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Changes in Arbuscular Mycorrhizal Fungus Community Along an Exotic Plant *Eupatorium adenophorum* Invasion in a Chinese Secondary Forest[§]

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Knowledge of the changes in arbuscular mycorrhizal (AM) fungi is fundamental for understanding the success of exotic plant invasions in natural ecosystems. In this study, AM fungal colonization and spore community were examined along an invasive gradient of the exotic plant *Eupatorium adenophorum* in a secondary forest in southwestern China. With increasing *E. adenophorum* invasion, the density of arbuscules in the roots of *E. adenophorum* significantly increased, but the AM root colonization rate and the densities of vesicles and hyphal coils in roots of *E. adenophorum* were not significantly different. A total of 29 AM fungi belonging to nine genera were identified based on spore morphology. *Claroideoglomus etunicatum*, *Funneliformis geosporus*, and *Glomus aggregatum* were the most common AM fungal species. The *E. adenophorum* invasion significantly decreased the AM fungal spore density in the soil. Furthermore, with increasing of *E. adenophorum* invasion the spore densities of *C. etunicatum*, *G. aggregatum*, and *G. arenarium* significantly decreased, whereas *F. geosporus* significantly increased. Non-metric multidimensional scaling demonstrated that the AM fungus community composition was significantly different (P=0.003) in the different invasive levels of *E. adenophorum*, and significantly correlated with plant species richness, soil total P, and soil NO_3^--N. The results suggest that the alteration in AM fungus community might be caused by *E. adenophorum* invasion via changing the local plant community and soil properties in a Chinese secondary forest ecosystem.

Keywords: Arbuscular mycorrhiza, spore community composition, Crofton weed, invasion density

Introduction

Invasive plants can dramatically alter the above-ground plant community and threaten biodiversity and ecosystem function throughout the world (Pejchar and Mooney, 2009). In addition, an increasing number of studies have shown that exotic invasive plants can change both the structure and function of soil microbial communities (Batten *et al*., 2006; Elgersma and Ehrenfeld, 2011; Lankau, 2011; Arthur *et al*., 2012). Soil microbes, as an important below-ground community, can regulate plant competition, diversity and composition (Klironomos, 2002; Wolfe and Klironomos, 2005; van der Heijen, 2008). Therefore they have the potential to affect the process of exotic plant invasion and the ultimate effects of invaders on the resident plant community and associated ecosystem function (Callaway *et al*., 2004).

Arbuscular mycorrhizal (AM) fungi, as an important component of the soil microbial community, form symbioses with most terrestrial plants (Smith and Read, 2008). AM fungi play a key role in the function and performance of plants through increasing nutrient acquisition as well as drought and disease resistance (Smith and Read, 2008). Several previous studies demonstrated significant changes in biomass and composition of AM fungus communities during exotic plant invasion (Hawkes *et al*., 2006; Mummey and Rillig, 2006; Wilson *et al*., 2012). The identity of specific AM fungal species and the individual combination of AM fungi with host plant species have different effects on the performance of plants. AM fungus community would therefore be expected to differentially influence plant competitive relationships and potentially facilitate plant coexistence (Bray *et al*., 2003; Stampe and Daehler, 2003; Scheublin *et al*., 2007). As a result, AM fungus communities may affect the success of invasive species by altering the competitive interactions between invasive and native plants (Stinson *et al*., 2006; Vogelsang and Bever, 2009; Harner *et al*., 2010).

Eupatorium adenophorum Spreng. (Crofton weed), which is native to Mexico and Costa Rica, is a worldwide noxious invasive weed. This plant has invaded southern China since the 1940s and become one of the most economically destructive invaders in China (Sun *et al*., 2004). Several mechanisms have been proposed to explain the success of this highly invasive species. These include extraordinary biological characteristics including high seed production and germination (Lu *et al*., 2008; Li and Feng, 2009), rapid genetic differentiation (Wan *et al*., 2010), high phenotypic plasticity (Zhao *et al*., 2012) and the production of allelopathic compounds that reduce the seed germination and growth of native plants (Baruah *et al*., 1994; Zhang *et al*., 2012). However, except for a few studies concerning the microbial community on leaves (Zhou *et al*., 2010) and the soil bacterial community

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(Yu *et al.*, 2005; Xu *et al.*, 2012), little information is available on the response of AM fungus community to *E. adenophorum* invasion.

