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High rock content enhances plant resistance to drought in saline topsoils

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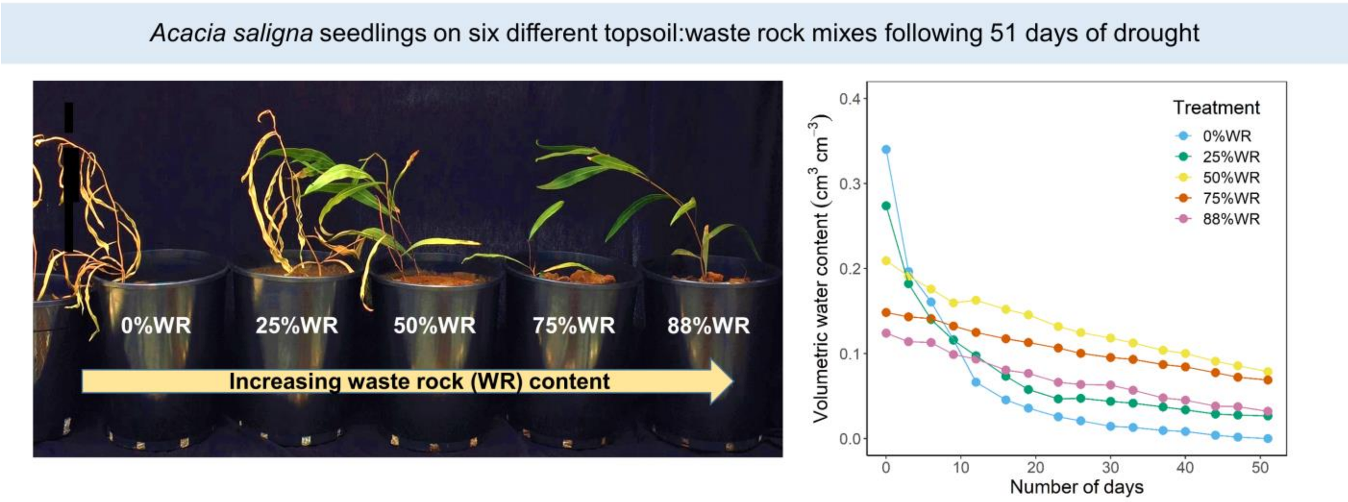
Graphical Abstract



Abstract

Mining represents a major disturbance to natural ecosystems, with cumulative impacts leading to large areas requiring rehabilitation or ecological restoration. Successful 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 are a key limiting factor to vegetation establishment. Additionally, saline soils are a common feature of these regions and pose an additional stressor to vegetation establishment. Crushed waste rock is abundantly available at iron ore mine sites. We examined if topsoil amendment with waste rock may be acceptable as a strategy to augment the amount of substrate available for plant growth. In a pot study, we tested the growth and development of a species with known salt tolerance, on saline topsoil incorporating 25%, 50%, 75% and 88% waste rock. Soil water content and plant water use were examined to determine how waste rock content affects plant-water relations and if it is a limiting factor to plant growth and development. Under well-watered conditions higher percentages 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*. Under drought conditions, however, the lower rate of water loss in the presence of waste rock allowed stomatal conductance to be maintained over a longer period. There was no significant increase, decrease or optimal relationship between waste rock content and plant growth. Final biomass was on average 46% higher in 25% waste rock, 22% lower in 50% waste rock, 48% lower in 75% waste rock and 3% lower in 88% waste rock. These results show that addition of waste rock to topsoil has complex effects on plant-water relations and growth. Altered patterns of plant water use under drought can enhance survival despite lower water availability in rock-amended topsoil. We demonstrate that augmentation of limited topsoil resources with iron-ore mine waste rock is a promising option for mine site restoration, favouring increased plant resistance to drought.

Keywords

Drought stress, mine restoration, plant water use, plant growth, salinity, topsoil, waste rock

Introduction

Since 1984, total mining production globally has increased six-fold (IOCWMC 2019). Worldwide, it is now estimated that over 57,000 km^2 of land is affected by mining disturbance (Maus et al. 2020). The extraction and processing of natural resources has been identified as one of the main contributors to land-use related biodiversity loss (Oberle et al. 2019), and the potential for mining to disturb large areas has increasingly placed greater importance around the need for ecological function and structure to be restored after the cessation of mining (DISI 2017). Successful revegetation and restoration of post-mining areas presents a number of significant challenges, however.

One such challenge 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 (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 availability of water and nutrients than subsoil or crushed waste rock (Machado et al. 2013, Festin et al. 2019). In order for topsoil to be utilized in restoration, it must be stripped and stockpiled prior to mining activities commencing (Golos et al. 2016). Often the surface area of disturbed areas exceeds that of the pre-mined surface area, resulting in a topsoil deficit. 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 (Kneller et al. 2018). Waste rock arises from mining as coarse, broken, partly weathered rock (Blight 2010), is heterogeneous in size and shape, and often comprises large rock fragments in a matrix of finer particles (<2 mm). It is common practice for mining operations to utilise waste rock percentages >50% (Tiemann 2015) in topsoil mixes, however few studies have analysed the effects of incorporating large amounts of waste rock on plant growth and development, despite previous studies showing the rate of plant establishment to be higher on topsoil mixed with waste rock than without (Golos et al. 2019).

For restoration to be successful it is imperative that substrates used in topsoil mixes are conducive to plant growth. Water availability is one of the most important physical factors affecting plant growth (Kirkham 2005). This is especially true in arid and semi-arid environments where rainfall is limited or highly seasonal (less than 250 mm per annum) (Rodriguez-Iturbe and Porporato 2007) and evaporative loss of soil moisture is high (Bell 2001). Several studies have observed a covering of rock fragments to contribute to a reduction in evaporation via capillary loss, as a consequence of having less soil exposed at the soil surface (e.g. Van Wesemael et al. 1996, Tetegan et al. 2015). Others have suggested that porous rock fragments in soils can retain available water and act as a reservoir during drought (Tetegan et al. 2015, Korboulewsky et al. 2020). It is important to understand how the augmentation of topsoil with waste rock influences soil water content and plant water use, in order to better guide mine restoration outcomes.

Soil salinity is commonly encountered in the restoration of arid and semi-arid post-mining landscapes world-wide (Tordoff et al. 2000). Salt-affected soils and landforms can originate from use of hypersaline groundwater for mineral processing, or through natural release of salt from parent rocks or ancient drainage basins (Jordán et al. 2004). In Australia, salt has been present in the landscape for a long time (Crowley 1994) and most terrestrial plants have evolved mechanisms to tolerate varying levels of salinity (Munns and Tester 2008). *Acacia saligna* (Labill.) H.L Wendl. (Fabaceae) is native to Western Australia but has been introduced to other parts of the world. The species can play an important role in the restoration of degraded landscapes, including wastes dumps, due to its capacity to fix nitrogen, and grow in saline soils or environments receiving less than 250 mm rainfall per annum (Gwaze 1987, Brockwell et al. 2005, Amrani et al. 2010)In this study, we intend to test the growth and physiological performance of *A. saligna* on saline topsoil mixes incorporating up to 88% waste rock.

