DENDROBIOLOGY
2012, vol. 67, 3–13

*Wenjuan Ding, Renqing Wang, Yifu Yuan, Xiaoqin Liang, Jian Liu**

Effects of nitrogen deposition on growth and relationship of *Robinia pseudoacacia* and *Quercus acutissima* seedlings

Received: 13 June 2011; Accepted: 25 October 2011

Abstract: Increased nitrogen deposition and biological invasions may be two crucial consequences of global change. Exotic plants often have adverse effects on native plants. However, there are reports of occasional positive interaction between them. Increased nitrogen deposition enhances nitrogen availability in soil, which may facilitate the growth of some exotic plant and decrease the competitive advantage of native plants because of their adaptation to low-nutrient environments. Consequently, increased nitrogen deposition may regulate the relationship between exotic and native plants. A greenhouse experiment was, thus, conducted to examine the effects of increased nitrogen deposition on the relationship between the native *Quercus acutissima* and the exotic *Robinia pseudoacacia*, which are both widely distributed in North China. Seedlings of both species were grown in monoculture and mixture arrangements in a pot experiment with different nitrogen addition levels (i.e., 0, 2, 5, and 10 g N m^{-2} a^{-1}).

Nitrogen addition altered the biomass partitioning of *Q. acutissima* and *R. pseudoacacia* seedlings, with more biomass allocated to leaves and less to roots. Although *R. pseudoacacia* seedlings always had growth predominance over *Q. acutissima* seedlings, *Q. acutissima* was not threatened by *R. pseudoacacia* at the four levels of nitrogen addition during the three-month duration of the experiment. A positive interaction between the seedlings of *R. pseudoacacia* and *Q. acutissima* was found, and increased nitrogen deposition did not affect the positive interaction. Further studies are necessary to determine whether or not *R. pseudoacacia* is harmful to native ecosystems and should be limited for forestation in North China.

Additional key words: Biological invasions; Competition; Facilitation; Nitrogen fixing; Positive interaction

Addresses: W.J. Ding, Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University, Jinan 250100, P. R. China
R.Q. Wang, Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University, Jinan 250100, P. R. China, Institute of Environmental Research, Shandong University, Jinan 250100, P.R. China, Shandong Provincial Engineering and Technology Research Center for Vegetation Ecology, Shandong University, Jinan 250100, P. R. China
Y.F. Yuan, Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University, Jinan 250100, P. R. China
X.Q. Liang, Institute of Environmental Research, Shandong University, Jinan 250100, P.R. China
*(Corresponding author) J. Liu, Institute of Environmental Research, Shandong University, Jinan 250100, P.R. China, E-mail: ecology@sdu.edu.cn

Introduction

Over the last century, human activities such as fossil fuel combustion and agricultural fertilizer use have contributed to a three- to five-fold increase in nitrogen emissions and elevated nitrogen depositions toward land and oceans (Reay et al. 2008). Evidence of the corresponding increase of atmospheric nitrogen depositions has been observed in North America, Europe, and Asia (Matson et al. 2002; Holland et al. 2005; Mo et al. 2008). And such increases are expected to continue in the future (Galloway et al. 2004; Reay et al. 2008). Consequently, the effects of nitrogen deposition on terrestrial ecosystems have aroused widespread concern from ecologists (Palátová 2004; Mo et al. 2008; Bedison and McNeil 2009; Maskell et al. 2010). In general, permanent nitrogen addition could increase plant growth and net primary production in N-limited temperate ecosystems (Aber et al. 1993; Magill et al. 1997). On the other hand, over-enrichment of nitrogen in soil causes loss of nitrogen retention capacity, soil eutrophication, acidification, and the enhancement of carbon dioxide emissions to the environments; all may interact to regulate species relationships and result in the alteration of species composition and ecosystem decline (Matson et al. 2002; Maskell et al. 2010).

Biological invasions may occur when an organism is transported to a new range and are made to settle there due to human migration and commerce (Mack et al. 2000). Hence, biological invasions have also been widely investigated in recent years (Callaway and Aschehoug 2000; Vilà and Weiner 2004; Funk and Vitousek 2007; Bradley et al. 2010). Exotic plants can alter hydrology, nutrient cycling, and plant productivity in an ecosystem, suppress native plants, resulting in the loss of their abundance and biodiversity, and may replace dominant species to threaten entire natural systems (Mack et al. 2000). When such plants are introduced into a community, exotic and native plants often coexist in the ecosystems, and competition for limited resources (e.g., light, water, or soil nutrients) is likely to become the first interaction between them (Vilà and Weiner 2004). Apart from this negative interaction (competition), co-occurring plants may also have a positive interaction (facilitation), in which at least one plant benefits and no harm is done to either plant (Bruno et al. 2003; Valiente-Banuet and Verdú 2008). Competition and facilitation may be balanced in plant populations and may be affected by the gradients of abiotic environments (Callaway and Walker 1997; Chu et al. 2008).

Nitrogen availability in soil has increased by elevated atmospheric nitrogen deposition (Bradley et al. 2010). Whereas nitrogen is the primary limiting resource for plant growth in many ecosystems, as articulated in LeBauer and Treseder (2008), increased atmospheric nitrogen deposition may also affect the interaction between exotic and native plants (Brooks 2003; Scherer-Lorenzen et al. 2007). Native plants, which usually adapt to low resource availability, often exhibit competitive advantage under low-nutrient conditions (Perry et al. 2010). However, high nutrient levels generally benefit growth and facilitate invasions for many exotic plants that have high relative growth rates and competitive advantage in high-nutrient environments, whereas native plants are less competitive under high-nutrient conditions than exotic species (Lowe et al. 2003; Bradley et al. 2010). Therefore, exotic plants may suppress native species and dominate plant communities under high-nutrient conditions. An investigation of the effects of nitrogen deposition on the interaction between exotic and native species will provide valuable data on the prediction of some possible future effects of exotic plants on native plants in the context of global change, for which only few relevant studies exist.

