# $^{15}$N natural abundances in two podsol soils of two spruce forests differing in their atmospheric N deposition conditions

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**ABSTRACT**

This study aims to investigate the changes in isotope ratios in foliage and soils of the two spruce forests [*Picea abies* (L.) Karst.] differing greatly in their atmospheric N deposition and climatic conditions. As expected, both N concentrations and $\delta^{15}$N values in both needles and litter were found to be significantly higher in the Solling stand (N-saturated) compared to the Hyytialä stand (N-poor). For the N-limited site (Hyytialä plot), a typical vertical gradient of the soil $^{15}$N-enrichment (both in organic and mineral soil) was observed. The N-saturated site (Solling) differs from the N-limited site (Hyytialä) with respect to the $^{15}$N abundance trend in organic layer. In the upper organic layer up to O-f horizon, i.e. mor layer (0–3.5 cm depth) of Solling plot, there is almost a trend of slight soil $^{15}$N-depletion with increasing depth, and then there is a $^{15}$N-enrichment from O-h horizon (humus layer) of organic layer to mineral soil horizons. This is explained by the presence of prominent $NO_3^-$ leaching at this plot.

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

The characteristics of the isotopic compositions of pollutants can provide useful information with regards to its source and quantity in an environment. Over decades, N deposition has affected forest areas of Europe and North America (van Egmond et al. 2002, Matson et al. 2002). Nitrogen saturation is associated with the increased rates of N cycling and losses of $NO_3^-$ to percolation water (Lajtha et al. 1995, Matson et al. 2002). Several ecosystem researchers (Nadelhoffer and Fry 1994) have 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 in $^{15}$N than those from a declining stand receiving increased N and S depositions (Gebauer and Schulze 1991, Gebauer et al. 1994).

$^{15}$N-enrichment of the foliage can occur as N supply increases following fertilizer applications as demonstrated in agricultural systems (Meints et al. 1975) and forest ecosystems (Högberg 1990), or chronic atmospheric N-pollution (Gebauer and Schulze 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 (Garten and Miegroet 1995).

Most of the studies in the past have been limited to the temperate spruce forests under different atmospheric N-deposition gradients. A comparative study of spruce forests under different atmospheric N deposition loads and climatic variations have rarely been studied. This study is concerned with the comparative study of the two spruce forests (*Picea abies*, Karst.) differing greatly in their atmospheric N deposition and climatic conditions. One spruce stand is temperate and highly N-saturated, which is situated in Solling, central Germany and other spruce stand is boreal and has a very low atmospheric N-deposition situated in Hyytialä, southern Finland. This study aims to investigate the changes in isotope ratios in foliage and soils of both spruce stands resulting from N-deposition. This study hypothesizes that the isotopic N composition of the both ecosystems will act as an environmental indicator of the N saturation.

## MATERIAL AND METHODS

### Sites selection and their description

Two spruce forest research stands, one temperate stand at Solling (F1-plot), central Germany, and other boreal stand at Hyytialä, southern Finland have been selected for the present study. The purpose of selection was to compare the natural abundance of the same forest tree species under the different load of atmospheric N-deposition and climatic differences. 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 sandstone bedrock. The spruce forest (F1-plot) is 110 years old and is situated at 504 m altitude and the vegetation mainly comprises of mainly spruce [*Picea abies* (L.) Karst.]. Solling bears the average annual precipitation of 1142 mm and the mean annual air temperature of 6.4°C. The open field precipitation N-deposition (between 1981 to 1994) has been found 20 kg N/ha/year (Messenburg et al. 1995). The nitrogen deposition in throughfall (1969–1985) was found to be 40.8 kg N/ha/year. The nitrogen leaching from seepage water was strongly high 14.9 kg N/ha/year.

The other spruce forest site studied is boreal forest and is about 130 years old. It comprises of two dominant conifer tree species: Scot pine (*Pinus sylvestris* L.) and Norway spruce [*Picea abies* (L.) Karst.]. The research site is located near the Hyytiälä Forestry Filed Station of Helsinki University (61°48′N, 24°19′E). The average annual precipitation is 709 mm. The annual mean temperature during the growing seasons (May–September) is 12.6°C. The research site is located at about 150 m altitude. The soil is haplic podzols aged about 10 000 years (FAO classification). The bedrock is mainly acidic granite. The soil pH ($H_2O$) is highly acidic of about 4.4. The atmospheric N-deposition (1988–1996) is very low (6 kg N/ha/year) (Kumala et al. 1998). The nitrogen leaching is also of negligible amount.

