

Article

Arbuscular Mycorrhizal Fungi Modulate Root Growth and Intraspecific Interaction in Invasive *Nicotiana glauca* Graham Seedlings

Abdelmalik M. Adam ^{1,*} , Thobayet S. Alshahrani ^{1,*} , Abdulaziz A. Alqarawi ¹ and Ibrahim A. Abdelfadeel ² 

¹ Department of Plant Production, Faculty of Food & Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; aalqarawi@ksu.edu.sa

² Department of Soil Sciences, Faculty of Food & Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; 442106997@student.ksu.edu.sa or 442106007@student.ksu.edu.s

* Correspondence: aadam1@ksu.edu.sa (A.M.A.); talshahrani@ksu.edu.sa (T.S.A.)

Abstract

This study examined the influence of arbuscular mycorrhizal fungi (AMF) on root system development of the invasive *Nicotiana glauca* Graham (Solanaceae) under intraspecific competition. Seedlings were grown with or without AMF inoculation at four planting densities (1–4 plants per pot) in a factorial experiment arranged in a completely randomized design under greenhouse conditions. After six months, shoot and root fresh and dry biomass were measured, along with root-to-shoot ratio. Root morphological traits, including total root length, surface area, volume, mean diameter, and number of root tips, were quantified and further analyzed across five levels of root diameter (0–1 mm at 1 mm intervals). Root nitrogen, phosphorus and potassium content, AMF colonization, and spore density were also assessed. Results showed that all measured traits of non-mycorrhizal seedlings declined markedly with increasing plant density. In contrast, AMF-inoculated seedlings maintained higher root biomass, total root length, surface area, and root tip numbers, particularly at low density. Across all treatments, roots < 1 mm contributed the highest proportion of total root length, surface area, and tip number, with AMF significantly enhancing these fine-root traits. Mycorrhizal seedlings also exhibited higher root-to-shoot ratios and improved N, P, and K content compared to non-inoculated controls. Multivariate analyses further indicated that AMF inoculation was the primary factor structuring root trait variation across planting densities. Overall, AMF inoculation promoted a finer and more extensive root system under increasing intraspecific competition, indicating a key role in enhancing resource acquisition and potentially supporting the competitive ability and invasiveness of *N. glauca*.



Keywords: root parameters; plant density; invasive plants; diameter classes

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1. Introduction

Biological invasion is widely recognized as one of the most pressing ecological challenges, posing serious threats to biodiversity and altering the structure of native plant communities worldwide [1]. The invasions of plants across different environments is often attributed to high resource-acquisition efficiency and strong competitive ability under varied environmental range [2]. *Nicotiana glauca* Graham (Solanaceae) (tree tobacco) is a perennial tree native to South America that has become invasive in many arid and semi-arid regions [3]. This species has the ability to form dense monospecific stands, leading

to the displacement of native vegetation, reductions in biodiversity, and alterations in soil properties [4]. For example, *N. glauca* in Saudi Arabia is regarded as one of the six top aggressive invasive plants, having expanded its range and dominated natural habitats formerly occupied by native plant species. The invasion by this plant might result in economic losses through reduced land productivity and higher management costs. On the other hand, *N. glauca* has potential economic value as a source of bioactive compounds and as a promising bioenergy plant due to its high biomass [5]. Moreover, the establishment of *N. glauca* can influence the composition and functioning of soil microbes [6]. Despite this, an increasingly recognized yet still underexplored factor contributing to invasion success is the plant–soil–microbe interaction, particularly arbuscular mycorrhizal fungi (AMF) [7].

It is reported that AMF are associated with approximately 80% of plants on the Globe through colonizing plant roots and extending extensive hyphal networks into the surrounding soil, thereby enhancing water and nutrient uptake [8]. Invasive plant species have frequently been reported to form mutualistic relationships with AMF, which may increase their competitive advantage over native plants [9,10]. In general, invasive plants can markedly influence the richness and diversity of microbes within the plant rhizosphere, including AMF [11]. Beyond their nutritional role, AMF strongly affect plant morphological and physiological processes, particularly root system architecture, by increasing root length, surface area, and branching intensity [12]. Such modifications can substantially alter plant competitive behavior and improve plant ability to establish in new ecological niches. AMF hyphae extend beyond the root surface, allowing colonized plants to access soil resources that would otherwise remain unavailable, thereby improving nutrient and water acquisition [13]. Moreover, AMF promote plant development and biomass production while enhancing tolerance to both biotic and abiotic stresses [14]. Consequently, the presence or absence of AMF can influence plant community succession and restoration processes, as well as the survival and establishment of invasive species such as *N. glauca* [15].

Invasive plants may derive disproportionate benefits from associations with AMF compared to neighboring species, thereby enhancing their competitive dominance through these symbiotic relationships [16]. By improving nutrient-uptake efficiency and modifying root morphology, AMF can mediate plant–plant interactions, including both interspecific and intraspecific competition [17]. Within plant communities, intraspecific competition is considered a key process shaping population structure and regulating density-dependent growth [18]. AMF can either alleviate or intensify such competition by influencing root spatial distribution and nutrient-foraging capacity [19]. However, most studies addressing mycorrhizal mediation of competition have focused primarily on interactions between invasive and native species, whereas intraspecific effects remain comparatively underexplored, particularly at the seedling stage [20]. For example, a controlled greenhouse study involving *Schizachyrium scoparium*, *Rudbeckia hirta*, and *Plantago lanceolata* demonstrated that soil microbial communities enhanced the relative strength of intraspecific competition compared to interspecific competition; however, this effect was strongly dependent on soil water availability [21]. Under conditions of AMF presence combined with reduced soil moisture, the intensity of intraspecific competition was further increased [22].

To better understand plant invasiveness, it is essential to evaluate interaction effects, fungal associations, and the functional role of invasive plant root systems [23]. Roots with small diameters represent the most permeable element of the plant root structure and play a central role in water and nutrient absorption [24]. Root functions are strongly diameter-dependent: coarse roots (greater than 2 mm in diameter) primarily provide anchorage and transport water from deeper soil layers, whereas fine roots (less than 2 mm in diameter) are chiefly responsible for nutrient and water uptake [25]. Understanding mycorrhizal influences on root system dynamics is therefore critical, as roots are the

primary organs governing resource acquisition and belowground competition. Arbuscular mycorrhizal fungi-induced modifications in root morphology—such as increases in root length, volume, and fine-root density—can alter nutrient-foraging efficiency and the degree of root overlap among conspecific individuals [26]. As a result, AMF may regulate the intensity of intraspecific competition by shaping how invasive seedlings partition soil space and available resources. Investigating these processes in *N. glauca* can provide valuable insight into the role of mutualistic interactions in enabling the persistence and extent of invasive plants within resource-limited ecosystems.

Despite evidence that *N. glauca* is capable of forming synergetic relations with AMF [27], the functional role of these symbioses in enhancing the invasive performance of this species—particularly under intraspecific competition—remains largely unexplored. In addition, the root system of *N. glauca* has received comparatively less attention than its shoot system, largely due to the methodological challenges associated with observing root morphological changes under conditions with and without AMF. Accordingly, our study attempts to investigate root responses of *N. glauca* seedlings to increasing plant density in the existence and nonexistence of AMF. Although the involvement of AMF in plant interactions is well established, it remains essential to clarify the direction and magnitude of their effects in the context of plant invasion. The findings drawn from our study are expected to increase our understanding of how root systems of *N. glauca* seedlings respond to intraspecific competition when associated with AMF. Furthermore, this work may provide deeper insight into the role of mycorrhizal associations in regulating intraspecific competition and belowground resource dynamics in invasive plant species.

2. Materials and Methods

2.1. Plant and Soil Materials

Mature fruits of *N. glauca* were collected from a naturalized population in the Al-Baha region (Southwest Saudi Arabia; 41°21'51" E, 20°13'18" N). Healthy, fully developed capsules were sampled from multiple individuals to ensure adequate genetic diversity. Seeds were air-dried and stored at 4 °C until use. Furthermore, soil samples containing AMF were collected from the upper soil layer beneath *N. glauca* shrubs for the isolation of AMF spores and hyphae used as mycorrhizal inoculum.