In order to understand the effect of *E. adenophorum* invasion on AM fungus community, in this study we selected three invasive levels of *E. adenophorum* in a secondary forest of Yunnan Province, southwestern China. The AM colonization structures and spore community were examined and the objectives of the study were to understand how the AM fungal colonization structures and community composition changed along the invasive gradient of *E. adenophorum.* Therefore, this study will provide the primary data for further understanding of the interactions between AM fungi and *E. adenophorum* invasion in ecosystems.

Materials and Methods

Study site and sampling

The study was conducted in an evergreen mixed forest in Yunnan Province, southwest China (24°58′33.1″ N, 102°37′04.9″ E), with an average elevation of 2,200 m above sea level, a mean annual precipitation of 933 mm, and a mean annual temperature of 15.6°C. This location used to be dominated by native plant communities, but has been invaded by *E. adenophorum* for the last 30 years.

The expansion of *E. adenophorum* has obviously started from roadside to hillside with different displacement intensities, i.e. the relative abundance of *E. adenophorum* decreased from the roadside to the hillside. Three sites with different relative abundance of *E. adenophorum* were selected. In the first site, there was no *E. adenophorum* invasion and this was termed the *E. adenophorum* non-invaded site (hereafter referred to as NI). In this site, there were about 18 plant species, including three woody trees, 12 shrubs and three grasses dominated by *Myrsine africana* (59.0±5.8%, relative abundance), *Rhododendron speciferum* (8.0±2.0%), *Ilex chamaebuxus* (5.0±3.2%), *Rosa longicuspis* (4.0±4.0%), *Lindera communis* (3.0±3.0%), and *Rubus foliolosus* (3.0±2.0%). In the second site, the mean relative abundance of *E. adenophorum* was 48.6% and this was termed the *E. adenophorum* moderately invaded site (MI). In this site, there were about 19 plant species, including four woody trees, 12 shrubs and three grasses dominated by *M. africana* (12.2±2.4%, relative abundance), *Cupressus duclouxiana* (5.2±1.6%), *Viburnum foetidum* (5.0±3.2%), *I. chamaebuxus* (4.0±2.9%), and *Rumex hastatus* (4.0±1.9%). In the third site, the mean relative abundance of *E. adenophorum* was 73.8% and this was termed the *E. adenophorum* heavily invaded site (HI). In this site, there were about 16 plant species, including four woody trees, nine shrubs, and three grasses dominated by *M. africana* (5.6±1.7%, relative abundance), *Coniogramme intermedia* (3.0±1.0%), *Prinsepia utihis* (2.6±1.9%), and *R. speciferum* (2.4±1.9%). The distances between the sites were more than 100 m.

Three plots (20 m × 20 m each) were randomly set in each site and the distances between plots were more than 100 m. Five composite soil samples, each consisting of three soil cores (3.5 cm in diameter, 15 cm deep), were randomly collected from each plot in April 2008. The soil samples were immediately transported to the laboratory and sieved (2-mm mesh) to remove plant roots and debris. A part of each soil sample was air-dried for AM fungal spore isolation. The other part was stored at 4°C to determine soil chemical properties. Roots of *E. adenophorum* were collected from the MI and HI sites and were stored at -20°C for AM colonization observation. For each plot, soil (organic matter [SOM], total nitrogen [N], NO_3^--N, NH_4^+-N, total phosphorus [P], available P, and pH) and plant (species abundance and richness) variables were determined.

Root staining

Root samples were rinsed with tap water, cleared in 10% (w/v) KOH (20 min, 92°C), acidified in lactic acid (3 min), and stained (20 min, 92°C) with 0.5% acid fuchsin (Berch and Kendrick, 1982). Fifty fine root fragments (*ca.* 1 cm long) were mounted on slides in a polyvinyl alcohol solution (Koske and Tessier, 1983) and examined with a compound microscope at 100–400× for the presence of AM structures. The percentage of root length colonized by AM fungal structures was determined by using the magnified line-intersect method (McGonigle *et al.*, 1990).

Spore isolation and identification of AM fungi

AM fungal spores were isolated from 100 g air-dried soil of each composite sample. Spores were extracted by wet sieving and decanting, followed by sucrose centrifugation (Gerdemann and Nicolson, 1963), modified by Daniels and Skipper (1982). AM fungal species were identified based on spore morphological features such as color, size, wall structure, and hyphal attachment, following the descriptions of Schenck and Pérez (1990), information from the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/), and the original species descriptions with their emendations.

