Journal of Plant Biology, September 2005, 48(3) : 332-337

Diurnal and Seasonal Patterns of Nitrogen Fixation in an *Alnus hirsuta* Plantation of Central Korea

Yoon Young Lee and Yowhan Son*
Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Korea

We used an acetylene reduction assay to measure rates of nitrogen fixation on a 38-year-old *Alnus hirsuta* plantation in central Korea. The diurnal pattern of acetylene reduction changed significantly during May, August, and October, typically varying by 3-fold throughout the course of the day. Maximum rates occurred at 3 p.m. in May and October, but at 6 p.m. in August. Increasing trends were evident during the early growing season, with sustained high rates from mid-May through late September; July had the highest rates, averaging 7.2 μmole g^{-1} dry nodule h^{-1}. The average nodule biomass for this plantation was 220 kg ha^{-1}. Rates of acetylene reduction were related to soil temperature, but not to soil moisture content. Combining these nodule biomass calculations with seasonal average acetylene reduction rates yielded an estimate of current annual nitrogen fixation of 60 kg N ha^{-1} for the plantation. This rate of annual nitrogen addition was very large in relation to the yearly nitrogen requirements of coniferous and deciduous forests in central Korea.

Keywords: *Alnus hirsuta*, diurnal variation, nitrogen fixation, seasonal variation

Because low soil fertility hampered reforestation efforts on devastated lands in Korea several decades ago, nitrogen-fixing trees, such as *Robinia pseudoacacia* L. and *Alnus* spp., were widely planted to enhance soil nitrogen content. The most successful species, *Alnus hirsuta* Rupr., is indigenous to northeast Asia, shows rapid growth rates in central Korea, and re-sprouts after cutting (Krussmann, 1984). *Alnus* species have been studied extensively in other countries for their commercial uses and as sources of biologically fixed nitrogen (Trappe et al., 1968; Binkley et al., 1994; Hibbs et al., 1994; Fisher and Binkley, 2000; Anderson et al., 2004; Razgulin and Bogatyrev, 2004). However, very limited information is available on the ecology and management of *A. hirsuta* (Chae and Kim, 1977; Mun et al., 1977) or other nitrogen-fixers in central Korea (Koo et al., 1982, 1996; Lee, 1984, 1988).

Understanding the influences of nitrogen-fixing species on stand structure and growth, and on soil properties is important for proper management of forest ecosystems (Rhoades et al., 2001; Rothe et al., 2002; Deal et al., 2004). The complex ecophysiological factors that control nitrogenase activity have been studied, with the acetylene reduction method being widely used to measure nitrogen fixation by *Alnus* spp. (Teklehaimanot and Martin, 1999; Hurd et al., 2001; Anderson et al., 2004; Razgulin and Bogatyrev, 2004; Yamanaka et al., 2005). However, estimates of nitrogen fixation and its diurnal and seasonal patterns by *A. hirsuta* using that assay have not been reported. Furthermore, the effect of environmental parameters on such nitrogen fixation has not been examined. Therefore, the objectives of the current study were to 1) investigate diurnal and seasonal patterns of nitrogen fixation, 2) examine the relationship between nitrogen fixation and soil conditions, e.g., soil temperature and moisture content, 3) measure the nodule biomass for this species, and 4) estimate the annual rate of nitrogen fixation using the acetylene reduction technique for a 38-year-old *A. hirsuta* plantation in central Korea.

MATERIALS AND METHODS

This study was conducted on an *A. hirsuta* plantation in central Korea (37°47′32″ N, 127°48′48″ E). The average annual temperature and precipitation are 10.9°C and 1266 mm, respectively, in that region, with about 70% of the annual precipitation falling between June and August. The silt loam soil has developed from granite gneiss, with a pH of 5.2 (measured in H_2O), 0.45% Kjeldahl N, 18.8 mg kg^{-1} available P, and 5.9 meq exchangeable K kg^{-1}. In

*Corresponding author; fax +82-2-928-0852
e-mail yson@korea.ac.kr

2003, the average diameter at breast height (DBH) and height of this 38-year-old plantation were 20.0 cm and 17.8 m, respectively, and stand density was approximately 700 trees ha^{-1}. Three 10 m × 10 m research plots were established within the plantation.