Experiment soil was collected from a wadi near Riyadh and was characterized as sandy loam with low organic matter content and low available phosphorus (pH 7.2; EC, 0.51 dS m⁻¹; available, N 150 mg kg⁻¹; available P, 13 mg kg⁻¹). This soil samples were dried under air, sieved by a 2 mm mesh, and sterilized by autoclaving at 120 °C for 2 h on three consecutive days to eliminate indigenous soil microorganisms [28]. Following sterilization, the soil was thoroughly mixed with autoclaved sand at a 2:1 (v/v) ratio to improve soil physical properties.

2.2. AMF Inoculum Preparation

The arbuscular mycorrhizal inoculum is a mixture consisting of *Rhizophagus irregularis* and *Claroideoglomus etunicatum*, two widely distributed and well-characterized AMF species known to associate with a broad range of plant species in arid environments [2,29]. A mixed inoculum was selected to better represent the diverse AMF communities commonly found in natural soils and to maximize the likelihood of successful root colonization and plant response. The original inoculum, obtained from *N. glauca* stands, was propagated on a host plant (*Sorghum bicolor*) for three months at shade house using sterilized sandy loam soil. The resulting AMF inoculum contained spores, mycelium, and colonized roots, thereby ensuring the presence of viable mycorrhizal propagules.

2.3. Seedlings Establishment, Growth Condition, and Design of Experiment

To assess the intensity of intraspecific competition, *N. glauca* seedlings were grown with (AMF+) or without (AMF−) arbuscular mycorrhizal fungi at four planting densities: one, two, three, and four plants per pot. These densities were selected to simulate increasing levels of intraspecific competition that may occur during population establishment and expansion under natural conditions. For seedling establishment, seeds were sown in experimental pots (16 × 50 cm) filled with sterilized silty sand soil (3:1), with four replicates per treatment. In pots assigned to the AMF treatment, soil was inoculated prior to seed sowing by adding AMF inoculum (25 g of inoculum soil with fine roots) encompassing 250 AMF spores. Pots of the experiment were kept under controlled greenhouse conditions at the Department of Plant Production, King Saud University, from January to July 2024.

A completely randomized factorial design was employed with two main factors: (1) AMF inoculation (two levels: AMF-inoculated and non-inoculated control), and (2) intraspecific interaction (four levels: solitary growth—one seedling per pot; paired growth—two seedlings per pot; three seedlings per pot; and four seedlings per pot). Four replicates were considered for each treatment, resulting in a total of 32 experimental units. All pots were filled with the sterilized soil mixture; in AMF treatments, 25 g of live inoculum was thoroughly mixed into the upper soil layer, whereas control pots received no inoculum. Experimental pots were maintained in a greenhouse at approximately 30 °C under natural daylight conditions.

2.4. Data Collection

After the application of the experimental treatments, the following data were collected.

2.4.1. AMF Colonization and Spore Extraction

AMF colonization was assessed in the roots of *N. glauca* seedling root samples (root segments) at each treatment, following the protocol that stated by [30]. Roots were carefully passed through distilled water (4 °C) to eliminate adhering particles, cleared in 10% KOH, and subsequently stained with trypan blue in lactophenol, according to the procedure of [31]. The stained segments of roots were examined under a light microscope at 400× magnification. The presence of AMF structures, including hyphae, vesicles, and arbuscules, was recorded, and the percentage of root colonization was identified using the equation below:

$$\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segment studied!}} \times 100$$

The spores of AMF were extracted from the soil of seedling using the wet sieving method reported by [32]. Briefly, 100 g of soil from each treatment was suspended in 1 L of water, thoroughly mixed, and allowed to settle for 3–5 min. The suspension was then passed through a series of sieves (ASTM-60, ASTM-100, and ASTM-240) to recover AMF spores. The retained material was further examined under a stereomicroscope to confirm spore presence. Spores were collected on Whatman No. 1 filter paper and counted under a microscope at 25× magnification. Spore density was expressed as the number of spores per 100 g of dry soil, and AMF species were identified based on morphological characteristics.

2.4.2. Root Harvesting and Morphological Analysis

After six months of growth, seedlings were harvested from the pots, and roots were gently separated from the soil under running tap water. In treatments with two, three, or four plants per pot, roots of individual seedlings were manually separated. Root samples were stained with a colored dye to enhance contrast and evenly spread on a flatbed scanner.

Root samples were scanned by a root scanner (resolution of 600 dpi), and images were kept in TIFF format. Root images were analyzed using WinRHIZO (version 2004a) software (Regent Instruments Inc., Québec, QC, Canada) to determine root parameters such as total root length, total root surface area (cm²), root volume (cm³), mean root diameter (mm), and the number of root tips. These root traits were further classified into five root-diameter classes: 0–1, 1–2, 2–3, 3–4, and 4–5 mm. Root fresh weight was recorded immediately after harvesting, and root dry weight was determined after oven drying at 70 °C for 48 h.

2.4.3. Measurements of Vegetative Parameters

Shoots were separated from roots, and shoot fresh weight was measured using a digital balance. Shoot dry weight was identified after drying (at 70 °C for 48 h using oven) until constant weight was achieved.

2.4.4. Estimation of NPK Content in Seedling Roots

Roots of *N. glauca* seedlings were thoroughly washed to remove adhering soil particles, dried in an oven at 48 °C until constant weight, and ground into a fine powder. Subsamples (5–10 mg for nitrogen and 50–100 mg for phosphorus) were analyzed on a dry-weight basis. Total nitrogen content was determined using the dry combustion (Dumas) method, with an elemental analyzer, calibrated against certified reference materials. Phosphorus content was quantified following wet acid digestion (H₂SO₄–H₂O₂ or HNO₃–H₂O₂), using the molybdenum-blue colorimetric method, and measured with a spectrophotometer. Potassium (K) concentration in roots was determined using a flame photometer. Nitrogen, phosphorus, and potassium concentrations were expressed as percentages of dry mass.

2.5. Data Arrangement and Statistical Analysis

The data were organized and subjected to two-way analysis of variance (ANOVA). Mean differences among treatments were evaluated at $p < 0.05$ using the Least Significant Difference (LSD) test. Pearson correlation analysis was conducted to assess relationships among AMF colonization, root traits, biomass variables, and nutrient parameters. All statistical analyses were performed using Statistix software version 10.0 (Analytical Software, Tallahassee, FL, USA). In addition, correlation analyses were applied to examine relationships among root traits and their distribution across different diameter classes in *N. glauca*. Principal component analysis (PCA) was performed using OriginPro (Version 2026) software to summarize multivariate patterns and explain variation among treatments based on all measured traits and AMF treatments.

3. Results

3.1. Mycorrhizal Colonization and AMF Spore's Density

AMF successfully colonized the roots of *N. glauca* seedlings in all AMF-inoculated treatments across the different plant densities, while no AMF structures were detected in the non-inoculated control seedlings. Microscopic examination revealed the presence of characteristic mycorrhizal structures, including mycelium, vesicles, and arbuscules, distributed throughout fine root segments (Figure 1).

Mean AMF colonization rates were 62.7%, 56.6%, 57.5%, and 57.2% at planting densities of one, two, three, and four plants per pot, respectively. AMF spore densities in soils associated with *N. glauca* were 98, 89, 103, and 85 spores per 100 g of soil at planting densities of one, two, three, and four plants per pot, respectively (Table 1).