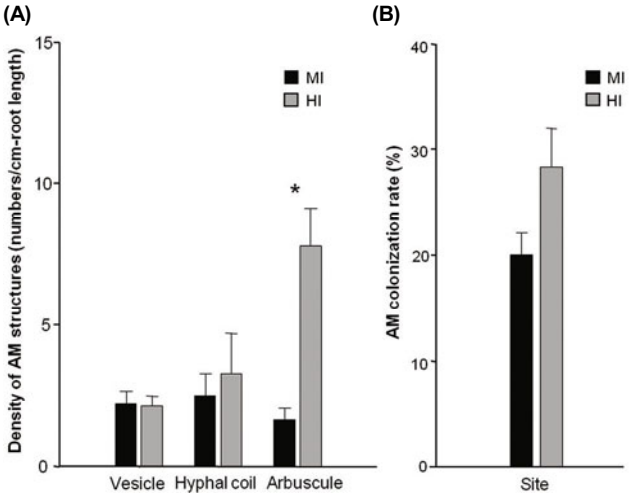

Fig. 1. Arbuscular mycorrhizal (AM) colonization structures (A) and overall AM colonization rate (B) of the roots of *E. adenophorum*. MI, *E. adenophorum* moderately invaded site; HI, *E. adenophorum* heavily invaded site. Asterisk indicates significant difference between two invasive levels by two-sample t-test at $P<0.05$ (means±SE, n=3).

Data analysis

The densities of arbuscules, vesicles and hyphal coils in *E. adenophorum* roots were defined as the number of arbuscules, vesicles and hyphal coils per cm-root length. Frequency was defined as the number of samples from which spores of a given AM fungal taxon was isolated divided by the total number of samples. Spore density was the number of spores per 100 g air-dried soil. Species richness was defined as the number of AM fungal species per soil sample (Koske, 1987). The Shannon diversity index (H') of AM fungi was calculated according to the formula:

$$H' = -\sum_{i=1}^{k} P_i \times \ln P_i$$

where k is the total number of fungal species, and P_i is the proportion of individuals that species i contributes to the total (Pielou, 1975). One-way ANOVA followed by a Least Square Difference (LSD) *post hoc* test was used to test the differences in spore densities among the three levels of *E. adenophorum* invasion. Two-sample t-tests were used to test differences in AM colonization structures of *E. adenophorum* roots between MI and HI. Distance matrices of AM fungus community (spore density dataset, wisconsin-sqrt transformed) were generated by calculating Bray-Curtis dissimilarity. Non-metric multidimensional scaling (NMDS) was carried out to visualize the distance matrices of AM fungus community of all plots. *Eupatorium adenophorum* invasive levels were fitted as centroids, and soil (SOM, total

Table 1. The frequency, spore density, species richness and Shannon diversity index (H') of arbuscular mycorrhizal (AM) fungi in the different *E. adenophorum* invaded sites

