# Effects of Arbuscular Mycorrhizal Inoculation on Growth, Photosynthesis and Antioxidant Enzymatic Activity of *Euonymus Maackii* Rupr. Under Gradient Water Deficit Levels

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# Abstract

The role of arbuscular mycorrhizal (AM) fungus (*Rhizophagus intraradices*) in amelioration of water deficit mediated negative influence on growth, photosynthesis and antioxidant system in *Euonymus maackii* Rupr. was examined. *E. maackii* seedlings were subjected to 5 water deficit levels: soil water contents of 20 %, 40 %, 60 %, 80 % and 100 % field capacity (FC) respectively, and 2 inoculation treatment: with and without AM inoculation. Water deficit increasingly limited seedlings growth of height, biomass accumulation of shoot and root, chlorophyll content, gas exchange and chlorophyll fluorescence parameters along the increase of water deficit level. In addition, Water deficit stimulated the activities of antioxidant enzymatic activities, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of both shoot and root, except under 20 % FC condition. *E. maackii* seedlings under all water deficit conditions formed AM symbiosis well with AM fungi, which ameliorated the drought mediated negative effect significantly, especially under 40 % and 60 % FC conditions. Under 40 % to 80 % FC conditions, AM formation improved seedlings growth and photosynthesis by significantly enhancing biomass accumulation, chlorophyll content and assimilation. Mycorrhizal seedlings showed better tolerance and less sensitive to water deficit, reflected in lower SOD activities of shoot and root, and CAT activity of shoot under 40 % and 60 % FC conditions. Down-regulation of antioxidant system in mycorrhizal seedlings suggested better maintenance of redox homeostasis and protection of metabolism, including biomass accumulation and assimilation. All the results advocated the positive role of *R. intraradices* inoculation in *E. maackii* against water deficit, which suggested the potential role of AM fungi in ecological restoration in arid region.


# 1. Background

The continued economic activities have induced severe environmental concerns, such as soil salinization, soil erosion, water deficit, which seriously limited plant growth (Du et al. 2008). Water deficit is one of the major limiting factors on agricultural and forestal yield (Solomon et al. 2007). Water deficit has emerged as a global alarming stress factor, causing excessive loss of agriculture production and ecosystem damage. In the area of arid and semi-arid regions, incidence of water deficit episodes increased in the last decades (Zhu et al. 2012). Even in the non-arid region, seasonal drought could not be avoided (Rouphael et al. 2015).

*Euonymus maackii* Rupr. is an important ecological restoration tree species, and is widely cultivated in northwest China. *E. maackii* is economically important as material for biodiesel (Liu et al. 2019). However, it is a high water-consuming species, and severely limited by water deficit. Drought caused severe damage on plant growth by influencing antioxidant enzymatic activity, photosynthesis capacity and mineral nutrient uptake (Farooq et al. 2009; Ahanger & Agarwal 2017). Water deficit induced stomatal closure and photosystem I and II, resulting in limited access to $CO_2$. Drought-mediated decrease in growth and biomass accumulation results from photosynthesis decline (Laleel et al. 2007).

When water deficit occurred, reactive oxygen species (ROS) excessively accumulated in plant tissue, causing serious oxidative damage, structural and functional integrity damage of cellular organelles, and up-regulated antioxidant enzymatic activities, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Ahanger & Agarwal 2017). The antioxidant system is considered to eliminate the excess accumulation of ROS to protect plant metabolism. The negative effect by water deficit stress on plants depend on the severity and duration of the stress (Farooq et al. 2009).

Arbuscular mycorrhizal (AM) symbiosis occurred in almost all terrestrial ecosystems, and have been widely reported to form mutually beneficial relationship (Smith & Read 2008). A range of different plants and AM fungal species have been reported that AM symbiosis improved plant capacity of tolerating water deficit through a variety of ways, such as enhancing water and mineral nutrient uptake, improving photosynthesis and up-regulating antioxidant system and the gene expression of stress responsive genes (Ruiz-Lozano 2003; Li et al. 2015; Smith & Read 2008). AM mediated regulations in plant morphological and physio-chemical processes, such as improvement in water uptake and mineral assimilation, are reflected in better performance in photosynthesis and biomass accumulation (Ruiz-Lozano 2003).

To investigate the effect of AM inoculation treatment on *E. maackii* when subjected to different water deficit levels, we set this study and hypothesize that: 1) water deficit would limit growth, photosynthesis and stimulate antioxidant system of *E. maackii*; 2) the negative effect by drought would increase with water deficit level increase; 3) AM inoculation treatment would enhance plant to cope with water deficit. Furthermore, different attributes between moderate and severe water deficit result in different responses of seedlings, which could ultimately result in distinct AM performance in drought tolerance. Thus, to test the potential role of AM fungi in ecological restoration in arid region is another aim of this study.

# 2. Methods

## 2.1 AM inoculum

*Rhizophagus intraradices* [Schenck & Smith (BGC BJ09)], provide by Beijing Academy of Agriculture and Forestry Science) was used as AM inoculation in this study. The AM inoculum was expanding propagated with *Trifolium repens*, and consisted of AM spores (about 50 spores per gram of incolum), mycelia, root fragments and soil.

## 2.2 Plant and soil treatment

Full seeds of *E. maackii*, provided by Shaanxi Provincial Forestry Technical Extension Station, were selected and disinfected in 0.5% $KMnO_4$ solution for 20 min, and washed 3 times with sterile water, and soaked in sterile water for 24 h. Seeds were transferred on a salver covered with the wet gauze and kept at 25 °C, 12 h light in a illumination incubator for rapid germination. After the seeds germinated and grew to 1 cm, the seedling pot (47 cm × 33 cm, 66 holes) filled with sterilized vermiculite were used for further cultivation. One germinated seed was put in one hole, and the seedling pot were placed at 25 °C, 12 h

light in an illumination incubator. The seeds were watered every morning (20 ml/hole) for 1 month, and fully grown seedlings of similar growth were transplanted to the pots descripted as following.

Topsoil of 5–20 cm in depth was collected from a field of *E. maackii* growing. The soil, sieved through a 2 mm sieve, were mixed with fine washed sand (v: v = 1: 1). The mixed substrate was then autoclaved at 0.11 MPa and 121 °C for 20 min for further pot experiment. The topsoil physicochemical properties were as follows: soil organic carbon (SOC), 16.37 g·kg$^{-1}$; available K, 111.08 g·kg$^{-1}$; available N, 33.36 mg·kg$^{-1}$; available P, 10.25 mg·kg$^{-1}$; pH value (soil: water = 1: 5), 7.9. The field capacity (FC) of mixed substrate was 22.50%. Compared with soil, nutrition of fine washed sand used was very few and could be ignored.