To assess the effect of augmentation of topsoil with waste rock on plant water relations and plant growth, we designed a pot study whereby *A. saligna* seedlings were grown on four different topsoil mixes including topsoil amended with 25%, 50% 75% or 88% waste rock. We monitored both soil water content, and plant physiological responses (including stomatal conductance, leaf water potential, growth) under well-watered and drought conditions. We hypothesised that 1) in substrates with higher rock content, reduced soil volume and reduced water storage capacity will limit plant growth, especially in the absence of watering and 2) when relying on stored soil water, plants in substrates with higher rock content will deplete the available water more rapidly, and will have a faster reduction of their stomatal conductance and water potential than plants in substrates with low rock content.

Materials and Methods

Study area

Soils 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) (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 sand over loam/clay on lower slopes (DPIRD 2019). Mining activity occurs within the Banded Ironstone Formation in which iron occurs as magnetite and various 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). 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 (Bureau of Meteorology 2019).

Growth substrates

Topsoil was stripped with a skid steer loader from the upper 15 cm of the soil profile in a 10 m x 10 m plot at the mine site, that had previously been restored in 2013. Texture of topsoil stripped from the 10 m x 10m plot was determined to be red loamy sand, based on results of particle size analysis (Appendix A). Topsoil typically contained small ironstone rock fragments (4-20mm) which were removed prior to use.

Waste rock was collected from the surface of a 1 m high stockpile containing previously dumped waste rock. Waste rock was predominantly large ironstone rocks and boulders in a matrix of fines. Waste rock was sieved to discard fines (<4 mm). The size of waste rock fragments varied between 4 mm and 100 mm. The particle 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). To estimate specific density, rock fragment volumes were measured using the water displacement method (Archimedes' principle).

Table 1. Particle size distribution and density of waste rock fragments in a random sample of 589 rock fragments

Feret’s diameter	4-10 mm	10-20 mm	20-30 mm	30-40 mm	>40 mm
n	*383*	*94*	*47*	*30*	*35*
% of total volume	3	4	4	9	80
Mean density (g/cm^3)	3.5±0.23	3.3±0.17	3.6±0.23	3.2±0.21	3.2±0.16

An overview of the physical properties and bulk chemical properties of growth media is presented in Appendix A. Physical and chemical properties of the growth media 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 Appendix A) were undertaken by ChemCentre (Bentley, Western Australia) following the methods of Rayment and Lyons (2011). The extent of element enrichment in the topsoil was assessed via use of the Geochemical Abundance Index (GAI) (Berkman 2011) (Appendix B).

Study species

The species employed in this study was *Acacia saligna* (Figure 1). Approximately 20-week old *A. saligna* seedlings were sourced from a local nursery for the study. All seedlings of *A. saligna* had phyllodes and had shed the true leaves of their juvenile stage.

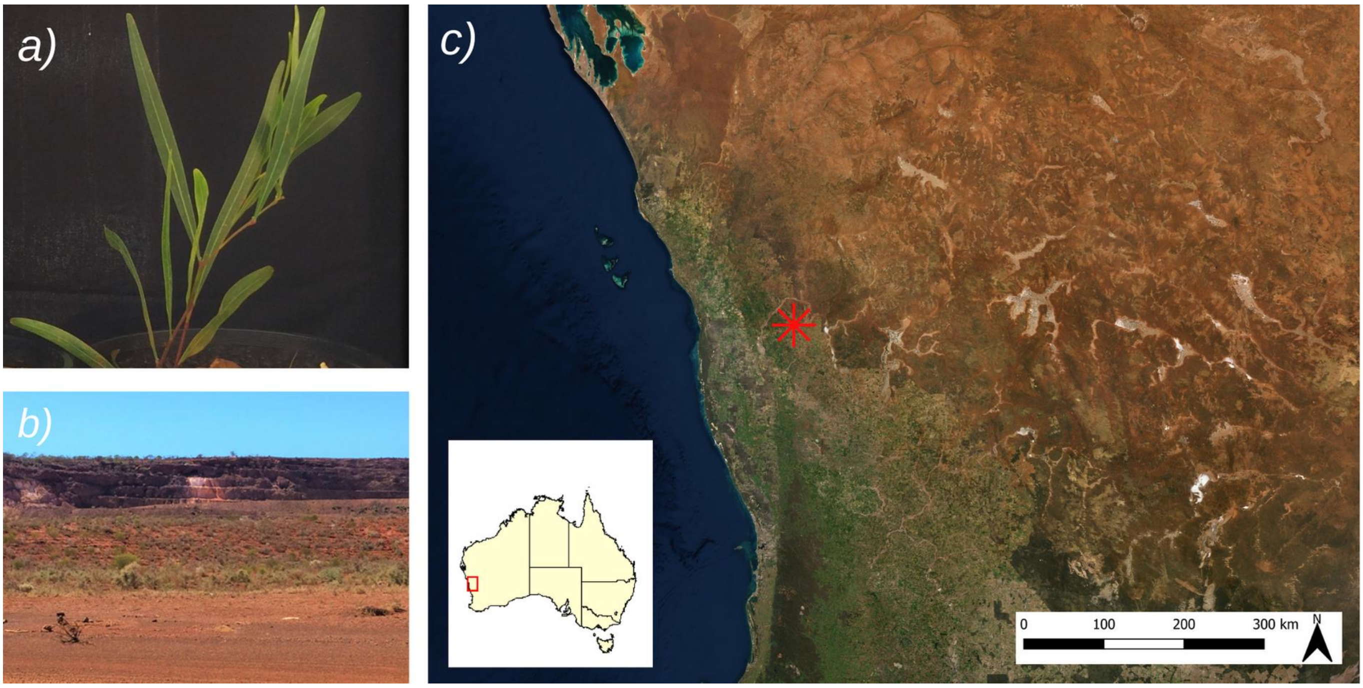


Figure 1. (a) Species used in study, *Acacia saligna*, (Labill.) H.L. Wendl. (b) Mine site where substrates were collected for use in study; (c) Location of mine site (asterix) where substrates were collected for use.

