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Does biochar improve establishment of tree seedlings in saline sodic soils?

Land Degradation & Development, 2016; 27(1):52-59

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which has been published in final form at <http://dx.doi.org/10.1002/ldr.2374>

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23 August 2021

<http://hdl.handle.net/2440/100868>

1 **DOES BIOCHAR IMPROVE ESTABLISHMENT OF**
2 **TREE SEEDLINGS IN SALINE SODIC SOILS?**

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18

19 Running Title: REFORESTATION, BIOCHAR AND SALINE SODIC SOILS.

20

21

22 **Abstract**

23 Reforestation of saline sodic soil is increasingly undertaken as a means of reclaiming
24 otherwise unproductive agricultural land. Currently, restoration of degraded land is
25 limited to species with high tolerances of salinity. Biochar application has the
26 potential to improve physical, biological and chemical properties of these soils to
27 allow establishment of a wider range of plants. In a glasshouse trial, we applied
28 biochar made from *Acacia pycnantha* (5 t ha⁻¹) or no biochar to either a low (EC_e 4.75
29 dS m⁻¹, ESP 6.9), moderate (EC_e 27.6 dS m⁻¹, ESP 29.3) or high (EC_e 49.4 dS m⁻¹,
30 ESP 45.1) saline sodic soil. The regional common reforestation species *Eucalyptus*
31 *viminialis* and *Acacia mearnsii* were planted as tubestock in to the soils. Early
32 establishment indicators, including growth, plant condition and nutrition were
33 assessed at the end of a simulated growing season, 108 days after biochar application.
34 Application of biochar increased height, and decreased root:shoot and the
35 concentration of Mn, N and S in plants of *E. viminialis* when grown in the highly-
36 saline sodic soil. Biochar application increased the concentration of B in leaves of *E.*
37 *viminialis* and increased the concentration of P, K and S in leaves of *A. mearnsii* when
38 grown in the low saline sodic soil. The results confirm that there is potential for
39 biochar to assist in reforestation of saline sodic soils.

40

41

42 **Keywords:** amelioration, afforestation, biochar, nutrition, remediation, revegetation

43

44 **Introduction**

45 Over 30% of the world's soils are saline and/or sodic making them unproductive, with
46 the area of salinized land continually increasing (Rengasamy, 2006). Reforestation is
47 an important tool for mitigating dryland salinity and land degradation (George, *et al.*,
48 2012). In addition, reforestation improves biodiversity and conservation values,
49 provides income to land holders through forest resources, carbon credits and offset
50 schemes, and successfully remediates and reclaims land that is otherwise
51 unproductive (Bartle, *et al.*, 2007; Lal, 2008; Schirmer & Bull, 2013; Smith, 2008).
52 As soil degradation is a global issue, there is currently much research into reclamation
53 and amelioration of saline and/or sodic soils (e.g. Ahmad, *et al.*, 2011; Oo, *et al.*,
54 2013; Srivastava, *et al.*, 2014). Reforestation of saline and sodic soils is an approach
55 that can be applied internationally (Lal, 2008).

56 The high cation content of saline and/or sodic soils limits plant growth, making them
57 unusable for production agriculture (Naidu, *et al.*, 1995; Rengasamy, 2006). Plants
58 living in sodic and/or saline soils are likely to experience conditions outside of the
59 normal range, including both nutrient toxicity and deficiencies due to excess sodium
60 and other cations in the soil, a high pH, reduced redox potential (Curtin & Naidu,
61 1998), and increased osmotic stress (Semple, *et al.*, 2008; Stiller, 2009). Sodic soils,
62 which have weak structure, also contain water and nutrients that are inaccessible to
63 plants, and provide poor root zone aeration (Curtin & Naidu, 1998; Rengasamy, 2006).
64 Together, this can result in damaged and poor root growth, reduced shoot growth,
65 necrosis and ultimately plant death (Curtin & Naidu, 1998; Rengasamy, 2006; Semple,
66 *et al.*, 2008; Stiller, 2009).

67 Some species can tolerate saline and sodic soil conditions, making them suitable for
68 reforestation. This includes some species from, but are not limited to, the following
69 genera: *Eucalyptus* and *Acacia* (Dale & Dieters, 2007; Jackson & Bird, 2008; Marcar
70 & Crawford, 2004), *Casuarina* and *Melaleuca* (Dunn, *et al.*, 1994; Marcar &
71 Crawford, 2004); *Albizia*, *Azadirachta*, *Dalbergia*, *Terminalia* (Tripathi & Singh,
72 2005); *Prosopis* (Bhojvaid & Timmer, 1998); *Atriplex* (Nedjimi, 2014; Smith, 2008);
73 and *Taxodium* specifically bred for salt tolerance (Stiller, 2009). There are species
74 within these genera that are more sensitive to saline and sodic soils (Dunn, *et al.*,
75 1994; Stiller, 2009). These plants can show visible indicators of stress and poor plant
76 health (e.g. chlorosis, wilting, poor growth, abscission, necrosis and death) (Jackson
77 & Bird, 2008; Marcar & Crawford, 2004), related to the characteristics of saline and
78 sodic soils. The success of reforestation on saline sodic soils depends on careful
79 species selection for soil and site conditions (Jackson & Bird, 2008) and can also be
80 improved through soil amelioration prior to reforestation.