# 2.3 Experimental design

The experimental design included 2 factors: inoculation treatment (inoculated with *R. intraradice*s or autoclaved inoculum); gradient of water deficit (20%, 40%, 60%, 80% and 100% FC). The seedlings of similar growth were planted in 1 l plastic pots filled with 1 kg of preprocessed substrate and grown in the artificial climate greenhouse at 25–30 °C, with 12 h light per day and stable humidity of 50%. 15 replications of each treatment were used in this experiment, totaling 150 seedlings (2 × 5 × 15). Half of the pots were inoculated with 10 g of AM inoculum, and the remaining pots were non-mycorrhizal control and inoculated with 10 g of autoclaved inoculum. All the seedlings were well watered for the first 3 months with sterile water, and added 150 ml of Hoagland's complete nutrient solution every 2 weeks. After 3 months, pots subjected to 20%, 40%, 60%, 80% FC treatments remained unwatered until their soil water content reached desired FC levels. The rest of the pots were subjected 100% FC treatment, and were kept well-watered. Pots were arranged in a randomized complete block design. All pots were kept at stable water content for 30 d and then harvested. All pots were weighed and watered every day at 17:00 to maintain the water content at the desired FC levels. All the indexes were measured with 6 randomly selected seedlings of each treatment.

# 2.4 AM colonization rate and AM dependency measurement

The AM colonization rate was measured as described by Phillip and Hayman (1970). At harvest, the seedlings with substrate on roots were moved gently from the pot. The roots were washed carefully with flowing tap water to remove the substrate, and then rinsed 3 times with distilled water. Fine roots (< 2 mm) were cut into 1-cm-long segments and bleached in 5% KOH at 90 °C in a water bath. Then the cleared root segments were acidified in 1% HCl solution for 5 min and stained with trypan blue, and placed on the glass slide for colonization determination. A total of 150 root segments per treatment were examined under the microscope to determine the proportion of the root length that had been colonized by the AM fungi. The presence or absence of AM fungi in roots from inoculated and uninoculated seedlings were further confirmed by a nested-PCR using the primer pairs: SSUmAf/LSUmAr and SSUmCf/LSUmBr (Krüger et al. 2009). AM dependency was calculated by determining the ratio of dry weight of mycorrhizal seedling to the dry weight of non-mycorrhizal one.

# 2.5 Growth index and biomass measurement

At the beginning and end of the last 30-day water treatment, height was measured by tape. Growth of height (GH) was recorded as the average amount of growth every day. The fully expanded leaf (near the apex) was used to measure the relative chlorophyll content by a SPAD meter (SPAD-502, Minolta, Tokyo, Japan).

At harvest, seedlings were cut into shoot and root part, placed at 105 °C for 20 min in an oven to destroyed the enzymes, and then dried at 80 °C to constant weight to determine the biomass of shoot and root. The Root/Shoot ratio was calculated by determining the ratio of root biomass to shoot biomass.

# 2.6 Gas exchange measurement

Within a week before harvest, the net photosynthesis (Pn), stomatal conductance (Gs), intercellular $CO_2$ concentration (Ci) and transpiration rate (E) of the fully expanded leaves of six randomly selected seedlings in each treatment were measured at a light intensity of 1,000 μmol (photon) $m^{-2}$ $S^{-1}$ between 08:00 and 11:30 h using a *Li- 6400* portable photosynthesis measuring system (Li-Cor Inc., Lincoln, NE, USA). The parameters were set as following: temperature, 30 °C; leaf-air vapor pressure deficit, 1.5 ± 0.5 kPa; relative humidity, 50% and ambient $CO_2$, 350 μmol $mol^{-1}$.

# 2.7 Chlorophyll fluorescence measurement

The fully expanded leaves near the apex of six randomly selected plants of each treatment were placed in the dark for 30 min at room temperature before Chlorophyll fluorescence measurement. The maximal fluorescence (Fm) and the minimum fluorescence (Fo) yields were determined using a modulated chlorophyll fluorometer (MINI-Imaging-PAM, Walz, Germany). The actual quantum yield of photosystem II (PSII) (ΦPSII), the maximum quantum yield of PSII (Fv/Fm), the non-photochemical quenching (qN) and the photochemical quenching (qP) were calculated as following:

Fv/Fm = (Fm − Fo)/Fm;

ΦPSII = (Fm'−F)/Fm';

qN = 1−(Fm'−Fo')/(Fm−'Fo);

qP = (Fm'−F)/(Fm'−Fo').

# 2.8 Statistical analysis

All the data were analyzed using statistical analysis software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Two-way analyses of variance (ANOVA) was adapted to evaluate the significance of effects of water deficit, AM inoculation and their interaction on seedlings at the significant level of $P = 0.05$. The means were compared using Duncan's multiple range test and HSD test ($P = 0.05$). Principal component analysis (PCA) was implemented to reduce all the parameters to the fewest dimensions keeping the eigenvalue > 1. Before PCA, the correlation analyses were performed by Pearson correlation coefficients, and highly

correlated variables were removed (only one remain), and all remaining data were standardized and used for PCA. All figures were made using Origin 9.1.

# 3. Results

## 3.1 Water regimes in weight loss of pots

In the first 3 months, the pots lost 45–55 g in weight every day, and it took about 4 d that the water content in water deficit treatments changed from 100% to 20% of FC before water deficit treatment. During the water deficit treatment period, the average weights lost every day in 20%, 40%, 60%, 80% and 100% FC treatments were about 32, 37, 41, 45 and 50 g respectively, and showed slight increases with time.

## 3.2 AM inoculation status

Seedlings that had received AM inoculation treatment formed typical AM structure, and in all non-mycorrhizal treatments, no AM structure was detected in plant roots (Fig. 1). Water deficit decreased the AM inoculation rate from 78.40% to 58.00%, and improved the AM dependency from 103.46% to 143.13% (Fig. 2). There was no significant difference between AM inoculation rates and AM dependencies of seedlings in 100%, 80% and 60% FC treatments. ANOVA results indicated that the significant effects of water deficit levels on AM inoculation rate and AM dependency.

## 3.3 Growth and biomass accumulation

Seedlings were affected by water deficit and showed significant gradually reduced growth of height (GH) and SPAD compared with seedlings of 100% FC (Fig. 3). Compared with non-mycorrhizal seedlings, mycorrhizal seedlings showed significant higher GH under moderate drought conditions (40%, 60% and 80% FC), and higher SPAD of seedlings in 60% and 80% FC treatment. Meanwhile, under extreme drought treatment (20% FC), no obvious difference was detected between mycorrhizal and non-mycorrhizal seedlings in GH and SPAD. ANOVA results suggested GH and SPAD were significantly affected by AM inoculation and drought and their interaction.

Compared with seedlings of 80% and 100% FC, the biomass of the shoot (BS), the biomass of the root (BR), and the total biomass (TB) of seedlings that had been subjected to the more serious water deficit treatment (20%, 40% and 60% FC) were significantly lower (Fig. 4). Effect of AM inoculation was not obvious in 80% and 100% FC treatments in BS, BR and TB. Furthermore, seedlings grown under water deficit conditions of 40% and 60% FC that had significantly lower BS, BR and TB than seedlings that had received the inoculation treatment. Besides, water deficit decreased Root/Shoot ratio (RSR) gradually, whereas the effect of AM inoculation was not significant. ANOVA results suggested the significant effect of AMF and water deficit on BS, BR and TB, and the significant effect of interaction of AMF and water deficit on BS, TB and RSR.

