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Arbuscular mycorrhizal fungi differ in affecting the flowering of a host plant under two soil phosphorus conditions

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Abstract

Aims

Studies have showed that arbuscular mycorrhizal fungi (AMF) can greatly promote the growth of host plants, but how AMF affect flowering phenology of host plants is not well known. Here, we conducted a pot experiment to test whether life cycle and flowering phenology traits of host plant *Medicago truncatula* Gaertn can be altered by AMF under low and high soil phosphorus (P) levels.

Methods

The experiment was conducted in a greenhouse at Zhejiang University in China (120°19′E, 30°26′N) and had a completely randomized design with two factors: AMF treatments and soil P levels. Six AMF species (*Acaulospora scrobiculata*, As; *Gigaspora margarita*, Gma; *Funneliformis geosporum*, Fg; *Rhizophagus intraradices*, Ri; *Funneliformis mosseae*, Fmo and *Glomus tortuosum*, Gt.) were used, and two soil P levels (24.0 and 5.7 mg kg^{-1} Olsen-soluble P) were designed. The six AMF species were separately inoculated or in a mixture (Mix), and a non-AMF control (NAMF) was included. When plants began to flower, the number of flowers in each pot was recorded daily. During fruit ripening, the number of mature fruits was also recorded daily. After ~4 months, the biomass, biomass P content and AMF colonization of host plant were measured. Correlation between root colonization and first flowering time, or P content and first flowering time was analyzed.

Important Findings

Under the low P level, first flowering time negatively correlated with root colonization and biomass P. Only host plants with AMF species As, Fg, Ri, or Mix were able to complete their life cycle within 112 days after sowing. And treatment with AMF species Fg, Gt, or As resulted in two periods of rapid flower production while other fungi treatments resulted in only one within 112 days after sowing. The cumulative number of flowers produced and biomass P content were highest with species Fg. Host biomass allocation significantly differed depending on the species of AMF. Under both soil P levels, the host plant tended to allocate more biomass to fruits in the Mix treatment than in the other treatments. These results indicated that the effects of AMF on host flowering phenology and biomass allocation differed depending on AMF species and soil P levels.

Keywords: biomass allocation, flowering phenology, life cycle, *Medicago truncatula* Gaertn, Mycorrhizae

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INTRODUCTION

Flowering phenology is an important ecological trait for plants and their populations (Forrest and Miller-Rushing 2010). Although flowering phenology is genetically determined in general (Brachi *et al.* 2010), it can be changed by abiotic factors, such as photoperiod (Garner and Allard 1920), temperature (Aikawa *et al.* 2010), water availability (Borchert *et al.* 2004; Crimmins *et al.* 2013) and soil fertility (Gaur *et al.* 2000), as well as biotic factors, including pathogen infection (Korves and Bergelson 2003), herbivory (Brys *et al.* 2011) and soil microbes (Wagner *et al.* 2014).

The symbiosis between roots and arbuscular mycorrhizal fungi (AMF) in soil can help host plants acquire nutrients

and water from soil and can therefore enhance plant growth (Newsham *et al.* 1995; Smith and Smith 2012). Whether AMF can alter host plant flowering phenology and fruiting, however, has been debated (Koide 2010). Some studies have reported that AMF caused earlier flowering, increased the total duration of flowering and increased flower number (Conversa *et al.* 2013; Derelle *et al.* 2015; Gaur 2000; Lu and Koide 1994; Trimble *et al.* 1995; Vaingankar and Rodrigues 2014). Other experiments, in contrast, indicated that AMF did not affect flowering time or flower number (Bryla and Koide 1990; Nakatsubo 1997; Philip *et al.* 2001). AMF-enhanced fruit production (e.g. resulted in larger fruits and greater numbers of fruits) in most studies (Conversa *et al.* 2013; Derelle *et al.* 2015; Stanley *et al.* 1993; Trimble *et al.* 1995) and failed to increase fruit biomass (or even decreased fruit biomass) in only a few cases (Lu and Koide 1991; Koide and Lu 1992).

Although most AMF exhibiting a low level of host specificity (specificity means a restricted host range usually) has been documented for some host-fungal pairings (Bever 2002; Bidartondo *et al.* 2002; Kapulnik 2010; Martinez-Garcia and Pugnaire 2011; Zhang *et al.* 2010), in some cases, the effects of a specific AMF may also differ among hosts. For example, the effects of the AMF *Glomus etunicatum* Becker and Gerd. on host flowering characteristics differed among four *Lycopersicon esculentum* genotypes (Bryla and Koide 1990). At the same time, a host plant may respond differently to different AMF species (Helgason *et al.* 2002; Zhang *et al.* 2010). For example, host *Geranium sylvaticum* L. flowered 20 days earlier when inoculated with *Glomus hoi* than with *Claroideoglomus claroideum* (Varga *et al.* 2013). The sporulation rates of AMF were also found to be host-dependent in a laboratory system (Bever *et al.* 1996). It is not well known, however, whether the effects on the reproductive traits of host plants differ depending on AMF species.

