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# Soil N chemistry in oak forests along a nitrogen deposition gradient

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**Abstract.** Anthropogenic N deposition may change soil conditions in forest ecosystems as demonstrated in many studies of coniferous forests, whereas results from deciduous forests are relatively scarce. Therefore the influence of N deposition on several variables was studied *in situ* in 45 oak-dominated deciduous forests along a N deposition gradient in southern Sweden, where the deposition ranged from 10 to 20 kg N ha$^{-1}$ year$^{-1}$. Locally estimated $NO_3^-$ deposition, as measured with ion-exchange resins (IER) on the soil surface, and grass N concentration (%) were positively correlated with earlier modelled regional N deposition. Furthermore, the $\delta^{15}N$ values of grass and uppermost soil layers were negatively correlated with earlier modelled N deposition. The data on soil $NO_3^-$, measured with IER in the soil, and grass N concentration suggest increased soil N availability as a result of N deposition. The $\delta^{15}N$ values of grass and uppermost soil layers indicate increased nitrification rates in high N deposition sites, but no large downward movements of $NO_3^-$ in these soils. Only a few sites had $NO_3^-$ concentrations exceeding 1 mg N l$^{-1}$ in soil solution at 50 cm depth, which showed that N deposition to these acid oak-dominated forests has not yet resulted in extensive leaching of N. The $\delta^{15}N$ enrichment factor was the variable best correlated with $NO_3^-$ concentrations at 50 cm and is thus a variable that potentially may be used to predict leaching of $NO_3^-$ from forest soils.

## Introduction

Increased N deposition due to human activities to terrestrial ecosystems may markedly change soil conditions and is sometimes followed by leaching of nitrate with eutrophication of recipient waters and health risks to humans as the most serious consequences. The response of deciduous forests to N deposition is less well studied than that of coniferous forests, and the effects of N deposition are reported to differ between these two forest types. For example, higher N retention capacities were found in mixed deciduous stands in northeastern USA than in adjacent pine plantations (Aber et al. 1998; Magill et al. 2000). Deciduous forest soils in Europe had higher net nitrification rates (Persson et al. 2000) and higher nitrate concentrations (Kristensen et al. 2004) than coniferous forest soils. The C/N ratio of the soil organic layer, that often is a good predictor of the potential risk of N leaching from coniferous forest soils (e.g. Gundersen et al. 1998), could not be used as a predictor of N leaching from deciduous forests (Kristensen et al. 2004) which may be due to the lack of a well-developed organic layer in many temperate deciduous forests.

Soil pH is related to many soil characteristics and affects several soil processes, including N transformations. One reason for the observed higher nitrification rates in deciduous forests relative to coniferous forest along a European N deposition gradient is higher soil pH (Persson et al. 2000), but different soil pH cannot entirely explain variation in nitrification in forest soils (De Boer and Kowalchuk 2001). Nitrification in acid forest soils (pH < 5) is more often detected in deciduous forest (in 80% of the sites included in a meta-analysis), compared to in coniferous forests (35%) (Robertson 1982). In a study of soils from more than 500 oak-dominated deciduous forests in southern Sweden, effects of N deposition such as increases in potential net N mineralization and nitrification rates and decreases in the soil C/N ratios was most pronounced in the most acid soils (Falkengren-Grerup and Diekmann 2003). In addition, the highest leaching of $NO_3^-$ was observed from some of the most acidified soils of investigated oak forests in southwest Sweden (Falkengren-Grerup et al. 2006). Thus, low pH soils might also leach nitrate although the determining factors for the extent of leaching is not well understood. This lack of knowledge, in combination with a possible increase in deciduous tree species distribution as an effect of climate change (Izaurralde et al. 2005), makes it important to study deciduous forest soil responses to N deposition, especially on the most acid soils.

In the present study we therefore evaluated the influence of N deposition to acid deciduous forest soils using *in situ* methods, with the main aim to identify possible leaching of N and factors that could be related to this. For this purpose we investigated 45 oak-dominated forest sites with similar low soil pH (from 3 to 4) along a 300 km N deposition gradient in southern Sweden where deposition changes from 10 to 20 kg N $ha^{-1}$ $year^{-1}$. We estimated N deposition locally, using ion-exchange resins (IER) on the soil surface, and soil availability of N, from IER in the soil and from grass N concentrations. These estimates were related to earlier modelled estimates of N deposition valid for larger regional scales and to earlier data on potential net N mineralization from soil incubations. The seasonal variations in local N deposition and soil N availability were monitored over the course of one year to evaluate e.g. the influence of climatic variation and plant activity on soil N availability. We measured $NO_3^-$ concentrations in soil solutions below the main rooting zone to detect any possible leaching of N, and tried to find potential factors able to predict leaching of $NO_3^-$ from these acid deciduous forest soils. The $\delta^{15}N$ values can be used to estimate soil processes integrated over several years. Nitrification results in $NO_3^-$ being depleted in $^{15}N$ while the remaining $NH_4^+$ becomes enriched. Nitrification followed by $NO_3^-$ movements down the soil profile is suggested to deplete deeper soil horizons in $^{15}N$ (Högberg 1997). In addition, the $\delta^{15}N$ enrichment factor, the difference in $\delta^{15}N$ in plants that take up $NO_3^-$ and the soil, was used as an indicator of the degree of nitrification. For this reason we analysed the $\delta^{15}N$ values of a grass found at most sites; *Deschampsia flexuosa* (L.) Trin.

