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Arbuscular Mycorrhizal Fungal Composition Affects the Growth and Nutrient Acquisition of Two Plants from a Karst Area

(Kesan Komposisi Kulat Mikoriza Arbuskul terhadap Pertumbuhan dan Pemerolehan Nutrien oleh Dua Tumbuhan dari Kawasan Karst)

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ABSTRACT

How the composition of the arbuscular mycorrhizal (AM) fungal community affects plant traits of different plant species in karst environments is poorly understood. Broussonetia papyrifera *(a woody shrub) and* Bidens pilosa *(a herbaceous plant) growing in pots in limestone soil were inoculated with an AM fungus, either* Funneliformis mosseae (FM), Diversispora versiformis (DV) *or* Glomus diaphanum (GD) *or with an inoculum mixture of all three AM fungi (bn).* B. papyrifera *and* B. pilosa *seedlings inoculated with AM fungi showed a significant increase in biomass and nitrogen and phosphorus acquisition compared with the controls, which lacked mycorrhiza. Mixed fungal inoculations significantly enhanced biomass and nitrogen and phosphorus acquisition by* B. papyrifera *seedlings compared with single fungal inoculations. Nitrogen and phosphorus acquisition by* B. papyrifera *mycorrhizal seedlings was significantly greater than that of* B. pilosa *mycorrhizal seedlings. Fungal composition significantly influenced the mycorrhizal benefits of biomass and phosphorus acquisition and mixed fungal inoculations enhanced nitrogen acquisition. Plant species significantly affected nitrogen acquisition but did not have an effect on biomass and phosphorus benefits. We concluded that AM fungal associations increased plant growth and nutrient absorption and that in general a mixed inoculation of AM fungi enhanced biomass and nutrient acquisition more than a single AM fungal inoculation. In addition, a mycorrhizal association was more beneficial for* B. papyrifera *seedlings in terms of biomass and nutrient acquisition than for* B. pilosa *seedlings.*

Keywords: Arbuscular mycorrhizae; fungal composition; karst environments; nutrient acquisition

ABSTRAK

Pengaruh komposisi komuniti kulat mikoriza arbuskul (AM) terhadap sifat tumbuhan daripada spesies tumbuhan berbeza dalam persekitaran kars masih kurang difahami. Broussonetia papyrifera *(pokok renek berkayu) dan* Bidens pilosa *(herba) yang tumbuh di dalam pasu dalam tanah batu kapur telah diinokulat dengan kulat AM seperti* Funneliformis mosseae (FM), Diversispora versiformis (DV) *atau* Glomus diaphanum (GD) *atau campuran inokulum ketiga-tiga kulat AM (MI). Benih* B. papyrifera *dan* B. pilosa *yang diinokulat dengan kulat AM menunjukkan kenaikan ketara dalam pemerolehan biojisim, nitrogen dan fosforus berbanding dengan sampel kawalan yang kurang mikoriza. Inokulasi kulat campuran telah menyebabkan peningkatan pemerolehan biojisim, nitrogen dan fosforus yang ketara oleh benih* B. papyrifera *berbanding inokulasi kulat tunggal. Pemerolehan nitrogen dan fosforus oleh benih mikoriza* B. papyrifera *jauh lebih ketara daripada benih mikoriza* B. pilosa. *Komposisi kulat memberi kesan ketara terhadap pemerolehan biojisim dan fosforus mikoriza manakala inokulasi fungus campuran telah meningkatkan pemerolehan nitrogen. Spesies tumbuhan memberi kesan ketara terhadap pemerolehan nitrogen tetapi tidak mempunyai kesan ke atas pemerolehan biojisim dan fosforus. Kesimpulannya, hubungan kulat AM telah meningkatkan pertumbuhan tumbuhan dan penyerapan nutrien dan secara amnya ialah inokulasi campuran kulat AM telah meningkatkan pemerolehan biojisim dan nutrien lebih daripada inokulasi kulat AM tunggal. Di samping itu, hubungan mikoriza lebih bermanfaat untuk benih* B. papyrifera *daripada segi pemerolehan biojisim dan nutrien daripada benih* B. Pilosa.

Kata kunci: Komposisi kulat; mikoriza arbuskul; pemerolehan nutrien; persekitaran kars

INTRODUCTION

Mycorrhizal symbiosis is the most ancient and widespread fungal symbiosis in nature (Brachmann & Parniske 2006). Eighty percent of terrestrial plants establish a mutual relationship with mycorrhizal fungi (AM) (Brundrett 2009). Mycorrhizal symbiosis involves the mutual exchange of benefits between the plant and the mycorrhizal fungus: Carbohydrates derived from the plant photosynthetic products are exchanged for mineral nutrients taken up from the soil by the extra-radical mycelium of the AM fungus (Koide & Mosse 2004; Smith & Read 2010). AM fungi also provide their host with other advantages such as protection against biotic and abiotic stresses and the improvement of the soil structure (Smith & Read 2010). AM fungi can also influence plant species diversity by affecting ecosystem properties via their widespread underground extra-radical

mycelial net (Bever et al. 2010; Grime et al. 1987; Nelson & Sommers 1982; Van Tuinen et al. 1998; Vogelsang et al. 2006), which can influence vegetation succession.