## 3.4 Gas exchange

The gas exchange measurement recorded for mycorrhizal and non-mycorrhizal seedlings grown under water deficit were similar: Pn, Gs, Ci and E were limited gradually by gradient water deficit (Fig. 5). Inoculation of seedlings with AM fungi had no significant effect on Gs and E. In 20%, 80% and 100% FC treatments, no significant difference was detected between mycorrhizal and non-mycorrhizal seedlings in Pn and Ci. Meanwhile, compared with non-mycorrhizal seedlings, mycorrhizal seedlings showed significantly higher Pn and Ci.

ANOVA results suggested that AM inoculation had significant effect on Pn, Ci and E, and the effect of water deficit were significant on all gas exchange parameters detected. However, the interaction of AM inoculation and water deficit only affected Pn and Gs significantly.

## 3.5 Chlorophyll fluorescence

By the end of the water deficit treatment period, seedlings that grown under gradient water deficit showed gradually decreasing qN, qP, Fv/Fm and ΦPSII (Fig. 6). The qN among seedlings that inoculated with AM fungi was significantly higher than uninoculated seedlings under 60% FC condition. Seedlings that received AM inoculation treatment had significantly higher qP under 40% and 60% FC compared with uninoculated seedlings. Under 40%, 60% and 80% FC conditions, inoculated seedlings showed significantly higher ΦPSII compared with uninoculated seedlings. However, no significant effect of AM inoculation was detected in Fv/Fm of seedlings that grown under all water deficit conditions. ANOVA results indicated that the effects of AM inoculation, water deficit and their interaction were significant on all these gas exchange parameters.

## 3.6 Antioxidant enzyme activities

Compared with growth indexes detected above, the trend of changes in antioxidant enzymatic activities along with water deficit levels was complicated (Fig. 7). The SOD, POD and CAT activities of shoot and root showed similar changes along with water deficit levels: increased from treatment of 100% to 60% FC, peaked under 40% or 60% FC conditions, and decreased under 20% FC condition. However, SOD, POD and CAT showed different responses to AM inoculation treatment. SOD activities of shoot and root among seedlings that received AM inoculation treatment were significantly lower than uninoculated seedlings under 20% and 40% FC conditions. By contrast, AM inoculation significantly improved SOD activity of shoot under 80% FC condition.

In shoot, POD activity of seedlings that received AM inoculation treatment had no difference with that of non-mycorrhizal seedlings. Furthermore, in root, AM inoculation significantly increased POD activity under 40% and 60% FC conditions, whereas contrary effect of AM inoculation treatment was detected under 80% and 20% FC conditions. Besides, the intensity of change of POD activities along with water deficit levels in root was greater than that in shoot. In shoot, seedlings that received AM inoculation treatment

showed significantly lower CAT activity under 40% and 60% FC conditions. For CAT activity in root, AM inoculation had no effect.

ANOVA results indicated that AM inoculation effect was significant on SOD activities of shoot and root and CAT activity of root; water deficit treatment had significant effect on SOD, POD and CAT activities; the interaction of AM inoculation and water deficit treatment affected all the enzymatic activities (except CAT activity in root) detected significantly.

## 3.7 PCA results

To investigate the effect of AM inoculation on the growth, photosynthesis and antioxidant enzymatic avtivity of *E. maackii* under gradient water deficit levels, PCA was performed using all the experimental data sets. Before PCA, correlation analysis was calculated and highly correlated variables were removed. GH and CAT activity in root were selected as presentation for following PCA process. Among all seedlings, principal component 1 (PC1) and principal component 2 (PC2) accounted for 73.97% and 17.15% of the variance, respectively (Fig. 8). PC1 tended to separate the water deficit effects, which suggested greater effect of water deficit compared with AM inoculation. In PC1, under 20%, 40%, 60% and 80% FC conditions, seedlings that received AM inoculation treatment were closer to seedlings under 100% FC condition than non-mycorrhizal seedlings, which meant that AM inoculation had positive effect on growth, photosynthesis and antioxidant enzymatic activity of *E. maackii* under gradient water deficit levels.

# 4. Discussion

Water deficit is one of the major limitations on plant growth and as a result of anthropogenic disturbance and predicted global climate changes, drought conditions are expected to increase in the near future (Solomon et al. 2007). During the last few decades, a large amount of studies focused on implementing and validating tools and techniques to prevent drought-mediated damages were reported (Baslam & Goicoechea 2011). In this study, we have investigated the effect of AM fungi species, *R. intraradices* in improving the drought tolerance mechanisms in *E. maackii* under gradient water deficit conditions.

Water deficit had negative effect on AM inoculation (Ryan & Ash 1996), which supported our findings that AM inoculation rates in seedlings along with increasing water deficit decreased gradually. ter deficit was the key limitation in plant growth. Zhang et al. (2013) reported that the main limiting factor in growth was the seasonal drought. Plants have evolved kinds of mechanisms avoiding damages by water deficit, including forming symbiosis with AM fungi. The improvement in plant growth in AM inoculation treatment could be attributed to the maintenance of increased mineral availability for absorption and water content in plant tissue (Ahanger et al. 2014). Many researchers have reported the positive role of AM symbiosis in plant growth under drought stress (Chitarra et al. 2016; Li et al. 2015). Among AM inoculated damask rose, significant increase in uptake of essential mineral elements were detected compared with non-mycorrhizal plants under severe water deficit, reflected in growth and drought tolerance improvement (Abdel-Salam et al. 2018). Under water deficit conditions, AM fungi could modify

the root morphology, such as increased root fineness, root/shoot ratio, and the length of hair root, resulting in improvement of mineral elements uptake (Hetrick 1991; Begum et al. 2019).

Water deficit had directly negative effect on plant photosynthesis, resulting from greater water absorption pressure and reduction in coupling factors (Tezara et al. 1999). Begum (2019) suggested that the severe water deficit reduced the pigment accumulation and photosynthesis attributes, which was alleviated by AM inoculation. AM-mediated increase in chlorophyll content and photosynthesis rate in damask rose under water deficit was reported (Abdel-Salam et al. 2018). Similar to our findings, improvement in gas exchange and PSII activity in seedlings that received AM inoculation treatment positively enhanced the photosynthesis function over water deficit and non-mycorrhizal seedlings.

Under moderate water deficit conditions (40%, 60% and 80% FC), seedlings that received AM inoculation treatment showed better performance in gas exchange and chlorophyll fluorescence indexes compared to the non-mycorrhizal treatment. This confirmed the report of Li et al. (2015), suggesting the role of AM fungi in substantially increasing the host plant's drought tolerance. Formation of AM symbiosis could increase the root hydraulic conductivity, stomatal regulation in host plant, improving contact with soil particles and water extraction, which supplied better water regime for photosynthesis (Augé 2001).