## Materials and methods

### *Forest sites*

This study was performed in deciduous forests dominated by oak (*Quercus robur* L.) in four regions (Öland, eastern Småland, Skåne and Halland) along a N deposition gradient in southern Sweden (Figure 1). Total

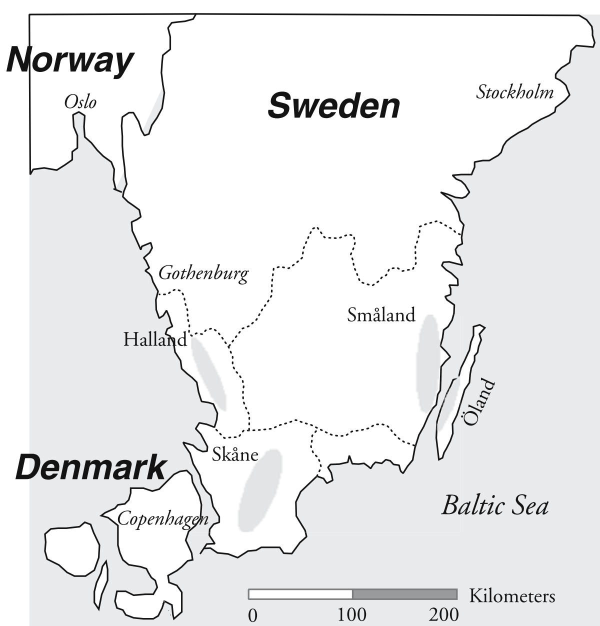


*Figure 1.* Sampling sites in southern Sweden. The 45 oak-dominated deciduous forest sites investigated are located (within shaded areas in the map) in regions in with high N deposition (Skåne, $n = 11$; and Halland, $n = 13$) and in regions with low N deposition (Öland, $n = 10$; and Småland, $n = 11$).

inorganic N deposition was about twice as high in two of the regions (hereafter called 'regions with high N deposition'; Skåne and Halland) than in the other two regions ('regions with low N deposition'; Öland and Småland) (Table 1). The four regions are climatically quite uniform with similar mean temperatures ($\sim$7 °C) and growing seasons, but differ somewhat in mean annual precipitation (Skåne, 600 mm; Halland, 700 mm; Öland and Småland, 500 mm (Falkengren-Grerup and Diekmann 2003)). We selected 10–13 forests in each of the four regions out of more than 500 oak forest sites earlier studied in these regions (Falkengren-Grerup et al. 1998). Forest sites were chosen based on similarity in soil acidity ($pH_{KCl}$ 3–4 of soils 0–5 cm below the litter layer), but despite the large number of sites to choose from it was not possible to find sites with exactly similar soil pH (Table 1), due to anthropogenic acidification of the forest soils in the region with highest N deposition. The soils are characterized as cambisols with a varying but usually low clay content and having no or only a thin humus layer. The texture is mainly fine sand with a low boulder content except in Halland and Småland where moraines are found. The sampled soils were, according to our field experience, judged to be well-drained down to at least 1 m. However, when digging the soil profiles it became clear that a few sites had a ground water table at about 0.3 m and they were therefore omitted in the analyses of nitrate leaching.

### *N deposition and soil inorganic N*

Inorganic N concentrations in precipitation and soil were estimated using IER-filled bags made of acid-washed nylon stockings, filled with 35 g of a mixed IER (Duolite® MB 6113, BDH, Poole, UK) and sealed with nylon threads at both ends. Before the experiment the IER-filled bags were washed once in 2 M $CaCl_2$ and three times in 2 M NaCl and were rinsed with Milli-Q water (Millipore Co, Billerica, MA, USA) after each wash.

IER-filled bags used to estimate N deposition locally were placed on the soil surface in plastic pots ($6\times6\times6$ cm) with holes in the bottom to facilitate water drainage. A nylon mesh (mesh size 0.5 mm) was placed in the bottom of each pot to avoid root ingrowth and on the top to avoid impurities from litter, invertebrates, etc.

To obtain an estimate of the available inorganic N in the soil (soil $N_i$) we buried IER-filled bags in the soil. This soil $N_i$ can be assumed to include N in water running through the soil, diffusion of mineralised N close to the IER-filled bags and diffusion of excess mineralised N not immobilised by plants or microbes. IER-filled bags used to estimate soil $N_i$ were buried in the topsoil at approximately 5 cm depth in plastic rings (2 cm high; diameter 7.2 cm) in order to facilitate calculations per unit area.

Five pairs of IER bags (one on the ground and one in the soil) were placed randomly, with a distance of $\sim$5 m between each pair, in each forest plot (approximately $10\times10$ m). The IER-filled bags were replaced three times during the year in order to study seasonal changes in N deposition and soil $N_i$. We estimated N deposition and soil $N_i$ during the summer (4 months, from April to August 2001), the autumn (three months, from August to November 2001) and during the winter (5 months, from November 2001 to April

*Table 1.* Soil pH, soil C/N ratio, potential net N mineralization rate and nitrification ratio (%) from soil incubations in laboratory, and estimated N deposition.

| Region | $pH_{KCl}$[a] | C/N ratio[a] | N mineralization[a] ($\mu g\ g^{-1}\ day^{-1}$) | Nitrate (%)[a] of total N mineralized | Estimated N deposition[b] ($kg\ ha^{-1}\ year^{-1}$) |
|---|---|---|---|---|---|
| Öland | 3.9 ± 0.1 (10) | 26 ± 3 (10) | 8.6 ± 1.0 (10) | 49 ± 10 (10) | 10.2 ± 0.2 (10) |
| Småland | 3.9 ± 0.1 (11) | 22 ± 2 (10) | 11.3 ± 1.2 (11) | 46 ± 9 (11) | 10.0 ± 0.2 (11) |
| Skåne | 3.7 ± 0.0 (11) | 19 ± 2 (11) | 17.6 ± 0.9 (11) | 58 ± 4 (11) | 17.3 ± 0.4 (11) |
| Halland | 3.3 ± 0.1 (13) | 24 ± 1 (13) | 16.8 ± 1.3 (13) | 37 ± 5 (13) | 17.9 ± 0.3 (13) |

Mean values ± SE and number of replicates (*n*) for sites used in the present study.
[a]Data from Falkengren-Grerup and Diekmann (2003).
[b]Data from Diekmann and Falkengren-Grerup 2002, based on Langner et al. 1995.

2002). Winter values were only recorded at selected sites (four sites in Skåne and Småland for N deposition, and four to six sites in each region for soil $N_i$) to obtain an average estimate. The winter data were not included in the statistical tests.

After collection in the field the IER-filled bags were stored cool (at 5 °C) for about 1 week until analysis. Before opening, each IER-filled bag was first rinsed with Milli-Q water, to prevent contamination from soil particles, and was thereafter placed on a nylon mesh until dripping ceased (30–60 min). IER from five IER-filled bags per forest site was mixed carefully and a subsample of 35 g IER was placed in a 250 ml flask. NaCl (2 M, 150 ml) was added and the flasks were shaken for 2 h on a rotary shaker. The extracts were filtered and thereafter immediately frozen. The extracts were analysed on $NH_4^+$ and $NO_3^-$ by flow-injection analysis (FIA). The water content of the IER was measured and used to correct the $NH_4^+$ and $NO_3^-$ values.

