

Nodulation Alleviates Water Stress in *Vachellia Sieberiana*

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1 **Nodulation alleviates water stress in *Vachellia sieberiana***

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14 Abstract

15 Species in the genus *Vachellia* (Fabaceae) have a global tropical and sub-tropical distribution.
16 Numerous *Vachellia* species are currently observed to be expanding their indigenous ranges and
17 increasing in dominance globally, suggesting an overarching driver. Most *Vachellia* species enhance
18 nitrogen uptake mutualistically via specialized root nodule structures. Nodules contain N₂-fixing
19 rhizobia that consume host supplied carbon to catalyse atmospheric N₂ into a plant useable form, a
20 key element in plant growth. The rhizobial mutualism of some *Vachellia* species may be vital to
21 understanding changing patterns of ecological success observed across the savanna precipitation
22 gradient.

23 Here, we investigated how the seedling root development and physiology of two dominant savanna
24 woody species, the arid-adapted *Vachellia erioloba* and the mesic-adapted *Vachellia sieberiana*,
25 responded to simulated drought events. Seedlings of both species were grown at 4%, 8% and 16%
26 soil moisture content (SMC) for four months. Seedling growth and allometry of arid-adapted *V.*
27 *erioloba* was unresponsive to water stress treatments, and no nodulation was observed, reflecting a
28 fixed higher relative investment in belowground biomass. In contrast, *V. sieberiana* roots were
29 nodulated, but developed the highest nodule biomass and growth rate when grown at the lowest
30 soil moisture (4% SMC). These patterns suggest that effective life history strategies for the arid-
31 adapted species precludes the need for rhizobial mutualism, possibly due to more “open” N cycling
32 and lower competitive interactions in arid systems, while the more “closed” N cycling in mesic
33 savannas, and higher competitive stress, may favour nodulation, especially under low water supply
34 that limits root access to soil nitrogen, and signals a more competitive environment and an
35 advantage from N₂-fixing.

36 Keywords – Nodules, *Vachellia*, drought, soil moisture, savanna, N₂-fixation

37 Introduction

38 Savannas account for a fifth of the earth's land surface across four continents, including half of Africa
39 (Sankaran et al., 2005; Scholes & Archer, 1997), and are “open ecosystems” (Bond, 2019),
40 characterised by discontinuous woody cover and a continuous herbaceous ground layer (House et
41 al., 2003; Scholes & Archer, 1997). A major threat to savanna “open ecosystem” function is woody
42 encroachment, defined as the increase in dominance and cover of woody species (Devine,
43 McDonald, Quaife, & Maclean, 2017; Stevens, Lehmann, Murphy, & Durigan, 2017). Across Africa,
44 woody cover is increasing at an average rate of ~2.4% per decade (Venter, Cramer, & Hawkins,
45 2018); threatening biodiversity, grazing provision, hydrology, and nutrient cycling (Honda & Durigan,
46 2016; Lehmann & Parr, 2016; Leitner, Davies, Parr, Eggleton, & Robertson, 2018; Parr, Gray, & Bond,
47 2012; Stevens et al., 2017). Woody encroachment has been attributed to interactions among
48 changing climates, decline in browsing herbivores, fire suppression, and an increase in global
49 atmospheric CO₂ concentrations altering woody plant growth and survival (Archer et al., 2017;
50 Venter et al., 2018). However, it remains unclear why only a small number of species are responsible
51 for encroachment (Liu et al., 2013).

52 In Africa, the majority of encroaching woody species are legumes, belonging to the genera *Vachellia*
53 and *Senegalia* (Stevens et al., 2017). Many of these species have the capacity to fix atmospheric
54 nitrogen (N₂) via a rhizobial mutualism (Scholes & Archer, 1997; Sprent, 1995), a trait that possibly
55 influences their regional dominance (Cramer, Chimphango, Van Cauter, Waldram, & Bond, 2007).
56 The rhizobial mutualism manifests via the formation of root nodules; where N₂ is transformed into
57 plant useable NH₄ (Colebatch et al., 2004; Kambatuku, Cramer, & Ward, 2013). Previous experiments
58 have shown that the legume-rhizobia symbiosis is sensitive to climatic extremes such as extreme
59 aridity (Deans, Ali, & Lindley, 1993; Laris, 2008) where the mobility of rhizobia is impeded by low soil
60 moisture, reducing symbiosis establishment (Fall et al., 2011; Swaine, Swaine, & Killham, 2007). This
61 may be why non-nodulating woody legumes, such as *Vachellia erioloba*, are more common in arid

62 savanna (<450mm mean annual precipitation (MAP)) (Hean & Ward, 2012; Milton & Dean, 1995;
63 Seymour, 2008; Sprent & Gehlot, 2010). It has also been suggested that if N is not a limit on plant
64 growth, the ability to nodulate is of limited advantage (Sprent & Gehlot, 2010), as substantial energy
65 must be invested in traits that facilitate survival in water limited conditions. Arid savannas leaf N¹⁵
66 reflects an “open” N cycle (Aranibar et al., 2004; Midgley, Aranibar, Mantlana, & Macko, 2004) .
67 Nodulating legumes, such as *Vachellia sieberiana*, are more common in mesic savannas (~800-
68 1000mm MAP) (Figure 1.c). Mesic savannas are typically N limited; due to high rates of soil nutrient
69 leaching (Zahran, 1999), competition with grasses (due to increased productivity associated with
70 higher annual rainfall) (Cramer et al., 2007; Cramer, Van Cauter, & Bond, 2010) and frequent fire
71 leading to N loss (Archibald & Hempson, 2016; Pellegrini, Staver, Hedin, Charles-Dominique, &
72 Tourgee, 2016). Their leaf N¹⁵ values reflect an “closed” N cycle (Aranibar et al., 2004; Midgley et al.,
73 2004). Further, nodulation enables woody legume seedlings to compensate for limited soil N in a
74 competitive environment at a critical, but vulnerable establishment phase (Kambatuku et al., 2013).

75 Savanna ecosystems are typified by bottlenecks in recruitment (Bond & Midgley, 2003). Plant
76 survival is determined by interacting factors that vary in their relative importance along productivity
77 gradients including water stress, herbivory, and fire (Kraaij & Ward, 2006; Sankaran et al., 2005).
78 Hence, understanding seedling growth and recruitment of *Vachellia* species could help understand
79 the key traits that underlie varying savanna vegetation dynamics across rainfall gradients. A seldom
80 considered aspect of savanna seedling success is how water stress (Sankaran, 2019) alters the
81 functionality of the legume-rhizobium symbiosis (Serraj, 2003). Previously it has been proposed that
82 water stress can reduce N₂-fixation by reducing carbon nodule metabolism, introducing oxygen
83 limitation, thus causing reduction of N₂-fixation product transport (Serraj, 2003). Understanding
84 interactions between water stress and nodule production related to plant growth will help
85 determine the functional role of N₂-fixation in seedling success (Krug, 2017; Wonkka, Twidwell,
86 Trenton, Taylor, & Rogers, 2016). Additionally, climate change predictions suggest that Africa will
87 become drier and hotter, with changing patterns of seasonal rainfall and experience an increase in

88 drought events (Engelbrecht & Engelbrecht, 2016; IPCC, 2014; Mbokodo, Bopape, Chikoore,
89 Engelbrecht, & Nethengwe, 2020).