### Sampling

For Solling site, samples of current year needles were collected during spring season in 1986 from the different heights (bottom, middle and upper parts). Litter fall was collected weekly in 15 litter traps in autumn season (September to November, 1990–1994) and were mixed to one sample for each month. 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 replicates) was extracted with a steel soil core sampler (30 cm long and 8 cm diameter) up to the soil depth of 14 cm at random. The extraction of samples followed in the year 2001. The soil cores were cut into 0.5–2 cm slices for the laboratory analysis.

For Hyytialä, samples of current year's needles were taken during spring season (in 1997) from three different heights of the trees: bottom, middle heights and top of the trees. For the above-mentioned sampling, eight trees of each species were selected at random from the stand. Litter fall was sampled in a circular 12 collectors of size (0.5 $m^2$) in autumn season (in 1997). In order to assess the vertical distribution of stable isotopes in the soil, undisturbed soil cores ($n$ = 10) were taken at random with a steel soil core sampler (49.5 deep × 5 cm diameter). The soil cores were cut into 1–2 cm slices for the laboratory analysis.

### 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 analyzer for conversion of N into $N_2$, C into $CO_2$. Results of the IRMS measurement were given in δ notation. The δ values of isotopes of N is expressed as parts per 1000 differences from a standard atmospheric isotopes (Shearer and Kohl 1993):

$$\delta^{15}N = \{[(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{air})] - 1\} \times 1000$$

(‰) air = reference standard gas

## RESULTS

### Natural $^{15}N$ abundance in the needle

Table 1 presents the data on the $\delta^{15}N$ values and N% in the needles and in their litter of both spruce forests. From this table it is evident that Solling spruce needles were significantly ($P \leq 0.05$) more $^{15}N$-enriched (–1.70‰, $SE$ ± 0.18‰) than that of the Hyytiälä spruce needles (–4.0‰, $SE$ ± 0.20‰). Similarly, N concentrations of the needle of Solling site (1.53%, $SE$ ± 0.20%) were significantly ($P \leq$ 0.05) higher (about 2 times more) than that found for the Hyytialä site (0.79%, $SE$ ± 0.02%). A similar trend also existed for the litter of both stands. The Solling site has significantly ($P \leq 0.05$) higher N concentrations (0.91%, $SE$ ± 0.02%) in its litter compared to that of the Hyytialä site (0.70%, $SE$ ± 0.04%). The litter of both spruce stands contained lower N concentrations than the green needles. The retranslocation of N (calculated as the percentage difference of N between oldest age class green needle and the litter (i.e. oldest age class green needle-litter/oldest age class green needle × 100) by the spruce needle litter of the Solling site was significantly higher (40%) as compared to the spruce needle litter (12%) of the Hyytialä site. Both stands differed markedly in their $^{15}N$ abundance in their

Table 1. δ$^{15}$N values and N concentrations in the needles, branches and litter of the spruce trees of both Hyytiälä ($n$ = 48, ± $SE$ values) and Solling ($n$ = 10, ± $SE$ values)

| Vegetation components | Hyytialä | | Solling | |
|---|---|---|---|---|
| | N (%) | δ$^{15}$N (‰) | N (%) | δ$^{15}$N (‰) |
| 1. Green needles | 0.79 (± 0.02) | –4.0 (± 0.2) | 1.53 (± 0.20) | –1.70 (± 0.18) |
| 2. Needle-litter | 0.70 (± 0.04) | –3.2 (± 0.6) | 0.91 (± 0.02) | –2.86 (± 0.18) |
| 3. Living branches | 0.64 (± 0.05) | –4.6 (± 0.1) | – | – |
| 4. Retranslocation of nitrogen from litter[1] | 12%* | – | 40%** | – |

[1]calculated as the percentage difference of N between green needle and the litter (i.e. older age green needle – litter/green needle × 100); *3 years old green needle; **current year needle

litter. The litter of the Solling site was $^{15}$N-depleted compared to its green needle whereas the litter of Hyytiälä site was $^{15}$N-enriched compared to its green needle. The living branches of spruce trees (–4.6‰, $SE$ ± 0.1) of Hyytiälä site were depleted in $^{15}$N in comparison to green needles and litter. N concentrations were also significantly lower in the living branch compared to its needles and litter. No such data exists for the Solling site.

