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# Nitrogen fixation in lysimeter-grown grey alder (*Alnus incana* (L.) Moench.) saplings - influence of nitrogen fertilization

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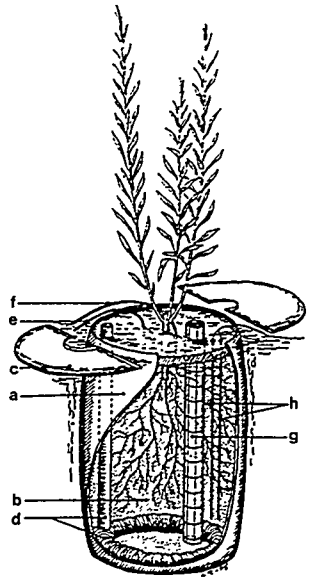


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## Contents

## Abstract

A lysimeter study was started in order to test if nitrogen fixation as well as biomass production in grey alder plantations (*Alnus incana* (L.) Moench.) can be stimulated by daily addition of small N doses. One-year-old grey alder saplings were planted in undrained lysimeters, each filled with 200 litres of quartz sand. Water and a low-concentrated balanced nutrient solution were distributed daily via a drip irrigation system. In this paper a complete N budget for the first growing season is presented. The results showed that presence of mineral N in the growth substrate had no beneficial effect on growth during the first growing season. The capacity of the plants to make use of the daily N additions was overestimated in this investigation. Almost twice as much N was added with fertilizers as the amount of N accumulating in the tissues. Consequently, the N concentration in the drainage water increased and the annual rate of $N_2$-fixation was strongly suppressed, 31 mg N plant$^{-1}$ (1 kg N ha$^{-1}$) versus 1700 mg N plant$^{-1}$ (32 kg N ha$^{-1}$) in the controls. However, no harmful effect of the elevated soil-N concentration on nodule development could be detected.

**Key words:** biomass distribution, computer-controlled water and nutrient supply, lysimeter, nitrogen budget, nitrogen losses

## Introduction

Despite the limited contribution to the standing tree biomass in forests, 1.2% of the total stem volume in Sweden (Eriksson 1991), grey alder (*Alnus incana* (L.) Moench.) is a species that has attained growing interest the last 20 - 30 years. The species has been included in the Swedish Energy Forestry Programme since the late 1970s. The recent interest in the use of grey alder is not only attributable to its $N_2$-fixing capacity. Characters such as high production potential (Rytter *et al.* 1989, Granhall and Verwijst 1994), low preference for browsing (Danell *et al.* 1991) and high frost tolerance (Christersson and von Fircks 1984) are also important. Field observations indicate that this species is self-supporting with nitrogen and only marginally better growth performances have been reported where nitrogen has been added as fertilizer (Rytter *et al.* 1989, Granhall and Verwijst 1994). In contrast to field observations grey alder seedlings grown under laboratory conditions responded strongly and positively to mineral nitrogen additions. Ingestad (1980) found that the rate of $N_2$-fixation and growth increased with an increased supply of mineral N up to near-optimum addition rates. Only at high N-flux rates, when the N concentration in the nutrient solution became high (6 - 12 mM), were inhibitory effects on $N_2$-fixation seen. Thus, high concentrations of inorganic N seem to depress nodulation and nodule function whereas low N concentrations seem to have the opposite effect. Similar positive effects from small doses of inorganic N have been reported by Bond *et al.* (1954), Stewart and Bond (1961) and Kohls and Baker (1989). One explanation to the differences obtained between field and lab in growth and $N_2$-fixation in response to N-fertilization may be attributable to the problems associated with controlling the N concentration in a complex growth substrate. In general, it is always difficult to transform knowledge received *in vitro* to *in situ* conditions and particularly to extrapolate results obtained with small seedlings to large trees. The use of lysimeters, in which the water and nutrient content can be carefully controlled and where the growth above- as well as below ground can be followed from establishment to canopy closure, may bridge the existing gap between lab and field studies (Rytter *et al.* 1998).