As reported, reduced water availability limited the expression of growth associated genes, like tubulin and cyclin genes, reflected in reduced cellular division and proliferation (Setter & Flannigan 2001). Similar to our results, decreased biomass accumulation along increased water deficit level is directly regulated by declined photosynthesis functioning. Besides, AM inoculation could significantly ameliorate the induced declines in growth and biomass accumulation by water deficit (Zhang et al. 2019), which supported our findings that AM inoculation alleviated negative effect by water deficit in BS, BR and TB under 20%, 40% and 60% FC conditions. Leaf was more sensitive to drought compared with root, which was in line with our results that RSR increased slightly along with increasing water deficit level.

Drought mediated unbalance in reactive oxygen species (ROS), such as $H_2O_2$ and $O_2^-$, causing severe oxidative damage, and up-regulated antioxidant enzymatic activities (Kapoor et al. 2013; Ahanger & Agarwal 2017). Similar to our results, water deficit induced SOD, POD and CAT activities of both shoot and root improvement from 100% FC treatment to 40% FC treatment. However, significant decreased in SOD, POD and CAT activities at severe water deficit condition (20% FC), which may be caused by severe drought limitation. AM inoculation showed limitation in SOD activities of both shoot and root under 20%, 40% and 60% FC conditions, which suggested less sensitive of mycorrhizal seedlings to water deficit compared with non-mycorrhizal seedlings (Begum et al. 2019). Maintaining lower antioxidant enzymatic activity suggested lower ROS concentration, which benefits seedlings in regulating development processes, such as root growth, signaling, stomatal functioning and cell senescence (Miller et al. 2010). However, roots of seedlings that had received AM inoculation treatment showed significant higher POD activity under 40% and 60% FC conditions, which was in line with research of Begum et al. (2019). Furthermore, no significant difference between mycorrhizal and non-mycorrhizal seedlings in CAT activity. Loss of antioxidant enzymatic stability intensified the reduction in photosynthesis, which resulted from

up-regulating chlorophyllase activity and reducing Rubisco synthesis by environmental stress (Fatma et al. 2014; Dalal & Tripathy 2012).

Many studies suggested better performance of AM fungi under moderate drought stress and ineffective role under severe drought condition (Bryla & Duniway 1997). Severe water deficit caused the reduction of effectiveness of AM inoculation in wheat, which in turn resulted in prolonging water stress (Ryan & Ash 1996), which supported our findings that AM inoculation benefited in growth, photosynthesis and antioxidant enzymatic activity of seedlings that had been subjected moderate water deficit, especially under 40%, 60% and 80% FC conditions, whereas AM inoculation was ineffective under 20% FC condition.

# 5. Conclusion

In conclusion, water deficit induced negative influence on the growth and development of *E. maackii*, reflected in limitation in growth and biomass accumulation, photosynthesis decline and antioxidant enzymatic activity improvement. Inoculation of AM fungus (*R. intraradice*s) alleviated these negative effects and benefited *E. maackii*, especially under moderate water deficit stress (40% and 60% FC), which suggested the potential role of AMF in application for ecological stability suffered from drought.

# Abbreviations

AM, arbuscular mycorrhizal; FC, field capacity; ROS
reactive oxygen species; BS, biomass of the shoot; BR, biomass of the root; TB, total biomass; RSR, Root/Shoot ratio; PC, principal component; Pn, net photosynthesis; Gs, stomatal conductance; Ci, intercellular $CO_2$ concentration; E, transpiration rate; Fm, maximal fluorescence; Fo, minimum fluorescence; PSII, photosystem II; ΦPSII, actual quantum yield of PSII; qN, non-photochemical quenching; qP, photoc hemical quenching; Fv/Fm, maximum quantum yield of PSII; ANOVA, analyses of variance; PCA, principal component analysis; SOC, soil organic carbon; GH, growth of height; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase.

# Declarations

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Competing interests:** The authors declare that they have no competing interests.

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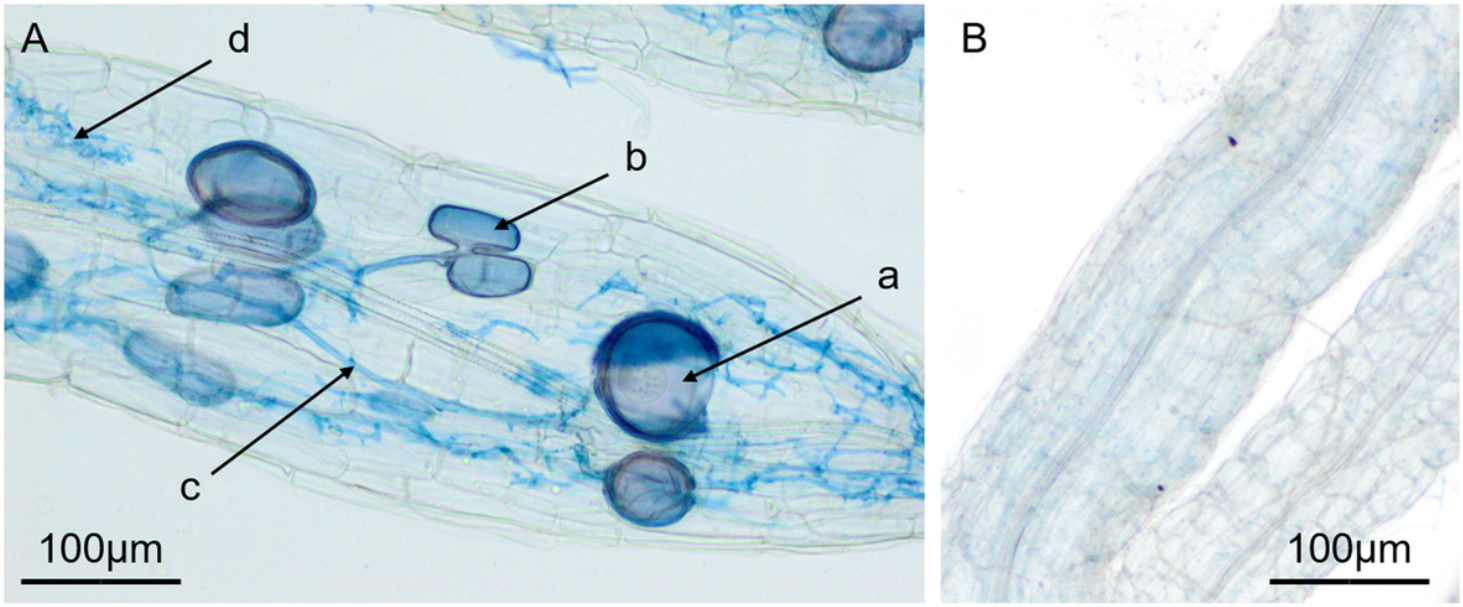
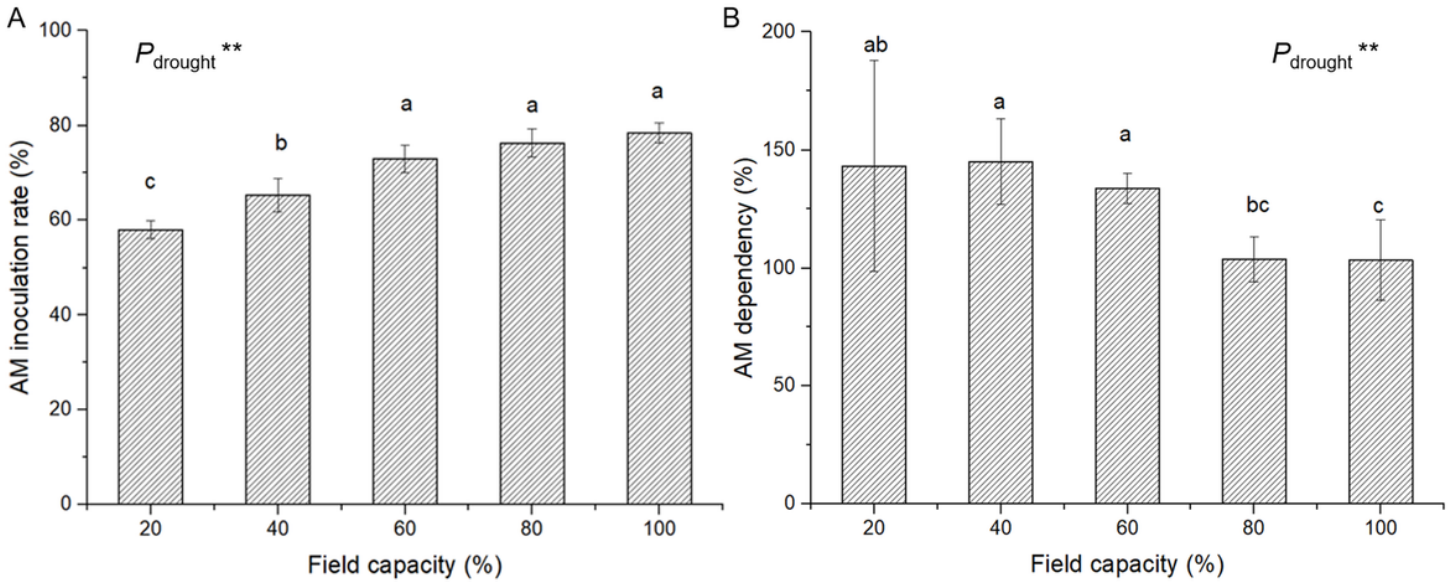
# Figures



