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ORIGINAL PAPER

Arbuscular mycorrhizal mediation of biomass–density relationship of *Medicago sativa* L. under two water conditions in a field experiment

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Abstract The biomass–density relationship (whereby the biomass of individual plants decreases as plant density increases) has generally been explained by competition for resources. Arbuscular mycorrhizal fungi (AMF) are able to affect plant interactions by mediating resource utilization, but whether this AMF-mediated interaction will change the biomass–density relationship is unclear. We conducted an experiment to test the hypothesis that AMF will shift the biomass–density relationship by affecting intraspecific competition. Four population densities (10, 100, 1,000, or 10,000 seedlings per square meter) of *Medicago sativa* L. were planted in field plots. Water application (1,435 or 327.7 mm/year) simulated precipitation in wet areas (sufficient water) and arid areas (insufficient water). The fungicide benomyl was applied to suppress AMF in some plots ("low-AMF" treatment) and not in others ("high-AMF" treatment). The effect of the AMF treatment on the biomass–density relationship depended on water conditions. High AMF enhanced the decrease of individual biomass with increasing density (the biomass–density line had a steeper slope) when water was sufficient but not when water was insufficient. AMF treatment did not affect plant survival rate or population size but did affect absolute competition intensity (ACI). When water was sufficient, ACI was significantly higher in the high-AMF treatment than in the low-AMF treatment, but ACI was unaffected by AMF treatment when water was insufficient. Our results suggest that AMF status did not impact survival rate and population size but did shift the biomass–density relationship via effects on intraspecific competition. This effect of AMF on the biomass–density relationship depended on the availability of water.

Keywords Plant density · Individual plant biomass · Mycorrhizal colonization · Intraspecific competition · Water availability

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Introduction

Arbuscular mycorrhizal fungi (AMF), forming symbiotic associations with roots of most plant species in terrestrial ecosystems (Smith and Read 1997), play an important role in plant population process (Koide 1991) and in plant biodiversity (O'Connor et al. 2002). AMF can affect both interspecific and intraspecific interactions between plants (Daleo et al. 2008) by mediating plant acquiring water and nutrients. For example, AMF changed the outcome of plant competition by altering the nutrient distribution between co-occurring plants (van der Heijden et al. 2003; Scheublin et al. 2007). By having different effects on different host plants, AMF also mediated plant competition under arid (Allen and Allen 1986), shading (Landis et al. 2005), and low-nutrient (Eriksson 2001) conditions.

AMF mediation of intraspecific competition was affected by host-plant density (Eissenstat and Newman 1990; Koide 1991; Hartnett et al. 1993; Moora and Zobel 1996; Facelli et al. 1999; Ronsheim and Anderson 2001). For

example, AMF infection increased the size and size inequality when plant densities were low but not under high plant densities (Allsopp and Stock 1992; Facelli et al. 1999; Facelli and Facelli 2002). Other studies have also reported that high plant density can reduce the beneficial effects of AMF (Koide 1991; Ayres et al. 2006). Moreover, the plant density impact the effects of AMF depending on resource levels. In a microcosm experiment, plant biomass decreased more rapidly with increasing density of *Otholobium hirtum* and *Aspalathus linearis* with mycorrhiza than without mycorrhiza (Allsopp and Stock 1992). In a field experiment, however, AMF increased the fecundity of *Abutilon theophrasti* Medic. regardless of plant density (Shumway and Koide 1995). The different results obtained in microcosm vs. field experiments involving AMF and competition are likely to be due to the density of colonization achieved in these situations (Koide and Dickie 2002) or to species-specific effects of the fungi. As the nutrient depletion tended to occur when plant density increased in the controlled microcosm experiments (Facelli and Facelli 2002), the differences in microcosm and field experiments may also be due to the differences in resources (Ayres et al. 2006).

Biomass–density relationship that the mass of individual plants decreases with increasing plant density is an important density-dependent process of plant population (Westoby 1984; Petraitis 1995). Scaling exponent of this biomass–density relationship was previously considered a constant value: geometric model predicted it to be −3/2 (Yoda et al. 1963) and metabolic theory predicted it to be −4/3 (Enquist et al. 1998). However, recent studies indicated that biomass–density exponent deviated from a constant value along abiotic environmental gradient such as soil fertility (Morris 2002, 2003) and water availability (Deng et al. 2006). Experiments also showed that this negative relationship between biomass and density resulted from increases in intraspecific competition as population density increases (Westoby 1984; Petraitis 1995). Thus, abiotic and biotic factors affecting intraspecific competition could change biomass–density relationship (Shumway and Koide 1995; Deng et al. 2006; Chu et al. 2008). AMF have been shown to affect intraspecific competition (Ronsheim and Anderson 2001), but whether this AMF effect on intraspecific competition can shift biomass–density relationship has seldom been studied. Also unclear is whether this AMF effect on the biomass–density relationship is affected by resource levels.

