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## **Three Years of Increased Nitrogen Deposition Do not Affect the Vegetation of a Montane Forest Ecosystem**

By

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**K e y w o r d s :** Nitrogen deposition, forest ecosystem, *Picea abies*, foliar chemistry, tree growth, vegetation.

### S u m m a r y

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Nitrogen was added over three years to a spruce-dominated (*Picea abies*) forest at Alptal, central Switzerland. The site (altitude 1200 m) is moderately affected by atmospheric nitrate and ammonium deposition (12 kg N ha<sup>-1</sup> year<sup>-1</sup>). Gleysols occur atop a Flysch substratum and bear different humus types with various botanical associations. This patchy vegetation is related to the micro-topography and to light conditions. An ammonium nitrate solution was chronically sprinkled over a plot (approx. 1500 m<sup>2</sup>) to simulate a deposition increase of 30 kg N ha<sup>-1</sup> year<sup>-1</sup>. This treatment was compared to both a control plot and a year of pre-treatment measurements.

The ground vegetation was monitored in permanent quadrates. Its botanical composition has remained unaffected to date. During the first two years, foliage analyses of the most abundant species showed only inconsistent variations in their nitrogen content. Tree growth was measured by dendrometers installed on trunks and by photographs of crowns. Leaf area index (3.8 in average) was measured with a plant canopy analyser and litterfall collected fortnightly. None of these parameters revealed any effect of nitrogen addition. Current-year spruce needles contained 1.1% N. In spite of this slight N deficiency, no increase was measured during 3 years of N addition.

Two factors probably explain the unchanged N content in plants. First, N taken up from the treatment was diluted into a large biomass, delaying any effect on the trees and on the ground vegetation (all perennial species). Second, the uptake itself was small: 12% of the N addition going into aboveground biomass, as shown by <sup>15</sup>N labelling. This, in turn, is attributed to other factors limiting root growth and nitrogen uptake: short period of vegetation, soil anaerobity, phosphorus deficiency, nitrate leaching, denitrification and effective fixation of ammonium in clay minerals. The relative importance of these factors is discussed for this site.

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## Introduction

Emissions of inorganic nitrogen (N) by human activities has greatly increased over the past decades. The resulting atmospheric deposition of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) causes an unintended fertilisation of natural or semi-natural ecosystems. This supplementary N can induce a higher primary productivity in normally N-limited systems, which is the case for most forests in temperate climates (TAMM 1992). It has therefore been postulated that N deposition is at least partly responsible for observed increases in tree growth rates (BECKER & al. 1990). Nitrogen may, however, also induce nutritional disbalances, increase the sensitivity of plants to biotic and abiotic stresses and alter the competition between species (reviews by FANGMEIER & al. 1994, ORTLOFF & SCHLAEPFER 1996). Along with  $\text{NO}_3^-$  leaching, these are all matters of concern for the stability of forest ecosystems.

Botanical changes in the ground vegetation of forests were reported by several authors (ELLENBERG 1985, KUHN & al. 1987, THIMONIER & al. 1992, other works reviewed by KELLNER & REDBO-TORSTENSSON 1995). The analysis of time series is, however, often hindered by the confounding of several factors. This is also the case for diachronical botanical studies (KISSLING & al. 1988) and a shift towards more N-demanding species may also reflect other changes than N deposition (climate, stand ageing, silvicultural practices). This difficulty in interpretation is overcome by synchronical studies comparing adjacent plots with different N inputs.

Standard fertilisation experiments, however, are not suitable to simulate atmospheric deposition to forests. Depending on the rate and frequency of application, the same amount of nutrient can indeed induce different effects (PERSSON 1981). This may be due both to the toxicity of high nutrient concentrations and to an altered partitioning between plant species, soil, soil microorganisms and leaching (JOHNSON 1992). Within the European project NITREX, nitrogen deposition was manipulated to simulate higher or lower deposition rates to coniferous forests (WRIGHT & VAN BREEMEN 1995, EMMETT & al. 1998 a). Nitrogen was either chronically added or removed (by means of sub-canopy roofs) from ambient throughfall. After 3 to 5 years of treatment on six sites, only one site (with N removal) showed a change in its ground vegetation. On three sites, the manipulation modified the N content of tree needles but this did not affect the growth of the trees (BOXMAN & al. 1998, EMMETT & al. 1998b).