Experimental design

The experiment was conducted under controlled greenhouse conditions from June to October 2018 when minimum and maximum temperature averaged 7.4°C and 29.6°C respectively. Cylindrical 10.5-litre (25 cm diameter x 27.5 cm depth) free-draining pots were filled with one of five topsoil mixes, including either topsoil only (0%WR), topsoil mixed with 25% waste rock (25%WR), topsoil mixed with 50% waste rock (50%WR), topsoil mixed with 75% waste rock (75%WR) or topsoil mixed with 88% waste rock (88%WR). The percentages in these treatment abbreviations depict the relative volumes of the rock component, in its unmixed form. After mixing and settling, soil fills macropores between rocks, causing the proportion of rocks to decrease. Actual waste rock volumes in each pot after settling were estimated based on their weight and specific density, after which topsoil was assumed to take up the remainder of the volume (Table 2). The bottom of the pots was lined with a fine synthetic mesh to retain soil. Smaller 6 L (20 cm diameter by 18 cm depth) cylindrical pots were used for a shallow topsoil treatment, in which the volume of topsoil equaled that used in the 50%WR treatment (approximately 5.25 L), to examine if the effect of adding waste rock on plant development could be fully explained by the reduction of soil volume (hereby referred to as H0%WR). The 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 block design on the glasshouse floor, and pots of each treatment were randomly placed within each block.

Table 2. Mean volume of total topsoil mix, topsoil and waste rock fractions after settling (mean ± SE). Total mix is the volume of the topsoil mix after settling, waste rock fraction is the percentage of waste rock and topsoil fraction is the volume of total mix minus waste rock volume. 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

Mean volume (cm^3 cm^{-3})	H0%WR	0%WR	25%WR	50%WR	75%WR	88%WR
n	*9*	*9*	*12*	*8*	*6*	*13*
Total mix	4980±60.1	7651±95.0	8456±41.0	8414±111.5	8550±70.2	8696±68.8
Topsoil volume	4980±60.1	7651±95.0	7012±41.0	5526±111.5	4219±70.2	3614±68.8
Waste rock volume	0	0	1444±0	2887±0	4331±0	5082±0
Estimated % waste rock	0	0	17	34	51	58

One *A. saligna* seedling was planted into each pot in June 2018. Pots without plants were used to estimate water loss via evaporation (one per treatment). Nursery potting mix was removed from seedlings prior to planting by soaking roots in water for up to 20 minutes (to ensure that plants were rooted in the experimental soil/rock mix only). Physiological measurements undertaken during the experimental period were conducted on fully grown phyllodes. Phyllodes will be referred to as leaves hereafter.

Following the planting of seedlings, pots were fully 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 allowed to reach constant water content of 80% FC, and from then onwards water was withheld. At the start of the well-watered phase each topsoil mix included 15 replicates. Some mortality occurred during the well-watered phase, leaving 6-9 replicates per treatment.

Water content of topsoil mixes

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

$$\theta_m\ (cm^3\ cm^{-3}) = Vw/Vm$$

$$\theta_s\ (cm^3\ cm^{-3}) = Vw/Vs$$

Where *Vw* = volume of water, *Vm* = volume of the total soil mix (topsoil and rock fraction) and *Vs* = volume of topsoil fraction. Mean volumetric water content was determined every 3-4 days during the dry-down period. The calculation of θ_s used the soil volumes listed in Table 2, and assumed that the rock fraction held no water. To check this assumption, mean θ of 100% waste rock was determined after oven-dried (105°C) waste rock was submerged in water for 12 hours, drained and dry-patted. Mean volumetric content of waste rock was 0.10 cm^3 cm^{-3}.

Bulk density of topsoil mixes

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

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

Where R_m = percentage of rock, BD_m = total bulk density of the soil mix and BD_r = mean density of the waste rock fragments (3.33 g cm^3).

Plant water relations

Stomatal conductance (g_s; mmol m^{-2} s^{-1}) 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 to 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 fully expanded leaves contained within the upper third of the plant. During the measurement period (30 August to 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 µmol m^2 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 mmol m^{-2} 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 pressure 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. Log transformations were undertaken on RGR, biomass and leaf water potential measurements. Where transformed data indicated normality, differences between treatments were tested using a one-way analysis of variance (ANOVA) with substrate treatment as the main effect, followed by Tukey-honestly significant difference (HSD) tests for post hoc mean comparisons. Data for g_s and bulk density were 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 (BH) 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 θ_m and θ_s. The same analysis was conducted to test the effect of θ_m on g_s and the interaction between treatment and θ_m. Regression analysis was conducted to determine the relationships between leaf water potential and θ_m. The relationship between g_s, θ_m and θ_s

was fit with a three parameter log-logistic function. All analyses were performed with R software version 1.1.463 (RStudio, 2018). Data presented is non-transformed for ease of interpretation.

Results

Topsoil was classified as saline (electrical conductivity (ECe) > 4 dSm) as defined by the Soil Science Society of America (2020) (Appendix A). Assessment of element enrichment via the GAI indicated that elements in topsoil were present at concentrations similar to or less than the median abundance and thus were unlikely to have toxic effects on plant growth (Appendix B). Experimental plants showed no signs of nutrient deficiency or toxicity during the well-watered period.

Bulk density

Total bulk density of soil mixes was dependent on waste rock content (χ^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 3).

Table 3. Mean bulk density of topsoil mixes in the experiment. Data is presented as mean ± 1 s.e. Annotated lettering represents the results of Kruskal-Wallis test, with BH test for comparison. Values followed by the same letters are not significantly different (α = 0.05). 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

Topsoil Mix	H0%WR	0%WR	25%WR	50%WR	75%WR	88%WR
n	*9*	*9*	*12*	*11*	*8*	*13*
Total bulk density (g/cm³)	1.6±0.02^{e}	1.9±0.03^{d}	2.1±0.02^{c}	2.3±0.02^{a}	2.3±0.02^{a}	2.2±0.03^{b}
Bulk density topsoil fraction (g/cm³)	1.6±0.02^{b}	1.9±0.03^{a}	1.8±0.02^{a}	1.8±0.02^{a}	1.2±0.01^{c}	0.6±0.02^{d}

Soil moisture content

During the well-watered period, when all pots were maintained at 80% field capacity, total absolute water content (cm^3 per pot) was significantly lower in pots with a higher proportion of waste rock ($F_{5, 51}$ = 67.4, P <0.0001) (Table 4). In drought induced conditions, the decline of θ_m varied with both waste rock content and time ($F_{5,51}$ = 79.18, P<0.0001) (Figure 2). Here the decline in θ_m was slower in topsoil mixes with waste rock compared to those without. Those treatments that contained no waste rock (H0%WR and 0%WR) exhibited the most rapid decline in θ_m when compared to initial values, with approximately 70-80% reduction in mean θ_m in the first 10 days of dry-down. A slower decline in θ_m was exhibited in 50%WR, 75%WR and 88%WR with mean θ_m reducing by 10-20% in the first 10 days compared to initial values.

