern, though to a lesser degree. It has been observed on other mine sites that Couch can cover large areas and produce reduced native species diversity within the area. Couch, being native and a good soil stabiliser due to its creeping habit, is a desired plant and many mine rehabilitation sites deliberately include it in seeding mixes. Nevertheless, when the rehabilitation target is biodiversity and sustainable native plant populations, it should be avoided as much as possible.

Other weeds are usually not a problem in the long term as these have been observed to diminish considerably after 3 years on other rehabilitation sites. By February 2016 only 21 exotic species were frequently observed on the site. Some additional exotic grasses (*Setaria gracilis* in particular) will have to be observed over time and an application of herbicide planned if they become too aggressive.



**Plate 5.3: Exotic plants on the experimental area January 2013 & April 2013.** A) Japanese millet (Jan 2013), disappeared naturally from the site. B) *Galenia pubescens* seedling. C) Hand weeding for *Galenia* (April 2013). This species remains a problem on the site. D) *Ricinus communis* plant was removed (Jan 2013) and no other plants have been observed since.



**Plate 5.4: Exotic plants on the experimental area 2015.** A) Couch (*Cynodon dactylon*) rapidly turns into a monoculture with a capacity to remain in place for many years as the plant is clonal. The same applies to B) Rhodes grass and C) Kikuyu (*Chloris gayana* and *Pennestum clandestinum*).

### 5.1.2 Species richness on substrate treatment plots

In November 2015, a full survey of the experimental plots was conducted. Data on species richness, abundance, cover, reproduction, and tree heights was collected. Overall 48 of the 50 seeded species were found, 69 additional species including the 6 planted species (Group D see Section 4.3.1.) and 55 exotic weed species (Table 5.1)

The treatment that had best seeded species success was subsoil amended with mulch. Overall it was also the treatment with the most varied species composition, totalling 98 species across 6 blocks. One 10x10m plot had 81 species on it alone. The contribution by species germinating from a soil seed bank was quite large but some of these species won't form long term viable populations because numbers of individuals are too low. Adding OGM to subsoil decreased

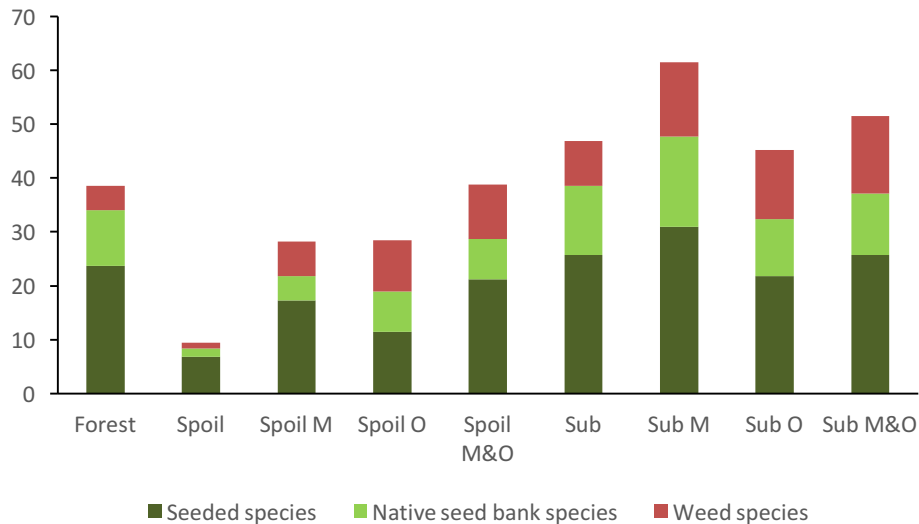


species diversity. Overall subsoil treatments performed better than treatments based on spoil, but on spoil OGM allowed more species to establish than without amendment, but even just adding mulch to spoil increased diversity. Forest topsoil overall performed only slightly less well than subsoil by itself or with mulch. In terms of attaining biodiversity goals, using either subsoil with mulch, subsoil alone or forest topsoil still remains the best option. Adding OGM to subsoil treatments gives no extra benefit in terms of diversity, but as we shall discuss further down, may impact on site vegetation cover. In contrast, when spoil is used adding any source of organics (be it mulch or OGM) increases diversity of species established. Some seed bank species are also observed in the spoil treatments. Rather than being from an actual seed bank these are species that have either dispersed into the plot over time, or have been introduced as contaminants in the seeded species: for example grass seed frequently had a mix of species in it some of which were not in the seeded list.

In terms of weed species, the percentage values in Table 5.1 show that overall 30% of the species diversity is exotic, a value that is consistent over most treatments (23-36%) despite some statistical differences having been detected when considering the average of weed species presence. The relatively uniform distribution of weeds testifies to their good dispersal capacities (invasiveness) and to the difficulty of controlling weeds. This is an undesirable situation but may be transitory as other studies have found that exotic weeds may diminish after the initial flush of weeds at site set-up. This survey was conducted at 2 years from set-up and tendencies shown in the previous section (Figure 5.1) are already showing a reduction in species visible in the standing vegetation. It remains to be seen whether weed diversity continues to diminish as time progresses. Nevertheless, some of the species identified in the previous section could potentially jeopardize the development of an EEC-like vegetation on the rehabilitation site. Invasive, clonal grasses like Rhodes grass and Kikuyu (and also the native Couch) can cover large areas effectively excluding other species mainly by shading out any seedlings and smothering small plants. Weed control needs to be conducted if the values for exotic species usually accepted for analogue sites is to be attained.

**Table 5.1. Totals and average number of seeded, seed bank and weed species for each treatment combining all replicates. Letters in the average columns refer to statistical difference at  $p < 0.05$  and are specific to the column. Letters that are different indicate treatments that differ significantly from each other. The percentage value of Weed species is also given the week total species column in brackets. A visual representation is shown in Figure 5.2.  $n=6$ .**

Treatment	Seeded species		Native seed bank species		Weed species		Total
	Total	Average	Total	Average	Total and (%)	Average	
Forest	36	23.6 <sup>bc</sup>	31	10.3 <sup>bc</sup>	11 (14)	4.5 <sup>ef</sup>	78
Spoil	14	6.8 <sup>f</sup>	5	1.5 <sup>e</sup>	6 (24)	1.2 <sup>f</sup>	25
Spoil Mulch	28	17.3 <sup>d</sup>	13	4.5 <sup>de</sup>	16 (28)	6.3 <sup>cde</sup>	57
Spoil OGM	23	11.5 <sup>e</sup>	15	7.5 <sup>cd</sup>	21 (35)	9.5 <sup>abcd</sup>	59
Spoil Mulch & OGM	34	21.2 <sup>c</sup>	20	7.5 <sup>cd</sup>	24 (30)	10.2 <sup>abcd</sup>	78
Subsoil	39	25.6 <sup>b</sup>	37	12.8 <sup>ab</sup>	26 (27)	8.3 <sup>bcde</sup>	102
Subsoil Mulch	44	31 <sup>a</sup>	49	16.6 <sup>a</sup>	33 (23)	13.8 <sup>a</sup>	126
Subsoil OGM	34	21.8 <sup>c</sup>	28	10.5 <sup>bc</sup>	30 (32)	12.8 <sup>ab</sup>	92
Subsoil Mulch & OGM	37	25.6 <sup>b</sup>	26	11.5 <sup>bc</sup>	36 (36)	14.8 <sup>a</sup>	99
All treatments	48		69		55 (31)		172



*Figure 5.2. Average number of species for each treatment divided into seeded species, native seed bank species and weed species. Statistical differences are shown in Table 5.1.*

### 5.1.3 Species richness compared with EEC lists

As the goal of Experiment 1 was to establish an EEC complex that is recognisable as such, we compared the results from this rehabilitation site with the EEC lists produced by OEH-NSW and known species lists of the area. We used Multi-Dimensional Scaling (Primer 6.0) to analyse the treatments for similarity with the reference lists and found that overall, the treatments clustered away from the reference lists (Figure 5.3). This at first appears to be a very poor result as the aim of the project is to produce endangered ecological communities, however, rather than being a negative result it highlights the dangers of using restrictive lists of recommended species that may not be area appropriate and do not consider the degraded and modified state of most vegetation in the Hunter Valley. Typically, EEC lists contain only the most characteristic species and are taken out of context of the local supporting vegetation from which they are created. It is a common failing to think that these lists are sufficient to establish self-sustaining ecosystems. This is partially demonstrated by the spoil treatment in Figure 5.3 being closest to the EEC lists. As very low external inputs happened for this substrate (only seeded species, very little invasion and no seed bank) it most closely reflects the seeded list which was built on the EEC lists. This, in contrast to the subsoil treatments and the Master and Northern off-set list, (which are all vastly different from each other) reflects a more realistic local species diversity.

The phenomenon of finding hidden species in the seed bank versus the visible standing vegetation is common where ecosystem dynamics have been disrupted. What regulates the re-appearance of these species is disturbance and fire dynamics. Whilst fire is not recommended in these depauperate weed invaded and young vegetation areas, disturbance at small scales can be encouraged by building the ecosystems to include ground disturbing native animals like bandicoots and diverse ant populations. Small scale disturbances of this type can promote species re-emergence at a sustainable level as well as promoting de-compaction with all its follow-on benefits for soil health.

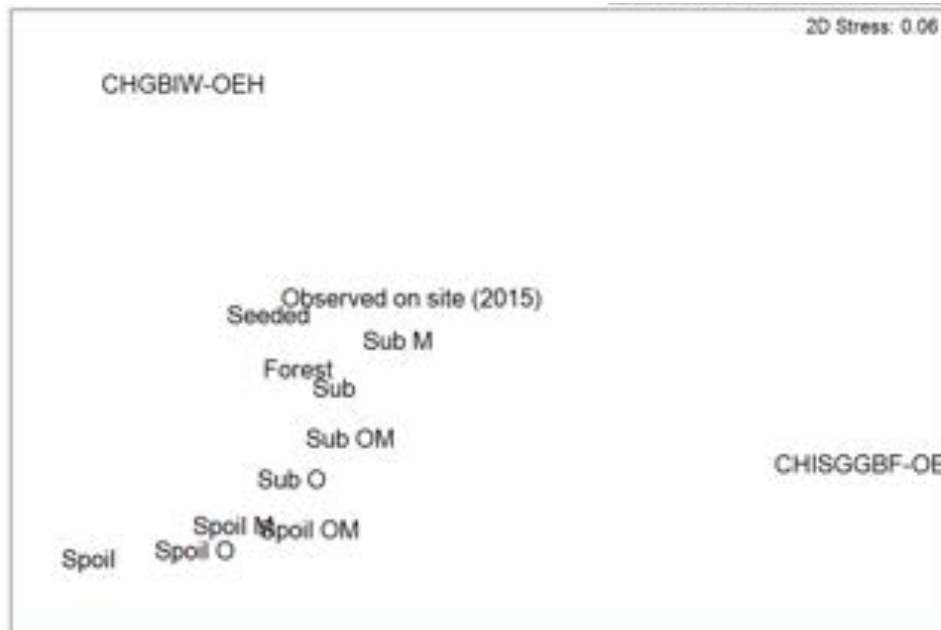


**Figure 5.3. Multi-Dimensional Scaling of the treatments and reference lists** (Table 5.2) based on the native plant species present. This model was produced using presence/absence analysis as the reference lists do not give information on how common a species may be. (D1 Euclidean Distance was used to generate the distribution).

**Table 5.2. Number of species for reference lists used in Figure 5.3** Refer to Table 5.1 for number of species per treatment.

Treatment / Reference	Description	Number of Species
Seeded	Species used to seed the experimental site	50
All Treatments	Combined species list from plot surveys	120
Master List*	List extracted from original Master List, see Appendix B, to only include EEC species, species from the North Off-set and seeded species.	180
North Offset	Species list for the North offset on Ravensworth Operations	133
CH GB I W	Central Hunter Grey Box Ironbark Woodland EEC list	37
CH I SG GB F	Central Hunter Ironbark Sotted Gum Grey Box Forest EEC list	42
CEC	Central Hunter Valley Eucalypt Forest and Woodland Critically Endangered Community list	77

If the analysis is repeated excluding all species that are not on the two EEC lists the results reflect seed availability and establishment success. In Figure 5.4 we can see that the treatments have affected which species are able to establish in which substrates that maximise the desired outcome can be identified. Subsoils in general without major amendments (like OGM) and forest topsoil are still the best for diversity despite recognised deficiencies. The reason so many of the desired species are present on these substrates could have a lot to do with the appropriate microbial remnants being available in the soils that help sustainable nutrient acquisition and cycling. Whilst OGM is marketed as having microbes beneficial for plant growth, these are not necessarily the most appropriate ones as certain levels of specificity have been demonstrated between plant species and their symbionts (for example Fisher, 2010).



**Figure 5.4.** Multi-Dimensional Scaling of the treatments and references based only on the EEC species. Observed on site = combined species list from plot surveys.

By examining which plants were most common on each treatment we can determine which species are most suitable for that substrate. By choosing the correct plants when seeding areas, costs can be saved. When deciding which species successfully made it into the community we needed to define successful and this is discussed further down (Section 5.5.).

## 5.2 Cover Assessment

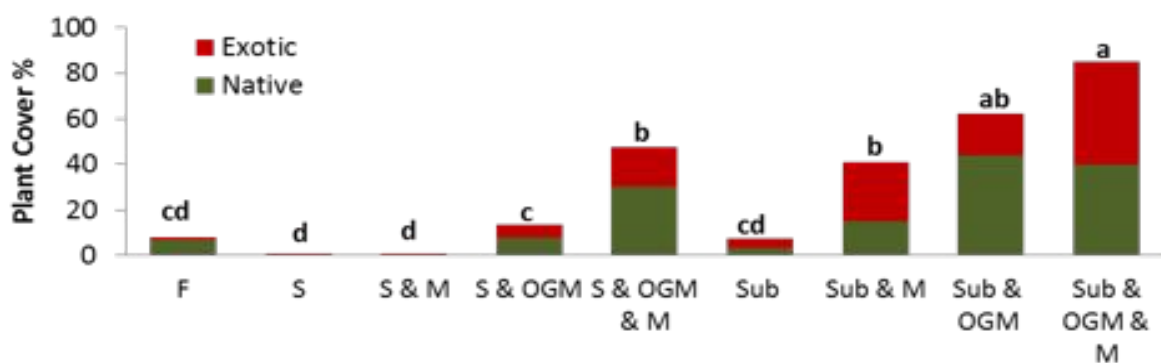
### 5.2.1 Cover assessment at 6 months

In April 2014, at 6 months from seeding, we assessed the whole site for plant cover. A visual assessment by 2 people was made to determine within each 10x10m plot the cover of native plant species and the cover of exotic species. Invasive exotic grass presence was also noted. In general, OGM plots and some mulch plots had more cover (Plate 5.5; Figure 5.5). Unfortunately, large proportions of this was contributed by exotic weeds, especially from the weed contaminated mulch. Nevertheless, some native species like *Einadia nutans* var. *linearis* and *Atriplex semibractata* revealed themselves as excellent cover plants for early rehabilitation. How much they interfere with the establishment of canopy and shrubs needs to be determined. Another very successful plant was *Chloris truncata*. This species proliferated on subsoil treatment plots. The forest topsoil treatment is ecologically best as it had few weeds, a regular spread of native species and additional species from the forest seed bank. These seemed mainly to be *Acacia amblygona*, but other species like *Zornia dictyocarpa*, *Tempeltonia stenophylla* and *Sida corrugata* were present on forest plots and also in subsoil plots (see section 5.1.1).





**Plate 5.5** Aerial of experimental site taken in June 2014. Refer to Figure 3.1 for position of treatments. The orange coloured subsoil is visible on this aerial, and the OGM treated areas have the highest plant cover as does the mulch treatment on blocks 1 and 4 (red arrow). Treatments are not perfectly contained within their theoretical outlines and contamination of some plots is evident.



**Figure 5.5:** Plant cover for exotic and native species on the experimental site treatments at 6 months from seeding. F = forest topsoil; S = spoil; M = mulch; OGM = organic growth medium; Sub = subsoil. Letters show significant differences between treatments for overall cover (n=6).

