NIPHYS - Nitrogen Physiology of Forest Plants and Soils

E.-D. Schulze (coordinator)

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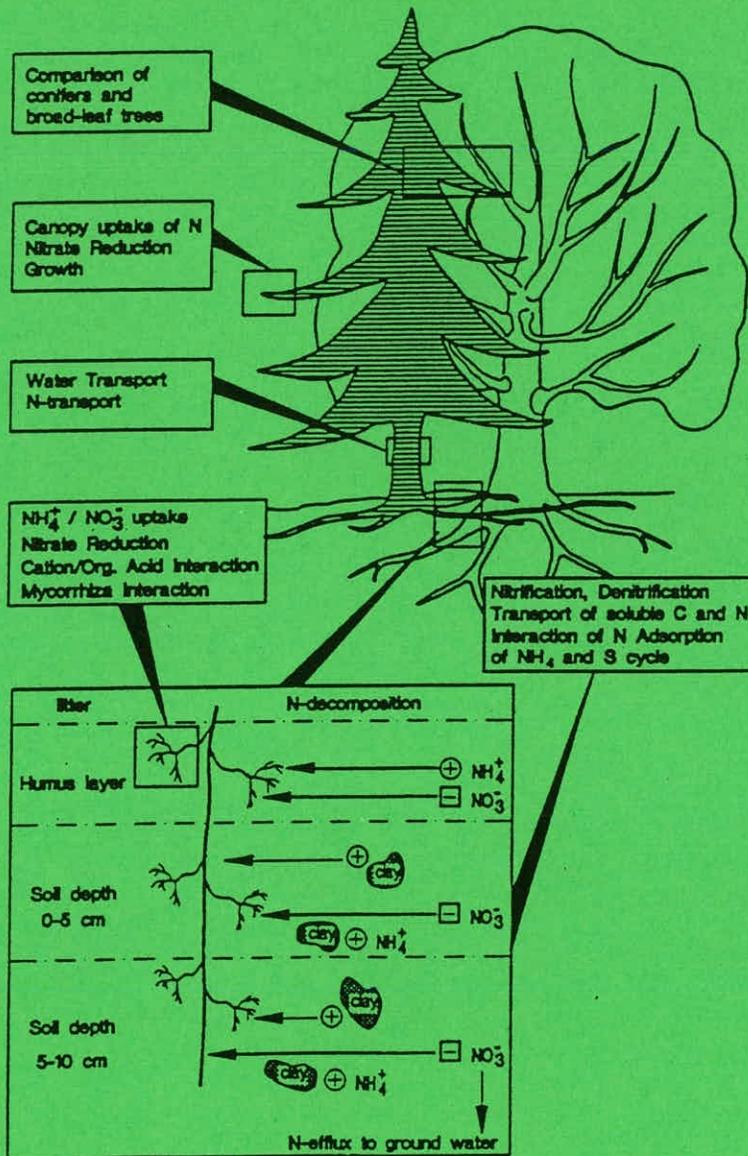### E.-D. Schulze (coordinator)

