

**School of Molecular and Life Sciences**

**Centre for Mine Site Restoration**

**Mine Site Restoration - Maximisation of Topsoil in Restoration of  
Semi-arid Lands**

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**Declaration**

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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## **Abstract**

Over the last fifty years, global mining production has doubled. Increasing societal and governmental expectations have placed greater pressure on mining companies to restore land to pre-mining conditions once mining is completed. The process of ecological restoration can be costly, take significant time and be wrought with challenges. One of these challenges is the availability of topsoil. Topsoil deficits often occur when the surface area of post-disturbance landforms exceeds the pre-mined surface area. Secondary materials produced during mining operations such as waste rock, are commonly added to topsoil to bulk up the volume of material available for restoration. Although this procedure is common practice, little is known about whether the practice facilitates or is deleterious to restoration capability.

To address this knowledge gap the following research questions were investigated:

1. How does the addition of waste rock to topsoil cover mixes influence physical characteristics (e.g. soil water content);
2. How does the addition of waste rock to topsoil cover mixes influence chemical characteristics (e.g. salinity);
3. What are the physiological responses of plants to topsoil mixes incorporating greater than 25% waste rock; and
4. What effect does the inclusion of waste rock have on early seedling recruitment and plant establishment?

This study analysed the physical and chemical properties of soil mixes incorporating 25%, 50%, 75% and 88% waste rock and tested the influence of these properties on plant growth and development (Chapter 2) and early seedling growth (Chapter 3). Overall our results indicate that the incorporation of waste rock changes physical and chemical properties of soil mixes, which can have complex effects on plant development and seedling emergence depending on waste rock percentage. We showed increasing waste rock content to be positively correlated with soil salinity which caused declines in early seedling emergence. Increasing waste rock content also lowered soil water availability and this had a negative effect on the growth and development of established plants up to percentages of 75%. Amendment of topsoil with 88% waste rock had negligible effect on plant growth. Although dilution of topsoil with waste rock reduced soil water content, incorporation of waste rock improved soil water conservation during times of water deficit, prolonging plant survival. This study helps to enhance knowledge about the importance of considering waste rock content in topsoil mixes and it is expected that the results will facilitate optimisation of restoration techniques in Australia and elsewhere.

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## **Thesis structure**

This thesis is presented in the form of a ‘traditional thesis’ in accordance with Curtin University *Guidelines for Thesis Preparation*. The two core chapters of this thesis have been prepared for publication in peer-reviewed journals, in each case following the conventions of the relevant journal, except for minor changes to achieve consistency in formatting between chapters. Chapter 1 provides a general introduction to the thesis and Chapter 4 presents an overall discussion of it.

## **Chapter 1: General Introduction**

Chapter One introduces the concept of soil cover preparation in the restoration of lands disturbed by mining and the conventional practice involving mixing topsoil and waste rock substrate. It provides a synthesis on what is known of waste rock and its effect on the chemical and physical properties of substrates based on current literature. The review concludes with a summary of the potential implications for plant development and early seedling growth, and presents the research objectives of this thesis.

### **Overview of mine restoration**

Over the last fifty years, global mining production has doubled (Reichl and Schatz 2019). The extraction and processing of natural resources is now one of the main contributors to land-use related biodiversity loss worldwide (Oberle et al. 2019). In Western Australia 17% of the states total land mass is potentially open to mining with 44.2 million hectares of land under mining tenements in 2017 (DMIRS 2018). In consideration of this scale of disturbance, mining and other extractive industries are increasingly required to rehabilitate<sup>1</sup> or restore<sup>2</sup> land upon conclusion of mineral extraction and cessation of mining activities.

According to McDonald et al. (2016) restoration comprises the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. Reconstruction of a stable land surface followed by re-establishment of vegetation is generally the first step in creation of functional ecosystems post-mining (Festin et al. 2019). Restoration of landscapes after mining disturbance can pose substantial challenges, however (Festin et al. 2019), and despite significant effort and investment to restore post-mining landforms worldwide (Bullock et al. 2011), there is limited evidence of successful restoration in areas that have been subject to large-scale mining (James et al. 2013, Environmental Protection Authority 2014, Stevens and Dixon 2017).

One of the challenges in mine restoration is the availability of topsoil (i.e. the upper 5–10 cm of the soil profile). Covering disturbed areas with topsoil is a common technique used for restoring post-mining landscapes (Rokich et al. 2000, Golos and Dixon 2014, Golos et al. 2019). Numerous studies have highlighted natural topsoil as being the best substrate for plant development in post-mining ecological restoration as it provides a source of propagules via the soil seed bank (Merino-Martín et al. 2017) in addition to favourable chemical, physical

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<sup>1</sup> Rehabilitation is defined as ‘direct or indirect actions with the aim of reinstating a level of ecosystem functionality where ecological restoration is not sought, but rather renewed and ongoing provision of ecosystem goods and services’ (McDonald et al. 2016).

<sup>2</sup> Restoration is defined as ‘the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed’ (McDonald et al. 2016). The term restoration is used in this thesis as it encompasses the process of rehabilitation and is more established in literature.



and microbiological properties (Kumaresan et al. 2017, Cross et al. 2018, Kneller et al. 2018). Unfortunately topsoil deficits are a common problem encountered in the global mining sector, because the surface area of post-disturbance landforms such as tailings storage facilities and waste rock dumps often exceeds that of the pre-mined surface (Ghose 2001). One of the procedures used to overcome topsoil shortages is the inclusion of secondary materials, such as waste rock, into the topsoil mix. This bulks up the volume of substrate available for use and allows topsoil to be spread across a larger area (Machado et al. 2013, Muñoz-Rojas et al. 2016a).

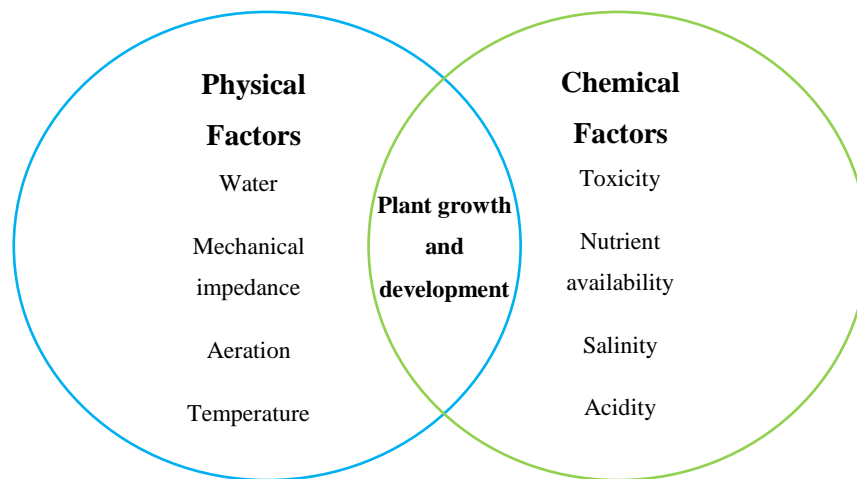
Waste rock is the residuum of mineral processing activities and consists of coarse, broken, partly weathered rock (Blight 2010a). Waste rock fragments are commonly heterogeneous in size and shape, and exist within a matrix of finer particles (<2 mm). Unlike topsoil, the availability of waste rock is unconstrained. Open-cut mines need to remove large volumes of overburden and/or waste rock to access the mineral resource (Golos 2013), and stripping ratios can be as high as 3:1 (waste rock:ore) (Ecologia Environment 2008). Previous studies explore implications associated with topsoil mixes incorporating 25% waste rock in the surface cover layer (3:1 rock:topsoil) (Merino-Martín et al. 2017, Golos et al. 2019). Although it is common practice for mining operations to utilise waste rock percentages greater than 50% (Tiemann 2015). Little is known whether this practice facilitates or is deleterious to restoration capability. For this reason it is important to understand how waste rock content influences physio-chemical characteristics of soil mixes, in order to better guide restoration outcomes.

### **Influence of rock on physical and chemical characteristics**

The list of factors known to affect plant growth is diverse and numerous and as such is beyond the scope of this thesis. Instead, attention is paid to physical and chemical properties that are most influenced by the presence of rock (including both natural rock fragments and waste rock fragments) with a focus on examining the current state of research about how changes in these properties can affect plant development and early seedling growth.

#### *Soil moisture*

Of the edaphic factors that affect plant growth water is the most important (Kirkham 2005) (Figure 1). In Western Australia, the majority of mining operations are located in arid/ semi-arid environments where the availability of water is a major limiting factor to plant establishment (Rodriguez-Iturbe and Porporato 2004, Wang et al. 2017). Primarily this is a result of these environments receiving limited rainfall (less than 250mm per annum) (Muñoz-Rojas et al. 2016b) and most of precipitation being lost to evaporation (Bell 2001, Helfer et al. 2012).



**Figure 1.** Some chemical and physical factors affecting plant growth and development (Kirkham 2005, Gregory and Nortcliff 2013)

In general, plants respond to water stress through a wide range of physiological responses. Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata and alteration of photosynthesis (Kramer and Boyer 1995). Leaf water potential is the thermodynamic expression of water status of leaves, and can be used to provide indication of changes in plant tissue water status (Gregory and Nortcliff 2013). Stomata close in response to either a decline in leaf turgor and/or water potential (Maroco et al. 1997). Photosynthesis often decreases in parallel with or more than stomatal conductance, as closure of the stomata causes a decrease in internal CO<sub>2</sub> concentrations (Bhattacharjee and Saha 2014). Because plant growth is reliant on photosynthesis for cell division and enlargement, water stress directly reduces growth by decreasing CO<sub>2</sub> assimilation (Bhattacharjee and Saha 2014).

Rock fragments<sup>3</sup> (>2 mm diameter) are known to have important effects on hydrological processes (Brakensiek and Rawls 1994). The influence of rock fragments on hydrological processes is dependant on several parameters: (i) the origin and porosity of the rock fragments, (ii) the size of the rock fragments, (iii) the position of the rock fragments and finally (iv) the volumetric percentage of the rock fragments (Cousin et al. 2003). Poesen and Lavee (1994) compared water holding capacity of over 10 different types of rocks and found water retention properties varied based on origin. For example water content of chalk at saturation was reported to be 90% whereas water content of flintstone was reported to be 0.2%.

Several studies have shown that water held within or on rock fragments can account for a significant part of the soil water reserves in soils (Gras and Monnier 1963, Poesen and Lavee

<sup>3</sup> Rock fragments are defined by as unattached pieces of rock 2 mm in diameter or larger that are strongly cemented or more resistant to rupture (Soil Science Society of America 2020)

1994, Tetegan et al. 2015). However whether or not plants can access these reservoirs is still a subject of study. Korboulewsky et al. (2020) recently showed that water stored in porous limestone rock fragments is available and is used by plants. They supported this finding with evidence that (i) plants grown in porous limestone pebbles compared to nonporous pebbles (quartz) exhibited less water stress (evidenced by higher leaf stomatal conductance) and that this observation was more significant as rock content increased. Korboulewsky et al. (2020) hypothesised that this water could have been accessed by plants using either direct (plant roots directly access water from pores of rock) or indirect (where water moves from the rocky phase to the fine soil phase) methods. A higher plant root:shoot ratio for plants grown on low porous rock contents (20%) compared to non-porous rock supported the direct access theory however they found no significant difference in plant root:shoot at higher rock contents (40%). Bornyasz et al. (2005) and Querejeta et al. (2006) suggest that plant roots are confined to the fractures of rock and physically restricted from the matrix micropores (in this case weathered granitic bedrock) and that as a result, water reservoirs within rock can only be accessed via symbiotic interactions between plant and fungi (specifically Mycorrhizae). Here they showed Mycorrhiza initiate hyphal extension into rock micropores, providing a mechanism for water transport between fracture-confined roots and water reservoirs.

The size of rock fragments can also influence dynamics of water movement. Katra et al. (2008) observed the effects of rock fragment size on water redistribution following rainfall. They found that large, rock fragments (75-100mm) affect the water redistribution more than small ones (50-75mm). This is because there is better absorption and retention of water in the soil under relatively large rock fragments. This outcome was surmised to be an effect of (i) the positive relationships with the amount of water encountering the rock fragment, so that larger rock fragments enabled larger amounts of stone flow to reach the soil beneath them (Lavee and Poesen 1991) and (ii) the lower periphery effect (i.e., the effect of the ratio between the periphery and the area of the rock fragment) on moisture losses associated with large than with small rock fragments (Katra et al. 2008). Zhou et al. (2009, 2011) studied the effect of rock size on water movement and found that that water transport through the soil:rock matrix was more inhibited as rock fragments increased in size for a given rock fragments content. Similarly, De Figueiredo and Poesen (1998) found that infiltration of water through the soil profile was greatest in soil with smallest rock fragments, which is a consequence of the tortuosity and increasing discontinuity of water flow with larger rock fragments.

The position of rock fragments, covering the soil surface or within the soil is also important. Rocks reduce the volume of soil exposed at the soil surface and therefore evaporation via capillary loss (Unger 1971b, Ingelmo-Sanchez et al. 1980, Van Wesemael et al. 1996) and it has been shown that a 5 cm gravel mulch can reduce the annual evaporation by 85% (Kemper

et al. 1994). Katra et al. (2008) found that rock fragments lying on and embedded in the surface of the soil increased interception of water, evidenced by soil moisture contents under rock fragments being higher than in bare soil areas on the first day after rainfall. However comparison of soil moisture content with each rock position showed that water was retained for longer around embedded rock fragments in comparison to fragments lying on this surface. This effect hypothesised to be a consequence of greater contact of embedded rock to the soil which protected the soil from water loss caused by wind and solar radiation (Katra et al. 2008). Preisler et al. (2019) surmised that survival of Aleppo pine was greater during extended periods of drought on soils where rock fragments were present both within and on top of the soil surface. This finding was supported by Aleppo pine trees on rocky soils having higher summer pre-dawn leaf water potential ( $\Psi_{pd}$ ) than those on non-rocky soils (Preisler et al. 2019).

With regards to volumetric content, an increase in rock content is generally associated with a decline in water content, particularly when the water retention of rock is less than soil (Mi et al. 2016). This effect can lead to plants transpiring less. Mi et al. (2016) reported a 18% and 34% reduction of mean daily transpiration of plants in the soils with 30 and 50% rock fragment contents respectively. Reductions in transpiration are synonymous with declines in stomatal conductance, and these responses can coincide with a decrease in biomass accumulation (Mi et al. 2016). The presence of rocks in soil can also alter the movement of water, and these feedbacks can vary depending on rock content (Zhang et al. 2016). When rock fragment content is low, rock fragments most likely reduce the available area for water flow which increases the tortuosity of the water flow, resulting in lower hydraulic conductivity (whereby the time it takes for water to infiltrate all soil pores is longer) (Magier and Ravina 1984, Brakensiek and Rawls 1994, Novák et al. 2011). In contrast, the space (or voids) between rock fragments and surrounding soil is increased as rock content increases, creating preferential flows which can increase hydraulic conductivity (Cerdà 2001, Shi et al. 2008, Zhou et al. 2009). For example (Zhou et al. 2011) showed hydraulic conductivity to decline up to rock fragment content of <40% (v/v) and increase when rock fragment content reached >40%.

These findings suggest that the influence of waste rock on hydrological processes is likely to have implications for plant growth and development in that (i) availability of water may vary depending on the volumetric percentage of rock fragments and rock size, and (ii) varying ranges of rock content and sizes lead to different levels of continuity in water flow, causing a mosaic-like pattern of water distribution. The potential for rocks to promote water conservation may be especially beneficial in restoration of semi-arid environments where water is a major limiting factor to plant growth. Here the potential for rocks to reduce evaporation and provide a reservoir of water may prolong plant survival in times of water deficit.

### *Mechanical impedance*

Secondary to water availability, mechanical impedance can be a significant physical constraint to plant development and growth (Gregory and Nortcliff 2013). Mechanical impedance exists in the form of soil compaction which is usually exhibited by a high bulk density or soil strength (Stirzaker et al. 1996, Benigno et al. 2012). Soil compaction creates unfavourable growing conditions for plant roots as supplies of oxygen, water and nutrients are reduced (Houlbrooke et al. 1997). Results of previous studies suggest that soils containing rock are less susceptible to compaction. Saini and Grant (1980) and Magier and Ravina (1984) found rock fragments prevented soil from being compacted either; at the soil surface by acting as a mulch or in the soil by acting as a skeleton. Total bulk density of soil and rock mixes is dependent on the amount of rock fragments (Danalatos et al. 1995b). Poesen and Lavee (1994) showed that total soil bulk density reached a maximum when gravimetric rock fragment content reached approximately 40–50%, beyond which it decreased. However, increases to total soil bulk density following incorporation of rock do not necessarily indicate a poor environment for root growth. This is because there is no increase in bulk density of the soil fraction (in between the rocks) and thus plant root growth related to the soil fraction should remain unaffected (Stewart et al. 1970, Poesen and Lavee 1994).

Merino-Martín et al. (2017) evaluated soil strength in topsoil mixes with and without waste rock, reporting that surface soil strength was higher in topsoil mixes containing 25% waste rock and this coincided with lower seedling emergence. They also found that, drivers of seedling emergence were hierarchical and multivariate analysis revealed that surface soil moisture had a stronger influence on seedling emergence than soil strength (Merino-Martín et al. 2017). In contrast Golos et al. (2019) reported soil surface impedance to be lower in topsoils containing rock compared with topsoil without rock, and appeared to promote greater opportunity for seedling emergence. Valentin (1994) observed that rock fragments protect the soil surface against raindrop impact forces and thus prevent soil surface sealing and crusting. These results suggest that although rocks change the physical characteristics of soil mixes, these changes might not be deleterious to plant growth and development.

### *Nutrient supply*

The availability of mineral nutrients to plants in soil and rock mixes is dependent on water availability (Gregory and Nortcliff 2013). This is because soil water plays an important role in every process of nutrient acquisition, including nutrient mobilisation from the solid phase and translocation to shoot and root systems (Kautz et al. 2013). Consequently, lower water availability decreases nutrient use efficiency in plants, resulting in reduced growth (Vitousek and Farrington 1997). Where the presence of rocks changes hydraulic properties of soils, it can also be expected to influence nutrient availability (Carrick et al. 2013).

The presence of rock fragments also decreases the soil volume for nutrient supply (Childs and Flint, 1990). Kneller et al. (2018) found levels of total organic carbon and total nitrogen to be significantly lower when topsoil and waste rock was mixed in proportions of 50:50 (%). Similarly, Muñoz-Rojas et al. (2016b) reported availability of nitrogen and organic carbon to reduce by more than 50% in topsoil and waste rock proportions of 50:50 (%) and 25:75 (%). Although rock fragments are regarded as a potential reservoir and source of available nutrients for plant growth (Tetegan et al. 2015), evidence suggests that this resource can only be exploited via symbiosis with microorganisms (such as ectomycorrhizal fungi and bacteria) which facilitate nutrient absorption and release from rocks (Bornyasz et al. 2005, Adeleke et al. 2012, Wu et al. 2017). Coleman et al. (1983) and Reddell and Milnes (1991) noted the absence of soil fauna and microflora in mine waste rock to be major limitation to successful vegetation establishment as a result of diminished plant nutrition and nutrient cycling processes. The presence of rocks in soils is, therefore, likely to adversely affect plant development by restricting the nutritional capacity of the soil (Poesen and Lavee 1994).

#### *EC, pH, carbon and toxic metals*

Merino-Martín et al. (2017) studied differences in physical and chemical traits between topsoil, waste rock and topsoil mixes with 25% waste rock and their influence on seedling emergence. They found seedling emergence to differ when physical traits of substrates were similar, suggesting that chemical traits (i.e. EC, pH, soil organic carbon, sodium and trace elements) also played a role in influencing seedling emergence. Which of these traits is the most influential remains an open question. Metals released from iron ore bodies (Co, Cu, Mn and Zn) play key physiological functions in plants (Nagajyoti et al. 2010) and can influence seedling metabolism, resulting in deficiency symptoms and potentially affecting seedling emergence. In addition, soil organic carbon is known to alter hydrological properties (e.g., available water content) (Hudson 1994) and this effect has previously been linked with declines in seedling recruitment on topsoil:waste rock mixes where waste rock substrates are highly deficient in soil organic carbon (Muñoz-Rojas et al. 2016b).

Sodium content and soil electrical conductivity are key factors influencing seed germination and seedling survival (García-fayos et al. 2000). Notably, salinity is a common feature of semi-arid and arid regions world-wide (Jordán et al. 2004, DPIRD 2019a) and in Western Australia, the majority of mining operations occur in these regions. Here, salt in the soil is mostly derived from the weathering of rocks and primary minerals, which is formed in situ or transported by water or wind (Allbed and Kumar 2013).

If saline soils have the potential to occur in semi-arid and arid areas then adding waste rock could potentially affect salt transfer processes. This is because the accumulation of salts is

influenced by water movement (Corwin et al. 2007, Rengasamy 2010). More specifically, to prevent the accumulation of excessive salts in soils, enough water must pass through the root zone to leach soluble salts (Corwin et al. 2007). If adding rock to soil has the potential to affect water movement by either increasing or decreasing the flow of water through soil, as discussed previously, then its presence may also affect the movement of salts.

### **Effects on rock on early seedling growth and plant establishment**

Existing literature suggests that the presence of rocks is likely to compromise plant establishment by limiting the supply of nutrients required for plant growth. Numerous studies have confirmed this effect. Cross et al. (2018) reported that root development was reduced by around 50% in 100% waste rock mixes, an effect they associated with decreased availability of nitrogen and organic carbon. Similarly, Muñoz-Rojas et al. (2016b) and Kneller et al. (2018) showed that seedling recruitment on topsoil mixes incorporating waste rock can be challenged, primarily due to lower availability of organic carbon, which diminishes water holding capacity. Du et al. (2017) observed that increasing rock fragment content adversely affected above ground biomass in alpine steppe vegetation, and identified negative correlations between increasing rock content and soil organic carbon.

