Effects of Water and Soil Microorganisms on Growth Traits of *Albizia kalkora* and *Leucaena leucocephala* in Dry-Hot Valleys: Postprint

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Abstract

Vegetation restoration is an effective approach for ecological restoration in dry-hot valleys, and many exotic species have been introduced during the ecological restoration of dry-hot valleys. To compare the differential responses of native and exotic introduced species to water and microorganisms, this study selected the local indigenous species Albizia kalkora and the exotic species Leucaena leucocephala, simulated conditions of Yunnan' s dry-hot valley region using a climate chamber, and investigated the effects of water and respective soil microorganisms on the growth and development of the two leguminous plants through soil moisture control and microbial inoculation. The results showed that: (1) Compared with Leucaena leucocephala, Albizia kalkora exhibited 58.82%, 76.13%, 90.59%, and 18.42% higher seed germination rate, root biomass, root-to-shoot ratio, and N:P ratio, respectively ($P<0.05$); (2) Under drought conditions, root biomass and nitrogen concentration of Albizia kalkora were 33.67% and 8.65% lower than under moist conditions ($P<0.05$), while plant height, aboveground biomass, and root biomass of Leucaena leucocephala under drought conditions were 39.32%, 40.06%, and 39.61% lower than under moist conditions ($P<0.05$), indicating that drought inhibited plant growth and nutrient absorption; (3) Albizia kalkora had more nodules under drought conditions than under moist conditions ($P<0.05$), whereas no significant difference in nodule number was observed between moist and drought conditions for Leucaena leucocephala; (4) Except for the proportion of dead leaf biomass, soil microbial inoculation and the interaction between soil microorganisms and moisture did not have significant effects on the growth of either Albizia kalkora or Leucaena leucocephala. These findings suggest that Albizia kalkora may be more suitable for arid environments than Leucaena leucocephala, providing a theoretical basis for species selection in vegetation restoration of dry-hot valleys, though the

effects of soil microorganisms on plant growth warrant further exploration and research.

Full Text

Effects of Water and Soil Microorganisms on Growth Traits of Albizia kalkora and Leucaena leucocephala in a Dry-Hot Valley

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Abstract

Vegetation restoration is an effective approach for ecological rehabilitation in dry-hot valleys, yet this process has introduced numerous alien species. To compare the differential responses of native and introduced species to water and microbial factors, we selected the native species *Albizia kalkora* and the alien species *Leucaena leucocephala*, both legumes. Using climate chambers to simulate conditions in Yunnan' s dry-hot valley region, we investigated the effects of soil moisture control and microbial inoculation on the growth and development of these two species. The results showed: (1) Compared with *L. leucocephala*, *A. kalkora* exhibited significantly higher seed germination rate, root biomass, root-shoot ratio, and nitrogen-to-phosphorus ratio by 58.82%, 76.13%, 90.59%, and 18.42%, respectively ($P<0.05$); (2) Under drought conditions, root biomass and nitrogen concentration in *A. kalkora* decreased by 33.67% and 8.65% compared with moist conditions ($P<0.05$), while plant height, aboveground biomass, and root biomass in *L. leucocephala* decreased by 39.32%, 40.06%, and 39.61%, respectively ($P<0.05$), indicating that drought inhibited plant growth and nutrient uptake; (3) *A. kalkora* produced more root nodules under drought than under moist conditions ($P<0.05$), whereas no significant difference in nodule number was observed for *L. leucocephala* between water regimes; (4) Except for dead leaf biomass proportion, neither soil microbial inoculation nor its interaction with water had significant effects on the growth of either species. These findings suggest that *A. kalkora* may be more suitable for arid environments than *L. leucocephala*, providing a theoretical basis for species selection in dry-hot valley vegetation restoration. However, the effects of soil microorganisms on plant growth warrant further investigation.

