

SOIL MICROBES AND SUCCESSFUL INVASIONS OF AN EXOTIC WEED *EUPATORIUM ADENOPHORUM* L.

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Abstract

The effects of soil microbes collected from the two invasive species *Eupatorium adenophorum* and *E. odoratum* and the two native species *E. japonicum* and *E. chinense* on the growth and biomass of *E. adenophorum* was examined to explore a possible link between soil microbes and successful invasions of the weed species *E. adenophorum*. In most cases, plant height, stem diameter, root number and root length were significantly enhanced when *E. adenophorum* was grown in sterilized soils compared with those when one was grown in non-sterilized soils collected from the rhizosphere of *E. adenophorum*, *E. japonicum* and *E. chinense*. In contrast, the growth and biomass of *E. adenophorum* were apparently inhibited when grown in soils collected from the rhizosphere of *E. odoratum*. Plant height, stem diameter, leaf area per plant and root length of *E. adenophorum* was greater when it was grown in soils collected from the rhizosphere of *E. adenophorum* compared with those when it was grown in soils collected from the rhizosphere of *E. odoratum*, but the enhancement considerably greater when it was grown in soils collected from the rhizosphere of *E. japonicum* and *E. chinense* compared with those when it was grown in soils collected from the rhizosphere of *E. adenophorum*. In addition, the biomass allocation of *E. adenophorum* was not significantly affected by soil microbes and soil sources. These results suggest that although the competitive advantage of the invasive weed *E. adenophorum* is not achieved solely by soil microbes, successful invasions of *E. adenophorum* may result partly from its release from the harmful soil microbes in its native range and the positive feedbacks of soil microbes from itself and the native species in its invading range.

Key words: *Eupatorium adenophorum*, Weed, Enemy release hypothesis, Soil microbe, Feedback effect.

Introduction

Biological invasions are one of the five major causes of biodiversity loss, alongside habitat destruction, over-exploitation, climate change and pollution. Scientists have raised a wide range of hypothesis to answer the question of why exotic plants can successfully invade new ranges. Of the numerous hypotheses, the enemy release hypothesis (Darwin, 1859; Williams, 1954; Elton 1958; Keane & Crawley 2002) is highly influential and has been widely cited. Since the invasive species are released from their predators and the natives are under pressure from their native predators, the invasive have a comparably competitive advantage (Memmot *et al.*, 2000; Wolfe 2002; Mitchell & Power 2003; Jakobs *et al.*, 2004). Exotic plants are free from aboveground predators, and thus a strategy for biologically controlling invasive plants is to introduce its aboveground predator from its native range to its invasive range. This strategy is widely adopted because people are accustomed to investigating visible, aboveground biology. However, there is a lot of evidence that plant performance is strongly controlled by the below ground biological and non-biological processes in an ecosystem (Brown, 1958; Garrett, 1963; Hudson, 1968; Janos, 1980; Westover & Bever, 2001; Reynolds *et al.*, 2003). For example, it has been confirmed that plant specific soil borne diseases contribute to succession in foredune vegetation (Van der Putten *et al.*, 1993), and the biodiversity of belowground mycorrhizal fungi determine that of aboveground plant species (Van der Heijden *et al.*, 1998). Hence, research focusing solely on aboveground plant-herbivore interactions and not below ground plant-

soil microbe interactions cannot fully explain the successful invasion of exotics.

The soil microbe community is an important biological factor in mediating plant growth and competition (Westover & Bever 2001; Reynolds *et al.*, 2003). The effects of soil microbes on the success of the invasive are long underestimated owing to the limitation of research methodology. Soil microbes are an agent in the interaction between the exotics and natives, which are impacted by exotic plant invasion and, in turn, alter the invasion process by feedback effects (Kourtev *et al.*, 2002 & 2003; Li *et al.*, 2006; Niu *et al.*, 2007). It has been accepted that the negative feedbacks between plants and soil microbes result from accumulations of fungal pathogens, harmful mycorrhizae and detrimental fungi (Mills & Bever 1998; Westover & Bever 2001; Bever 2002). Correspondingly, the positive feedbacks result from accumulations of microbes facilitating plant growth, such as mycorrhizal fungi and nitrogen-fixing bacteria. However, positive feedback might excessively facilitate plant growth of one species, consequently resulting in a loss of plant community biodiversity. It has been reported that the relative richness of one species is related to the strength and direction of feedbacks, and that rare species show a negative feedback and common species does a positive feedback (Klironomos, 2002). Feedbacks to plants are predominately negative from soil microbes in a natural ecosystem (Janzen, 1970; Connell, 1971). This effectively regulates the size of different plant population and thus advantageously maintains plant community diversity (Florence, 1965; Augspurger & Kelly 1984; van der Putten *et al.*, 1993; Mills & Bever, 1998; Klironomos, 2002; Bever, 2003).

Previous research has paid less attention to whether negative feedbacks still function in the process of exotic plant invasion. However, research has found that there exists an unexpected positive feedback between soil microbes and the exotic plant *Centaurea maculosa* in its invading ranges. In its native European soils, *Centaurea* cultivates soil microbes with increasingly negative effects on its growth, but in the North American soils it invades, *Centaurea* cultivates soil biota with increasingly positive effects on itself (Callaway *et al.*, 2004). This suggests that the direction of the feedback effect is altered in the transition from the native ranges to the invading ranges. On the other hand, feedback effects were eliminated in all sterilized soils from its native Europe or invading North America, indicating that soil microbes can indeed regulate plant growth. In a similar study, it was observed that invasion of *Eupatorium adenophorum* might alter the soil microbe community in its invading ranges and that the altered soil microbes inhibit the growth of the native and yet boost the growth of the exotic (Niu *et al.*, 2007). Unfortunately, the effects of soil microbes on the invader's success have been estimated only in a small amount of plant species.

More importantly, the positive feedbacks observed in some invaders do not hold true in all invasive plants (Rout & Callaway, 2012; Birnbaum & Leishman, 2013). For example, the germination rate of seeds was reduced by 12–16%, the survival rate of seedlings by 7–13% and the biomass of root and stem by >80% when *Ammophila arenaria* was grown in non-sterilized soils compared with that in sterilized soils from its invasive range in North America (Beckstead & Parker, 2003). It has also been observed that survival rates of seedlings and growth rates were enhanced when *Prunus serotina* was grown in sterilized soils compared with that in non-sterilized soils from native ranges, and that growth rate was only elevated by soil microbes from its invasive range and survival rate of seedlings was not impacted (Reinhart *et al.*, 2003). In another study, the growth of *Acer platanoides* and *A. negundo* was inhibited by soil microbes from its native range but was promoted by soil microbes from the rhizosphere of native plants in its invasive range and yet was inhibited by soil microbes from its own rhizosphere in its invasive range (Reinhart & Callaway, 2004). Overall, similar studies are limited, and thus the effects of soil microbes on exotic plant invasion are not fully understood.