Early seedling growth is expected to be reduced by rock content as a result of lower water availability. At 25% waste rock content, Golos et al. (2019) found that seedling emergence was improved >2.5-fold as a result of sub-soil moisture being retained for longer periods. Merino-Martín et al. (2017) found similarities in hydrological properties (e.g. soil moisture, hydraulic conductivity, infiltration rate) supported no significant difference in emergence between topsoil and topsoil incorporating 25% waste rock. At 100%WR content Merino-Martín et al. (2017) observed low soil moisture content to cause decline in seedling emergence, suggesting that increasing waste rock can adversely affect seedling emergence as a result of declining water content. The effect of rock fragments on water availability for plants should also affect plant development. Mi and Liu (2016) found plant height, basal stem diameter and biomass did not differ significantly between soil rock contents of 0% and 30% but were lower at 50%. Here the decline in plant biomass at 50% was explained by decreases in plant water consumption as a result of decreased water content and inaccessibility of the rock matrix to roots (Mi et al. 2016). This implies that beyond an optimal rock fragment content, plant development is adversely affected.

Overall these findings indicate that increasing waste rock has the potential to decrease plant growth, primarily as a result of limitations to nutrient and water availability. This effect may be disadvantageous to plant development because in these soil conditions, plants tend to take longer to reach reproductive stages such as flowering and seed production (Gregory and

Nortcliff 2013). At the same time, plants that grow faster are also more susceptible to water stress. A plant will suffer when its water demand is greater than the amount of water available in the soil (Gregory and Nortcliff 2013). This then raises the question of whether the presence of waste rock increases plant survival under drought induced conditions as a result of either: i) reduced plant size which reduces plant water requirements, or ii) the potential for rock to improve the conservation of water. In semi-arid and, arid areas, where water is the most limiting factor to plant development, the effect of rock on water conservation and its influence of plant survival, are particularly relevant. Notably, the author could not find any studies that tested the affect of waste rock on plant survival under simulated drought conditions. This suggests that there is a significant gap in this area of knowledge.

Movement of water, and subsequently movement of salts, should also be affected by rock content. A review of existing literature found no studies have been reported on this area of study. With these gaps in knowledge in mind, it is important that further research is conducted to improve our understanding of how addition of waste rock to saline soils affects the restoration trajectory.

### **Rationale, aim and thesis outline**

The overarching objective of this research was to optimise principles and techniques currently used for restoration of landscapes disturbed by mining activity in semi-arid regions. Importantly, the results of this research have the potential to be applicable to restoration of semi-arid regions, not only in Australia, but also across the globe.

This thesis intends to contribute to addressing significant knowledge gaps in mine restoration by breaking new ground in the study of the effect of waste rock content on physio-chemical characteristics and, vegetation establishment. The influence of waste rock content on soil water availability and salt transfer process is particularly relevant given saline soils and water availability are two factors known to affect vegetation establishment in semi-arid and arid areas.

In pursuit of addressing the previously highlighted knowledge gaps, two research questions were posed:

1. How do soil water dynamics of topsoil:waste rock mixes incorporating 25%, 50%, 75% and 88% waste rock influence plant growth and physiology (Chapter 2); and
2. How do physio-chemical properties of topsoil:waste rock mixes incorporating 25%, 50%, 75% and 88% waste rock influence early seedling growth (Chapter 3).

These questions were examined through the testing of seven hypotheses:



1. Water content is greatest in the topsoil fraction when compared with waste rock fraction (Chapter 2);
2. The addition of waste rock would have no negative impact on plant growth and development at low percentages (up to 25%) (Chapter 2);
3. At high percentages (>25%), increasing rock fraction will begin to restrict plant access to water, causing a negative impact to plant growth and development (Chapter 2);
4. That salinity levels of topsoil:waste rock mixes will increase as waste rock content is increased (Chapter 3);
5. that there will be a negative relationship between germination and early seedling growth of *Acacia* species and levels of soil salinity (Chapter 3);
6. That the effect of salt on germination and early seedling growth can be separated into osmotic and specific (Chapter 3); and
7. That sensitivity to salt stress in seed germination and seedling development is less pronounced for a broadly distributed species than a narrowly distributed species (Chapter 3).

In answering the research questions, this thesis highlights the implications of restoring post-mining areas with topsoil mixes amended with >25% waste rock. It first builds an understanding of how and why waste rock is used in mine site restoration and identifies several changes to physio-chemical properties that occur with the presence of waste rock and how this may potentially impact plant development and early seedling growth. Secondly, it shows that increasing waste rock content has a variable effect on plant growth and development, but that it can alter patterns of plant water use under simulated drought, prolonging plant survival. Thirdly, we that show increasing waste rock content is associated with accumulation of salt, and that, while native species exhibit some tolerance to salt, these increases in salinity lead to reduced seedling emergence. Finally, the in-depth discussion of the overall findings of the thesis brings to light further directions for research and, its significance and broader relevance to restoration practice.

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## **Chapter 2: High rock content enhances plant survival under drought in saline topsoils**

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## **Abstract**

Mining represents a major disturbance with cumulative impacts leading to large areas requiring restoration. Success of mine site restoration in semi-arid regions is limited by availability of topsoil and water. Topsoil shortages are frequently encountered when post-mining areas exceed the pre-mined surface area and hot, dry summers prolong periods of water deficits. Additionally, saline soils are a common feature of these regions. We examined a novel means to maximise topsoil effectiveness through the amendment of saline topsoil with waste rock sourced from an iron mine in semi-arid Western Australia. We tested the growth and development of a species with known salt tolerance on saline topsoil incorporating between 25% and 88% waste rock. Hydrological properties of each of the different topsoil mixes were examined to determine how waste rock content affects plant water relations and if it is a limiting factor to plant growth and development.

In well-watered conditions the addition of waste rock lowered the volumetric water content of the total soil mix, causing a reduction in stomatal conductance of the test species *A. saligna*. In drought conditions the lower rate of water loss in the presence of waste rock allowed stomatal conductance to be maintained over a longer period. The effect of waste rock on growth and development of *A. saligna* varied depending on waste rock content. Final biomass of *A. saligna* decreased with increasing waste rock content up to percentages of 75%, beyond which it increased. These results show that addition of waste rock to topsoil has complex effects on plant performance. Altered patterns of plant water use under drought can enhance survival despite lower water availability in rock-amended topsoil. Through our study we demonstrate that waste rock can be used effectively to optimise limited topsoil resources in mine restoration of semi-arid areas.

## **Introduction**

Since 1984 total mining production globally has increased by 60% (IOCWMC 2019). In Western Australia 17% of the states total land mass is potentially open to mining with 44.2 million hectares of land under mining tenements in 2017 (DMIRS 2018). The potential for mining to disturb large areas of land has in recent years, placed greater importance around the need for ecological function and structure to be restored (McDonald et al. 2016, DISI 2017) with restoration being defined as ‘the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed’ (McDonald et al. 2016). Restoration of mining areas faces a number of significant challenges, however.

One of these challenges is the availability of topsoil. Covering disturbed areas with topsoil (i.e. the upper 5–10 cm of the soil profile) is a common technique used for restoring post-mining landscapes (Rokich et al. 2000, Golos and Dixon 2014, Golos et al. 2019). Natural



topsoil is a key resource that provides the most effective means of re-establishing vegetation as it is a better source of propagules and has higher water holding capacity than subsoil or crushed waste rock (Muñoz-Rojas et al. 2016b, Merino-Martín et al. 2017) in addition to favourable chemical, physical and microbiological properties (Kumaresan et al. 2017, Cross et al. 2018, Kneller et al. 2018). Unfortunately topsoil deficits are a common problem encountered in the global mining sector. This is because the surface area of disturbed land and post-mining landforms such as tailings storage facilities and waste rock dumps often exceeds the pre-mined surface area (Ghose 2001). One of the procedures used to overcome topsoil shortages is the inclusion of inert waste materials produced during mining into the topsoil mix, such as waste rock. Waste rock arises as coarse, broken, partly weathered rock (Blight 2010b), heterogeneous in size and shape and often existing in a matrix of finer particles (<2 mm). The amendment of topsoil substrates with waste rock allows topsoil to be spread across a larger area (Machado et al. 2013, Muñoz-Rojas et al. 2016b) and facilitates improved stability of landform slopes (Howard 2016). A general approach when restoring landforms is to deliver topsoil and waste rock to the tipping edge (such as the top of a waste rock dump) and use machinery to push topsoil and waste rock across post-mining surfaces, allowing for mixing of substrates (Tiemann 2015). However little is known whether such a practice facilitates or negates restoration capability.

For restoration to be successful it is imperative that substrates used in topsoil mixes demonstrate competency to support plant growth. Of the four soil physical factors that affect plant growth (mechanical impedance, water, aeration and temperature) water availability is often the most important (Kirkham 2005). Long-term deficits in water supply can be highly deleterious to plant growth (Gregory 2013). This is particularly the case in arid and semi-arid environments where rainfall is limited (less than 250mm per annum) (Rodríguez-Iturbe, I. Porporato 2007, Muñoz-Rojas et al. 2016b, Wang et al. 2017) and evaporative loss of soil moisture is high (Bell 2001, Helfer *et al.* 2012). The presence of rock fragments in soils significantly affects hydrological functions such as water storage, infiltration and evaporation and soil hydraulic properties such as hydraulic conductivity and water retention (e.g. Van Wesemael et al. 1996, Novák et al. 2011, Tetegan et al. 2015). Several studies have observed a covering of rock fragments to contribute to a reduction in evaporation (e.g. Unger 1971, Van Wesemael et al. 1996, Tetegan et al. 2015) and others have suggested that rock fragments in soils could promote water conservation in arid and semi-arid regions due to greater availability of water at low moisture contents (Gras and Monnier 1963, Poesen and Lavee 1994). As far as we know only one study has assessed differences in hydrological characteristics of topsoil mixes containing waste rock specifically. Merino-Martín et al. (2017) found no significant difference in hydrological properties (e.g. soil moisture, hydraulic conductivity, infiltration



rate) between topsoil including 25% waste rock and standard topsoil. The hydrological performance of topsoil mixes incorporating greater than 25% waste rock remains to be tested and is particularly relevant given the practice of mining operations to utilise waste rock percentages greater than 50%.

Similarly little is known about how waste rock content influences plant growth and development, particularly at percentages >25%. Craw et al. (2007) observed plant establishment to be higher on abandoned waste dump slopes with <15% rock fragment content compared to slopes with >35% rock content. This suggests that physical features (such as rock content) play a major role in influencing vegetation establishment. Others studies observing the effect of rock fragments (but not necessarily waste rock) found that increasing rock content (i.e. >30%) could adversely affect plant establishment by decreasing nutrient and water supply (Childs and Flint 1990, Mi et al. 2016) or restricting rooting space (Lutz and Chandler 1946). Despite rock content causing a reduction in final plant size, Machado et al. (2013) reported growth of species in waste rock to have no negative affect on plant survival. Hallett et al. (2014) highlighted the importance of considering biotic and abiotic factors over time for assessing restoration success. In this study, it is proposed that plant growth and development can be used as an indicator of biotic response to changes in soil water dynamics associated with increasing rock content.

Salinity is commonly encountered in restoration of post-mining landscapes (Tordoff et al. 2000, Reichman et al. 2006). Salt-affected soils and landforms can originate from use of hypersaline groundwater for mineral processing, or natural release of salt from parent rocks or ancient drainage basins (Barrett 2000, Jordán et al. 2004). Salt has been present in the landscape for a long time (Crowley 1994a, 1994b, Doran and Turnbull 1997) and most terrestrial plants have evolved mechanisms to tolerate varying levels of salinity (Munns and Tester 2008, Bartels and Dinakar 2013). To date, examination of growth and development of plants on rock amended saline topsoil mixes has been limited. In this study, tests are conducted on the growth and development of a moderately salt tolerant species on saline topsoil mixes incorporating up to 88% waste rock.

The aims of this study are to investigate the effects of the addition of small to very large amounts of waste rock substrate on plant water relations and plant growth and development. We hypothesised that 1) water content is greatest in the topsoil fraction when compared with waste rock fraction, 2) the addition of waste rock would have no negative impact on plant growth and development at low percentages (up to 25%), and 3) at high percentages (>25%), increasing rock fraction will begin to restrict plant access to water, causing a negative impact to plant growth and development. Rock additions were investigated in terms of volumetric

water content and plant physiological responses (including stomatal conductance, leaf water potential, growth) through inclusion of 25-88% waste rock into the topsoil mix.

## Materials and methods

### *Growth substrates*

Growth substrates were obtained from an iron ore mine site 160 km south-east of Geraldton in Western Australia (29°11'05, 116°12'06) (Figure 1). The mine site is situated in the Koolanooka land system which includes the Koolanooka Hills, a range of rolling to very steep low hills. Soils are a matrix of rock and sandy loam on upper slopes and loamy earths and duplexes on lower slopes (DPIRD 2019b). Mining activity occurs within the Banded Ironstone Formation in which iron occurs largely in the magnetite and the amphiboles (ATA Environmental 2004). The climate of the study site is semi-arid with mild, wet winters and hot, dry summers (Bureau of Meteorology 2019).

Topsoil was stripped with a skid steer loader from the upper 15 cm of the soil profile in a previously restored 10 m x 10 m area at the mine site. Waste rock was collected from the surface of a 1 m high stockpile containing previously dumped waste rock. To ensure that topsoil mixes accurately reflected waste rock and topsoil components, waste rock was sieved to discard fines (<4 mm) and topsoil was sieved to discard rock fragments >4 mm. Topsoil was then air dried for up to 14 days prior to use. Waste rock size varied between 4 mm and 100 mm. The size distribution of waste rock was calculated based on Feret's diameter using a binary image of a random sample of 589 rock fragments analysed in the program ImageJ (Ferreira and Rasband 2012) (Table 1). Rock fragment density was measured using the water displacement method (Archimedes' principle).

**Table 1.** Size distribution and density of waste rock fragments

Feret's diameter	4-10 mm	10-20 mm	20-30 mm	30-40 mm	>40 mm
<i>n</i>	383	94	47	30	35
% of number fragments	65	16	8	5	6
<i>n</i>	6	6	6	5	11
Mean density (g/cm <sup>3</sup> )	3.5±0.23	3.3±0.17	3.6±0.23	3.2±0.21	3.2±0.16

The physio-chemical properties of the topsoil and waste rock were determined by analysing three 500 g samples taken from a bulked sample of each substrate collected in May 2018. Soil samples were stored dry at ambient temperature (ca. 25 °C) prior to analytical determination of chemical factors in June 2018. Analyses (see Table 2) were undertaken by ChemCentre

(Bentley, Western Australia) following the methods of Rayment and Lyons (2011). Soil texture of the topsoil determined to be loamy sand based on results of particle size analysis. Topsoil was classified as saline (electrical conductivity (EC) > 4 dSm) according to definition provided by Soil Science Society of America (2020).

### *Study species*

The species we chose for this study was *Acacia saligna* (Labill.) H.L. Wendl (Figure 1). *A. saligna* occurs widely throughout the southwest of Western Australia in both temperate and semi-arid climates (Western Australian Herbarium 2020). It is categorized as a pioneer species (Skoglund 2017) known to inhabit a variety of habitats including moderately saline soils (Thomson 1987, Haas 1993, El Mghadmi 2011) and nitrogen poor soils, as a result of symbiotic association with rhizobia (Amrani et al. 2010).

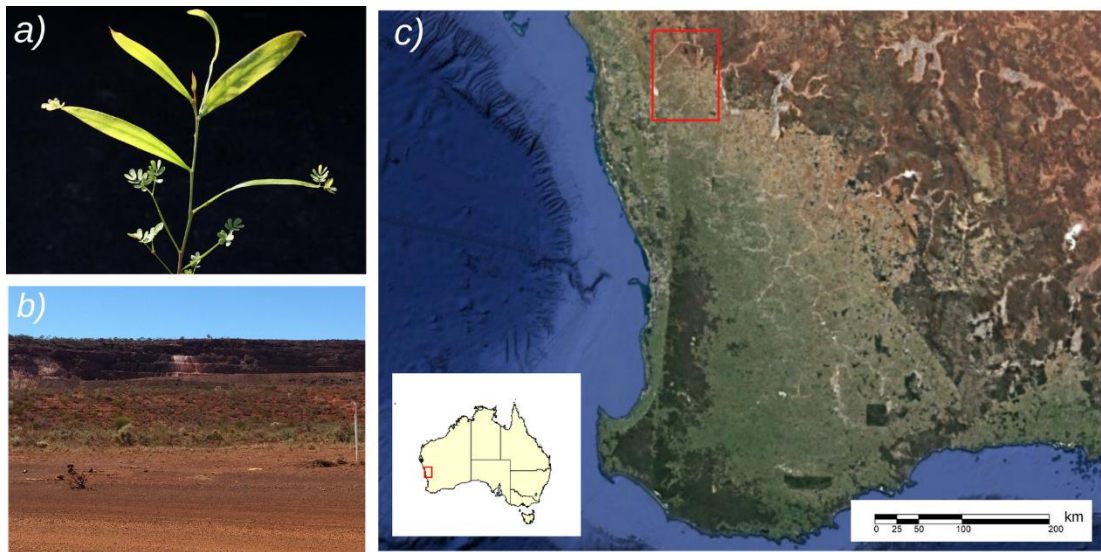
### *Experimental design*

The experiment was conducted under controlled greenhouse conditions from June to October when minimum and maximum temperature averaged 7.4°C and 29.6°C respectively. Plants were grown in cylindrical 10.5 litre (25 cm diameter x 27.5 cm depth) free draining pots (Figure 2). The bottom of the pots was lined with a fine synthetic mesh to retain soil. Pots were filled with one of five topsoil mixes (Table 2). Smaller 6 L (20 cm diameter by 18 cm depth) cylindrical pot were used for a shallow topsoil treatment, in which the volume of topsoil equalled that used in the 50% WR treatment (approximately 5.25L), to examine if the effect of adding waste rock on plant development is a result of there being less soil volume (hereby referred to as H0% WR). To mimic the mixing of substrates during the covering of mining landforms, the appropriate topsoil and waste rock volumes for each ratio were poured into a revolving drum mixer and rotated for up to 30 seconds prior to potting. Pots were set up in a standard block design on the glasshouse floor and pots of each treatment were randomised within each block. Actual topsoil and waste rock volumes in each pot were re-calculated after settling to take into account porosity of the waste rock at the time of mixing (Table 3).

Approximately 20-week old *A. saligna* seedlings were sourced from a local nursery and one seedling was planted in each pot. Pots without plants were used to estimate water loss via evaporation. Nursery potting mix was removed from seedlings prior to planting by soaking roots in water for up to 20 minutes (to ensure that plants did not benefit from nursery sourced soil during the experimental period). All seedlings of *A. saligna* had phyllodes and had shed the true leaves of their juvenile stage. Physiological measurements undertaken during the experimental period were conducted on fully grown phyllodes. Phyllodes will be referred to as leaves hereafter.

**Table 2.** Physical and chemical characteristics of topsoil and waste rock (crushed and grinded). Data are presented as means  $\pm$  1 s.e. (n=3). T values and P values represent the results of pairwise comparison of topsoil and waste rock based on two sample t-test. Blank cells indicate no tests run.

Parameter	Method	Unit	Topsoil	Waste Rock	T value	P value
<b>Physical Properties</b>						
Texture			Loamy sand	-		
Particle size distribution						
Stones (>2 mm)	Sieve	w/w	10.2 $\pm$ 0.65	100	-138	<0.05
Sand (0.2-2 mm)	Fraction	w/w	73.0 $\pm$ 0.58	0	126	<0.05
Silt (0.002–0.2 mm)	Fraction	w/w	5.13 $\pm$ 0.07	0	77.0	<0.05
Clay (<0.002 mm)	Fraction	w/w	11.7 $\pm$ 0.17	0	67.5	<0.05
<b>Chemical Properties</b>						
EC	1:5 water	dS/m	5.38 $\pm$ 0.24	0.36 $\pm$ 0.02	94.2	<0.05
pH	H <sub>2</sub> O		5.37 $\pm$ 0.03	7.33 $\pm$ 0.06	-26.3	<0.001
Organic carbon	Walkley-Black	%	0.33 $\pm$ 0.02	0.12 $\pm$ 0.00	10.5	<0.05
Total nitrogen	Kjeldahl digest	mg/kg	0.02 $\pm$ 0.00	<0.005	35.5	<0.05
Total phosphorus	Kjeldahl digest	mg/kg	186 $\pm$ 3.33	606 $\pm$ 89.87	-4.67	<0.05
Available phosphorus	HCO <sub>3</sub>	mg/kg	3 $\pm$ 0	2 $\pm$ 0	6.84	<0.05
Al	Mehlich-3	mg/kg	316 $\pm$ 3.33			
B	Mehlich-3	mg/kg	2.30 $\pm$ 0.15			
Ca	Mehlich-3	mg/kg	526 $\pm$ 23.33			
Cd	Mehlich-3	mg/kg	<0.01			
Co	Mehlich-3	mg/kg	0.06 $\pm$ 0.00			
Cu	Mehlich-3	mg/kg	0.30 $\pm$ 0.00			
Fe	Mehlich-3	mg/kg	28.0 $\pm$ 0.00			
K	Mehlich-3	mg/kg	216 $\pm$ 3.33			
Mg	Mehlich-3	mg/kg	810 $\pm$ 10.0			
Mn	Mehlich-3	mg/kg	14.3 $\pm$ 0.33			
Mo	Mehlich-3	mg/kg	<0.01			
Na	Mehlich-3	mg/kg	>1000	226 $\pm$ 31.7	24.3	<0.05
Ni	Mehlich-3	mg/kg	0.20 $\pm$ 0			
P	Mehlich-3	mg/kg	1.70 $\pm$ 0.33			
S	Mehlich-3	mg/kg	>250	0.07 $\pm$ 0.01	17201	<0.05
Zn	Mehlich-3	mg/kg	0.43 $\pm$ 0.03			
As	Mehlich-3	mg/kg	<0.01			
Pb	Mehlich-3	mg/kg	1.43 $\pm$ 0.03			
Se	Mehlich-3	mg/kg	<0.10			



**Figure 1.** (a) Species used in study, *Acacia saligna*, Orange Wattle (FIO 2019); (b) Mine site where substrates were collected for use in study; (c) Location of mine site (circle) where substrates were collected for use.