Robinia pseudoacacia L., a nitrogen-fixing deciduous species of the Leguminosae family, native to North America and now widely distributed over Europe, Asia, and Australia (Von Holle et al. 2006), is the second most abundant deciduous tree in the world and was declared as one of the most aggressive invaders worldwide (Boring and Swank 1984a; Scherer-Lorenzen et al. 2007). Weber et al. (2008) listed *R. pseudoacacia* as one of the predominant plants in China that invade nutrient-poor ecosystems, thereby increasing soil nitrogen availability, enhancing nitrogen return in litterfall, and elevating nitrogen mineralization rates (Boring and Swank 1984b; Rice et al. 2004; Von Holle et al. 2006). On the other hand, *Quercus acutissima* Carr. belongs to the Fagaceae family, and is the predominant native broad-leaved deciduous tree species in North China (Wang and Zhou 2000). Increased atmospheric nitrogen deposition may have different effects on the growth and interactions of these species. We conducted a greenhouse experiment to study the effects of nitrogen deposition on the growth of *R. pseudoacacia* and *Q. acutissima* seedlings and their interaction. Generally, seedling development is the most critical period in the life history of trees and this period is sensitive to environmental changes (Xu et al. 2009). Seedlings of both species were grown in single and mixed-species arrangements, with nitrogen addition at four levels: 0, 2, 5, and 10 g N m^{-2} a^{-1}.

Based on the above objectives, the following research questions were addressed: (1) Does increased nitrogen deposition affect the aboveground and belowground biomass of *Q. acutissima* and *R. pseudoacacia* seedlings? (2) Does increased nitrogen deposition affect seedling interaction between the two species?

Materials and methods

Site description

The experiment was conducted at the Fanggan Research Station of Shandong University, Shandong Province, China (36°26'N, 117°27'E). The area is characterized by a warm temperate monsoon climate, with a mean annual temperature of 13°C ± 1°C. Annual average precipitation is approximately 600 mm to 850 mm, most of which falls from June to September. The area has a terrain typically comprising of hills and mountains with the altitude ranging 300 m to 450 m. The soil type of this region is yellow cinnamon, and the parent material is limestone (Xu et al. 2009).

Mixed forests of the warm temperature zone are the predominant vegetation in this area. The canopy of the forests is up to 14 m in height and consists of three evergreen coniferous species (*Pinus densiflora* Sieb. et Zucc., *Pinus thunbergii* Parl., and *Platycladus orientalis* (L.) Franco) and two deciduous broadleaf species (*Robinia pseudoacacia* L. and *Quercus acutissima* Carr.). *Vitex negundo* L. var. *heterophylla* (Franch.) Rehd. and *Zizyphus jujube* Mill. var. *spinosa* (Bge.) Hu ex H. F. Chow are abundant shrubs understory (Wang and Zhou 2000, Xu et al. 2009).

Plant materials

Acorns of *Q. acutissima* and seeds of *R. pseudoacacia* were collected from a hill near the research station during the autumn of 2009 and were put to germinate in April 2010 in a greenhouse of the research station. When radicles reached 10mm to 20 mm in length, the seedlings were transplanted into plastic pots (24 cm diameter × 25 cm height) containing 3:2 mixture of loam and humic soil. Both species were planted in monoculture (two individuals of one species per pot) and mixture arrangement (two individuals per pot: one *Q. acutissima* and one *R. pseudoacacia*). During the experiment, all pots were watered every two days to keep the soil water content saturated and subjected to weed and insect control.

Experimental design

Two factors, plant cultivation and nitrogen addition, were conducted for the plants in the experiment. Four nitrogen-addition treatments [0 (control, without nitrogen added), 2, 5, and 10 g N m^{-2} a^{-1}] were applied to all three plant cultivation treatments, each having a monoculture of two *Q. acutissima* individuals, a monoculture of two *R. pseudoacacia* individuals, and a mixture of one *Q. acutissima* and one *R. pseudoacacia*. Six replicates for each of the twelve treatment combinations were applied in total.

The nitrogen-addition treatments began on June 5, 2010. For each nitrogen-addition treatment, equal amounts of ammonium nitrate (NH_4NO_3) solution were added to each pot every 15 days during the period of the experiment. As there were a total of 24 NH_4NO_3 solution applications in one year, the quantity of each application was equivalent to 1/24 of the total amount applied in one year. The fertilizer was weighed, dissolved in 500 ml of water, and applied to each pot except for the control pots. The control pots were watered with 500 ml of water without nitrogen. The experiment was completed on September 15, 2010.

Measurements

Heights of the seedlings were measured at the beginning of every month during the experiment starting from the first nitrogen-addition treatment on June 5, 2010 to the last on September 5, 2010. Height increments for each month were calculated in June, July, and August. At the end of the experiment, the plants were harvested, and soil from the roots was carefully rinsed. The harvested plant materials were divided into three parts consisting of the leaf, stem, and root parts. The roots were further divided into the main roots (MR) and the lateral roots (LR). The samples were oven-dried at 80°C for approximately 48 h to obtain constant mass, and then weighed. Root-shoot ratio was calculated and biomass allocation in each part was expressed in terms of the proportion of total biomass. The MR/LR ratio was represented as MR divided by LR. Biomass in each pot of the monoculture treatment was averaged from the biomass of both plants in the pot. The relative dominance index (RDI) was used to estimate the dominance of *Q. acutissima* and *R. pseudoacacia* when they were grown in a mixed setting (Myers and Bazely 2003; Niu et al. 2007). The RDI formula used in the computation is as follows:

One species RDI = biomass of this species /total biomass of the two species

After oven-drying, mature leaves were selected for leaf nitrogen concentration analysis using the Kieldahl method; the procedure was conducted at Shandong Agricultural University. In pots with two monoculture individual seedlings, only one seedling was analyzed to represent the leaf nitrogen concentration. Available soil nitrogen before and after the experiment was analyzed using the alkali-diffusion method, and the analysis was also performed at Shandong Agricultural University. Three soil samples were selected before the experiment to represent soil background values. Soil samples from each pot were collected after experiment.

Statistical analysis

Two-way ANOVA was performed separately on *Q. acutissima* and *R. pseudoacacia* to examine the effects of plant cultivation and nitrogen addition on biomass,

biomass partitioning, and leaf nitrogen concentration. Biomass values obtained from two seedlings of *R. pseudoacacia* were found to be too extreme for inclusion in the experimental data, and were thus excluded from further analyses. Species RDI with a one-way ANOVA was also expressed separately for *Q. acutissima* and *R. pseudoacacia*. Paired t-test for RDI between the two species was also conducted at each nitrogen level. Repeated-measures ANOVAs were conducted to test the effects of the factors, including growth period, plant cultivation, and nitrogen addition, on height increment. Available soil nitrogen at different nitrogen levels was also examined separately using one-way ANOVA against three plant cultivation treatments. All analyses were conducted using SPSS Version 13.0 (SPSS Inc., Chicago, IL) and performed at a level of significance of 0.05. All figures were drawn using the OriginPro Version 7.5 (OriginLab Co., Northampton, MA).