AM fungus	NI[a]		MI[b]		HI[c]	
	Density[d]	Frequency (%)[e]	Density	Frequency (%)	Density	Frequency (%)
Acaulospora capsicula Błaszk.	0.6±0.4	6.7	0.0±0.0	0.0	0.0±0.0	0.0
A. mellea Spain & N.C. Schenck	0.8±0.5	6.7	1.8±0.8	13.3	4.4±3.0	11.1
A. scrobiculata Trappe	0.0±0.0	0.0	0.8±0.6	6.7	0.8±0.4	11.1
A. spinosa C. Walker & Trappe	0.0±0.0	0.0	0.0±0.0	0.0	0.4±0.4	4.4
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler	0.0±0.0	0.0	0.0±0.0	0.0	2.0±1.1	11.1
C. etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler	84.0±31.9[a]	33.3	21.6±4.4[b]	31.1	24.4±8.0[b]	31.1
C. luteum (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüßler	0.2±0.2	4.4	0.0±0.0	0.0	0.0±0.0	0.0
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	0.4±0.4	4.4	0.0±0.0	0.0	0.2±0.2	4.4
Funneliformis constrictum (Trappe) C. Walker & A. Schüßler	0.4±0.3	6.7	0.0±0.0	0.0	0.0±0.0	0.0
F. geosporus (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	7.4±2.0[a]	24.4	6.0±1.5[a]	24.4	18.6±3.1[b]	31.1
F. mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	0.8±0.5	6.7	0.6±0.3	11.1	0.2±0.2	4.4
Glomus aggregatum N.C. Schenck & G.S. Sm.	13.0±3.7[a]	31.1	10.8±3.2[a]	26.7	4.0±1.3[b]	20.0
G. albidum C. Walker & L.H. Rhodes	3.2±1.2	17.8	5.8±3.0	17.8	2.8±1.7	11.1
G. ambisporum G.S. Sm. & N.C. Schenck	4.0±2.1	13.3	0.6±0.4	6.7	3.0±1.3	13.3
G. arenarium Błaszk., Tadych & Madej	8.0±2.8[a]	24.4	2.4±1.1[b]	13.3	1.2±0.7[b]	11.1
G. aureum Oehl & Sieverd.	12.4±7.5	13.3	2.2±1.3	11.1	0.0±0.0	0.0
G. deserticola Trappe, Bloss & J.A. Menge	0.2±0.2	4.4	0.0±0.0	0.0	0.0±0.0	0.0
G. hoi S.M. Berch & Trappe	0.0±0.0	0.0	0.0±0.0	0.0	1.4±1.0	6.7
G. multiforum Tadych & Błaszk.	0.0±0.0	0.0	0.8±0.6	6.7	0.0±0.0	0.0
G. tortuosum N.C. Schenck & G.S. Sm.	0.4±0.3	6.7	0.0±0.0	0.0	0.0±0.0	0.0
Glomus sp. 1	0.0±0.0	0.0	1.0±0.7	6.7	5.0±5.0	4.4
Glomus sp. 2	0.4±0.4	4.4	0.0±0.0	0.0	1.0±0.7	6.7
Glomus sp. 3	0.4±0.4	4.4	0.0±0.0	0.0	0.4±0.4	4.4
Glomus sp. 4	1.8±1.1	11.1	1.6±0.8	11.1	0.0±0.0	0.0
Gigaspora margarita W.N. Becker & I.R. Hall	0.2±0.2	4.4	1.0±0.6	11.1	0.2±0.2	4.4
Paraglomus laccatum (Błaszk.) Renker, Błaszk. & Buscot	0.0±0.0	0.0	0.0±0.0	0.0	0.2±0.2	4.4
P. occultum (C. Walker) J.B. Morton & D. Redecker	0.0±0.0	0.0	0.4±0.3	6.7	0.0±0.0	0.0
Rhizophagus clarus (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler	0.4±0.4	4.4	0.0±0.0	0.0	0.0±0.0	0.0
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	0.4±0.3	6.7	0.0±0.0	0.0	0.8±0.6	6.7
Species richness[f]	6.8±1.0		5.9±0.7		5.6±0.7	
H'[g]	1.3±0.2		1.3±0.1		1.1±0.1	
Total spore density	139.4±18.2[a]		58.4±4.7[b]		71.0±5.5[b]	

[a] *E. adenophorum* non-invaded site.
[b] *E. adenophorum* moderately invaded site.
[c] *E. adenophorum* heavily invaded site.
[d] The number of spores per 100 g air-dried soil.
[e] The number of samples from which spores of a certain AM fungal taxon was isolated divided by the total number of samples.
[f] The number of AM fungal species per soil sample.
[g] Calculated according to the formula: $H' = -\sum_{i=1}^{k} P_i \times \ln P_i$, , where k is the total number of fungal species, and P_i is the proportion of individuals that species i contributes to the total.
Values with different letters in the same row indicate significant difference at $P<0.05$ (means±SE, n=3).

N, NO_3^--N, NH_4^+-N, total P, available P, and pH) and plant (abundance, species richness) variables were fitted as vectors onto the ordination plot using '*envfit*' in the *Vegan* package (Oksanen *et al.*, 2009). All statistical analyses were carried out in R 2.15.1 (R Development Core Team, 2011).

Results

AM colonization

There were no significant differences in the AM root colonization rate, vesicle density and hyphal coil density in the roots of *E. adenophorum* between MI and HI (Fig. 1). For example, the AM root colonization rate was 20.0±1.97% in MI and 28.3±3.5% in HI. The vesicle density was 2.2±0.5 in MI and 2.1±0.4 in HI, and the hyphal coil density was 2.5±0.7 in MI and 3.3±1.3 in HI. However, the density of arbuscules was significantly higher in HI (7.8±1.2) than in MI (1.6±0.4) (F=41.86, P<0.001).