**Figure 2.** Pots used in the experiment

**Table 3.** Mean volume of total topsoil mix, topsoil and waste rock fractions after settling. H0% WR – 5.25 L topsoil, 0% WR – 10.5 L topsoil, 25% WR – 3:1 topsoil:waste rock, 50% WR – 1:1 topsoil:waste rock, 75% WR – 1:3 topsoil:waste rock, 88% WR – 1:7 topsoil:waste rock (mean  $\pm$  SE). Where total mix is the volume of the topsoil mix after settling, waste rock fraction is the percentage of waste rock minus porosity and topsoil fraction is the volume of total mix minus waste rock volume.

Mean volume ( $\text{cm}^3 \text{ cm}^{-3}$ )	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
<i>n</i>	9	9	12	8	6	13
Total mix	4980 $\pm$ 60.1	7651 $\pm$ 95.0	8456 $\pm$ 41.0	8414 $\pm$ 111.5	8550 $\pm$ 70.2	8696 $\pm$ 68.8
Topsoil fraction	4980 $\pm$ 60.1	7651 $\pm$ 95.0	7012 $\pm$ 41.0	5526 $\pm$ 111.5	4219 $\pm$ 70.2	3614 $\pm$ 68.8
Waste rock fraction			1444 $\pm$ 0	2887 $\pm$ 0	4331 $\pm$ 0	5082 $\pm$ 0

Following the planting of seedlings, pots were saturated, and field capacity (also known as drained upper limit) was determined according to Gardner (1985). During the experimental period all plants were subjected to a well-watered period (70 days) followed by a dry-down period (51 days). During the well-watered period, plants were hand-watered to 80% of field capacity (80% FC), a point determined via daily weighing of pots. Prior to the dry-down period all pots were taken to a constant water content of 80% FC and from then onwards all watering was withheld. At the start of the well-watered phase each topsoil mix included 15 replicates. Some mortality occurred during the well-watered phase, reducing the minimum number of replicates to nine, except for the 50% WR and 75% WR treatments which had eight and six replicates respectively.

#### *Water content of topsoil mixes*

The water content of each pot was determined by weighing of each pot. This was then converted to volumetric water contents of the mix ( $\theta_m$ ) or of the soil fraction only ( $\theta_s$ ):

$$\theta_m (\text{cm}^3 \text{ cm}^{-3}) = V_w/V_m$$

$$\theta_s (\text{cm}^3 \text{ cm}^{-3}) = V_w/V_s$$

Where  $V_w$  = volume of water,  $V_m$  = volume of the total soil mix (topsoil and rock fraction) and  $V_s$  = volume of topsoil fraction. Mean volumetric water content was determined every 3-4 days during the dry-down period. The calculation of  $\theta_s$  used the soil volumes listed in Table 2, and assumed that the rock fraction held no water. As a check on this assumption, mean  $\theta$  of 100% waste rock was determined after waste rock was dried over 48 hours at 105°C, submerged in water for 12 hours, drained and patted dry.

### *Bulk density of topsoil mixes*

Total bulk density of the soil ( $BD_t$ ) was calculated for each of the different soil mixes, after soil mixes had been allowed to settle in pots, by dividing dry weight ( $Ws$ ) by soil volume ( $Vm$ ). Bulk density of the topsoil fraction ( $BD_s$ ) was calculated using the equation:

$$BD_s \text{ (g/cm}^3\text{)} = \frac{(1 - R_m)}{\left(\frac{1}{BD_t}\right) - \left(\frac{R_m}{BD_r}\right)}$$

Where  $R_m$  = percentage of rock,  $BD_t$  = total bulk density of the soil and  $BD_r$  = mean density of the waste rock fragments (3.33 g/cm<sup>3</sup>).

### *Plant water relations*

Stomatal conductance ( $g_s$ ; mmol m<sup>-2</sup> s<sup>-1</sup>) measurements were taken from three plants in each treatment once at the end of the well-watered period and thereafter every 3-4 days during the dry-down period. Measurements were taken mid-morning (9.00-11.00 am) using a SC-1 Leaf Porometer (Decagon Devices Inc., Washington, USA), calibrated before each use. Stomatal conductance readings were taken from the down-facing side of the leaf, on leaves contained within the upper third of the plant. During the measurement period (30 August – 25 October 2018), mid-morning light levels were recorded every 15 minutes with a handheld light meter (LI-COR LI-250, Nebraska, USA). Plants received maximum natural light of 1800  $\mu\text{mol m}^2 \text{ s}^{-1}$  during measurements which peaked on average at 11.30 am.

Pre-dawn water potential was measured using a Pressure Chamber (Model 1000, PMS Instruments, Oregon, USA). Measurements were taken once at the end of the well-watered period and once in the dry-down phase, when stomatal conductance reached a pre-defined drought condition of  $< 40 \text{ mmol m}^{-2} \text{ s}^{-1}$ , a cut-off point determined from a pilot trial where plants began to exhibit signs of water stress such as drying or wilting of leaves. One leaf was excised from each plant from the upper third of the plant. The cut leaf was then enclosed in a polyethylene bag and immediately inserted into the chamber. After measurement, these leaves were saved and dried for later inclusion in biomass data.

### *Plant growth*

Growth measurements including leaf count and plant height were taken at the start of the well-watered period, the end of the well-watered period (70 days) and again at the end of the dry-down (121 days) on all individuals. Plant height was determined as the distance from hypocotyl to shoot apical meristem. Relative growth rate (RGR) was calculated for leaf count and height for each treatment during both the well-watered and drought period using the equation

$$RGR = (\ln X_2 - \ln X_1)/(t_2 - t_1)$$

Where  $t_1$  is time one,  $t_2$  is time two,  $X_1$  is leaf count or plant height at  $t_1$  and  $X_2$  is leaf count or plant height at  $t_2$ . Leaf length and plant biomass was recorded at the end of the experiment. Leaf length was determined as the distance from base to tip, for the largest leaf. Shoot (total aboveground) and root dry weight was determined at final harvest. Substrate was washed from the roots. Shoots and roots were then dried at 70°C for 24 hours, and then weighed.

#### *Data analysis*

All variables were tested for normality and homogeneity of variance and log transformations undertaken where appropriate. Where transformed data indicated normality, differences between treatments were tested using a one-way analysis of variance (ANOVA), followed by Tukey-honestly significant difference (HSD) tests for post hoc mean comparisons. Data for  $g_s$  and bulk density was non-normal and resistant to transformation so we used the non-parametric Kruskal-Wallis test. P-values for  $g_s$  and bulk density were adjusted using Benjamini-Hochberg method.

For repeated measurements taken over the dry-down period, linear mixed effects models were used to test the interaction between treatment and time and its effect on  $\theta_m$  and  $\theta_s$ . The same analysis was conducted to test the effect of  $\theta_m$  on  $g_s$  and the interaction between treatment and  $\theta_m$ . All analyses were performed with R software version 1.1.463 (RStudio, 2018). Data presented is non-transformed for ease of interpretation.

## **Results**

### *Bulk density*

Total bulk density of soil mixes was dependent on waste rock content ( $\chi^2_5 = 650.75$ ,  $P < 0.0001$ ). Total soil bulk density reached a maximum when waste rock content increased to approximately 50 – 75%, beyond which it decreased. Similarly, bulk density of the topsoil fraction remained constant up to approximately 50% waste rock content, beyond which it decreased (Table 4).



**Table 4.** Mean bulk density of topsoil mixes during the experimental period. Data is presented as mean  $\pm$  1 s.e. Annotated lettering represents the results of Kruskal-Wallis test. Values followed by the same or no letters are not significantly different (at P=0.05).

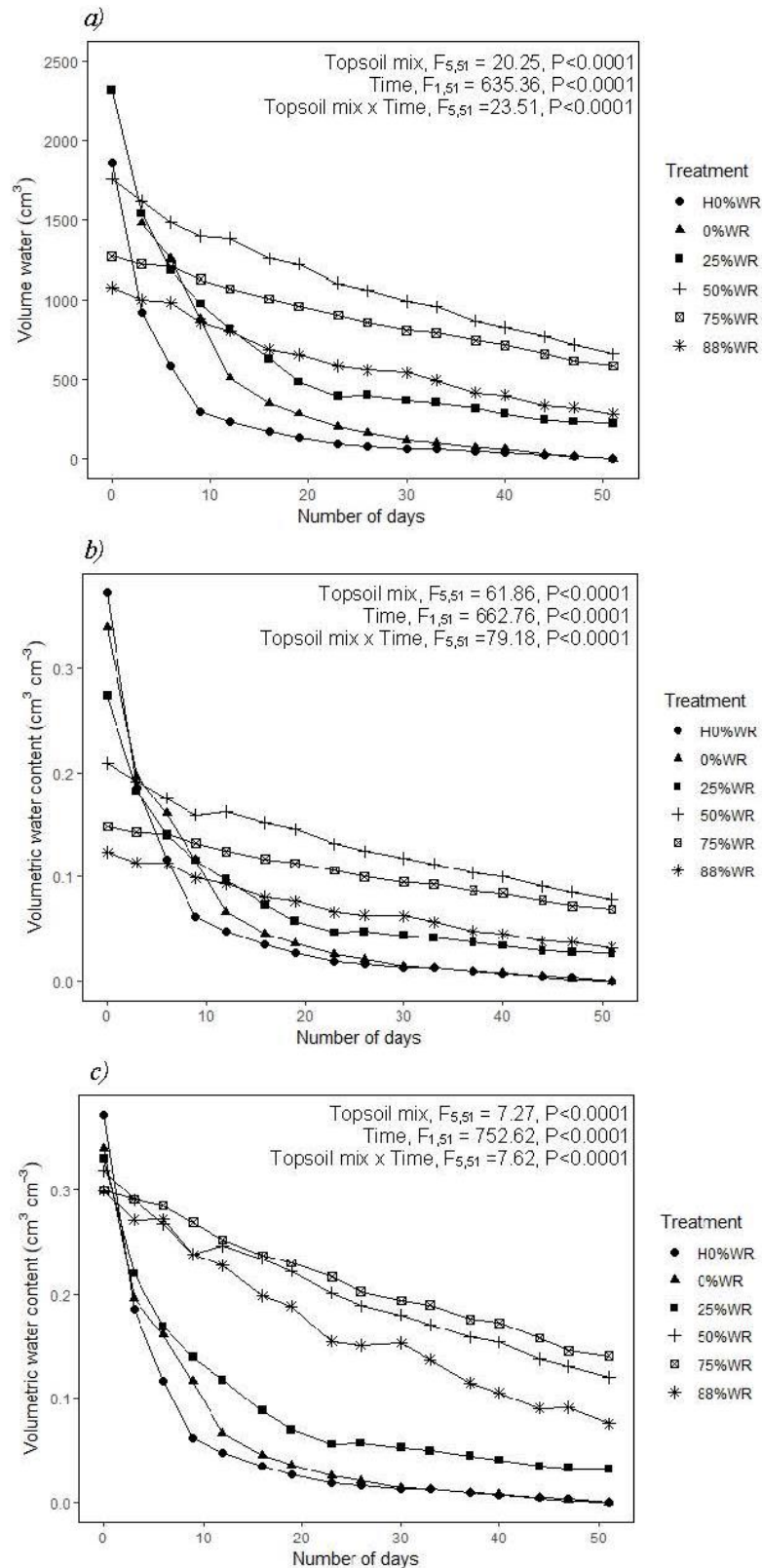
Topsoil Mix	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
<i>n</i>	9	9	12	11	8	13
Total bulk density (g/cm <sup>3</sup> )	1.6 $\pm$ 0.02 <sup>e</sup>	1.9 $\pm$ 0.03 <sup>d</sup>	2.1 $\pm$ 0.02 <sup>c</sup>	2.3 $\pm$ 0.02 <sup>a</sup>	2.3 $\pm$ 0.02 <sup>a</sup>	2.2 $\pm$ 0.03 <sup>b</sup>
Bulk density topsoil fraction (g/cm <sup>3</sup> )	1.6 $\pm$ 0.02 <sup>b</sup>	1.9 $\pm$ 0.03 <sup>a</sup>	1.8 $\pm$ 0.02 <sup>a</sup>	1.8 $\pm$ 0.02 <sup>a</sup>	1.2 $\pm$ 0.01 <sup>c</sup>	0.6 $\pm$ 0.02 <sup>d</sup>

#### *Soil moisture content*

During the well-watered period, when all pots were maintained at 80% field capacity, there were significant reductions in total absolute water content (cm<sup>3</sup> per pot) as the proportion of waste rock increased ( $F_{5, 51} = 67.4$ ,  $P < 0.0001$ ) (Table 5). In drought induced conditions, the decline of  $\theta_m$  varied with both waste rock content and time ( $F_{5,51} = 79.18$ ,  $P < 0.0001$ ) (Figure 3). Here the decline in  $\theta_m$  was slower in topsoil mixes with waste rock compared to those without. Treatments H0%WR and 0%WR exhibited the most rapid decline in  $\theta_m$  when compared to initial values, with approximately 70-80% reduction in mean  $\theta_m$  in the first 10 days of dry-down. A slower decline in  $\theta_m$  was exhibited in 50%WR, 75%WR and 88%WR with mean  $\theta_m$  reducing by 10-20% in the first 10 days when compared to initial values.

**Table 5.** Differences in mean water content of topsoil mixes during the well-watered phase of the experiment (at 80% of field capacity). Data is presented as mean  $\pm$  1 s.e. Annotated lettering represents the results of one-way ANOVA. Values followed by the same or no letters are not significantly different (at P=0.05).

Topsoil Mix	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
<i>n</i>	9	9	12	8	6	13
Absolute water content (cm <sup>3</sup> )	1858 <sup>b</sup>	2607 <sup>a</sup>	2315 <sup>a</sup>	1759 <sup>b</sup>	1270 <sup>c</sup>	1076 <sup>c</sup>
$\theta_m$ (cm <sup>3</sup> cm <sup>-3</sup> )	0.37 $\pm$ 0.01 <sup>a</sup>	0.34 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.00 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>c</sup>	0.15 $\pm$ 0.00 <sup>d</sup>	0.12 $\pm$ 0.00 <sup>d</sup>
$\theta_s$ topsoil fraction (cm <sup>3</sup> cm <sup>-3</sup> )	0.37 $\pm$ 0.01 <sup>a</sup>	0.34 $\pm$ 0.02 <sup>ab</sup>	0.33 $\pm$ 0.01 <sup>ab</sup>	0.32 $\pm$ 0.02 <sup>ab</sup>	0.30 $\pm$ 0.02 <sup>b</sup>	0.30 $\pm$ 0.01 <sup>b</sup>



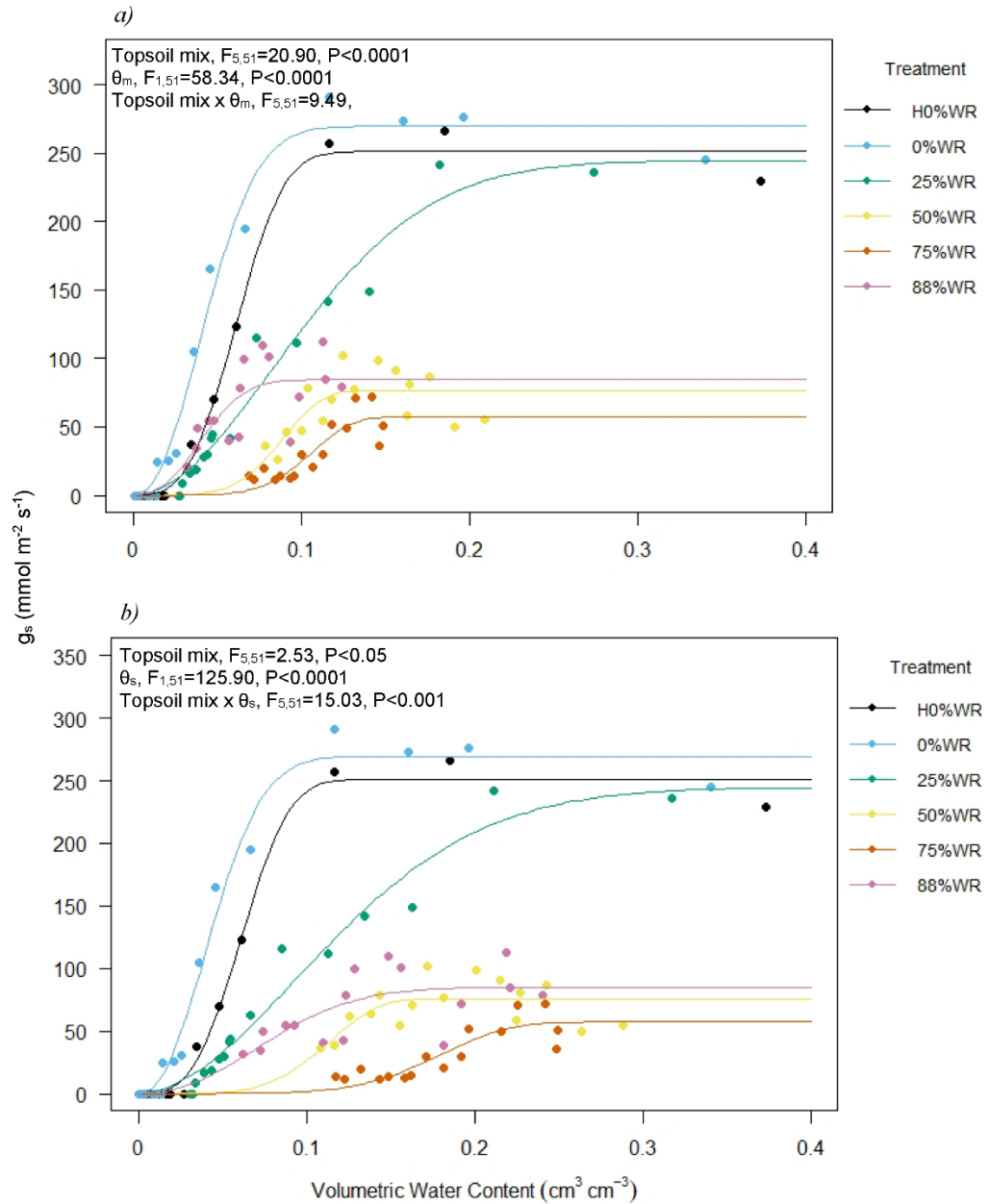
**Figure 3** (a) Mean absolute water content ( $\text{cm}^3$  per pot), (b) mean volumetric water content of the total soil mix ( $\theta_m$ ) and (c) mean volumetric water content of topsoil fraction only ( $\theta_s$ ) across six different topsoil mixes, including ANOVA statistics for effects of topsoil mix, time and the interaction.

### *Physiological performance*

Stomatal conductance ( $g_s$ ) of *A.saligna* tended to decrease as the proportion of waste rock increased and this trend coincided with decreases in  $\theta_m$  and  $\theta_s$  ( $p < 0.001$  for both). When plants were well-watered, those mixes with more waste rock had significantly lower stomatal conductance ( $F_{5,51}=8.53$ ,  $P < 0.0001$ ), with 50% WR, 75% WR and 88% WR exhibiting 80-68% lower  $g_s$  when compared to mean values of the control (0% WR) (Table 6). In the dry-down phase plants on high waste rock mixes exhibited slower decline in  $g_s$  when compared to plants on H0% WR, 0% WR, 25% WR (Figure 4) with patterns in conductance significantly effected by interactions between  $\theta_m$  and waste rock content ( $F_{5,51}=9.49$ ,  $P < 0.001$ ). The most rapid decline in  $g_s$  was exhibited by plants on the H0% WR and 0% WR which reached the pre-determined drought-stressed condition at approximately 13 days and 24 days respectively. A slower decline in  $g_s$  was exhibited by plants on the 25%, 50%, 75% and 88% WR treatments which reached drought-stress at approximately 26 days, 51 days, 47 days and 42 days respectively.

**Table 6.** Mean stomatal conductance for *Acacia saligna* recorded during the well-watered period for six different topsoil mixes. Data is presented as mean  $\pm$  1 s.e. Annotated lettering represents the results of Kruskal-Wallis test. Values followed by the same or no letters are not significantly different (at  $P < 0.05$ ).

Topsoil Mix	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
<i>n</i>	9	9	12	11	8	13
Mean $g_s$ (mmol $m^{-2} s^{-1}$ )	229 $\pm$ 14.4 <sup>a</sup>	245 $\pm$ 14.4 <sup>a</sup>	236 $\pm$ 12.5 <sup>a</sup>	55 $\pm$ 15.3 <sup>b</sup>	51 $\pm$ 17.7 <sup>b</sup>	79 $\pm$ 12.0 <sup>b</sup>



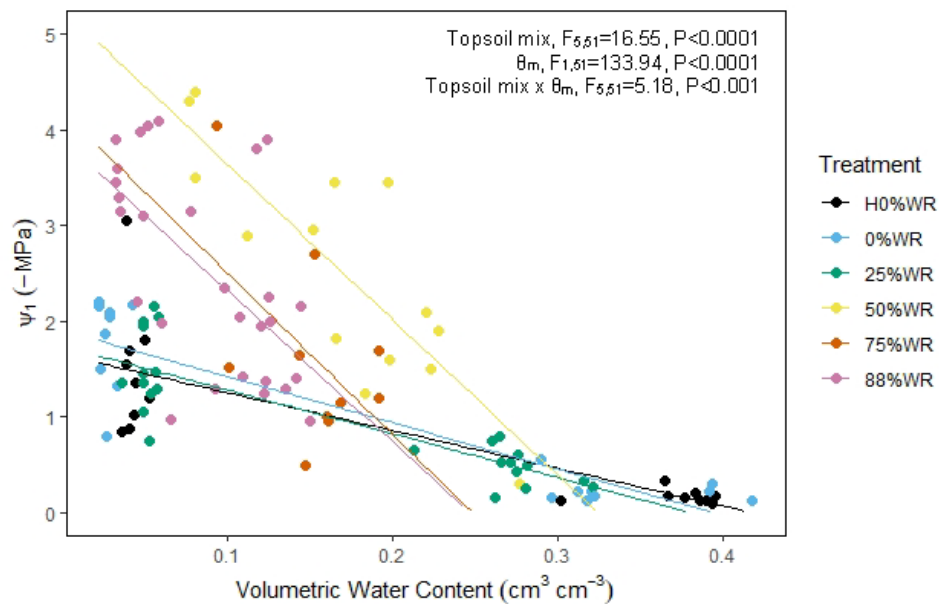
**Figure 4.** Mid-morning (09:00-11:00) stomatal conductance ( $g_s$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) as a function of (a) mean volumetric water content of the total soil mix ( $\theta_m$ ) and (b) mean volumetric water content of topsoil fraction only ( $\theta_s$ ) for *Acacia saligna* across six topsoil mixes, including ANOVA statistics for effects of topsoil mix,  $\theta_m$ ,  $\theta_s$  and interactions.