Table 4. 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 ± 1 s.e. Annotated lettering represents the results of one-way ANOVA, with Tukey's HSD test for comparison. Values followed by the same letters are not significantly different (α = 0.05). 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

Topsoil Mix	H0%WR	0%WR	25%WR	50%WR	75%WR	88%WR
n	*9*	*9*	*12*	*8*	*6*	*13*
Absolute water content (cm^3)	1858^{b}	2607^{a}	2315^{a}	1759^{b}	1270^{c}	1076^{c}
θ_m (cm³ cm^{-3})	0.37±0.01^{a}	0.34±0.02^{a}	0.27±0.00^{b}	0.21±0.01^{c}	0.15±0.00^{d}	0.12±0.00^{d}
θ_s topsoil fraction (cm³ cm^{-3})	0.37±0.01^{a}	0.34±0.02^{ab}	0.33±0.01^{ab}	0.32±0.02^{ab}	0.30±0.02^{b}	0.30±0.01^{b}

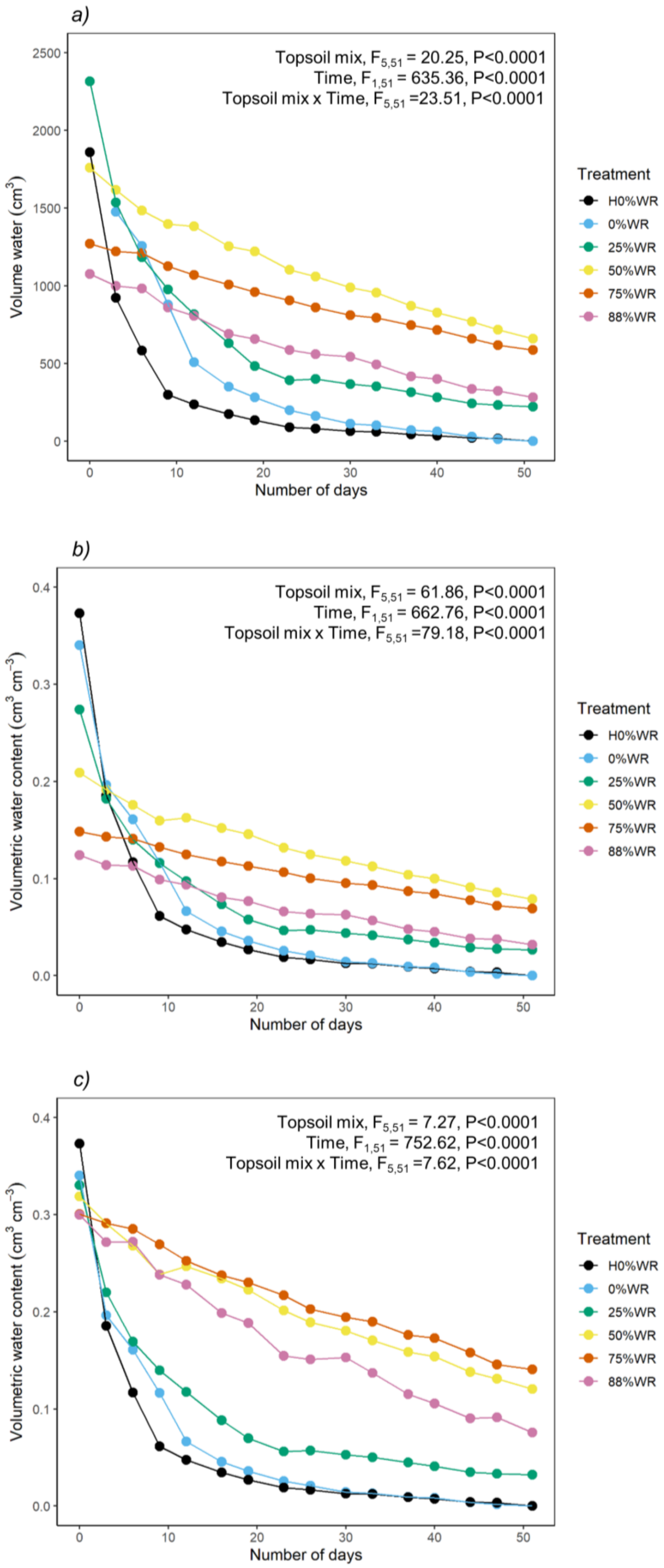


Figure 2 (a) Mean absolute water content (cm^3 per pot), (b) mean volumetric water content of the total soil mix (θ_m) and (c) mean volumetric water content of topsoil fraction only (θ_s) across six different topsoil mixes, during 51 days of dry-down, including ANOVA statistics for effects of topsoil mix, time and the interaction. 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

Physiological performance

In well-watered conditions, stomatal conductance (g_s) of *A. saligna* tended to decrease as the proportion of waste rock increased and this trend coincided with decreases in θ_m and θ_s ($P<0.001$ for both). When plants were well-watered, mixes with >50% 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 5). However, during the dry-down phase, plants on mixes with >50% waste rock exhibited less decline in g_s over time when compared to plants on H0%WR, 0%WR, 25%WR (Figure 3). Here, patterns in stomatal conductance were significantly affected by interactions between θ_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 (g_s=40 mmol m^{-2} s^{-1}) 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 the pre-determined drought-stress condition at approximately 26 days, 51 days, 47 days and 42 days respectively.

Table 5. Mean stomatal conductance for *Acacia saligna* recorded during the well-watered period for six different topsoil mixes. Data is presented as mean ± 1 s.e. Annotated lettering represents the results of Kruskal-Wallis test, with BH for comparison. Values followed by the same letters are not significantly different (α = 0.05). 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

