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Examensarbeten **2010:28**
Institutionen för skogens ekologi och skötsel

Stem injection of different nitrogen forms into young Norway spruce

Foto: Torgny Näsholm

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Sveriges Lantbruksuniversitet Skogligt magisterprogram/Jägmästarprogrammet
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Injektion av olika kväveformer i stammar av unga granar

Anna Petersson

Nyckelord / *Keywords:*
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I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handletts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc/BSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examinator. However, the author is the sole responsible for the content.

Content

Summary

This master thesis has been a pilot study preceding a forthcoming project of a larger scale with the long term objective to separate the direct effect of added nitrogen on soil processes from indirect effects via trees. The aim of this study has been to investigate the allocation of nitrogen following direct injection of liquid solutions into the xylem of 40 year old Norway spruce. The field site is located at Flakaliden (64°07'N, 19°27'E), approximately 60 km northwest of Umeå, Sweden. A total of 18 trees were selected for treatment, equally divided between three treatments, potassium nitrate, glutamine and water (control). The solutions were labelled with ^{15}N in order to enable assessment of how the injected nitrogen compounds are distributed within the crown, as well as speed of translocation. Allocation was measured over time during three subsequent sampling occasions as well as through spatial distribution in the canopy.

Needle samples were taken on two different levels and four different directions within the crown of each tree, at three subsequent sampling occasions after stem injection on August 25, 2009. Needles were analysed through an EA-IRMS *(Elemental Analyzer - Isotope Ratio Mass Spectrometer)*, for atom percent ^{15}N and total nitrogen concentration.

No significant differences were found regarding sampling direction or interactions between sampling direction and level, treatment nor sampling occasion. For neither of the treatments was labelled nitrogen detected anywhere in the foliage until the second sampling occasion, 13 days after stem injection. Labelling was consistently significantly higher for the trees treated with nitrate compared to the trees treated with glutamine and more labelled nitrogen was detected in the lower third of the foliage compared to the upper third. For the glutamine treatment only the lower canopy was successfully labelled. No significant increases for either level between the second or the third sampling occasion were detected.

Considerable rises in atom percent ^{15}N were detected for both treatments but not in total nitrogen concentrations. A comparison between the theoretical increases in nitrogen concentrations, based on the amount of added nitrogen, and the observed increases showed that the theoretical increase for both crown levels were considerably higher than the achieved rise in nitrogen concentration. Possible explanations are high variations in natural concentrations of nitrogen between and within individual trees and seasonal fluctuations in nitrogen levels in needles.

Svensk sammanfattning

Detta examensarbete har varit en pilotsudie inför ett kommande projekt i större skala med det långsiktiga målet att särskilja den direkta effekten av kvävegödsling på markprocesser från den indirekta effektan via träden. Syftet med denna studie har varit att undersöka kvävets allokering efter direkt injektion in i 40-åriga träds xylem. Försöksområdet är beläget vid Flakaliden (64°07'N, 19°27'E), cirka 60 kilometer nordväst om Umeå, Sverige. Sammanlagt utvaldes 18 provträd, jämnt fördelade mellan behandlingarna kaliumnitrat, glutamin och vatten (kontroll). Lösningarna var inmärkta med ^{15}N för att göra det möjligt att spåra hur det injicerade kvävet spreds inom kronan, liksom att bedöma spridningshastighet. Allokering mättes över tid samt i olika positioner inom kronan.

Barrprover togs på två olika höjdnivåer samt fyra olika riktningar inom kronan på varje träd, vid tre på varandra följande tillfällen efter staminjktion den 25 augusti 2009. Barren analyserades med hjälp av EA-IRMS *(Elemental Analyzer - Isotope Ratio Mass Spectrometer)* för atomprocent ^{15}N samt totalkväve.

Inga signifikanta skillnader upptäcktes gällande provtagningsriktning eller interaktionen mellan riktning, nivå, behandling eller provtagningstillfälle. Inte för någon av behandlingarna kunde något inmärkt kväve detekteras i barren förrän vid det andra provtagningstillfället, 13 dagar efter behandling. Inmärkningen var signifikant högre för träd injicerade med nitrat jämfört med glutamin och mer inmärkt kväve återfanns i den nedre tredjedelen av kronan jämfört med den övre tredjedelen. För de träd behandlade med glutamin kunde inmärkning endast detekteras i den nedre tredjedelen av kronan. Inga signifikanta skillnader för någon av nivåerna kunde ses mellan det andra och det tredje provtagningstillfället.

Markanta förhöjningar gällande atomprocent ^{15}N kunde detekteras för såväl nitrat som glutamin, men ej gällande totalkvävekoncentrationer. En jämförelse mellan den teoretiskt möjliga ökningen i kvävekoncentration, baserat på mänden injicerat kväve, och den verkligt uppmätta ökningen visade att den teoretiska ökningen för båda nivåerna betydligt översteg den åstadkomna ökningen. Möjliga förklaringar till detta är stora variationer i den naturligt förekommande kvävekoncentrationen mellan och inom trädindivider liksom barrs årstidsvariationer gällande kvävekoncentrationer.