Keywords: dry-hot valley; *Albizia kalkora*; *Leucaena leucocephala*; soil moisture; soil microorganisms

The dry-hot valley of Yuanmou, Yunnan, is located in the subtropical humid and semi-humid region of the Yunnan Plateau, in the lower reaches of the Longchuan River (a primary tributary of the Jinsha River), between 101°35 -102°05 E and 25°25 -26°07 N (Zhao et al., 2006). This region experiences year-round high temperatures, abundant sunlight, and aridity, with nearly 16 hours of daylight and an average annual temperature no lower than 20 °C, providing ample heat for plant growth. However, the environmental conditions are harsh, characterized by drought, poor soil, low vegetation coverage, severe soil erosion, and fragile ecosystems. Vegetation restoration represents an effective pathway for ecological rehabilitation in dry-hot valleys, and since 1992, numerous studies have focused on species screening and restoration techniques (Yang et al., 2007). Species selection has primarily emphasized drought tolerance, leading to the introduction of many alien species such as eucalyptus, *Leucaena leucocephala*, moringa, neem, and acacia. Restoration techniques have concentrated on soil preparation, seedling cultivation, and drought-resistant water conservation (Zhou et al., 2021; Zhao and Yang, 2023). Drought is the primary environmental stressor in dry-hot valleys, and improving soil moisture conditions is critical for degraded ecosystem recovery (Zhong, 2000). The dominant soil type, dry red soil, is characterized by nitrogen deficiency, low phosphorus, and poor organic matter content (Zhang et al., 2005). Under such impoverished conditions, plant-soil microorganism relationships become particularly important (Sardans & Peñuelas, 2013). While numerous studies have examined water stress effects on plant physiological and growth characteristics in dry-hot valleys, the impacts of soil microorganisms on plant growth remain poorly documented.

Soil moisture content directly affects plant growth and development. As a major component of plant tissues, water enables cell elongation and expansion; when soil moisture is insufficient and turgor pressure decreases, plant growth slows or ceases (Lei et al., 2009). Soil water content also influences plant development indirectly by affecting soil fertility (Li and Han, 2019). Consequently, research on plant drought resistance has emerged, with growth traits serving as indicators of drought adaptation capacity (Kudoyarova et al., 2013). Studies have demonstrated that both aboveground and belowground biomass decrease under drought stress, with aboveground biomass showing greater reduction (Haffani et al., 2014). Soil microorganisms constitute an important component of soil living organisms, closely related to plant growth and nutrient uptake. Their species composition, abundance, and activity all affect plant development. Microbial influences can be direct (e.g., mycorrhizal fungi, nitrogen-fixing symbionts, pathogens) or indirect through free-living microorganisms (Wang et al., 2017). These effects can be positive or negative. Positive effects typically arise from beneficial bacteria such as nitrogen-fixing rhizobia and saprotrophic microorganisms that mineralize organic matter into inorganic nutrients. Negative effects are primarily caused by pathogens. Research indicates that plant adaptation to drought depends largely on soil microbial responses, suggesting that plants facing environmental stress may benefit from rapid responses in their surrounding biological communities beyond mere adaptation or migration (Lau & Lennon, 2012). However, the interactive effects of soil microorganisms and water on plant growth remain unclear.

Albizia kalkora (native) and *Leucaena leucocephala* (alien) both belong to the Leguminosae family. *A. kalkora* grows along streams, roadsides, and slopes, serving as a common native species and pioneer tree for afforestation in the dry-hot valleys of central Yunnan Plateau (Cui et al., 2010). *L. leucocephala*, native to tropical America, was introduced to China on a large scale beginning in the 1960s. Due to its strong environmental adaptability and low water requirements, it has been promoted as an excellent afforestation species for dry-hot valleys. However, studies have also identified its high transpiration rate, low water use efficiency, and potential invasive characteristics, leading to its classification as an invasive plant (Zhao et al., 2019). Duan et al. (2013) analyzed water use efficiency dynamics in both species, finding that *A. kalkora* had slightly larger ground diameter but smaller plant height and crown width than *L. leucocephala*. In terms of water use efficiency, *A. kalkora* outperformed *L. leucocephala* during the dry season but underperformed during the wet season, though their specific drought response characteristics remain unclear. Moreover, legumes form intimate relationships with soil microorganisms, establishing arbuscular mycorrhizal fungi (AMF)-legume-rhizobia triple symbioses—a special type of plant-microorganism association (Igiehon & Babalola, 2018) that is highly sensitive to soil microbial changes (Yang et al., 2020). Currently, no studies have examined the responses of *A. kalkora* and *L. leucocephala* to soil microorganisms and their interactions with water.