Results

Biomass and biomass allocation

Nitrogen addition significantly affected biomass allocation for *Q. acutissima* (Table 1). Root-shoot ratios in the groups with 5 and 10 g m^{-2} a^{-1} nitrogen addition were 23% and 32% lower, respectively, than that in the control group (Table 2). As the amount of applied nitrogen increased, the aboveground biomass allocation also increased and the root biomass allocation decreased. Compared with the control group, the groups with 5 and 10 g m^{-2} a^{-1} levels of nitrogen addition registered 14% and 16% higher leaf biomass ratios, and 9% and 13% lower root biomass ratios, respectively (Table 2). MR/LR ratios in the seedlings of *Q. acutissima* significantly decreased as the levels of nitrogen addition increased (Table 2), whereas other biomass components, such as total biomass and individual-part biomass, did not change significantly (Table 1). Biomass and biomass allocation of *Q. acutissima* were not significantly different whether grown in the monoculture or mixture with *R. pseudoacacia* (Table 1). Also, no significant interaction between plant cultiva-

Table 1. Results of two-way ANOVA for the effects of plant cultivation (C) and nitrogen addition (N) on biomass and biomass allocation in *Quercus acutissima* and *Robinia pseudoacacia*

Measurements	C		N		C × N	
	df	*F*	*df*	*F*	*df*	*F*
Quercus acutissima						
Total biomass	1	0.087	3	0.059	3	2.187
Leaf biomass	1	0.044	3	1.054	3	1.418
Stem biomass	1	0.586	3	0.440	3	2.940*
Root biomass	1	0.892	3	0.501	3	1.816
Root-shoot ratio	1	1.025	3	4.169*	3	0.392
Leaf biomass ratio	1	0.396	3	3.210*	3	0.454
Stem biomass ratio	1	2.922	3	1.726	3	1.491
Root biomass ratio	1	1.772	3	4.062*	3	0.367
MR biomass	1	0.999	3	0.993	3	1.431
LR biomass	1	0.202	3	0.560	3	2.347
MR/LR	1	0.053	3	4.623**	3	1.566
Robinia pseudoacacia						
Total biomass	1	5.027*	3	0.980	3	0.768
Leaf biomass	1	4.409*	3	1.298	3	1.635
Stem biomass	1	3.631	3	0.986	3	0.607
Root biomass	1	7.088*	3	0.755	3	0.416
Root-shoot ratio	1	6.251*	3	3.768*	3	1.956
Leaf biomass ratio	1	3.141	3	1.059	3	0.225
Stem biomass ratio	1	0.001	3	0.675	3	0.464
Root biomass ratio	1	5.801*	3	3.972*	3	1.826
MR biomass	1	7.896**	3	0.890	3	0.635
LR biomass	1	5.862*	3	0.911	3	0.487
MR/LR	1	1.040	3	2.072	3	1.373

*Significance level at $p < 0.05$. **Significance level at $p < 0.01$.

Table 2. Root-shoot ratio, leaf biomass ratio, and root biomass ratio at four nitrogen addition levels for *Quercus acutissima* and *Robinia pseudoacacia*, respectively. Means ± SE. Different letters in a column represent significant difference among four nitrogen treatments at $p < 0.05$ by Duncan's test

Nitrogen level (g m^{-2} a^{-1})	Root-shoot ratio	Leaf biomass ratio	Root biomass ratio	MR/LR
Quercus acutissima				
0	0.151 ± 0.03 a	0.263 ± 0.01 b	0.585 ± 0.01 a	0.820 ± 0.04 a
2	0.087 ± 0.03 ab	0.294 ± 0.01 ab	0.549 ± 0.02 ab	0.706 ± 0.03 b
5	0.057 ± 0.02 b	0.305 ± 0.01 a	0.532 ± 0.01 b	0.678 ± 0.02 b
10	0.027 ± 0.02 b	0.311 ± 0.01 a	0.516 ± 0.01 b	0.695 ± 0.03 b
Robinia pseudoacacia				
0	0.512 ± 0.04 a	0.380 ± 0.01 a	0.335 ± 0.01 a	0.766 ± 0.04 a
2	0.481 ± 0.04 ab	0.370 ± 0.02 a	0.321 ± 0.02 ab	0.640 ± 0.06 a
5	0.394 ± 0.02 b	0.411 ± 0.02 a	0.281 ± 0.01 b	0.586 ± 0.05 a
10	0.409 ± 0.04 b	0.401 ± 0.02 a	0.286 ± 0.02 b	0.775 ± 0.09 a

