Nodulation Alleviates Water Stress in *Vachellia Sieberiana*

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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 N^{15}
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 N^{15} 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 N_2 -fixation by reducing carbon nodule metabolism, introducing oxygen
83 limitation, thus causing reduction of N_2 -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 N_2 -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

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

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

Materials and Methods

Description of study species

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

Bothma, 1984). Where it is found, herbivore densities are low and fire is infrequent (M. E. Barnes, 2001; Canadell et al., 1996; Seymour, 2003; Seymour & Alias, 2003; Seymour & Huyser, 2008). *Vachellia sieberiana* is herbivore dispersed species found across regions where rainfall ranges from 800-1100mm MAP (Figure 1.a & 1.c) (Bunney, 2013) and is a known nodulator (Sprent, 2009). *V. sieberiana* grows on deep, well-drained, light sandy and medium loamy acid soils (Tadesse, Desalegn, & Alia, 2007). The species is fast growing (Sunmonu & Van Staden, 2014), and considered a vicious bush encroacher (Russell et al., 2019; Sabiiti & Wein, 1987; Stevens et al., 2017). Browse pressure is primarily on adult trees by giraffes (*Giraffe camelopardalis*) (Zinn, Ward, & Kirkman, 2007). Within its range, fire is frequent and fuelled by highly productive grasses (Bunney, 2013; February, Higgins, Bond, & Swemmer, 2013; Sabiiti & Wein, 1987; Zinn et al., 2007).

Experimental design

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

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

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

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

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

Soil moisture treatments

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

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

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

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

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

Plant and leaf measurements at harvest

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

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

Nodulation in relation to age and water availability

As expected, *V. erioloba* did not develop nodules regardless of water treatment. The nodule count and nodule biomass in *V. sieberiana* increased with age (df=2, F=129.194, p<0.001), across the three harvesting efforts (Figure 4.a, Table 3 & 4). There was an 800% increase in the number of individuals that nodulated between Harvest 1 (two months old) and Harvest 2 (three months old) (Table 4). There was an 11% increase in the number of individuals that nodulated between Harvest 2 (three months old) and Harvest 3 (four months old) (Table 4).

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

Discussion

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

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

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

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

al., 2013; Seymour, 2003, 2008). Rigid patterns of belowground investment allows mature *V. erioloba* individuals to survive in a consistently water limited environment (M. E. Barnes, 2001).

Alternatively, *V. erioloba* extends into most arid parts of the African desert; more than any other tree species (R. D. Barnes, Fagg, & Milton, 1997). Therefore, perhaps it was unaffected by water treatment because the seedlings were not drought stressed. We suggest that future experiments using *V. erioloba* incorporate a more severe drought treatment to better understand their growth traits relating to their arid niche.

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

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

Future research

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

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

Conclusion

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

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

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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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Tables

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

<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

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

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	
	df	Mean Sq	F	p
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	
	df	Mean Sq	F	p
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	
	df	Mean Sq	F	p
Treatment	2	0.186	1.45	0.242
Species	1	17.839	138.31	0.000****
Residuals	59	0.129		

Table 3 The coefficients (β), lower (CI 2.5%) and upper (CI 97.5%) confidence intervals for the biomass of nodules harvested at two months, three months and four months old. Treatment refers to the water availability treatment. Species refers to *V. erioloba* (VE) and *V. sieberiana* (VS). The output from 2 way ANOVA for testing the effect of water availability treatment on nodule biomass harvested at two months (harvest 1), three months (harvest 2) and four months old (harvest 3). Significance is 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		

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

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).

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).

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).