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Response of *Alhagi sparsifolia* Seedlings to AMF Inoculation and Nitrogen Addition under Drought Stress

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Abstract: Riparian forest veg etation in the lower Tarim River desert often faces a water and nitrogen deficiency. To investigate the ecological effects of drought stress and nitrogen limitation of arbuscular mycorrhizal fungi (AMF) on *Alhagi sparsifolia* seedlings at the vulnerable stage of growth, a control experiment was conducted on *Alhagi sparsifolia* seedlings with indoor potted plants. The main findings are as follows: drought stress inhibited the normal growth and development of *Alhagi sparsifolia* seedlings. When *Alhagi sparsifolia* seedlings were inoculated with AMF and at the N1 (50 mmol·L^{-1}) nitrogen addition level, the mycorrhizal infection rate of *Alhagi sparsifolia* seedlings was the best: 84.44% under sufficient moisture content and 77.78% under drought stress. Under the same nitrogen treatment, the relative growth rate of *Alhagi sparsifolia* seedling height and base diameter, plant biomass, root system indicators (total root length, root surface area, root volume, average root diameter, the number of tips, and root shoot ratio), chlorophyll content (except for the chlorophyll a/b content at the N0 (0 mmol·L^{-1}) nitrogen addition level), *Fv*/*Fm*, total nitrogen content, idole acetic acid (IAA) and gibberellic acid (GA) content were initially decreased, then increased and finally decreased again. Each of these indicators was increased significantly after being inoculated with AMF, and they reached their maximum value under the normal moisture and AMF treatment; however specific root length (except for at the N0 (0 mmol·L^{-1}) nitrogen addition level), minimal fluorescence (*Fo*), antioxidant enzyme activity, the contents of osmotic regulation substances, abscisic acid (ABA) and strigolactones (SLs) contents were initially increased, then decreased and finally increased again, and they reached their maximum value under drought stress and AMF treatment. Under the same CK (black control), D (drought stress), CK + A (inoculated with AMF under black control), and D + A (inoculated with AMF under drought stress) treatments, all of the above indicators, except for specific root length, chlorophyll a/b content, minimal fluorescence (*Fo*), maximum fluorescence (*Fm*) and malondialdehyde (MDA) content initially increased and then decreased with the increasing nitrogen addition rate, and they reached the maximum value at the N1 (50 mmol·L^{-1}) nitrogen addition level. Therefore, in the arid and N-deficient lower Tarim River region, *Alhagi sparsifolia* seedlings established an efficient symbiotic structure with AMF, which improved the drought resistance of seedlings and promoted the rapid passage of seedlings through the growth vulnerability period. This indicates that AMF inoculation is a key link in the survival strategy of *Alhagi sparsifolia*.

Keywords: the Tarim River; *Alhagi sparsifolia*; arbuscular mycorrhizal fungi; growth and physiology

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1. Introduction

Environmental emissions and consequently climate change have negative impacts on crop production [1–5]. In particular, the reduction of effective water in the soil is considered one of the key abiotic factors affecting plant growth and physiology [6]; drought stress has certain negative effects on plant growth and production [7]. Drought stress significantly reduces plant photosynthesis, disrupts metabolism, destroys water relations and osmoregulation in plants, and leads to the excessive production of reactive oxygen species (ROS) and increased membrane lipid peroxidation by affecting metabolic processes [8,9]. In addition, drought stress can cause nutrient disorders in plants, reduce the effectiveness of nutrients in the soil, and decrease the uptake of phosphorus, potassium, and other nutrients in the root system as well as the transport and distribution to the above-ground parts [10,11]. In contrast, proper nutrient intake or the use of beneficial microorganisms as inoculants can promote plant growth and improve plant resistance to drought stress [12]. As Sheteiwy et al. showed, the AMF inoculation improved CAT and POD in the seeds and decreased MDA under drought stress, further enhancing the positive effects of drought on antioxidant and osmoprotectant levels, and there was a decrease in the level of ABA and increases in GA and IAA in the inoculated plants [13–15]. Plants have many survival strategies, including regulating morphological, physiological, and molecular levels to cope with drought stress; the ability of plants to fully utilize these survival strategies determines the effectiveness of plant protection systems [16].

As a beneficial nutrient necessary for plant growth and development, the role of nitrogen in improving plant stress resistance cannot be underestimated [17,18]. Plant roots take up nitrogen in the form of nitrate (NO_3^-) or ammonium (NH_4^+) and actively use it to promote the completion of metabolic processes, growth, and development of the plant [19]. The amount of nitrogen applied and soil moisture conditions have a strong influence on plant growth, exogenous nitrogen application promotes chlorophyll synthesis, increases leaf area, basal diameter, and internode distance, positively affects plant height, chlorophyll fluorescence, and water status under drought stress, excessive N application; on the other hand, it can lead to soil acidification and pollute the atmosphere, which shows that the appropriate amount of irrigation combined with the application of nitrogen can enhance photosynthesis, promote plant growth and increase yield [20–22]. Despite the positive effect of exogenous N application on plant growth, plants can only absorb 30–50% of the supplied N depending on soil type, environmental conditions, and plant populations [19]. Therefore, it is necessary to inoculate beneficial plant symbionts such as arbuscular mycorrhizal fungi to counteract the inhibitory effects of drought stress on plant growth.

Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with the root systems of most vascular plants, mycorrhizal associations help improve and protect the soil structure, improving the efficiency of nutrient uptake by plants via increasing the uptake area of nutrient sources, enabling plants to better access resources from the soil through their extensive mycelial network [11,23]. The symbiotic relationship between AMF and plants can significantly promote the growth of seedlings, reduce the production of malondialdehyde (MDA) in seedlings, increase the accumulation of antioxidant enzymes and osmotic substances, reduce drought damage to plants, thus improving the tolerance of plants to drought stress; the relative water content of all organs of root, stems, and leaves of plants colonized by AM fungi were higher than those of non-colonized plants [24,25]. *Alhagi sparsifolia* belongs to the Fabaceae family, and is a perennial desert herb [26]. It is a dominant established species of plants in the southern margin of the Taklamakan Desert, and is also a dominant species and a key mycorrhizal plant in the riparian forest of the lower Tarim River desert [27]. *Alhagi sparsifolia* plays an important role in maintaining the ecological security of oases, preventing wind and sand, and stabilizing and improving the fragile ecological environment of desert areas [28]. As a member of the Fabaceae family, *Alhagi sparsifolia* can convert atmospheric N_2 into carbohydrates for easy uptake by plant roots via establishing a symbiotic relationship with microorganisms in the soil, which is also an important survival strategy for *Alhagi sparsifolia* to overcome the low-nitrogen environment [29]. However, the amount of carbon may not be sufficient to invest in root secretion and biological nitrogen fixation under drought stress [30].

In different plants, the combined use of N and AMF was more effective against various abiotic stresses than alone [31]. Bahadur et al. investigated the synergistic effect between N application and AMF inoculation on biomass, nutrient acquisition, and competitive intensity in Fabaceae and Gramineae [32]. Azcon et al. observed that AMF inoculation and nitrogen application increased the drought resistance of lettuce by increasing glutamine synthetase activity, nitrate reductase activities, and proline content under drought stress [33]. The individual and interactive effects of nitrogen application and AMF inoculation have been evaluated in many plants; however, there have been few studies on the combined effects of exogenous nitrogen application and AMF inoculation on *Alhagi sparsifolia* under drought stress.

In this study, we investigated the effects of different treatments of water, nitrogen and AMF on seedling growth, photosynthesis, nutrition, and stress resistance physiology, using *Alhagi sparsifolia* as experimental material in indoor pots. *Alhagi sparsifolia* is a dominant herb in the riparian forest of the lower Tarim River desert; the ability of seedlings to grow rapidly and successfully through the vulnerable stage is of great importance for the regeneration of its populations. However, under soil N deficiency and extreme drought conditions, the dependence or reciprocal promotion of the early growth of seedlings of the mycorrhizal plant *Alhagi sparsifolia* on AMF is unclear, and whether AMF has a decisive role in the seedling stage in this plant survival strategy is uncertain. Therefore, the objective of this study was to further analyze the survival strategy of this plant in drought and nitrogen-deficient environments, in order to provide a scientific basis for the restoration and conservation of the riparian forest in the lower Tarim River desert.