For the acetylene reduction assays, nodulated root pieces were carefully excavated from the soil and enclosed in either 312-mL or 600-mL wide-necked glass bottles (depending on nodule size) that were fitted with rubber stoppers. The procedure involved incubating the nodules in the field for 1 h in an atmosphere of 10% acetylene (Knowles, 1987; Weaver and Frederick, 1987; Rice and Olsen, 1993). 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 conducted with a Porapak R column (80 to 100 mesh). Acetylene reduction rates were expressed in micromoles of ethylene produced per gram dry weight of nodule tissue per hour. The quantity of nitrogen fixation was calculated by assuming 1 mole of nitrogen for 3 moles of acetylene reduced (Hardy et al., 1973).

Diurnal patterns were determined by measuring nitrogen fixation on three occasions (25 May, 14 August, and 25 October 2003), when the nodules were incubated at 3-h intervals over a 24-h period. To obtain the rates of seasonal nitrogen fixation, nodule samples were assayed from April to December of 2003. Measurements were generally done between midmorning and noon on all measuring dates; the diurnal trends had indicated that this time period would produce readings close to, or slightly below, the 24-h average. The total biomass of nodules was calculated by excavating three to four 30 cm × 30 cm × 30 cm soil pits per plot (total 9 to 10 pits) on 2 April, 17 May, 6 July, 15 August, and 28 September 2003. After the dead material was excluded, the nodules were oven-dried at 75°C and weighed to determine their biomass. At the time of each nitrogen fixation measurement, the soil moisture content was determined gravimetrically and the soil temperature was monitored with a probe at 15 cm depth.

We assumed that the measured acetylene reduction rates approached the average 24-h rates. Therefore, the overall growing-season level of nitrogen fixation was calculated by multiplying the specific nitrogenase activity by the number of hours in the measuring period and by the nodule biomass per unit area (Binkley, 1981). The annual nitrogen fixation per unit area was estimated by summing the values for growing-season nitrogen fixation, assuming that rates were negligible in winter. That is, the acetylene reduction rates from 17 December through 1 April were assumed to be zero based on the data for seasonal nitrogenase activity gained from this study. Statistical analyses were performed using SAS 6.03 software (SAS, 1988). Analysis of variance was used to test mean differences in diurnal and seasonal nitrogen fixation among the measurements. In addition, we conducted regression analysis to quantify the relationship among nitrogen fixation and soil moisture and temperature. Results were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

During the growing season, the nodule biomass (kg ha^{-1}, mean ± 1 standard error) on the *Alnus hirsuta* plantation was 192.6 ± 133.7 (2 April), 200.0 ± 62.6 (17 May), 257.8 ± 157.0 (6 July), 220.1 ± 87.6 (15 August), and 229.8 ± 108.9 (28 September 2003). Biomass did not differ significantly among those five sampling times ($p=0.3$), with an overall average of 220 kg ha^{-1}. Although few previous studies have measured this parameter in pure *Alnus* species under field conditions, their nodule biomass has ranged from 30 to 450 kg ha^{-1}, depending on the species and the age of the stand (summarized by Binkley, 1981; Sharma and Ambasht, 1984; Binkley et al., 1992). Our values fell with this range and were close to those for stands of similar age.

Distinct diurnal patterns in *A. hirsuta* appeared for acetylene reduction and soil temperature at the 15-cm depth (Fig. 1). In general, acetylene reduction rates increased from the early morning, peaked in the mid- to late afternoon, then decreased throughout the night. Maximum rates occurred at 3 p.m. in the spring and fall, but at 6 p.m. in the summer. These diurnal patterns, possibly related to soil temperature, are very similar to those reported for *A. rubra* (Tripp et al., 1979) and *A. glutinosa* (Dawson and Gordon, 1979). An alternative explanation may be that these patterns simply tracked daily photosynthetic activity in the canopy (Wheeler, 1969; Tripp et al., 1979; Binkley et al., 1994). Mean acetylene reduction rates (mmole g^{-1} dry nodule h^{-1}) over individual 24-h test periods were 4.94 for 25 May, 7.30 for 14 August, and 3.41 for 25 October.

