

1 **Climate and soil factors influencing seedling recruitment of**  
2 **plant species used for dryland restoration**

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12

13 **Abstract**

14 Land degradation affects 10-20% of drylands globally. Intensive land use and management, large scale  
15 disturbances such as extractive operations, and global climate change, have contributed to degradation of these  
16 systems worldwide. Restoring these damaged environments is critical to improve ecosystem services and  
17 functions, conserve biodiversity, and contribute to climate resilience, food security, and landscape sustainability.  
18 Here, we present a case study on plant species of the mining intensive semi-arid Pilbara region in Western  
19 Australia that examines the effects of climate and soil factors on the restoration of drylands. We analysed the  
20 effects of a range of rainfall and temperature scenarios and the use of alternative soil materials on seedling  
21 recruitment of key native plant species from this area. Experimental studies were conducted in controlled  
22 environment facilities where conditions simulated those found in the Pilbara. Soil from topsoil (T) stockpiles  
23 and waste materials (W) from an active mine site were mixed at different proportions (100% T, 100% W, and  
24 two mixes of topsoil and waste at 50:50 and 25:75 ratios) and used as growth media. Our results showed that  
25 seedling recruitment was highly dependent on soil moisture and emergence was generally higher in the topsoil,  
26 which had the highest available water content. In general, responses to the climate scenarios differed  
27 significantly among the native species which suggest that future climate scenarios of increasing drought might  
28 affect not only seedling recruitment but also diversity and structure of native plant communities. The use of  
29 waste materials from mining operations as growth media such could be an alternative to the limited topsoil.  
30 However, at early plant stages a successful seedling recruitment can be challenging in the absence of water.  
31 These limitations could be overcome by using soil amendments but the cost associated to these solutions at large  
32 landscape scales needs to be addressed.

33

34 **Keywords**

35 Mine rehabilitation, seedling emergence, native plants, global change, growth media, soil water retention.

36 **1. Introduction**

37 Land degradation affects nearly two billion hectares of land globally, with 25% of the total global land  
38 considered degraded to some extent (Barbero-Sierra et al., 2015; Bisaro et al., 2014; Brevik et al., 2015; Miao et  
39 al., 2015; Stanturf et al., 2015; Torres et al., 2015; Wang et al., 2015). Restoring these damaged environments is  
40 critical to improve ecosystem services and functions, conserve biodiversity, and contribute to climate resilience,  
41 food security and landscape sustainability at the local, regional and global level (de Moraes Sá et al., 2015;  
42 Minnemeyer et al., 2011; Perring et al., 2015; Prosdocimi et al., 2015; Roa-Fuentes et al., 2015; Zucca et al.,  
43 2015). Drylands, which include semi-arid and arid environments, are particularly vulnerable to land degradation  
44 with estimates suggesting 10-20% of these ecosystems are degraded globally (Millennium Ecosystem  
45 Assessment 2005; Safriel et al., 2005), and continue to be degraded across millions of hectares every year  
46 (Brauch and Spring, 2009; Wang et al., 2015; Yan et al., 2015). Intensive land use and management, large scale  
47 disturbances, such as extractive operations (e.g. mining), and global climate change have contributed to  
48 degradation of these systems worldwide (Anaya-Romero et al., 2011; Keesstra et al., 2016a ; Kildisheva et al.,  
49 2016).

50 When attempting to restore degraded land, arid ecosystems face the challenges of limited rainfall, high  
51 temperatures, and soils with low nutrient levels and water holding capacity (Anaya-Romero et al., 2015; Muñoz  
52 Rojas et al., 2016a). Thus, despite the efforts and investments to restore these systems worldwide (Keesstra et

53 al., 2016a), restoration of drylands has low rates of success (James et al., 2013; Sheley et al., 2011). To improve  
54 our capacity to reinstate biodiverse, viable plant communities, there is a strong need to advance our  
55 understanding of how these systems function and the effects that environmental and edaphic factors have on  
56 processes such as seedling emergence and plant growth and survival (Perring et al., 2015). For example,  
57 changes in soil water availability as a consequence of reduced rainfall and evaporation, or increases in  
58 temperature due to global warming, may affect restoration outcomes through influencing seedling recruitment  
59 (Cochrane et al., 2015; Lloret et al., 2004) or the composition and distribution of plant species (Lai et al., 2015).  
60 But the impact of environmental factors on restoration can be also compounded by unfavourable edaphic  
61 conditions (Audet et al., 2013; Muñoz-Rojas et al., 2015; Thomas et al., 2015). Thus, improving soil physical  
62 and chemical properties can be decisive for successful revegetation (Machado et al., 2013), which is important  
63 in extractive industries operating in dryland environments.

64 During open-cut and strip mining operations, the top layer of soil is commonly removed and stockpiled before  
65 starting the extraction and then respread before seeding the target sites for restoration (Lamb et al. 2015; Rivera  
66 et al., 2015). This topsoil is an important source of seeds, nutrients and microorganisms (Erickson et al. 2016a;  
67 Golos et al., 2014; Koch, 2007; Muñoz-Rojas et al., 2016b) but its use in restoration is often limited by its  
68 scarcity and the detrimental conditions that topsoil stockpiling can have on soil functionality (Keipert et al.  
69 2002). Waste materials produced in mining operations provide alternative substrates that are currently being  
70 used as growth media in restoration (Machado et al., 2013; Muñoz-Rojas et al., 2016b; Thomas et al., 2015).  
71 These substrates can integrate coarser materials that help to reduce slope instability and prevent erosion  
72 processes, but they are often highly deficient in organic matter which can reduce soil water retention (Shrestha  
73 and Lal, 2006). In addition, developing appropriate soil structures for restoration, for example technosoils, can  
74 be expensive and demanding in terms of time and natural resources (Rivas-Pérez et al., 2016).