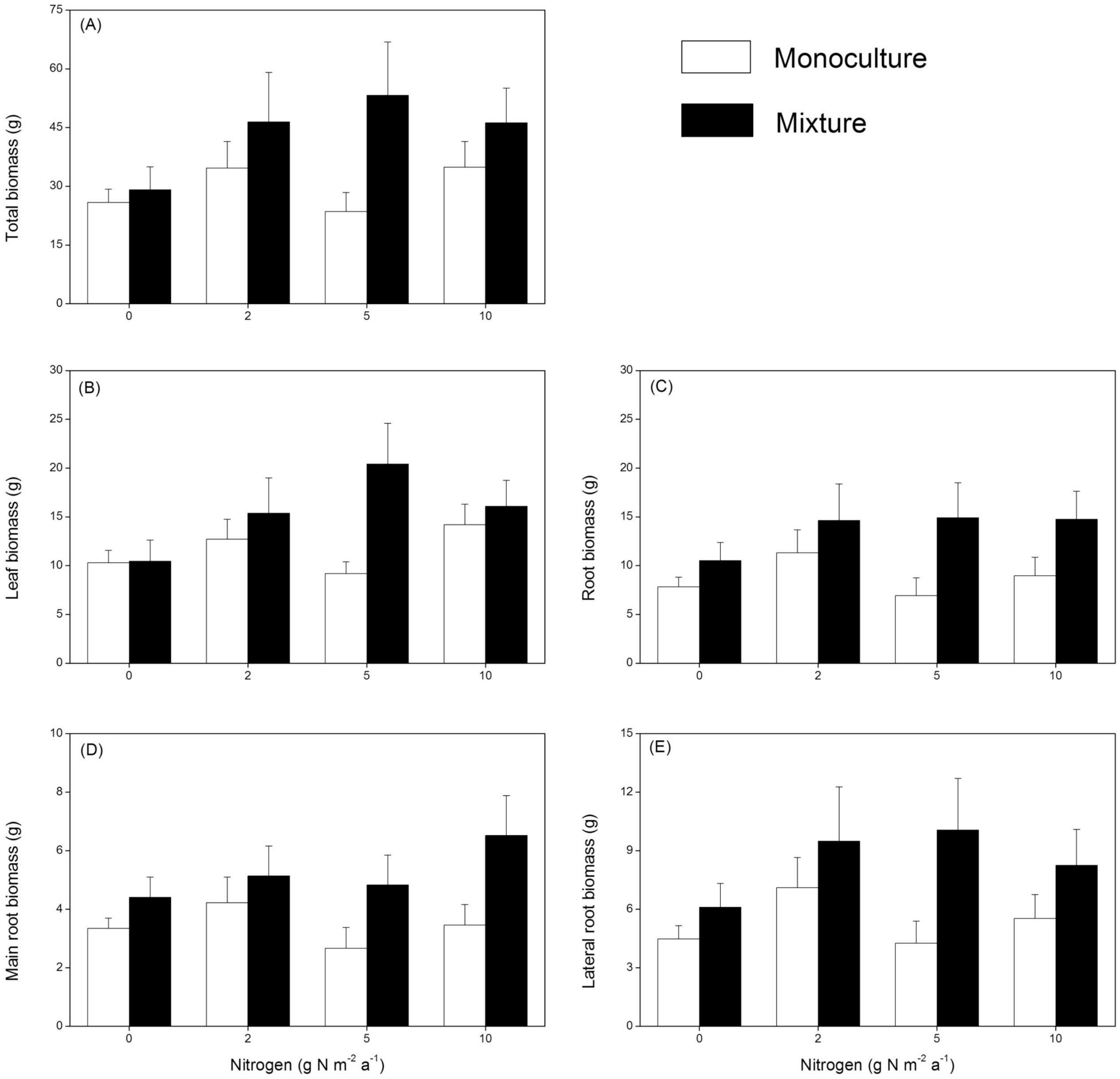

Fig. 1. Biomass in *Robinia pseudoacacia* grown in monoculture and mixture at the four nitrogen addition levels: (A) total biomass, (B) leaf biomass, (C) root biomass, (D) MR biomass and (E) LR biomass. Values are presented as mean + SE

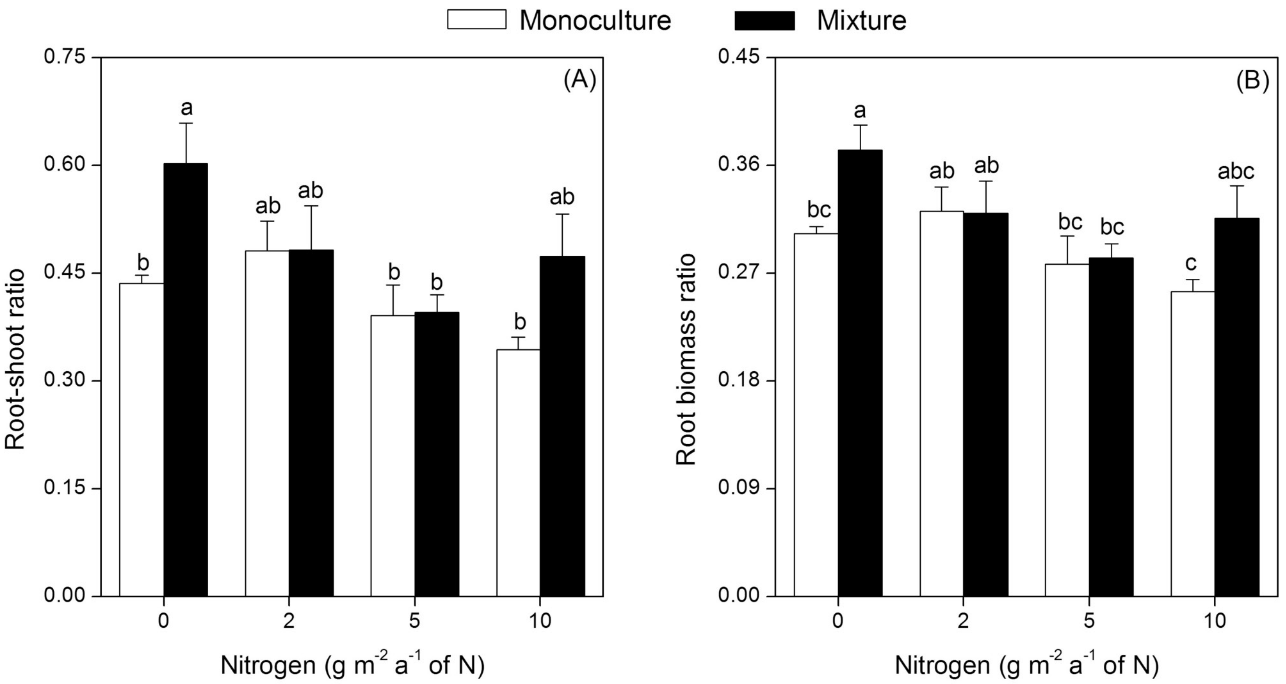

Fig. 2. Biomass allocation in *Robinia pseudoacacia* grown in monoculture and mixture at four nitrogen addition levels: (A) root-shoot ratio and (B) root biomass ratio. Values are presented as mean + SE. Different letters denote significant differences at $p < 0.05$ by Duncan's test

tion and nitrogen addition on biomass and biomass allocation in *Q. acutissima* was confirmed (Table 1).