In order to assess possible effects of increased N deposition on a forest with natural regeneration and a well-developed herb layer, we added nitrogen to small plots at Alptal, central Switzerland. We report here on measurements and observations done on the spruce trees and on the ground vegetation of this site.

## Material and Methods

The geological parent material of the Alptal valley is Flysch, a formation typical of the Prealps, the northern edge of the Alps. The experimental site is located at 47°03'N and 8°33'E, at an altitude of 1200 m. Soils are umbric Gleysols with a west aspect and a slope of 20%. The climate is

cool and wet (6°C average temperature and 2300 mm precipitation per year). The bulk deposition of inorganic N is 12 kg ha<sup>-1</sup> year<sup>-1</sup>, equally partitioned between NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> (SCHLEPPI & al. 1998 b). The throughfall deposition is 17 kg ha<sup>-1</sup> year<sup>-1</sup>, the 5 kg difference corresponding to a previous estimate of the dry deposition (FISCHER-RIEDMANN 1995).

At the research site, the landscape consists of naturally regenerating forest and wet grassland. The tree species are predominantly Norway spruce (*Picea abies* (L.) Karst) with 15% silver fir (*Abies alba* Miller). They are up to 250 years old. With 430 stems ha<sup>-1</sup> (diameter >10 cm), the density of the canopy is low; the basal area is 41 m<sup>2</sup> ha<sup>-1</sup>. The vegetation and the humus types of the Gleysols are closely linked to the micro-topography and therefore to the water regime of the site. Three different plant associations were found (MULLER 1997, SCHLEPPI & al. 1998 b).

The experimental forest plots (approximately 1500 m<sup>2</sup> each) were two small catchments from which water runoff was monitored (SCHLEPPI & al. 1998 b). An NH<sub>4</sub>NO<sub>3</sub> solution was sprinkled over the treated plot while the control received only water collected from a polyethylene sheet (300 m<sup>2</sup>) spread outside the forest (SCHLEPPI & al. 1998 a). This resulted in a precipitation supplement of 10% on each plot. The small but frequent additions simulated an atmospheric deposition increase of 30 kg N ha<sup>-1</sup> year<sup>-1</sup> to the treated plot. During the winter the automatic irrigation was replaced by the occasional application of a concentrated NH<sub>4</sub>NO<sub>3</sub> solution on the snow using a backpack-sprayer.

Dendrometers made of stainless steel bands and springs were installed on half of the trees larger than 10 cm in diameter (29 and 24 on the control and treated plot, respectively). Because dendrometers tend to underestimate growth rates the first year (BOWER & BLOCKER 1966), this was checked by installing a second band on 17 trees in 1995. Old and new dendrometers showed consistent readings already after one month of active growth. The height increment of dominant or co-dominant spruce trees was estimated using photographs of crowns taken annually from a tower. Four trees on the N-treated plot were compared with 5 trees of the surrounding forest (the control plot being too far from the tower). Growth rates were compared before and after the beginning of the N addition and N effects statistically tested in analyses of variance as the interaction between plots and years. Twigs were taken from the 7th whorl of five dominant trees per plot each winter (between November and January). They were separated in the most recent 5 age classes. Dried and ground needles, as well as twigs and wood, were analysed by mass spectrometry because their <sup>15</sup>N content also had to be determined (SCHLEPPI & al. 1999). Leaf area index (LAI) of the trees was measured for each plot using a plant canopy analyser (LAI 2000, Li-Cor, Lincoln, NE, USA). These measurements were done on grid points (5.65 x 5.65 m apart), once or twice every summer, before sunrise or after sunset and by clear sky. As recommended by the manufacturer, results were corrected after GOWER & NORMAN 1991 to take the structure of a conifer canopy into account. Litterfall was sampled every second week from four collectors (0.12 m<sup>2</sup> each) per plot. Changes in the amount of litterfall were tested by a random intervention analysis of the obtained time series.