90 Here we investigated how soil moisture relates to the growth and nodulation response of two
91 woody *Vachellia* species, one arid and one mesic, both considered encroachers (Hauwanga,
92 McBenedict, & Strohbach, 2018; Russell, Tedder, & Demmer, 2019). We asked (1) How does water
93 availability affect seedling growth rates? We anticipated that the growth of the mesic *V. sieberiana*
94 would be positively correlated with water availability (Kraaij & Ward, 2006; Vadigi & Ward, 2013). In
95 contrast, we predicted the growth of arid *V. erioloba* would be largely unaffected by soil moisture,
96 and would have a larger relative investment in belowground biomass (Moustakas, 2006). (2) Is there
97 a correlation between soil moisture content and nodulation? We predicted *V. sieberiana* would have
98 the lowest level of nodulation (nodule biomass) when grown in the lowest soil moisture treatment,
99 as the movement of rhizobia would be limited (Fall et al., 2011; Swaine et al., 2007). We supposed
100 that *V. erioloba* would not develop nodules (R. D. Barnes, 2001), regardless of soil moisture, due to a
101 fixed phenotypic response that excludes nodulation.

102

103 Materials and Methods

104 Description of study species

105 *Vachellia erioloba* is herbivore dispersed and is found across savannas where rainfall is less than
106 ~450mm MAP (Figure 1.a & 1.b) (Seymour, 2008). This species is considered to have lost the ability
107 to nodulate (Sprent 1995). *V. erioloba* is a drought resistant, slow growing species characteristic of
108 acidic sandy soils (Milton & Dean, 1995; Moustakas, 2006), and is recognised as a mild bush
109 encroacher (Hauwanga et al., 2018; Stevens et al., 2017). Recruitment of the species is generally
110 episodic, and wet season dependent (Seymour, 2008; Van Rooyen, Van Rensburg, Theron, &

111 Bothma, 1984). Where it is found, herbivore densities are low and fire is infrequent (M. E. Barnes,
112 2001; Canadell et al., 1996; Seymour, 2003; Seymour & Alias, 2003; Seymour & Huyser, 2008).

113 *Vachellia sieberiana* is herbivore dispersed species found across regions where rainfall ranges from
114 800-1100mm MAP (Figure 1.a & 1.c) (Bunney, 2013) and is a known nodulator (Sprent, 2009). *V.*
115 *sieberiana* grows on deep, well-drained, light sandy and medium loamy acid soils (Tadesse,
116 Desalegn, & Alia, 2007). The species is fast growing (Sunmonu & Van Staden, 2014), and considered
117 a vicious bush encroacher (Russell et al., 2019; Sabiiti & Wein, 1987; Stevens et al., 2017). Browse
118 pressure is primarily on adult trees by giraffes (*Giraffe camelopardalis*) (Zinn, Ward, & Kirkman,
119 2007). Within its range, fire is frequent and fuelled by highly productive grasses (Bunney, 2013;
120 February, Higgins, Bond, & Swemmer, 2013; Sabiiti & Wein, 1987; Zinn et al., 2007).

121

122 Experimental design

123 Seeds were purchased from SilverHill Seeds (<http://www.silverhillseeds.co.za>) (Cape Town, South
124 Africa). Seeds of both species had coat-imposed dormancy, and pre-germination treatments were
125 required. Seeds were soaked in Sodium hypochlorite (NaClO) for two minutes, to reduce fungal and
126 mould growth (Materechera & Materechera, 2001) and then soaked in boiled water for 10 minutes
127 (Bodede, Shaik, & Moodley, 2018; Cramer et al., 2007). Treated seeds were germinated in petri
128 dishes containing 10% Agar gel placed in a growth chamber (Conviron A1000, Conviron Europe Ltd,
129 Isleham, Cambridgeshire, B7 5RJ, UK) at 30°C for three-four days until germination occurred. There
130 was approximately 90% germination success in both species.

131 Seedlings were grown from May – September 2018 (winter) at the University of Stellenbosch
132 glasshouses heated using two standard garden infra-red heaters mounted three metres apart across
133 the glasshouse ceiling. The average glasshouse temperature 25°C and soil temperatures between

134 17°C- 35°C. Temperature was measured using Thermochron iButtons (Thermochron, Baulkham Hills,
135 Australia).

136 At the beginning of the experiment, 90 newly germinated seedlings of each species were
137 transplanted into individual two litre pots with soil packed tightly around with the shoot. Soil was a
138 mixture of a native alluvial sand aggregate and vermiculate (two parts sand: one part vermiculate).
139 Seedlings were randomly distributed in the glasshouse and were moved every three days to ensure a
140 homogenised growing environment. In total, the experiment consisted of 180 plants of two species.

141 All seedlings were provided with a 5ml of water soluble 3:2:2 nitrogen: phosphorous: potassium
142 fertiliser. Soil was not inoculated with rhizobia, rather we relied upon the rhizobia already present in
143 the native sands. Given the limited understanding of in-situ growth traits of *Vachellia* species (Krug,
144 2017; Winters et al., 2018) we chose to use already present free-living rhizobia in the soil. We
145 believed that this was more likely to provide an accurate representation of the natural ability of
146 rhizobia to survive water limited conditions (Shetta, 2015).

147 Soil moisture treatments

148 We imposed three watering treatments (high, medium and low) on each species. Plants were
149 harvested in three sequential phases (i.e., at three ages), providing ten replicates per species x water
150 treatment x harvest. During the first two weeks 200ml of water was given to each pot every two
151 days to ensure establishment (Kraaij & Ward, 2006; Mucunguzi & Oryem-Origa, 1996). In weeks
152 three and four seedlings received 200ml water every three days. At the beginning of week five
153 (approximately one month after being transplanted into individual pots) soil moisture treatments
154 were imposed. For each species, 30 individuals were watered at an average of 4% soil moisture
155 content (SMC) ($0.100 \text{ m}^3/\text{m}^3$); average 8% SMC ($0.180 \text{ m}^3/\text{m}^3$); and, average 16% SMC ($0.280 \text{ m}^3/\text{m}^3$).

156 The appropriate SMC for this experiment was determined through a pilot study involving planting
157 already germinated seedlings of the fast growing *Vachellia exuvialis*. Here, ten *V. exuvialis* seedlings

158 per water treatment, water treatments were applied of: no water, 0.020 m³/m³, 0.080 m³/m³, 0.100
159 m³/m³, 0.120 m³/m³, 0.180 m³/m³, 0.220 m³/m³, 0.280 m³/m³, 0.330 m³/m³ and 0.380 m³/m³. After
160 three weeks *V. exuvialis* seedlings were removed, and the soil from each pot weighed and oven
161 dried at 105°C to determine appropriate SMC for watering treatment using the following equation:

$$162 \quad P = W/D \times 100$$

163 Where P is the percentage of water in the soil, W is the mass of the original soil sample and D is the
164 mass of the dried soil sample (ASTM, 2010). The survival of *V. exuvialis* was noted throughout the
165 pilot study. The driest treatment was decided by observing what was within the range of survival of
166 the *V. exuvialis* in the pilot study, which was 4% SMC (0.100 m³/m³). The wettest treatment was
167 chosen in line with Shetta (2015) where plants were watered every three days and calculated to be
168 an average of 16% SMC (0.280 m³/m³). A third water treatment with the average of 8% SMC (0.180
169 m³/m³) showed a median drought effect. Soil moisture was recorded throughout the main
170 experiment and pilot study using an HS2 HydroSense II Display (Campbell Scientific Ltd,
171 Loughborough, UK). Soil moisture readings were collected every three days and the pots were
172 watered accordingly.

173

174 Plant and leaf measurements at harvest

175 Height (mm) of each seedling was measured weekly. Ten seedlings of each species of each
176 treatment were harvested at three points after commencing the water treatments. Harvests were at
177 one (Harvest 1), two (Harvest 2) and three months (Harvest 3) post water treatment. Seedlings were
178 separated into above and belowground biomass and roots were carefully washed to maintain fine
179 root mass. Nodules were removed from the roots using forceps and cleaned using a paint brush. For
180 each harvested plant, the final plant height, dry aboveground and belowground biomass (g), and
181 nodule dry biomass (g) were recorded. All plant material was oven dried at 65°C for 36 hours
182 (Kambatuku et al., 2013).