*A. saligna* seedlings on waste rock mixes typically had lower leaf water potentials ( $\Psi_1$ ) (Table 7, Figure 5). This pattern persisted as soils dried down and was influenced by interactions between waste rock content and  $\theta_m$  ( $F_{5,51} = 5.18$ ,  $P<0.001$ ). When subjected to drought-stressed conditions the largest reduction in  $\Psi_1$ , occurred in plants on H0% WR and 0% WR. On these treatments  $\Psi_1$  decreased by approximately 600-800% when compared to well-watered values. In comparison, plants on 50%WR, 75%WR and 88%WR exhibited much lower

reductions in  $\Psi_1$ , with values decreasing by 48%, 108% and 25% respectively when compared to well-watered values.

**Table 7.** Mean leaf water potential ( $\Psi_1$ , -MPa) of *Acacia saligna* seedlings across six different topsoil mixes at the end of the well-watered period and at a pre-determined cut-off point of  $< 40 \text{ mmol m}^{-2} \text{ s}^{-1}$  during the dry-down period. Data is presented as mean  $\pm$  1 s.e. Annotated lettering represents the results of one-way ANOVA. Values followed by the same or no letters are not significantly different (at  $P < 0.05$ ).

Topsoil Mix	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
<i>n</i>	9	9	12	11	8	13
Well-watered						
Mean $\Psi_1$ (-MPa)	0.1 $\pm$ 0.02 <sup>c</sup>	0.2 $\pm$ 0.04 <sup>c</sup>	0.4 $\pm$ 0.06 <sup>b</sup>	2.0 $\pm$ 0.42 <sup>a</sup>	1.3 $\pm$ 0.31 <sup>a</sup>	2.1 $\pm$ 0.27 <sup>a</sup>
Dry-down						
Mean $\Psi_1$ (-MPa)	1.2 $\pm$ 0.19 <sup>b</sup>	2.1 $\pm$ 0.07 <sup>ab</sup>	1.4 $\pm$ 0.05 <sup>b</sup>	3.0 $\pm$ 0.45 <sup>a</sup>	2.7 $\pm$ 0.43 <sup>a</sup>	2.6 $\pm$ 0.44 <sup>a</sup>



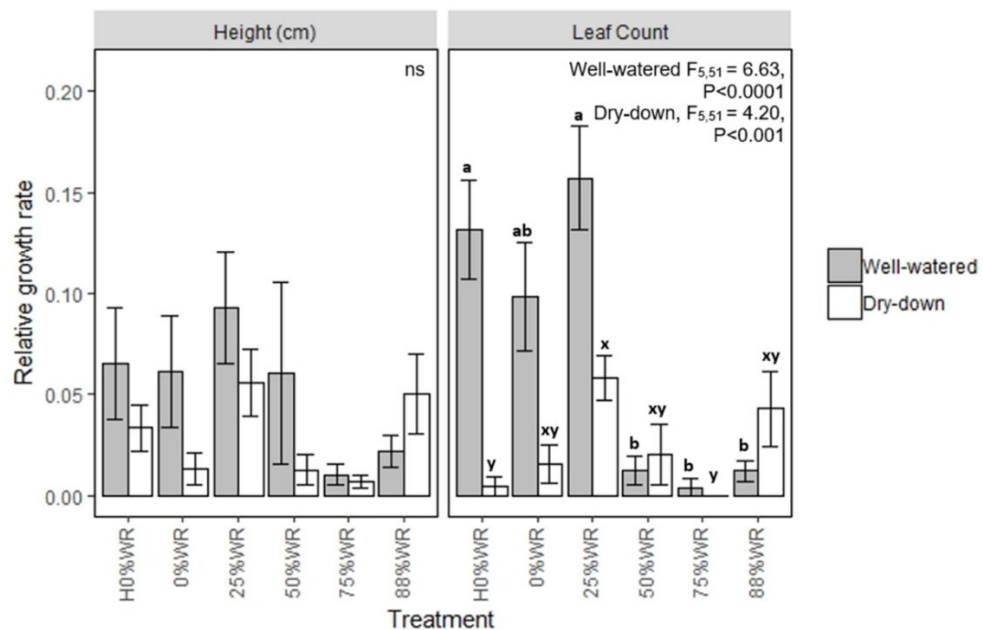
**Figure 5.** Mean leaf water potential ( $\Psi_1$ , -MPa) of *Acacia saligna* seedlings across six different topsoil mixes at the end of the well-watered period and at a pre-determined cut-off point of  $< 40 \text{ mmol m}^{-2} \text{ s}^{-1}$  during the dry-down period, including ANOVA statistics for effects of topsoil mix, total volumetric water content ( $\theta_m$ ) and the interaction.

#### Growth and morphology

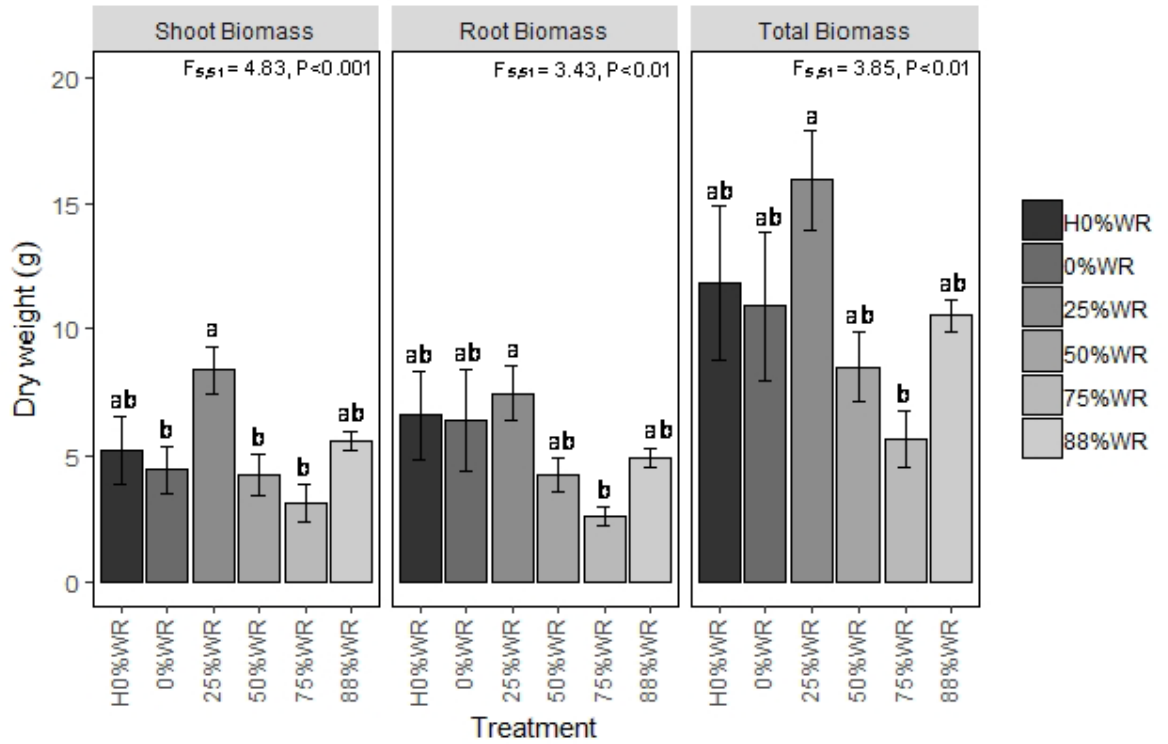
Overall RGR based on leaf count was greatest at waste rock percentages of 25% for both well-watered and dry-down conditions. In well-watered conditions, inclusion of  $>50\%$  waste rock content caused a significant reduction in RGR based on leaf count ( $F_{5,51}= 6.63$ ,  $P < 0.0001$ )

(Figure 6). Lowest RGR values overall were recorded from plants on 75% WR. In drought conditions reductions in RGR based on leaf count occurred across all treatments except 50% WR and 88% WR, which recorded increased RGR. These differences did not vary considerably from those recorded on 0% WR however ( $p>0.05$ ). No significant difference in RGR based on height occurred across treatments, but waste rock content did cause a reduction in height of *A.saligna* at final harvest for 75% WR and 88% WR relative to 25% WR ( $F_{5,51}=3.54$ ,  $P<0.01$ ). There was no significant difference in mean length of longest leaf (at final harvest) of *A.saligna* on topsoil mixes containing waste rock compared to those without ( $p>0.05$ ).

Total plant biomass of *A.saligna* was dependent on waste rock content ( $F_{5,51}=3.85$ ,  $P<0.01$ ). The presence of 25% waste rock had a positive effect on biomass with mean biomass being  $5\text{ g} \pm 1.81$  greater than 0% WR (Figure 7). At waste rock percentages  $>50\%$ , biomass of *A.saligna* was lower but remained similar to that recorded on topsoil without rock. 75% WR caused a significant reduction in biomass of *A.saligna* relative to 25% WR, but 50% WR and 88% WR did not. Similarly, increasing waste rock content had a negative effect on root growth but root growth did not differ statistically to that recorded on 0% WR. Only 75% WR caused a significant reduction in root growth of *A.saligna* relative to 25% WR ( $F_{5,51}=3.43$ ,  $P<0.01$ ). Shoot growth on 25% WR was significantly greater compared to 0% WR, 50% WR and 75% WR ( $F_{5,51}=4.80$ ,  $P<0.001$ ).



**Figure 6.** Mean relative growth rate (RGR) of *Acacia saligna* in six topsoil mixes recorded at two time intervals during both the well-watered phase and dry-down phase. Letters indicate significant differences in RGR ( $P<0.05$ ) among treatments following results of one-way ANOVA. For height, there were no significant differences (ns).



**Figure 7.** Shoot, root and total biomass of *A. saligna* at the end of the 120 day growth period, on six different topsoil mixes. Letters indicate significant differences in dry weight (g) ( $P < 0.05$ ) between treatments following results of one-way ANOVA.

## Discussion

This study analysed how the amendment of saline topsoil with waste rock affects soil water dynamics and plant growth and development of a moderately salt tolerant species. We tested the hypothesis that water content is greatest in the topsoil fraction when compared with waste rock fraction. This hypothesis was accepted based on our results which showed the presence of waste rock significantly reduced the absolute water content of the pot ( $\text{cm}^3$ ) and  $\theta_m$  of the total topsoil mix. The overall effect was that the test species, *A. saligna*, responded to lower water availability through a reduction in physiological functions ( $g_s$  and leaf water potential). We observed a non-linear relationship between waste rock content and plant growth and development however. Here plant growth and development of *A. saligna* decreased with increasing waste rock content up to percentages of 75%, beyond which it increased. These results partially support acceptance of our hypothesis that at high percentages (>25%), reductions in soil water supply would have an inhibitory effect on plant growth and development. No observable decline in plant growth and development occurred on 25% WR, supporting our additional hypothesis that the inclusion of small amounts of waste rock has no negative impact on plant growth and development.

The presence of waste rock at percentages of 25% can be observed to be beneficial for plant growth and development for two reasons. Firstly, at this level absolute water content of the pot was not significantly different compared to topsoil and secondly, decline in water content was slower which prolonged water availability. Several other studies also reported rock fragment to be optimal at percentages close to 25%. Mi et al. (2016) found that rock fragment volumes of up to 30% had a positive effect on plant growth and biomass for a peashrub species inhabiting semi-arid regions. Similarly Voiculescu et al. (1983) found soils containing up to 20% rock fragments were favourable for growth of walnut trees and Magier and Ravina (1984) reported an optimum rock fragment volume of 25-30% for apple tree development and yield. At waste rock percentages >50%, the influence of waste rock on water content led to *A. saligna* seedlings to exhibit significantly lower  $g_s$ ,  $\Psi_l$  and RGR, even under well-watered conditions. These findings support the premise that beyond optimal rock fragment contents, plant productivity is adversely affected (Poesen and Lavee 1994).

Our results indicated that at percentages of 50% and 75%, waste rock content had a negative effect on plant growth and biomass. This effect may be related to reductions in absolute water content of the pot ( $\text{cm}^3$ ) and  $\theta_m$  following amendment with waste rock. Decreases in plant growth and development are a common effect of limited soil water supply (Sharp and Davies 2008) and plants have been shown to slow growth early in anticipation of unfavourable conditions, in order to reduce their water requirement and therefore the impact of low water availability (Stirzaker et al. 1996). The compressive strength of waste rock may



also be a limiting factor to plant growth. In this study, root biomass of *A. saligna* was lower in high waste rock mixes even though root growth occurred throughout the pot in all topsoil mixes (e.g. 35% reduction in root growth on 50% WR compared to H0% WR). These findings reflect those of previous studies where root growth was observed to be lower in soils with rock. For example Babalola and Lal (1977) observed root development of maize seedlings to be adversely affected by rock fragments when rock mass exceeded 10-20% and Lutz and Chandler (1946) observed rock volumes of more than 20% to produce unfavourable effects on plant development as a result of restricted root space. Mi and Liu (2016) also observed root biomass to be lower in soil rock volumes of 50% and related this result to inaccessibility of the rock matrix to roots. Although plants can grow in both soil and rock (Zhang et al. 2016), the distribution of root systems is influenced by the compressive strength of rock (Estrada-Medina et al. 2013) and root growth is restricted when rock penetration resistance is higher than soil (>4 MPa) (Arshad et al. 1996, Schwinning 2013). Reductions in root development can adversely affect overall plant growth and development because the uptake of water and nutrients becomes limited (Stirzaker et al. 1996).

This trend is confounded, however, by growth of plants on 88% WR. Final biomass of *A. saligna* was higher on 88% WR compared to plants on 50% and 75% WR. We suggest the main explanation for this result is an increase in the presence of macropores (>50  $\mu\text{m}$  diameter) at 88% waste rock content (evidenced by lower total bulk density). As the volume of waste rock increases, the likelihood of macropores being created also increases. This is most likely because, there is insufficient material present to plug voids created from increased rock fragment-to-fragment contact (Zhou et al. 2009). It is hypothesised that an increase in the number and size of macropores within the soil matrix led to either of two effects. The first is that the presence of structural voids encouraged greater proliferation of plant roots (Estrada-Medina et al. 2013) by providing them with a path of least resistance, in addition to increased levels of water and nutrients (Ingelmo et al. 1994, Stirzaker et al. 1996). Although greater presence of macropores did not increase root weight in this study, it may have increased root length, allowing plants on 88% WR to access water held at the bottom of a pot. The second is that a greater presence of macropores improved air flow within the soil matrix, leading to increased fixation of nitrogen from the atmosphere. Notably, *A. saligna* is part of the legume (Fabaceae) family, which is known to form a symbiosis in its root nodules with nitrogen fixing microbes (rhizobia) (Marsudi et al. 1999, Amrani et al. 2010, Bruning and Rozema 2013). The symbiotic rhizobia in root nodules can take up gaseous dinitrogen ( $\text{N}_2$ ) from the air and 'fix' the nitrogen into molecules that can be assimilated by the plant (Bruning and Rozema 2013). Mansouri (1998) showed that an increase in the fixation of nitrogen by rhizobia has a positive effect on total plant biomass of *A. saligna*. As we did not measure parameters of root growth

(i.e. root length), or rates of nitrogen fixation, future studies should consider the effects of high waste rock content on these factors.

Exposure of plants to water-limited conditions may also account for differences in plant biomass across treatments. Topsoil mixes with 88% waste rock recorded the lowest absolute water content and  $\theta_m$  in well-watered conditions. This factor might have resulted in either of two outcomes; (i) that plants were of smaller size going into the dry-down and consequently required less water, evidenced by a low RGR in the well-watered phase or, (ii) that these plants were already pre-disposed to low water availability which may have buffered against the effects of water deficit during the dry-down phase. Some studies have found that pre-disposure to drought contributes to improved growth in future drought events (Valliere et al. 2019). This response has been attributed to the ability of a plant to maintain higher water use efficiency, allowing plants to maintain higher  $g_s$  under periods of stress (Vilagrosa et al. 2003). In our study, *A.saligna* plants exhibited slightly higher  $g_s$  on 88% WR compared to plants on 50% and 75% WR, even though soil water content was significantly less. Differences in  $g_s$  were not clearly separated, however, and further studies are needed to further test this hypothesis. Drought conditioning is dependent on previous experience to stressors (e.g. low water availability) causing a “priming effect” or “stress memory” that facilitates protection from future stress events (Novoplansky 2009, Tanou et al. 2012, Walter et al. 2013) and thus is likely to be species specific (Driessche 1991, Landhäusser et al. 1996, Valliere et al. 2019). Nonetheless, if the performance of plants on 88% WR is related to pre-disposure to low water conditions then this could be especially beneficial in restoration of semi-arid environments where water is a limiting factor to plant establishment.

The possibility that exposing plants to small volumes of topsoil promoted the extraction of water from waste rock fragments was also examined. Weighing of waste rock that had been drained for 12 hours after saturation and patted dry revealed the potential for waste rock to absorb some water ( $0.10 \text{ cm}^3 \text{ cm}^{-3}$ ). This value was higher than that recorded for basalt (0), flintstone (0) and diorite (0.03), comparable to weathered schist (0.10) and siltstone (0.10) but less than that recorded for weathered sandstone (0.13) and limestone (0.35-0.5) (Poesen and Lavee 1994, Querejeta et al. 2006). Rock fragments have been shown to be a potential water reservoir in soils (Gras and Monnier 1963, Poesen and Lavee 1994). Tetegan et al. (2015) estimated that when both the volume and the hydric properties of fragments were ignored, the available water capacity of the soil was underestimated by 20%. Additionally, increased presence of voids and macropores at high rock contents promotes greater retention of water, especially at low matric potentials (Baetens et al. 2009, Khetdan et al. 2017). Plants roots of *A.saligna* were observed to grow between rock:soil interfaces and within the cracks and fissures of waste rock at time of harvest. Other studies reported similar findings, where plant

roots were observed to penetrate highly weathered rock fragments and gaps between rocks (Zhang et al. 2016), including weathered granitic and limestone bedrock. At bedrock interfaces, trees and shrubs have been shown to take up substantial amounts of water after soil water has become depleted (Querejeta et al. 2006, McCole and Stern 2007, Ruiz et al. 2010). Observations of root growth in this study suggest that plants might have utilized rocks as an additional source of water. Whether plants utilized water from the surface of waste rock or within the waste rock itself and how much water was utilized is unknown. The mechanisms behind plant-rock interactions are only just beginning to be understood (Schwinning 2010). Further research is required to examine the potential for water transfers to occur between waste rock fragments and plant roots.

In this study we observed that water conservation is improved when topsoil was amended with waste rock. Decreases in soil water content under drought conditions was slowed by the addition of waste rock and was not confounded by soil volume (see 50%WR - H0%WR comparisons) nor impacted by plant size (see Figure S1). These findings reflect those found in other studies that also identified the potential for rock fragments to have a significant role in water conservation during periods of plant growth (Danalatos et al. 1995a, Van Wesemael et al. 1996, Tetegan et al. 2015). The positive relationship between waste rock and soil water conservation can be explained by two mechanisms: the addition of waste rock fragments reducing the rate of evaporation by reducing the volume of soil exposed at the soil surface and therefore capillary loss (Unger 1971b, Ingelmo-Sanchez et al. 1980, Van Wesemael et al. 1996) and/or waste rock fragments within the soil mix slowing the movement of water (i.e. hydraulic conductivity) by reducing the available area for water flow and forcing water to move around fragments laterally, (Magier and Ravina 1984, Brakensiek and Rawls 1994, Shi et al. 2008, Zhou et al. 2011, Mi et al. 2016). Which of the two influences is most important and whether this phenomenon is identical for all waste rock contents has yet to be established.

## **Conclusion**

Overall, this study highlights that restoration outcomes of salt-affected landscapes are intimately linked to the hydrological characteristics of re-made topsoil mixes. We tested plant water relations and growth and development of a salt tolerant species on saline topsoil amended with 0%, 25%, 50%, 75% and 88% waste rock. The results of the analysis support the premise that increasing waste rock content reduces water content of topsoil mixes but at the same time slows declines in water loss. The main effect of this is a reduction in plant physiological function but improvement in plant survival under drought. Reductions in water content with increasing waste rock were observed to have a negative effect on plant growth and development. The exception to this was when large waste rock contents were present. This is most likely due to the greater availability of soil macropores that is predicted to promote

greater access to water through increased root length or greater access to nutrients through increased nitrogen fixation. These results improve our understanding of how waste rock content influences soil water dynamics and facilitates optimization of restoration practices, specifically in semi-arid and arid regions. The potential for waste rock amendment to improve availability of water to plants under drought as a result of either; altering soil water dynamics or pre-disposing plants to drought stress highlights the complexity behind plant-rock interactions and further research into these mechanisms remains relevant, especially under field settings.