75 Where topsoil is limiting and waste materials form the substrate for plant growth, direct seeding is the most  
76 feasible means of reinstating biodiverse plants communities, particularly at larger scales (Ceccon et al., 2015;  
77 Erickson et al. 2016a; James et al., 2011; Perring et al., 2015; Porensky et al., 2014). However, direct seeding is  
78 inefficient in terms of the proportion of seeds that produce an established seedling ; in arid ecosystems it is  
79 common for only 2-7% of seeds to establish (Chambers, 2000; James et al. 2011; Larson et al. 2015), although  
80 the use of biochar has shown to increase these percentages (Drake et al., 2016) . The early developmental life-  
81 stages of plants are usually more sensitive to environmental or edaphic constraints than are the adult stages  
82 (Standish et al., 2014) and the transition from germinated seed to emerged seedling has been identified as the  
83 life-stage transition most limiting the success of direct seeding (James et al. 2011). As these first stages of plant  
84 regeneration fundamentally influence the composition of the future plant community (Jiménez-Alfaro et al.,  
85 2016), characterising abiotic factors of the edaphic environment and their effects on seeds and seedlings is  
86 necessary for developing seeding practices that can achieve the desired restoration outcomes. For example,  
87 highly erodible soils have proved to be an additional challenge for seed germination and consequently for  
88 obtaining an adequate plant cover (Bochet, 2015; Cerdà et al., 1997; Cerdà et al., 2002 ; Wang, et al., 2014).

89 With the numerous potential drivers of global change comes a wide range of potential climate change scenarios  
90 (IPCC, 2014). This hinders the incorporation of future climate predictions into restoration programs (Standish et  
91 al., 2014). In this context, more experimental studies are needed to accurately evaluate the effects of altered  
92 climatic conditions on seedling recruitment and subsequent vegetation community structure and function, all of  
93 which, in turn, are strongly linked to soil conditions (Audet et al., 2013). Such experimental approaches can be

94 effectively addressed by manipulation of combinations of climate and soil factors under controlled conditions  
95 (Lloret et al., 2004; Muñoz-Rojas et al., 2015). Here, we present a case study on plant species of the Pilbara  
96 region in the northwest of Western Australia, where we assess the effects of climate and soil factors on the  
97 restoration of semi-arid ecosystems. The Pilbara (22°03'S, 118°07'E to 23°19'S, 119°43'E) is a vast (179,000  
98 km<sup>2</sup>) and biodiverse (c. 1800 plant species) semi-arid ecosystem (Erickson et al. 2016a; McKenzie et al., 2009).  
99 The region is subjected to intensive mining, particularly of iron ore, and ecological restoration following mining  
100 commonly requires re-introducing plant propagules to vastly altered growth substrates (Erickson et al. 2016a).  
101 Using five native plant species that form key elements of the vegetation of this ecosystem, the specific  
102 objectives of this study were to: (i) analyse the effects of a range of climate scenarios (rainfall and temperature)  
103 and the use of alternative soil materials on seedling emergence of key native plant species from the Pilbara, (ii)  
104 determine the effects of the climate and soil scenarios on the time to emerge of these plant species, and (iii)  
105 assess the implications of these climate and soil factors on improving the restoration potential in semi-arid  
106 environments.

## 107 **2. Methods**

### 108 **2.1 Experimental design**

109 This study was conducted between August and December 2014 in a controlled environment room (CER) at The  
110 University of Western Australia (UWA) and a glasshouse facility at Kings Park and Botanic Garden in Perth,  
111 Western Australia. Five native plant species from five families were selected as representative of a diverse range  
112 of life-forms (e.g. perennial grass, shrub and tree components) that commonly contribute to the mature plant  
113 communities found throughout the mining intensive Pilbara region of Western Australia (Erickson et al. 2016b).  
114 These study species comprised *Acacia hilliana* Maiden (Fabaceae), *Eucalyptus gamophylla* L'Her. (Myrtaceae),  
115 *Gossypium robinsonii* F.Muell. (Malvaceae), *Grevillea pyramidalis* R.Br. (Proteaceae) and *Triodia epactia*  
116 S.W.L.Jacobs (Poaceae). Soil materials commonly used in mine restoration operations in the Pilbara (Bateman  
117 et al., 2016; Muñoz-Rojas et al., 2016b) were collected from an active mining site in the southern part of the  
118 region and used as growth media. These materials consisted of topsoil retrieved from previously stockpiled  
119 material and an overburden waste material commonly used in landform reconstruction due to its erosive stability  
120 and physical competency.

121 Two experimental studies were carried out to test different climate and soil scenarios. The climate in the Pilbara  
122 region is semi-arid with mean annual rainfall ranging between 250 and 400 mm, mostly concentrated in the  
123 summer months (December to March), accounting for approximately 72% of the total annual rainfall. This  
124 rainfall originates from sporadic summer convection thunderstorms and tropical cyclones. Mean annual  
125 temperatures range between 19.4 and 33.2 °C with average maximums over 40 °C in the summer period  
126 (Bureau of Meteorology, 2015). For the climate scenario experiment we selected a range of precipitation  
127 conditions representative of those of the Pilbara during the summer (growing season) based on the rainfall pulse  
128 duration and the amount of precipitation falling in each event (Bureau of Meteorology, 2015; CSIRO, 2007).  
129 From this selection we developed four simulated rainfall treatments, and a control (e.g. maintained at field  
130 capacity) (Table 1). Three temperature conditions were selected based on daily average temperatures in the  
131 study area (25, 30 and 35°C). These combined rainfall (n=5) and temperature (n=3) treatments resulted in 15  
132 climate scenarios and were evaluated solely in topsoil.

133 For the soil scenario experiment, a range of growth media blends were evaluated to assess the feasibility of  
134 using growth media mixes in restoration sites. These growth media consisted of four different blends of the soil  
135 materials collected from the mining sites: 100% topsoil (T), 100% waste (W), and two mixes of topsoil and  
136 waste at 50:50 (TW50:50) and 25:75 (TW25:75) ratios. Also, two watering scenarios were set up consisting of a  
137 well watered treatment (WW) and a water deficit treatment (WD). Both treatments were watered 25 ml for 3 d,  
138 then 25 ml every 3<sup>rd</sup> day for WW and every 6<sup>th</sup> day for WD for a total duration of 16 d.

