horticulturae

MDPI

Article

The Effect of Arbuscular Mycorrhizal Fungi on the Canopy and Root Growth of *Opuntia ficus-indica* (L.) Mill. Potted Plants

Giuseppe Greco [1], Francesco Gargano [1], Guido Lingua [2], Nadia Massa [2], Raimondo Gaglio [1], Luca Settanni [1], Paolo Inglese [1] and Giorgia Liguori [1,*]

[1] Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Ed. 4, 90128 Palermo, Italy; giuseppe.greco03@unipa.it (G.G.); francesco.gargano07@unipa.it (F.G.); raimondo.gaglio@unipa.it (R.G.); luca.settanni@unipa.it (L.S.); paolo.inglese@unipa.it (P.I.)

[2] Department of Science and Technological Innovation, University of Piemonte Orientale (UPO), Viale Teresa Michel 11, 15121 Alessandria, Italy; guido.lingua@uniupo.it (G.L.); nadia.massa@uniupo.it (N.M.)

* Correspondence: giorgia.liguori@unipa.it

Abstract

Cactus pear (*Opuntia ficus-indica* (L.) Mill.) is increasingly recognized as a climate-resilient crop in arid and semi-arid regions, yet its performance is often constrained by poor soil fertility and limited external inputs. Arbuscular mycorrhizal fungi (AMF) are known to enhance phosphorus uptake, water relations, and stress tolerance in many species, but their contribution to cactus pear growth remains largely unexplored. One-year-old cladodes were grown in pots filled with sandy loam soil, either inoculated with a mixed AMF consortium or kept as non-inoculated controls. Plant growth was assessed after 6 and 12 months by measuring cladode number and surface area, shoot and root dry weight, and biomass allocation indices. Inoculated plants produced more cladodes, developed a larger canopy surface area, and accumulated greater root and shoot biomass than controls. These gains reflected an overall acceleration of growth, while biomass partitioning (root-to-shoot balance) remained stable. AMF inoculation substantially enhanced the vegetative growth of *O. ficus-indica*, pointing to its promise as a sustainable practice for improving cactus pear cultivation in nutrient-poor and water-limited soils.

Keywords: cactus pear; AMF; biomass allocation; canopy development; root growth; sustainable agriculture

Academic Editor: Qiang-Sheng Wu

Received: 24 October 2025
Revised: 15 November 2025
Accepted: 17 November 2025
Published: 18 November 2025

Citation: Greco, G.; Gargano, F.; Lingua, G.; Massa, N.; Gaglio, R.; Settanni, L.; Inglese, P.; Liguori, G. The Effect of Arbuscular Mycorrhizal Fungi on the Canopy and Root Growth of *Opuntia ficus-indica* (L.) Mill. Potted Plants. *Horticulturae* **2025**, *11*, 1392. https://doi.org/10.3390/horticulturae11111392

1. Introduction

Opuntia ficus-indica (L.) Mill., commonly known as cactus pear, is a perennial CAM species widely cultivated in arid and semi-arid regions for its fruits, cladodes, and ecosystem services [1–4]. Its high water-use efficiency, drought tolerance, and ability to thrive on marginal soils make it strategically valuable for sustainable agriculture in drylands, where it contributes to soil conservation, carbon storage, and rural livelihoods [5–7]. Mature cladodes serve as forage for livestock, providing water, carbohydrates, and minerals while supporting agricultural resilience in drought-prone areas [8].

Despite these advantages, cactus pear performance is often constrained by poor soil fertility and limited water availability, as fertilizer inputs are rarely feasible in marginal lands [7,9]. Low-input strategies are therefore needed to sustain growth and productivity.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plant roots that enhance nutrient acquisition—particularly phosphorus—improve water relations, increase

resilience under drought or salinity stress [10–15], and improve quality traits [16,17]. AMF are increasingly recognized as natural biofertilizers for resource-limited agroecosystems, enhancing nutrient-use efficiency in both major and niche crops.

While AMF benefits are well-documented in staples like cereals, legumes, and horticultural species such as tomato and maize [18,19], studies on AMF interactions with cactus pear remain scarce, particularly regarding juvenile plant stages and their inoculation responses in sandy-textured soils. Indeed, most mycorrhizal research has focused on mature plants or long-established field conditions, with fewer studies addressing the colonization dynamics and growth responses of young plantlets during their critical establishment phase. A recent study reported that AMF inoculation improves the nutritional quality of *O. ficus-indica* fruits and cladodes [20,21], but ecological and growth responses remain underexplored.

This gap is particularly relevant for *O. ficus-indica*, since sandy loam and sandy soils—characterized by limited fertility, low organic matter, and moderate water-holding capacity—dominate the Mediterranean and semiarid regions where cactus pear orchards are established. Under such conditions, the capacity of young *O. ficus-indica* cladodes to respond to AMF inoculation may differ substantially from mature plants, and the underlying mechanisms remain unexplored. Furthermore, the microbial community assembly and its dynamics in sandy substrates following AMF inoculation have not been previously characterized for this species, particularly regarding the three-way interactions between plant, AMF hyphae, and heterotrophic bacteria that shape nutrient cycling in the rhizosphere.

Considering that limitations of the soil volume available to the root system result in a continuous root turnover, while the canopy volume stops its growth [6], this study evaluated the effects of AMF inoculation on canopy development, root biomass, and biomass allocation in young *O. ficus-indica* plants grown in pots with sandy soil under greenhouse conditions. It was hypothesized that AMF would enhance shoot and root growth while maintaining stable biomass partitioning, supporting cactus pear cultivation in nutrient-poor, water-limited soils.

2. Materials and Methods

2.1. Experimental Site and Soil Characterization

The experiment was conducted in the greenhouse facilities of the Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Italy (38°07′ N, 13°02′ E; 29 m a.s.l.). The growth substrate was a sandy loam soil collected from the Botanical Garden of Palermo. Particle size distribution was determined by the hydrometer method [22]. Soil pH was measured in a 1:2.5 soil-to-water suspension [23]. Organic matter (OM) was determined by the Walkley–Black oxidation method [24], and total nitrogen (N) by the Kjeldahl procedure [25]. Bulk density (BD) was measured using the core method [26]. The soil water retention curve was determined up to −100 kPa using the hanging water column method on porous plates [27]. Undisturbed soil cores were equilibrated sequentially at decreasing matric potentials, and gravimetric water content was obtained by oven drying. Conversion to volumetric water content was then derived using the van Genuchten [28] water retention model. Because the permanent wilting point conventionally defined at −1500 kPa lies outside the measurement range of this technique, it was neither measured nor extrapolated.

To manage irrigation, field capacity (FC) was operationally set at −10 kPa, in accordance with the sandy loam texture of the substrate, while −33 kPa was taken as the lower threshold of the readily available water (RAW) range. Volumetric water contents (θv, cm^3 cm^{-3}) measured along the retention curve were converted into absolute volumes using the relation:

$$\boldsymbol{V_{(water)} = \theta v \times Vpot}$$

where ***Vpot*** is the pot volume (7.5 L = 7500 cm^3).

2.2. Plant Material and Experimental Design

One-year-old cladodes of *Opuntia ficus-indica* (L.) Mill. cv. Gialla were collected from a commercial orchard located in Roccapalumba (Palermo province, Italy; 37°48′ N, 13°38′ E; 540–560 m a.s.l.). Cladodes were standardized by size (mean length 37.5 ± 1.2 cm, width 19.1 ± 0.8 cm, thickness 1.9 ± 0.1 cm) and fresh weight (1.08 ± 0.06 kg). After a two-week curing period under shaded and ventilated conditions, cladodes were individually planted in 7.5 L plastic pots (60 in total) filled with the sandy loam soil described above. A two-month establishment period was allowed before treatments were imposed. Pots were then randomly assigned to two groups: AMF-inoculated (INOC, n = 30) and non-inoculated control (CTR, n = 30). Plants were maintained under greenhouse conditions, irrigated to keep soil moisture close to field capacity, and received no fertilization throughout the experiment.

2.3. Inoculum Preparation and Application

The AMF inoculum was produced on *Sorghum bicolor* L. grown under greenhouse conditions at the University of Piemonte Orientale (UPO, Alessandria, Italy). It consisted of a mixed community of Glomeromycota, including *Viscospora viscosa* (formerly *Glomus/Septoglomus viscosum*), *Rhizoglomus intraradices*, *R. irregulare*, *R. aggregatum*, and *Glomus* spp., according to the current MycoBank classification and recent taxonomic updates [29,30]. The inoculum contained spores, root fragments, and extraradical hyphae, with an estimated density of ~85,000 infective propagules L^{-1}. At planting, 500 mL of inoculum was applied around the cladode base in the inoculated treatment (INOC). Control pots (CTR) received the same substrate without inoculum.

2.4. Canopy and Root Measurements

Six and 12 months after planting, 15 plants per sampling date per treatment (CTR and INOC) were dug out carefully and separated into shoots (mother and first-generation cladodes) and roots. Samples were oven-dried at 65 °C to constant weight, and biomass components were determined as total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), mother cladode dry weight, and first-generation cladode dry weight. In addition, the number of cladodes per plant was recorded at each harvest. Cladode surface area was measured by image analysis from scanned/photographed silhouettes using ImageJ v1.54 (National Institutes of Health, Bethesda, MD, USA); total canopy area per plant was computed as the sum across cladodes.

2.4.1. Growth Response and Effect Size Indices

Growth responses to AMF inoculation were quantified using several effect size indices. The mycorrhizal growth response (MGR%) was calculated as the relative difference in biomass between inoculated and control plants [31,32]. In addition, the log response ratio (lnRR) was computed for total (lnRRt), shoot (lnRRs), and root biomass (lnRRr) [33,34], with 95% confidence intervals estimated using the delta method. Relative growth rate (RGR) between 6 and 12 months was estimated at the population level, based on mean biomass values from the two destructive harvests, following standard growth analysis procedures [35–37].

