J. Plant Biol. (2010) 53:61–69
DOI 10.1007/s12374-009-9088-9

ORIGINAL RESEARCH

Comparison of Nitrogen Fixation for North- and South-facing *Robinia pseudoacacia* Stands in Central Korea

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Received: 8 April 2009 / Revised: 3 August 2009 / Accepted: 5 October 2009 / Published online: 15 December 2009

Abstract The nitrogenase activity, root nodule biomass, and rates of nitrogen (N) fixation were measured in 25-year-old pure north- and south-facing *Robinia pseudoacacia* stands in an urban forest of Seoul (Kkachisan Mountain) in central Korea. The nitrogenase activity was estimated using an acetylene reduction (AR) assay, which showed an increasing trend during the early growing season, with sustained high rates from June through to September with a decrease thereafter. July had the highest nitrogenase activity rate (micromoles C_2H_4 per gram dry nodule per hour), averaging 95.8 and 115.1 for the north- and south-facing stands, respectively. The maximum root nodule biomass (kilograms per hectare) was 45.7 and 9.1 for the north- and south-facing stands in July, respectively. The AR rate appeared to be strongly correlated to the soil temperature (r^2=0.68, P<0.001) and soil pH (r^2=0.59, P<0.001) while root nodule biomass was correlated to the soil temperature (r^2=0.36, P<0.01) and water content (r^2=0.35, P<0.05). The soil temperature showed clear differences between seasons, while there was a significant difference in soil pH, organic matter, total N concentrations, and available phosphorus between the north- and south-facing stands. The N_2 fixation rates during the growing season varied from 0.1 to 37.5 kg N $ha^{-1}month^{-1}$ depending on the sampling location and time. The annual N_2 fixation rate (kg N per hectare per year) was 112.3 and 23.2 for the north- and south-facing stands, respectively. The differences in N_2 fixation rate between the two stands were due mainly to the differences in total nodule biomass.

Keywords Acetylene reduction · Aspect · Nitrogen fixation · Nodule biomass · *Robinia pseudoacacia*

Introduction

Nitrogen (N) can mainly be added to terrestrial ecosystems through biological N_2 fixation, dry and wet deposition, and industrial N_2 fixation. Among these inputs, biological N fixation is still one of the most important processes for introducing combined N into nature (Bøckman 1997; Waring and Running 1998; Graham and Vance 2000). It is also the process of reducing atmospheric N_2 to ammonia and other biochemicals, which is available for plants. The process can only be performed by certain microbes. Some microbes fix N_2 without the need for higher plants while others make symbiotic relationships with plants. However, it is very difficult to quantify N_2 fixation rate and generalize it due to the lack of information on biological N_2 fixation for forest ecosystems (Boring and Swank 1984a; Ntayombya and Gordon 1995; Pearson and Vitousek 2001).

Low soil fertility initially hindered reforestation efforts on devastated lands due to overuse and mismanagement in Korea several decades ago. Consequently, N_2-fixing trees, such as black locust (*Robinia pseudoacacia* L.) and alders (*Alnus* spp.), were planted widely to enhance the soil N

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content. In particular, *R. pseudoacacia* was utilized for soil conservation or fuel wood as well as for early reforestation due to its rapid growth and adaptation to barren soil and devastated sites (Kim et al. 1986; Park 1996).

However, *R. pseudoacacia* has been devaluated economically and ecologically in the region. The N_2 fixation rate by the species has not been studied thoroughly, even though *R. pseudoacacia* fixes N_2 and enhances the soil nutrient content (Ntayombya and Gordon 1995; Berthold 2005). Hong and Song (1990) reported that *R. pseudoacacia* has high nitrogenase activity but that it was very difficult to estimate the total nodule biomass because of the small root nodules. In addition, there are differences in the nodule biomass and biological activity according to the micro-topography, such as the aspect and slope, which can affect solar radiation, soil temperature, water content, and nutrient content (Mueller et al. 1999; Koponen et al. 2003; Florinsky et al. 2004).

Generally, soil temperature affects not only the physiological reaction rates of a cell but also most of the physicochemical characteristics of the environment. In addition, soil moisture and pH affect the nitrogenase activity because excessive or deficient value generally causes dehydration and inhibits enzyme activity. Moreover, extreme acidity can cause protein denaturation and enzyme inhibition (Paul and Clark 1996). The formation and development of root nodules are also influenced by environmental conditions, such as soil temperature, soil pH, dissolved organic carbon, nitrate level, and N and P concentrations (Röhm and Werner 1991; Uselman et al. 2000; Gentili and Huss-Danell 2003; Brockwell et al. 2005). However, there is still limited information on the root nodule biomass of woody N_2-fixers particularly *R. pseudoacacia*. Furthermore, the effect of the micro-topography on the N_2 fixation rate has not been examined. Therefore, an examination of the relationships between N_2 fixation and these environmental factors will provide important information for understanding N_2 fixation of forest ecosystems.