## 139 **2.2. Experimental methods**

### 140 **2.1.1 Soil analyses and measurements**

141 Topsoil and waste material from the mine site were collected and transported to the CER facilities at UWA and  
142 Kings Park in 200 l drums. To create each growth media combination, one drum of topsoil (ca. 350 kg) and one  
143 drum of waste (ca. 225 kg) were mixed thoroughly into the different blend proportions required (e.g. T, W,  
144 TW50:50 and TW25:75, Table 2), ensuring each blend was homogeneous once blended. From each of these  
145 growth media blends, three composited soil samples of 500 g were taken, air-dried, and sieved (2 mm mesh) for  
146 physical and chemical analysis. Soil pH and electrical conductivity (EC) were calculated in deionised water  
147 (1:2.5 and 1:5, w/v, respectively), with a AD8000 microprocessor-based pH. Organic C (OC) was measured by  
148 dichromate oxidation (Walkley and Black, 1934) and total N with the Kjeldahl method (Bremner and Mulvaney,  
149 1982). Particle size was analysed by laser diffraction using a Mastersizer 2000 (Malvern Instruments, Malvern,  
150 England) after removing the organic matter with H<sub>2</sub>O<sub>2</sub>. Bulk density (BD) was determined according to the  
151 method proposed by Rawls (1983).

152 Soil hydrological parameters (Table 2) were determined according to Conant et al. (2014) using a pressure plate  
153 device at four tensions between saturation (-0.001 kPa) and wilting point (-1500 kPa) including field capacity (-  
154 10 kPa) (Table 2). Briefly, soil samples were saturated and placed in the pressure plates and then weighed to  
155 determine moisture content after hydrostatic equilibrium was reached at each water potential.

### 156 **2.1.2 Experimental set up**

157 Seeds for each species were obtained from commercially collected seeds supplied to the mining industry for use  
158 in Pilbara restoration programs. Upon receipt at Kings Park and Botanic Garden, seeds of *A. hilliana*,  
159 *Eucalyptus gamophylla*, *Gossypium robinsonii*, and *Grevillea pyramidalis* were cleaned of any non-seed  
160 material (e.g. chaff in *Eucalyptus* collections) and then x-rayed to remove any empty, partially filled, or clearly  
161 non-viable seeds (Faxitron MX-20 x-ray cabinet, Tucson, Arizona, USA) following Erickson et al. (2016a). A  
162 seed was deemed filled/viable when the x-ray images showed no abnormalities and the image was uniform  
163 white/grey in colour. For *Triodia epactia*, a seed is dispersed in an indehiscent floret and requires removal of the  
164 floret to maximise the chances of germination (Erickson et al. 2016b). Therefore, seeds were cleaned from the  
165 covering florets structures by carefully rubbing florets on a ribbed rubber mat and separating the seed from the  
166 floret debris using vacuum separation ('Zig Zag' Selecta, Machinefabriek BV, Enkhuizen, The Netherlands).  
167 Seeds were examined under the microscope to ensure no embryo damage occurred. These cleaning processes  
168 ensured only > 95% filled/viable material was used in each experiment and removed seed quality as a potential  
169 cause of reduced emergence.

170 To maximise the germination potential of each batch and accommodate seeds with primary dormancy, seed pre-  
171 treatments followed pre-treatment recommendations in Erickson et al. (2016a). Seeds of *A. hilliana* and *G.*

172 *robinsonii* were treated for 1-2 mins at 90°C to break physical dormancy. Seeds of *E. gamophylla* and *G.*  
173 *pyramidalis* were non-dormant and did not require a pre-treatment. Once cleaned from florets, seeds of *T.*  
174 *epactia* were soaked for 24 h in a 1µM concentration of karrikinolide (KAR<sub>1</sub>; 3-methyl-2H-furo[2,3-c]pyran-2-  
175 one, synthesised following Flematti et al. (2005)) and re-dried at 15°C / 15% relative humidity for at least 2-3 d  
176 prior to sowing.

177 The climate scenario experiment was conducted in the CER at UWA, where temperature, CO<sub>2</sub> and relative  
178 humidity were controlled and monitored routinely. The CER was set to a constant 12 h day and night cycle for  
179 the duration of the experiment, where day-time temperature was the treatment temperature (25, 30 and 35 °C)  
180 and night-time temperature was set at 20° C for all three temperature treatments. Relative humidity was  
181 maintained at 50% and CO<sub>2</sub> at 400 ppm. The soil scenario experiment was conducted in the glasshouse facilities  
182 of Kings Park and Botanic Garden where air temperatures where on average 30 °C and relative humidity ca.  
183 50%.

184 For both experiments, pots of 25cm<sup>2</sup> surface by 12 cm height were assorted in a randomised block design and  
185 replicated 12 times. Five seeds were sown into each pot and watering regimes were imposed on day 1 of the  
186 experiments and applied manually using a 50ml syringe. Volumetric soil moisture was continuously monitored  
187 across all treatments in three additional ‘dummy’ pots. An ECHO EC-5 moisture sensor (Decagon Devices,  
188 Inc.) connected to a HOBO micro station data logger (Onset Computer Corporation, Massachusetts, USA) was  
189 inserted completely into the soil surface. Measurements of volumetric soil moisture content were recorded every  
190 5 mins for the duration of the experiment, and were later averaged for daily moisture contents (Fig S1 and Fig  
191 S2). Air temperature was also logged in both experiments.

192 Seedling emergence was recorded daily in each pot for 16 d. Final emergence (%) was determined as the  
193 average emergence per pot after 16 d divided by five (the number of seeds per pot) and mean emergence time  
194 (MET) was calculated using the following equation adapted from Ellis and Roberts (1980):

$$195 \quad MET = \frac{\sum Dn}{\sum n} \quad (1)$$

196 Where n is the number of seedlings that emerged on day D, and D is the number of days counted from the  
197 beginning of emergence.

198

### 199 **2.1.3 Statistical analyses**

200 Differences in seedling emergence (final proportion of emerged seedlings among climate and soil scenarios) and  
201 time to emergence among treatments were tested using analysis of variance (ANOVA). Comparisons between  
202 means were performed with the Tukey’s HSD (honestly significant difference) test ( $P < 0.01$ ). Before ANOVA  
203 testing, the analysed variables were tested for normality and variance homogeneity using the Shapiro-Wilk and  
204 Levene tests, and data were log transformed as necessary (presented data are non-transformed). All analyses  
205 were performed with R statistical software version 3.1.2 (R Core Team 2014).

