# Isotope ratios and concentration of N in needles, roots and soils of Norway spruce (*Picea abies* [L.] Karst.) stands as influenced by atmospheric deposition of N

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ABSTRACT: This study aims to investigate the changes in isotope ratios in foliage and soils of two Norway spruce

(*Picea abies* [L.] Karst.) forests greatly differing in their atmospheric N deposition and climatic conditions. As expected, both N concentrations and $\delta^{15}N$ values in needles and roots were found to be significantly higher in the Solling stand (N-saturated) compared to the Hyytiälä stand (N-poor). For both stands a typical vertical gradient of the soil $^{15}N$-enrichment was observed. As expected, the soil of N-polluted site (Solling) was $^{15}N$-enriched significantly more than that of N-limited site (Hyytiälä) and this is explained by the presence of marked $NO_3-$ leaching at the Solling site. Although the annual trends (1990–1994) of N concentration in the foliage of spruce trees remained almost constant, their $\delta^{15}N$ values significantly decreased with the increasing years of sampling. The $^{15}N$-depletion in spruce needle litter from 1990 to 1995 was by 2.0‰. This is explained by a slight decrease in N deposition at Solling site during this period.

Keywords: N-deposition; $^{15}N$; nitrogen; spruce; soil

The use of natural abundance of stable isotopes to elucidate physiological processes in plants is one of the most common and of the oldest applications of isotope analysis in ecology. The characteristics of the isotopic composition of pollutants can provide useful information on their source and quantity in the environment. Over decades, N deposition has affected forest areas of Europe and North America (ᴠᴀɴ Eɢᴍᴏɴᴅ et al. 2002; Mᴀᴛsᴏɴ et al. 2002). As nitrogen cycles through the ecosystem, slight fractionation or discrimination against the heavier isotope $^{15}N$ is usually observed (Nᴀᴅᴇʟʜᴏғғᴇʀ, Fʀʏ 1994). Fractionation is particularly important during nitrification, denitrification and ammonia volatilisation processes (Mᴀʀɪᴏᴛᴛɪ et al. 1981) and can result in $^{15}N$ enrichment of the soil N pool (Hᴏ̈ɢʙᴇʀɢ 1990). As a consequence, $^{15}N$ enrichment of the foliage can occur as N supply increases following fertiliser applications as demonstrated in agricultural systems (Mᴇɪɴᴛs et al. 1975) and forest ecosystems (Hᴏ̈ɢʙᴇʀɢ 1990), or chronic atmos-

pheric N-pollution (Gᴇʙᴀᴜᴇʀ, Sᴄʜᴜʟᴢᴇ 1991). This has been attributed to accelerated nitrification in N rich sites leading to constant and increasing uptake of $^{15}N$-enriched $NH_4$-N by vegetation as $^{15}N$-depleted $NO_3$-N is leached from the system (Gᴀʀᴛᴇɴ, Mɪᴇɢʀᴏᴇᴛ 1995).

Several ecosystem researchers (Nᴀᴅᴇʟʜᴏғғᴇʀ, Fʀʏ 1994) predicted that $^{15}N$ natural abundance values would increase for systems approaching N-saturation. Furthermore, the $^{15}N$ natural abundance technique has been used in forest ecosystem health studies, for example, needles from a healthy Norway spruce stand were more depleted of $^{15}N$ than those from a declining stand receiving increased N and S depositions (Gᴇʙᴀᴜᴇʀ, Sᴄʜᴜʟᴢᴇ 1991; Gᴇʙᴀᴜᴇʀ et al. 1994).

A majority of the studies in the past was focused on the temperate spruce forests under different atmospheric N-deposition gradients. Comparative studies of spruce forests under different atmospheric N deposition loads and climatic conditions have been

scarce. This paper presents a comparative study of two Norway spruce (*Picea abies* [L.] Karst.) forests differing greatly in their atmospheric N deposition and climatic conditions. One spruce stand that is situated in Solling, Central Germany, is temperate and highly N-saturated while the other spruce stand, situated near Hyytiälä, Southern Finland, is boreal and has a very low atmospheric N-deposition. This study aims to investigate the changes in isotope ratios in foliage, roots and soils of both spruce stands resulting from N-deposition. This study hypothesises that the isotopic N composition of both ecosystems will act as an environmental indicator of N saturation.