Total biomass of *R. pseudoacacia* in the mixture was 48% higher than that in the monoculture (Fig. 1A), leaf biomass and root biomass in the mixture were 35% and 57% higher than those in the monoculture, respectively (Figs. 1B and 1C). The main effect of plant cultivation in *R. pseudoacacia* was significant on MR and LR biomass, which were 52% and 59% greater in the mixture than in the monoculture, respectively (Table 1, Figs. 1D and 1E). Root-shoot and root biomass ratios were significantly influenced by plant cultivation and nitrogen addition, independently (Table 1). *R. pseudoacacia* grown in the mixture had higher root biomass allocation compared to that in the monoculture, especially in the control group (Fig. 2). As nitrogen addition increased, root biomass ratio decreased by 14% and 16% in the groups with 5 and 10 g m^{-2} a^{-1} nitrogen addition levels, respectively (Table 2). No significant interaction between plant cultivation and nitrogen addition on biomass and its allocation in *R. pseudoacacia* was confirmed (Table 1).

Height increment

Height increment for *Q. acutissima* was significantly different during the three-month duration (Table 3). The largest height increment was observed in July, whereas the lowest was in August (Table 4). Nitrogen addition significantly affected the height increment during the three months (Table 3). In June, when ni-

Table 3. Results of repeated-measures ANOVA for the effects of growth period (P), plant cultivation (C) and nitrogen addition (N) on the height increment in *Quercus acutissima* and *Robinia pseudoacacia*

Source	*Quercus acutissima*			*Robinia pseudoacacia*		
	df	*F*	*p*	*df*	*F*	*p*
P	2	34.580	**<0.001**	2	46.155	**<0.001**
P × C	2	2.502	0.086	2	0.511	0.601
P × N	6	3.094	**0.007**	6	1.073	0.382
P × C × N	6	2.000	0.070	6	0.843	0.539
Error (P)	128			128		
C	1	1.353	0.249	1	3.098	0.083
N	3	1.656	0.185	3	0.633	0.596
C × N	3	1.865	0.144	3	0.284	0.837
Error	64			64		

Figures in bold were significant at the level of 0.05.

Table 4. Height increment (mm) for three months at four nitrogen addition levels for *Quercus acutissima* and *Robinia pseudoacacia*, respectively. Means ± SE. Different letters in a column represent significant difference among four nitrogen treatments at $p < 0.05$ by Duncan's test

Nitrogen level (g m^{-2} a^{-1})	June	July	August
Quercus acutissima			
0	113 ± 10.4 a	104 ± 11.2 a	23 ± 6.6 b
2	69 ± 10.3 b	108 ± 12.7 a	57 ± 13.2 a
5	82 ± 6.0 b	93 ± 10.1 a	46 ± 12.4 ab
10	89 ± 9.8 ab	127 ± 10.1 a	70 ± 9.4 a
Robinia pseudoacacia			
0	257 ± 3.9 a	159 ± 33.6 a	122 ± 18.6 a
2	344 ± 3.7 a	163 ± 29.8 a	159 ± 38.9 a
5	358 ± 4.3 a	178 ± 32.2 a	150 ± 27.8 a
10	334 ± 4.1 a	157 ± 25.1 a	180 ± 33.2 a

trogen was applied, height increment of the other groups was lower than that in the control group, but a significant increase was observed with nitrogen application in August (Table 4).

In case of *R. pseudoacacia*, the height increments gradually decreased during the three-month period following nitrogen addition (Table 3 and Table 4). Neither plant cultivation nor nitrogen addition significantly affected height increments during the three months (Table 3).

Leaf nitrogen concentration

The leaf nitrogen concentration of *Q. acutissima* was significantly affected by nitrogen ($F_{3,40} = 9.34$, $p < 0.001$), plant cultivation ($F_{1,40} = 11.87$, $p = 0.001$) and their interaction ($F_{3,40} = 6.92$, $p = 0.001$). When nitrogen level was increased to 5 and 10 g m^{-2} a^{-1}, leaf nitrogen concentration in *Q. acutissima* significantly increased compared with the control values (Fig. 3A). Only the two highest nitrogen addition levels in the *Q. acutissima* grown in mixture indicated significantly enhanced leaf nitrogen concentration compared to that in monoculture (Fig. 3A). Leaf nitrogen concentration of *R. pseudoacacia* was not significantly altered either by nitrogen ($F_{3,40} = 0.22$, $p = 0.88$) or by plant cultivation ($F_{1,40} = 2.18$, $p = 0.15$).

Dominance of *Q. acutissima* and *R. pseudoacacia*

RDI for *Q. acutissima* was lower than *R. pseudoacacia* in all four nitrogen addition levels, especially at 0 and 5 g m^{-2} a^{-1} (Table 5). The findings indicated that