206

## 207 **3. Results and discussion**

### 208 **3.1 Climate effects on seedling emergence**

209 Our results showed that seedling emergence of the Pilbara native plant species was highly dependent on soil  
210 water content in the topsoil growth media (Table 3). Total emergence varied significantly across plant species  
211 and water treatments ( $P < 0.001$ , Table 3; Fig. 1) and, although we did not find significant differences between  
212 temperature scenarios, interactions of temperature, water and plant species were significantly different ( $P <$   
213  $0.001$ , Table 3).

214 Seedling emergence for *A. hilliana* ranged between 10 and 45% (Fig. 1) and higher values were obtained in the  
215 control and the R1 and R2 treatments (pulse watering treatments of 10 mm and 20 mm daily for 6 d,  
216 respectively). The maximum number of emerged seedlings was recorded at a day temperature of 35°C. Seedling  
217 emergence of *E. gamophylla* followed the same trend with higher emergence in the control, R1 and R2 watering  
218 treatments compared to R3 and R4 watering treatments. For this species, seedling emergence was  $20.1 \pm 3.8\%$  on  
219 average and up to  $40.1 \pm 6.1\%$  with available water (R1 and R2) and at 35°C. In contrast, emergence of *G.*  
220 *robinsonii* was lower and differences were not significant across water and temperature treatments. Seedlings of  
221 *G. robinsonii* did not emerge at 35°C with short initial pulses of watering (R3 and R4 watering treatments).  
222 However, maximum emergence occurred under this 35°C temperature scenario with the 6-day pulse regime (R1  
223 and R2). Although the maximum seedling emergence recorded for *G. pyramidalis* was higher than the other  
224 species (above 80% in the 30°C scenario), seedlings only emerged with continuous irrigation (control  
225 conditions); suggesting, in terms of seedling emergence, that this species has the lowest tolerance to drought.  
226 Patterns of seedling emergence for *T. epactia* were irregular, but in general, the seeds also proved to be  
227 dependent on higher amounts of water, and emergence generally decreased as temperature increased. Lower  
228 simulated rainfall pulse amounts seemed to be more beneficial for this species (R2 and R4).

229 Overall, our results showed that rainfall patterns had a large influence on seedling emergence across the five  
230 native species and suggest that seedling recruitment of these native plants may decrease in a climate scenario of  
231 increasing drought. These results are broadly consistent with other similar studies conducted in seasonally dry  
232 environments. For example, Lewandrowski (2016) found that seedling emergence of *Triodia* species decreased  
233 with water stress and high temperatures (35- 40° C). Similarly, in a study of Mediterranean shrubland of Eastern  
234 Spain, Lloret et al (2004) applied a range of warming treatments with temperature increments of 0.19-1.12 °C to  
235 analyse seedling emergence of native species. They found a moderate decrease in seedling recruitment in the  
236 warming treatments compared to the control, but differences were not statistically significant. Hogenbirk and  
237 Wein (1992) obtained larger seedling emergence at higher temperatures, but only for weedy species, suggesting  
238 that climate changes can favour weedy species over native plants. In general, the climate effect on seedling  
239 emergence seems to be more closely connected to water availability than to warming, and temperature is likely  
240 to be less of a limiting factor in the seedling emergence phase for most species (Lloret et al, 2004; Perring and  
241 Hoevenden, 2012; Woods et al., 2010).

242 In our study, seedling emergence responses to the watering regimes differed significantly among the five  
243 species. We found significantly decreased emergence of seedlings of *G. pyramidalis* and *G. robinsonii* under  
244 water-limited treatments, which suggest that changes in precipitation patterns can have a critical effect on the  
245 recruitment of these species. Plant species producing fewer recruits have been proposed to be more likely to  
246 disappear with drier conditions in future climate scenarios, with a consequent impact on diversity and structure  
247 of native plant communities (Lloret et al. 2004). Thus, the ability of seedlings to make use of the reduced  
248 amount of precipitation for emergence and subsequent survival will be a determinant of their distribution (Lai et  
249 al., 2015).

250 The mean time for emergence of the five plant species was significantly different across temperature and rainfall  
251 treatments with slightly shorter times recorded under higher temperatures, particularly in *A. hilliana* and *T.*  
252 *epactia* (Fig. 2); results that are in agreement with some previous studies (De Frenne et al., 2012; Richter et al.,  
253 2012). However, in the southwest of Western Australia, Cochrane et al. (2015) found that emergence of  
254 seedlings was delayed with warmer conditions, compared to control. It has been previously suggested that early  
255 emergence is a strong determinant of seedling vigour and can significantly increase plant biomass (Verdú and  
256 Traveset 2005).

257 Regardless of plant species or temperature conditions, our results showed significantly higher rates of emerged  
258 seedlings with longer pulses of simulated rainfall (6 d compared to 2 d) with the same amount of accumulated  
259 water during the treatment (60 ml over the irrigation phase). Semi-arid ecosystems are particularly influenced by  
260 precipitation patterns, and water availability in these environments can be highly pulsed with discrete rainfall  
261 events followed by drought periods (Miranda et al, 2011). Therefore, changes in precipitation frequency, such as  
262 rainfall pulses, can have a stronger effect than rainfall quantity in these environments (Woods et al., 2014).

263 Another factor that might affect plant production in global climate change scenarios is the elevated  
264 concentration of atmospheric CO<sub>2</sub> (IPPC, 2007). However, we have not considered this effect in this study since  
265 it is unlikely that CO<sub>2</sub> had any direct impact at the seedling emergence stage (Classes et al., 2010). A number of  
266 studies have previously analysed the possible impacts of CO<sub>2</sub> in seedling recruitment but most of them found  
267 that the response of seedling to changes in atmospheric CO<sub>2</sub> are constrained by changes in precipitation patterns  
268 (Garten et al., 2008; Kardol et al., 2010).