1. Introduction

Nitrogen has long been recognized as an important nutrient, often limiting growth of plants in natural ecosystems. Traditionally, plant acquisition of nitrogen was believed to occur in the forms of nitrate and ammonium only. More and more of recent studies now suggest that amino acids and other organic nitrogen compounds may play an important role as well (Kielland 1994; Näsholm *et al*. 1998; Lipson and Näsholm 2001; Finzi *et al*. 2005). Commercial fertilizers today are based on the former two forms of nitrogen, not on amino acids, and add approximately 150 kg nitrogen per hectare, according to the present recommendations from the Swedish Forest Agency (www.skogsstyrelsen.se). Following forest fertilization much of the added nitrogen, however, cannot be found anywhere in the tree, and is still unaccounted for. Usually, only around 20% of the added nitrogen ends up in the trees (Melin *et al.*, 1983). Nitrogen fertilization also has a strong impact on carbon allocation of trees and on rates of soil respiration of the stand (Olsson *et al*., 2005). The extent to which these effects are directly related to tree nitrogen status vs. related to specific metabolites in the tree is, however, unknown.

This master thesis has been a pilot study preceding a project of a larger scale which will be initiated later in 2010. The long term objective of that project will be to separate the direct effect of added nitrogen on soil processes from indirect effects via trees. In order to design the forthcoming project correctly and reaching the long term objective that has been set up, a number of short term objectives have been identified. These short term objectives are the focus of this study. To eliminate the direct effects of adding nitrogen through soil fertilization, nitrogen will be added to the sample trees though stem injection of an artificial sap solution. A small amount of labelled nitrogen will be added to each tree to enable detection of the movement of the added nitrogen. By means of using labelled nitrogen the spatial distribution patterns after stem injection into young Norway spruce will be investigated, as well as the speed of translocation. Additionally, possible differences between organic (glutamine) and inorganic (nitrate) nitrogen form will be studied.

Isotopic labelling is a method used for a wide variety of scientific research, e. g. medicine, agriculture, geology, biology and forestry. In forestry, the method has been used for e. g. investigations of water-conducting pathways in living trees (Umebayashi *et al*., 2007). The technique involves tracing a substance through a system. The substance is labelled by substituting one atom of the molecule of interest for an atom of the same chemical element, but of a different isotope (number of neutrons). The labelled molecules will chemically and functionally behave as unlabelled compounds. The difference in number of neutrons, and thereby in mass, allows separate detection from the other atoms of the same element, which means that they must have come from the added, labelled, substance. In this case the system studied is a tree and the substances are two different forms of nitrogen, potassium nitrate and L-glutamine. These two nitrogen forms were chosen because glutamine is one of the most common nitrogen transportation and storage compounds found in the xylem sap in conifers (Dickson, 1989; Näsholm & Ericsson, 1989; Nordin *et al*., 2001; Persson *et al*., 2006). Nitrate, on the other hand, may be present after forest fertilization as more than 50% of the nitrogen in commercial fertilizers is nitrate. Regarding root uptake, the preferred order of

uptake by most trees is ammonium, followed by amino acids and the lowest rates of uptake usually regards nitrate (Näsholm *et al.*, 2009).

2. Materials and Methods

2.1 Site and stand description

The field site was located at Flakaliden (64°07'N, 19°27'E), 310-320 m a.s.l., approximately 60 kilometres north west of Umeå, Sweden. The climate is boreal with monthly mean temperature varying from -8.7°C in February to 14.4°C in July. The annual precipitation is approximately 600 mm, of which more than one-third falls as snow. The soil is a podzolic sandy glacial till (Bergh *et al*., 1999).

The stand in which the experiment was performed was planted in 1963 with 4-year-old seedlings of a local provenance of Norway spruce (*Picea abies* (L.) Karst).

2.2 Experimental design and treatment

Amino acid analysis

Prior to initiation of the main stem injection experiment a trial including six trees (three of each treatment) was performed to ensure the appointed levels of nitrogen to inject was appropriate and establish the levels of glutamine before and after stem injection, respectively. Injections were made on August 21 and needle samples were collected three days after treatment. The same concentrations were used as in the main experiment and the same procedures surrounding stem injection and needle sampling were applied (see below).

Labelled nitrogen and total nitrogen

A total of 18 trees were selected for treatment, average height being 7.8 m and average diameter being 8.7 cm (*Table 1*). The trees were divided equally between three treatments, potassium nitrate, L-glutamine and water (control). The six trees within each treatment were placed in two clusters of three. The three trees within the first cluster received 1 l solution through one injection point whereas the trees in the second cluster had two injection points and received in total 2 l of artificial sap solution. Needle samples were collected at three subsequent occasions following stem injection. *Table 1* displays collected data for all trees (diameter and height), dry weight of needles according to Ulvcrona (after Andersson) and assigned treatment.