183

184 Statistical analyses

185 All analyses was conducted using R 3 5.1 (R Core Team, 2020). The weekly measurements of seedling
186 height were used to create a linear mixed model, using the lme4 package (Bates, Maechler, Bolker,
187 & Walker, 2015). A mixed model was chosen due to the hierarchical nature of the data. Week and
188 treatment were fixed effects, and individual tag number (Species-Treatment-Pot) as a random effect.
189 Due to the large difference in the niches occupied by these two species they were separated when
190 creating the model assessing height related to water treatment.

191 Seedling biomass and allocation data (below ground biomass, above: belowground ratio, and nodule
192 biomass) were analysed using a two-way ANOVA. Data were checked for normality and log
193 transformed if it did not meet assumptions. Post-hoc Tukey significant difference (HSD) test was
194 carried out to separate the effects of water availability on each species, taken from the
195 MultcompView: Visualizations of Paired Comparisons package (Graves, Piepho, Selzer, & Dorai-Raj,
196 2019). To check homogeneity of variance a Levene's test was used as a robust test of deviations
197 from normality using the Car: Companion to Applied Regression package (Fox & Weisberg, 2019).

198

199 Results

200 Biomass, height and growth related to water availability

201 The final height and aboveground biomass of the four-month-old *Vachellia sieberiana* seedlings
202 (collected in harvest 3) were affected by water treatment ($df= 6, p<0.010$) (Table 1). Contrary to
203 initial predictions, *V. sieberiana* seedlings grown in the driest conditions (4% SMC) were significantly
204 taller (Figure 2 & Table 1) and had the largest aboveground biomass (Figure 3.b & Table 2) than
205 seedlings grown at 8% SMC and 16% SMC. In contrast, the belowground biomass of *V. sieberiana*
206 was not affected by water availability ($df= 2, F=1.45, p>0.050$) (Figure 3.b & Table 2).

207 The growth and allocation patterns of the arid *V. erioloba* did not vary across water treatment (df=
208 2, F=1.45, p>0.050) (Figure 3.a, Figure 3.b & Table 2). Relative to *V. sieberiana*, *V. erioloba* seedlings
209 were shorter (df= 2, p>0.050) (Figure 2 & Table 1), but proportionally had a larger belowground
210 biomass ($\beta = -0.963$) (df=1, F=57.42, p<0.010) (Figure 3.c & Table 2).

211

212 Nodulation in relation to age and water availability

213 As expected, *V. erioloba* did not develop nodules regardless of water treatment. The nodule count
214 and nodule biomass in *V. sieberiana* increased with age (df=2, F=129.194, p<0.001), across the three
215 harvesting efforts (Figure 4.a, Table 3 & 4). There was an 800% increase in the number of individuals
216 that nodulated between Harvest 1 (two months old) and Harvest 2 (three months old) (Table 4).

217 There was an 11% increase in the number of individuals that nodulated between Harvest 2 (three
218 months old) and Harvest 3 (four months old) (Table 4).

219 Nodule biomass and count was affected by water treatment (df=2, F=4.40, p<0.050) (Figure 4.a &
220 Table 3). These patterns fluctuated across the experiment with three seedlings grown in 8% SMC
221 developing nodules at around two months old, seedlings grown in 16% SMC collectively producing
222 the highest nodule count across all three harvests. However, seedlings grown in 4% SMC developed
223 nodules with the highest nodule biomass in Harvest 3 (four years old) (Table 4). Despite these
224 complex patterns there was a strong positive linear relationship between belowground biomass and
225 nodule biomass (Figure 4.b).

226

227 Discussion

228 (1) How does water availability affect seedling growth rates?

229 Contrary to expectation we found that the growth of mesic *V. sieberiana* seedlings increased with a
230 decline in water availability. However, links between reduced water availability and increased

231 growth/success have been seen in other *Vachellia* species: rapid above and belowground growth in
232 *Vachellia tortilis* and *Vachellia raddiana* has been found to occur during the dry season in Southern
233 Israel (Winters et al., 2018). These two species are also known nodulators (Sprent, 2009), and are
234 native to the semi-arid (500-1000 MAP) savanna (Ludwig, Dawson, Kroon, Berendse, & Prins, 2003).
235 Although we only recorded changes in the aboveground growth, we suggest that for semi- arid and
236 mesic *Vachellia* species flexible growth patterns are influenced by water availability. Further, these
237 patterns are supported by additional N provided by nodulation (Boonman et al., 2019). A soil water
238 deficit has been found to lower *Vachellia* nutrient absorption, due to decreased mobility of nutrients
239 to the root surface (Moura & Vieira, 2020). The slow nutrient diffusion from the soil to the root
240 surface under drought conditions reduces the nutrient translocation speed to the leaves (Vieira,
241 Andrade Galvão, & Barros, 2019). Thus, reducing the mobility of N, unless the plant responds with
242 accessing additional sources of N photosynthetic rates and enzymatic activity could decline, and a
243 consequent reduction in growth (Moura & Vieira, 2020). In addition, it is possible that the lower soil
244 water availability may signal a more competitive environment, and thus trigger a greater investment
245 in nodulation in order to enhance plant growth under competitive conditions.

246 As predicted the growth and allometry of *V. erioloba* remained unaffected by drought conditions.
247 Plant growth rate traits are only one of many elements of species life history strategy and must be
248 considered alongside the ability to survive and reproduce under a range of environmental conditions
249 (Adams, Turnbull, Sprent, & Buchmann, 2016). Arguably, flexible growth patterns that increase
250 water uptake under water-stricken conditions would not benefit *V. erioloba* survival. For arid
251 adapted species it is the ability to overcome severe water deprivation that poses the biggest
252 challenge. Therefore traits favouring slow growth, that require limited water, are possibly the most
253 advantageous to their longevity (Seymour, 2003). A better use of resources is the maintenance of a
254 non-plastic phenotypically fixed belowground network that facilitates the growth of large tap roots
255 that increases soil moisture access and drought avoidance. This lack of root plasticity is further
256 demonstrated as tap root construction is favoured even under well-water conditions (February et

257 al., 2013; Seymour, 2003, 2008). Rigid patterns of belowground investment allows mature *V.*
258 *erioloba* individuals to survive in a consistently water limited environment (M. E. Barnes, 2001).
259 Alternatively, *V. erioloba* extends into most arid parts of the African desert; more than any other
260 tree species (R. D. Barnes, Fagg, & Milton, 1997). Therefore, perhaps it was unaffected by water
261 treatment because the seedlings were not drought stressed. We suggest that future experiments
262 using *V. erioloba* incorporate a more severe drought treatment to better understand their growth
263 traits relating to their arid niche.

264 (2) Is there a correlation between soil moisture content and nodulation?

265 Increases in *V. sieberiana* plant height and total biomass in the driest soil conditions (4% SMC) were
266 correlated with an increase in nodulation (nodule biomass). Similar patterns of increasing nodulation
267 with plant biomass are reflected in *Albizia saman* and *Leucaena leucocephala* (Azad, Mondol, &
268 Matin, 2013). An increase in nodule biomass is indicative of increased N₂-fixing bacteria
269 concentration within the nodules (Gwata et al., 2004; Voisin et al., 2003), and hence increased
270 activity which in turn could lead to increased available N for plant growth. We propose this pattern
271 of increased biomass in *V. sieberiana* under drought conditions is mediated through enhanced
272 nodulation, and may be triggered by low water availability that signals a more competitive
273 environment below ground. Possibly, drought conditions result in plant tissue damage which can
274 trigger an increase in plant jasmonic acid levels (Hause & Schaarschmidt, 2009; Oka-Kira &
275 Kawaguchi, 2006; Sun et al., 2006) which in turn increases nodulation (Hause & Schaarschmidt,
276 2009; Sun et al., 2006). If the plant has sufficient C reserves to maintain the increased rhizobia
277 mutualism, this could increase the amount of plant available N for growth. This was contrary to what
278 was predicted as we believed that the movement of rhizobia would be impinged by a lack of water
279 (Fall et al., 2011; Swaine et al., 2007) and highlights remaining uncertainties to understanding N₂-
280 fixation (Soper et al., 2021).