81 Biochar is often promoted as a way of ameliorating degraded soils (Atkinson, *et al.*,
82 2010; Barrow, 2012; Lehmann, *et al.*, 2011). Biochar is produced by the pyrolysis of
83 naturally derived organic matter, such as manure or wood chip, in a low oxygen
84 environment to form a high-carbon product that can be applied to soil (Lehmann &
85 Joseph, 2009). Biochar has been shown to increase mesoporosity, field available
86 water and reduce bulk density of saline sodic bauxite wastes (Jones, *et al.*, 2010).
87 Biochar has been found to reduce salt stress through sorption, improving productivity
88 of *Prunella vulgaris* and *Abutilon theophrasti* (Thomas, *et al.*, 2013). Application of
89 biochar to soils can also improve nitrogen and phosphorous availability (Atkinson, *et*
90 *al.*, 2010; Barrow, 2012; Joseph, *et al.*, 2010), which is important for nutrient-limited
91 sodic soils. Biochar amendments are known to increase abundance of mycorrhizas

92 and improve microbial community structure (Lehmann, *et al.*, 2011). Increased
93 arbuscular mycorrhizal (AM) associations are linked directly to improved plant growth
94 in saline soils (Al-Karaki, 2006; Ahanger *et al.*, 2014).

95 Research in forest ecosystems has focused on changes to soil physiochemical
96 properties due to charcoal produced following fires in plantations and native forests
97 (Atkinson, *et al.*, 2010; DeLuca, *et al.*, 2006) and not the addition of biochar. The
98 limited studies of saline sodic soils amended with biochar have focused on the yield
99 and productivity of crop and other herb species (e.g. Lashari *et al.* 2013; Thomas *et al.*
100 2013). Until now, there have been no tests of benefits of biochar addition to improve
101 the success (growth, condition or nutrition of plants) of reforestation and particularly
102 not on saline sodic soils.

103 Here, we determined if the application of biochar improves growth, condition and
104 nutrition of seedlings during establishment of tree plantings on three different saline
105 sodic soils. Seedlings of two species commonly used in reforestation in southeastern
106 Australia, *Acacia mearnsii* and *Eucalyptus viminalis*, were grown in a glasshouse trial,
107 with and without the addition of biochar derived from a local native species *Acacia*
108 *pycnantha*. This trial will provide crucial information on the potential for a combined
109 amendment-reforestation method to reclaim salinized land.

110

111 **Methods and materials**

112 **Site description**

113 Soils and plants used in this study were sourced from western Victoria, Australia (see
114 Table 1), due to its prevalence of saline sodic soils. The climatic averages for the

115 hottest month of the year (February) are 22.3 – 26.3 °C maximum and 11.6 – 13.3 °C
116 minimum, and a rainfall of between 578 - 909 mm year⁻¹ (Bureau of Meteorology,
117 2014). Temperatures increase and rainfall further from the coast. The focus of the
118 soils in this research are Sodosols (Isbell, 1996), which are soils with an exchangeable
119 sodium percentage (ESP) > 6%. The soils used in this trial are predominantly grazed
120 for meat and fibre (cattle and sheep), and dairy production, with limited remnant
121 forest or reforestation. The areas that were sampled were historically classed as
122 Grassy Woodlands, previously dominated by *Eucalypt sp.* with an open canopy and
123 ground cover including grasses and herbs. Otherwise they were historically classed as
124 wetlands that were predominately grassed with scattered trees.

125

126 **Soil collection and preparation**

127 Saline sodic soils used in this study were from three sites in the regions of Darlington,
128 Dundonald and Grassmear (Table 1). At each of the three sites, approximately 300 kg
129 of soil from the 0 – 10 cm layer was collected from a single location using a tractor.
130 The soil from each site was placed into its own bulk bag, with all soils kept separate
131 for the duration of the experiment, including preparation prior to use. Any large
132 organic debris, including maize residue, was removed, and the soil crushed and sieved
133 to < 10 mm and homogenised thoroughly. The soil was then air-dried prior to
134 experimental set up.

135 Key physicochemical properties of these soils (Table 1) were analysed as follows: pH
136 (1:5 water), EC (1:5), exchangeable Na and exchangeable sodium percentage (ESP),
137 exchangeable K, B and Mn, Colwell-P (Rayment & Higginson, 1992); total carbon

138 (TC) and total nitrogen (TN) by LECO CNS2000 Analyser; sulfur, ammonium and
139 nitrate-N (modified Morgan extract)(Wolf & Beegle, 2009).

140 All three soils were saline sodic, with an ESP > 6 and a saturated electrical
141 conductivity (EC_e) > 4 dS m⁻¹, based on the definitions of Rengasmy (2006) and
142 Murphy (2002). The texture class, using the ribbon test, was determined as being
143 loam for all soils. EC_e was determined using the standard equation and conversion
144 table in Hazelton and Murphy (2007), where $EC_e = EC_{1:5} \times 9.5$ (Table 1). All three
145 soils had varying amounts of salinity and sodicity (Table 1), and included: a low
146 saline sodic (LS), moderately saline sodic (MS), and highly saline sodic (HS).