### 269 **3.2 Soil type effects on seedling emergence**

270 Seedling emergence differed significantly between growth media types, watering treatments and plant species,  
271 but the effect of water inputs seemed to be a larger driver of emergence than growth media type ( $P < 0.0001$ ,  
272 Table 4). With the higher soil moisture treatment (WW treatment), differences between soil materials were not  
273 significant at the  $P=0.0001$  level for *E. gamophylla*, *G. robinsonii* and *G. pyramidalis*, but emergence of *T.*  
274 *epactia* seedlings was significantly ( $P < 0.0001$ ) higher in the topsoil ( $56.7 \pm 7.1\%$ ) and the 50:50 topsoil:waste  
275 blend ( $65.1 \pm 7.1\%$ ), as compared to the 25:75 topsoil:waste blend ( $23.3 \pm 6.9\%$ ) and the waste ( $25.1 \pm 5.6\%$ )  
276 (Fig.3). Similarly, emergence of *A. hilliana* seedlings showed a progressive decline as the amount of topsoil  
277 decreased, ranging from  $58.3 \pm 6.3\%$  in the topsoil to  $33.3 \pm 7.1\%$  in the waste material. In the WD scenario,  
278 seedling emergence was lower for all species with total emergence varying between  $1.7 \pm 1.0\%$  in *G.*  
279 *pyramidalis* and  $40.1 \pm 7.1\%$  in *T. epactia* in the topsoil growth media. In this water limited scenario, seedlings  
280 of *G. pyramidalis* and *G. robinsonii* did not emerge in any growth media apart from the 100% topsoil soil type.  
281 Mean time to emergence did not differ across growth media types (Table 4) or in any of the interactions between  
282 growth media type, water, and plant species.

283 The analyses of soil physio-chemical properties showed lower contents of sand in the topsoil growth media  
284 ( $70.5 \pm 0.7\%$ ) consistently increased with increasing fractions of waste in the blend (Table 2). The influence of  
285 soil texture on soil water retention has been largely investigated (Saxton and Rawls, 2006) with different  
286 responses in seedling emergence (Cortina et al., 2011). Soil water holding capacity is generally higher in soils  
287 with larger clay and low sand content (Rawls, 2003). Higher nutrient retention in these soils rich in clay may  
288 increase seedling emergence and seedling root growth, allowing an easier extraction of water from deeper layers  
289 of the soil profile (Woodall, 2010). However, some studies showed that higher infiltration rates in soils with



290 elevated contents of sand may increase seedling emergence allowing plants to effectively extract water  
291 following precipitation (Cortina et al., 2011).

292 Our study showed that seedling emergence across the five plant species was higher in the topsoil growth media  
293 which might be explained by the greater water availability as a consequence of larger amounts of organic C  
294 content (Table 2). Although additional factors, such as adequate nutrient levels in the soil, can be necessary for  
295 plant establishment in degraded soils (Valdecantos et al., 2006; Brevik et al., 2015), water availability seems to  
296 be more critical at early plant life stages, particularly in semi-arid environments (Cortina et al., 2011; Miranda et  
297 al, 2011).

### 298 **3.3. Implications for restoration of degraded lands**

299 The use of growth media such as waste materials has proved to be a competent alternative to the original soil  
300 (i.e. topsoil) in restoration of degraded semi-arid areas (Machado et al., 2013; Muñoz-Rojas et al., 2015, 2006b;  
301 Rivera et al., 2014). Muñoz-Rojas et al. (2016b) showed that soil functions in a rehabilitated area of northwest  
302 Western Australia, with the use of mine waste material, can reach levels of microbial activity and organic C  
303 similar to those of topsoil once vegetation was established. However, here we show that at the early stages of  
304 plant recruitment, the use of alternative substrates depleted of organic materials can be challenging for  
305 successful seedling recruitment in the absence of water. Low contents of soil OC have been commonly  
306 associated to the loss of soil structure, which as a consequence, diminishes water holding capacity, increases  
307 bulk density, and accordingly produces soil compaction (Lal, 2004; Willaarts et al., 2015).

308 Overall, the results obtained in this study provide evidence that the availability of water in the soil system is a  
309 key determinant factor for increasing seedling recruitment and, therefore, optimising restoration of semi-arid  
310 lands such as the Pilbara. The application of irrigation has been proposed in restoration of semi-arid systems to  
311 control watering inputs (Bainbridge et al., 2002). There are several types of irrigation systems available that  
312 could effectively increase seedling recruitment, particularly in plant species most sensitive to water limitations  
313 (Padilla et al., 2009). However, there are higher costs associated with this alternative that makes its use  
314 impractical at the landscape level (Cortina et al., 2011).

315 Degraded soils – frequently infertile and depleted of organic materials – can respond positively to the addition  
316 of amendments (Cortina et al., 2011; Keesstra et al., 2016b; Lozano-García et al., 2011; Valdecantos et al.,  
317 2006). Soil amendments have been commonly used in restoration to improve soil structure, restore the  
318 hydrological balance and increase the mineral nutritional capacity (Hueso-González et al., 2014; Jordán et al.,  
319 2011). Inorganic amendments (e.g. fertilisers) are usually applied to overcome plant nutritional deficiencies or  
320 physical limitations. However, the use of organic amendments such as mulch or manure has proved to increase  
321 soil water retention in soils with poor structure with a consequent increase of plant survival in mine restoration  
322 (Benigno et al., 2013). Even low doses of composted organic waste applied in degraded soils have shown to  
323 support seedling response for long periods (Fuentes et al., 2010; Yazdanpanah et al., 2016). Nevertheless, the  
324 application of organic amendments can have several implications such as competition with existing species  
325 which is compounded by the high costs of these practices at large scales in mine restoration (Cortina et al.,  
326 2011).

327 Since seedling establishment from seeds can be challenging in restoration (James et al., 2011), increasing seed  
328 input, or enhancing the availability of suitable micro-sites for seedling emergence through modifying the soil  
329 environment or alternatively improving the regenerative capacity of seeds represent alternative strategies for

330 those species with limited recruitment (e.g. *G. pyramidalis* or *G. robinsonii*). Such approaches will involve new  
331 technologies for improving seed handling, processing and quality evaluation and the use of seed treatments to  
332 overcome dormancy and improve seedling resilience and vigour germination (Merritt et al. 2007, Turner et al.  
333 2013). For example, though in its infancy, seed coating procedures for native species offer promise of  
334 overcoming recruitment bottlenecks by ‘empowering’ the seed through coating, pelleting and aggregate  
335 technologies (Madsen et al., 2014; Madsen et al. 2016). Our results highlight the critical impact of soil water  
336 availability for seedling recruitment and the need to address this limitation, but further studies are needed to  
337 develop suitable applications and techniques in drylands restoration at a management scale. It would be useful  
338 to transfer the experiments reported here to larger-scale field trials to effectively assess applicability of the  
339 findings into restoration programs.