2. Materials and Methods

2.1. Experimental Materials

Seeds of the experimental plant *Alhagi sparsifolia* Shap. and the experimental soil were collected from the lower Tarim River in the distribution area of the *Alhagi sparsifolia* community. Seeds of *Alhagi sparsifolia* were surface-sterilized with 75% ethanol solutions for 10 min, rinsed well with deionized water 3~5 times, and then rubbed with sandpaper for germination. The experimental sand was sieved with a 2 mm sieve to remove impurities, then rinsed 3 times with running water and once with deionized water. In a pot containing 3 kg of a 1:1 mixture of sands and vermiculite, sand was autoclaved for 2 h, at 0.11 Mpa and 121 °C, and pots were disinfected by wiping with 75% ethanol solutions. The field water holding capacity of the experimental substrate in the pots was measured at 21.10% before the start of the control experiment. The characteristics of the experimental substrate used here were pH 8.69, 0.073 $g \cdot kg^{-1}$ total N, 0.505 $g \cdot kg^{-1}$ total P, 22.9 $g \cdot kg^{-1}$ total K, 13.2 $mg \cdot kg^{-1}$ available N, 2.44 $mg \cdot kg^{-1}$ available P, and 82.3 $mg \cdot kg^{-1}$ available K. The experimental AMF was a mixture of *Claroideoglomus etunicatum* (BGC XJ04B) and *Funneliformis mosseae* (BGC XJ02), purchased from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry, and the inoculum was a mixture of internal spores and extra-rooted mycelium, with a spore density of 14–20 spores g^{-1}. In this experiment, no bacterial agent was added except for AMF.

2.2. Experimental Design

Experiments were conducted at the indoor nursery culture experimental site from March 2021 to September 2021. In March 2021, seeds of *Alhagi sparsifolia* seedlings were selected and sown in plastic trays for germination. In June 2021, three *Alhagi sparsifolia* seedlings of similar size and healthy growth were transferred into plastic pots. Soil drought stress experiments were set up with 2 moisture treatments, a black control group (CK) with soil water content of (70 ± 5)% of the field holding capacity and an experimental group (D) with soil water content of (30 ± 5)% of the field holding capacity; and 2 inoculation treatments, inoculation with AMF and no inoculation with AMF. Then, 20 g of AMF inoculum was accurately weighted and placed 3 cm below the roots of seedlings, and an equal amount of experimental substrate was added in a 1:1 ratio mixture of sand to vermiculite in the control group [34]. Nitrogen was added as inorganic nitrogen NH_4Cl, and the levels of nitrogen applied included three levels: 0 $mmol \cdot L^{-1}$, 50 $mmol \cdot L^{-1}$, and 500 $mmol \cdot L^{-1}$, expressed as N0, N1, and N2, respectively. To avoid excessive N toxic effects, every 15 days a quantitative amount of N solution was added to the pots after dissolving in water, and the set addition amount was reached after 5 additions. Watering was applied close to the pot wall to avoid experimental errors caused by contact between the nitrogen solution and plant leaves. The soil water content was measured using a WET-2 portable moisture rapid measuring instrument, weighed and replenished with an electronic scale at 19:30 daily, and the relative soil water content was adjusted to within the range of each drought stress treatment. Drought stress and nitrogen addition were carried out simultaneously for 60 d. During this period, seedlings were grown normally, and functional leaves were collected from the apical part of the new shoot down to 3–4 nodes to determine each physiological index.

2.3. Sample Measurement

2.3.1. Mycorrhizal Root Colonization Determination

The mycorrhizal colonization was performed using the Trypan blue staining method [35]:

$$\text{mycorrhizal colonization (\%)} = (\text{number of infested root segments}/\text{number of all root segments}) \times 100\% \quad (1)$$

2.3.2. Plant Growth Parameters

The plant height and basal diameter of *Alhagi sparsifolia* seedlings were measured directly using a tape measure and vernier caliper, respectively, before and after 60 d from the drought stress treatment:

$$\text{The relative growth rate of plant height} = (\text{Final plant height} - \text{Initial plant height})/\text{Initial plant height} \times 100\% \quad (2)$$

$$\text{Relative growth rate of basal diameter} = (\text{Final plant basal diameter} - \text{Initial plant basal diameter})/\text{Initial plant basal diameter} \times 100\% \quad (3)$$

The root system was scanned with an EPSON root scanner and analyzed with the accompanying Win-Rhizo software. Root samples were sterilized in an oven at 105 °C for 10 min; then, the temperature was adjusted to 75 °C until it had dried to a constant weight. The above-ground and below-ground biomass valve were measured to calculate the root shoot ratio.

$$\text{Root shoot ratio} = \text{below-ground biomass}/\text{above-ground biomass} \quad (4)$$

$$\text{Specific root length } (\text{m}\cdot\text{g}^{-1}) = \text{root length}/\text{below-ground biomass} \quad (5)$$

2.3.3. Photosynthetic Index

Chlorophyll content was determined by acetone ethanol extraction [36]. Fully-opened leaves of *Alhagi sparsifolia* seedlings were selected between 09:00 and 18:00 on a sunny day using a Junior-Pam portable modulated chlorophyll fluorometer; the minimal fluorescence (Fo) and maximum fluorescence (Fm) of different loci of different leaves from top to bottom of the same plant of *Alhagi sparsifolia* seedlings under light acclimation were measured every 3 h; then, the maximum photochemical efficiency (Fv/Fm) was calculated; each treatment was replicated 6 times.

2.3.4. Nutrient Contents

The total nitrogen content was determined by the Kjeldahl method [37].

2.3.5. Physiological Index

Superoxide dismutase (SOD) activity and peroxidase (POD) activity were determined by the nitrogen blue tetrazolium method [38] and guaiacol method [38], respectively; catalase (CAT) activity was determined by the trace method [39]; the malondialdehyde (MDA) content was determined by the thiobarbituric acid method [40], the proline (Pro) content was determined by the sulfosalicylic acid method [41], and the soluble sugar (SS) content was determined by the anthrone colorimetric method [42]. The determination of idole acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and strigolactones

(SLs) was carried out by ELISA kits, which were purchased from Shanghai Enzyme Link Biotechnology Co. Specific experimental procedures are described in the corresponding kit instructions.

2.4. Statistical Analysis

Microsoft Excel 2019 software was used to organize the data of all indicators of *Alhagi sparsifolia* seedlings; one-way ANOVA and multi-way ANOVA were performed using SPSS 26 software; and Origin 2021 was used for graphing. All indicators except chlorophyll fluorescence were repeated three times, and the results were expressed as the mean ± standard error.

3. Results

3.1. Effect of AMF Inoculation and N Addition on the Inoculation Rate of Alhagi sparsifolia Seedlings under Drought Stress

Under uninoculated AMF conditions, the root inoculation rate of *Alhagi sparsifolia* seedlings was 0 for each level of nitrogen addition, and after inoculation with AMF, AMF was able to form a symbiotic structure with the seedlings (Figure 1). As shown in Table 1, in the CK + A treatment and D + A treatment, the AM fungal inoculation rate of seedling roots initially increased and then decreased with the increasing nitrogen addition rate, and reached maximum values at the N1 nitrogen addition level, 84.44% and 77.78%, respectively; both N2 nitrogen addition levels were lower than the N0 no nitrogen addition level. The root inoculation rate of *Alhagi sparsifolia* seedlings in the CK treatment inoculated with AMF was consistently higher than that of the D treatment inoculated with AMF under the same nitrogen treatment. This showed that the root system of *Alhagi sparsifolia* seedlings could form a good symbiosis with clumping mycorrhizae, and the growth of *Alhagi sparsifolia* seedlings and AMF was better at the N1 nitrogen addition level. Excessive nitrogen addition inhibited root growth and AMF reproduction in *Alhagi sparsifolia* seedlings and reduced the inoculation rate instead.

Table 1. The effect of AMF inoculation and N addition on the inoculation rate of *Alhagi sparsifolia* seedlings under drought stress.