147

148 **Biochar**

149 A biochar was produced from wood chip of *Acacia pycnantha* (Golden Wattle) in a
150 continuous reactor pyrolyser operated by Biochar Energy Systems, Australia.
151 Approximately 15 kg of dry *A. pycnantha* wood chips were placed in a mixer.
152 Phosphoric acid (20%, 500 mL) was sprayed onto the surface of the wood chip, and
153 500 g bentonite (Arumpo Bentonite Pty Ltd, Victoria, Australia) was added to the
154 wood chip. Phosphoric acid (10%, 1:1 solution to biochar) was added to the blends to
155 oxidise the surface of the biochar whilst concomitantly stabilising carbonyl groups
156 and improving loss of H from the biochar surface (Chia *et al.*, 2014). Bentonite was
157 added to the biochar during production to increase dehydration and oxidation (Chia *et*
158 *al.*, 2012). The mixture was then homogenised in the mixer for 30 min. The wood
159 chip, clay and phosphoric acid mix was then added to the pre-heated pyrolyser (550
160 °C) for carbonisation in an oxygen-limited environment. The charring temperature
161 was between 450 - 480 °C. Large particles of the denser/wetter wood chip that were

162 not fully pyrolysed were discarded. The general characteristics of the biochar are in
163 Table 1, and methods of analysis were the same as for soil.

164

165 **Plants**

166 Seedlings of two species, *A. mearnsii* (a nitrogen fixer) and *E. viminalis* were chosen
167 for this experiment. The chosen species are common in tree plantings in Western
168 Victoria. A variant of *E. viminalis* was found to have a salinity tolerance of between 4
169 - 8 dS m⁻¹ (Jackson & Bird, 2008). *Acacia mearnsii* has a salinity tolerance between
170 EC_e 2 - 4 dS m⁻¹, with reduced growth expected when EC_e > 4 dS m⁻¹ and reduced
171 survival when EC_e > 10 dS m⁻¹ (Jackson & Bird, 2008; Marcar & Crawford, 2004;
172 Marcar, *et al.*, 2003).

173 Seedlings were sourced from the Franklin Native Nursery in western Victoria. The
174 seedlings were of the same local provenance to the soils used in this study, which is
175 the typical approach used by land managers. At the start of the experiment, the 36
176 seedlings of *A. mearnsii* were 16 weeks old and the 36 seedlings of *E. viminalis* were
177 12 weeks old.

178

179 **Experimental design**

180 A randomised one-way treatment design was used in the glasshouse trial. Soils (LS,
181 MS, HS) were used as a block, and located in three separate areas (left, middle and
182 right) in the glasshouse. Within each block, half were treated as control (0 t ha⁻¹) and
183 5 t ha⁻¹ were incorporated to the other half of the soils. Two tubestock species (*A.*
184 *mearnsii* or *E. viminalis*) planted into each soil and biochar combination. This gave a
185 total of four treatments (+/- biochar with either *A. mearnsii* or *E. viminalis*), which

186 were then replicated six times within each soil. Randomisation of pots within soil
187 blocks was done by species and biochar combination, where three replicate sets of the
188 treatments were positioned at the back of the glasshouse, and three at the front of the
189 glasshouse. Positions within the replicate set were fully randomised by treatment.

190 Free draining plastic pots (20 cm tall, radius of 9.8 cm), with a layer of gauze placed
191 in the base to limit soil loss, were filled with 5 kg of either LS, MS or HS soils.

192 Biochar was incorporated into the top 10 cm of the soil at a rate of 5 t ha⁻¹ (15.1 g per
193 pot) to half of each soil. The application rate of 5 t ha⁻¹ biochar was chosen as an
194 economically feasible rate for farmer application. These pots were then watered to
195 100% of field capacity (FC), pots were then weighed every second day, and allowed
196 to equilibrate for 10 d to reach 60% FC. The weight of the plant was also subtracted
197 from the pots prior to calculating the FC. A FC of 60% was chosen to ensure
198 sufficient available water for the native plants (Wegglar, *et al.*, 2008), whilst avoiding
199 waterlogging. On the same day, each soil had six seedlings planted into soil with
200 biochar, and six into soil without biochar. Potting mix surrounding the seedlings roots
201 was retained during planting, as this reflects the common method used in field. The
202 experiment began on the day of the planting and finished 106 d later.

203 During the trial, pots were weighed and watered every 2 - 3 d to maintain 60% FC
204 and no less than 50% FC at any given time, with weight of the plant removed prior to
205 FC calculation. The pots were maintained in glasshouse conditions for the entirety of
206 the experiment. The climatic conditions were maintained at 25 °C during the day, and
207 10 °C at night, with natural light and a day length of between 9.5 – 11.5 hours during
208 the study period (June to September of 2012). These parameters are similar to
209 growing season conditions of the field site. Relative humidity was maintained at 60%.

210

211 **Plant monitoring**

212 Growth, condition and nutrition of plants were measured to understand the effect of
213 biochar on improving establishment in early stages of reforestation. Plants were
214 assessed at the time of planting (time 0 hereafter) and again at 106 d. Condition
215 assessments followed those used by Marcar et al. (1989; 1995), and in this research
216 included: a) percentage of leaves that had necrosis; b) percentage of leaves that had
217 chlorosis; c) presence of abscission; d) presence of other diseases or stress indicators
218 including yellow spots, not related to mites, and leaf curl; e) overall presence of
219 disease and stress, as a total of the presence or absence of all indicators. Necrosis and
220 chlorosis was calculated as the percentage of leaves affected by each condition
221 compared to overall number of leaves. Abscission and other diseases were determined
222 as either absence or presence for each plant.

223 Growth indicators measured included plant height, root and shoot biomass. Plant
224 height was measured from the base of the stem at the soil surface to the top of the
225 highest branch, just before the leaf petiole. Plants were cut at the soil surface and
226 anything below this point was considered roots. Roots were washed thoroughly to
227 remove potting mix and soil, with some loss of fine roots. This was unavoidable given
228 the clay present in the soils. Root and shoot biomass was dried at 60 °C for 72 hr prior
229 to weighing for dry biomass. Root to shoot ratio was then calculated.