Eupatorium adenophorum, native to Central and South America, belongs to a family of Compositae and is a noxious and invasive weed in China. It widely forms a mono-plant community in its invasive range of southwest China, leading to a loss of species diversity in the native ecosystem. Previous study has focused on the biological and physiological traits (Liu *et al.*, 1989; Zu *et al.*, 2005), distribution (Lu & Ma 2004), control methods (Yang & Guo, 2008), ecological plasticity (Zhang *et al.*, 2009), competitive capacity (Jiang *et al.*, 2008a & b) and allelopathy (Yu *et al.*, 2004; Yang, 2006) of *E. adenophorum*. Only one study has observed that *E.*

adenophorum benefits from the soil microbes of its own rhizosphere in its invasive ranges (Niu *et al.*, 2007). However, the feedback effects of soil microbes from the rhizospheres of native species on *E. adenophorum* are still unknown. In addition, little is known about the benefits of soil microbes from plants of the same genus in the same invasive range on *E. adenophorum*. This would contribute to a full understanding of feedback effects.

The two invasive weed *E. adenophorum* and *E. odoratum* and the two native weed *E. japonicum* and *E. chinense* were selected to investigate effects of soil microbes from the rhizosphere of identical genus plants on *E. adenophorum*. *E. odoratum* is also native to Central and South America and has also successfully invaded southwest China. In a field condition, *E. odoratum* is not competitively replaced by *E. adenophorum* and vice versa; however, the two native species are competitively replaced by *E. adenophorum*. The objective of the present study was to explore the effects of soil microbes from the rhizosphere of two invasive and two native plants on the growth and biomass of the invasive *E. adenophorum*. The hypothesis was that *E. adenophorum* might benefit from the soil microbes from its own rhizosphere and from the soil from the rhizosphere of the natives, but suffer from the soil microbes from the rhizosphere of the invasive *E. odoratum*.

Material and Methods

Site description: The study was conducted at the teaching and experimental farm of Yunnan Agricultural University in Kunming, Yunnan, China. The average annual temperature is 15°C and annual precipitation is 1000 mm. The dry season is from November to April and the well-defined wet season is from May to October in which 80% of the annual rainfall occurs.

Soil collection: In sites heavily invaded with *E. adenophorum* and *E. odoratum*, a mono-plant community of *E. adenophorum* and *E. odoratum* was observed and no native plants existed within it. The native plants *E. japonicum* and *E. chinense* seldom form a mono-plant community, and thus sites where *E. japonicum* and *E. chinense* are dominant were selected for soil collection. Soils were collected from the rhizosphere of *E. adenophorum*, *E. odoratum*, *E. japonicum* and *E. chinense*. All field soil was prepared by dicing the roots and crumbling the soil until it passed through a 1 cm sieve. In order to avoid microbial cross-contamination, separate sampling tools were used for each rhizosphere soil, and the chosen distance between the four sites was relatively far.

Soil sterilization: Soils were collected and immediately subjected to slow air drying to mimic the drying conditions that would occur during natural drought. After drying, the soil was sieved and separated into two parts. One half of the soils were sterilized by triple autoclaving. All tools, pots and surfaces in contact with non-sterile soil were sterilized to avoid cross-contamination by one of five methods (Reinhart & Callaway, 2004): autoclaving

for 60–180 min, flame sterilization, surfaces sprayed or material soaked in ≥10% bleach solution (itself 5.25% aqueous NaOCl), surfaces sprayed with 70% ETOH solution, or material heated at ≥110°C for 16 h in a dry oven. Moreover, a separate suit of tools was used for each plant source soil.

Pot experiment: A pot experiment was conducted to examine the effects of soil microbes on growth and biomass of *E. adenophorum*. Sixty 20 cm deep and 20 cm internal diameter pots were used for each plant rhizosphere soil, making a total of 240 pots. One half of the pots were filled with sterilized soils. The sterilized and non-sterilized soils both were mixed with sterilized river sand (5:1 w/w) to fill pots. To eliminate potentially allelopathic chemicals, activated carbon was added to the mixtures of soil and sand (2%, w/w). Activated carbon has a high affinity for organic compounds and a weak affinity for inorganic electrolytes such as those found in nutrient solution (Mangle *et al.*, 2008), and has been shown to reduce the negative effects of root exudates from other species (Mahall & Callaway, 1992; Callaway & Aschehoug, 2000).

The pots were placed in a growth house where total photon exposure per day was equivalent to 85% of that in the full sunlight. The growth house was created using agricultural film on a steel frame and provides shelter from rain but is ventilated. The pots received identical natural temperature and humidity. Seeds of *E. adenophorum* were collected from numerous mother plants and numerous seeds were sowed in a pot. After germination, seedlings (3–5 cm in height) were thinned to one plant per pot. Seedlings were well watered every 2 days with sterilized tap water (100–150 mL) and fertilized once every 15 days with 400 ml of 0.25 strength Hoagland's solution for 265 days.

Plant morphology: Plant height and stem diameter were measured, and leaf number was counted before harvest. Root number was counted and the length of total root was measured after harvest. Leaf area per plant was measured *In vitro* with a leaf area meter (Li-COR).

Plant biomass: After measurement of plant morphology, plants were divided into roots, stems and leaves. Fresh samples were oven dried at 70°C to constant weight and then weighed. Root, stem and leaf mass ratio was defined by the ratio of root, stem, and leaf biomass, respectively to total biomass. Leaf area ratio was defined by the ratio of leaf area per plant to total biomass.

Statistical analysis: In a comparison of effects of non-sterilized and sterilized soils, and of effects of *E. adenophorum* and *E. odoratum*, *E. japonicum* and *E. chinense* soils, a *t*-test was used for examining the effect of soil microbes on growth and biomass of *E. adenophorum*. Before harvest, some individuals died owing to an unknown reason. However, at least 23 individuals remained, so 23 individuals were harvested in all treatments. Means ± SD were given ($n = 23$), and *t* and *P* values were also shown. Statistical significance

was defined as $p \leq 0.05$, unless otherwise noted in the text. All data were analyzed using SPSS for Windows (SPSS, 18.0).

Results

Plant morphology was obviously impacted by soil microbes from different plant sources (Fig. 1). Plant height was significantly elevated when *E. adenophorum* was grown in non-sterilized soils collected from the rhizosphere of itself but apparently inhibited by soil microbes from the rhizosphere of *E. odoratum* (Fig. 1a). Soil microbes from the rhizosphere of *E. japonicum* and *E. chinense* enhanced plant height to a lesser extent. Stem diameter of *E. adenophorum* was significantly increased by soil microbes from the rhizosphere of itself and *E. japonicum* but was significantly decreased by those from the rhizosphere of *E. odoratum* (Fig. 1b) and not significantly enhanced by those from the rhizosphere of *E. chinense*. There was not a significant difference in leaf number between *E. adenophorum* grown in non-sterilized and sterilized soils (Fig. 1c). Soil microbes from the rhizosphere of itself significantly elevated leaf area per plant of *E. adenophorum*, but soil microbes from the rhizosphere of *E. japonicum* and *E. chinense* did not and those from the rhizosphere of *E. odoratum* significantly inhibited it (Fig. 1d). Soil microbes from the rhizosphere of itself, *E. japonicum* and *E. chinense* significantly enhanced root number of *E. adenophorum* but those from the rhizosphere of *E. odoratum* considerably depressed it (Fig. 1e). Root length of *E. adenophorum* was markedly promoted by soil microbes from the rhizosphere of itself, *E. japonicum* and *E. chinense*, but was clearly restrained by those from the rhizosphere of *E. odoratum* (Fig. 1f).