Tree ID	Measured height *(m)*	Measured diameter *(cm)*	Dry weight needles *(kg)*	Treatment
17	8.4	8.3	4.40	0.05 M L-Gln
21	7.7	7.3	3.40	"
25	5.7	7.3	2.76	"
171	7.7	8.5	4.28	"
172	7.7	9.3	4.91	"
176	6.6	8.3	3.68	"
114	8.6	9.7	5.71	0.1 M KNO_3
125	6.3	7.4	3.01	"
126	8.2	10	5.76	"
231	7.9	8.5	4.36	"
232	8.3	9.1	5.03	"
235	7.8	8.5	4.32	"
50	7.5	8	3.83	H_2O
51	8.2	8.5	4.49	"
53	7.8	9.5	5.12	"
313	9.1	10.2	6.47	"
315	8.6	10.2	6.18	"
316	8.1	8.4	4.37	"
Average	***7.8***	***8.7***	***4.56***	

Table 1. *Measured values for the sample trees (diameter, height), estimated dry weight of needles and assigned treatment.*

Stem Injections

The study started on August 25th when all trees were injected with an artificial sap solution. Six trees received water, whereas 12 trees received solutions containing nitrogen in the forms of either 0.1 M KNO_3 or 0.05 M L-glutamine (Gln contains 2 N per molecule). The amount of added nitrogen, 100 mM for each treatment, was equivalent to 1.4 g N per tree. A 6 mm diameter hole was drilled into the trunk base approximately 50 cm above ground, a little over half-way into the tree at a straight angle. A tube of equal diameter was then immediately inserted into the hole, connected to a bottle containing the artificial sap solution. The bottle was attached to the tree at a distance above the injection point (*Fig. 1*). A small hole was then drilled into the bottle, now turned upside down, to prevent vacuum. To minimize ^{15}N inputs to soil, the labelled nitrogen was then added to the solution with a pipette through the hole. The tracer solutions contained 3.7 mg ^{15}N-potassium nitrate or 3.7 mg ^{15}N L-glutamine, dissolved in deionised water. This level was chosen as it is 10^3 times the detection level for ^{15}N (0.1 ‰ of the natural abundance). The amount of labelled nitrogen added to the trees in the injected solutions corresponds to 8.88 mg ^{15}N (5.18 mg from natural abundance and 3.7 mg from added tracer).

Figure 1. *Injected tree at the field site.*

2.3 Needle sampling

Labelled nitrogen and total nitrogen

Needle samples were taken at three subsequent occasions following stem injection (August 25 to October 23, 2009). The three different sample occasions occurred 3, 13 and 59 days, respectively, after injection. Due to a heavy infection of spruce needle rust (*Chrysomyxa ledi*) on the current year needles (*C, 2010*), samples were instead taken from the previous year (*C+1*) to prevent any contamination.

Samples were taken on two different levels and four different directions within the crown of each tree (*Fig. 2a-b*). The two levels are *Upper* and *Lower*, the first meaning the upper third of the crown and the latter meaning the middle third of the crown. The four sample directions were denoted *R1-R4* (*Fig. 2b*), *R1* always being the direction of injection.

Out of the 12 trees that were labelled, two did not absorb all the artificial sap solution. One of the trees treated with nitrate left 1/5 of the solution in the bottle and one of the trees treated with glutamine left 1/2 plus 1/4 of the solution (two injection points). This was noticed already at the first sampling occasion and stayed unchanged throughout the experiment. This fact was accounted for when compiling and analyzing data by multiplying the values from the analysis with the factors 1.2 and 1.415, respectively as well as the measured value for natural abundance of ^{15}N (*See 3.5*).

A total of 8 samples were taken from each tree at each sampling occasion. All samples were kept in a refrigerator until preparation for further analyses.

Amino acid analysis

The same procedure as for collecting samples for the labelled nitrogen analysis was applied when collecting samples for amino acid analysis. A total of 8 samples were taken from each tree at each sampling occasion. All samples were kept in a refrigerator until preparation for further analyses.

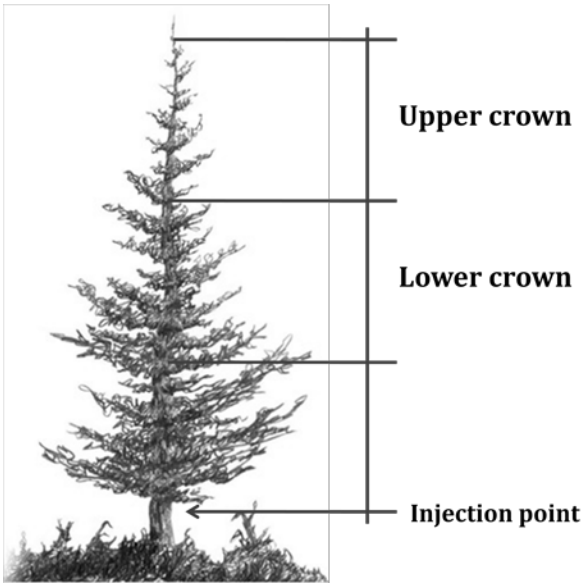

Figure 2a. *Injection point and sampling levels.*

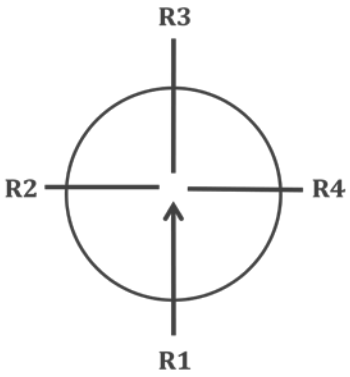

Figure 2b. *Sampling directions. Arrow indicates direction of injection.*

2.4 Analyses

Stable isotope analysis and total nitrogen

The shoot samples were briefly submerged in liquid nitrogen, after which separation between needles and branches was easily done. The needles were then dried at a temperature of 65 degrees Celsius for approximately 36 hours and were then allowed to cool in an exicator to ensure equal water content in all samples. After having dried, all samples were ground to a fine powder in a ball mill. An amount of approximately 5 mg (target mass) of each sample was weighed into tin capsules and run through an Elemental Analyzer - Isotope Ratio Mass Spectrometer (EA-IRMS). The results relevant for this experiment are given in atom percent ^{15}N.