Here, we hypothesize that AMF affect the biomass–density relationship by mediating intraspecific competition and that this effect is resource dependent. To test these hypotheses, we conducted a field experiment under sufficient and insufficient water conditions and with *Medicago sativa* L. as the model plant population.

Materials and methods

Study site

The experiment was conducted on an experimental farm (30.217° N, 12.025° E) of the Zhejiang Forestry Academy in Zhejiang Province, southeastern China. The site is located in a hilly area and has a subtropical monsoon climate with a mean annual air temperature of 17–18°C and a mean annual precipitation of 1,435 mm. The soil has a bulk density of 1.1±0.1 g cm^{-1}. Total porosity is 52.8%. The percentages of sand (2~0.05 mm), sand–clay (0.05~0.002 mm), and clay particles (<0.002 mm) are 60.05%, 25.66%, and 14.29%, respectively. The soil has a pH of 5.9 and contains 224 mg kg^{-1} total N, 27.99 mg kg^{-1} extractable P, 318.37 mg kg^{-1} extractable K, and 16.76 g kg^{-1} organic matter.

Experimental design

The experiment used a split–split plot design with two water levels or treatments ("sufficient water" and "insufficient water" treatments) as the main plots; two AMF levels ("low AMF," obtained by applying a fungicide (benomyl) that suppresses the AMF naturally present in soil, and "high AMF", obtained by not applying fungicide) as the split plots; and four densities of *M. sativa* L. (10, 100, 1,000, and 10,000 *M. sativa* seeds per square meter) as split–split plots. The sufficient water treatment simulated precipitation in a moist area (Hangzhou, 30.217° N, 12.025° E) with 1,435 mm annual precipitation, and the insufficient water treatment simulated precipitation in a dry area (Lanzhou, 36.02°N, 103.78° E) with 327.7 mm annual precipitation. In this dry area, the precipitation limits allocation of biomass to aboveground plant parts (Deng et al. 2006). The mycorrhizal potential before and after benomyl treatment was assessed by the soil dilution method (Requena et al. 1996). Data (Fig. 1) indicated that two AMF levels (low AMF and high AMF) can be obtained by applying or not applying benomyl.

The main plots were randomly assigned to the three replicates (blocks), such that each block contained two main plots, one with the insufficient water treatment and the other with the sufficient water treatment. Each block was 14.2 m×1.4 m with a 0.6-m space between blocks. The main plot unit was 6.6 m×1.4 m with a 1-m space between main plots. Each main plot contained two 3 m×1.4 m split plots (the low- and high-AMF treatments, randomly assigned), and each split plot contained four 0.8 m×1.4 m split–split plots (four plant densities, randomly assigned). Adjacent split plots were separated by 0.6 m, and adjacent split–split plots were separated by 0.2 m. To avoid the natural rainfall, a 3-m-high roof with polycarbonate sheet

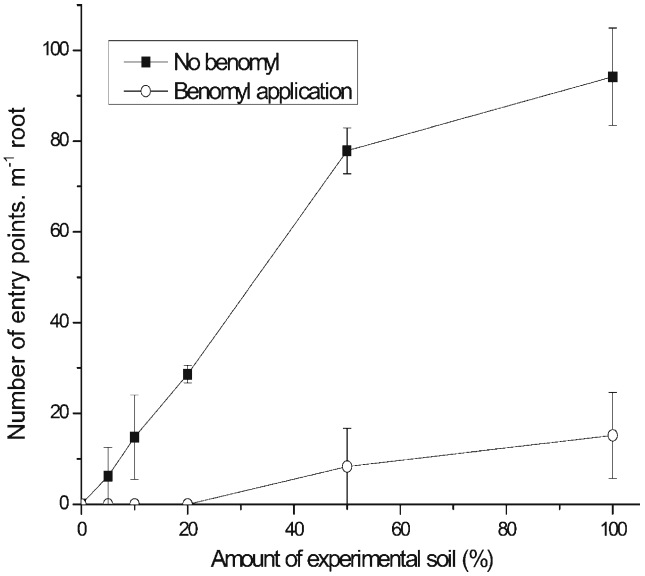

Fig. 1 Infectivity of soil samples from high-AMF (no benomyl application) or low-AMF (benomyl application) treatment, determined by the soil dilution method. Results are expressed as the number of entry points per meter of root. Values are means ± SE. Difference is significant between two AMF treatments (LSD $P<0.05$)

was installed over the whole experimental area (25 m× 10 m) during the experiment. Tubes were fixed in the lower end of the roofs to direct the rainfall 0.8 m away from the plots. To prevent water uptake by plants with side roots, a 20-cm-deep fens was cut into the soil vertically around the sheltered area. The roofs reduced light intensity by 24.6%.