### Natural $^{15}$N abundance in the soil

The δ$^{15}$N values of the uppermost soil surface of Solling spruce stand (–1.48‰, $SE$ + 1.11) was significantly higher than that observed for the Hyytialä stand (–4.53‰, SE ± 0.27). The range of δ$^{15}$N in the different depths of the mineral soil layers, for both spruce stands, was in between –4.53‰ to + 5.33‰. Both stands however, differed in their humus depth gradient of δ$^{15}$N abundance (Figure 1). The Hyytialä spruce stand showed a typical humus and mineral soil $^{15}$N-enrichment with increasing soil depth. In contrast, Solling spruce stand revealed first a slight soil $^{15}$N-depletion within the upper soil organic layers (i.e. O-l and O-f up to 3.5 cm of the soil profile), and then below O-f layer (i.e. O-h and mineral soil layer), similar to the Hyytialä stand, it showed a $^{15}$N-enrichment with increasing mineral soil depth (up to 12 cm). While the mineral soil layer of both stands showed more or less similar δ$^{15}$N values, but the organic layer of both stands differed substantially in their δ$^{15}$N values; the organic layer of Solling spruce stand values were significantly ($P \leq 0.05$) more $^{15}$N-enriched than observed for the Hyytialä spruce stand values. For both stands, the increasing $^{15}$N values in the soil profile with increasing depth corresponded to a decrease in the total nitrogen, carbon concentrations and C/N ratio (Figures 2 and 3). However, similar to $^{15}$N, N concentrations in all soil depths of Solling was significantly higher ($P \leq 0.05$) in comparison to that of Hyytialä. On the contrary, C concentrations were slightly but significant

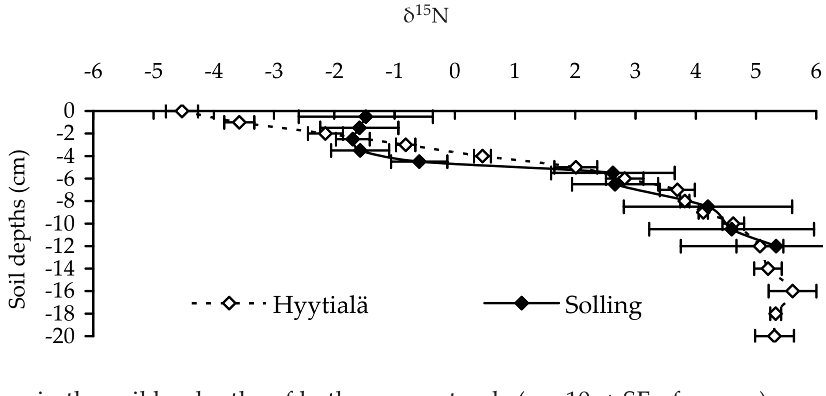


Figure 1. δ$^{15}$N values in the soil by depths of both spruce stands ($n$ = 10, ± SE of means)

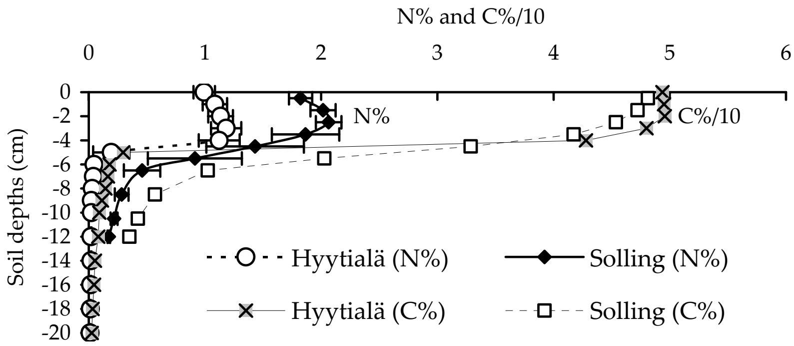


Figure 2. C% and N% in the soil by depths of both spruce stands (*n* = 10, ± SE of means)

higher in all soil depths of Hyytialä compared to that of Solling. In the organic layers of both stands, C/N ratio decreased with the increase in depth (from 26.4 to 20.3 at Solling site and from 49.7 to 21.6 at Hyytialä site). This shows that C/N ratio in the organic layer was significantly higher for the Hyytialä site relative to that of Solling, but both sites did not differ much in C/N ratio in the mineral soil. C/N ratio showed a large spatial variability for the Hyytialä site compared to that of the Solling site. In the mineral soil of both sites, C/N ratio decreased markedly and remained constant afterwards.