## MATERIALS AND METHODS

### Selection of sites and their description

Two spruce research stands, a temperate forest stand at Solling, Central Germany, and a boreal stand at Hyytiälä, Southern Finland, were selected for the present study. The purpose of selection was to compare the natural abundance of the same forest tree species under different loads of atmospheric N-deposition and climatic conditions. Solling is located in the mountainous Solling area of Germany at 51°46´N, 9°34´E. The Solling area is a part of the Weser river mountain range. The soil is strongly acidified (pH in CaCl$_2$ = 3.0), dystric Cambisol (FAO Classification), which has developed in a loess solifluction layer overlying the sandstone bedrock. The spruce forest is 110 years old and it is situated at an altitude of 504 m and the vegetation mainly consists of Norway spruce (*Picea abies* [L.] Karst.). Solling has the average annual precipitation of 1,142 mm and the mean annual air temperature of 6.4°C. The open field precipitation N-deposition (between 1981 and 1994) was found to amount to 20 kg N/ha/yr (MESSENBURG et al. 1995). The nitrogen deposition in throughfall (1969–1985) was found to be 40.8 kg N/ha/yr. The nitrogen leaching from seepage water was strongly high 14.9 kg N/ha/yr.

The other spruce forest site under study is a boreal forest about 120 years old. It is mainly composed of Norway spruce (*Picea abies* [L.] Karst.). The research site is located near the Hyytiälä Forestry Field Station of Helsinki University (61°48´N, 24°19´E). The average annual precipitation is 709 mm. The annual mean temperature during the growing season (May–September) is 12.6°C. The research site is located at an altitude of about 150 m. The soil is haplic podzols aged about 10,000 years (FAO Classification). The bedrock is mainly acidic granite. The soil pH (H$_2$O) is highly acidic of about 4.4. The atmospheric N-deposition (1988–1996) is very low (6 kg N/ha/yr) (KUMALA et al. 1998). The nitrogen leaching is also of negligible amount.

### Sampling

At Solling site, samples of current year needles were collected during the spring season in 1986 from different heights (bottom, middle and upper parts). Litter fall was collected weekly in 15 litter traps in the autumn season (September to November, 1990–1994). All plant samples were dried at 80°C and milled using planetary mills. In order to assess the vertical distribution of stable isotopes in the soil, an undisturbed soil core (in 10 replications) was extracted with a steel soil core sampler (30 cm long and 8 cm in diameter) up to the soil depth of 14 cm at random. The extraction of samples followed in 2001. The soil cores were cut into different horizons OL+F, OH, and mineral soils (5cm slices up to 40cm depth) for the laboratory analysis. Fine root material was collected separately from the soil layers O$_P$, O$_H$, 0–5, 5–10, 10–20, 20–30 and 30–40 cm. For our present $^{15}$N study we only considered the soil layers OL+F, H, 0–5, 5–10 and 10–40 cm. Samples from organic layers and 10 to 40cm soil layers were mixed due to an insufficient amount of sample material for all further analyses. Fine roots (≤ 2 mm) from different soil depths were sampled in October 2001 (MURACH 1984).

For Hyytiälä, samples of current year's needles were taken during spring season (in 2004). For the above-mentioned sampling, eight trees of each species were selected at random from the stand. In order to assess the vertical distribution of stable isotopes in the soil, undisturbed soil cores (*n* = 5) were taken at random with a steel soil core sampler (49.5 deep × 5 cm diameter). The soil cores were cut into different horizons as mentioned above for the laboratory analysis. The fine roots (≤ 2 mm) were also collected from the different soil horizons.

### Analytical methods

All the dried and ground samples were measured for $^{15}$N on a Finnigan MAT Delta plus stable isotopic ratio mass spectrometer (IRMS) equipped with an elemental analyser for conversion of N into N$_2$. The results of IRMS measurements were given in δ notation. The δ values of isotopes of N are expressed as parts per 1,000 differences from standard atmospheric isotopes (SHEARER, KOHL 1993).

$$\delta^{15}N = \{[(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{air})] - 1\} \times 1,000$$

where: (‰) air – reference standard gas.