The aims of this study are as follows: (1) to investigate the nitrogenase activity using the acetylene reduction (AR) technique; (2) to estimate the root nodule biomass; (3) to examine the relationship between the AR rate and root nodule biomass as well as the environmental factors, such as soil temperature, soil moisture content, soil pH, and N availability; and (4) to estimate the annual rate of N_2 fixation for 25-year-old north- and south-facing *R. pseudoacacia* stands in an urban forest in central Korea

Materials and Methods

Study Site

This study examined the north- and south-facing *R. pseudoacacia* stands in an urban forest of Seoul (Kkachisan Mountain) in central Korea (37°28′53–55″N, 126°57′46–49″E). The distance between the two stands was 40 m, and the elevation of the plots was similar (100–120 m a.s.l.). The average annual temperature and precipitation were 12.2°C and 1,344 mm, respectively, with approximately 70% of the annual precipitation falling between June and August. Two 20×20 m plots were established within each aspect, and the characteristics of the study stands were investigated. In 2005, the average diameter at breast height (DBH, >5 cm) and the height of *R. pseudoacacia* in the study sites were 18.6 cm and 16.9 m for the north-facing and 18.6 cm and 16.8 m for the south-facing stands, respectively. The stand density for all trees (DBH>5 cm) was 1,022 and 1,178 trees ha^{-1} for the north- and south-facing stands, respectively. The slope (in degrees) ranged 18 to 28 and 10 to 30 for the north- and south-facing stands, respectively. The understories were dominated by *Quercus mongolica* for the north-facing stand and *Lespedeza crytobotrya* and *Forsythia koreana* for the south-facing stand.

Soil Characteristics

The soil characteristics were examined using five samples of 15-cm depth soil for each plot, which were collected using a hand-driven soil sampling corer (Hwang et al. 2001). The soil pH and organic carbon concentration were determined in a 1:5 soil H_2O suspension as well as by the Walkley–Black method (Nelson and Sommers 1996), respectively. The total soil N and phosphorus (P) concentrations were determined using a Lachat continuous flow ion autoanalyzer (QuikChem AE, Lachat Instruments, WI, USA) following wet digestion in concentrated H_2SO_4 in a block digester. The available P was determined using Bray no.1 method (Kuo 1996). The cation exchange capacity was determined using 1-N NH_4OAc extractions (Sumner and Miller 1996). The soil texture was determined by the hydrometer method (Grossman and Reinsch 2002). The soil pH was lower for the north-facing stand (4.32) than for the south-facing stand (4.59) (Table 1). In addition, organic C, total N, and available P concentrations, and CEC for the north-facing stand were higher than those for the south-facing stand, while soil physical characteristics for both stands were similar (Table 1).

Nitrogenase Activity

An acetylene reduction (AR) assay was used to measure the nitrogenase activity (Knowles 1987; Rice and Olsen 1993; Weaver and Danso 1994; Coyne 1999; Fisher and Binkley 2000). The seasonal patterns of nitrogenase activity were determined by measuring the AR rate monthly, and the diurnal patterns were determined by measuring the AR on

Table 1 Stand and soil characteristics of *R. pseudoacacia* study sites

Characteristics	Aspect	
	North	South
Stand		
DBH (cm)	18.6	16.9
Height (m)	18.6	16.8
Stand density (trees ha^{-1})	1,022	1,178
Slope (°)	18-28	10-30
Soil		
pH	4.32b	4.59a
Organic carbon (%)	4.12a	1.96b
Total N (%)	0.24a	0.08b
Total P (%)	0.05	0.04
Available P (mg kg^{-1})	18.44a	9.06b
CEC ($cmol^{+}$ kg^{-1})	15.11a	10.98b
Texture (sand: silt: clay)	Silt loam (59 : 35 : 6)	Loam (70 : 28 : 2)
Bulk density (g cm^{-3})	1.09	1.10
Rock fragment (>2 mm, %)	26.21	25.21

The means with the different letter denote a significant difference between the north- and south-facing stands at the $P<0.05$ level

five separate occasions, when the nodules were incubated for 1 h at 4-h intervals over a 24-h period. In order to minimize the errors for estimating the level of N_2 fixation from the diurnal variation during the incubation time, the nitrogenase activity was measured using random-sized nodules between 1 and 4 p.m. avoiding dry and rainy days (Waughman 1972; Boring and Swank 1984a).