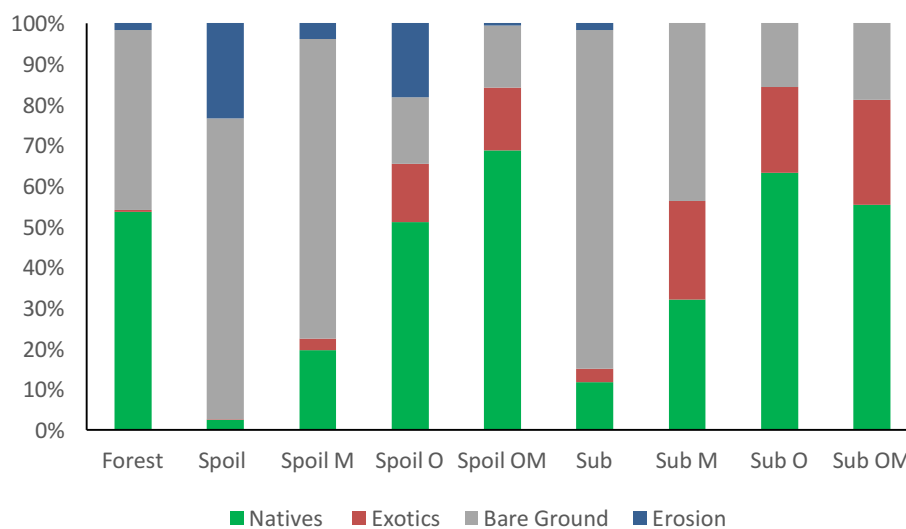


**Plate 5.6** Photos of some experimental substrates at 6 months from seeding. A) Forest topsoil with native plants, B) Spoil and Mulch, a spoil with no additions can be seen in the background, C) Subsoil with OGM and Mulch with a combination of weeds and native plants growing profusely.

### 5.2.2. Cover at final survey – 2 years

During the full survey at 2 years from seeding, cover of species and other ecosystem characteristics like presence of bare ground and extent of erosion were evaluated.

Subsoil treatments were not significantly different spoil treatments for native or exotic cover, but were significantly lower for erosion. Mulch also didn't significantly affect cover of plants, but seemed to significantly reduce erosion. OGM significantly increased native and exotic cover and therefore reducing bare ground. OGM was not a significant factor in the existence of erosion. This data indicates that as seen on Plate 5.7 and Plate 5.8, best cover is achieved when using OGM, but as forest topsoil and subsoil have no severe erosion issues, cover could be sacrificed in favour of having less exotic species. The presence of erosion must also be considered from a landscape perspective as very subtle changes in slope can have a great effect on where erosion occurs and will be independent of the substrate.



**Figure 5.6: Percentage of Average Native, Exotic, Bare Ground and Erosion Cover at 2 years on different treatments.** See Table 5.3 for non-adjusted values and significant differences between treatments.

**Table 5.3: Average Native, Exotic, Bare Ground and Erosion Cover at 2 years on different treatments.** Letters indicate significant difference at  $p > 0.005$ . Note: for average cover of exotics no significant difference found despite large difference in values. This is due to very high variance. In other words, the plots were not consistently high or low for cover values on a given substrate. For example, on Subsoil mulch, cover values for exotics were approximately 1, 2, 10, 12, 50, 74 for the  $n=6$  plots.

Treatment	Average Cover of Natives	Average Cover of Exotics	Average Bare Ground	Average Erosion
Forest	52ab	0.4a	43b	2ab
Spoil	3c	0.1a	96a	31a
Spoil Mulch	20c	3a	76a	4ab
Spoil OGM	58a	16a	19bc	21ab
Spoil Mulch & OGM	64a	14a	14c	0.5b
Subsoil	11c	3a	82a	2ab
Subsoil Mulch	28bc	21a	38bc	0b
Subsoil OGM	60a	20a	15c	0b
Subsoil Mulch & OGM	51ab	24a	18bc	0b



**Plate 5.7 Areal of experimental site taken in late 2015.** Refer to Figure 3.1 for position of treatments. The orange coloured subsoil is visible on this aerial, and the OGM treated areas have the highest plant cover and mulch and forest treatments are also high in cover. Treatments are not perfectly contained within their theoretical outlines and contamination of some plots is evident as are some erosion channels.

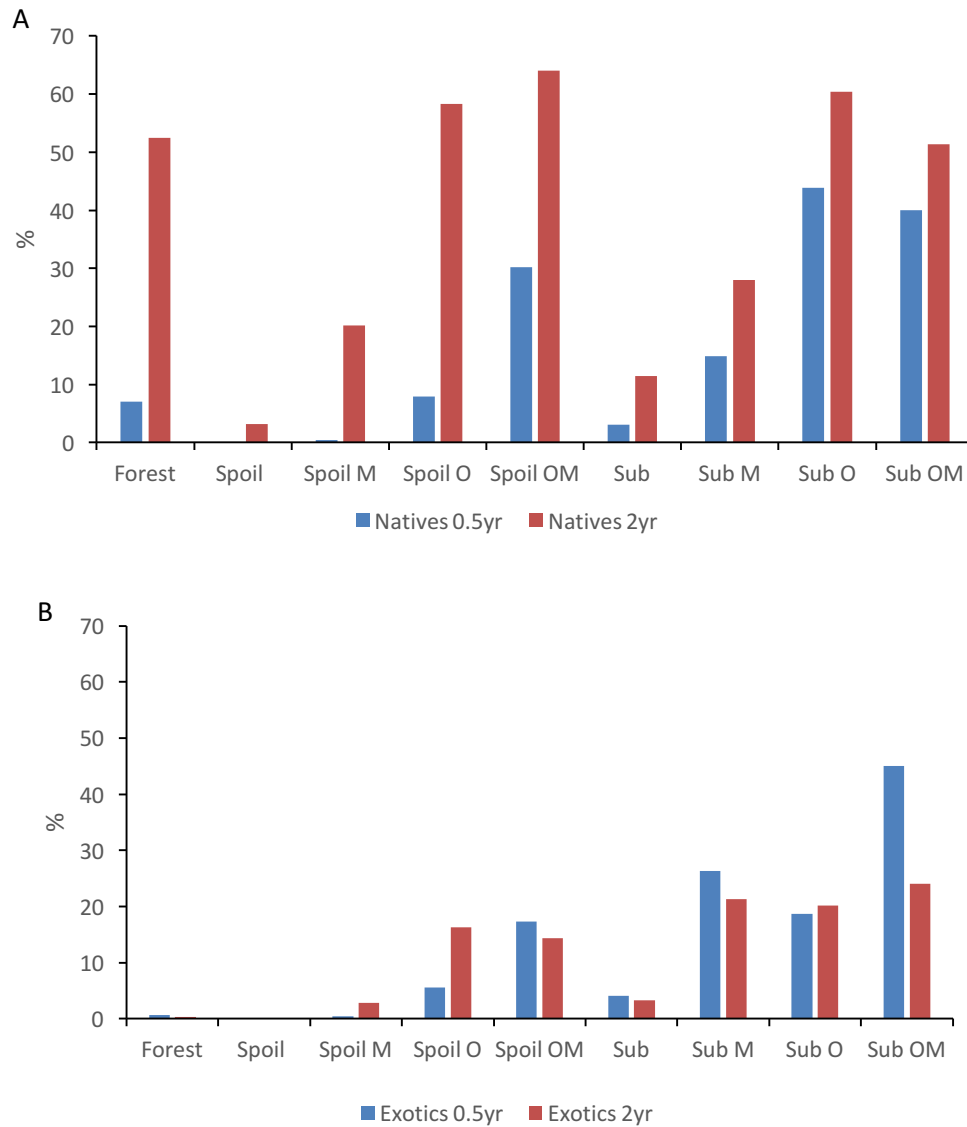


**Plate 5.8: Photos of some experimental substrates at 2 years from seeding.** A) Forest topsoil with native plants, B) Spoil and Mulch with chenopod ground covers and some erosion. A spoil plot with no additions can be seen in the background, C) Subsoil with OGM and Mulch, strong eucalypt growth is seen and acacias in the foreground.

### 5.2.3. Comparison between 6 month and 2 year cover data

A comparison between the two survey periods reveals some interesting trends. Native cover (Figure 5.7a) has greatly increased between the two surveys especially in OGM treated plots. The species mainly responsible for this were the chenopods *Einadia nutans* var. *linifolia*, *E. nutans* var. *nutans*, *E. hastata*, *Enchylaena tomentosa*, *Atriplex semibracata* and the grass *Cynodon dactylon* (Couch). Many chenopods (and Couch) are salt tolerant and intolerant of mycorrhizal infections. Whilst we cannot confirm this, it is likely that the combination of the two factors has promoted the development of these species. This phenomenon is worthy of further research as a method may be developed where substrates lacking in organic content and high in salt could be ameliorated with OGM and chenopods to initiate soil building and soil stabilisation. Research into the successional development after the initial phase observed on this site (2 years) would determine what other interventions are necessary to build a native ecosystem. This topic is particularly interesting as the chenopods could be nearing the end of their life cycles and we don't know what will replace them. Cover on forest topsoil was due to different species, the main one being *Acacia amblygona*, large amounts of which were contributed to by seed bank sources.



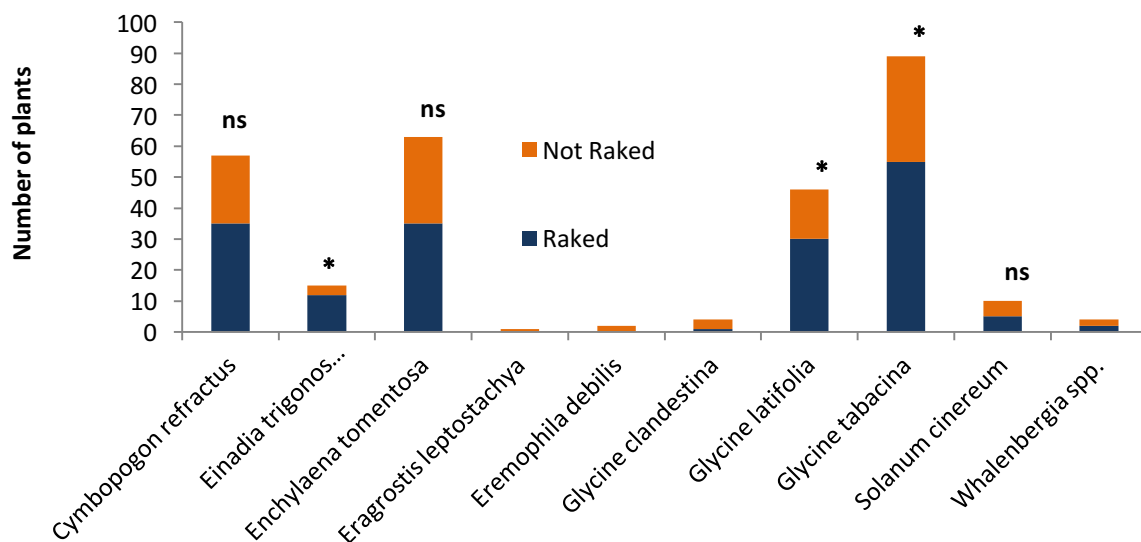


**Figure 5.7: Percentage of Native and Exotic Cover on different treatments at 0.5 and 2 years.**

Exotic cover (Figure 5.7b), on the other hand, mainly trended towards diminishing cover but only by small amounts. The increasing contribution to cover by aggressive invasive grasses like Rhodes and Kikuyu are slowing down a potentially larger trend mirrored by species diversity reduction (Figure 5.1). Some plots, spoil mulch and spoil OGM are still increasing in exotic diversity. This could have two explanations: one is that cover is still increasing because in these locations cover has evolved at a much slower pace, the other is that native species cover is different and not potentially inhibiting further extension of exotic cover. These plots contain exotic species like *Galenia pubescence* and Rhodes grass. Both potentially can grow well on spoil. These plots also have very few trees, so shading and root competition is not occurring. Both these observations could be indicating that plots like these could transform into weedy areas rather than the desired EEC.

### 5.3. Effect of decompacting seed bed on seedling emergence

The species sown for the “hot spot” trial were assessed in April 2014, six months after seeding. Because mine rehabilitated soil often crusts, the blocks were divided into two, and half of the seeding areas were hand raked to loosen the crust. The other half had seed sown directly onto the surface. In Figure 4.1 the seeding areas in each plot are shown. Of the 10 species used only 6 had 10 or more seedlings emerge and/or establish (Figure 5.8). A chi square goodness of fit was used to evaluate the difference between treatments for those species where enough data was available. Of these *Einadia trigonos*, *Glycine latifolia* and *Glycine tabacina* were significantly affected by the raking, showing more emergence in these plots than in their non-raked counterparts. *Cymbopogon refractus*, *Enchylaena tomentosa* and *Solanum cinereum* showed the same tendency but were not significantly different. This analysis, although not optimal as the numbers that emerged were still quite low and no attempt to differentiate the treatments has been made, shows that the surface texture is crucial to the success of a seeding program. Another aspect that was noted during the setup of the trial was that the addition of OGM, and even mulch, had drastic effects on the crusting of the surface, reducing it notably and making hand raking much easier. This has not been evaluated formally at this time but we are planning to investigate this more in the future.

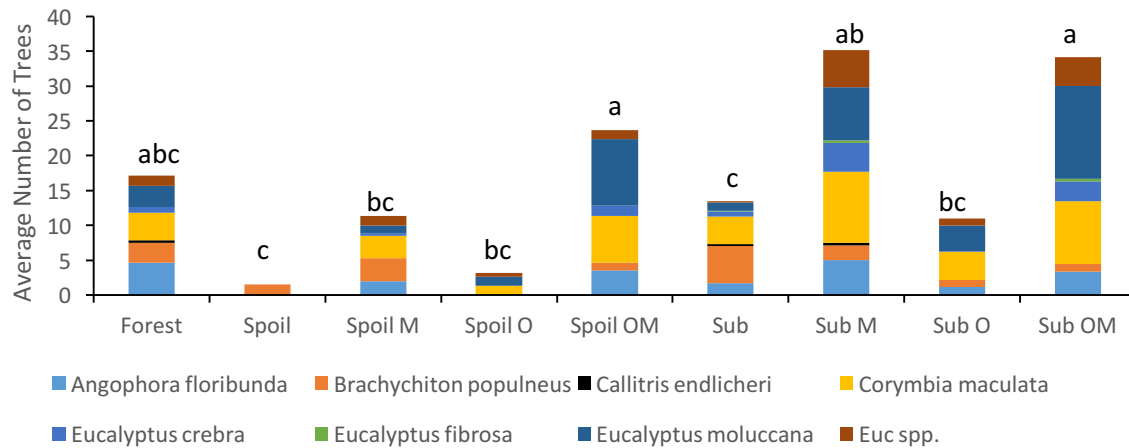


**Figure 5.8:** Number of plants of each of 10 hot spot species emerged at 6 months from sowing onto substrates that had been hand raked to loosen the soil crust or not. Six species were evaluated using a Chi square goodness of fit. Of these, \* indicates a significant effect at  $p < 0.01$  and “ns” indicates those where no significant statistical difference was detected.

### 5.4. Canopy establishment and height

The establishment of trees is important as an indication of the ability to produce a viable canopy for ecosystem development as well as for aesthetic reasons as the general public typically associate restoration success solely by the success of canopy species. In addition to counting the overall number of trees on each treatment at 2 years from seeding we also measured the heights of trees that were greater than 1.5m as an indication of growth potential. Examining the average number of trees shows a very clear indication that the use of mulch in combination with subsoil is very beneficial in supporting the germination and continued survival of the tree species (Figure 5.9). Even Spoil, remediated with both OGM and mulch attains has a reasonable number of trees, even more so than the forest soil treatment.