Seasonal patterns were also monitored for soil temperature and acetylene reduction by *A. hirsuta* (Fig. 2). Our values represented the average for 9 to 10 points or trees at each measuring time; they were not

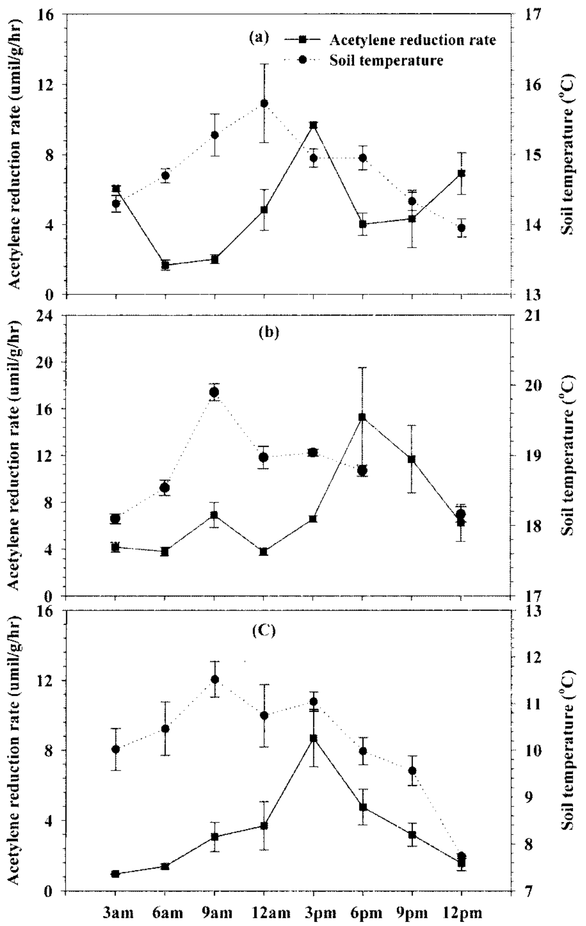

Figure 1. Diurnal pattern of acetylene reduction and soil temperature by *A. hirsuta* for May 25 (a), August 14 (b), and October 25 (c).

adjusted to account for the daily variations in acetylene reduction shown in Figure 1. The seasonal average fixation rate of 3.9 μmole g^{-1} dry nodule h^{-1} was lower than those (5 to 10 μmole g^{-1} dry nodule h^{-1}) reported for *Alnus* species older than 15 years (Binkley et al., 1992). In general, our data revealed similar seasonal patterns for acetylene reduction and soil temperature, both of which increased from spring, remained high through mid-summer, and decreased thereafter. This same seasonal pattern for acetylene reduction was reported by Bormann and Gordon (1984), Sharma and Ambasht (1984), and Binkley et al. (1992). In the current study, acetylene reduction rate was strongly correlated with soil temperature (see below). Some seasonal variations were evident in acetylene reduction, with the greatest variability occurring in September and December when the 95% confidence limits were 37 to 38% of the means. However, on other measuring dates, these were 10 to 20%. Our values were lower than those reported by

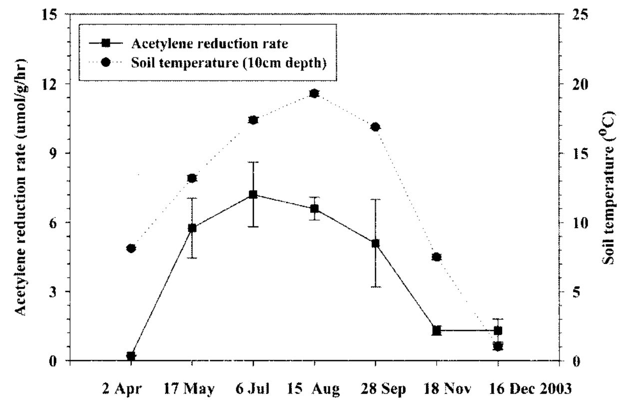

Figure 2. Seasonal pattern of acetylene reduction by *A. hirsuta* and soil temperature at 7 time points.

Tripp et al. (1979); some of this variation might have been related to seasonal changes in nodule biomass.