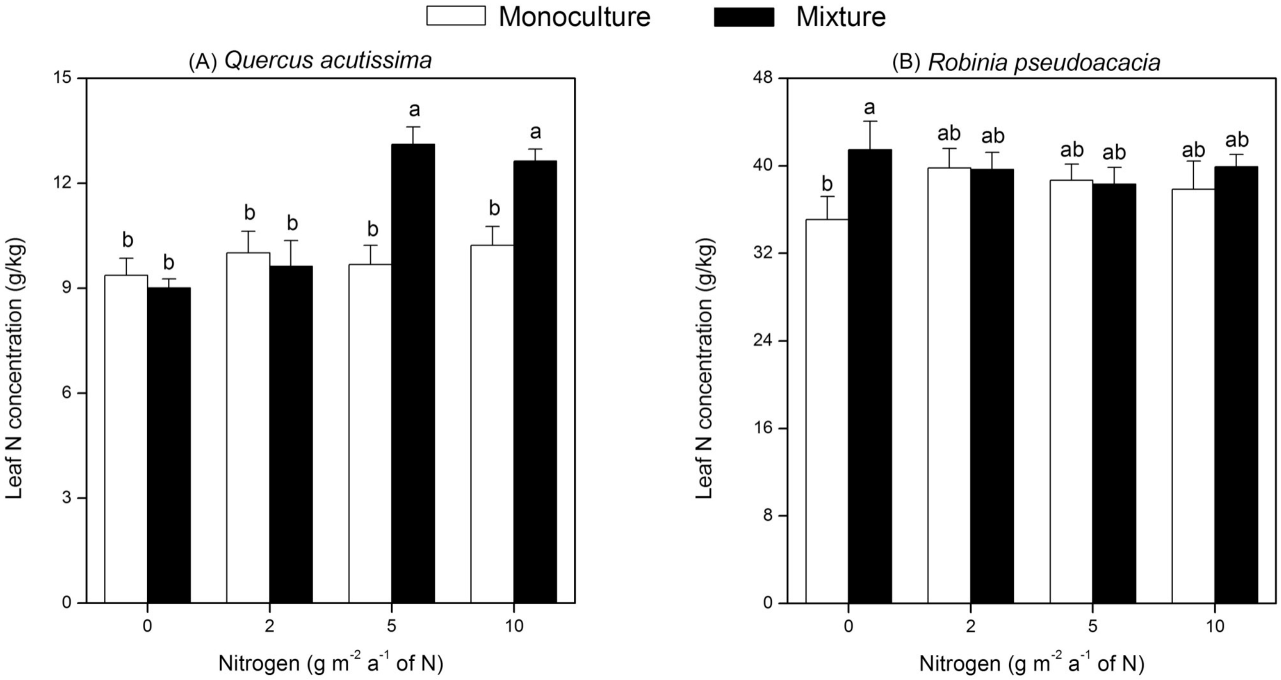

Fig. 3. Leaf nitrogen concentration of (A) *Quercus acutissima* and (B) *Robinia pseudoacacia* grown in monoculture and mixture at the four nitrogen addition levels. Values are presented as mean + SE. Different letters denote significant differences at $p < 0.05$ by Duncan's test

Table 5. RDI of *Quercus acutissima* and *Robinia pseudoacacia* at four nitrogen addition levels. Means ± SE. Different letters in a column represent significant difference between two species at $p < 0.05$ by t-test

Species	Nitrogen level (g m^{-2} a^{-1})			
	0	2	5	10
Quercus acutissima	0.382 ± 0.07 a	0.415 ± 0.08 a	0.377 ± 0.07 a	0.388 ± 0.08 a
Robinia pseudoacacia	0.618 ± 0.07 b	0.585 ± 0.08 a	0.623 ± 0.07 b	0.612 ± 0.08 a

Table 6. Available soil nitrogen after the experiment under three plant cultivation methods and at four nitrogen addition levels. Means ± SE

Nitrogen level (g m^{-2} a^{-1})	Monoculture (g/kg) (*Quercus acutissima*)	Mixture (g/kg) Both species	Monoculture (g/kg) (*Robinia pseudoacacia*)
0	70.07 ± 4.13	99.69 ± 9.52	85.65 ± 8.98
2	79.25 ± 3.13	73.89 ± 4.94	80.14 ± 5.92
5	74.66 ± 5.50	85.49 ± 8.66	75.42 ± 3.84
10	89.87 ± 8.79	84.59 ± 4.83	83.45 ± 1.68

growth of *R. pseudoacacia* was dominant when grown with *Q. acutissima* in the mixture. Moreover, RDI was not significantly different at the four nitrogen levels for *Q. acutissima* and *R. pseudoacacia* ($F_{3,23} = 0.051$, $p = 0.984$). The dominance of *R. pseudoacacia* was not significantly affected by nitrogen addition.

Available soil nitrogen

Available soil nitrogen before the experiment was 83.07 ± 1.99 g/kg. After the experiment, available soil nitrogen was not significantly different among the four nitrogen addition levels regardless of plant cultivation method (Table 6). Significant differences in available soil nitrogen before and after the experiment were examined using Duncan's test.

Discussion

Effects of nitrogen addition on the growth of *Q. acutissima* and *R. pseudoacacia*

Nitrogen addition did not affect total biomass accumulation of *Q. acutissima* and *R. pseudoacacia* seedlings after more than three months of treatment (Table 1), whereas biomass allocation was altered as the level of nitrogen addition increased, with more biomass invested to leaves and less to roots at high nitrogen-addition levels (Table 2). The changed biomass allocation in our study was consistent with the plant optimal partitioning theory (Bloom et al. 1985; Chapin et al. 1987), in which biomass allocation in three annual plants of same age, *Abutilon theophrasti*, *Chenopodium album*, and *Polygonum pensylvanicum*, increased in leaves and decreased in roots under low light and high nutrient conditions (McConnaughay and Coleman 1999). Similar effects on biomass allocation were reported among 12 herbaceous plants in the study of Müller et al. (2000). Trees absorb nitrogen from the soil that mainly depends upon fine roots, which play an important role in plant growth and photosynthesis (Pregitzer et al. 2000, 2002). Because fine roots of trees are concentrated on lateral roots, the morphology and development of fine roots are partly similar to those of lateral roots. Pregitzer et al. (2000) found that fine root biomass increased in high-N compared with low-N soil. Similar results appeared in our study, where *Q. acutissima* seedlings demonstrated increased biomass allocation into their lateral root through MR/LR ratio comparison when nitrogen was added (Table 2).

In our study available soil nitrogen after the nitrogen-addition experiment was not different from that before the experiment. This phenomenon may be explained from the perspective that the added nitrogen during the experiment may have been completely absorbed by the plants because leaf nitrogen concentration of *Q. acutissima* was enhanced by increased nitrogen addition levels. The three-month nitrogen addition period may be too short a time for leaving redundant nitrogen in soil. Therefore, the short length of time may be why simulated nitrogen deposition wielded minimal effects on the biomass accumulation of *Q. acutissima* and *R. pseudoacacia* seedlings. Soil nitrogen content before the experiment was only about 0.1%, which is comparatively lower than the 0.14% nitrogen content of the nutrient-poor, upland forested ecosystem in the study of Von Holle et al. (2006) and is lower than the 0.2% – 0.3% nitrogen content in the forests near the research station. Although nitrogen availability in natural conditions is relatively low, nitrogen may not be the limiting factor for the growth of *R. pseudoacacia* owing to nitrogen fixation from symbiotic rhizobia (Von Holle et al. 2006); thereby providing another reason for the slight effect of increased simulated nitrogen deposition on biomass of *R. pseudoacacia* seedlings.

The response of the ecosystem to permanent nitrogen addition could be highly nonlinear over time in the scale of years (Magill et al. 1997). In our experiment, the effect of nitrogen deposition on species growth was also different in the scale of months (Table 4). Increased nitrogen deposition promoted the increment of seedling height for *Q. acutissima* in the third month (August), whereas no effects were found in the first two months. No effects of nitrogen deposition on height increment of *R. pseudoacacia* seedlings were found during all three months. Therefore, nitrogen deposition at a larger temporal scale may have different effects from that of a three-month period on the growth of *Q. acutissima* and *R. pseudoacacia*.

