

# Arbuscular mycorrhizal fungi improved plant growth and nutrient acquisition of desert ephemeral *Plantago minuta* under variable soil water conditions

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**Abstract:** Desert ephemeral plants play an important role in desert ecosystem. Soil water availability is considered as the major restrictive factor limiting the growth of ephemeral plants. Moreover, arbuscular mycorrhizal fungi (AM fungi) are widely reported to improve the growth of desert ephemerals. The present study aimed to test the hypothesis of that AM fungi could alleviate drought stress of desert ephemeral *Plantago minuta*, and AM fungal functions reduced with the improvement of soil water content. A pot experiment was carried out with three levels of soil water contents (4.5%, 9.0%, and 15.8% (w/w)), and three AM inoculation treatments (*Glomus mosseae*, *Glomus etunicatum* and non-inoculation). The results indicate that mycorrhizal colonization rate decreased with the increase of soil water availability. Inoculation improved plant growth and N, P and K acquisition in both shoots and roots regardless water treatments. When comparing the two fungi, plants inoculated with *G. mosseae* performed better than those inoculated with *G. etunicatum* in terms of plant growth and nutrient acquisition. These results showed that ameliorative soil water did not suppress arbuscular mycorrhizal fungal functions in improving growth and nutrient acquisition of desert ephemeral *Plantago minuta*.

**Keywords:** *Plantago minuta*; soil water availability; nutrient acquisition; desert ephemeral; Junggar Basin

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Desert ephemeral plants are specially adapted to harsh desert environments. Generally, they remain dormant until a rare rainfall event occurs where they emerge with the appearance of short, wiry grasses and delicate flowers. These plants grow and flower quickly before the desert soil dries up again. Desert ephemeral plants usually have a very short epigeous phase ranged from 40 to 90 days with a mean of 76 days (Mao and Zhang, 1994; Zhang and Chen, 2002; Ramawat, 2010).

In China, desert ephemeral plants are mainly distributed in North Xinjiang with the easternmost limit at the eastern edge of the Junggar Basin (Mao and Zhang,

1994). In Junggar Basin, ephemerals emerge in March and disappear between May and June. In early spring, ephemeral plants are dominant in the plant community and form a synusia, with the fresh weight of ephemerals accounting for over 60% of the total community yield (Zhang and Chen, 2002). Ephemerals play a key role in dune stabilization and can reduce the intensity of wind erosion (Wang et al., 2003) and desert ecosystem stability (Qian et al., 2007; Wang et al., 2009).

Desert soil is typically deficient in soil water availability and nutrients. For example, the mean

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annual precipitation ranges from 100 to 200 mm with a high surface evaporation of 1,000–1,700 mm (Wang et al., 2003). Soil water contents in soil layer of 0–30 cm are 2%–5% and the available P is less than 2 mg/kg soil (Wang et al., 2004; Shi et al., 2013). Ephemeral plants can survive and complete their life cycles in such harsh conditions. Therefore, to explore the adapted strategies of ephemeral plants under desert conditions has triggered the interest of the scientific community (Lan and Zhang, 2008; Yuan and Tang, 2010).

Arbuscular mycorrhizal fungi (AM fungi; Phylum Glomeromycota) are significant members of the soil microbial community, which form symbiotic relationships with the majority of higher plants (Smith and Read, 2008). AM fungi are important components of virtually all terrestrial ecosystems and are especially critical in improving plant nutrient and water uptake under semi-arid conditions (van der Heijden et al., 2006; Allen, 2011). AM fungi can improve plant resistance to soil water deficit (Lambers et al., 2008; Smith and Read, 2008; Apple, 2010; Ruiz-Lozano and Aroca, 2010). The underlying mechanisms are most likely to be a combination of nutritional and non-nutritional host-plant benefits. Non-nutritional mechanisms may include: (1) hormonal effects (particularly abscisic acid) due to mycorrhizal colonization; (2) direct water uptake by improved soil–hyphal contacts (especially important during soil drying) leading to more effective scavenging for water in micropores; and (3) increased photosynthesis through sink stimulation (Kaschuk et al., 2009; Smith et al., 2010). There is also evidence of plant interconnectivity facilitated by AM fungi connecting plant roots via a common mycorrhizal network, where inter-plant resources are transferred through the network along a source-sink gradient (Kiers et al., 2011). Augé (2004) investigated mycorrhizal network ability to alter moisture retention properties of soils through an increase in soil aggregation, such that non-mycorrhizal plants growing in a mycorrhizal soil benefit through enhanced plant water availability.