2.4.2. Biomass Allocation Indices

Root Mass Fraction (RMF) was calculated as the proportion of root biomass relative to total plant biomass, providing a measure of carbon allocation to belowground organs:

$$RMF = \frac{RDW}{TDW}$$

where RDW is root dry weight and TDW is total dry weight.

Shoot Mass Fraction (SMF) was defined as the proportion of shoot biomass relative to total biomass and was obtained as:

$$SMF = \frac{SDW}{TDW} = 1 - RMF$$

where SDW is shoot dry weight.

Root-to-Shoot ratio (R:S) was used as an indicator of biomass allocation balance between below- and aboveground organs and was calculated as:

$$R{:}S = \frac{RDW}{SDW}$$

where RDW is root dry weight and SDW is shoot dry weight [34–36].

These indices are standard metrics in plant growth analysis [35–38].

2.4.3. Allometric Analysis

Root–shoot allometry was analysed by fitting log–log relationships between root and shoot biomass using Standardized Major Axis (SMA) regression [39]:

$$ln(RDW) = \alpha + \beta ln(SDW)$$

where α is the intercept and β the scaling exponent. Slopes were compared between treatments, and intercepts were tested where slopes were homogeneous.

2.4.4. Photosynthetic Area Indices

Specific Cladode Area (SCA) was calculated as the ratio of total cladode surface area to shoot dry weight, providing an estimate of photosynthetic surface deployed per unit biomass [38,39]:

$$SCA = \frac{CanopyArea}{SDW}$$

Leaf Area Ratio (LAR) was defined as the ratio of total cladode surface area to total dry weight, reflecting the relative investment in photosynthetic area per unit of whole-plant biomass [40,41]:

$$LAR = \frac{CanopyArea}{TWD}$$

Specific Cladode Mass (SCM), the inverse of SCA, was calculated as the ratio of shoot dry weight to total cladode surface area, indicating the structural investment per unit photosynthetic surface [40,41].

$$SCM = \frac{SDW}{CanopyArea} = SCA^{-1}$$

2.5. Microbial Quantification and Derived Indices

At 0, 6, and 12 months, rhizosphere soil samples (n = 3 per treatment) were collected. Serial decimal dilutions prepared in Ringer's solution (Sigma-Aldrich, St. Louis, MO, USA) were plated on Nutrient Agar (NA; Sigma-Aldrich, St. Louis, MO, USA) for total

bacteria and Potato Dextrose Agar (PDA; Sigma-Aldrich, St. Louis, MO, USA) for total filamentous fungi to ensure selective growth, NA was supplemented with cycloheximide (Sigma-Aldrich, St. Louis, MO, USA) (10 mg mL^{-1}) to inhibit fungal proliferation, while PDA was enriched with chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA) (0.1 g/L) to suppress bacterial development. Plates were incubated at 30 °C for 3 days (bacteria) and 7 days (fungi), and colony-forming units (CFU) were counted. Microbial abundances were expressed as $\log_{10}$ CFU g^{-1} dry soil [42,43]. The AMF inoculum itself contained a baseline microbial load of 6.43 ± 0.15 $\log_{10}$ CFU g^{-1} for bacteria and 4.78 ± 0.10 $\log_{10}$ CFU g^{-1} for fungi.

From raw counts, Δlog CFU (difference in $\log_{10}$ CFU between treatments or sampling times), the log response ratio (lnRR) [42,43], the area under the microbial curve (AUMC) [42,44], and the fungal share (%) were calculated. While Δlog CFU and lnRR are standard in microbial and ecological studies, the AUMC was originally developed in predictive food microbiology [44–47] and was here adapted to soil microbial counts.

Fungal share (%) was calculated as the relative proportion of filamentous fungi in the total microbial community, according to the formula:

$$\textbf{\textit{Fungal share \%}} = \frac{\textbf{\textit{TMF}}}{(\textbf{\textit{TMB}} + \textbf{\textit{TMF}})} \times \mathbf{100}$$

where ***TMB*** and ***TMF*** are bacterial and filamentous fungal counts ($\log_{10}$ CFU g^{-1} dry soil), respectively.

This index was introduced in this study as a simple descriptor of microbial balance between bacteria and fungi and does not derive from a previously established metric.

2.6. Root Colonization Analysis

The frequency (F%) and intensity (M%) of root colonization by arbuscular mycorrhizal fungi (AMF) were determined according to the method [48,49]. Fine roots were gently washed to remove soil particles, cut into ~1 cm segments, and stained following the original protocol, which involves tissue clearing in 10% (*w*/*v*) KOH, acidification with acetic acid, and subsequent staining with blue ink in an acidic medium.

For each sample, thirty root fragments were randomly selected and observed microscopically (×100–400 magnification). Each fragment was assigned to one of six colonization classes ranging from 0 (no fungal structures visible) to 5 (>90% of the root cortex colonized). Based on this scoring, two quantitative indices of mycorrhizal colonization were calculated:

$$\boldsymbol{F}(\%) = \frac{\boldsymbol{N_{myc}}}{\boldsymbol{N_{tot}}} \times \mathbf{100}$$

where $\boldsymbol{N_{myc}}$ is the number of colonized root fragments (scores 1–5) and N_{tot} is the total number of fragments observed.

$$\boldsymbol{M}(\%) = \frac{\mathbf{95}\boldsymbol{n_5} + \mathbf{70}\boldsymbol{n_4} + \mathbf{30}\boldsymbol{n_3} + 5\boldsymbol{n_2} + \boldsymbol{n_1}}{\boldsymbol{N_{tot}}}$$

where $\boldsymbol{n_1}$–$\boldsymbol{n_5}$ are the numbers of root fragments in classes 1–5, respectively.

The frequency (F%) describes the occurrence of AMF within the root system, while the intensity (M%) represents the mean degree of fungal colonization across all observed root fragments. Both parameters provide complementary information on the extent and distribution of mycorrhizal infection.

2.7. Statistical Analysis

All analyses were performed in R v.4.3.3 (R Core Team, Vienna, Austria).

For biomass traits (TDW, SDW, RDW, mother and first-generation cladodes, canopy area, and derived indices SCA, LAR, SCM), data were analysed using two-way ANOVA with Treatment (CTR vs. INOC) and Time (6 vs. 12 months) as fixed factors. Assumptions of normality and homogeneity of variances were checked with Shapiro–Wilk and Levene's tests, respectively; when violated, results were confirmed with robust ANOVA using heteroscedasticity-consistent (HC3) standard errors. Cladode number, as count data, was analysed with a generalized linear model (GLM) assuming Poisson distribution and log link, with dispersion checked and pairwise contrasts adjusted using Tukey correction.

Root–shoot allometry was tested with Standardized Major Axis (SMA) regression (smart package v3.4-8). Slopes were compared between treatments; when homogeneous, intercepts (elevations) were tested for differences.

Effect sizes were computed as Mycorrhizal Growth Response (MGR%), log response ratio (lnRR) for total, shoot, and root biomass, and canopy area, and Relative Growth Rate (RGR) between 6 and 12 months. Confidence intervals were estimated by bootstrap resampling (10,000 iterations) or by the delta method, as appropriate.

Microbial counts (TMB and TMF) were analysed with two-way ANOVA including Treatment (CTR vs. INOC) and Period (0, 6, 12 months) as fixed factors. Derived indices (Δlog CFU, lnRR, AUMC, fungal share) were calculated according to standard microbiological modelling approaches.

Root colonization (frequency, F%, and intensity, M%) was analysed with one-way ANOVA with Treatment (CTR vs. INOC) as the fixed factor, after verifying normality (Shapiro–Wilk) and homogeneity (Levene's test).

Results are presented as means ± SE (n = 15 per treatment and time for plant traits and root colonization; n = 3 for microbial counts). Post hoc comparisons were carried out with emmeans v1.10.0 (estimated marginal means) and Tukey adjustment.

Figures adopt a consistent color scheme: CTR = light grey, INOC = dark green (plants, bacteria), and purple (fungi). Significance is reported as ns (not significant), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or with homogeneous-group letters.

3. Results

3.1. Soil Characterization

The growth substrate was classified as a sandy loam, with 77.2% sand, 13.0% silt, and 9.8% clay (Figure 1A). It had a bulk density of 1.38 ± 0.05 g cm^{-3}, a slightly sub-neutral pH (6.9 ± 0.1), and low organic matter and nitrogen contents (8.2 ± 0.3 and 1.3 ± 0.2 g kg^{-1}, respectively), consistent with coarse-textured soils of limited fertility.

The soil water retention curve showed a volumetric water content of 35.7 ± 4.4 cm^3 cm^{-3} at saturation, decreasing to 26.2 ± 2.2 cm^3 cm^{-3} at −10 kPa, which was taken as field capacity (FC) given the sandy texture (Figure 1B). At −33 kPa, the water content was 19.7 ± 1.3 cm^3 cm^{-3}, while at −100 kPa it further declined to 14.3 ± 1.6 cm^3 cm^{-3}. These values confirm the moderate water-holding capacity typical of sandy loam soils, with most of the plant-available water stored between 0 and −33 kPa.

In line with the sandy texture, field capacity was set at −10 kPa, corresponding to a volumetric water content of 0.262 (1.97 L per pot), while −33 kPa corresponded to 0.197 (1.48 L per pot). The difference between these two thresholds defined the readily available water, equivalent to ~0.49 L per pot. Each irrigation event supplied ~1.6 L per pot, thus exceeding the RAW volume and including a small leaching fraction before equilibrating near FC. Each irrigation corresponded on average to ~6-day intervals, which were seasonally adjusted to every 5 days in summer, 7–8 days in spring and autumn, and about 8 days in winter.