281

282 Future research

283 If projections that Africa will experience an increase in drought frequency are realized (Engelbrecht
284 & Engelbrecht, 2016; Mbokodo et al., 2020), the evidence described here suggests that both species
285 may see enhanced encroachment across the savanna. For arid-origin species such as *V. erioloba*,
286 whose allometry remains unaffected by drought, a hotter, drier climate may permit an extension of
287 geographical range. However, *V. sieberiana*'s ability to nodulate could allow it to take advantage of
288 drought conditions that may compromise competing species; encroaching further across the
289 savanna landscape, despite being a mesic-origin species. The relationship between savanna tree
290 cover and water availability is not straight-forward (Sankaran et al., 2005), as it is influenced by
291 external top-down drivers; such as fire and herbivory (Venter et al 2018). We suggest that future
292 experiments elucidating the effect of drought conditions on *Vachellia* species combine water
293 availability with these top-down drivers.

294 Finally, as arid adapted woody legume species (such as *Vachellia*) are thought to establish only in
295 years of above-average rainfall (Seymour, 2008) an increase in drought events could reduce the
296 establishment of *Vachellia* seedlings (Van Der Merwe, Van Rooyen, Bezuidenhout, Du, & Van
297 Rooyen, 2020). In this experiment, to allow seedlings the opportunity to establish they were exposed
298 to four weeks of continuous watering before drought treatments were imposed. We suggest that
299 future experiments could identify the minimum watering period for seedling establishment to
300 understand how seedlings will adapt to these climatic changes.

301

302 Conclusion

303 Drought has a wide range of effects on closely related *Vachellia* species relative to their
304 environmental niche and associated growth traits. A high growth rate is not necessarily an indicator
305 of plant success (Adams et al., 2016); it must be considered alongside the ability of a plant to survive

306 and reproduce under a range of environmental conditions. For *V. sieberiana* to be successful in the
307 mesic environment it requires extended periods of growth to escape fire and compete with grass.
308 Nodulation enables a flexible N supply to enhance growth over such time periods. In this experiment
309 *V. sieberiana's* increased nodulation triggered by drought stress suggests that nodulation assists the
310 withstanding of water stress within its environmental niche. The growth traits of *V. erioloba*,
311 remained unaffected by drought stress, potentially attributable to its high tolerance to aridity (R. D.
312 Barnes et al., 1997). Perhaps, the measure of success for *V. erioloba* is not rapid growth but being
313 able survive in a water limited environment via methods of below ground investment. These
314 patterns suggest that effective life history strategies for the arid-adapted species precludes the
315 requirement for rhizobial mutualism, due to more "open" N cycling and lower competitive
316 interactions in arid systems (Aranibar et al., 2004). Whereas in the mesic savanna, the more "closed"
317 N cycling and higher competitive stress, may favour nodulation, especially under low water supply
318 that limits root access to soil nitrogen, and signals a more competitive environment and an
319 advantage from N₂-fixing (Aranibar et al., 2004; Veldhuis, Hulshof, Fokkema, Berg, & Olf, 2016).

320

321

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327 Author contributions

328 Conceptualization: all authors; Data collection: EMT, NS; Statistical analyses: EMT, NS, CERL; Writing:
329 all authors

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334 Availability of Data

335 See online resources and Github folder: https://github.com/Elizabeth261191/Masters_dream

336 Conflict of interest

337 The authors declare that there is no conflict of interest.

338 Ethical approval

339 This study was approved by University of Edinburgh School of GeoScience Research Ethics & Integrity
340 Committee.

341

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595

597 Tables

598 **Table 1** The output of a mixed model for the effect of water treatment on the growth (height) of *V.*
 599 *erioloba* (VE) and *V. sieberiana* (VS) seedlings. 4%, 8% and 16% correspond the soil moisture content
 600 the seedlings were grown in. These measurements were taken three weeks following germination for
 601 15 weeks. Soil moisture treatments were applied at week two. The individual seedling was tested as
 602 a random effect. The standard deviation is represented in brackets. Significance is indicated as follows:
 603 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

<i>Vachellia erioloba</i> Height (mm)							
Coefficients							
Treatment 8% SMC	4.313 (7.038)						
Treatment 16% SMC	0.772 (6.667)						
Week	11.402 *** (0.262)						
Constant	-10.744* (5.401)						
Observation	390						
Log likelihood	-1778.609						
Akaike Inf. Crit.	3571.218						
Bayesian Inf Crit.	3595.015						
	<i>df</i>	AIC	BIC	LogLik	Deviance	Chisq	<i>p</i> (>Chisq)
VE Week	4	3581.60	3597.46	-1786.80	3573.60		
VE Week + Treatment	6	3585.11	3608.91	357	3575.11	0.49	0.7837
<i>Vachellia sieberiana</i> Height (mm)							
Coefficients							
Treatment 8% SMC	-26.172 * (10.239)						
Treatment 16% SMC	-30.872 ** (10.239)						
Week	16.355 *** (0.322)						
Constant	-8.291 (8.055)						
Observation	420						
Log likelihood	-2022.771						
Akaike Inf. Crit.	4057.542						
Bayesian Inf Crit.	4081.783						
	<i>df</i>	AIC	BIC	LogLik	Deviance	Chisq	<i>p</i> (>Chisq)
VS Week	4	4079.79	4095.95	-2035.9	4071.79		
VS Week + Treatment	6	4074.2	4098.44	-2031.1	4062.20	9.59	0.0083

605 **Table 2** The coefficients (β), lower (CI 2.5%) and upper (CI 97.5%) confidence intervals from the whole
606 plant dry biomass (grams), below ground biomass (grams) and the root: shoot ratio of seedlings
607 harvest at four months old (during Harvest 3). Treatment refers to the water availability treatment.
608 Species refers to *V. erioloba* (VE) and *V. sieberiana* (VS) and The output from 2 way ANOVA for testing
609 the effect of water availability treatment and differences between species in the whole plant biomass
610 (grams), below ground biomass (grams) and above: below ground ratio of *V. erioloba* (VE) and *V.*
611 *sieberiana* (VS) seedlings that were harvested at four months old (during Harvest 3). Treatment refers
612 to the water availability treatment. Significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$,
613 *** $p < 0.001$, **** $p < 0.0001$.

Below ground biomass (g)				
	Coefficient	CI 2.5%	CI 97.5%	
Intercept	-0.734	-0.835	-0.634	
Treatment 8%	-0.083	-0.203	0.037	
Treatment 16%	-0.120	-0.141	0.101	
Species VS	0.378	0.278	0.477	
	<i>df</i>	Mean Sq	<i>F</i>	<i>p</i>
Treatment	2	0.037	0.78	0.462
Species	1	2.744	57.24	0.000****
Residuals	59	0.048		
Above ground biomass (g)				
	Coefficient	CI 2.5%	CI 97.5%	
Intercept	-0.426	-0.153	0.068	
Treatment 8%	0.016	-0.094	0.128	
Treatment 16%	0.059	-0.052	0.172	
Species VS	-0.088	0.012	0.164	
	<i>df</i>	Mean Sq	<i>F</i>	<i>p</i>
Treatment	2	0.024	0.859	0.4277
Species	1	0.148	5.279	0.024*
Residuals	59	0.028		
Above: below ground ratio				
	Coefficient	CI 2.5%	CI 97.5%	
Intercept	1.076	0.911	1.241	
Treatment 8%	0.128	-0.068	0.325	
Treatment 16%	0.132	-0.067	0.331	
Species VS	-0.963	-1.126	-0.799	
	<i>df</i>	Mean Sq	<i>F</i>	<i>p</i>
Treatment	2	0.186	1.45	0.242
Species	1	17.839	138.31	0.000****
Residuals	59	0.129		

