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Competition and soil fungi affect the physiological and growth traits of an alien and a native tree species

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Abstract

Competition plays an important role in the replacement of native species by alien plants. A greenhouse experiment was conducted to investigate whether the competition pattern of alien *Robinia pseudoacacia* L. and native *Quercus acutissima* Carr. is affected by soil sterilization. Physiological traits, such as gas-exchange parameters and chlorophyll (Chl) content, and growth traits, such as the biomass accumulation of the two species, were examined in natural soil or in soil sterilized with benomyl. The results show that native *Q. acutissim*a inhibits the growth of *R. pseudoacacia* in natural soil. When the two plants coexisted and competed under sterilization treatment, *R. pseudoacacia* was less inhibited by *Q. acutissima* and the competition of *R. pseudoacacia* decreased the growth of *Q. acutissima* in terms of biomass, Chl *a*, Chl *b*, total Chl, and Chl *a/b.* These results suggest that soil sterilization benefits the growth of *R. pseudoacacia* and changes the competition pattern by the changed soil biota. Soil sterilization increased the biomass of root nodules, which ultimately benefits the growth of *R. pseudoacacia* and root nodule bacteria may be important in the dispersal and invasion process of nitrogen-fixing alien plants such as *R. pseudoacacia*.

Additional key words: chlorophyll; competition; photosynthesis; *Quercus acutissima*; *Robinia pseudoacacia*; root nodule.

Introduction

Alien plants may replace native plants *via* competition (Chittka and Schurkens 2001, Littschwager *et al.* 2010, Shochat *et al.* 2010). Previous studies found that disturbance, parasitic plants, level of nutrients, and soil biota might change the competition outcome of competition between alien and native plants (Van der Putten and Peters 1997, Corbin and D'Antonio 2004, Liancourt *et al.* 2005, Xu *et al.* 2007, Cui and He 2009, Yu *et al.* 2009). Investigations on competition of alien plants have mainly focused on alien herbs, rarely on alien shrubs or trees.

Considering that the function traits of alien plants, such as physiological and growth traits, are assumed to relate to invasiveness, the traits of alien species have been investigated to predict invasions (Kuhn *et al.* 2004, Kyle and Leishman 2009, Leishman *et al.* 2007, Kuster *et al.* 2008, Chen *et al.* 2011). Invasive alien species often have higher photosynthetic ability and biomass accumulation than native species (Pattison *et al.* 1998, Wang *et al.* 2008, Zheng *et al.* 2009). Higher photosynthetic ability and biomass accumulation is beneficial for alien plants when replacing native plants through competition.

The transformation of alien plants from relatively low dominance in native habitats to preponderant species in invaded habitats is frequently the process of escape from natural enemies. In this process, alien plants escape aboveground herbivores and harmful soil biota (Beckstead and Parker 2003, Reinhart *et al.* 2003, Knevel *et al.* 2004). Plants could be negatively influenced by harmful fungi, and also be positively influenced by beneficial arbuscular mycorrhizal (AM) fungi and root

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; g_s – stomatal conductance; L_s – stomatal limitation; NST – nonsterilization treatment; P_N – net photosynthetic rate; ST – sterilization treatment; WUE – instantaneous water-use efficiency.

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nodule bacteria (Niu *et al.* 2007, Amaya-Carpio *et al.* 2009, Šraj-Kržič *et al.* 2009). Root nodule bacteria can efficiently improve soil structure, increase the level of organic carbon and nitrogen content in the soil, mobilize phosphorus, and facilitate absorption by plants (Killingbeck 1996, Dakora and Keya 1997, Dutta and Agrawal 2002). For example, *Myrica faya* could alter nitrogen cycling and it has greatly altered native plant communities in Hawaiian ecosystems through nitrogen fixation (Reinhart and Callaway 2006). Soil fungi might play an important role in the competition between alien and native plants, often facilitating the growth of alien plants, which is one of the drivers of the invasion process (Callaway *et al.* 2001, 2004; Levine *et al.* 2004). However, the relationship between soil fungi and the physiological traits of alien and native plants is rarely studied.

In the present study, a greenhouse experiment is conducted to investigate the effects of soil fungi on the competition pattern between alien *R. pseudoacacia* L. and native *Q. acutissima* Carr. Physiological traits, such as gas-exchange parameters and Chl content, and growth traits, such as the biomass accumulation of *R. pseudoacacia* and *Q. acutissima*, are investigated. We aim to answer the following questions: (*1*) Whether competition affects the physiological and growth traits of the alien *R. pseudoacacia* and the native *Q. acutissima* and (*2*) whether soil sterilization affects the physiological and growth traits of *R. pseudoacacia* and *Q. acutissima*?

Materials and methods

Plants and soil: *R. pseudoacacia* is a nitrogen-fixing, leguminous deciduous species, considered to be one of the 100 worst woody plant invaders worldwide (Cronk and Fuller 1995, Von Holle *et al.* 2006, Wei *et al.* 2009). This species is native to North America, but it is now distributed in Europe, Asia, and Australia (Von Holle *et al.* 2006). *R. pseudoacacia* has invaded a wide range of habitats, such as mountain valleys, mountain sides, rivers, and coasts (Rice *et al.* 2004, Landgraf *et al.* 2005, Jung *et al.* 2009). The harmful fungi accumulated by *R. pseudoacacia* have inhibited the growth of some native species, such as Japanese black pine (*Pinus thunbergii*) and the effects of harmful fungi are beneficial to replacement (Taniguchi *et al.* 2008). As a widely distributed alien plant in China (Wang and Zhou 2000), the influence of *R. pseudoacacia* on native plants and ecological systems has rarely been studied.

Q. acutissima is the main deciduous broad-leaved species in northern China, widely distributed on mountains and hills in northern China (Wang and Zhou 2000). *Q. acutissima* has high plasticity and stress tolerance to light, water, and other environmental factors, and coexists with *R. pseudoacacia* in multiple habitats in North China (Woo *et al.* 2003, Noh *et al.* 2007).