Amino acid analysis

Needles were kept frozen during the whole procedure from collection to grinding. Separation between needles and branches was achieved using liquid nitrogen as described above. Derivatives from the amino acids in the needle samples were then made according to Water's method. The amino acids present were then identified using UPLC (Ultra Performance Liquid Chromatography). The results from the amino acid analysis did not give any cohesive results and will therefore not be presented further.

2.5 Statistical analyses

Statistical analyses were performed with Mystat 12 for Windows™.

Comparing results between sampling occasions, levels and directions for the different treatments were done by means of ANOVA and paired t-tests.

Sampling occasion, treatment, level and direction were initially included as variables in the ANOVA-model.

3. Results

3.1 Sampling occasion

On the first sampling occasion, three days after injection, no significant difference was detected between labelled trees of either treatment and the control, consisting of water *(p=0.078)*. The second and third sampling occasions, 13 and 59 days after injection respectively, displayed significant differences between the two labelled treatments compared with the control, which indicated successful labelling *(p=0.000, p=0.009)*.

3.2 Injection points

Whether or not the trees had one or two injection points had no significant effect on response time or level of labelling for any of the treatments.

3.3 Directions

No significant differences were found regarding sampling direction *(p=0.520)* or interactions between sampling direction and level *(p=0.798)*, treatment *(p=0.868)* nor sampling occasion *(p=0.581)*. Therefore, average values of all directions on each level were decided to be used in further analysis.

3.4 Levels

Results from paired t-tests from the first sampling occasion showed no difference between detected labelling in the lower compared to the upper parts of the canopies for either treatment *(p=0.961)*. However, significant differences were found on the second and third sampling occasion between levels for both nitrate *(p=0.012*, *p=0.041)* and glutamine *(p=0.010*), *p=0.027)*. For both treatments, significantly more labelled nitrogen was detected in the lower third of the foliage compared to the upper third.

3.5 Water

The control trees had an average ^{15}N atom percent of 0.36516 *(Table 2)*, which is somewhat lower than the reference natural abundance of ^{15}N in N_2 in the atmosphere (0.3663 atom percent). This value was then used to correct the values for those trees that did not absorb all the artificial sap fluid (*see 2.4*). Average values for the control trees from the first and second sampling occasion are shown in Table 3, as well as values for the two labelled treatments from the third sampling occasion.

Tree ID	^{15}N atom %	SE
50	0.366168	2.62E-05
51	0.364827	2.90E-05
53	0.365793	2.72E-05
313	0.364657	3.17E-05
315	0.364735	3.13E-05
316	0.364661	4.84E-05
Overall average	***0.365164***	***8.99E-05***

Table 2. *Measured atom percent ^{15}N in control trees, including standard errors.*

Tree ID	Treatment	Upper		Lower	
		N (%)$_{korr}$	Atom%	N (%)$_{korr}$	Atom%
17	0.05 M L-Gln	0.8840	0.3655	0.9261	0.3720
21	"	0.8370	0.3655	0.8321	0.3658
25	"	0.9746	0.3670	1.0935	0.3686
171	"	0.9116	0.3661	0.9242	0.3669
172	"	1.0767	0.3652	0.9492	0.3668
176	"	1.0046	0.3659	0.9888	0.3692
114	0.1 M KNO_3	0.9704	0.3696	0.8911	0.3812
125	"	1.0294	0.3760	0.9475	0.4056
126	"	1.0291	0.3700	0.9005	0.3722
231	"	1.1450	0.3720	0.9650	0.3823
232	"	1.0179	0.3682	1.1121	0.3713
235	"	0.9500	0.3746	-	-
50	H_2O	0.8889 ± 0.00913	0.3662 ± 4.017E-05	0.8053 ± 0.01453	0.3662 ± 3.58E-05
51	"	1.2217 ± 0.02729	0.3649 ± 4.771E-05	1.1894 ± 0.01459	0.3648 ± 3.58E-05
53	"	0.9625 ± 0.02153	0.3659 ± 2.492E-05	0.9977 ± 0.04204	0.3647 ± 3.27E-05
313	"	0.9396 ± 0.02152	0.3647 ± 3.976E-05	1.0120 ± 0.02380	0.3646 ± 4.44E-05
315	"	0.9897 ± 0.02457	0.3648 ± 3.869E-05	0.9772 ± 0.01855	0.3646 ± 2.06E-05
316	"	0.9663 ± 0.01497	0.3647 ± 8.005E-05	0.9579 ± 0.03323	0.3646 ± 5.54E-05

Table 3. *Data for labelled and control trees from the third sampling occasion. Values for the water control are average values from the first and the second sampling occasion, including standard errors.*

3.7 Nitrate

Figure 3 below displays measured values for the nitrate treatment. For the first set of measurements there were no differences between directions *(p=0.906)* or levels *(0.142)* and no difference in ^{15}N levels compared to the control *(p=0.078)*, i.e. no labelling detected anywhere in the foliage after three days.