Previous studies have shown that a root fragment root can be colonized by multiple AM fungal species and that an AM fungal species can infect different plant species representing different functional types (Urcelay & Diaz 2003). The interactions between plant traits and AM fungi are an important aspect of soil microbial ecology (Powell et al. 2013). The host plant is a biotic driver for the formation of AM fungal communities, either as a function of plant species identity (Martinez-Garcia & Pugnaire 2011; Sanchez-Castro et al. 2012; Vandenkoornhuyse et al. 2003) or as a plant functional group (Davison et al. 2011; Scheublin et al. 2004). Çakan and Karataş (2006) and Lopez-Garcia et al. (2014) suggested that the type of plant life form influenced the colonization of AM fungal species during the vegetation succession of sand dunes. However, Urcelay et al. (2009) concluded that there was no relationship between plant life form and AM fungal community by using a removal method to analyze plant functional groups. Recently, Yang et al. (2012) reported a high specificity of plant functional type for AM fungi at different scales on the basis of their life forms as grasses, forbs and woody plants. Furthermore, Lekberg et al. (2013) proposed that AM fungal abundance was based on a ratios of forbs and grasses and suggested that plant species may have an effect on mycorrhizal fungal composition in natural ecosystems. However, does the AM fungal composition influence the benefits conferred on different host plants?

In many limestone regions of the world, including the widespread karst landscape in southwest China (Wang et al. 2004), the supply of soil nutrients is strongly constrained (Liu et al. 2014). However, soil microbial diversity improves with the developmental succession of the plant community in Karst areas (Zhu et al. 2012). Wei (2012) has detected 68 AM fungal species in virgin forest soil in the Maolan Karst region of southwest China, where *Glomus* was the dominant genus. Bavaresco et al. (2000) and Likar et al. (2013) also reported that *Glomus* was stable and dominant in Karst areas and influenced plant growth traits during the succession process. Zhang et al. (2014) studied the effects of AM fungi on the drought tolerance of *Cyclobalanopsis glauca* seedlings in karst soil; however, their investigation was not primarily focused on the effects of AM fungal composition on different plant species of different life forms during vegetation succession in a karst environment.

A plant species can be colonized by multiple AM fungal species and an AM fungal species can colonize many different plant species (Urcelay & Diaz 2003). Therefore, plant traits could be affected by fungal species, fungal composition and plant species. For instance, the nutritional uptake of plants greatly depends on plant functional types in Karst habitats (Liu et al. 2014). One of the mycorrhizal functions is the facilitation of nutrient acquisition (Smith & Read 2010), thus AM fungi could change the nutritional traits of plants. In karst ecosystems, many plant species with different functions exist at each successional stage. There is a great need to understand better how fungi and fungal composition affect plant traits such as growth, nitrogen acquisition and phosphorus acquisition. To investigate this problem, we conducted a greenhouse experiment using two typical plant species that grow in karst habitats in southwest China (a mid-successional stage species with a woody plant function type and an early-successional stage species with an herbaceous plant functional type). The two plant species were inoculated with a single AM fungus or a mixture of three AM fungi.

Materials and Methods

Limestone soil and seeds of *Broussonetia papyrifera* and *Bidens pilosa* were collected at Jigong Mountain, Beibei of Chongqing city in China, a typical karst ecosystem with many highly adapted woody and herbaceous plants, including *B. papyrifera* (a mid-successional stage plant with a woody plant function type) and *B. pilosa* (an early-successional stage plant with a herbaceous functional type). Each pot (19.0 cm in diameter, 15.0 cm in deep) was autoclaved at 0.14 Mpa and 124°C for 1 h and then filled with 3.5 kg of substrate (2:1:1 w/w/w of limestone soil, sand and quartz). The growth substrate had a pH of 6.81 and contained 6.1 g/kg of organic carbon (ORC), 463.5 mg/kg of total nitrogen, 33.3 mg/kg of available nitrogen, 158 mg/kg of total phosphorus and 1.81 mg/kg of available phosphorus. *B. papyrifera* and *B. pilosa* seeds were surface sterilized by placing in 10% H_2O_2 for 10 min. Then, six seeds of either *B. papyrifera* or *B. pilosa* were planted in each pot. The inoculum used in this experiment was purchased from the Institute of Plant and Natural Resources, Beijing Academy of Agriculture and Forestry Science of China. The arbuscular mycorrhizal fungi had been isolated from a karst site in Guizhou Province in southwest China and identified and characterized according to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu). The experiment involved four AM fungal inoculum treatments. Three of these treatments consisted of 20 g of culture media containing approximately 200 spores, hyphae and colonized root pieces of *Funneliformis mosseae* (FM), *Diversispora versiformis* (DV) or *Glomus diaphanum* (GD). The fourth treatment (MI) consisted of 6.67 g of culture media of each of the three AM fungi and contained approximately 200 spores. The inoculum was spread over the substrate in each pot and then covered by an additional 2 cm thick layer of substrate. The control treatment (M^-), which lacked mycorrhizal fungi, consisted of a 10 mL solution of AM fungal inoculum slurry that had been filtered using a 25 mm filter paper to remove the mycorrhizal fungi while allowing other soil microorganisms, such as potential pathogenic bacteria and fungi, to remain in the solution. This solution was sprayed onto the substrate and then