Mature seeds of *R. pseudoacacia* were collected near the Fanggan Research Station of Shandong University in November 2008 and stored at 0–4°C throughout the winter. They were sterilized by soaking in 3% H_2O_2 for 30 min and then soaked in distilled water for 24 h to stimulate germination. The seeds were germinated on moist pledgets, and then healthy and uniform germinated seeds were selected and sown in 9-L plastic pots (32 cm × 29 cm, height × diameter) on June 15th, 2009. The *Q. acutissima* seedlings, which germinated this year, were gathered in the *Q. acutissima* forest near the Fanggan Research Station of Shandong University on June 24th. The seedlings were gathered on similar size of germinated *R. pseudoacacia* seedlings. *Q. acutissima* and *R. pseudoacacia* were planted alone and together in 9-L plastic pots on June 25th. *Quercus acutissima* and *R. pseudoacacia* competed with each other in the same plastic pots until November 2nd. Before planting into the plastic pots, the *Q. acutissima* seedlings were sterilized by soaking in 3% H_2O_2 for 2 min.

All the seedlings were cultivated in the greenhouse of the Fanggan Research Station. The average photosynthetic active radiations (PAR) measured once an hour from 07:00 to 16:00 h was 361 ± 17 μmol m^{-2} s^{-1} in the greenhouse. The mean temperature in the greenhouse was 32.4 ± 0.4ºC. Each pot was given 400 ml of water every afternoon during the period of the experiment. At the end of the experiment, the mean heights of *R. pseudoacacia* and *Q. acutissima* were 40–60 cm and 15–20 cm, respectively, the mean number of leaves of *R. pseudoacacia* and *Q. acutissima* were 150–230 and 10–20, respectively.

The soil in the experiment was collected near the Fanggan Research Station of Shandong University. A 5-mm sieve was used to remove rocks and the remains of plants and animals. Given that the effects of soil biota on plant interaction might vary with resource availability (Johnson *et al.* 1997, Simard *et al.* 1997), no additive nutrient solutions were added in the experiment. The mixture weighed about 9.5 kg of dry mass per pot, and the chemical properties were as follows: organic matter 8.53 g kg^{-1}, pH 7.05, total N 0.38 g kg^{-1}, total P 0.76 g kg^{-1}, and total K 24.82 g kg^{-1}.

Experimental design: The soil was divided into two treatments: sterilized and unsterilized soil. Sterilization of the soil was done by applying 50 mg (benomyl) kg^{-1}(soil) in 100 ml of water per pot on July 20th according to previous studies (Hetrick *et al.* 1989, Callaway *et al.* 2003). Sterilization of the soil was carried out every two weeks until November 2nd. There were 14 replicates in every treatment.

Benomyl is a systemic fungicide used worldwide to sterilize harmful fungi. Benomyl inhibits fungal cell growth during mitosis of the cell cycle (Smith *et al.* 2000, Gupta *et al.* 2004), and has minimal direct effect on plants (Paul *et al.* 1989, 2001). Benomyl is widely used to investigate the effect of soil fungi on the interaction of alien and native plants in the dispersal process of alien plants (Callaway *et al.* 2001, 2003, 2004).

All pots were randomly arranged in the same greenhouse. To avoid the effects of possible environmental patchiness within the greenhouse, all pots were rotated every month so each pot experienced all possible conditions.

Gas-exchange measurements: Leaf gas exchange was measured during sunny days between 9:30 and 11:30 h, and 13:30 and 15:00 h from 17th to 19th October. Stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), and ambient CO_2 concentration (C_a) were measured with a portable leaf gas-exchange system (*GFS-3000*, *Walz GmbH*, Effeltrich, Germany) at a PPFD of 1,000 µmol m^{-2} s^{-1} (a saturated light derived from a pre-experiment). The ambient CO_2 concentration (C_a) was about 360 µmol mol^{-1}. Three fully expanded fresh leaves were selected in each pot and alternately measured between treatments. Instantaneous water-use efficiency (WUE) was calculated as P_N/E, and stomatal limitation (L_s) was calculated as $1 - C_i/C_a$ for each leaf sampled (He and Dong 2003). During the measurement, mean air temperature and relative humidity inside the chamber were 30.6°C and 60.2%, respectively.

Chl analysis: Six seedlings per treatment were chosen to determine leaf Chl content. Ten leaf disks from each plant were removed with a cork-borer 1 cm in diameter, and 20 ml ethanol (95%, v/v) in a labeled vial was used to extract Chl. The samples were kept in the dark for 24 h before Chl analysis when the leaf surfaces were completely white. The absorption spectra of the supernatant liquid was spectrophotometrically measured at wavelengths of 665 and 649 nm to calculate Chl *a* and Chl *b* concentrations, as described by Lichtenthaler and Wellburn (1983). The Chl *a/b* ratio was calculated as Chl *a/b* = Chl *a*/Chl *b*.

Biomass measurement: At the end of the experiment, all the seedlings were harvested. The roots were washed thoroughly and carefully with tap water. Root nodules were separated from the roots of *R. pseudoacacia*. All the plant parts were dried at 85°C for 48 h and weighed.

Statistical analysis: The data were subjected to two-way *ANOVA* for each species, where competition and sterilization were considered fixed factors with Type III Sum of Squares. One-way *ANOVA* and *Duncan*'s Significant Difference tests were performed to determine the differences among treatments in each species. The differences were considered significant at $P \le 0.05$ in all the tests. All statistical analyses were performed using *SPSS 13.0* software package (*SPSS Inc.*, IL, USA). All graphs were drawn using *Origin 7.5* (*OriginLab Co.*, Massachusetts, USA).

Results

The effects of soil sterilization on the physiological and growth traits of *R. pseudoacacia* and *Q. acutissima*: The sterilization of soil treatments significantly affected the biomass of the root nodules and the total biomass accumulation of *R. pseudoacacia* (Table 1). The biomass of the root nodules and the whole *R. pseudoacacia* plant with or without *Q. acutissima* in the sterilization treatment (ST) were significantly greater

Table 1. Results of two-way *ANOVA* on biomass accumulation, gas exchange, and chlorophyll content of *Robinia pseudoacacia* and the biomass of *Rhizobium*, with competition (C) and soil sterilization (ST) treatments as fixed factors. P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; L_s – stomatal limitation; WUE – instantaneous water-use efficiency; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; total Chl – Chl (*a* + *b*); Chl *a/b* – Chl *a*/Chl *b*. *F* – *F* values of *ANOVA*; *P* – the significance levels. The effects are significant at the $P<0.05$ level.