Therefore, this study investigated the native legume *A. kalkora* and the alien legume *L. leucocephala* using local dry red soil as the experimental substrate and controlled indoor climate chamber cultivation. By examining the effects of water, soil microorganisms, and their interactions on plant growth, we addressed two questions: (1) Do *A. kalkora* and *L. leucocephala* differ in their responses to water stress? (2) Do soil microorganisms affect plant growth traits and influence plant responses to water stress? Both species are dominant legumes in dry-hot valleys with similar morphological characteristics, but their contrasting native/alien status provides a valuable comparison for informing species selection in vegetation restoration. Additionally, by focusing on both water and microbial effects, this study aims to provide scientific support for regulating and utilizing soil microbial functions in restoration techniques.

1.1.1 Plant Seeds

Seeds of *A. kalkora* and *L. leucocephala* were collected from the dry-hot valley region during the mature season, air-dried, and stored in cloth bags. The 100-seed weight was 8.689\$±0.003*gfor* ∗ *A.kalkora* ∗ *and*5.460±\$0.016 g for *L. leucocephala*. Healthy, uniform seeds were selected for the experiment.

1.1.2 Potting Soil

Experimental soil was collected from dry red soil in areas where both species naturally occurred. Field surveys were conducted in a typical Yuanmou dry-hot valley region (101.81° E, 25.67° N) where both species co-occurred with minimal environmental variation. At 5-10 sampling points for each species, soil was excavated to approximately 50 cm depth around plant stems using a cross-pattern approach. To eliminate existing microorganisms, soil samples were subjected to moist heat sterilization in an autoclave (121 °C, 103.5 kPa) for 1 hour, repeated three times with 24-hour intervals between sterilizations. High-pressure sterilization is an effective soil sterilization method with minimal impact on soil physicochemical properties (Berns et al., 2008). Zheng et al. (2017) demonstrated that three autoclaving cycles with 24-hour intervals achieve excellent sterilization. After sterilization, equal masses of *A. kalkora* and *L. leucocephala* soils were mixed to create a uniform substrate, eliminating species-specific soil preferences and ensuring consistent physicochemical properties across treatments. The mixed substrate was placed in sterilized pots (121 °C, 20 min). The basic physicochemical properties of the substrate were: pH 6.20\$$\pm 0.01, organiccarbon 1.55\pm 0.47 g \cdot kg^{\{-1\}, totalnitrogen 0.43\pm 0.05 g \cdot kg}\{-1\}, totalphosphorus 0.01\pm 0.00 g\cdot kg^{\{-1\}, totalpotassium 0.58\pm 0.02 g \cdot kg}\{-1\}, availablenitrogen 15.16\pm 3.31 mg\cdot kg^{\{-1\}, availablephosphorus 0.34\pm 0.02 mg \cdot kg}\{-1\}, andavailablepotassium 57.26\pm 2.29 mg\cdot kg\hat{}\{-1\}$\$.

On January 5, 2022, fresh soil from 80-day-old *A. kalkora* and *L. leucocephala* plants was collected for microbial inoculation. High-throughput sequencing revealed the microbial community composition and diversity (Table 1). Fresh soil was stored at -20 °C until use.

1.2 Experimental Design

The experiment comprised three factors: plant species, water, and soil microorganisms. Plant species included *A. kalkora* (S) and *L. leucocephala* (Y). Water treatments were controlled using the traditional gravimetric method by maintaining different field capacity (FC) levels (Wang et al., 2016; Liu et al., 2023). The FC of local dry red soil was determined to be 12%, establishing two gradients: moist (W) at 80–85% FC and drought (D) at 40–45% FC, corresponding to soil water contents of 9.6-10.2% and 4.8–5.4%, respectively. Microbial treatments included non-inoculated (M0) and inoculated with soil microorganisms (M). Because different plant species harbor distinct soil microbial communities with varying feedback effects, we focused on the feedback effects of each species' native soil microorganisms. Thus, *A. kalkora* treatments received *A. kalkora* soil microorganisms (MS), and *L. leucocephala* treatments received *L. leucocephala* soil microorganisms (MY), yielding eight treatments: SDM0, SWM0, SDMS, SWMS, YDM0, YWM0, YDMY, and YWMY, with four replicates per treatment.