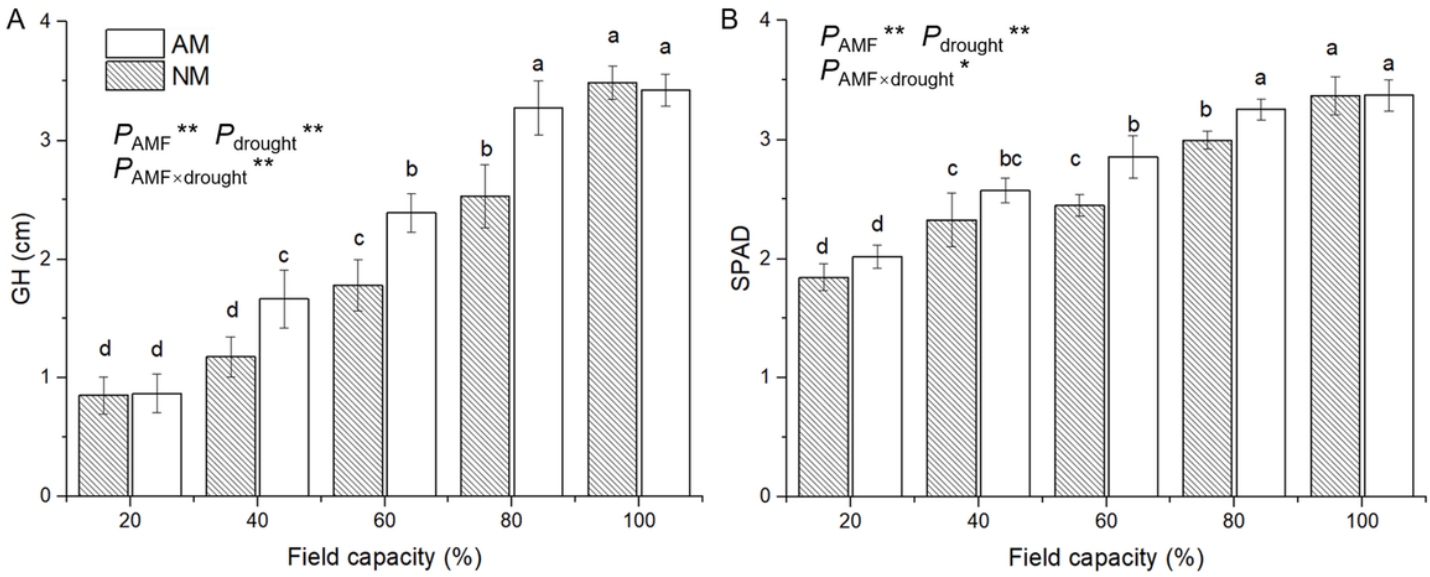

## Figure 1

AM structures detected in mycorrhizal root (A) and root of non-mycorrhizal seedlings (B). a: spore; b: vesicle; c: hypha; d: arbuscule.



