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Dinitrogen (C_2H_2) fixation in relation to nitrogen fertilization of grey alder [*Alnus incana* (L.) Moench.] plantations in a peat bog

L. Rytter[1], A.S. Arveby[1], and U. Granhall[2]

[1] Department of Ecology and Environmental Research, P.O. Box 7072
[2] Department of Microbiology, P.O. Box 7025, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

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Summary. Nitrogenase activity was measured in young grey alder plantations in a peat bog in central Sweden. The stands were treated in three ways: (1) daily irrigation during the growing season with a complete nutrient solution, including N; (2) application of bark ash or wood ash before planting; and (3) fertilization every second year with solid PK fertilizers. Acetylene reduction assays were performed on (1) detached nodules and attached nodules, either on (2) whole enclosed plants or (3) enclosed nodules. The acetylene reduction rate for the enclosed plants showed a maximum in July when mean values of nearly 80 μmol C_2H_4 (g nodule dry matter)$^{-1}$ h^{-1} were reached. No diurnal patterns were observed. The irrigated stands, with an N supply, showed overall nitrogenase activities that corresponded well with those of the other treatments. Only in the case of temporarily increased soil nutrient concentrations in the irrigated stands did the nitrogenase activity fall considerably. In 6- to 7-year-old intensively managed irrigated stands N_2-fixation was estimated as 85–115 kg N ha^{-1} year^{-1} which was about 55% of the total N uptake of the trees.

Key words: Acetylene reducing activity – *Alnus incana* – *Frankia* – Nodules – Sphagnum peat – N_2 fixation

Introduction

The potential for N_2 fixation in the genus *Alnus*, by the actinomycete *Frankia*, is one of the main reasons for the interest shown in alders in temperate silviculture during recent decades. Conclusive evidence of N_2-fixing properties in *Alnus glutinosa* (L.) Gaertn. was presented by Bond et al. (1954), and later surveys showed that nearly all alder species investigated were nodulated (Bond 1976). Estimates of the amount of N_2 fixed by the alder vary from a few kg to more than 300 kg N ha^{-1} year^{-1} (Silvester 1977; Binkley 1981).

Offprint requests to: L. Rytter

In Scandinavia there are two alder species, black alder (*A. glutinosa*) and grey alder [*A. incana* (L.) Moench.]. The latter is considered the most useful species for intensive cultivation since it is frost-hardy, and grows more rapidly when young than the black alder. Grey alder was included in the Swedish Energy Forestry Research Programme, mainly as a species for cultivation on low-fertility sites with a severe climate (Rytter et al. 1989).

It has often been claimed that N fertilization suppresses the nitrogenase activity of root nodules, but there are also reports that small applications of N have a positive effect, both on nodulation and fixation (Bond et al. 1954; Stewart and Bond 1961; Kohls and Baker 1989). Ingestad (1980) found that the N_2-fixation rate increased with an increased supply of N up to near-optimum addition rates under laboratory conditions. Only at high N-flux rates, when the N concentration in the nutrient solution became high, were inhibitory effects seen. Thus, high concentrations of inorganic N seem to depress nodulation and nodule function whereas low concentrations seem to have the opposite effect.

Among the different methods for estimating N_2 fixation (Rennie and Rennie 1983), the acetylene reduction assay is favoured because it is highly sensitive and requires only simple and inexpensive equipment. The method is indirect, however, and a conversion factor between C_2H_2 and N_2 therefore needs to be estimated (Witty and Minchin 1988) for the particular study object.

In the acetylene reduction assay detached root nodules have often been used. This could lead to a considerable reduction in the estimated level of nitrogenase activity compared with undisturbed systems (Wheeler et al. 1978; Huss-Danell and Ahlqvist 1984). In the present study, detached nodules, attached nodules of enclosed plants and attached nodules in cuvettes were used.

The main purpose of the present study was to estimate the amount of N made available to the plant annually by actinorrhizal N_2 fixation in grey alder plantations under different treatments, especially with N fertilization.

Table 1. Treatments and N_2-fixation measurements of grey alder stands; liming and rotovation were carried out before planting

Stand	IL-80A	IL-80B[a]	IL-81A	AF-80	AF-82	F-80
Provenance	Siljansfors	Siljansfors	Ockelbo	Siljansfors	Siljansfors	Siljansfors
Source of *Frankia*	Ag + Ai	Ag + Ai	Ai	Ag + Ai	Ai	Ag + Ai
Year of planting	1980	1980	1981	1980	1982	1980
Amounts of lime (t ha^{-1})	4	4	12	4	4	9
N_2-fixation measurement						
Year and method	1982 dn	–	–	1982 dn	–	1982 dn
	1983 dn	–	1983 dn + ep	1983 dn	1983 dn + ep	1983 dn
	1986 en	–	–	–	–	–
N supply (kg ha^{-1})						
1980	90	59	0	0	0	30
1981	86	86	92	0	0	0
1982	320	329	188	0	0	0
1983	350	350	297	0	0	0
1984	164	164	108	0	0	0
1985	249	246	195	0	0	0
1986	223	216	222	0	0	0