Before sowing, all the aboveground vegetation and roots were removed and the soil was turned and leveled by hand. A thin layer of soil was sieved over the plots to provide a smooth surface to minimize spatial heterogeneity. Seeds were mixed with sand and sown with a sieve to achieve a random spatial pattern. To set up a "no intraspecific competition treatment" in the split–split plots with 10 seeds per square meter, two plants were randomly chosen as target plants, and neighbors within 60 cm were removed. The canopies of target plants were less than 60 cm in diameter, and interactions between *M. sativa* plants ≥60 cm distant were assumed to be insignificant. The target plants were used to calculate competition intensity, as described later in the "Methods." Weeds were removed manually, and no insect herbivory occurred during the experiment.

Seeds of *M. sativa* were supplied by the Zhejiang Forestry Academy and were stored at 4°C for 1 week before sowing. In the first month after sowing, plants were watered once each week to maintain soil moisture at 70–90% of water-holding capacity and to ensure germination. After germination, the water treatments (sufficient water and insufficient water) were established by applying water to simulate precipitation in moist and dry areas during the *M. sativa* growing period. For "sufficient water" treatment, the applied rainfall in December, January, February, March, April, and May were 34.7, 39.0, 58.8, 81.2, 102.3, and 114.5 mm, respectively. For "insufficient water" treatment, the applied rainfall was 1.1, 1.1, 2.4, 8.9, 19.1, and 38.2 mm, respectively. There was no natural rainfall during the whole experiment. For the low-AMF treatment, the fungicide benomyl (2 g dissolved in 6 L of tap water) was applied to the soil weekly in the first month after sowing to suppress AMF (Helgason et al. 2007). In the remaining 24 plots (those that received the high-AMF treatment), the same amount of tap water without fungicide was added. After the insufficient water treatment was initiated, benomyl was not applied.

Sampling and measurements

Plant number and total aboveground biomass were determined 126 days after seeding, when the vegetation growth of the plants was vigorous, and 165 days after seeding, when the plants were flowering. Three sampling squares, each with an area of 100 cm^2, were designated in each split–split plot with a seeding density of 100, 1,000, and 10,000 plants per square meter; all plants in these sampling squares were removed from the soil, counted, and taken to the laboratory. In split–split plots with a density of 10 plants per square meter, one of the two target plants was randomly selected, removed from the soil, and taken to the laboratory.

In the laboratory, the plants were oven dried at 80°C for 48 h and then weighed. Additional root samples from each split–split plot were taken at both sampling times to evaluate the effectiveness of the fungicide. The proportion of AMF colonization was estimated using a dissection microscope (20×40) after cleaning the roots in 10% KOH (*w*/*v*) and staining them in acid fuchsine. The gridline intersection method was used to determine the presence or absence of mycorrhizal associations (hyphae or arbuscules or vesicles or hyphal coils; Giovannetti and Mosse 1980).

Calculations

AMF colonization rate was calculated as: (1) total AMF colonization (%) = number of intersections colonized (hyphae, arbuscules, vesicles, and hyphal coils)/total number of intersections examined × 100%; (2) arbuscular colonization rate (%) = number of intersections with arbuscules/total number of intersections examined × 100%.

Survival rate was calculated as: survival rate = NS/NF × 100%, where NF is the plant number in the first sampling, and NS is the plant number in the second sampling.

The response to mycorrhizal infection was calculated as the relative biomass response (RBR) as described by Facelli et al. (1999): RBR = (HM − LM)/LM, where HM is the biomass of an individual plant from the high-AMF treatment and LM is the biomass of an individual plant

from the low-AMF treatment. The relative competition intensity (RCI) was calculated as: RCI = ($S - C$)/S, where S is the biomass of a target plant without neighbors in low-density plots (10 plants per square meter) and C is the average biomass of individual plants grown in high-density plots (Facelli et al. 1999). The absolute competition intensity (ACI) was calculated as ACI = $S - C$, where S and C have the same meaning as in RCI. Positive values of RCI and ACI indicate competition and negative values indicate facilitation; high values mean high competition (Facelli et al. 1999).