Relationship between *Q. acutissima* and *R. pseudoacacia*

Through the RDI comparison between *Q. acutissima* and *R. pseudoacacia* seedlings in the mixture, *R. pseudoacacia* was found to exhibit growth predominance in our study. Growth of *R. pseudoacacia* is rapid during the early sprout growth period, with heights reaching up to 8 m in 3 years (Boring and Swank 1984b). In our experiment, after 3 months of treatment, biomass and height increment of *R. pseudoacacia* seedlings were 1.6 and 2.6 times higher than those of *Q. acutissima*, respectively. Therefore, with rapid growth, *R. pseudoacacia* seedlings had absolute dominance over *Q. acutissima* seedlings. Moreover, total biomass, including aboveground and belowground biomass of *R. pseudoacacia* was always 1–3 times higher than those of *Q. acutissima*. The aforementioned ratios, together with RDI, were not significantly different even at different nitrogen levels. However, increased nitrogen addition did not significantly change the growth dominance of *R. pseudoacacia* seedlings over *Q. acutissima* seedlings.

Although growth of *R. pseudoacacia* seedlings was observed to be always dominant over *Q. acutissima*, such dominance did not threaten the native *Q. acutissima* at the studied nitrogen addition levels during the three-month period. *Q. acutissima* grown in the mixture with *R. pseudoacacia* was not different from that of *Q. acutissima* grown in the monoculture in our study. Moreover, *Q. acutissima* seedlings benefited from *R. pseudoacacia* seedlings at high nitrogen levels. Leaf nitrogen concentration of *Q. acutissima* was higher in the mixture than that in the monoculture at high nitrogen levels (Fig. 3), which indicates that *R. pseudoacacia* provided *Q. acutissima* with nitrogen when they grew in the mixture. According to Uselman et al. (1999), nitrogen-fixing trees are able to exude dissolved organic nitrogen from roots or nodules and rapidly transfer nitrogen to neighboring trees, and these reported characteristics of nitrogen-fixing trees is consistent with the results of our study. Many studies found that leaf nitrogen concentration correlated positively with plant photosynthesis rates (Mitchell and Hinckley 1993; Yuan et al. 2007). Therefore, in our study, higher leaf nitrogen concentration in the mixture for *Q. acutissima* may be interpreted as a matter of greater photosynthetic rate in the mixture than that in the monoculture at high nitrogen addition levels. The afore-stated condition suggests that higher leaf nitrogen concentration is favorable for the growth of *Q. acutissima*. Additionally, *R. pseudoacacia* seedlings grew better in the mixture than in the monoculture (Fig. 1). It may, therefore, be stated that positive interaction (facilitation) exists between seedlings of *R. pseudoacacia* and *Q. acutissima* and it is not influenced by the simulated nitrogen deposition during the three-month period. Our results are different from the findings of Hughes and Denslow (2005) and Von Holle et al. (2006), both of which indicated that the exotic nitrogen-fixing tree enhanced soil nutrient and decreased proportion of native species in the ecosystem.

Nitrogen-fixing plant species could introduce more fixed-N to ecosystems and reduce N limitation for net primary production under elevated CO_2 (Zanetti et al. 1997). Moreover, plant biomass stimulated by elevated CO_2 was higher under high-N supply than low-N supply (Reich et al. 2006). Therefore, nitrogen-fixing abilities of *R. pseudoacacia* could increase nitrogen supply and CO_2 sequestration in forest ecosystems, and consequently decrease CO_2 under the background of global warming. In addition, *R. pseudoacacia* is widely used for forestation in China and Korea because of its fast growth and ability to fix nitrogen (Lee et al. 2004). Interestingly, there were conflicting accounts regarding the invasion of *R. pseudoacacia* in China, which was listed as an important invasive species in Weber et al. (2008), but not in Li and Xie (2002), Liu et al. (2005), and Feng and Zhu (2010). Given that *R. pseudoacacia* is extensively used for forestation in North China (Wang and Zhou 2010), the judgment of its hazard has important implications in forest management. On the basis of our study, native species *Q. acutissima* was not threatened by exotic species *R. pseudoacacia*, and *R. pseudoacacia* grew well in the mixture. It is not, however, possible to extrapolate our results to native forests in the field because we only tested tree seedlings at controlled nitrogen conditions for three months. Therefore, further studies with more complicated experiments or field experiments are necessary to determine whether or not *R. pseudoacacia* is harmful to native ecosystems and should be limited for forestation in North China.

Acknowledgements

We thank two anonymous reviewers for their insightful and helpful comments. We are grateful to Dr. Wu Daqian for his advices regarding the experiment and the manuscript; to Professor Cheinchi Chang of University of Maryland for his advices to the manuscript; to Luo Yujie, Guo Xiao, and Tan Xiangfeng for their assistance in the experiment; to Professor Lui Chunsheng of Shandong Agricultural University for his help in the leaf and soil nitrogen measurement; and to a professional editing company "Enpapers" for language editing. This study was supported by The National Natural Science Foundation of China (No. 30970166), Key Science and Technology Project of Shandong Province (No. 2011GGH21605), Ministry of Education on Doctorial Discipline (No.20090131 110066), and Natural Science Foundation of Shandong Province, China (No. 2009ZRB01875; ZR2010 CM062).

References

Aber J.D., Magill A., Boone R., Melillo J.M., Steudler P., Bowden, R. 1993. Plant and soil responses to chronic nitrogen additions at the Harvard forest, Massachusetts. Ecological Applications 3: 156–166.

Bedison J.E., McNeil B.E. 2009. Is the growth of temperate forest trees enhanced along an ambient nitrogen deposition gradient? Ecology 90: 1736–1742.

Bloom A.J., Chapin F.S., Mooney H.A. 1985. Resource limitation in plants – an economic analogy. Annual Review of Ecology and Systematics 16: 363–392.

Boring L.R., Swank W.T. 1984a. Symbiotic nitrogen fixation in regenerating black locust (*Robinia pseudoacacia* L.) stands. Forest Science 30: 528–537.

Boring L.R., Swank W.T. 1984b. The role of black locust (*Robinia pseudoacacia*) in forest succession. Journal of Ecology 72: 749–766.

Bradley B.A., Blumenthal D.M., Wilcove D.S., Ziska L.H. 2010. Predicting plant invasions in an era of global change. Trends in Ecology and Evolution 25: 310–318.

Brooks M.L. 2003. Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. Journal of Applied Ecology 40: 344–353.

Bruno J.F., Stachowicz J.J., Bertness M.D. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18: 119–125.