For the second and third set of measurements there was a significant difference between the nitrate treatment and the control *(p=0.000)*, i.e. labelled nitrogen was first detected in the foliage 13 days after stem injection.

For the second and third set of measurements there were significant differences between levels *(p=0.012, p=0.041).* Significantly more labelled nitrogen was found in the lower parts of the canopy compared to the upper parts. However, no significant increase for either level between the second or the third sampling occasion was detected *(p(upper)=0.641, p(lower)=0.365).*

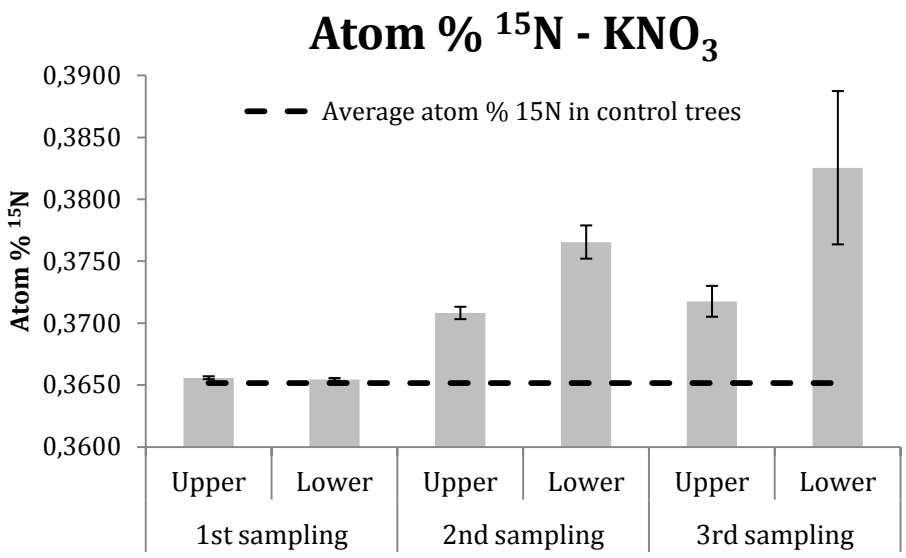

Figure 3. *Atom % ^{15}N from the nitrate treatment for all levels and sampling occasions. Bars are standard errors.*

3.8 Glutamine

For the first set of measurements there were no differences between directions *(p=0.968)* or levels *(p=0.377)* and no difference in ^{15}N levels compared to the control *(p=0.078)*, i.e. no labelling detected anywhere in the foliage after three days *(Figure 4).*

For the second and third set of measurements there were significant differences compared to the water control only for the samples from the lower level in the canopies *(p=0.003, p=0.009)*, i.e. no labelling in the upper parts of the canopies at any time *(p=0.320, p=0.105).* For the second and third set of measurements there were significant differences between levels *(p=0.011, p=0.033).* Significantly more labelled nitrogen was found in the lower parts of the canopy compared to the upper parts.

There was no significant increase of the level of labelling between sampling occasions two and three for the lower parts of the canopies *(p(upper)=0.294, p(lower)=0.515).*

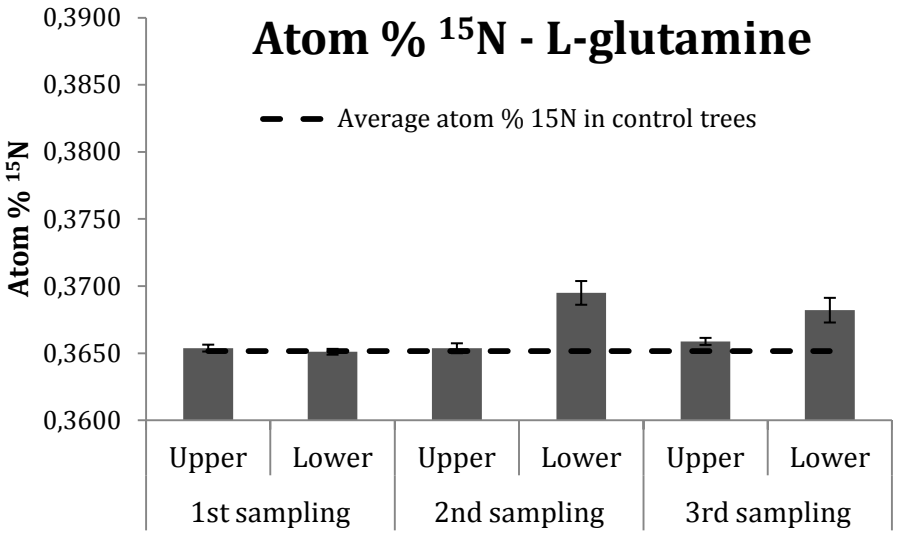

Figure 4. *Atom % ^{15}N from the L-glutamine treatment for all levels and sampling occasions. Bars are standard error.*

3.9 Comparison between nitrate and glutamine

For neither of the treatments was labelled nitrogen detected anywhere in the foliage until the second sampling occasion, 13 days after stem injection *(Figure 5)*.