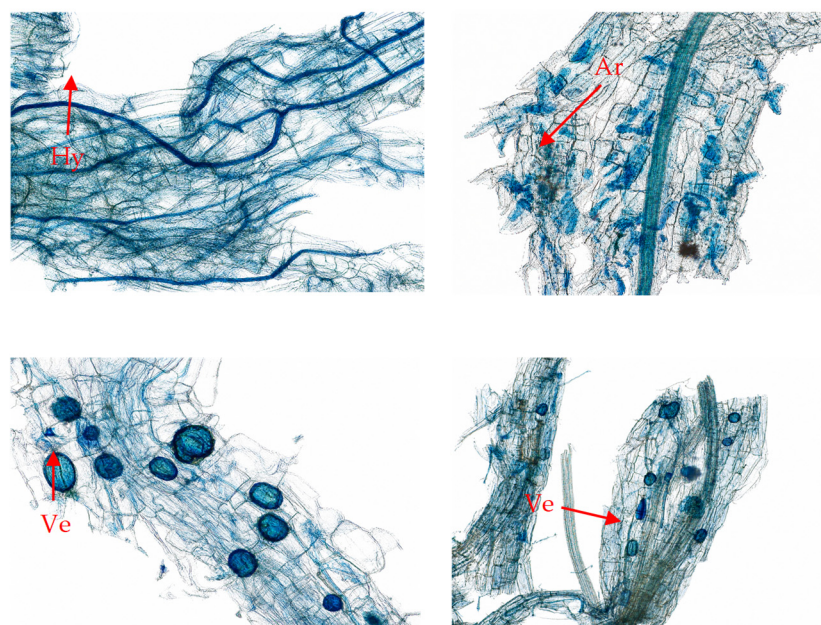


Figure 1. AMF colonization structures (Hy, hyphae; Ar, arbuscules; and Ve, vesicles) in roots of *N. glauca* seedlings. Scale = 100 μ m.

Table 1. AMF colonization rate and spore number in *N. glauca* seedlings under different densities.

Seedling Density	AMF (%)	Spore Number
1	62 $\pm$ 4.7	98 $\pm$ 5.0
2	56 $\pm$ 6.5	89 $\pm$ 4.4
3	57 $\pm$ 8.4	103 $\pm$ 10.0
4	57 $\pm$ 5.0	85 $\pm$ 3.3

3.2. Effect of AMF and Plant Density on SFW, SDW, RFW, RDW, and the Collar Diameter of *N. glauca* Seedlings

Shoot fresh weight (SFW) and shoot dry weight (SDW) of *N. glauca* seedlings varied significantly with AMF inoculation ($p = 0.002$ and 0.035 for SFW and SDW, respectively) and plant density ($p = 0.0001$ for both parameters), whereas no significant interaction between AMF and plant density was detected ($p = 0.23$ and 0.20 for SFW and SDW, respectively). Seedlings inoculated with AMF exhibited higher SFW and SDW values than non-inoculated seedlings. Increasing seedling density led to progressive decline in both SFW and SDW, particularly at densities of two, three, and four plants per pot, with a more pronounced reduction observed in non-inoculated seedlings (Figure 2A,B).

RFW and RDW were also considerably impacted by AMF inoculation ($p = 0.01$ and 0.003 for RFW and RDW, respectively) and plant density ($p = 0.0001$ for both variables). Across treatments, AMF-inoculated seedlings showed higher RFW and RDW values than non-inoculated seedlings, particularly at the lowest planting density (one plant per pot). Both RFW and RDW decreased with increasing plant density; however, the magnitude of this reduction was greater in non-inoculated seedlings compared with AMF-treated seedlings (Figure 2C,D).

Collar diameter of *N. glauca* seedlings was significantly impacted by AMF inoculation ($p = 0.0007$) and plant density ($p = 0.057$). No interaction effect between AMF inoculation and seedlings density for collar diameter ($p = 0.63$). As shown in Table 1, increasing plant density resulted in a reduction in collar diameter. Across all densities, AMF-inoculated seedlings exhibited significantly larger collar diameters than non-inoculated seedlings. In comparison to non-inoculated seedlings, collar diameter in seedlings with AMF increased

by 40.3% at one plant per tube, 26.9% at two plants per tube, 52.2% at three plants per tube, and 52.5% at four plants per tube (Figure 2E).

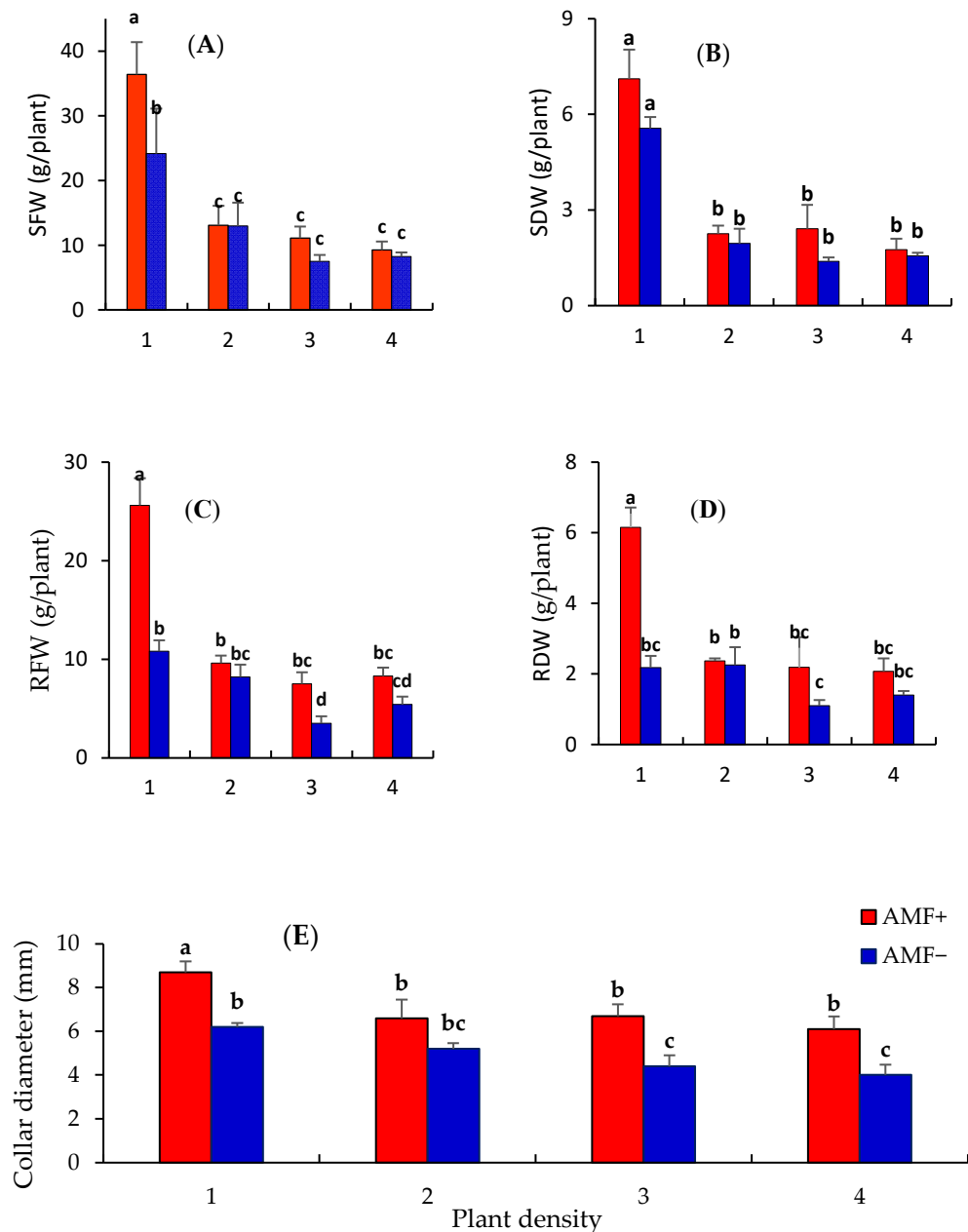


Figure 2. Effect of AMF inoculation and plant density on shoot fresh weight (A), shoot dry weight (B), root fresh weight (C), root dry weight (D), and collar diameter (E) of *N. glauca* seedlings. Data are displayed as means and $\pm$ standard errors of mean. Letters above columns indicate significant differences between means across treatments according to the least significant difference (LSD) at significance level of $p < 0.05$.