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

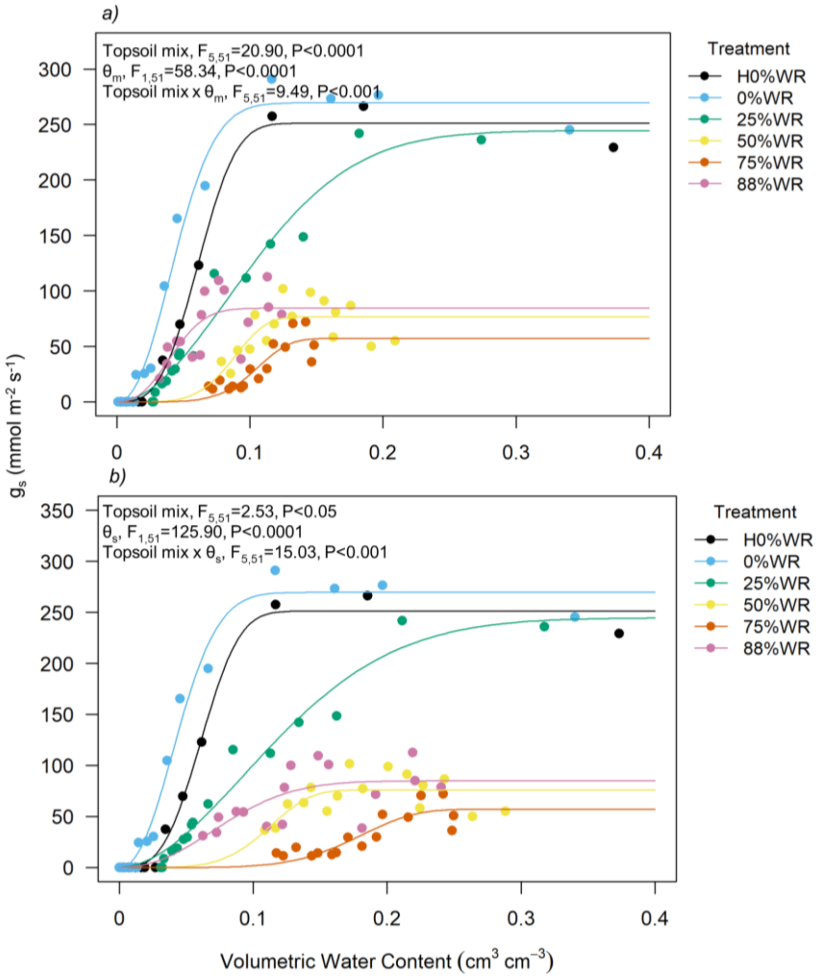


Figure 3. Non-linear regression analysis to explore the relationship between mid-morning (09:00-11:00) stomatal conductance (g_s; mmol m^{-2} s^{-1}) and (a) mean volumetric water content of the total soil mix (θ_m)

and (b) mean volumetric water content of topsoil fraction only (θ_s) for *Acacia saligna* across six topsoil mixes, over 51 days of dry-down, including ANOVA statistics for effects of topsoil mix, θ_m, θ_s and interactions.

A. saligna seedlings on waste rock mixes typically had lower leaf water potentials (Ψ_l) (Table 6, Figure 4). This pattern persisted as soils dried down and was influenced by interactions between waste rock content and θ_m ($F_{5,51}$ = 5.18, P<0.001). When subjected to drought-stressed conditions, the largest reduction in Ψ_l occurred in plants on H0%WR and 0%WR. On these treatments Ψ_l decreased by approximately 600% and 800% respectively, when compared to well-watered values. In comparison, plants on 50%WR,75%WR and 88%WR exhibited much lower reductions in Ψ_l, with values decreasing by 48%, 108% and 25% respectively when compared to well-watered values.

Table 6. Mean leaf water potential (Ψ_l, -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 mmol m^{-2} s^{-1} during the dry-down period. Data is presented as mean ± 1 s.e. Annotated lettering represents the results of one-way ANOVA, with Tukey's HSD test for comparison. Values followed by the same letters are not significantly different (α = 0.05). 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

Topsoil Mix	H0%WR	0%WR	25%WR	50%WR	75%WR	88%WR
n	*9*	*9*	*12*	*11*	*8*	*13*
			Well-watered			
Mean Ψ_l (-MPa)	0.1±0.02^c	0.2±0.04^c	0.4±0.06^b	2.0±0.42^a	1.3±0.31^a	2.1±0.27^a
			Dry-down			
Mean Ψ_l (-MPa)	1.2±0.19^b	2.1±0.07^{ab}	1.4±0.05^b	3.0±0.45^a	2.7±0.43^a	2.6±0.44^a

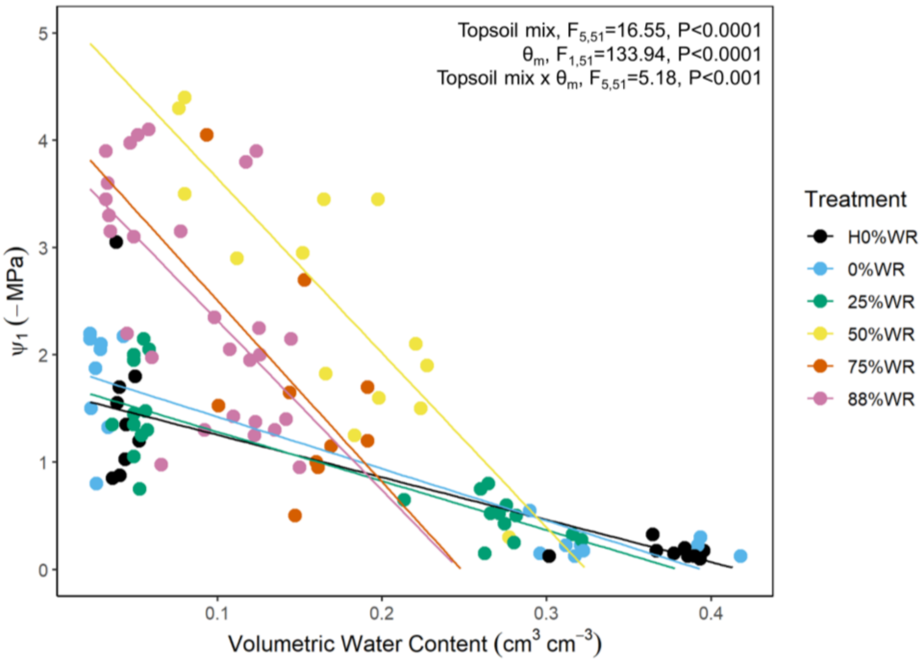


Figure 4. Linear regression analysis to explore the relationship between mean leaf water potential (Ψ_l, -MPa) and mean volumetric water content of *Acacia saligna* seedlings across six different topsoil mixes. Measurements were taken at the end of the well-watered period and at a pre-determined cut-off point of < 40 mmol m^{-2} s^{-1} during the dry-down period. Included are ANOVA statistics for effects of topsoil mix, total volumetric water content (θ_m) and the interaction on Ψ_l. 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

Growth and morphology

Overall, RGR based on leaf count was greatest in plants grown on waste rock percentages of 25% during both well-watered and dry-down conditions. In well-watered conditions, plants grown on waste rock proportions >50% recorded significant reductions in RGR based on leaf count ($F_{5,51}$= 6.63, P<0.0001) (Figure 5). The lowest RGR values overall were recorded from plants grown in 75%WR. Compared to well-watered conditions, drought conditions reduced RGR based on leaf count across all treatments except 50%WR and 88%WR, where RGR increased under drought. No significant difference in RGR based on height occurred across treatments during the well-watered period, but comparison of absolute growth showed *A. saligna* plants on 75%WR and 88%WR had reduced height growth relative to those on 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* varied with 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 g greater than plants grown in 0%WR (Figure 6). At waste rock percentages 50%, mean 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% and 88%WR did not. Similarly, increasing waste rock content tended to decrease root growth of *A. saligna* but root growth in higher rock contents 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). Shoot growth on 88%WR was not significantly different to other treatments. Differences in final biomass (Figure 6) roughly matched the observed RGRs based on non-destructive measurements during the well-watered and dry-down phases (Figure 5).