Abbreviations: IL, irrigated and fertilized; AF, ash-fertilized; F, solid fertilized excluding N; Ag, *Alnus glutinosa*; Ai, *Alnus incana*; dn, detached nodules; en, enclosed nodules; ep, enclosed plants

[a] No measurements made. The stand was used for comparisons with the IL-80A stand

Materials and methods

Plant material

Grey alder stands were established in 1980–1982 (Table 1). The plants were raised from local seeds (provenances Siljansfors and Ockelbo) in a glasshouse during winter and spring, and were fertilized and irrigated until planting. Since there appeared to be no naturally occurring infective *Frankia* in the peat of the study site (Arveby and Huss-Danell 1988) the plants were inoculated. Additions of N were stopped a few weeks before planting and the seedlings were inoculated with *Frankia* by watering them with a suspension of crushed root nodules (Rytter et al. 1989). The nodules had been collected from grey alder stands within a 2-km radius of the study site, and in 1980 also from a black alder stand at Studsvik (58°50′ N; 17°30′ E; altitude 5 m). Planting was carried out between late June and early August when the seedlings were 10–15 cm high.

Site description and treatments

The field site is situated within Jädraås Research Station in central Sweden (60° 49′ N; 16°30′ E; altitude 185 m). It consists of a raised sphagnum bog with a peat layer of at least 1.5 m. All experimental plots (Table 1) were limed and rotovated before planting (Elowson and Rytter 1986; Rytter et al. 1989). The stands were 30×30 m or 30×15 m in area, with a density of four plants m^{-2}, and were treated in three different ways (Table 1).

In the first treatment, the stands (IL-80A, IL-80B, IL-81A) were irrigated and fertilized daily during the growing season with a complete nutrient solution. The amounts of fertilizer applied, together with assumed mineralization/immobilization rates, were estimated according to the level of nutrient use of the trees, the ground vegetation, and microorganisms (Ingestad 1987). The fertilizer contained all essential elements in balanced proportions by weight (Ingestad 1971), except Ca and Mg, which were mainly given as dolomite (Elowson and Rytter 1988). Fertilizer N was applied as NH_4^+-N (40%) and NO_3^--N (60%). The fertilizer regime was checked during the growing seasons of 1981–1985 by weekly conductivity analyses of the soil. On each sampling occasion soil cores were collected from three randomly chosen areas in each stand. The cores were divided into 3-cm sections down to a soil depth of 9 cm. The contents of each 3-cm cylinder (ca. 19 ml) were mixed with 80 ml of distilled water and the solution was left overnight at 21 °C before the conductivity was measured.

For the second treatment, no irrigation was carried out. The stands (AF-80, AF-82) were fertilized with 50 kg ha^{-1} of P as superphosphate and 5 t ha^{-1} of bark ash (AF-80) or wood ash (AF-82) before planting.

For the third treatment, also, there was no irrigation. The single stand (F-80) was fertilized in early summer every second year with solid PK fertilizers which also contained microelements (Rytter et al. 1989). During the year of establishment, a small amount of N (30 kg ha^{-1}) was applied.

Determination of acetylene reducing activity

Measurements of acetylene reducing activity were made on field material in three ways: (1) on detached nodules during 1982–1983; (2) on attached nodules of enclosed plants in 1983; and (3) on attached and enclosed nodules in 1986 (Table 1).

Detached nodules. A pilot study was conducted in 1982. In June, plastic tubes, 40 cm in diameter and 50 cm in length, were pushed into the soil so that they enclosed the entire root systems of plants in the IL-80A, AF-80, and F-80 stands. Three tubes per stand were used for the measurements. In October the tubes were removed intact, together with plants and peat (Rytter 1989). In the laboratory the tubes were withdrawn, leaving peat cylinders with complete root systems. Each peat cylinder was divided into sections 5–10 cm long. Nodules from the different layers were collected and the acetylene reducing activity was measured on detached nodules in 30-ml glass bottles with rubber membranes, at 21 °C. Gas samples were collected after 2–3 h and immediately analysed. The gas environment in the bottles comprised ambient air supplemented with 10% v:v of acetylene. Measurements of pH were carried out on soil samples from the centre of the peat cylinder from each soil layer.