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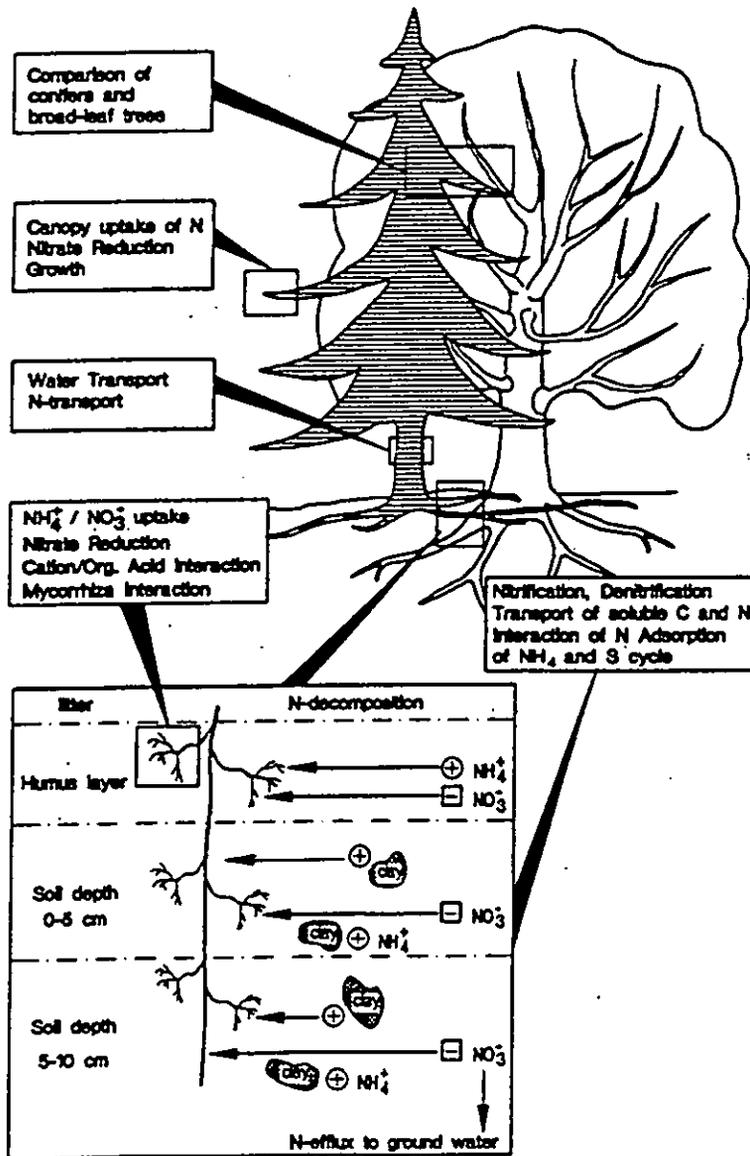## Final Report for 1995

E. - D. Schulze (coordinator)

Executive Summary for NIPHYS

The NIPHYS consortium discussed the results of the last three years of research in a workshop held at Thurnau, near Bayreuth, from February 12 to 14, 1996. The group endorces results of general importance for science and environmental policy.

NIPHYS was designed to study ecosystem consequences of breaking the nitrogen cycle by investigating fluxes and transformations of nitrogenous compounds in different trophic levels. Nitrogen in plant material (litter) may either be used directly by mycorrhizal fungi and returned to the plants or it is mineralized to ammonium and then to nitrate. Both inorganic nitrogen forms may again be used by the plants. Some nitrate is denitrified by microorganisms and returned into the atmosphere, or nitrate is leached to ground water. During the transformation of ammonium into nitrate and during denitrification of nitrate, NO_x and N_2O trace gases may be formed which are of concern because of their potential long-term effects on climate. Therefore, the formation and the fate of nitrate has major environmental implications. It makes a great difference for the environment, if nitrate enhances soil acidification and impacts drinking water quality, or if it transforms into a greenhous-gas, or if it is returned into the biological cycle.

Results of general importance for environmental policy:

- The broken nitrogen cycle:

No nitrate is normally leached from boreal forests on acid soils at sites with low atmospheric input of nitrogen because of a very tight, closed nitrogen cycle. In these forests a rich mycorrhizal flora is using the resources in litter directly and returns to the plant cover bypassing the microbial population. There is normally no nitrifier activity in such acid boreal soils. If nitrification is being activated (by liming, fertilizer treatments) a diverse herbaceous flora will possibly take up this nitrate from the soil.

