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Arbuscular mycorrhizal fungus identity and diversity influence subtropical tree competition

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi can influence plant nutrient uptake and, therefore, may alter interspecific plant competition. However, the role of AM fungi in subtropical tree competition is poorly understood. In this study, we investigated the effects of AM fungus identity (four species) and diversity (a mixture of the same four species) on the competitive relationships between seedlings of a pioneer tree *Rhus chinensis* and a late-pioneer tree *Celtis sinensis*, and between *R. chinensis* and a mid-successional tree *Cinnamomum camphora*. In seedlings, AM fungi significantly promoted a competitive advantage of *R. chinensis* over both *Ce. sinensis* and *Ci. camphora*. Furthermore, the extent to which AM fungi affected interspecific plant competition outcomes was dependent on AM fungus identity, and the effect of AM fungus diversity on interspecific competition outcomes may derive from the most beneficial AM fungal species.

1. Introduction

Plant competition is considered to be one of the main biotic factors shaping plant communities (Mangla et al. 2011; Martorell and Freckleton, 2014). The drivers of plant competition have long been debated, but plant–soil microbe feedbacks have been shown to be an important mechanism driving the assembly of plant communities (Kardol et al. 2007; Mangan et al. 2010; Bever et al. 2012). Of the soil microorganisms, arbuscular mycorrhizal (AM) fungi form symbiotic associations with most terrestrial plant species (Smith and Read, 2008). In AM associations, fungi exchange soil-derived nutrients for carbohydrates from plants and are beneficial to their host plant's performance (Vannette and Hunter, 2011; Doubková et al. 2013). Therefore, AM fungi may play important ecological roles in inter- or intra-specific interactions and maintenance of plant community diversity in ecosystems (Read, 1997; Simard et al. 2002).

Previous studies have shown that AM fungal presence alters plant competition (West, 1996; Facelli et al. 2010; Gross et al. 2010; Rinaudo et al. 2010; Daisog et al. 2012; Sabais et al. 2012; Mariotte et al. 2013; Birhane et al. 2014). In microcosm studies, AM fungus identity has been shown to influence plant community composition (Hart et al. 2003; Stampe and Daehler, 2003; Vogelsang et al. 2006). However, the direct effects of AM fungus identity on plant competition have been less well documented (Scheublin et al. 2007; Rinaudo et al. 2010; Wagg et al. 2011). For example, AM fungal species differed in their ability to influence plant competition between *Lolium multiflorum* and *Trifolium pratense* in a Switzerland grassland (Wagg et al. 2011), between a legume and a grass or a forb in a Netherlands dry dune grassland (Scheublin et al. 2007), between genotypes of tomato (Facelli et al. 2010), and between genotypes of *Medicago truncatula* (Facelli et al. 2014). However, another study recorded similar effects of different AM fungal species on the interspecific competition between sunflower (*Helianthus annuus*) and six weed species (Rinaudo et al. 2010), suggesting that some AM fungi may have similar physiological effects on plant competition.

The relationship between biodiversity and ecosystem functioning has received considerable attention in previous studies (Loreau and Hector, 2001; Cardinale et al. 2006). In natural ecosystems, AM fungi do not occur as single species, but instead there is a high diversity of AM fungi in soil (Zhang et al. 2004; Davison et al. 2012) or in a root system (Öpik et al. 2013). Previous studies have shown that the composition and diversity of AM fungal

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communities influences plant diversity, productivity, and community composition (van der Heijden et al. 1998; Vogelsang et al. 2006), as a result of the added beneficial effect of each single AM fungal species facilitating more efficient exploitation of soil nutrients and better use of the resources available in the system (Drew et al. 2003). However, few studies have investigated the direct effect of AM fungus diversity on plant competition (Facelli et al. 2010; Rinaudo et al. 2010; Wagg et al. 2011). For example, Wagg et al. (2011) studied the effect of AM fungus diversity on the competitive interaction between *L. multiflorum* and *T. pratense* and found that AM fungus diversity (a mixture of four species) generally enhanced the competitive ability of *T. pratense* more than single AM fungal species in low-sand soil. However, no complementary effect of AM fungus diversity was observed in competition between sunflower and weeds (Rinaudo et al. 2010) or between competing genotypes of tomato (Facelli et al. 2010).

Most studies of the impact of AM fungi on plant competition have focused on herbaceous plants (Scheublin et al. 2007; Gross et al. 2010; Wagg et al. 2011; Sabais et al. 2012), and only a few have considered tropical trees (Guadarrama et al. 2004; Danieli-Silva et al. 2010). It is likely that AM fungi play important roles in determining plant competition in other plant community types as well, but studies from a variety of systems are necessary to determine the general importance of AM fungi (Hart et al. 2003). Tropical and subtropical forest ecosystems support a high diversity of woody plant species (Wills et al. 2006; Bruelheide et al. 2011) and AM fungal species (Zhang et al. 2004; Öpik et al. 2013). Previous studies have shown inconsistent shifts in mycorrhizal responsiveness associated with different plant successional stages (Janos, 1980; Siqueira et al. 1998; Zangaro et al. 2003; Middleton and Bever, 2012; Koziol and Bever, 2015). For example, the AM responsiveness of grasses and shrubs increased from pioneer to early and late successional stages (Middleton and Bever, 2012; Koziol and Bever, 2015), whereas woody plant responsiveness to AM fungi decreased from pioneer to early, late and climax species during succession in tropical ecosystems (Janos, 1980; Siqueira et al. 1998; Kiers et al. 2000; Zangaro et al. 2000, 2003). Thus, we propose that AM responsiveness of woody plants may also decrease as secondary succession progresses in subtropical forest ecosystems. However, a major unknown is the effect of AM fungi on tree species competition from different successional stages in subtropical forests.