The study employed indoor cultivation using climate chambers to simulate dry-hot valley temperature and humidity conditions, excluding topographic effects. The experiment began at the end of February 2022. Empty pot weights were recorded, and sterilized pots (121 °C, 20 min) were filled with 400 g each of sterilized *A. kalkora* and *L. leucocephala* soils (800 g total per pot) and moistened with sterile water. Healthy, plump seeds were treated with concentrated sulfuric acid for 5 minutes to break dormancy, rinsed thoroughly with sterile water, and sown at 10 seeds per pot. After covering with sterilized substrate, pots were placed in a climate chamber (16 h/8 h day/night, 35 °C/25 °C, 15,000 lx light intensity). The 35 °C/25 °C temperature regime approximated air temperatures during the growing season in dry-hot valleys (Wang et al., 2017). Adequate watering promoted germination, and seedlings were thinned to four per pot once most pots contained four seedlings. After additional growth, microbial inoculation was performed.

Soil microbial inoculation followed van de Voorde et al. (2012) using soil suspension. Fresh soil from each species (80 g, 10% of substrate mass) was mixed with sterile water at a 1:1 ratio and stirred for 2 minutes. After 15 minutes, the suspension was restirred for 2 minutes and left for another 15 minutes. The supernatant was then sieved through a 0.5 mm mesh to produce the inoculum, which was rinsed to a standard volume for each pot. Control treatments received an equivalent volume of sterilized soil suspension. One month after inoculation, water stress was imposed by maintaining target FC levels through daily weighing and supplementation with sterile water, with watering amounts recorded. Dead leaves were collected promptly, and plant conditions were monitored. After 72 days of water stress, plants and soil samples were harvested for analysis.

1.3 Measurement Methods

(1) **Plant growth and biomass:** Plant height was measured with a ruler. Aboveground and root components were separated, oven-dried at 65 °C to constant weight, and weighed for biomass determination. Dead leaves collected during the experiment were also oven-dried at 65 °C and weighed. For relative water content, 20 healthy leaves per pot were weighed fresh (WF), soaked in deionized water at 5 °C in darkness for 12 hours, surface moisture removed, and weighed saturated (WR), then oven-dried at 65 °C to constant weight (WD). Relative water content (%) = [(WF-WD)/(WR-WD)] × 100.

(2) **Plant nutrients:** Aboveground tissues were oven-dried, ground, and analyzed for nitrogen and phosphorus content using the Kjeldahl method for nitrogen and vanadium molybdate yellow spectrophotometry for phosphorus.

(3) **Soil properties:** Soil water content was determined gravimetrically. Bacterial, fungal, and actinomycete populations were quantified using dilution plating: bacteria on beef extract agar (10^{-4} dilution), actinomycetes on

Gause' s No. 1 medium (10^{-2} dilution), and fungi on rose bengal agar (10^{-2} dilution). Available nitrogen was measured by alkali diffusion, and available phosphorus by molybdenum-antimony colorimetry.

(4) **Symbionts:** Root nodules were counted during root sampling. AMF colonization was assessed using ink staining (Yang et al., 2010) by counting hyphal, vesicular, and arbuscular intersections under high magnification.

1.4 Data Analysis

Data were analyzed using Excel and SPSS 22.0. Independent samples t-tests first compared differences between species. Because each species received its own soil microbial community requiring separate comparisons, two-way ANOVA was used to test water and microbial effects and their interactions for each species. Multiple comparisons were performed using Turkey' s HSD test at $\alpha = 0.05$.

2.1 Water Control and Plant Water Status

The experiment successfully established distinct drought (DM0, DM) and moist (WM0, WM) conditions controlled gravimetrically. Daily watering maintained clear separation between treatments (Figure 1 [Figure 1: see original paper]). At experiment termination (after 72 days of water treatment), soil water content differed significantly between drought (0.95%) and moist (14.46%) treatments (Figure 2 [Figure 2: see original paper]), confirming a clear moisture gradient. Plant relative water content also showed distinct differences, being 56% higher under moist than drought conditions (Figure 3 [Figure 3: see original paper]).