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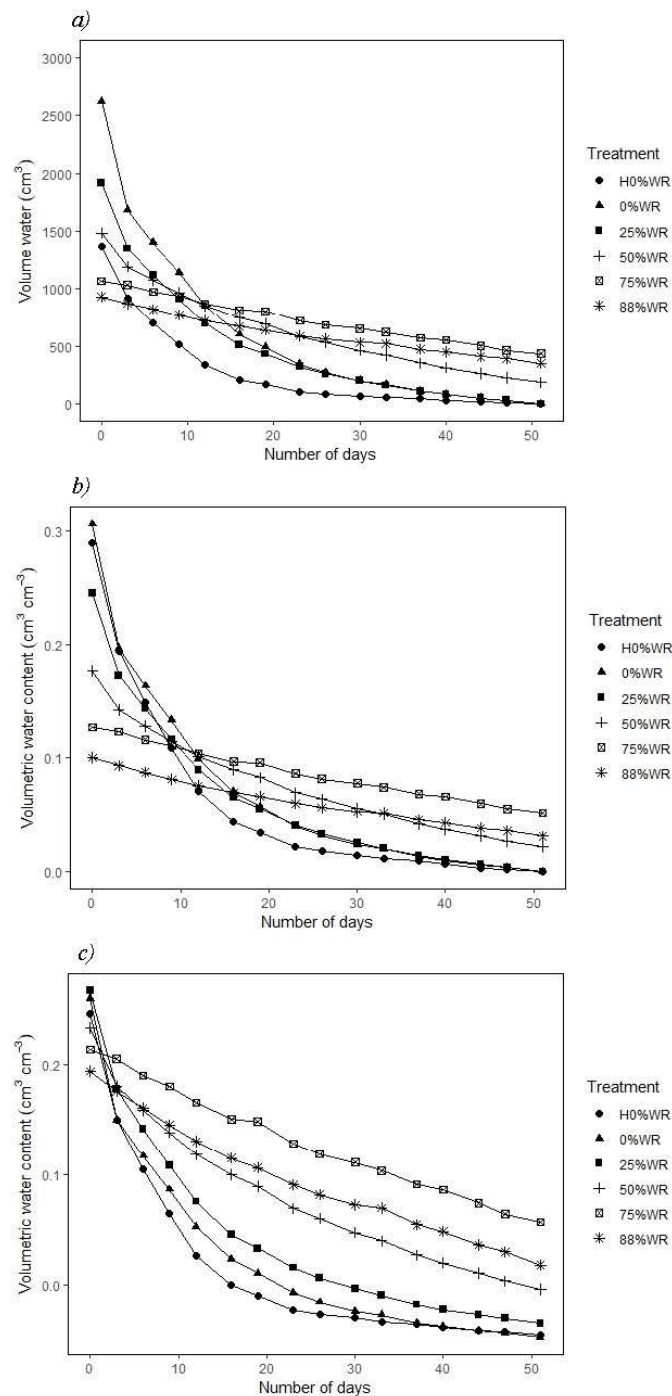
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## Appendix S1 Water content of evaporation pots



**Figure S1.** (a) Mean absolute water content, (b) mean volumetric water content of the topsoil mix and (c) mean volumetric water content of topsoil fraction only for evaporation pots across six different topsoil mixes. H0%WR – 5.25L topsoil, 0%WR – 10.5 L topsoil, 25%WR – 3:1 topsoil:waste rock, 50%WR – 2:2 topsoil:waste rock, 75%WR – 1:3 topsoil:waste rock, 88%WR – 1:7 topsoil:waste rock

### **Chapter 3: Salinity impact on early seedling growth varies with rock content of substrate in mine-site restoration**

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## **Abstract**

A number of mining operations are located in semi-arid environments. Post-mining restoration of these areas faces significant challenges. One of these challenges is the availability of topsoil. With topsoil deficits frequently encountered, secondary materials such as waste rock, are often added to increase the volume of substrate available for use by plants. Semi-arid areas are also known to contain saline soils, that also have the potential to limit restoration success. With recent evidence suggesting anthropogenic related climate change could expand the spatial distribution of salt affected soils, we aimed to test the extent to which waste rock content could influence salinity and affect early seedling emergence of two *Acacia* species known to inhabit semi-arid areas; *Acacia aneura* and *Acacia burkittii*.

We analysed early seedling growth and physical properties of topsoil mixes incorporating between 25% and 88% waste rock. The results showed that waste rock content influenced salinity and this caused differences in germination and seedling emergence. Electrical conductivity ( $EC_{1:5}$ ) was observed to increase by three-fold and five-fold at waste rock content of 50% and 75%, respectively and these increases coincided with a reduction in seedling emergence. Increases in  $EC_{1:5}$  appear to be related to a reduction in leaching potential with increasing rock content. The lowest  $EC_{1:5}$  and greatest seedling emergence was recorded on standard topsoil. This highlights the superiority of this substrate over other secondary materials. Replication of salt levels under laboratory conditions confirmed that germination of *A. aneura* and *A. burkittii* was inhibited by increasing levels of NaCl, with the osmotic component of NaCl having the greatest effect on germination. Overall the results of this study show the importance of physical properties of topsoil mixes in restoration of semi-arid areas, particularly the conditions under which germination and early seedling emergence can be supported when saline topsoil is amended with mine waste rock substrates.

## **Introduction**

Over the last fifty years, global mining production has doubled (Reichl and Schatz 2019). The extraction and processing of natural resources is now one of the main contributors to land-use related biodiversity loss (Oberle et al. 2019). Increasingly, mining operations must address mine closure at the completion of extraction, in accordance with local regulatory requirements (ICMM 2019). A critical part of mine closure is ensuring that post-mining areas are restored in accordance with public interest (Wilson 1999) and a substantial effort is now invested in re-establishing the ecological trajectory of restored mine sites (Stevens and Dixon 2017). The effort expended in restoring sites can range from minimal to extremely extensive (Palmer et al. 1997) focusing on the restoration of a group of individual species or suits of species. Regardless of the level of effort invested, however, covering re-constructed land surfaces with

topsoil is generally considered the first step in creating functional ecosystems post-mining (Bell 2001, Festin et al. 2019, Golos et al. 2019)

Where appropriate topsoil handling techniques are considered (Golos et al. 2016), natural topsoil (i.e. the upper 5–10 cm of the soil profile) contains a source of plant propagules via the soil seed bank (Merino-Martín et al. 2017) and chemical, physical and microbiological properties supportive to plant development (Kumaresan et al. 2017, Cross et al. 2018, Kneller et al. 2018). One of the most critical challenges faced in mine-site restoration of semi-arid lands however, is the limited availability of topsoil (Golos and Dixon 2014, Muñoz-Rojas et al. 2016, Merino-Martín et al. 2017). In order for topsoil to be utilized in restoration, it must be stripped and stockpiled prior to mining activities commencing (Kneller et al. 2018). The technical difficulties in correctly performing these processes on site can significantly reduce the amount of topsoil available for restoration (Merino-Martín et al. 2017). Additionally, the surface area of post-disturbance landforms often exceeds the pre-mined surface area (Ghose 2001).

Secondary materials produced during mining activities, such as mine waste rock can be used to alleviate topsoil deficits (Bateman et al. 2018, Kneller et al. 2018). Waste rock is the residuum of mineral processing activities and consists of coarse, broken, partly weathered rock (Blight 2010), often existing within a matrix of finer particles (< 2 mm). A general approach when restoring landforms is to dump pre-mixed topsoil and waste rock onto surfaces or use machinery to push and mix topsoil and waste rock down slopes (Golos et al. 2019). Studies testing incorporation of up to 25% waste rock in topsoil mixes found seedling emergence either improved (Golos et al. 2019) or remained similar (Merino-Martín et al. 2017) when compared to emergence on topsoil only. Although it is common practice for mining operations to utilise waste rock percentages greater than 50% (Tiemann 2015) limited study has been undertaken on the effect of waste rock content on early seedling growth at percentages >25%.

The success of mine-site restoration can also be compromised by the presence of saline soils. Salinity is a common feature of semi-arid regions (Schofield and Kirkby 2003, Jordán et al. 2004, Corwin et al. 2007, Allbed and Kumar 2013). Salt can originate naturally from parent rocks, which release salts as they get wet, or ancient drainage basins and inland seas that left behind salt deposits as they evaporated (Jordán et al. 2004). In these areas, salt dynamics are most influenced by temperature or rainfall (Schofield and Kirkby 2003). Where rainfall is insufficient to leach soluble salts from the soil or evapotranspiration increases, there is potential for salts to accumulate at high levels (Rengasamy 2010, Bartels and Dinakar 2013). In addition, recent evidence has suggested that anthropogenic related climate change could expand the spatial distribution of salt-affected soils by changing patterns of precipitation and

evapotranspiration (Schofield and Kirkby 2003). The amendment of saline topsoil with waste rock has the potential to affect salt dynamics. This is because the presence of rock has the potential to create preferential flow paths (Nichol et al. 2005, Zhang et al. 2016) which can increase or decrease leaching efficiency (hydraulic conductivity) depending on rock content (Corwin et al. 2007, Zhou et al. 2011). The influence of rock content on salinity remains relevant, given waste rock is frequently used to alleviate topsoil deficits.

Sodium content and soil electrical conductivity are key factors influencing seed germination and seedling survival (García-fayos et al. 2000). Seed germination can be affected by salts in two ways: the first is by inhibiting water uptake due to osmotic pressure of saline soil solution lowering its potential energy (water always moving from a higher to lower potential energy levels) and the second is by causing accumulation of specific ions leading to ion toxicity or ion imbalance (Munns and Tester 2008). Inhibition of water uptake by seeds can delay or prevent germination, depending on the extent of reduction in water potential (Alam et al. 2002). Toxicity of salt ions can cause damage to the seed metabolism (Shannon and Francois 1977) and intracellular compartments (Katembe et al. 1998) and numerous studies have reported declines in seedling growth following exposure to salinity stress (Alam et al. 2002, Bajji et al. 2002, Masondo et al. 2018). Most terrestrial plants display varying levels of salt tolerance (Bartels and Dinakar 2013). This is because salt has been present in the landscape for a long time (Crowley 1994a, 1994b, Doran and Turnbull 1997), especially in low relief landscapes where saline ground and surface water systems have persisted for millennia (Hopper 2009). Recent advances in modelling have shown soil salinity to be a driving factor in the distribution and diversification of the *Acacia* genus (Bui 2013) and several *Acacia* species are capable of growing in salt-affected areas (Bui and Henderson 2003, Joseph et al. 2015). The degree to which salinity affects germination and early seedling emergence of *Acacia* species and whether tolerance to salt stress differs between species is still a subject of study.

This study arose from the need to understand the extent to which the success of mine-site restoration in semi-arid regions was affected by waste rock content and/or salinity. To this end, the following hypotheses were tested: 1) that salinity levels of topsoil:waste rock mixes will increase as waste rock content is increased; 2) that there will be a negative relationship between germination and early seedling growth of *Acacia* species and levels of soil salinity; 3) that the effect of salt on germination and early seedling growth can be separated into osmotic and specific and; 4) that sensitivity to salt stress in seed germination and seedling development is less pronounced for a broadly distributed species than a narrowly distributed species.

We tested the hypotheses in glasshouse and laboratory experimental conditions. The first experiment tested seedling emergence of two *Acacia* species in glasshouse conditions, on saline topsoil incorporating up to 88% waste rock. To investigate the effects of increasing salt on germination and early seedling growth, a second experiment was set up under laboratory conditions where seeds of the same *Acacia* species were germinated under a gradient of NaCl. Additionally, osmotic effects were separated from ionic effects through comparison of NaCl and equivalent metabolically inactive osmoticum, polyethylene glycol (PEG 8000) concentrations.

## **Materials and methods**

### *Study Site*

Topsoil and waste rock were obtained from an iron ore mine site 160 km south-east of Geraldton in Western Australia (29°11'05, 116°12'06) in April 2018. The mine site is situated in the Koolanooka land system which includes the Koolanooka Hills, a range of rolling to very steep low hills. Soils are a matrix of rock and sandy loam on upper slopes and loamy earths and duplexes on lower slopes (DPIRD 2019). Mining activity occurs within the Banded Ironstone Formation in which iron occurs largely in the magnetite and the amphiboles (ATA Environmental 2004). The climate of the study site is semi-arid with cool, mild winters and hot, dry summers (Bureau of Meteorology 2019). Mean annual rainfall ranges between 240 mm to 460 mm and is mostly concentrated in the winter months (May to August), accounting for approximately 60% of total annual rainfall. During the winter months maximum temperatures range between 19.3 to 22.9°C and mean minimum temperatures range between 6.3 and 9.9 °C (Bureau of Meteorology 2019).

### *Study Species*

In order to test if species sensitivity to salt was influenced by distribution patterns; two *Acacia* species with contrasting habitat preferences were chosen for this study: *Acacia burkittii* and *Acacia Aneura*. *Acacia burkittii* is known to occur on sandhills and ridgelines and *A. aneura* occurs on slopes and crests of rocky outcrops (Woodman Environmental Consulting 2012). *Acacia aneura* is widely distributed throughout the arid interior of Australia and *A. burkittii* is distributed throughout the arid/semi-arid interior of Australia from Western Australia to Victoria (Atlas of Living Australia 2020a, 2020b). Seed of the two species was obtained from a commercial seed supplier. Prior to experimental use filled seed was separated from unfilled seed with a vacuum-aspirator (“Zig Zag” Selecta, Machinefabriek BV, Enkhuisen, the Netherlands) and then x-rayed (MX-20 digital X-ray cabinet, Faxitron, Tucson, USA) to determine seed fill. Seeds were identified as filled if the embryo and endosperm appeared under x-ray as uniform white-grey in colour, showed no signs of internal damage and was not

shrivelled or retracted from the testa. Filled seed was pre-treated prior to sowing by soaking in near boiling water (90°C) for two minutes.

*Experiment 1: manipulating topsoil and waste rock substrates to test seedling emergence*

Topsoil was stripped with a skid steer loader from the upper 15 cm of the soil profile in a 10 m x 10 m area of the mine site that had previously been restored in 2015. Waste rock was collected from the surface of a 1 m high stockpile containing previously dumped waste rock. To ensure that topsoil:waste rock mixes accurately reflected waste rock and topsoil components, waste rock was sieved to discard fines (<4 mm) and topsoil was sieved to discard rock fragments >4 mm. Topsoil was then air dried for minimum 14 days prior to use. Waste rock size varied between 4 mm and 100 mm. The size distribution of waste rock was calculated based on Feret's diameter using a binary image of a random sample of 589 rock fragments analysed in the program ImageJ (Ferreira and Rasband 2012) (Table 8). Rock fragment density was measured using the water displacement method (Archimedes' principle).

**Table 8.** Size distribution and density of waste rock fragments

Feret's diameter	4-10 mm	10-20 mm	20-30 mm	30-40 mm	>40 mm
<i>n</i>	383	94	47	30	35
% of number fragments	65	16	8	5	6
<i>n</i>	6	6	6	5	11
Mean density (g/cm <sup>3</sup> )	3.5±0.23	3.3±0.17	3.6±0.23	3.2±0.21	3.2±0.16

The physio-chemical properties of the topsoil were determined from three 500 g bulked samples collected in May 2018. Soil samples were stored dry at ambient temperature (ca. 25 °C) one month prior to analytical determination of chemical factors. Analyses (see Table 9) were undertaken by Chem Centre (Bentley, Western Australia) following the methods of Rayment and Lyons (2011). Soil texture of the topsoil determined to be loamy sand based on results of particle size analysis. Topsoil was classified as saline (electrical conductivity (EC) > 4 dSm) according to definition provided by Soil Science Society of America (2020).



**Table 9.** Physical and chemical characteristics of topsoil and waste rock (crushed and grounded). Data are presented as means  $\pm$  1 s.e. (n=3). T values and P values represent the results of pairwise comparison of topsoil and waste rock based on two sample t-test. Blank cells indicate no tests run.

Parameter	Method	Unit	Topsoil	Waste Rock	T value	P value
<b>Physical Properties</b>						
Texture			Loamy sand	-		
Particle size distribution						
Stones (>2 mm)	Sieve	w/w	10.2 $\pm$ 0.65	100	-138	<0.05
Sand (0.2-2 mm)	Fraction	w/w	73.0 $\pm$ 0.58	0	126	<0.05
Silt (0.002–0.2 mm)	Fraction	w/w	5.1 $\pm$ 0.07	0	77.0	<0.05
Clay (<0.002 mm)	Fraction	w/w	11.7 $\pm$ 0.17	0	67.5	<0.05
<b>Chemical Properties</b>						
EC	1:5 water	dS/m	5.4 $\pm$ 0.25	0.36 $\pm$ 0.02	94.2	<0.05
pH	H <sub>2</sub> O		5.4 $\pm$ 0.03	7.3 $\pm$ 0.06	-26.3	<0.005
Organic carbon	Walkley-Black	%	0.33 $\pm$ 0.02	0.12 $\pm$ 0.00	10.5	<0.05
Total nitrogen	Kjeldahl digest	mg/kg	0.02 $\pm$ 0.00	<0.005	35.5	<0.05
Total phosphorus	Kjeldahl digest	mg/kg	186 $\pm$ 3.33	606 $\pm$ 89.87	-4.67	<0.05
Available phosphorus	HCO <sub>3</sub>	mg/kg	3 $\pm$ 0	2 $\pm$ 0	6.84	<0.05
Al	Mehlich-3	mg/kg	316 $\pm$ 3.33			
B	Mehlich-3	mg/kg	2.3 $\pm$ 0.15			
Ca	Mehlich-3	mg/kg	526 $\pm$ 23.3			
Cd	Mehlich-3	mg/kg	<0.01			
Co	Mehlich-3	mg/kg	0.06 $\pm$ 0.00			
Cu	Mehlich-3	mg/kg	0.30 $\pm$ 0.00			
Fe	Mehlich-3	mg/kg	28.0 $\pm$ 0.00			
K	Mehlich-3	mg/kg	216 $\pm$ 3.33			
Mg	Mehlich-3	mg/kg	810 $\pm$ 10.00			
Mn	Mehlich-3	mg/kg	14.3 $\pm$ 0.33			
Mo	Mehlich-3	mg/kg	<0.01			
Na	Mehlich-3	mg/kg	>1000	226 $\pm$ 31.7	24.3	<0.05
Ni	Mehlich-3	mg/kg	0.20 $\pm$ 0			
P	Mehlich-3	mg/kg	1.7 $\pm$ 0.33			
S	Mehlich-3	mg/kg	>250	0.07 $\pm$ 0.01	17201	<0.05
Zn	Mehlich-3	mg/kg	0.43 $\pm$ 0.03			
As	Mehlich-3	mg/kg	<0.01			
Pb	Mehlich-3	mg/kg	1.4 $\pm$ 0.03			
Se	Mehlich-3	mg/kg	<0.10			

To test the effects of waste rock content on soil salinity and early seedling growth four topsoil mixes with varying percentages of waste rock were created including 25%, 50%, 75% and 88% waste rock. Percentage of waste rock was added based on volume (v/v) required. Topsoil and waste rock quantities were mixed in a 40 L revolving drum for up to 30 seconds to ensure each blend was homogenous. Approximately 5 L of each topsoil mix was then added to a 24 cm height by 17 cm width square pot lined with polyethylene with four drainage holes punctured in the bottom. Actual topsoil and waste rock volumes in each pot were re-calculated after settling to take into account porosity of the waste rock at the time of mixing (Table 10). Three treatments were left without waste rock for comparison (0%WR, H0%WR and Q0%WR). Smaller square pots approximately 16 cm x 16 cm and 10 cm x 15 cm (height by width) were used for the H0%WR and Q0%WR treatments, respectively. Here the volume of topsoil in the 50%WR (approximately 2.5 L) and 75%WR treatments (approximately 1.25 L) was placed in these pots, to examine if the effect of adding waste rock on seedling emergence is a result of there being less soil volume.

**Table 10.** Total and fractional volumes of topsoil and waste rock used in used in Experiment 1. Q0%WR – 1.25 L topsoil , H0%WR – 2.5 L topsoil, 0%WR – 5 L topsoil, 25%WR, 50%WR , 75%WR, 88%WR (mean  $\pm$  SE,  $n=20$ ). Where total mix is the volume of the topsoil mix after settling, waste rock fraction is the percentage of waste rock minus porosity and topsoil fraction is the volume of total mix minus waste rock volume.

Mean volume ( $\text{cm}^3 \text{ cm}^{-3}$ )	Q0% WR	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
Total mix	1388 $\pm$ 23.5	2811 $\pm$ 70	5175 $\pm$ 108.5	5038 $\pm$ 48.9	4732 $\pm$ 45.5	4937 $\pm$ 77.1	5283 $\pm$ 83.1
Topsoil fraction	1388 $\pm$ 23.5	2811 $\pm$ 70	5175 $\pm$ 108.5	4351 $\pm$ 48.9	3357 $\pm$ 45.5	2875 $\pm$ 77.1	2863 $\pm$ 83.1
Waste rock fraction	0	0	0	688 $\pm$ 0	1375 $\pm$ 0	2063 $\pm$ 0	2420 $\pm$ 0

The experiment was conducted when glasshouse daily temperatures were similar to those experienced in mid-west of Western Australia during peak growing season (June and July). Mean maximum and minimum glasshouse temperature varied between 23.9°C and 6.1°C and mean humidity varied between 35.8% relative humidity (rH) and 99.8% rH respectively. For each treatment seeds were sown in 20 replicate pots. Each pot contained 20 seeds of *A. aneura* and 30 seeds of *A. burkittii*. An additional two pots were left unsown across all treatments for continuous measurement of volumetric water content. Pots were given 10 mm of water daily for three days and 5 mm every third day thereafter for 21 days. Electrical conductivity ( $\text{EC}_{1:5}$ ) of irrigation water was 0.38  $\text{dS m}^{-1}$ . Final seedling emergence was recorded at 21 days.

Seedlings were considered fully emerged when hypocotyl erection and cotyledon development was exhibited.

EC<sub>1:5</sub> of soil mixes at the stage of final seedling emergence was measured with deionised water (1:5 w/v) at 25°C using an EC tester (HI98130, Hanna Instruments Ltd, VIC, Australia). For this analysis, eight 5 g soil samples were taken from the centre of the pot, in-between seedlings, at a depth where the majority of seedling roots resided (maximum 3 cm from soil surface). Only the topsoil fraction was sampled during this process. Samples were dried in a drying oven (40°C for 48 hours), submerged in 25 ml deionised water, agitated and left to settle for 3 h prior to taking measurements. Minimum and maximum EC<sub>1:5</sub> was 0.22 and 8.24 dS m<sup>-1</sup>, respectively. If salinity was due to NaCl only, this level of salinity based on a gravimetric water content of 0.68 g g<sup>-1</sup>, equates to concentrations of approximately 1.78 (~13 mM NaCl) and 65 dS m<sup>-1</sup> (~700 mM) soluble salts in soil water (EC<sub>e</sub>) (Slavich and Petterson 1993, CRC 2019).