Soil nutrients can affect flowering phenology (Achor *et al.* 1997; Salazar-Garcia and Lovatt 1998). Soil phosphorus (P), for example, can affect flowering duration, numbers of flowers and fruit set, and seed traits (Achor *et al.* 1997; Landis and Fraser 2008; Salazar-Garcia and Lovatt 1998). Because AMF can enhance the uptake of P and other nutrients by host plants (Smith and Smith 2012), AMF and soil P level may interactively affect host flowering phenology and biomass allocation. In the current study, we hypothesize that AMF species differ in helping host to acquire soil P, and thus differ in affecting the flowering phenology of host plants. We use *Medicago truncatula* Gaertn as host plant and six AMF species to test the hypothesis in this study.

MATERIALS AND METHODS

AMF, host plant and soil

The six AMF used in this experiment were *Acaulospora scrobiculata* (As, BGC, HK02A), *Gigaspora margarita* (Gma, BGC, ZJ03), *Funneliformis geosporum* (Fg, BGC, GZ01), *Rhizophagus intraradices* (Ri, BGC, BJ09), *Funneliformis mosseae* (Fmo, BGC, XJ01) and *Glomus tortuosum* (Gt, BGC, NM03A). The original AMF isolates were provided by Glomales Germplasm Bank in China (Institute of Plant Nutrient & Resources, Beijing Academy of Agriculture & Forestry Sciences). The inoculum was prepared by infecting sterilized sand in pots with each pure AMF spores and then growing two host plants (maize and *Kummerowia striata*) and pots were maintained in a greenhouse with watered properly every day. After 5 months, the inoculum of each AMF species, which consisted of spores, extraradical mycelium and mycorrhizal roots, was harvested. Then, we used molecular tool and morphological method to check the purity of each inoculum. Before the inoculum for each AMF species was used in experiments, it was stored for several months at ambient air temperature to break the dormancy of the spores (Zhang *et al.* 2010).

The host plant used in the experiment was *M. truncatula* Gaertn. 'Jemalong' (line A17), which was provided by the Rujin Chen laboratory, Samuel Roberts Noble Foundation, USA. *M. truncatula* (Medicago) is a well-established model for studying symbiotic associations because it has a high colonization rate and a short seed-to-seed generation time (of ~3 months in long day conditions; Barker *et al.* 1990). *M. truncatula* is also widely used to study P-deficiency tolerance in plants (Jain *et al.* 2007).

The soil used in the experiment was a sandy loam obtained from a rice field at the Experimental Farm of Zhejiang University in Changxing County (119°91′E, 30°01′N). Soil total P was 236.75 mg kg^{-1}, total N was 1323.75 mg kg^{-1}, Olsen-soluble P was 5.7 mg kg^{-1} and the pH was ~7. The soil was autoclaved twice at 121°C for 30 min each time before it was placed in 3-l plastic pots, with 2 kg of soil per pot.

Experimental design

The experiment was conducted in a greenhouse at Zhejiang University in China (120°19′E, 30°26′N) and had a completely randomized design with two factors: AMF treatments and soil P levels. There were eight AMF treatments: six single-species treatments, one mixed-species treatment (Mix) and a non-AMF control (NAMF). Each pot was infected with inoculum which was mixed throughout the pot soil before the experiment began. Inoculum comprised of sand: soil mixture containing colonized roots, hyphae and spores. Spore numbers in the 50-g inoculum of species were 584 in As, 1137 in Gma, 1750 in Ri, 342 in Fg, 1046 in Fmo and 498 in Gt, respectively. For the treatment of AMF species monoculture, 33 g of As, 17 g of Gma, 57 g of Fg, 11 g of Ri, 19 g of Fmo and 40 g of Gt were added into the corresponding pots to reach an equal number (400 spores) in each pot. For the species mixture, each pot also contained 400 spores (67 spores per fungus species). For non-AMF treatment, each pot was not inoculated but was treated with 50 ml of filtrate from the mixed AMF inoculum. And all pots were treated with 50 ml of filtrate from the field soil.