## DISCUSSION

### Natural $^{15}N$ abundance in the needle and litter

The range of natural $^{15}N$ abundance values in the green foliage of our spruce plots from –4.3 to –2.6‰ were within the values observed for other conifer forests (Koopmann et al. 1997, Emmett et al. 1998). As expected, N concentrations in both needles and litter are significantly higher in the Solling stand (N-saturated) compared to the Hyytialä stand (N-poor) and we assume this to be due to the higher N deposition at the Solling site.

In general, the foliage $^{15}N$-enrichment might be due to several reasons such as (1) the source(s) of N, such as, 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 $^{15}N$ fractionations, the foliage $^{15}N$-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, $^{15}N$-enrichment of green needles of N-saturated Solling site may be attributed to an accelerated nitrification in soils of this site, which results in the $^{15}N$-enriched $NH_4$-N production in soil. As a result, there is a constant and increasing uptake of $^{15}N$-enriched $NH_4$-N by vegetation as $^{15}N$-depleted $NO_3$-N is leached off from the system. The $NO_3^-$ leaching at the Solling site has been found very high (14.9 kg/ha/hr). But in the stand of Hyytialä, since the $NO_3^-$ leaching is insignificant (Sah and Ilvesniemi, personal communi-

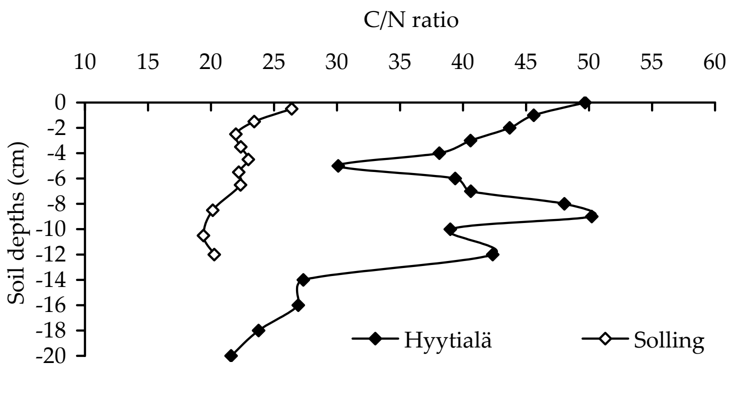


Figure 3. C/N ratio in soil by depths of both spruce forests

cation); trees needle should take more $^{15}N$-depleted nitrogen. Therefore, we presume that the higher N concentrations and more $^{15}N$-enrichment in the spruce needle of Solling site might have been due to the higher N-deposition in this stand. Similar results were reported from several ecosystem and they have predicted that $^{15}N$ natural abundance values would increase for systems approaching N-saturation researchers (Nadelhoffer and Fry 1994). Needles from a healthy Norway spruce stand with low N-deposition were reported to be more depleted in $^{15}N$ than those from a declining stand with high N and S depositions (Gebauer and Schulze 1991, Gebauer et al. 1994). $^{15}N$-enrichment of the foliage has also been found due to increase in N supply through fertilizer 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 has been observed for several vegetation types (Johanisson and Högberg 1994). 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 (Garten and Miegroet 1995).

For Hyytialä stand, in addition to the insignificant $NO_3^-$ leaching, the other reason for the $^{15}N$-depletion in the green needle might also be due to the higher proportion of mycorrhizal N-uptake. We do not have so far the data on the mycorrhizal abundances in our study sites. So, we can only make only speculations on this aspect. But certainly, the role of mycorrhizal plant root uptake of N in the depletion of needle of N-limited site of Hyytialä cannot be ignored (Hobbie et al. 2000, Kitayama and Iwamoto 2001). This might be attributed to the higher proportion of mycorrhizal N uptake as soil N source in spruce trees of Hyytialä, as the isotopic fractionation during N-transfer from mycorrhizas to plants leads to depletion of $\delta^{15}N$ 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 Hyytialä, 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 declined (Brandrud and Timmermann 1998) and hence there should be lower proportion of myccorhizal N-uptake.

The needle-litter of the Hyytialä site, as in other author's studies, was found to be much more enriched in $^{15}N$ compared to the green needle, which is attributed to a redistribution of N from the needles before needle litterfall (Gebauer and Schulze 1991, Gebauer et al. 1994). However, the needle litter of N-saturated site of Solling spruce trees were significantly more $^{15}N$-depleted in comparison to its green needle. The observed depletion of $^{15}N$ in litterfall in our study remains largely unexplained.

