Negative effect of N-fertilisation on N-fixation in *Alnus-Frankia* symbiosis as shown by ^{15}N incorporation

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Abstract

The *Alnus incana* study plants were fertilised for two weeks with 5 different concentrations of NH_4-NO_3 which were equivalents of N-fertilising loads of 0, 25, 50, 100 and 150 kg N ha^{-1} y^{-1}, respectively. ^{15}N concentrations (^{15}N‰) in leaves, current year shoots and nodules were measured after 10-d ^{15}N (N_2) incubation period. As for the leaves and shoots the highest ^{15}N‰ were found in case of no mineral nitrogen addition, 15,67‰ ± 3,45; 16,82‰ ± 3,14 in leaves and shoots, respectively (± indicates the 95% confidence limits). At 150 kg N ha^{-1} y^{-1} the ^{15}N‰ of leaves was significantly lower than at any other treatment staying as low as 2,62‰ ± 1,12. The activity of nitrogen fixation over the 10-d ^{15}N-incubation period was calculated (mg ^{15}N per gram of nodule dry weight). Fixation activity at N addition rate of 150 kg N ha^{-1} y^{-1} was significantly lower than at N addition rates 0, 50, 100 kg N ha^{-1} y^{-1}.

Key words: *Alnus incana*,*Frankia*, ^{15}N (N_2) incorporation, N_2 fixation

Results

As expected, fertilising-induced positive effect on plant growth was not detected as a result of too short exposure to the added mineral nitrogen. Also, no statistically relevant decrease in nodule dry mass upon high N loads occurred. That was, neither in absolute nodule mass terms, nor in relation to total aboveground biomass of which the nodules comprised 2,33% ± 0,91 (± indicates the 95% confidence limits). The nodule dry mass of the control plants was similar to that of study plants.
The overall ^{15}N concentrations (^{15}N‰) of study plants were significantly higher than these of control plants ($p < 0.0001$) showing active nitrogen fixation in the ^{15}N enriched experiment pot (data not shown).
As for the leaves and shoots the highest ^{15}N‰ were found in case of no mineral nitrogen addition, 15,67‰ ± 3,45; 16,82‰ ± 3,14 in leaves and shoots respectively. At 150 kg N ha^{-1} y^{-1} the ^{15}N‰ of leaves was significantly lower than at any other treatment staying as low as 2,62‰ ± 1,12. ^{15}N concentration in shoots was also the lowest at 150 kg N ha^{-1} y^{-1} being 3,23‰ ±1,78, but the difference with ^{15}N‰ at 25 kg N ha^{-1} y^{-1} was at 95% confidence limits not relevant. At 25, 50, 100 kg N ha^{-1} y^{-1} the corresponding ^{15}N concentrations were as follows, in leaves: 7,51‰ ± 3,35; 9,25‰ ± 0,99; 8,03‰ ± 2,81 and in shoots: 6,81‰ ± 3,62; 10,58‰ ± 0,78; 7,94‰ ± 2,37. Generally the different N loads did not notably affect the ^{15}N‰ of the nodules. Only statistically relevant difference in ^{15}N‰ of the nodules was found between treatments 50 and 150 kg N ha^{-1} y^{-1}. ^{15}N‰ of the nodules were in general higher than these of other studied plant fractions (except for 0 kg N ha^{-1} y^{-1}) (Figure 1).
The calculated absolute amounts (mg) of ^{15}N incorporated into analysed plant fractions are shown on table 1. As for the nodules the only statistically relevant difference was detected between the treatments 50 and 150 kg N ha^{-1} y^{-1}, where in case of the latter fertilisation rate the nodules had incorporated significantly less ^{15}N. The non-fertilised plants incorporated more ^{15}N into shoots, than plants grown at N addition rates 25, 100 and 150 kg N ha^{-1} y^{-1}. The amount of incorporated ^{15}N in leaves of the alders grown at the highest N load was significantly smaller compared to those of fertilising loads 0, 50, 100 kg N ha^{-1} y^{-1}.
The activity of nitrogen fixation over the 10-d ^{15}N-incubation period was calculated (mg ^{15}N per gram of nodule dry weight). Fixation activity at N addition rate 150 kg N ha^{-1} y^{-1} was significantly lower than at N addition rates 0, 50, 100 kg N ha^{-1} y^{-1}.