Statistical analysis

The data were analyzed using the DPS statistical package for analysis of split–split plot design (DPS, V7.05, Tang and Feng 2007). Water level (sufficient water vs. insufficient water) was the main-plot treatment, fungicide application (low AMF vs. high AMF) was the split plot treatment, and plant densities (10, 100, 1,000, and 10,000 seeds per square meter) was the split–split plot treatment. Main and interactive effect means were compared by the least significant difference (LSD) test with a 0.05 significance level. Normality tests were performed by Kolmogorov–Smirnov. Slopes and the intercepts of biomass–density relationships were estimated by the standardized major axis (SMA, SMATR Version 2.0, Warton et al. 2006) regression on log-transformed data. Comparisons of slopes between high AMF and low AMF were done in SMATR.

Results

Mycorrhizal colonization

Benomyl application significantly decreased total AMF colonization of roots. At the first sampling time, benomyl application reduced total AMF colonization by 75% (df=1, 4, F=211.84, P=0.0001). Total AMF colonization was 24±5% without benomyl application and 6.5±0.9% with benomyl application. Interaction between benomyl treatment and water level was not significant (df=1, 4, F=0.418, P=0.5532). Arbuscular colonization rate was also decreased by benomyl colonization from 16±3% to 4.6±1% (df=1, 4, F=229.08, P=0.0001). Interaction between benomyl treatment and water level was not significant (df=1, 4, F=0.188, P=0.687).

At the second sampling time, benomyl application (df=1, 4, F=794.54, P=0.0001) and the interaction between benomyl and water (df=1, 4, F=31.21, P=0.005) significantly influenced total AMF colonization. Benomyl application decreased AMF colonization by 69% with the sufficient water treatment and 80% with the insufficient water treatment. In the high-AMF treatment, AMF colonization was 20±3% with sufficient water and 23±2% with insufficient water; in the low-AMF treatment, total AMF colonization was only 6.2±2% with sufficient water and 4.5±2.1% with insufficient water. Arbuscular colonization rates were also significantly lower in low-AMF treatment than those in high-AMF treatment (df=1, 4, F=416.88, P=0.0001). Interaction between benomyl treatment and water level was significant (df=1, 4, F=19.53, P=0.01). Under sufficient water, arbuscular colonization rate was 12±3% in high-AMF treatment, and 4±0.8% in low-AMF treatment (benomyl application decreased arbuscular colonization about 67%). Under insufficient water treatment, arbuscular colonization rate was 15±1.8% in high-AMF treatment, and 2.8±1.4% in low-AMF treatment (benomyl application decreased arbuscular colonization about 81%).

Plant densities, water levels, and two-way and three-way interactions did not significantly affect total mycorrhizal colonization or arbuscular colonization at either sampling time (P>0.05).

Plant number and survival rate

Plant density was significantly affected by seeding rate at the first sampling time (df=2, 16, F=1,461.77, P=0.0001) and second sampling time (df=2, 16, F=1,583.85, P=0.0001; Fig. 2). AMF level, water treatment, and the two-way and three-way interactions did not significantly influence plant density (P>0.05).

Plant survival rate was significantly higher in the sufficient water treatment than in the insufficient water treatment (Fig. 3, df=1, 2, F=85.515, P=0.015). Survival rate was lower in the high-AMF than in the low-AMF

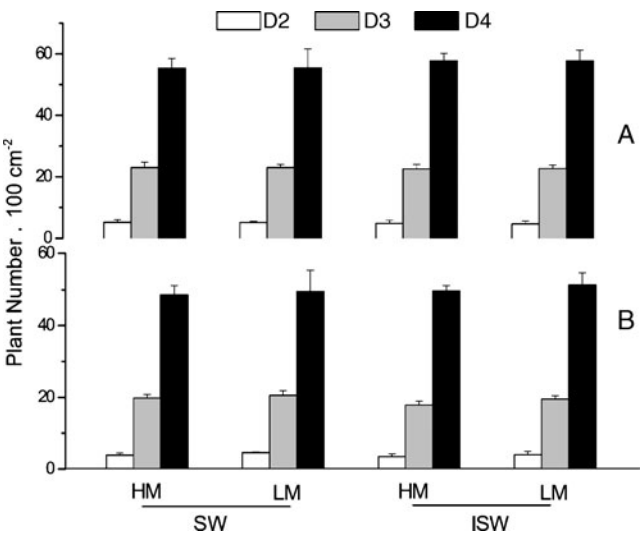

Fig. 2 Plant numbers in a 10 cm×10 cm square at the first sampling (**A**) and at the second sampling (**B**). *SW* sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment. *D2*, *D3*, and *D4* indicate planting density (100, 1,000, and 10,000 seeds per square meter, respectively). Values are means ± SE