covered with a 2 cm thick layer of substrate (Johnson 1993). There were five replicates of each treatment. All the pots were placed in a plastic greenhouse at Southwest China University, Chongqing, China (106°22′ E, 29°49′ N, altitude 300 m). This region has a subtropical monsoon climate with mild and humid winters and hot summers with periodic drought. Following seed germination, which took approximately a month, three plants were retained in each pot. All the seedlings were harvested after 3 months growth, dried at 105°C for 48 h and then weighed to obtain the dry mass of the roots, leaves, stems and total biomass.

MYCORRHIZAL ROOT COLONIZATION AND BIOMASS

Fresh roots of *B. papyrifera* and *B. pilosa* seedlings were washed and cut into lengths of about 1 cm. All samples were immersed in 5% (w/v) KOH at 95°C for 40 min to clear the roots and then stained by placing the samples in 0.05% magenta at 95°C for 40 min, followed by a 2% lactic solution for 3 min for neutralization. The stained roots were placed on a Petri dish with gridlines and examined under the microscope at ×40 magnification (Kormanik et al. 1980). Mycorrhizal colonization was expressed as the proportion of roots that were colonized by the AM fungus at all the root-grid intersection points (Brundrett et al. 1984; Giovannetti & Mosse 1980). Harvested plants were dried at 105°C for 48 h. Then, each part of each plant (root, leaf and shoot) was separated and weighed.

NITROGEN AND PHOSPHORUS DETERMINATIONS

Total nitrogen concentration was determined using Kjeldahl measurements after digesting each sample with sulfuric acid followed by distillation using a Büchi Distillation Unit B-324 (Switzerland). Total phosphorus concentration was determined using a molybdenum-antimony anti-spectrophotometric method (Bao 2000). Nutrient (nitrogen and phosphorus) acquisition by individual plants was determined via nutrient concentration multiplied by biomass.

STATISTICAL ANALYSIS

Statistical analyses were conducted using SPSS 13.0 (SPSS, Chicago, IL, USA). The least significant difference (LSD) test was applied to compare the different treatments. A P value of ≤ 0.05 was considered to be significant for all tests. A t-test was applied to compare differences in biomass and nitrogen and phosphorus acquisition between *B. papyrifera* and *B. pilosa* seedlings. Two-way ANOVAs of the main effects and interacting effects were performed to analyze mycorrhizal benefits in terms of biomass and nitrogen and phosphorus acquisition. Three-way ANOVAs of the main effects and interacting effects were performed to analyze the effects of biomass and nitrogen and phosphorus acquisition. The individual plants that were used to provide measurements were randomly selected at the beginning of the experiment.

RESULTS AND DISCUSSION

EFFECTS OF FUNGI, FUNGAL COMPOSITION AND SPECIES ON PLANT GROWTH AND NUTRIENT ACQUISITION

B. papyrifera and *B. pilosa* seedlings that received an AM fungal treatment showed positive feedback in terms of biomass and nitrogen and phosphorous acquisition (Figure 1(a), 1(b), 1(c)), which was consistent with the results reported in many related studies showing that AM fungi change the growth and nutrient uptake of their host plants (Gustafson & Casper 2006; Hart et al. 2013; Jansa et al. 2008; Kiers et al. 2011; Thonar et al. 2011). Differences in the influence of an AM fungal association on the performance of plant traits for biomass and nitrogen and phosphorus acquisition depending on the plant species and the AM fungal composition have previously been explained as a method of avoiding competition between individuals (Jansa et al. 2008; Kiers et al. 2011).

Previous studies have shown that inoculating plants with multiple AM fungi was beneficial for the nutritional uptake of the host plant (Gustafson & Casper 2006; Thonar et al. 2011), which was consistent with our results for the *B. papyrifera* seedlings that received the MI treatment (Figure 1(b), 1(c)). However, Edathil et al. (1996) and Hart et al. (2013) argued that inoculating plants with a single AM fungus can derive the biggest benefit. This idea is partly supported by our findings for *B. pilosa* seedlings, which did not derive greater benefits in terms of biomass or nutrient acquisition when inoculated with a mixed inoculum of several AM fungi compared with those inoculated with a single AM fungus (Figure 1). Individual plant roots are often infected by multiple AM fungi in natural ecosystems (Yang et al. 2010), which has also been reported to occur in Karst ecosystems. Furthermore, Karst ecosystems have been found to have a greater AM species diversity than normal land forms (Wei 2012). Kiers et al. (2011) have suggested that individual AM species might use different strategies to supply resources and benefits to host plants when multiple AM species share an individual root system. The relationship between different AM fungal species, such as synergy, competition or antagonist and the effect of that relationship on a plant species is difficult to determine (Krak et al. 2012), especially in karst habitats. Mixed inoculation was better than single inoculation for enhancing the plant traits for nutrient acquisition and biomass accumulation of *B. papyrifera* seedlings (Figure 1), which suggested that there could be a synergistic relationship among the AM fungi species. Wagg et al. (2011) suggested the synergistic relationship could be changed by various environmental conditions or the host plant, but Koide (2000) argued that functional complementarity benefits the host plant. Individual AM fungal species have been reported to affect the growth traits of different host plant species in different ways (Sanders 2003), which supports our findings that fungal composition significantly affected biomass, phosphorus and nitrogen acquisition traits (Table 1).