#### 340 **4. Conclusions**

341 Seedling recruitment of the five native plants was highly dependent on soil moisture and temperature did not  
342 have a significant effect in the number of emerging seedlings. Emergence across the five plant species was  
343 higher in the topsoil growth media compared to the other soil materials, most likely due to its larger available  
344 water content as a consequence of increased amounts of organic C. Overall, under drought scenarios total  
345 seedling emergence was below 40% for all species and growth media types. In general, responses to the climate  
346 scenarios differed significantly among the five native species suggesting that future climate scenarios of  
347 increasing drought might affect not only seedling recruitment, but also diversity and structure of native plant  
348 communities. In particular, we found significantly decreased emergence rates in seedlings of *G. pyramidalis* and  
349 *G. robinsonii* under water limited treatments meaning that changes in precipitation patterns may have a critical  
350 affect on the recruitment of these species. The use of growth media such as waste materials from mining  
351 operations could be an alternative to the scarce topsoil. However, at early plant stages the use of these  
352 alternative substrates that are depleted of organic materials can be challenging for successful seedling  
353 recruitment in the absence of water. These limitations could be overcome by using soil amendments but the cost  
354 associated to these solutions at large landscape scales needs to be addressed.

355

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581 **Tables**

582 **Table 1.** Simulated rainfall (watering) treatments utilised in this study. Pulse durations and rainfall amounts  
583 were selected from interrogating long-term weather data accessed from the Newman Airport weather station  
584 (CSIRO 2007; Bureau of Meteorology, 2015). Simulated rainfall treatments (R1 – R4) comprised repeat daily  
585 applications of water for either 6 d or 3 d and two different rainfall amounts (20mm or 10mm). The total  
586 irrigation amount of 50ml or 25ml matched the pot sizes used in this study and rainfall amount treatments  
587 required to simulate the desired simulated rain conditions

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Treatment	Control	R1	R2	R3	R4
Pulse duration (days)	-	6	6	3	3
Rainfall amount (mm)	-	20	10	20	10
Irrigation (ml)	50	50	25	50	25

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591 **Table 2.** Soil physicochemical and hydraulical properties of growth media types (mean  $\pm$ SE, n=3).EC: electrical  
 592 conductivity, OC: organic C; N: total N, FC: field capacity, WP: wilting point, AWC: available water content  
 593 (difference between FC and WP).

Soil	pH	EC (ms/m)	OC (%)	N (%)	Clay (%)	Silt (%)	Sand (%)	Bulk Density (g/cc)	FC (%)	WP (%)	AWC (%)
Topsoil	7.8 $\pm$ 0.1	46.7 $\pm$ 0.8	0.8 $\pm$ 0.1	0.10 $\pm$ 0.01	4.6 $\pm$ 0.1	24.9 $\pm$ 0.7	70.5 $\pm$ 0.7	1.55 $\pm$ 0.01	28.7 $\pm$ 0.2	8.9 $\pm$ 0.1	19.5 $\pm$ 0.1
TW (50:50)	7.6 $\pm$ 0.1	38.5 $\pm$ 2.5	0.4 $\pm$ 0.1	0.03 $\pm$ 0.01	3.1 $\pm$ 0.1	21.9 $\pm$ 1.7	75.0 $\pm$ 1.6	1.57 $\pm$ 0.01	19.1 $\pm$ 0.4	9.1 $\pm$ 0.1	10.0 $\pm$ 0.3
TW (25:75)	7.8 $\pm$ 0.1	38.9 $\pm$ 2.9	0.3 $\pm$ 0.1	0.02 $\pm$ 0.01	2.4 $\pm$ 0.1	12.9 $\pm$ 0.7	84.7 $\pm$ 0.9	1.57 $\pm$ 0.01	17.1 $\pm$ 0.3	87 $\pm$ 0.2	8.1 $\pm$ 0.2
Waste	7.3 $\pm$ 0.1	55.7 $\pm$ 10.7	0.1 $\pm$ 0.1	0.01 $\pm$ 0.01	2.1 $\pm$ 0.3	11.8 $\pm$ 1.0	86.0 $\pm$ 1.0	1.57 $\pm$ 0.01	12.4 $\pm$ 0.4	9.1 $\pm$ 0.2	5.4 $\pm$ 0.2

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597 **Table 3.** Effects of climate factors (temperature and water) and plant species types, and interactive effects of these  
 598 factors on total emergence and mean time to emerge. Statistical significance levels: NS: not significant, \*\*\* $P < 0.001$ ,  
 599 \*\* $P < 0.01$ , \*  $P < 0.05$ .

Factor	Total emergence		Mean time to emerge	
	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Temperature (T)	2.7802	NS	15.5427	***
Water (W)	107.5179	***	18.0772	***
Plant species (P)	27.9409	***	67.2350	***
T x P	3.4951	**	3.2449	*
W x P	19.6585	***	3.8249	***
T x W	2.8951	*	0.9380	NS
T x W x P	3.2669	***	1.3067	NS

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602 **Table 4.** Effects of soil or growth media type, water treatments and plant species, and interactive effects of these  
 603 factors on total emergence and mean time to emerge. Statistical significance levels: NS: not significant, \*\*\* $P < 0.001$ ,  
 604 \*\* $P < 0.01$ , \*  $P < 0.05$ .