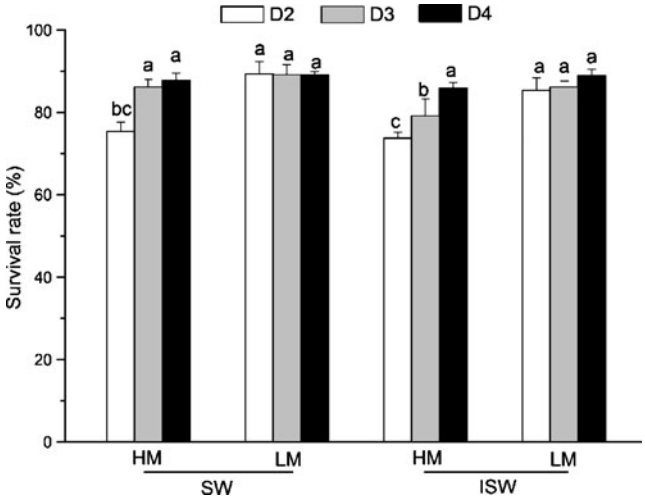

Fig. 3 Survival rate as affected by water level, mycorrhizal level, and plant density. *SW* sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment. *D2*, *D3*, and *D4* indicate planting density (100, 1,000, and 10,000 seeds per square meter, respectively). Values are means ± SE. Different *letters* represent significant differences across all treatments

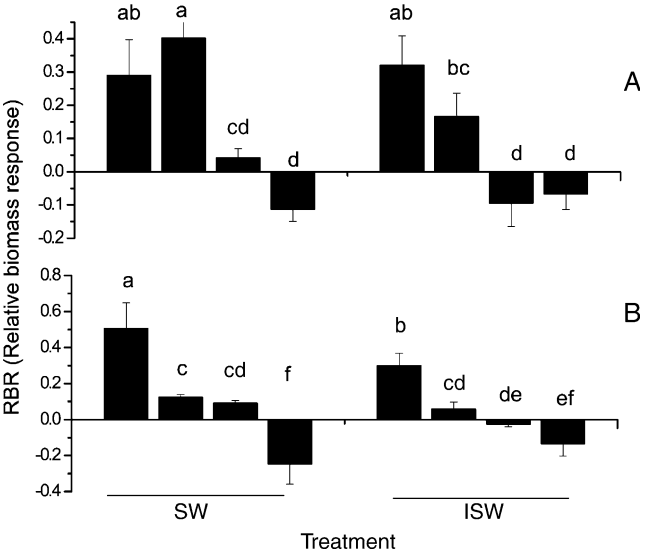

Fig. 4 The response of shoot biomass to mycorrhiza at the first sampling (**A**) and at the second sampling (**B**). *SW* sufficient water treatment, *ISW* insufficient water treatment. *D1*, *D2*, *D3*, and *D4* represent the four planting densities, from the lowest to the highest. Values are means ± SE. Different *letters* represent significant differences across all treatments

treatment (*df*=1, 4, *F*=42.599, *P*=0.0028), but this effect was density dependent (*df*=2, 16, *F*=17.5423, *P*=0.0001). At the highest plant density, the effect of AMF treatment on survival was not significant (Fig. 3). Survival rate increased with density (*df*=2, 16, *F*=28.712, *P*=0.0001). The two- and three-way interactive effects were not significant (*P*> 0.05).

Relative biomass response to mycorrhiza

At the first sampling time, RBR to mycorrhizal level was affected by plant density (*df*=3, 12, *F*=47.108, *P*=0.0001) and the interaction of water level and density (*df*=3, 12, *F*=5.143, *P*=0.016) (Fig. 4). RBR decreased with density (Fig. 4). At high density, RBR was less than zero. The effects of water level on RBR depended on density. In the lowest-density (100 seedlings per square meter) treatment, RBR was significantly higher with the sufficient water treatment than with the insufficient water treatment (*P*<0.05, Fig. 4).

At the second sampling time, RBR was affected by water level (*df*=1, 2, *F*=26.098, *P*=0.036), plant density (*df*=3, 12, *F*=56.800, *P*=0.0001), and the interaction between water level and plant density (*df*=3, 12, *F*=4.238, *P*=0.029).

Relative competition intensity

RCI was increased by the high-AMF treatment (for the first sampling: *df*=1, 4, *F*=8.469, *P*=0.044; for the second sampling: *df*=1, 4, *F*=12.63, *P*=0.024) and by density (for the first sampling: *df*=2, 16, *F*=76.495, *P*=0.0001; for the second sampling: *df*=2, 16, *F*=353.89, *P*=0.0001) at both sampling times (Fig. 5).

At the first sampling, the interaction between mycorrhizal level and plant density on RCI was not significant (*df*= 2, 16, *F*=1.58, *P*=0.24). Interaction between mycorrhizal level and water level on RCI was not significant (*df*=1, 4, *F*=2.14, *P*=0.22).