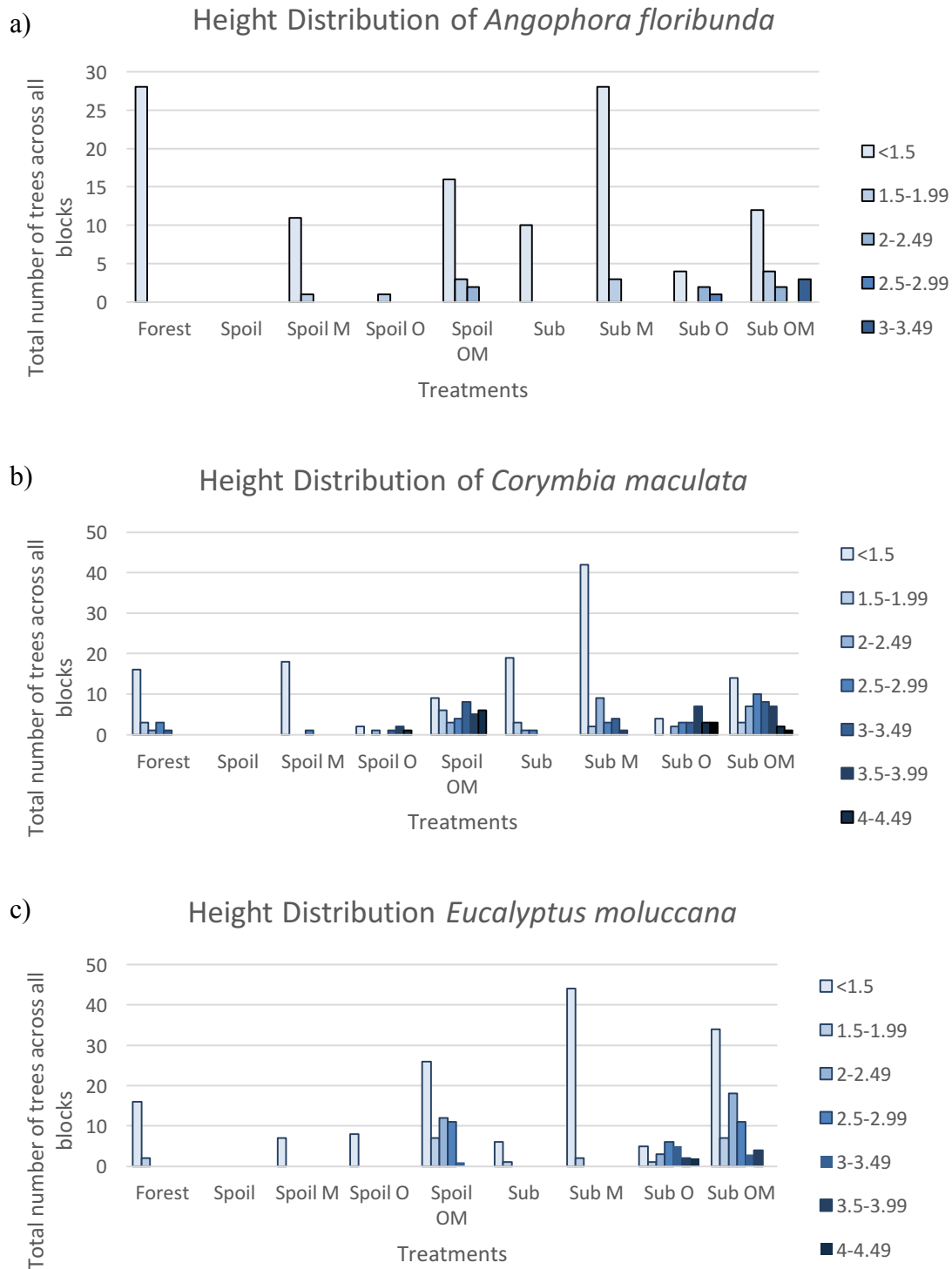
**Figure 5.9.** Average number of trees larger than 1.5m in each treatment at 2 years from seeding for all species across the six blocks. Statistical analysis using Tukey Kramer post hoc tests was used to show statistical difference between treatments. Treatments with the same letter are statistically not different while treatments that do not share a letter are different. Some Eucalyptus individuals were not identified and are included here as “Euc spp.”.

The heights achieved by the trees is an indication of how much readily available nutrients are found in each treatment. Statistical analysis showed that OGM produced a significant increase in tree heights for three species (*B. populneus*  $p = <0.0001$ , *C. maculata*  $p = 0.0021$ , *E. moluccana*  $p = 0.0035$ ). This is an interesting result as it suggests that the mulch promotes survival while OGM promotes rapid growth. Whether the rapid growth will have deleterious effects as the stands mature will need to be assessed. Species specific effects can be seen for the three most abundant species on the rehabilitation area (Figure 5.10). *Angophora floribunda* does best on forest soil and subsoil with mulch, but subsoil with OGM and mulch has larger trees. *Corymbia maculata* grows highest on the sites remediated with OGM but is also abundant on mulch when combined with subsoil. *Eucalyptus moluccana* benefits most from OGM for growth in combination with mulch. Overall all three species perform well on subsoil mixed with OGM and mulch.

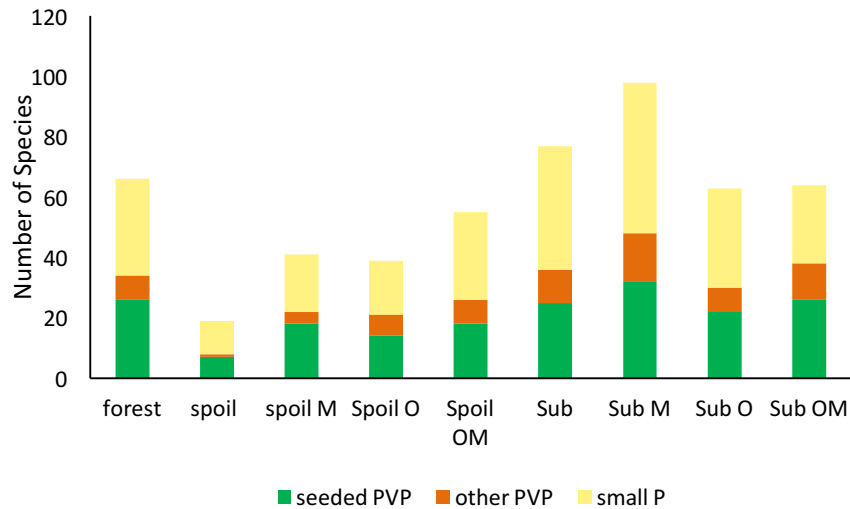
### 5.5. Species reproduction

During the survey, individual plants were inspected for flowering, seeding, or evidence of having done either in the time frame previous to the survey. Plants were also classified into adults, juveniles and seedlings. Seedlings are not considered in the plant abundance evaluation, but are considered in this section to demonstrate possible sustainability of populations and substrate effects. Data has been presented here (Figure 5.11 and Table 5.4) by filtering for species which have populations which could be sustainable.

In total 36 seeded species and 29 seed bank species had populations greater than 6 individuals each (average = 1). We have decided to present the data in this way because it is half way between a conservative and optimistic evaluation. In the absence of any information on the size of viable plant populations (a population which has enough genetic variability to be sustainable over time), or the success and behaviour of local pollinators (presence, capacity for locating flowers, etc.) we have opted for the number 6 as being a possibly viable population from a genetic point of view. Some plant species can be viable and sustainable with a starting population of just one individual, other species need populations in the hundreds, but as no information is available we are using a best guess for the time being. This data will become more important at a later date when failure and success of species needs to be explained.



**Figure 5.10.** Height distributions of a) Rough barked apple (*Angophora floribunda*), b) Spotted Gum (*Corymbia maculata*) and c) Grey Box (*Eucalyptus moluccana*). The data is divided up in two ways, firstly by treatment but also by colour where the darker colour indicates individuals that are taller. Height categories are in metres.



**Figure 5.11.** Number of native species for each treatment divided into seeded species possibly forming viable populations (seeded PVP), other non-seeded species possibly forming viable populations (other PVP) and species with small populations (small P) having low numbers of individuals which may or may not become viable populations. A Viable population is a group of plants that are capable of sustainable existence over time.

The ability of a species to reproduce is a desirable outcome of a species restoration project as it is the first step that leads to a sustainable population. Both seeded and seed bank species are showing evidence of reproduction. Some species seem to show preferences for certain substrates. For example, seeded acacias that have flowered only on forest and subsoil plots (Note: other acacias that haven't flowered at all require longer to reach reproductive age). Overall, subsoil based treatments and forest topsoil have the highest numbers of reproducing species (Table 5.4) with subsoil and mulch performing best with 31 species showing evidence of reproduction. It is important to note here that because of the nature of a survey within a limited time period, not all species were observed to reproduce.

Some evidence of second generation seedlings is also already available from this survey event. Determining whether seedlings are late emerging individuals from the seeding or are real second generation is impossible, so some exclusion rules have been applied to reduce the error. Species that have been observed to reproduce and have seedlings have been classified as having second generation individuals. Species that have not been observed to reproduce but do have seedlings have been assumed to have delayed first generation seedling emergence and are not shown. Some of the latter are also possibly species that have dispersed into the plot from outside. An example is *Salsola australis*, which grows well on spoil substrates and disperses over the whole mine site by wind. These kinds of species are technically sustainable but have been excluded from the analysis at this time for consistency reasons. Subsoil by itself and subsoil with mulch produced most species with seedlings. It is worthwhile to note though that one survey event, and just analysing seedling presence is not telling the whole story. As an example, *Linum marginale*, a native grassland herb which emerged from the seed bank, rapidly expanded its population within the 2 year period, but has not been accounted for in the seedling survey because all seedlings that survived had converted into adults or juveniles. This point illustrates the importance of having a good knowledge of the species that are being surveyed and that more than one survey over time will be necessary to capture sustainability indicators and changes in population size. Part of the success of these substrates is the openness of the vegetation. Other factors harder to prove are beneficial interactions with soil microbes and seedling safe-sites conferred by the texture of the substrates.

Seeded Species	Forest	Spoil	Spoil M	Spoil O	Spoil OM	Sub	Sub M	Sub O	Sub OM
Acacia ambylygona	115.00 ✓	1.17 ✗	3.83 ✗		2.50 ✗	43.50 ✓	34.50 ✓	11.67 ✓	8.50 ✓
Acacia decora	24.33 ✗	1.17 ✗	4.33 ✗	1.00 ✗	4.50 ✗	21.83 ✓	26.50 ✓	22.33 ✓	19.17 ✗
Acacia falcata	37.67 ✓		1.00 ✗			11.83 ✓	27.17 ✓	8.83 ✓	8.83 ✓
Acacia implexa	2.83 ✗		1.17 ✗	1.00 ✗	2.67 ✗	4.17 ✗	3.83 ✗	2.33 ✗	1.33 ✗
Acacia salicina	8.17 ✗	2.83 ✗	3.33 ✗	4.00 ✗	4.67 ✗	5.17 ✗	5.17 ✗	5.67 ✗	5.17 ✗
Angophora floribunda	4.67 ✗		2.00 ✗		3.50 ✓	1.67 ✗	5.00 ✗	1.17 ✓	3.33 ✗
Aristida sp.	1.17 ✓		2.00 ✓			1.33 ✓	8.67 ✓	1.00 ✓	4.00 ✓
Atriplex semibraccata	5.67 ✗	5.83 ✗	21.17 ✓	71.00 ✓	21.50 ✓	14.33 ✓	7.50 ✓	15.67 ✓	14.50 ✗
Austrostipa scabra	6.33 ✓		4.17 ✓	3.83 ✓	5.00 ✓	5.83 ✓	13.00 ✓	1.67 ✓	1.50 ✓
Austrostipa verticillata				1.33 ✓	3.00 ✓			3.00 ✓	
Brachychiton populneus	2.83 ✗	1.50 ✗	3.33 ✗		1.17 ✗	5.33 ✗	2.17 ✗	1.00 ✗	1.17 ✗
Chloris truncata	4.00 ✗	6.67 ✓	10.33 ✓	31.50 ✓	20.17 ✓	11.33 ✓	16.67 ✓	32.00 ✓	5.00 ✓
Corymbia maculata	4.00 ✓		3.17 ✓	1.17 ✗	6.67 ✗	4.00 ✗	10.17 ✗	4.00 ✓	8.33 ✗
Daviesia genistifolia	4.00 ✓		3.67 ✗			2.00 ✓	8.50 ✓		
Daviesia ulicifolia							2.67 ✓		
Dichondra repens	7.17 ✗					8.50 ✗	12.83 ✗	5.33 ✓	7.17 ✗
Dodonaea viscosa	4.83 ✓					2.33 ✗	3.50 ✗	2.83 ✓	2.00 ✓
Einadia hastata				1.17 ✓					
Enchylaena tomentosa	4.33 ✗	10.83 ✓	6.00 ✓	5.83 ✗	6.67 ✗	4.83 ✗	2.17 ✓	8.83 ✗	2.00 ✓
Eragrostis brownii	1.00 ✓						2.33 ✓		
Eragrostis leptostachya	2.33 ✓						1.33 ✓		
Eremophila debilis				1.17 ✗					
Eucalyptus crebra					1.50 ✗		4.17 ✗		2.83 ✗
Eucalyptus moluccana	3.00 ✗		1.17 ✗	1.33 ✗	9.50 ✗	1.17 ✗	7.67 ✗	3.67 ✗	13.33 ✗
Glycine latifolia							1.33 ✗		1.33 ✗
Glycine tabacina	1.83 ✗					3.50 ✗	5.83 ✗	3.00 ✗	1.50 ✗
Hardenbergia violacea	7.17 ✓		1.33 ✗		1.33 ✓	3.83 ✓	5.33 ✓	1.50 ✓	1.83 ✓
Indigofera australis						1.50 ✓	2.50 ✓		2.83 ✓
Kennedia rubicunda	2.33 ✓		1.17 ✗			1.33 ✓	2.00 ✓	1.17 ✓	2.00 ✓
Olearia elliptica				1.17 ✗	1.67 ✗		2.33 ✗		1.00 ✗
Panicum effusum							1.50 ✓		
Pultenaea microphylla	8.33 ✓					2.50 ✗	5.50 ✓		
Rytidosperma fulvum	2.17 ✓			3.33 ✓	1.17 ✓	2.17 ✓	10.00 ✓		1.00 ✓
Themeda australis	7.50 ✓		1.50 ✓			2.17 ✓	9.17 ✓	1.50 ✓	1.67 ✓
Vittadinia pterochaeta					4.67 ✓	5.83 ✓	7.17 ✓	2.50 ✓	4.67 ✓
Wahlenbergia gracilis	1.67 ✓								
<b>Total Seeded Species</b>	<b>26</b>	<b>7</b>	<b>18</b>	<b>14</b>	<b>18</b>	<b>25</b>	<b>32</b>	<b>22</b>	<b>26</b>

Non-Seeded Species	Forest	Spoil	Spoil M	Spoil O	Spoil OM	Sub	Sub M	Sub O	Sub OM
Acacia irrorata	3.00 ✓					1.83 ✓	3.00 ✗		
Bothriochloa spp.						4.50 ✓	19.67 ✓		3.83 ✓
Chamaesyce spp.					1.50 ✓	32.17 ✗	62.17 ✗	7.67 ✗	45.00 ✗
Chloris divaricata							1.83 ✓		
Chloris verticillata								1.00 ✓	
Cynodon dactylon	2.00 ✓		1.17 ✓	4.83 ✓	5.17 ✓	3.17 ✓	17.00 ✓	4.17 ✓	3.50 ✓
Cyperus gracilis								2.00 ✗	
Desmodium varians							1.83 ✗		
Digitaria ramularis	1.00 ✓								
Einadia nutans subsp. linifolia				3.33 ✓	8.67 ✓	2.33 ✓	2.83 ✓	8.17 ✓	12.17 ✓
Einadia sp2				6.33 ✓	9.00 ✗			5.83 ✓	5.33 ✓
Entolasia stricta	3.17 ✓		1.67 ✓			1.00 ✓	6.17 ✓		1.83 ✓
Euchiton involucratu								3.33 ✓	
Erodium crinitum									1.17 ✓
Euc spp.	1.50 ✗		1.17 ✗		1.17 ✗		4.50 ✗		3.33 ✗
Lepidium pseudohyssopifolium				3.00 ✓	1.50 ✓				
Linum marginale							16.17 ✓		
Lomandra multiflora							1.83 ✓		
Maireana microphylla				2.50 ✗					
Paspalidium distans	1.50 ✓					7.83 ✓	5.83 ✓		
Phyllanthus virgatus							1.33 ✓		
Salsola australis		16.50 ✗	1.83 ✗	24.00 ✗		5.83 ✗			
Senecio quadridentatus				8.67 ✓	3.83 ✓				1.50 ✓
Sida corrugata						1.00 ✓			1.50 ✓
Solanum prinophyllum						1.33 ✓	1.33 ✓		
Templetonia stenophylla	1.83 ✓								
Unknown Native grass spp.							2.17 ✗		1.50 ✓
Vittadinia pustulata					1.33 ✗	10.17 ✓	4.67 ✓	12.67 ✓	4.83 ✓
Zornia dyctiocarpa	1.00 ✓								
<b>Total Non-seeded Species</b>	<b>8</b>	<b>1</b>	<b>4</b>	<b>7</b>	<b>8</b>	<b>11</b>	<b>16</b>	<b>8</b>	<b>12</b>
<b>Grand Total of Species</b>	<b>34</b>	<b>8</b>	<b>22</b>	<b>21</b>	<b>26</b>	<b>36</b>	<b>48</b>	<b>30</b>	<b>38</b>
Number reproducing	22	2	9	11	13	23	31	22	23
Number with seedlings	2	0	2	0	0	5	5	1	2

**Table 5.4. Average abundance and evidence of reproduction.** Average is only shown if greater than 1. Ticks and crosses indicate whether the species was seen to present evidence of reproduction within the treatment during the survey (tick for yes, cross for no), and green shaded squares indicate where second generation seedlings were found. The numbers indicate the average number of individuals per plot for each treatment. This data represents the communities that may form on each treatment.