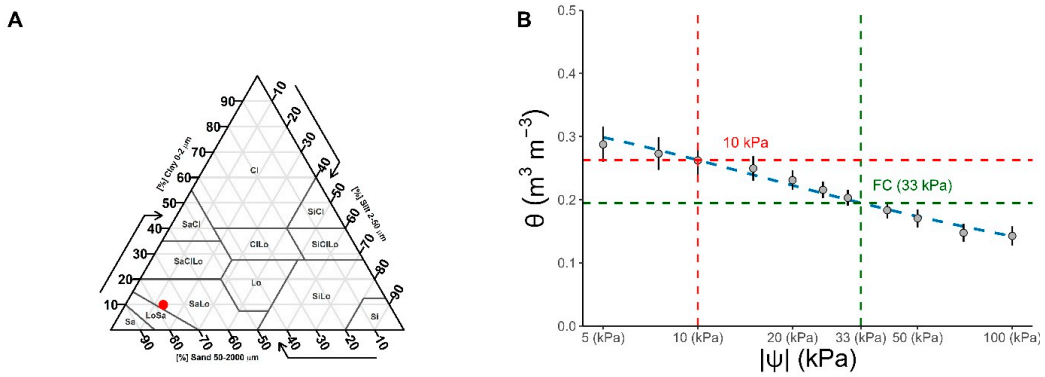

Figure 1. (**A**) USDA soil texture triangle showing the position of the experimental sandy loam soil (red dot). (**B**) Soil water retention curve of the experimental sandy loam. Symbols represent mean volumetric water content (θ, cm^3 cm^{-3}) ± SE (n = 3) measured at increasing matric potentials (0 to −100 kPa). The dashed blue line indicates the van Genuchten fit (θr = 0.0000, θs = 0.3528, α = 0.21069 kPa^{-1}, n = 1.297), while the dashed green and red lines mark soil water content at −33 kPa (field capacity, FC) and –10 kPa, respectively.

3.2. Biomass Accumulation

AMF inoculation markedly increased total biomass across harvests (Treatment: $F_{1,56}$ = 185.60, $p < 0.001$, η^2_p = 0.77), with plants also growing between 6 and 12 months (Time: $F_{1,56}$ = 41.98, $p < 0.001$) and no Treatment × Time interaction ($F_{1,56}$ = 2.64, p = 0.11). TDW averaged 221.3 ± 6.5 g vs. 133.6 ± 3.9 g at 6 months (+65.7%, lnRR = 0.51) and 311.3 ± 8.8 g vs. 168.3 ± 12.3 g at 12 months (+84.9%, lnRR = 0.62). Cumulative gains were +90.0 g in inoculated plants versus +34.8 g in controls, indicating a persistent size advantage without changes in trajectory (Figure 2). This corresponded to a mycorrhizal growth response (MGR%) of +65.7% at 6 months and +84.9% at 12 months, with lnRR values of 0.51 and 0.62, respectively (Table 1).

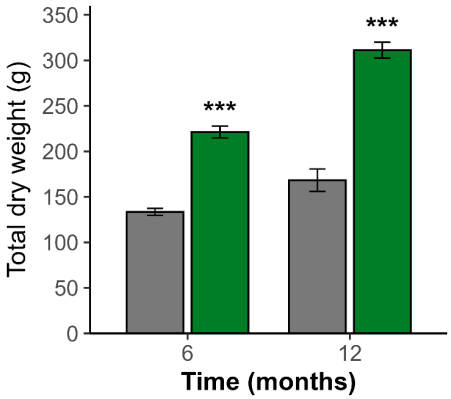

Figure 2. Total dry weight (TDW) of *Opuntia ficus-indica* plants grown with (INOC, dark green) or without (CTR, grey) AMF inoculation at 6 and 12 months. Bars represent means ± SE (n = 15). Asterisks indicate significant differences between treatments at each sampling time (*** $p < 0.001$; Tukey test).

Table 1. Effect size of AMF inoculation on biomass traits of *Opuntia ficus-indica*. Values are expressed as mycorrhizal growth response (MGR%) and log response ratio (lnRR), with 95% confidence intervals (CI) based on bootstrap or delta method estimates.

Variable (Name)	Time (Months)	MGR (%)	95% CI (MGR%—%)	lnRR	95% CI (lnRR—%)
TDW (Total dry weight)	6	65.7	53.7–79.5	0.51	0.43–0.59
	12	84.9	59.7–113.9	0.62	0.47–0.76

Table 1. *Cont.*

Variable (Name)	Time (Months)	MGR (%)	95% CI (MGR%—%)	lnRR	95% CI (lnRR—%)
SDW (Shoot dry weight)	6	61.6	48.7–76.9	0.48	0.40–0.57
	12	85.3	59.7–114.7	0.62	0.47–0.76
RDW (Root dry weight)	6	92.1	65.9–122.9	0.65	0.51–0.80
	12	82.4	36.9–144.3	0.60	0.31–0.89

3.2.1. Canopy and Root Dry Weight

AMF inoculation strongly increased both shoot (SDW; $F_{1,56}$ = 167.59, p < 0.001, η^2_p = 0.75) and root biomass (RDW; $F_{1,56}$ = 37.24, p < 0.001, η^2_p = 0.40). Time significantly affected SDW ($F_{1,56}$ = 42.65, p < 0.001) but not RDW ($F_{1,56}$ = 2.12, p = 0.15), and no Treatment × Time interactions were detected (p > 0.05). After 6 months, SDW averaged 186.6 ± 5.7 g vs. 115.5 ± 4.0 g (+61.6%) and RDW 34.7 ± 1.9 g vs. 18.1 ± 1.0 g (+92.1%) in inoculated versus control plants. At 12 months, SDW reached 268.6 ± 8.5 g vs. 144.9 ± 10.6 g (+85.3%), and RDW 42.7 ± 3.9 g vs. 23.4 ± 2.9 g (+82.4%) (Figure 3).

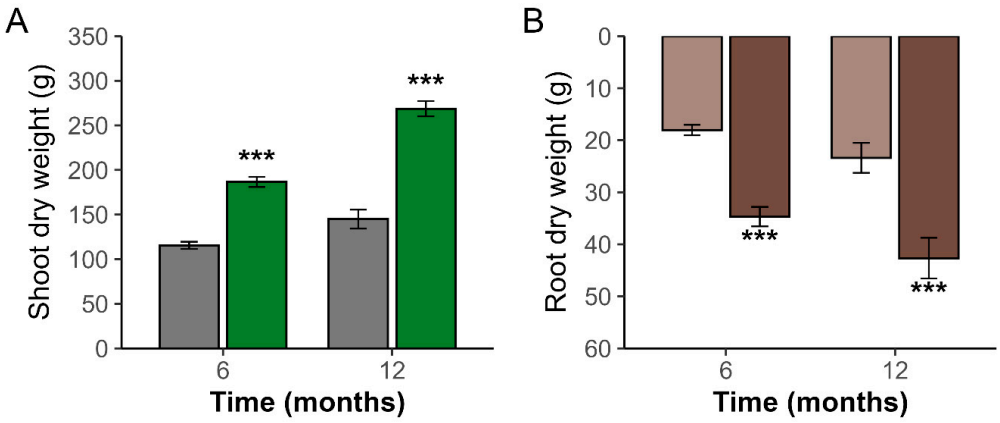

Figure 3. Shoot (**A**) and root (**B**) dry weight of *Opuntia ficus-indica* plants grown with (INOC, dark green in (**A**); dark brown in (**B**)) or without (CTR, grey in (**A**); light brown in (**B**)) AMF inoculation at 6 and 12 months. Bars represent means ± SE (n = 15). Asterisks indicate significant differences between treatments at each sampling time (*** p < 0.001; Tukey test).

Derived effect sizes further illustrate the positive influence of inoculation. For SDW, the mycorrhizal growth response (MGR%) was +61.6% (95% CI: 48.7–76.9%) at 6 months and +85.3% (95% CI: 59.7–114.7%) at 12 months, with lnRR values of 0.48 (95% CI: 0.40–0.57) and 0.62 (95% CI: 0.47–0.76). For RDW, MGR% was even higher at 6 months (+92.1%, 95% CI: 65.9–122.9%) and remained elevated at 12 months (+82.4%, 95% CI: 36.9–144.3%), with lnRR values of 0.65 (95% CI: 0.51–0.80) and 0.60 (95% CI: 0.31–0.89) (Table 1).

3.2.2. Mother and First-Generation Cladodes

AMF inoculation significantly increased the biomass of both mother cladodes ($F_{1,56}$ = 84.67, p < 0.001, η^2_p = 0.61) and first-generation cladodes ($F_{1,56}$ = 70.05, p < 0.001, η^2_p = 0.57). Time effects were weaker for mother cladodes ($F_{1,56}$ = 6.82, p = 0.012) but strong for first-generation cladodes ($F_{1,56}$ = 42.64, p < 0.001), with a significant interaction for mother cladodes only ($F_{1,56}$ = 4.35, p = 0.042). After 6 months, inoculated plants averaged 118.3 ± 3.1 g vs. 78.9 ± 4.2 g (+50.0%) for mother cladodes and 68.3 ± 5.1 g vs. 36.6 ± 3.5 g (+87.0%) for first-generation cladodes. At 12 months, differences widened further, with mother cladodes reaching 157.2 ± 6.8 g vs. 84.9 ± 8.3 g (+85.0%) and first-generation cladodes 111.5 ± 4.5 g vs. 60.0 ± 5.2 g (+85.6%) (Figure 4).