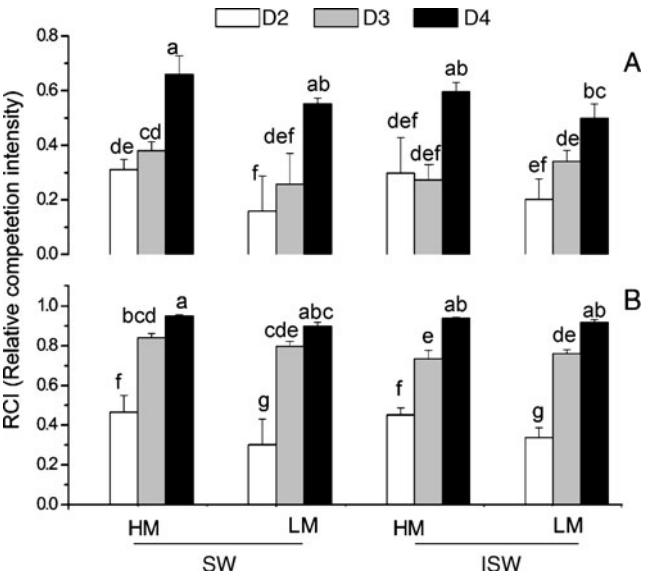

Fig. 5 Relative plant competition intensity at the first sampling (**A**) and at the second sampling (**B**). *SW* sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment. *D2*, *D3*, and *D4* indicate planting density (100, 1,000, and 10,000 seeds per square meter, respectively). Values are means ± SE. Different *letters* represent significant differences across all treatments

At the second sampling, the interaction between mycorrhizal level and plant density was significant (df=2, 16, F=5.369, P=0.016). A significant effect of mycorrhizal level on RCI occurred with 100 seedlings per square meter. Water level (df=1, 2, F=1.61, P=0.33), interaction between water level and mycorrhizal level (df=1, 4, F=2.02, P=0.23), and the three-way interactions (df=2, 16, F=0.12, P=0.89) did not significantly affect RCI.

Absolute competition intensity

Mycorrhizal level, water level, and plant density significantly influenced ACI (Fig. 6). At the first sampling time, ACI was increased by the high-AMF treatment (df=1, 4, F=40.59, P=0.003) and by increasing plant density (df=2, 16, F=77.77, P=0.0001). Insufficient water decreased ACI (df=1, 2, F=54.61, P=0.018). ACI was significantly affected by interactions between water level and mycorrhizal level (df=1, 4, F=8.74, P=0.041) and between water level and density (df=2, 16, F=8.122, P=0.0037).

At the second sampling time, the high-AMF treatment significantly increased ACI (df=1, 4, F=226.23, P=0.0001). Insufficient water decreased ACI (df=1, 2, F=49.51, P=0.0015). ACI significantly increased with plant density (df=2, 16, F=263.25, P=0.0001). ACI was significantly affected by the interactions between water level and mycorrhizal level (df=1, 4, F=92.09, P=0.0007) and between water level and density (df=2, 16, F=27.34, P=0.0001).

Biomass–density relationship

The relationship between mean individual biomass and plant density was significantly affected by the water and mycorrhizal treatments (Fig. 7, Table 1). Under sufficient water treatment, slope of biomass–density relationship was significantly steeper in the high-AMF treatment than in the low-AMF treatment (for the first sampling: test statistic=3.770, P=0.042; for the second sampling: test statistic=3.472, P=0.041; Fig. 7, Table 1). This indicated that individual biomass declined faster with density in high-AMF treatment than in low-AMF treatment. While under insufficient water treatment, difference between slopes of the two AMF treatment was not significant (for the first sampling: test statistic=0.054, P=0.815; for the second

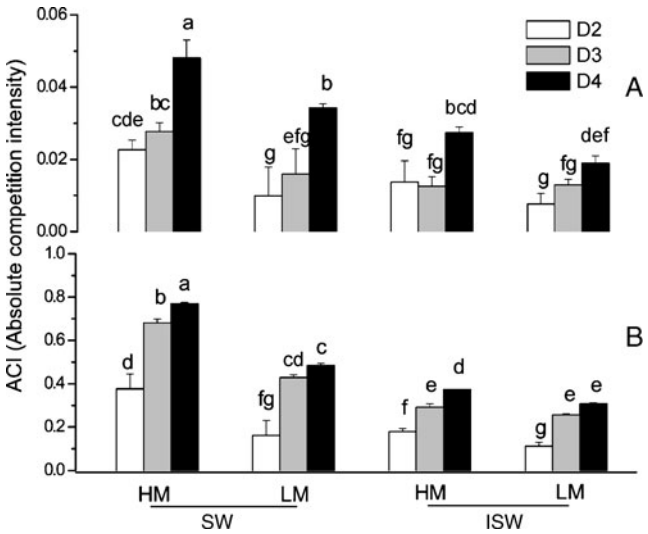

Fig. 6 Absolute competition intensity at the first sampling (**A**) and at the second sampling (**B**). *SW* sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment. *D2*, *D3*, and *D4* indicate planting density (100, 1,000, and 10,000 seeds per square meter, respectively). Values are means ± SE. Different *letters* represent significant differences across all treatments