Labelling was consistently significantly higher for the trees treated with nitrate compared to the trees treated with glutamine.

Higher levels of labelling were consistently found in the lower parts of the canopies for both treatments. For the glutamine treatment only the lower canopy was successfully labelled.

For both the nitrate and the glutamine treatment there were no significant differences between the second and the third sampling occasion for either level.

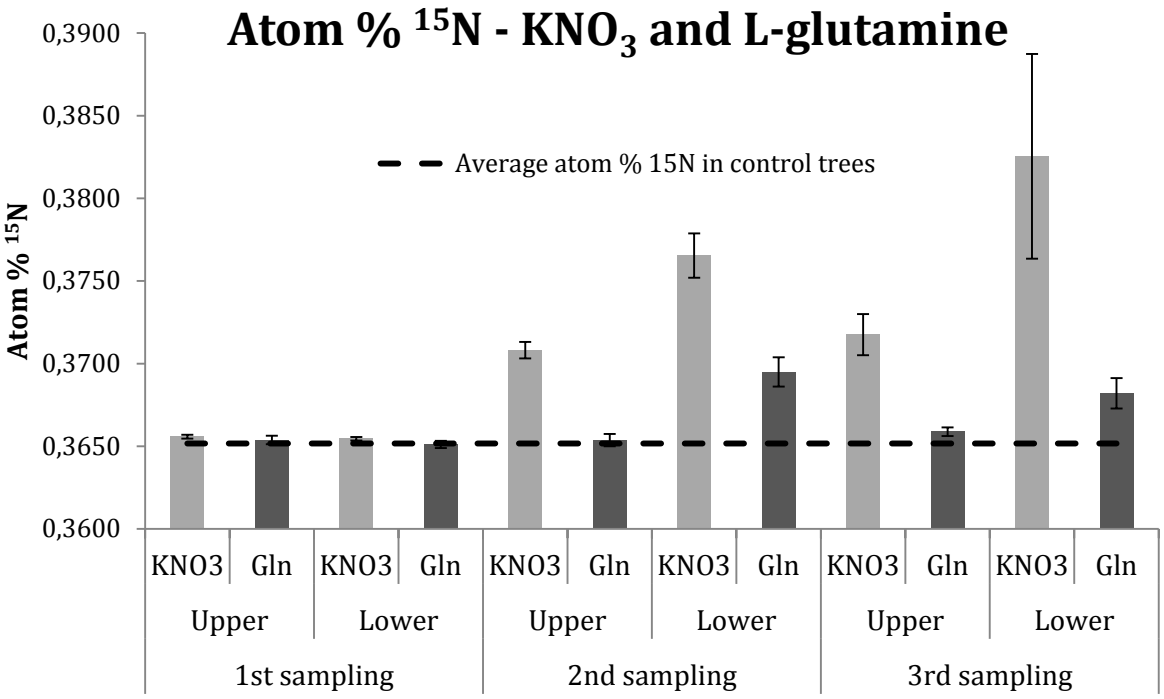

Figure 5. *Atom % ^{15}N from all treatments, all levels and all sampling occasions. Bars are standard error.*

3.6 Labelling in needles

The differences in atom percent ^{15}N from the first and third sampling occasions (*Δ Atom %*) in all labelled trees, both for the upper and the lower levels, were all positive values *(Table 4)*. The difference in nitrogen content (*Δ* N_{korr}) however, displayed an increase of 0.0033 for the upper levels and a decrease of 0.0186 for the lower levels during the same period of time. The *theoretically possible* increase in nitrogen level due to injected nitrogen was calculated as;

$$\Delta\ Atom\%\ ^{15}N_{(Set3-Set1)} *\ \ N\%korr_{(Set3)} * \frac{injected\ N}{injected\ ^{15}N\ excess},$$

where injected N equals 1.4 g and injected ^{15}N excess equals $3.7 * 10^{-3}$ g. A comparison between this theoretical increase and the observed increase shows that the theoretical increases in nitrogen concentration for the upper and lower levels are considerably higher, 0.010 and 0.0024, respectively *(Table 4)*. In *Figure 3* the theoretical increase in nitrogen content is plotted against the difference between the first sampling occasion and the last sampling occasion *(Set3-Set1)* regarding µg ^{15}N *(grey)* and atom percent ^{15}N *(black)*.