Treatments/%	CK + A	CK	D + A	D
N0	75.56 ± 2.22 abc	0 ± 0 d	64.44 ± 8.89 abc	0 ± 0 d
N1	84.44 ± 5.88 a	0 ± 0 d	77.78 ± 9.69 ab	0 ± 0 d
N2	62.22 ± 11.76 bc	0 ± 0 d	55.56 ± 12.37 c	0 ± 0 d

Note: CK + A represents inoculation with AMF under sufficient moisture; CK represents sufficient moisture; D + A represents inoculation with AMF under drought stress; D represents drought stress. N0, N1, and N2 represent three levels of nitrogen addition: 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the inoculation rate of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

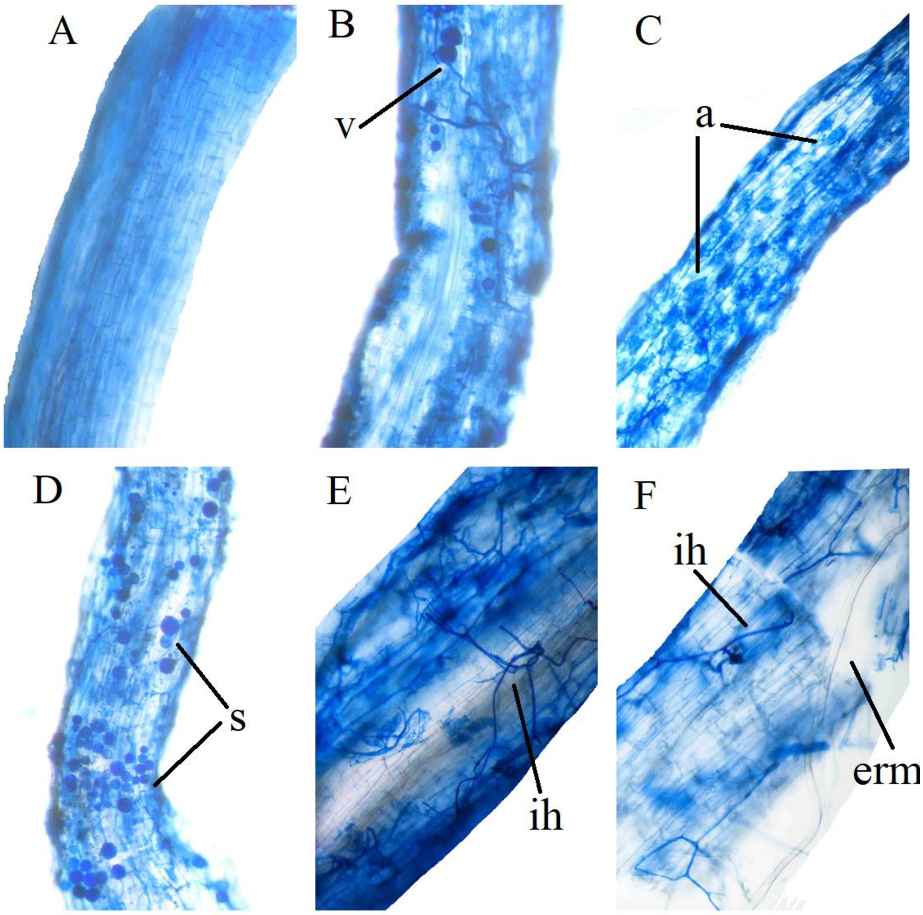

Figure 1. Structures of arbuscular mycorrhizal fungi (AMF) in *Alhagi sparsifolia* seedling roots inoculated with AMF and N addition under drought stress. Note: (**A**) non-treated control root, in which no structures such as vesicles and arbuscules were found; (**B**,**C**) D + A treatment at the N1 nitrogen addition level; (**D**–**F**) CK + A treatment at the N1 nitrogen addition level. v: vesicles, a: arbuscules, s: spores, ih: internal hyphae, erm: extraradical mycelium.

3.2. Effect of AMF Inoculation and N Addition on the Relative Growth Rate of Height, Base Diameter, and Biomass of Alhagi sparsifolia Seedlings under Drought Stress

Drought stress reduced the relative growth rate of the height and base diameter of *Alhagi sparsifolia* seedlings (Figure 2). The relative growth rate of height and basal diameter of *Alhagi sparsifolia* seedlings inoculated with AMF were significantly higher than those of the non-inoculated group at different nitrogen addition levels and under moisture treatments. The relative growth rate of height and base diameter of *Alhagi sparsifolia* seedlings in the CK, D, CK + A, and D + A treatments were initially increased and then decreased with the increasing nitrogen addition rate; they reached the maximum value at the N1 nitrogen addition level, and the lowest at the N2 nitrogen addition level. The relative growth rate of height and base diameter of *Alhagi sparsifolia* seedlings were initially decreased then increased and decreased with CK, D, CK + A, and D + A treatments under the same nitrogen treatment; they reached the maximum value under normal moisture and AMF treatment; followed by the drought stress and AMF treatment. It was shown that AMF inoculation and moderate nitrogen addition facilitated the increase in the relative growth rate of height and base diameter of *Alhagi sparsifolia* seedlings.

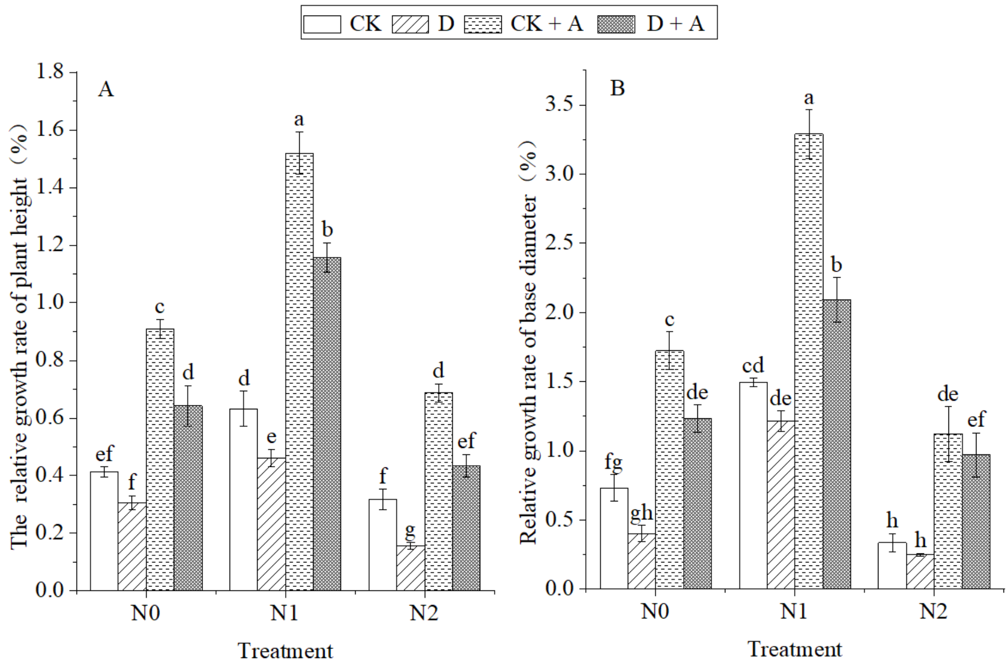

Figure 2. Effect of AMF inoculation and N addition on the relative growth rate of plant height and base diameter of *Alhagi sparsifolia* seedlings under drought stress. Note: subfigures **A** represents the relative growth rate of plant height; subfigures **B** represents the relative growth rate of plant base diameter; CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the relative growth rate of height and base diameter of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

The above-ground biomass of *Alhagi sparsifolia* seedlings was significantly higher than the below-ground biomass (Figure 3). Except for the N2 nitrogen addition level, drought stress significantly reduced the biomass of above and below-ground parts of *Alhagi sparsifolia* seedlings. The biomass of above and below-ground parts of *Alhagi sparsifolia* seedlings inoculated with AMF was significantly higher than that of the non-inoculated group in different nitrogen addition level and moisture treatments. The biomass of above and below-ground parts of *Alhagi sparsifolia* seedlings in the CK, D, CK + A, and D + A treatment were initially increased and then decreased with the increasing nitrogen addition rate, reaching the maximum value at the N1 nitrogen addition level, followed by N0, and significantly decreasing at the N2 application compared with the N1 application. The biomass of above and below-ground parts of *Alhagi sparsifolia* seedlings increased significantly after inoculation with AMF, reaching a maximum in the CK + A treatment, followed by the D + A treatment, were initially decreased then increased and decreased with CK, D, CK + A, and D + A treatment under the same nitrogen treatment. It was shown that AMF inoculation could effectively increase the above and below-ground biomass of *Alhagi sparsifolia* seedlings, and the promotion effect was better with the N1 nitrogen addition level, whereas excessive nitrogen addition would inhibit the growth of above and below-ground parts.