2.2 Plant Growth Characteristics

Statistical analysis (Table 2) and trait comparisons (Figure 4 [Figure 4: see original paper]) revealed significant differences between species in germination rate, root biomass, and root-shoot ratio ($P<0.01$). *A. kalkora* showed 54.76% germination versus 34.48% for *L. leucocephala*, with 76.13% and 90.59% higher root biomass and root-shoot ratio, respectively. For *A. kalkora*, water significantly affected root biomass and root-shoot ratio ($P<0.01$) and dead leaf biomass and proportion ($P<0.05$), with moist conditions increasing these traits by 50.76%, 46.25%, 54.13%, and 70.27% compared with drought. For *L. leucocephala*, water significantly impacted plant height, aboveground biomass, root biomass, and dead leaf biomass ($P<0.01$), with moist conditions increasing these by 64.79%, 66.83%, 65.59%, and 117.27% versus drought. Microbial treatment alone did not significantly affect any traits, though the water × microorganism interaction influenced dead leaf proportion in *A. kalkora* ($P<0.05$), with microbial inoculation significantly increasing dead leaf proportion under moist conditions.

2.3 Plant Nutrient Characteristics

Nitrogen content, phosphorus content, and N:P ratios are shown in Figure 5 [Figure 5: see original paper]. Species differed significantly in phosphorus content and N:P ratio ($P<0.05$). *L. leucocephala* had 21.2% higher phosphorus content ($0.5903\ \mathrm{g \cdot kg^{-1}}$) than *A. kalkora* ($0.4872\ \mathrm{g \cdot kg^{-1}}$), while *A. kalkora* had a significantly higher N:P ratio (45) than *L. leucocephala* (38). Water significantly affected nitrogen content in *A. kalkora* ($P<0.05$), with moist conditions increasing nitrogen by 9.47% (23.13 vs. $21.13\ \mathrm{g \cdot kg^{-1}}$). Neither microbial treatment nor its interaction with water significantly affected nutrient metrics ($P>0.05$).

2.4 Symbiont Characteristics

Root nodule counts under microbial treatment (Figure 6 [Figure 6: see original paper]) revealed that *A. kalkora* produced more nodules under drought (11) than moist conditions (3.5) ($P<0.05$), while *L. leucocephala* showed no significant difference between water regimes (Figure 7 [Figure 7: see original paper]). Microscopic examination detected AMF colonization in only a few samples (Figure 8 [Figure 8: see original paper]), with most roots of both species showing no AMF infection.

2.5 Soil Characteristics

ANOVA results (Table 3) and microbial community data (Figure 9 [Figure 9: see original paper]) showed that soils growing different plants differed in fungal abundance ($P<0.05$), with *A. kalkora* soils containing 80.81% more fungi than *L. leucocephala* soils. For *A. kalkora*, water significantly affected bacterial abundance ($P<0.01$), with drought increasing bacteria by 171.18% compared with moist conditions, while microbial treatment significantly affected fungal abundance ($P<0.01$), with non-inoculated soils containing 272.84% more fungi than inoculated soils. For *L. leucocephala*, microbial treatment affected bacterial abundance ($P<0.05$), with non-inoculated soils containing 91.10% more bacteria than inoculated soils.

Soil nutrient analysis (Table 3 ; Figure 10 [Figure 10: see original paper]) revealed species differences in available phosphorus ($P<0.05$), with *A. kalkora* soils containing 43.50% more available phosphorus than *L. leucocephala* soils. For *A. kalkora*, the water × microorganism interaction significantly affected available nitrogen ($P<0.01$), with non-inoculated drought soils showing significantly higher available nitrogen than other treatments. For *L. leucocephala*, both water and microbial treatment significantly affected available phosphorus ($P<0.05$), with drought increasing available phosphorus by 72.04% and microbial inoculation decreasing it by 45.32% compared with respective controls. The water × microorganism interaction also significantly affected available nitrogen in *L. leucocephala* soils ($P<0.05$). Compared with pre-planting substrate, post-harvest soils showed 26.98% lower available nitrogen but 391.18% higher available phosphorus.