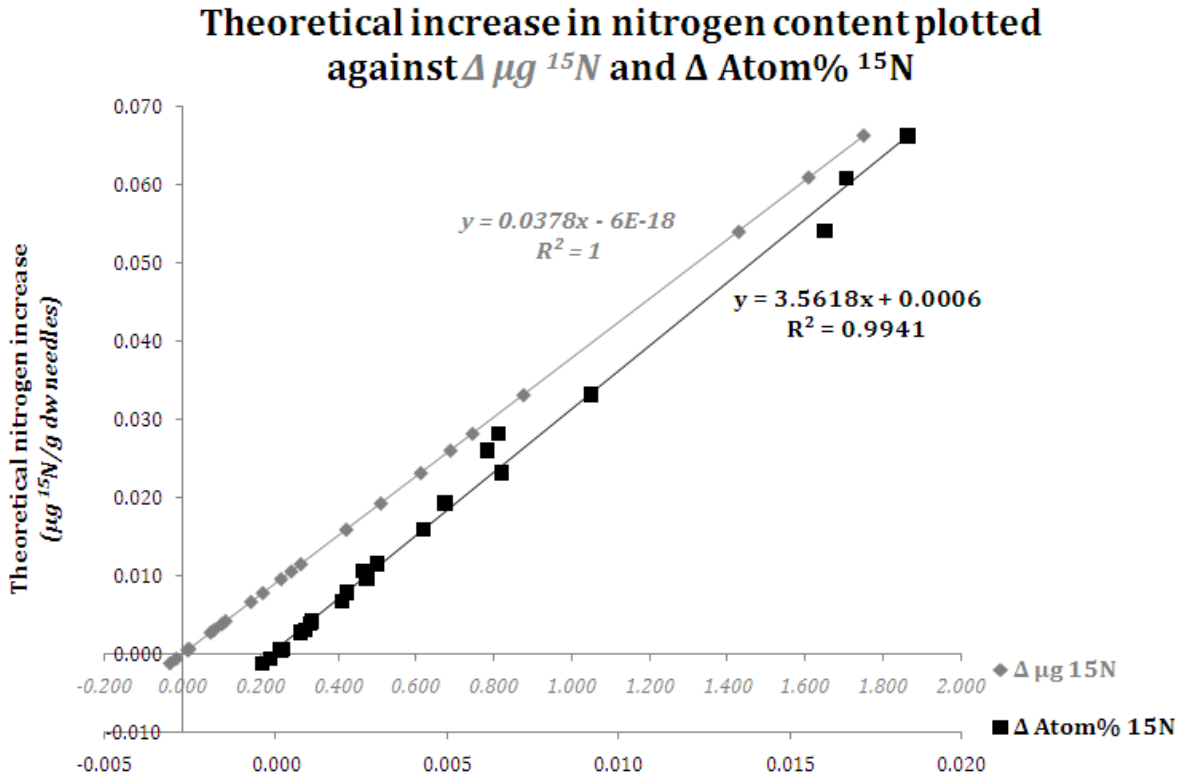

Figure 3. *Theoretical increase of nitrogen content following stem injection plotted against Δ µg ^{15}N (grey) and Δ Atom% ^{15}N (black). Δ denotes the difference in values from the first and the last sampling occasion (Set3-Set1).*

Tree ID	Measured height *(m)*	Measured diameter *(cm)*	Est. Dry weight needles [1)] *(kg)*	Treatment	Δ Atom% (Set3 - Set1) *Upper*	Δ Atom% (Set3 - Set1) *Lower*	Δ N% $_{korr}$ (Set3 - Set1) *Upper*	Δ N% $_{korr}$ (Set3 - Set1) *Lower*	Theoretical N increase [2)] *Upper (%)*	Theoretical N increase [2)] *Lower (%)*
17	8.4	8.3	4.40	0.05 M L-Gln	0.000	0.007	0.0244	0.0246	0.000	0.023
21	7.7	7.3	3.40	"	0.000	0.000	-0.0376	-0.0110	-0.001	0.001
25	5.7	7.3	2.76	"	0.001	0.003	0.0659	-0.0881	0.003	0.011
171	7.7	8.5	4.28	"	0.000	0.001	-0.0139	0.0238	0.000	0.003
172	7.7	9.3	4.91	"	0.000	0.003	-0.0868	-0.0870	-0.001	0.010
176	6.6	8.3	3.68	"	0.001	0.002	-0.0215	-0.0841	0.004	0.008
114	8.6	9.7	5.71	0.1 M KNO_3	0.004	0.016	-0.0255	0.0000	0.016	0.054
125	6.3	7.4	3.01	"	0.005	0.018	-0.0395	-0.0338	0.019	0.066
126	8.2	10	5.76	"	0.001	0.002	0.0272	0.0967	0.004	0.007
231	7.9	8.5	4.36	"	0.006	0.017	-0.0325	-0.0376	0.028	0.061
232	8.3	9.1	5.03	"	0.003	0.006	0.1774	-0.0079	0.011	0.026
235	7.8	8.5	4.32	"	0.009	-	0.0019	-	0.033	-
50	7.5	8	3.83	H_2O	-	-	-	-	-	-
51	8.2	8.5	4.49	"	-	-	-	-	-	-
53	7.8	9.5	5.12	"	-	-	-	-	-	-
313	9.1	10.2	6.47	"	-	-	-	-	-	-
315	8.6	10.2	6.18	"	-	-	-	-	-	-
316	8.1	8.4	4.37	"	-	-	-	-	-	-
Average	***7.8***	***8.7***	***4.56***		***0.003***	***0.007***	***0.0033***	***-0.0186***	***0.010***	***0.024***
Overall average					***0.005***		***-0.0072***		***0.017***	

Table 4. *Measured, estimated and calculated data for sample trees. [1)] Estimated dry weight of needles according to Ulvcrona (19XX). [2)] Theoretical nitrogen increase was calculated as Δ Atom% (Set3-Set1) * N%$_{korr}$ (Set3) * (injected N / injected ^{15}N excess).*

4. Discussion

A uniform distribution pattern was detected in all crowns following labelling with stem injection, even though trees were only injected in one or two directions. No differences were found regarding sampling direction. These were expected results but still needed to be confirmed in order to be ruled out as variables. For both treatments however, significantly more of the labelled nitrogen was detected in the lower parts of the foliage. This was also the pattern found by Swanston and Myrold (1997) in red alder trees.