Parameter	C		ST		C × ST	
	F	*P*	*F*	*P*	*F*	*P*
Total biomass [g]	9.940	0.003	11.548	0.001	0.001	0.972
Rhizobium [g]	9.818	0.003	11.850	0.001	0.040	0.843
P_N [µmol m^{-2} s^{-1}]	7.824	0.006	3.440	0.066	5.191	0.024
E [mmol m^{-2} s^{-1}]	8.120	0.005	3.270	0.073	5.383	0.022
g_s [mmol m^{-2} s^{-1}]	0.569	0.452	3.825	0.052	10.893	0.001
C_i [µmol mol^{-1}]	0.740	0.391	11.765	0.001	0.287	0.593
L_s [%]	0.628	0.429	11.046	0.001	0.003	0.960
WUE [mmol mol^{-1}]	8.706	0.004	0.536	0.465	9.877	0.002
Chl *a* [mg g^{-1}]	4.268	0.054	2.768	0.113	6.704	0.019
Chl *b* [mg g^{-1}]	0.777	0.390	0.490	0.493	0.131	0.721
Total Chl [mg g^{-1}]	4.442	0.049	0.552	0.467	4.576	0.046
Chl *a/b*	0.001	0.980	2.408	0.138	0.182	0.675

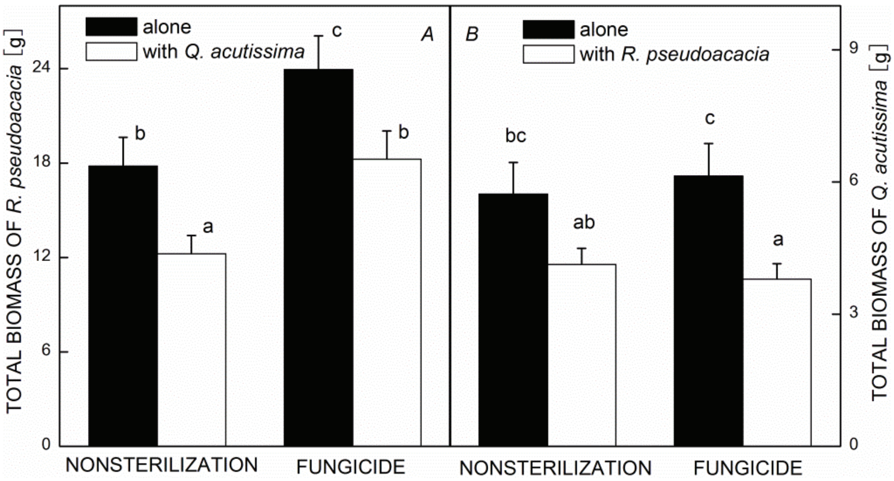

Fig. 1. Biomass of *Robinia pseudoacacia* (*A*) and *Quercus acutissima* (*B*) in response to the competition and soil sterilization treatments. The different *lowercase letters* represent significant differences among treatments in each species ($P \leq 0.05$, *Duncan*'s test). Error bars indicate one standard error of the mean. $n = 11–13$ for nonsterilization and fungicide treatments.

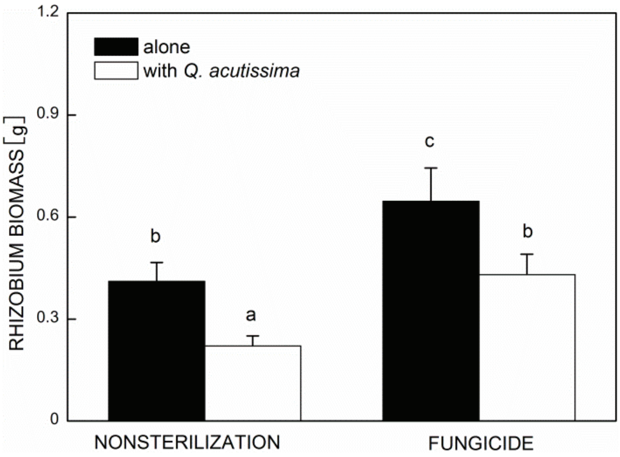

Fig. 2. Biomass of *Rhizobium* in the roots of *Robinia pseudoacacia* in response to the competition and soil sterilization treatments. The different *lowercase letters* represent significant differences among treatments ($P \leq 0.05$, *Duncan*'s test). Error bars indicate one standard error of the mean ($n = 11–13$).

than those in the nonsterilization treatment (NST) (Figs. 1, 2). Soil sterilization benefited the growth of *R. pseudoacacia*, whether it competed with *Q. acutissima* or not. The C_i and L_s of *R. pseudoacacia* were significantly affected by the soil sterilization, and the effect of sterilization on P_N, E, and g_s were approximately significant (Table 1). When *R. pseudoacacia* grew alone, its P_N, E, g_s, and L_s in ST were significantly greater than those in the NST (Fig. 3). While its C_i in ST was significantly lower than that in NST (Fig. 3). Sterilization had no significant effect on Chl *a*, Chl *b*, total Chl, and Chl *a/b* of *R. pseudoacacia*.

Soil sterilization had no significant effect on the total biomass of *Q. acutissima* (Table 2). Sterilization significantly affected the C_i, Chl *b*, and Chl *a/b* of *Q. acutissima* (Table 2). When *Q. acutissima* competed with *R. pseudoacacia*, its C_i in ST was profoundly lower than that in NST. When *Q. acutissima* grew alone, its Chl *b* in the ST was apparently greater than that in NST and the Chl *a/b* of *Q. acutissima* in ST was apparently lower than that in NST (Fig. 5).