Table 1. The mean %N and $^{15}N$ in the green foliage of both stands ($n$ = 12, ±SE)

|  | %N | $\delta^{15}N$ |
|---|---|---|
| Solling | 1.30 (± 0.04) | −3.32 (± 0.18) |
| Hyytiälä | 0.99 (± 0.04) | −4.89 (± 0.16) |

## RESULTS

### N concentrations and natural $^{15}N$ abundance in needles

The data on $\delta^{15}N$ values and N% in the needles of both spruce forests are presented in Table 1. This table shows that Solling spruce needles were $^{15}N$-enriched significantly ($P \leq 0.05$) more (−3.32‰, SE ± 0.18‰) than those of the Hyytiälä spruce forest (−4.89‰, SE ± 0.16‰). Similarly, N concentrations of the needles of Solling site (1.30%, SE ± 0.04%) were significantly ($P \leq 0.05$) higher than those found for the Hyytiälä site (0.99%, SE ± 0.04%).

### Natural $^{15}N$ abundance in roots versus soil

The $\delta^{15}N$ values of the uppermost soil surface (OL+F) of Solling spruce stand (−2.83‰, SE ± 0.58) were significantly higher than those observed for the Hyytiälä stand (−3.84‰, SE ± 0.22) (Figs. 1 and 2). The range of $\delta^{15}N$ at the different depths of the mineral soil layers, for Solling spruce stand, was between +2.71‰ and + 7.12‰, significantly higher than that found for the Hyytiälä spruce stand (+1.18 to 2.28‰). Similarly, Solling spruce stand consisted of

significantly ($P \leq 0.05$) more $^{15}N$-enriched organic layer than that observed for Hyytiälä spruce stand. Both stands showed a typical $^{15}N$-enrichment with increasing soil depth. For both stands, the increasing $\delta^{15}N$ values in the soil profile with increasing depth corresponded to a decrease in the total nitrogen (Figs 1 and 2). However, similar to $^{15}N$, N concentrations at all soil depths of Solling were significantly higher ($P \leq 0.05$) in comparison with those of Hyytiälä.

In general, the roots of Solling site showed a higher $^{15}N$-enrichment than it was analysed for Hyytiälä site (Figs. 1 and 2). From these figures it is also evident that $\delta^{15}N$ values of roots increased with the depth of mineral soil. Furthermore, it was also observed that on all plots the vertical soil gradient of the $^{15}N$-enrichment of roots followed the vertical $^{15}N$ enrichment of the soil matrix. The content of nitrogen in roots was always found depleted in $N^{15}N$ compared to the soil nitrogen and this difference increased with soil depth. Furthermore, a significant correlation between $\delta^{15}N$ in the soil matrix and the living fine root fraction was found to be positive at both sites (Hyytiälä, $r$ = 0.83 and Solling, $r$ = 0.95).

### Annual variations of $\delta^{15}N$ and N concentrations in needle litter

Fig. 3 shows the annual variations of N and its $\delta^{15}N$ of Solling spruce stand. In addition, the figure also includes such data on a beech forest stand just close (ca. 100 m away) to the spruce stand. No such data has been collected for Hyytiälä stand. This figure indicates that although the annual trends of N con-
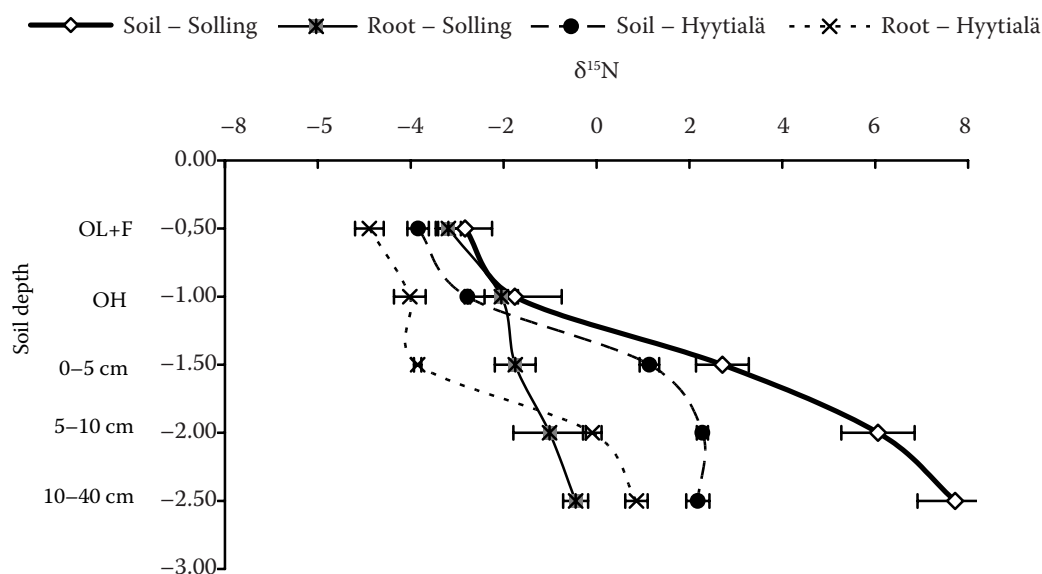