Factor	Total emergence		Mean time to emerge	
	<i>F value</i>	<i>P value</i>	<i>F value</i>	<i>P value</i>
Soil (S)	10.5853	***	0.4043	NS
Water (W)	301.1846	***	75.6453	***
Plant species (P)	19.3987	***	85.6517	***
S x P	3.07	***	0.8914	NS
W x P	12.1949	***	1.3579	NS
S x W	1.2097	NS	0.5689	NS
S x W x P	3.0291	***	1.9029	NS

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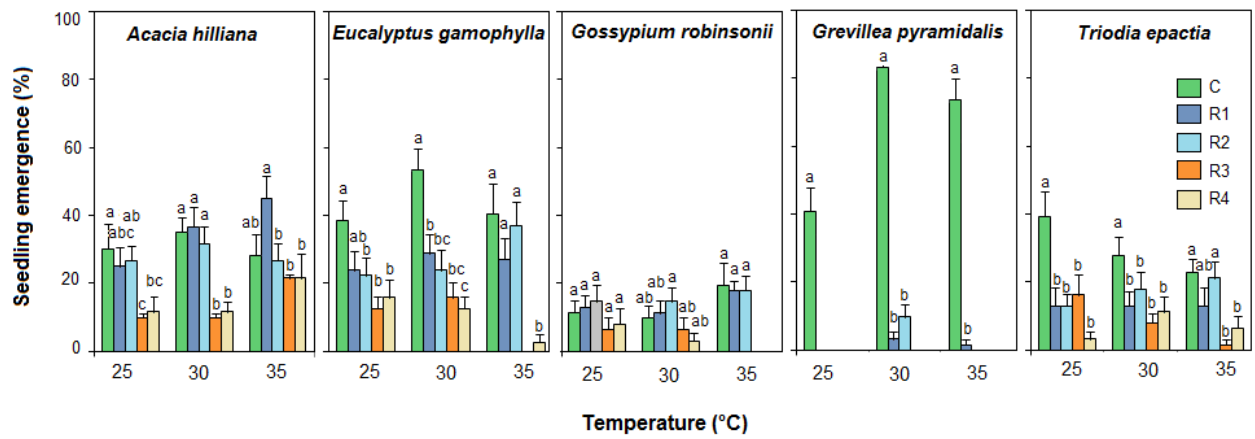
607 **Figure captions**

608 **Figure 1.** Total seedling emergence (% , mean± SE, n=12) of Pilbara native plant species under climate scenarios  
609 (temperature and rainfall). Different letters indicate significant differences over time among watering treatments (C,  
610 R1, R2, R3 and R4) for each temperature scenario (LSD post hoc test,  $P < 0.05$ ). Watering treatments as described in  
611 Table 1.

612 **Figure 2.** Mean time to emergence (days, mean± SE, n=12) of Pilbara native plant species under climate scenarios  
613 (temperature and rainfall). Watering treatments (C, R1, R2, R3 and R4) as described in Table 1.

614 **Figure 3.** Total seedling emergence (% , mean± SE, n=12) of Pilbara native plant species for different growth media  
615 types (T: 100% topsoil, TW 50:50: mix of topsoil and waste at 50:50 ratio, TW 25:75: mix of topsoil and waste at  
616 75:25 ratio and W: 100% waste) and watering treatments (WW; well watered and WD: water deficit). Different letters  
617 indicate significant differences over time among watering treatments for each temperature scenario (LSD post hoc  
618 test,  $P < 0.01$ ).

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620

621 **Figure 1.**Total seedling emergence (% , mean± SE, n=12) of Pilbara native plant species under climate scenarios  
 622 (temperature and rainfall). Different letters indicate significant differences over time among watering treatments (C,  
 623 R1, R2, R3 and R4) for each temperature scenario (LSD post hoc test,  $P < 0.05$ ). Watering treatments as described in  
 624 Table 1.

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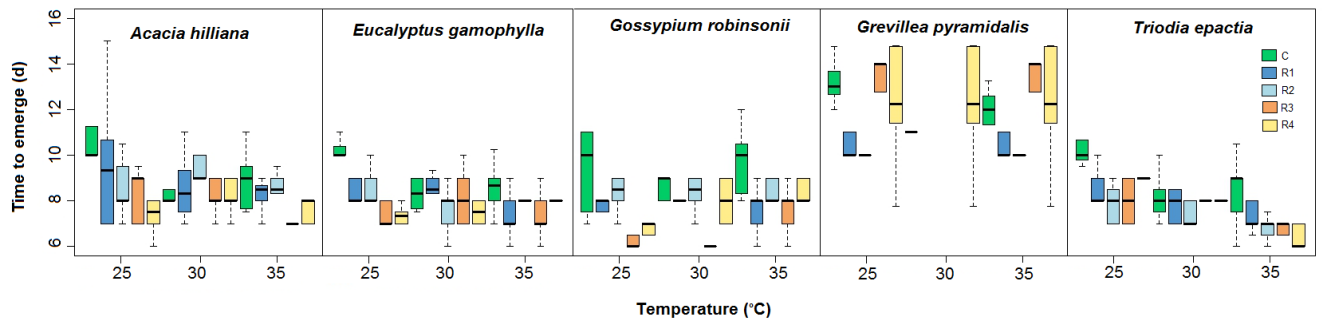
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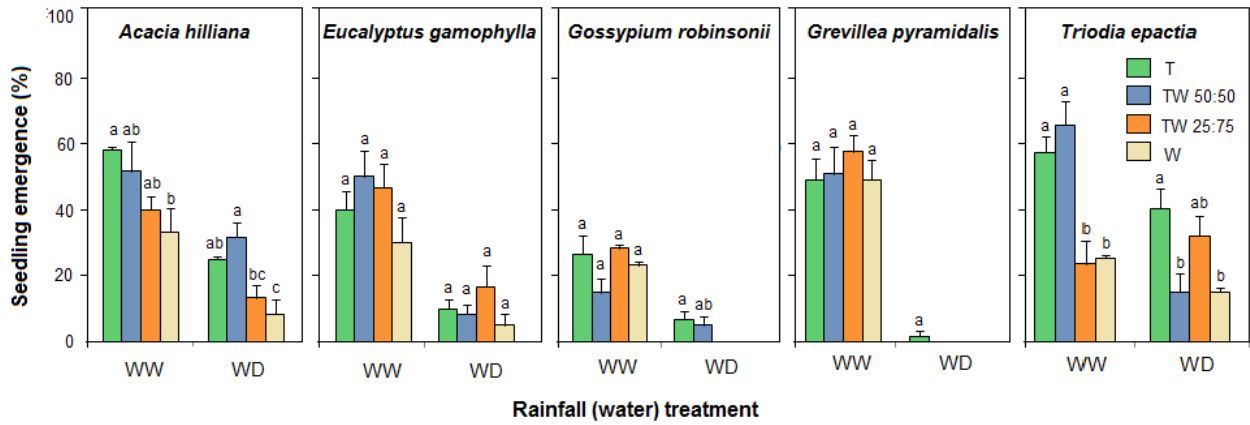
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639 **Figure 2.** Time to emergence (days, mean± SE, n=12) of Pilbara native plant species under climate scenarios  
 640 (temperature and rainfall). Watering treatments (C, R1, R2, R3 and R4) as described in Table 1.