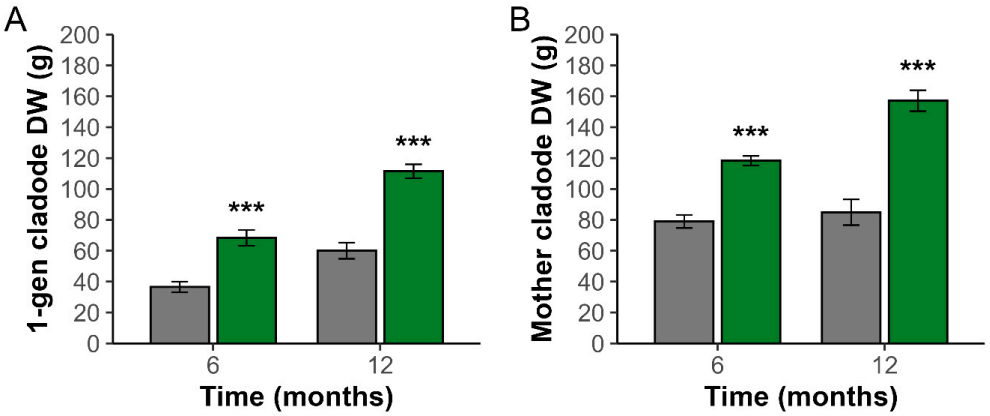

Figure 4. Dry weight of first-generation cladodes (**A**) and mother cladodes (**B**) of *Opuntia ficus-indica* plants after 6 and 12 months. Bars represent means ± SE (n = 15). Grey = uninoculated controls (CTR), dark green = AMF-inoculated plants (INOC). Asterisks indicate significant differences between treatments within each sampling time (*** $p < 0.001$; Tukey test).

Derived effect sizes further confirmed the magnitude of these responses. For mother cladodes, MGR% increased from +50.0% (95% CI: 35.7–65.6%) at 6 months to +85.0% (95% CI: 61.5–114.3%) at 12 months, with lnRR values of 0.42 (95% CI: 0.28–0.56) and 0.62 (95% CI: 0.47–0.76). For first-generation cladodes, MGR% was +87.0% (95% CI: 63.8–116.4%) at 6 months and +85.6% (95% CI: 59.7–115.1%) at 12 months, with lnRR values of 0.66 (95% CI: 0.45–0.87) and 0.62 (95% CI: 0.46–0.77) (Table 2).

Table 2. Effect size of AMF inoculation on mother and first-generation *Opuntia ficus-indica* cladodes. Values are expressed as mycorrhizal growth response (MGR%) and log response ratio (lnRR) with 95% confidence intervals (CI).

Variable (Name)	Time (Months)	MGR (%)	95% CI (MGR%—%)	lnRR	95% CI (lnRR—%)
Mother cladode	6	50.0	35.7–65.6	0.42	0.28–0.56
	12	85.0	61.5–114.3	0.62	0.47–0.76
First-generation cladodes	6	87.0	63.8–116.4	0.66	0.45–0.87
	12	85.6	59.7–115.1	0.62	0.46–0.77

3.2.3. Biomass Allocation

Biomass allocation patterns remained unaffected by AMF inoculation. Two-way ANOVA detected no significant effects of treatment, time, or their interaction on root mass fraction (RMF), shoot mass fraction (SMF), or the root-to-shoot ratio (R:S) (all $p > 0.19$). At 6 months, RMF averaged 0.137 ± 0.009 in controls and 0.157 ± 0.007 in inoculated plants, while SMF values were 0.863 ± 0.009 and 0.843 ± 0.007, respectively. Similar values were observed at 12 months, with RMF of 0.137 ± 0.013 and 0.136 ± 0.013, SMF of 0.863 ± 0.013 and 0.864 ± 0.013, and R:S ratios of 0.162 ± 0.018 and 0.161 ± 0.016 in controls and inoculated plants (Table 3), respectively.

3.2.4. Root–Shoot Allometry

Standardized Major Axis (SMA) regression revealed positive allometric relationships between root and shoot biomass, with slopes significantly greater than unity (CTR: 1.66, 95% CI: 1.18–2.36; INOC: 1.92, 95% CI: 1.33–2.79). Slopes did not differ between treatments ($\chi^2 = 0.32$, $p = 0.57$), indicating that AMF inoculation did not alter the scaling between canopy and root biomass.

Table 3. Root mass fraction (RMF), shoot mass fraction (SMF), and root-to-shoot ratio (R:S) of *Opuntia ficus-indica* plants under control (CTR) and AMF-inoculated (INOC) conditions at 6 and 12 months. Values are means ± SE (n = 15).

Treatment	Time (Months)	RMF (Mean ± SE)	SMF (Mean ± SE)	R:S (Mean ± SE)
CTR	6	0.137 ± 0.009	0.863 ± 0.009	0.161 ± 0.012
	12	0.137 ± 0.013	0.863 ± 0.013	0.162 ± 0.018
INOC	6	0.157 ± 0.007	0.843 ± 0.007	0.187 ± 0.010
	12	0.136 ± 0.013	0.864 ± 0.013	0.161 ± 0.016

ANCOVA confirmed a significant effect of shoot biomass ($p = 0.029$) and treatment ($p = 0.041$), whereas the interaction was not significant ($p = 0.48$). This suggests that inoculated plants had a higher elevation (intercept) of the root–shoot relationship, reflecting greater root biomass for a given shoot biomass, without changes in the proportional scaling. Overall, AMF inoculation increased the absolute investment in roots but preserved the underlying allometric balance between organs (Figure 5).

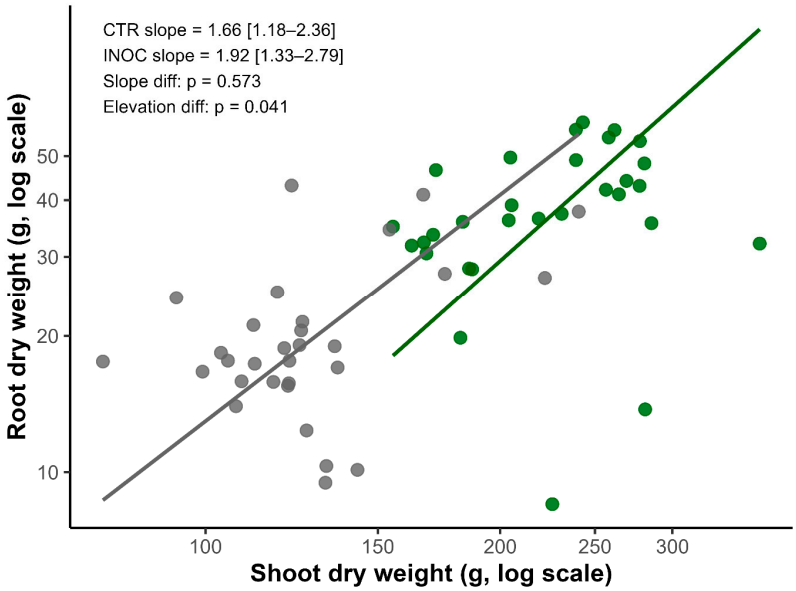

Figure 5. Standardized Major Axis (SMA) regressions between shoot dry weight (SDW) and root dry weight (RDW) of *Opuntia ficus-indica* plants after 6 and 12 months under control (CTR, grey) and AMF-inoculated (INOC, dark green) conditions. Points represent individual plants (n = 15 per treatment × time). Both groups showed slopes significantly greater than unity (CTR = 1.66 [95% CI: 1.18–2.36]; INOC = 1.92 [95% CI: 1.33–2.79]), with no difference between treatments ($p = 0.57$). ANCOVA indicated a significant elevation shift ($p = 0.041$), reflecting greater root biomass for inoculated plants at a given shoot biomass.

3.3. Cladode Development

AMF inoculation significantly increased cladode production (Treatment: $\chi^2_1 = 15.97$, $p < 0.001$), with a modest effect of time ($\chi^2_1 = 3.95$, $p = 0.047$) and no interaction ($p = 0.88$). At 6 months, inoculated plants bore 3.20 ± 0.11 cladodes compared with 1.67 ± 0.21 in controls, and at 12 months they had 4.27 ± 0.27 vs. 2.33 ± 0.21, respectively (Table 4). Post hoc tests confirmed significant differences between treatments at both harvests ($p < 0.05$), while both treatments showed a small, non-significant increase from 6 to 12 months (Table 4), with a slightly larger absolute gain in inoculated plants.

Table 4. Number of cladodes per plant in *Opuntia ficus-indica* after 6 and 12 months under control (CTR) and AMF-inoculated (INOC) conditions. Values are means ± SE (n = 15). Δ% (Treatment) represents the relative increase of inoculated plant compared with controls at each time point; Δ% (Time) represents the relative increase from 6 to 12 months within each treatment.

Treatment	Time (Months)	Cladodes (Mean ± SE)	Δ% (Treatment)	Δ% (Time)	*p*-Value
CTR	6	1.67 ± 0.2	–	–	–
	12	2.33 ± 0.21	–	+39.5% (ns)	0.573
INOC	6	3.20 ± 0.11 *	+91.6%	–	0.041
	12	4.27 ± 0.27 *	+83.3%	+33.4% (ns)	0.021

Asterisks denote significance levels (* $p < 0.05$; ns = not significant) according to Tukey's post hoc tests.

3.4. Canopy Surface Expansion

AMF inoculation markedly enhanced canopy expansion ($F_{1,56} = 173.94$, $p < 0.001$, $\eta^2_p = 0.76$), with a further effect of time ($F_{1,56} = 32.99$, $p < 0.001$) but no interaction ($p = 0.37$). At 6 months, inoculated plants had a canopy area of 2682.8 ± 93.3 cm^2 compared with 1540.8 ± 83.1 cm^2 in controls, and at 12 months the difference increased to 3647.8 ± 125.3 vs. 1925.0 ± 102.5 cm^2 (Figure 6). Effect size analysis confirmed this trend, with MGR% of +74.1% (95% CI: 54.7–97.1%; lnRR = 0.56) at 6 months and +89.5% (95% CI: 68.6–114.0%; lnRR = 0.64) at 12 months (Figure 6).