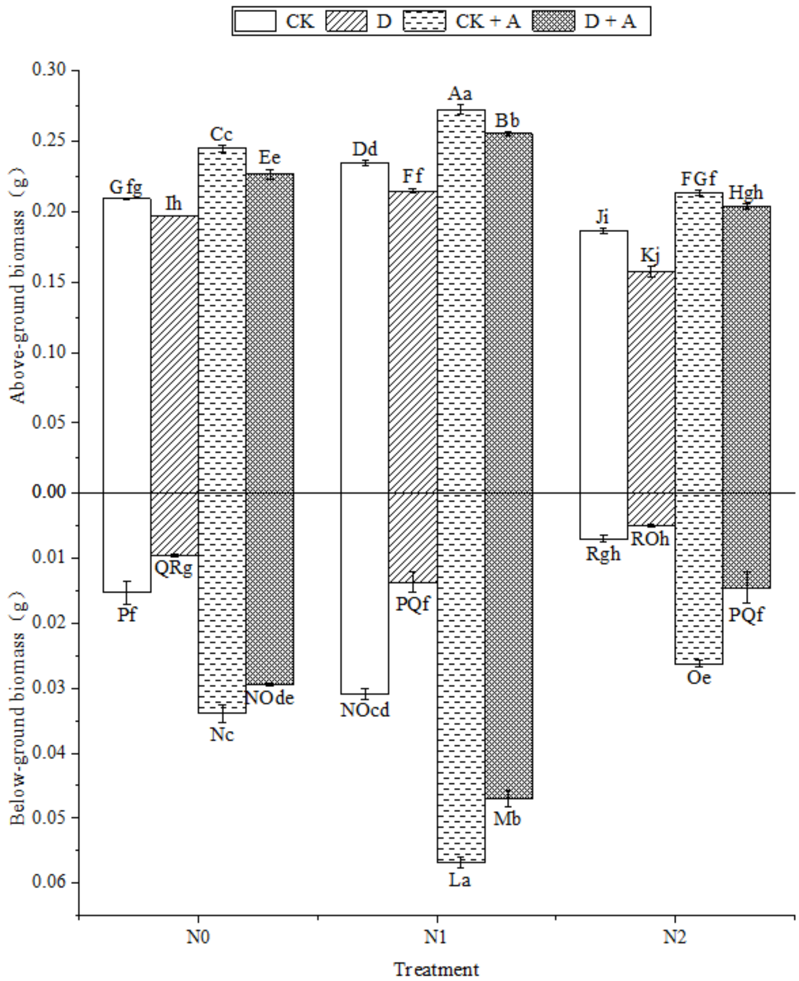

Figure 3. Effect of AMF inoculation and N addition on the biomass of *Alhagi sparsifolia* seedlings under drought stress. Note: CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in above and below-ground biomass of *Alhagi sparsifolia* seedlings at different levels of nitrogen addition ($p < 0.05$); different capital letters indicate significant differences in the above and below-ground biomass of *Alhagi sparsifolia* seedlings at the same level of nitrogen addition ($p < 0.05$).

3.3. Effect of AMF Inoculation and N Addition on the Root System of Alhagi sparsifolia Seedlings under Drought Stress

Drought stress reduced the root growth of *Alhagi sparsifolia* seedlings (Table 2). In the CK, D, CK + A, and D + A treatments, the total root length, root surface area, root volume, average root diameter, root tip number, root shoot ratio, and specific root length (except for the CK and D + A treatment) of *Alhagi sparsifolia* seedlings initially increased and then decreased with the increasing nitrogen addition rate. At the same level of nitrogen addition, all the above indices of *Alhagi sparsifolia* seedlings, except for the specific root length, were significantly higher after inoculation with AMF than the uninoculated treatment, and the maximum value was found in the CK + A treatment, followed by D + A and the smallest value in the D treatment, showing a trend of first decreasing, then increasing and then decreasing, while the specific root length showed a trend of first increasing, then decreasing and then increasing, except for in the N0 nitrogen addition level.

Table 2. Effect of AMF inoculation and N addition on the root system of *Alhagi sparsifolia* seedlings under drought stress.

Treatment		Total Root Length (cm)	Root Surface Area (cm^2)	Root Volume (cm^3)	Average Root Diameter (mm)	Number of Tips	Root Shoot Ratio	Specific Root Length
N0	CK	25.791 ± 0.720 h	6.499 ± 0.073 f	0.183 ± 0.008 f	0.484 ± 0.008 def	175.000 ± 6.083 f	0.073 ± 0.008 d	17.327 ± 2.126 bcd
	D	21.006 ± 0.378 i	5.595 ± 0.137 g	0.145 ± 0.011 g	0.382 ± 0.006 def	139.000 ± 5.132 g	0.048 ± 0.001 e	22.124 ± 0.435 b
	CK + A	39.030 ± 1.819 e	8.350 ± 0.108 e	0.243 ± 0.000 d	0.563 ± 0.017 cde	256.000 ± 10.017 de	0.138 ± 0.004 c	11.582 ± 0.865 d
	D + A	33.930 ± 1.513 f	8.070 ± 0.118 e	0.228 ± 0.000 e	0.511 ± 0.010 def	232.667 ± 2.603 e	0.130 ± 0.001 c	11.525 ± 0.474 d
N1	CK	51.271 ± 0.673 c	11.633 ± 0.495 c	0.277 ± 0.001 c	0.778 ± 0.003 bc	346.667 ± 17.295 c	0.132 ± 0.002 c	16.623 ± 0.632 bcd
	D	46.078 ± 1.043 d	9.428 ± 0.101 d	0.256 ± 0.003 d	0.628 ± 0.020 cd	276.000 ± 16.503 d	0.064 ± 0.007 d	34.604 ± 4.425 a
	CK + A	78.377 ± 0.876 a	16.080 ± 0.294 a	0.331 ± 0.004 a	1.637 ± 0.276 a	574.667 ± 6.119 a	0.209 ± 0.002 a	13.810 ± 0.156 cd
	D + A	66.994 ± 1.359 b	14.635 ± 0.588 b	0.304 ± 0.002 b	0.942 ± 0.020 b	414.000 ± 7.767 b	0.184 ± 0.005 b	14.265 ± 0.497 cd
N2	CK	21.364 ± 0.270 i	4.692 ± 0.340 h	0.093 ± 0.004 i	0.306 ± 0.004 ef	87.667 ± 4.842 ij	0.038 ± 0.003 ef	30.623 ± 1.813 a
	D	15.961 ± 0.461 j	3.841 ± 0.075 i	0.062 ± 0.003 j	0.288 ± 0.007 f	72.667 ± 2.028 j	0.032 ± 0.001 f	31.893 ± 1.921 a
	CK + A	30.077 ± 0.282 g	7.904 ± 0.051 e	0.123 ± 0.005 h	0.360 ± 0.003 ef	118.333 ± 4.702 gh	0.123 ± 0.002 c	11.488 ± 0.271 d
	D + A	26.172 ± 0.695 h	6.303 ± 0.142 fg	0.107 ± 0.001 i	0.322 ± 0.003 ef	110.667 ± 8.452 hi	0.071 ± 0.012 d	19.186 ± 3.19 bc

Note: CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the root systems of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

AMF inoculation significantly promoted the root growth of *Alhagi sparsifolia* seedlings; AMF could effectively alleviate the inhibitory effect of drought stress on the root growth of *Alhagi sparsifolia* seedlings, even at high nitrogen addition levels, and the effect was better than that of D + A after CK + A treatment, whereas appropriate amounts of nitrogen addition could significantly improve the root growth of *Alhagi sparsifolia* seedlings.

3.4. Effect of AMF Inoculation and N Addition on the Nitrogen Uptake of Alhagi sparsifolia Seedlings under Drought Stress

The nitrogen content of *Alhagi sparsifolia* seedlings was significantly lower ($p < 0.05$) under drought stress conditions than under sufficient moisture conditions. The nitrogen contents of *Alhagi sparsifolia* seedlings in the CK, D, CK + A, and D + A treatments were initially increased and then decreased with the increasing nitrogen addition rate, reaching a maximum valve at the N1 nitrogen addition rate, indicating that the low nitrogen treatment was more beneficial to the accumulation of nitrogen content in *Alhagi sparsifolia* seedlings (Figure 4). The nitrogen content of *Alhagi sparsifolia* seedlings initially decreased then increased and decreased at the same rate of nitrogen addition, and the nitrogen content was significantly higher in the N1 nitrogen addition rate CK + A and D + A treatments than the CK and D treatments by 51.84% and 56.40%, respectively. AMF inoculation and low nitrogen treatment can enhance the promotion of nutrient accumulation and improve the drought resistance of *Alhagi sparsifolia* seedlings, thus helping *Alhagi sparsifolia* seedlings to successfully pass through the survival vulnerability period.