Plant biomass was affected to a greater extent by the source of the soil microbes (Fig. 2). Root biomass of *E. adenophorum* was significantly boosted by soil microbes from the rhizosphere of itself, *E. japonicum* and *E. chinense*; however, it was depressed by those from the rhizosphere of *E. odoratum* (Fig. 2a). Similarly, stem biomass was more accumulated when *E. adenophorum* was grown in non-sterilized than in sterilized soils from the rhizosphere of itself, *E. japonicum* and *E. chinense*, but the opposite hold true with those from the rhizosphere of *E. odoratum* (Fig. 2b). Leaf biomass of *E. adenophorum* was comparably less facilitated by soil microbes from the rhizosphere of itself, *E. japonicum* and *E. chinense* and also relatively less inhibited by those from the rhizosphere of *E. odoratum* (Fig. 2c). In general, soil microbes from the rhizosphere of itself, *E. japonicum* and *E. chinense* significantly facilitated the accumulation of *E. adenophorum* total biomass, and, however, those from the rhizosphere of *E. odoratum* drastically reduced it (Fig. 2d).

Biomass allocation of *E. adenophorum* was not significantly affected by soil microbes from the rhizosphere of itself, *E. odoratum*, *E. japonicum* and *E. chinense* (Fig. 3a, b & c), suggesting that root, stem and leaf might be equally enhanced or inhibited by soil microbes. The leaf area ratio of *E. adenophorum* was also not impacted by soil microbes (Fig. 3d).

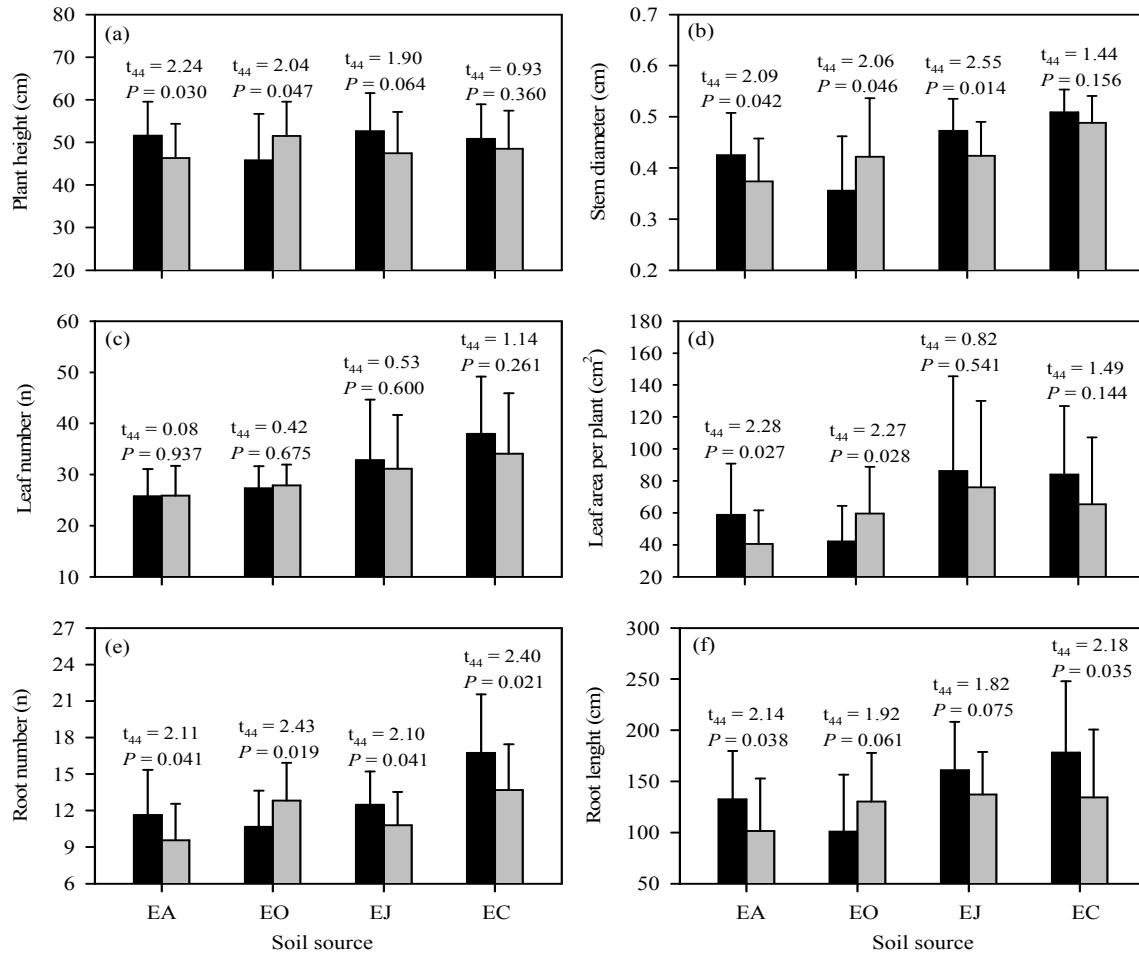


Fig. 1. Plant height (a), stem diameter (b), leaf number (c), leaf area per plant (d), root number (e), and root length (f) of *Eupatorium adenophorum* grown in non-sterilized (black bar) and sterilized (gray bar) soil from the rhizosphere of *E. adenophorum* (EA), *E. odoratum* (EO), *E. japonicum* (EJ), and *E. chinense* (EC). Values are means \pm SD (n=23), and t and P values are given.