Nitrate leaching in Central European soils results from atmospheric inputs (N-saturation), from decreasing capacity for denitrification in acidified soil, and from land-use changes replacing deciduous trees by conifers. Conifers have more than 10 times less capacity to use nitrate than do deciduous trees or herbaceous plants respectively and thus cannot cope with nitrate deposition or with nitrate being formed in Central European soils. In addition, beech has more fine roots in deep soil than spruce enabling beech to use N from deeper soil layers. The ecological conditions, which allow a closed N-cycle in a boreal coniferous forest, do not exist in Central Europe. In addition, soil acidification and an impoverished mycorrhizal flora and alternative pathways in the denitrifying process cause excess nitrate to be formed and leached. This may enhance soil acididfication to a level at which even nitrification is inhibited and ammonium accumulates. Under theses conditions not only aluminum is mobilized but also losses of dissolved organic nitrogen and carbon occur, which may act as source for nitrification in deeper soil horizons.

- Forest instability and biodiversity

Forests of Central Europe are shown to have increased growth rates even with soil acidification. The causes and consequences are manifold. Growth rates are related to the increased nitrogen gain from atmospheric trace gases, which are directly utilized by trees in the canopy bypassing the soil and root function. It is estimated that more than 20% of the forest N-requirement may come from nitrogenous gas uptake. This decreases the requirement for old needles, which serve as storage organs, resulting in a changed allocation of carbon towards increased shoot/root ratios. The requirement for cations appears to be met (in contrast to the earlier periods of forest decline) in part by an increasing ammonium to nitrate ratios in soils (decreased nitrification, increased input). The growth response of spruce bears major long-term risks, such as (i) cation mining of forest sites, (ii) increased probability of outbreaks of pests because of high amino acid concentrations in xylem water, (iii) instability of trees against wind and drought, (iv) decreased biodiversity of soil organisms (microbes and mycorrhizae) with the associated decrease in ecosystem services, (v) forest practice does not allow growth of herbaceous plants, which may otherwise act to ameliorate N-deposition. At the same time, forest management will more frequently disturb the humus layer because of decreasing rotation periods.

- Carbon storage

Forests are considered as major carbon sinks which are important in a world of increasing atmospheric CO_2 and urgent demand for a closed carbon cycle. NIPHYS shows that the rate of mineralization is related to the soil carbon pool. The increased C-fixation at high N-deposition is balanced by high rates of mineralization. Thus, the long-term ecosystem productivity for carbon is lower than previously thought. The ecosystem carbon balance is also determined by disturbances (such as forest harvest), which will increase in frequency under conditions of N-depoition and increased forest growth. Decomposition is also strongly increased by a temperature rise, increasing also N-leaching.

- Monitoring Nitrogen saturation

Monitoring amino acids and stable isotopes will be more powerful tools to assess N-imbalance rather than monitoring N-concentrations and nitrate leaching.

- Critical Loads

The Critical Load Concept has proven to be a very powerful and valid concept, but it needs to be based on real soil processes in the future in order to account for short-term changes in storage, depletions and recovery. Previous critical load models were underestimating anthropogenic effects because of neglecting the flux of dissolved organic carbon and nitrogen.

Results of general scientific importance

FOREST PLANTS

- Trees generally prefer NH_4 over NO_3, but uptake depends on the NH_4/NO_3 ratio. Deciduous trees have a less strong preference for NH_4. Herbaceous species are the main nitrate user. Beech has more fine roots at greater soil depth and may thus be more capable to utilize nitrate in deep soil than spruce.

- Aerial uptake of nitrogen gases and solutes from antropogenic sources is important (more than 20% of demand) and may even be damaging to trees. There is a distinct shift in isotopic composition of ammonium and nitrate percolating through forest canopies, indicating differences in deposition and metabolization of ammonium and nitrate by tree crowns.

- Forests grow more than indicated by their former classification into yield classes due to canopy uptake and throughfall of N. This process may also be enhanced by CO_2 fertilization and improved forest practice.

- Conifers need fewer needles than formerly for the same productivity. There is increasing evidence that old needles serve as storage organs, which are no longer needed under conditions of increased N-deposition.

- Despite high N-input, the ratio between N and other nutrients are generally not unbalanced because the N-concentrations are regulated by wood growth.

MYCORRHIZA

- Mycorrhizae take up ammonium just as effectively as roots and deliver nitrogen to the tree (this may, in fact, explain the high isotopic ratios of fungi). Hyphal growth is initially enhances by N-deposition but decreases beyond an optimum.