Callaway R.M., Aschehoug E.T. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290: 521–523.

Callaway R.M., Walker L.R. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78: 1958–1965.

Chapin F.S., Bloom A.J., Field C.B., Waring R.H. 1987. Plant responses to multiple environmental factors. BioScience 37: 49–57.

Chu C.J., Maestre F.T., Xiao S., Weiner J., Wang Y.S., Duan Z.H., Wang G. 2008. Balance between facilitation and resource competition determines biomass–density relationships in plant populations. Ecology Letters 11: 1189–1197.

Feng J.M., Zhu Y.Y. 2010. Alien invasive plants in China: risk assessment and spatial patterns. Biodiversity and Conservation 19: 3489–3497.

Funk J.L., Vitousek P.M. 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature 446: 1079–1081.

Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth R.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., Karl D.M., Michaels A.F., Porter J.H., Townsend A.R., Vörösmarty C.J. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153–226.

Holland E.A., Braswell B.H., Sulzman J., Lamarque J-F. 2005. Nitrogen deposition onto the United States and Western Europe: synthesis of observations and models. Ecological Applications 15: 38–57.

Hughes R.F., Denslow J.S. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forests of Hawaii. Ecological Applications 15: 1615–1628.

LeBauer D.S., Treseder K.K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89: 371–379.

Lee C.S., Cho H.J., Yi H. 2004. Stand dynamics of introduced black locust (*Robinia pseudoacacia* L.) plantation under different disturbance regimes in Korea. Forest Ecology and Management 189: 281–293.

Li Z.Y., Xie Y. 2002. Invasive alien species in China. China Forestry Publishing House, Beijing.

Liu J., Liang S.C., Liu F.H., Wang R.Q., Dong M., 2005. Invasive alien plant species in China: regional distribution patterns. Diversity and Distributions 11: 341–347.

Lowe P.N., Lauenroth W.K., Burke I.C. 2003. Effects of nitrogen availability on competition between *Bromus tectorum* and *Bouteloua gracilis*. Plant Ecology 167: 247–254.

Mack R.N., Simberloff D., Lonsdale W.M, Evans H., Clout M., Bazzaz F.A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10: 689–710.

Magill A.H., Aber J.D., Hendricks J.J., Bowden R.D., Melillo J.M., Steudler P.A. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7: 402–415.

Maskell L.C., Smart S.M., Bullock J.M., Thompson K., Stevens C.J. 2010. Nitrogen deposition causes widespread loss of species richness in British habitats. Global Chang Biology 16: 671–679.

Matson P., Lohse K.A., Hall S.J. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. Ambio 31: 113–119.

McConnaughay K.D.M., Coleman J.S. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80: 2581–2593.

Mitchell A.K., Hinckley T.M. 1993. Effects of foliar nitrogen concentration on photosynthesis and water use efficiency in Douglas-fir. Tree Physiology 12: 403–410.

Mo J.M., Zhang W., Zhu W.X., Gundersen P., Fang Y.T., Li D.J., Wang H. 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Chang Biology 14: 403–412.

Müller I., Schmid B., Weiner J. 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics 3: 115–127.

Myers J.H., Bazely D. 2003. Appendix-Some tools for studying plant populations. In: Ecology and control of introduced plants. Cambridge University Press, pp. 255.

Niu H.B., Liu W.X., Wan F.H., Liu B. 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. Plant and Soil 294: 73–85.

Palátová E. 2004. Effect of increased nitrogen depositions and drought stress on the development of young Norway spruce *Picea abies* (L.) Karst. stands. Dendrobiology 51 Supplement: 41–45.

Perry L.G., Blumenthal D.M., Monaco T.A., Paschke M.W., Redente E.F. 2010. Immobilizing nitrogen to control plant invasion. Oecologia 163: 13–24.

Pregitzer K.S., Zak D.R., Maziasz J., DeForest J., Curtis P.S., Lussenhop J. 2000. Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. Ecological Applications 10: 18–33.

Pregitzer K.S., DeForest J.L., Burton A.J., Allen M.F., Ruess R.W., Hendrick R.L. 2002. Fine root architecture of nine North American trees. Ecological Monographs 72: 293–309.

Reay D.S., Dentener F., Smith P., Grace J., Feely R.A. 2008. Global nitrogen deposition and carbon sinks. Nature Geoscience 1: 430–437.

Reich P.B., Hobbie S.E., Lee T., Ellsworth D.S., West J.B., Tilman D., Knops J.M.H., Naeem S., Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440: 922–925.

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

Scherer-Lorenzen M., Venterink H.O., Buschmann H. 2007. Nitrogen enrichment and plant invasions: the importance of nitrogen-fixing plants and anthropogenic eutrophication. In: Biological invasions. Nentwig W. (eds). Springer-Verlag, Berlin, Heidelberg, pp. 163–180.

Uselman S.M., Qualls R.G., Thomas R.B. 1999. A test of a potential short cut in the nitrogen cycle: The role of exudation of symbiotically fixed nitrogen from the roots of a N-fixing tree and the effects of increased atmospheric CO_2 and temperature. Plant and Soil 210: 21–32.

Valiente-Banuet A., Verdú M. 2008. Temporal shifts from facilitation to competition occur between closely related taxa. Journal of Ecology 96: 489–494.

Vilà M., Weiner J. 2004. Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. Oikos 105: 229–238.

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

Wang R.Q., Zhou G.Y. 2000. The Vegetation of Shandong Province. Shandong Science and Technology Publisher, Jinan, pp. 259–264.

Weber E., Sun S.G., Li B. 2008. Invasive alien plants in China: diversity and ecological insights. Biological Invasions 10: 1411–1429.

Xu F., Guo W.H., Wang R.Q., Xu W.H., Du N., Wang Y.F. 2009. Leaf movement and photosynthetic plasticity of black locust (*Robinia pseudoacacia*) alleviate stress under different light and water conditions. Acta Physiologiae Plantarum 31: 553–563.

Yuan Z.Y., Liu W.X., Niu S.L., Wan S.Q. 2007. Plant nitrogen dynamics and nitrogen-use strategies under altered nitrogen seasonality and competition. Annals of Botany 100: 821–830.

Zanetti S., Hartwig U.A., van Kessel C., Lüscher A., Hebeisen T., Frehner M., Fischer B.U., Hendrey G.R., Blum H., Nösberger J. 1997. Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? Oecologia 112: 17–25.