Fig. 1. Living roots $\delta^{15}N$ versus soil $\delta^{15}N$ in both stands (n = 5, error bars represent standard error of means)
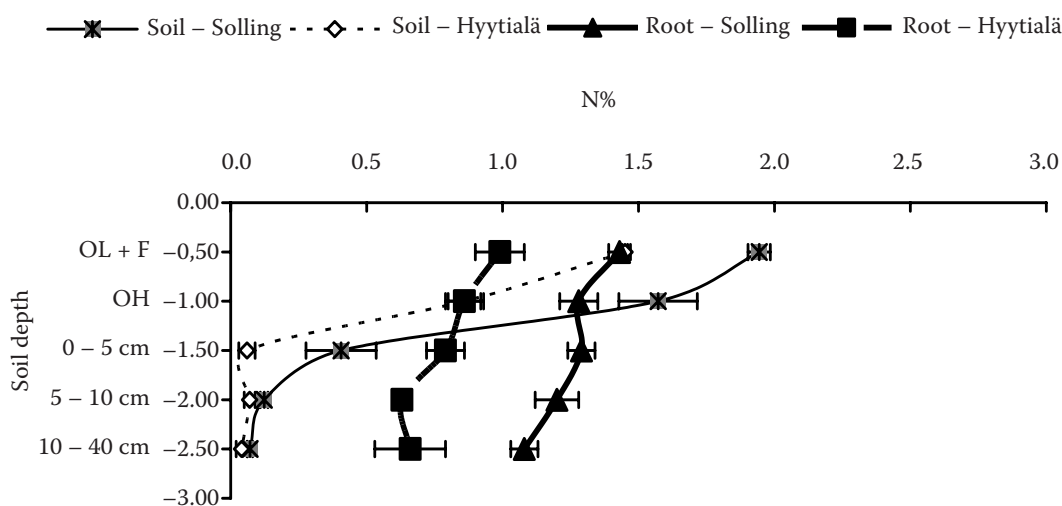
Fig. 2. Living roots %N versus soil %N in both stands (*n* = 5, error bars represent standard error of means)

centration in the foliage of both tree species (beech and spruce) remained almost constant, their δ¹⁵N values significantly decreased with the increasing years of sampling. The ¹⁵N-depletion from 1990 to 1995 was about 1.0‰ in beech foliage litter and 2.0‰ in spruce needle litter.

## DISCUSSION

### Natural ¹⁵N abundance and N concentrations in needles

The range of natural ¹⁵N abundance values in the green foliage of our spruce plots from –5.6 to –2.5‰ corresponded to the values reported for other conifer forests (KOOPMANN et al. 1997; EMMETT et al. 1998). As expected, N concentrations in the needles are significantly higher in the Solling stand (N-saturated) compared to the Hyytiälä stand (N-poor) and we assume this to be due to the higher N deposition at Solling site.

In general, the foliage ¹⁵N-enrichment might be due to several reasons such as (1) the source(s) of N, nitrogen obtained directly from the soil via root uptake or nitrogen obtained from mycorrhizal fungi, (2) the depth(s) in the soil from which N is taken up, (3) the form(s) of soil N used (organic N, $NH_4^+$, and $NO_3^-$).