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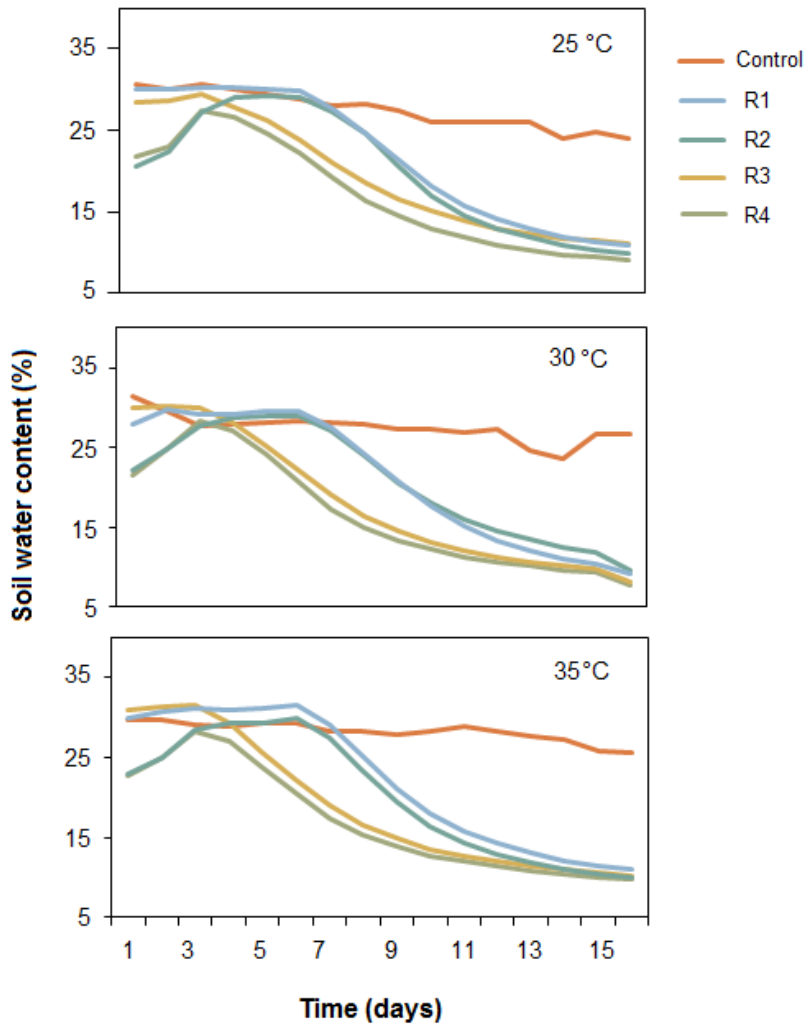


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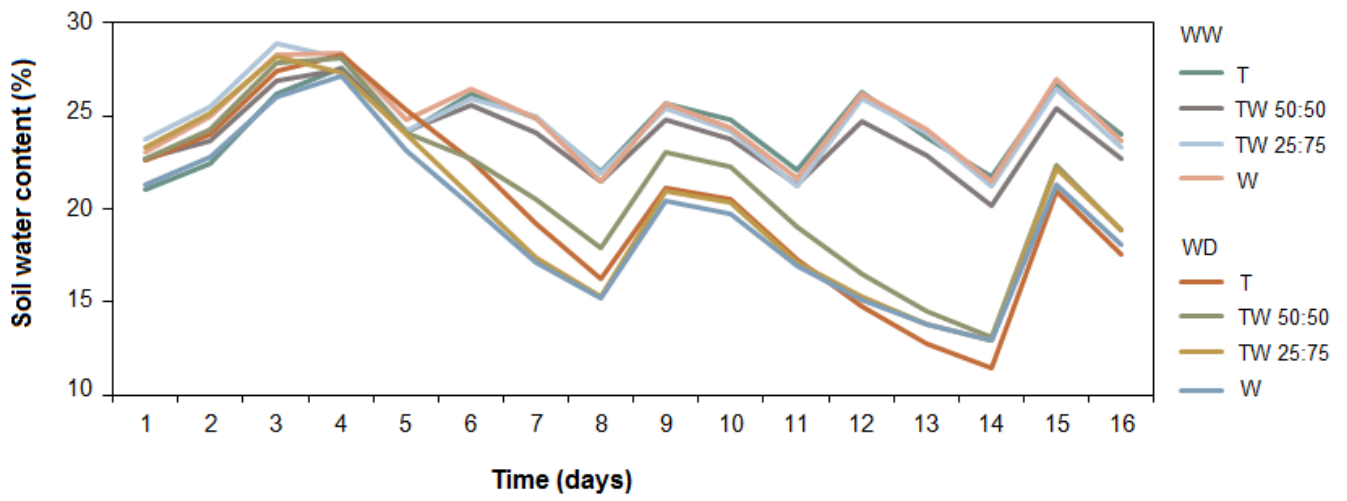
644 **Figure 3.** Total seedling emergence (% mean  $\pm$  SE, n=12) of Pilbara native plant species for different growth media  
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 646 75:25 ratio and W: 100% waste) and watering treatments (WW; well watered and WD: water deficit). Different letters  
 647 indicate significant differences over time among watering treatments for each temperature scenario (LSD post hoc  
 648 test,  $P < 0.01$ ).



Supplementary material



**Figure S1.** Variation in soil water content (%) with time (days) for climate scenarios (T= 25, 30 and 35 °C). Watering treatments (control, R1, R2, R3 and R4) as described in Table 1.



**Figure S2.** Variation in soil water content (%) with time (days) for different watering treatments (WW; well watered and WD: water deficit) and growth media types (T: 100% topsoil, TW 50:50: mix of topsoil and waste at 50:50 ratio, TW 25:75: mix of topsoil and waste at 75:25 ratio and W: 100% waste).