In Junggar Basin, the majority of desert ephemerals form mutualisms with AM fungi (Shi et al., 2006, 2007; Zhang et al., 2011, 2012a, b), and mycorrhizal colonization increased plant growth, nutrition uptake, productivity and community restoration (Chen et al., 2008; Sun et al., 2008; Zhang et al., 2011, 2012a).

However, the major factor limiting ephemeral plant growth in the desert ecosystem is water availability (Wang et al., 2004; Sun et al., 2009). Through a glasshouse experiment, the present study aimed to investigate the effects of soil water availability and AM fungi on the growth of ephemeral plants under different soil water conditions.

## 1 Materials and methods

### 1.1 Plant and fungal species

Two AM fungal species, *Glomus mosseae* BEG167 (G.m) and *Glomus etunicatum* BEG168 (G.e) were previously propagated in pot culture on maize (*Zea mays*) and clover (*Trifolium pretense*) plants grown in sand for 12 weeks. Inocula from the pot culture comprised a mixture of spores, mycelium, sand and maize and clover root fragments and contained approximately 1,000 spores per 100 g.

Seeds of *P. minuta* were collected from the Gurbantunggut Desert in the Junggar Basin in May and June of 2004. The seeds were stored in 4°C until use. Before sowing, seeds were surface sterilized with 10% (v/v) hydrogen peroxide for 10 min, washed with sterile water and germinated in the dark on moistened filter paper at 28°C for 3 days. Germinated seeds in uniform size were selected for planting.

Soil used in this experiment was collected from the Gurbantunggut Desert with the following properties: pH (water:soil ratio 5:1) 8.54, organic matter 1.43 g/kg, total salt 0.73 g/kg, available N 6.92 mg/kg, Olsen P 1.78 mg/kg, available K 73.00 mg/kg and electrical conduct 0.178 ms/cm. The soil was sieved (1-mm), steam-sterilized (121°C for 30 min) and air-dried prior to potting.

### 1.2 Experimental design

The glasshouse experiment used a randomized block design consisting of three soil water regimes and three inoculation treatments. Soil water contents were 4.5%, 9.0% and 15.75%, equivalent to 20%, 40% and 70% of field capacity, respectively. Plants were inoculated with *Glomus mosseae* BEG167, *Glomus etunicatum* BEG168, or treated with non-inoculation, respectively. There were six replicate pots per treatment.

Fifty grams of fungal inoculum was mixed with 550 g of soil in each pot. Sterilized inoculum was used

as non-inoculant control. Ten pre-germinated seeds of *P. minuta* were transplanted into each pot covered by another 50 g of non-inoculant sand. Seedlings were thinned to five per pot after emergence.

Plants were grown in a sunlit greenhouse with day/night temperature of 25–30°C/18–22°C. The soil water content was maintained by water to weight at 08:00 and 18:00 daily. Hoagland's nutrient solution with 1/2P was added every two weeks. Plants were harvested 8 weeks after sowing.

### 1.3 Harvest and sample analysis

Whole plant was harvested after 8 weeks sowed. Then, shoots and roots were separated. The roots were carefully washed free of soil. Sub-samples of roots were collected for determination of mycorrhizal colonization rate using the acid fuchsin staining-grid intersect method (Kormanik and McGraw, 1982). Both shoots and roots were dried at 70°C to determine dry weights. The tissue N concentration was determined by the Kjeldahl method; P concentration was measured with spectrophotometry by the molybdenum blue method after digested with concentrated H<sub>2</sub>SO<sub>4</sub> and 30% H<sub>2</sub>O<sub>2</sub>; and K concentrations was determined by flame photometry (Lu, 2000).