In our studies, of the above-mentioned reasons of ¹⁵N fractionations, the foliage ¹⁵N-natural abundance cannot be related to the rooting depth as trees at both sites are rooted primarily in the upper organic soil horizon. In our study, ¹⁵N-enrichment of green needles of N-saturated Solling site may be attributed to accelerated nitrification in soils of this site, which results in the ¹⁵N-enriched $NH_4$-N production in soil. As a result, there is a constant and increasing uptake of ¹⁵N-enriched $NH_4$-N by vegetation as ¹⁵N-depleted $NO_3$-N is leached off from the system. The $NO_3$ leaching at Solling site was found very high (14.9 kg/ha/hr). But in the stand of Hyytiälä, where the $NO_3^-$ leaching is insignificant (Sah and Ilves-
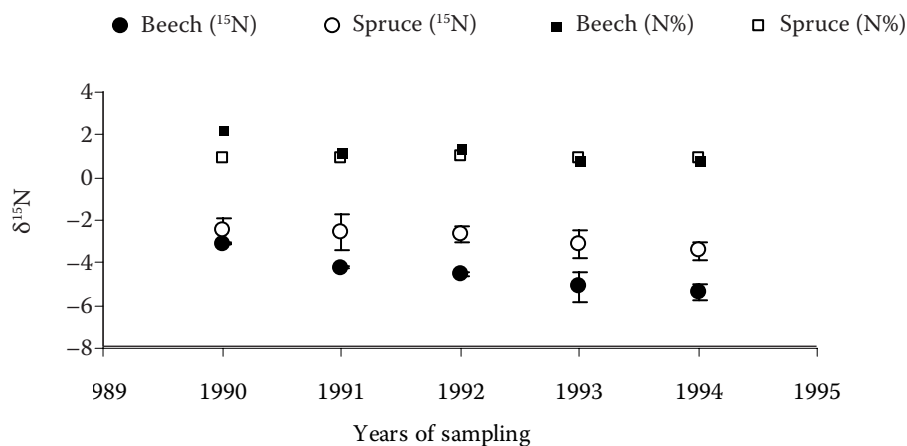


Fig. 3. δ15N and N % in the foliage litter of spruce and beech trees by years of sampling

niemi, data unpubl.), tree needles should take more $^{15}N$-depleted nitrogen. Therefore, we presume that the higher N concentrations and higher $^{15}N$-enrichment in the spruce needles of Solling site might be due to the higher N-deposition in this stand. Similar results were reported from several ecosystems and the researchers predicted that $^{15}N$ natural abundance values would increase for systems approaching N-saturation (NADELHOFFER, FRY 1994). Needles from a healthy Norway spruce stand with low N-deposition were reported to be more depleted of $^{15}N$ than those from a declining stand with high N and S depositions (GEBAUER, SCHULZE 1991; GEBAUER et al. 1994). $^{15}N$-enrichment of the foliage was also found to be due to an increase in N supply through fertiliser applications as demonstrated in agricultural systems (MEINTS et al. 1975) and forest ecosystems (HÖGBERG 1990). A positive relationship between N supply and an increase in the relative $^{15}N$-enrichment of vegetation to soil was observed for several vegetation types (JOHANISSON, HÖGBERG 1994). This was attributed to accelerated nitrification in N rich sites leading to constant and increasing uptake of $^{15}N$-enriched $NH_4$-N by vegetation as $^{15}N$-depleted $NO_3$-N is leached from the system (GARTEN, MIEGROET 1995).

For Hyytiälä stand, in addition to the insignificant $NO_3^-$ leaching, the other reason for the $^{15}N$-depletion in green needles might also be the higher proportion of mycorrhizal N-uptake. We do not have any data on the mycorrhizal abundances in our study sites so far. So we can only make speculations on this aspect. Certainly, the role of mycorrhizal plant root uptake of N in the depletion of needles of N-limited site of Hyytiälä cannot be ignored (KITAYAMA, IWANOTO 2001; HOBBIE et al. 2000). This might be attributed to the higher proportion of mycorrhizal N uptake as a soil N source in spruce trees of Hyytiälä, as the isotopic fractionation during N-transfer from mycorrhizas to plants leads to a decrease in $\delta^{15}N$ values compared to the source soil N. Because much of the plant N passes first through mycorrhizal fungi, the foliar $\delta^{15}N$ is depleted relative to available N (HOBBIE et al. 2000). In the case of Hyytiälä, since the atmospheric deposition of N is of negligible amount and the site is N-limited, the role of mycorrhizas appears to be important causing lower foliar $\delta^{15}N$ values and N concentrations in this site. In the case of spruce of the Solling stand (under high N deposition), mycorrhizal diversity should be greatly decreased (BRANDRUD, TIMMERMANN 1998) and hence there should be a lower proportion of myccorhizal N-uptake.