The effect of competition on the physiological and growth traits of *R. pseudoacacia* and *Q. acutissima*: Competition had a significant effect on the biomass of root nodules and biomass accumulation of *R. pseudoacacia* (Table 1). In both soil treatments, the biomass of the root nodules and the whole *R. pseudoacacia* that grew alone, were distinctly greater than those of the *R. pseudoacacia* that competed with *Q. acutissima* (Fig. 1). The P_N, E, and WUE of *R. pseudoacacia* were significantly affected by the competition of *Q. acutissima* ($P<0.05$) (Table 1). In NST, the P_N and E of *R. pseudoacacia* with *Q. acutissima* were significantly greater than those of the *R. pseudoacacia* without *Q. acutissima* and the WUE of the *R. pseudoacacia* with *Q. acutissima* was significantly lower than that of the *R. pseudoacacia* without *Q. acutissima* (Fig. 3). In the ST, the P_N, E, C_i, L_s, and WUE of the *R. pseudoacacia* with and without *Q. acutissima* were not significantly different. The total Chl of *R. pseudoacacia* was significantly affected by the competition, and the competition effect of *Q. acutissima* on the Chl *a* of *R. pseudoacacia* was approximately significant (Table 1). In the NST, the total Chl and Chl *a* of the *R. pseudoacacia* with *Q. acutissima* were greater than those of the *R. pseudoacacia* without *Q. acutissima* (Fig. 4). In ST, the Chl *a*, Chl *b*, total Chl, and Chl *a/b* of the *R. pseudoacacia* with and without *Q. acutissima* had no significant differences.

The effect of competition affected the biomass accumulation of *Q. acutissima* significantly (Table 2). In ST, the biomass of the *Q. acutissima* that grew alone was significantly greater than that with *R. pseudoacacia* (Fig. 1). The P_N, E, g_s, C_i, L_s, and WUE of the *Q. acutissima* were not affected by the competition effect of *R. pseudoacacia*. Chl *a*, Chl *b*, and total Chl of the *Q. acutissima* were significantly affected by competition with *R. pseudoacacia* (Table 2). In NST, Chl *a*, Chl *b*, total Chl, and Chl *a/b* of the *Q. acutissima* with and

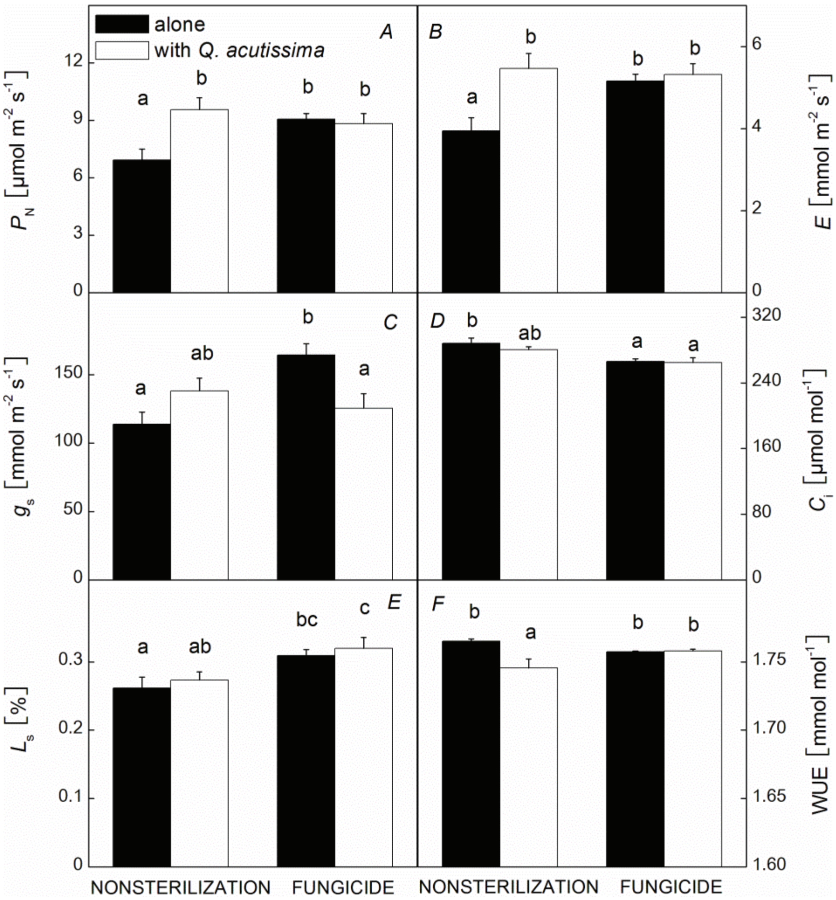

Fig. 3. Gas-exchange parameters (*A–F*) of *Robinia pseudoacacia* in response to the competition and soil sterilization treatments. *A*: net photosynthetic rate (P_N); *B*: transpiration rate (E); *C*: stomatal conductance (g_s); *D*: intercellular CO$_2$ concentration (C_i); *E*: stomatal limitation (L_s); *F*: instantaneous water-use efficiency (WUE). The different *lowercase letters* represent significant differences among treatments ($P \leq 0.05$, *Duncan*'s test). Error bars indicate one standard error of the mean (n = 33–39 for the nonsterilization and fungicide treatments).

Table 2. Results of two-way *ANOVA* on biomass accumulation, gas exchange, and chlorophyll content of *Quercus acutissima*, with competition (C) and soil sterilization (ST) treatments as fixed factors. P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO$_2$ concentration; L_s – stomatal limitation; WUE – instantaneous water-use efficiency; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; total Chl – Chl (*a* + *b*); Chl *a/b* – Chl *a*/Chl *b*. *F* – *F* values of *ANOVA*; *P* – the significance levels. The effects are significant at the $P<0.05$ level.

