J. Ecol. Field Biol. 29 (2): 151～155, 2006

Root Nodule Biomass of *Robinia pseudoacacia* and *Amorpha fruticosa* Seedlings with Fertilization Treatments

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ABSTRACT: Root nodule biomass, and seedling biomass and growth were examined for 2-year-old *Robinia pseudoacacia* and *Amorpha fruticosa* seedlings following fertilization treatments. Organic fertilizer, solid combination fertilizer, and organic fertilizer plus solid combination fertilizer were used for the study. Root nodule biomass (g/plant) ranged from 3.00 to 7.06 for *R. pseudoacacia* and varied from 1.52 to 2.32 for *A. fruticosa*, respectively. In all treatments, root nodule biomass of *R. pseudoacacia* was significantly higher than those of *A. fruticosa*. Fertilization significantly increased root nodule biomass for only *R. pseudoacacia*, however, there were no significant differences in root nodule biomass among fertilization treatments. Root nodule biomass was not influenced by soil nitrogen (N) and phosphorous (P) concentrations following fertilization treatments. Seedling biomass (components and total) and growth (diameter at root collar and height) were strongly correlated with root nodule biomass for the two N fixing tree species.

Key words: *Amorpha fruticosa*, Fertilization, *Robinia pseudoacacia*, Root nodule biomass, Seedling biomass and growth

INTRODUCTION

Biological nitrogen (N) fixation is one of the most important nutrient cycling processes and the safest method for the introduction of combined N into natural environments (Waring and Running 1998). In agricultural field, there are many studies on N fixation, and those have contributed to gain the effect of soil improvement (Bøckman 1997, Abberton et al. 2000, Graham and Vance 2000). However, in forestry field, there are relatively few studies about nitrogenase activity with several woody species. To understand the role of biological N fixation in forest ecosystems, more information about root nodule biomass as well as N fixation activity with different species is needed. Root nodule growth may be linked to total plant growth (Mengel 1994) and depend on environmental factors (e.g. nitrate supply, salt stress and drought stress) directly (Streeter 1998). Relationships between plants and nodules are also affected by nitrate (Leidi and Rodriguez-Navarro 2000), but are still unclear. In addition, very limited information on root nodule biomass is available for quantitative study of ecology. *Robinia pseudoacacia* L. had been planted widely for erosion control and *Amorpha fruticosa* L. has been known for a dominant species in variable destroyed ecosystems. Both species play an important role in vegetation succession and contribute to soil conservation as N fixation species. However, there are very few studies about root nodule for these species (Hong and Song 1990). Especially the influence of soil fertility on root nodule biomass is unclear (Ekbland and Huss-Danell 1995, Tobita et al. 2005). The objectives of study were to 1) measure root nodule biomass and 2) examine the relationships between root nodule biomass and seedling biomass and growth of *R. pseudoacacia* and *A. fruticosa* seedlings following fertilization treatments.

METERIALS AND METHODS

Two year old (1-1) seedlings of *Robinia pseudoacacia* and *Amorpha fruticosa* were planted in plastic pots (ϕ35 × ϕ25 × h30cm) in April 2005. Pots were filled with soil, which is loamy sandy soil with a soil pH of 7.2, 0.033% of total N, 0.048% of total P (determined by the Kjeldahl method), 0.40% of organic carbon (determined by the Walkley-Black method), 88.72 mg/kg of available P (determined by the Bray No.1 method), and 9.08 mg/kg CEC (determined by Brown method). After planting, four types of fertilization treatments were applied: no fertilization (C), organic fertilizer (O; 200 g per pot, N:P:K:organic matter:zeolite = 4:2:1:70:20:3), solid combination fertilizer (S; 15 g per pot, N:P:K = 3:4:1) and organic fertilizer plus solid combination fertilizer (OS; O: 200 g + S: 15 g). The organic fertilizer was applied on 29 April, and solid combination fertilizer was applied on 20 May. Four replicates were applied for each treatment. Pots were watered every 2～3 days de-

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pending on weather conditions.

On 16 September 2005, three seedlings per treatment were sampled, and height and diameter at root collar of seedlings were measured. Seedlings were carefully separated into shoots, leaves, roots and root nodules, and the belowground part was washed free of soil. Biomass was determined after oven drying at 75℃. All soil samples after seedling harvest were taken from each pot, and also used for total N and P analyses. General linear model and Duncan's multiple range tests were employed to determine differences in root nodule biomass, seedling component biomass and seedling growth following fertilization treatments. Regression analysis was used to analyze the relationships between root nodule biomass and seedling component biomass and seedling growth. All statistical analyses were conducted using the SAS program (SAS Institute Inc. 2000).

RESULTS AND DISCUSSION

Root Nodule Biomass

Root nodule biomass (g/plant) ranged from 3.00 to 7.06 for *R. pseudoacacia* and ranged from 1.52 to 2.32 for *A. fruticosa* seedlings depending on fertilization treatment, respectively (Table 1). Root nodule biomass was highest for the OS treatment in *R. pseudoacacia* (7.06 g/plant). Root nodule biomass of *R. pseudoacacia* for the control was higher than 4.0 g/plant for 16-month-old *Leucaena leucocephala* (Kadiata et al. 1995) and was lower than 8.9 g/plant for 1-year-old *Alnus rubra* (Koo et al. 1996). Fertilization significantly increased root nodule biomass for *R. pseudoacacia*, however, there were no significant differences in root nodule biomass among different types of fertilization. In *A. fruticosa*, there was no significant difference in root nodule biomass following fertilization treatments. Seedling root biomass and total seedling biomass did not change following fertilization for both species.