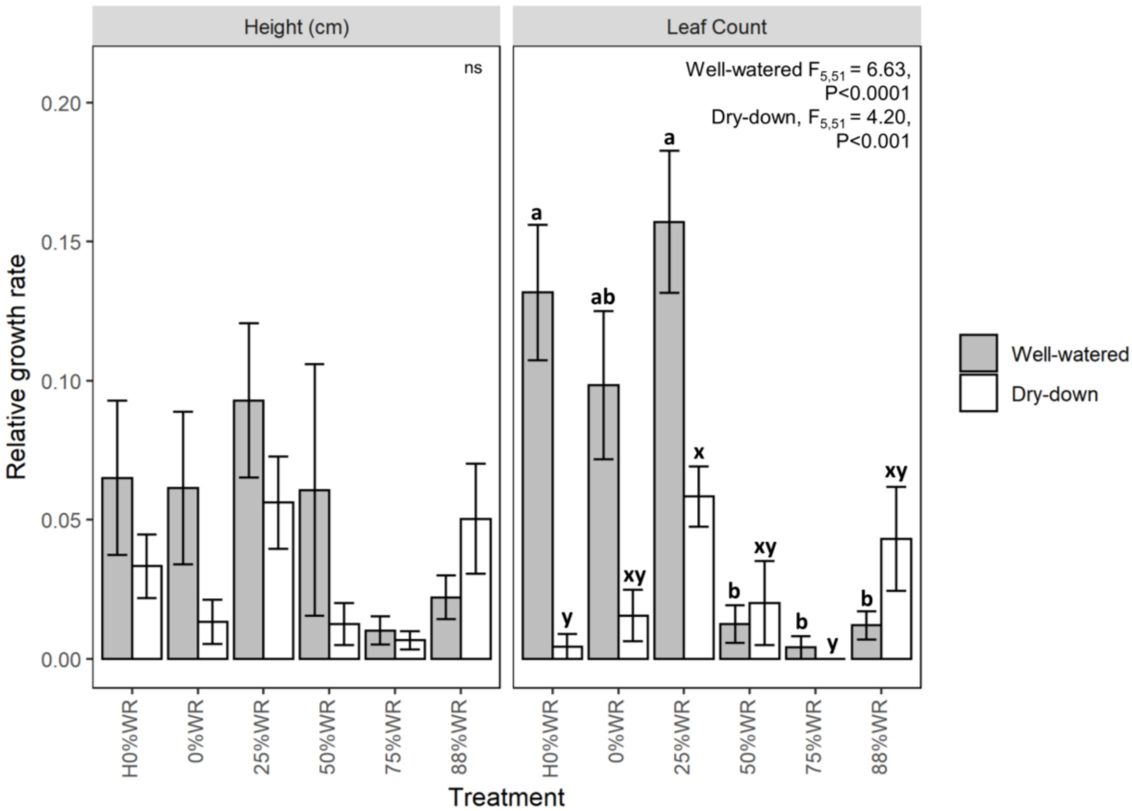


Figure 5. Mean relative growth rate (RGR) of *Acacia saligna* in six topsoil mixes recorded at two time intervals during both the well-watered phase (70 days) and dry-down phase (51 days). Letters indicate significant differences in RGR (α = 0.05) among treatments following results of one-way ANOVA, with Tukey's HSD test for comparison. For height, there were no significant differences (ns). 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

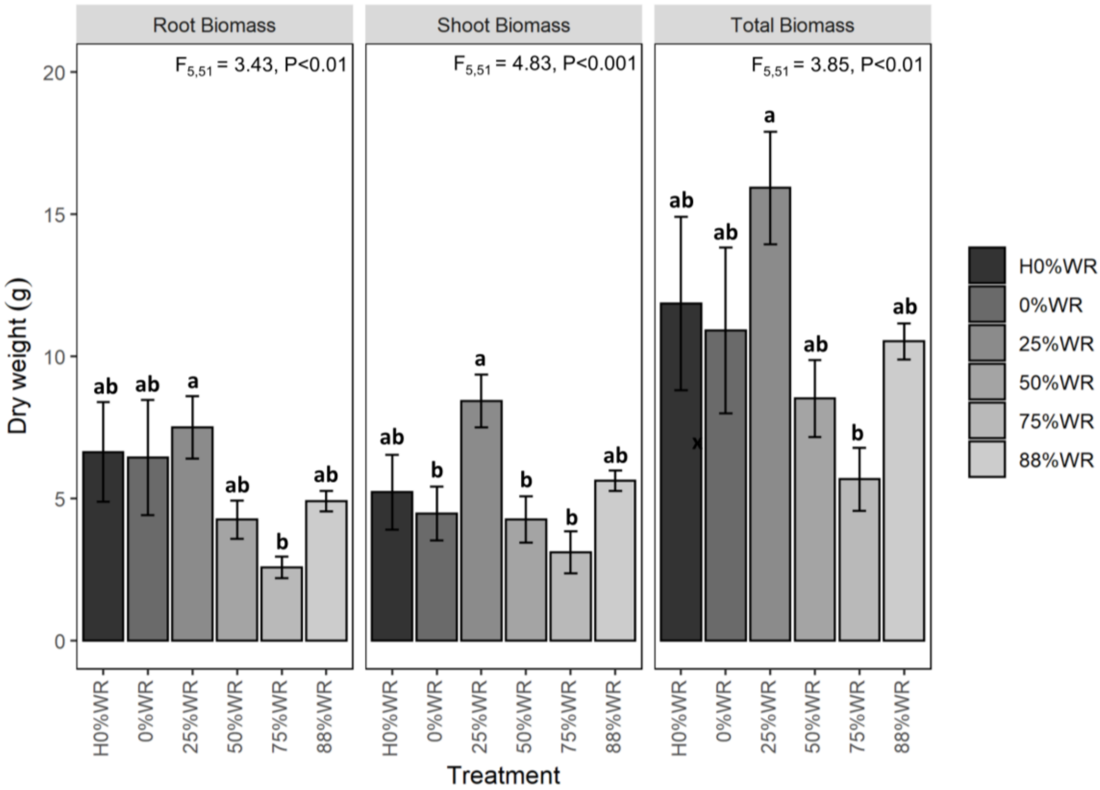


Figure 6. Shoot, root and total biomass of *A. saligna* at the end of the 121-day growth period, on six different topsoil mixes. Letters indicate significant differences in dry weight (g) (α = 0.05) between treatments following results of one-way ANOVA, with Tukey's HSD test for comparison. H0%WR – 5.25L 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