Parameter	C		ST		C × ST	
	F	*P*	*F*	*P*	*F*	*P*
Total biomass [g]	10.580	0.002	0.004	0.951	0.376	0.543
P_N [µmol m^{-2} s^{-1}]	1.826	0.178	1.817	0.179	12.949	<0.001
E [mmol m^{-2} s^{-1}]	1.447	0.231	2.819	0.095	13.145	<0.001
g_s [mmol m^{-2} s^{-1}]	2.474	0.118	1.159	0.283	6.752	0.010
C_i [µmol mol^{-1}]	0.862	0.354	5.085	0.025	0.922	0.338
L_s [%]	0.569	0.452	3.194	0.076	1.339	0.249
WUE [mmol mol^{-1}]	1.375	0.243	3.115	0.079	13.130	<0.001
Chl *a* [mg g^{-1}]	14.118	0.001	0.000	0.993	0.856	0.366
Chl *b* [mg g^{-1}]	19.086	<0.001	5.726	0.027	6.318	0.021
Total Chl [mg g^{-1}]	18.586	<0.001	0.698	0.413	2.431	0.135
Chl *a/b*	3.599	0.072	13.780	0.001	2.725	0.114

without *R. pseudoacacia* had no significant differences. In ST, Chl *a*, Chl *b*, and total Chl of the *Q. acutissima* with *R. pseudoacacia* were significantly lower than those of the *Q. acutissima* without *R. pseudoacacia* while the Chl *a/b* of the *Q. acutissima* with *R. pseudoacacia* were significantly greater than that of the *Q. acutissima* without *R. pseudoacacia* (Fig. 5).

Discussion

The results suggest that the more intense competition effect of *R. pseudoacacia* on *Q. acutissima* in ST was caused by the greater growth of *R. pseudoacacia*, which was benefited by soil sterilization.

The effects of soil sterilization on the root nodules of *R. pseudoacacia*: The root nodule biomass of *R. pseudoacacia* with or without competition with *Q. acutissima* in ST was greater than that in NST. Many fungi have the

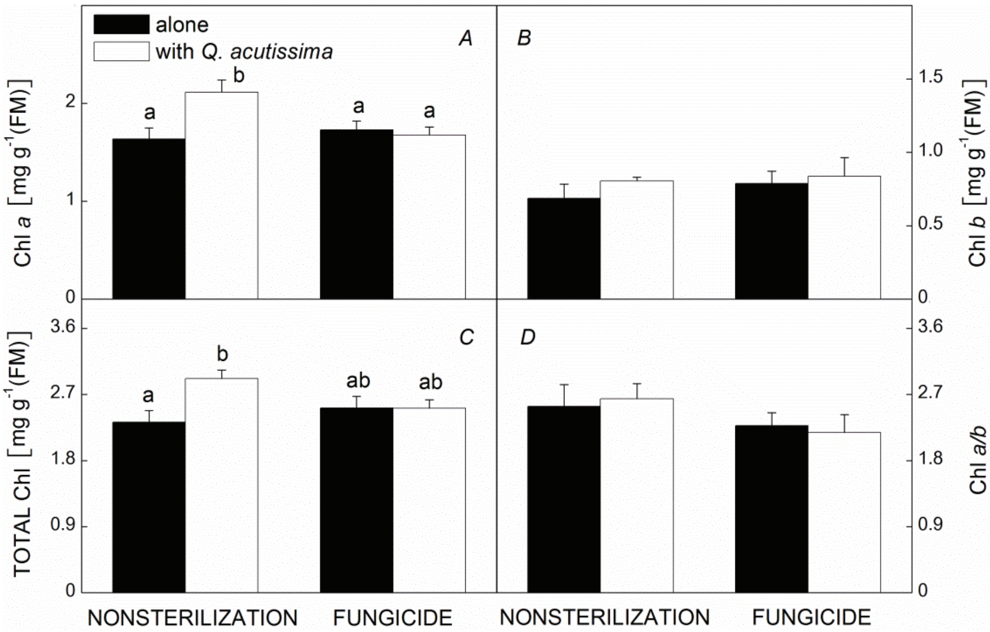

Fig. 4. Chlorophyll (Chl) content of *Robinia pseudoacacia* in response to the competition and soil sterilization treatments. The different *lowercase letters* (*A*,*C*) represent significant differences among treatments ($P \leq 0.05$, *Duncan*'s test). Error bars indicate one standard error of the mean ($n = 18$ for the nonsterilization and fungicide treatments).

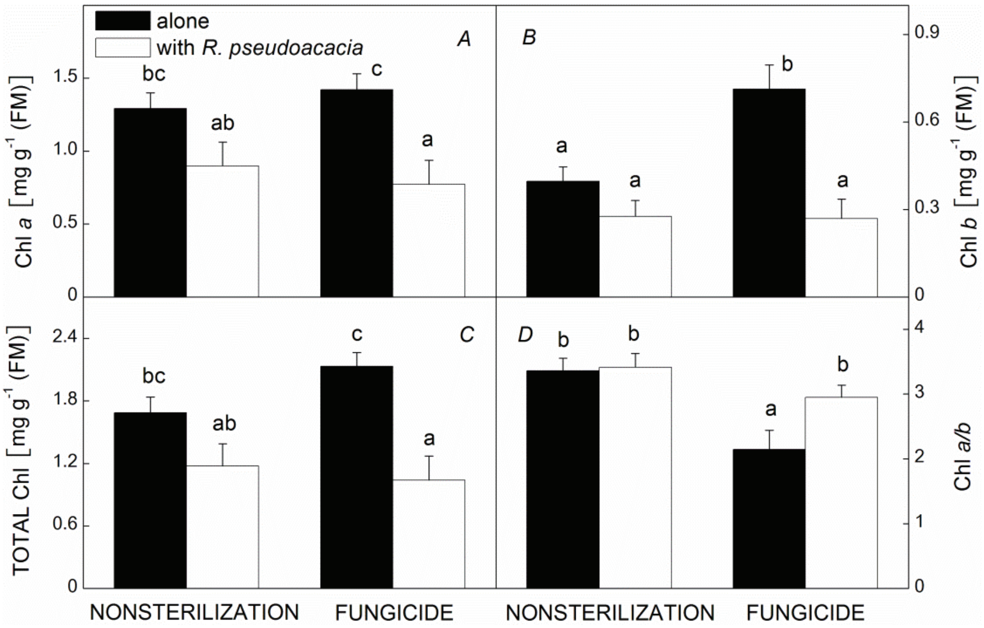

Fig. 5. Chlorophyll (Chl) content of *Quercus acutissima* in response to the competition and soil sterilization treatments. The different *lowercase letters* represent significant differences among treatments ($P \leq 0.05$, *Duncan*'s test). Error bars indicate one standard error of the mean ($n = 18$ for the nonsterilization and fungicide treatments).