3.3. Effect of AMF and Seedling Density on the Root Traits of *N. glauca* Seedlings

AMF had a significant impact on total root length of *N. glauca* seedlings ($p = 0.0003$). In contrast, plant density and its interaction with AMF did not significantly affect root length ($p = 0.80$ and $p = 0.96$, respectively). Across all planting densities, AMF-inoculated seedlings exhibited greater mean root length than non-inoculated seedlings (Figure 3A).

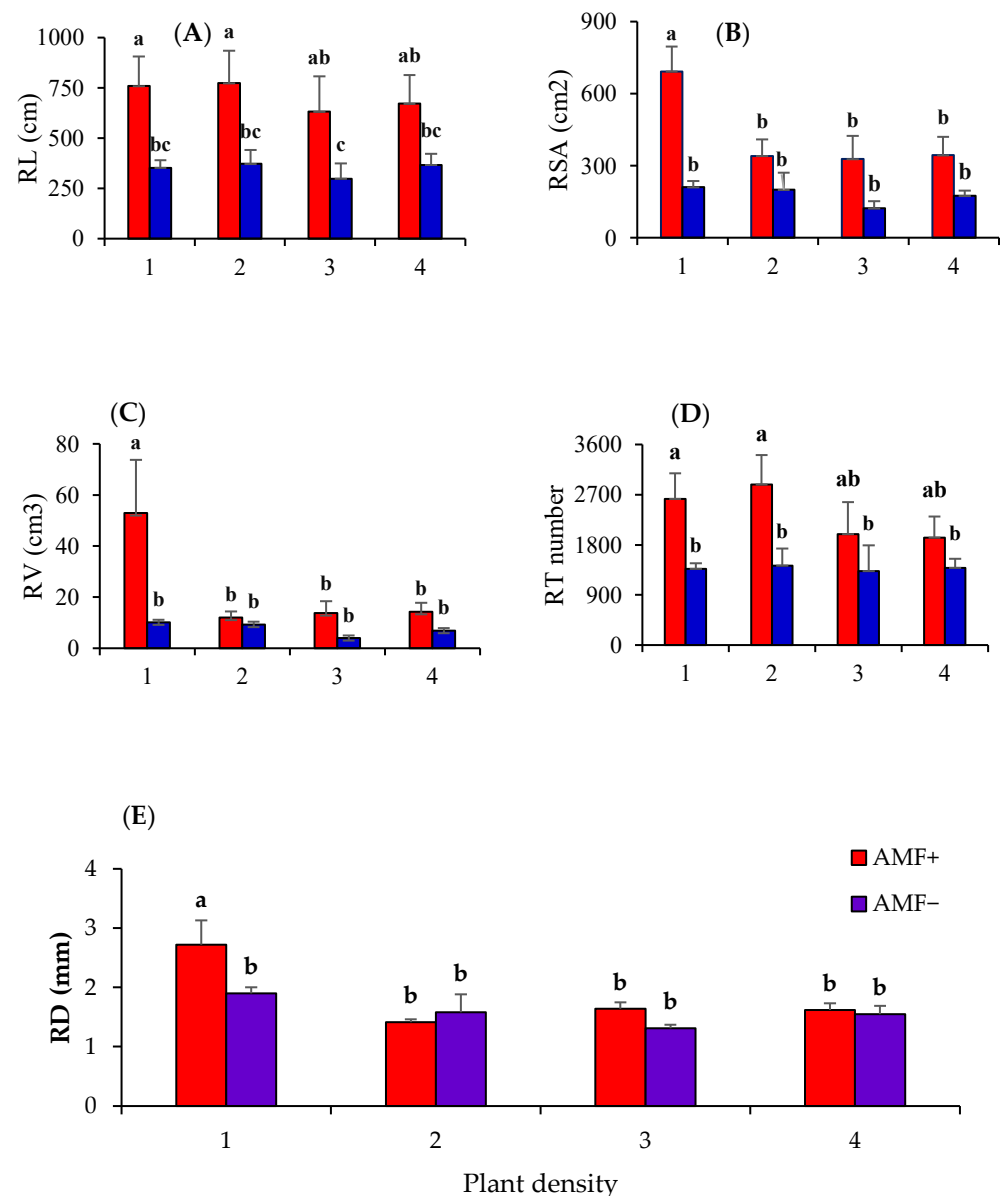


Figure 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and plant density on root length (A), root surface area (B), root volume (C), root tip numbers (D), and root diameter (E) of *N. glauca* seedlings. Data are presented as means and standard errors. Data are displayed as means and $\pm$ standard errors of mean. Letters above columns indicate significant differences between means across treatments according to the least significant difference (LSD) at significance level of $p < 0.05$.

Inoculation of AMF had a significant effect on root surface area of *N. glauca* seedlings ($p = 0.0009$). In contrast, plant density and its interaction with AMF did not significantly influence root surface area ($p = 0.09$ and $p = 0.25$, respectively). AMF-inoculated seedlings exhibited the greatest root surface area at one seedling per pot (Figure 3B).

Root volume of *N. glauca* seedlings was also impacted by AMF inoculation ($p = 0.009$) and seedling density ($p = 0.023$). The highest root volume was recorded at one seedling per pot in AMF-inoculated seedlings. Increasing plant density to two, three, and four plants per pot led to a progressive reduction in root volume in AMF-inoculated and non-inoculated seedlings (Figure 3C). This reduction was more pronounced in non-inoculated seedlings. No interaction effect between AMF inoculation and plant density was detected for root volume (Figure 3C).

AMF inoculation significantly affected the number of root tips in *N. glauca* seedlings ($p = 0.002$). On the other hand, plant density did not show a significant effect on root tip number ($p = 0.52$). Similarly, no interaction effect between AMF and plant density was detected for root tip number ($p = 0.61$). The highest number of root tips in AMF-inoculated seedlings was observed at two seedlings per pot (Figure 3D).

AMF inoculation had a significant effect on root diameter of *N. glauca* seedlings ($p = 0.0008$). In contrast, neither plant density nor its interaction with AMF significantly influenced root diameter ($p = 0.07$ and $p = 0.10$, respectively). Across treatments, AMF-inoculated seedlings generally exhibited larger root diameters, except at two seedlings per pot. The biggest root diameter was recorded in the treatment containing one seedling per pot, regardless of AMF treatment. Non-inoculated seedlings at treatment with three plants per pot showed the smallest root diameter, whereas AMF-inoculated seedlings exhibited the lowest root diameter at treatment, with two plants per pot (Figure 3E).

3.4. Effect of AMF Inoculation and Plant Density on Root Length Distribution Across Five Diameter Classes in *N. glauca* Seedlings

AMF inoculation significantly influenced root length across most root diameter classes, with the exception of the 4–5 mm diameter class. Specifically, AMF inoculation had significant effects on total root length within the 0–1, 1–2, 2–3, 3–4, and 4–5 mm diameter classes, with p -values of 0.0001, 0.05, 0.01, 0.02, and 0.07, respectively (Table 2). Across all diameter classes, AMF-inoculated seedlings exhibited greater root length per diameter class than non-inoculated seedlings. In both AMF-inoculated and non-inoculated seedlings, root length gradually decreased with increasing root diameter class (Table 2), with the majority of total root length concentrated in the smallest diameter class (0–1 mm). Plant density had no significant effect on root length within any diameter grade ($P = 0.52, 0.94, 0.73, 0.37$, and 0.16 for diameter grades 0–1, 1–2, 2–3, 3–4, and 4–5 mm, respectively). Similarly, no significant interaction between AMF inoculation and plant density was detected for root length across diameter grades ($p = 0.75, 0.48, 0.52, 0.31$, and 0.45 for diameter classes 0–1, 1–2, 2–3, 3–4, and 4–5 mm, respectively) (Table 2).

Table 2. Effect of arbuscular mycorrhizal fungi inoculation and plant density on root length (cm) distribution across five root diameter grades (0–1, 1–2, 2–3, 3–4, and 4–5 mm) in *N. glauca* seedlings.