Species frequencies were counted three times per vegetation period on three permanent quadrates (50 x 50 cm) per plot and per botanical association (MULLER 1997). An analysis of principal components was performed on the obtained frequency table. Ecological indicator values (LANDOLT 1977) were used for the interpretation. The effect of the treatment was then assessed on the principal components rather than on individual species (BECKER & al. 1992). The 10 most important species were sampled from both plots to analyse the N content of aboveground tissues.

## Results

The dendrometer readings showed differences in growth between years and plots, but no significant effect of the N addition (Fig. 1). The same is true for the apical growth measured on photographs of crowns. The leaf area index (Table 1) was 3.8 on average, reflecting the relatively open structure of the canopy. Variations between years were parallel in both plots.

Collected litterfall from four years (including one as pre-treatment) was

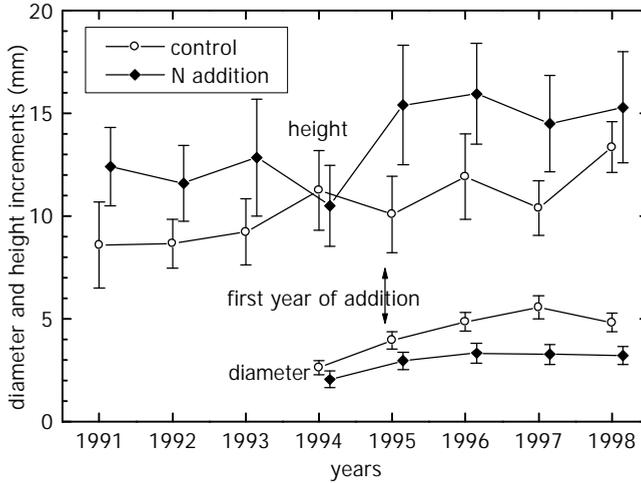


Fig. 1: Height growth and diameter increments of spruce trees. Means  $\pm$  standard errors;  $n = 8$  (height, control), 5 (height, N addition), 29 (diameter, control) and 24 trees (diameter, N addition).

Table 1. Leaf area index (LAI) of the forested plots. Means  $\pm$  standard errors.

plot	1995	1996	1997	1998
control	$3.66 \pm 0.11$	$3.50 \pm 0.08$	$3.49 \pm 0.14$	$3.22 \pm 0.13$
N addition	$4.32 \pm 0.11$	$4.16 \pm 0.11$	$4.11 \pm 0.14$	$3.78 \pm 0.11$

$0.91 \pm 0.11 \text{ kg m}^{-2} \text{ year}^{-1}$  on the control,  $1.12 \pm 0.15$  on the N-addition plot. No significant effect of the treatment could be detected by the random intervention analysis.

Spruce needles from the current year had between 10 and 12  $\text{mg N g}^{-1}$  (Fig. 2). This content was slightly declining with increasing age of the needles. Here again, in spite of variations from year to year, no consistent effect of the addition of N could be detected. By  $^{15}\text{N}$  labelling (SCHLEPPI & al. 1999), it was possible to show that only  $0.14 \text{ g N m}^{-2}$  (5% of the  $3.1 \text{ g m}^{-2}$  added the first year) reached the needles of the trees, while  $0.11 \text{ g m}^{-2}$  (3%) ended up in the trunk and branches. Since labelled N may partly replace unlabelled N and because it may be diluted in an increased biomass, this gives an upper limit to estimate the increase in N content. The obtained figures are  $0.2 \text{ mg N g}^{-1}$  for current-year needles,  $0.1 \text{ mg g}^{-1}$  for older needles,  $0.03 \text{ mg g}^{-1}$  for the bark and  $0.005 \text{ mg g}^{-1}$  for the wood.