#### *Experiment 2: testing the effects of soil salinity on seed germination*

Salinity levels recorded on soil mixes were recreated in laboratory conditions, to test the effect of salinity on seed germination and seedling vigour. Different iso-osmotic concentrations were imposed on *A. aneura* and *A. burkittii* seeds using NaCl (Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia) and polyethylene glycol (PEG) (PEG-8000; Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia). Prior to the germination test, seeds were sterilised using 2% (w/v) calcium hypochlorite [Ca(OCl)<sub>2</sub>] solution for 30 minutes under 10 minute cycles of alternating vacuum (e.g. on/off/on at -70 kPa) and then rinsed in deionised water for three minutes. Physical dormancy was broken by treating seeds in hot water (90°C) for 2 minutes.

Four replicates of 25 seeds were sown onto 90 mm petri dishes lined with filter paper and irrigated with 8 mL of NaCl or PEG at osmotic potential of -0.232 (50 mM NaCl), -0.457 (100 mM NaCl), -0.906 (200 mM NaCl), -1.310 (300 mM NaCl), -1.661 (400 mM NaCl) or -1.985 MPa (500 mM NaCl) or a control solution of sterile, deionized water. The concentration of PEG-8000 needed to produce equivalent osmotic potential was prepared according to Michel (1983) and validated with a dew point psychrometer (WP4C Dew Point PotentialMeter; Decagon Devices, Inc., Pullman, WA, USA). Petri dishes were wrapped in cling film to prevent evaporation and placed in a growth chamber (Contherm Scientific Ltd., Petone, NZ) with 12/12 h light/dark regime (60 μmol m<sup>-2</sup> s<sup>-1</sup>, 400–700 nm, cool-white fluorescent light). Seeds were incubated at 20°C and germination scored daily up until the point at which no further germination had occurred in the control for two weeks. Seeds were considered germinated when radicle protrusion was ≥ 2 mm long. A seed was considered to show abnormal germination if shoot growth occurred in the absence of radicle extension and thus

was excluded from counts. To assess radicle protrusion length relative to salinity or osmotic stress, 25 seeds were randomly selected from each treatment and protrusion length measured at 48 hours post germination using a digital electronic caliper (EC799B Series, Starrett, MA, USA). A cut test was performed at the end of the germination period and sample sizes adjusted based on the number of seeds with non-viable embryos.

### *Data Analysis*

All analyses were performed with R Studio statistical software version 1.1.463 (RStudio, 2018). For the emergence study, generalised linear models (GLM) were fitted to binomial data using the ‘glm’ function in R (RStudio, 2018) and a logit-link function. The full model was used to test the interaction between treatment and EC<sub>1.5</sub> and its effect on seedling emergence. Wald chi-squared test was used to determine statistical significance for each of the independent variables. Means across treatments were compared within species using Fisher least significant test (LSD) for seedling counts and Tukey-honestly significant difference (HSD) for EC<sub>1.5</sub>. For the germination study, a three parameter log-logistic function was used to fit germination data within a time-to-event structure using the ‘drm’ function available in the ‘drc’ package in R (Ritz and Strebig 2005):

$$F(x) = \frac{d}{1 + \exp[b(\log(x) - \log(e))]}$$

Where (d) is the parameter for maximum germination, (b) the slope of the curve F(x) and (e) time to 50% germination (t<sub>50</sub>) or the 50% point of the curve. Thresholds for germination under different osmotic and ionic conditions were calculated using mean germination from the final day with treatment standardised. Parameter estimates were compared by t-test of ratios using the ‘CompParm’ function (Ritz and Strebig 2005). Differences in radicle protrusion length across different iso-osmotic concentrations and between species were tested with GLM fitted with Gaussian distribution. Before analysis with GLM variables were tested for homogeneity of variance with the Levene test. For all nonlinear models Akaike Information Criterion index was used to determine best fit (Sakamoto et al. 1986).

## **Results**

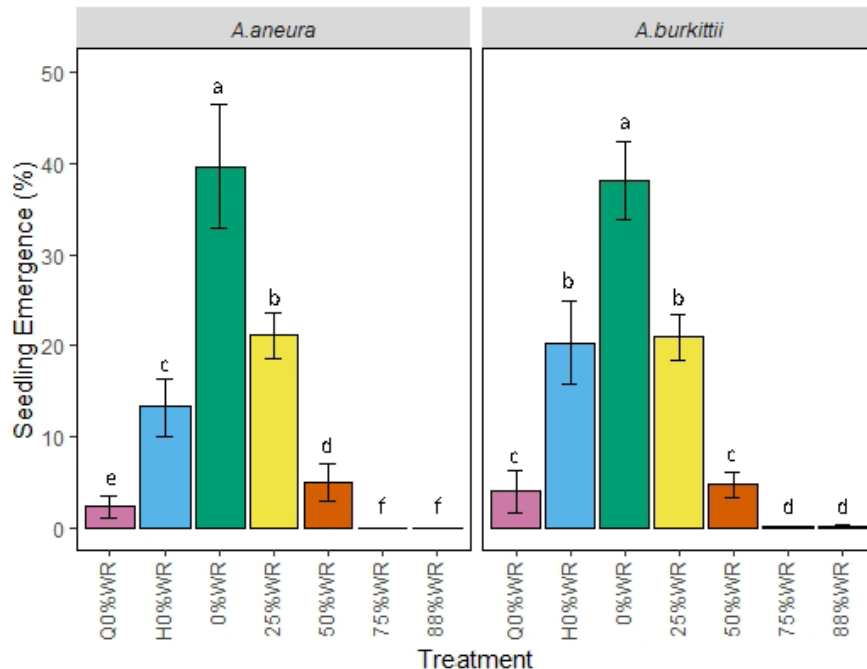
### *Experiment 1: manipulating topsoil and waste rock substrates to test seedling emergence*

Increasing the waste rock content of topsoil mixes generally led to lower seedling emergence of both *A. aneura* and *A. burkittii* ( $\chi^2(6) = 433.9$ ,  $P < 0.001$ ) (Figure 1). The 25% WR treatment recorded the highest emergence percentage ( $21 \pm 1.8\%$ ) out of the four topsoil mixes that contained waste rock. The highest mean emergence overall occurred on 0% WR ( $39 \pm 4.0\%$ ), which had no added waste rock. Lowest mean emergence was measured from 75 – 88% WR

(1 ± 0.1%). At three weeks post emergence 100% of seedlings on the 88% and 75% WR treatments and 70% of seedlings on the 50% WR treatment experienced mortality.

Lower seedling emergence also occurred on H0%WR and Q0%WR when compared to 0%WR. These treatments contained smaller topsoil volumes. When compared to 0%WR, seedling emergence on H0%WR and Q0%WR lowered by approximately 57% and 92% respectively for both *Acacia* species. At three weeks post emergence seedling mortality was 3% on H0%WR and 25% on Q0%WR. Although seedling emergence differed statistically across treatments ( $\chi^2(6) = 433.9, P<0.001$ ), trends in seedling emergence did not differ significantly between *A. aneura* and *A. burkittii*. ( $\chi^2(1) = 2.23, P>0.14$ ).

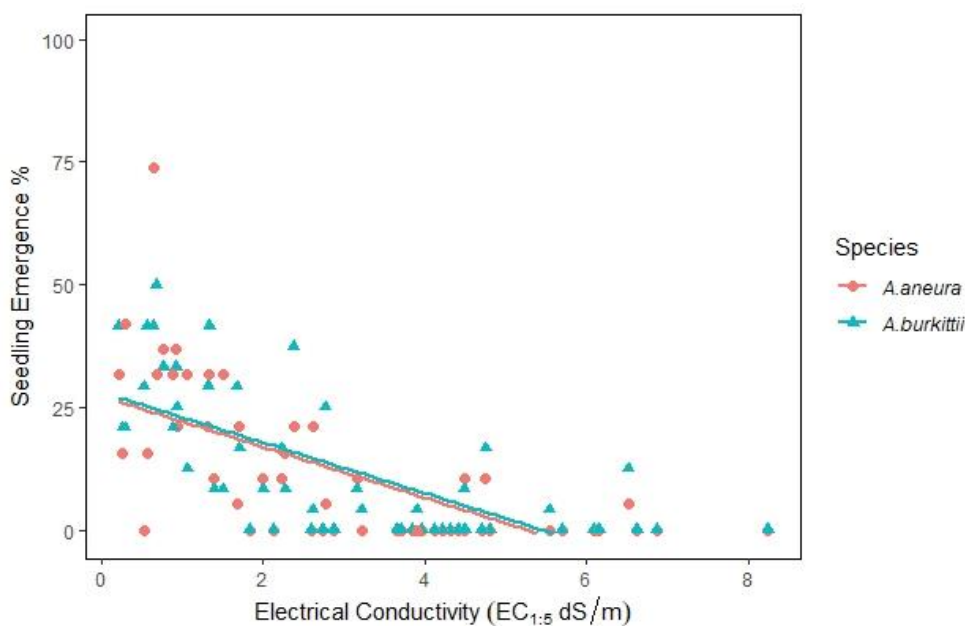
Incorporation of waste rock to topsoil mixes coincided with an increase in salinity ( $\chi^2(1) = 26.3, P<0.001$ ) (Figure 2, Table 11). The highest mean EC<sub>1:5</sub> was measured from 75%WR at 5.0 dS m<sup>-1</sup>. This reading was five times higher than mean EC<sub>1:5</sub> recorded on 0%WR (0.95 dS m<sup>-1</sup>). Treatments including smaller topsoil volumes also recorded higher EC<sub>1:5</sub> readings than those recorded on 0%WR, with mean EC<sub>1:5</sub> being 2.5±0.71 and 4.47±0.69 dS m<sup>-1</sup> for H0%WR and Q0%WR respectively. Topsoil mixes exhibiting higher EC<sub>1:5</sub> also recorded lower seedling emergence ( $\chi^2(1)= 68.7, P<0.0001$ ) with patterns in emergence influenced by interactions between EC<sub>1:5</sub> and waste rock content ( $\chi^2(6) = 26.2, P<0.001$ ).



**Figure 1.** Total seedling emergence (% , mean ± SE, n=20) of *A. aneura* and *A. burkittii* across seven different topsoil mixes. Letters indicate significant differences in seedling emergence between treatments (Fisher LSD, P<0.05). Q0%WR – 1.25 L topsoil , H0%WR – 2.5 L topsoil, 0%WR – 5 L topsoil, 25%WR, 50%WR, 75%WR, 88%WR

**Table 11.** EC<sub>1:5</sub> (mean ± SE, n=8) of topsoil fraction sampled from seven different topsoil mixes at 21 days of seedling emergence. Letters indicate significant differences in seedling emergence between treatments (HSD, P<0.05). Q0% WR – 1.25 L topsoil , H0% WR – 2.5 L topsoil, 0% WR – 5 L topsoil, 25% WR, 50% WR, 75% WR, 88% WR

Topsoil Cover Mix	Q0% WR	H0% WR	0% WR	25% WR	50% WR	75% WR	88% WR
Mean EC <sub>1:5</sub> (dS m <sup>-1</sup> )	4.5±0.69 <sup>b</sup>	2.5±0.71 <sup>ab</sup>	0.95±0.24 <sup>a</sup>	1.2±0.26 <sup>a</sup>	3.6±0.63 <sup>b</sup>	5.0±0.70 <sup>b</sup>	3.7±0.35 <sup>b</sup>



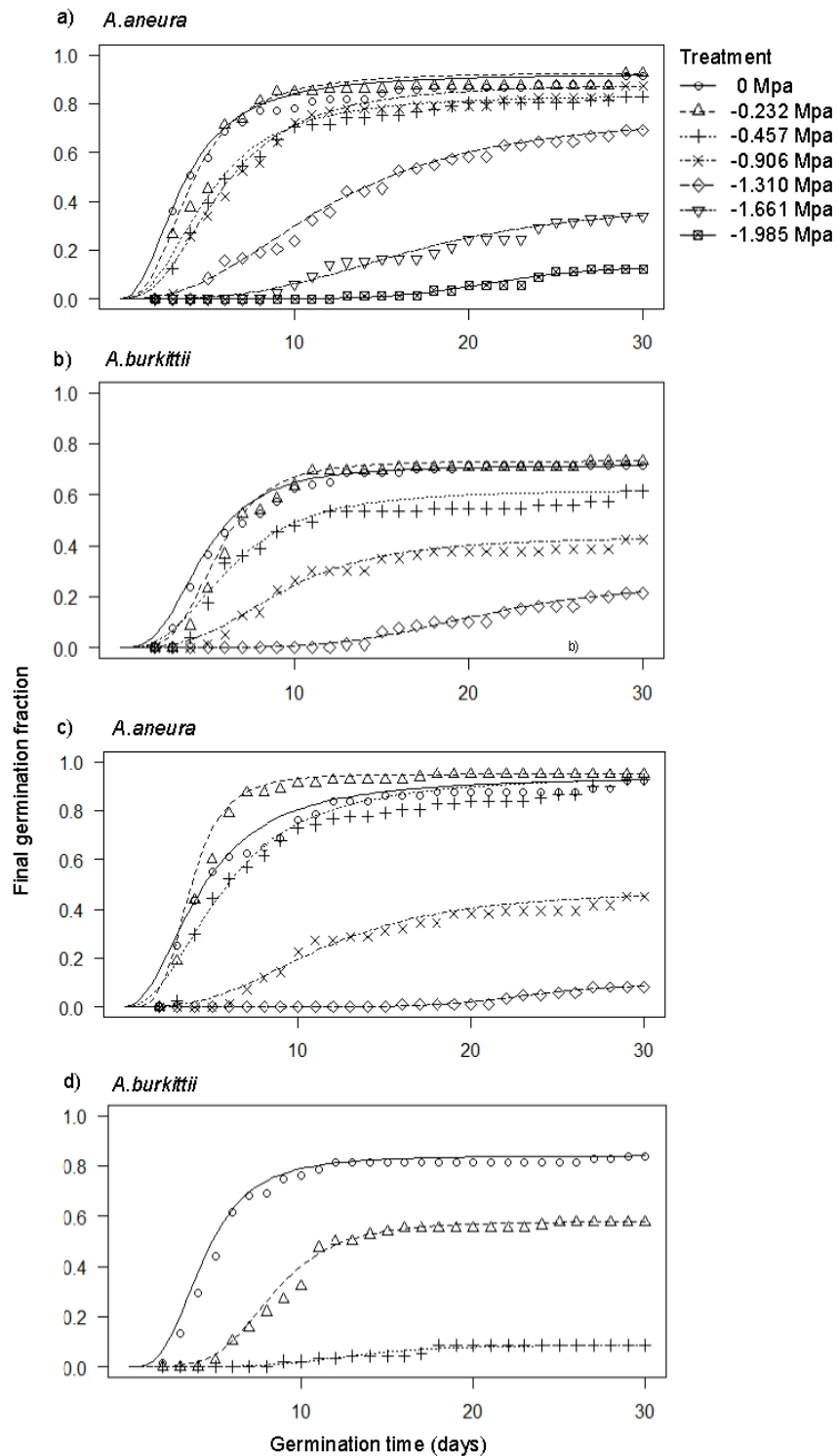
**Figure 2.** Mean seedling emergence (%) of *A. aneura* and *A. burkittii* as a function of salinity (EC<sub>1:5</sub> dS m<sup>-1</sup>), n=8.

*Experiment 2: testing the effects of salinity stress on seed germination*

Replication of soil saline conditions in laboratory setting, showed increasing concentrations of NaCl progressively reduced germination in both *A. aneura* and *A. burkittii* (p<0.001 for both, see Appendix 1). At low salinity levels, (-0.232 – -0.457 Mpa) maximum germination reached approximately 70-90% in both species (Figure 3a and 3b). At moderate salt levels (-0.906 – -1.310 Mpa) maximum germination was reduced by half. Germination was completely inhibited at -1.661 and -1.985 Mpa for *A. burkittii* and *A. aneura*, respectively. Sensitivity to increasing concentrations of NaCl differed between species with *A. burkittii* exhibiting a significant decline in maximum germination at -0.906 Mpa ( $t_{6,21} = 2.60$ , P<0.05) while *A. aneura* reached -1.661 Mpa NaCl before differences were observed ( $t_{6,21} = 2.99$ , P<0.001). Time to germination (t<sub>50</sub>) was affected at lower NaCl concentrations than maximum germination with significant differences in t<sub>50</sub> observed at salt levels of -0.457 Mpa (P<0.05 for both species). At -1.310 Mpa, t<sub>50</sub> increased three-fold (from approximately 4 to 12 days)

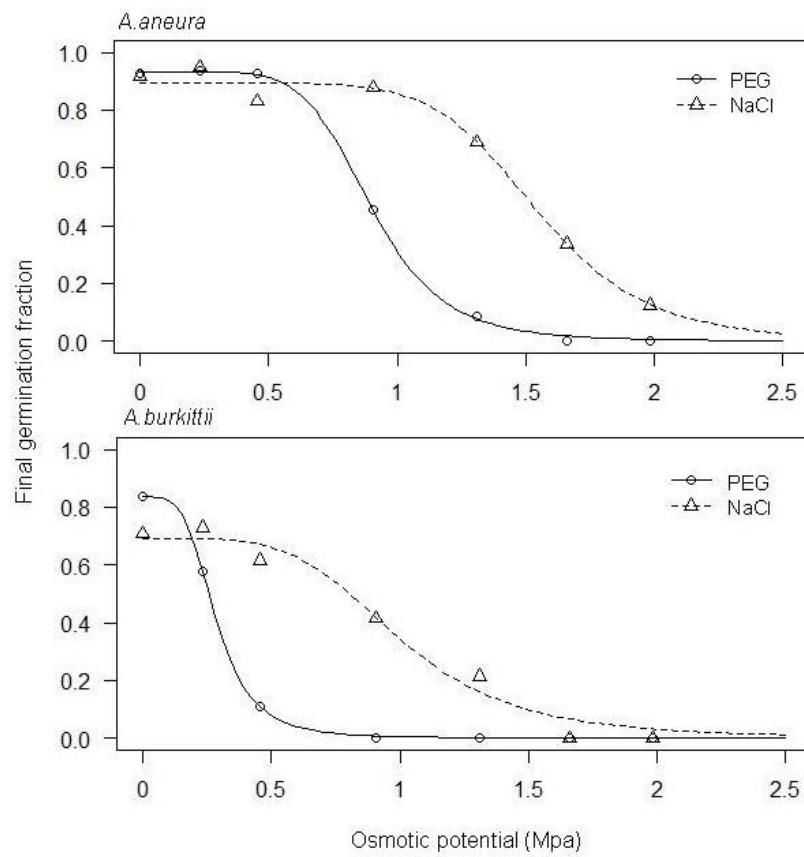
and four-fold (from approximately 5 to 20 days) for *A. aneura* and *A. burkittii*, respectively. Seeds of *A. burkittii* were slower to germinate under effect of NaCl with germination delayed at -0.232 Mpa and -0.457 Mpa for *A. burkittii* and *A. aneura*, respectively ( $t_{13,42} = 3.76$ ,  $P < 0.001$ ).

Maximum germination was affected at lower osmotic potentials of PEG than NaCl, although this difference was not statistically significant ( $t_{13,98} = 0.86$ ,  $P > 0.39$ ) (see Appendix S1). When seeds were germinated in PEG, significant differences in maximum germination were observed at -0.232 MPa for *A. burkittii* ( $t_{6,21} = 2.85$ ,  $P < 0.001$ ) and -0.906 MPa for *A. aneura* ( $t_{6,21} = 3.72$ ,  $P < 0.001$ ). Under the effect of PEG, germination was completely inhibited at -1.661 MPa in *A. aneura* and -0.906 MPa in *A. burkittii* (Figure 3c and 3d). The most significant effect of PEG was on speed of germination ( $t_{50}$ ) where imbibition was significantly slower in both species compared to NaCl ( $t_{13,98} = -8.95$ ,  $P < 0.001$ ) (Figure 4). These differences were pronounced at -0.457 MPa for *A. aneura* and at -0.232 MPa for *A. burkittii*. At -0.232 MPa the number of days taken to reach maximum germination was doubled for *A. burkittii* ( $t_{6,21} = -19.4$ ,  $P < 0.001$ ). *A. aneura* maintained similar germination speed up to osmotic potential of -0.457 MPa ( $t_{6,21} = 10.4$ ,  $P < 0.001$ ). At -0.457 MPa the number of days taken to reach maximum germination was increased by one day. Again, *A. burkittii* exhibited a higher sensitivity to increasing osmotic potential of PEG both in maximum germination ( $t_{13,42} = 2.39$ ,  $P < 0.05$ ) and germination speed ( $t_{13,42} = 12.7$ ,  $P < 0.001$ ) when compared to *A. aneura*.



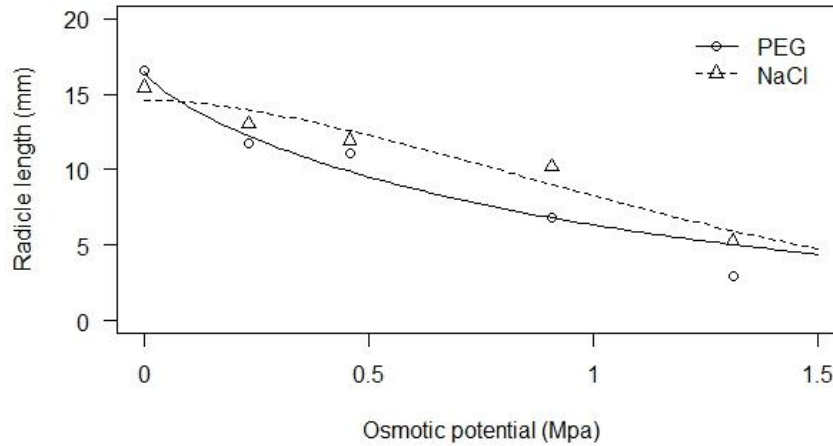
**Figure 3.** (a) Germination responses for *A. aneura* and (b) *A. burkittii* across an osmotic potential gradient (Mpa) created with varying concentrations of NaCl. (c) Germination responses for *A. aneura* and (d) *A. burkittii* across an osmotic potential gradient (Mpa) created with varying concentrations of polyethylene glycol. Seeds were germinated under 12 h alternating light/dark for 30 days. Data were excluded from graphs where no germination occurred (i.e. at -1.661 Mpa or -1.985). Full model parameters are available in Appendix S1.