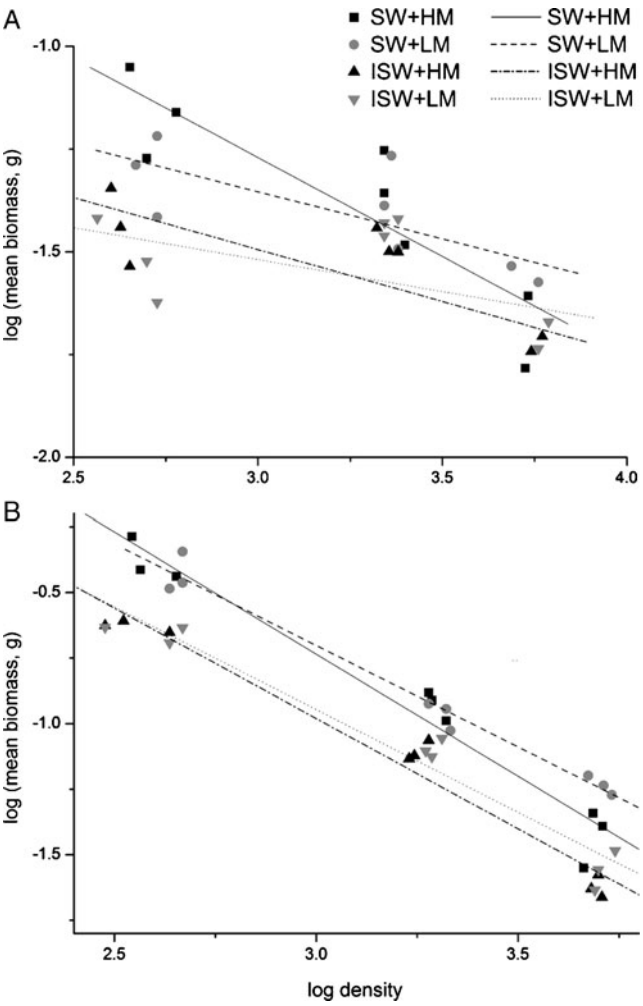

Fig. 7 Biomass–density relationship in *M. sativa* populations as affected by mycorrhizal level and water level at the first sampling (**A**) and at the second sampling (**B**). *SW* sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment. Parameters of biomass–density lines fit by the standardized major axis are given in Table 1

Table 1 Regression parameter estimates (standardized major axis regression) of log (shoot biomass) on log density in populations of *M. sativa* L. in high-AMF and low-AMF treatments under two water levels

Treatments			Number	Intercept	Slope	95% CI of slope	r^2
The first sampling	SW	HM	9	0.4203	−0.5594	(−0.8180,−0.3825)	0.810
		LM	9	−0.4402	−0.2985	(−0.5148,−0.1731)	0.590
	ISW	HM	9	−0.5309	−0.3161	(−0.5284,−0.1891)	0.639
		LM	9	−0.6221	−0.2876	(−0.5762,−0.1436)	0.292
The second sampling	SW	HM	9	2.124	−0.9516	(−1.1423,−0.7927)	0.958
		LM	9	1.643	−0.7815	(−0.8860,−0.6892)	0.980
	ISW	HM	9	1.588	−0.8557	(−1.0078,−0.7265)	0.966
		LM	9	1.471	−0.8045	(−0.9829,−0.6585)	0.949

SW sufficient water treatment, *ISW* insufficient water treatment, *HM* high-AMF treatment, *LM* low-AMF treatment

sampling: Test statistic=0.288, P =0.584; Fig. 7, Table 1). Individual biomass was greater in the high-AMF treatment than in the low-AMF treatment at low plant density but the opposite was true at high plant density (Fig. 7).