As mentioned above, N retranslocation from the litter is also relatively significant higher in the Solling spruce (40%) in comparison to the Hyytialä spruce (12%). These characteristics all appear to contribute to spruce trees of Solling site abilities to both acquire more N and use it more effectively.

### The 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: branches < green needle < needle litter < uppermost soil organic layer < mineral soil. This typical $^{15}N$-enrichment from the vegetation to soil observed in our study is in 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 (Gebauer and Schulze 1991, Nadelhoffer and Fry 1994, Högberg et al. 1996, Koopman et al. 1997, Emmet et al. 1998). 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 and Fry 1994).

For the N-limited site of Hyytialä plot, a typical vertical gradient of the soil $^{15}N$-enrichment was observed. In this plot, the $^{15}N$-enrichment of nitrogen in the soil increased from –4.53‰ at 0 cm to +5.31‰ in 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 of total soil C and N with the depth. Similar values of enrichment of $^{15}N$ in lower soil depths (+5 and +10‰) has been reported from the most of studies (Gebauer and Schulze 1991, Nadelhoffer and Fry 1994, Högberg et al. 1996, Koopman et al. 1997, Emmet et al. 1998). The N-saturated site of Solling differs from the N-limited site of Hyytialä with respect to the $^{15}N$ abundance trend in the organic layer. In the upper organic layer up to O-f horizon (0–3.5 cm depth) of Solling plot, there is almost a trend of slight soil $^{15}N$-depletion with increasing depth, and then there is a $^{15}N$-enrichment from O-h horizon of organic layer to mineral soil horizons. This observation is not in agreement with the other field studies and this is explained by the presence of prominent $NO_3^-$ leaching at this plot (for details, see below).

### $^{15}$N natural abundance as indicator of N-saturation of ecosystems

Our observations have found the natural abundance of $^{15}$N as an indicative parameter of the N-status of both studied spruce forest as follows:

In N-saturated systems, N input promotes nitrification, which in turn results into $^{15}$N-enriched $NH_4$-N production in soil. Plants preferentially taking $^{15}$N-enriched $NH_4$-N as N source will progressively enrich the surface soil; in other words, the fresh needle/litter, which accumulates on the uppermost soil surface, is always more enriched in $^{15}$N compared to the needle/litter of previous year, because each year there is a production of more $^{15}$N-enriched needle than the previous year due the N saturation in the system. Therefore, we have decreasing trend of soil $\delta^{15}$N in the organic layer with the depth. This is only my speculation, since I have no long-term data on needle/litter to prove this.

In N-limited systems there are low rates of nitrification, which means there is no isotopic enrichment of $NH_4$-N. If $NH_4$-N is preferred for trees uptake, isotopically N-depleted foliage/litter is produced, which progressively depletes the uppermost soil surface compared to lower soil depth. The highly N-saturated Solling site and the relatively N-limited Hyytialä site support our above mentioned speculations.

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**ABSTRAKT**

**Přirozené obohacení izotopem $^{15}N$ dvou podzolových půd smrkových lesů lišících se podmínkami atmosférické depozice dusíku**

Na dvou odlišných stanovištích (Solling, Německo a Hyytialä, Finsko) s různými klimatickými podmínkami a velikostí atmosférické depozice byly sledovány změny izotopického poměru dusíku v jehličí a půdě. Obsah N i hodnoty $\delta^{15}N$ ve smrkových jehlicích i v rostlinném pokryvu půdy byly významně vyšší na stanovišti Solling, kde byla zjištěna i vyšší depozice N. Hyytialä jako stanoviště s nízkým obsahem N vykazovalo vertikální gradient obohacení $^{15}N$ půdy (byl sledován organický i minerální horizont). Trend obohacení $^{15}N$ se na stanovišti Solling lišil v organickém horizontu. Ve svrchní organické vrstvě (hloubka 0–3,5 cm) se obsah $^{15}N$ postupně slabě snižoval, zatímco obohacení $^{15}N$ rostlo od O-h horizonu (humusová vrstva organického horizontu) směrem k horizontům minerálním. Důvodem může být vyplavování $NO_3^-$ na tomto stanovišti.

**Klíčová slova:** atmosférická depozice dusíku; $^{15}N$; dusík; smrk; půda

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