Because of their visibility, canopy species are given special consideration in this paragraph. Across the site we observed 26 individual trees that were either budding or flowering from two species, *A. floribunda* and *C. maculata* (Plate 5.9). This was not expected as the trees are only 2 years old and as such would typically still be juveniles. All trees that produced buds and flowers were on OGM treatments. This is of concern as the early flowering could indicate that the trees are stressed and may die in the near future. This a common response for short lived herbs under stress as it allows them to spread their genetic material in adverse conditions so that the future generation will have a chance to survive. Unexpectedly, there were significantly more *C. maculata* reproducing on treatments without mulch ( $p = 0.0007$ ) and based on the current data it is likely that with more samples *A. floribunda* will show the same trend. The reason this is unexpected is that the treatments without mulch have thus far shown to be less desirable for growing plants. Data also suggests that the ideal environment for germinating seeds is on treatments with mulch, therefore were the trees to reproduce and their offspring not travel outside the plot, it would lead to poor second generation germination rates.



*Plate 5.9: Photos of A) Angophora floribunda buds and B) Corymbia maculata buds in November 2015.*

## 5.6 Summary of treatment effects

In an attempt to summarise the large volume of data obtained in this study, a simple index was devised to aid decision making for outcomes on substrate types. It was assumed that for a rehabilitation site which aims to reconstruct an EEC that biodiversity, sustainability and low weed cover would be major goals. Also, given the nature of the deconsolidated surface of mine rehabilitation areas, that avoiding erosion would be another major goal. Many other factors could be included like ecosystem function, ecosystem structure, etc. (SERA 2015) but more data needs to be collected. Subsoil with mulch and forest topsoil are the best performing substrates for the goals stated above (Table 5.4). Nevertheless, other subsoil combinations and spoil ameliorated with mulch and OGM follow close behind. The reason the subsoils and forest are more successful is because of higher seed bank input, and probably better plant survival as remnant microbial populations would have been present which can positively interact with the plants. The extra input of nutrients by the OGM is not resulting in extra value except in a scenario where spoil is the only substrate available, but even then only in combination with mulch which reduces the nitrogen loads and the salt content.



**Table 5.5. Ranked substrates for rehabilitation goals and measures and a composite decision making index based on data for 2 year old rehabilitation using the current species mix and rates. Ranks in each column: 1=best, 9=worst. Index is the sum of the 4 ranks considered (diversity, sustainability potential, exotic cover and amount of bare ground) divided by 4 and rounded to one unit. The sustainability potential is a composite of 3 variables: population size larger than 6, presence of reproduction and presence of second generation seedlings all ranked, averaged and final value ranked. Possible treatments which optimise all three rehabilitation goals have been highlighted in dark green. Ranks based on data shown in Table 5.1, Table 5.3 and Table 5.4.**

Ecosystem Goals:	Biodiversity	Sustainability	Low Threats	Low Erosion	Index
Measure:	Diversity	Sustainability potential	Low Exotic cover	Low Bare ground	
Forest	5	4	2	6	4
Subsoil	3	3	4	8	5
Subsoil & Mulch	1	1	8	5	4
Subsoil & OGM	4	5	7	2	5
Subsoil & Mulch & OGM	2	4	9	3	5
Spoil	9	9	1	9	7
Spoil & Mulch	8	8	3	7	7
Spoil & OGM	7	7	6	4	6
Spoil & Mulch & OGM	6	6	5	1	5

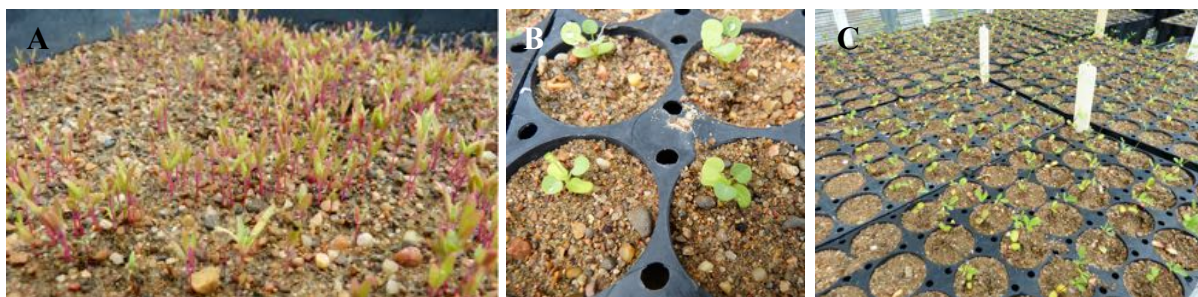
## 6. Sustainability and Niche of Herbaceous Species (Experiment 2)

### 6.1 Experimental Herbaceous Species

Six herbaceous perennial species were targeted for detailed experimental and niche assessment. As a group they represent combinations of different characteristics such as type of root associations, seed dispersal types and success on existing rehabilitation areas (Table 6.1). All but one of these species, *Desmodium brachypodum*, seem to have low establishment, or have disappeared from rehabilitation areas over time at Mount Owen mine and so were chosen as target species. These species were grown at The University of Newcastle's Plant Growth Facility (Plate 6.1) until large enough to be planted (Plate 6.2).

**Table 6.1: List of species utilised for herbaceous plant experiments and some of their ecological characteristics.**  
*A: For only 5 of these species are root association status known: 2 have endomycorrhizae, symbiotic fungi which help in phosphorus acquisition among other things, 2 have rhizobia, symbiotic bacteria which form nodules on roots and fix atmospheric nitrogen which can then be used by the plant, and one which is known to be able to survive without any symbionts. B: Most of these species have been observed to produce seed at Mount Owen mine rehabilitation areas. This indicates that pollination should not be an obstacle to sustainability of these populations C: These species have different seed dispersal vectors: broadly they fall into wind dispersed and animal dispersed species. Animals can disperse seed by attachment to the body, swallowing it, or actively carrying it away (ants). D: Selection of species has also been based on how these species are surviving at Mount Owen Mine rehabilitation area based on plot data and on observation of seedlings.*

Species	A: Probable root associations	B: Production of seed at Mount Owen mine rehabilitation area	C: Probable dispersal vector	D: Presence on Mount Owen mine rehabilitation area
<i>Calotis lappulaceae</i>	Endo-mycorrhizae	yes	animal	low numbers
<i>Chrysocephalum apiculatum</i>	Endo-mycorrhizae	yes	wind	low numbers
<i>Desmodium brachypodum</i>	rhizobia	yes	animal	establishment & recruitment observed
<i>Einadia nutans</i>	none	yes	animal	low numbers, disappears
<i>Hypericum gramineum</i>	unknown	unknown	wind	low numbers
<i>Swainsona galegifolia</i>	rhizobia	yes	wind	no second generation

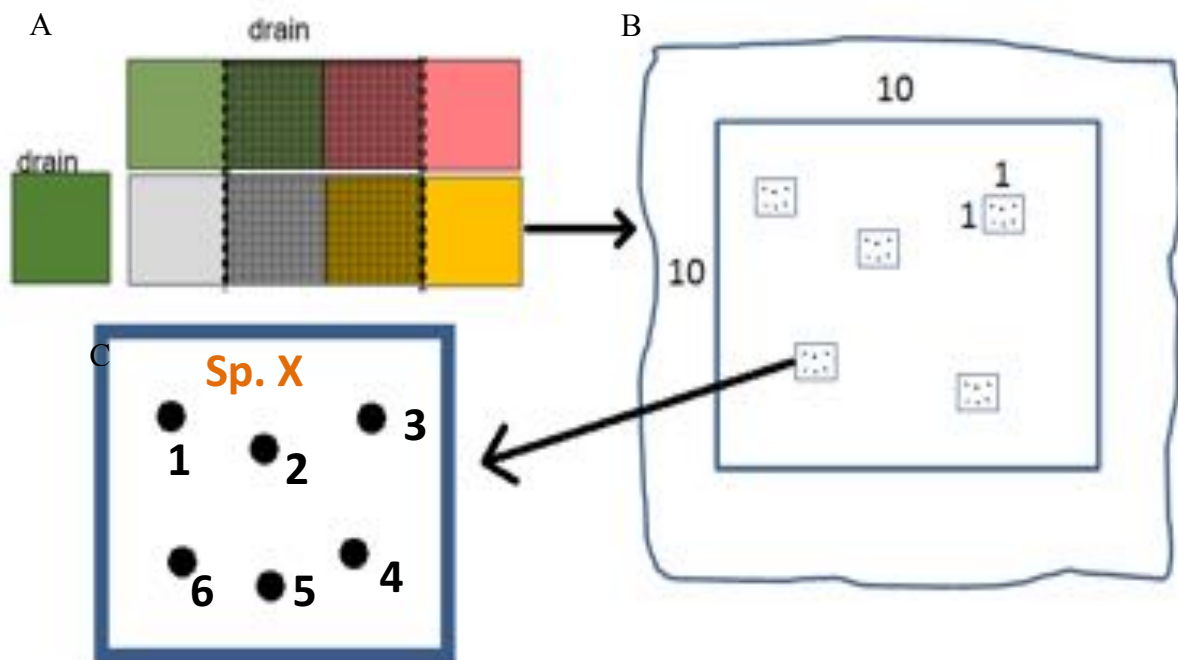


**Plate 6.1: Photos of seedlings for herbaceous plant experiment.** A) tray with emerging *Einadia nutans*. B) potted up *Desmodium brachypodum* seedlings. C) Potted up *Swainsona galegifolia* seedlings in hiko trays.

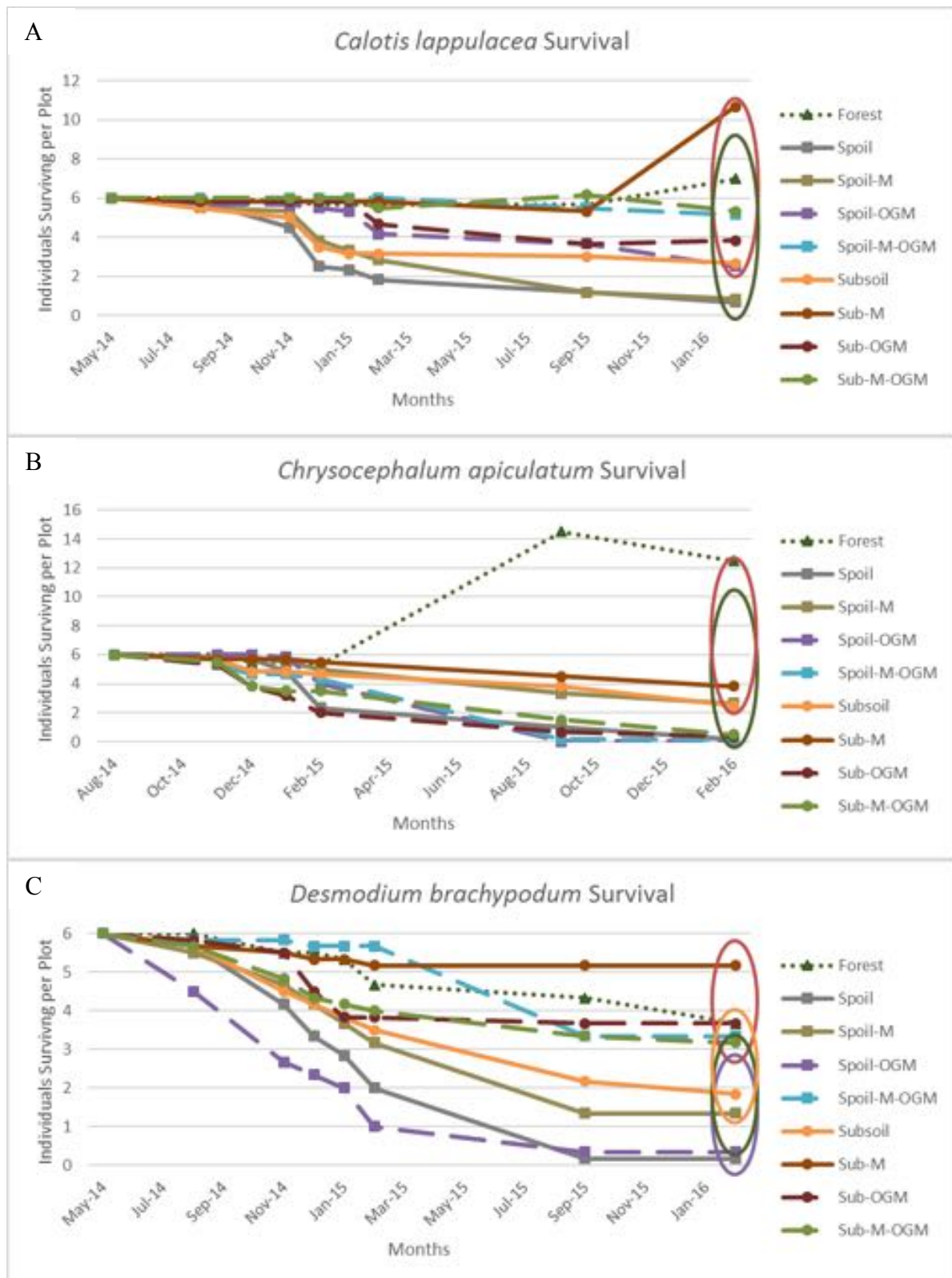


**Plate 6.2 Photo of planting in the field.** A) Hikos with 4 different species at seedling stage from back to front *Swainsona galegifolia*, *Desmodium brachypodum*, *Einadia nutans* and *Calotis lappulacea*. B) planting seedlings in groups of six inside 1x1m areas marked by short white stakes.