It is also possible that the low rate of acetylene reduction early in the growing season partly resulted from low moisture content, moisture stress being

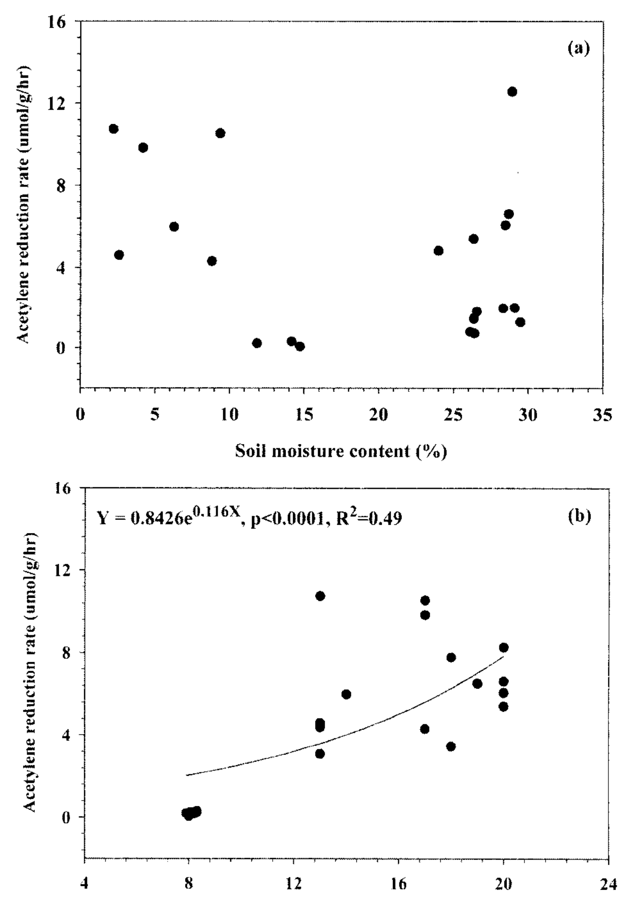

Figure 3. Relationship between acetylene reduction and soil moisture content (a) and soil temperature (b) for *A. hirsuta*.

Table 1. Annual nitrogen fixation rates in *Alnus* species ecosystems, as measured by the acetylene reduction technique.

Species	Age (yr)	Rate (kg ha^{-1} yr^{-1})	Reference
A. hirsuta, pure stand	38	60	Current study
A. glutinosa, pure stand	5-20	58	Cited by Binkley, 1981
A. incana, pure stand	6-7	85-115	Rytter et al., 1991
	30	43-	Johnsrud, 1978
	-	37	Hurd et al., 2001
A. rubra, pure stand	5	50-106	Cited by Binkley, 1981
	2-4	62	Tripp et al., 1979
	5-55	60-150	Reviewed by Binkley et al., 1994
A. nepalensis, mixed with *Amomum subulatum*	5-40	52-155	Sharma et al., 2002
A. rubra, mixed with *Pseudotsuga menziesii*	15-20	130	Binkley, 1981
A. sinuate, mixed with *Pseudotsuga menziesii*	15-20	20	Binkley, 1981

common in that study region (Binkley et al., 1992). However, seasonal changes in photoassimilates might also have influenced acetylene reduction rates (Wheeler, 1971; Tripp et al., 1979; Huss-Danell et al., 1992; Ekblad et al., 1994).

Soil moisture content was low between spring and early summer (range of 3.7 to 13.6%), but rapidly increased and remained high from mid-summer throughout the rest of the season (26.5 to 28.2%). Although Binkley et al. (1994) have concluded that seasonal dynamics in soil moisture likely affect nitrogen fixation for *A. rubra*, here we observed no significant correlation between acetylene reduction rate and soil moisture content for *A. hirsuta* ($p>0.1$) (Fig. 3a). Similar seasonal patterns of acetylene reduction and soil temperature have suggested that temperature plays an important role in controlling nitrogen fixation (Binkley et al., 1994); we also found a significant correlation between acetylene reduction and soil temperature ($p<0.0001$, $r=0.70$) (Fig. 3b).