For the AR assays, the nodulated root pieces were excavated carefully from the five 50×50 cm soil pits with 20 cm depth per plot and enclosed in 100 ml tubes (considering the nodule size), which were fitted with rubber stoppers. The procedure involved incubating the nodules in the field in an atmosphere containing 10% acetylene (C_2H_2) for 1 h. The gas subsamples were then removed and stored in Vacutainer tubes for later analysis by gas chromatography (Model 6890, Hewlett Packard, USA). Gas separation was carried out on a Porapak R column (80 and 100 mesh) and He (carrier gas). The oven temperature was 190°C, and the injection port and detector temperature was 200°C. The AR rates are expressed in micromoles of ethylene produced per gram dry weight of nodule tissue per hour.

During the AR measurements, the soil temperature and moisture were measured at adjacent soil pits. The soil temperature was monitored with a probe at a depth of 12 cm (Digi-sense Type K, Cole-Parmer Ins., USA) and the soil water content was determined gravimetrically using the soils sampled by a core sampler.

Nitrogen Availability

The nitrogen availability was measured from May 2005 through May 2007 using ion exchange resin bags (Garcia-Montiel and Binkley 1998). Five resin bags per plot were placed 10 cm below the surface of the mineral soil, every 2 m along a single transect across each plot (Son et al. 2007). The resin bags were retrieved and replaced approximately every 60 days except for winter. After extracts from the retrieved resin bag containing 2 M KCl were settled for 24-h and filtered, the NH_4^+ and NO_3^- concentrations in the extracts were determined using a Lachat continuous flow ion autoanalyzer (QuikChem AE, Lachat Instruments, WI, USA).

Root Nodule Biomass

A single transect was located across each plot and five soil pits were excavated every 3 m along the transect (Lee and Son 2005). The root nodule biomass was calculated from the five soil pits per plot monthly from May 2005 to April 2006. Some of the root nodules were used to measure the AR rate. The other nodules were taken to the laboratory. After excluding the dead material, the nodules were oven-dried at 75°C and weighed to determine their biomass.

Nitrogen Fixation

Nitrogen fixation was calculated by multiplying the following three factors (Binkley 1981): the average AR rate per unit nodule mass, the nodule biomass per unit area, and the conversion factor (3:1; Hardy et al. 1973; Coyne 1999). The annual N fixation per unit area was estimated by summing the values for monthly N fixation. Although the AR assay has an uncertainty in the ideal conversion factor under different environmental conditions for the incubation,

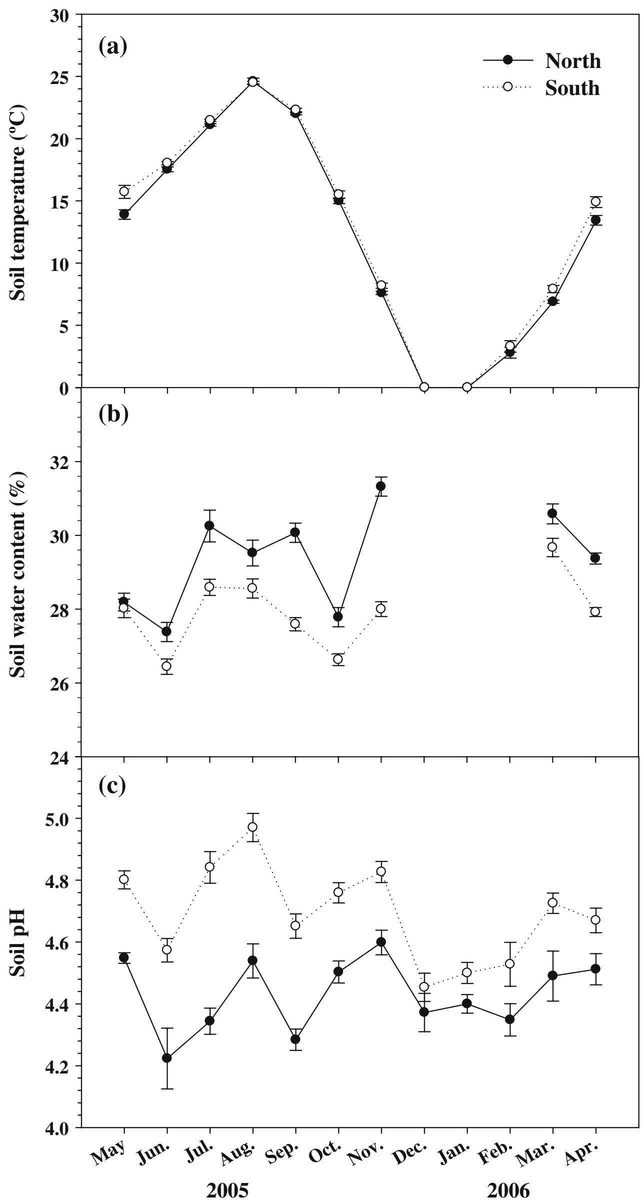

Fig. 1 Seasonal soil temperature (**a**), soil moisture content (**b**), and soil pH **c** for the north- and south-facing *R. pseudoacacia* stands. The vertical lines denote one standard error of the mean

it can still be useful when examining potential value of N_2 fixers in unexplored areas (Danso 1995; Fisher and Binkley 2000).