615 **Table 3** The coefficients (β), lower (CI 2.5%) and upper (CI 97.5%) confidence intervals for the biomass
616 of nodules harvested at two months, three months and four months old. Treatment refers to the
617 water availability treatment. Species refers to *V. erioloba* (VE) and *V. sieberiana* (VS). The output from
618 2 way ANOVA for testing the effect of water availability treatment on nodule biomass harvested at
619 two months (harvest 1), three months (harvest 2) and four months old (harvest 3). Significance is
620 indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

	Nodule biomass (g)			
	Coefficient	CI 2.5%	CI 97.5%	
Intercept	-8.089	-8.743	-7.435	
Treatment 8%	1.063	0.376	1.751	
Treatment 16%	0.638	-0.065	1.341	
Harvest 2	4.899	4.166	5.633	
Harvest 3	5.129	4.440	5.819	
	<i>df</i>	Mean Sq	<i>F</i>	<i>P</i>
Treatment	2	9.91	4.398	0.015*
Harvest	2	291.23	129.194	0.000***
Residuals	104	2.25		

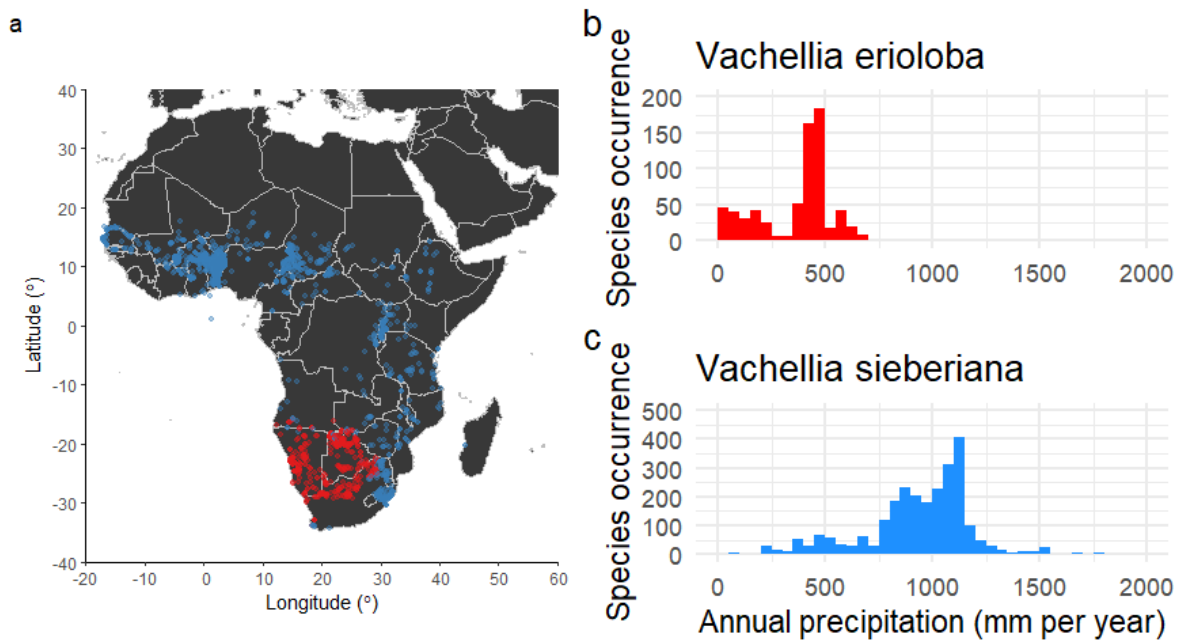
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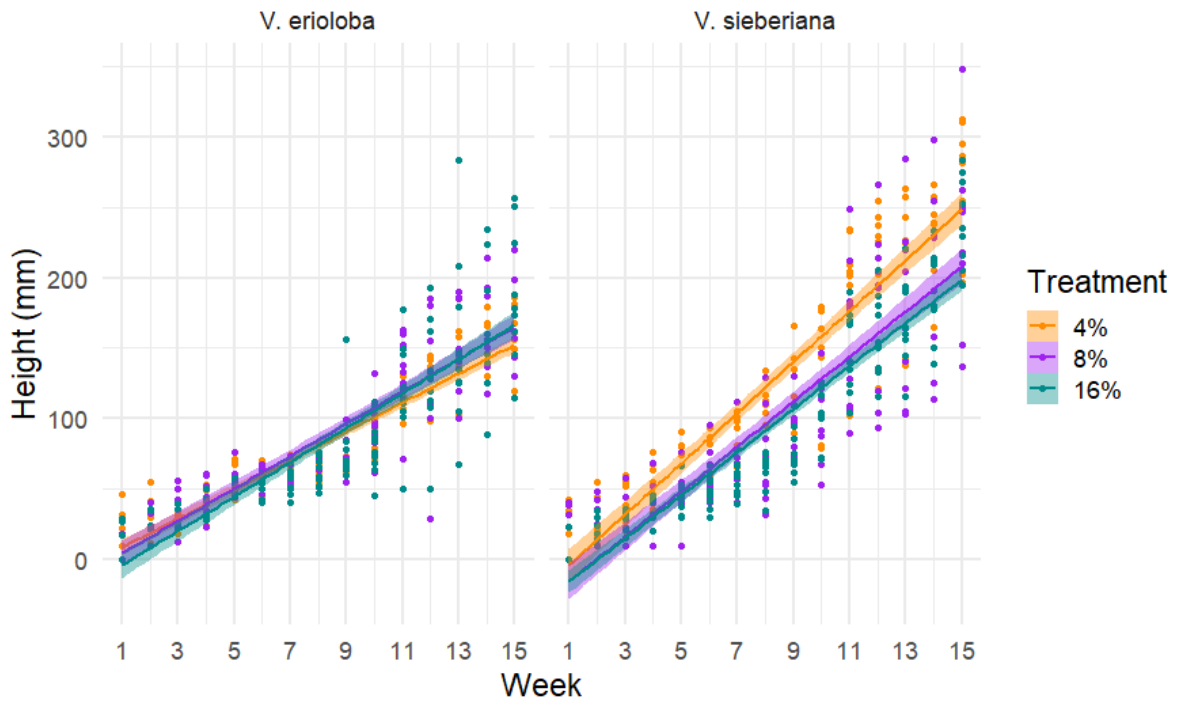
623 **Table 4** The proportion (%) of *V. sieberiana* seedlings that had nodules on their roots. Treatments
 624 correspond to the water. Treatment refers to the water availability treatment (SMC = soil moisture
 625 content). The seedlings were harvested at two months (harvest 1), three months (harvest 2) and
 626 four months old (harvest 3).