### 1.4 Mycorrhizal dependency

Mycorrhizal dependency at the given soil water content was calculated as:

Mycorrhizal dependency (%) = (biomass of inoculated AM fungi – biomass of CK) / biomass of CK × 100%.

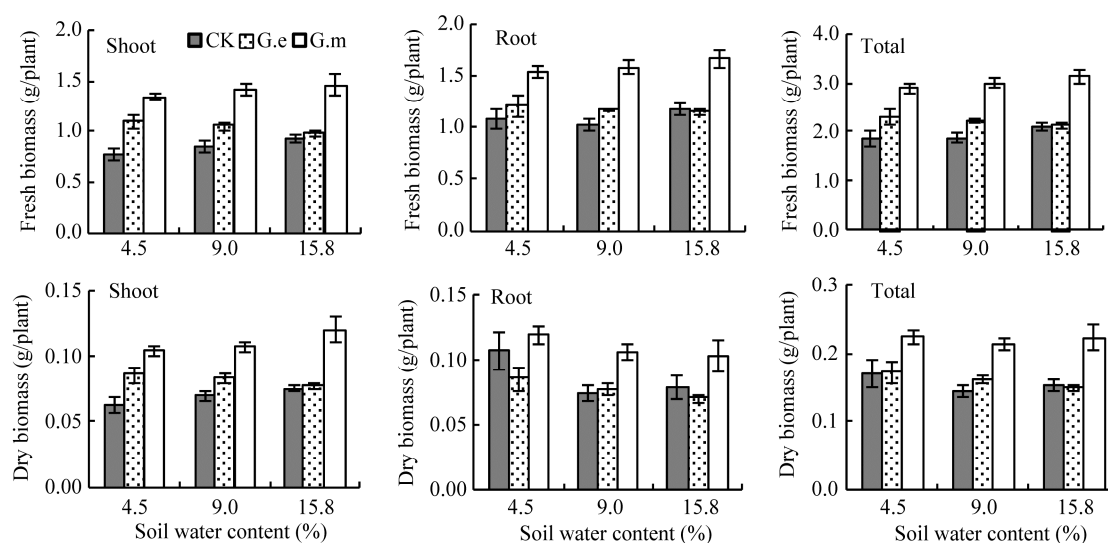
## 1.5 Statistical analysis

The differences in percentage of root length colonized, shoot and root biomass, and N, P and K concentrations of shoots and roots were subjected to one-way analysis of variance by least significant difference (LSD) at the 5% level for significant differences between the means in all treatments using the SPSS software package version 16.0 (SPSS, Chicago IL). The effects of soil water content and AM fungi, and their interaction were subjected to two-way analysis of variance by the Univariate Analysis of Variance of General Linear Model.

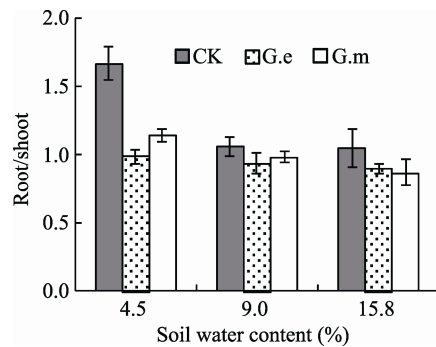
## 2 Results

### 2.1 Plant growth

Both fresh and dry weights of plants (both shoots and roots) inoculated with G.m were significantly higher than those of the controls in all water treatments (Fig. 1). The fresh and dry weights of G.m-inoculated plants were 1.2, 1.4, and 1.5 times those of G.e treatment in 4.5%, 9.0% and 15.8% of soil water contents, respectively. When the effects of different AM fungi species were considered, biomass of G.m treatments was remarkably higher than these of G.e except for the dry weight of roots and whole plant in the treatment of 4.5% water. By comparing the same AM fungi treatments in different water conditions, there were not significant differences among them. The root to shoot ratio showed no significant difference between fungal species in the same water condition except for 4.5% water treatment (Fig. 2).