The analysis of principal components reflected well the previous classification of the ground vegetation into three plant communities (MULLER 1997). The first component was correlated with the indicator values R (soil pH,  $r=0.52$ ), N (nutrients,  $r=0.46$ ), L (light,  $r=0.39$ ), F (humidity,  $r=0.26$ ), D (soil density,  $r=0.28$ ) and negatively with H (humus,  $r=-0.33$ ). It clearly separated the plant community classified as *Bazzanio-Abietum* (number 46 in ELLENBERG & Klötzli 1972). This community is dominated by *Vaccinium sp.* (L.) shrubs and occupies, along with the trees, the acidified mounds of the site. The remaining observations corresponded to the nutrient richer, wet depressions. They were

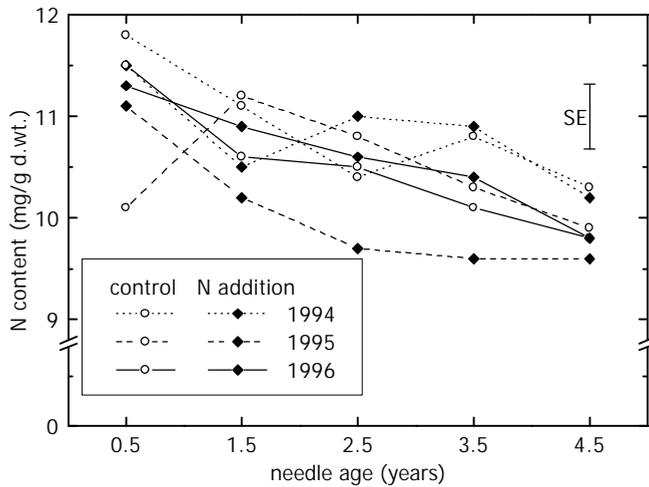


Fig. 2: Nitrogen content of spruce needles as a function of their age. Samples harvested in the winter from the 7th whorl of 5 trees per plot. Means and standard error (SE).

discriminated into two further communities by the second component, linked to the L values ( $r=0.36$ ). These communities are Equiseto-Abietum (number 49), dominated by *Caltha palustris* (L.) and *Petasites alba* ((L.) Gaertner) and Carici remotae-Fraxinetum (number 27), dominated by *Poa trivialis* (L.) and *Carex ferruginea* (Scop.). The first grows more in the shade and the second in canopy gaps. The second component was also changing with the month of observation. Further components had only weak correlations ( $r<0.25$ ) with indicator values and are therefore not easily interpretable. The ten first components, representing together 57% of the variance, were checked for an interaction between plots and years. None showed any statistical significance, neither individually nor taken together in a multivariate analysis of variance. There was thus no measurable response to the addition of nitrogen.

Analyses of N in plant tissues gave only inconsistent results (MULLER 1997). The moss *Thuidium tamarisnicum* ((Hedw.) B.S.G.) was an exception as the N content of its dry matter increased by approximately  $1.3 \text{ mg g}^{-1}$  (7%). For vascular plants, analyses of  $^{15}\text{N}$  give an average increase lower than or equal to  $0.7 \text{ mg N g}^{-1}$ .

## Discussion

The absence of nitrogen effects is in contradiction with many fertilisation experiments in coniferous forests. Even in small amounts ( $15 - 31 \text{ kg ha}^{-1} \text{ year}^{-1}$  split in 3 applications), N was found by McNULTY & ABER 1993 to increase foliar N contents of red spruce (*Picea rubens* Sarg.) at a montane site. An increased basal area growth but no effects on litterfall were reported. On the contrary, ABER & al. 1993 reported only an increased litterfall in a red pine (*Pinus resinosa* Soland.)

stand. They, however, used larger amounts of N (50 and 150 kg ha<sup>-1</sup> year<sup>-1</sup> in 6 applications) and foliar N was significantly changed only at the higher dosage.