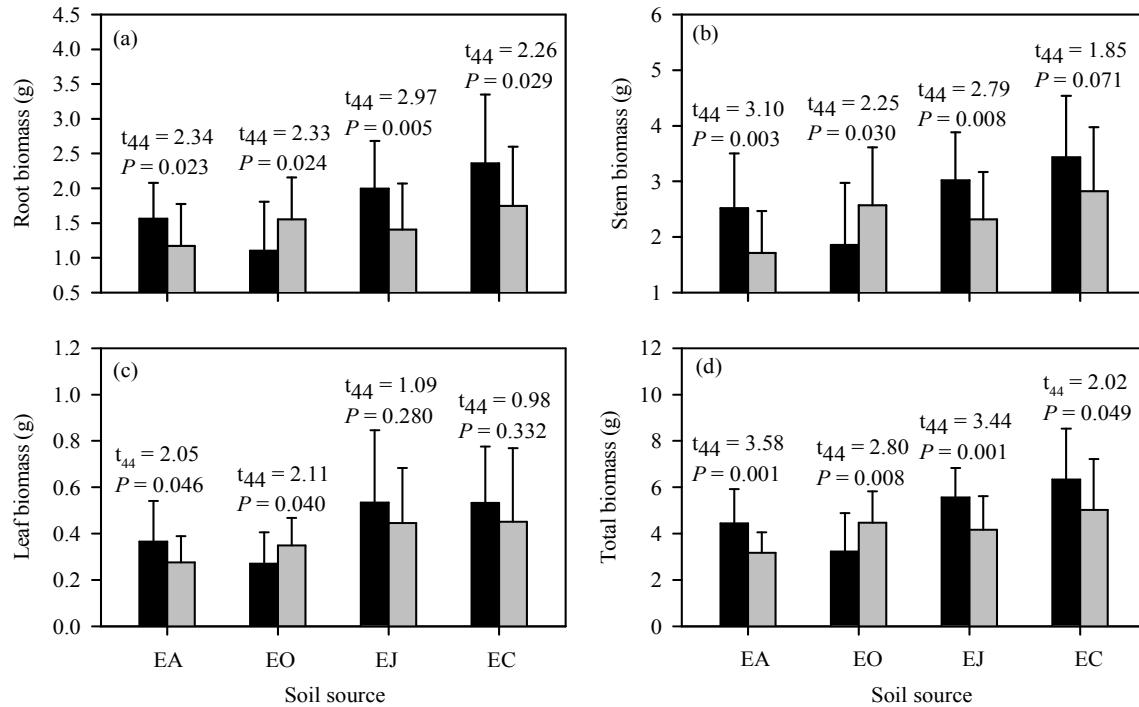


Fig. 2. Root biomass (a), stem biomass (b), leaf biomass (c), and total biomass (d) of *Eupatorium adenophorum* grown in non-sterilized (black bar) and sterilized (gray bar) soil from the rhizosphere of *E. adenophorum* (EA), *E. odoratum* (EO), *E. japonicum* (EJ), and *E. chinense* (EC). Values are means \pm SD (n=23), and t and P values are given.

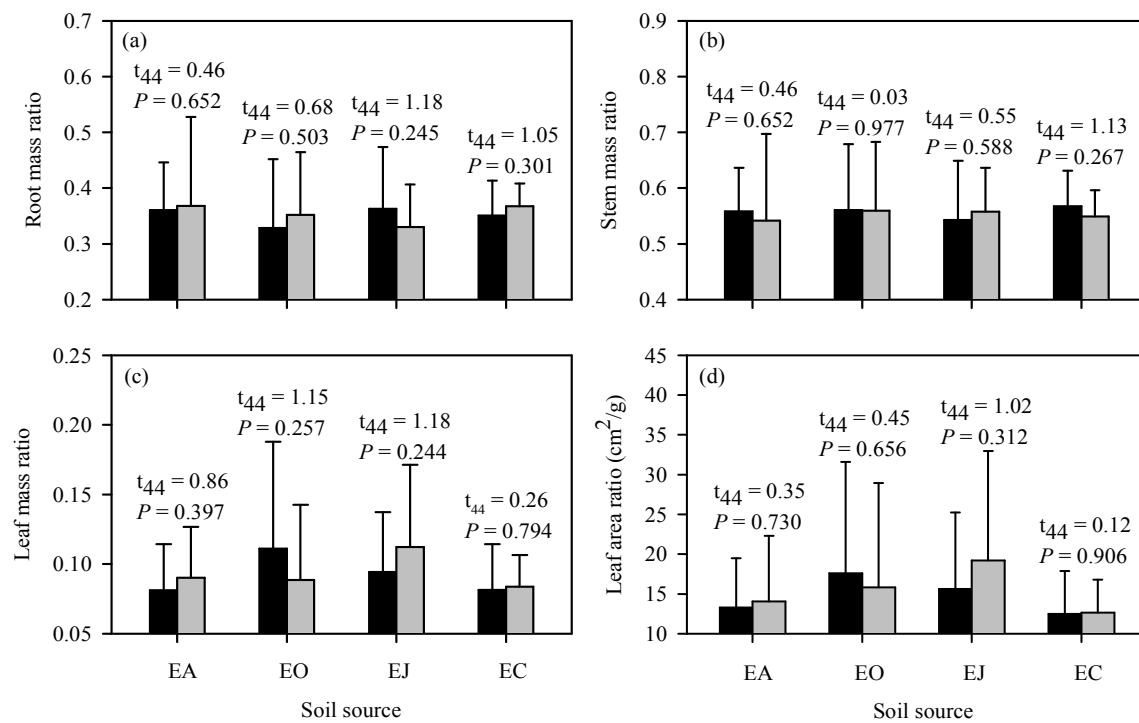


Fig. 3. Root biomass ratio (a), stem biomass ratio (b), leaf biomass ratio (c), and leaf area ratio (d) of *Eupatorium adenophorum* grown in non-sterilized (black bar) and sterilized (gray bar) soil from the rhizosphere of *E. adenophorum* (EA), *E. odoratum* (EO), *E. japonicum* (EJ), and *E. chinense* (EC). Values are means \pm SD ($n=23$), and t and P values are given.

Plant height was greater when *E. adenophorum* was grown in its own soils than in *E. odoratum* soils; however, there was not a significant difference in plant height between *E. adenophorum* grown in its own soil and *E. japonicum* and *E. chinense* rhizosphere soils (Fig. 4a). Stem diameter was significantly wider when *E. adenophorum* was grown in its own soils than in *E. odoratum* soils but even wider when grown in *E. japonicum* and *E. chinense* soils (Fig. 4b). There was not a difference in leaf number between *E. adenophorum* grown in its own soil than in *E. odoratum* soils, but leaf number was much higher when grown in *E. japonicum* and *E. chinense* soils (Fig. 4c). In comparison with *E. adenophorum* grown in its own soil, *E. adenophorum* grown in *E. odoratum* soils had a lesser value of leaf area per plant but a greater value when grown in *E. japonicum* and *E. chinense* soils (Fig. 4d). Root number of *E. adenophorum* was greater in *E. japonicum* and *E. chinense* soils than in its own soil (Fig. 4e and f).

There was a significant difference in plant biomass between *E. adenophorum* grown in its own soil and grown in *E. odoratum*, *E. japonicum* and *E. chinense* soils (Fig. 5). In comparison with root, stem, leaf, and total biomass of *E. adenophorum* grown in its own soil, those grown in *E. odoratum* soil had significantly reduced values, and those grown in *E. japonicum* and *E. chinense* soils had largely elevated values. There was not a statistical difference in root and leaf mass ratio and leaf area ratio between *E. adenophorum* grown in its own soil and in *E. odoratum*, *E. japonicum* and *E. chinense* soil (Fig. 6a, c, and d). Meanwhile, stem mass ratio was hardly affected by the different soils (Fig. 6b).