**Fig. 1** Fresh and dry weights of *Plantago minuta* with different water and inoculation treatments. Data were means ± SE (n=6). CK, non-inoculation treatment; G.e, *Glomus etunicatum*; G.m, *Glomus mosseae*.



**Fig. 2** The root to shoot ratio of dry weight of *Plantago minuta* with different water and inoculation treatments. Data were means $\pm$ SE ( $n=6$ ). CK, non-inoculation treatment; G.e, *Glomus etunicatum*; G.m, *Glomus mosseae*.

AM fungi significantly increased plant biomass and the root/shoot ratio (Table 1). Soil water content influenced significantly dry biomass of roots and the ratio of root to shoot. Further, there was significant interaction between water and AM fungi on root/shoot mass ratio (Table 1).

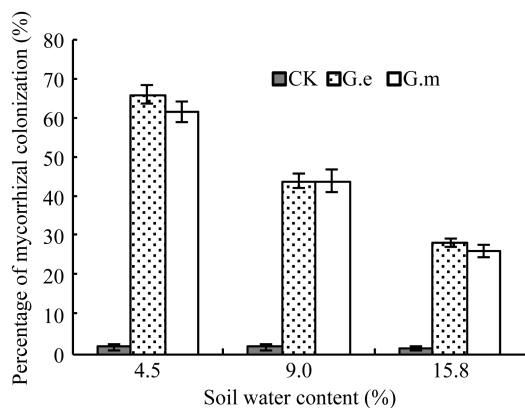
## 2.2 Mycorrhizal colonization rate

In all water treatments, inoculated plants had higher colonization rates than the non-inoculation treatment ( $F=475.39$ ,  $P<0.001$ ), with those inoculated with G.e under 4.5% soil water content (the highest is 15.8%; Fig. 3). The colonization rate decreased significantly

**Table 1** The effect of water and arbuscular mycorrhizal fungi on biomass of *Plantago minuta*

Factor	Fresh biomass			Dry biomass			Root/shoot
	Shoot	Root	Whole plant	Shoot	Root	Whole plant	
Water	ns	ns	ns	ns	**	ns	**
AM fungi	**	**	**	**	**	**	**
Water $\times$ AM fungi	ns	ns	ns	ns	ns	ns	**

Note: \*\* indicates significance at  $P<0.01$  level; ns, not significant.



**Fig. 3** Colonization rates of arbuscular mycorrhizas in roots of *Plantago minuta*. Data were means $\pm$ SE ( $n=6$ ). CK, non-inoculation treatment; G.e, *Glomus etunicatum*; G.m, *Glomus mosseae*.

with the increase of soil water content ( $F=116.98$ ,  $P<0.001$ ). AM fungi species, soil water content and their interactions affected significantly the mycorrhizal colonization rate of *P. minuta* ( $F=26.70$ ,  $P<0.01$ ; Fig. 3).

## 2.3 Plant dependency on mycorrhiza

In each soil water treatment, the based dependency of *P. minuta* to G.m was higher than that to G.e, respectively. The highest mycorrhizal dependency of shoots, roots and the whole plant in G.m was 83.3% (under 4.5% water treatment), 57.1% (9.0% water treatment), and 64.29% (15.8% water treatment), respectively.