**Figure 4.** Germination responses for *A. aneura* and *A. burkittii* across an osmotic potential gradient (MPa) created by different concentrations of polyethylene glycol and NaCl. Seeds were germinated under 12 h alternating light/dark for 30 days. Full model outputs are available in Appendix S1.

Declines in maximum germination under saline conditions also coincided with a decrease in radicle protrusion ( $\chi^2(10) = 203$ ,  $P < 0.001$ ) (Figure 5). For both species mean radicle length was reduced by more than 60% at osmotic potentials greater than -1.310 Mpa. Seeds germinated under PEG recorded lowest radicle length ( $\chi^2(1) = 13.6$ ,  $P < 0.001$ ) with mean radicle length  $12.6 \pm 0.36$  mm under NaCl and  $11.9 \pm 0.38$  mm under PEG. Radicle growth was significantly reduced at -0.232 MPa for both *A. burkittii* ( $\chi^2(6) = 24.3$ ,  $P < 0.001$ ) and *A. aneura* ( $\chi^2(6) = 64.0$ ,  $P < 0.001$ ) under effect of PEG. A slightly higher level of NaCl (-0.457 Mpa) was required to reduce radicle protrusion in both species ( $P < 0.001$  for *A. burkittii* and *A. aneura*). Radicle protrusion in *A. burkittii* was consistently lower under iso-osmotic concentrations of PEG and NaCl when compared to *A. aneura* ( $\chi^2(1) = 6.75$ ,  $P < 0.01$ ) with mean radicle length  $10.8 \pm 0.42$  for *A. burkittii* and  $11.5 \pm 0.34$  for *A. aneura*.



**Figure 5.** Mean radicle protrusion length at 48 hours post germination for *A. aneura* and *A. burkittii* across an osmotic potential gradient (MPa) created by different concentrations of polyethylene glycol and NaCl.

### Discussion

In this study, incorporation of waste rock with topsoil coincided with an increase in  $EC_{1.5}$  and a reduction in seedling emergence. Analysis of results showed that declines in seedling emergence were most likely due to increasing levels of salt delaying or inhibiting seed germination. This result supports the first and second hypotheses that salinity levels increase as waste rock content increases and that increasing levels of salinity have a negative effect on germination and early seedling growth. The factors that lead to the observed salt accumulation in topsoil mixes might be related to increased evaporation or decreased leaching potential. It was found that, accumulation of salt did not correspond with increased evaporation (Appendix S2) and thus could only be related to decreased leaching potential. The mechanisms behind the observed increase in EC might therefore be explained with reference to depth of soil in the experimental conditions employed in this study. In order to alleviate impacts of salts on plant growth, salts must be leached below the root zone (Rengasamy 2010). The mean EC of topsoil prior to incorporation with waste rock was  $5.38 \pm 0.24 \text{ dS m}^{-1}$ . Sampling of substrate from the root zone after 21 days of irrigation, showed mean EC of 0%WR was  $0.95 \pm 0.24 \text{ dS m}^{-1}$ , indicating that some leaching of salts occurred over the irrigation period. Treatments Q0%WR and H0%WR contained smaller volumes of topsoil, which resulted in these treatments containing lower soil depths compared to 0%WR. It is possible that where leaching occurred on these topsoil volumes, the depth of soil was not sufficient enough to maintain adequate separation between salts accumulated at deeper soil depths and the root zone.

With regard to treatments with waste rock, the addition of rock fragments may have altered soil hydrological processes, causing a decrease in leaching efficiency. Rengasamy et al. (2003) contends that in order for salt to be leached from the soil, salt must be moved downwards as water passes through the soil solution. As waste rock content increases, the frequency of rock-to-rock contact increases (Zhang et al. 2016) and there is potential for macropores to develop when the space among rock fragments is not completely filled by soil or when larger rock fragments prevent the surrounding soil matrix from packing (Nasri et al. 2015). The creation of these macropores has the potential to develop continuous preferential flow paths (Cerdà 2001, Zhou et al. 2011). The associated increases in preferential flow through these paths can affect the leaching process when all or part of the infiltrating water passes through a portion or all of the soil profile via large pores or cracks without contacting or displacing water present within finer pores or soil aggregates (Bouma 1991, Corwin et al. 2007). The result of this effect is that some resident salt is not miscibly displaced by incoming water (Corwin et al. 2007). This reduces the leaching efficiency and increases the amount of salt retained within substrates. Similar mechanisms have been used to explain solute transport in mine waste rock piles. For example, exchange of water or tracer (chloride) between small and large pores was reported to be limited in waste rock piles as a result of water flow being dominated by preferential flow paths after infiltration events (Nichol et al. 2005). It should be noted here that while the physical mechanisms leading to salt accumulation as described were not observed in this study, they can be considered reasonable hypotheticals that are worthy of further research to test their veracity.

The third hypothesis of this study, that the effects of salinity on germination and early seedling growth of *Acacia* species can be separated into osmotic and specific was accepted. It was found that delays in germination were greater when seeds were germinated under iso-osmotic solutions of PEG. These findings are consistent with previous studies where the osmotic effect of NaCl was observed to inhibit the germination of desert shrub species (Gorai et al. 2014) and crop species such as wheat, rice and *Brassica* species (Hampson and Simpson 1990a, Huang and Redmann 1995, Almansouri et al. 2001, Alam et al. 2002). Some studies noted ion toxicity to have greater effect on germination of halophytes (e.g. Katembe et al. 1998, Duan et al. 2004) although this result is likely due to the tolerance of these plant species to high osmotic stress (Alam et al. 2002). The greater inhibitory effect of osmotica on germination speed can be related to its effect on mechanisms of water entry. This is because in order for germination to occur, a threshold level of hydration is required (Ramagopal 1990). PEG is a non-penetrating solute that inhibits external water content at increasing concentrations, leading to lower diffusion of water through the seed coat (Bajji et al. 2002, Khayatnezhad and Gholamin 2011). By contrast, NaCl may readily cross the cell membrane

into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions (Katembe et al. 1998). Dodd and Donovan (1999) explained that penetrating ions could contribute to a decrease in the internal osmotic potential of the seed, leading to increased water uptake efficiency and initiation of germination. A rapid response from seeds exposed to low water potentials is an important adaptation since it ensures that ungerminated seeds exposed to saline conditions can germinate during periods of precipitation when stress was temporarily alleviated by leaching (Katembe et al. 1998).

Exposure of seeds to PEG also had greater effect on radicle growth. Average radicle length was measured to be 0.7 mm lower under PEG compared to NaCl. This observation is consistent with Almansouri et al. (2001) and Farid and Ridwan (2018) who found osmotic stress to have greater effect on radicle growth for wheat and rice cultivars. In contrast, Roundy et al. (1985) and Hampson and Simpson (1990b) reported NaCl to be more inhibitory. This is because Na<sup>+</sup> ions displaces Ca<sup>2+</sup> in root cells, resulting in disruption of membrane integrity and leakage of K<sup>+</sup>, of which adequate concentrations are essential for continued root growth (Cramer et al. 1985, Alam et al. 2002). The explanation for greater root growth under NaCl might lie in improved water balance, as presence of ions in water solution maintains water-absorbing ability of plant roots (Farid and Ridwan 2018). Nonetheless, the adverse affects of salt on seedling vigour have the potential to increase species exposure to water stress. This is because exposure to osmotic stress causes reductions in root cell turgor (Pritchard et al. 1991). These reductions make it more difficult for plant roots to penetrate soils, leading to declines in overall root growth (Triboulot et al. 1995). This effect can increase the susceptibility of seedlings to water stress when reductions in root growth prevent plants from seeking out larger volumes of soil for water under drought (Cochrane 2018).

In hypothesis four, it was predicted that sensitivity to salt stress in seed germination and seedling development is less pronounced for a broadly distributed species than a narrowly distributed species. This hypothesis was accepted. The results showed that the widely-distributed *A. aneura* was more tolerant to salt than the more narrowly distributed *A. burkittii*. In the case of *A. aneura*, germination was completely inhibited at -1.661 Mpa compared to -0.906 Mpa for *A. burkittii*. *Acacia burkittii* also displayed lower seedling vigour (as indicated by radicle protrusion) than *A. aneura* under increasing osmotic potential. We suggest that the lower sensitivity of *A. aneura* to salt is likely to arise due to this species having greater exposure to habitats of low water availability where exaptation to salt has occurred, rather than adaptation to water stress. Notably, *A. aneura* is known to occur in mulga communities that dominate the arid interior of Australia (Atlas of Living Australia 2020a, Bureau of Meteorology 2020). Previous studies have shown *Acacia* species inhabiting desert ecosystems are more likely to continue to germinate under higher salt thresholds (i.e. >400 mM NaCl), for

example, *A. rostellifera*, *A. raddiana* and *A. decurrens* (Khalil et al. 2016, Kheloufi et al. 2016). The potential for species inhabiting arid areas to demonstrate higher salt tolerance is not surprising, as water deficits are a common feature of these areas and plants have evolved mechanisms to tolerate low soil water potentials caused by drought as well as salinity (Munns and Tester 2008). Exaptation to salt might also be related to a species preferred habitat. Rajapakshe et al. (n.d.) found that that *Eucalyptus* species restricted to rocky outcrops germinated over a broader range of water stress levels because skeletal and shallow soils of rocky outcrops have a lower water retention capacity than the surrounding environment. Notably, *A. aneura* occurs on shallow rocky soils on hills or sandy soils in areas of low relief while *A. burkittii* most commonly occurs on sand hills and ridgelines (Bui 2013, Atlas of Living Australia 2020b). Thus it is possible that the lower sensitivity of *A. aneura* to salt in germination originated from greater exposure to either: arid habitats or rocky soils where there is low water retention capacity.

The results of this analysis suggest that tolerance to salt in germination does not preclude the possibility that seedlings become sensitive to salt post-emergence. In this study 25% of seedlings on Q0% WR and more than 70% of seedlings on 50%, 75% and 88% WR treatments experienced mortality beyond three weeks post-emergence. These treatments recorded higher salt levels compared to other treatments (between 3-5 dS m<sup>-1</sup>). Madsen and Mulligan (2006) found similar effects in three *Eucalyptus* sp. where seedling survival decreased over time when seedlings germinated and emerged in saline soils equivalent to 100 mM. One possible explanation is that seedlings could become more susceptible to salt accumulation once seed reserves have been depleted. Croser et al. (2001) suggests that since growing seedlings are more dependent on photosynthesis, rather than stored food for their source of energy, they transpire at higher rates. Consequently, more salts are able to enter the plant in the transpiration stream leading to salt accumulation and seedling mortality. The implications of this is that salt tolerance of *A. aneura* and *A. burkittii* is likely to be determined by their ability to withstand excessive accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions, rather than their ability to tolerate osmotic stress. Overall the results showed survival of emerged seedlings to be highest up to 2.52 dS m<sup>-1</sup> (~180 mM NaCl), implying that *A. aneura* and *A. burkittii* express sensitivity to salt at concentrations greater than 200 mM NaCl. These results relate to germination and early seedling growth. Further research is required to examine the possibility that seedling survival is also adversely affected by salt exposure in later stages of growth.

## **Conclusion**

This study examined the conditions under which germination and early seedling emergence can be supported when mine waste rock substrates are utilised in restoration of saline soils. A major impediment to successful restoration is when salts accumulate in soil at

a level that causes recruitment failure. The results demonstrated that salt concentrations within the root zone of seedlings at surfaces could be reduced by leaching but that leaching processes could potentially be impeded by mixing of topsoil and waste rock substrates. The main effect of exposure to saline conditions was a reduction in osmotic potential that delayed or inhibited seed germination. This response may promote emergence when conditions are more favourable (when salt stress is temporarily alleviated by leaching). For those seeds that germinate regardless of saline conditions, a reduction in seedling vigour has potential to increase sensitivity to water stress and further work is necessary to determine how seedlings are affected by salinity in later stages of growth. Nonetheless, this study showed that two *Acacia* species known to inhabit semi-arid areas (*A. aneura* and *A. burkitii*), are most likely to express sensitivity to salt at concentrations of 200 mM. These results contribute to improving our understanding of plant salt tolerances which can facilitate better species selection in restoration of semi-arid regions.

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## Appendix S1 Regression parameters estimated from the three-parameter log-logistic model

**Table S1.1** Regression parameters estimated from the three-parameter log-logistic model (where b = slope, d = maximum germination and e = t50) for analysis of time-to-event germination of *A. aneura* at all concentrations of PEG and NaCl. Summary statistics (t-statistics and P-values) are shown for comparison with control only and are based on the approximate t test using the “CompParm” function in the ‘drc’ package (RStudio, 2018). Asterisks show the significance level at  $P < 0.001$  (\*\*\*)).

Regression parameter estimates					
Treatment	Species	b ( $\pm$ SE)	d ( $\pm$ SE)	e ( $\pm$ SE)	
0 mM	<i>A. aneura</i>	-2.25 (0.25)	0.92 (0.03)	3.57 (0.33)	
50 mM	<i>A. aneura</i>	-2.72 (0.31)	0.92 (0.03)	4.05 (0.31)	
100 mM	<i>A. aneura</i>	-2.47 (0.29)	0.84 (0.04)	4.99 (0.45) <sup>t=-3.06**</sup>	
200 mM	<i>A. aneura</i>	-2.55 (0.29)	0.87 (0.04)	5.75 (0.47) <sup>t=-4.92***</sup>	
300 mM	<i>A. aneura</i>	-2.49 (0.37)	0.77 (0.07)	11.93 (1.37) <sup>t=-15.9***</sup>	
400 mM	<i>A. aneura</i>	-3.24 (0.71)	0.40 (0.07) <sup>t=2.99**</sup>	16.88 (2.48) <sup>t=-21.4***</sup>	
500 mM	<i>A. aneura</i>	-7.0 (2.47) <sup>t=-5.72***</sup>	0.14 (0.04) <sup>t=2.72**</sup>	21.59 (2.01) <sup>t=-38.2***</sup>	
-0.232	Mpa	<i>A. aneura</i>	-3.97 (0.42) <sup>t=-5.47***</sup>	0.95 (0.02)	3.74 (0.20)
-0.457	Mpa	<i>A. aneura</i>	-2.25 (0.26)	0.95 (0.03)	5.61 (0.54) <sup>t=-2.11*</sup>
-0.906	Mpa	<i>A. aneura</i>	-2.95 (0.52)	0.48 (0.06) <sup>t=3.72***</sup>	11.40 (1.30) <sup>t=-10.6***</sup>
-1.310	Mpa	<i>A. aneura</i>	-8.53 (4.01) <sup>t=-6.28***</sup>	0.09 (0.04) <sup>t=2.18*</sup>	23.16 (2.33) <sup>t=-30.4***</sup>
-1.661	Mpa	<i>A. aneura</i>	n.a	n.a	n.a
-1.985	Mpa	<i>A. aneura</i>	n.a	n.a	n.a

**Table S1.2** Regression parameters estimated from the three-parameter log-logistic model (where b = slope, d = maximum germination and e = t50) for analysis of time-to-event germination of *A. burkittii* at all concentrations of PEG and NaCl. Summary statistics (t-statistics and P-values) are shown for comparison with control only and are based on the approximate t test using the “CompParm” function in the ‘drc’ package (RStudio, 2018). Asterisks show the significance level at  $P < 0.001$  (\*\*\*).

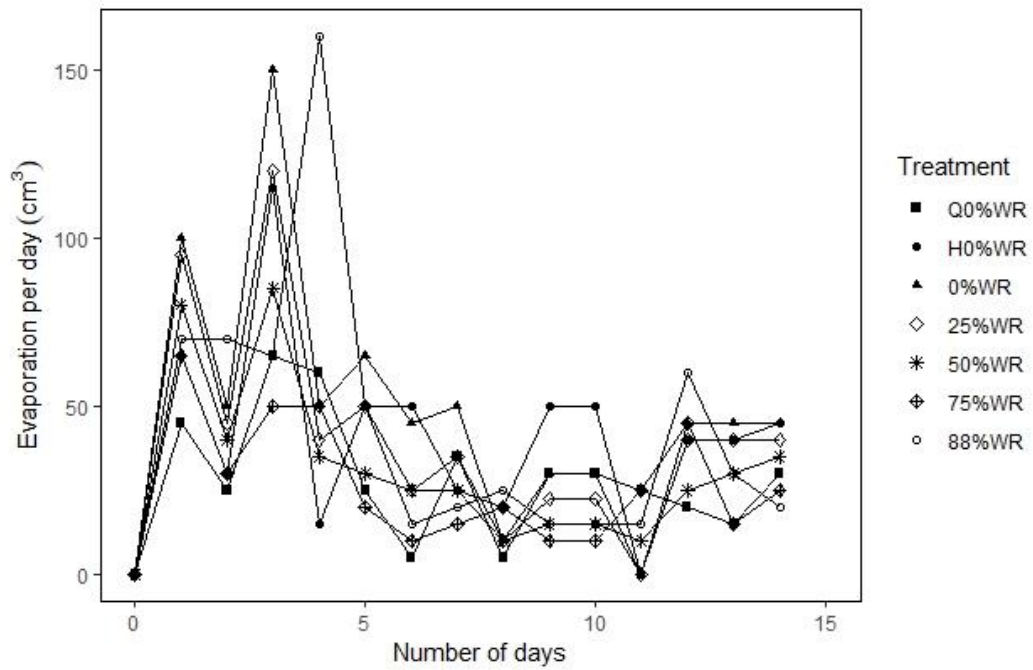
Treatment	Regression parameter estimates			
	Species	b ( $\pm$ SE)	d ( $\pm$ SE)	e ( $\pm$ SE)
0 mM	<i>A. burkittii</i>	-3.0 (0.37)	0.72 (0.05)	4.75 (0.38)
50 mM	<i>A. burkittii</i>	-4.0 (0.47)	0.73 (0.05)	5.57 (0.32)
100 mM	<i>A. burkittii</i>	-2.83 (0.41)	0.62 (0.06)	6.27 (0.59) <sup>t=-2.58**</sup>
200 mM	<i>A. burkittii</i>	-3.13 (0.55)	0.44 (0.06) <sup>t=2.60**</sup>	9.25 (0.95) <sup>t=-7.27***</sup>
300 mM	<i>A. burkittii</i>	-4.78 (1.41)	0.26 (0.07) <sup>t=2.27*</sup>	20.72 (2.87) <sup>t=-21.0***</sup>
400 mM	<i>A. burkittii</i>	n.a	n.a	n.a
500 mM	<i>A. burkittii</i>	n.a	n.a	n.a
-0.232 Mpa	<i>A. burkittii</i>	-4.58 (0.62) <sup>t=-2.44*</sup>	0.58 (0.06) <sup>t=2.86**</sup>	8.37 (0.48) <sup>t=-10.4***</sup>
-0.457 Mpa	<i>A. burkittii</i>	-4.95 (1.59)	0.09 (0.03) <sup>t=2.61**</sup>	12.92 (1.70) <sup>t=-13.5***</sup>
-0.906 Mpa	<i>A. burkittii</i>	n.a	n.a	n.a
-1.310 Mpa	<i>A. burkittii</i>	n.a	n.a	n.a
-1.661 Mpa	<i>A. burkittii</i>	n.a	n.a	n.a
-1.985 Mpa	<i>A. burkittii</i>	n.a	n.a	n.a

**Table S1.3** Akaike information criterion (AIC) comparison of the three-parameter log-logistic model for mean germination on final day for *A. aneura* and *A. burkittii* under osmotic potential gradient.

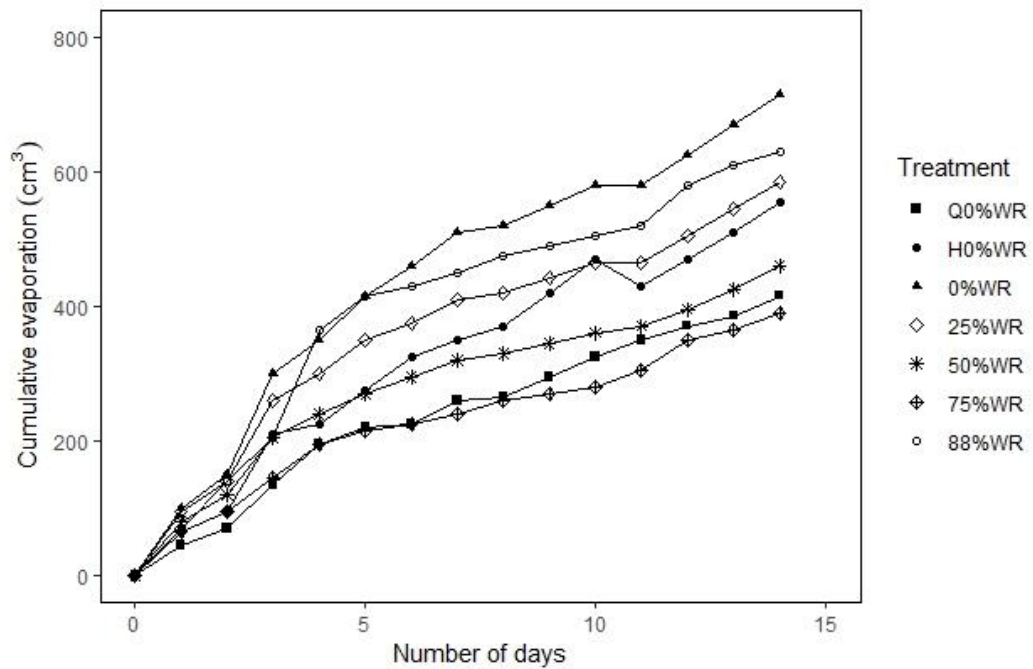
	Residual deviance	AIC	df
NaCl	107	-12.7	4
NaCl + species	49	-62.0	8
PEG	52	-11.2	4
PEG + species	49	-136	8
Osmotic potential	107	11.4	4
Osmotic potential + effect (NaCl or PEG)	104	-17.0	8
Osmotic potential	107	11.4	4
Osmotic potential + species	104	-43.9	8

## Appendix S2 Evaporation per day and cumulative evaporation

a)



b)



**Figure S2.** (a) Evaporation per day and (b) cumulative evaporation of evaporation pots across seven different treatments. Q0%WR – 1.25L topsoil H0%WR – 2.5L topsoil, 0%WR – 5 L topsoil, 25%WR, 50%WR, 75%WR, 88%WR

## Chapter 4: Discussion and Conclusion

### Synthesis of results

A significant number of mining operations are located in semi-arid regions where saline soils are present and water deficits are frequent. This underlines the importance of conducting this study. Although it is common practice for companies to supplement topsoil with waste rock to overcome topsoil shortages, little is known about how this practice affects the physio-chemical properties of topsoil:waste rock mixes and whether these effects are a limiting factor to vegetation establishment. This thesis reviewed the existing literature, and found two significant gaps in the research about the effects of waste rock content on the establishment of vegetation in semi-arid regions. The first is that little is known about the influence of high waste rock content on soil water availability and the second is that there is need to augment our understanding of the influence of waste rock content on salt transfer processes.