Statistical Analyses

All statistical analyses were carried out using the general linear models procedure of the SAS 9.12 software (SAS 2004). Duncan's multiple range tests were used to determine if the mean differences in the diurnal or seasonal changes in the environmental factors, root nodule biomass, AR rate and N_2 fixation rate were significant between the north- and south-facing stands. In addition, regression analysis was carried out to determine the relationship between the AR and environmental conditions. A P value<0.05 was considered significant.

Results

Diurnal and Seasonal Variation of Acetylene Reduction Rate

The soil temperature for the north- and south-facing stands increased from December, peaked in August, and decreased until December (Fig. 1a). The mean soil temperature (°C, one standard error) during the study period was 13.2±2.4 and 13.8±2.4 for the north- and south-facing stands, respectively. The soil moisture varied from 9% to 22%, and the soil pH ranged from 4.2 to 5.0 (Fig. 1b and c). There were no distinct seasonal patterns. However, there was a significant difference in mean soil water content (%, one standard error) and soil pH between the two stands (P< 0.05); 29.4±0.2 and 4.32 for the north-facing and 27.9±0.1 and 4.69 for south-facing stands, respectively.

Table 2 shows the diurnal patterns of the AR rates for the *R. pseudoacacia* stands. During the growing season, the AR rate (micromoles C_2H_4 per gram dry nodule per hour) over the 24-h test periods varied from 51.0 to 238.9 according to the measuring times. The AR rates generally increased from the early morning, peaked in the mid- to late afternoon, then decreased throughout the night. In our study, the maximum AR rates were observed at 10 a.m. in June and August, and at 2 p.m. in July and September.

The AR rates (micromoles C_2H_4 per gram dry nodule per hour) measured monthly during daylight ranged from 0.0 to 295.6 with an overall average of 96.6 (Fig. 2). The

Table 2 Diurnal variation of acetylene reduction rates (micromoles C_2H_4 per gram dry nodule per hour) for *R. pseudoacacia* in 2005

Time of day	9 June	8 July	15 August	12 September
06:00–07:00	–	50.97 (0.48)c	63.15 (10.00)c	–
10:00–11:00	230.89 (25.49)a	198.35 (9.14)a	209.61 (23.98)a	202.46 (19.55)a
14:00–15:00	151.75 (19.39)b	238.85 (22.41)a	142.12 (11.67)a	224.85 (6.33)a
18:00–19:00	130.71 (29.68)b	224.59 (18.29)a	88.30 (5.77)b	109.02 (13.62)b
22:00–23:00	138.57 (16.12)b	74.03 (5.38)c	69.94 (10.13)c	81.56 (28.71)b
02:00–03:00	–	148.29 (8.34)b	59.50 (15.58)c	92.59 (39.40)b

The means with a different letter denote a significant difference between the times at the P<0.05 level

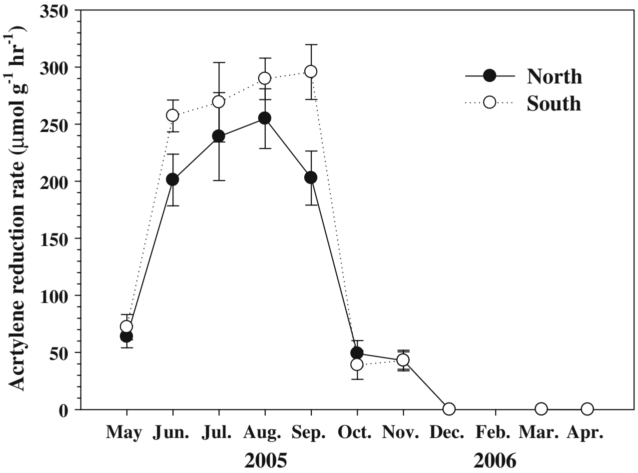

Fig. 2 Seasonal pattern of the acetylene reduction rate for the north- and south-facing *R. pseudoacacia* stands. The *vertical lines* are one standard error of the mean

seasonal AR rates showed an increasing trend during the early growing season with sustained high rates from June through to September. In particular, the AR rate of the south-facing stand during the high temperature season, from June to September was higher than that of the north-facing stand: annual mean AR rates (micromoles C_2H_4 per gram dry nodule per hour) was 87.8 and 105.5 for the north- and south-facing stands, respectively (P<0.05).