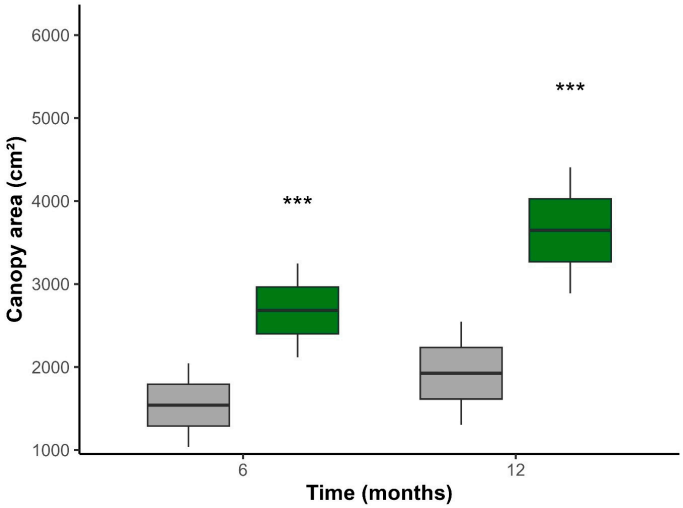

Figure 6. Canopy surface area of *Opuntia ficus-indica* plants after 6 and 12 months under control (CTR, grey) and AMF-inoculated (INOC, dark green) conditions. Boxplots show median, quartiles, and range; *** indicates significant differences between treatments within each time point (Tukey test, *** $p < 0.001$).

3.5. Physiological Responses

Indices of canopy physiology, including Specific Canopy Area (SCA), Leaf Area Ratio (LAR), and Specific Cladode Mass (SCM), did not vary significantly with AMF inoculation or time (all $p > 0.5$; Table 5). Average SCA values ranged between 11.7 and 12.3 $cm^2\ g^{-1}$, LAR between 13.7 and 14.6 $cm^2\ g^{-1}$, and SCM between 0.071 and 0.079 $g\ cm^{-2}$ across treatments and sampling dates. Partial η^2 values were consistently < 0.01, indicating negligible effect sizes. These results suggest that AMF effects on overall plant growth (biomass accumulation, cladode number, and canopy expansion) were not accompanied by detectable shifts in morphological efficiency indices at the whole-canopy level.

Table 5. Physiological indices of *Opuntia ficus-indica* plants after 6 and 12 months under control (CTR) and AMF-inoculated (INOC) conditions. Values are means ± SE (n = 15). SCA = Specific Canopy Area; LAR = Leaf Area Ratio; SCM = Specific Cladode Mass.

Treatment	Time (Months)	SCA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SCM ($g\ cm^{-2}$)
CTR	6	11.73 ± 0.76	13.68 ± 0.97	0.0787 ± 0.0058
	12	12.31 ± 1.14	14.27 ± 1.31	0.0789 ± 0.0072
INOC	6	12.30 ± 0.60	14.60 ± 0.72	0.0710 ± 0.0037
	12	11.87 ± 0.56	13.74 ± 0.59	0.0749 ± 0.0036

No significant effects of treatment, time, or their interaction were detected (two-way ANOVA, all $p > 0.5$).

3.6. Microbial Responses

Total mesophilic bacteria (TMB) showed clear temporal dynamics and a transient response to AMF inoculation. Two-way ANOVA detected a significant effect of time ($F_{2,12} = 5.99$, $p = 0.016$) and a near-significant treatment × time interaction ($p = 0.061$). At baseline (0 months), TMB were similar across treatments (6.97 vs. 6.78 log CFU g^{-1}). After 6 months, controls declined to 5.68 ± 0.24 log CFU g^{-1}, whereas inoculated plants maintained higher values (6.75 ± 0.24 log CFU g^{-1}), corresponding to a lnRR of 2.41 (95% CI: 1.47–3.42; +1013%). Effect sizes were calculated using the natural-log response ratio ($lnRR = \ln(X_1/X_2)$). At 12 months, bacterial counts partially recovered in controls (5.99 ± 0.24) but remained slightly higher in inoculated plants (6.23 ± 0.24), though the difference was not significant (lnRR = 0.36, 95% CI: −0.57–1.55) (Figure 7A). Cumulative abundance (AUMC) confirmed this pattern, with inoculated plants hosting 5.69 × 10^7 CFU g^{-1}, about 68% more than controls (3.38 × 10^7 CFU g^{-1}) (Table 6).

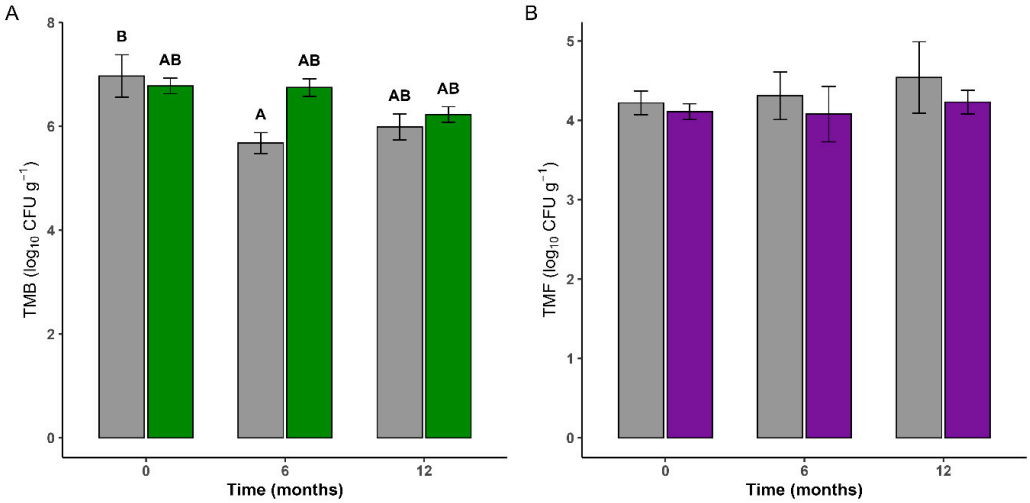

Figure 7. Rhizosphere microbial counts in *Opuntia ficus-indica* plants after 0, 6, and 12 months. (**A**) Total mesophilic bacteria (TMB) under control (CTR, light grey) and AMF-inoculated (INOC, dark green) conditions. (**B**) Total mesophilic fungi (TMF) under control (CTR, light grey) and AMF-inoculated (INOC, purple) conditions. Bars represent means ± SE (n = 3). Different letters indicate significant differences among treatment × time combinations according to Tukey's HSD post hoc test ($p < 0.05$).

Total mesophilic fungi (TMF) were unaffected by treatment or time (all $p > 0.3$), with average counts remaining between 4.1 and 4.5 log CFU g^{-1} across sampling dates and treatments (Figure 7B). Effect size analysis yielded non-significant lnRR values (−27 to −77%), and AUMC was lower in inoculated (1.62 × 10^5) than control plants (2.76 × 10^5).

Table 6. Derived microbial indices in the rhizosphere of *Opuntia ficus-indica* under control (CTR) and AMF-inoculated (INOC) conditions. lnRR = log response ratio (INOC/CTR) with 95% confidence interval; AUMC = area under the microbial curve; fungal share = proportion of fungal CFU relative to total microbial CFU.

Variable	Time (Months)	lnRR [95% CI]	Effect (%)	AUMC ($\times 10^7$ CFU g^{-1} d^{-1})	Fungal Share (%)
TMB	6	2.41 [1.47, 3.42]	1013%	CTR: 3.38–INOC: 5.69	–
TMB	12	0.36 [−0.57, 1.55]	+43.5%	–	–
TMF	6	−0.39 [−2.36, 1.23]	−32.5%	CTR: 0.028–INOC: 0.016	CTR: 4.5–INOC: 0.3
TMF	12	−1.47 [−2.54, 1.05]	−77.0%	–	CTR: 4.3–INOC: 1.0

The relative fungal share of the total microbial community was consistently low (<5%), increasing modestly in controls from 0.2% at baseline to 4.5% at 6 months, while remaining ≤1.0% in inoculated plants (Table 6).

3.7. Root Colonization Analysis

Root colonization analysis confirmed the establishment of arbuscular mycorrhizal fungi (AMF) in inoculated *Opuntia ficus-indica* plants (Figure 8).

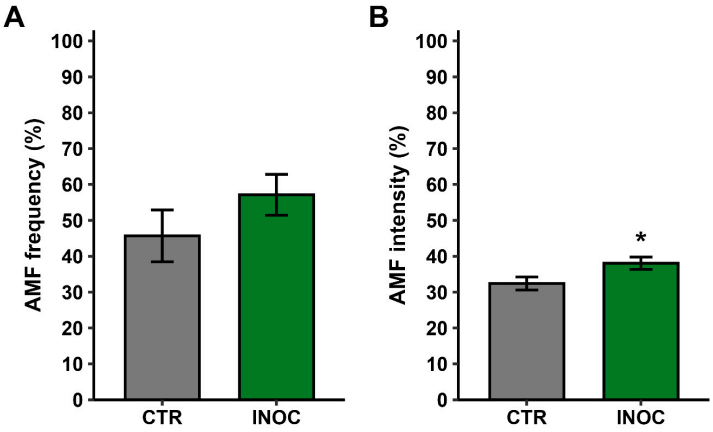

Figure 8. (**A**) Frequency and (**B**) intensity of AMF root colonization in *Opuntia ficus-indica* plants after 12 months under control (CTR, light grey) and AMF-inoculated (INOC, dark green) conditions. Bars represent means ± SE (n = 15). Asterisks indicate significant differences between treatments ($p < 0.05$; Tukey test). CTR = uninoculated control; INOC = plants inoculated with a mixed AMF consortium.