During 1983, excised rootlets with root nodules were collected in five stands (IL-80A, IL-81A, AF-80, AF-82, and F-80). On each of four sampling occasions a 1/4 sector consisting of the upper 5 cm of the root system within a radius of 20 cm from the stem base was excavated. Three plants in each plot were sampled. The roots and their nodules were kept moist, immediately cleaned of peat soil, and trimmed so that about 2 cm of the roots remained laterally and distally of the nodules. The nodules were arbitrarily sorted according to size and each size class was then incubated for 2 h in 30-ml glass bottles containing 90% air and 10% acetylene. During the incubation, the bottles were kept buried in the top 5 cm of the peat soil. Gas samples in duplicate were taken after 1 and 2 h using 3-ml Venoject evacuated blood-collecting tubes (Terumo Europe, Belgium) with double-sided injection needles. The 3 ml removed from the incubation vessels was substituted with an equal amount of ambient air. Allowance was made for the dilution in the concentration of C_2H_4. After incubation the root nodules were dried at 85 °C for 2 days and weighed. Estimates of nodule biomass per ground area were made by correcting the biomass of the 1/4 sector with the nodule distribution reported by Rytter (1989).

Attached nodules of enclosed plants. As for the pilot study of detached nodules, plastic tubes were buried around plants in each of the IL-81A and AF-82 stands, after leaf emergence in mid-June, 1983. Acetylene reducing activity was measured on two plants per stand once a month during the period June-October. The start and end points of the N_2-fixing periods in 1983 were defined by the first emergence of leaves on May 20 in both stands and the completion of leaf-fall by October 12 and 22 in the IL-81A and AF-82 stands, respectively. During sampling, the plants were enclosed in 50- to 75-litre gas-tight plastic bags (Cryovac three-layered barrier bags, Grace Duncan Co., USA) sealed around the upper part of the tube by a firmly attached and stretched rubber tube. A rubber stopper was mounted on the bags for injections and samplings. This system was incubated in ambient air supplement with 10% v:v of acetylene and 1 ml propane as tracer gas. Gas samples were taken hourly in duplicate Venoject tubes. The total incubation time was 24 h for the first two samplings but later reduced to 4 h. The soil temperature was recorded in both stands at the start of the incubation (around noon) at a soil depth of 5 cm outside the tubes. After the growing season the plants were harvested and root nodules were collected, dried at 85 °C for 2–3 days, and weighed.

Attached and enclosed nodules. Measurements were made with cuvettes on attached nodules in the IL-80A stand on four occasions during 1986, 10–12 June, 16–17 July, 20–21 August, and 23–25 September. The start and end points of the season for this stand were defined by the first emergence of leaves on May 27 and the completion of leaf-fall by October 15.

On each sampling occasion, the measurements were separated into two series, dawn to noon (a.m.) and noon to dusk (p.m.). In each series five cuvettes of 130 ml were mounted around carefully exposed roots with nodules (Fig. 1) in the evening before the a.m measurements and in the morning before the p.m. measurements. The two parts of the cuvette were fixed together by hose clips. The root entries and exits were sealed with rubber stoppers and an adhesive mass. There was an additional rubber stopper with double walls for injections and for sampling of gases and water. The cuvettes were covered with peat before and between samplings. At the start of the experiment some air was removed and acetylene (14%) and propane (0.15% v:v) were injected. In each time series five or six gas samples in duplicate were collected in 3-ml Venoject tubes from each cuvette at about hourly intervals. Water was added to the cuvettes in order to maintain the atmospheric pressure and checks were made to see that no nodules inside the cuvettes were covered with water. The soil temperature was measured at a depth of about 5 cm during each gas collection. After the assay the nodules were excised and weighed before and after drying at 85 °C for 2 days.

Comparative study between the enclosed plant and the enclosed nodule method

A study was undertaken to compare acetylene reducing activity as measured by the enclosed nodule method with that of enclosed plants. Selected nodulated grey alder seedlings (provenance Siljansfors) of about equal size were transferred into buckets (volume 12 litres) filled with a mixture of peat and sand. The plants were supplied with a balanced nutrient solution (Ingestad 1971) at an addition rate of 7% day^{-1} which is below the rate that reduces the nodule activity in grey alder (Ingestad 1980). The light in the glasshouse was maintained at about 200 μEm^{-2} s^{-1} for 20 h per day.

The experiment was carried out after 6 weeks of growth. The plants were divided into two groups. In one group the plants, including bucket and soil, were enclosed in gas-tight plastic bags 1–2 h before the start of incubation (as described above). The air volume of the bags was 10–14 litres. In the other group, roots with nodules were carefully exposed and one cuvette (Fig. 1) was mounted around one root with nodules on each plant. The installation took place either 4 or 15 h before the start of incubation.

At the start of the experiment, a syringe was used to add acetylene to the bags and cuvettes to a concentration of 10–15% v:v. Propane was added at a concentration of 0.10–0.15% v:v to control gas leakage. Gas samples were collected in Venoject tubes from the bags and cuvettes in duplicate at hourly intervals for 5 h. Water was added to the cuvettes to maintain atmospheric pressure. The experiment was carried out on two subsequent days in March 1986. Seven and eight plants, respectively, were used for acetylene reducing activity screening in the enclosed nodule method and the enclosed plant method. After the experiment the plants were harvested and the nodules were separated, dried at 85 °C for 2 days, and weighed.