## *Grass N concentration, $^{15}N$ in grass and soil*

The N concentration (%) and values of $\delta^{15}N$ in leaf blades of the grass *Deschampsia flexuosa* and the $\delta^{15}N$ values in the soil down through the soil profile were measured in order to estimate the N status of the oak forest soils. Leaf blades of *Deschampsia flexuosa* from three to five plants per site were sampled in August 2001. *D. flexuosa* was found in only three sites per region in Öland and Småland, and totally in 27 sites (Table 2). Leaf blades of *D. flexuosa* were dried (40 °C) and about 0.2 g was analysed with regard to total N by Kjeldahl digestion, followed by FIA analysis. Soil samples from the organic layer and mineral soils from 0–5 cm, 5–10 cm, and 25–30 cm were sampled in November 2002. In many sites the mineral soil was often found directly below the litter layer. Therefore samples of organic soils had to be collected wherever they could be found within the site. Analyses of $^{15}N$ in the grass blades and of soil samples were performed at the Department of Forest Ecology at the Swedish University for Agricultural Sciences at Umeå, using an online, continuous-flow C and N analyser connected to an isotope mass spectrometer.

Results of $^{15}N$ analyses are expressed in the standard notation ($\delta^{15}N$) in parts per thousand (‰) relative to the international standard N in air (Högberg 1997). The difference in $\delta^{15}N$ in the substrate (the soil) and the product (the plant) is described by a $\delta^{15}N$ enrichment factor ($\varepsilon_{p/s}$) (Mariotti et al. 1981) here defined as: $\varepsilon_{p/s} = \delta^{15}N_{plant} - \delta^{15}N_{soil}$.

## *$NO_3^-$ at 50 cm depth*

Leaching of $NO_3^-$ was estimated from soil samples taken early in the winter season at 50 cm depth ($[NO_3^-]_{50\ cm}$). This sampling depth was shown to be useful for estimates of $NO_3^-$ leaching (Callesen et al.

*Table 2.* $\delta^{15}N$ values of *D. flexuosa* leaf blades and soils from the organic layer, the enrichment factor, and the $\delta^{15}N$ range.

| Region | $\delta^{15}N$ in grass | $\delta^{15}N$ in organic layer[a] | $\delta^{15}N$ enrichment[b] | $\delta^{15}N$ range[c] |
|---|---|---|---|---|
| Öland | 0.0 ± 1.0 (3)a | −1.9 ± 0.8 (3)ab | 1.9 ± 0.8 (3)a | 6.4 ± 0.5 (7)b |
| Småland | 1.2 ± 0.3 (3)a | −1.1 ± 0.4 (3)a | 2.2 ± 0.3 (3)a | 4.6 ± 0.2 (7)a |
| Skåne | −3.1 ± 0.2 (11)b | −2.6 ± 0.3 (11)b | −0.5 ± 0.3 (11)b | 8.5 ± 0.4 (11)c |
| Halland | −3.7 ± 0.3 (10)b | −3.1 ± 0.4 (10)b | −0.5 ± 0.4 (10)b | 9.7 ± 0.4 (12)d |
| ANOVA | $p < 0.001$ | $p = 0.04$ | $p = 0.002$ | $p < 0.001$ |
| *Planned contrast* | $p < 0.001$ | $p = 0.01$ | $p < 0.001$ | $p < 0.001$ |

Mean values ± SE, and number of replicates (*n*). Significant differences between regions are indicated by differing letters ($p < 0.05$). Planned contrasts are used to test for differences between regions with high N deposition (Skåne and Halland) and low N deposition (Öland and Småland).

[a]Only including values from sites where *D. flexuosa* was present.

[b]Enrichment factor – the difference in $\delta^{15}N$ values of grass and soils from the organic layer ($\varepsilon_{p/s} = \delta^{15}N_{plant} - \delta^{15}N_{soil}$).

[c]$\delta^{15}N$ range – the difference in $\delta^{15}N$ values of soil from the organic layer and of mineral soils at 25–30 cm depth.

1999). One sample per site was taken in December 2002, when root and microbial activities are low. About 200 g (< 6 mm) soil at field moisture content was centrifuged at 13,000 rpm ($G = 14{,}200$) at 10 °C for 1.5 h (essentially according to Giesler and Lundström 1993). The pH was measured in the soil solution whereafter the solution was syringe-ultrafiltered (Acrodisc PF, 0.8 $\mu$m prefilter, 0.2 $\mu$m Supor, Pall Corp., East Hills, NY, USA) and analysed for nitrate by FIA analysis. Some of the soil samples were excluded from the analysis of $[NO_3^-]_{50\ cm}$ and $^{15}N$ down the soil profile due to ground water levels at ~30 cm depth.

### *Data analysis and statistics*

Data from sites in the four regions were tested using analysis of variances (ANOVA). Data on N deposition and soil $N_i$ were tested by repeated measurement (RM-) ANOVA for summer and autumn values (Figures 2 and 3) at all sites. *A priori* planned contrasts were performed to test for differences in values between regions with high N deposition (Halland and Skåne) and regions with low N deposition (Öland and Småland) and pairwise *post-hoc* tests where performed to test for differences between regions and seasons (LSD, when significant ANOVAs). Statistical tests were performed on log transformed values when necessary to achieve equal variance. Correlations using data on deposition of N and soil $N_i$ were done on summer + autumn values, unless anything else is noted.

## Results

### *N deposition*

Deposition of $NO_3^-$ during the summer and the autumn seasons differed between regions ($p = 0.001$), with higher deposition of $NO_3^-$ to the forest sites in regions with high N deposition (Skåne and Halland) than in regions with low N deposition (Öland and Småland) ($p < 0.001$) (Figure 2a). Deposition of $NO_3^-$ did not differ between seasons. Deposition of $NO_3^-$was positively correlated with earlier modelled N deposition (see Table 1) (summer + autumn values: $R^2 = 0.24$, $p = 0.001$; autumn values: $R^2 = 0.51$, $p < 0.001$).