Plant Density	Diameter Classes									
	0–1 mm		1–2 mm		2–3 mm		3–4 mm		4–5 mm	
	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–
1	546 ±85	240 ±16	103 ±19	50 ±15	40 ±15	20 ±7	19 ±8	11 ±2	48 ±20	30 ±8
2	652 ±148	255 ±36	73 ±18	68 ±17	27 ±6	21 ±9	7 ±4	9 ±3	12 ±7	17 ±3
3	439 ±123	214 ±63	87 ±25	48 ±13	49 ±15	12 ±3	21 ±6	5 ±1	33 ±11	18 ±4
4	652 ±103	241 ±33	81 ±17	73 ±15	45 ±10	27 ±5	24 ±6	10 ±3	41 ±13	14 ±1
LSD	412		85		48		24		48	

3.5. Effect of AMF Inoculation and Plant Density on Root Surface Area Distribution Across Five Diameter Classes in *N. glauca* Seedlings

AMF showed significant impact on root surface area (RSA) across all root diameter classes. Specifically, AMF inoculation significantly influenced RSA within the 0–1, 1–2,

2–3, 3–4, and 4–5 mm diameter classes, with p -values of 0.0001, 0.05, 0.01, 0.02, and 0.07, respectively (Table 3). Across all diameter classes, AMF-inoculated seedlings exhibited greater RSA per diameter class than non-inoculated seedlings. In both AMF-inoculated and non-inoculated seedlings, RSA increased with increasing root diameter class (Table 3), with the highest RSA observed in the largest diameter class (4–5 mm). Plant density had no significant effect on RSA within any diameter class ($p = 0.52, 0.94, 0.73, 0.37$, and 0.16 for the 0–1, 1–2, 2–3, 3–4, and 4–5 mm diameter classes, respectively) (Table 3).

Table 3. Effect of AMF inoculation and plant density on root surface area (cm²) distribution across five root diameter grades (0–1, 1–2, 2–3, 3–4, and 4–5 mm) in *N. glauca* seedlings.

Plant Density	Diameter Classes									
	0–1 mm		1–2 mm		2–3 mm		3–4 mm		4–5 mm	
	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–
1	65.2 ±11.2	29.4 ±3.1	45.2 ±9	22.5 ±7.2	31.0 ±12.3	15.9 ±5.7	22.7 ±8.7	12.4 ±3.0	177.4 ±69.9	109.6 ±25.9
2	66.4 ±14.6	29.2 ±5.1	32.6 ±8.2	30.5 ±8.2	23.4 ±5.3	16.8 ±7.4	8.1 ±5.1	10.4 ±3.9	87.6 ±22.2	61.2 ±18.4
3	48.9 ±13.8	21.9 ±5.3	39.7 ±11.2	22.5 ±6.3	38.0 ±12.4	9.2 ±2.5	23.5 ±6.7	6.1 ±1.4	99.7 ±34.1	50.9 ±15.7
4	53.1 ±10.4	27.5 ±3.9	36.6 ±7.8	34.4 ±6.9	34.6 ±8.2	21.1 ±4.0	26.8 ±6.8	11.5 ±4.3	112.8 ±32.7	36.1 ±1.6
LSD	27.7		24.1		23.4		16.0		97.3	

3.6. Effect of AM Inoculation and Plant Densities on Root Volume Distribution Across Five Diameter Class in *N. glauca* Seedling

AMF inoculation influenced root volume (RV) across root diameter classes. Specifically, AMF inoculation had significant effects on RV within the 0–1, 1–2, 2–3, 3–4, and 4–5 mm diameter classes, with $p = 0.0001, 0.05, 0.01, 0.02$, and 0.07 , respectively (Table 4). Across all diameter classes, AMF-inoculated seedlings exhibited higher RV per diameter class than non-inoculated seedlings. In both AMF-inoculated and non-inoculated seedlings, RV increased with increasing root diameter class (Table 4), with the greatest RV observed in the largest diameter class (4–5 mm). Plant density had no significant effect on RV within any diameter class ($p = 0.52, 0.94, 0.73, 0.37$, and 0.16 for the 0–1, 1–2, 2–3, 3–4, and 4–5 mm diameter classes, respectively) (Table 4).

Table 4. Effect of AMF inoculation and seedling density on root volume distribution across five root diameter grades (0–1, 1–2, 2–3, 3–4, and 4–5 mm) in *N. glauca* seedlings.

Plant Density	Diameter Classes									
	0–1 mm		1–2 mm		2–3 mm		3–4 mm		4–5 mm	
	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–
1	0.4418 ±0.076	0.2092 ±0.030	0.8169 ±0.171	0.4207 ±0.142	0.9612 ±0.387	0.4960 ±0.175	0.9961 ±0.378	0.5391 ±0.134	60.487 ±24.4	38.313 ±25.4
2	0.4058 ±0.091	0.1968 ±0.039	0.5960 ±0.150	0.5605 ±0.158	0.7307 ±0.165	0.5256 ±0.232	0.355 ±0.222	0.453 ±0.166	9.526 ±6.89	17.740 ±8.85
3	0.3168 ±0.091	0.1405 ±0.032	0.7447 ±0.207	0.4273 ±0.125	1.1899 ±0.395	0.2814 ±0.074	1.0137 ±0.291	0.2664 ±0.061	25.677 ±13.12	7.5802 ±3.59
4	0.3455 ±0.064	0.1869 ±0.028	0.6824 ±0.1433	0.6638 ±0.129	1.0740 ±0.254	0.6485 ±0.130	1.1653 ±0.296	0.5049 ±0.189	20.63 ±6.11	5.4250 ±0.431
LSD	0.1819		0.4544		0.7344		0.6924		41.216	

3.7. Effect of AMF Inoculation and Plant Densities on Root Tip Distribution Across Five Diameter Classes in *N. glauca* Seedlings

Inoculation of AMF had a significant impact on root tip number of *N. glauca* seedlings within the 0–1 mm ($p = 0.002$) and 3–4 mm ($p = 0.03$) root diameter classes. In contrast, AMF inoculation did not significantly affect root tip number within the 1–2 mm ($p = 0.21$), 2–3 mm ($p = 0.13$), or 4–5 mm ($p = 0.18$) diameter classes (Table 5). Across diameter classes, the greatest number of root tips was detected in the smallest diameter class (0–1 mm), followed by a progressive decline with increasing root diameter. Within each diameter class, AMF-inoculated seedlings consistently exhibited a higher number of root tips than non-inoculated seedlings (Figure 4). At comparable planting densities, AMF-inoculated seedlings showed greater root tip numbers than non-inoculated seedlings. Seedling density had no significant impact on root tip number across any of the diameter classes. Nevertheless, planting densities of three and four plants per pot exhibited lower root tip numbers compared with pots containing one and two seedlings (Table 5).

Table 5. Effect of AMF inoculation and plant density on root tip number distribution across five root diameter grades (0–1, 1–2, 2–3, 3–4, and 4–5 mm) in *N. glauca* seedlings.

Plant Density	Diameter Classes									
	0–1 mm		1–2 mm		2–3 mm		3–4 mm		4–5 mm	
	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–	AMF+	AMF–
1	2587 ±446	1338 ±98	22 ±6	18 ±4	9 ±4	4 ±1	3 ±1	2 ±0.6	4 ±1	5 ±0.9
2	2864 ±525	1402 ±305	16 ±3	15 ±3	3 ±1	3 ±0.9	1 ±0.7	1 ±0.5	2 ±0.4	3 ±1
3	1955 ±565	1309 ±463	20 ±7	11 ±3	7 ±2	4 ±1	3 ±1	0.25 ±0.02	6 ±2	3 ±0.5
4	1900 ±374	1365 ±164	14 ±2	11 ±1	6 ±1	6 ±1	2 ±0.8	1 ±0.6	7 ±3	1 ±0.6
LSD	1167.5		12.8		5.9		2.8		5.2	