	Treatment	Number of reps	Number of plants nodulated	Nodule biomass (grams)	Nodule count	Percentage of plants nodulated	Percentage increase between harvests
Harvest 1	4% SMC	10	0	0.000	0	0%	-
Harvest 1	8% SMC	10	3	0.006	18	30%	-
Harvest 1	16% SMC	10	0	0.000	0	0%	-
Harvest 2	4% SMC	10	8	0.040	120	80%	800
Harvest 2	8% SMC	10	10	0.090	230	100%	233
Harvest 2	16% SMC	10	9	0.142	417	80%	900
Harvest 3	4% SMC	10	10	0.249	379	100%	25
Harvest 3	8% SMC	10	10	0.215	571	100%	0
Harvest 3	16% SMC	10	10	0.239	658	100%	11

627



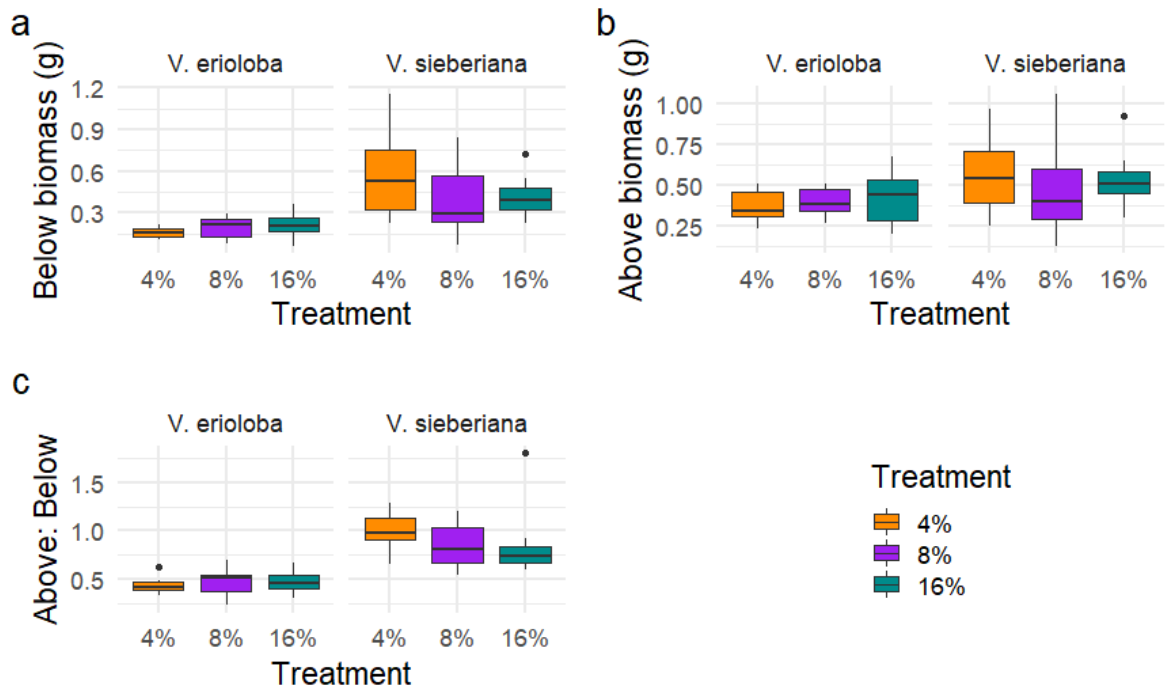
629
630 **Figure 1 (a)** A map showing the distribution of *Vachellia erioloba* (red) and *Vachellia sieberiana* (blue)
631 across Africa (Bivand & Nicholas, 2019; GBIF.org, 2020). Histograms of species distribution of **(b)**
632 *Vachellia erioloba* and **(c)** *Vachellia sieberiana* in relation to mean annual precipitation (MAP) (mm)
633 (GBIF.org, 2020; Hijmans, 2020). *V. erioloba* occurrence is highest at ~ MAP 500 mm and *V. sieberiana*
634 is highest at ~ MAP 1000 mm.



636

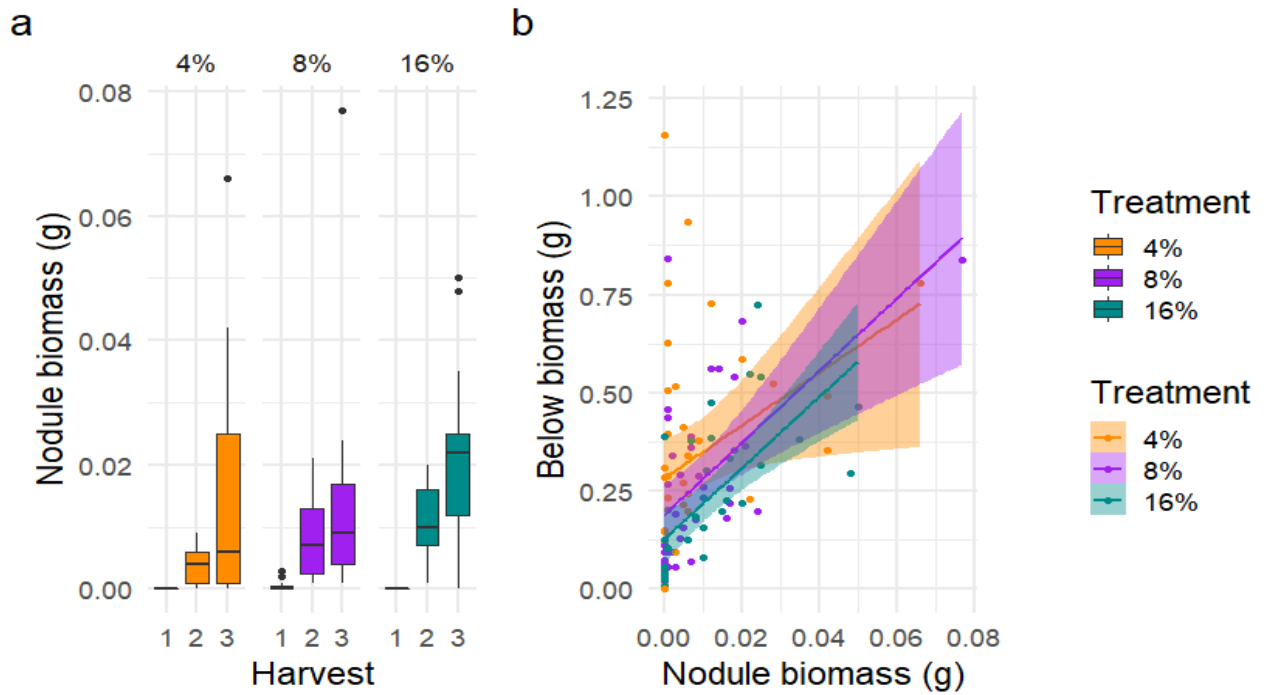
637

638 **Figure 2** The height of *V. erioloba* and *V. sieberiana* measured at weekly intervals over a four-month
639 period. Treatment 4% (orange), 8% (purple) and 16% (blue) correspond the soil moisture content the
640 seedlings were grown in. These measurements were taken three weeks following germination. Soil
641 moisture treatments were applied at week 2 (Wickham, 2016).



642

643 **Figure 3** (a) The belowground biomass, (b) The aboveground biomass, (c) The root: shoot ratio of *V.*
 644 *erioloba* *V. sieberiana* and harvested during Harvest 3 at four months old. The water treatment
 645 corresponds to 4%, 8% and 16% soil moisture content (Wickham, 2016).



646

647 **Figure 4 (a)** The nodule biomass (grams) of *V. sieberiana* seedlings. Nodules were not present during
 648 Harvest 1 for *V. sieberiana* seedlings grown in 4% SMC (orange) and 16% SMC (blue). **(b)** The
 649 belowground biomass (grams) plotted again the nodule biomass (grams). The data points from all *V.*
 650 *sieberiana* across all three harvests. The zeros are plants from Harvest 1 that did not develop
 651 nodules. (Wickham, 2016).