Mycorrhizal fungi significantly affected the biomass and nitrogen acquisition of both plant species ($p<0.001$), but did not significantly affect phosphorus acquisition (Table 1). Biomass, nitrogen acquisition and phosphorus acquisition were higher in the M^+ treatments with AM fungi than that in M^- treatments without AM fungi ($p<0.05$), while the differences between M^+ and M^- treatments were significant except for phosphorus acquisition by *B. pilosa* seedlings that received the DV treatment (Figure 1). These analyses showed that AM fungi positively facilitated biomass accumulation and the absorption of nitrogen and phosphorus by *B. papyrifera* and *B. pilosa* seedlings.

Fungal composition significantly influenced biomass, nitrogen acquisition and phosphorus acquisition (Table 1). *B. papyrifera* seedlings that received the MI treatment consisting of an inoculum mixture of *F. mosseae*, *D. versiformis* and *G. diaphanum* showed significantly greater biomass, nitrogen acquisition and phosphorus acquisition than seedlings that received the FM, DV or GD treatment, which consisted of a single inoculum species. These results indicate that the mixed AM fungal inoculum greatly increased biomass accumulation and nutrient absorption. Seedlings that received the FM, DV or GD treatment did not show significantly different biomass or nitrogen acquisition (Figure 1(a) and 1(b)), while phosphorus acquisition by seedlings that received the GD treatment was significantly greater than by seedlings that received the FM or DV treatments (Figure 1(c)). *B. pilosa* seedlings that received the MI treatment were not significantly different to those that received the GD treatment in terms of biomass, nitrogen acquisition and phosphorus acquisition (Figure 1) but were significantly different to those that received the DV or FM treatment. However, seedlings that received the FM or DV treatments were not significantly different; phosphorus acquisition by seedlings that received the GD treatment was significantly greater than by those that received the DV or FM treatment (Figure 1(c)). These results indicated that AM fungal composition had different effects on the plant growth and nutrient absorption of the two plant species under investigation and that the mixed fungal treatment improved the performance of *B. papyrifera* seedlings in terms of biomass, nitrogen and phosphorus acquisition.

Plant species significantly influenced nitrogen acquisition and phosphorus acquisition but did not influence the biomass of seedlings that received the FM, DV or GD treatment (Table 1). However, the biomass of *B. papyrifera* seedlings that received the MI treatment was significantly greater than that of *B. pilosa* seedlings (Figure 1(a)); furthermore, all the *B. papyrifera* seedlings that received a mycorrhizal treatment acquired significantly more nitrogen and phosphorus than the *B. pilosa* seedlings (Figure 1(b) and 1(c)). The results indicated that the influence of arbuscular mycorrhizal fungi on plant traits might be different in different plant species.

The interaction of fungi and fungal composition (F×C) significantly affected biomass but did not significantly affect nitrogen and phosphorus acquisition ($p<0.05$); the interaction of fungi and plant species (F×S) significantly affected nitrogen acquisition but did not significantly affect biomass and phosphorus acquisition ($p<0.01$); and fungal composition and plant species (C×S) significantly affected phosphorus but did not significantly affect biomass and nitrogen acquisition. Furthermore, the interaction between fungi, fungal composition and plant species (F×C×S) did not significantly affect biomass or nitrogen and phosphorus acquisition (Table 1).

EFFECTS OF FUNGAL COMPOSITION AND PLANT SPECIES ON THE BENEFITS OF GROWTH AND NUTRIENT ACQUISITION OF B. PAPYRIFERA AND B. PILOSA SEEDLINGS

Plant species with different life forms display different traits, including the mycorrhizal benefits of biomass, nitrogen and phosphorus acquisition (Figure 2, Table 2), which may be attributed to functional traits in morphological taxonomy according to the studies of Díaz and Cabido (1997) and Lavorel et al. (1997). Çakan and Karataş (2006) have suggested that the mycorrhizal infection level depends on the life form of the plant species that occur at different stages of succession. Yang et al. (2012) have reported that a global meta-analysis showed that host plants had a high level of specificity for AM fungi for functional selection based on their life forms such as grasses, forbs and woody plants. *B. papyrifera* is a perennial woody plant appearing in the mid-successional stage of karst areas whereas *B. pilosa* is an annual herb that appears at an early stage of succession as a pioneer plant species in southwest China. *B. papyrifera* seedlings inoculated with AM fungi acquired more nutrient benefits (i.e. nitrogen and phosphorus) to maintain growth compared with the *B. pilosa* seedlings. We speculate that one of the reasons that woody plants replace herbs in plant successions in karst communities is due to the mycorrhizal benefit of nutrient supply, which would explain why *B. pilosa* gradually disappears from plant communities but *B. papyrifera* appears in the mid- or late-stages of succession in karst areas.