Acetylene Reduction Rate, Root Nodule Biomass, and Environmental Factors

There was a significant correlation between the AR rate and soil temperature (r^2=0.68, P<0.001; Fig. 3). In addition, the AR rate showed a strong correlation with soil pH (r^2= 0.59, P<0.001) and increased with soil pH (Fig. 3c). However, there was no correlation between the AR rate and soil water content in this study site (Fig. 3b).

The root nodule biomass (kilogram per hectare) ranged from 0.0 to 45.7 depending on the location and season (Fig. 4). The root nodule biomass increased gradually from early spring until July, decreased from August, and was very low during winter (Fig. 4).

There was a significant difference in root nodule biomass between the north- and south-facing stands (P< 0.01); the overall monthly average was 14.8 kg ha^{-1} and 2.6 kg ha^{-1} for the north- and south-facing stands, respectively. In addition, there were significant differences in soil pH, soil organic carbon, total N concentrations, and available P between the north- and south-facing stands (P< 0.05; Table 1). The soil temperature (P<0.01) and water content (P<0.05) were found to be related to the root nodule biomass, while the soil pH was not (Fig. 5).

Nitrogen Fixation and N Availability

The nitrogen fixation rate during the growing season varied from 0.1 to 37.5 kg N $ha^{-1}month^{-1}$ depending on the sampling location and season. The annual N_2 fixation rates (kilogram N per hectare per year) for the north- and south-facing stands were 112.3 and 23.2, respectively (Table 3).

Figure 6 shows the resin NH_4^+ and NO_3^- for the six incubation periods (May 2005–April 2007). The resin NH_4^+ and NO_3^- concentrations (milligrams N per bag) showed a clear seasonal trend of increases during the early growing season, peaks during the mid growing season, and decreases from the late growing season. In this study, the relationship was not clear for *R. pseudoacacia*. There were no significant differences in the resin NH_4^+ and NO_3^- between the two stands; 24.51 mg N bag^{-1} and 24.46 mg N bag^{-1} for the north- and south-facing stands, respectively. The nitrogen

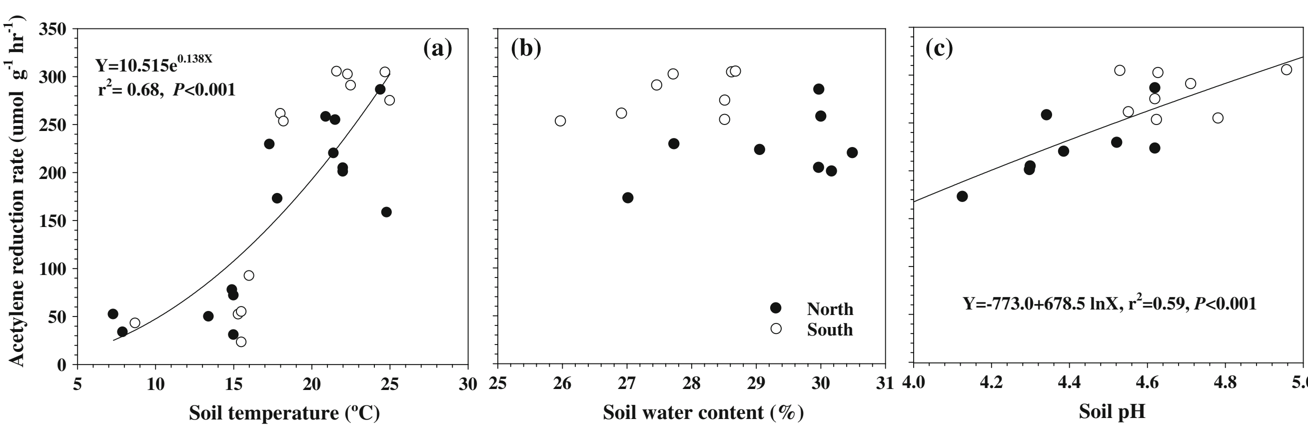

Fig. 3 Relationship between the acetylene reduction rate and soil temperature (**a**), soil water content (**b**), and soil pH for the north- and south-facing *R. pseudoacacia* stands. Each point represents the mean of three plots for each measuring time