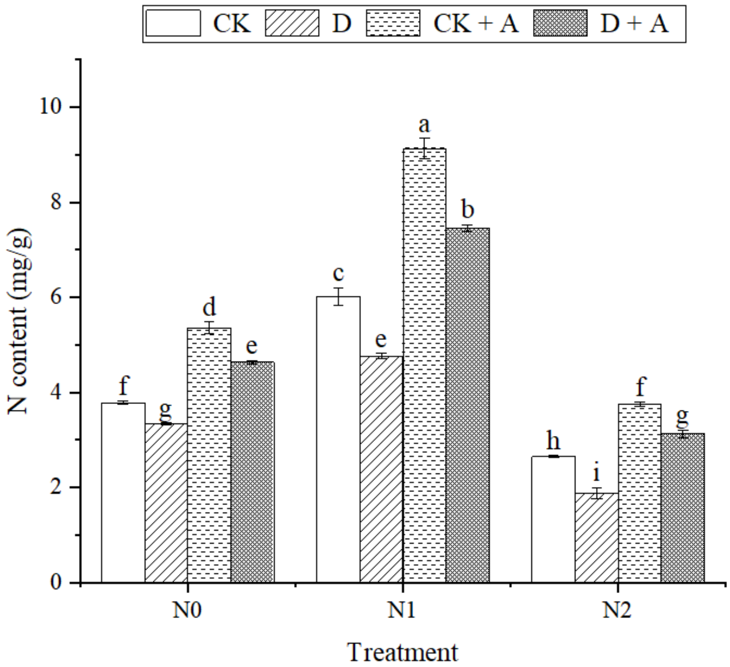

Figure 4. Effect of AMF inoculation and N addition on the nitrogen uptake of *Alhagi sparsifolia* seedlings under drought stress. Note: CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the nitrogen uptake of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

3.5. Effect of AMF Inoculation and N Addition on the Photosynthesis of Alhagi sparsifolia Seedlings under Drought Stress

Drought stress reduced chlorophyll a, b, total chlorophyll content, and the chlorophyll a/b content of *Alhagi sparsifolia* seedlings to different degrees, although the differences were not significant; the *Fo* of *Alhagi sparsifolia* seedlings was significantly increased ($p < 0.05$), and the *Fm* and *Fv/Fm* were significantly reduced ($p < 0.05$) (Figure 5). Chlorophyll a, b, total chlorophyll content, and *Fv/Fm* of *Alhagi sparsifolia* seedlings in CK, D, CK + A, and D + A treatment were initially increased and then decreased with the increasing nitrogen addition rate, and reached a maximum valve at the N1 nitrogen addition rate; the chlorophyll a/b content showed a decreasing trend except for the CK + A treatment; *Fo* showed a trend of decreasing and then increasing, and was the minimum at N1 nitrogen addition rate; and *Fm* showed no significant pattern (Figure 5). The chlorophyll a, b, total chlorophyll content, and *Fv/Fm* of *Alhagi sparsifolia* seedlings initially decreased then increased and decreased at the same level of nitrogen addition rate. The chlorophyll a/b content of *Alhagi sparsifolia* seedlings was also initially decreased then increased and decreased at the same level of nitrogen addition rate, except for the N0 nitrogen addition rate. *Fm* decreased with the N0 and N2 nitrogen addition rate, and showed a trend of decreasing, then increasing and then decreasing at the N1 nitrogen addition, while *Fo* showed a trend of increasing, then decreasing and then increasing. *Alhagi sparsifolia* seedlings can improve their photosynthetic performance by increasing the chlorophyll content, *Fm*, and *Fv/Fm*, and decreasing *Fo* through AMF inoculation and low nitrogen treatment; and the maximum value was reached at the N1 nitrogen addition rate.

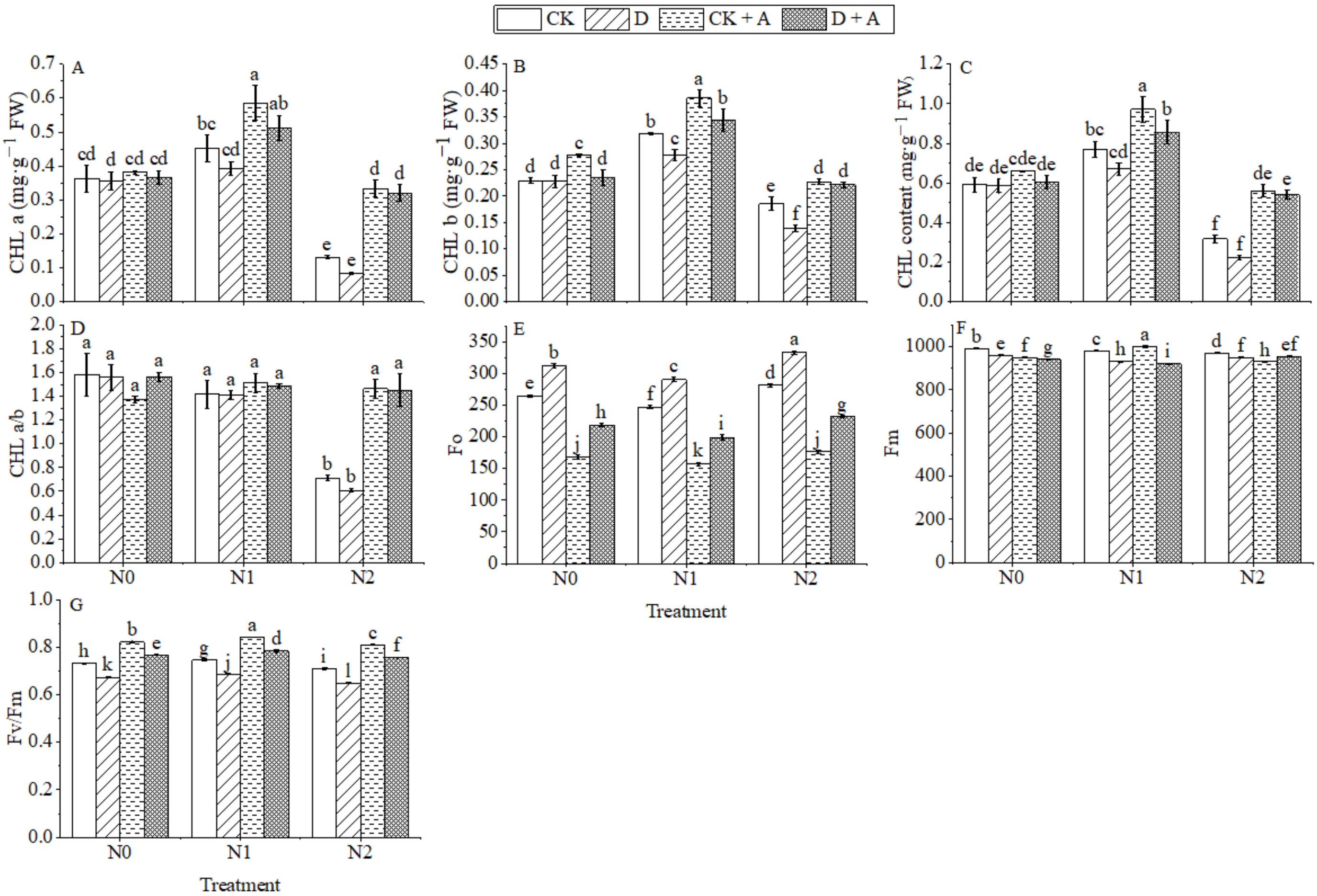

Figure 5. Effect of AMF inoculation and N addition on the photosynthesis of *Alhagi sparsifolia* seedlings under drought stress. Note: (**A**) represents Chlorophyll a content; (**B**) represents Chlorophyll b content; (**C**) represents Chlorophyll content; (**D**) represents Chlorophyll a/b content; (**E**) represents minimal fluorescence (*Fo*); (**F**) represents maximum fluorescence (*Fm*); (**G**) represents maximum photochemical efficiency; CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 $mmol \cdot L^{-1}$, 50 $mmol \cdot L^{-1}$, and 500 $mmol \cdot L^{-1}$, respectively. Different lowercase letters indicate significant differences in the photosynthesis of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

3.6. Effect of AMF Inoculation and N Addition on the Antioxidant Enzymes, Malondialdehyde, Osmoregulatory Substances of Alhagi sparsifolia Seedlings under Drought Stress

Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities, malondialdehyde (MDA), proline (Pro), and soluble sugar (SS) contents of *Alhagi sparsifolia* under drought stress conditions were significantly higher than those of the CK treatment, except for the SOD activity at the N2 nitrogen addition rate and the POD activity at the N0 nitrogen addition rate, which were significantly higher in the drought stress (D) than in the sufficient moisture (CK) treatment (Figure 6). The SOD, POD, and CAT activities and Pro, SS contents of *Alhagi sparsifolia* seedlings were initially increased and then decreased with the increasing nitrogen addition rate, and reached a maximum valve at the N1 nitrogen addition rate in CK, D, CK + A, and D + A treatment, while the MDA contents of *Alhagi sparsifolia* seedlings were initially decreased and then increased with the increasing nitrogen addition rate, and reached a maximum valve at the N2 nitrogen addition rate in the CK, D, CK + A, and D + A treatments. This may be due to the maximum enzymatic activity of N1 nitrogen addition rate to *Alhagi sparsifolia* seedlings, which reduced the MDA content in the plants. The contents of SOD, POD, CAT, MDA, Pro, and SS of *Alhagi sparsifolia* seedlings were initially increased then decreased and increased at the same level of nitrogen addition rate. The increase in antioxidant enzyme activity in *Alhagi sparsifolia* seedlings after AMF inoculation reduced the MDA content and alleviated the degree of cell injury in *Alhagi sparsifolia* seedlings. The cellular osmotic pressure was maintained through the accumulation of osmotic substances, which, in turn, enhanced the cellular osmoregulatory capacity and the ability of *Alhagi sparsifolia* to cope with adversity; and its value was greatest at the N1 nitrogen addition rate when inoculated with AMF.