Better simulations of N deposition were achieved within the NITREX project since the nutrients were always given in small but frequent amounts. No effect on the growth of conifers could be reported from these experiments (BOXMAN & al. 1998). Within 3 to 5 years, only half of the sites exhibited changes in foliar N as a result of manipulated inputs. This slow reaction may be due to an increased competition with soil microorganisms when nitrogen is given in small frequent amounts (JOHNSON 1992). TIETEMA & al. 1995 and EMMETT & al. 1998 also stressed the importance of this time-lag before a change in N deposition affects the trees, even if the soil chemistry is rapidly changed.

In our experiment, the addition of NH<sub>4</sub>NO<sub>3</sub> let the nitrate concentrations increase markedly in the soil solution collected from the top soil (HAGEDORN & al. 1997) and in the runoff water (SCHLEPPI & al. 1998 a). This, however, cannot be interpreted as a nitrogen saturation of the ecosystem, because nitrate leaching is, at least partly, hydrologically driven, i.e. due to a preferential flow regime in the soil (FEYEN & al. 1996). For this reason, part of the deposited nitrate is leached before the plants have any chance to take it up. The treatment induced an increase in nitrate leaching of 0.3 g N m<sup>-2</sup> year<sup>-1</sup> or 10% of the added N (SCHLEPPI & al. 1999). A further 0.12 g m<sup>-2</sup> year<sup>-1</sup> (4%) was denitrified (MOHN 1999).

Inorganic N was added during the whole year while the period of active vegetation is short (5 months). This certainly puts the plants at a disadvantage in their competition with soil microorganisms for N uptake. In spite of a relatively low soil C/N ratio of 18, as much as 63% of the added N is stored in the soil for at least one year (SCHLEPPI & al. 1999). Beside the incorporation into organic matter, part of this storage probably comes from the adsorption of ammonium on clay minerals (45% of the soil mineral fraction according to DISERENS 1992).

At Alptal, roots and mycorrhiza are found only in the upper 10-25 cm of the soil, depending on the micro-topography and depth of water table. Root growth is limited by anaerobic conditions often prevailing in the mineral soil. This adverse environment is probably a further factor limiting the uptake of nitrate and ammonium by the plants. Another point to consider is the slight phosphorus deficiency exhibited by the trees (SCHLEPPI & al. 1998 b). The response of the vegetation to the addition of N is certainly not as pronounced as it would be if N was the only factor limiting below- and above-ground growth.

Because of the large biomass of the trees, the proportion of new N taken up each year is only about 15% their total content (MULLER 1997). Due to its high mobility within the plants, N is redistributed between new and old tissues, as was clearly shown by our analyses of <sup>15</sup>N (SCHLEPPI & al 1999). This dilution effect explains, along with all the factors limiting the uptake itself, why no increase in N content could be detected by chemical methods. The analytical errors are indeed larger than the possible changes. It would therefore be necessary to continue the treatment for several more years to induce a measurable accumulation of N in the foliage. Only then may physiological effects also arise.

In the ground vegetation, the dilution effect is less. However, because all species are perennial, nitrogen is also stored over the winter, in roots, stolons, bulbs or shoots. The uptake of labelled N was close to the minimum amount which could be detected chemically. Some effects of nitrogen on the growth of transplanted plants were shown by MULLER 1997 in a competition study. There were, however, no significant interactions between N and competition. It may, therefore, also take many years before any change in the botanical composition of the herb layer appears. The situation is different for mosses because they rely more directly on deposition for their nutrient uptake. This explains the reported N accumulation in *T. tamarisnicum*. Another bryophyte, *Sphagnum nemoreum* (Scop.), appeared to contain less photosynthetic pigments as a result of the N treatment. Further physiological investigations have been undertaken since it is likely that the first botanical changes will affect mosses.

#### A c k n o w l e d g e m e n t

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