An investigation was initiated in 1991 in which the influence of inorganic N on growth and $N_2$-fixation in grey alder was studied under field conditions. Our aim was to test whether the large increase in growth and $N_2$-fixation reported by Ingestad (1980) in response to small and repeated nitrogen doses can be achieved also under field conditions and with larger trees. Lysimeters, with a volume of 200 litres, and with one tree each, were submerged in the soil in stands consisting of equally aged and sized grey alders. The duration of the experiment was 4 years. The stands were harvested after the third growing season and the impact from this management operation on $N_2$-fixation was studied the following year. In this paper a complete N budget from the first year of this experiment will be presented and discussed.

## Material and Methods

The experiment was carried out at Ultuna research station (lat. 59°49'N; long. 17°40'E; alt 5 m) south of Uppsala in east-central Sweden. One year old grey alder saplings (*Alnus incana* (L.) Moench.) were planted in undrained containers of 200 litres in May 1991. Data on height, stem diameter and estimated weights (based on 10 destructively harvested saplings) of the different plant parts at time of planting are given in Table I for the trees harvested after the first growing season. Quartz sand with an average particle size of 0.58 mm and initially free from humus was used as growth substrate. A pipe connected to a circular drainage-tube on the bottom of the lysimeters made it possible to remove excessive water. The lysimeters used in this study differed in three respects from the system described in detail by Rytter *et al.* (1998); they were not equipped with covers (protecting from precipitation) and minirhizotrons as well as sensors for measuring the soil water content. The lysimeters were kept weed free during the whole experiment.

Two treatments were applied, (i) optimal supply of water and liquid fertilizers containing nitrogen (+N treatment) and a balanced set of all other essential mineral elements (Rytter and Ericsson 1993), (ii) optimal supply of water and liquid fertilizers without N (-N treatment). These treatments were applied to 10 m x 15 m large plots. The submerged lysimeters, 33 per treatment in three rows, were placed in a stand structure with a spacing of 0.7 m x 0.7 m. Each row of lysimeters was surrounded on each side by 2 - 3 rows of equally aged trees planted directly in the soil. Water and nutrients were distributed via drip-tubes. The supply of water and nutrients was directed from a computer program which worked on a daily basis and compensated for precipitation. Water (128 mm season$^{-1}$) and nutrients (2768 mg N plant$^{-1}$ or 55 kg N ha$^{-1}$ season$^{-1}$) were given from late May until the end of August. The daily doses were adjusted to the requirements set by growth, i.e. the smallest amounts were given early and late in the season whereas the largest additions were made at time of maximum growth (mid July). The water table was checked regularly and kept at a minimum in order to avoid anaerobic conditions in the soil. When raising, excessive water was pumped up, quantified and analysed on $NH_4^+$ and $NO_3^-$ (Bifok FIA 5010, Tecator Höganäs, Sweden).

Leaf litter from the trees to be harvested in the autumn was collected at 1 - 2 weeks intervals during the second half of the growing season from thin light-permeable litter-nets of nylon enclosing the canopies. The stems were cut from the stumps at the end of the growing season after measurements of heights and diameters (5 cm above soil surface). The below ground plant parts were washed free from sand and divided into stump, coarse roots (> 1 mm), fine roots (< 1 mm) and root nodules. Root samples for nutrient analysis were taken from each category prior to this preparation with a soil corer. This was done in order to avoid uncontrolled nutrient losses of easily mobile ions from the tissues when washing the roots free from sand. Dry weights of above and below ground plant parts were

determined after 48 h at 70 °C and the nitrogen concentration of the different plant parts was determined with a Carlo Erba NA 1500 (Carlo Erba Strumentatzione, Milan, Italy) .

Treatment differences in absolute and relative terms were tested, for parts of and whole trees, by one way ANOVA (Statgraphics 1988). The confidence level was 95%.