Approximately 60 kg N ha^{-1} was fixed annually by our pure *A. hirsuta* plantation. This value was within the ranges identified from other *Alnus* species. However, Binkley et al. (1994) have reported that a pure *A. rubra* stand can show higher values (100 to 200 kg ha^{-1} yr^{-1}) than those measured in a mixed *A. rubra* stand (50 to 100 kg ha^{-1} yr^{-1}) (Table 1). Likewise, Mun et al. (1977) have estimated annual, per-hectare values for nitrogen requirements (120 kg), retranslocation (31 kg), and return (89 kg) on a 12-year-old *A. hirsuta* plantation with a stand density of 1700 trees ha^{-1}. Considering the age and stand density in that previous study, the 60 kg ha^{-1} yr^{-1} estimated for nitrogen input in our study plantation would be substantial and might be more than half of its annual nitrogen requirement. Most of the nitrogen necessary for tree growth is generally supplied through N mineralization in the soil. In Korea, estimated rates of this mineralization have ranged from 42 to 92 kg ha^{-1} yr^{-1} for coniferous species (*Pinus rigida* and *Larix leptolepis*) to 98 to 127 kg ha^{-1} yr^{-1} for the deciduous *Quercus* spp. (Son and Lee, 1997; Son, unpublished data, 2004). Therefore, the rate of nitrogen fixation by *A. hirsuta* may be half or more of the common rates of soil nitrogen mineralization. Given that this is an annual rate of input, the long-term increase in soil nitrogen supply probably rises dramatically under the influence of *A. hirsuta*, supporting the feasibility of using this species as source of nitrogen enrichment in the forests of central Korea.

ACKNOWLEDGEMENTS

We thank Drs. M.J. Yi and C.D. Koo for their help in the field and laboratory during the study. Dr. Dan Binkley provided helpful comments on earlier drafts. This work was supported by a Korea Research Foundation Grant (KRF-2002-041-F00022).

Received June 8, 2005; accepted July 22, 2005.

LITERATURE CITED

Anderson MD, Ruess RW, Uliassi DD, Mitchell JS (2004) Estimating N_2 fixation in two species of *Alnus* in interior Alaska using acetylene reduction and N^{15} uptake. EcoScience 11: 102-112

Binkley D (1981) Nodule biomass and acetylene reduction rates of red alder and Sitka alder on Vancouver Island, B.C. Can J For Res 11: 281-286

Binkley D, Cromack K, Baker DD (1994) Nitrogen fixation by red alder: Biology, rates, and controls. *In* DE Hibbs, DS DeBell, RF Tarrant, eds, The Biology and Management of Red Alder. Oregon State University Press, Corvallis, pp 57-72

Binkley D, Sollins P, Bell R, Sachs D, Myrold D (1992) Biogeochemistry of adjacent conifer and alder-conifer stands. Ecology 73: 2022-2033

Bormann BT, Gordon JC (1984) Stand density effects in young red alder plantations: Productivity, photosynthate partitioning, and nitrogen fixation. Ecology 65: 394-402

Chae MI, Kim JH (1977) Comparison of biomass, productivity and productive structure between Korean alder and oak stands. Kor J Ecol 1: 57-65

Dawson JO, Gordon JC (1979) Nitrogen fixation in relation to photosynthesis in *Alnus glutinosa*. Bot Gaz 140S: 70-75

Deal RL, Hennon PE, Orlikowska EH, D'Amore DV (2004) Stand dynamics of mixed red alder-conifer forests of southeast Alaska. Can J For Res 34: 969-980

Ekblad A, Lundquist PO, Sjostrom M, Huss-Danell K (1994) Day-to-day variation in nitrogenase activity of *Alnus incana* explained by weather variables: A multivariate time series analysis. Plant Cell Environ 17: 319-325

Fisher DF, Binkley D (2000) Ecology and Management of Forest Soils, Ed 3. Wiley, New York, pp 352-353

Hardy RWF, Burns RC, Holsten RD (1973) Application of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol Biochem 5: 47-81

Hibbs DE, DeBell DS, Tarrant RF (1994) The Biology and Management of Red Alder. Oregon State University Press, Corvallis

Hurd TM, Raynal DJ, Schwintzer CR (2001) Symbiotic N_2 fixation of *Alnus incana* spp. *rugosa* in shrub wetlands of the Adirondack Mountains, New York, USA. Oecologia 126: 94-103

Huss-Danell K, Lundquist PO, Ohlsson H (1992) N_2 fixation in a young *Alnus incana* stand, based on seasonal and diurnal variation in whole plant nitrogenase activity. Can J For Res 70: 1537-1544

Johnsrud SC (1978) Nitrogen fixation by root nodules of *Alnus incana* in a Norwegian forest ecosystem. Oikos 30: 475-479

Knowles R (1987) Free-living dinitrogen-fixing bacteria. *In* AL Page, RH Miller, DR Keeney, eds, Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, Ed 2. Soil Science Society of America, Madison, pp 1072-1092