230 Dried samples of the plants shoots (stems and leaves) were ground to a fine powder in
231 a ball mill prior to analysis for plant nutrition. TN, TC and S were analysed by a
232 LECO CNS2000 Analyser. Phosphorus, Ca, Na, K, Mg, Zn, Fe, Mn, Cu, B and Mo
233 were analysed by undertaking microwave digestion with nitric acid and read on an
234 ICP-MS.

235 **Data Analysis**

236 All data analysis was undertaken using SPSS Version 21 (IBM Corporation, 2012). A
237 restricted maximum likelihood (REML) was used to determine if there were
238 significant effects of biochar on species response variables, including plant growth,
239 condition and nutrition indicators. Growth variables included shoot and root mass,
240 root:shoot ratio and the final plant height. Condition variables included % necrosis, %
241 chlorosis, and presence or absence at end of the trial of abscission, other diseases, and
242 overall disease and stress indicators. Nutrient variables included concentrations of N,
243 P, K, S, C, Ca, Mg, Na, Cu, Zn, Mn, Fe, B and Mo. A REML was chosen for analysis
244 due to heterogeneity of variance, as determined using residual plots. Unlike analysis
245 of variance (ANOVA) that requires parametric and homogenous data, REML analysis
246 can use heterogeneous non-parametric data. The REML was applied to each soil and
247 plant species combination; being low, medium and highly saline sodic soil, with
248 either *A. mearnsii* or *E. viminalis*, giving a total of six REMLs. Plant and soil
249 combinations were not compared. Biochar was the fixed factor, and side and position
250 of the replicates in the glasshouse as random effects. The species and soils are highly
251 dissimilar, and thus analysed individually to ensure there is no masking of effects.
252 Pairwise comparisons were undertaken using least significant differences (LSDs).
253 Where P-values were significant, F-values were checked against appropriate
254 orthogonal contrasts.

255

256 **Results**

257 The results are only discussed as the effect of biochar on the individual soil-plant type
258 combinations, and no comparison is made between plant or soil types. The addition of
259 biochar to the highly saline sodic (HS) soil significantly ($P = 0.018$) increased the
260 height of *E. viminalis* by an average of 5.1 cm and also significantly ($P = 0.001$)
261 reduced the root:shoot ratio by 0.1 (Table 2). Biochar had no other effect on plant
262 growth variables (height, biomass, root:shoot) or condition indicators (necrosis,
263 chlorosis, abscission, other and overall) in any of the soil (LS, MS or HS) and plant
264 type (*E. viminalis* or *A. mearnsii*) combinations (Table 2). During root cleaning,
265 nodules were only observed in two seedlings of *A. mearnsii* grown in LS and these
266 nodules were confined to the original potting mix, which was retained when planting
267 the seedlings. No other plants in any soils had nodules.

268 Biochar application had significant effects on some aspects of plant nutrition, which
269 were soil-plant specific (Tables 3 and 4). In LS, *A. mearnsii* also had significantly
270 higher concentrations of leaf tissue P ($P = 0.02$), K ($P = 0.004$) and S ($P < 0.001$) with
271 biochar application than without (Table 3). Also in LS, *E. viminalis* had significantly
272 ($P = 0.032$) higher B concentration in leaf tissue with biochar application, with an
273 increase of 2.8 mg kg⁻¹ compared to plants without biochar (Table 4).

274 In HS, *A. mearnsii* had significantly ($P = 0.048$) higher concentrations of Na in leaf
275 tissue, by a factor of 4.2, with biochar application compared to without (Table 3). The
276 Na in plant tissue ranged from 0.3 – 0.8% in low and moderate soils (Table 3), similar
277 to the 0.8% in the tissue of *A. mearnsii* from highly saline sodic soil without biochar
278 addition. Also in HS, *E. viminalis* with biochar application had significantly lower
279 concentrations of TN ($P = 0.031$), S ($P = 0.003$) and Mn ($P = 0.01$) in leaf tissue

280 compared to those without biochar (Table 4), with these averages (2% N, 0.19% S,
281 76.8 mg kg⁻¹ Mn) closer to plant tissue concentrations in the LS (1.9% N, 0.2% S,
282 23.8 mg kg⁻¹ Mn) and MS (2.1% N, 0.2% S, 83.7 mg kg⁻¹ Mn), irrespective of biochar
283 addition. There were no significant changes in nutrition of either species in the MS
284 soil (Table 3 and 4).

285

286 **Discussion**

287 The international importance of large-scale development of reforestation to reclaim
288 salinized land has been discussed in the Introduction. The adverse effects of high
289 salinity have been shown in the reduced growth of *Eucalypt sp.* (Niknam & McComb,
290 2000), and in the observations of necrosis and death of *E. viminalis* when irrigated
291 with ≥ 300 mol m⁻³ NaCl (Marcar, 1989). No amelioration attempts to improve
292 *Eucalyptus* and *Acacia sp.* reforestation in saline sodic soils have been reported, and
293 the effects of biochar appear to have been restricted to reports on the improved
294 growth of the herb *Prunella vulgaris* and yield increases of wheat grown in saline
295 soils (Lashari, *et al.*, 2013; Thomas, *et al.*, 2013). The increased height and decreased
296 root:shoot response of *E. viminalis* to biochar addition in the highly saline sodic soil
297 are thus similar to previous findings (Lashari, *et al.*, 2013; Thomas, *et al.*, 2013). The
298 improvement in growth is most likely related to sorption of salts by the biochar.