Mycorrhizal frequency (F%) averaged 57.1 ± 5.7% in inoculated plants and 45.7 ± 7.2% in controls, showing a higher mean value in the inoculated treatment, although the difference was not statistically significant ($p = 0.22$). Mycorrhizal intensity (M%) was significantly greater in inoculated roots (38.1 ± 1.7%) compared with controls (32.4 ± 1.8%, $p = 0.032$), confirming the establishment of the symbiosis.

4. Discussion

AMF inoculation nearly doubled plant growth compared with non-inoculated controls, with total biomass increasing by 66–85% and canopy surface by up to 90% within the first year. These responses are among the strongest AMF-induced growth effects reported for cactus species and align with earlier studies showing enhanced survival and nutrient uptake in *O. albicarpa* plantlets [21], increased biomass, photosynthesis, and water-use efficiency in *O. ficus-indica* under contrasting water regimes [50,51], and improvements in cladode and fruit nutritional quality [20]. Taken together, these findings highlight the remarkable responsiveness of young *O. ficus-indica* to symbiotic inoculation when grown on nutrient-poor sandy soils. The strong stimulation of growth observed in inoculated plants is consistent with the well-established capacity of AMF to enhance nutrient acquisition—particularly phosphorus and nitrogen—while improving plant water relations through

extraradical hyphal exploration, increased root absorptive surface, and enhanced hydraulic conductivity [10–15]. Such improvements in resource acquisition and water relations likely reflect an effective root colonization by AMF, which enhances both nutrient foraging and hydraulic capacity. Similar responses have been documented in other *Opuntia* species, where AMF improved drought tolerance, photosynthesis, and nutrient uptake in *O. ficus-indica*, *O. albicarpa*, and *O. streptacantha* under contrasting environmental conditions [21,50–53]. Similarly, studies on *Nopalea cochenillifera* have reported significant increases in fresh and dry biomass following inoculation with Glomus spp. [54] other research reported that inoculation with arbuscular mycorrhizal fungi promoted vegetative growth on *Hylocereus* spp. [55]. However, some research shows more moderate or context-dependent effects, depending on the environmental conditions and fungal strain used [34].

From an agronomic perspective, AMF inoculation emerges as a valuable tool to strengthen cactus-based systems. By promoting biomass and canopy growth without altering partitioning, inoculated plants can sustain greater photosynthetic capacity and higher cladode yields, a trait of particular relevance for semiarid forage production [56]. Despite these gains, biomass allocation patterns remained stable, with root mass fraction (0.14–0.16), shoot mass fraction (0.84–0.86), and root-to-shoot ratio (0.16–0.19) showing negligible effect sizes. These values are consistent with previous reports for *O. ficus-indica* under greenhouse conditions, where roots typically account for ~13% of total biomass [57]. The relatively low RMF and R:S confirm the limited root investment characteristic of CAM plants, while the dominant shoot fraction reflects the multifunctional role of cladodes as photosynthetic and storage organs [58,59]. At the canopy level, the low specific surface (SCA, LAR) and high tissue density (SCM) further align with strategies of slow-growing succulents compared with fast-growing herbs [37,60–62].

The allometric analysis further confirmed that AMF inoculation increased both root and shoot biomass proportionally, shifting the elevation of the root–shoot relationship without altering its slope. This indicates a conservative response, where overall growth was amplified but the structural balance between below- and above-ground organs remained unchanged. Such stability contrasts with other functional groups, where AMF frequently induce plastic reallocations of biomass. In grasses and annuals, colonization often increases root fractions and R:S ratios [63]. In maize subjected to drought, AMF have been shown to promote root proliferation at the expense of shoot growth [64]. Similarly, tree seedlings can display plastic shifts in leaf area ratios in response to symbiosis [65]. In *O. ficus-indica*, by contrast, the symbiosis boosted absolute biomass production while preserving conservative allocation patterns, underscoring the robustness of its growth strategy under mycorrhizal inoculation.

The magnitude of the response observed here can also be interpreted considering the general functional diversity of the AMF taxa present in the inoculum, without implying species-specific effects.

Viscospora viscosa (formerly *Glomus/Septoglomus viscosum*) is known to enhance phosphorus uptake and to promote glomalin production, which improves soil structure and water retention, thereby supporting biomass accumulation in nutrient-poor substrates [66,67]. *Rhizoglomus intraradices* has been extensively documented to increase nutrient acquisition, particularly phosphorus through its extraradical hyphae, while also conferring tolerance to abiotic stresses such as drought and metal(loid) contamination [68–70]. Similarly, *Rhizoglomus irregularis*, the genomic model species of AMF and the most widely propagated in commercial inoculants, is recognized for its ability to improve plant performance under nutrient and water limitations, and for its remarkable plasticity in symbiotic development [71,72]. *Rhizoglomus aggregatum* has been shown to enhance host performance under environmental stress, improving biomass accumulation, chlorophyll concentration, and stress tolerance in

both millets exposed to high temperatures and sunchoke subjected to drought, confirming its role as an effective AMF species under adverse conditions [73,74]. Finally, *Glomus* species remain among the most widely studied AMF taxa, frequently reported to increase plant nutrient acquisition, biomass production, and stress resilience across diverse hosts. Strains such as *G. hoi* and *G. etunicatum* have been associated with improvements in phosphorus and nitrogen contents, tuber yield, and inulin stability even under drought, highlighting the broad ecological versatility of *Glomus spp*. and their relevance for crop resilience in water-limited and nutrient-poor environments [75,76]. Taken together, the complementary functions of these AMF taxa provide a mechanistic explanation for the exceptional biomass and canopy gains achieved in *O. ficus-indica*.

The transient microbial response observed in this study further highlights the tripartite nature of AM symbioses. At 6 months, total mesophilic bacteria (TMB) were more than one order of magnitude higher in inoculated plants (lnRR = 2.41, 95% CI: 1.47–3.42; +1013%), whereas controls showed a marked decline. Cumulative abundance (AUMC) confirmed this enrichment, with inoculated plants hosting 5.69×10^7 CFU g^{-1}, ~68% more than controls (3.38×10^7 CFU g^{-1}). By contrast, total mesophilic fungi (TMF) did not respond to inoculation, showing non-significant lnRR values (-0.39 to -1.47) and even lower cumulative abundances in inoculated plants. As a result, the fungal share of the rhizosphere community remained $\leq$1% in inoculated plants, compared with up to 4–5% in controls.

The reduction in total fungal counts in soil following AMF inoculation could be attributed to competition or dominance phenomena exerted by AMF in the rhizosphere. Several studies suggest that these symbioses can exert a repressive effect on the growth of other fungi, both through direct competition for the root substrate and through the release of antimicrobial compounds or modulation of the rhizosphere chemical environment [77,78]. This behavior is known as "fungal exclusion" and can provide AMF with a competitive edge, particularly in poor and marginal soils, as in the current study.

These dynamics align with the concept of mycorrhiza-associated bacteria (MHB), which can proliferate in the hyphosphere by feeding on carbon-rich exudates released by AM hyphae and, in turn, support fungal colonization and nutrient cycling [76]. Similar transient bacterial stimulations have been reported in root-free systems, where AMF such as Glomus hoi selectively enriched Firmicutes while reducing *Actinobacteria* and *Comamonadaceae*, thereby restructuring microbial community assembly [79]. In parallel, AMF hyphae can also act as nutrient mediators: *G. hoi* was shown to transfer 2–3% of plant nitrogen from decomposing litter to the host while altering the flow of litter-derived carbon through saprotrophic fungi and bacteria [80]. Moreover, hyphal exudates of *Glomus* spp. contain low-molecular-weight sugars and organic acids that stimulate bacterial growth and vitality by more than one order of magnitude, with clear shifts in bacterial community composition [80]. At the same time, other experiments demonstrated that AMF hyphae can suppress co-occurring microbial groups, with *G. mosseae* and *G. intraradices* reducing Gram-positive and Gram-negative bacteria as well as saprotrophic fungi [81].

Although the frequency of mycorrhizal colonization (F%) did not differ significantly between inoculated and control plants, the higher intensity (M%) observed in the inoculated roots indicates a denser intraradical colonization rather than a broader spatial spread of infection sites. This pattern suggests that native AMF present in the sandy loam substrate likely colonized control roots to some extent, whereas inoculation enhanced the functional development of existing symbioses rather than increasing the number of infection events.

5. Conclusions

AMF inoculation turned young *O. ficus-indica* into far more vigorous plants, nearly doubling their growth while preserving the conservative balance of a CAM succulent.

These gains rest on the hidden work of fungal partners and their bacterial allies, showing that the cactus–AMF symbiosis is not only a dyad but a wider community shaping life in poor soils.

For farmers in drylands, this means a tangible opportunity: more biomass, more cladodes, more resilience. For science, it opens a frontier still largely unexplored—the long-term fate of these tripartite alliances in the field, their role in nutrient cycles, and their power to sustain agroecosystems under stress.

These results indicate that improving cactus pear performance depends on the functional integration between AMF and the associated rhizosphere microbiota that regulate nutrient acquisition and soil processes in resource-poor environments. This is particularly important in the poor soils of most of the conditions in which cactus pear orchard systems are established, whatever the crop destination. Furthermore, recent works (*pers.comm*.) demonstrate the ability of cactus pear systems, which include the trees and the associated bioma, to improve soil conditions over time, even after 30 years of cropping. Future research should follow those threads, testing AMF inoculation across soils, climates, and management systems, and tracing not only plant growth but also the microbial ecologies that sustain it.