Figures

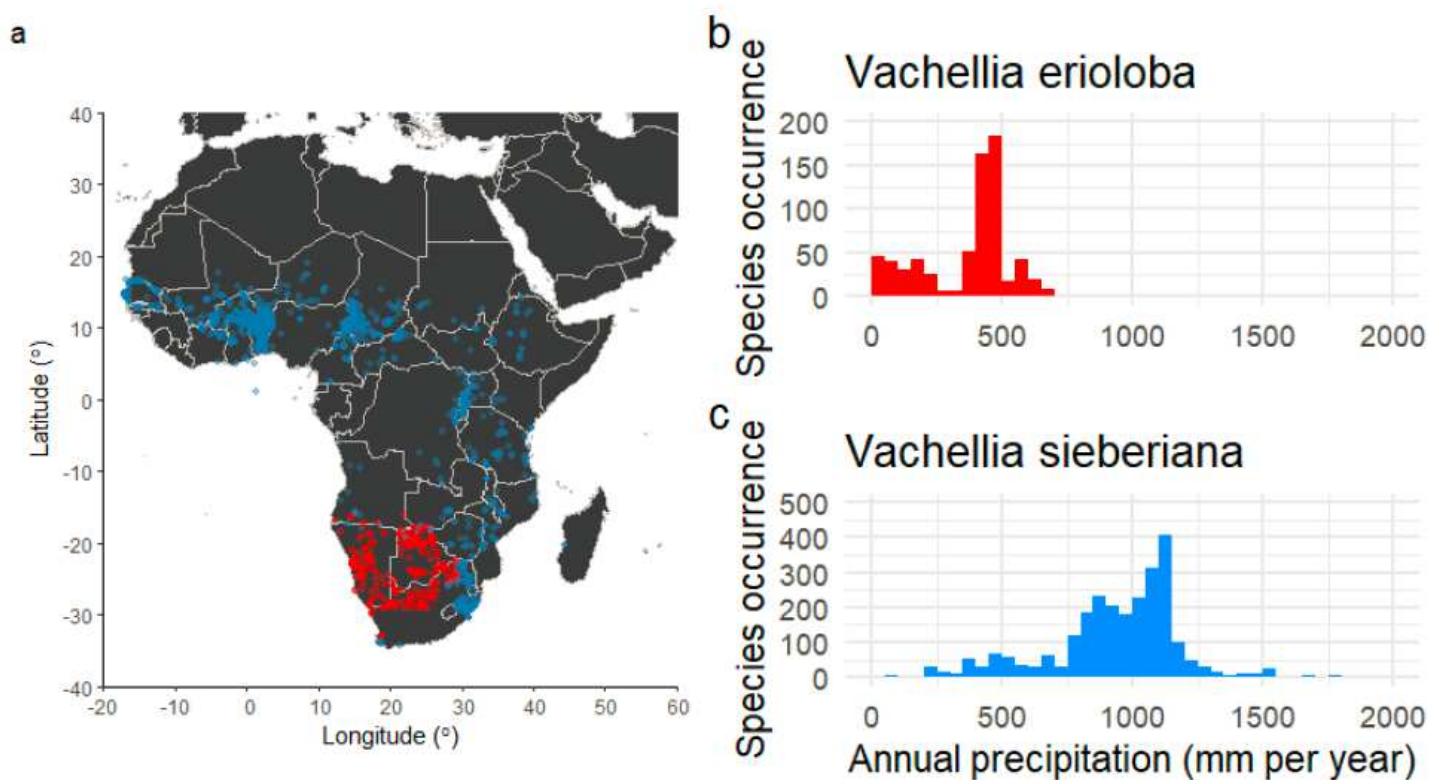


Figure 1

(a) A map showing the distribution of *Vachellia erioloba* (red) and *Vachellia sieberiana* (blue) across Africa (Bivand & Nicholas, 2019; GBIF.org, 2020). Histograms of species distribution of (b) *Vachellia erioloba* and (c) *Vachellia sieberiana* in relation to mean annual precipitation (MAP) (mm) (GBIF.org, 2020; Hijmans, 2020). *V. erioloba* occurrence is highest at ~ MAP mm and *V. sieberiana* is highest at ~ MAP 500 mm. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

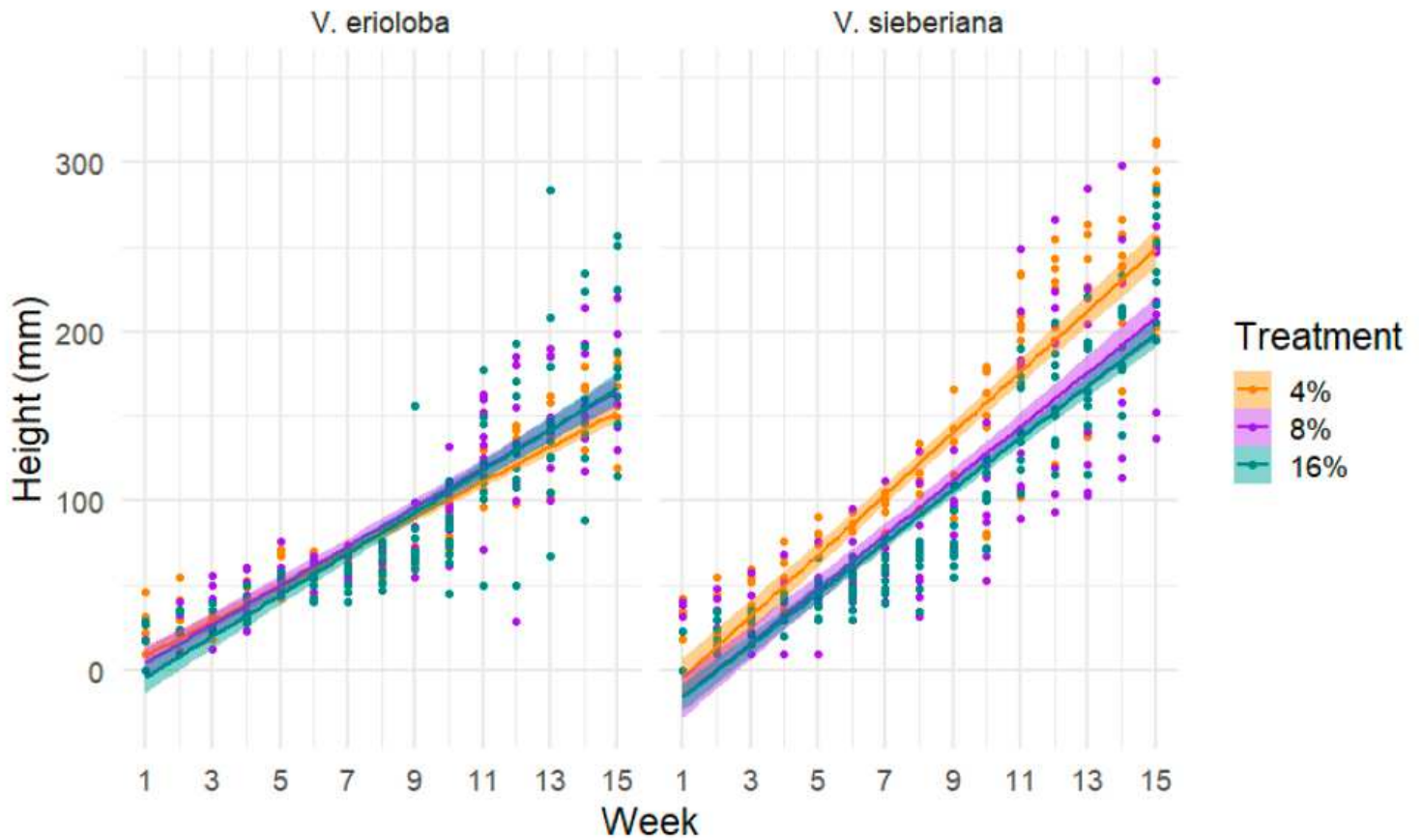


Figure 2

The height of *V. erioloba* and *V. sieberiana* measured at weekly intervals over a four-month period. Treatment 4% (orange), 8% (purple) and 16% (blue) correspond to the soil moisture content the seedlings were grown in. These measurements were taken three weeks following germination. Soil moisture treatments were applied at week 2 (Wickham, 2016).