**Results**

The tree height increased during the first growing season with 35 cm (-N) and 45 cm (+N) and the stem diameter 5 cm above ground was doubled (Table I). The total plant biomass on a dry weight basis increased from ~12 g to slightly more than 90 g per tree during the same period. No statistically significant differences between treatments were obtained in these plant characters. Neither did additions of mineral N significantly influence the mass of the different plant components compared with plants whose only source of N came from $N_2$-fixation (Table I). However, on a relative basis, the fraction of tree mass consisting of stem and stump was significantly larger in presence of mineral N, whereas the coarse root fraction increased significantly in absence of fertilizer N (Figure 1). Presence of mineral N in the root substrate had no effect on the absolute mass of fine roots and root nodules or on their share of the total tree mass (Table I, Figure 1).

**Table I.** *Shoot length, diameter (at 5 cm above soil surface) and dry weights of the different plant parts, at the beginning and at the end of the first growing season, of grey alder saplings cultured in 200 litres lysimeters. The treatments were: 55 kg N $ha^{-1}$ (+N) and no nitrogen supply (-N). Otherwise both treatments received all other essential nutrients and water in non-growth-limiting amounts. Standard deviations are included. n = 6*

| Plant character | Treatments | | | |
|---|---|---|---|---|
| | Spring | | Autumn | |
| | +N | -N | +N | -N |
| Stem height, cm | 90.5 ±5.2 | 90.0 ±9.8 | 135.8 ±14.4 | 124.0 ±15.2 |
| Diameter, mm | 7.6 ±0.6 | 7.4 ±0.4 | 15.0 ±1.7 | 14.7 ±2.2 |
| Stem weight, g | 7.5 ±1.4 | 7.2 ±1.2 | 45.5 ±10.4 | 43.2 ±10.4 |
| Stump weight, g | - | - | 3.7 ±0.8 | 2.6 ±1.0 |
| Coarse roots, g | 2.2 ±0.4 | 2.1 ±0.3 | 26.5 ±7.3 | 31.4 ±10.6 |
| Fine roots, g | 1.7 ±0.4 | 1.6 ±0.3 | 14.6 ±4.5 | 15.3 ±3.9 |
| Root nodules, g | 0.5 ±0.08 | 0.5 ±0.06 | 1.5 ±0.36 | 1.6 ±1.61 |
| Total weight, g | 12.5 ±2.3 | 11.9 ±1.9 | 91.9 ±22.9 | 94.1 ±24.4 |

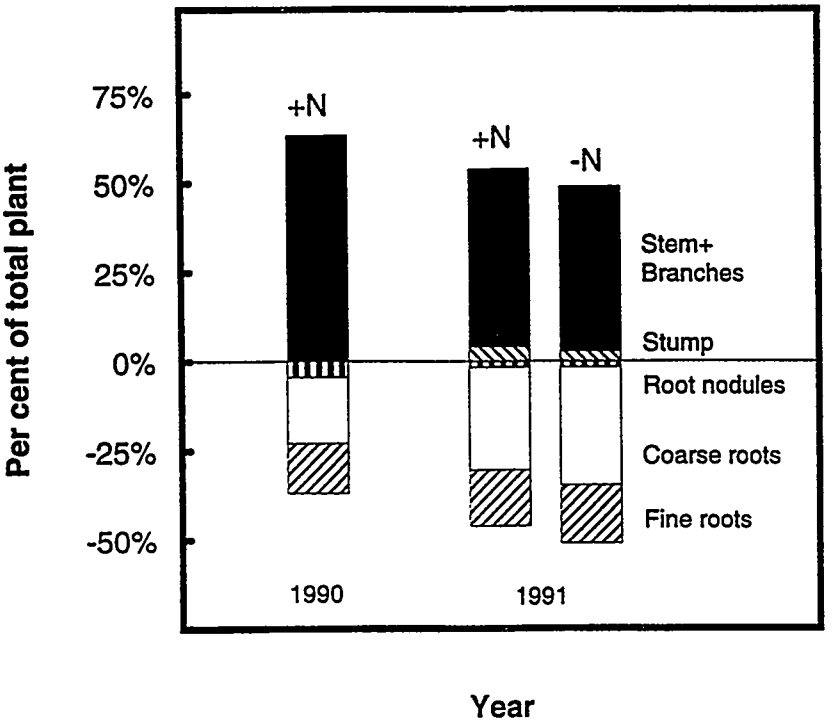


**Figure 1.** *Partitioning of growth and nodulation of grey alder saplings at time of planting (one-year-old) and after one growing season in lysimeters. The saplings received daily irrigation and liquid fertilization with (+N, 2768 mg N plant$^{-1}$ or 55 kg N ha$^{-1}$) or without N (-N) included. The treatments lasted from late May to end of August. n = 6.*