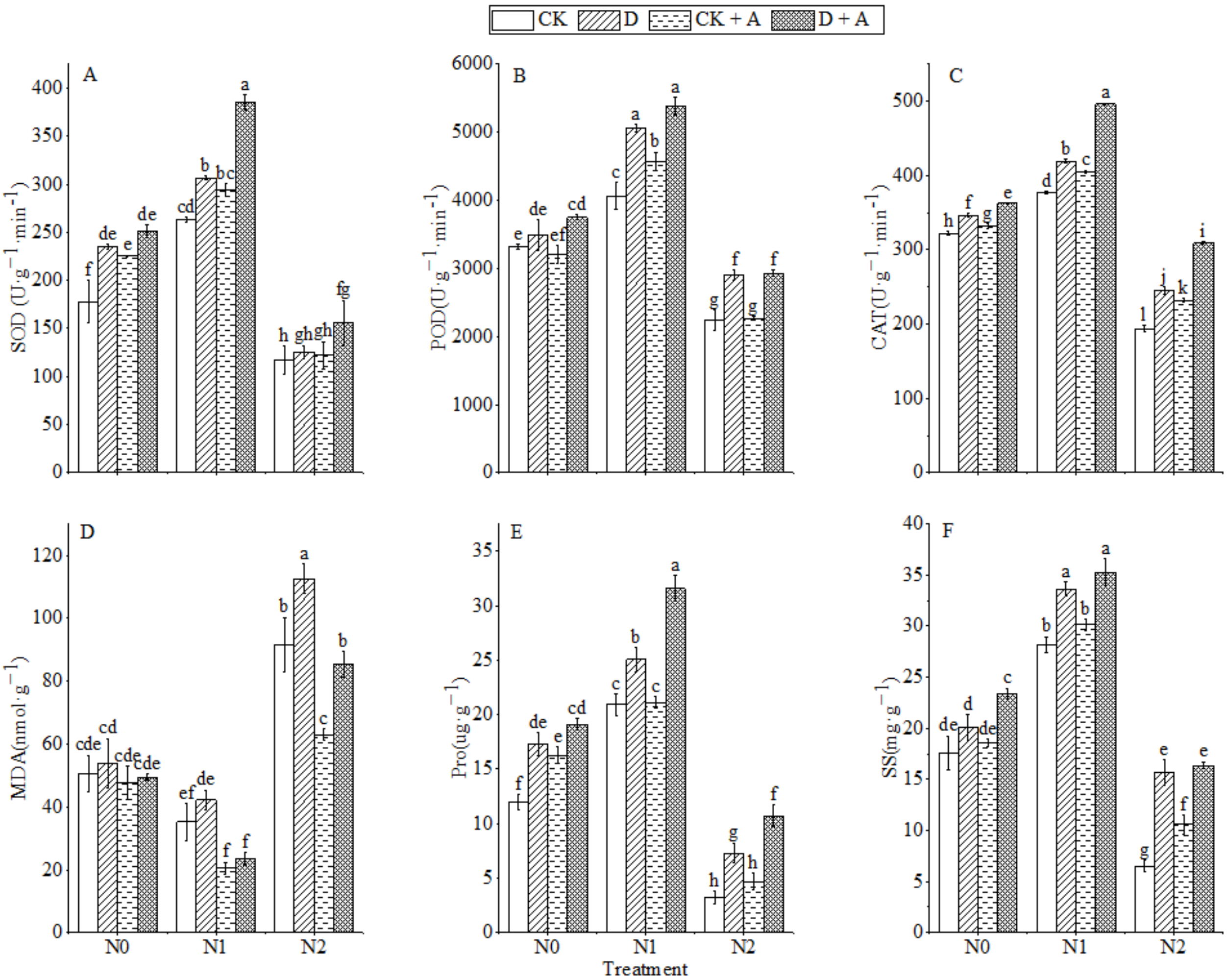

Figure 6. Effect of AMF inoculation and N addition on the antioxidant enzymes, malondialdehyde, osmoregulatory substances of *Alhagi sparsifolia* seedlings under drought stress. Note: (**A**) represents superoxide dismutase activity; (**B**) represents peroxidase activity; (**C**) represents catalase activity; (**D**) represents malondialdehyde content; (**E**) represents proline content; (**F**) represents soluble sugar content; CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the antioxidant enzymes, malondialdehyde, osmoregulatory substances of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

3.7. *Effect of AMF Inoculation and N Addition on the Hormone Content of Alhagi sparsifolia Seedlings under Drought Stress*

Drought stress induced a decrease in the leaf growth of indole acetic acid (IAA) and gibberellic acid (GA) contents, an increase in the abscisic acid (ABA) and strigolactone

(SLs) content of *Alhagi sparsifolia* seedlings (Figure 7). The leaf IAA, GA, ABA, and SLs contents of *Alhagi sparsifolia* seedlings inoculated with AMF were significantly higher than those of the uninoculated treatment at all nitrogen addition rates, whether for sufficient moisture or drought stress treatment. The contents of IAA, GA, ABA and SLs in the leaves of *Alhagi sparsifolia* seedlings were initially increased and then decreased with the increasing nitrogen addition rate, reaching a maximum valve at the N1 nitrogen addition rate in CK, D, CK + A, and D + A treatments. The leaf IAA and GA contents of *Alhagi sparsifolia* seedlings initially decreased then increased and decreased, while the contents of ABA and SLs initially increased then decreased and increased at the same level as the nitrogen addition rate. Drought inhibits the accumulation of IAA and GA, and induces the increase in ABA and SLs, which adversely affects the growth and development of seedlings, while the inoculation of AMF can effectively promote the accumulation of the above hormones in seedlings, and reduce the damage caused by drought stress to plants.

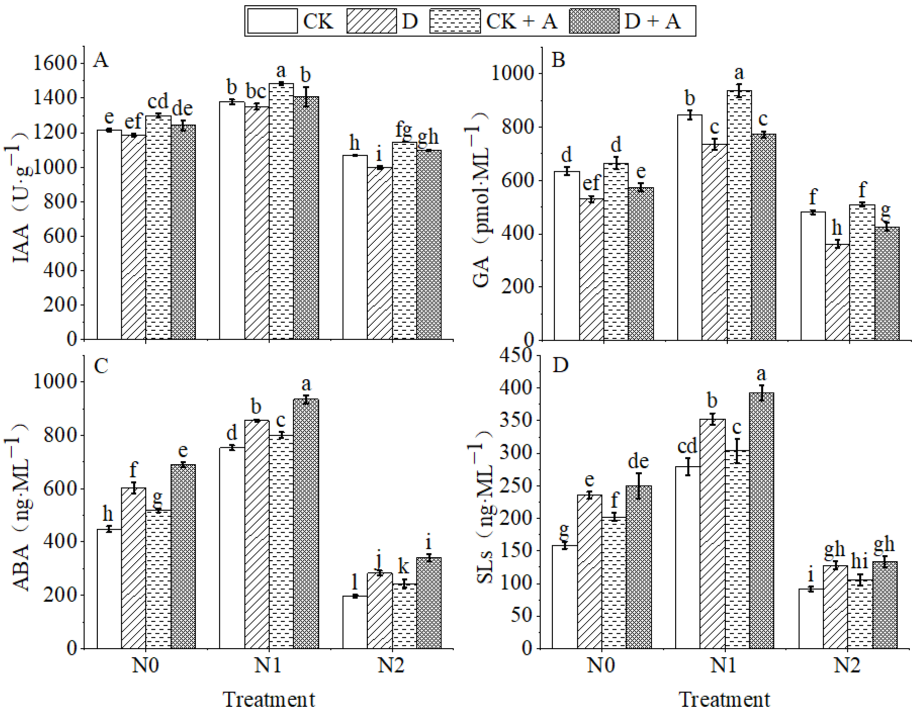

Figure 7. Effect of AMF inoculation and N addition on the hormone content of *Alhagi sparsifolia* seedlings under drought stress. Note: (**A**) represents indole acetic acid content; (**B**) represents gibberellic acid content; (**C**) represents abscisic acid content; (**D**) represents strigolactone content; CK represents sufficient moisture; D represents drought stress; CK + A represents inoculation with AMF under sufficient moisture; D + A represents inoculation with AMF under drought stress; N0, N1, and N2 represent three levels of nitrogen addition, 0 mmol·L^{-1}, 50 mmol·L^{-1}, and 500 mmol·L^{-1}, respectively. Different lowercase letters indicate significant differences in the hormone content of *Alhagi sparsifolia* seedlings at different levels of CK + A, CK, D + A, D, and nitrogen addition ($p < 0.05$).