3.1 Effects of Water on Plants

Plants under moist conditions exhibited superior performance in height, biomass, and nitrogen concentration compared with drought-stressed plants, confirming that drought inhibits growth and development. Soil moisture content in the Jinsha River dry-hot valley often drops below 5% during the dry season (Wei and Ye, 1991), matching our drought treatment level. Our moisture levels also approximated those in *L. leucocephala* forests during rainy and dry seasons (Han et al., 2019).

Differences in drought resistance between native and alien species have been documented. Li et al. (2008) found that the invasive species *Merremia boisiana* showed superior physiological adaptation to varying moisture conditions compared with the native early-invader *Pueraria lobata*. Chen and Wang (2008) reported that the native species *Oxalis corniculata* was more sensitive to water stress than the alien *O. corymbosa*. Zhou and Zhang (1998) identified alien species (*L. leucocephala*, *Albizia procera*, *Pinus caribaea*) as suitable for Yuanmou dry-hot valley afforestation, contrasting with our findings. We found *A. kalkora* had higher germination rate, root biomass, and root-shoot ratio than *L. leucocephala*, plus more root nodules under drought. Allocating more biomass belowground helps meet nutrient and water demands, representing an important survival strategy under water deficit that enhances drought resistance (Zhang et al., 2004; Zhou et al., 2014). The greater root biomass and root-shoot ratio in *A. kalkora* suggest this native species may be better adapted to drought stress than the alien *L. leucocephala*. Furthermore, water availability strongly influences mineral nutrient absorption, with nutrient deficiency often becoming a secondary effect of drought stress (da Silva et al., 2011). The higher nodule number in drought-stressed *A. kalkora* could enhance nitrogen fixation, alleviating local nitrogen limitation. Combined with Duan et al.’ s (2013) finding that *A. kalkora* had higher water use efficiency than *L. leucocephala* during the dry season, our results indicate the native species is better suited to local arid conditions and vegetation restoration.

Yang et al. (2016) noted that dry-hot valleys have become severely affected by alien plant invasions, and Yang et al. (2007) advocated prioritizing native tree species for restoration. Zhao et al. (2019) cautioned against using *L. leucocephala* in dry-hot valley restoration due to its high transpiration, low water use efficiency, and invasive potential. Our study supports these recommendations, suggesting *A. kalkora* as the preferred species.

3.2 Effects of Soil Microorganisms on Plants

Microbial inoculation may influence soil nutrients, plant biomass, and soil microbial populations through several mechanisms: (1) Inoculated microorganisms may include bacteria that promote nutrient uptake, such as rhizobia; (2) Microorganisms can improve nutrient availability to enhance plant growth or cause nutrient loss through biochemical processes that inhibit growth. Shen and Zhao (2015) identified microorganisms that form symbiotic relationships with roots or inhabit the rhizosphere, affecting nutrient acquisition, including nitrogen-fixing bacteria, AMF, and plant growth-promoting rhizobacteria. Bi et al. (2014) demonstrated that microbial inoculation significantly increased plant biomass and nutrient content while improving mineral element absorption and utilization, particularly phosphorus. Soil microorganisms can differentially affect native and alien species, often benefiting aliens while negatively affecting natives. Zhang et al. (2012) found positive microbial effects on the invasive species *Sapium sebiferum*, with greater seedling performance in fresh soil compared with native species. Liang et al. (2016) reported that soil microorganisms significantly affected both the invasive *Ageratina adenophora* and native *Rabdosia amethystoides*, enhancing the invader’ s competitive advantage. However, our study found no such effects, with microbial treatment showing no significant impact on either species.

Several factors may explain this discrepancy. First, contamination by airborne microorganisms may have occurred. Airborne microbes exist as aerosols with multiple sources, diverse species, variable activity, three-dimensional dispersal, regenerative deposition, and widespread infection (Sun et al., 2010). Uninoculated samples could have been colonized by airborne microbes, particularly saprophytes, competing for nutrients. Our finding that non-inoculated soils had significantly higher fungal abundance suggests possible airborne fungal contamination. In inoculated samples, airborne microbes may have competed with introduced microbes, affecting both nutrient utilization and beneficial microbial functions.