Discussion

The enemy release hypothesis proposes that the invaders have a comparatively competitive advantage over the natives because of their releases from their herbivores of their origin ranges (Keane & Crawley, 2002). One of the possible mechanisms is that invader's resources are more invested in growth than defenses owing to this partial release from environmental pressure (Blossey & Nötzold, 1995; Herms & Mattson, 2002), so some exotic plants grow taller and larger in their invading ranges than in their native ranges (Blossey & Nötzold, 1995; Siemann & Rogers, 2001; Joshi & Vrieling, 2005; Rogers & Siemann, 2005; Zou *et al.*, 2007; Feng *et al.*, 2009). It should be noted that the releases from soil microbes harmful to plants maybe also give the invader a competitive advantage in its invasive range. There has been evidence that soil microbes contribute to successful invasion of exotic plants (e.g. Callaway *et al.*, 2004, 2008, & 2011). In the present study, it was found that *E. adenophorum* grew significantly taller and larger in the non-sterilized than sterilized soils from the rhizosphere of itself (Figs. 1 & 2). This indicates that although feedback effects of soil microbes was not examined in its native range and the magnitude of the release from harmful soil microbes is also unknown, the invasive plant *E. adenophorum* obviously benefits from its own rhizosphere soil microbes in its invasive range. Soil microbes beneficial to plant growth necessarily accelerate successful invasion of *E. adenophorum* in the invading ranges.

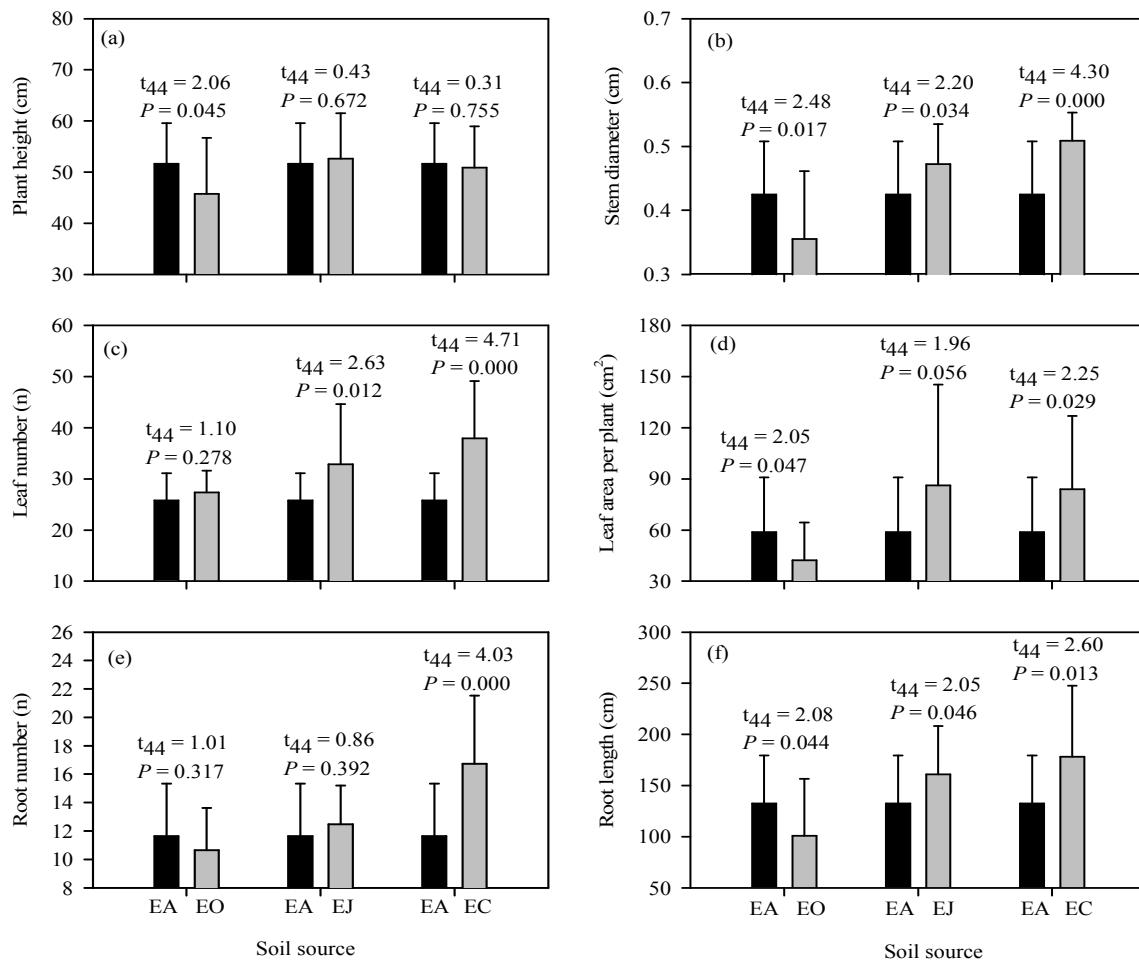


Fig. 4. Plant height (a), stem diameter (b), leaf number (c), leaf area per plant (d), root number (e), and root length (f) of *Eupatorium adenophorum* grown in soil from the rhizosphere of *E. adenophorum* (EA, black bar), *E. odoratum* (EO, gray bar), *E. japonicum* (EJ, open bar), and *E. chinense* (EC, fine bar). Comparisons between effect of EA soil and effect of EO, EJ, and EC soil on plant morphology were analyzed by *t*-test. Values are means \pm SD ($n=23$), and *t* and *P* values are given.