ability to secrete metabolites that have antibacterial effects to decrease bacteria content, so after sterilization, the fungi in the soil decreased and the root nodules, which are constituted by various bacteria, increased because of less antibacterial fungal metabolites (Zhang *et al.* 2008a, Zhang *et al.* 2008b, Zhao and Zhou 2008, Popova *et al.* 2009, Qi *et al.* 2009, de Boer *et al.* 2010, Karaman *et al.* 2010). In both soil treatments, the root nodule biomass of *R. pseudoacacia* that grew alone was greater than that of the *R. pseudoacacia* that competed with *Q. acutissima.* This finding indicates that the effect of competition with *Q. acutissima* on *R. pseudoacacia* inhibited root nodule growth. The tendency of root nodule biomass was in accordance with total biomass of *R. pseudoacacia* in different treatments. It is generally considered that nitrogen fixation plays an important role in nutrient absorption and in increasing growth rate of leguminous plants. The restriction length fragment polymorphism (RFLP) analysis and the sequencing of 16S rRNA of bacteria in the root nodules isolated from the *R. pseudoacacia* distributed in China show that the root nodule bacteria were not diffused from America or Europe, but Chinese local bacteria that formed root nodules by specific adaption (Wei *et al.* 2009). The alien plant *R. pseudoacacia* in China might benefit from the local bacteria that constitute root nodules. Competition with *Q. acutissima* and sterilization affected the local root nodules, which ultimately influenced the growth of *R. pseudoacacia*.

The effects of soil sterilization on *R. pseudoacacia* and *Q. acutissima*: Although the biomass of the *R. pseudoacacia* in ST was greater than that in NSTs, its Chl content had no significant differences with that of the *R. pseudoacacia* without *Q. acutissima* in NSTs. Soil sterilization benefited the growth of root nodules, which provided more nutrients that contributed to the formation of Chl. Ultimately, *R. pseudoacacia* attained relatively higher photosynthetic activity to accumulate biomass.

The biomass of *Q. acutissima* with or without *R. pseudoacacia* in ST had no differences with those in NST, which suggests that the change in soil biota caused by the sterilization had no evident effect on the growth of *Q. acutissima*. When *Q. acutissima* grew alone, its Chl *b* in ST was greater than that in NST. This result is in agreement with that of *R. pseudoacacia*, and it indicates that after sterilization, the nutrient supply to plants is more abundant.

The different responses of *R. pseudoacacia* and *Q. acutissima* to sterilization support the finding that soil sterilization has no direct effect on the growth of plants, which is in agreement with previous studies and the sterilization increased the biomass of root nodules, which ultimately benefited nutrient absorption and the growth of *R. pseudoacacia* (Paul *et al.* 1989, 2001). The symbiosis of *R. pseudoacacia* with *Rhizobium* made it more sensitive to alteration of soil biota than *Q. acutissima*.

The competition between *R. pseudoacacia* and *Q. acutissima*: In NST, the existence of *Q. acutissima* decreased the growth of *R. pseudoacacia*, suggesting that *Q. acutissima* had evident inhibition effects on *R. pseudoacacia* in natural conditions. After sterilization of the soil, the existence of *Q. acutissima* still decreased the growth of *R. pseudoacacia* in terms of biomass, suggesting that the inhibition effect of *Q. acutissima* on *R. pseudoacacia* was not decreased even with the helpful effect of the sterilization on *R. pseudoacacia*.

After soil sterilization, the effect of competition with *R. pseudoacacia* decreased the growth of *Q. acutissima* in terms of biomass, Chl *a*, Chl *b*, and total Chl of *Q. acutissima*. This finding showed that after sterilization, the existence of *R. pseudoacacia* had an obvious inhibition effect on *Q. acutissima* and affected biomass accumulation and formation of Chl. The more intense competition effect of *R. pseudoacacia* after the sterilization was caused by the greater growth of *R. pseudoacacia*, which was benefited by sterilization of the soil.

The investigation of the effect of soil biota on the competition pattern of *R. pseudoacacia* and *Q. acutissima* was conducted in a greenhouse, thus, spatial space was limited. However, in the field, *Robinia pseudoacacia*, as a clonal plant, can produce massive clonal ramets and has the ability of clonal integration to explore resources in greater space (Zhang *et al.* 2006). The competition effect of *R. pseudoacacia* on *Q. acutissima* may be more intense in nature.

Conclusion: This study found the different responses of *R. pseudoacacia* and *Q. acutissima* to sterilization, which indicated that symbiosis of *R. pseudoacacia* with *Rhizobium* made it more sensitive than *Q. acutissima* to alteration of soil biota. Native *Q. acutissima* had a negative effect on the growth and dispersal of *R. pseudoacacia* in natural soil. However, after sterilization of the soil, *R. pseudoacacia* was less inhibited by *Q. acutissima*, and the competition of *R. pseudoacacia* decreased the growth of *Q. acutissima*. The more intense effect of competition after sterilization was caused by the greater growth of *R. pseudoacacia*, which was benefited by the soil sterilization. Soil sterilization increased the nodule biomass, which ultimately benefited the growth of *R. pseudoacacia*. Root nodule bacteria may be important in the dispersal and invasion process of nitrogen-fixing alien plants such as *R. pseudoacacia*.

References

Amaya-Carpio, L., Davies, F.T., Fox, T., He, C.: Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* ssp. *fistulosa*. – Photosynthetica **47**: 1-10, 2009.

Beckstead, J., Parker, I.M.: Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens? – Ecology **84**: 2824-2831, 2003.

Callaway, R.M., Mahall, B.E., Wicks, C., Pankey, J., Zabinski, C.: Soil fungi and the effects of an invasive forb on grasses: Neighbor identity matters. – Ecology **84**: 129-135, 2003.