- Base-cations are probably more important for mycorrhizal and microbial activity than for trees.

- Over 90% of all root tips at all sites were ectomycorrhizal. This suggestes that trees are stongly dependent on mycorrhizal fungi for the acquisition and primary assimilation of nitrogen and other elements.

- The taxonomic and genetic diversity of fungi involved in mycorrhiza formation is large, but is significantly greater in the north than in the south of the NIPHYS gradient. This decline in diversity is accompanied by increases in single species dominance, and this is paralled by changes in the nitrogen nutrition of the fungi involved. Characteristic fungal species of northern sites, which are able to utilize organic N, decrease in number as availability of mineral N increases.

- Nitrate can adversely affect the ability of some fungi, typically those of the northern sites, to form mycorrhizas. At the same time the addition of nitrate to sources of organic N adversely affects the ability of some mycorrhizal fungi to utilize organic nitrogen.

MICROBIAL PROCESSES

- CO_2 evolution rate/decomposition rate decrease with decreasing C:N ratio, i.e. N additions will cause (at least temporarily) an increased retention of C and (perhaps) N in soil.

- Uptake of organic N is probably the dominating N uptake mechanism in soils poor in N. Besides that almost no free NH_4^+ or NO_3^- is found in this type of soil and the decomposer organisms are N-limited and do not mineralize N during the first period after a clear-cut. Leaching of inorganic N remains negilible. Increased N deposition will affect the organic N uptake negatively, resulting in more NH_4^+ being produced.

- Provided that trees/ground vegetation have the capacity to take up all NH_4^+ produced, nitrifiers will have low populations/low activity. Under conditions of excess NH_4^+ and soil acidification, acid sensitive nitrifiers may survive and be active even in B-horizons of low pH, probably by adhesion to weatherable minerals.

- In areas with permanent high levels of NH_4^+, an adaptation or selection of more acid-tolerant forms of nitrifiers is possible (Aubure and Fichtelgebirge). In some stands (e.g. Aubure, 40-y-old spruce) nitrifiers seem to be restricted by some unknown factor(s).

- There is a higher nitrification potential in beech than in corresponding spruce stands. This, however, does not lead to an increased as NO_3 leaching.

- The emission rate of N_2O from beech and spruce forest soils in Denmark is low and at the same level: 0.2 - 1 kg N_2O-N ha^{-1} y^{-1}. No correlation was found between the number of nitrate reducers and denitrifiers and the rate of N_2O/N_2. Bacteria are present at all seasons. The denitrification process appears to be carbon limited. The end product of denitrification in acidic forest soils is not N_2.

SOILS

- Observations along the climate transect in combination with soil transplant experiments can separate the temperature factor from other climatic and pedogenic factors. Climate change will influence transfer of N in soils and its leaching to ground water

- Ammonium sorption in soils is linearly increasing with ammonium supply (in exchange with cations).

- Nitrate leaching in deep soil layers reaches a distinct maximum in Central Europe.

- Organic N leaching is related to the organic C transport. The total loss is significant. Accounting for organic C and N loss may require to lower the established critical loads which are based only on inorganic nitrogen.

- NH_4^+ fixation in soils varies along the North-south transect due to differences in pedogenesis and pollution levels

- Increased temperature will accelerate N leaching due to effects of temperature on microbial activity probably by a factor of 5 per 10°C. At the same time, temperature increase will decrease sulfur leaching because of increased S-fixation in the organic layer.

ENVIRONMENTAL MONITORING

- Amino acids are a better indicator of N-saturation than N-concentrations

- The stable isotopes N serve as an additional important tool for the identification of pollutant inputs

GENERAL COMMENT

- Studies which were carried out along the continental transect yielded more general answers than studies at local scale, although the variability of processes may be just as high at local and continental scale.