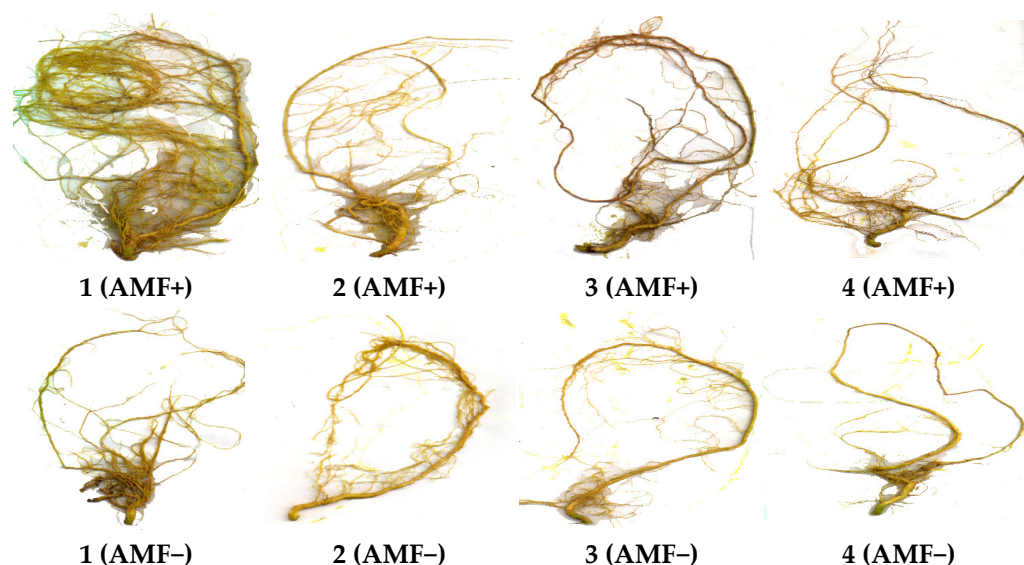


Figure 4. Effect of AMF inoculation and plant density on root morphological traits of *N. glauca* seedlings. Scale, 1:5.

3.8. Effect of AMF and Plant Density on Root-to-Shoot Ratio in *N. glauca* Seedlings

AMF inoculation and plant density significantly affected the root-to-shoot ratio of *N. glauca* seedlings. Across all planting densities, AMF-inoculated seedlings consistently exhibited higher root-to-shoot ratios than non-inoculated seedlings (Figure 5).

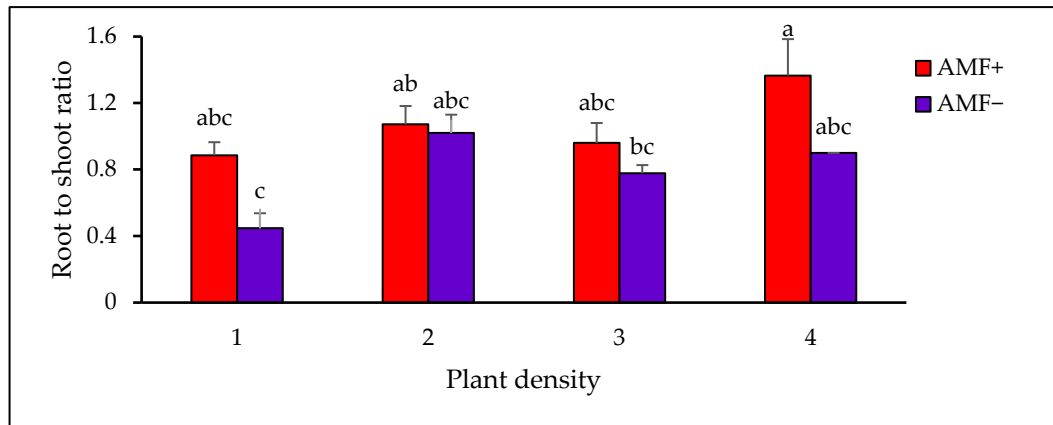


Figure 5. Effect of AMF and plant density on the root-to-shoot ratio of *N. glauca* seedlings. Data are displayed as means and $\pm$ standard errors of mean. Letters above columns indicate significant differences between means across treatments according to the least significant difference (LSD) at significance level of $p < 0.05$.

In AMF-inoculated seedlings, the root-to-shoot ratio increased progressively with increasing plant density, reaching the highest value at the density treatment containing four plants per pot. In contrast, non-inoculated seedlings exhibited relatively minor changes in root-to-shoot ratio across planting densities, with a slight increase from one to three plants per pot, followed by stabilization or a slight decline at four plants per pot. At low planting density (one plant per pot), differences in root-to-shoot ratio between AMF-treated and non-treated seedlings were small; however, this difference became more pronounced with increasing plant density, particularly at four plants per pot. Overall, AMF-inoculated seedlings maintained higher root-to-shoot ratios than non-inoculated seedlings across all planting densities (Figure 5).

3.9. Effect of AMF Inoculation and Plant Density on Nitrogen, Phosphorus, and Potassium Content in Roots of *N. glauca* Seedlings

AMF inoculation significantly affected root nitrogen (N%) and phosphorus (P%) content of *N. glauca* seedlings, with responses varying across planting densities (Table 6). Root phosphorus content (P%) was consistently higher in seedlings with AMF than in non-AMF seedlings across all planting densities, with the highest values recorded at densities of one and three plants per pot. Root nitrogen content (N%) was higher in AMF-inoculated seedlings at density treatments of one, two, and four plants per pot. At a planting density of three plants per pot, AMF-inoculated seedlings exhibited lower root N% compared with non-inoculated seedlings. Also, potassium was higher in seedlings treated with AMF across different densities compared to untreated counterparts. Overall, AMF inoculation increased root N, P, and K content at most planting densities, although the magnitude of the response differed among densities (Table 6).

Table 6. Effect of AMF inoculation and plant density on nitrogen and phosphorus content in roots of *N. glauca* seedlings.

Plant Density	N %		P %		K %	
	AMF+	AMF−	AMF+	AMF−	AMF+	AMF−
1	1.899 ±0.029	0.420 ±0.057	0.1353 ±0.011	0.0910 ±0.001	1.645 ±0.001	0.792 ±0.022
2	1.978 ±0.059	1.558 ±0.059	0.1088 ±0.000	0.0726 ±0.000	1.415 ±0.033	1.057 ±0.043
3	0.981 ±0.000	1.619 ±0.029	0.1428 ±0.000	0.1119 ±0.011	1.110 ±0.041	1.010 ±0.060
4	2.040 ±0.030	1.138 ±0.059	0.1083 ±0.012	0.0817 ±0.000	1.807 ±0.085	0.886 ±0.084
LSD	0.1334		0.0248		0.1763	

3.10. Correlation Analysis Between Root Traits of *N. glauca* Seedlings

Based on the correlation analysis of seedlings traits, results showed strong and significant positive relationships among most root traits of *N. glauca* seedlings. In particular, total RL, RSA, RV, and RT numbers were highly correlated with one another (Figure 6). These positive correlations were observed under both AMF-inoculated and non-inoculated conditions and across different plant densities. Root traits within finer diameter classes (1–3 mm) showed stronger correlations compared with those within coarser diameter classes (4–5 mm). Overall, correlation patterns indicated a high degree of association among multiple root traits across treatments (Figure 6).

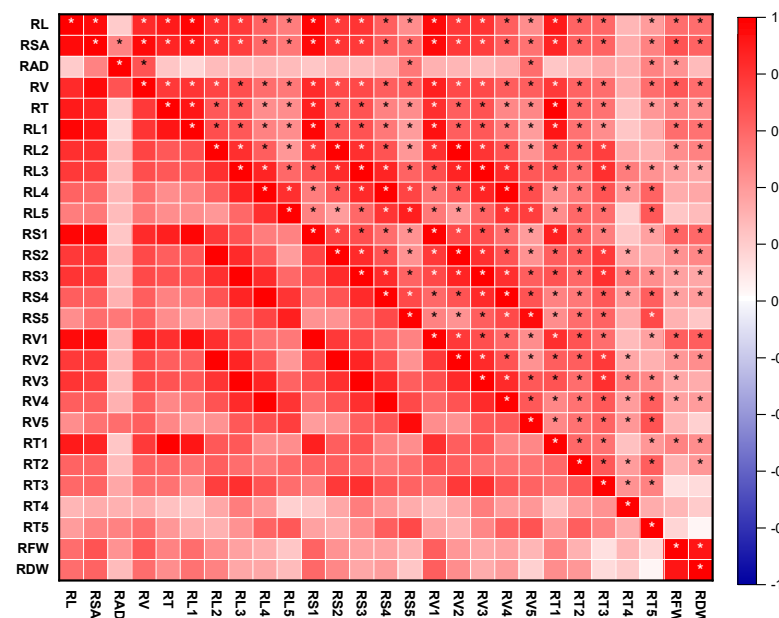


Figure 6. Correlations among root traits of *N. glauca* seedlings grown with and without AMF under varying plant densities. RL, root length; RS, root surface area; RV, root volume; RAD, mean root diameter; RT, root tip number. RL1–5, root length within diameter classes 1–5 mm; RV1–5, root volume within diameter classes 1–5 mm; RS1–5, root surface area within diameter classes 1–5 mm; RT1–5, root tip number within diameter classes 1–5 mm. * indicates a significant difference ($p < 0.05$).