Callaway, R.M., Newingham, B., Zabinski, C.A., Mahall, B.E.: Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbours. – Ecol. Lett. **4**: 429-433, 2001.

Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W., Gannon, J.E.: Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. – Ecology **85**: 1062-1071, 2004.

Chen, H., Liu, J., Zhang, Y.L., Wang, Q., Ge, X.L., Wei, Y.H., Wang, R.Q.: Influence of invasive plant *Coreopsis grandiflora* on the functional diversity of soil microbial communities. – J. Environ. Biol. **32**: 567-572, 2011.

Chittka, L., Schurkens, S.: Successful invasion of a floral market - An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. – Nature **411**: 653-653, 2001.

Corbin, J.D., D'Antonio, C.M.: Competition between native perennial and exotic annual grasses: Implications for an historical invasion. – Ecology **85**: 1273-1283, 2004.

Cronk, Q.C.B., Fuller, J.L.: Plant Invaders: The Threat to Natural Ecosystems. Chapman & Hall, London. 1995.

Cui, Q.G., He, W.M.: Soil biota, but not soil nutrients, facilitate the invasion of *Bidens pilosa* relative to a native species *Saussurea deltoidea*. – Weed Res. **49**: 201-206, 2009.

Dakora, F.D., Keya, S.O.: Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. – Soil Biol. Biochem. **29**: 809-817, 1997.

de Boer, W., Folman, L.B., Gunnewiek, P.J.A.K., Svensson, T., Bastviken, D., Oberg, G., del Rio, J.C., Boddy, L.: Mechanism of antibacterial activity of the white-rot fungus *Hypholoma fasciculare* colonizing wood. – Can. J. Microbiol. **56**: 380-388, 2010.

Dutta, R.K., Agrawal, M.: Effect of tree plantations on the soil characteristics and microbial activity of coal mine spoil land. – Tropical Ecol. **43**: 315-324, 2002.

Gupta, K., Bishop, J., Peck, A., Brown, J., Wilson, L., Panda, D.: Antimitotic antifungal compound benomyl inhibits brain microtubule polymerization and dynamics and cancer cell proliferation at mitosis, by binding to a novel site in tubulin. – Biochemistry **43**: 6645-6655, 2004.

He, W.M., Dong, M.: Plasticity in physiology and growth of *Salix matsudana* in response to simulated atmospheric temperature rise in the Mu Us Sandland. – Photosynthetica **41**: 297-300, 2003.

Hetrick, B.A.D., Wilson, G.W.T., Hartnett, D.C.: Relationship between mycorrhizal dependence and competitive ability of two tallgrass prairie grasses. – Can. J. Bot. **67**: 2608-2615, 1989.

Johnson, N.C., Graham, J.H., Smith, F.A.: Functioning of mycorrhizal associations along the mutualism-parasitism continuum. – New Phytol. **135**: 575-586, 1997.

Jung, S.C., Matsushita, N., Wu, B.Y., Kondo, N., Shiraishi, A., Hogetsu, T.: Reproduction of a *Robinia pseudoacacia* population in a coastal *Pinus thunbergii* windbreak along the Kujukurihama Coast, Japan. – J. Forest Res.-JPN **14**: 101-110, 2009.

Karaman, M., Jovin, E., Malbasa, R., Matavuly, M., Popovic, M.: Medicinal and edible lignicolous fungi as natural sources of antioxidative and antibacterial agents. – Phytother Res. **24**: 1473-1481, 2010.

Killingbeck, K.T.: Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. – Ecology **77**: 1716-1727, 1996.

Knevel, I.C., Lans, T., Menting, F.B.J., Hertling, U.M., van der Putten, W.H.: Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. – Oecologia **141**: 502-510, 2004.

Kuhn, I., Durka, W., Klotz, S.: BiolFlor - a new plant-trait database as a tool for plant invasion ecology. – Diversity Distributions **10**: 363-365, 2004.

Kuster, E.C., Kuhn, I., Bruelheide, H., Klotz, S.: Trait interactions help explain plant invasion success in the German flora. – J. Ecol. **96**: 860-868, 2008.

Kyle, G., Leishman, M.R.: Functional trait differences between extant exotic, native and extinct native plants in the hunter river, NSW: a potential tool in riparian rehabilitation. – River Res. Appl. **25**: 892-903, 2009.

Landgraf, D., Wedig, S., Klose, S.: Medium- and short-term available organic matter, microbial biomass, and enzyme activities in soils under *Pinus sylvestris* L. and *Robinia pseudoacacia* L. in a sandy soil in NE Saxony, Germany. – J. Plant Nutr. Soil Sc**.** **168**: 193-201, 2005.

Leishman, M.R., Haslehurst, T., Ares, A., Baruch, Z.: Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. – New Phytol. **176**: 635-643, 2007.

Levine, J.M., Adler, P.B., Yelenik, S.G.: A meta-analysis of biotic resistance to exotic plant invasions. – Ecol. Lett. **7**: 975-989, 2004.

Liancourt, P., Callaway, R.M., Michalet, R.: Stress tolerance and competitive-response ability determine the outcome of biotic interactions. – Ecology **86**: 1611-1618, 2005.

Lichtenthaler, H.K., Wellburn, A.R.: Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. Trans. **11**: 591-592, 1983.

Littschwager, J., Lauerer, M., Blagodatskaya, E., Kuzyakov, Y.: Nitrogen uptake and utilisation as a competition factor between invasive *Duchesnea indica* and native *Fragaria vesca*. – Plant Soil **331**: 105-114, 2010.

Niu, H.B., Liu, W.X., Wan, F.H., Liu, B.: 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, 2007.

Noh, N.J., Son, Y., Kim, R.H., Seo, K.W., Koo, J.W., Park, I.H., Lee, Y.J., Lee, K.H., Son, Y.M.: Biomass accumulations and the distribution of nitrogen and phosphorus within three *Quercus acutissima* stands in Central Korea. – J. Plant Biol. **50**: 461-466, 2007.

Pattison, R.R., Goldstein, G., Ares, A.: Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. – Oecologia **117**: 449-459, 1998.