299 The application of biochar to highly sodic soils decreased the concentration of Mn, N
300 and S in *E. viminalis* plants, which may have been related to improved fungal
301 associations with biochar addition. *Eucalyptus* species are not known to
302 physiologically regulate S uptake, and low S can lead to reductions in leaf area and
303 height (Wilson & Murray, 1994). Furthermore, Mn toxicity is known to cause reduced

304 biomass in *Eucalyptus* species (Guo, *et al.*, 2002). Poorer growth indicators with
305 higher plant tissue Mn and S were found here for *E. viminalis* plants without biochar
306 addition. This lack of regulation is the result of mechanisms in *Eucalyptus* species
307 that minimise loss and maximise uptake of nutrients due to their adaptation to nutrient
308 poor environments (Wilson & Murray, 1994; Guo, *et al.*, 2002). However,
309 ectomycorrhizas have associations with *Eucalyptus* species (Kariman, *et al.*, 2012)
310 and are well known to down regulate nutrients when the plant may not have its own
311 mechanism (Jourand, *et al.*, 2014; Lehto, *et al.*, 2010). The harsh conditions of saline
312 soils are known to limit fungal and bacterial associations with plants (Ahanger, *et al.*,
313 2014; Nadeem, *et al.*, 2014), and this association may have been absent in the highly-
314 saline sodic soil. As the low and moderate saline sodic soils both had higher
315 concentration of Mn (Table 1) and lower leaf tissue Mn than the highly saline sodic
316 soil, this suggests the lower ESP and EC_e of these soils allowed for mycorrhiza
317 associations that regulate Mn uptake. As biochar addition to soil improves habitat and
318 conditions for fungal or bacterial associations (Warnock *et al.* 2007; Lehmann *et al.*
319 2011) and reduces salinity through sorption (Lashari, *et al.*, 2013; Thomas, *et al.*,
320 2013), this may have created conditions that promote fungal associations in the highly
321 saline sodic soil. This would have resulted in the down regulation of N, Mn and S
322 closer to the concentrations in leaves of *E. viminalis* in the low and moderately saline
323 sodic soils, and improved growth. The exact mechanism involved in the reduction of
324 plant tissue Mn, N and S is unclear and warrants further examination.

325 There was an increased uptake of B in *E. viminalis* in the low sodic saline soil (Table
326 4), indicating changes to available B with biochar addition. Biochar application is
327 known to increase plant available nutrients in soil (Atkinson, *et al.*, 2010; Chan, *et al.*,
328 2008; Joseph, *et al.*, 2010), and the exchangeable B present in the biochar (Table 1)

329 would have contributed to the soils B availability. As there was 11.9 mg kg⁻¹
330 exchangeable B in the biochar and 1.1 mg kg⁻¹ exchangeable B in the low saline sodic
331 soil (Table 1), there would be a an absolute maximum of 0.2 mg kg⁻¹ increase in
332 exchangeable B with a biochar addition of 5 t ha⁻¹, increasing exchangeable B to 1.3
333 mg kg⁻¹. However, the increase of B in plant tissue was 2.8 mg kg⁻¹, which is higher
334 than the maximum amount related to biochar input (0.2 mg kg⁻¹), and thus another
335 biochar-soil interaction must have contributed. Ectomycorrhizas are known to have
336 associations with *Eucalyptus* species (Kariman, *et al.*, 2012) and regulate B uptake in
337 forest tree species (Lehto, *et al.*, 2010). In conjunction with this, ectomycorrhizas are
338 known to increase in abundance and/or plant associations with the addition of biochar
339 (Warnock, *et al.*, 2007). Thus, the potential for an increased association with
340 ectomycorrhizas with biochar addition could explain the additional increase in the
341 uptake of B in *E. viminalis*.

342 The increased plant tissue P, K and S in *A. mearnsii* in the low saline sodic soil (Table
343 3) is related to an increase in available nutrients with biochar application, as has been
344 reported previously (e.g. Chan, *et al.*, 2008; Joseph, *et al.*, 2010; Tagoe, *et al.*, 2008).
345 With biochar applied at a rate of 5 t ha⁻¹ to the soil, there would be a maximum
346 increase of 21.4 mg kg⁻¹ Colwell-P, 6.3 mg kg⁻¹ S and 3.42% K (using data from
347 Table 1), which is sufficient to explain the increases in plant tissue P, K and S in *A.*
348 *mearnsii*.

349 The addition of biochar to the highly-saline sodic soil resulted in a dramatic increase
350 in the concentration of Na in *A. mearnsii* plant tissue. Similar to the concentration of
351 Mn and S in *E. viminalis* plant tissue, the concentration of Na in plant tissue from low
352 and moderate saline sodic soils was similar to that from highly saline sodic soil
353 without biochar addition. This suggests that the biochar in the highly saline sodic soil