The ratio of root nodule biomass to root biomass (RNR, %) varied from 8.4 to 21.0 for *R. pseudoacacia* and varied from 3.4 to 4.3 for *A. fruticosa* and the ratio of root nodule biomass to total biomass (RNT, %) ranged from 2.6 to 4.0 for *R. pseudoacacia* and ranged from 0.9 to 1.3 for *A. fruticosa*, respectively. These results were similar to those for previous studies; Temperton et al. (2003) reported 3.0～7.6 of RNR for 1-to 4-year-old *Alnus glutinosa* seedlings and Koo et al. (1996) indicated 3.5 of RNT for 1-year-old *Alnus rubra* seedling. RNR was significantly different among the treatments only in *R. pseudoacacia*. However, there was no difference in RNT for both species.

In all treatments, root nodule biomass of *R. pseudoacacia* was significantly higher than those of *A. fruticosa*. However, root biomass for the solid combination fertilizer and organic fertilizer plus solid combination fertilizer treatments was significantly higher in *A. fruticosa* than in *R. pseudoacacia* while total seedling biomass for the control and organic fertilizer treatment was significantly higher in *A. fruticosa* than in *R. pseudoacacia*. RNR and RNT were significantly higher in *R. pseudoacacia* than in *A. fruticosa* because of higher root nodule biomass for *R. pseudoacacia*.

Root Nodule Biomass and Soil N and P Concentrations

Leidi and Rodriguez-Navarro (2000) reported that the nodule formation and growth in bean were affected by NO_3^- concentration and nodulation was slightly improved by increases in P supply. It was also reported that N or P supply by fertilizers increased or decreased root nodule biomass and plant growth (Gentili and Huss-Danell 2003). We analyzed the relationship between root nodule biomass and soil N and P concentrations after fertilization treatments. However, our study indicated that root nodule biomass was not

Table 1. Root nodule biomass, root biomass, total biomass and the ratios of root nodule biomass to root biomass (RNR, %) and total biomass (RNT, %) for *R. pseudoacacia* and *A. fruticosa* seedlings. One standard error of the means is in parentheses. The small letters indicate differences among treatments within a species, and the capital letters indicate differences between two species within the same treatment

Species	Treatments	Root nodule biomass (g)	Root biomass (g)	Total biomass (g)	RNR (%)	RNT (%)
R. pseudoacacia	C	3.00bA (0.00)	32.68aA (0.00)	111.11aB (0.00)	8.4bA (0.0)	2.7aA (0.0)
	O	5.82aA (1.76)	40.22aA (15.75)	133.76aB (25.01)	13.1abA (1.6)	3.2aA (0.2)
	S	4.34aA (0.80)	23.66aB (7.41)	165.60aA (36.66)	16.0abA (1.8)	2.6aA (0.1)
	OS	7.06aA (0.63)	26.90aB (3.23)	181.33aA (16.29)	21.0aA (3.5)	4.0aA (0.7)
A. fruticosa	C	1.52aB (0.41)	36.74aA (11.61)	178.13aA (55.25)	4.1aB (0.4)	0.9aB (0.1)
	O	2.15aB (0.40)	58.95aA (9.54)	251.21aA (64.79)	3.5aB (0.1)	0.9aB (0.2)
	S	2.32aB (0.41)	54.82aA (13.78)	196.51aA (53.42)	4.3aB (0.8)	1.3aB (0.3)
	OS	1.76aB (0.24)	57.06aA (18.53)	177.71aA (30.53)	3.4aB (0.8)	1.0aB (0.1)

significantly influenced by soil N and P concentrations following fertilization (Fig. 1). There was a possibility that the combination of N and P influenced the results (Koo et al. 1996). Fertilization with single element seemed to be necessary to elucidate the effects of fertilization on root nodule biomass for the two species.

Fig. 1. Relationships between soil N and P concentration and root nodule biomass for *R. pseudoacacia* (Rp) and *A. fruticosa* (Af).

Root Nodule Biomass and Seedling Biomass and Growth

The seedling biomass was correlated with root nodule biomass for both species (p<0.005) (Fig. 2). Especially, total seedling biomass for *A. fruticosa* was strongly correlated with root nodule biomass (r^2=0.76) (Fig. 2b). These results indicated that root nodule biomass might be influenced by plant growth as previous studies reported (Kadiata et al. 1995, Koo et al. 1996). The seedling growth for *R. pseudoacacia* and *A. frutisosa* was correlated with root nodule biomass (Fig. 3). Especially, seedling height growth for *A. fruticosa* was strongly correlated with root nodule biomass (r^2=0.69, p<0.005). Two-year-old seedlings were used for the current study, and it has been known that nodulation was variable with ages (Klucas 1974, Boring and Swank 1984, Frank and Salas 2003). Therefore, our results could not apply over the different growth stages. However, our data strongly indicated that root nodule growth was linked to seedling growth for both species (Mengel 1994, Koo et el 1996). In this regard, if further investigations on overall ages and seasonal patterns of root nodule biomass were conducted, we might estimate the root nodule biomass of these two N fixing tree species.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Environment (The Eco-technopia 21 project).

Fig. 2. Relationships between root nodule biomass and seedling biomass for *R. pseudoacacia* (a) and *A. fruticosa* (b) (◆ total, □ shoot ▲ stem ○ leave * root).

Fig. 3. Relationships between root nodule biomass and seedling diameter at root collar (a) and seedling height (b) for *R. pseudoacacia* and *A. fruticosa.*

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(Received April 10, 2006; Accepted April 24, 2006)