Fungal composition significantly increased biomass and phosphorus acquisition but did not significantly affect nitrogen (Table 2). First, *B. papyrifera* seedlings that received the MI treatment showed a significantly greater increase in biomass than the seedlings that received the FM, DV or GD treatments, which were not significantly different from each other. *B. pilosa* seedlings that received the MI treatment showed a significantly greater biomass than the seedlings that received the DV or FM treatment but not the GD treatment. Furthermore, seedlings that received the DV treatment produced significantly less biomass than those that received the FM or GD treatment, which were not significantly different from each other in terms of biomass production (Figure 2(a)). Second, *B. papyrifera* seedlings that received the MI treatment showed significantly greater nitrogen acquisition than those that received the FM, DV or GD treatment and the difference between the DV and GD treatments was not significant, whereas the difference between the FM and GD treatments was significant. *B.*

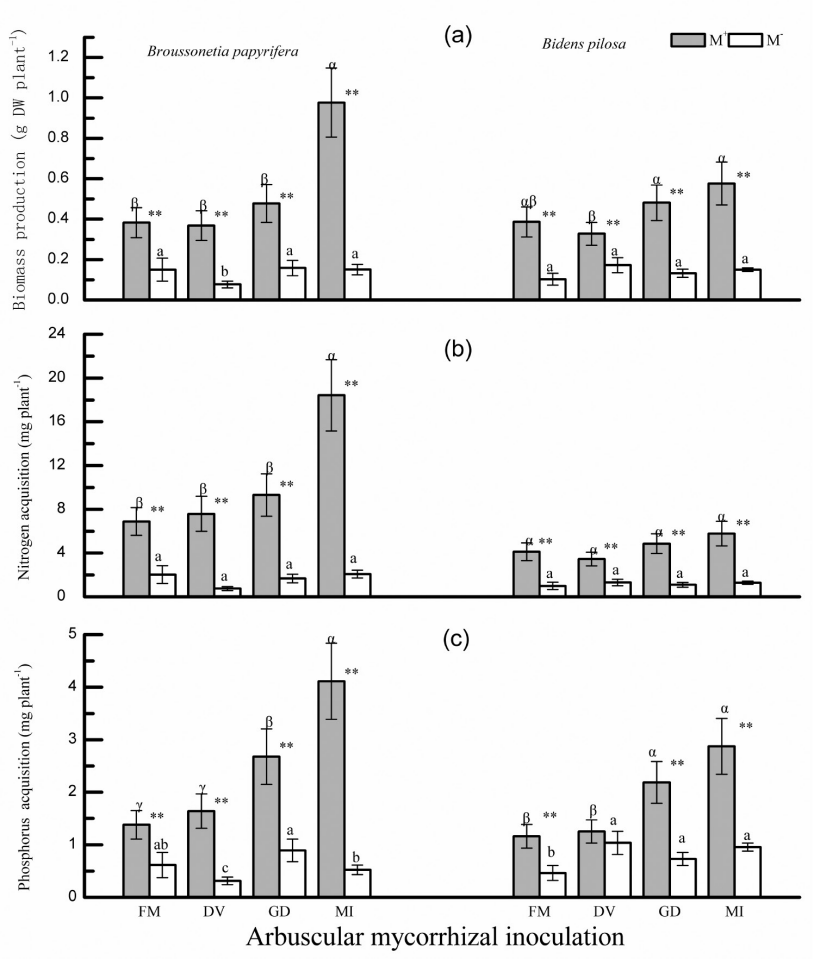

FIGURE 1. Effects of fungi (without vs. with mycorrhizal fungi) and fungal composition (*Funneliformis mosseae* vs. *Diversispora versiformis* vs. *Glomus diaphanum* vs. the AM fungal mixture) on the growth and nutrient acquisition of *Broussonetia papyrifera* and *Bidens pilosa* seedlings

FM = *F. mosseae;* DV = *D. versiformis;* GD = *G. diaphanum*; MI = mixture of all three AM fungi; M^+ = with mycorrhizal fungi; M^- = without mycorrhizal fungi. Different Greek letters (α, β, γ) above the bars of M^+ treatments and different lowercase letters (a, b, c) above the bars of M^- treatments indicate a significant difference in the biomass of *B. papyrifera* or *B. pilosa* seedlings using the LSD test at a p level of <0.05. ** indicates a significant difference between the M^+ and M^- treatments at a p level of <0.01

TABLE 1. ANOVAs for effects of fungi (without vs. with mycorrhizal fungi), fungal composition (*Funneliformis mosseae* vs. *Diversispora versiformis* vs. *Glomus diaphanum* vs. the AM fungal mixture) and species (*Broussonetia papyrifera* vs. *Bidens bipinnata*) on the growth and nutrient acquisition of *B. papyrifera* and *B. pilosa* seedlings

Indexes	Fungi (F)	Composition (C)	Species (S)	F×C	F×S	C×S	F×C×S
Biomass	46.108***	3.763*	0.943	2.973*	1.152	0.914	0.958
Nitrogen	10.697***	2.835*	38.768***	1.411	7.851**	2.217	1.236
Phosphorus	0.620	5.412**	38.078***	0.282	2.779	3.976**	0.592