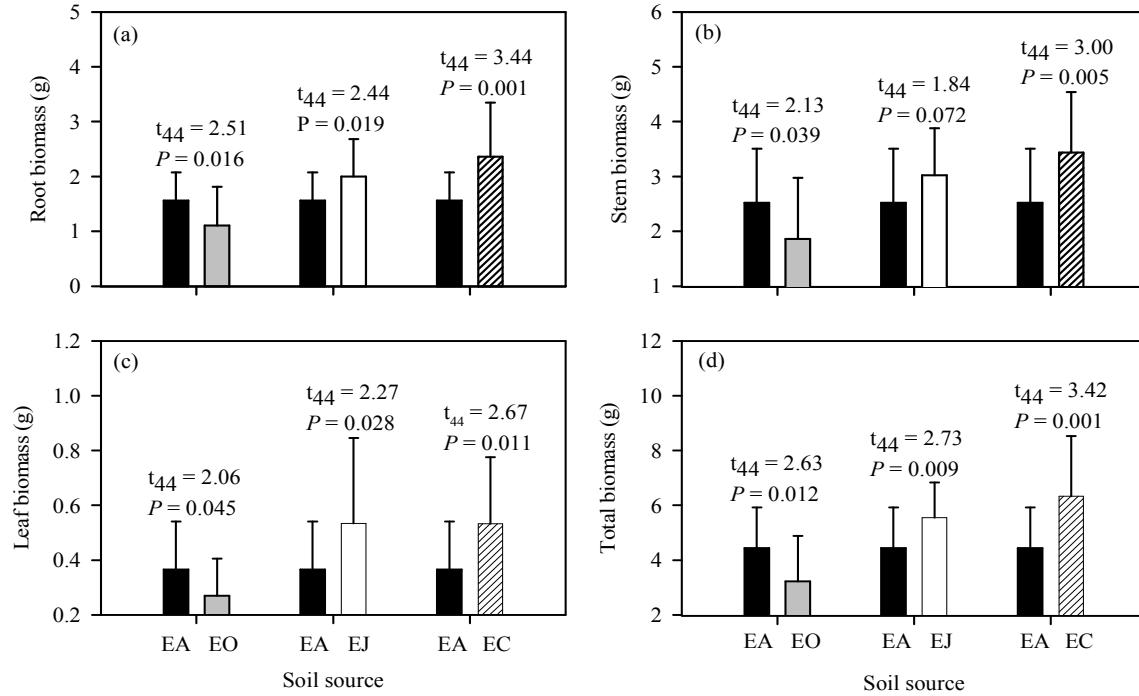


Fig. 5. Root biomass (a), stem biomass (b), leaf biomass (c), and total biomass (d) of *Eupatorium adenophorum* grown in soil from the rhizosphere of *E. adenophorum* (EA, black bar), *E. odoratum* (EO, gray bar), *E. japonicum* (EJ, open bar), and *E. chinense* (EC, fine bar). Comparisons between effect of EA soil and effect of EO, EJ, and EC soil on plant biomass were analyzed by *t*-test. Values are means \pm SD ($n=23$), and *t* and *P* values are given.

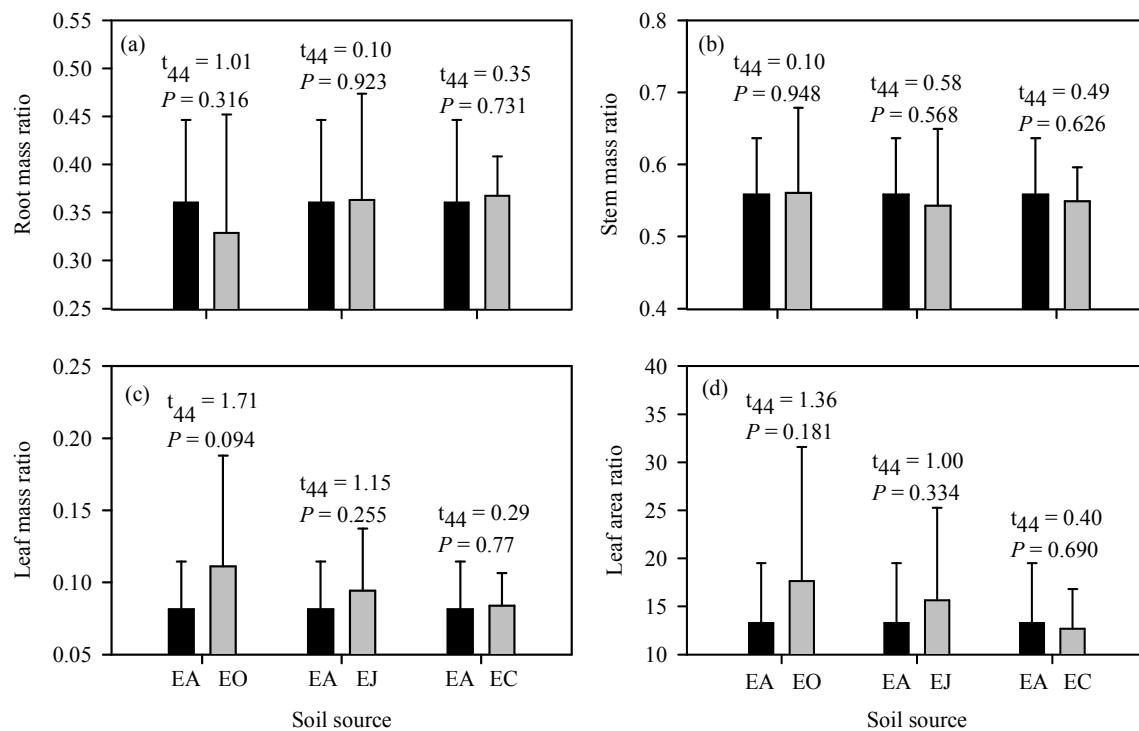


Fig. 6. Root biomass ratio (a), stem biomass ratio (b), leaf biomass ration (c), and leaf area ratio (d) of *Eupatorium adenophorum* grown in soil from the rhizosphere of *E. adenophorum* (EA, black bar), *E. odoratum* (EO, gray bar), *E. japonicum* (EJ, open bar), and *E. chinense* (EC, fine bar). Comparisons between effect of EA soil and effect of EO, EJ, and EC soil on biomass allocation were analyzed by *t*-test. Values are means \pm SD ($n=23$), and *t* and *P* values are given.

E. odoratum, as the congener of *E. adenophorum*, is also native to Central and South America and a noxiously invasive weed in Southwest China. It has been believed that among exotic plants, those lacking native congeners should have a greater advantage owing to the absence of very similar competitors (Darwin, 1859; Rejmánek & Richardson, 1996; Rejmánek, 1999; Daehler, 2001). This suggests that closely related species are likely to be attacked by common consumers (Connor *et al.*, 1980; Agrawal *et al.*, 2005). In this case, it should be accepted that *E. odoratum* has very similar soil microbes in the invasive ranges, and releases from the same harmful soil microbes from its native range as do *E. adenophorum*. Thus, it was originally anticipated that *E. adenophorum* would benefit from soil microbes from the rhizosphere of *E. odoratum*. Unexpectedly, soil microbes from the rhizosphere of *E. odoratum* were unfavorable for the growth of *E. adenophorum* (Figs. 1 & 2). *E. adenophorum* cannot form a mono-plant community in its native range, indicating that its expansion is hindered by other species native to its range, possibly including *E. odoratum*. In addition, the two closely related weeds can co-occur but are not competitively replaced by each other in their invasive range (personal observation), suggesting that the expansion of *E. adenophorum* is still controlled by *E. odoratum* even in their invasive range. Overall, the fact that the plant *E. adenophorum* flourishes particularly well in southwest China may be related to positive feedbacks of soil microbes from the rhizosphere of itself.