3.11. Results of Principal Component Analysis (PCA)

Results of PCA showed a clear difference between AMF-inoculated and non-inoculated seedlings along the first principal component analysis (PC1), which explained 60.28% of

the total variance (Figure 7). AMF-inoculant treatments were primarily associated with the positive side of PC1 and showed strong loadings for root-related traits, including total root length (RL), root volume (RV), root surface area (RS), root tip number (RT), and root and shoot biomass. In contrast, non-inoculated seedlings were grouped on the left (negative) side of PC1. The second PC2 represented 16.11% of the total difference and was essentially linked with variant in root-to-shoot ratio. Several AMF-inoculated treatments exhibited higher scores along PC2 (Figure 7). Overall, the first two principal components summarized the multivariate variation in seedling traits across AMF inoculation treatments and planting densities.

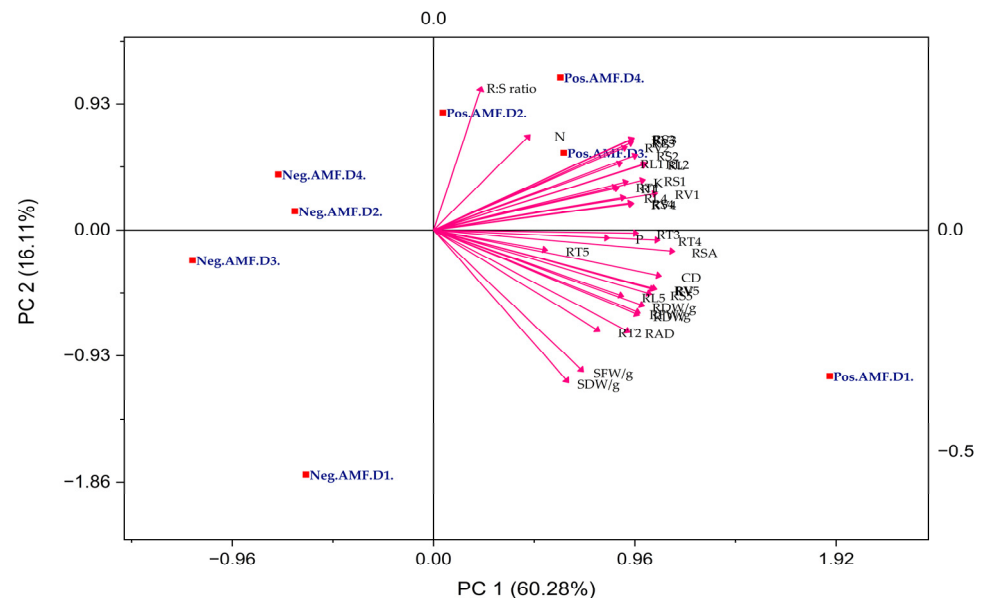


Figure 7. PCA demonstrating associations between seedling traits of *N. glauca*, AMF inoculation, and planting density treatments. Pos.AMF.D1–Pos.AMF.D4 represents AMF-inoculated seedlings grown at densities 1–4, while Neg.AMF.D1–Neg.AMF.D4 represents non-inoculated seedlings grown at densities 1–4. RL, total root length; RS, root surface area; RV, root volume; RAD, mean root diameter; RT, root tip number; CD, collar diameter. RL1–5, root length within diameter classes 1–5 mm; RV1–5, root volume within diameter classes 1–5 mm; RS1–5, root surface area within diameter classes 1–5 mm; RT1–5, root tip number within diameter classes 1–5 mm. N, nitrogen; P, phosphorus; RFW, root fresh weight; RDW, root dry weight; SFW, shoot fresh weight; SDW, shoot dry weight; R:S ratio, root-to-shoot ratio.

4. Discussion

4.1. AMF and Root System Development

The findings of this study demonstrate that AMF inoculation substantially enhanced key root parameters of *N. glauca* seedlings, including all root parameters across all planting densities when compared with non-inoculated counterparts. Such enhancement can be attributed to mycorrhizal colonization, which extends the effective absorptive surface of the root system through extraradical hyphal networks, thereby improving soil nutrient acquisition and stimulating root growth [33]. Similar findings have been stated in other invasive and native plant species. For example, both the invasive plant *Bidens alba* and the native *Bidens biternata* exhibited significant improvements in root traits following AMF inoculation under monoculture conditions, as reported by several studies [34]. These findings support the general role of mycorrhizal symbiosis in facilitating root system development by enhancing belowground resource access. In the present study, AMF-inoculated seedlings showed pronounced increases in total root length and root tip number within the finest root diameter class (0–1 mm) across different planting densities. This

response suggests a strong association between *N. glauca* seedlings and AMF at the fine-root level, where nutrient uptake is most efficient. Previous research has similarly revealed that AMF can promote the growth of invasive plants via aggregate fine-root development and functional efficiency [35]. An increase in fine-root length and root tip abundance has been linked to improved soil exploration capacity and enhanced nutrient acquisition, reinforcing the competitive performance of invasive species in resource-limited environments.

Consistent with the findings of the present study, seedlings inoculated with mycorrhizal fungi exhibited enhanced root system development, characterized by larger root traits. Previous studies have similarly reported that AMF-treated seedlings show significantly greater root parameters compared with non-inoculated controls under both normal and stress conditions [36]. In addition to structural root traits, AMF have been shown to stimulate root hair development in several plant species, such as trifoliate orange, through mycorrhiza-mediated regulation of auxin synthesis and transport, thereby enhancing seedling tolerance to environmental stress [37]. Such hormonal modulation contributes to improved root functionality and increases the efficiency of soil resource exploitation. The improved growth performance of mycorrhizal seedlings has also been linked to enhanced water uptake capacity, which results from increased root hydraulic conductivity, expanded absorptive root surface area, and a greater ability to access water retained in fine soil pores [38]. Together, these mechanisms show the important role of AMF in promoting root development and improving seedling performance under varying environmental conditions. Fungal species can modify plant root system architecture through alterations in endogenous auxin levels, which regulate primary root elongation and root hair development [39,40]. In addition to hormonal regulation, these changes are further influenced by environmental factors, including water availability and nutrient accessibility in the soil [41].

The observed increases in root parameters such as length, volume, surface area, and tip number across diameter classes in AMF-inoculated seedlings reflect a strategic adjustment of root parameters to optimize the gaining of growth-limiting resources. In particular, seedlings allocated a greater proportion of biomass to fine roots, which are primarily responsible for water and nutrient uptake. Root length has an important role in determining plant water efficiency and nutrient acquisition, as longer roots enhance soil exploration capacity [42]. Consistent with this pattern, previous research on *Quercus suber* has shown that root length decreases progressively with increasing root diameter class, highlighting the functional importance of finer roots [43]. Variations in root length among diameter classes can directly affect seedling performance, as root length is closely associated with root longevity and the ability of seedlings to acquire and transport soil nutrients [44]. Moreover, finer roots with a higher number of tips have been shown to increase the size of soil explored, thereby enhancing resource-uptake efficiency [45]. The increased number of root tips observed in AMF-inoculated seedlings under competitive conditions supports the role of biological interactions in improving plant tolerance to stressful environments, in agreement with previous findings [46]. At low planting density (one plant per pot), the higher root biomass of AMF-inoculated seedlings likely reflects greater access to soil resources. Similar responses have been reported in *Carthamus tinctorius* L., where AMF inoculation increased root length under non-stress conditions compared with stressed plants [47]. Overall, the enhanced growth of AMF-treated seedlings indicates improved plant water status under stress, reinforcing the role of AMF in promoting plant growth and stress tolerance [48].