The stem injections were performed on August 25th, which must be considered fairly late in the growing season, particularly as the field site is located in the north of Sweden. This fact might have had an effect on the speed of translocation of the labelled nitrogen through the trees. Injecting the trees in May rather than August, and also monitoring the movement of the labelled nitrogen for a longer period of time, might result in faster response times and records of successful labelling in the upper parts of the crowns as well as the lower parts.

The fact that the current year needles had been infected with spruce needle rust, making them inappropriate to sample, probably resulted in lower levels of labelling being detected for both nitrate and glutamine. According to Melin *et al*. (1983), the highest concentrations of newly acquired nutrients such as nitrogen are found in the current year needles. From there, there is a gradient of falling concentrations with older needles. Sampling current year needles would therefore have given higher levels of labelling.

According to the results, only a small fraction of the added nitrogen was recovered in the year-old needles. The question then remains where the bulk of the labelled nitrogen was allocated. A number of possible explanations exist. A substantial amount of nitrogen may, as mentioned above, have been transported to the current year needles and have thus not been sampled. What might be more plausible is that the majority of the added labelled nitrogen has been transported to the root system, and possibly even to mycorrhizal structures. According to a recent stem injection study by Garten and Brice (2009), a relatively large amount of injected nitrogen (ammonium) was traced to the roots. Another possibility is that the labelled nitrogen can be found in stem wood and/or branches.

Contrary to most previous stem injection trials, the injection holes were not drilled under constant artificial sap flow (Garten and Brice, 2009), submerged in artificial sap or water (Postlethwait and Rogers, 1958; Umebayashi *et al.*, 2007), pressurized (Sachs *et al*., 1977) or sealed with tight septum (Swanston and Myrold, 1997) to avoid that the water columns in the tree trunks will break. Nor was additional unlabelled sap fluid added after the labelled sap fluid had entered the trees, which have been done previously (Swanston and Myrold, 1997; Garten and Brice 2009), or the trees sealed after injection (Brockerhoff and Ho, 1997). Nevertheless, injection was considered successful.

The comparison between trees treated with nitrate and glutamine showed that, during the time of this particular experiment, only the labelled nitrate reached the upper level of the trees. This might be an effect of the fact that nitrate seems to travel faster in the xylem fluid than

amino acids (Schmidt and Stewart, 1999; Persson et al., 2006). A previous injection study by Horwath et al. (1998) also suggests that nitrogen in the form of nitrate was more efficient in initial labelling than ammonium. It could also be attributed to the fact that nitrate and glutamine are partitioned to different parts of the tree, with a higher relative distribution of acquired nitrogen into needles. Had the experiment run over a longer period of time the differences in levels of labelling would probably have evened out and had been more uniformly distributed, as was the case for Horwath (1998) three months after labelling.

The fact that changes due to the stem injection are detectable when considering atom percent ^{15}N but not total nitrogen content has a number of explanations. The most obvious is probably the high variation in concentration of nitrogen between individual trees as well as within individual trees as displayed in *Table 3*, which shows values from the third sampling occasion. It is likely that this big variation overshadows possible trends in the present data. Seasonal changes of nitrogen concentration in *C* and *C+1*needles are another aspect that may influence the results. The levels of glutamine in needles in conifers have been recorded to be the highest in spring and early summer and then decrease (Näsholm and Ericsson, 1989). Since the period between the first and the third and last sampling occasion was 59 days the natural variation of nitrogen content might have surpassed the change expected from the stem injection of labelled nitrogen. Also, changes within individual trees could be attributed to the fact that neither the same needles nor branches were sampled at the three different sampling occasions.

In conclusion, the project has successfully determined that uniform labelling of Norway spruce through stem injection is possible. The actual technique used for stem injection needs to be refined, concerning quality as well as execution. Proper sampling procedures concerning crown positions and time of year as well as having an understanding of the seasonal variations of nitrogen concentrations and the spatial variations in nitrogen within crowns is important knowledge to collect relevant data and correctly evaluate it. These are vitally important aspects to consider before initiation of the large scale trial later this year. The atom percent ^{15}N was effectively raised even though concentrations of total nitrogen did not notably increase. The fact that the levels of labelling recorded were lower than predicted raises questions whether the injected nitrogen spreads more evenly across needles from different years than before anticipated (Melin *et al*, 1983) or are diluted depending on crown size (Swanston and Myrold, 1997), as well as if the amount and/or concentrations of added nitrogen may be increased for added effect. In forthcoming projects it would be desirable to harvest whole trees, including roots when sampling, in order to investigate the allocation patterns of the injected nitrogen. Also, additional information regarding e. g. effects of stem injected nitrogen on soil respiration and growth effects would contribute to the understanding of the relationships between sources of nitrogen and allocation patterns. To compare these parameters from plots with stem injected/fertilized trees with plots treated with ordinary solid fertilizer would also benefit in achieving the long term goals that have been set up.

5. Acknowledgements

6. References

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