During the experiment, we did not use any exact rhizobium inoculum in all treatments. There were two levels of Olsen-soluble P: low P (5.7 mg kg^{-1}) and high P (24 mg kg^{-1}; Sulieman *et al.* 2013). The low P level was obtained

by using the field soil without adding P. The high P level was obtained by adding 0.14 g of calcium superphosphate to each pot before the experiment began. The soil pH was adjusted primarily after adding calcium superphosphate. Before seeds were sown in the pots, each pot was treated with 50 ml of filtrate from the mixed AMF inoculum and 50 ml of filtrate from the field soil (Zhang *et al.* 2010). These filtrates, which did not contain AMF spores (filter pore size = 20 μm), were added to obtain a similar microbial community (minus AMF) in each pot. Each of the 16 combinations of AMF treatment and P level was represented by five replicate pots.

Before germination, the *M. truncatula* seeds were soaked for 7 min in 98% concentrated sulfuric acid and rinsed three times with sterile water; they were then placed in sodium hypochlorite for 30 s and rinsed three times with sterile water. Seeds were germinated in the dark on moist filter paper in Petri dishes at 20°C. After 18 h, five germinated seeds were planted in each pot. Plants were grown in the greenhouse with ambient air temperature (mean 25.6°C). Pots were watered to field capacity every day. After 4 weeks, the seedlings were thinned to one per pot. And up to harvesting time, all the plant survived.

Measurements

When plants began to flower, the number of flowers in each pot was recorded daily. During fruit ripening, the number of mature fruits was also recorded daily. After ~4 months, shoots were cut at the soil surface, weighed, oven-dried at 65°C, and then weighed again. The roots were washed free of soil, air dried, and weighed. P content of each sample was extracted by using H_2SO_4–H_2O_2 method (Avio *et al.* 2006). P in the extraction was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES; Optima 3300 DV, PerkinElmer). The fine roots were cleared in 10% (w/v) KOH at 70°C for 5 min and then soaked in 3% HCL (v/v) for 3 min. The roots were rinsed three times with water and then stained with acid fuchsin (0.1% w/v) for 30 min. Mycorrhizal structures were observed (arbuscules, vesicles or hyphae) at 100× magnification with the aid of a microscope. The fractional root length colonized by AM fungi was assessed using the McGonigle *et al.*'s (1990) approach, observing 200 magnified root intersections per sample.

Statistical analysis

Analysis of variance (ANOVA) was performed with SPSS 16.0 software. For colonization rate, biomass P, total flowers and biomass (shoot, root, fruit and total), a two-way ANOVA was conducted with AMF treatments and soil P levels as factors. Means were separated with multiple comparisons (post-hoc Tukey HSD, $P < 0.05$); this was done separately for low and high P levels. The correlation (Pearson) of the flowering time with root colonization, or with biomass P was analyzed separately for low and high P level treatments by using the data of all AMF treatments.

RESULTS

Colonization

AMF colonization was significantly affected by AMF treatment ($F_{7,64}$ = 15.768, $P < 0.05$), soil P level ($F_{1,64}$ = 6.375, $P < 0.05$) and their interaction ($F_{7,64}$ = 5.958, $P < 0.05$; Fig. 1). Colonization was generally higher under the high than under the low P level except for the Mix treatment (Fig. 1). Under the low P level and among all AMF treatments, colonization was highest ($P < 0.05$) for Mix (Fig. 1). For treatments with a single AMF, colonization was highest under the low P level for Gma and was highest ($P < 0.05$) under the high P level for Gma and Fmo (Fig. 1).

Life cycle completion

The ability of the host to complete its life cycle was affected by AMF treatments and soil P levels (Fig. 2). Under the low P level, only host plants treated with As, Fg, Ri or Mix completed their life cycles; host plants treated with Gma, Fmo or Gt did not fruit and host plants treated with NAMF did not flower or fruit (Fig. 2). Under the high P level, host plants treated with As, Fg, Ri, Gma, Gt or Mix completed their life cycles (Fig. 2), but host plants treated with Fmo or NAMF did not produce any fruit (Fig. 2).

Flowering pattern

The timing of flowering and the cumulative number of flowers produced differed among AMF treatments and soil P levels (Fig. 3). Host plants flowered from 10 June to 28 July under

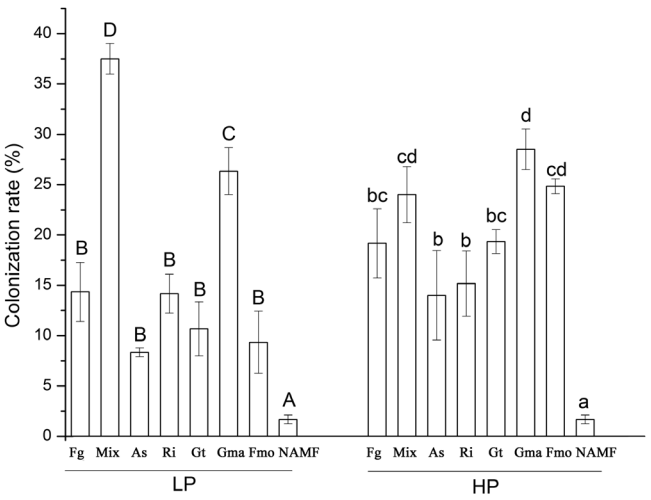

Figure 1: colonization of host plant roots by AMF as affected by eight AMF treatments and two soil P levels. Values are means (±SE, n = 5). Bars topped by the same letter do not differ significantly at $P \leq 0.05$ by multiple comparisons test [uppercase letters (for the low P level) or lowercase letters (for the high P level)]. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; HP = high phosphorus; LP = low phosphorus; SE = standard error.