In this study, experimental analysis was conducted to examine the relationships between soil water content, soil salinity and vegetation establishment on saline topsoil amended with 0%, 25%, 50%, 75% and 88% waste rock. The analysis showed that increasing waste rock content caused an increase in soil salinity, a decline in soil water content but an improvement in soil water conservation. Tests were conducted to ascertain the extent to which effects of increasing waste rock content differed according to phases of plant establishment. This included germination and emergence and later seedling growth. It was found that increasing waste rock content had a greater negative effect on early seedling growth than it had on plant growth post-emergence. Germination and emergence was completely inhibited at waste rock contents of 75% while growth and development of post-emerged seedlings continued in waste rock contents of up to 88%. These findings reflect those of other studies which found the developmental stages of seed germination and seedling emergence to be particularly vulnerable to soil salinity (Houle et al. 2016) and low soil water (Chambers 2000, Standish et al. 2012, Larson et al. 2015). The implications of these findings, and how they relate to broader restoration outcomes, are summarised as follows.

#### *Salinity impact on early seedling growth varies with rock content of substrate in mine-site restoration*

In Chapter 3 it was hypothesised that salinity of topsoil:waste rock mixes would increase with increasing waste rock content and this this would have an adverse effect on seedling emergence. This hypothesis was accepted in that the correlation between waste rock content and  $EC_{1.5}$  had a negative effect on seedling emergence. Out of the four waste rock percentages, emergence was greatest on 25% waste rock. At this percentage, there was no significant difference in soil salinity compared to standard topsoil. At 50% waste rock, soil salinity was

increased by three-fold when compared to standard topsoil. At these levels of salinity mean seedling emergence was less than 10%. Less than 1% seedling emergence was recorded at waste rock percentages beyond 75%. This low emergence level was a result of  $EC_{1.5}$  increasing by up to five-fold compared to standard topsoil. This findings concur with those of García-fayos et al. (2000), who found increasing soil salinity to be a limiting factor on seedling recruitment.

A comprehensive search of published research literature prior produced no studies that identified an interaction between rock fragment content of soil and levels of soil salinity. The strong correlation between  $EC_{1.5}$  and increasing waste rock content found in this study was theorised to be a consequence of reduced leaching efficiency. This contention arises from research evidence that there is a tendency for rock fragments in soils to create preferential flow paths (Nasri et al. 2015). These preferential flow paths reduce the amount of water infiltrating through the soil fraction that in turn decrease the amount of salt that can be displaced from finer pores or soil aggregates (Rengasamy et al. 2003, Corwin et al. 2007). The mechanisms behind salt accumulation in soils containing rocks have yet to be described in literature and further characterisation of topsoil mixes is critical to support this hypothesis. In particular, testing of  $EC_{1.5}$  throughout the soil profile (including lower depths) would help to determine the effectiveness of leaching processes in topsoil mixes where waste rock has been added.

In this study, two *Acacia* species from semi-arid, arid regions were found to have moderate salt tolerance (i.e. the ability to germinate, emerge and survive in salt levels up to 200 mM NaCl). These findings support those of others (e.g. Khalil et al. 2016, Kheloufi et al. 2016) who found *Acacia* species inhabiting semi-arid and arid regions are likely to exhibit exaptation to salt as a result of exposure to habitats of low water availability. The implication of these findings is that beyond 200 mM NaCl, survival of these species is likely to be significantly reduced. This creates the potential for species assemblages on post-mining areas to potentially be altered, as species with greater salt tolerance are increasingly selected as waste rock content and soil salinity increases. Restricting species assemblages to highly salt tolerant species may not always be synonymous with pre-disturbance biodiversity levels. This outcome may conflict with societal and government expectations of restoration, especially where mining occurs within or near conservation significant areas. Characterisation of substrates prior to use might therefore be critical for improving the optimisation of topsoil resources.

Notably, other studies analysing seedling emergence on topsoil incorporating 50% and 75% waste rock found negligible increase in salinity when waste rock was mixed with non-saline topsoil ( $EC_{1.5}$   $0.47 \pm 0.008$  dS/m) (Muñoz-Rojas et al. 2016b). With no increase in salinity, seedling emergence declined but was not significantly different to that recorded on standard

topsoil (Muñoz-Rojas et al. 2016b). Not all topsoil occurring at the mine site chosen for this study was saline. Topsoil located approximately 1 km north west of the study area was reported to contain very low levels of salt ( $EC_{1:5} 0.43 \pm 0.03$  dS/m) (Merino-Martín et al. 2017). Where both saline and non-saline soils occur at site, characterisation of electrical conductivity prior to use would allow these soils to be differentiated and stockpiled separately. This process would provide several advantages in that: i) non-saline soil could be amended with a higher percentages of waste rock than saline soil, maximising water conserving properties of rock content (as demonstrated in Chapter 2), and ii) greater use of non-saline soil on post-mining areas could alleviate the use of saline soil or alleviate the need to amend saline soil with high amounts of waste rock. Fortunately, improvements in technology are increasingly allowing mining companies to track use of rehabilitation materials (DMIRS 2017).

#### *High rock content enhances plant survival under drought in saline topsoils*

In Chapter 2 water content of topsoil mixes decreased as the proportion of waste rock increased. The overall effect was that the test species, *A. saligna*, responded to lower water availability through a reduction in stomatal conductance. As expected, the net outcome was a negative relationship between plant development and the percentage of waste rock in soil beyond 25%. However, this relationship was found to be non-linear. At 25% waste rock content a positive impact on biomass of *A. saligna* was observed. This was due to soil moisture being retained for longer without any significant reduction to volumetric water content occurring. This finding supports the premise that amendment of topsoil with small amounts of waste rock causes no negative impact to hydrological properties of topsoil mixes (Merino-Martín et al. 2017, Golos et al. 2019).

A progressive decline in biomass of *A. saligna* was observed up to waste rock content of 75%. At 88% waste rock, the biomass of *A. saligna* increased relative to 50% and 75% waste rock. It is postulated that this result was an outcome of high waste rock mixes containing higher macro porosity that might have promoted greater access to water through increased root length (which was not measured in this study) or greater access to nutrients through increased nitrogen fixation. The literature search conducted for this study found no other published research that has observed a non-linear relationship between plant growth and increasing rock content in soil. Previous studies report rock fragments to have a positive effect on plant growth up to percentages of between 20 – 30%, beyond which plant growth is adversely affected (Poesen and Lavee 1994). The results of this research provide evidence that high percentages of rock fragments in soil could also be used. This is promising for restoration of mine-sites in semi-arid regions, especially the finding that the amendment of topsoil with waste rock has potential to prolong plant survival during drought, either by reducing water loss via evaporation or by slowing the movement of water through soil. The positive effect of rock



content on patterns of plant water use under drought supports and expands on the results of other studies that found the presence of rock to be beneficial for conservation of water (Gras and Monnier 1963, Poesen and Lavee 1994).

### **Application of findings to restoration practices**

Broadly speaking, the findings of this study can be applied to a number of scenarios that may occur within a mine-site restoration field setting. Table 12 outlines a number of restoration approaches that may be undertaken dependant on the volume of materials available for use or the salinity of materials. Advantages and disadvantages associated with each approach have also been identified, based on observations from this study and others. It is hoped that future research will confirm the applicability of results from this study to field settings.

**Table 12. Suggested waste rock contents and plant establishment methods to be used in mine-site restoration practice based on findings of study**

Scenario	Topsoil Salinity	Suggested waste rock content	Suggested method of plant establishment	Opportunities	Limitations
Significant topsoil deficit	Non-saline (EC <sub>1:5</sub> <2 d/Sm)	88%	Seeding	<ul style="list-style-type: none"> <li>Improved conservation of soil water</li> <li>Plant function sustained for longer during drought</li> <li>Reduced plant water requirements as a result of smaller plant size</li> </ul>	<ul style="list-style-type: none"> <li>Lower seedling emergence</li> <li>Reduced plant growth and development</li> <li>Lower total water content of topsoil:waste rock mix</li> <li>Possible early senescence of plants due to continual limited water and nutrient supply</li> </ul>
	Saline (EC <sub>1:5</sub> >2 dS/m)	88%	Planting with salt tolerant or halophyte species	<ul style="list-style-type: none"> <li>Improved conservation of soil water</li> <li>Plant function sustained for longer during drought</li> </ul>	<ul style="list-style-type: none"> <li>Skewed plant community composition</li> <li>Reduced plant diversity</li> </ul>
Minor topsoil deficit	Non-saline (EC <sub>1:5</sub> <2 d/Sm)	25%	Seeding	<ul style="list-style-type: none"> <li>Increased plant growth and development</li> <li>Faster rate of vegetation establishment</li> </ul>	<ul style="list-style-type: none"> <li>Faster rate of soil water loss as a result of either increased evaporation or increased plant size</li> <li>Increased susceptibility of plants to water stress during drought</li> </ul>
	Saline (EC <sub>1:5</sub> >2 dS/m)	25%	Seeding	<ul style="list-style-type: none"> <li>Increased germination, emergence and survival</li> <li>Increased plant diversity</li> <li>Less effect on seedling vigour</li> </ul>	<ul style="list-style-type: none"> <li>Slight decline in seedling emergence compared with topsoil only</li> <li>Faster rate of soil water loss as a result of increased evaporation</li> </ul>

### **Additional areas of research**

While making some significant findings, this study also raises questions worthy of further research. The first relates to finding the optimal trade-off between the costs and benefits of including varying levels of waste rock content in mine-site restoration. The second question arises from the observation that topsoil is a source of seed propagules and, the implication of diluting topsoil with high amounts of waste rock is that there will be lower levels of seedling recruitment. Future studies are needed to determine the extent to which including waste rock in soil compensates for lower emergence from soil seed bank through increased water conservation, and therefore plant survival. The third question stems from the finding that root growth of *A.saligna* was compromised in the presence of waste rock (evidenced by 35% reduction in root growth on 50% WR compared to H0% WR). This finding reflects those of previous studies where root growth was observed to be lower in soils with rock (e.g. Mi et al. 2016). Generally, reductions in root growth prevent plants from seeking out larger volumes of soil for water under drought (Cochrane 2018) and this effect can be particularly disadvantageous in early seedling growth in water-limited semi-arid and arid ecosystems where seedling mortality is high during the first onset of drought (Chesson et al. 2004). In this study, the incorporation of waste rock was observed to slow declines in water loss meaning that there could be more water available for early seedling growth than previously thought. Research that examines the effects of waste rock content on early seedling survival under simulated drought could help determine if improved conservation of soil water compensates for reductions in root growth.

It is also yet to be established whether an improvement in conservation of water from the addition of high waste rock amounts in soil is capable of supporting resilient vegetation. This is because dilution of soil volumes decreases nutrient supply (Childs and Flint 1990, Cross et al. 2018). Since the regulatory biochemistry of photosynthesis is reliant on adequate supply of water and mineral nutrients, plants under continuing water and nutrient stress are more likely reach pre-mature senescence (where plants begin to age) because plant productivity cannot be maintained (Gregory and Nortcliff 2013). As nutrient availability and uptake was beyond the scope of this study, future research could be conducted to establish the long term effects associated with exposing plants to decreased nutrient supply as a result of increased rock content in soil.

In light of the results of this study, hypothetical mechanisms for observed trends in germination and plant growth were explored and presented that warranted further investigation. In particular, future research into mechanisms influencing root:rock interactions, remain especially relevant. In this study it was theorised that the extraction of water from rock might have been an additional mechanism contributing to increased biomass

of *A. saligna* on 88% waste rock. Previous research has shown that water transfers can occur between plant roots and natural rock fragments in soils (Querejeta et al. 2006, McCole and Stern 2007, Zhang et al. 2016). In this study, roots of *A. saligna* were observed growing in between rock:soil interfaces and within the cracks and fissures of waste rock. However, whether roots extract water from the inside or surface of the rock and whether this phenomenon also applies to waste rock has yet to be established. In addition, it is not yet known to what extent plants are reliant on water reservoirs within rock. Research evidence suggests that significant amounts of water can be accessed from rock via symbiotic interactions between plant and fungi (specifically Mycorrhizae) which can initiate hyphal extension into rock micropores, providing a link between rock water resources and plants (Bornyasz et al. 2005, Querejeta et al. 2006). Given this interaction is common to over 90% of the natural land plants and is the means by which associated plants supplement nutrient and water requirements (Brownlee et al. 1983, Smith and Read 1997, Auge 2001) plants may rely on symbiotic associations to facilitate extraction of water from rock more than we think. There is potential for future studies to investigate the mechanisms behind water extraction from rock and the influence of symbiotic interactions. This research is particularly relevant to arid and semi-arid mine-site restoration, where rainfall is limited and significant amounts of waste rock are available for use.

The implementation of field trials would also help examine the observations made in this study about the influence of waste rock on water conservation. Glasshouse temperatures during experimental periods were restricted to  $<30^{\circ}\text{C}$ . In semi-arid environments in Western Australia, maximum daytime temperatures typically exceed  $30^{\circ}\text{C}$  (Bureau of Meteorology 2019), especially in summer, when water deficits are most likely to occur. Reductions in water loss under the presence of waste rock is theorised to occur as a result of waste rock reducing bare soil evaporation (Unger 1971b, Ingelmo-Sanchez 1980, Van Wesemael et al. 1996) or slowing the overall movement of water through soil (Ravina and Magier 1984, Brakensiek and Rawls 1994, Shi et al. 2008, Zhou et al. 2009, Mi et al. 2016). Water balances undertaken on pots without plants in this study indeed showed evaporation to be less when waste rock was present (Appendix 1). The effect of rock fragments on evaporation has been suggested to be dependent on climatic conditions, as evaporation increases under dry, hot conditions as a result of rocks increasing soil temperatures (Saini and Grant 1980, Poesen and Lavee 1994, Danalatos et al. 1995b, Cousin et al. 2003). Further field trials would help discount this effect and differentiate between the mechanisms accounting for reductions in water loss. Additionally, it is noted that the use of glasshouse trials limited the size of waste rock that could be studied. Diameters of plant pots used in the trials varied between 200-250mm, and consequently maximum rock size (based on Feret's diameter) that could be incorporated into

topsoil mixes was 100mm. Mine waste rock sizes in the field, have been known to vary between 2mm and 300mm, with some rocks >500mm (Golos et al. 2019, Merino et. al. 2017). The completion of field trials would allow for larger sizes (>10mm) of waste rock typical at mine sites to be incorporated into topsoil:waste rock mixes. Allowing for this factor is important for future studies, as the presence of larger rock in the topsoil would influence spatial heterogeneity of nutrient and water content in topsoil (Katra et al. 2008, De Figueiredo and Poesen, 1998), which may play important roles in determining the habitation and growth of plants.

Finally, research into the ways in which different species respond to varying topsoil mixes is critical to support the results of this analysis about the effects of waste rock content on plant development. It is noted that the *Acacia* species chosen for study in Chapter 2 is nitrogen-fixing. Had another species been chosen, its growth might have been constrained by the low nutrient availability that comes as a consequence of topsoil dilution (Childs and Flint 1990). This is not to say that rock fragments have a negligible effect on nutrient content. Some studies have shown that rock fragments in soils (including basalt, granite and schist) can contribute significantly to nutrient content and cation exchange capacity (Munn et al. 1987, Burghel et al. 2015). Nonetheless, in order to discount the influence of waste rock content on this effect, further testing must be undertaken on other plant species, particularly those exhibiting varying life forms, rooting strategies and forms of nutrient acquisition. Field trials undertaking seeding of topsoil covers with multiple species would be particularly valuable in this space and would allow restoration practitioner to gain a better understanding on how waste rock content affects resultant species composition.

### **Concluding remarks**

While this thesis is only a preliminary study the analysis of the effects of adding waste rock to topsoil supports the argument that mine-site restoration outcomes are intimately linked to the hydrological, physical and chemical characteristics of re-made topsoil mixes and paves the way for efforts to optimise restoration practices. Overall, the analysis reinforces the contention that amendment of saline topsoil with waste rock challenges early seedling growth more than it does later plant growth, but prolongs plant survival under drought. The observed positive effect of waste rock amendment on water conservation may be especially relevant to improving restoration outcomes in semi-arid and arid environments where availability of water is the main limiting factor to plant establishment. Further research that focuses on the effect of waste rock content on germination and plant growth will help to support and expand the findings of this study.

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# Appendices

## Appendix 1 Water content of evaporation pots

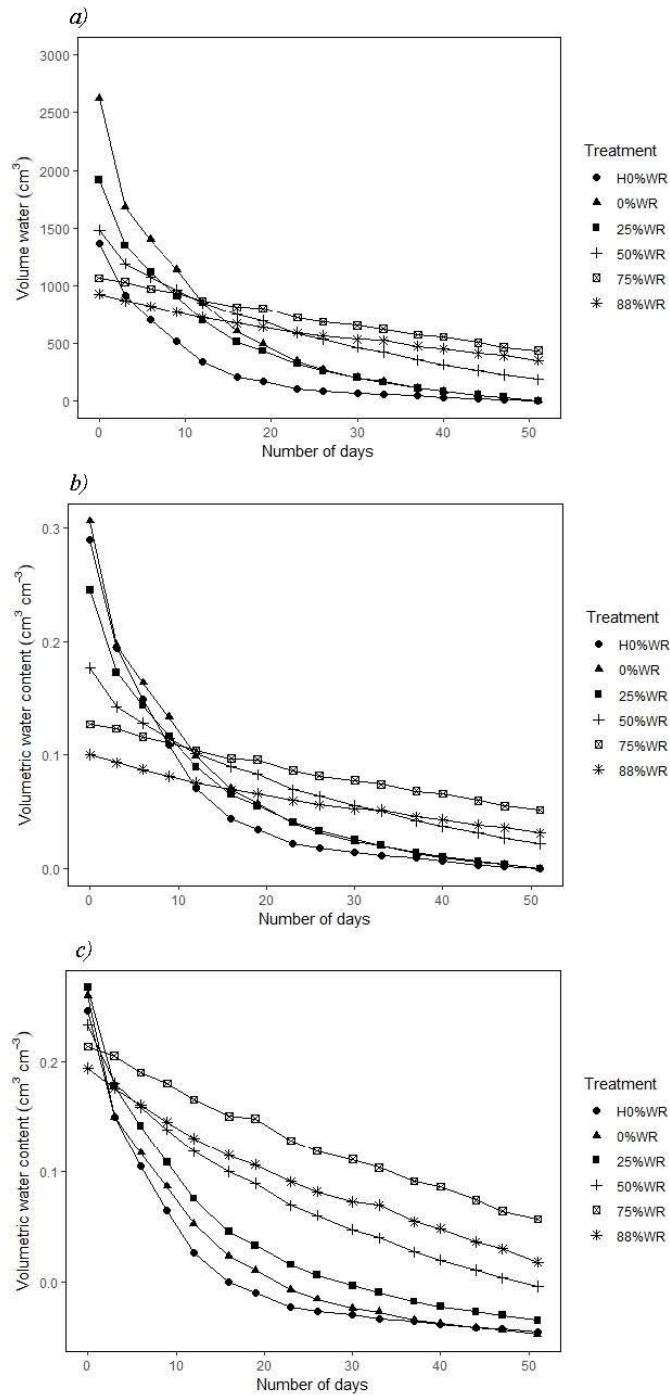


Figure A1. (a) Mean absolute water content, (b) mean volumetric water content of the topsoil mix and (c) mean volumetric water content of topsoil fraction only for evaporation pots across six different topsoil mixes. H0%WR – 5.25L topsoil, 0%WR – 10.5 L topsoil, 25%WR – 3:1 topsoil:waste rock, 50%WR – 2:2 topsoil:waste rock, 75%WR – 1:3 topsoil:waste rock, 88%WR – 1:7 topsoil:waste rock

## **Appendix 2 Statement of candidate contribution**

This thesis contains manuscripts in preparation for publication that are co-authored. The citations and breakdown of authorship contribution are listed below.

-Lison, C.A.; Cross, A.T.; Stevens, J.C.; Valliere, J.M.; Dixon, K.W.; and Veneklaas, E. High rock content enhances plant survival under drought in saline topsoils.

I contributed 80% to this manuscript, including conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation. A.T.C., J.C.S., E.V., J.V and K.W.D. contributed to methodology and visualisation. E.V. and J.V. contributed to the conception and analysis. A.T.C., J.C.S., K.W.D., E.V. and J.V contributed to review and editing.

-Lison, C.A.; Cross, A.T.; Stevens, J.C.; Valliere, J.M.; Lewandrowski, W; and Veneklaas, E. Salinity impact on early seedling growth varies with rock content of substrate in mine-site restoration.

I contributed 75% to this manuscript, including conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation. A.T.C., J.C.S., E.V., J.V and W.L. contributed to methodology and visualisation. E.V., W.L. and J.V. contributed to the analysis. A.T.C., J.C.S., W.L., E.V. and J.V contributed to review and editing.

To Whom it May Concern

I, Christine Lison, contributed in conception of idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation for the manuscript *High Rock Content Enhances Plant Survival Under Drought in Saline Topsoils*

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Signature of candidate

I, as co-author, indicate that the level of contribution by the candidate listed above is appropriate.

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Adam Cross

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Erik Veneklaas

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Jason Stevens

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Kingsley Dixon

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Justin Valliere

To Whom it May Concern

I, Christine Lison, contributed in conception of idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation for the manuscript *Salinity Impact on Early Seedling Growth Varies With Rock Content of Substrate in Mine-site Restoration*.

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Signature of candidate

I, as co-author, indicate that the level of contribution by the candidate listed above is appropriate.

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Jason Stevens

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