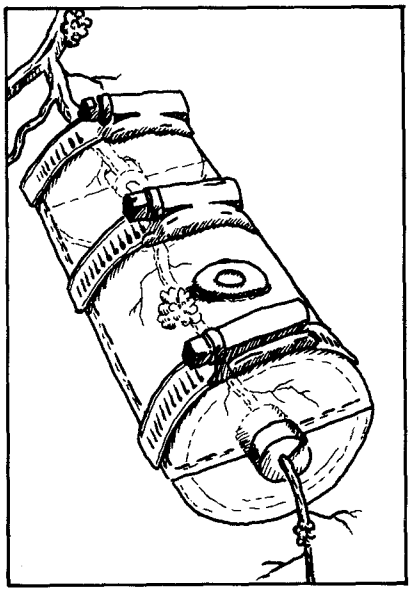

Fig. 1. Cuvette used for acetylene reduction measurements of root nodules attached to the tree; the cuvettes were used in 1986, both in the field and for the glasshouse measurements, and had an inner volume of 130 ml. The figure shows the cuvette with injection and gas-sampling port before it was sealed with an adhesive mass

Analyses of gas samples

The gas samples were stored at about 5 °C until analysed. The propane and ethylene contents were measured in a Perkin-Elmer 428 gas chromatograph with flame ionization detector (Lindberg and Granhall 1984). In the attached nodule methods, where propane was measured, a Durapak column was used while in the detached nodule method a Porapak column was used. Controls and gas blanks were included and corrections were made for them. Large and irregular losses of propane were considered to be uncontrolled leakage. Any such cuvette or vacuum tube was discarded and was not included in the results presented. Where whole plant systems were incubated in open-ended tubes the peat absorbed considerable quantities of ethylene and propane in fairly equal amounts ($C_2H_4 : C_3H_8 = 0.9 : 1$). About 35% of the ethylene produced was absorbed in this way (A.-K. Freij and U. Granhall, unpublished data 1982) and all field data for enclosed plants were corrected accordingly. Measurements of acetylene reduction in the cuvettes were adjusted for the corresponding propane leakage, assuming a 1:1 relationship.

Statistics

When calculating the acetylene reducing activity in terms of ground area, mean values of nitrogenase activity were calculated as weighted averages, thus taking into account the individual size of the nodule sample. Diurnal trends in acetylene reducing activity (both in the glasshouse and under field conditions), changes in the ratio of nodule dry weight to fresh weight over time, and correlations between nodule dry weight and acetylene reducing activity were tested by regression analysis. Mean differences between measurement methods or time of application cuvettes in the glasshouse experiment were tested by one-way analysis of variance. The confidence level was 95%.

Table 2. Root nodule biomass per plant and soil pH in relation to soil depth from plants grown in tubes in the field; grey alders from stands IL-80A, AF-80, and F-80 were analysed in October 1982, and IL-81A and AF-82 alders in October 1983; data for IL-80A from Rytter (1989)

Stand	Age (years)	Number of plants	Soil depth (cm)	pH	Nodule biomass (g dry matter plant^{-1})	Nodule distribution with soil depth (%)
IL-80A	C+2	5	0–5	5.76±0.22	2.954±0.693	97.1
			5–10	5.19±0.16	0.084±0.068	2.8
			10–20	5.11±0.11	0.004±0.003	0.1
			20–30	5.11±0.07	0	0
			30–40	5.01±0.06	0	0
IL-81A	C+2	10	0–5	–	0.609±0.234	89.6
			5–10	–	0.065±0.054	9.6
			10–20	–	0.006±0.005	0.9
			20–30	–	0	0
			30–40	–	0	0
AF-82	C+1	2	0–5	7.10±0.25	2.510±0.210	74.6
			5–10	6.03±0.34	0.745±0.255	22.1
			10–20	4.87±0.12	0.046±0.001	1.4
			20–30	–	0.064±0.064	1.9
			30–40	–	0	0
AF-80	C+2	3	0–5	6.40±0.18	2.205±0.618	81.8
			5–15	4.51±0.23	0.490±0.193	18.2
			15–25	3.83±0.12	0.002±0.002	0.1
			25–35	3.95±0.13	0	0
			35–45	4.03±0.07	0	0
F-80	C+2	3	0–5	5.00±0.32	2.178±1.137	73.8
			5–15	4.06±0.20	0.772±0.389	26.2
			15–25	3.75±0.10	0	0
			25–35	3.84±0.06	0	0
			35–45	4.02±0.07	0	0

Means ± SE. C, current year; for other abbreviations, see Table 1

Results

Determination of acetylene reducing activity from field samples

Detached nodules. In the pilot study in 1982 only biomass data and potential nodule activity could be assessed because of the late start (October). Potential nodule activity was detectable in fully defoliated IL-80A and F-80 alders when incubated at 21 °C. The trees in stand AF-80 still had some leaves at the time of harvest and accordingly had a somewhat higher level of activity than the other stands. The plants in the IL-80A stand were harvested from early October to early November. Nodule activity decreased rapidly after the first harvest but was detectable even after frosts in early November (about 1% of that in early October). The root nodules had a shallow distribution (Table 2), and 74–82% of nodule dry weight was found in the 0–5 cm soil layer of the non-irrigated stands. In the irrigated stands, the corresponding figure was 90–97%.