**Table 2** The dependency of *Plantago minuta* to AM fungi in different soil water treatments

Treatments		Mycorrhizal dependency (%)		
Soil water content (%)	AM fungi	Shoot	Root	Whole plant
4.5	CK	0.00	0.00	0.00
	<i>Glomus etunicatum</i>	33.33	37.50	35.71
	<i>G. mosseae</i>	83.33	50.00	64.29
9.0	CK	0.00	0.00	0.00
	<i>Glomus etunicatum</i>	14.29	14.29	14.29
	<i>G. mosseae</i>	57.14	57.14	57.14
15.8	CK	0.00	0.00	0.00
	<i>Glomus etunicatum</i>	0.00	12.50	0.00
	<i>G. mosseae</i>	50.00	25.00	46.67

## 2.4 The nutrient concentration of *P. minuta*

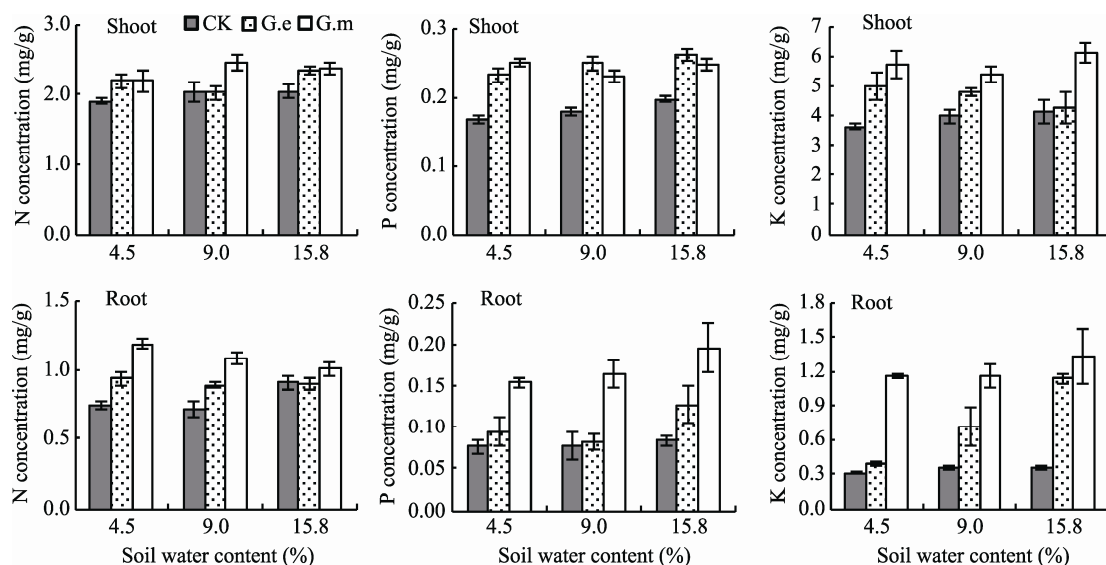
The shoot P and root K of inoculated plants were significantly higher than those of non-mycorrhizal controls in each soil water condition (Fig. 4). Shoot N and K concentration followed the similar trend as root P concentration, i.e. significantly higher concentration in the G.m-inoculated plants than in the non-mycorrhizal controls, respectively. However, plants inoculated with G.e affected shoot N and K and root P concentrations compared to non-inoculated treatments. The root N concentrations of inoculated plants were higher than that in controls under 4.5% and 9.0% soil water conditions. The changes of nutrient concentration either in shoots or in roots were not significant among varied soil water content regardless of the difference of AM fungi species.

When the water and AM fungi were considered simultaneously, AM fungi increased markedly the N, P and K concentration of *P. minuta* in both shoots and roots (Table 3). However, soil water content influenced significantly P in shoots and N and K in roots. Further, the interaction of water and AM fungi

affected remarkably the P concentrations in shoots and K concentrations in roots (Table 3).

## 3 Discussion and conclusion

This study examined the combined effects of soil water content and AM fungi colonization on a desert spring ephemeral plant. Our results showed that AM fungi played vital roles in growth and nutrition absorption under varying soil water conditions, which indicated that the effects of AM fungi to *P. minuta* did not depend on soil water content given in this experiment. However, Wang et al. (2004) reported that the coverage of desert ephemeral plants presented a positive correlation with soil water content at a range from 0.45% to 4.92% based on a filed investigation in the Gurbantunggut Desert. The possible reason is AM fungi can help host plants to uptake enough water for their growth even under drought condition because AM fungi can increase the absorption area of hosts (Li et al., 1991). Additionally, the colonization rate decreased with the increase of soil water content in our study.