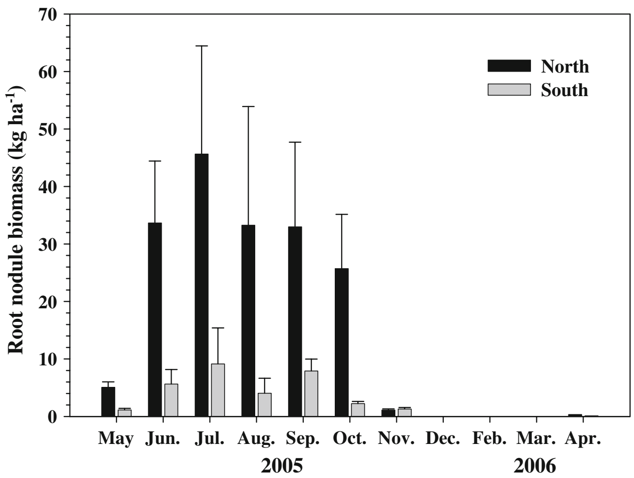

Fig. 4 Seasonal pattern of nodule biomass for the north- and south-facing *R. pseudoacacia* stands. The vertical lines denote one standard error of the mean

fixation rate was also not significantly associated with the NH_4^+ and NO_3^- concentrations (P>0.05).

Discussion

The diurnal patterns of the AR rates for *R. pseudoacacia* stand were unclear, even though the AR rates differed significantly among several measurement times (Teklehaimanot and Martin 1999; Pearson and Vitousek 2001). However, these results showed that the AR rates might be favored by sunlight (Binkley et al. 1994) and the diurnal changes in photoassimilates (Wheeler 1971; Huss-Danell et al. 1992). In addition, a similar seasonal pattern for the AR rate was reported by Fisher and Binkley (2000). The range of mean monthly AR rates (0–296 μmol C_2H_4 g^{-1} dry nodule h^{-1}) was also similar to the values (50–246) measured for young *R. pseudoacacia* stands in the southern part of Korea (Hong and Song 1990), and the overall mean AR rate (96.6) was relatively higher than those for other N-fixing trees (Binkley 1981; Zitzer and Dawson 1989; Son et al. 2007).

In particular, the south-facing stand had higher AR rates than the north-facing stand. The AR rate could be influenced by the form and size of the nodules (Boring and Swank 1984a). During the study period, the root nodules was mostly small (<2 mm) for the south-facing stand while the size varied (1–15 mm) for the north-facing stand. In this study site, the AR rate was negatively correlated to root nodule size (P<0.001; Fig. 7).

In addition, the AR rate is generally correlated to the soil temperature (Lee and Son 2005; Son et al. 2007). The same phenomenon was observed in this study. Because the nitrogenase activity can be limited by the loss of fluidity for a cellular membrane at low temperatures, while it increases at moderate temperatures (McColl and Edmonds 1986; Uliassi and Ruess 2002). The AR rate was also correlated to soil pH. Hong and Song (1990) observed the maximum nitrogenase activity at pH 7 and it was limited at pH greater than 9 for a young *R. pseudoacacia*. However, there was no relationship between the AR rate and soil water content. In this study, the ranges of soil water contents for the north- and south-facing stands did not appear to limit the AR rate.

There is limited information on the nodule biomass of *R. pseudoacacia* because of the small root nodule size (<0.5 mm). Several studies reported that the root nodule biomass for woody legume species varied from 0 to 106 kg ha^{-1} depending on the species, stand age, and density (Boring and Swank 1984b; Bormann et al. 1993). In this study, the nodule biomass for the north-facing stand in July was high (45.7 kg ha^{-1}). It was lower than the 106 kg ha^{-1} reported for a 17-year-old *R. pseudoacacia* stand in Southern Appalachian hardwood forests (Boring and Swank 1984b), while it was similar to the 51± 16 kg ha^{-1} measured for a 4-year-old *Leucaena* spp.