The significant positive correlations observed among root traits under both AMF inoculation and varying plant density indicate a high degree of integration between structural and functional components of the root system. The strong associations among fine-root

traits (diameter classes 1–3) highlight their central role in resource acquisition and suggest enhanced absorptive efficiency, particularly under AMF symbiosis. In contrast, weaker correlations among coarse-root traits (diameter classes 4–5) reflect their primarily structural and supportive functions within the root system. Together, these patterns indicate that AMF association contributes to maintaining a coordinated root system organization, enabling seedlings to optimize resource uptake and sustain performance under increasing intraspecific competition.

4.2. Role of AMF in Enhancing Fresh, Dry Weights, and Shoot-to-Root Ratio of *N. glauca* Seedlings

Our results indicate that AMF inoculation and plant density significantly influenced root biomass in *N. glauca* seedlings. The marked increase in root biomass observed under AMF inoculation suggests that mycorrhizal symbiosis enhances the capacity of seedlings to acquire water and nutrients, particularly under conditions of low intraspecific competition. This response is consistent with previous findings showing that AMF exploit extensive extraradical hyphal networks to expand the effective root absorption zone, thereby improving access to phosphorus and other essential nutrients. Enhanced nutrient acquisition, in turn, promotes greater root production and increased carbon allocation to belowground biomass [2].

Our results further showed that increasing plant density led to a progressive decline in RFW and RDW [49] across all treatments, reflecting intensified intraspecific competition for soil resources. Despite this overall decline, AMF-inoculated seedlings consistently maintained higher root biomass than non-inoculated seedlings across all planting densities, indicating that AMF inoculation partially mitigated the negative effects of intraspecific competition. Results from different studies have similarly reported that AMF-associated plants often exhibit improved growth performance and enhanced resource-use efficiency under competitive conditions, largely due to improved nutrient acquisition and increased tolerance to environmental stress [47].

The root-to-shoot ratio of *N. glauca* seedlings was influenced by AMF inoculation and plant density (Figure 7), indicating a mycorrhiza-mediated shift in biomass allocation toward the root system. This shift can be attributed to improved nutrient and water uptake, particularly phosphorus, which stimulates root growth. Enhanced acquisition of essential nutrients, especially phosphorus, nitrogen, and potassium, has been shown to stimulate root production in AMF-associated plants [2]. In the present study, increasing plant density resulted in higher root-to-shoot ratios in AMF-inoculated seedlings, suggesting that AMF facilitate adaptive biomass partitioning toward root growth under intensified intraspecific competition. Such allocation patterns enhance seedlings' ability to explore limited soil nutrients more effectively [50]. In contrast, non-inoculated seedlings exhibited only minor changes in root-to-shoot ratio across planting densities, indicating reduced plasticity in biomass allocation. The interaction between AMF inoculation and plant density further suggests that the beneficial influences of AMF become more evident under high-density conditions, where competition for nutrients and space is intensified. These results are consistent with varied studies demonstrating that AMF enhance seedling tolerance to competitive stress by improving nutrient-use efficiency and sustaining root growth under resource-limited environments [51].

4.3. AMF Mediated Modulation of Intraspecific Interaction

In our study, the plant densities used (1 to 4 plants/pot) were selected to create a controlled gradient of intraspecific competition and to evaluate how increasing neighbor abundance affects seedling growth. Although these densities do not directly represent specific field population densities, they reflect conditions that may occur during different

stages of population establishment and local expansion. In general, in natural invasions, plant performance is influenced by varied factors, including competition with other species, herbivory, pathogen pressure, and resource availability [52]. A key finding of this study is that AMF modulate the intensity of intraspecific competition among *N. glauca* seedlings. Although increasing plant density generally reduced seedling growth, the presence of AMF attenuated the magnitude of this reduction, resulting in more balanced root development and biomass allocation among individuals. This observation is consistent with previous studies demonstrating that AMF can either amplify or mitigate plant–plant interactions, depending on species identity and availability of soil resources [53]. In the *N. glauca* case, the alleviative effect observed under AMF inoculation suggests that mycorrhizal symbiosis may promote competitive neutrality among conspecific individuals. Such neutrality is likely mediated through enhanced phosphorus, nitrogen, and potassium acquisition efficiency within the rhizosphere, reducing disparities in resource uptake among neighboring plants [54]. Improved nutrient sharing and reduced resource-use asymmetry may, in turn, facilitate coexistence among individuals under high-density conditions [55]. This mechanism may be particularly advantageous for *N. glauca* and other invasive species, as it supports the establishment and persistence of dense monospecific stands under competitive environments.

This study demonstrated strong positive correlations particularly among root traits. The uniformly positive correlations likely reflect coordinated root development, whereby AMF colonization promotes simultaneous increases in root size, branching, nutrient acquisition, and biomass, leading to concurrent improvement across multiple root attributes [56]. The PCA results indicate that AMF symbiosis represents the primary factor structuring the performance of *N. glauca* seedlings under intraspecific competition. AMF-inoculated seedlings clustered closely with key indicators of enhanced root traits, along with greater shoot and root biomass, reflecting a coordinated improvement in below- and aboveground growth. These results are in parallel with studies showing that AMF enhance nutrient uptake and root foraging capacity, resulting in substantial improvements in plant growth [2]. In contrast, non-inoculated seedlings formed a distinct cluster characterized by lower values for most growth-related parameters, suggesting that the absence of AMF limits resource acquisition. This pattern agrees with earlier reports indicating that the lack of mycorrhizal association can constrain root development and reduce competitive ability, particularly under nutrient-limited conditions [57]. Furthermore, the second principal component highlighted the contribution of AMF to nitrogen uptake and more balanced biomass allocation. Similar PCA-based patterns have been reported in other plant species, where AMF symbiosis enhances nitrogen acquisition and influences carbon partitioning between roots and shoots [58]. Although increasing plant density slightly reduced the magnitude of AMF-associated benefits, inoculated seedlings consistently outperformed non-inoculated counterparts across all densities. This pattern indicates that AMF alleviate competitive stress by increasing access to otherwise unavailable soil nutrients [59].

5. Conclusions

This study demonstrates that AMF exert strong effects on multiple root morphological traits of the invasive species *N. glauca*. Fine roots within the smallest diameter class (0–0.1 mm) accounted for the largest proportion of total root length, and root length within this class was strongly correlated with root surface area and root tip number, underscoring the functional importance of fine-root development. AMF-induced enhancements in root morphological traits, particularly fine-root length and branching, improve the ability of *N. glauca* seedlings to compete intraspecifically under conditions where growth-limiting resources remain available. Accordingly, AMF inoculation emerges as a potential key factor

shaping intraspecific competitive performance and resource-use strategies of *N. glauca* seedlings, especially under high-density conditions. The positive correlations observed between AMF symbiosis and root traits further highlight a close link between symbiotic association and host functional performance. This relationship suggests a high degree of plasticity in the responsiveness of *N. glauca* to AMF colonization, a trait that may potentially contribute to its broad ecological tolerance and invasive success. Moreover, AMF played a crucial role in mitigating the negative effects of intraspecific competition by enhancing phosphorus, nitrogen, and potassium acquisition and promoting greater biomass allocation to root systems. Such shifts in root growth and biomass accumulation likely support persistence and competitive dominance of *N. glauca* under stressful and resource-limited conditions. Overall, these findings improve our understanding of how belowground biotic interactions influence the success of invasive plants and emphasize the importance of incorporating mycorrhizal ecology into studies of plant invasion processes. Further field studies incorporating natural community interactions and environmental variability would be valuable for validating the ecological relevance of these findings.

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