AM fungus community

A total of 29 AM fungi belonging to nine genera were found based on spore morphology, of which four taxa belonged to *Acaulospora*, three to *Claroideoglomus*, one to *Entrophospora*, three to *Funneliformis*, 13 to *Glomus*, one to *Gigaspora*, two to *Paraglomus*, one to *Rhizophagus*, and one to *Scutellospora* (Table 1 and Supplementary data Fig. S1). *Claroideoglomus etunicatum*, *Funneliformis geosporus*, and *Glomus aggregatum* were the most common species. Of the 29 AM fungi, 21 were isolated from NI, 15 from MI, and 19 from HI. The AM fungal species richness (6.8±1.0, 5.9±0.7, 5.6±0.7) and Shannon diversity index (1.3±0.2, 1.3±0.1, 1.1±0.1) were not significantly different among the NI, MI, and HI (Table 1).

The mean spore density of AM fungi was significantly different among the NI, MI, and HI (F=3.86, P=0.034). For example, the mean AM fungal spore density was significantly higher in NI (139.4±18.2) than in MI (58.4±4.7) and HI (71.0±5.5), but there was no significant difference between MI and HI (Table 1). Furthermore, with increasing *E. adenophorum* invasion the spore densities of the AM fungi, *C. etunicatum* (F=3.38, P=0.040), *G. aggregatum* (F=3.21, P=0.043), and *G. arenarium* (F=4.04, P=0.029) significantly decreased, but *F. geosporus* (F=8.84, P=0.001) significantly increased. In addition, six AM fungal species occurred only in NI, including *Acaulospora capsicula*, *Claroideoglomus luteum*, *Funneliformis constrictum*, *Glomus deserticola*, *G. tortuosum*, and *Rhizophagus clarus*. In contrast, eight AM fungal species appeared only in invaded sites, including *Acaulospora scrobiculata*, *A. spinosa*, *Claroideoglomus claroideum*, *Glomus hoi*, *G. multiforum*, *Glomus* sp. 1, *Paraglomus occultum*, and *P. laccatum*.

NMDS analysis indicated that the AM fungus community composition was significantly different among NI, MI, and HI (r^2=0.33, P=0.003). Furthermore, AM fungus community composition was significantly correlated with plant species richness (r^2=0.23, P=0.020), soil total P (r^2=0.30, P=0.005) and soil NO_3^--N (r^2=0.20, P=0.043) (Fig. 2).

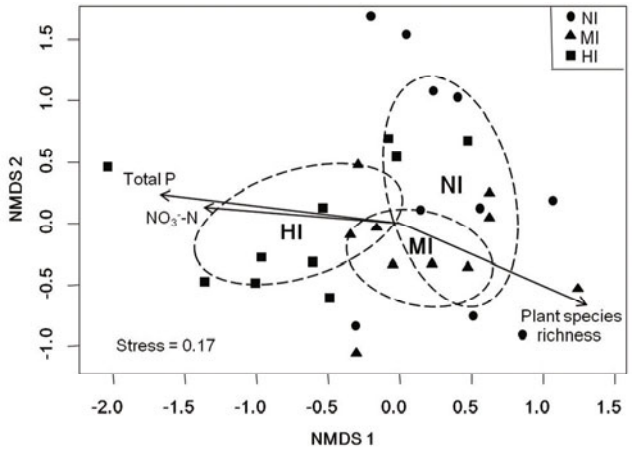

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination of AM fungus community composition in the three sites. NI, *E. adenophorum* non-invaded site; MI, *E. adenophorum* moderately invaded site; HI, *E. adenophorum* heavily invaded site. Soil (total P and NO_3^--N) and plant (species richness) vectors shown on the ordination plot were significant at P<0.05.

Discussion

AM fungal structures, such as arbuscules, vesicles, hyphal coils, and internal non-septate hyphae, commonly occurred within the roots of *E. adenophorum*. In particular, the density of arbuscules significantly increased with *E. adenophorum* invasion. Mycorrhizae have been reported to improve the uptake of nutrients and arbuscules, which are highly branched tree-like structure, are considered to be the major site of the carbon and nutrient exchange between the AM fungi and the host plants (Smith and Read, 2008). Therefore, it is possible that AM fungi can promote the competitive ability of *E. adenophorum* by increasing nutrient acquisition.