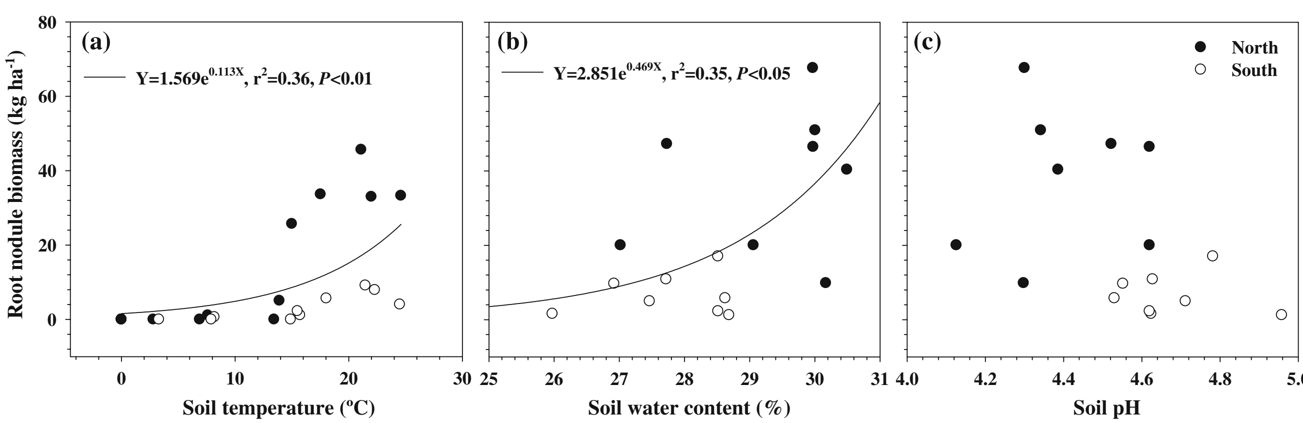

Fig. 5 Relationship between the root nodule biomass and soil temperature (**a**), soil water content (**b**), and soil pH (**c**) for the north- and south-facing *R. pseudoacacia* stands. Each point represents the mean of three plots for each measuring time

Table 3 Annual symbiotic nitrogen fixation rates for the stands dominated by woody leguminous N fixers, measured using the acetylene reduction technique

Species	Age (year)	Rate (kg N ha^{-1}year^{-1})	Aspect	Slope (°)	Altitude (m)	Mean annual temperature (°C)	Precipitation (mm)	Location	Reference
Acacia dealbata	1–3	12–32	S	moderate	400	5–25	1,750	Melbourne (37°52′S, 145°45′E)	Adams and Attiwill 1984
Acacia pennatula	20	34	–	–	1225	19	1,758	Mexico (19°27′N, 96°57′W)	Roskoski et al. 1982
Acacia koa	6–20	1.5–23.0	E	6	1700–1850	–	1,800	Island of Hawaii (19°30′N, 155°20′W)	Pearson and Vitousek 2001
Leucaena leucocephala	4	110±30	Sandbox experiment		500	24.4	870	Tanzania (6°50′S, 37°38′E)	Högberg and Kvarnström 1982
Robinia pseudoacacia	4	30	E	31	868–914	13	1,810	NC, USA (35°N, 83°W)	Boring and Swank 1984a
R. pseudoacacia	6	94	Sandbox experiment		–	18 (Jul.)	1,400	Hubbard Brook Experimental Forest	Bormann et al. 1993
R. pseudoacacia	25	112.3 23.2	N S	18–28 10–30	100–120	12.2	1,344	Republic of Korea (37°28′N, 126°57′E)	This study

N north-facing stand, *S* south-facing stand

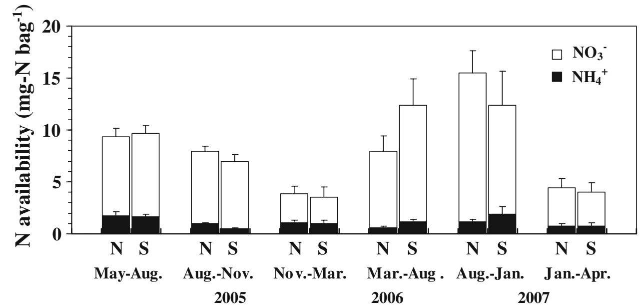

Fig. 6 Seasonal pattern of ion exchange resin NH_4^+ and NO_3^- concentrations (milligrams N per bag) measured from May, 2005 through to April, 2007 for the north- (N) and south (S)-facing *R. pseudoacacia* stands. The vertical lines denote one standard error of the mean

(Fabaceae) stand in Tanzania (Högberg and Kvarnström 1982), and was higher than the 26 kg ha^{-1} measured for a 6-year-old *Acacia* spp. stand in Hawaii (Pearson and Vitousek 2001). The seasonal pattern of root nodule biomass of *R. pseudoacacia* was similar to that of the AR rate (Figs. 1 and 4). However, the seasonal variables during the growing season were different from the pattern of *Alnus hirsuta* reported by Son et al. (2007; Fig. 4). The root nodule biomass for the north- and south-facing *R. pseudoacacia* stands increased rapidly with soil temperature within the range of 10°C to 25°C (Fig. 5a). The relationship between the root nodule biomass and soil temperature also reflected the seasonal changes. A lower moisture level was sufficient to limit nodule abundance and nodulation (Sprent and Sprent 1990; Pacchioli and Hower 2004). The dry root nodules and limited nodulation for the south-facing stands