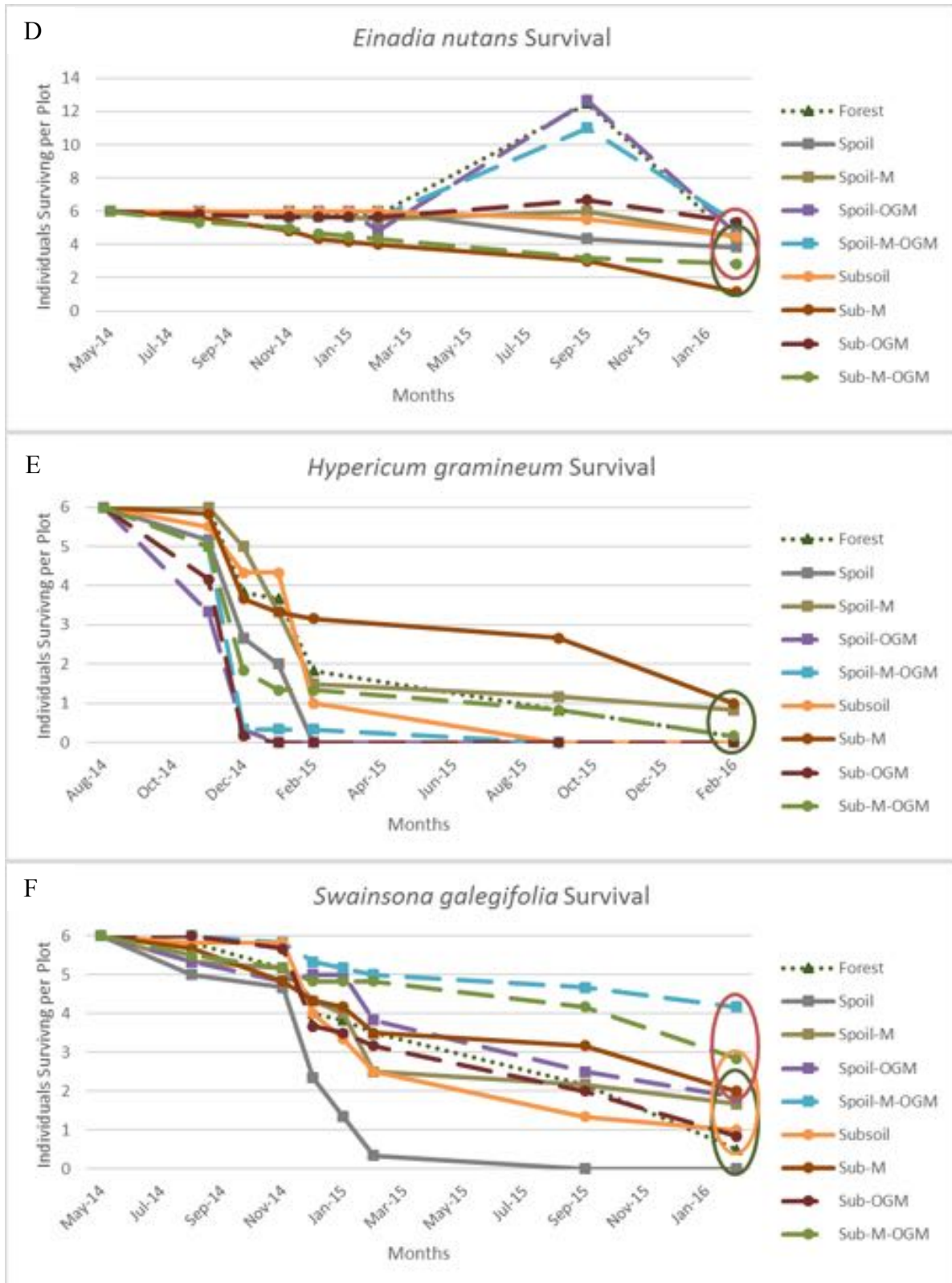
Between April 2014 and June 2014 four of these species, *Calotis lappulacea*, *Desmodium brachypodum*, *Einadia nutans* and *Swainsona galegifolia* were planted into 1x1m subplots (Plate 6.2, Figure 6.1) in each of the treatments over all six blocks. The final two species, *Chrysocephalum apiculatum* and *Hypericum gramineum*, were similarly planted in August 2014. Six plants were planted in each subplot and then mapped to allow re-identification in field surveys.



**Figure 6.1: Experimental layout.** On each of the 9 treatments (A) in the 6 blocks of the experimental site 6 smaller plots 1x1m in size (B) were planted with 6 individuals of one species per plot (C). The position of species within the plots was semi random.







**Figures 6.2 A- F.** Survival of planted individuals and second generation seedlings from the six target species has been observed from mid-2014 through to February 2016. M = Mulch, OGM = Organic Growth Medium, Sub = Subsoil. All spoil treatments are represented by a square symbol, Subsoils have a circle symbol and treatments with OGM use a dashed line as opposed to a solid line for other treatments. The forest treatment is represented by a triangle and a dotted line. The ovals encompassing several points at Feb 2016 represent statistical difference between treatments. If two treatments are encompassed by the same oval, then they are not statistically different. Where species populations increase this indicates that seedlings were found for the species.



Surveys were performed in August, November, December 2014, January, February, September 2015 and February 2016. During the survey each planted individual from the six species was evaluated for survival, evidence of reproduction and establishment of second generation seedlings (Figure 6.2 a-f). *Desmodium brachypodum*, *Hypericum gramineum* and *Swainsona galegifolia* did not increase in size. Whilst all flowered and produced seed, none germinated. *Calotis lappulacea*, *Chrysocephalum apiculatum* and *Einadia nutans* all flowered and produced seed and seedlings allowing some planted populations to increase in size. Some species showed preferences for certain substrates and is discussed below.

A proportional hazards model was developed for the survival of each species on the different treatments. Similar to the results shown in Scanlon (2015), three species preferred subsoil based treatments and the other three were indifferent to substrate (Table 6.2) and five of the six species had a much higher chance of survival when mulch was used. Response to OGM was variable with two species performing better without it and three others performing better with it. This type of analysis allows us to match each species to an ideal substrate. For example, *Chrysocephalum apiculatum* can be grown on either spoil or subsoil but has higher survival when mulch is used and OGM is not used. *Desmodium brachypodum* survives preferentially in subsoil and mulch but is indifferent to the presence of OGM.

**Table 6.2. Proportional hazards analysis for the six targeted herbaceous perennials.** Green highlighting indicates increased survival when on a substrate compared to its alternative (substrate tested / alternative substrate). Red highlighting indicates decreased survival when on a substrate compared to its alternative. This has been updated since it was first published in Scanlon (2015) to include the more recent surveys.

Species	Death Risk for Base		Death Risk for Mulch		Death Risk for OGM	
	p value	Subsoil / Spoil	p value	Mulch Present / Mulch Absent	p value	OGM Present / OGM Absent
<i>C. lappulacea</i>	0.0002	0.49	<0.0001	0.46	0.0025	0.56
<i>C. apiculatum</i>	0.101	0.8	0.0003	0.6	<0.0001	2
<i>D. brachypodum</i>	<0.0001	0.38	<0.0001	0.44	0.1416	1.28
<i>E. nutans</i>	0.1077	1.48	0.0348	1.66	0.0083	0.54
<i>H. gramineum</i>	0.0195	0.75	<0.0001	0.55	<0.0001	2.4
<i>S. galegifolia</i>	0.7099	0.94	<0.0001	0.45	<0.0001	0.47

## 6.2 Seedlings

During each survey, the site, particularly the 1x1m subplots, was examined for seedlings of the 6 target species. The first seedlings were found in the September 2015 survey and these individuals were mapped and resurveyed at the final survey in February 2016. Most of the seedlings found have been from *E. nutans* however we also found several smaller groups of seedlings from *C. lappulacea* and one from *C. apiculatum* (Table 6.3). This is considered a fantastic result as seedlings are the base requirement for the maintenance of plant population and life cycling. However, as seen in the data, many individuals do not survive to reach the juvenile or adult stage so it is still not known if there is a stable population for any species. The loss of large numbers of seedlings is not unexpected and explained by the natural loss of the individuals over time and the high level of competition that they will be exposed to, both from their parents (particularly in *E. nutans* as it can smother seedlings) and from other species in the plots.

**Table 6.3.** Number of seedlings from each treatment where seedlings were found across all six blocks. Searches for the seedlings were conducted in areas that were considered to be the most likely locations, around and within the 1x1m subplots. No seedlings were found in Spoil and Subsoil plots.

Treatment	Species	Block	Seedlings Sept 2015	Seedlings Feb 2016
Forest	<i>C. lappulacea</i>	3	0	13
Forest	<i>C. apiculatum</i>	4	60	43
Forest	<i>E. nutans</i>	4	41	1
Spoil Mulch	<i>E. nutans</i>	6	3	0
Spoil OGM	<i>E. nutans</i>	4	26	0
Spoil OGM	<i>E. nutans</i>	5	23	9
Spoil OGM Mulch	<i>C. lappulacea</i>	2	0	3
Spoil OGM Mulch	<i>E. nutans</i>	1	1	1
Spoil OGM Mulch	<i>E. nutans</i>	4	6	0
Spoil OGM Mulch	<i>E. nutans</i>	5	22	2
Spoil OGM Mulch	<i>E. nutans</i>	6	3	0
Subsoil Mulch	<i>C. lappulacea</i>	4	0	4
Subsoil Mulch	<i>C. lappulacea</i>	5	0	17
Subsoil Mulch	<i>C. lappulacea</i>	6	0	1
Subsoil OGM	<i>C. lappulacea</i>	2	0	2
Subsoil OGM	<i>E. nutans</i>	5	2	0
Subsoil OGM	<i>E. nutans</i>	6	5	0
Subsoil OGM Mulch	<i>C. lappulacea</i>	2	10	5
Subsoil OGM Mulch	<i>C. lappulacea</i>	5	0	6

Interestingly no seedlings were found in the subsoil mulch treatments until the latest survey and even then they were restricted to only one species, *C. lappulacea*. This is considered odd as, based on current data, the subsoil mulch treatment appears to be the most beneficial for survival of native herbs in general. This could be due to the low amounts of seed dispersed by the species examined as the species that have produced seed are known to produce small amounts. It could also be that the soil, while the most suitable for survival, is not the most suitable for reproduction, germination and / or establishment.

### 6.3 Niche Characteristics

It was hoped that the six herb species would reproduce early on in the experiment and that numerous seedlings could be mapped and followed through to adulthood. As part of this process the niche characteristics of each second generation juvenile could have been identified so that the landscape could be manipulated to encourage the establishment of herbs. Unfortunately, the late germination and high mortality of the seedlings has led to this data not being captured. A detailed analysis of the niche characteristics of each treatment is presented in Scanlon (2015).

## 6.4 Long Term Sustainability

Predicting the long term sustainability of a population is always difficult and in novel ecosystems such as a coal mine rehabilitation area the challenges only increase. However, some inferences can be made from the data at hand. *Hypericum gramineum* is considered highly unlikely to remain on site in the future due to its low and disperse population and lack of offspring germination. *Calotis lappulacea* however is a species that we expect to remain on site as there are several meta-populations with seedlings/juveniles. *Einadia nutans* is another species that is likely to maintain its populations into the future however it may be threatened by competition from both native and exotic species. *Chrysocephalum apiculatum* may be able to establish itself on site given that it has reproduced and there are also populations in the nearby Northern Offset area, however it's populations are dropping and recruitment over the next year is vital. *Desmodium brachypodum* and *Swainsona galegifolia* are unknowns, although they both have fair populations in some treatments they have yet to produce offspring and as such may not survive in to the future.

## 7. Recommendations and Avenues of Further Research

### 7.1 General Recommendations

Some general recommendations can be made.

A. Design landscapes that minimise gully erosion. Once erosion is deep enough that it has reached the spoil layer gully erosion will quickly continue and while stabilisation of the ground will occur with plants in place, severe gullies and gullies forming early in the reclamation process will have a large impact on the restoration works.

B. Subsoil is chemically and physically closest to forest topsoil and even though both these are deficient in many ways, they have produced species rich rehabilitation areas. Adding clean coarse wood mulch further enhances results. Where ever possible, these substrates should be favoured when ecologically sustainable ecosystems is the desired outcome.

C. Ensure that substrates aren't contaminated by weeds (OGM, topsoil, mulch stockpiles). Fresh material should be checked for contamination before use while regular and frequent monitoring early on to spot spray or remove known threats. Consider burying to depths >5cm substrates that are suspected to be carrying a high weed seed bank.

D. Increased numbers of ground cover native species need to be incorporated in seeding mixes.

### 7.2 Avenues of Further Research

Further research needs to be done into long term effects of substrates, ameliorants, seed mixes and ecosystem development. We have observed second generation seedlings for some herbaceous species but how many of the seedlings will survive and establish as adult plants? Is there an effect of treatment in the establishment of seedlings? Is there a nurse effect and will it influence the ability of seedlings to survive to adulthood?

Once a rehabilitation area is established how will the species diversity evolve? How can missing species be incorporated into existing rehabilitation areas? What methods are most appropriate and can species be classed into groups requiring different methods? For example, some seed require light to germinate and some require dark or a switch from dark to light like what would occur in a natural system when animal activity buries and re-exposes seed.

What is the long term soil sustainability of the created system? Are there nitrogen fixing bacteria? Are there phosphorus fixing endo-mycorrhiza? Are there decomposing fungi? Are there invertebrates such as earthworms and dung beetles?

Is the OGM going to have a long term negative effect on the ecosystem due to either stalling of vegetative growth or toxicity to invertebrates from the plastic content?

### 7.3 Recommended Rehabilitation Plan and Outcomes

The objective of the recommended Rehabilitation Plan is to establish vegetation communities that align with the Central Hunter Grey Box-Ironbark Woodland and the Central Hunter Ironbark-Spotted Gum-Grey Box Forest EECs. In addition, consideration was given to the early stabilisation of the rehabilitation sites and the inclusion of species found in proximity to the rehabilitation site.

In Table 7.1 suggested steps to be followed in new rehabilitation areas are given. In Table 7.2 the events which are expected to occur on a rehabilitation site using the suggestions for rehabilitation from Table 7.1 are presented. As this project terminated at 2 years of vegetation development, the projections for years 3 to 5 are based on observations on the site and experience on other sites. Continued monitoring of the experimental site would inform at least 2-3 years ahead of time what the overall rehabilitated ecosystem is likely to become and allow for forward planning of actions. Table 7.3 shows recommended seeding rates or modifications to the current practice and Table 7.4 shows additional species that could be sourced for this rehabilitation.

**Table 7.1: Suggested Rehabilitation Plan.** This table should be read in conjunction with Table 7.2

Phase 1: Site set-up	
1	<b>Use subsoil.</b> Subsoil has been shown repeatedly to be beneficial for native vegetation establishment. Remnant soil biology and remnant seed banks are present and contribute to both diversity and nutrient cycling. Dispersiveness of subsoil will be reduced when used in conjunction with mulch and OGM.
2	<b>Use clean coarse wood Mulch.</b> Wood mulch will protect from surface erosion, add humidity to substrate increasing water infiltration, create microsites for germination, create micro shelter for soil fauna and act as a food source for fungi stimulating nutrient cycling. The main issue is to avoid using mulch containing exotic species and weeds. This will save a lot of money down the track in weed control.
3	<b>Add OGM at 50dt/ha.</b> Application rates in the Ravensworth Operations Experimental site were probably too high (100dt/ha) as they encouraged profuse invasive grass development. Research into optimal application rates, including as low as 10dt/ha, would be beneficial.
4	<b>Spread OGM in Patches.</b> Not the whole area should be covered homogenously with OGM. A large number of small areas (15x15m) should be left free of OGM to allow slower growing natives (e.g. <i>Callitris</i> , <i>Allocasuarina</i> , <i>Cassinia</i> ...) to establish without competition from the more aggressive chenopods and grasses. These areas in later years can act as dispersal sources for species into the general rehabilitation area from which they may have disappeared due to the high plant competition on OGM areas.
5	<b>Add rock and wood piles.</b> Habitat areas will increase usage by animals and promote dispersal of plants into the rehabilitation area. Control weeds around these but be aware that native species can be dispersed into these piles by birds (e.g. <i>Myoporum</i> , <i>Breynia</i> , <i>Notelaea</i> ....)
Phase 2: Seeding Plan	
1	<b>Continue with current Seed mix.</b> Current species mix was quite successful. Would suggest increasing grass species rates. Other under-storey species could be added. Many additional species emerged from the seed bank and some of these could be sourced for adding to the seeding list.
2	<b>Add more EEC species.</b> Many of these were not included as seed was unavailable. Active sourcing should be conducted and orders placed with seed collectors well in advance.
3	<b>Add new seeding procedures.</b> Species for which low amounts of seed are available should be included in the rehabilitation area but not spread in the seed mix as the resulting individuals may be too spread out to reproduce effectively. These species should be spread in several patches



	across the rehabilitation area. More than one patch is advisable as soil conditions may not be favourable everywhere for germination.
<b>Phase 3: Weed control and Adaptive management</b>	
1	<b>MUST control weeds.</b> Controlling invasive grasses is an absolute must. Most of these are clonal and so will not die out (Kikuyu, Rhodes, and maybe Couch (native) if too abundant). Other invasive grasses affect the soil chemistry and inhibit all other species. Some of these are Coolatai, Whiskey grass, African love-grass, etc...
2	<b>Apply Adaptive Management.</b> After monitoring, it may be seen that some species have failed to establish. A decision will need to be made about re-seeding, or planting new individuals or if a different set of species should be trialed, equally by re-seeding or planting into existing rehabilitation. Research on introducing species to established but depauperate rehabilitation is lacking in the Hunter Valley. But see section 5.3 in this report.