Our results indicated that *E. adenophorum* invasion significantly decreased the spore density of AM fungi. Similarly, some previous studies have reported that AM fungal biomass was reduced by exotic plant invasion (Batten *et al.*, 2006; Sanon *et al.*, 2009; Vogelsang and Bever, 2009). For example, the spore density of AM fungi was significantly lower in an *Amaranthus viridis* invaded site than an un-invaded site (Sanon *et al.*, 2009). Batten *et al.* (2006) demonstrated that *Centaurea solstitialis* invasion significantly reduced biomass of AM fungi based on phospholipid fatty acid analysis. In Californian grasslands, AM fungal infection potential was suppressed in soil by invasion of the exotic plant *Carduus pycnocephalus* (Vogelsang and Bever, 2009). It appears that invasive plant species can get a competitive advantage through interfering with mutualistic interactions between AM fungi and local plants; or by reducing the AM fungal abundance on which the native plants rely. This proposed concept has been defined as the degraded mutualism hypothesis by Vogelsang and Bever (2009).

The spore densities of AM fungi, *C. etunicatum*, *G. arenarium*, and *G. aggregatum*, significantly decreased with *E. adenophorum* invasion, whereas *F. geosporus* significantly increased. Similarly, the spore density of *Funneliformis mosseae* (syn. *Glomus mosseae*) increased with the invasion

time of the exotic plant *Solidago canadensis*, while *Funneliformis constrictum* (syn. *Glomus constrictum*) decreased (Jin *et al.*, 2004). Therefore, the results suggest that different AM fungal species have different competitive abilities in the process of exotic plant invasion (Jin *et al.*, 2004).

Our results demonstrated that the AM fungus community composition was significantly affected by *E. adenophorum* invasion. Changes in AM fungal composition in the process of plant invasions have been reported in some previous studies (Hawkes *et al.*, 2006; Mummey and Rillig, 2006; Zhang *et al.*, 2010). For example, Mummey and Rillig (2006) found that the AM fungus community composition in soil was significantly affected by *C. maculosa* invasion in a grassland in Missoula. Hawkes *et al.* (2006) reported that the AM fungus community composition in native plant roots was dramatically changed by the presence of the invasive plants *Avena barbata* and *Bromus hordeaceus* in grasslands in California and Utah, USA. The alteration in AM fungus community composition may be caused by the effects of changes in plant community (Burrows and Pfleger, 2002; Johnson *et al.*, 2004; Hausmann and Hawkes, 2009). In the present study, NMDS indicated that the AM fungus community composition was significantly related to plant species richness (Fig. 2). Different plant communities are likely to produce litter, root exudates and organic matter that differ in their chemical composition and role in maintaining nutrient pools (Eskelinen *et al.*, 2009), thus the AM fungus community could be affected by *E. adenophorum* invasion via changing local plant community.

Another possible reason is that *E. adenophorum*, as a strong allelopathic plant (Baruah *et al.*, 1994; Zhang *et al.*, 2012), produces high levels of allelopathic substances which can change the AM fungal composition as reported in previous studies on exotic plant invasion (Zhang *et al.*, 2007). In addition, our study found that AM fungus community composition was significantly correlated with soil NO_3^--N and total P, which were significantly enhanced by increased *E. adenophorum* invasion. Similar changes of soil chemical properties in plant invasions were reported in previous studies, for example Liao *et al.* (2008) reviewed 94 experimental studies concerning variation of soil N availability caused by plant invasion and concluded that soil NO_3^--N content was 30% higher in invaded ecosystems than in native ecosystems. Sanon *et al.* (2009) found that *Amaranthus viridis* invasion significantly increased concentration of soil total P. The changed soil chemical properties might be another possible reason for the alteration of AM fungus community composition (Oehl *et al.*, 2010; Ji *et al.*, 2012).

In summary, with *E. adenophorum* invasion the density of arbuscules significantly increased, whereas the spore density of AM fungi significantly decreased. Some AM fungal species showed different responses to *E. adenophorum* invasion. The AM fungus community composition changed significantly along the *E. adenophorum* invasion. This study provides fundamental data for the further understanding the interactions between AM fungi and *E. adenophorum* invasion in ecosystems. However, the investigation of AM fungi in the present study was based only on spore isolation and therefore cannot provide a complete picture of the AM fungus community. Therefore, in order to completely understand the change of AM fungus community in the process of *E. adenophorum* invasion, molecular techniques should be used in future studies.

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