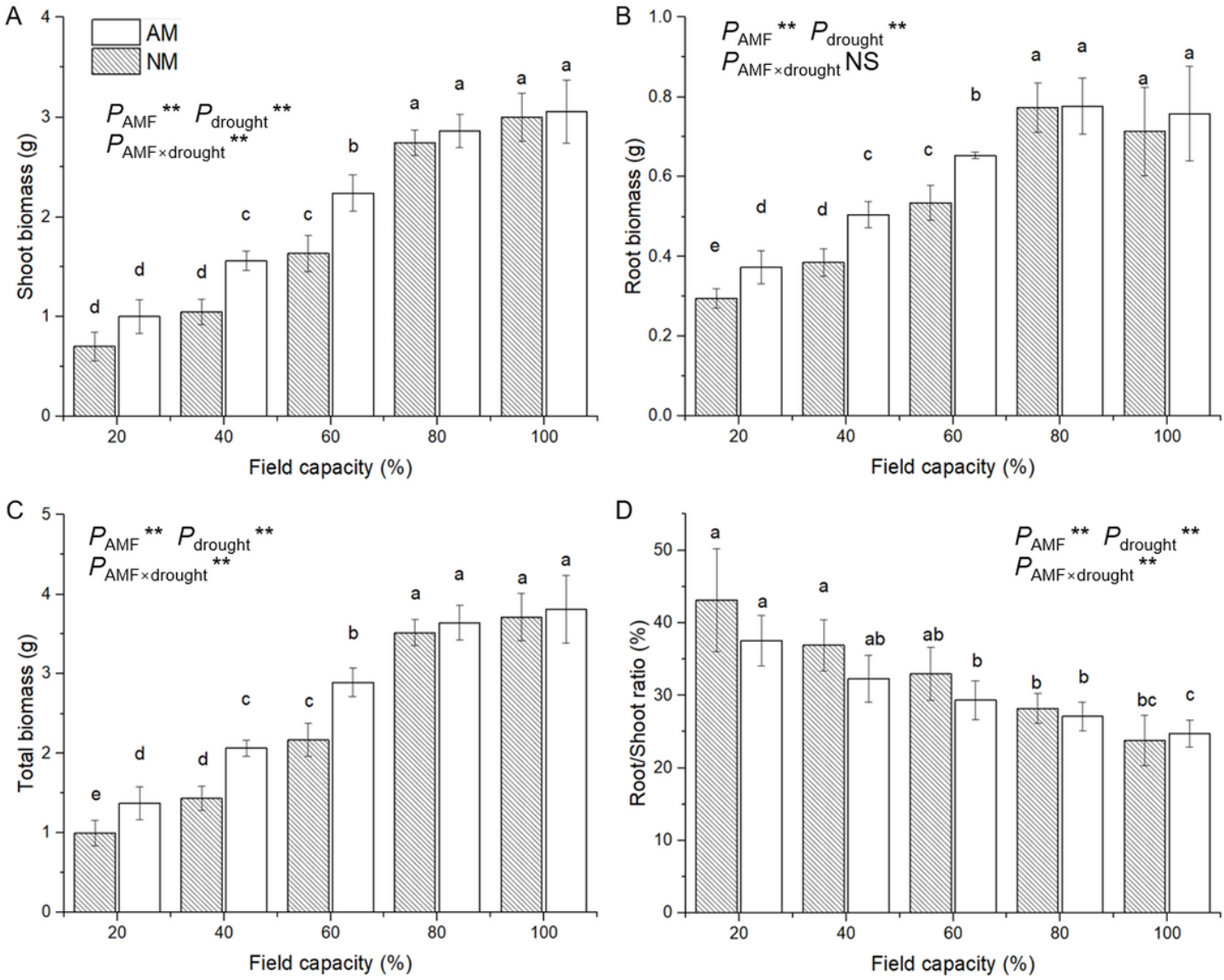

## Figure 2

AM colonization rate (A) and AM dependency (B) in E. maackii under water deficit. AM: AM inoculated treatment; NM: uninoculated treatment; **: P ≤ 0.01. Means with a letter in common within each variable can't be considered different at P ≤ 0.05.



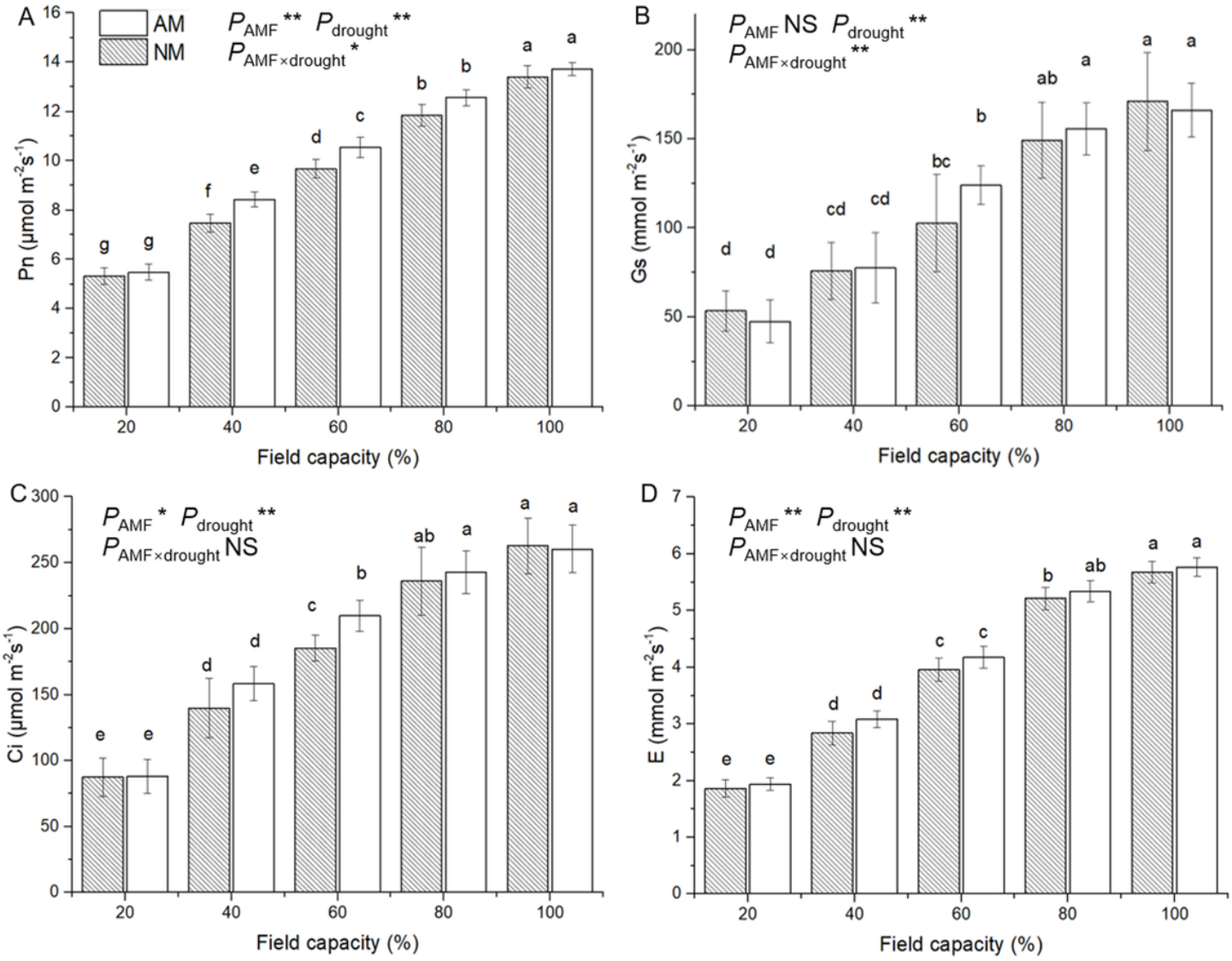

## Figure 3

Effect of AM inoculation on GH (A) and SPAD (B) of E. maackii under water deficit. AM inoculated treatment; NM: uninoculated treatment; AMF: effect of AM inoculation; drought: effect of water deficit; AMF×drought: effect of interaction of AM inoculation and water deficit treatment; *: $P \leq 0.05$; **: $P \leq 0.01$. Means with a letter in common within each variable can't be considered different at $P \leq 0.05$.