**Table 7.2: Projected Development of the Rehabilitation area using “Suggested Rehabilitation Plan” over 5 years. Note that this scenario assumes a similar seeding mix and availability of substrates as used in the experiment reported here. Projections for Yr 3-5 are not yet backed by local evidence.**

Year	Observed or Predicted	Action required
1	Most seeded species germinate.	
	Chenopods* will progressively cover site where OGM is used.	
	Isolated areas of invasive grasses will appear	Spot spray
	Noxious weeds will appear	Spot spray or hand remove
2	Chenopods will dominate OGM areas and protect against erosion	
	Emergent trees and mid-storey in all areas will be obvious	
	Non OGM areas will contain most other under-storey species and species from the seed bank	
	Continue control of invasive grasses	Spot spray
	If high, control some areas of Couch	Spot spray
	Other noxious weeds	Spot spray or hand remove
3-5	Should see a decrease in cover by chenopods and new plants will be smaller	
	For other ground cover species especially native grasses there are 2 possible outcomes: A: species re-emerge from a seed bank B: no species re-emerge	B: Re-seed in open areas (consider loosening soil surface) or plant foci of species.
	Re-evaluate trajectory	Monitoring

\*Chenopods: *Einadia spp.*, *Enychlaena tomentosum*, *Atriplex semibractata*,...

**Table 7.3: Recommended seeding rates and other methods with comments and justifications.** “Successful seeded species” lists the species that formed larger populations using the protocols in this project and “Species requiring modification” lists suggested variations from the protocol for species which established less well. The two EEC listings (CHGBIW and CHISGGBF) and the CEC list (see Section 3 and Appendix B) are included to aid decision making when buying new seed. The “Rav Ops plants/ha” column gives the number of plants per hectare obtained on the experimental site using the seed rates reported in “Seeding rates used kg/ha”. The recommendations column recommends continuing with current practice or modifying practice by increasing seeding rates or planting patches of species and Comments or Justifications are given. It is assumed that the best substrates be used as per Table 5.5 to obtain a similar result when using the seeding rates shown here (results from year to year will vary depending on seed quality, soil conditions and weather).

	CH GBI W	CHI SGG BF	CEC	Rav Ops plants/ha	Seeding rates used kg/ha	Recom- mendation	Comments/justification
<b>Successful seeded species</b>							
<i>Acacia amblygona</i>			1	1893	0.25	seed	additional seed bank contribution
<i>Acacia decora</i>			1	1073	0.64	seed	
<i>Acacia falcata</i>		1	1	826	0.53	seed	
<i>Acacia implexa</i>			1	169	0.30	seed	
<i>Acacia parvipinnula/irrorata</i>		1	1	93	0.29	seed	
<i>Acacia salicina</i>			1	379	0.60	seed	
<i>Angophora floribunda</i>	1		1	184	0.37	seed	
<i>Aristida ramosa</i>	1		1	161	0.19	seed	
<i>Atriplex semibractata</i>				1519	0.09	seed	good ground cover
<i>Austrostipa scabra</i>	1		1	359	0.93	seed	
<i>Austrostipa verticillata</i>				79	0.19	seed	
<i>Brachychiton populneus</i>	1		1	159	2.43	seed	
<i>Chloris truncata</i>				1180	0.57	seed	good ground cover
<i>Corymbia maculata</i>		1	1	361	0.19	seed	
<i>Daviesia genistifolia</i>			1	173	0.11	seed	
<i>Dichondra repens</i>	1	1	1	361	0.20	seed	
<i>Dodonaea viscosa</i>	1		1	144	0.11	seed	
<i>Enchylaena tomentosa</i>				441	0.002	seed	hot spot species - seed bank contribution
<i>Eucalyptus crebra</i>	1	1	1	90	0.50	seed	
<i>Eucalyptus moluccana</i>	1	1	1	350	0.74	seed	

	CH GBI W	CHI SGG BF	CEC	Rav Ops plants/ha	Seeding rates used kg/ha	Recom- mendation	Comments/justification
<i>Glycine tabacina</i>	1	1		140	0.05	seed	hot spot species - seed bank contribution
<i>Hardenbergia violacea</i>				199	0.22	seed	increase nitrogen fixing diversity
<i>Indigofera australis</i>				70	0.19	seed	increase nitrogen fixing diversity
<i>Kennedia rubicunda</i>				93	0.10	seed	increase nitrogen fixing diversity
<i>Olearia elliptica</i>			1	63	0.37	seed	
<i>Pultenaea microphylla</i>				144	0.09	seed	increase nitrogen fixing diversity
<i>Rytidosperma fulvum</i>				197	0.19	seed	locally native grass successful on rehab.
<i>Themeda australis</i>		1	1	209	3.71	seed	
<b>Species requiring modification</b>							
<i>Cymbopogon refractus</i>	1		1	6	0.09	increase rate*	hot spot species
<i>Einadia trigonos subsp leicarpon</i>				9	0.09	increase rate	hot spot species - good ground cover
<i>Eragrostis brownii</i>				29	0.19	increase rate	may have been misidentified in field
<i>Eragrostis leptostachya</i>	1		1	36	0.02	increase rate	hot spot species
<i>Eucalyptus fibrosa</i>		1		7	0.67	increase rate	under-performed: site unsuitable?
<i>Eucalyptus tereticornis</i>		1	1	16	0.37	increase rate	under-performed: site unsuitable?
<i>Glycine clandestina</i>		1	1	20	0.05	increase rate	hot spot species - viability may have been lower than reported.
<i>Glycine latifolia</i>			1	41	0.05	increase rate	hot spot species - low viability of seed batch
<i>Microlaena stipoides</i>	1	1	1	10	1.11	increase rate	viability may have been lower than reported.
<i>Panicum effusum</i>				21	0.80	increase rate	viability may have been lower than reported.
<i>Senna artemisioides</i>				4	0.04	increase rate	low viability, good pollinator plant
<i>Solanum cinereum</i>	1		1	23	0.06	increase rate	hot spot species
<i>Wahlenbergia gracilis</i>		1	1	23	0.05	increase rate	hot spot species
<i>Eremophila debilis</i>	1	1	1	34	0.05	seed / plant patches	hot spot species - will establish a population from existing plants and dispersal by animals
<i>Allocasuarina luehmannii</i>	1	1	1	1	0.19	plant patches*	seed viable but low in field establishment

	CH GBI W	CHI SGG BF	CEC	Rav Ops plants/ha	Seeding rates used kg/ha	Recom- mendation	Comments/justification
<i>Bursaria spinosa</i>	1	1	1	1	0.20	plant patches	seed viable but low in field establishment
<i>Callitris endlicheri</i>	1			7	0.56	plant patches	viability may have been lower than reported.
<i>Cassinia quinquefaria</i>	1		1	1	0.04	plant patches	seed viable but low in field establishment
<i>Kunzea ambigua</i>				0	0.04	plant patches	seed viable but no field establishment; good pollinator plant; distribution marginal - replace with local species
<i>Ozothamnus diosmifolius</i>				0	0.19	plant patches	seed viable but no field establishment; good pollinator plant
<i>Daviesia ulicifolia</i>		1	1	36	0.11	trial	under-performed compared to <i>D. genistifolia</i> . May not have established appropriate N-fixing symbiosis.
<i>Vittadinia pterochaeta</i>				219	0.09	trial	seed supplied as <i>Vittadinia</i> spp. - replace with local species

\*Although increasing seeding rates is recommended, if large amounts of seed are unavailable, consider seeding small patches spread across the rehab. Planting or seeding in patches is recommended to increase the chance of individuals of the desired species outcrossing and producing better second generation seed. Not all species need to be treated in this fashion, but as we have limited knowledge of which species do or don't, this procedure acts as a fail-safe. Some species have been recommended to plant rather than seed. This is based on low establishment rates from seed in the field combined with a high viability and germinability in shade-house conditions.



**Table 7.4: Recommended species for inclusion in rehabilitation areas and comments and justifications.** Species have been ordered by ease of adding to rehabilitation. The two EEC listings (CHGBIW and CHISGGBF) and the CEC list (see Section 3 and Appendix B) are included to aid decision making when buying new seed. The “Rav Ops plants/ha” column gives the number of plants per hectare found on the experimental site. These species were not intentionally seeded and were either contaminants in the seed batches for other species, emerged from the soil seed bank, or were part of the herbaceous plant experiments.

Other Species belonging to EECs which should be trialed	CHGBIW	CHISGG BF	CEC	Rav Ops plants/ha	Recommendation	Comments/justification
<i>Acacia cultriformis</i>	0	0	1		seed	
<i>Aristida vagans</i>	0	0	1		seed	
<i>Bothriochloa spp.</i>	1	0	0	251	seed	present as contaminant in seed batch
<i>Calotis cuneifolia</i>	0	1	1		seed	
<i>Calotis lappulacea</i>	1	0	1	20	seed	from seed bank
<i>Chloris ventricosa</i>	1	0	0	9	seed	present as contaminant in seed batch
<i>Dodonaea viscosa subsp. cuneata</i>	0	0	1		seed	
<i>Echinopogon caespitosus var. caespitosus</i>	0	1	1		seed	
<i>Einadia nutans var linifolia</i>	1	0	0	331	seed	present as contaminant in seed batch
<i>Einadia nutans var nutans</i>	0	0	1		seed	good ground cover
<i>Entolasia stricta</i>	0	1	1	143	seed	present as contaminant in seed batch
<i>Eucalyptus punctata</i>	0	0	1		seed	koala food
<i>Hakea sericea</i>	0	1	1		seed	nesting bush, good pollinator plant
<i>Paspalidium distans</i>	0	1	1	139	seed	present as contaminant in seed batch
<i>Pratia purpurescence</i>	0	1	1		seed	
<i>Pultenaea spinosa</i>	0	1	1		seed	nesting bush, good pollinator plant
<i>Solanum brownii</i>	0	0	1		seed	
<i>Solanum prinophyllum</i>	0	1	1	36	seed	from seed bank
<i>Sporobolus creber</i>	1	0	1	6	seed	present as contaminant in seed batch
<i>Ajuga australis</i>	1	0	1		seed in patches	
<i>Brachyscome multifida</i>	0	1	1		seed in patches	
<i>Brunoniella australis</i>	1	1	1		seed in patches	
<i>Cyperus enervis/gracilis</i>	1	0	1	23	seed in patches	from seed bank

Other Species belonging to EECs which should be trialed	CHGBIW	CHISGG BF	CEC	Rav Ops plants/ha	Recommendation	Comments/justification
<i>Desmodium brachypodum</i>	0	0	1		seed in patches	
<i>Desmodium varians</i>	1	1	1	17	seed in patches	from seed bank
<i>Fimbristylis dichotoma</i>	0	0	1		seed in patches	
<i>Hypericum gramineum</i>	0	1	1	1	seed in patches	from seed bank
<i>Opercularia diphylla</i>	0	1	1		seed in patches	
<i>Pomax umbellata</i>	0	1	1		seed in patches	
<i>Stackhousia viminea</i>	0	1	1		seed in patches	
<i>Vernonia cinerea var cinerea</i>	0	1	1		seed in patches	
<i>Vittadinia cuneata</i>	1	0	1		seed in patches	
<i>Wahlenbergia communis</i>	0	1	1	6	seed in patches	from seed bank
<i>Breynia oblongifolia</i>	1	1	1		plant in patches	birds consume fruit
<i>Cheilanthes distans</i>	1	0	1		plant in patches	fern - no seed available
<i>Cheilanthes sieberi</i>	1	1	1		plant in patches	fern - no seed available
<i>Dianella revoluta var. revoluta</i>	0	1	1		plant in patches	birds may consume fruit
<i>Geijera parviflora</i>	0	0	1		plant in patches	birds consume fruit
<i>Laxmannia gracilis</i>	0	1	1	4	plant in patches	from seed bank
<i>Lissanthe strigosa</i>	0	1	1		plant in patches	birds may consume fruit
<i>Lomandra multiflora</i>	1	1	1	24	plant in patches	from seed bank
<i>Melicrus ureceolatus</i>	1	1	1		plant in patches	birds may consume fruit
<i>Myoporum montanum</i>	0	0	1		plant in patches	birds consume fruit
<i>Notelaea microcarpa var. microcarpa</i>	1	0	1		plant in patches	birds consume fruit
<i>Sida corrugata</i>	1	0	1	23	plant in patches	from seed bank
<i>Acacia bulgaensis (ROTAP-2RC)</i>	1	0	1			Use only if appropriate
<i>Acacia pendula (E population)</i>	1	0	1			Use only if appropriate
<i>Eucalyptus dawsonii</i>	0	0	1			Use only if appropriate
<i>Eucalyptus glaucina (ROTAP-3VCa)</i>	0	1	1			Use only if appropriate

## 8. References:

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## Appendix 1: Pre-OGM application soil analysis.



### REPORT OF ANALYSIS

**Laboratory Reference:** A13/3876 [R00 ]

**Client:** University of Newcastle  
Biology Building  
Callaghan NSW 2308

**Contact:** Yvonne Neuberger

**Order No:**  
**Project:** Soil Analysis  
**Sample Type:** Soil  
**No. of Samples:** 4  
**Date Received:** 14/08/2013  
**Date Completed:** 21/08/2013

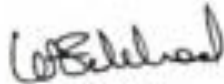
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**Laboratory Contact Details:**

**Client Services Manager:** Daniel Um  
**Technical Enquiries:** Ian Eckhard  
**Telephone:** +61 2 9888 9077  
**Fax:** +61 2 9888 9577  
**Email:** daniel.um@advancedanalytical.com.au

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**Attached Results Approved By:**



Ian Eckhard  
Technical Director

**Comments:**

All samples tested as submitted by client. All attached results have been checked and approved for release. This is the Final Report and supersedes any reports previously issued with this reference number. Accredited for compliance with ISO/IEC 17025. This document shall not be reproduced, except in full.



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Issue Date: 17 September 2013

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Laboratory Reference: A13/3876 [R00]  
 Project: Soil Analysis

Laboratory Reference:	-	-	A13/3876/1	A13/3876/2	A13/3876/3	A13/3876/4
Client Reference:	-	-	Spoil block 1	Spoil block 4	Subsoil block	Subsoil block
Date Sampled:	-	-	-3	-4	1-3	4-6
Analysis Description	Method	Units	08/08/2013	08/08/2013	08/08/2013	08/08/2013
<b>Total Solids</b>						
Total Solids	04-004	%	90.0	89.8	87.9	87.9
<b>Trace Elements</b>						
Arsenic	04-001	mg/kg	7.5	11	6.1	7.9
Cadmium	04-001	mg/kg	<1.0	<1.0	<1.0	<1.0
Chromium	04-001	mg/kg	6.4	5.5	9.4	10
Copper	04-001	mg/kg	26	28	7.5	8.2
Lead	04-001	mg/kg	12	13	11	13
Mercury	04-002	mg/kg	<0.1	<0.1	<0.1	<0.1
Nickel	04-001	mg/kg	17	16	5.8	7.2
Selenium	04-001	mg/kg	<1.0	<1.0	<1.0	<1.0
Zinc	04-001	mg/kg	80	80	32	33
<b>Organochlorine Pesticides</b>						
alpha-BHC	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Hexachlorobenzene	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
beta-BHC	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
gamma-BHC	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
delta-BHC	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Heptachlor	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Aldrin	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Heptachlor epoxide	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
trans-Chlordane	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
cis-Chlordane	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
alpha-Endosulfan	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
pp-DDE	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Dieldrin	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Endrin	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
beta-Endosulfan	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
pp-DDD	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Endrin aldehyde	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Endosulfan sulphate	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
pp-DDT	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Methoxychlor	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05

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Laboratory Reference: A13/3876 [R00]  
 Project: Soil Analysis

Laboratory Reference:	-	-	A13/3876/1	A13/3876/2	A13/3876/3	A13/3876/4
Client Reference:	-	-	Spoil block 1	Spoil block 4	Subsoil block	Subsoil block
Date Sampled:	-	-	-3	-6	1-3	4-6
Analysis Description	Method	Units	08/08/2013	08/08/2013	08/08/2013	08/08/2013
Endrin ketone	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Oxychlorfane	04-024	mg/kg	<0.05	<0.05	<0.05	<0.05
Surrogate Recovery	04-024	%	107	90	94	97
Date Extracted	04-024	-	19/08/2013	19/08/2013	19/08/2013	19/08/2013
Date Analysed	04-024	-	20/08/2013	20/08/2013	20/08/2013	20/08/2013
<b>PCB by Aroclor</b>						
PCB by Aroclor	04-070	mg/kg	<0.10	<0.10	<0.10	<0.10
Type of Aroclor	04-070	-	NA	NA	NA	NA
Surrogate 1 Recovery	04-070	%	96	98	96	104
Date Extracted	04-070	-	19/08/2013	19/08/2013	19/08/2013	19/08/2013
Date Analysed	04-070	-	20/08/2013	20/08/2013	20/08/2013	20/08/2013

Method	Method Description
04-004	Total Solids by gravimetric, %
04-001	Metals by ICP-OES, mg/kg
04-002	Mercury by CVAAS, mg/kg
04-024	OC & OP Pesticides by GCMS
04-070	PCB Aroclors in sediments and soils by GCMS, mg/kg

**Result Comments**

- [<] Less than
- [INS] Insufficient sample for this test
- [NA] Test not required

\*Analyte is not covered by NATA scope of accreditation.