In the 1983 measurements (data not shown) there was a large variation in acetylene reducing activity over the season. The only consistent change during the growing season was a reduced activity at the end (October). No correlation was found between treatments and acetylene reducing activity, or between nodule size and acetylene reducing activity. Specific nodule activity was generally much lower (ca. 10%) than the corresponding acetylene reducing activity of enclosed plant systems.

Attached nodules of enclosed plants. Only insignificant gas leakage was found in this system, even when the assay lasted as long as 24 h. Ethylene formation was linear over time, with no diurnal pattern during this period. The later assays were therefore limited to 4 h.

Nitrogenase activity varied during the growing season, with a peak in July for the AF-82 stand (Fig. 2A). Activity in the IL-81A stand was depressed at this time, coinciding with a sharp increase in soil conductivity (Fig. 2B). At this time the fertilization rate was high but in the following week it was much reduced (Fig. 2C). The peak levels of nitrogenase activity were 56 and 79 µmol C_2H_4 (g nodule dry matter)$^{-1}$ h^{-1} for the IL-81A (August) and AF-82 (July) stands, respectively. Low values were recorded in the autumn when a large part of the leaves had fallen. In 1983 the soil temperature at noon was highest in July (18 °C). In June, August, September, and October the soil temperatures at noon were 16, 10, 12, and 4 °C, respectively, in both stands.

Attached and enclosed nodules. A large variation in acetylene reducing activity between cuvettes was seen on each sampling occasion in the IL-80A stand during 1986. Although there were some variations in nodule activities within individual cuvettes during the day, no detectable diurnal pattern was observed. The variation in nodule activity increased irregularly for most cuvettes after about 5 h, partly due to variations in leakage as judged from the disappearance of propane. Therefore, the diurnal average values of ethylene production were calculated from the

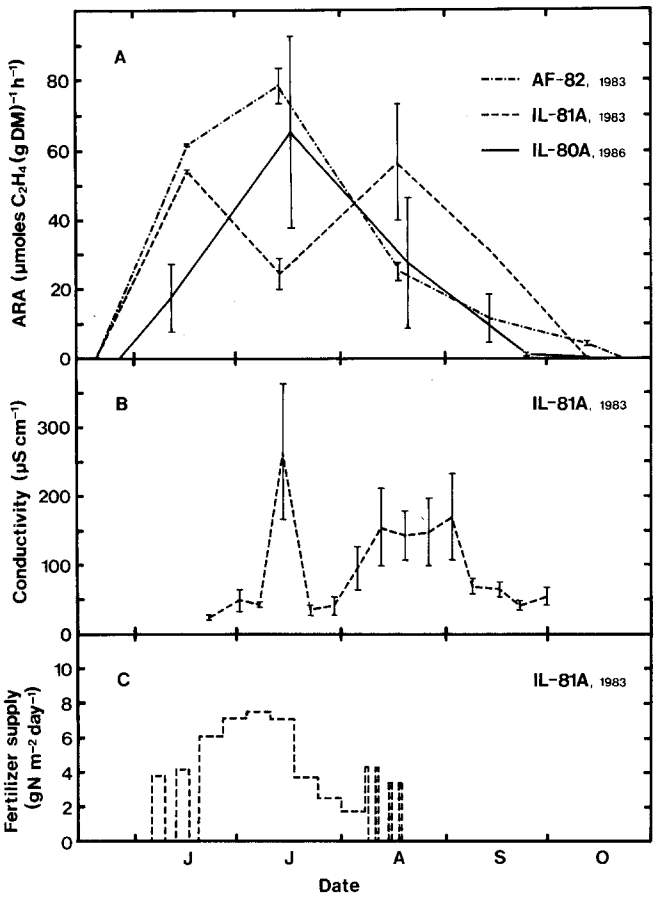

Fig. 2. **A** Acetylene reducing activity (*ARA*) in grey alder nodules from the AF-82, IL-81A, and IL-80A stands (AF, ash-fertilized; IL, irrigated and fertilized). Mean values with standard errors of nodule activity per day are shown. In 1983 enclosed plant systems from AF-82 and IL-81A ($n = 2$) were assayed while in 1986 nodules enclosed in cuvettes from IL-80A ($n = 8-10$) were used. **B** Conductivity of the soil in the IL-81A stand during 1983; each point in the figure consists of mean values with standard errors from three cores with three soil layers, 0–3, 3–6, and 6–9 cm soil depth ($n = 9$). **C** Fertilization regime of the IL-81A stand during 1983; at the end of the fertilization period nutrients were supplied twice a week

first 4 h of each assay. The peak value in July was 65 μmol C_2H_4 (g nodule dry matter)$^{-1}$ h^{-1} (Fig. 2A). The average nodule activity was lower in June and September of 1986 than in 1983. During the vegetation period of 1986 the ratio between the root nodule dry weight and fresh weight increased significantly, from 0.22 in June to 0.27 in September, indicating that the nodules were more lignified at the end of the season.