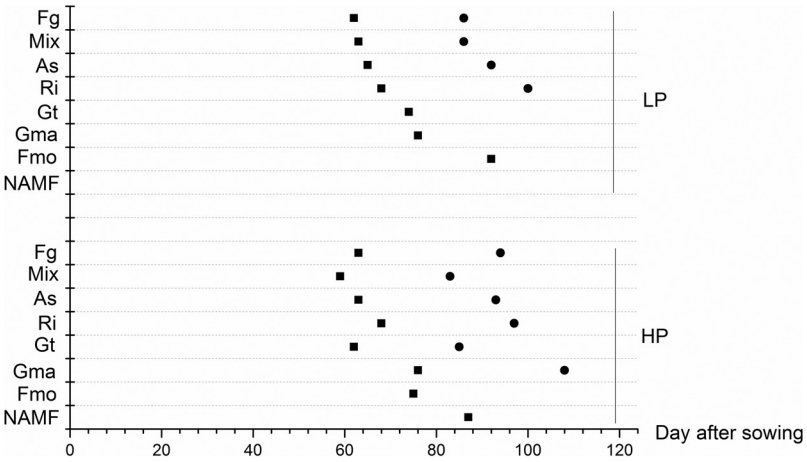

Figure 2: host plant life cycle completion as affected by eight AMF treatments and two soil P levels. Square represents the day of first flower arising and circle represents the day of first fruit mature arising each treatment. All treatments were harvested at 112 days after sowing. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; LP = low phosphorus. HP = high phosphorus.

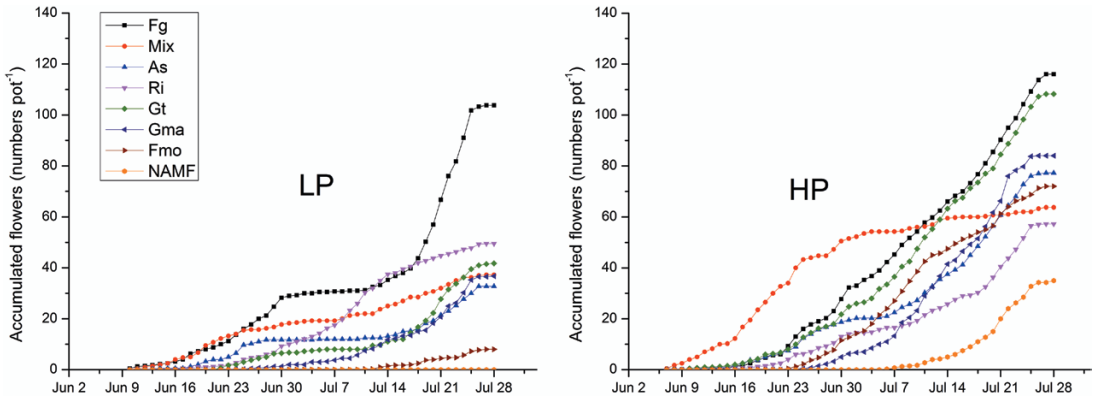

Figure 3: the flowering pattern of host plants as affected by eight AMF treatments and two soil P levels. The points show the means ($n = 4$) of accumulated flower numbers in each treatment. Flowering occurred from June 10 to July 28 with LP and from June 7 to July 28 with HP. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; LP = low phosphorus. HP = high phosphorus.

the low P treatment and from 7 June to 28 July under the high P treatment (Fig. 3). Under the low P level, host plants treated with Fg, Gt or As had two stages of increased flowering; those treated with Gma, Fmo, Ri or Mix had only one stage of increased flowering and no flowering occurred with NAMF (Fig. 3). Under the high P level, host plants treated with As or Ri had two stages of increased flowering, while host plants in the other treatments had only one stage of increased flowering (Fig. 3).