# Fenamiphos not determined in matrix spike due to matrix effect, Oxychlorfane not present in matrix spike

Issue Date: 17 September 2013

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Laboratory Reference: A13/3876 [R00]  
 Project: Soil Analysis

QUALITY ASSURANCE REPORT

TEST	UNITS	Blank	Duplicate Sm#	Duplicate Results
Total Solids	%	[NA]	A13/3876-1	90.0 90.3 RPD:0

TEST	UNITS	Blank	Duplicate Sm#	Duplicate Results	Spike Sm#	Spike Results
Arsenic	mg/kg	<1.0	A13/3876-1	7.5 7.3 RPD:3	A13/3876-1	100%
Cadmium	mg/kg	<1.0	A13/3876-1	<1.0 <1.0	A13/3876-1	102%
Chromium	mg/kg	<1.0	A13/3876-1	6.4 6.3 RPD:2	A13/3876-1	98%
Copper	mg/kg	<1.0	A13/3876-1	26 26 RPD:0	A13/3876-1	95%
Lead	mg/kg	<1.0	A13/3876-1	12 12 RPD:0	A13/3876-1	89%
Mercury	mg/kg	<0.1	A13/3876-1	<0.1 <0.1	A13/3876-1	113%
Nickel	mg/kg	<1.0	A13/3876-1	17 17 RPD:0	A13/3876-1	90%
Selenium	mg/kg	<1.0	A13/3876-1	<1.0 <1.0	A13/3876-1	95%
Zinc	mg/kg	<1.0	A13/3876-1	80 81 RPD:1	A13/3876-1	86%

TEST	UNITS	Blank	Duplicate Sm#	Duplicate Results	Spike Sm#	Spike Results
alpha-BHC	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	92%
Hexachlorobenzene	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	91%
beta-BHC	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	99%
gamma-BHC	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	96%
delta-BHC	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	90%
Heptachlor	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	94%
Aldrin	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	96%
Heptachlor epoxide	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	101%
trans-Chlordane	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	111%
cis-Chlordane	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	106%
alpha-Endosulfan	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	107%
pp-DDE	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	106%
Dieldrin	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	116%
Fenitn	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	107%
beta-Endosulfan	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	106%
pp-DDD	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	106%
Fenitn aldehyde	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	102%
Endosulfan sulphate	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	100%
pp-DDT	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	97%
Methoxychlor	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	96%
Fenitn ketone	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	102%
Oxychlorane	mg/kg	<0.05	[NT]	[NT]	A13/3913-B-1	[NT]
Surrogate Recovery	%	100	[NT]	[NT]	A13/3913-B-1	100%



**Laboratory Reference:** A13/3876 [R00 ]  
**Project:** Soil Analysis

TEST	UNITS	Blank	Duplicate Sm#	Duplicate Results	Spike Sm#	Spike Results
PCB by Aroclor	mg/kg	<0.10	A13/3876-1	<0.10   <0.10	A13/3876-1	96%
Type of Aroclor	-	NA	A13/3876-1	NA   NA	A13/3876-1	1254%
Surrogate 1 Recovery	%	89	A13/3876-1	96   94   RPD: 2	A13/3876-1	89%

**Comments:**

RPD = Relative Percent Deviation

[NT] = Not Tested

[N/A] = Not Applicable

∅ = Spike recovery data could not be calculated due to high levels of contaminants

Acceptable replicate reproducibility limit or RPD: Results < 10 times LOR: no limits.  
Results > 10 times LOR: 0% - 50%

Acceptable matrix spike & LCS recovery limits:  
Trace elements 70-130%  
Organic analyses 50-150%  
SVOC & speciated phenols 10-140%  
Surrogates 10-140%

When levels outside these limits are obtained, an investigation into the cause of the deviation is performed before the batch is accepted or rejected, and results are released.



**Appendix 2: Species List for Ravensworth Operations.** See section 4.1 for details. Species list is compiled from several sources: <sup>a</sup>= North Offset species list from Umwelt 2010; <sup>b,c,f</sup> & <sup>g</sup> from surveys by Stephen Cox on quadrats in target communities at Ravensworth Mine (Rav) and Bulga Mine (Bul). <sup>d</sup>= MU10 community type and <sup>h</sup>= MU27 as described by Peak (2006); <sup>e</sup> and <sup>l</sup>= lists from the OEH web page. Species are ordered by Taxonomic group, family and genus. ROTAP species are indicated in bold lettering (note that *Bothriochloa biloba* has been removed from ROTAP). Rows coloured light blue indicate widespread species, light green CHBIW predominant species and yellow CHISGGBF predominant species. Life forms for each species have been indicated and some notes added respecting if the plant produces fleshy fruit or if it is possibly out of range for this area.

		C. Hunter Box Ironbark Wood - EEC				C. Hunter Ironbark Spotted Gum Box Forest - EEC				CE C	Experimenta l site Rehab North			
		10 q	7 q	70 q		2 q	1 q	38 q						
Fam .	Species	North Offset	CHBIW-Rav	CHBIW-Bul	MU10	CHGBIW-OEH	CHISGGBF-Rav	CHISGGBF-Bul	MU27	CHISGGBF-OEH		seeded/planted	Observed on site (2013-2016)	Life Form
	<b>Filicopsida (Ferns)</b>													
Adi	<i>Cheilanthes distans</i>	1	1	1	1	1	1	1	1	1	1			fern
Adi	<i>Cheilanthes sieberi</i>	1	1	1	1	1	1	1	1	1	1			fern
	<b>Cycadopsida (Cycads)</b>													
Zam	<i>Macrozamia</i> sp.							1						subshrub
	<b>Coniferopsida (Conifers)</b>													
Cup	<i>Callitris endlicheri</i>				1	1						1	1	tree

	<b>Magnoliopsida (Flowering Plants) - Magnolidae (Dicots)</b>													
Aca	<i>Brunoniella australis</i>	1	1	1	1	1	1	1	1	1	1			forb
Aca	<i>Brunoniella pumilio</i>								1					forb
Aca	<i>Rostellularia adscendens</i>	1	1		1									forb
Ama	<i>Alternanthera denticulata</i>												1	forb
Ama	<i>Alternanthera</i> sp A		1											forb
Api	<i>Centella asiatica</i>	1											1	forb
Api	<i>Daucus glochidiatus</i> forma F			1					1					forb
Api	<i>Hydrocotyle laxiflora</i>			1	1									forb
Apo	<i>Parsonsia lanceolata</i>			1				1						twiner
Apo	<i>Sarcostemma brunonianum</i> ( <i>Cynanchum viminale</i> sub sp australe)				1									subshrub
Asc	<i>Marsdenia viridiflora</i>		1											twiner
Ast	<i>Brachyscome ciliaris</i> var <i>ciliaris</i>		1											forb
Ast	<i>Brachyscome multifida</i>			?				1	1	1				forb
Ast	<i>Calocephalus citreus</i>		1	1	1								1	forb
Ast	<i>Calotis cuneifolia</i>			1	1			1	1	1				forb
Ast	<i>Calotis lappulaceae</i>	1	1	1	1	1	1	1	1	1	p	1	1	forb
Ast	<i>Cassinia aculeata</i>							1						shrub
Ast	<i>Cassinia arcuata</i>	1	1	1										subshrub
Ast	<i>Cassinia quinquefaria</i>		1		1	1				1	1	1	1	shrub
Ast	<i>Chrysocephalum apiculatum</i>	1	1	1	1	1		1	1	1	p	1	1	forb
Ast	<i>Chrysochalum semipapposum</i>	1	1											forb
Ast	<i>Cotula australis</i>	1		1	1									forb
Ast	<i>Cymbonotus lanatus</i>												1	forb
Ast	<i>Epaltes australis</i>							1						forb
Ast	<i>Euchiton gymnocephalus</i>	1	1											forb
Ast	<i>Euchiton involucratus</i>						1	?					1	forb
Ast	<i>Euchiton sphaericus</i>			1				1						forb
Ast	<i>Glossogyne tannensis</i> = <i>Glossocardia bidens</i>	1	1	1	1		1	1					1	forb
Ast	<i>Lagenifera gracilis</i> = <i>Lagenophora gracilis</i>				1		1							forb

Ast	<i>Leiocarpa leptolepis</i>				1									forb
Ast	<i>Olearia elliptica</i> var. <i>elliptica</i>		1		1			1			1	1	1	shrub
Ast	<i>Ozothamnus diosmifolius</i>	1	1					1			1			subshrub
Ast	<i>Pseudognaphalium luteo-album</i>							1						forb
Ast	<i>Senecio linearifolius</i>	1												subshrub
Ast	<i>Senecio quadridentatus</i>												1	subshrub
Ast	<i>Sigesbeckia orientalis</i> subsp. <i>orientalis</i>				1									forb
Ast	<i>Solenogyne bellioides</i> = <i>Lagenifera</i> sp. A	1	1		1									forb
Ast	<i>Triptilodiscus pygmaeus</i>				1									forb
Ast	<i>Vernonia cinerea</i> var. <i>cinerea</i>			1	1			1	1	1				forb
Ast	<i>Vittadinia condyloides</i>	1	1											forb
Ast	<i>Vittadinia cuneata</i>				1	1		?		1				forb
Ast	<i>Vittadinia cuneata</i> var. <i>hirsuta</i>	1	1											forb
Ast	<i>Vittadinia muelleri</i>												1	
Ast	<i>Vittadinia pterochaeta</i>		1	1			1					1	1	forb
Ast	<i>Vittadinia pustulata</i>												1	
Ast	<i>Vittadinia sulcata</i>	1	1										1	forb
Bor	<i>Cynoglossum australe</i>	1												forb
Bra	<i>Lepidum pseudohypsopifolium</i>												1	forb
Cam	<i>Wahlenbergia communis</i>	1	1		1			1	1	1			1	forb
Cam	<i>Wahlenbergia gracilis</i>	1	1	1	1		1	1	1	1	1	1	1	forb
Cam	<i>Wahlenbergia luteola</i>				1									forb
Car	<i>Polycarpaea corymbosa</i> var. <i>minor</i>	1												forb
Cas	<i>Allocasuarina luehmannii</i>	1	1	1	1	1	1		1	1	1	1	1	small tree
Cas	<i>Casuarina glauca</i>	1												small tree
Cel	<i>Maytenus silvestris</i>			1				1	1					shrub
Che	<i>Atriplex semibractata</i>	1	1	1								1	1	forb
Che	<i>Chenopodium crinitum</i>												1	forb
Che	<i>Chenopodium melanocarpum</i>				1									subshrub
Che	<i>Einadia hastata</i>	1	1		1								1	forb
Che	<i>Einadia nutans</i> subsp. <i>linifolia</i>	1	1	1	1	1						1	1	forb
Che	<i>Einadia nutans</i> subsp. <i>nutans</i>	1	1				1				1	p	1	forb

Che	<i>Einadia polygonoides</i>			1	1		1							forb
Che	<i>Einadia trigonos</i> subsp. <i>leiocarpa</i>				1							1	1	forb
Che	<i>Enchylaena tomentosa</i>	1	1	1	1		1					1	1	forb
Che	<i>Maireana enchylaenoides</i>				1									forb
Che	<i>Maireana microphylla</i>	1	1	1	1								1	subshrub
Che	<i>Salsola australis</i> (=kali)		1										1	
Clo	<i>Spartothamnella juncea</i>	1	1		1									subshrub
Clu	<i>Hypericum gramineum</i>		1	1	1			1	1	1	p		1	forb
Con	<i>Convolvulus angustissimus</i> subsp. <i>angustissimus</i>	1											1	forb
Con	<i>Convolvulus erubescens</i>	1			1								1	forb
Con	<i>Dichondra repens</i>	1	1	1	1	1	1	1	1	1	1	1	1	forb
Con	<i>Evolvulus alisnoides</i>		1											forb
Con	<i>Polymeria calycina</i>												1	forb
Cra	<i>Crassula sieberiana</i>		1		1									forb
Dil	<i>Hibbertia diffusa</i>							1						subshrub
Dil	<i>Hibbertia linearis</i>							1						subshrub
Dil	<i>Hibbertia obtusifolia</i>				1			1						subshrub
Dil	<i>Hibbertia fasciculata</i>							1						subshrub
Dro	<i>Drosera pygmaea</i>							1						forb
Epa	<i>Acrotriche rigida</i>				1									subshrub
Epa	<i>Astroloma humifusum</i>			1										subshrub
Epa	<i>Leucopogon juniperinus</i>							1						subshrub
Epa	<i>Lissanthe strigosa</i>			1			1	1	1	1				subshrub
Epa	<i>Melicrus ureceolatus</i>					1		1	1	1				subshrub
Eup	<i>Breynia oblongifolia</i>				1	1		1	1	1				shrub
Eup	<i>Chamaesyce drummondii</i>	1			1								1	forb
Eup	<i>Euphorbia planiticola</i>				1									forb
Fab	<i>Chorizema parviflorum</i>		1				1						1	subshrub
Fab	<i>Daviesia genistifolia</i>			1						1	1	1	1	subshrub
Fab	<i>Daviesia ulicifolia</i>	1	1					1	1	1	1	1	1	subshrub
Fab	<i>Desmodium brachipodum</i>		1		1			1		1	p		1	twiner



Fab	<i>Desmodium gunnii</i>		1									1	forb	
Fab	<i>Desmodium rhytidophyllum</i>	1		1									forb	
Fab	<i>Desmodium varians</i>			1	1	1			1	1	1		1	twiner
Fab	<i>Glycine clandestina</i>	1	1	1	1				1	1	1	1	1	twiner
Fab	<i>Glycine latifolia</i>			1							1	1	1	twiner
Fab	<i>Glycine microphylla</i>							1						twiner
Fab	<i>Glycine stenophita</i>				1									twiner
Fab	<i>Glycine tabacina</i>	1	1	1	1	1	1		1	1		1	1	twiner
Fab	<i>Hardenbergia violacea</i>				1				1			1	1	twiner
Fab	<i>Hovea longipes</i>				1									shrub
Fab	<i>Indigofera australis</i>			1					1			1	1	shrub
Fab	<i>Jacksonia scoparia</i>				1									small tree
Fab	<i>Kennedia rubicunda</i>		1									1	1	twiner
Fab	<i>Pultenaea microphylla</i>								1			1	1	shrub
Fab	<i>Pultenaea spinosa</i>							1	1	1	1			shrub
Fab	<i>Pultenaea sp.</i>												1	
Fab	<i>Rhynchosia minima</i>				1									twiner
Fab	<i>Senna artemisioides</i> ssp. <i>zygophylla</i>				1							1	1	shrub
Fab	<i>Swainsona galegifolia</i>				1							p	1	subshrub
Fab	<i>Templetonia stenophylla</i>				1								1	subshrub
Fab	<i>Zornia dyctiocarpa</i>												1	forb
Gen	<i>Centaurium spicatum</i> ( <i>Schenkia spicata</i> )	1	1											forb
Ger	<i>Erodium crinitum</i>												1	forb
Ger	<i>Gernaium solanderii</i>		1	1									1	forb
Goo	<i>Goodenia hederacea</i> subsp. <i>hederacea</i>		1				1		1					forb
Goo	<i>Goodenia pinnatifida</i>			1										forb
Goo	<i>Goodenia rotundifolia</i>							1						forb
Hal	<i>Haloragis serra</i>				1									forb
Lam	<i>Ajuga australis</i>	1		1	1	1			1		1			forb
Lam	<i>Mentha diemenica</i>	1												forb
Lam	<i>Mentha satuireiodes</i>			1										forb
Lam	<i>Salvia plebeia</i>				1									forb