The deposition of $NH_4^+$ was several times higher than the deposition of $NO_3^-$ ($p < 0.001$). The deposition of $NH_4^+$ did not differ between regions, but there was a significant difference between the seasons ($P < 0.001$) (Figure 2b). Of the total amount of $NH_4^+$ deposited throughout the year most was deposited during the summer (about 70%) and least was deposited during the winter (about 5%) (as estimated from 4 + 4 sites in Skåne and Småland). High deposition of $NH_4^+$ in Skåne in the summer was the main reason for the significant interaction term (region × season) ($p = 0.035$). Since the deposition of $NH_4^+$ was much larger than that of $NO_3^-$, the total deposition of N ($NO_3^- + NH_4^+$) was mainly influenced by $NH_4^+$ (Figure 2a and b). There was thus no significant difference in total deposition of N between the regions, while seasons differed ($p < 0.001$) with higher total deposition of N during summer than during autumn.

### *Soil inorganic N*

Soil $NO_3^-$ during the summer and the autumn seasons, estimated using IER-filled bags buried in the soil, differed between the regions ($p = 0.05$), with higher soil $NO_3^-$ in the regions with high N deposition (Skåne and Halland) than in the regions with low N deposition (Öland and Småland) ($p = 0.017$) (Figure 3a). Soil $NH_4^+$ showed a tendency to differ between regions ($p = 0.09$), being highest in Halland (one of the regions with high N deposition), and there was a difference in soil $NH_4^+$ between seasons ($p = 0.005$), with the highest values during the autumn (Figure 3b). Total soil $N_i$ ($NO_3^-$ and $NH_4^+$) did not differ between regions.

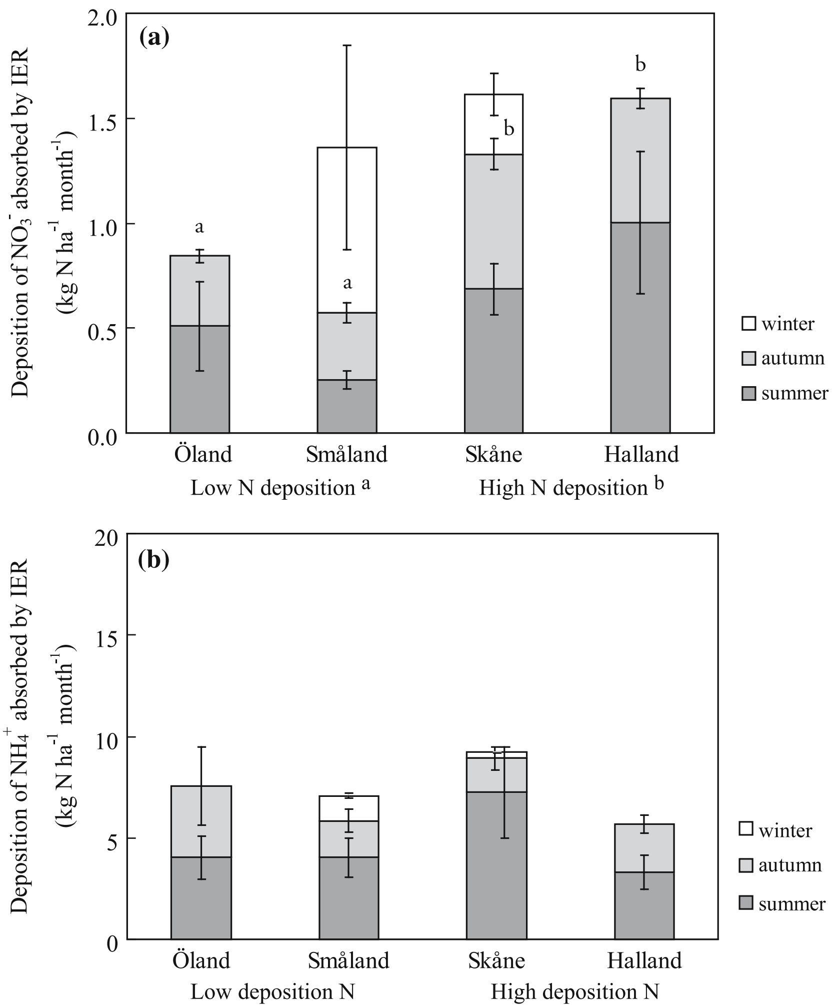


*Figure 2.* Deposition of (a) $NO_3^-$ and (b) $NH_4^+$ (kg N $ha^{-1}$ $month^{-1}$) estimated using IER placed on the soil surface during summer (4 months) and autumn (3 months). Winter values (5 months) were only obtained from a few sites in Skåne and Småland, and were therefore not included in the statistical analysis. Means ± SE. Note the difference scales on the *y*-axes. RM-ANOVA (on log-transformed values): (a) regions, $p < 0.001$; seasons, ns; interaction r×s, ns, (b) regions, ns; seasons, $p < 0.001$; interaction r×s, $p = 0.035$. A priori planned contrasts were performed to test for differences between regions with high N deposition and regions with low N deposition. Pairwise *post-hoc* tests where performed to test for differences between regions and seasons (LSD, when significant ANOVAs). Significant differences between regions are indicated by differing letters ($p < 0.05$).

## *Grass N concentration, values of $\delta^{15}N$ of grass and soil*

The N concentration (%) of *Deschampsia flexuosa* leaf blades differed significantly between the regions ($p < 0.001$) (Figure 4). Grass N was higher in the regions with high N deposition (Halland and Skåne) than in the regions with low N deposition (Öland and Småland) ($p = 0.025$) with N concentrations being twice as high in Halland (2.7 ± 0.1%) as in Öland (1.3 ± 0.1%). Grass N was positively correlated with earlier modelled N deposition for the sites (see Table 1) ($R^2 = 0.62$; $p < 0.001$) and with deposition of $NO_3^-$ measured using IER ($R^2 = 0.33$, $p = 0.002$). Grass N was also positively correlated with soil $NO_3^-$ ($R^2 = 0.37$, $p < 0.001$) and soil $N_i$ ($R^2 = 0.41$, $p < 0.001$), but not with soil $NH_4^+$, estimated using IER.