Discussion

Effects of fungicide on AMF

Benomyl application was shown to suppress AMF colonization in this experiment. Although some experiments have shown that benomyl can also affect pathogenic fungi (Callaway et al. 2004) and other soil organisms like root-feeding nematodes (van der Putten et al. 1990), other experiments reported that benomyl application has little or no effect on nonmycorrhizal plant and bacterial community (Daleo et al. 2008). Benomyl application caused no difference on plant growth compared to pasteurized soil with other soil microflora added back (Hetrick et al. 1986). If pathogenic fungi were affected by benomyl more strongly than AMF were affected, plant growth should be promoted, not suppressed (Hartnett and Wilson 1999). Although we did not re-establish AMF potential in low-AMF (benomyl-treated) plots by controlled inoculation to verify the effects of the reduced AMF by benomyl in our study, the decreases in plant growth and mycorrhizal colonization by benomyl application implied that the primary effects of benomyl on plant growth are due to suppressed AMF colonization. There are contradictory reports of benomyl application for Rhizobiaceae when host plants are Leguminosae. Some experiments showed that benomyl application inhibited Rhizobiaceae (Hashem et al. 1997; Campo et al. 2009), but others showed that benomyl application could promote Rhizobiaceae (Hossain and Alexander 1984a, b). In a previous study, we found that benomyl had no effect on nodule number of *M. sativa* under the concentration we used (19.9±2.9 per plant without benomyl vs. 21.2±3.6 per plant with benomyl application, unpublished data).

Effects of AMF on biomass–density relationship

Our results demonstrated that the biomass–density relationship, as represented by a plot of log mean plant biomass on log plant density, was influenced by mycorrhiza. The biomass–density relationship has been shown to be affected by mycorrhiza in non-self-thinning plant populations, i.e., plant populations in which the probability of mortality does not increase with population density (Koide 1991; Allsopp and Stock 1992). Shumway and Koide (1995) reported, however, that mycorrhizal colonization did not influence the biomass–density relationship in self-thinning *A. theophrasti* populations. In our experiment with *M. sativa*, the biomass–density relationship had a steeper slope (a greater decline with density) in the high-AMF treatment than in the low-AMF treatment. In addition, both the RCI and the ACI were higher in the high-AMF treatment than in the low-AMF treatment. These results indicate that mycorrhiza affected the biomass–density relationship by enhancing competition between individual plants.

Morris (2002, 2003) reported that fertilizer levels can affect biomass–density relationships. In our study, the effects of higher AMF level are similar to the effects of higher nutrient level because, with their hyphae extending into the soil, AMF can enhance water and nutrient uptake by host plants. Mycorrhiza can also increase shoot branching (Shumway and Koide 1995), canopy radius (Yang 2007), and above-ground biomass. These enhancements of individual plant growth can increase the competition among individual plants within a population. Thus, we infer that higher intraspecific competition induced by mycorrhiza led to a decrease in the biomass of individual plants with increasing plant density, i.e., a shift in the line describing the relationship between biomass and density (Morris 2002).

Lower survival rate caused by intensive intraspecific competition in a self-thinning population can also shift the biomass–density relationship (Morris 1999) because lower survival rate which means higher mortality can increase the individual biomasses of the surviving plants (Weiner et al. 2001; Stoll et al. 2002). In our experiment, the mycorrhizal

treatment did not reduce plant survival rate when plant density was high. This result suggests that mycorrhiza did not affect the biomass–density relationship by changing plant survival rate or population size. Again, we suggest that mycorrhiza changes the relationship by enhancing intraspecific competition.

Effects of water and AMF interaction on biomass–density relationship

By supporting abundant growth, high levels of nutrients, water, and other resources increase competition among plants (Gurevitch et al. 1990; Deng et al. 2006) and thus generate biomass–density lines with more negative slopes (Deng et al. 2006). In this experiment, we found interactive effects of mycorrhiza and water levels on the biomass–density relationship. Although slopes of the biomass–density relationship were more negative in the high-AMF than in the low-AMF treatment in both the sufficient water and insufficient water treatments, the effect of the AMF treatment on the slope was greater in the sufficient water treatment than in the insufficient water treatment. Both sufficient water and mycorrhiza promoted plant growth. Also, ACI and RCI were higher in the sufficient water and high-AMF treatments than in the insufficient water and low-AMF treatments. These results indicate that sufficient water can amplify the effects of mycorrhiza on the biomass–density relationship by promoting individual plant growth and further enhancing intraspecific competition.

In summary, our results support the hypotheses that mycorrhiza affects the biomass–density relationship and that this effect is resource dependent. A high-AMF level shifted the biomass–density relationship by promoting the growth of individual plants and thereby increasing intraspecific competition. Water level influenced the effects of mycorrhiza on the biomass–density relationship in that the mycorrhizal effect on the biomass–density relationship was greater when water was sufficient than when it was insufficient because sufficient water increased intraspecific competition.

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