Lau	<i>Cassytha</i> sp.							1							twiner
Lin	<i>Linum marginale</i>	1												1	forb
Lob	<i>Pratia purpurescence</i>	1		1	1				1	1	1				forb
Log	<i>Mitrasacme alsinoides</i>								1						forb
Lor	<i>Amyema bifurcatum</i>			1											Mistletoe
Lor	<i>Amyema cambagei</i>	1	1		1										Mistletoe
Lor	<i>Amyema gaudichaudi</i>				1										Mistletoe
Lor	<i>Amyema miquelii</i>				1										Mistletoe
Lor	<i>Amyema pendulum</i> var <i>pendulum</i>				1				1						Mistletoe
Lor	<i>Dendrophthoe vitellina</i>								1						Mistletoe
Lor	<i>Lysiana exocarpi</i> subsp <i>tenuis</i>	1			1										Mistletoe
Lor	<i>Lysiana linearifolia</i>	1													Mistletoe
Lor	<i>Muellerina eucalyptoides</i>								1						Mistletoe
Mal	<i>Hibiscus sturtii</i> var <i>sturtii</i>			1											subshrub
Mal	<i>Malvastrum coromandelianum</i>				1										forb
Mal	<i>Melhaniania oblongifolia</i>				1										subshrub
Mal	<i>Sida corrugata</i>	1	1	1	1		1				1		1	1	forb
Mal	<i>Sida cunninghamii</i>	1													subshrub
Mal	<i>Sida filiformis</i>	1													subshrub
Mal	<i>Sida hackettiana</i> - previously <i>Sida subspicata</i>													1	
Mal	<i>Sida trachopoda</i>	1													subshrub
Mim	<i>Acacia amblygona</i>	1	1		1		1	1	1		1	1	1	1	shrub
Mim	<i>Acacia bulgaensis</i> (2RC-)				1	1					1				small tree
Mim	<i>Acacia cultriformis</i>										1				shrub
Mim	<i>Acacia dealbata</i>			1											small tree
Mim	<i>Acacia decora</i>				1						1	1	1	1	shrub
Mim	<i>Acacia doratoxylon</i>	1													small tree
Mim	<i>Acacia falcata</i>	1	1		1		1	1	1	1	1	1	1	1	shrub
Mim	<i>Acacia filicifolia</i>			1											small tree
Mim	<i>Acacia gunnii</i>				1										shrub
Mim	<i>Acacia implexa</i>		1		1						1	1	1	1	small tree
Mim	<i>Acacia irrorata</i>													1	small tree

Mim	<i>Acacia melvillei</i>				1									shrub
Mim	<i>Acacia paradoxa</i>				1									shrub
Mim	<i>Acacia parvopinnula</i>								1	1	1		1	small tree
Mim	<i>Acacia pendula (E population)</i>				1	1					1			small tree
Mim	<i>Acacia pravifolia</i>				1									shrub
Mim	<i>Acacia salicina</i>	1	1	1	1						1	1	1	small tree
Myo	<i>Eremophila debilis</i>	1	1	1	1	1	1		1	1	1	1	1	forb
Myo	<i>Myoporum montanum</i>		1	1	1						1			shrub
Myr	<i>Angophora floribunda</i>	1		1	1	1					1	1	1	tree
Myr	<i>Corymbia maculata</i>							1	1	1	1	1	1	tree
Myr	<i>Eucalyptus albens</i>										1			tree
Myr	<i>Eucalyptus blakelyi</i>			1							1			tree
Myr	<i>Eucalyptus canaliculata</i>								1					tree
Myr	<i>Eucalyptus crebra</i>	1	1	1	1	1		1	1	1	1	1	1	tree
Myr	<i>Eucalyptus dawsonii</i>										1			
Myr	<i>Eucalyptus fibrosa</i>						1		1	1		1	1	tree
Myr	<i>Eucalyptus glaucina</i>									1	1			tree
Myr	<i>Eucalyptus melliodora</i>	1												tree
Myr	<i>Eucalyptus moluccana</i>	1	1	1	1	1		1	1	1	1	1	1	tree
Myr	<i>Eucalyptus punctata</i>										1			
Myr	<i>Eucalyptus tereticornis</i>	1	1	?					1	1	1	1	1	tree
Myr	<i>Kunzea ambigua</i>								1			1		shrub
Myr	<i>Melaleuca uncinatus</i>						1							shrub
Nyc	<i>Boerhavia dominii</i>				1									forb
Ole	<i>Jasminum suavissimum</i>				1									vine
Ole	<i>Notelaea microcarpa var. microcarpa</i>	1	1		1	1					1			shrub
Oxa	<i>Oxalis chnoodes</i>								1					forb
Oxa	<i>Oxalis exilis</i>			1					1					forb
Oxa	<i>Oxalis perennans</i>	1	1	1			1		1				1	forb
Oxa	<i>Oxalis radicata</i>				1									forb
Oxa	<i>Oxalis rubens</i>								1					forb
Phy	<i>Phyllanthus hirtellus</i>			1			1		1					shrub

Phy	<i>Phyllanthus virgatus</i>	1	1		1	1	1		1		1		1	forb
Pit	<i>Bursaria spinosa var. spinosa</i>	1	1	1	1	1		1	1	1	1	1	1	shrub
Pla	<i>Plantago debilis</i>	1	1	1	1		1		1				1	forb
Pla	<i>Plantago gaudichaudii</i>	1							1					forb
Pla	<i>Plantago hispida</i>				1									forb
Pol	<i>Persicaria lapathifolia</i>												1	forb
Pol	<i>Persicaria orientalis</i>												1	forb
Pol	<i>Rumex brownii</i>	1	1	1	1									forb
Pro	<b><i>Grevillea montana (2VC)</i></b>							1	1					shrub
Pro	<i>Hakea sericea</i>								1	1	1			shrub
Pro	<i>Persoonia linearis</i>								1					small tree
Pro	<b><i>Persoonia pauciflora (2E)</i></b>								1					small tree?
Ran	<i>Clematis aristata</i>	1			1									twiner
Rha	<i>Cryptandra amara</i>		1											shrub
Ros	<i>Acaena sp (agnipila?)</i>												1	forb
Rub	<i>Asperula conferta</i>	1		1	1				1					forb
Rub	<i>Canthium odoratum (Psydrax odorata)</i>				1									small tree
Rub	<i>Galium propinquum</i>			1										forb
Rub	<i>Galium sp.</i>		1											forb
Rub	<i>Opercularia aspera</i>	1	1				1							forb
Rub	<i>Opercularia diphylla</i>	1	1	1			1		1	1	1			forb
Rub	<i>Opercularia hispida</i>		1											forb
Rub	<i>Pomax umbellata</i>								1	1	1			forb
Rut	<i>Geijera parviflora</i>		1								1			small tree
San	<i>Choretrum sp. A</i>			1			1							small tree
San	<i>Exocarpus cupressiformis</i>		1											small tree
San	<i>Santalum lanceolatum</i>				1									small tree
Sap	<i>Dodonaea triquetra</i>							1						shrub
Sap	<i>Dodonaea viscosa</i>			1	1	1		1			1	1	1	shrub
Sap	<i>Dodonaea viscosa subsp. cuneata</i>										1			
Scr	<i>Veronica plebeia</i>	1	1	1	1		1		1					forb

Sol	<i>Nicotiana megalosiphon</i> subsp <i>megalosiphon</i>				1									sub
Sol	<i>Solanum brownii</i>									1				
Sol	<i>Solanum cinereum</i>	1	1	1	1	1				1	1	1		subshrub
Sol	<i>Solanum elegans</i>				1									subshrub
Sol	<i>Solanum opacum</i>	1	1											forb
Sol	<i>Solanum papaverifolium</i>								1					forb
Sol	<i>Solanum prinophyllum</i>						1	1	1	1			1	forb
Sol	<i>Solanum pungetium</i>	1	1											forb
Sta	<i>Stackhousia muricata</i>	1	1	1	1		1						1	forb
Sta	<i>Stackhousia viminea</i>				1		1	1	1	1				forb
Ste	<i>Brachychiton populneus</i> subsp <i>populneus</i>		1	1	1	1				1	1	1		tree
Thy	<i>Pimelea curviflora</i>				1									subshrub
Thy	<i>Pimelea curviflora</i> var <i>sericea</i>				1									subshrub
Thy	<i>Pimelea stricta</i>			1										shrub
Ver	<i>Verbena gaudichaudii</i>				1									subshrub
Vit	<i>Cayratia clematidea</i>						1							vine
Zyg	<i>Zygophyllum glaucum</i>				1									forb
	<b>Magnoliopsida (Flowering Plants) - Lilidae (Monocots)</b>													
Ant	<i>Arthropodium milleflorum</i>			1	1				1					forb
Ant	<i>Arthropodium</i> sp. B						1							forb
Ant	<i>Laxmania compacta</i>				1									forb
Ant	<i>Laxmannia gracilis</i>	1	1	1					1	1	1		1	forb
Ant	<i>Tricoryne elatior</i>	1	1											forb
Asp	<i>Bulbine bulbosa</i>				1									forb
Com	<i>Commelina cyanea</i>	1			1				1				1	forb
Cyp	<i>Carex fascicularis</i>				1									sedge
Cyp	<i>Carex inversa</i>	1	1	1	1								1	sedge
Cyp	<i>Cyperus enervis</i>	1	1										1	sedge
Cyp	<i>Cyperus gracilis</i>		1		1	1				1			1	sedge
Cyp	<i>Cyperus imbecilis</i>	1												sedge
Cyp	<i>Fimbristylis dichotoma</i>	1	1		1				1		1		1	sedge



Cyp	<i>Gahnia aspera</i>			1	1			1							sedge
Cyp	<i>Lepidosperma laterale</i>							1	1						sedge
Cyp	<i>Ptilothrix deusta</i>		1												sedge
Cyp	<i>Schoenus apogon</i>												1		sedge
Cyp	<i>Scleria mackaviensis</i>				1								1		sedge
Iri	<i>Hypoxis hygrometrica</i>												1		forb
Jun	<i>Juncus subsecundus</i>			1											sedge
Lom	<i>Lomandra bracteata</i>				1										forb
Lom	<i>Lomandra confertifolia</i>		1				1	1							forb
Lom	<i>Lomandra confertifolia</i> subsp. <i>pallida</i>	1	1		1										forb
Lom	<i>Lomandra filiformis</i> subsp. <i>filiformis</i>	1	1	1	1		1	1	1						forb
Lom	<i>Lomandra glauca</i>	1													forb
Lom	<i>Lomandra longifolia</i>								1						forb
Lom	<i>Lomandra multiflora</i> var. <i>multiflora</i>	1	1	1	1	1	1	1	1	1	1		1		forb
Luz	<i>Geitonoplesium cymosum</i>				1										vine
Orc	<i>Calochilus</i> sp.				1										orchid
Orc	<i>Diuris punctata</i> var. <i>punctata</i>								1						orchid
Orc	<i>Microtis unfolia</i>				1										orchid
Orc	<i>Pterostylis curta</i>				1										orchid
Orc	<i>Pterostylis cycnocephala</i>								1						orchid
Pho	<i>Dianella caerulea</i> var. <i>caerulea</i>	1	1	1	1				1						forb
Pho	<i>Dianella caerulea</i> var. <i>producta</i>	1													forb
Pho	<i>Dianella longifolia</i> var. <i>longifolia</i>	1	1	1	1				1						forb
Pho	<i>Dianella revoluta</i> var. <i>revoluta</i>				1				1	1	1				forb
Poa	<i>Aristida benthamii</i> var. <i>benthamii</i>		1												grass
Poa	<i>Aristida calycina</i> var. <i>calycina</i>		1												grass
Poa	<i>Aristida lignosa</i>								1						grass
Poa	<i>Aristida personata</i>		1	1											grass
Poa	<i>Aristida ramosa</i>	1	1	1	1	1		1	1		1	1	1		grass
Poa	<i>Aristida</i> sp.												1		grass
Poa	<i>Aristida vagans</i>	1	1	1	1		1	1			1				grass
Poa	<i>Austrodanthonia auriculata</i>	1													grass

Poa	<i>Austrodanthonia bipartita</i>				1									grass
Poa	<i>Austrodanthonia caespitosa</i>		1											grass
Poa	<i>Austrodanthonia fulva</i> ( <i>Rytidosperмум</i> )			1	1			1	1			1	1	grass
Poa	<i>Austrodanthonia racemosa</i> var. <i>racemosa</i>				1									grass
Poa	<i>Austrodanthonia richardsonii</i>			1										grass
Poa	<i>Austrodanthonia setaceae</i>		1											grass
Poa	<i>Austrodanthonia tenuior</i>	1	1				1		1					grass
Poa	<i>Austrostipa aristiglumis</i>				1									grass
Poa	<i>Austrostipa scabra</i>	1	1	1	1	1	1				1	1	1	grass
Poa	<i>Austrostipa trichophylla</i>		1											grass
Poa	<i>Austrostipa verticillata</i>	1	1	1	1		1					1	1	grass
Poa	<i>Bothriochloa biloba</i> ( <i>V-delisted</i> )		1		1									grass
Poa	<i>Bothriochloa dicepiens</i>	1	1	?	1	1	1		1			?	1	grass
Poa	<i>Bothriochloa macra</i>	1			1				1			?	1	grass
Poa	<i>Bothriochloa</i> sp.												1	grass
Poa	<i>Chloris divicariata</i>	1	1				1						1	grass
Poa	<i>Chloris truncata</i>		1		1							1	1	grass
Poa	<i>Chloris ventricosa</i>	1	1	1	1	1					1		1	grass
Poa	<i>Cymbopogon refractus</i>	1	1	1	1	1	1	1	1		1	1	1	grass
Poa	<i>Cynodon dactylon</i>		1	1	1								1	grass
Poa	<i>Dichanthium sericeum</i>	1	1	1	1							?	1	grass
Poa	<i>Dichelachne inaequiglumis</i>	1												grass
Poa	<i>Dichelachne micrantha</i>				1				1					grass
Poa	<i>Digitaria breviglumis</i>	1												grass
Poa	<i>Digitaria brownii</i>				1									grass
Poa	<i>Digitaria diffusa</i>			1	1									grass
Poa	<i>Digitaria parviflora</i>								1					grass
Poa	<i>Echinochloa colona</i>												1	grass
Poa	<i>Echinopogon caespitosus</i> var. <i>caespitosus</i>							1	1	1				grass
Poa	<i>Echinopogon ovatus</i>		1		1				1					grass
Poa	<i>Elymus scaber</i>	1												grass
Poa	<i>Entolasia marginata</i>								1					grass

Poa	<i>Entolasia stricta</i>								1	1	1		1	grass
Poa	<i>Eragrostis brownii</i>			1	1				1			1	1	grass
Poa	<i>Eragrostis leptocarpa</i>								1					grass
Poa	<i>Eragrostis leptostachya</i>	1		1	1	1			1		1	1	1	grass
Poa	<i>Eragrostis molybdea</i>				1									grass
Poa	<i>Eriochloa procera</i>												1	grass
Poa	<i>Eriochloa pseudoacrotricha</i>		1											grass
Poa	<i>Heteropogon contortus</i>				1									grass
Poa	<i>Microlaena stipoides var. stipoides</i>	1	1	1	1	1			1	1	1	1	1	grass
Poa	<i>Notodantonia longifolia</i>				1									grass
Poa	<i>Panicum effusum</i>				1			?	1			1	1	grass
Poa	<i>Panicum simile</i>		1	1										grass
Poa	<i>Panicum subxerophilum</i>				1									grass
Poa	<i>Paspalidium aversum</i>								1					grass
Poa	<i>Paspalidium distans</i>	1	1						1	1	1		1	grass
Poa	<i>Paspalidium gracile</i>	1	1		1									grass
Poa	<i>Sporobolus creber</i>	1	1	1	1	1			1		1		1	grass
Poa	<i>Themeda australis</i>			1	1				1	1	1	1	1	grass
Poa	<i>Tragus australianus</i>				1									grass
Xan	<i>Xanthorrhoea acaulis</i>				1									subshrub

**Appendix 3: SWEP analysis of treatments.** See section 3.3. Three blocks of nine treatments were analysed for micro and macronutrients.

**SEE ATTACHMENT**