Introduction

High concentration of inorganic nitrogen is generally accepted to be inhibitory to symbiotic nitrogen fixation efficiency in actinorhizal systems. Yet, there are indications that small applications of N have a positive effect both on nodulation and fixation (Stewart and Bond, 1961; Ingestad, 1980; Kohls and Baker, 1989). Ingestad (1987) concluded that as long as the capacity of the trees or plants to make use of N is not exceeded the harmful effects of an increased N concentration in the growth medium could be avoided.
In majority of studies addressing N-fixation activity under different conditions acetylene reduction assay has been used as the method to estimate N-fixation. Acetylene itself is inhibitory to nitrogenase and induces what is called acetylene-induced decline. It is stated that the initial (2-3 min) peak rate of acetylene reduction is the most accurate measure of nitrogenase activity (Schwintzer and Tjepkema, 1997). Earlier studies have generally used longer acetylene exposure times.
^{15}N incorporation is a direct method to assess N-fixation over a certain time period. Techniques using ^{15}N have been widely used to calculate the conversion factor between acetylene reduction and N_2 reduction (Sellstedt, 1986 and references therein). In the present study we use $^{15}N_2$ to confirm the negative effect of added mineral nitrogen to nitrogen fixation activity in *Alnus-Frankia* symbiosis.

Materials and methods

Stainless steel was used to construct a 1,1 m^2 quadrangular pot, which was divided into five rows of seven hexagonal wells (a = 11cm, h = 20cm). The wells of a row were not partitioned allowing free mixture of soil water throughout the row while soil and soil water penetration between rows was impossible. The experimental device was filled with loamy sand soil originating from humus horizon of ~20 year *Alnus incana* stand.
3-4 year *Alnus incana* bare-root seedlings were collected from abandoned cultivated grassland and transferred into the experiment pot (1 plant per well). Additionally, 10 control plants were planted outside the pot in a furrow filled with soil of the same source as used in the experiment pot. All trees were of generative origin. Soil deriving from the plant source site was carefully removed from the root systems (to the reasonable extent not to damage the roots or nodules). The plants were grown for four-week adjustment period during which some of the trees had to be replaced due to little or no vitality.
During the following two weeks each of the five rows received ammonium nitrate (NH_4-NO_3) solution of different concentrations equivalent to fertilising loads 0, 25, 50, 100 and 150 kg N ha^{-1} y^{-1}. The fertilisation was carried out in three steps with six-day intervals as 0; 0,183; 0,367; 0,733; 1,10 g N per row was respectively added each time. Control plants were not fertilised.
For ^{15}N incubation the pot was hermetically sealed using 0,5 cm transparent polymethyl-methacrylate (Plexiglas) plates and silicon mass. Sealing was implemented 2 cm above the row partitions, thus creating a conjoint air layer for all rows but leaving the shoots in the open (Figure 2,3,4). 2L of ^{15}N N_2 gas (99,95% ^{15}N, CK Gases, USA) was entered through a valve. As a result, about 2% of the gas volume in the pot comprised ^{15}N. Control plants did not receive additional ^{15}N.
After 10-d ^{15}N incubation the plants were harvested. Five trees out of seven per each row and three control plants were taken for further analysis. Fresh weight, height and stem diameter at root collar of the trees was measured. Dry biomass of stems, branches, nodules, leaves, and shoots (current year) was analysed. N and ^{15}N concentrations (^{15}N atom‰) were determined in the samples of three latter sample groups. Analysis was carried out in three repetitions using isotope mass-spectrometer “Finigan S” connected to elemental analyser “Vario EL” (calibration at 0,399 atom%). Some trees were left out from further analysis, as ^{15}N transport to shoots did not occur (most probably due to stem damage by Plexiglas plates).Materials and methods

Fig. 1.
^{15}N concentrations (atom‰) in leaves, shoots (primary growth of the branches) and nodules of 2-3 year *Alnus incana* trees after 10-d incubation of ca. 2% ^{15}N (N_2) at five different ammonium-nitrate (NH_4-NO_3) fertilisation rates. Error bars indicate 95% confidence limits.

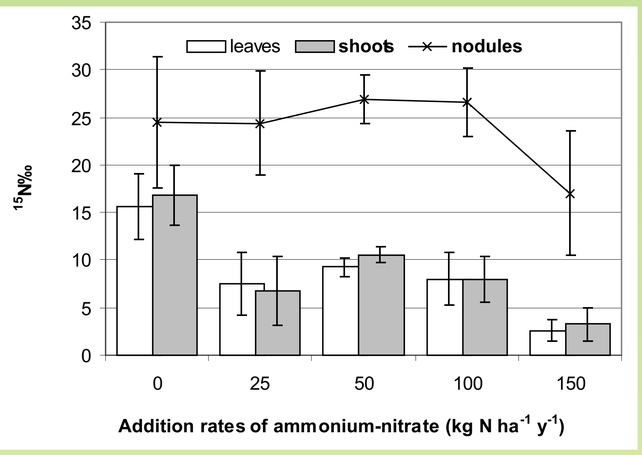
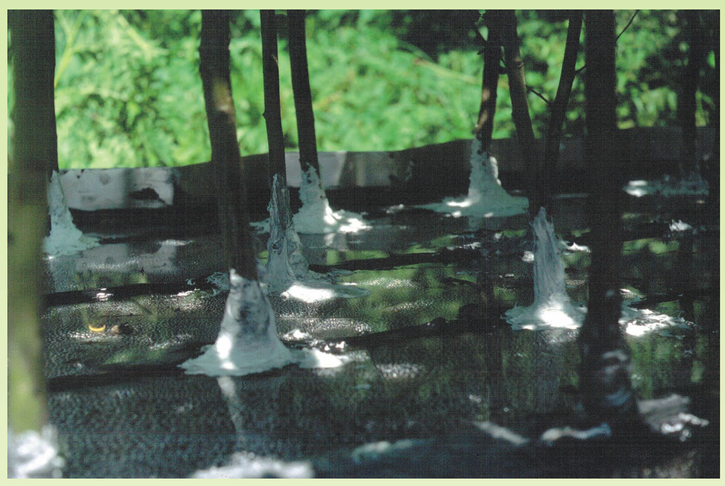