To better understand the effects of AM fungus identity and diversity on interspecific plant competition in relation to succession, we studied competition between seedlings of a pioneer tree *Rhus chinensis* and a late-pioneer tree *Celtis sinensis* or a mid-successional tree *Cinnamomum camphora* in a subtropical forest. These three plant species were cultivated in monocultures and mixtures uninoculated or inoculated with AM fungi (single species, mixture of four species) in a pot experiment. In this study, we hypothesized that: (1) AM fungi would promote a competitive advantage of *R. chinensis* over *Ce. sinensis* or *Ci. camphora*, because the effect of AM fungi on seedling growth has been shown to decrease from pioneer to early, late and climax woody plant species (Siqueira et al. 1998; Kiers et al. 2000; Zangaro et al. 2000, 2003); (2) the outcomes of interspecific competition would be dependent on AM fungus identity; and (3) AM fungus diversity could enhance the competitiveness of *R. chinensis* more effectively than single AM fungal species.

2. Materials and methods

2.1. Plants and AM fungal inocula

In this study, we selected three tree species: *R. chinensis*, *Ce. sinensis* and *Ci. camphora*, which represent a pioneer, late-pioneer and mid-successional species in a Chinese secondary subtropical forest, respectively. These three plant species are relatively common and coexist in different successional stages in this forest ecosystem. During 2011, seeds of these three plant species were collected from a subtropical forest in the Gutianshan National Nature Reserve (GNNR), in southeast China (29°15′6″–29°15′21″ N, 118°07′01″–118°07′24″ E). The site has an annual mean temperature of 15.38 °C and annual mean precipitation of 1964 mm. Plant seeds were stored in moist vermiculite at 4 °C for 20 weeks until used in the experiment. The top soil (20 cm deep) was collected from the forest in the GNNR, and sieved through a 1 cm mesh to remove large stones and root fragments. Soil chemical analysis showed a pH of 4.6, 43.6 g kg^{-1} total carbon, 2.2 g kg^{-1} total nitrogen (N), 0.1 g kg^{-1} total phosphorus (P), 1.2 mg kg^{-1} Mehlich-3 extractable P, 34.9 mg kg^{-1} available N. Subsequently, the soil was mixed with sand and vermiculite (1:1:1 by volume), autoclaved for 2 h at 120 °C and used as the growing medium in the following experiment. Three weeks prior to the experiment, seeds were surface sterilized twice with 0.5% NaOCl for 5 min and rinsed five times with distilled water, and then sown individually in nursery flats containing autoclaved substrate to germinate.

AM fungal species *Diversispora eburnea* BGC HK02C, *Claroideoglomus lamellosum* BGC NM03E, *Funneliformis mosseae* BGC YN05 and *Diversispora* sp. BGC BJ04B used in the present experiment were supplied by the Bank of Glomeromycota in China (BGC) in Beijing Academy of Agriculture and Forestry, China. AM fungus *D. eburnea* BGC HK02C was isolated from rhizosphere soil of *Lagerstroemia indica* in Hong Kong, *C. lamellosum* BGC NM03E from rhizosphere soil of *Medicago sativa* in Inner Mongolia, *F. mosseae* BGC YN05 from rhizosphere soil of *Colocasia esculenta* in Yunnan Province, and *Diversispora* sp. BGC BJ04B from rhizosphere soil of *Amygdalus persica* in Beijing, China. These four AM fungal species were identified based on morphological characteristics and 18S rDNA sequence analysis, and these sequences have been deposited in GenBank (accession number KT152856 for *C. lamellosum*, KT152857 for *F. mosseae*, KT152858 for *D. eburnea* and JF439136 for *Diversispora* sp.). Inocula of these AM fungal species were multiplied by co-culture with *Zea mays* in the mixed substratum described above after growing for 6 months in a greenhouse. To eliminate potential contamination, the aerial part of the plant and top 2 cm of the sand substratum were discarded. We extracted AM fungal spores from the substratum using sucrose gradient centrifugation (Morton, 1995) and counted the number of AM fungal spores under a stereomicroscope before AM fungal inoculation.

2.2. Experimental design

A pot experiment was carried out in a greenhouse for 6 months from 20 April to 20 October 2012. The experiment was laid out according to a randomized complete block design with two factors. One factor, AM fungi, contained six treatments: *D. eburnea*, *C. lamellosum*, *F. mosseae*, *Diversispora* sp., a 1:1:1:1 mixture of these four AM fungal species, and a non-mycorrhizal control. The second factor, plant competition, involved five treatments: *R. chinensis* monoculture, *Ce. sinensis* monoculture, *Ci. camphora* monoculture, *R. chinensis* + *Ce. sinensis* mixture, and *R. chinensis* + *Ci. camphora* mixture. There were four replicates (blocks) for each of the 30 treatment combinations, resulting in a total of 120 pots (13 cm diameter and 15 cm height, containing 1.15 kg autoclaved mixed substrate). In each pot, we added *ca.* 1000 spores in 100 ml sterilized water for each single AM fungal treatment, *ca.* 250 spores in 25 ml sterilized water for each AM fungal species to give a total of *ca.* 1000 spores for the four-species AM fungal mix (1:1:1:1) treatment, and 100 ml sterilized water (no AM fungal spores) for the control treatment. In all cases the liquid was mixed throughout