Discussion

In this study we provide evidence that augmentation of saline topsoil with waste rock may alter patterns of plant water use in a way that increases plant resistance to drought. We tested the hypothesis that in substrates with higher rock content, reduced soil volume and reduced water storage capacity will limit plant growth, especially in the absence of watering. The results showed that the presence of waste rock significantly reduced the volume of water held in the pot (cm^3) and the volumetric water content of the total topsoil mix. In response, the test species, *A. saligna*, showed signs of lower water availability namely a reduction in physiological functions (stomatal conductance and leaf water potential). Plants grown on larger proportions of waste rock showed slower growth in well-watered conditions as a result of lower water availability and this was evidenced by lower RGR values for plants grown in >50%WR. Given that water storage capacity was limited by the presence of waste rock, we expected an absence of watering to have a greater effect on the growth of plants on augmented topsoil compared to plants on topsoil only. Comparison of final biomass at the end of a 121-day growth period, that included a 70-day well-watered phase followed by a 51-day dry-down phase, showed that growth of *A. saligna* across treatments was not significantly different. The relationship between waste rock content and plant growth, was observed to be non-linear. The augmentation of soil with small amounts of waste rock (25%) had a positive effect on the growth of *A. saligna*, with plants on this treatment being significantly larger than plants grown on topsoil only. Augmentation of topsoil with waste rock at percentages >50% had a negative effect on the growth of *A. saligna* with plants on these treatments tending to be smaller than plants grown on 25%WR.

The presence of small amounts of waste rock may be beneficial for plant growth for two reasons. Firstly, at this level absolute water content of the pot was not significantly different compared to pots with topsoil only. Secondly, the presence of waste rock slowed the rate of dry-down, prolonging soil water availability. Other studies have 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 *Caragana korshinskii*, a peashrub species inhabiting semi-arid regions. Similarily, 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, Ψ_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 >50%, waste rock content had a negative effect on plant growth and biomass. This effect may be related to lower absolute water content of the pot (cm^3) and θ_m in the presence of large proportions of 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 (via root signals), 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 root growth. In this study, root biomass of *A. saligna* tended to be lower in high waste rock mixes. These findings reflect those of previous studies where root growth was observed to be lower in rocky soils. For example Babalola and Lal (1977) and Mi and Liu (2016) observed unfavourable effects on root development as a result of restricted rooting space at rock volumes of more than 20–50%. 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).

A fundamental gap in current knowledge is how waste rock content influences plant water use. When relying on stored soil water, we hypothesised that plants in substrates with higher rock content would deplete the available water more rapidly, causing a faster reduction of their stomatal conductance and water potential than plants in substrates with low rock content. Under prolonged water deficit, declines in stomatal conductance and leaf water potential were less severe in plants grown on high proportions of waste rock, even though these treatments recorded lower absolute water content and θ_m in well-watered conditions. This finding rejects our second hypothesis that plants in substrates with higher rock content will deplete the available water more rapidly. Pre-exposure of plants to water-limited conditions may account for the observed differences in plant water use across treatments, in that plants grown on high proportions of waste rock were either; (i) 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) 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) and this response has been attributed to the ability of a plant to maintain higher water use efficiency, allowing plants to maintain higher stomatal conductance under periods of stress (Vilagrosa et al. 2003). In our study, *A. saligna* plants exhibited slightly higher stomatal conductance on 88%WR compared to plants on 50% and 75%WR, even though this treatment had significantly less soil water content. Differences in stomatal conductance were not clearly separated however, and further studies are needed to 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) and thus is likely to be species-specific (Driessche 1991, Valliere et al. 2019). Nonetheless, if the performance of plants on high proportions of waste rock is related to a pre-exposure 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.

In this study, we observed similar reduction in evaporative water loss from pots without plants compared to pots with plants (Appendix C). 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 in Figure 2) nor impacted by plant size (Appendix C). 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. 1995). The positive relationship between waste rock and soil water conservation can be explained by rock fragments at the soil surface reducing the volume of soil exposed to evaporation (Unger 1971, Van Wesemael et al. 1996) and/or waste rock fragments within the soil mix slowing the upward movement of water through the soil (Ravina and Magier 1984). Both of these effects may assist conservation of soil water by reducing the rate of water loss via capillary rise. Further field trials would help to differentiate between the mechanisms accounting for reductions in water loss.

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 cm^3 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 (Korboulewsky et al. 2020). 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%. 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). 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) and further research is required to examine the potential for water transfers to occur between waste rock fragments and plant roots.

Conclusion

Overall, this study shows that augmentation of saline topsoil with iron-ore mine waste rock is beneficial for improving conservation of soil water under prolonged periods of water deficit, which may increase plant resistance to drought. We tested growth and physiology of a salt tolerant species, *A. saligna*, on saline topsoil amended with 0%, 25%, 50%, 75% and 88% waste rock. The results of this work support the premise that increasing waste rock content reduces water content of topsoil mixes but at the same time slows declines in water availability over time. While limitations in water availability may cause reductions in plant growth and physiological functions, we showed that plant growth was not significantly compromised by the presence of high proportions of waste rock. Future research that explores the effect of waste rock content on a greater diversity of species and plant functional groups under field settings will be useful in determining the application of these results in a wider setting. The results of this study improve our understanding of how plants respond to changes in soil water dynamics caused by waste rock content and highlights the importance of taking into account the waste rock fraction of topsoil mixes, specifically in restoration of post-mining areas in semi-arid and arid regions.

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Appendix A

Physical and chemical characteristics of topsoil and waste rock (crushed and ground) utilized in experiments. Data are presented as means ± 1 s.e. (*n* = 3 for each substrate). T and P values represent the results of pairwise comparison of topsoil and waste rock based on two-sample T-tests. Blank cells indicate no tests were run.