The $\delta^{15}N$ values differed between regions both in grass ($p < 0.001$, mean values ranging from $-3.7‰$ to $+1.2‰$) (Table 2) and in soils from the organic layer ($p < 0.001$, means of all samples ranging from $-3.1‰$ to $-0.3‰$) (Figure 5). Both grass and soils from the organic layer were more $^{15}N$-depleted in the

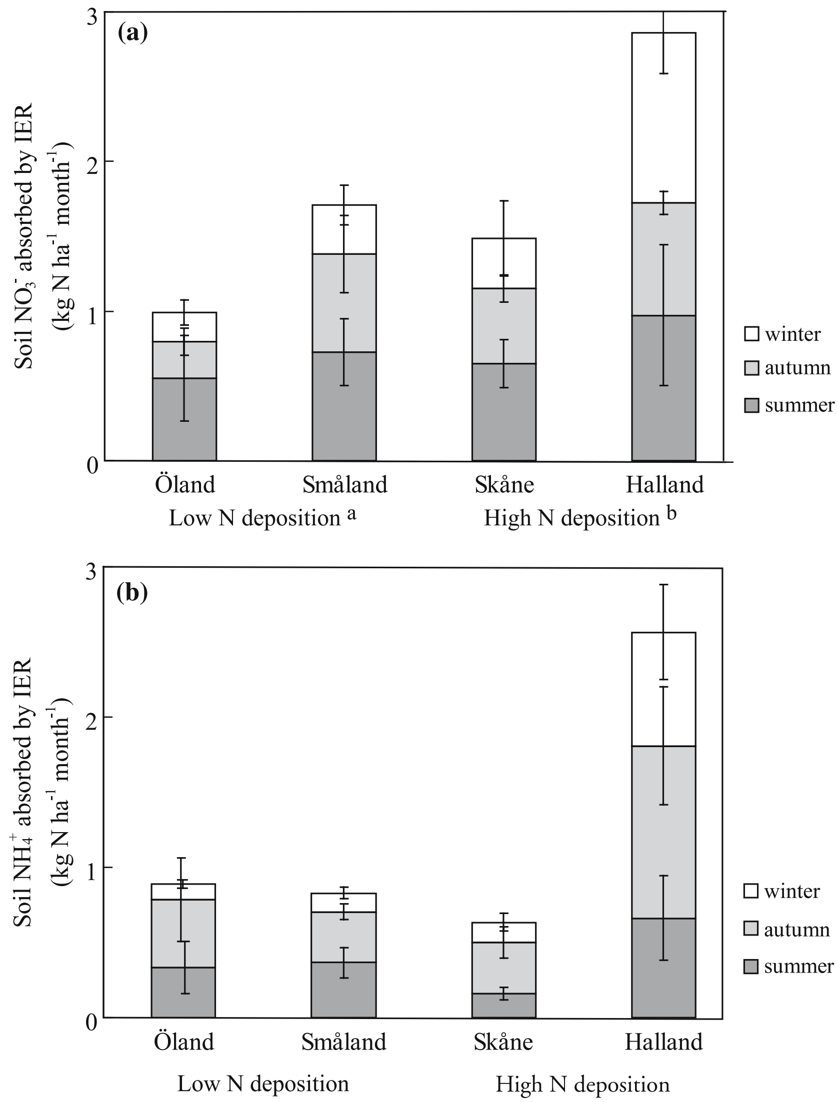


*Figure 3*. Soil N as (a) $NO_3^-$ and (b) $NH_4^+$ (kg N $ha^{-1}$ $month^{-1}$) measured using IER buried in the soil during summer (4 months) and autumn (3 months). Winter values (5 months) were only obtained from a few sites in each region, and were therefore not included in the statistical analysis. Means ± SE. RM-ANOVA (on log-transformed values): (a) regions, $p = 0.05$; seasons, ns; interaction r × s, ns ($p = 0.09$) and (b) regions, ns ($p = 0.09$); seasons, $p = 0.005$; interaction r × s, ns. *A priori* planned contrasts were performed to test for differences between regions with high N deposition and regions with low N deposition. Pairwise *post-hoc* tests where performed to test for differences between regions and seasons (LSD, when significant ANOVAs). Significant differences between regions are indicated by differing letters ($p < 0.05$).

regions with high N deposition (Halland and Skåne) than those with low N deposition (Öland and Småland) (grass: $p < 0.001$; soils: $p = 0.01$). The $\delta^{15}N$ enrichment factor differed between the regions ($p < 0.001$), with plants in regions with high N deposition (Skåne and Halland) being depleted in $^{15}N$ relative to soil (−0.5‰), while plants in regions with low N deposition (Öland and Småland) were enriched in $^{15}N$ relative to soil (1.9‰ and 2.2‰ respectively) ($p < 0.001$) (Table 2). The $\delta^{15}N$ values increased with soil depth in all regions (Figure 5). The range, i.e. the difference in $\delta^{15}N$ values between soils from the organic layer and mineral soils at 25–30 cm depth, differed significantly between regions ($p < 0.001$), with greater ranges in $\delta^{15}N$ values in regions with high N deposition (Halland and Skåne) than in regions with low N deposition (Öland and Småland) (Figure 5; Table 2).

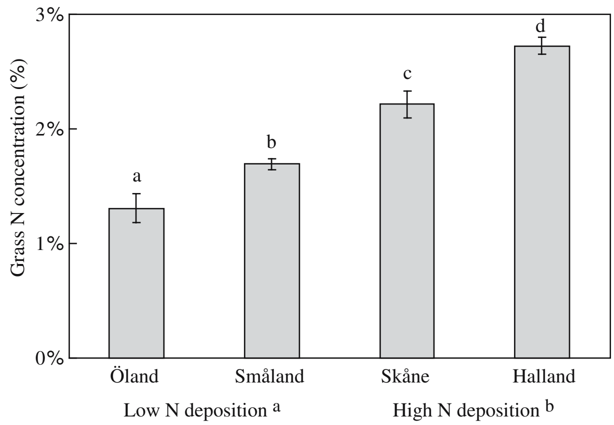


*Figure 4*. N concentration (%) of *Deschampsia flexuosa* leaf blades. Means ± SE. ANOVA (on log-transformed values): $p < 0.001$. *A priori* planned contrasts were performed to test for differences between regions with high N deposition and regions with low N deposition. Pairwise *post-hoc* tests where performed to test for differences between regions (LSD, when significant ANOVAs). Significant differences between regions are indicated by differing letters ($p < 0.05$).