Author Contributions: Conceptualization, G.L. (Giorgia Liguori) and G.L. (Guido Lingua); methodology, G.L. (Giorgia Liguori), G.L. (Guido Lingua) and L.S.; software, G.G.; validation, G.L. (Giorgia Liguori), G.L. (Guido Lingua), L.S. and P.I.; formal analysis, G.G., F.G., R.G. and N.M.; investigation, G.L. (Giorgia Liguori), G.G., F.G., R.G. and N.M.; resources, G.L. (Guido Lingua); data curation, G.G. and G.L. (Giorgia Liguori); writing—original draft preparation, G.G., G.L. (Giorgia Liguori), G.L. (Guido Lingua), P.I. and L.S.; writing—review and editing, G.G., G.L. (Giorgia Liguori), G.L. (Guido Lingua), P.I. and L.S.; visualization, G.G., G.L. (Giorgia Liguori), G.L. (Guido Lingua), P.I. and L.S.; supervision, G.L. (Guido Lingua). All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the ProsmallAgriMed PRIMA project "Promoting soil fertility, yield and income in smallholder agriculture of semiarid and arid Mediterranean regions by management of beneficial soil microbiota, conservation agriculture and intercropping" funded by the European Union.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Nobel, P.S. (Ed.) *Cacti: Biology and Uses*; University of California Press: Berkeley, CA, USA, 2002.
2. Nobel, P.S.; Bobich, E.G. Environmental biology. In *Cacti: Biology and Uses*; Nobel, P.S., Ed.; University of California Press: Berkeley, CA, USA, 2002; pp. 57–74.
3. Inglese, P.; Basile, F.; Schirra, M. Cactus pear fruit production. In *Cacti: Biology and Uses*; Nobel, P.S., Ed.; University of California Press: Berkeley, CA, USA, 2002; pp. 163–183.
4. Mizrahi, Y.; Nerd, A. Climbing and columnar cacti: New arid land fruit crops. In *Perspectives on New Crops and New Uses*; Janick, J., Ed.; ASHS Press: Alexandria, VA, USA, 1999; pp. 358–366.
5. Liguori, G.; Inglese, P.; Sortino, G.; Inglese, G. Sustainable use of cactus pear (*Opuntia ficus-indica* L. Mill.) biomass in Mediterranean agro-ecosystems. *J. Arid Environ.* **2013**, *98*, 135–145.
6. Hassan, S.; Liguori, G.; Inglese, P.; Louhaichi, M.; Sortino, G. The effect of soil volume availability on *Opuntia ficus-indica* canopy and root growth. *Agronomy* **2020**, *10*, 635. [CrossRef]
7. Inglese, P.; Saenz, C.; Mondragón, C.; Nefzaoui, A.; Louhaichi, M. *Crop Ecology, Cultivation and Uses of Cactus Pear*; FAO & ICARDA: Rome, Italy, 2017.
8. Dubeux, J.C.B.; dos Santos, M.V.F.; da Cunha, M.V.; dos Santos, D.C.; de Almeida Souza, R.T.; de Mello, A.C.L.; de Souza, T.C. Cactus (*Opuntia* and *Nopalea*) nutritive value: A review. *Anim. Feed Sci. Technol.* **2021**, *275*, 114890. [CrossRef]

9. Felker, P.; Inglese, P. Short-term and long-term research needs for *Opuntia ficus-indica* (L.) Mill. utilization in arid areas. *J. Prof. Assoc. Cactus Dev.* **2003**, *5*, 131–151.
10. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008.
11. Barea, J.M.; Ferrol, N.; Azcón-Aguilar, C.; Azcón, R. Mycorrhizal symbioses. In *The Ecophysiology of Plant-Phosphorus Interactions*; White, P.J., Hammond, J.P., Eds.; Springer: Dordrecht, The Netherlands, 2008; pp. 143–163.
12. Augé, R.M. Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza* **2001**, *11*, 3–42. [CrossRef]
13. Neumann, E.; George, E. Nutrient uptake: The arbuscular mycorrhiza fungal symbiosis as a plant nutrient acquisition strategy. In *Arbuscular Mycorrhizas: Physiology and Function*; Koltai, H., Kapulnik, Y., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 137–167.
14. Berruti, A.; Lumini, E.; Balestrini, R.; Bianciotto, V. Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Front. Microbiol.* **2016**, *6*, 1559. [CrossRef] [PubMed]
15. Sheng, M.; Tang, M.; Chen, H.; Yang, B.; Zhang, F.; Huang, Y. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* **2008**, *18*, 287–296. [CrossRef] [PubMed]
16. Bona, E.; Cantamessa, S.; Massa, N.; Manassero, P.; Marsano, F.; Copetta, A.; Lingua, G.; D'Agostino, G.; Gamalero, E.; Berta, G. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: A field study. *Mycorrhiza* **2015**, *27*, 1–9. [CrossRef] [PubMed]
17. Todeschini, V.; AitLahmidi, N.; Mazzucco, E.; Marsano, F.; Gosetti, F.; Robotti, E.; Bona, E.; Massa, N.; Bonneau, L.; Marengo, E.; et al. Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatilome. *Front. Plant Sci.* **2018**, *9*, 1611. [CrossRef]
18. van der Heijden, M.G.; Martin, F.M.; Selosse, M.A.; Sanders, I.R. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytol.* **2015**, *205*, 1406–1423. [CrossRef]
19. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Zhang, L. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. [CrossRef]
20. Labidi, S.; Servent, A.; Bouzoumita, G.; Julien, T.; Cazals, G.; Ibrahim, M.; Hammami, S.B.M.; Achir, N. Arbuscular mycorrhizal fungi inoculation enhances nutritional quality of prickly pear (*Opuntia ficus-indica*) fruits and cladodes. *Agriculture* **2025**, *15*, 1902. [CrossRef]
21. Estrada-Luna, A.A.; Davies, F.T., Jr. Mycorrhizal fungi enhance growth and nutrient uptake of prickly-pear cactus (*Opuntia albicarpa* Scheinvar 'Reyna') plantlets after ex vitro transplantation. *J. Hortic. Sci. Biotechnol.* **2001**, *76*, 739–745. [CrossRef]
22. Gee, G.W.; Bauder, J.W. Particle-size analysis. In *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph 9; Soil Science Society of America: Madison, WI, USA, 1986; pp. 383–411.
23. Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Sparks, D.L., Ed.; Soil Science Society of America: Madison, WI, USA, 1996; pp. 475–490.
24. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
25. Bremner, J.M.; Mulvaney, C.S. Nitrogen—Total. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Ed.; Agronomy Monograph 9; Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.
26. Blake, G.R.; Hartge, K.H. Bulk density. In *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph 9; Soil Science Society of America: Madison, WI, USA, 1986; pp. 363–375.
27. Klute, A. Water retention: Laboratory methods. In *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph 9; Soil Science Society of America: Madison, WI, USA, 1986; pp. 635–662.
28. van Genuchten, M.T. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci. Soc. Am. J.* **1980**, *44*, 892–898. [CrossRef]
29. da Silva, G.A.; de Assis, D.M.A.; Sieverding, E.; Oehl, F. Four New Families of Arbuscular Mycorrhizal Fungi Within the Order Glomerales. *Taxonomy* **2024**, *4*, 761–779. [CrossRef]
30. Bhunjun, C.S.; Chen, Y.J.; Phukhamsakda, C.; Boekhout, T.; Groenewald, J.Z.; McKenzie, E.H.C.; Crous, P.W. What are the 100 most cited fungal genera? *Stud. Mycol.* **2024**, *108*, 1–412. [CrossRef]
31. Cavagnaro, T.R.; Smith, F.A.; Ayling, S.M.; Smith, S.E. Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. *New Phytol.* **2003**, *157*, 127–134. [CrossRef] [PubMed]
32. Li, H.; Smith, F.A.; Dickson, S.; Holloway, R.E.; Smith, S.E. Plant growth depressions in arbuscular mycorrhizal symbioses: Not just caused by carbon drain? *New Phytol.* **2008**, *178*, 852–862. [CrossRef]
33. Hedges, L.V.; Gurevitch, J.; Curtis, P.S. The meta-analysis of response ratios in experimental ecology. *Ecology* **1999**, *80*, 1150–1156. [CrossRef]
34. Hoeksema, J.D.; Chaudhary, V.B.; Gehring, C.A.; Johnson, N.C.; Karst, J.; Koide, R.T.; Umbanhowar, J. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol. Lett.* **2010**, *13*, 394–407. [CrossRef]
35. Hoffmann, W.A.; Poorter, H. Avoiding bias in calculations of relative growth rate. *Ann. Bot.* **2002**, *90*, 37–42. [CrossRef]