The total flowers (all flowers that produced by host plants in their life cycle) were significantly affected by AMF treatment ($F_{7,48} = 5.871$, $P < 0.05$) and soil P level ($F_{1,48} = 25.178$, $P < 0.05$) but not by their interaction ($F_{7,48} = 1.126$, $P > 0.05$; Fig. 4). The total flowers were greater under the high P level than under the low P level (Fig. 4). Under the low P level, the total number of flowers was highest ($P < 0.05$) for host plants treated with Fg (Fig. 4). Under the high P level, the total number of flowers was greater ($P < 0.05$) for plants treated with Fg than for plants treated with Ri, NAMF and Mix, and did not differ between plants treated with Fmo, Gt, Gma and As (Fig. 4).

Biomass allocation

Total biomass was significantly affected by AMF treatment ($F_{7,64} = 10.117$, $P < 0.05$) and soil P level ($F_{1,64} = 14.696$, $P < 0.05$) but not by their interaction ($F_{7,64} = 0.650$, $P > 0.05$). For all AMF treatments, total biomass was greater under the high than the low P level (Fig. 5d). Under both low and high P levels, host plants with Fg tended to have the largest ($P < 0.05$) total biomass (Fig. 5d).

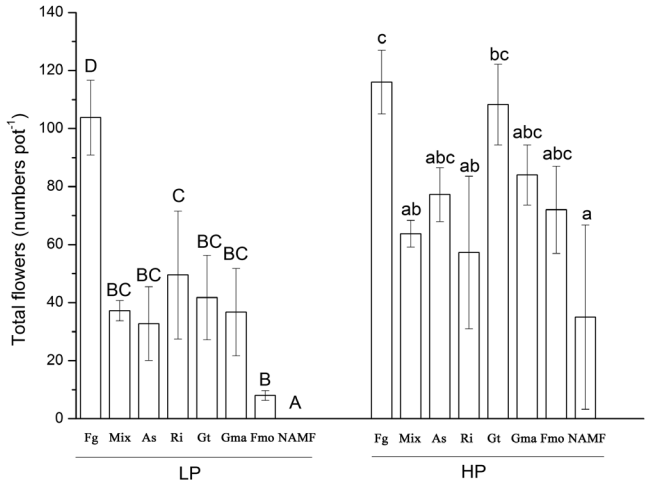

Figure 4: the total flower numbers of host plants as affected by eight AMF treatments and two soil P levels. Values are means (+SE, $n = 4$). Within each P level, bars topped by the same letter do not differ significantly at $P \leq 0.05$ by multiple comparisons test [uppercase letters (for the low P level) or lowercase letters (for the high P level)]. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; LP: low phosphorus. HP = high phosphorus; SE = standard error.

Soil P level and AMF treatment also significantly affected the allocation of biomass to shoots ($F_{1,64} = 11.652$, $P < 0.05$; $F_{7,64} = 9.755$, $P < 0.05$), roots ($F_{1,64} = 14.491$, $P < 0.05$; $F_{7,64} = 8.280$, $P < 0.05$) and fruit ($F_{1,64} = 9.963$, $P < 0.05$; $F_{7,64} = 13.375$, $P < 0.05$; Fig. 5a–c). And their interaction did not significantly affect the allocation of biomass to shoots ($F_{7,64} = 0.730$, $P > 0.05$), roots ($F_{7,64} = 0.742$, $P > 0.05$) and fruit ($F_{7,64} = 2.452$, $P > 0.05$). Under both P levels, shoot and root biomass was highest ($P < 0.05$) with Fg (Fig. 5a and b). Under both P levels, fruit biomass tended to be highest ($P < 0.05$) with Mix (Fig. 5c).

Biomass P

Biomass P (the total P content of host plant each pot) was significantly affected by AMF treatment ($F_{7,64} = 8.191$, $P < 0.05$) and soil P level ($F_{1,64} = 23.359$, $P < 0.05$) but not by their interaction ($F_{7,64} = 0.958$, $P > 0.05$). For all AMF treatments, biomass P was greater under the high P level than the low P level (Fig. 6). Under the low P level, the biomass P was highest ($P < 0.05$) for host plants treated with Fg (Fig. 6). Under the high P level, the biomass P was greater ($P < 0.05$) for plants treated with Fg than for plants treated with Fmo and NAMF, and did not differ between plants treated with other AMF species (Fig. 6).

Correlations between root colonization or P content and flowering time

Both root colonization and biomass P significantly correlated with the flowering time under low soil P level (Fig. 7a; for root colonization, Pearson correlation −0.338, $P < 0.05$; for biomass P, Pearson correlation −0.628, $P < 0.05$). And both root colonization and biomass P significantly correlated with the flowering time under high soil P level (Fig. 7a; for root colonization, Pearson correlation 0.429, $P < 0.05$; for biomass P, Pearson correlation −0.523, $P < 0.05$).