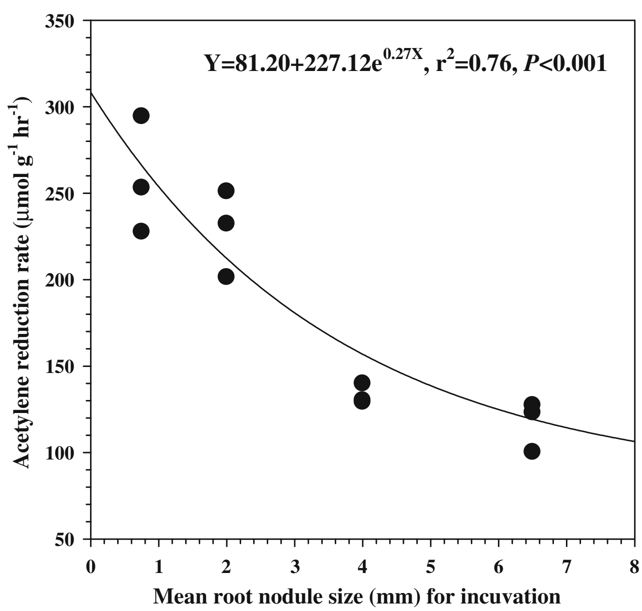

Fig. 7 Relationship between acetylene reduction rates and root nodule size for *R. pseudoacacia*

might be influenced by low water condition. In addition, a combination of low pH and low Ca delayed nodulation and strongly depressed the number of nodules and the nodule biomass (Alva et al. 1990). However, the north-facing stand had enough root nodules to fix N_2 despite of its low soil pH (4.32) because most known bacterial species can grow within the pH range of 4 to 9 (Paul and Clark 1996). Sometimes, soil acidity might be increased indirectly by biological N fixation of root nodules. Therefore, more studies will be needed to clarify the relationship between the environmental factors and nodule biomass.

The annual N_2 fixation rate for the north-facing *R. pseudoacacia* stand was higher than those measured in previous studies, while that for the south-facing stand was lower. These differences in N_2 fixation between the two stands were due mainly to the distinct difference in total nodule biomass. Although these even-aged stands were closely located, the environmental conditions from different aspects could affect the amount of N_2 fixation.

Nevertheless, these values fall not only within the ranges (10–160 kg N $ha^{-1}year^{-1}$) in ecosystems where N-fixing species are present during the early successional stages (Boring et al. 1988), but also within the range documented for other symbiotic leguminous N-fixing species (Table 3). There was a possibility that these values were overestimated because the AR rates measured during the daytime were used to calculate the N_2 fixation rates. However, it is difficult to measure the mean daily AR rate every measurement period. Therefore, we need additional studies on the estimation of the rates considering the relationship between the nitrogenase activity and soil temperature.

In addition, the N_2 fixation rate for the north-facing *R. pseudoacacia* stand was similar to the 120 kg N $ha^{-1}year^{-1}$ estimated for the annual N requirements in a 12-year-old *A. hirsuta* plantation (Mun et al. 1977) or for a 55-year-old forested ecosystem (Likens and Bormann 1977). On the other hand, the N_2 fixation rate for the south-facing stand was more than two thirds that of the annual N deposition input (30.1 kg N $ha^{-1}year^{-1}$) for urban forests in Seoul (Kim 2006). Previous studies also reported significant relationships between the N availability and N_2 fixation for *R. pseudoacacia* and *A. hirsuta* (Röhm and Werner 1991; Tobita et al. 2005). Although we could not find the relationship between those, some studies reported a decrease in N_2 fixation with increasing N availability whereas other studies concluded the opposite (Johnsen and Bongarten 1991; Kato et al. 2007).

Conclusion

In nature, it is difficult to interpret the interactions among temperature, moisture, soil pH, PAR, soil aeration, and soil type, because environmental factors seldom act independently. N_2 fixation would be influenced by all these interactions (Paul and Clark 1996). Therefore, more studies on N_2 fixation including root nodule biomass, denitrification, and leaching will help understand the N dynamics in forest ecosystems. In this study, the symbiotic N_2 fixation rates for the *R. pseudoacacia* showed great potential for the accretion of N in a forest ecosystem, while there was a significant difference of annual N_2 fixation rates between the south- and north-facing stands. These results also could be used to manage the *R. pseudoacacia* stands in Korea which have different micro-topographic conditions such as aspects.

Acknowledgments We thank Seong-Joon Kim for laboratory assistance. Funding for this study was provided by Korea University and the KOSEF A3 Foresight Program (Grant No. A307-K001).

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