F value significance levels ($^{***}p< 0.001$, $^{**}p< 0.01$, $^{*}p<0.05$) are given

pilosa seedlings that received the DV treatment acquired significantly less nitrogen than those seedlings that received the FM, GD or MI treatments, which were not significantly different from each other (Figure 2(b)). Third, *B. papyrifera* seedlings that received the MI treatment acquired significantly more phosphorus than those that received the FM, DV or GD treatment; the difference between the DV and GD treatments was not significant. Furthermore, phosphorus acquisition by seedlings that received the FM treatment was significantly different to those that received the GD treatment but was not significantly different to those that received the DV treatment. *B. pilosa* seedlings that received the MI treatment acquired significantly more phosphorus than those that received the FM or DV treatment but was not significantly different to those that received the GD treatment (Figure 2(c)). These results indicated

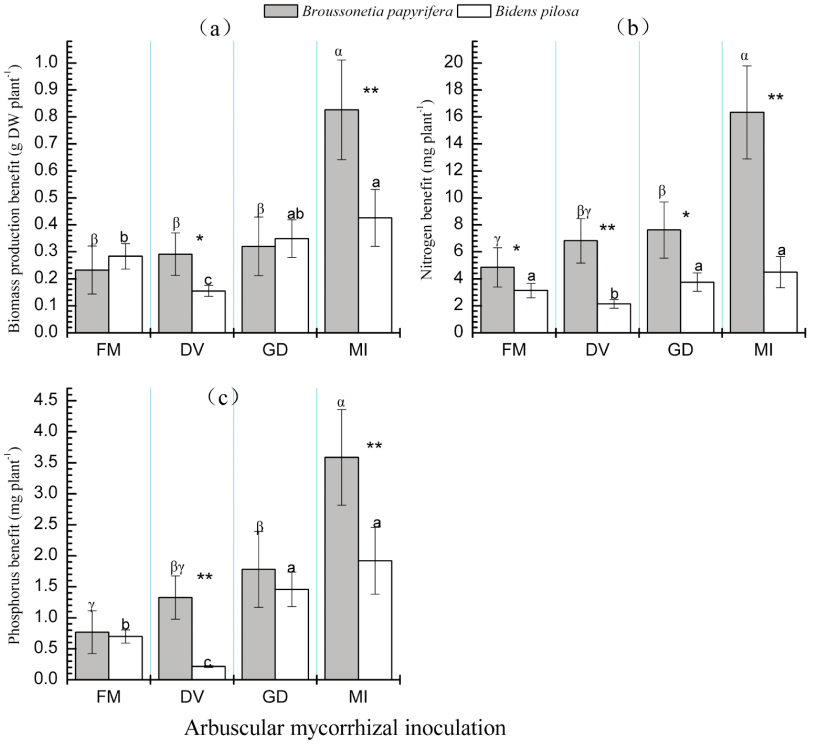

FIGURE 2. Effects of fungal composition (*Funneliformis mosseae* vs. *Diversispora versiformis* vs. *Glomus diaphanum* vs. the AM fungal mixture) on the benefits of growth and nutrient acquisition of *Broussonetia papyrifera* and *Bidens pilosa* seedlings

Refer to Figure 1 for an explanation of the FM, DV, GD and MI treatments. Values of mycorrhizal benefits in terms of biomass, nitrogen and phosphorus acquisition were obtained by calculating the M^+ biomass minus the M^- biomass. Different Greek letters (α, β and γ) above the bars representing the *B. papyrifera* seedlings and different lowercase letters (a, b and c) above the bars representing the *B. pilosa* seedlings indicate a significant difference in the biomass, nitrogen acquisition and phosphorus acquisition benefits determined using the LSD test at the <0.05 level. * indicates a significant difference between *B. papyrifera* and *B. pilosa* seedlings at a p level of <0.05, ** indicates a significant difference between *B. papyrifera* and *B. pilosa* seedlings at a p level of <0.01.

TABLE 2. ANOVAs for effects of fungal composition (*Funneliformis mosseae* vs. *Diversispora versiformis* vs. *Glomus diaphanum* vs. the AM fungal mixture) and species (*Broussonetia papyrifera* vs. *Bidens bipinnata*) on the benefits of growth and nutrient acquisition of *B. papyrifera* and *B. pilosa* seedlings

Indexes	Composition (C)	Species (S)	C×S
Biomass	2.765*	1.071	0.891
Nitrogen	2.071	7.336**	1.155
Phosphorus	3.688*	2.577	0.55

F value significance levels ($^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$) are given

that the mixed fungal inoculum had a beneficial influence on biomass and nitrogen and phosphorus acquisition by the seedlings.