DISCUSSION

Our experiment indicated that the effects of AMF on host plant life cycle completion depended on the AMF species and on the soil P levels (Fig. 2). One possible explanation for different effects of AMF species on host life cycle completion could involve the rapidity with which AMF colonized roots and form symbiotic relationships. Hart (2002), who investigated 21 AMF isolates from three families (*Acaulosporaceae*, *Gigasporacese* and *Glomacese*), found that 12 *Glomaceae* isolates had colonized roots by week four, while some *Gigasporaceae* and *Acaulosporaceae* isolates did not colonize roots until weeks 6–8. Another possible explanation for AMF species differed in affecting life cycle completion may simply be due to the effects of different AMF species on P uptake of host plants. Some AMF promote host growth by enhancing nutrient uptake, while others do so by promoting tolerance to pathogens, drought or other stresses (Newsham *et al.* 1995). This may explain the reason why host plant with Gma and Gt had high biomass P but did not complete their life cycle under the low P level.

Our experiment also indicated that both AMF species and soil P levels affected flowering pattern and the first flower time (Fig. 6). Correlation analysis further showed that the first flowering time negatively correlated with root colonization among the seven AMF treatments under low soil P level, but not under high soil P level (Fig. 7a and b), suggesting that host plants would flower early with a highly colonized AMF species when there was a P limitation in soil. Under low P soil level, host plant depends on AMF for P, while host plant can have enough P by roots themselves under high P soil level. As higher root colonization needs higher carbon supply (Fitter 2006), carbon for the host plant growth should be lower under low P soil level. Because better plant growth can advance flowering time (Sun *et al.* 2008), the first flowering time may later under low P soil level than under high soil level.

Significantly negative correlations between the first flowering time and biomass P under both soil low and high P levels were also found in our study (Fig. 7a and b). These evidences suggest that P uptake may be the key factor that different AMF species affect plant flowering. As P is necessary for host plants growth, increases in P uptake by roots or AMF can promote plant growth. Given that increase in leaf and root growth, an in nutrient uptake can advance flowering time (Sun *et al.* 2008), AMF may alter flowering pattern by affecting the vegetative growth and nutrient uptake of the host. Plants may begin to flower when a critical amount of carbohydrate is redirected from vegetative growth to