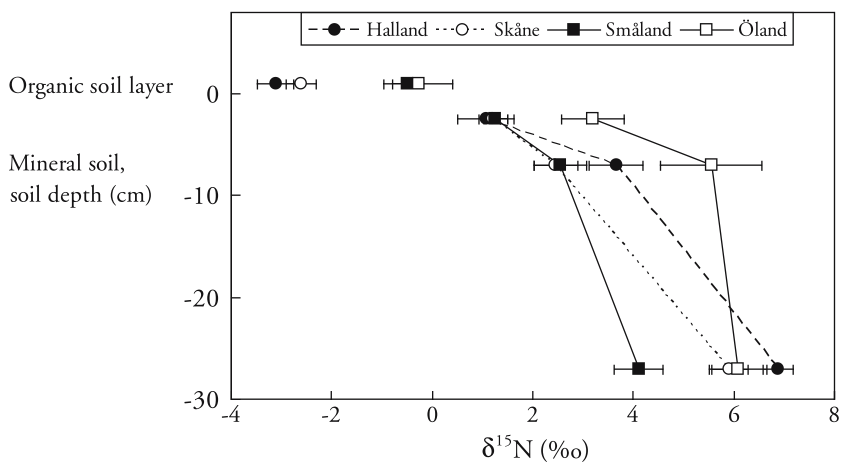


*Figure 5*. $\delta^{15}N$ values in soils from the organic layer, and in mineral soil from 0–5 cm, 5–10 cm and 25–30 cm depths. Mean values ± SE. ANOVA (regions): organic soil, $p < 0.001$; mineral soil from 0–5 cm, $p < 0.001$; 5–10 cm, $p < 0.001$; 25–30 cm, $p < 0.001$. See Table 2 for enrichment factor and range of $\delta^{15}N$ values.

The $\delta^{15}N$ values in grass (Table 2) and in soils from the organic layer (Figure 5), respectively, were negatively correlated with earlier modelled N deposition (Table 1) (grass: $R^2 = 0.74$, $p < 0.001$ (Figure 6); soils: $r^2 = 0.52$, $p < 0.001$) and with deposition of $NO_3^-$ (grass: $R^2 = 0.22$, $p = 0.01$; soils: $R^2 = 0.16$, $p = 0.01$ (autumn values)). The $\delta^{15}N$ enrichment factor was negatively correlated with earlier estimated potential net $NO_3^-$ mineralization (Table 1) ($R^2 = 0.24$, $p = 0.008$) and with soil $NO_3^-$ (%) (soil $NO_3^-$ of total soil $N_i$) (autumn values, Figure 3) ($R^2 = 0.31$, $p = 0.002$).

## $NO_3^-$ *concentration and pH at 50 cm soil depth*

The concentrations of $NO_3^-$ in soil solution in samples collected from 50 cm ($[NO_3^-]_{50\ cm}$) varied substantially, but in spite of this a significant difference between regions was found ($p = 0.05$). Lower $[NO_3^-]_{50\ cm}$ were found in Öland ($0.2 \pm 0.1$ mg $l^{-1}$) than in the other regions (Småland: $1.9 \pm 0.3$; Skåne:

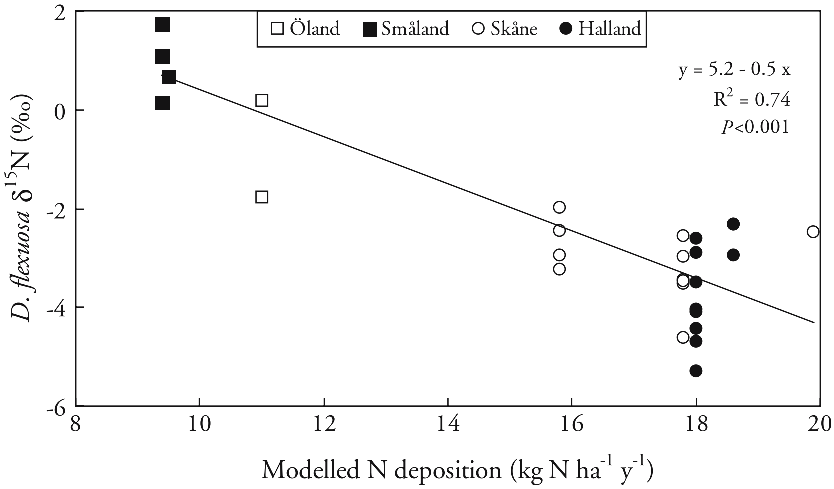


*Figure 6.* Correlation between earlier modelled N deposition (Table 1) and *Deschampsia flexuosa* $\delta^{15}$N values.

0.6 ± 0.1; Halland: 1.8 ± 0.7), but no difference was found between regions with high and low N deposition (Figure 7). All sites in Öland, and most sites in the other regions, had $[NO_3^-]_{50\ cm}$ of 1 mg $l^{-1}$ or lower. Only a few sites had values between 2 and 10 mg $l^{-1}$. Nitrate contributed to most of the N (62 ± 7%) found in the soil samples from 50 cm depth, and $NH_4^+$ did not differ between the regions (data not shown). There was a tendency towards differences in soil pH at 50 cm between regions ($p = 0.07$) with 5.2 ± 0.6 in Öland, 4.5 ± 0.1 in Småland, 4.4 ± 0.0 in Skåne and 4.5 ± 0.0 in Halland. Some of the most acid sites had $[NO_3^-]_{50\ cm}$ of more than 1 mg $l^{-1}$ (Figure 8a). We could not find any environmental factor, e.g. soil C/N ratio (Figure 8b), N deposition, net N mineralization, or nitrification ratio (Table 1) that was strongly correlated with high $[NO_3^-]_{50\ cm}$ and thus able to explain the nitrate leaching. However, a marginally significant ($R^2 = 0.19$, $p = 0.02$) negative correlation was found between the $\delta^{15}$N enrichment factor and $[NO_3^-]_{50\ cm}$.