354 influenced the uptake of Na, but only when Na levels in soil are already extremely
355 high (ESP > 40). However, the maximum increase of Na to soil from biochar can only
356 be 0.2 % (calculated using Table 1). As Na was 4.2 times higher in plants when
357 biochar was added to the soil, a change in a fungal and bacterial association that
358 regulates Na uptake is more likely. Fungal associations with plants are known to
359 regulate uptake of Na from saline soils (Ahanger, *et al.*, 2014; Al-Karaki, 2006;
360 Mardukhi, *et al.*, 2011; Nadeem, *et al.*, 2014). This regulation and association is most
361 likely to be occurring in this study, across all soils where Na in plant tissue is low (<
362 0.8%). However, biochar, is known to alter fungal associations, including changes in
363 the percentage presence of N-fixing and non-N-fixing AMF (Rondon *et al.*, 2007;
364 Warnock *et al.*, 2007). The addition of biochar in the highly saline sodic soil may
365 have resulted in a shift to species that do not regulate the uptake of Na, and thus
366 caused an increase in plant tissue Na. Although plant condition and growth in this
367 establishment phase was similar between all soils tested both with and without
368 biochar, accumulation of Na in plant tissue can cause poor condition, stunted growth
369 and eventually death (Jackson & Bird, 2008; Marcar & Crawford, 2004; Marcar, *et al.*,
370 2003). The response to excess Na in *A. mearnsii* may be delayed until they are
371 saplings. The mechanism related to the increased Na uptake in *A. mearnsii* with
372 biochar addition in the highly saline sodic soil is unclear and further research on this
373 mechanism is required.

374 Both species showed evidence of chlorosis, necrosis and leaf curl in the moderately
375 and highly sodic soils, which is suggestive of osmotic stress and nutrient limiting
376 impacts due to the saline sodic soil conditions (Marcar, 1989; Marcar, *et al.*, 1995;
377 Rengasamy, 2006). The application of 5 t ha⁻¹ biochar had no beneficial effect on
378 plant condition. An increased application rate of biochar may show further beneficial

379 effects on plant establishment, and have been reported to improve the ability of
380 *Prunella vulgaris* to grow, but only at an application rate of 50 t ha⁻¹ (Thomas, *et al.*,
381 2013). This would, however, reflect a ten-fold increase in biochar use compared to
382 what was undertaken here, and further consideration is necessary to determine if this
383 is economically feasible for landholders.

384 Although our short-term trial found benefits of adding biochar in the establishment
385 phase of reforestation, further beneficial effects of biochar application have been
386 found three years after application (Jones, *et al.*, 2012) and after a five-crop rotation
387 following a single biochar application (Liu, *et al.*, 2014). This suggests that the
388 benefits of biochar may persist, and possibly increase, during the early development
389 of these tree plantings on saline sodic soils.

390

391 **Conclusion**

392 We found that two important reforestation species in temperate Australia, *E. viminalis*
393 and *A. mearnsii*, had soil-specific responses to biochar addition. In low (EC_e 4.75 dS
394 m⁻¹, ESP 6.9) and highly (EC_e 49.4 dS m⁻¹, ESP 45.1) saline sodic soils, biochar
395 generally had a positive effect on conditions for plant establishment in reforestation at
396 commercially feasible application rates of 5 t ha⁻¹. Application of biochar to
397 moderately (EC_e 27.6 dS m⁻¹, ESP 29.3) saline sodic soils has no detrimental effect.
398 This finding is particularly important for highly saline sodic soil, which is extremely
399 degraded. This research demonstrates that biochar has the potential to improve
400 reforestation success on extremely degraded land, resulting in greater areas of land
401 that can be potentially reclaimed. Harnessing an understanding of the most

402 appropriate use of biochar to restore degraded lands can increase biodiversity, provide
403 alternative income sources and global carbon sinks.

404

405 **Acknowledgements**

406 This project was supported by funding from the Australian Government under its
407 Carbon Farming Initiative – Biochar Capacity Building Program. We also thank
408 Greening Australia and the three farms for their role in project facilitation and
409 engagement, and Stephen Joseph for his role in biochar manufacturing. We also thank
410 Katrina Wilson, Dr Marianne Hoogmoed and Stephanie Watts-Williams for
411 assistance in technical work, and Dr Ee Ling Ng for comments on the manuscript. We
412 thank the two anonymous reviewers for their comments that have greatly helped to
413 improve this manuscript. TRC thanks the Australian Research Council for supporting
414 his research (FT120100463).

415

416

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583

584

585 **Table Captions**

586

587 **Table 1 – Characteristics, including pH, electrical conductivity (EC), saturated electrical**
588 **conductivity (ECe), total carbon (TC), total nitrogen (TN), exchangeable sodium percentage**
589 **(ESP), nitrate-N (NO_3^- -N), ammonium-N (NH_4^+ -N), Colwell phosphorus (Colwell-P),**
590 **exchangeable potassium (K), sulfur (S), boron (B) and manganese (Mn), of the three**
591 **compromised soils including low saline sodic (LS), moderately saline sodic (MS), highly saline**
592 **sodic (HS), and the Golden Wattle biochar. The latitude and longitude and land use of each soil**
593 **are included. ^A The high Colwell-P value of the biochar is a result of phosphoric acid addition**
594 **during pyrolysis.**

595

596

597
598 **Table 2 – Average (mean \pm standard error, $N=6$) of plant growth and condition variables for each**
599 **soil and species combination by biochar rate (0 or 5 t ha⁻¹). This includes height, shoot and root**
600 **biomass, root:shoot, presence or absence of abscission or other conditions, presence or absence of**
601 **all conditions, percentage of leaves with necrosis or chlorosis. Soils are low sodic (LS),**
602 **moderately sodic (MS) and highly sodic (HS). Abscission, other and overall plant condition**
603 **indicators are presented as absence or presence. * is used when there is a significant difference (P**
604 **< 0.05) between biochar treatments.**