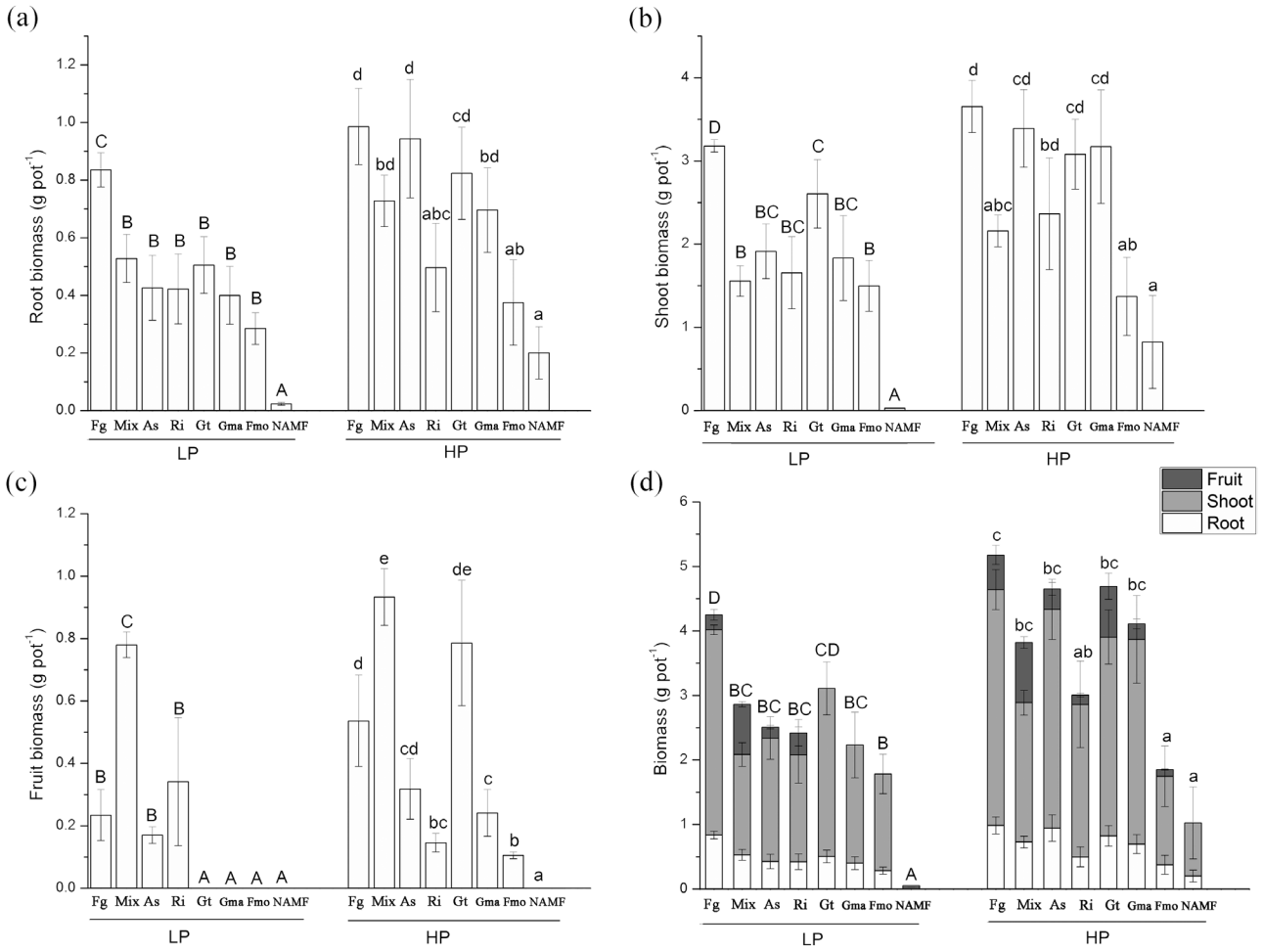

Figure 5: root (**a**), shoot (**b**), fruit (**c**) and total biomass (**d**) of host plants as affected by eight AMF treatments and two soil P levels. Values are means (±SE, $n = 5$). Total biomass is the sum of fruit, shoot and root biomass. Within each P level (except fruit), bars (represent total biomass) topped by the same letter do not differ significantly at $P \leq 0.05$ by multiple comparisons test [uppercase letters (for the low P level) or lowercase letters (for the high P level)]. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; LP: low phosphorus. HP = high phosphorus; SE = standard error.

reproductive growth (Lapointe 2001). Thus, species of AMF may have different effects on host flowering depending on how they affect the host's accumulation of photosynthates and nutrient (e.g. P) uptake. It was consistent with our observation that host plant with higher AMF colonization flowered early and had the highest total biomass and biomass P under the low P level (Figs. 4, 5d and 6).

The various flowering patterns (Fig. 6) may be due to the trade-off between the host's gain in nutrients and the host's carbon cost resulting from the symbiosis, if this trade-off differed among the AMF species. Fitter (2006) proposed that host plants would only allocate substantial amounts of carbon to AMF when the plants received P from the fungi. In our experiment, we found the biomass P, total flowers and total biomass were highest ($P < 0.05$) for host plants treated with Fg under the low P level (Figs. 3, 5d and 6). This means that the trade-off with Fg was best for host plant on growth and flowering in our study. Some species of AMF, however, may capture carbon from the host while supplying little or no P (Smith *et al.* 2009), resulting in a decrease in host plant growth and reproduction. This was similar to the results that host plant treated with Gma, which had a medium biomass P, highest colonization rate, only one stage of increased flowering and lower total biomass under low P level. (Figs. 1, 3, 5d and 6) This evidence could be that the trade-off with Gma was better for AMF because of more carbon divided to AMF by host plant. Similarly, using a ^{32}P isotope tracer technique, Pearson and Jakobsen (1993) found that *Glomus caledonium* provided 100% of the host's P requirement, while *Glomus invermaium* provided only 20% and *Scutellospora calospora* provided <10%.

The phenotypic and functional properties of the extraradical mycorrhizal mycelium differ among AMF species (Govindarajulu *et al.* 2005; Harrison and van Buuren 1995). This could be the key reason why P uptake by host plants can differ depending on AMF species (Ames *et al.* 1983; Hodge *et al.* 2001; Jakobsen *et al.* 1992; Mäder *et al.* 2000; Smith *et al.* 2004). *Glomus mosseae* and *Rhizophagus intraradices* differ in the extent and the interconnectedness of the extraradical mycelium and differ in total hyphal length, hyphal density and hyphal length per mm of colonized root (Avio *et al.* 2006). These differences were positively correlated with the changes in total shoot biomass and P content of *Medicago sativa* in response to AMF colonization (Avio *et al.* 2006).

Our study also found that AMF can mediate biomass allocation of the host (Fig. 5). Although increases in plant size often result in greater fecundity, the effects are not consistent (Jones and Smith 2004). Our results similarly indicated that the AMF treatment resulting in the greatest shoot biomass did not result in the greatest fruit biomass (Fig. 5). Van der Heijden *et al.* (1998) and Klironomos (2003) also showed that different species of AMF had different effects on the growth of the same species of host plant. Grace *et al.* (2009) documented large growth depressions in barley caused by *Funneliformis geosporum* and *Rhizophagus intraradices*. In the current study, host had highest total biomass, total flower number and biomass P under low soil P level (Figs. 4–6). In other words, the fitness of *M. truncatula* was increased more by Fg than by the other five AMF in this study.