The supply of mineral N did not significantly influence the tissue N-concentration of any plant part (data not shown). Neither were any statistically significant differences observed in the absolute amounts of N found in the different plant components in presence or absence of fertilizer N (Table II). However, the loss of N in the drainage water was considerably larger in the +N treatment, 1460 mg lysimeter$^{-1}$ compared to 203 mg N lysimeter$^{-1}$ in the -N treatment (Table II). Nitrogen fixation one year after planting (plant age = 2 years) was calculated as the difference between N found in sapling and in the drainage water collected during the season and N coming from fertilizers, irrigation, precipitation (data on precipitation were taken from the Ultuna area, whereas data on its N content are from Ryda Kungsgård 60 km south-west from Ultuna) and original seedlings. No significant increase in the soil N content was observed after the first growing season.

$$N_{fix} = N_{sapl.} + N_{\Sigma drain.} - (N_{fert.} + N_{irr.} + N_{prec.} + N_{seedl.})$$

The calculation revealed that 31 and 1607 mg N tree$^{-1}$ were fixed in the +N and -N treatments, respectively (Table II). These values correspond to a $N_2$-fixation rate ha$^{-1}$ of ~1 kg and 32 kg, respectively.

**Table II.** *A nitrogen budget, expressed as mg N plant$^{-1}$ ± standard deviation, of two-years-old grey alder saplings grown in lysimeters. The submerged and undrained lysimeters were filled with ~ 200 litres of quartz sand and placed in a stand of equally aged grey alder saplings. The plants received daily irrigation and liquid fertilization with (2768 mg N plant$^{-1}$ or 55 kg N ha$^{-1}$) or without N included. The treatments lasted from late May to end of August. n = 6.*

| | Treatments | |
|---|---|---|
| | +N | -N |
| **Recovered** | mg N plant$^{-1}$ | |
| Stem | 567±139 | 571±153 |
| Stump | 45±12 | 31±14 |
| Coarse roots | 455±123 | 537±172 |
| Fine roots | 277±78 | 286±68 |
| Root nodules | 44±11 | 48±11 |
| Leaf litter | 335±115 | 318±88 |
| **TOTAL PLANT** | **1723±445** | **1791±469** |
| Drainage water | 1460±925 | 203±154 |
| **TOTAL** | **3183** | **1994** |
| **Added** | | |
| Initial N in plant | 242±39 | 243±25 |
| Fertilizer-N | 2768 | 0 |
| Supplied water | 107 | 108 |
| Precipitation | 36 | 36 |
| **TOTAL** | **3153** | **387** |
| **Net difference (= $N_2$-fixation)** | 30 | 1607 |

## Discussion

The experimental technique applied in this investigation, i.e. daily additions of water and nutrients to field-grown plants cultured in submerged lysimeters, proved partly successful in creating controlled nutrient conditions. The use of an inert substrate, here quartz sand, also greatly facilitated the below ground biomass assessment. Soil water content was fairly well controlled during periods with sunny weather, but large fluctuations in the water table occurred during rainy days. The use of covers on top of the lysimeters is therefore recommended in future studies (see Rytter *et al.* 1998).

The capacity of the plants to make use of daily N additions was overestimated in this investigation. Almost twice as much N was added via the liquid fertilizer as the amount of N accumulated in the tissues during the growing season (Table II). As a consequence,

nitrogen concentration increased in drainage water and $N_2$-fixation rate was strongly suppressed. However, no harmful effect from the elevated soil N concentration on root nodule development could be detected (Table II). A strong negative relationship between the N-concentration in soil solution and nodulation is otherwise to be expected judged from literature data (e.g. Stewart and Bond 1961, Ingestad 1980). The absence of visuable damages on nodules in this investigation may be explained by the fact that the majority of the nodules were located in the upper soil horizon, i.e. some distance from the N-rich drainage water.