**Trend of $\delta^{15}N$ from needle-to-soil continuum**

Our results have shown that the natural abundances of $^{15}N$ in the vegetation and soil, in general, occurred in the following order: green needles < uppermost soil organic layer < mineral soil. This typical $^{15}N$-enrichment from vegetation to soil observed in our study is consistent with the other studies and this pattern reflects an isotopic discrimination of $\delta^{15}N$ during mineralisation of soil N as indicated by the decrease of total soil C, N and their ratio (C/N) with the depth (EMMET et al. 1998; NADELHOFFER, FRY 1994; GEBAUER, SCHULZE 1991; HÖGBERG et al. 1996; KOOPMAN et al. 1997). The mechanistic level is still controversial. $^{15}N$ discrimination during microbial decomposition and leaching of depleted nitrate may be the most important processes which result in the gradual $^{15}N$-enrichment of the residual organic matter (NADEHOFFER, FRY 1994).

For the Hyytiälä site, the $^{15}N$-enrichment of nitrogen in the soil increased from –3.84‰ in OL+F layer to +2.28‰ at lower soil depths (22 cm) and this pattern reflects an isotopic discrimination of $\delta^{15}N$ during mineralisation of soil N as indicated by the decrease in total soil N with the depth. The N-saturated site of Solling differs from the N-limited site of Hyytiälä with respect to the $^{15}N$ abundance trend both in the organic and mineral soil layer; $^{15}N$-enrichment of soil increased from –2.8 to +7.7‰, i.e. significantly more than that of Hyytiälä stand. This is assumed to be due to the higher N deposition at this site. Similar values of $^{15}N$ enrichment at lower soil depths (+5 and +10‰) were reported from the most of studies (EMMET et al. 1998; NADELHOFFER, FRY 1994; GEBAUER, SCHULZE 1991; HÖGBERG et al. 1996; KOOPMAN et al. 1997).

Is the annual $^{15}N$-depletion of foliage litter an indicator of decrease in annual atmospheric N deposition?

The data of our study suggests that there is a $^{15}N$-depletion of foliage litter N during the five years of observations from 1990 to 1994 (Fig. 3). This indicates that trees took up more $^{15}N$-depleted N in the consecutive years from 1990 to 1994 and this may be attributed to both 1) increase in mycorrhizal N uptake by tree roots proportionally, 2) decrease in N-deposition and/or decline in $NO_3^-$ leaching from soil. No study exists on the mycorrhiza of this stand and hence we can only speculate that the intensity of mycorrhizal infection should increase here due to a decrease in N deposition (BRANDRUD, TIMMERMANN 1998). The study of Solling site indicates that there was an increase in total N deposition from 1970 to 1980 (MATZNER 1989) and it was slightly reduced since then (MEESENBURG et al. 1995). A large fluctuation was observed for $NO_3$ leaching from soil

during the period 1990–1994 and it can be assumed that at least there was no significant increase in $NO_3^-$ leaching during this period. So we speculate on both these reasons, as mentioned above, behind the foliage litter ^{15}N-depletion with the time during the above-mentioned duration.

## CONCLUSIONS

In conclusion, the $\delta^{15}N$ natural abundance of undisturbed forest compartments can provide information about the N-status in forest ecosystems. The ^{15}N-enriched foliage of the polluted site of Solling indicates a higher rate of atmospheric N deposition and conversely, the ^{15}N-depleted foliage of the N-limited site of Hyytiälä indicates comparatively very low atmospheric N deposition.

## SUMMARY

The use of natural abundance of stable isotopes to elucidate physiological processes in plants is one of the most common and of the oldest applications of isotope analysis in ecology. The characteristics of the isotopic composition of pollutants can provide useful information on their source and quantity in the environment.