In our study, however, high AMF colonization may be not always with high growth and reproduction. For example, the colonization when inoculated Gma and Mix were higher than other species such as Gg, the effects of Gma on the total flower numbers (Fig. 4), root biomass (Fig. 5a), shoot biomass (Fig. 5b) and fruit biomass (Fig. 5c) were not strong and even lower than Gg. Some other studies also reported AMF colonization was not always consist with the host plant growth and reproduction (Fitter 2006). This evidence could be due to that the effects of AMF and host are not equal to each other. There may be a 'tricker' AMF species which obtained much carbon from host plant but gave litter P return (Denison 2011).

Summary, our results showed that AMF species differed in affecting the flowering phenology and reproduction growth of host plants. These effects were due to that AMF species differed in enhancing P uptake and altering the biomass allocation. These results suggest that the effect of AMF on host plant flowering depends on the species of AMF and the soil P level.

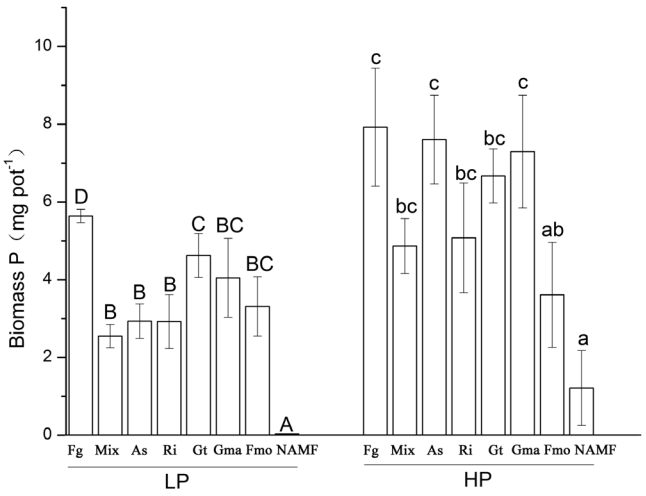

Figure 6: the biomass P of host plants as affected by eight AMF treatments and two soil P levels. Values are means (+SE, n = 5). Within each P level, bars topped by the same letter do not differ significantly at $P \leq 0.05$ by multiple comparisons test [uppercase letters (for the low P level) or lowercase letters (for the high P level)]. As: *Acaulospora scrobiculata*; Gma: *Gigaspora margarita*; Fg: *Funneliformis geosporum*; Ri: *Rhizophagus intraradices*; Fmo: *Funneliformis mosseae*; Gt: *Glomus tortuosum*, NAMF: non-AMF control; Mix: mixed AMF species. Abbreviations: AMF = arbuscular mycorrhizal fungi; LP: low phosphorus. HP = high phosphorus; SE = standard error.

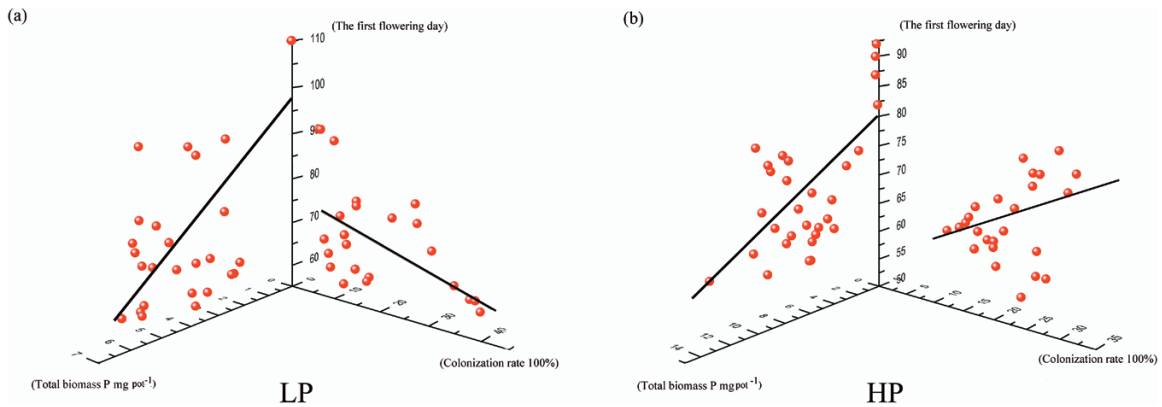

Figure 7: correlations between root colonization rate and the first flowering day and between biomass P and the first flowering day under low (**a**) and high (**b**) soil P level. Abbreviations: HP = high phosphorus; LP = low phosphorus.

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