The way of quantifying $N_2$-fixation in this investigation has advantages over the acetylene reduction assay, where a conversion factor between $C_2H_2$ and $N_2$ needs to be estimated for the particular object (Witty and Minchin 1988). Although the way of determining $N_2$-fixation from the difference between output and input of nitrogen is attractive and accurate, the technique has practical limitations. Only trees during early stages of development (1~10 years depending on species) can easily be handled. Denitrification, which was not measured, may give rise to uncontrolled N-losses and thereby errors in estimation of $N_2$-fixation. Although pH in drainage water was close to 7, the other conditions promoting denitrification , i.e. low oxygen content and high temperatures in the soil (Bremner and Shaw 1958), were not present in the lysimeters.

The amount of $N_2$ fixed in this investigation, 1607 mg $tree^{-1}$ or 32 kg N $ha^{-1}$ in the -N treatment is not high in comparison with literature data on grey alder, which ranges from 43 to 125 kg N $ha^{-1}$ (Stassen and Behrisch 1925, Ovington 1956, Johnsrud 1978, Rytter *et al.* 1991). But if taking into account the low age of the plants used in this investigation, 2 years, and that the stands had not reached canopy closure, the figures are more impressive. In an equally aged, but less densely spaced, grey alder stand, Huss-Danell *et al.* (1992) reported a $N_2$-fixation rate of 22 kg $ha^{-1}a^{-1}$.

No statistically significant differences in size of the different plant organs caused by treatment were obtained in this study. However, the fraction of total biomass consisting of coarse roots was significantly greater in grey alders receiving no mineral N. This result was not expected since nutrient status at the end of the growing season was the same in +N and -N plants. Neither did Ingestad (1980) found differences in relative biomass distribution between nodulated grey alder seedlings which received no or near optimum amounts of mineral N. We can not exclude the possibility that our test plants, particularly in the -N treatment, suffered from N shortage in the beginning of the growing season when the root systems were small in relation to soil volume. However, by the time of harvest roots had reached the bottom of the lysimeter, and because of improved access to added nutrients, the plants may have recovered from an eventual nutrient (N) shortage. The tissue nutrient (N) concentrations, particularly of young plants who are almost completely dependent on soil nutrients for growth, respond quickly to changes in the soil supply of nutrients, but it takes much longer time before a changed (here improved) nutrient (N) status is manifested in the shoot:root ratio. This may also explain why the root fraction of the total plant biomass in both treatments was larger after the first growing season than at time of planting (Figure 1).

Plant growth was not stimulated in this investigation, in opposite to expectations, by presence of mineral N in the growth substrate. Ingestad (1980) reported a doubling of the relative growth rate under laboratory conditions when grey alder seedlings used mineral-N instead of atmospheric-N as the dominating N-source. However, in the field only marginally better growth performances have been reported where nitrogen has been added as fertilizers (Rytter *et al.* 1989, Granhall and Verwijst 1994). It thus seems as if plant

size, or balance between assimilating and respiring (supporting, structural) tissues, modifies the beneficial effect from mineral N on growth. When the costs for maintenance respiration are low in relation to the carbon costs for growth, which is the case in small seedlings, access to mineral N greatly favours growth. As plants grow bigger proportionally less carbon will be available for structural growth because the costs for maintenance respiration continuously increases with plant size while the amount of $CO_2$ fixed by the canopy reaches a plateau at time of canopy closure. This may explain why application of N-fertilizers at this stage of plant development have only a minor stimulatory influence on growth of $N_2$-fixing tree species. Data collected from this experiment during the second and third growing season will allow us to test this hypothesis.