Since soil microbes from the rhizosphere of *E. odoratum* have negative feedback effects on *E. adenophorum*, and *E. adenophorum* benefits from its own soil microbes, it can be expected that *E. adenophorum*

will grow shorter and smaller in *E. odoratum* soils than in its own soil (Figs. 4 & 5). This further suggests that the expansion of the invader *E. adenophorum* can be restrained by *E. odoratum*. Results from this study might also partly explain why *E. adenophorum* hardly replaces *E. odoratum* in their invasive ranges. The two species with the same origin both successfully invade an identical range, but one species suffers from soil microbes from the rhizosphere of other species in the invading range. Unfortunately, similar experiments were not conducted in the original ranges of the two congeners, thus it is unknown if the growth of *E. adenophorum* would be reduced in *E. odoratum* soils in its native range. However, the extrapolation from the fact that the two congeners never form a mono-plant community in their native ranges, mean that the two species are likely controlled by similar natural enemies, including harmful soil microbes. This would indicate that, in the invading ranges, *E. odoratum* might not be released from harmful soil microbes that are also harmful to *E. adenophorum*, and the invasive mechanism of *E. odoratum* appears to be different from that of *E. adenophorum*. The results demonstrated here indirectly suggest that the escape from harmful soil microbes may contribute to successful invasions of *E. adenophorum*.

The positive feedbacks of soil microbes from the rhizosphere of itself might not grant the invasive *E. adenophorum* a competitive advantage over the native species, since other plants might also benefit from their own soil microbes but do not form a mono-plant community (Rout & Callaway, 2012). It was found that *E. adenophorum* grew smaller in non-sterilized than in sterilized soils from rhizosphere of the two natives *E.*

japonicum and *E. chinense* (Figs. 1 & 2). This suggests that the two native congeners share the same rhizospheric soil microbes as *E. adenophorum* does, and these soil microbes facilitate *E. adenophorum*. *E. japonicum* and *E. chinense* never form a denser community in the field, and are commonly replaced by *E. adenophorum*. Although it is unknown if the two natives benefit from soil microbes from their own rhizosphere, the invasive *E. adenophorum*, at least in part, obtains a comparably competitive advantage over the two natives by means of positive feedbacks of soil microbes from their rhizospheres. This advantage contributes to the replacement of the two natives by the invasive *E. adenophorum*. However, this does not mean that *E. adenophorum* outcompetes only the two natives to accomplish successful invasions.

On one hand, successful invasions of *E. adenophorum* are derived from positive feedback effects of soil microbes, but on the other it might be more dependent on the promotion effects of soil microbes from the natives. Previous work has found that rhizospheric soils of *E. adenophorum* facilitate itself, and yet inhibit the co-occurring natives *Lolium perenne*, *E. fortunei* and *Medicago sativa* (Niu *et al.*, 2007). This effect likely contributes to the competitive advantage of the invasive over the natives. In the present experiments, it has been observed in most cases that *E. adenophorum* grows significantly taller and larger in *E. japonicum* and *E. chinense* soils than in its own soils (Figs. 4 & 5). Based upon these results combined with the previous work, it is likely that successful invasions of *E. adenophorum* result partly from positive feedbacks of soil microbes from the invader itself and the natives, and from negative feedback effects of soil microbes from the invader on the natives. Thus, soil microbes might play an important role in successful invasions of *E. adenophorum*.

It was expected that the biomass allocation of the plant would be changed so long as *E. adenophorum* is affected by soil microbes irrespective of positive or negative feedbacks. Furthermore, it was expected that relatively more biomass would be allocated to root parts if *E. adenophorum* was negatively affected by soil microbes and vice versa. Surprisingly, biomass allocation of *E. adenophorum* was not significantly affected by soil microbes and soil sources (Figs. 3 & 6). It appears that soil microbes and soil nutrition have a different effect on the plants. If plant grows in barren soils, plant growth will be negatively affected by low soil nutrition and more biomass will be allocated to root parts for overcoming negative effects. However, a similar pattern was not observed in these experiments, and *E. adenophorum* did not respond to positive or negative feedbacks of soil microbes by means of biomass allocation. The underlying cause is not fully understood and needs to be studied further.

Conclusion

In general, it is certain that successful invasions of *E. adenophorum* result, in part, from its release from the harmful soil microbes from its native range, and from the positive feedbacks of soil microbes from itself and the native species in its invading range. Indeed, the competitive

advantage of the invader *E. adenophorum* over the natives is not achieved solely by soil microbes, and numerous mechanisms are involved in the replacement of the natives by the invasive *E. adenophorum* and in the formation of a mono-plant community. Indeed, information on soil microbe should be adequately examined on the further research.

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References

- Agrawal, A.A., P.M. Kotanen, C.E. Mitchell, A.G. Power, W. Godsoe and J.N. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology*, 86: 2979-2989.
- Augspurger, C.K. and C.K. Kelly. 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. *Oecologia*, 61: 211-217.
- Beckstead, J. and I.M. Parker. 2003. Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens? *Ecology*, 84: 2824-2831.
- Bever, J.D. 2002. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *P. Roy. Soc. Lond B Bio.*, 269: 2595-2601.
- Bever, J.D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol.*, 157: 465-473.
- Birnbaum, C. and M.R. Leishman. 2013. Plant-soil feedbacks do not explain invasion success of *Acacia* species in introduced range populations in Australia. *Biol. Invasions*, DOI: 201310.1007/s10530-013-0478-z.
- Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive non-indigenous plants: A hypothesis. *J. Ecol.*, 83: 887-889.
- Brown, J.C. 1958. Soil fungi of some British sand dunes in relation to soil type and succession. *J. Ecol.*, 46: 641-664.
- Callaway, R.M. and E.T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, 290: 521-523.
- Callaway, R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, 89: 1043-1055.
- Callaway, R.M., E.J. Bedmar, K.O. Reinhart, C. Gómez-Silvan and J. Klironomos. 2011. Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. *Ecology*, 92: 1027-1035.
- Callaway, R.M., G.C. Thelen, A. Rodriguez and W.E. Holben. 2004. Soil biota and exotic plant invasion. *Nature*, 427: 731-733.
- Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forests. In: (Eds.): den Boer, P.J. and G.R. Gradwell. Dynamics in Populations. the Netherlands: Center for Agricultural Publishing and Documentation, Wageningen, pp. 298-312.