Paul, A.L., Semer, C., Kucharek, T., Ferl, R.J.: The fungicidal and phytotoxic properties of benomyl and PPM in supplemented agar media supporting transgenic arabidopsis plants for a Space Shuttle flight experiment. – Appl. Microbiol. Biot. **55**: 480-485, 2001.

Paul, N.D., Ayres, P.G., Wyness, L.E.: On the use of fungicides for experimentation in natural vegetation. – Funct. Ecol. **3**: 759-769, 1989.

Popova, M., Trusheva, B., Gyosheva, M., Tsvetkova, I., Bankova, V.: Antibacterial triterpenes from the threatened wood-decay fungus *Fomitopsis rosea*. – Fitoterapia **80**: 263-266, 2009.

Qi, S.H., Xu, Y., Xiong, H.R., Qian, P.Y., Zhang, S.: Antifouling and antibacterial compounds from a marine fungus *Cladosporium* sp F14. – World J. Microb. Biot. **25**: 399-406, 2009.

Reinhart, KO, Callaway, RM: Soil biota and invasive plants. – New Phytol. **170**: 445-457, 2006.

Reinhart, K.O., Packer, A., Van der Putten, W.H., Clay, K.: Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. – Ecol. Lett. **6**: 1046-1050, 2003.

Rice, S.K., Westerman, B., Federici, R.: Impacts of the exotic, nitrogen-fixing black locust (*Robinia pseudoacacia*) on nitrogen-cycling in a pine-oak ecosystem. – Plant Ecol. **174**: 97-107, 2004.

Shochat, E., Lerman, S.B., Anderies, J.M., Warren, P.S., Faeth, S.H., Nilon, C.H.: Invasion, competition, and biodiversity loss in urban ecosystems. – Bioscience **60**: 199-208, 2010.

Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., Molina, R.: Net transfer of carbon between ecto-mycorrhizal tree species in the field. – Nature **388**: 579-582, 1997.

Smith, M.D., Hartnett, D.C., Rice, C.W.: Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. – Soil Biol. Biochem. **32**: 935-946, 2000.

Šraj-Kržič, N., Pongrac, P., Regvar, M., Gaberscik, A.: Photon-harvesting efficiency and arbuscular mycorrhiza in amphibious plants. – Photosynthetica **47**: 61-67, 2009.

Taniguchi, T., Tanaka, C., Tamai, S., Yamanaka, N., Futai, K.: Identification of *Cylindrocladium* sp causing damping-off disease of Japanese black pine (*Pinus thunbergii*) and factors affecting the disease severity in a black locust (*Robinia pseudoacacia*)-dominated area. – J. Forest Res.-JPN **13**: 233-240, 2008.

Van der Putten, W.H., Peters, B.A.M.: How soil-borne pathogens may affect plant competition. – Ecology **78**: 1785-1795, 1997.

Von Holle, B., Joseph, K.A., Largay, E.F., Lohnes, R.G.: Facilitations between the introduced nitrogen-fixing tree, *Robinia pseudoacacia*, and nonnative plant species in the glacial outwash upland ecosystem of cape cod, MA. – Biodivers. Conserv. **15**: 2197-2215, 2006.

Wang, N., Yu, F.H., Li, P.X., He, W.M., Liu, F.H., Liu, J.M., Dong, M.: Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress. –Ann. Bot. **101**: 671-678, 2008.

Wang, R.Q., Zhou, G.Y.: [The Vegetation of Shandong Province.] – Shandong Science Technology Publisher, Jinan 2000. [In Chin.]

Wei, G.H., Chen, W.M., Zhu, W.F., Chen, C., Young, J.P.W., Bontemps, C.: Invasive *Robinia pseudoacacia* in China is nodulated by *Mesorhizobium* and *Sinorhizobium* species that share similar nodulation genes with native American symbionts. – FEMS Microbiol. Ecol. **68**: 320-328, 2009.

Woo, S.Y., Kwon, K.W., Lee, J.C., Choi, J.H., Kang, B.S.: Recovery of net photosynthetic rate after SO_2 fumigation in *Quercus acutissima*, *Pinus densiflora*, *Populus alba* × *glandulosa*, and *Acanthopanax sessiliflorus*. – Photosynthetica **41**: 319-320, 2003.

Xu, C.Y., Griffin, K.L., Schuster, W.S.F.: Leaf phenology and seasonal variation of photosynthesis of invasive *Berberis thunbergii* (Japanese barberry) and two co-occurring native understory shrubs in a northeastern United States deciduous forest. – Oecologia **154**: 11-21, 2007.

Yu, H., He, W.M., Liu, J., Miao, S.L., Dong, M.: Native *Cuscuta campestris* restrains exotic *Mikania micrantha* and enhances soil resources beneficial to natives in the invaded communities. – Biol. Invasions **11**: 835-844, 2009.

Zhang, D., Yang, X., Kang, J.S., Choi, H.D., Son, B.W.: Chlorohydroaspyrones A and B, antibacterial aspyrone derivatives from the marine-derived fungus *Exophiala* sp. – J. Nat. Prod. **71**: 1458-1460, 2008a.

Zhang, H.Y., Xie, H.H., Qiu, S.X., Xue, J.H., Wei, X.Y.: Heteroatom-containing antibacterial phenolic metabolites from a terrestrial *Ampelomyces* fungus. – Biosci. Biotech. Bioch. **72**: 1746-1749, 2008b.

Zhang, X.Q., Liu, J., Welham C.V.J., Liu, C.C., Li, D.N., Chen, L., W, R.Q.: The effects of clonal integration on morphological plasticity and placement of daughter ramets in black locust (*Robinia pseudoacacia*). – Flora **201**: 547-554, 2006.

Zhao, J., Zhou, L. Antibacterial activity of endophytic fungi from rhizomes of *Paris polyphylla* var. *yunnanensis*. – Phytopathology **98**: S180-S180, 2008.

Zheng, Y.L., Feng, Y.L., Liu, W.X., Liao, Z.Y.: Growth, biomass allocation, morphology, and photosynthesis of invasive *Eupatorium adenophorum* and its native congeners grown at four irradiances. – Plant Ecol. **203**: 263-271, 2009.