3.8. The Effect of Drought Stress, N Addition, AMF Inoculation and Their Interaction on All Measured Response of Alhagi sparsifolia Seedlings Variables Tested by Two-Way ANOVAs

As shown in Table 3, in this study, drought stress, AMF *inoculation* and N addition had significant effects on the growth and physiology of *Alhagi sparsifolia* seedlings, although their interaction was not significant. The interactions were counteracted and balanced between the effects. Appropriate amounts of nitrogen addition and mycorrhizal colonization usually compensate for drought-stress-induced growth, photosynthesis, plant nutrient uptake, and physiological decline in *Alhagi sparsifolia* seedlings. AMF can transport the nitrogen source to the stem and leaves, which is more conducive to the growth and reproduction of *Alhagi sparsifolia* seedlings and resistance to drought stress. In contrast, when nitrogen was added in excess, *Alhagi sparsifolia* seedlings were in an environment rich in nitrogen sources. The nitrogen fixation capacity of legumes decreased, and the indexes also reduced; thus AMF inoculation did not have a synergistic effect at this time.

Table 3. The effect of drought stress, N addition, AMF inoculation, and their interaction on all measured responses of *Alhagi sparsifolia* seedlings variables tested by two-way ANOVAs.

Index	Significance						
	D	**N**	**AMF**	**D × N**	**D × AMF**	**N × AMF**	**D × N × AMF**
Root inoculation	1.186	2.941	350.206 ***	0.039	1.186	2.941	0.039
Plant height	73.422 ***	157.141 ***	396.306 ***	0.884	8.332 **	31.164 ***	0.319
Base diameter	37.112 ***	136.908 ***	208.868 ***	6.686 **	7.551 *	6.345 **	3.782 *
Above-ground biomass	186.82 ***	582.302 ***	780.206 ***	1.146	4.465 *	2.104	7.931 **
Below-ground biomass	51.2 ***	124.8 ***	320 ***	5.6 *	0.8	22.4 ***	5.6 *
Total root length	113.625 ***	1689.619 ***	769.939 ***	4.348 *	2.226	61.143 ***	4.304 *
Root surface area	60.302 ***	805.132 ***	438.769 ***	5.177 *	0.456	26.141 ***	2.38
Root volume	78.322 ***	1651.574 ***	369.208 ***	0.108	3.077	12.317 ***	2.32
Average root diameter	14.387 **	77.431 ***	27.891 ***	7.183 **	3.413	13.734 ***	4.121 *
Number of tips	102.186 ***	1206.351 ***	386.444 ***	38.755 ***	5.1 *	70.907 ***	10.43 **
Root shoot ratio	92.235 ***	214.235 ***	576.471 ***	7.176 **	0.471	9.294 **	17.765 ***
Specific root length	23.936 ***	16.363 ***	117.945 ***	3.42 *	5.901 *	4.195 *	9.985 **
CHLa	4.644 *	87.956 ***	52.771 ***	0.986	0.013	12.639 ***	0.209
CHLb	22.297 ***	164.287 ***	67.423 ***	0.88	0.001	3.768 *	3.56 *
CHL	9.729 **	125.066 ***	67.199 ***	1.132	0.007	10.96 ***	0.783
CHLa/b	0.005	32.437 ***	26.481 ***	0.65	0.84	29.146 ***	0.421
Fo	843.642 ***	124.953 ***	3339.817 ***	3.827 *	0.134	4.587 *	0.233
Fm	277.804 ***	10.419 ***	74.907 ***	142.903 ***	12.126 **	39.6 ***	44.977 ***
Fv/Fm	1027.49 ***	117.489 ***	2909.143 ***	0.232	1.231	4.089 *	0.26
N	220.313 ***	1440.796 ***	891.605 ***	20.116 ***	2.417	76.419 ***	1.915
SOD	41.146 ***	241.406 ***	26.301 ***	3.974 *	1.115	2.518	3.07
POD	81.951 ***	324.648 ***	6.008 *	5.125 *	0.244	3.028	1.353
CAT	1158.081 ***	4471.09 ***	629.922 ***	67.793 ***	76.877 ***	69.895 ***	15.553 ***
MDA	11.634 **	140.322 ***	31.923 ***	4.449 *	0.056	6.006 **	0.079
Pro	117.999 ***	435.715 ***	34.395 ***	3.655 *	3.66	0.29	6.369 **
SS	99.887 ***	430.479 ***	14.685 **	4.133 *	0.168	0.091	2.296
IAA	20.427 ***	277.361 ***	50.02 ***	0.166	0.566	0.219	0.837
GA	138.342 ***	531.272 ***	26.785 ***	1.684	0.002	0.65	1.976
ABA	345.769 ***	2418.311 ***	96.163 ***	9.865 **	2.106	1.296	0.234
SLs	88.022 ***	407.004 ***	14.511 **	5.213 *	0.365	1.244	1.057

F-values are followed by *p*-values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. The Inoculation Rate

The symbiotic relationship between plants and AMF is most beneficial during the early stages of plant growth and development, and the number of AMF propagules can be increased by preserving an intact fungal mycelium, which can lead to a faster and more effective establishment of the symbiotic relationship between the plant root and AMF [43]. In this study, drought stress led to a significant decline in the inoculation rate of the *Alhagi sparsifolia* seedlings, consistent with the findings of Abd_Allahet al. [44]. The root system of *Alhagi sparsifolia* seedlings did not exhibit structures such as root nodules, indicating the positive effect of soil and seed sterilization. AMF act as a specialized symbiont, obtaining energy such as carbohydrates and fatty acids from the host plant and transferring P, N, or other nutrients to the host plant for its growth and development through mycelium [45]. As described by Al-Malikiet al. [46], a positive effect of nutrient element application on AMF root inoculation rate can be observed even under drought stress conditions. In this study, we observed that the mycorrhizal inoculation rate of *Alhagi sparsifolia* seedlings ranged from 55.56% to 84.44%, initially increasing and then decreasing with the increasing nitrogen addition rate. It was shown that drought stress has a direct effect on the AMF developmental cycle, inhibiting spore germination, mycelial elongation, and spore production, thus reducing the inoculation rate [47]. Low nitrogen treatment increases the effective nitrogen available in the soil for direct plant uptake, promotes plant growth, correspondingly increases the carbon supply from plants to mycorrhizal fungi, and strengthening mycorrhizal symbiosis, which, in turn, promotes the effective nitrogen absorbed by plants through mycelial networks [48].

4.2. Growth and Plant N Uptake

Although drought stress inhibited the relative growth rate of height, basal diameter, and biomass of *Alhagi sparsifolia* seedlings in this study, inoculation with AMF significantly showed that their reduction is consistent with the findings of Xiao et al. [49], and the relative growth rate of height, basal diameter and biomass of *Alhagi sparsifolia* seedlings showed a single-peaked variation with the increasing nitrogen addition rate. In soil-plant systems, biomass, as the main input source to the soil carbon pool, is often used to assess the response of plant growth to environmental ecological conditions [50]. Excess nitrogen in the soil can inhibit plant growth and reduce its biomass by affecting mycorrhizal formation [51]. Notably, the above-ground biomass of *Alhagi sparsifolia* seedlings in this study was significantly greater than the below-ground portion, indicating that seedling roots significantly enhanced water and nutrient uptake in a low-nitrogen environment with the assistance of AMF [52]. In the lower Tarim River desert riparian forest, where water and nitrogen are deficient, *Alhagi sparsifolia* seedlings can expand their root mycelial network through effective AMF infestation, thus promoting the plant body to draw more much-needed water and nutrients from the soil and transport them to the above-ground parts so that the stems and leaves of the seedlings can grow rapidly. From the perspective of carbon allocation, seedlings are more capable of enhancing the accumulation of material in the aboveground parts, and rapidly increasing the photosynthetic area, thus promoting rapid growth of the plant body in adverse conditions [53].