- Connor, E.F., S.H. Faeth, D. Simberloff and P.A. Opler. 1980. Taxonomic isolation and the accumulation of herbivorous insects: a comparison of introduced and native trees. *Ecol. Entomol.*, 5: 205-211.
- Daehler, C.C. 2001. Darwin's naturalization hypothesis revisited. *Am. Nat.*, 158: 324-330.
- Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London.
- Elton, C.S. 1958. The ecology of invasions by animals and plants. Methuen, London.
- Feng, Y.L., Y.B. Lei, R.F. Wang, R.M. Callaway, A. Valiente-Banuet, Inderjit, Y.P. Li and Y.L. Zheng. 2009. Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. *Proc. Natl. Acad. Sci. USA*, 106: 1853-1856.
- Florence, R.G. 1965. Decline of old-growth redwood forests in relation to some soil microbiological processes. *Ecology*, 46: 52-64.
- Garrett, S.D. 1963. Soil Fungi and Soil Fertility. Pergamon Press, Oxford.
- Herms, D.A. and W.J. Mattson. 2002. The dilemma of plants: To grow or to defend? *Q. Rev. Biol.*, 67: 283-335.
- Hudson, H.J. 1968. The ecology of fungi on plant remains above the soil. *New Phytol.*, 67: 837-874.
- Jakobs, G., E. Weber and P.J. Edwards. 2004. Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. *Divers. Distrib.*, 10: 11-19.
- Janos, D.P. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, 61: 151-162.
- Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. *Am. Nat.*, 104: 501-528.
- Jiang, Z.L., W.X. Liu, F.H. Wan and Z.Y. Li. 2008a. Comparative studies on seasonal dynamics of soil enzymatic activities and soil nutrient Availability in mono- and mixed-culture plant communities of *Ageratina adenophora* and *Setaria sphacelata*. *J. Plant Ecol.* (Chinese version), 32: 900-907.
- Jiang, Z.L., W.X. Liu, F.H. Wan and Z.Y. Li. 2008b. Competitive effects between *Ageratina adenophora* (Asteraceae) and *Setaria sphacelata* (Gramineae). *Sci. Agric. Sin.*, 41: 1347-1354.
- Joshi, J. and K. Vrieling. 2005. The enemy release and EICA hypothesis revisited: Incorporating the fundamental difference between specialist and generalist herbivores. *Ecol. Lett.*, 8: 704-714.
- Keane, R.M. and M.J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.*, 17: 164-170.
- Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417: 67-70.
- Kourtev, P.S., J.G. Ehrenfeld and M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology*, 83: 3152-3166.
- Kourtev, P.S., J.G. Ehrenfeld and M. Häggblom. 2003. Experimental analysis of the effects of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biol. Biochem.*, 35: 895-905.
- Li, W.H., C.B. Zhang and H.B. Jiang. 2006. Changes in soil microbial community associated with invasion of the exotic weed, *Mikania micrantha* H.B.K. *Plant Soil*, 281: 309-324.
- Liu, L.H., W.Y. Liu, Z. Zheng and G.F. Jing. 1989. The characteristic research of autecology ecology of pamakan (*Eupatorium adenophorum*). *Acta Ecol. Sin.*, 9: 66-70.
- Lu, Z.J. and K.P. Ma. 2004. The influence of topographical factors on the invasion of the alien species, *Eupatorium adenophorum*. *Acta Phytoecol. Sin.*, 28: 761-767.
- Mahall, B.E. and R.M. Callaway. 1992. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology*, 73: 2145-2151.
- Mangle, S., Inderjit and R.M. Callaway. 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *J. Ecol.*, 96: 58-67.
- Memmot, J., S.V. Fowler, Q. Paynter, A.W. Sheppard and P. Syrett. 2000. The invertebrate fauna on bromm, *Cytisus scoparius*, in two native and two exotic habitats. *Acta Oecol.*, 21: 213-222.
- Mills, K.E. and J.D. Bever. 1998. Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology*, 79: 1595-1601.
- Mitchell, C.E. and A.G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature*, 421: 625-627.
- Niu, H.B., W.X. Liu, F.H. Wan and B. Liu. 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant Soil*, 294: 73-85.
- Reinhart, K.O. and R.M. Callaway. 2004. Soil biota facilitate exotic Acer invasions in Europe and North America. *Ecol. Appl.*, 14: 1737-1745.
- Reinhart, K.O., A. Packer, W.H. van der Putten and K. Clay. 2003. Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecol. Lett.*, 6: 1046-1050.
- Rejmánek, M. and D.M. Richardson. 1996. What attributes make some plant species more invasive? *Ecology* 77:1655-1661.
- Rejmánek, M. 1999. Invasive plant species and invasible ecosystems. In: *Invasive species and biodiversity management*. (Eds.): Sandlund, O.D., P.J. Schei and A. Viken. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 79-102.
- Reynolds, H.L., A. Packer, JD Bever and K. Clay. 2003. Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84: 2281-2291.
- Rogers, W.E. and E. Siemann. 2005. Herbivory tolerance and compensatory differences in native and invasive ecotypes of Chinese tallow tree (*Sapium sebiferum*). *Plant Ecol.*, 181: 57-68.
- Rout, M.E. and R.M. Callaway. 2012. Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is not everywhere'. *Ann. Bot.*, 110: 213-222.
- Siemann, E. and W.E. Rogers. 2001. Genetic differences in growth of an invasive tree species. *Ecol. Lett.*, 4: 514-518.
- Van der Heijden M.G.A, Klironomos J.N., Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.
- Van der Putten, W.H., C. Van Dijk and B.A.M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature*, 362: 53-56.
- Westover, K.M. and J.D. Bever. 2001. Mechanisms of plant species coexistence: complementary roles of rhizosphere bacteria and root fungal pathogens. *Ecology*, 82: 3285-3294.
- Williams, J.R. 1954. The biological control of weeds. In: Report of the Sixth Commonwealth Entomological Congress, London, United Kingdom, pp. 95-98.
- Wolfe, L.M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. *Am. Nat.*, 160: 705-711.

- Yang, G.Q. and J.Y. Guo. 2008. Biocontrol of *Ageratina adenophora*. In: *Biological Invasion: Biocontrol parts*. (Eds.): Wan, F.H., B.P. Li and J.Y. Guo. Science Press, Beijing, pp. 141-156.
- Yang, G.Q. 2006. Main allelochemicals isolated and identified from the leachates of *Ageratina adenophora* Sprengel (Asteraceae) and their action mechanisms on upland rice (*Oryza sativa*) seedlings. Chinese Academy of Agricultural Sciences Ph.D. Dissertation, Beijing.
- Yu, X.J., D. Yu and K.P. Ma. 2004. Relationships between allelopathy and invasiveness by *Eupatorium adenophorum* at different sites. *Acta Phytoecol. Sin.*, 28: 773-780.
- Zhang, C.L., Y.P. Li, Y.L. Feng, Y.L. Zheng and Y.B. Lei. 2009. Roles of phenotypic plasticity and local adaptation in *Eupatorium adenophorum* invasions in different altitude habitats. *Acta Ecol. Sin.*, 29: 1940-1946.
- Zou, J-W., W.E. Rogers and E. Siemann. 2007. Differences in morphological and physiological traits between native and invasive populations of *Sapium sebiferum*. *Funct. Ecol.*, 21: 721-730.
- Zu, Y.G., W.J. Wang, F.J. Yang, Z.H. Zhao and J.G. Cao. 2005. Stomatal regulation on the gas exchange of *Eupatorium adenophorum*: Implication on its invasive ability. *Sci. Silvae Sin.*, 41: 25-35.

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