The 1986 soil temperature showed big variations over time (mean values during the incubation periods were 14, 14, 10, and 4 °C for June, July, August, and September respectively), but not more than a 4 °C difference over each sampling occasion (2–3 days).

The nodule biomass in each stand was calculated in terms of ground area (Table 3), allowing for plant survival and the average tree size (Rytter 1989). There was no clear difference in nodule biomass between the different treatments but there was an increasing trend with increasing age. Relatively low values were found in the IL-81A stand in 1981. In the IL-80 stands relatively high values were recorded in 1984 and low values in 1985.

Table 3. Nodule biomass (g dry matter m^{-2}) of differently treated grey alder stands during 1982–1986. The samples were collected after the end of the growing season each year, in October

Stand	Age (years)					
	C+1	C+2	C+3	C+4	C+5	C+6
IL-80A	–	11 (5)[a]	14 (3)	29 (8)[a]	14 (8)[a]	48 (8)[a]
IL-80B	–	–	–	25 (8)[a]	17 (8)[a]	23 (8)[a]
IL-81A	–	3 (10)	–	–	–	–
AF-82	12 (5)	–	–	–	–	–
AF-80	–	11 (3)	12 (3)	–	–	–
F-80	–	5 (3)	20 (3)	–	–	–

Mean values are shown with the number of replicates within parentheses. For abbreviations see Tables 1 and 2.
[a] Data from Rytter (1989)

Comparative study in the glasshouse

There was a big variation in the activity between plants, independently of the measurement method used (Table 4). No time trend was observed for acetylene reducing activity for up to 5 h (insignificant slope). The mean acetylene reducing activity measured with the enclosed nodule method seemed to correspond to 70% of that measured by the enclosed plant method, but the difference between methods was not statistically significant. The length of time the cuvettes had been installed before the measurement were made (4 or 15 h) did not significantly influence the nodule activity recorded. We concluded that, if necessary, the cuvettes could be installed the day before the measurements were taken.

Discussion

High concentrations of N in the root medium may have harmful effects on the *Frankia* symbiosis, but low concentrations of inorganic N have shown positive effects on nodule number, weight, and activity. This has been reported both for NH_4^+-N (Bond et al. 1954; Stewart and Bond 1961) and NO_3^--N (Zavitkovski and Newton 1968; Kohls and Baker 1989). Ingestad (1980) showed that in nodulated grey alder seedlings N_2 fixation increased in absolute terms up to high rates of N addition. This means that while the amount of N supplied is of minor importance the way it is added is of paramount importance in view of negative effects on nodulation and nodule activity. As long as the capacity of the plants or trees to make use of N is not exceeded the harmful effects of an increased N concentration in the growth medium can be avoided (Ingestad 1987). In the irrigated and fertilized stands the principle of nutrient addition, including N, was to supply the elements at a rate that could be used by the ecosystem (Ingestad 1987; Elowson and Rytter 1988). However, in July 1983, nodule activity in the IL-81A stand dropped abruptly, and this coincided with a sharp increase in soil conductivity after a long period of a high fertilization rate (Fig. 2). In this case, the decreased nodule activity was directly associated with high nutrient concentrations, in which N was a major element. Thus, the capacity to use nutrients was probably exceeded

Table 4. Acetylene reducing activity (ARA) from a comparative study between the enclosed plant and the enclosed nodule method. The cuvettes of the enclosed nodule method were mounted either 15 or 4 h before, and the bags that enclosed whole plants 1 – 2 h before the start of measurements

	Method	
	Enclosed plants	Enclosed nodules
Number of plants	8	7
Mean plant weight, g (SE)	6.03 (0.59)	6.81 (0.55)
Mean nodule weight per plant, g (SE)	0.10 (0.01)	0.11 (0.02)
Linear regression of ARA with time		
Intercept	87.8	36.9
Slope (probability)	−4.15 (0.49)	3.38 (0.63)
Mean ARA, μmol C_2H_4 (g dry matter)$^{-1}$ h^{-1} (SE)		
Installation 15 h before	–	44.2 (35.0)
Installation 4 h before	–	48.6 (18.0)
Total	67.7 (15.5)	46.7 (16.4)

during this period. At all other times the nitrogenase activity of the irrigated and fertilized stands was closely comparable with that of the other treatments.