36. Hunt, R. *Basic Growth Analysis: Plant Growth Analysis for Beginners*; Springer Science & Business Media: Dordrecht, The Netherlands, 2012.
37. Poorter, H.; Sack, L. Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. *Front. Plant Sci.* **2012**, *3*, 259. [CrossRef] [PubMed]
38. Poorter, H.; Garnier, E. Ecological significance of inherent variation in relative growth rate and its components. In *Functional Plant Ecology*; Pugnaire, F.I., Valladares, F., Eds.; CRC Press: Boca Raton, FL, USA, 2007; pp. 67–100.
39. Warton, D.I.; Wright, I.J.; Falster, D.S.; Westoby, M. Bivariate line-fitting methods for allometry. *Biol. Rev.* **2006**, *81*, 259–291. [CrossRef]
40. Weiner, J. Allocation, plasticity and allometry in plants. *Perspect. Plant Ecol. Evol. Syst.* **2004**, *6*, 207–215. [CrossRef]
41. Evans, G.C. *The Quantitative Analysis of Plant Growth*; University of California Press: Berkeley, CA, USA, 1972; Volume 1.
42. Poorter, H.; Evans, J.R. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* **1998**, *116*, 26–37. [CrossRef] [PubMed]
43. Reynolds, J. Serial dilution protocols. *Am. Soc. Microbiol.* **2005**, 1–7.
44. Atlas, R.M. *Handbook of Microbiological Media*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2010.
45. Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; van 't Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875–1881. [CrossRef] [PubMed]
46. Maherali, H. Is there an association between root architecture and mycorrhizal growth response? *New Phytol.* **2014**, *204*, 192–200. [CrossRef]
47. Baranyi, J.; Roberts, T.A. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* **1994**, *23*, 277–294. [CrossRef]
48. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]
49. Trouvelot, A.; Kough, J.L.; Gianinazzi-Pearson, V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In *Physiological and Genetical Aspects of Mycorrhizae: Proceedings of the 1st European Symposium on Mycorrhizae, Dijon, 1–5 July 1985*; Gianinazzi-Pearson, V., Gianinazzi, S., Eds.; INRA: Paris, France, 1986; pp. 217–221.
50. Kebede, T.G.; Birhane, E.; Ayimut, K.M.; Egziabher, Y.G. Arbuscular mycorrhizal fungi improve biomass, photosynthesis, and water use efficiency of *Opuntia ficus-indica* (L.) Miller under different water levels. *J. Arid Land* **2023**, *15*, 975–988. [CrossRef]
51. Kebede, T.G.; Birhane, E.; Ayimut, K.M.; Egziabher, Y.G.; Belay, T. Arbuscular mycorrhizal fungi-induced resistance in *Opuntia ficus-indica* (L.) Miller plant against cochineal insects under various soil water levels. *Arthropod-Plant Interact.* **2024**, *18*, 253–264. [CrossRef]
52. Lahbouki, S.; Ben-Laouane, R.; Anli, M.; Boutasknit, A.; Ait-Rahou, Y.; Ait-El-Mokhtar, M.; Meddich, A. Arbuscular mycorrhizal fungi and/or organic amendment enhance the tolerance of prickly pear (*Opuntia ficus-indica*) under drought stress. *J. Arid Environ.* **2022**, *199*, 104703. [CrossRef]
53. Gardezi, A.K.; Berber, S.R.M.; Magdaleno, H.F.; Flores-Gallardo, H.; de la Cruz, M.F.S.; Buenfil, J.A.L.; Aguilar, G.H. Effect of endomycorrhiza (*Glomus intraradices*) and organic matter on the growth of cactus pear (*Opuntia albicarpa*) in two soil types. *Nova Scientia* **2022**, *14*, 1–13.
54. Camelo, D.; Dubeux, J.C.B., Jr.; dos Santos, M.V.F.; Lira, M.A., Jr.; Fracetto, G.G.M.; Fracetto, F.J.C.; de Freitas, E.V. Soil microbial activity and biomass in semiarid agroforestry systems integrating forage cactus and tree legumes. *Agronomy* **2021**, *11*, 1558. [CrossRef]
55. Dewir, Y.H.; Alsadon, A.A.; Ibrahim, A.A.; Youssef, S.A. Promising Application of Automated Liquid Culture System for Enhancing Micropropagation and Mycorrhization of Red Dragon Fruit (*Hylocereus polyrhizus*). *Horticulturae* **2023**, *9*, 178.
56. González-Monterrubio, C.F.; Martínez-Trujillo, M.; Martínez-Gómez, M.; Hernández-Santiago, J.; Maldonado-Mendoza, I.E. Influence of arbuscular mycorrhizal fungi (AMF) on *Opuntia streptacantha* Lem. plants exposed to drought under greenhouse conditions. *TIP Rev. Esp. Cienc. Quím.-Biol.* **2005**, *8*, 5–10.
57. Snyman, H.A. A case study on in situ rooting profiles and water-use efficiency of cactus pears. *Opuntia ficus-indica* and *O. robusta*. *J. Prof. Assoc. Cactus Dev.* **2005**, *7*, 1–21.
58. Nobel, P.S. *Environmental Biology of Agaves and Cacti*; Cambridge University Press: Cambridge, UK, 2003.
59. Nobel, P.S. Ecophysiology of *Opuntia ficus-indica*. *FAO Plant Prod. Prot. Pap.* **2001**, *169*, 1–42.
60. Poorter, H.; Remkes, C. Leaf area ratio and root–shoot partitioning in 24 wild species differing in relative growth rate. *Oecologia* **1990**, *83*, 553–559. [CrossRef]
61. Maiuolo, J.; Nucera, S.; Serra, M.; Caminiti, R.; Oppedisano, F.; Macrì, R.; Mollace, V. Cladodes of *Opuntia ficus-indica* (L.) Mill. possess important beneficial properties dependent on their different stages of maturity. *Plants* **2024**, *13*, 1365. [CrossRef]
62. Nobel, P.S.; Israel, A.A. Cladode development, environmental responses of CO_2 uptake, and productivity for *Opuntia ficus-indica* under elevated CO_2. *J. Exp. Bot.* **1994**, *45*, 295–303. [CrossRef]

63. Jakobsen, I.; Abbott, L.K.; Robson, A.D. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytol.* **1992**, *120*, 371–380. [CrossRef]
64. Subramanian, K.S.; Charest, C.; Dwyer, L.M.; Hamilton, R.I. Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling. *New Phytol.* **1995**, *129*, 643–650. [CrossRef]
65. Wilson, G.W.; Hartnett, D.C. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *Am. J. Bot.* **1998**, *85*, 1732–1738. [CrossRef] [PubMed]
66. Tarraf, W.; Ruta, C.; Tagarelli, A.; De Cillis, F.; De Mastro, G. Influence of arbuscular mycorrhizae on plant growth, essential oil production and phosphorus uptake of *Salvia officinalis* L. *Ind. Crops Prod.* **2017**, *102*, 144–153. [CrossRef]
67. Villani, A.; Tommasi, F.; Paciolla, C. The arbuscular mycorrhizal fungus *Glomus viscosum* improves the tolerance to verticillium wilt in artichoke by modulating the antioxidant defense systems. *Cells* **2021**, *10*, 1944. [CrossRef]
68. Fracasso, A.; Telò, L.; Lanfranco, L.; Bonfante, P.; Amaducci, S. Physiological beneficial effect of *Rhizophagus intraradices* inoculation on tomato plant yield under water deficit conditions. *Agronomy* **2020**, *10*, 71. [CrossRef]
69. Zhang, Q.; Gong, M.; Liu, K.; Chen, Y.; Yuan, J.; Chang, Q. *Rhizoglomus intraradices* improves plant growth, root morphology and phytohormone balance of *Robinia pseudoacacia* in arsenic-contaminated soils. *Front. Microbiol.* **2020**, *11*, 1428. [CrossRef]
70. Chen, L.; Zhang, D.; Yang, W.; Liu, Y.; Zhang, L.; Gao, S. Sex-specific responses of *Populus deltoides* to *Glomus intraradices* colonization and Cd pollution. *Chemosphere* **2016**, *155*, 196–206. [CrossRef] [PubMed]
71. Olsson, O.; Olsson, P.A.; Hammer, E.C. Phosphorus and carbon availability regulate structural composition and complexity of AM fungal mycelium. *Mycorrhiza* **2014**, *24*, 443–451. [CrossRef] [PubMed]
72. Kokkoris, V.; Banchini, C.; Paré, L.; Abdellatif, L.; Séguin, S.; Hubbard, K.; Stefani, F. *Rhizophagus irregularis*, the model fungus in arbuscular mycorrhiza research, forms dimorphic spores. *New Phytol.* **2024**, *242*, 1771–1784. [CrossRef] [PubMed]
73. Ndeko, A.B.; Founoune-Mboup, H.; Kane, A.; Cournac, L. Arbuscular mycorrhizal fungi alleviate the negative effect of temperature stress in millet lines with contrasting soil aggregation potential. *Gesunde Pflanzen* **2022**, *74*, 53–67. [CrossRef]
74. Nacoon, S.; Ekprasert, J.; Riddech, N.; Mongkolthanaruk, W.; Jogloy, S.; Vorasoot, N.; Boonlue, S. Growth enhancement of sunchoke by arbuscular mycorrhizal fungi under drought condition. *Rhizosphere* **2021**, *17*, 100308. [CrossRef]
75. Fernández, F.; Dell'Amico, J.M.; Angoa, M.V.; de la Providencia, I.E. Use of a liquid inoculum of the arbuscular mycorrhizal fungi *Glomus hoi* in rice plants cultivated in a saline Gleysol: A new alternative to inoculate. *J. Plant Breed. Crop Sci.* **2011**, *3*, 24–33.
76. Anand, K.; Pandey, G.K.; Kaur, T.; Pericak, O.; Olson, C.; Mohan, R.; Yadav, A.N. Arbuscular mycorrhizal fungi as potential biofertilizers for agricultural sustainability. *J. Appl. Biol. Biotechnol.* **2022**, *10*, 90–107. [CrossRef]
77. Bonfante, P.; Anca, I.A. Plants, mycorrhizal fungi, and bacteria: A network of interactions. *Annu. Rev. Microbiol.* **2009**, *63*, 363–383. [CrossRef]
78. Welc, M.; Ravnskov, S.; Kieliszewska-Rokicka, B.; Larsen, J. Suppression of other soil microorganisms by mycelium of arbuscular mycorrhizal fungi in root-free soil. *Soil Biol. Biochem.* **2010**, *42*, 1534–1540. [CrossRef]
79. Nuccio, E.E.; Hodge, A.; Pett-Ridge, J.; Herman, D.J.; Weber, P.K.; Firestone, M.K. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environ. Microbiol.* **2013**, *15*, 1870–1881. [CrossRef]
80. Herman, D.J.; Firestone, M.K.; Nuccio, E.; Hodge, A. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. *FEMS Microbiol. Ecol.* **2012**, *80*, 236–247. [CrossRef] [PubMed]
81. Toljander, J.F.; Lindahl, B.D.; Paul, L.R.; Elfstrand, M.; Finlay, R.D. Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* **2007**, *61*, 295–304. [CrossRef] [PubMed]