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**References**

Bond, G., Fletcher, W.W. and Ferguson, T.P. 1954. The development and function of the root nodules of *Alnus*, *Myrica* and *Hippophoë*. Plant and Soil 5: 309-323.

Bremner, J.M. and Shaw, K. 1958. Denitrification in soil: ll. Factors affecting denitrification. J. Agric. Sci. 51: 40-52.

Christersson, L. and von Fircks, H.A. 1984. Production losses in intensively cultivated energy plantations. Swed. Univ. Agric. Sci., Dept. Ecol. & Environ. Res., Uppsala, Sweden, Rep. 15, pp. 363-373.

Danell, K., Edenius, L. and Lundberg, P. 1991. Herbivory and tree stand composition: moose patch use in winter. Ecology 72: 1350-1357.

Eriksson, H. 1991. Vad vet vi idag om björkens och aspens produktion i Sverige? Skogsfakta Konferens 15, Swed. Univ. Agric. Sci., Uppsala, Sweden, pp. 73-83.

Granhall, U. and Verwijst, T. 1994. Grey alder (*Alnus incana*) - a $N_2$-fixing tree suitable for energy forestry. In Hall, D.O.; Grassi, G. and Scheer, H. (eds), Biomass for Energy and Industry, 7th E.C. Conference, Ponte Press, Bochum, Germany, pp. 409-413.

Huss-Danell, K., Lundquist, P.-O. and Ohlsson, H. 1992. $N_2$ fixation in a young *Alnus incana* stand, based on seasonal and dinural variation in whole plant nitrogenase activity. Can. J. Bot. 70: 1537-1544.

Ingestad, T. 1980. Growth, nutrition and nitrogen fixation in grey alder at varied rate of nitrogen addition. Physiol. Plant. 50: 353-364.

Johnsrud, S.C. 1978. Nitrogen fixation by root nodules of *Alnus incana* in a Norwegian forest ecosystem. Oikos 30: 475-479.

Kohls, S.J. and Baker, D.D. 1989. Effects of substrate nitrate concentration on symbiotic nodule formation in actinorhizal plants. Plant and Soil 118: 171-179.

Ovington, J.D. 1956, Studies of the development of woodland conditions under different trees. IV. The ignition loss, water, carbon and nitrogen content of mineral soil. J. Ecol. 44: 171-179.

Rytter, L. and Ericsson, T. 1993. Leaf nutrient analysis in *Salix viminalis* (L.) energy forest stands growing on agricultural land. Z. Pflanzenernähr. Bodenk. 156: 349-356.

Rytter, L., Arveby, A.S. and Granhall, U. 1991. Dinitrogen ($C_2H_2$) fixation in relation to nitrogen fertilization of grey alder (*Alnus incana* (L.) Moench.) plantations in a peat bog. Biol.Fert. Soils 10: 233-240.

Rytter, L., Slapokas, T. and Granhall, U. 1989. Woody biomass and litter production of fertilized grey alder plantations on a low-humified peat bog. For. Ecol. Manage. 28: 161-176.

Rytter, L., Ericsson, T., Lindroth, A. and Rytter, R.-M. 1998. A new lysimeter system - for controlled ecophysiological studies of young trees *in situ*. Swed. Univ. Agric. Sci., Dept. Short Rotation Forestry, Uppsala. Report 58.

Stassen and Behrisch, 1925. Über Aufforstungen von Kalködland, insbesondere in Bezug auf die Weisserle und Schwarzkiefer in der Klosteroberförsterei Göttingen. Zeitschrift für Forst- und Jagdwesen 57: 483-494.

Statgraphics 1988. Statistical Graphics System by Statistical Graphics Corporation. STSC Inc., Rockville, Maryland, USA.

Stewart, W.D.P. and Bond, G. 1961. The effect of ammonium on fixation of elemental nitrogen in *Alnus* and *Myrica*. Plant and Soil 14: 347-359.

Witty, J.F. and Minchin, F.R. 1988. Measurement of nitrogen fixation by root nodules of *Alnus rubra* Bong.: Effects of temperature and oxygen concentration. Plant and Soil 87: 91-107.