Table 1.
Incorporated amounts of ^{15}N into *Alnus incana* biomass fractions in relation to five different ammonium nitrate (NH_4-NO_3) addition rates. (± indicates 95% confidence limits.)

N fertilisation (kg N ha^{-1} y^{-1})	0	25	50	100	150
Leaves (mg ^{15}N)	2,47 ± 0,53	1,12 ± 0,62	1,86 ± 0,25	1,48 ± 0,56	0,4 ± 0,19
Shoots[1] (mg ^{15}N)	0,79 ± 0,19	0,3 ± 0,21	0,51 ± 0,10	0,28 ± 0,12	0,12 ± 0,06
Nodules (mg ^{15}N)	0,37 ± 0,14	0,3 ± 0,08	0,48 ± 0,10	0,29 ± 0,09	0,2 ± 0,14
Total[2] (mg ^{15}N)	3,63 ± 0,66	1,71 ± 0,89	2,85 ± 0,36	2,05 ± 0,70	0,71 ± 0,38
Activity (mg ^{15}N nodule g^{-1})	6,2 ± 3,08	2,5 ± 1,29	3,44 ± 0,52	3,75 ± 1,21	1,56 ± 0,48

[1] current year shoots
[2] leaves, shoots and nodules

Fig. 2,3,4
Different stages of sealing the experiment device are shown.(for further details see text “**Materials and methods**”



Discussion

The decrease in nodule mass in actinorhizal plants upon high doses of mineral nitrogen has been shown in previous studies (Baker et al., 1997a; Baker et al., 1997b). In this study, the two-week fertilising period prior to ^{15}N incubation was too short for any negative effect on nodule growth (e.g. nodule decay) at high nitrogen fertilisation rates to emerge. The fertilisation-induced positive effect on plant growth was not observed in any plant fraction. For the above-mentioned reason we were unable to detect the enhancement of nodule growth at low N addition rates as reported by others (Ingestad, 1980; Kohls and Baker, 1989; Lõhmus et al., 1996).
The results of this study are in general consistent with previous studies (Tjepkema et al., 1981; Huss-Danell et al., 1982; Huss-Danell and Hahlin, 1988; Rytter et al., 1991; Baker et al., 1997a; Baker et al., 1997b) showing the negative effect of combined nitrogen on nitrogen fixation by actinorhizal symbioses. Nitrogen fixation derived ^{15}N concentrations in two of the main sinks (leaves and shoots) of recently fixed nitrogen decreased as N addition rates increased. ^{15}N concentrations in leaves and shoots of the alders that received no additional mineral nitrogen were the highest over all other treatments. At 150 kg N ha^{-1} y^{-1} the concentration of ^{15}N in the leaves was the lowest. However, the ^{15}N concentrations in the nodules were not significantly affected by the different N addition rates. Excluding the non-fertilised trees, the ^{15}N concentrations in nodules were significantly higher than those in leaves and shoots. A possible mechanism accounting for this phenomenon suggests that the nodules might also serve as storage for the fixated nitrogen. In this case input (nitrogen fixation) and output (transport to shoots) exert minor influence on the ^{15}N concentration in the nodules at any given time.
The unexpected low nitrogen fixation activity at 25 kg N ha^{-1} y^{-1} could be due to an experiment error, which may have affected the normal xylem transportation system by squeezing the stems at root collar. Some trees in this group showed abnormally low efficiency to transport fixed ^{15}N to the shoots.
High variability of the results could be ascribed to two main factors. Firstly, the adjustment period after the planting of the trees into the experiment pot may have been too short. Hence, the influence of stress cannot be excluded. Secondly, the *Frankia* population was probably heterogeneous. This, on the other hand, may reflect the natural conditions more realistically.

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