Plant species significantly affected the nitrogen acquisition benefits derived from forming an association with AM fungi but did not affect biomass and phosphorus benefits (Table 2). *B. papyrifera* seedlings that received the MI or DV treatment derived significantly greater benefits in terms of biomass and phosphorus acquisition than those derived by the *B. pilosa* seedlings. However, *B. papyrifera* and *B. pilosa* seedlings that received the FM or GD treatment were not significantly different from each other in terms of biomass and phosphorus acquisition (Figure 2(a), 2(c)). *B. papyrifera* seedlings derived significantly more benefit from forming an association with one or more AM fungus (FM, DV, GD and MI treatments) in terms of nitrogen acquisition than the *B. pilosa* seedlings (Figure 2(b)). The results indicated that different plant species may derive different benefits as a result of an association with AM fungi.

The interaction between fungal composition and plant species (C×S) did not significantly affect the benefits of biomass, nitrogen acquisition or phosphorus acquisition (Table 2).

CONCLUSION

The results indicated that AM fungi positively facilitated biomass accumulation and the absorption of nitrogen

and phosphorus by *B. papyrifera* and *B. pilosa* seedlings. Different plant species significantly affected nitrogen acquisition but did not have an effect on biomass and phosphorus benefits, when nitrogen and phosphorus acquisition by *B. papyrifera* mycorrhizal seedlings was significantly greater than that of *B. pilosa* mycorrhizal seedlings as a result of an association with AM fungi. Fungal composition significantly influenced the biomass and the acquisitions of nitrogen and phosphorus and influenced the mycorrhizal benefits of biomass and phosphorus acquisition. Mixed fungal inoculations significantly enhanced biomass and nitrogen and phosphorus acquisition by *B. papyrifera* seedlings compared with single fungal inoculations and had a beneficial influence on biomass and nitrogen and phosphorus acquisition by the seedlings. We suggested that AM fungal associations increased the growth and nutrient absorption of *B. papyrifera* and *B. pilosa* seedlings and that in general a mixed inoculation of AM fungi enhanced biomass and nutrient acquisition more than a single AM fungal inoculation. Furthermore, a mycorrhizal association was more beneficial for *B. papyrifera* seedlings in terms of biomass and nutrient acquisition than for *B. pilosa* seedlings in this experiment.

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REFERENCES

Bao, S.D. 2000. Soil and agricultural chemistry analysis. *Agric. Press of China*. Beijing (in Chinese).

Bavaresco, L., Cantù, E. & Trevisan, M. 2000. Chlorosis occurrence, natural arbuscular-mycorrhizal infection and stilbene root concentration of ungrafted grapevine rootstocks growing on calcareous soil. *Journal Plant of Nutrition* 23: 1685-1697.

Bever, J.D., Dickie, I.A., Facelli, E., Facelli, J.M., Klironomos, J., Moora, M., Rillig, M.C., Stock, W.D., Tibbett, M. & Zobel, M. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* 25: 468-478.

Brachmann, A. & Parniske, M. 2006. The most widespread symbiosis on earth. *PLoS Biology* 4: e239.

Brundrett, M.C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* 320: 37-77.

Brundrett, M.C., Piché, Y. & Peterson, R.L. 1984. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany* 62: 2128-2134.

Çakan, H. & Karataş, Ç. 2006. Interactions between mycorrhizal colonization and plant life forms along the successional gradient of coastal sand dunes in the eastern Mediterranean, Turkey. *Ecological Research* 21: 301-310.

Davison, J., Opik, M., Daniell, T.J., Moora, M. & Zobel, M. 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbioogyl Ecology* 78: 103-115.

Diaz, S. & Cabido, M. 1997. Plant functional types and ecosystem function in relation to global change. *Journal of Vegetative Science* 8(4): 463-474.

Edathil, T.T., Manian, S. & Udaiyan, K. 1996. Interaction of multiple VAM fungal species on root colonization, plant growth and nutrient status of tomato seedlings (*Lycopersicon esculentum* Mill.). *Agriculture Ecosystem & Environment* 59: 63-68.

Giovannetti, M. & Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84: 489-500.

Grime, J., Mackey, J., Hillier, S. & Read, D. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328: 420-422.

Gustafson, D.J. & Casper, B.B. 2006. Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: Experimentally manipulating co-occurring Glomus species. *Plant Ecology* 183: 257-263.

Hart, M.M., Forsythe, J., Oshowski, B., Bücking, H., Jansa, J. & Kiers, E.T. 2013. Hiding in a crowd-does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis? *Symbiosis* 59: 47-56.

Jansa, J., Smith, F.A. & Smith, S.E. 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist* 177: 779-789.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* 3: 749-757.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M. & Bago, A. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333: 880-882.

Koide, R.T. 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. *New Phytologist* 147: 233-235.

Koide, R.T. & Mosse, B. 2004. A history of research on arbuscular mycorrhiza. *Mycorrhiza* 14: 145-163.

Kormanik, P.P., Bryan, W.C. & Schultz, R.C. 1980. Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. *Canadian Journal of Microbiology* 26: 536-538.

Krak, K., Janoušková, M., Caklová, P., Vosátka, M. & Štorchová, H. 2012. Intraradical dynamics of two coexisting isolates of the arbuscular mycorrhizal fungus Glomus intraradices sensu lato as estimated by real-time PCR of mitochondrial DNA. *Applied and Environmental Microbiology* 78: 3630-3637.