605
606

607 **Table 3 – Average (mean ± standard error, $N=6$) of nutrition variables for *Acacia mearnsii* in**
608 **each soil, by biochar rate (0 or 5 t ha⁻¹). These include nitrogen (N), phosphorus (P), potassium**
609 **(K), sulfur (S), carbon (C), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), Zinc (Zn),**
610 **manganese (Mn), iron (Fe), boron (B) and molybdenum (Mo). Soils are low sodic (LS),**
611 **moderately sodic (MS) and highly sodic (HS). * is used to denote a significant difference ($P < 0.05$)**
612 **between biochar treatments within the same soil.**

613

614 **Table 4 – Average (mean \pm standard error, $N=6$) of nutrition variables for *Eucalyptus viminalis* in**
615 **each soil, by biochar rate (0 or 5 t ha⁻¹). These include nitrogen (N), phosphorus (P), potassium**
616 **(K), sulfur (S), carbon (C), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), Zinc (Zn),**
617 **manganese (Mn), iron (Fe), boron (B) and molybdenum (Mo). Soils are low sodic (LS),**
618 **moderately sodic (MS) and highly sodic (HS). Soil is low sodic (LS), moderately sodic (MS) and**
619 **highly sodic (HS). * is used to denote a significant difference ($P < 0.05$) between biochar**
620 **treatments within the same soil.**

621

622 Tables – in order

623 Table 1

| | Soil | | | Biochar |
|--|-----------------------|------------------------|-------------------------|---------------------|
| | LS | MS | HS | |
| Latitude and Longitude | 37.93 °S 143.06 °E | -38.29 °S 142.53 °E | -37.47 °S 144.79 °E | NA |
| Land Use | Maize, Grazing | Restoration | Grazing, Restoration | NA |
| pH | 7.6 | 8.7 | 8.6 | 7.4 |
| EC (ds m⁻¹) | 0.5 | 2.9 | 5.2 | 1.1 |
| ECe (ds m⁻¹) | 4.75 | 27.6 | 49.4 | NA |
| TC (%) | 5.3 | 2.7 | 2 | 66 |
| TN (%) | 0.5 | 0.3 | 0.2 | 0.8 |
| ESP | 6.9 | 29.3 | 45.1 | 13.5 |
| NO₃⁻-N (mg kg⁻¹) | 64.9 | 4.7 | 11.9 | 28 |
| NH₄⁺-N (mg kg⁻¹) | 10.8 | 12.3 | 14.2 | 7.2 |
| Colwell-P (mg kg⁻¹) | 154.2 | 17.8 | 62.4 | 1427.4 ^A |
| K (%) | 1.6 | 4 | 4.5 | 22.8 |
| S (mg kg⁻¹) | 20.7 | 200.2 | 663.2 | 421.2 |
| B (mg kg⁻¹) | 1.1 | 3 | 5.71 | 11.9 |
| Mn (mg kg⁻¹) | 19.7 | 55.3 | 9.7 | 20.5 |

624 Table 2

| Soil Biochar Rate (t ha ⁻¹) | <i>A. mearnsii</i> | | | | | | <i>E. viminalis</i> | | | | | |
|---|--------------------|-----------|-------------|------------|------------|-------------|---------------------|-----------|-----------|-----------|------------|------------|
| | LS | | MS | | HS | | LS | | MS | | HS | |
| | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 |
| Height (cm) | 37.4±3.1 | 35.1±1.5 | 13.7±1.7 | 15.3±2.5 | 12.4±1.7 | 7.6±2.4 | 64.4±3.0 | 63.0±1.8 | 30.7±2.5 | 35.7±2.1 | 32.4*±1.8 | 37.5*±1.2 |
| Shoot Biomass (g) | 5.13±0.20 | 4.52±0.87 | 1.14±0.21 | 0.88±0.27 | 0.41±0.12 | 0.48±0.13 | 5.04±0.55 | 5.85±0.52 | 1.88±0.26 | 1.57±0.14 | 1.53±0.14 | 1.78±0.16 |
| Root Biomass (g) | 0.73±0.16 | 0.63±0.15 | 0.40±0.07 | 0.29±0.07 | 0.20±0.04 | 0.22±0.06 | 1.11±0.15 | 1.07±0.25 | 0.82±0.08 | 0.68±0.04 | 0.63±0.06 | 0.57±0.07 |
| Root:Shoot | 0.14±0.03 | 0.14±0.01 | 0.37±0.03 | 0.38±0.05 | 0.53±0.07 | 0.68±0.22 | 0.22±0.02 | 0.18±0.04 | 0.46±0.04 | 0.45±0.04 | 0.42*±0.02 | 0.32*±0.02 |
| Abscission Other | 0±0 | 0±0 | 0.33±0.21 | 0.33±0.21 | 0.67±0.21 | 0.50±0.22 | 0.33±0.21 | 0.17±0.17 | 0±0 | 0.17±0.17 | 0.67±0.21 | 0.33±0.21 |
| Other | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0.33±0.21 | 0.33±0.21 | 0.17±0.17 | 0.17±0.17 | 0.17±0.17 | 0±0 | 0±0 |
| Overall | 0±0 | 0±0 | 0.50±0.22 | 0.50±0.22 | 0.67±0.21 | 1.00±0.00 | 0.50±0.22 | 0.17±0.17 | 0.17±0.17 | 0.17±0.17 | 0.67±0.21 | 0.50±0.22 |
| Necrosis (%) | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 3.70±3.70 | 0±0 | 0±0 | 0±0 | 0±0 | 2.75±1.78 | 3.55±1.65 |
| Chlorosis (%) | 0±0 | 0±0 | 25.18±12.45 | 13.10±8.33 | 26.98±9.23 | 22.22±16.48 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |

625

626 **Table 3**

| Soil Biochar Rate (t ha ⁻¹) | LS | | MS | | HS | |
|---|-------------|-------------|-------------|-------------|------------|--------------|
| | 0 | 5 | 0 | 5 | 0 | 5 |
| N (%) | 3.1±0.1 | 3.1±0.1 | 2.7±0.1 | 2.7±0.1 | 2.9±0.1 | 2.8±0.1 |
| P (%) | 0.15*±0.005 | 0.16*±0.005 | 0.2±0.03 | 0.2±0.01 | 0.2±0.01 | 0.51±0.21 |
| K (%) | 0.9*±0.04 | 1.1*±0.04 | 0.9±0.09 | 1.0±0.1 | 0.9±0.08 | 0.76±0.09 |
| S (%) | 0.17*±0.003 | 0.20*±0.003 | 0.3±0.03 | 0.3±0.1 | 0.3±0.02 | 0.29±0.01 |
| C (%) | 44.3±0.2 | 44.5±0.2 | 45.2±0.5 | 45.2±0.3 | 44.6±0.3 | 43.7±0.4 |
| Ca (%) | 1.4±0.04 | 1.3±0.1 | 1.1±0.1 | 1.0±0.1 | 1.2±0.1 | 1.5±0.4 |
| Mg (%) | 0.2±0.01 | 0.2±0.01 | 0.4±0.03 | 0.4±0.04 | 0.3±0.01 | 0.44±0.12 |
| Na (%) | 0.3±0.02 | 0.3±0.04 | 0.8±0.28 | 0.6±0.1 | 0.8*±0.1 | 3.35*±1.06 |
| Cu mg kg⁻¹ | 4.9±0.4 | 5.6±0.6 | 13.4±1.3 | 12.6±0.5 | 12.1±1.5 | 21.7±8.3 |
| Zn mg kg⁻¹ | 16.8±1.0 | 17.8±0.9 | 22.6±3.2 | 19.8±1.3 | 22.0±3.0 | 65.1±30.5 |
| Mn mg kg⁻¹ | 11.9±0.8 | 10.5±0.8 | 34.7±6.9 | 27.4±4.9 | 18.7±1.3 | 28.8±7.5 |
| Fe mg kg⁻¹ | 194.8±19.7 | 208.8±26.1 | 523.0±206.1 | 474.8±184.5 | 382.7±55.1 | 1719.1±966.9 |
| B mg kg⁻¹ | 15.1±0.8 | 13.7±0.8 | 26.3±5.2 | 20.4±2.0 | 53.9±1.2 | 93.5±40.5 |
| Mo mg kg⁻¹ | 3.7±0.3 | 3.2±0.3 | 6.8±1.1 | 9.0±0.3 | 10.7±0.5 | 24.8±11.6 |

627

628

629 **Table 4**

| Soil Biochar Rate (t ha ⁻¹) | LS | | MS | | HS | |
|---|------------|------------|------------|-----------|------------|-------------|
| | 0 | 5 | 0 | 5 | 0 | 5 |
| N (%) | 1.9±0.2 | 2.1±0.1 | 2.1±0.1 | 1.9±0.1 | 2.3*±0.01 | 2.0*±0.01 |
| P (%) | 0.1±0.01 | 0.1±0.01 | 0.1±0.01 | 0.1±0.00 | 0.1±0.01 | 0.1±0.01 |
| K (%) | 0.9±0.04 | 0.9±0.1 | 0.7±0.03 | 0.7±0.02 | 0.6±0.04 | 0.7±0.1 |
| S (%) | 0.2±0.02 | 0.2±0.01 | 0.2±0.01 | 0.2±0.01 | 0.23*±0.01 | 0.19*±0.01 |
| C (%) | 45.0±0.3 | 45.6±0.3 | 47.0±0.2 | 47.0±0.2 | 45.9±0.4 | 46.1±0.4 |
| Ca (%) | 1.1±0.1 | 1.2±0.1 | 0.9±0.1 | 0.9±0.04 | 1.0±0.1 | 1.0±0.1 |
| Mg (%) | 0.2±0.01 | 0.3±0.01 | 0.3±0.03 | 0.4±0.01 | 0.3±0.01 | 0.3±0.02 |
| Na (%) | 0.2±0.03 | 0.2±0.01 | 0.3±0.02 | 0.4±0.01 | 0.6±0.04 | 0.7±0.1 |
| Cu mg kg⁻¹ | 6.5±0.6 | 11.4±3.7 | 9.8±0.1 | 10.0±0.6 | 10.3±0.6 | 12.3±1.3 |
| Zn mg kg⁻¹ | 31.9±3.3 | 38.4±2.4 | 26.4±2.1 | 24.0±0.9 | 25.6±1.9 | 25.6±1.2 |
| Mn mg kg⁻¹ | 23.8±2.1 | 29.1±3.4 | 83.7±12.3 | 85.3±3.5 | 116.1*±9.0 | 76.8*±9.6 |
| Fe mg kg⁻¹ | 172.4±95.5 | 131.0±20.6 | 155.2±23.8 | 128.0±8.7 | 141.2±11.1 | 387.7±121.7 |
| B mg kg⁻¹ | 17.3*±0.9 | 20.1*±1.1 | 23.8±1.8 | 27.8±1.6 | 48.4±2.9 | 43.7±5.9 |
| Mo mg kg⁻¹ | 0.9±0.2 | 0.9±0.1 | 1.4±0.1 | 1.4±0.1 | 1.8±0.1 | 3.0±0.6 |