The level of acetylene reducing activity estimated in the present study, nearly 80 μmol C_2H_4 (g nodule dry matter)$^{-1}$ h^{-1} in midsummer (Fig. 2A), was equal to or higher than that found for other alder species on other sites in Europe and North America. Tripp et al. (1979) recorded mean nodule activities of nearly 90 μmol C_2H_4 (g dry matter)$^{-1}$ h^{-1} in early summer in 3-year-old *Alnus rubra*, while Binkley (1981) reported 36 and 51 μmol C_2H_4 (g dry matter)$^{-1}$ h^{-1} in mid-July for 4 to 8-year-old *Alnus sinuata* (Regel) Rydb. and *Alnus rubra*, respectively. Johnsrud (1978) reported a low maximum mean acetylene reduction rate in July, 10 – 15 μmol C_2H_4 (g dry matter)$^{-1}$ h^{-1}, for 30-year-old *Alnus incana* in Norway.

However, in all the studies mentioned above, except for Johnsrud (1978), excised nodules, root pieces, or severely disturbed root systems were used. A strong decrease in acetylene reducing activity due to disturbance to the plant and the nodules was shown clearly by Wheeler et al. (1978) and Huss-Danell and Ahlqvist (1984), and was also seen in this study. This means that many values for the annual N_2 fixation by alder stands as calculated from detached nodules with the acetylene reduction method (Silvester 1977; Binkley 1981) could be underestimates.

In order to reduce the effects of harmful handling, whole plant systems and cuvette methods have been developed to take acetylene reducing activity measurements (Balandreau and Dommergues 1973; Winship and Tjepkema 1982; Granhall et al. 1983; Huss-Danell et al. 1989). In the present study, the 1983 measurements also included enclosed plant systems with minor disturbances. In 1986 the enclosed nodule method (Fig. 1) had to be developed because of increased tree size. The test comparing the methods (Table 4) showed an insignificantly lower degree of activity in the cuvettes than in the bags. The disturbance caused by the cuvettes was much less than that caused by detaching the nodules, where the activity was only about 10% of that in intact plants.

The overall nitrogenase activity seemed to be somewhat higher in 1983 than in 1986 (Fig. 2A), except for the strong reduction in activity in the IL-81A stand in July 1983. The methods, as discussed above, may be one reason for the differences between 1983 and 1986. However, nodules are perennial and it seems that older and more lignified nodules are less efficient than younger ones (Tripp et al. 1979; Schwintzer et al. 1982). During the 1986 season a significant increase in the ratio between nodule dry weight and fresh weight was found, indicating that the proportion of inactive woody material in the nodules had increased by the end of the season.

In young actinorrhizal seedlings diurnal patterns of nodule activity have been observed (Wheeler and Lawrie 1976; Dawson and Gordon 1979; Tripp et al. 1979). However, in older plants and trees, as in the present study, diurnal patterns do not seem to occur (Akkermans and van Dijk 1976; Wheeler and Lawrie 1976; Schwintzer 1979). This is probably due to the older and bigger trees having large carbohydrate reserves from which the root nodules can be continuously supplied and therefore being less directly dependent on daily photosynthesis. The long days of Swedish summers may also have contributed to the absence of diurnal variation.

Another factor that influences nitrogenase activity is temperature (Winship and Tjepkema 1985). Johnsrud (1978) reported that acetylene reducing activity was proportional to soil temperature in the range 9 – 16 °C for grey alder stands. The diurnal changes in soil temperature in the present study were small and did not allow any obvious diurnal pattern in nodule activity, while the larger temperature differences between the sampling occasions may explain some of the seasonal pattern in acetylene reduction rates.

N_2 fixation in alders takes place from leaf emergence in the spring until completion of leaf fall in the autumn (Johnsrud 1978; Tripp et al. 1979). However, in the present study, nitrogenase activity occurred in nodules from fully defoliated plants when the nodules were incubated at 21 °C. The activity after leaf fall fell rapidly with time but it is clear that stored carbohydrates were available for use. Temperature rather than leaf fall thus seems to deter-

Table 5. N in different biomass fractions of irrigated and fertilized grey alder stands, 1984–1986. The samples were collected after the end of the growing season each year, in October. The N concentrations of stem + branches and of below-ground parts have not previously been published; other data were given by Rytter (1989) and Rytter et al. (1989)