Lavorel, S., McIntyre, S., Landsberg, J. & Forbes, D. 1997. Plant functional classifications: From general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution* 12: 474-478.

Lekberg, Y., Gibbons, S.M., Rosendahl, S. & Ramsey, P.W. 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. *ISME Journal* 7: 1424-1433.

Likar, M., Hancevic, K., Radic, T. & Regvar, M. 2013. Distribution and diversity of arbuscular mycorrhizal fungi in grapevines from production vineyards along the eastern Adriatic coast. *Mycorrhiza* 23: 209-219.

Liu, C., Liu, Y., Guo, K., Wang, S. & Yang, Y. 2014. Concentrations and resorption patterns of 13 nutrients in different plant functional types in the karst region of south-western China. *Annals of Botany* 113: 873-885.

Lopez-Garcia, A., Palenzuela, J., Miguel Barea, J. & Azcon-Aguilar, C. 2014. Life-history strategies of arbuscular mycorrhizal fungi determine succession into roots of *Rosmarinus officinalis* L., a characteristic woody perennial plant species from Mediterranean ecosystems. *Plant and Soil* 379: 247-260.

Martinez-Garcia, L.B. & Pugnaire, F.I. 2011. Arbuscular mycorrhizal fungi host preference and site effects in two plant species in a semiarid environment. *Applied Soil Ecology* 48: 313-317.

Nelson, D. & Sommers, L.E. 1982. Total carbon, organic carbon, and organic matter. *Methods of Soil Analysis Part 2 Chemical and Microbiological Properties*. Location: Publisher. pp. 539-579.

Perez, M. & Urcelay, C. 2009. Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types. *Mycorrhiza* 19: 517-523.

Powell, J.R., Anderson, I.C. & Rillig, M.C. 2013. A new tool of the trade: Plant-trait based approaches in microbial ecology. *Plant and Soil* 365: 35-40.

Sanchez-Castro, I., Ferrol, N. & Barea, J.M. 2012. Analyzing the community composition of arbuscular mycorrhizal fungi colonizing the roots of representative shrubland species in a Mediterranean ecosystem. *Journal of Arid Environments* 80: 1-9.

Sanders, I.R. 2003. Preference, specificity and cheating in the arbuscular mycorrhizal symbiosis. *Trends in Plant Science* 8: 143-145.

Scheublin, T.R., Ridgway, K.P., Young, J.P.W. & van der Heijden, M.G.A. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology* 70: 6240-6246.

Smith, S.E. & Read, D.J. 2010. *Mycorrhizal Symbiosis*. New York: Academic Press.

Thonar, C., Schnepf, A., Frossard, E., Roose, T. & Jansa, J. 2011. Traits related to differences in function among three arbuscular mycorrhizal fungi. *Plant and Soil* 339: 231-245.

Urcelay, C. & Diaz, S. 2003. The mycorrhizal dependence of subordinates determines the effect of arbuscular mycorrhizal fungi on plant diversity. *Ecology Letters* 6: 388-391.

Urcelay, C., Díaz, S., Gurvich, D.E., Chapin Iii, F.S., Cuevas, E. & Domínguez, L.S. 2009. Mycorrhizal community resilience in response to experimental plant functional type removals in a woody ecosystem. *Journal of Ecology* 97: 1291-1301.

Van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A. & Gianinazzi-Pearson, V. 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology* 7: 879-887.

Vandenkoornhuyse, P., Ridgway, K.P., Watson, I.J., Fitter, A.H. & Young, J.P.W. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology* 12: 3085-3095.

Vogelsang, K.M., Reynolds, H.L. & Bever, J.D. 2006 Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist* 172: 554-562.

Wagg, C., Jansa, J., Schmid, B. & van der Heijden, M.G. 2011. Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecology Letters* 14: 1001-1009.

Wang, S.J., Liu, Q.M. & Zhang, D.F. 2004. Karst rocky desertification in southwestern China: Geomorphology, landuse, impact and rehabilitation. *Land Degradation & Development* 15: 115-121.

Wei, Y. 2012. Molecular diversity and distribution of arbuscular mycorrhizal fungi in karst ecosystem, Southwest China. *African Journal Biotechnology* 11: 14561-14568.

Yang, C., Hamel, C., Schellenberg, M.P., Perez, J.C. & Berbara, R.L. 2010. Diversity and functionality of arbuscular mycorrhizal fungi in three plant communities in semiarid Grasslands National Park, Canada. *Microbial Ecology* 59: 724-733.

Yang, H., Zang, Y., Yuan, Y., Tang, J. & Chen, X. 2012. Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: Evidence from ITS rDNA sequence metadata. *BMC Evolutionary Biology* 12: 50.

Zhang, Z., Zhang, J. & Huang, Y. 2014. Effects of arbuscular mycorrhizal fungi on the drought tolerance of *Cyclobalanopsis glauca* seedlings under greenhouse conditions. *New Forest* 45: 545-556.

Zhu, H., He, X., Wang, K., Su, Y. & Wu, J. 2012. Interactions of vegetation succession, soil bio-chemical properties and microbial communities in a Karst ecosystem. *European Journal of Soil Biology* 51: 1-7.

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