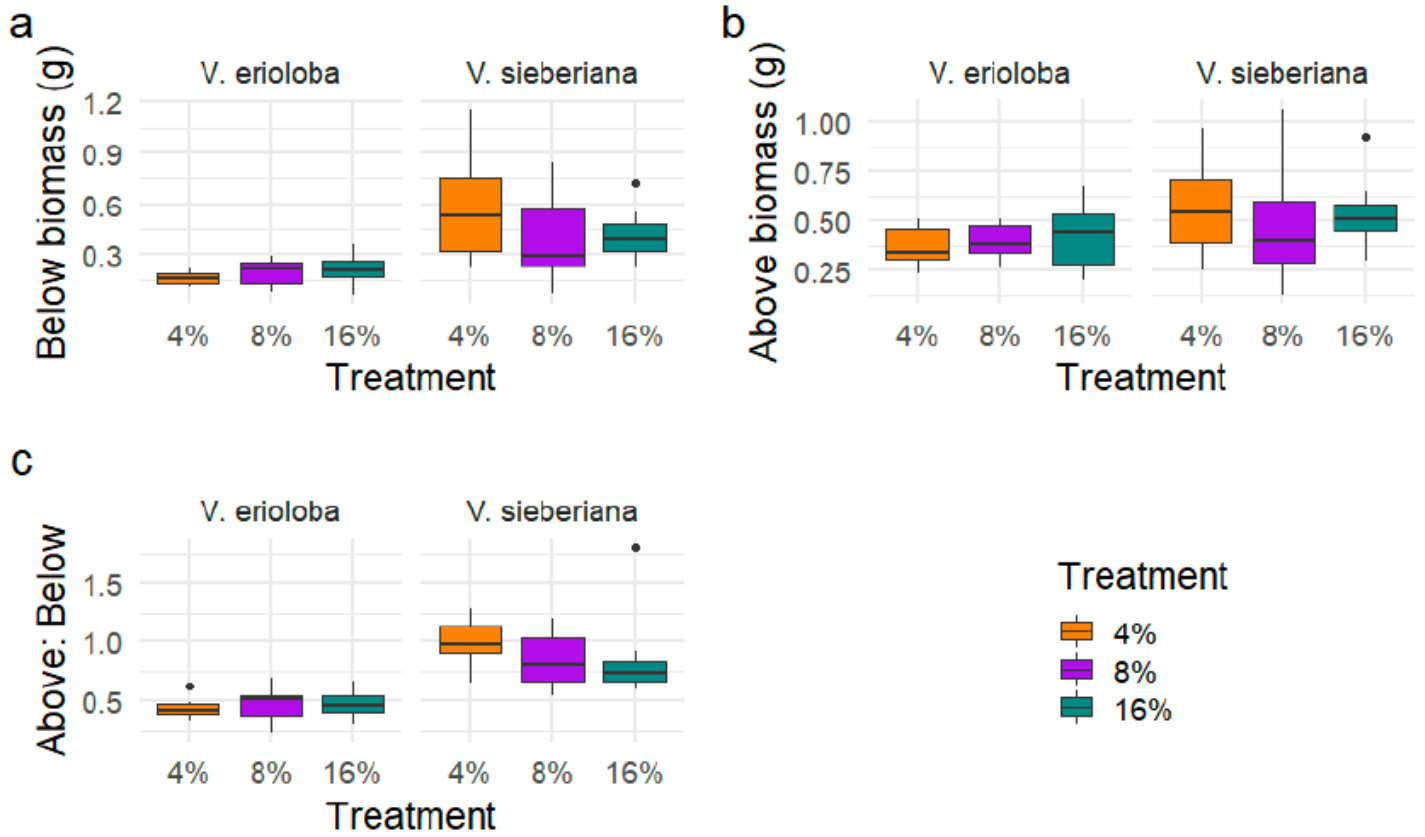


Figure 3

(a) The belowground biomass, (b) The aboveground biomass, (c) The root: shoot ratio of *V. erioloba* *V. sieberiana* and harvested during Harvest 3 at four months old. The water treatment corresponds to 4%, 8% and 16% soil moisture content (Wickham, 2016).

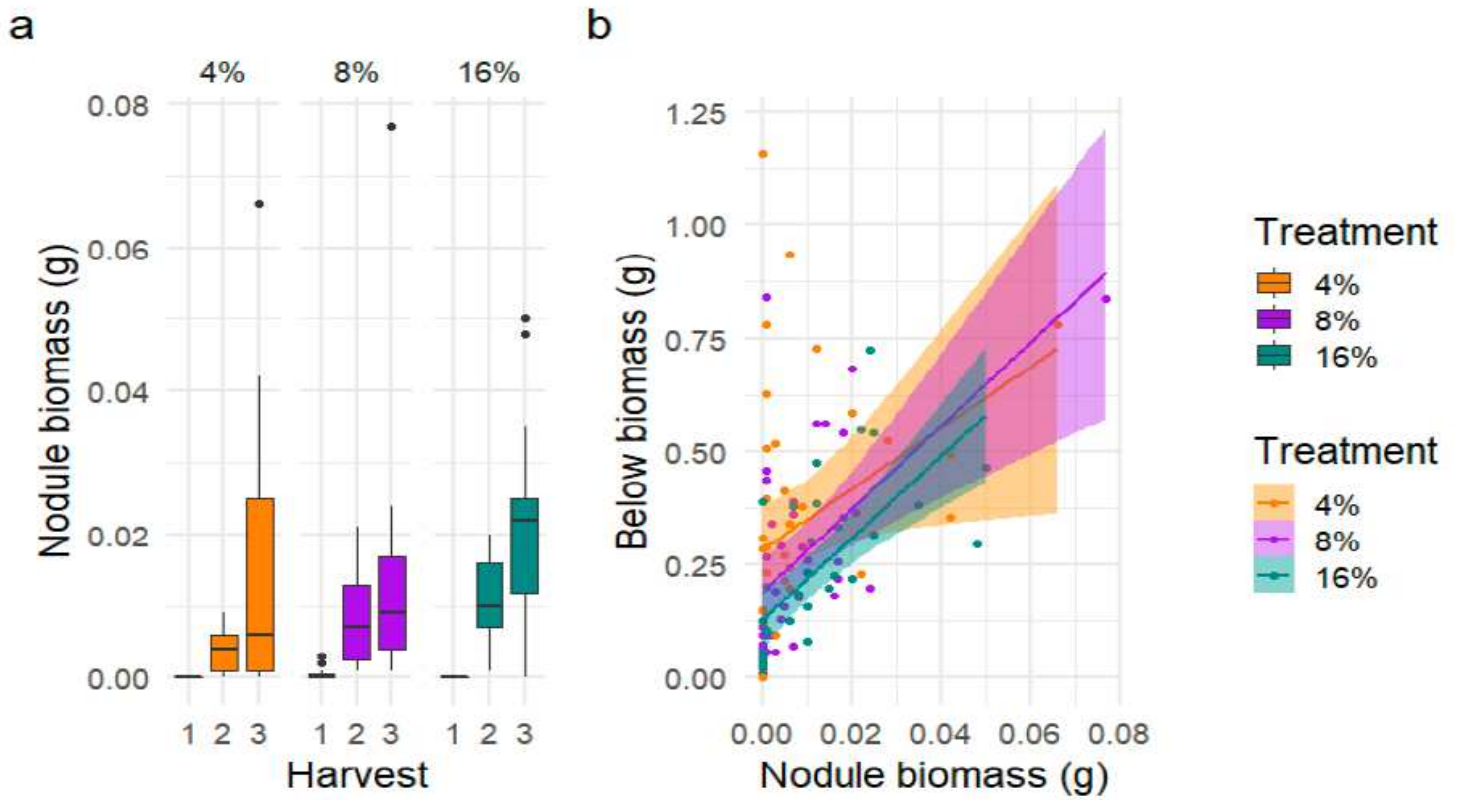


Figure 4

(a) The nodule biomass (grams) of *V. sieberiana* seedlings. Nodules were not present during Harvest 1 for *V. sieberiana* seedlings grown in 4% SMC (orange) and 16% SMC (blue). (b) The belowground biomass (grams) plotted against the nodule biomass (grams). The data points from all *V. sieberiana* across all three harvests. The zeros are plants from Harvest 1 that did not develop nodules. (Wickham, 2016).