Second, low-temperature storage may have reduced microbial activity. Zhou et al. (2015) reported that 4 months of storage at -20 °C significantly decreased microbial metabolic activity. Our inoculation soil was frozen for approximately two months, likely reducing microbial abundance and activity, thereby diminishing treatment effects.

Third, inoculation method may have influenced results. Soil microbial effects are typically studied through sterilization and inoculation. Since sterilization alters soil properties, Shaw et al. (1999) suggested that re-inoculating sterilized soil can serve as a non-sterile control, with inoculation densities of 1-15% affecting biomass (Pernilla et al., 2010). Methods include inoculating fresh soil passed through a 1 mm sieve, 1 mm soil suspension, or 20 m soil suspension, with the 1 mm fresh soil method having the strongest effect (van de Voorde et al., 2012). To avoid disturbing AMF and reduce soil fauna like nematodes, we used soil suspension inoculation. However, incomplete infiltration left some suspension on the soil surface, which may have dried under climate chamber conditions, preventing successful inoculation of some microorganisms and reducing inoculum density, potentially explaining the lack of significant differences.

Our inoculum contained rhizobia, yet final nodule numbers were low. In preliminary experiments under identical conditions, *A. kalkora* and *L. leucocephala* produced approximately 30 and 80 nodules, respectively, on dry red soil after

80 days—nearly 10-fold higher than observed here. Low inoculum density likely resulted in insufficient rhizobia for abundant nodulation. Additionally, legumes form AMF–legume–rhizobia triple symbioses. AMF are important components of dry-hot valley ecosystems, with most native species harboring abundant AMF (Li & Zhao, 2005; Li et al., 2010). Li and Zhao (2005) detected abundant AMF spores in rhizosphere soils of both species. However, we observed minimal AMF colonization. FUNGuild analysis revealed that symbiotic trophic types comprised less than 1% of the inoculum fungal community, with AMF relative abundances of only 4.2% and 13.8% in *A. kalkora* and *L. leucocephala* soils, respectively (unpublished data). Combined with reduced viability from frozen storage, the low initial AMF abundance likely resulted in poor colonization.

3.3 Effects of Plant Growth on Soil Available Nitrogen and Phosphorus

Growing both species reduced substrate available nitrogen but increased available phosphorus. The nitrogen reduction primarily reflects plant uptake, while phosphorus increases may relate to root exudates. Wu (2021) found that legumes secrete phosphatases and organic acids under low phosphorus stress to improve phosphorus availability. Acid phosphatases mineralize organic phosphorus, releasing phosphate ions (Xiao et al., 2014), while organic acids solubilize recalcitrant phosphorus, increasing available phosphorus (Shi, 2021). Legumes can thus produce large quantities of organic acids under phosphorus limitation, promoting rhizosphere acidification and enhancing phosphorus availability (Zhao et al., 2011). Dry-hot valley dry red soil strongly fixes phosphorus, resulting in low phosphorus availability. Our substrate contained only 0.34 $\mathrm{mg \cdot kg^{-1}}$ available phosphorus, likely creating phosphorus limitation. The average N:P ratio of 42 in both species exceeds the threshold of 20 that indicates phosphorus limitation (Zhang et al., 2015), confirming phosphorus stress. Therefore, both species likely increased soil phosphorus availability through root exudation of phosphatases, organic acids, and protons that solubilized adsorbed phosphorus.

In conclusion, drought inhibited plant growth, but *A. kalkora*' s greater root biomass and root-shoot ratio, along with increased nodule production under drought, enhance drought adaptation. We recommend the native species *A. kalkora* for dry-hot valley restoration. Additionally, both species substantially increased soil phosphorus availability, suggesting that leveraging legume effects to improve phosphorus availability in dry red soil warrants further exploration.

Despite significant results, limitations exist. The lack of microbial treatment effects may relate to inoculum source and handling. Future improvements should include: (1) Using fresh soil immediately after collection to ensure microbial viability; (2) Modifying inoculation methods, such as adding 10% fresh soil to increase microbial abundance and maximize benefits. Additionally, our climate chamber results may differ from field conditions; future studies should conduct in situ experiments to more realistically reflect species adaptation differences.

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Note: Figure translations are in progress. See original paper for figures.