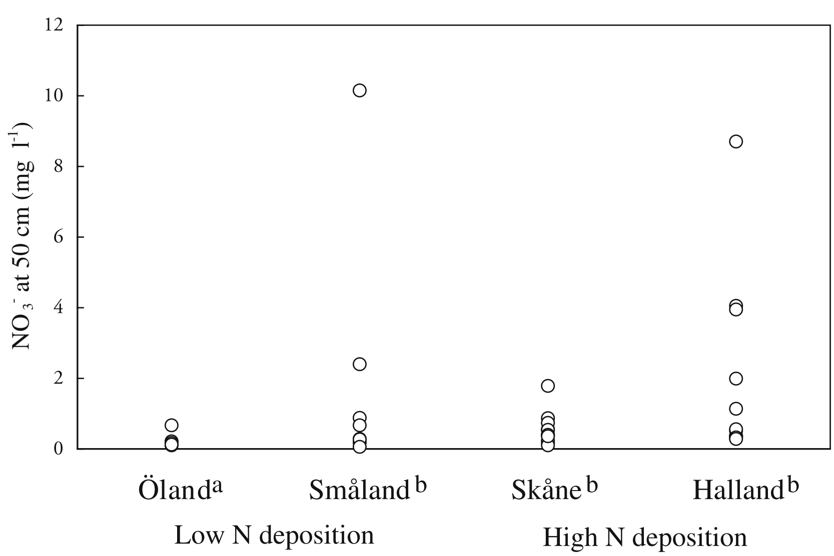


*Figure 7.* $NO_3^-$ concentration in soil samples from 50 cm depth. ANOVA: $p = 0.05$. *A priori* planned contrasts were performed to test for differences between regions with high N deposition and regions with low N deposition. Pairwise *post-hoc*-tests where performed to test for differences between regions (LSD, when significant ANOVAs). Significant differences between regions are indicated by differing letters ($p < 0.05$).

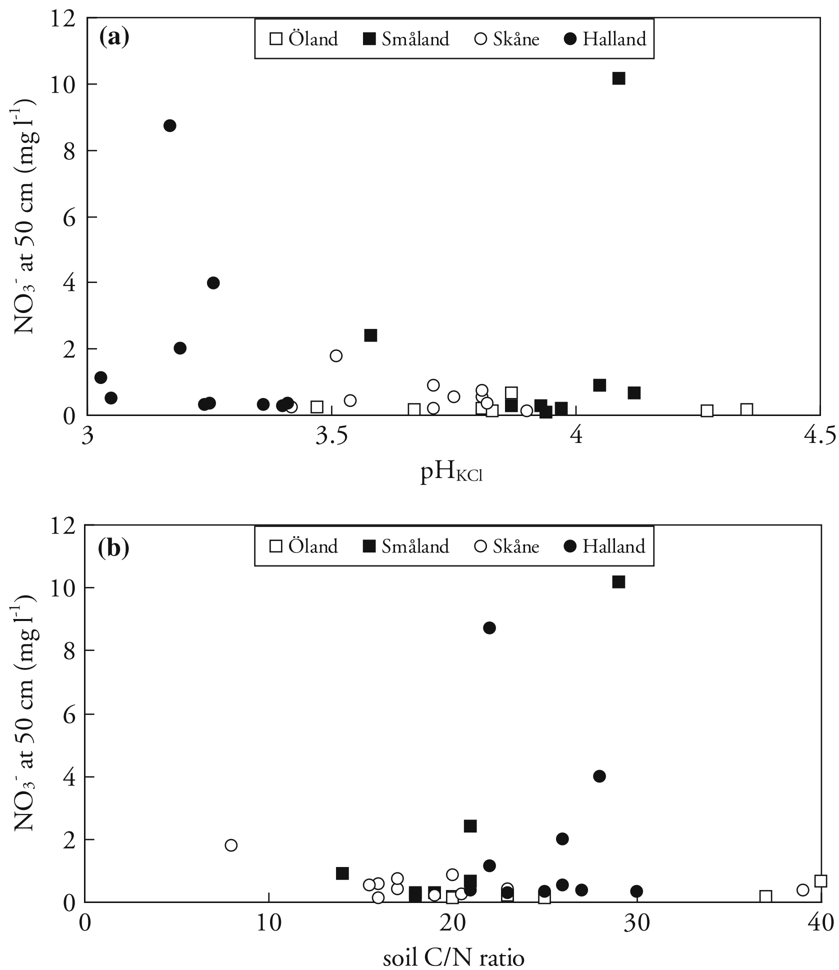


*Figure 8*. $NO_3^-$ concentration in soil samples from 50 cm depth plotted against (a) soil $pH_{KCl}$ (Table 1) and (b) soil C/N ratio (Table 1).

## Discussion

The positive correlation between measured $NO_3^-$ surface deposition and earlier modelled N deposition indicates that IER might be a useful method of estimating local deposition of $NO_3^-$. Besides, $NO_3^-$ deposition estimated by IER corresponded to roughly half the earlier estimates of total N deposition, in accordance with an often found contribution by $NO_x$–N of about half of the total N deposition in Sweden (Langner et al. 1995).

However, the very high amounts of $NH_4^+$ deposited locally, relative to $NO_3^-$ deposition, indicate that estimates of $NH_4^+$ deposition using IER were less reliable (Figures 2a and b), suggesting that further evaluations regarding precision and applicability of IER in estimating N deposition is necessary.

We found higher amounts of soil $NO_3^-$, estimated by IER, in the regions with high N deposition than in the regions with low N deposition (Figure 3a). This supports earlier results based on soil incubation, which demonstrated that elevated N deposition increased the available soil N pool and nitrification rates in acid oak forest soils in southern Sweden, (Table 1). IER buried in soils have been used previously for measuring N availability, and such data are reported to correlate with *in situ* mineralization of soil cores in forest soils (e.g. Binkley et al. 1986; Hanselman et al. 2004).

Anthropogenic deposition of N thus tends to increase soil pools of plant-available N, which was also reflected in a gradual increase in the N concentration of *Deschampsia flexuosa* leaf blades along the N deposition gradient (Figure 4). Close relationships between N deposition and plant N concentration have been found previously for e.g. *Deschampsia*, ericaceous shrubs and mosses in Great Britain (Baddeley et al. 1994; Hicks et al. 2000; Pitcairn et al. 1995), mosses along a European transect (Bragazza et al. 2005), and for plant communities in the N deposition gradient in southern Sweden (Falkengren-Grerup and Diekmann 2003). The positive correlations between the N concentration of *D. flexuosa* and N deposition, soil $N_i$, and potential net N mineralization, suggest that plant N concentration may be used as an indicator of N deposition and N availability in forest ecosystems. Substantial knowledge about the plant species and their N demand in relation to N supply is, however, essential since all species do not reflect the N availability in the soil under all soil conditions (Falkengren-Grerup et al. 2006).