**Fig. 4** Concentrations of N, P and K of *Plantago minuta* of different treatments by water and arbuscular mycorrhizal fungi. Data were means $\pm$ SE ( $n=6$ ). CK, non-inoculation treatment; G.e, *Glomus etunicatum*; G.m, *Glomus mosseae*.

**Table 3** The effect of water and arbuscular mycorrhizal fungi on nutrient concentration of *Plantago minuta*

Factor	Shoot			Root		
	N	P	K	N	P	K
Water	ns	**	ns	*	ns	**
AM fungi	**	**	**	**	**	**
Water $\times$ AM fungi	ns	**	ns	ns	ns	**

Note: \* and \*\* indicate significances at  $P<0.05$  and  $P<0.01$  levels, respectively; ns, not significant.

Many studies have showed that AM fungi colonization rate decreased under the water stress comparing to the normal water conditions (Khalvati et al., 2010; Ruiz-Lozano and Aroca, 2010; Jayne and Quigley, 2014). However, our results indicated that the mycorrhizal colonization of ephemeral plant *P. minuta* declined with the increase of the soil water content (Fig. 3). The lowest soil water content (4.5%) in the present study was close to the natural field water condition (Shi et al., 2013). The habitats of ephemerals are extremely adverse with low nutrient content of the substrate (quicksand) and low water content (2%–5%) in the top 30 cm of the sandy soil layer due to the lack of rainfall (annual precipitation of 100–120 mm) (Ji et al., 1995). In soil water deficit conditions, *P. minuta* may need to depend on AM fungi for accessing water directly through the hyphal network helping the host plant to survive in the adverse desert environment. In addition, the colonization rate of *P. minuta* was similar to that under natural field conditions (55%±8%) reported previously (Shi et al., 2006). With the increase of soil water content, the function provided by the AM fungi pathway in accessing soil water to alleviate plant water stress was not thus required. Further, the results of mycorrhizal dependency supported this conclusion (Table 2).

It is widely reported AM fungi can enhance the host plant growth and development whilst improving nutrient status of the host plant. Researchers show AM fungi can assist desert ephemeral plants by increasing the growth, development and nutrient status of the host plants (Chen et al., 2008; Sun et al., 2008; Zhang et al., 2011). Our results also showed that AM fungi can increase plant growth and nutrient concentrations (particularly P status). Biomass and nutrient concentrations of inoculated plants were not necessarily significantly higher than those of controls (Figs. 1 and 4), as the case in a report of Treseder (2013). In addition, plant growth was improved by AM fungi colonization with variation among AM fungi and among different water treatments. AM fungi significantly increased biomass and the concentrations of N, P and K in both shoots and roots, indicating their important nutritional role regardless of water conditions imposed in this study. This finding is consistent with a meta-analysis of Jayne and Quigley (2014). That is to say, the effect of AM fungi on plants of the high watered treatments was not different from that of the

low water treatment. When inoculated with AM fungi, the low-water treated plants grew as well as those under high-water conditions.

When the effects of water were considered, we found that higher soil water content did not suppress root dried biomass and the root/shoot ratio, but improved P concentration in shoots and N and K concentrations in roots. Further, plants depended on more roots for foraging water in drought condition than in well watered condition. Except for shoot P and root N and K, the influence of AM fungal inoculation had no significantly effect on other nutrient elements. Sun et al. (2009) showed that different host plants had various effects in N, P and K acquisition under different water treatments.

In conclusion, although the AM fungi dependency of *P. minuta* decreased with the increase of soil water content, the functions of AM fungi to *P. minuta* are not suppressed by improving soil available water. We inferred that the AM fungi is a vital factor for ephemeral plants to adapt to adverse soil water-deficit desert environment that is formed during the evolution process based on an equal term of trade agreement.

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