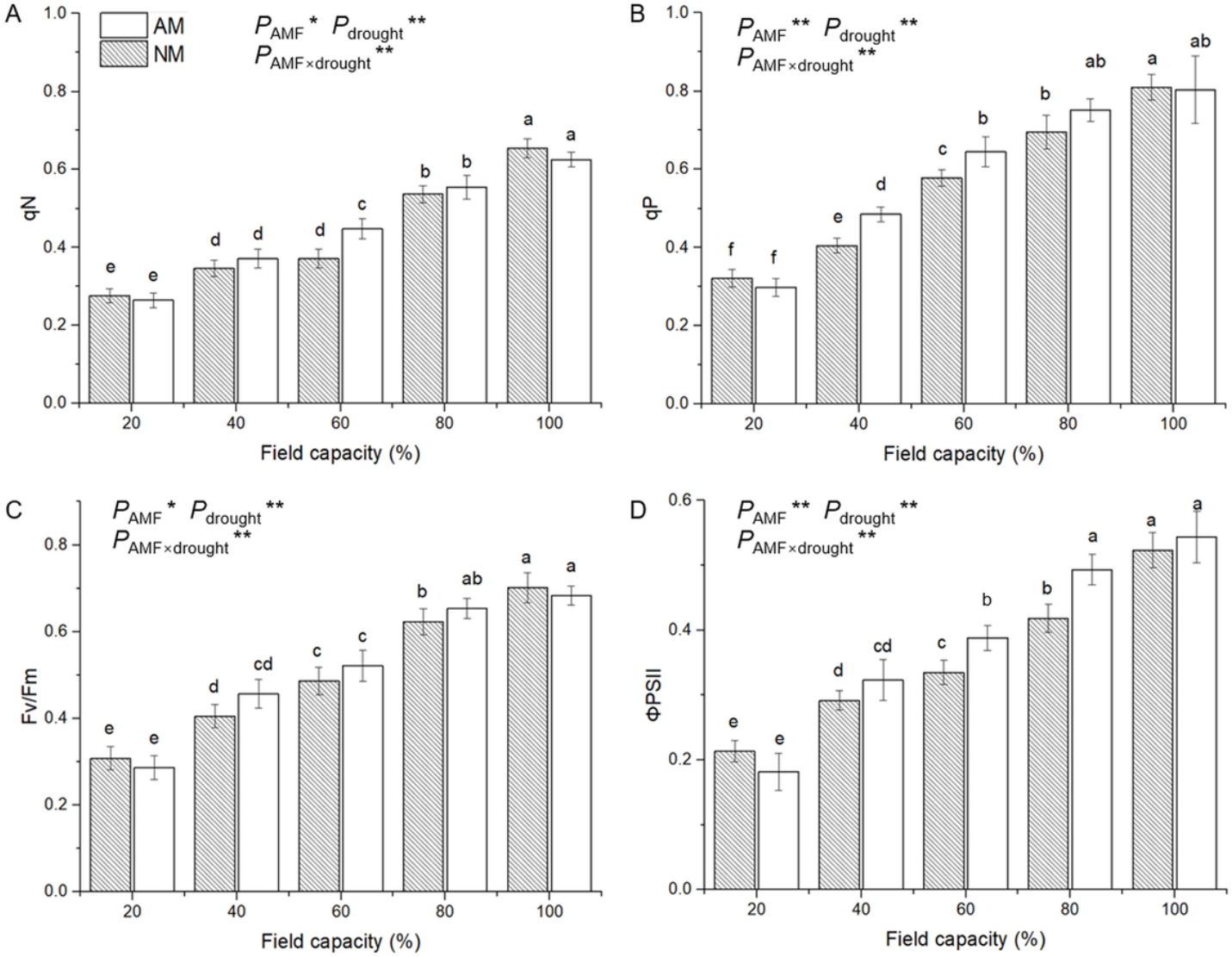

## Figure 4

Effect of AM inoculation on biomass of shoot (A), root (B) and total (C) and Root/Shoot ratio (D) of E. maackii under water deficit. AM inoculated treatment; NM: uninoculated treatment; AMF: effect of AM inoculation; drought: effect of water deficit; AMF×drought: effect of interaction of AM inoculation and water deficit treatment; NS: no significant effect; **: P ≤ 0.01. Means with a letter in common within each variable can't be considered different at P ≤ 0.05.