In this study, low-nitrogen and inoculation treatments increased the root growth of *Alhagi sparsifolia* seedlings under drought stress conditions, whereas high-nitrogen treatments produced an inhibitory effect. It was shown that AMF produces mycelium by infesting the root system of *Alhagi sparsifolia* seedlings, which rapidly grows and penetrates the subsoil layer to improve each root morphological index in low N and drought environments, which can maximize the root uptake area using limited carbon accumulation and enhance the deep rooting and uptake capacity of the root system in the soil, thus achieving a continuous increase in resilience [54]. Instead, high N conditions disrupted the symbiotic environment between AMF and *Alhagi sparsifolia* seedlings, eliminating soil N limitation, and reducing the root uptake range and root uptake capacity of *Alhagi sparsifolia* seedlings, thus reducing the allocation of below-ground biomass by *Alhagi sparsifolia* seedlings [55]. In addition, the results of nitrogen uptake in *Alhagi sparsifolia* seedlings showed that the combination of AMF and low-N treatment was beneficial to their nutrient uptake, significantly increasing the N content of *Alhagi sparsifolia* seedlings and peaking at low-N levels. It is evident that inoculation with mycorrhizal fungi and low-N treatment can promote the uptake of soil nutrients by affecting the root growth of *Alhagi sparsifolia* seedlings [56]. In contrast, high N treatment became the limiting factor and the N content of *Alhagi sparsifolia* seedlings decreased significantly, indicating that *Alhagi sparsifolia* seedlings weaken the mycorrhizal symbiosis by reducing the subsurface carbon allocation as a survival strategy under high N treatment, thus reducing the nutrient uptake capacity of the root system of *Alhagi sparsifolia* seedlings [57].

4.3. Photosynthesis

Chlorophyll, a pigment, plays a unique role in the Calvin cycle by absorbing light energy and producing biochemical energy during photosynthesis [58]. Chlorophyll fluorescence has been widely used to determine the effects of drought and other stresses on plant photosynthesis, because it reflects the "intrinsic nature" of photosynthesis [59,60]. In this study, the negative effects of drought stress on chlorophyll destruction and photosynthesis in *Alhagi sparsifolia* seedlings were significant, including a significant increase in seedling *Fo*, a decrease in chlorophyll a, b, total and chlorophyll a/b content, and decreases in *Fm* and *Fv/Fm*. However, *Alhagi sparsifolia* seedlings with AMF inoculation showed a significant decrease in *Fo* and a significant increase in both chlorophyll content and *Fv/Fm*, and both were optimal at low nitrogen levels. It was shown that drought stress causes damage to the chloroplast lamellar structure of *Alhagi sparsifolia* seedlings, causing water loss in the leaves, which affects chlorophyll synthesis, causes some degree of damage to the PS II system, and inhibits the heat dissipation function, ultimately leading to a decrease in photosynthesis [61]. Additionally, AMF symbiosis with *Alhagi sparsifolia* seedlings can help seedlings transport root water to the above-ground part for photosynthetic production, accelerate the cycle of related enzymes, water and protein during chlorophyll synthesis, decrease the rate of chlorophyll decomposition, and increase chlorophyll content and plant photosynthetic rate. Therefore, AMF inoculation and low-N treatment have become an important way to alleviate the impaired photosynthetic capacity of *Alhagi sparsifolia* seedlings [62].

4.4. Antioxidant Enzymes, Malondialdehyde, and Osmoregulatory Substances

Under normal conditions, the production and scavenging of reactive oxygen species ROS in plants are in dynamic balance [63]. In contrast, under drought stress, plant carbon assimilation processes are weakened, PS II activity is reduced, and free radicals accumulate in large quantities, causing membrane lipid peroxidation, and thus reducing the photosynthetic capacity of plant leaves [64,65]. In the present study, to prevent excessive reactive oxygen species production, the antioxidant enzymes and osmoregulatory systems were activated in the leaves of phytoplankton under drought stress, and the leaf antioxidant enzyme activity, osmoregulatory substance content and malondialdehyde content of *Alhagi sparsifolia* seedlings increased, indicating that the intracellular dynamic balance of the phytoplankton was disrupted. Superoxide dismutase (SOD), a key important oxygen radical scavenger, can dismutate superoxide radicals into H_2O_2 and O_2, while peroxidase (POD) and catalase (CAT) can assist SOD to effectively prevent the accumulation of H_2O_2, so that free radical scavenging capacity is enhanced and reactive oxygen species are in equilibrium, limiting potential oxidative damage and protecting photosynthesis [66]. Proline (Pro) and soluble sugar (SS), as energy sinks regulating redox potential, produce osmolytes to protect photosynthesis, maintain cell swelling, and avoid hydraulic failure during drought stress, improving plant drought resistance [67]. In this study, the trend of increasing antioxidant enzyme activity and osmoregulatory substances in *Alhagi sparsifolia* seedlings under drought stress was due to the stimulated activation of the antioxidant enzyme system to produce resistance and reduce the use of proline in the plant [68]. The antioxidant enzyme activity and osmoregulatory substance content were higher and the MDA content was lower in *Alhagi sparsifolia* seedlings after AMF inoculation, which is consistent with the findings of Zhang et al. [69]; the antioxidant enzyme activity and osmoregulatory substance content in *Alhagi sparsifolia* seedlings showed a single-peaked variation with the increasing nitrogen addition rate, and the MDA content showed a trend of decreasing and then increasing. It was shown that AMF with low-N treatment in a drought-deficient environment could induce an enzymatic defense system response, slow down oxidative damage caused by lipid peroxidation, facilitate root access to external water, promote the accumulation of organic matter produced by photosynthesis partially for leaf proline and soluble sugars, and maintain normal cellular functioning, thus preventing or reducing damage to *Alhagi sparsifolia* from drought stress, and helping *Alhagi sparsifolia* seedlings to survive the growth vulnerability period [70–74].

4.5. The Hormone Content

Indole acetic acid (IAA) and gibberellic acid (GA) are two extremely important endogenous hormones in plant growth and development [75]; IAA promotes not only root growth, but also stem elongation and fruit development [76], while GA promotes plant elongation, IAA accumulation, and inhibits plant senescence [77]. Abscisic acid (ABA) acts as a stress hormone that inhibits growth and promotes stomatal closure [78]. Strigolactones (SLs) are important molecules in the root interval that facilitate the establishment of AM symbiosis and can initiate a molecular dialogue between the plant root system and AMF [79]. In this study, IAA and GA contents of *Alhagi sparsifolia* seedlings decreased, ABA and SLs contents increased under drought stress, while inoculation with AMF significantly increased the accumulation of IAA, GA, ABA, and SLs, which is consistent with the findings of Wang et al. [80]. They improved the growth and development of seedlings; alleviated the negative effects of drought on seedlings under either drought stress or normal water; and the effects were more pronounced at low-N levels, indicating that endogenous plant hormones, as key physiologically active substances for plant growth and development, can act as signaling substances when subjected to drought stress and help plants to respond quickly and effectively to various environmental stresses [81]. Additionally, inoculating AMF can activate oxidative metabolism, coordinate various phytohormones, drive mycelial growth and branching increase, increase the chance of physical contact between mycelium and host plant roots, and promote the better and faster establishment of symbiotic relationships [82,83]. Low-nitrogen treatment can improve the growth and physiological characteristics of *Alhagi sparsifolia* seedlings [84], thus enhancing plant resistance to drought stress [85].

5. Conclusions

To investigate the ecological effects of Arbuscular mycorrhizal fungi (AMF) on *Alhagi sparsifolia* seedlings during the vulnerable growth period under drought stress and nitrogen limitation, this study was conducted to investigate the effects of different treatments of water, nitrogen, and AMF on the growth, photosynthesis, nutrition and stress tolerance of *Alhagi sparsifolia* seedlings through indoor pot experiments. In conclusion, drought stress obviously inhibited the rate of mycorrhizal inoculation, with an obvious reduction in plant relative growth rate of height, basal diameter, biomass, root system, photosynthesis, nitrogen content, and an obvious increase in plant membrane lipid peroxidation levels of drought stress as compared with plants exposed to CK (no inoculation and the soil water content of $70 \pm 5\%$ of the field holding capacity), thereby affecting the physiological activity of *Alhagi sparsifolia* seedlings. Inoculation with AMF and proper nitrogen addition improved the growth and physiology of *Alhagi sparsifolia* under drought stress. The inoculation of AMF enhances stem and leaf growth and photosynthesis by promoting the transport of water and nutrients from the seedling root system to the above-ground parts of the plant, and uses photosynthetic organic products to improve leaf enzyme activity, accumulate osmoregulatory substances and endogenous hormones, and reduce the degree of membrane lipid peroxidation, which ultimately improves the drought resistance of seedlings, thus helping seedlings to quickly pass through the vulnerable stage of growth. Therefore, AMF has an extremely important ecological role in the growth and development of *Alhagi sparsifolia* seedlings in a drought-deficient environment, and AMF inoculation is a key link in the survival strategy of *Alhagi sparsifolia*.

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