Parameter	Method	Unit	Topsoil	Waste Rock	T value	P value
Physical Properties						
Texture			Loamy sand	-		
Particle size distribution						
Stones (>2 mm)	Sieve	w/w	10.2 ± 0.65	100	-138	<0.001
Sand (0.2-2 mm)	Fraction	w/w	73.0 ± 0.58	0	126	<0.001
Silt (0.002–0.2 mm)	Fraction	w/w	5.13 ± 0.07	0	77.0	<0.001
Clay (<0.002 mm)	Fraction	w/w	11.7 ± 0.17	0	67.5	<0.001
Chemical Properties						
EC	1:5 water	dS/m	5.38 ± 0.24	0.36 ± 0.02	94.2	<0.001
pH	H_2O		5.37 ± 0.03	7.33 ± 0.06	-26.3	<0.001
Organic carbon	Walkley-Black	%	0.33 ± 0.02	0.12 ± 0.00	10.5	<0.01
Total nitrogen	Kjeldahl digest	mg/kg	0.02 ± 0.00	<0.005	35.5	<0.01
Total phosphorus	Kjeldahl digest	mg/kg	186 ± 3.33	606 ± 89.87	-4.67	<0.05
Available phosphorus	HCO_3	mg/kg	3 ± 0	2 ± 0	6.84	<0.05
Al	Mehlich-3	mg/kg	316±3.33			
B	Mehlich-3	mg/kg	2.30 ± 0.15			
Ca	Mehlich-3	mg/kg	526 ± 23.33			
Cd	Mehlich-3	mg/kg	<0.01			
Cl	Mehlich-3	mg/kg	6975 ± 1606			
Co	Mehlich-3	mg/kg	0.06 ± 0.00			
Cu	Mehlich-3	mg/kg	0.30 ± 0.00			
Fe	Mehlich-3	mg/kg	28.0 ± 0.00			
K	Mehlich-3	mg/kg	216 ± 3.33			
Mg	Mehlich-3	mg/kg	810 ± 10.0			
Mn	Mehlich-3	mg/kg	14.3 ± 0.33			
Mo	Mehlich-3	mg/kg	<0.01			
Na	Mehlich-3	mg/kg	3600 ± 850	226 ± 31.7	24.3	<0.01
Ni	Mehlich-3	mg/kg	0.20 ± 0			
P	Mehlich-3	mg/kg	1.70 ± 0.33			
S	Mehlich-3	mg/kg	>250	0.07 ± 0.01	17201	<0.001
Zn	Mehlich-3	mg/kg	0.43 ± 0.03			
As	Mehlich-3	mg/kg	<0.01			
Pb	Mehlich-3	mg/kg	1.43 ± 0.03			
Se	Mehlich-3	mg/kg	<0.10			

Appendix B

Elemental concentrations and Geochemical Abundance Index (GAI) index values for topsoil utilised in experiments (n=3). The GAI values are truncated to integer increments (0 through to 6) where a GAI of 0 indicates the element is present at a concentration similar to, or less than the median abundance, and a GAI of 6 indicates approximately a 100-fold or greater, enrichment above median abundance.

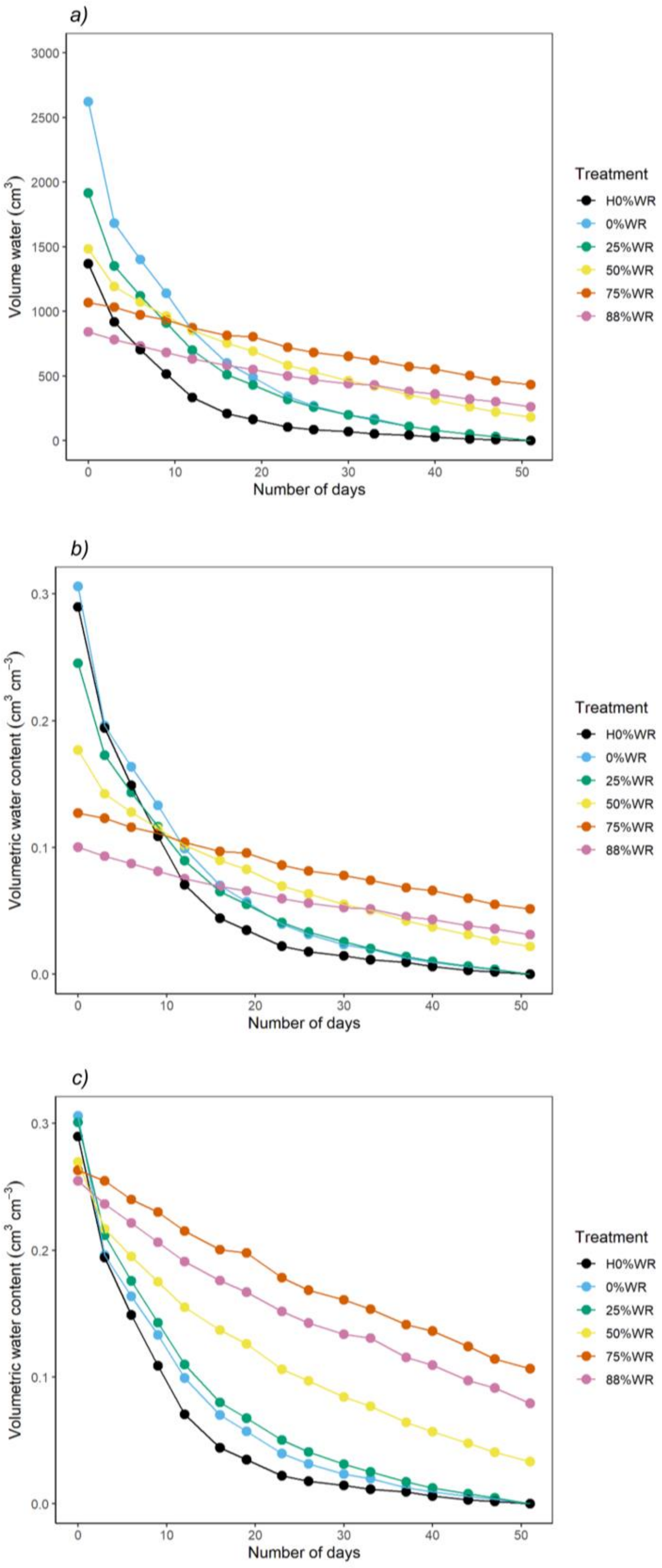
Element	Unit	Median Soil Content[1]	Elemental Concentration			GAI Value		
			Topsoil Sample 1	Topsoil Sample 2	Topsoil Sample 3	Topsoil Sample 1	Topsoil Sample 2	Topsoil Sample 3
Al	ppm	71000	310	320	320	0	0	0
As	ppm	5	<0.1	<0.1	<0.1	0	0	0
B	ppm	10	2	2.4	2.5	0	0	0
Cd	ppm	0.5	<0.01	<0.01	<0.01	0	0	0
Co	ppm	8	0.06	0.06	0.07	0	0	0
Cu	ppm	20	0.3	0.3	0.3	0	0	0
Fe	ppm	200	28	28	28	0	0	0
Mn	ppm	850	14	14	15	0	0	0
Mo	ppm	1.5	<0.01	<0.01	<0.01	0	0	0
Pb	ppm	40	0.2	0.2	0.2	0	0	0
Ni	ppm	10	1.5	1.4	1.4	0	0	0
Se	ppm	0.2	<0.1	<0.1	<0.1	0	0	0
Zn	ppm	50	0.4	0.5	0.4	0	0	0

[1]Median soil content data obtained from:

Berkman, D. A. 2011. *Field Geologists Manual*. 5th edition. Carlton, VIC, Australia: Australasian Institute of Mining and Metallurgy.

Bowen, H.J.M. 1979. *Environmental Geochemistry of the Elements*, Academic Press, London

Appendix C



(a) Mean absolute water content (cm^3 per pot), (b) mean volumetric water content of the total soil mix (θ_m) and (c) mean volumetric water content of topsoil fraction only (θ_s) 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