The main reason for the wider range in $\delta^{15}N$ values (in soils from the organic layer to minerals soil at 25–30 cm) in the regions with high N deposition than in the regions with low N deposition was that the uppermost soil layers were more $^{15}N$-depleted in the regions with high than in the regions with low N deposition (Figure 5). The low $\delta^{15}N$ values in the uppermost soils in Halland and Skåne are likely the result of the high N deposition in these regions. The isotopic composition of deposited N can according to the literature vary considerably (e.g. Koopmans et al. 1995; Poulson et al. 1995). However, since deposited $NO_3^-$ often has $\delta^{15}N$ values below zero, and deposited $NH_4^+$ often is even more depleted in $^{15}N$ (Yoneyama 1996), N deposition may result in $^{15}N$-depleted input.

The lower $\delta^{15}N$ enrichment factor in regions with high N compared with low N deposition (Table 2) is probably caused by higher uptake of $NO_3^-$–N by *D. flexuosa* in these regions. Nitrate is depleted in $^{15}N$ relative to total soil N and *D. flexuosa* take up nitrate, even though they benefit more from ammonium (Falkengren-Grerup and Lakkenborg-Kristensen 1994). Therefore the observed greater $^{15}N$ depletion in *D. flexuosa* relative to soil in the regions with high N deposition, and the correlation between soil $NO_3^-$ and the degree of $^{15}N$ depletion in *D. flexuosa*, may be seen as an indication of higher nitrification rates as a result of N deposition, similar as earlier found from net mineralization studies (Falkengren-Grerup and Diekmann 2003).

If high nitrification rates would have resulted in substantial leaching of $NO_3^-$, signs of $^{15}N$ depletion is suggested to be found down the soil profile, due to downward movements of $^{15}N$-depleted $NO_3^-$ (Högberg 1997). The observed $\delta^{15}N$ values down the soil profiles indicate no, or only minor, nitrate vertical movements even in the regions with high N deposition (Figure 5), in spite of higher nitrification rates. In addition to the $\delta^{15}N$ values, estimated $[NO_3^-]_{50\ cm}$ values (Figure 7) also indicate that nitrate movements generally were insignificant in these oak forests. Most of the investigated forest sites had $[NO_3^-]_{50\ cm}$ below 1 mg N $l^{-1}$, which is considered as minimum concentration in clearcuts, indicating no leaching of $NO_3^-$ (Löfgren and Westling 2002). Accordingly, we cannot conclude from this study that the risk of nitrate leaching in oak forests receiving 20 kg N $ha^{-1}$ $year^{-1}$ by deposition generally is higher than in forests receiving half of that N by deposition. It must, however, be remembered that these oak forests in southern Sweden receive relatively small amounts of N by deposition compared with many other forests, e.g. in central and eastern Europe (de Vries et al. 2003).

The risk of leaching of N from growing forests in southern Sweden has been found to be negligible (Akselsson et al. 2004) and risk of N losses is believed to occur mainly after harvest, especially in clear-cuts of N-fertilized forest stands (Ring et al. 2003; Ring 2004) or from damaged forest stands exposed to high N deposition (Akselsson et al. 2004). Our study shows, however, that N leaching ($[NO_3^-]_{50\ cm}$ above 1 mg N $l^{-1}$) can also be found occasionally from growing oak forest stands exposed to anthropogenic N deposition. Since much lower soil nitrate concentrations are found in oak forests than in maple, beech and birch forests (Lovett et al. 2004) the risk for N leaching from deciduous forest with other tree species in southern Sweden may be greater than found from the oak forests in the present study. More knowledge about how different tree species are affected by N deposition is necessary in order to evaluate the risk of N leaching from deciduous forests with other tree species than oak.

Similarly as in earlier studies of elevated N inputs to deciduous forests (e.g. Kristensen et al. 2004; Persson et al. 2000), we did not find correlations between neither N deposition nor the C/N ratio

(Figure 8b) of the organic layers and $[NO_3^-]_{50\ cm}$. This contrast to what is often found in coniferous forests (e.g. Dise and Wright 1995; Gundersen et al. 1998) where the risk of N leaching is considered to increase in soils with C/N ratios below 25. However, we found a negative, though weak, correlation between the enrichment factor and $[NO_3^-]_{50\ cm}$. A similar relationship has earlier been found in some European coniferous forests in a nitrogen deposition gradient (Emmett et al. 1998). In that study, however, enrichment in $^{15}N$ from soil to spruce trees was used (instead of grass in the present study) and the $^{15}N$ depletion may therefore rather have reflected differences in the relative uptake of N by ectomycorrhizal fungi, as suggested by Evans (2001) and Hobbie et al. (2000). The enrichment factor may thus be considered as a potential variable to predict nitrate leaching from forests, maybe also valid for deciduous forests.

We did not find soil $NO_3^-$ (Figure 3a) and $[NO_3^-]_{50\ cm}$ (Figure 7) to be lower in Halland than in Skåne, in spite of lower soil pH in Halland (Table 1), indicating that even very low soil pH could not mitigate nitrate leaching. Besides, N leaching was found from some of the most acid oak forest soils (Figure 8a), similar to observations in another recent study of oak forests in southern Sweden (Falkengren-Grerup et al. 2006). Nitrate leaching from very acid soils is probably a result of the elevated input of N by deposition leading to high availability of substrate for nitrification (ammonia), combined with the simultaneous acidification due to N deposition.

In conclusion, we found higher availability of N and indications of higher nitrification rates in oak forests soils in the regions were stands received about 20 kg N $ha^{-1}$ $year^{-1}$ by deposition than in stands with half of that N deposition. However, no signs of general downward nitrate movements were found, even in the regions with high N deposition. Nitrate leaching was, however, found from soils at some sites. The sites with the highest N deposition and thereby highest nitrification rates were also most acid ones, which indicates that nitrification and nitrate leaching can occur also in very acid soils.

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