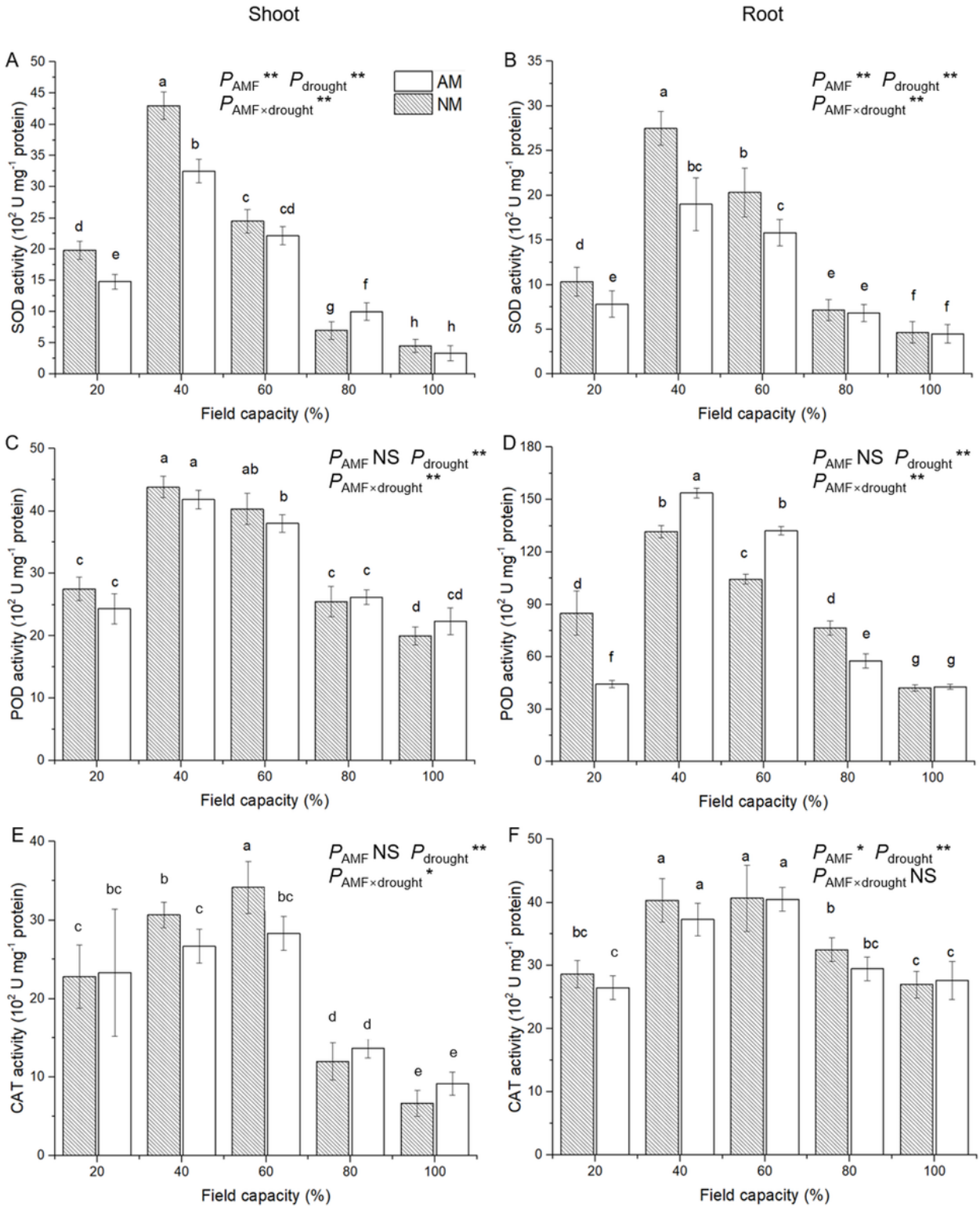

## Figure 5

Effect of AM inoculation on gas exchange indexes, Pn (A), Gs (B), Ci (C) and E (D) of E. maackii under water deficit. AM inoculated treatment; NM: uninoculated treatment; AMF: effect of AM inoculation; drought: effect of water deficit; AMF×drought: effect of interaction of AM inoculation and water deficit treatment; NS: no significant effect; *: P ≤ 0.05; **: P ≤ 0.01. Means with a letter in common within each variable can't be considered different at P ≤ 0.05.




## Figure 6

Effect of AM inoculation on chlorophyll fluorescence indexes, qN (A), qP (B), Fv/Fm (C) and ΦPSII (D) of E. maackii under water deficit. AM inoculated treatment; NM: uninoculated treatment; AMF: effect of AM inoculation; drought: effect of water deficit; AMF×drought: effect of interaction of AM inoculation and water deficit treatment; *: P ≤ 0.05; **: P ≤ 0.01. Means with a letter in common within each variable can't be considered different at P ≤ 0.05.




## Figure 7

Effect of AM inoculation on SOD (A, B), POD (C, D) and CAT (E, F) activities in shoot and root of E. maackii under water deficit. AM inoculated treatment; NM: uninoculated treatment; AMF: effect of AM inoculation; drought: effect of water deficit; AMF×drought: effect of interaction of AM inoculation and water deficit treatment; NS: no significant effect; *: $P \leq 0.05$; **: $P \leq 0.01$. Means with a letter in common within each variable can't be considered different at $P \leq 0.05$.

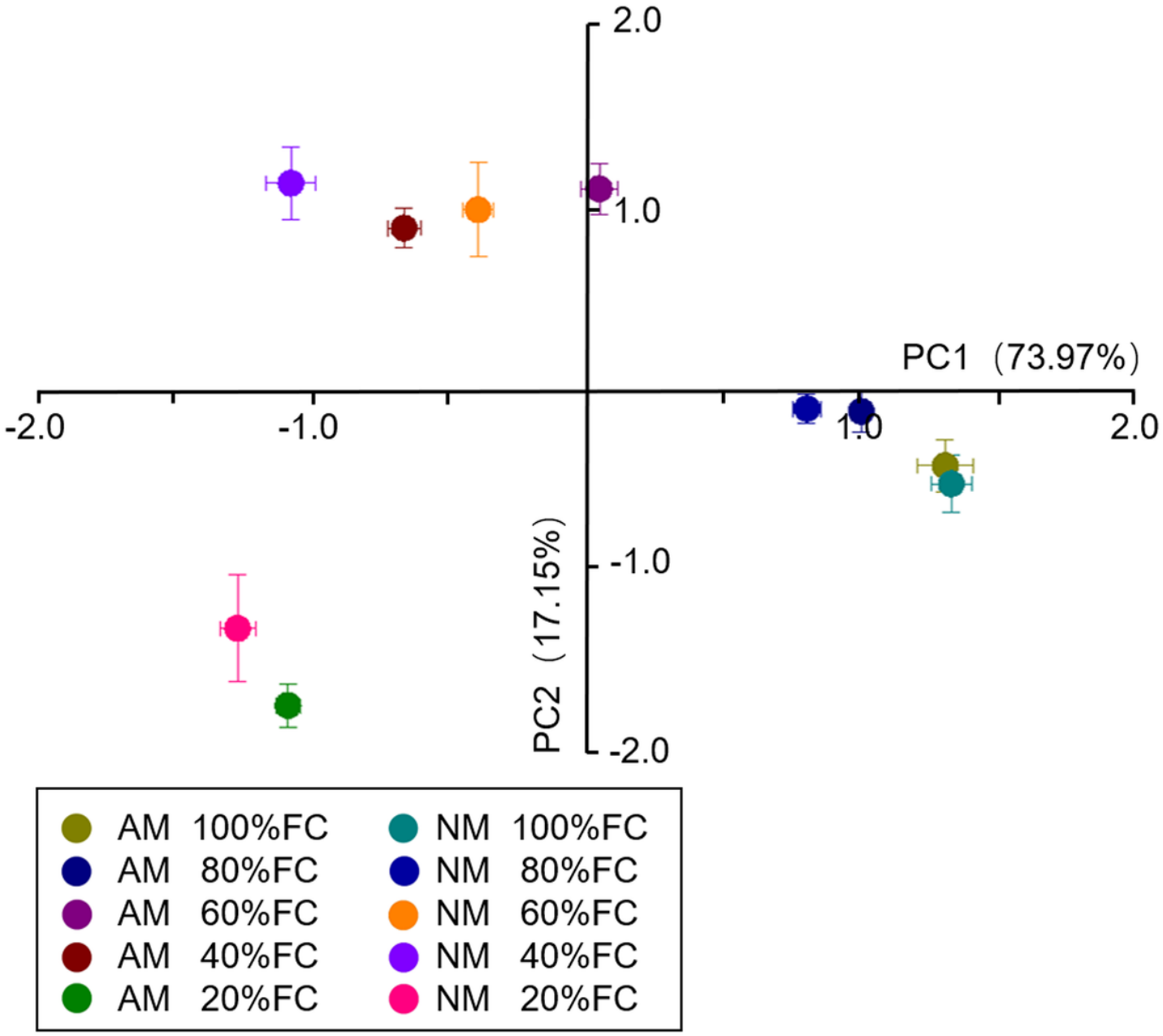



**Figure 8**

PCA results. AM inoculated treatment; NM: uninoculated treatment.