Koo CD, Lee KJ, Yim KB (1982) Growth stimulation of pines by artificial inoculation with mycorrhizal fungus, *Pisolithus tinctorius*. J Kor For Soc 55: 22-29

Koo CD, Molina RJ, Miller SL, Li CY (1996) Effects of nitrogen and phosphorus fertilization on ectomycorrhiza development, N-fixation and growth of red alder seedlings. J Kor For Soc 85: 96-106

Krussmann G (1984) Manual of cultivated broad-leaved trees and shrubs. Timber Press, Beaverton, Vol 1: 134

Lee KJ (1984) Growth response of *Pinus rigida* × *P. taeda* to mycorrhizal inoculation and efficiency of *Pisolithus tinctorius* at different soil texture and fertility with organic amendment. J Kor For Soc 64: 11-19

Lee KJ (1988) Growth stimulation of *Alnus firma* and *Robinia pseudoacacia* by dual inoculation with VA mycorrhizal fungi and nitrogen-fixing bacteria and their synergistic effect. J Kor For Soc 77: 229-234

Mun HT, Kim JM, Kim JH (1977) Distributions and cycling of nitrogen, phosphorus and potassium in Korean alder and oak stands. Kor J Biol 20: 109-118

Razgulin SM, Bogatyrev YG (2004) The nitrogen-fixing activity of gray alder (*Alnus incana*). Eurasian Soil Sci 37: 769-774

Rhoades C, Oskarsson H, Binkley D, Stottlemyer B (2001) Alder (*Alnus crispa*) effects on soils ecosystems of the Agashashok river valley, northwest Alaska. EcoScience 8: 89-95

Rice WA, Olsen PE (1993) Root nodule bacteria and nitrogen fixation. *In* MR Carter, ed, Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, pp 303-317

Rothe AR, Cromack K, Resh SC, Makineci E, Son Y (2002) Soil carbon and nitrogen changes under Douglas-fir with and without red alder. Soil Sci Soc Am J 66: 1988-1995

Rytter L, Arveby AS, Granhall U (1991) Dinitrogen (C_2H_2) fixation in relation to nitrogen fertilization of gray alder [*Alnus incana* (L.) Moench.] plantations in a peat bog. Biol Fertil Soils 10: 233-240

SAS (1988) SAS/STAT User's Guide. Ed. 6.03. SAS Institute, Cary, NC

Sharma E, Ambasht RS (1984) Seasonal variation in nitrogen fixation by different ages of root nodules of *Alnus nepalensis* plantations, in the Eastern Himalayas. J Appl Ecol 21: 265-270

Sharma G, Sharma R, Sharma E, Singh KK (2002) Performance of an age series of *Alnus* Cardamom plantation in the Sikkim Himalaya: Nutrient dynamics. Ann Bot 89: 273-282

Son Y, Lee IK (1997) Soil nitrogen mineralization in adjacent stands of larch, pine, and oak in central Korea. Ann For Sci 54: 1-8

Teklehaimanot Z, Martin R (1999) Diurnal and seasonal patterns of nitrogenase activity of red alder in comparison with white clover in silvopastoral agroforestry systems. Biol Fertil Soils 28: 267-270

Trappe JM, Franklin JF, Tarrant RF, Hansen GM (1968) Biology of Alder. Pacific Northwest Forest and Range Experiment Station, Portland

Tripp LN, Bezdicek DF, Heilman PE (1979) Seasonal and diurnal patterns and rates of nitrogen fixation by young red alder. For Sci 25: 371-380

Weaver RW, Frederick LR (1987) *Rhizobium*. *In* AL Page, RH Miller, DR Keeney, eds, Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, Ed 2.

Soil Science Society of America, Madison, pp 1043-1070
Wheeler CT (1969) The diurnal fluctuation in nitrogen fixation in the nodules of *Alnus glutinosa* and *Myrica gale*. New Phytol 68: 675-682
Wheeler CT (1971) The causation of the diurnal changes in nitrogen fixation in the nodules of *Alnus glutinosa*. New Phytol 70: 487-495
Yamanaka T, Akama A, Li CY, Okabe H (2005) Growth, nitrogen fixation and mineral acquisition of *Alnus sieboldiana* after inoculation of *Frankia* together with *Gigaspora margarita* and *Pseudomonas putida*. J For Res 10: 21-26