	N ($g\ m^{-2}$)			
	IL-80A stand		IL-80B stand	
	Biomass	Increase in year	Biomass	Increase in year
1984				
Stem + branches[a]	13.4	–	10.5	–
Leaf litter	10.6	–	8.3[b]	–
Twig litter	0.2	–	0.2[b]	–
Coarse roots	3.4	–	3.0	–
Fine roots	1.4	–	1.0	–
Root nodules	0.7	–	0.6	–
Total	29.7	–	23.6	–
1985				
Stem-branches[a]	16.8	3.4	16.1	5.6
Leaf litter	12.5	12.5	9.2	9.2
Twig litter	0.5	0.5	0.3	0.3
Coarse roots	3.3	−0.1	3.3	0.3
Fine roots	1.8	0.4	1.8	0.8
Root nodules	0.3	−0.4	0.4	−0.2
Total	35.2	16.3	31.1	16.0
1986				
Stem + branches[a]	24.0	7.2	20.2	4.1
Leaf litter	9.6	9.6	9.5	9.5
Twig litter	1.3	1.3	0.6	0.6
Coarse roots	5.0	1.7	3.9	0.6
Fine roots	2.4	0.6	1.5	−0.3
Roots nodules	1.0	0.7	0.5	0.1
Total	43.3	21.1	36.2	14.6

[a] Stump included
[b] Not measured, calculated from relationship between litter and stem + branches from the IL-80A stand

mine when nodule activity ceases, although low temperatures and completion of leaf fall usually coincide in the field.

The vertical distribution of the nodules was extremely shallow, with 90% or more above a soil depth of 5–6 cm in the irrigated stands (Table 2; Rytter 1989). The somewhat deeper nodule distribution in the non-irrigated stands may be explained by the drier conditions in these stands. The distribution was thus probably explained by the anaerobic conditions in the deeper peat layers (Akkermans and van Dijk 1976; Elowson and Rytter 1986). Nodulation was not correlated with the different pH levels found in the different treatments (Table 2). The relatively large nodule weight in the IL-80 stands in 1984 (over 4 years of age) were found in a year when the N supply was comparatively low (Table 3). The low level of *Frankia* infection in the IL-81A stand (provenance Ockelbo) remains unexplained.

Measurements of biomass production and N analyses of the biomass material (Rytter 1989; Rytter et al. 1989) showed that at least 211 kg N ha^{-1} was incorporated in the trees of the IL-80A stand during 1986 (Table 5). For the IL-80B stand of the same age and with nearly identical management, the corresponding figure was 146 kg N ha^{-1}.

By using the measurements of nodule activity obtained during 1986 (Fig. 2A) and of the standing crop of root nodules at the start (C+5) and end (C+6) of that growing season (Table 3), the annual input of N by N_2 fixation was estimated for the IL-80 stands in 1986. The measurement values were not corrected for any disturbance caused by the method used, but were weighted with respect to nodule sample weight. It was assumed that nodule growth followed the same seasonal pattern as shoot growth, which had been measured in detail in two previous years (L. Rytter, unpublished data 1982). With a theoretical C_2H_2/N_2 conversion factor of 4 (Witty and Minchin 1988), the IL-80A stand fixed about 115 kg N ha^{-1} $year^{-1}$. Assuming similar nodule activity in both IL-80 stands, an annual N_2 fixation of about 85 kg ha^{-1} was obtained for the IL-80B stand. Lack of stand data or of acetylene reducing activity measurements makes any estimates for other stands and years unreliable. An earlier estimate of N_2 fixation of about 70 kg ha^{-1} $year^{-1}$ in the IL-81A stand in 1983 (Rytter et al. 1989) seems, by a renewed analysis, somewhat overestimated.

During 1986, 223 kg N ha^{-1} was applied as fertilizer (Table 1), 115 kg N ha^{-1} may have been fixed from atmospheric N, and at least 211 kg N ha^{-1} was incorporated in the trees (Table 5) in the IL-80A stand. The corresponding figures for the IL-80B stand were 216, 85, and 146 kg N ha^{-1} $year^{-1}$, respectively. Therefore, of the total N uptake by the trees, around 55% could be related to N_2 fixation in the 7th year after planting in N fertil-

ized stands. The inflow of N was much higher than the amount incorporated in the tree biomass. The nutrients were, however, also consumed by the ground vegetation and the soil microorganisms. Since the fine root N turnover and the N uptake by ground vegetation were not included, more N was used than shown in Table 5. In addition, soil microoganisms immobilized a certain amount of N during this phase of building up site fertility (T. Slapokas, personal communication 1989). Thus, most added mineral N was probably used by vegetation and by soil microorganisms since only about 5–15 kg N ha^{-1} year leaked from the irrigated and fertilized stands during the period 1983–1986 (G. Wiklander, personal communication). Denitrification losses were probably small since conditions promoting the process (i.e. low oxygen availability, neutral pH, and high soil temperature) are seldom found in peat soil (Bremner and Shaw 1958).

The present study showed that high-producing grey alder stands (Rytter et al. 1989), both extensively grown with N-free fertilizers and intensively irrigated and fertilized with N, had a high N_2-fixing capacity. Nodule activity (Fig. 2) remained at a high level provided that high nutrient concentrations in the soil were avoided.

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