A majority of the studies in the past was focused on temperate spruce forests under different atmospheric N-deposition gradients. Comparative studies of spruce forests under different atmospheric N deposition loads and climatic conditions have been scarce. This paper presents a comparative study of two Norway spruce (*Picea abies* [L.] Karst.) forests differing greatly in their atmospheric N deposition and climatic conditions. One spruce stand that is situated in Solling, Central Germany, is temperate and highly N-saturated while the other spruce stand situated near Hyytiälä, Southern Finland, is boreal and has a very low atmospheric N-deposition. This study aims to investigate the changes in isotope ratios in foliage, roots and soils of both spruce stands resulting from N-deposition. This study hypothesises that the isotopic N composition of both ecosystems will act as an environmental indicator of N saturation.

The natural ^{15}N abundance values in the green foliage of our spruce plots from –5.6 to –2.5‰ were in the range of values observed for other conifer forests. As expected, N concentrations in the needles are significantly higher in the Solling stand (N-saturated) compared to the Hyytiälä stand (N-poor) and we assume this to be due to the higher N deposition at the Solling site. In our study, ^{15}N-enrichment of green needles of N-saturated Solling site may be attributed to accelerated nitrification in soils of this site, which results in the ^{15}N-enriched $NH_4$-N production in soil. But in the stand of Hyytiälä, since the $NO_3^-$ leaching is insignificant, tree needles should take more ^{15}N-depleted nitrogen. Therefore, we assume that the higher N concentrations and higher ^{15}N-enrichment in the spruce needles of Solling site might be due to the higher N-deposition in this stand. For Hyytiälä stand, in addition to the insignificant $NO_3^-$ leaching, the other reason for the ^{15}N-depletion in the green needles might also be due to the higher proportion of mycorrhizal N-uptake. We do not have any data on the mycorrhizal abundances in our study sites so far. So, we can only make speculations on this aspect.

For both stands a typical vertical gradient of the soil ^{15}N enrichment was observed. As expected, the soil of N-polluted site (Solling) was significantly more ^{15}N-enriched than that of N-limited site (Hyytiälä) and this is explained by the presence of marked $NO_3^-$ leaching at the Solling site. Although the annual trends (1990–1994) of N concentrations in the foliage of spruce trees remained almost constant, their $\delta^{15}N$ values significantly decreased with the increasing years of sampling. The ^{15}N-depletion in spruce needle litter from 1990 to 1995 was by 2.0‰. This is explained by a slight decrease in N deposition at the Solling site during this period. In conclusion, the ^{15}N-enriched foliage of the polluted site of Solling indicates a higher rate of atmospheric N deposition and conversely, the ^{15}N-depleted foliage of the N-limited site of Hyytiälä indicates comparatively very low atmospheric N deposition.

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## Poměry izotopů a koncentrace N v jehličí, kořenech a půdách porostů smrku ztepilého (*Picea abies* [L.] Karst.) pod vlivem atmosférických depozic N

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**ABSTRAKT**: Práce se zabývá zjišťováním změn poměru izotopů v jehličí a v půdách dvou porostů smrku ztepilého (*Picea abies* [L.] Karst.) se značně odlišnou atmosférickou depozicí N a klimatickými podmínkami. Jak koncentrace N, tak hodnoty $\delta^{15}$N v jehličí a kořenech byly podle očekávání významně vyšší v porostu Solling (nasyceném N) ve srovnání s porostem Hyytialä (chudým na N). Pro oba porosty jsme zjistili typický vertikální gradient obohacení půdy $^{15}$N. Obohacení půdy $^{15}$N bylo podle očekávání významně vyšší na stanovišti zatíženém N (Solling) než na stanovišti s limitovaným N (Hyytialä); lze to vysvětlit na základě výrazného vyplavování $NO_3^-$ na stanovišti Solling. Ačkoliv meziroční trendy (1990–1994) kon-

centrace N v jehličí smrků zůstávaly téměř konstantní, jejich hodnoty δ15N významně klesaly s přibývajícími roky odběru. Odčerpání 15N v jehličnatém opadu smrku v letech 1990–1995 činilo 2,0 ‰. Lze to vysvětlit mírným poklesem depozice N na stanovišti Solling, k němuž došlo v tomto období.

**Klíčová slova**: depozice N; 15N; dusík; smrk; půda

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