1. Prof. Dr. E.-D. Schulze (coordinator), Dr. G. Gebauer, G. Bauer

  Laboratory: Plant Ecology, University of Bayreuth
  Research Area: Natural abundance of ^{15}N and ^{18}O
  Ammonium/Nitrate uptake, transport and use in plants

Overall objectives of NIPHYS year 1 to 3

(Bayreuth plant ecology group according to Contract)

- to determine natural abundances of ^{15}N in needles and wood

- to determine nitratereductase activity (NRA) in needles and wood

- to measure actual uptake of ammonium and nitrate in old spruce

- to determine N-isotope ratios in aerosols

Specific Tasks in year 3 (according to contract):

1. to determine interactions of NRA in leaves with carbohydrate metabolism and environmental factors

2. to determine NRA in forest floor vegetation

3. to follow ^{15}N-label in an old spruce stand (Bayreuth site)

4. to measure isotope composition in aerosols

Methodology

The study of nutrient relations was carried out along the full NIPHYS transect, but including also the new CANIF site at Viterbo in Central Italy. Plant material was collected from the upper third of the suncrown after termination of the growing season. Only at the German site harvests were made at different times throughout the year. Fresh plant material was washed with deionized water, separated into different age classes and stored in liquid nitrogen. One part of the material was kept at -30°C until determination of leaf area. Another part was dried at 105°C for 48 hrs and used for determination of the weight per needle or leaf, and a third part was ground in a ball mill and stored in a desiccator until further analysis. Total nitrogen was determined with a C/N analyzer (Model 1500, Carlo ERBA, Italy) and amino acids by HPLC (Kontron). The elements S, P, K, Ca, and Mg were determined after acid digestion (100% HNO_3, 6 hrs at 170°C) using an IPC-AES (Model XMP, GBC, Australia). NRA was measured by an in-vivo test (Gebauer et al., 1988). Isotope ratios were measured by mass spectrometry (Gebauer and Schulze., 1991)

Results

1. Interactions between NRA in leaves with carbohydrate metabolism and environmental factors:

It was shown in the second NIPHYS report that (i) conifer have an intrinsic lower level of NRA than deciduous trees, and (ii) that the major proportion of nitrate which is taken up by roots is also metabolized by roots. The amount of nitrate which is transported in the xylem is close to the detection limit (Heim, 1996)

N-concentrations (N per dry weight) showed surprisingly little variation along the NIPHYS transect (Tab. 1; Bauer and Schulze, 1996). N-concentrations were not significant between Italy and S-Sweden and decreased only in the boreal region. At the same time, the regional and local variations of element concentrations were of similar order of magnitude as the variation along the whole European transect. For instance, the local variation of N-concentrations in spruce at the Waldstein site were of the same order as the regional variation in Bavaria or the variation along the transect. The observation of a large local and regional variation was also true for South- and North-Sweden.

Tab. 1: Variation in nitrogen nutrition of Picea abies at local and regional scale (from Bauer and Schulze, 1996)

| Location | Needle Nitrogen concentration (mmol g^{-1} dw) | | | Maximum | n |
	Miniumum	average	SD		
N Sweden		0.68	0.11		15
SW Sweden	0.54	0.78	0.09	1.36	60
Waldstein	0.56	0.94	0.30	2.12	5
Bavaria	0.70	0.98	0.10	1.21	22
NIPHYS	0.40	0.88	0.40	1.21	7

The interactions between N- and carbohydrate metabolism were studied based on the seasonal time course of needle development as a basis (Fig. 1). Three developmental stages of the needle N-pool became apparent: (i) The initial phase of needle growth was characterized by a rapid increase in total N per needle which reached 50% of the autumn maximum 2 to 3 weeks after bud break at the time when leaf area had reached 80% of its maximum. N-concentrations were very high in young needles and decreased with the development of dry weight which was slower than the development of needle area. (ii) The second phase is characterized by an increase of needle dry weight. It required about 2 months for needles to reach final weight. At the time when needles had reached full weight, the N-content had reached about 70% of the autumn maximum. (iii) In the third phase N-content (mol N per needle) increased without major changes in dry weight and N content reached an autumn maximum.
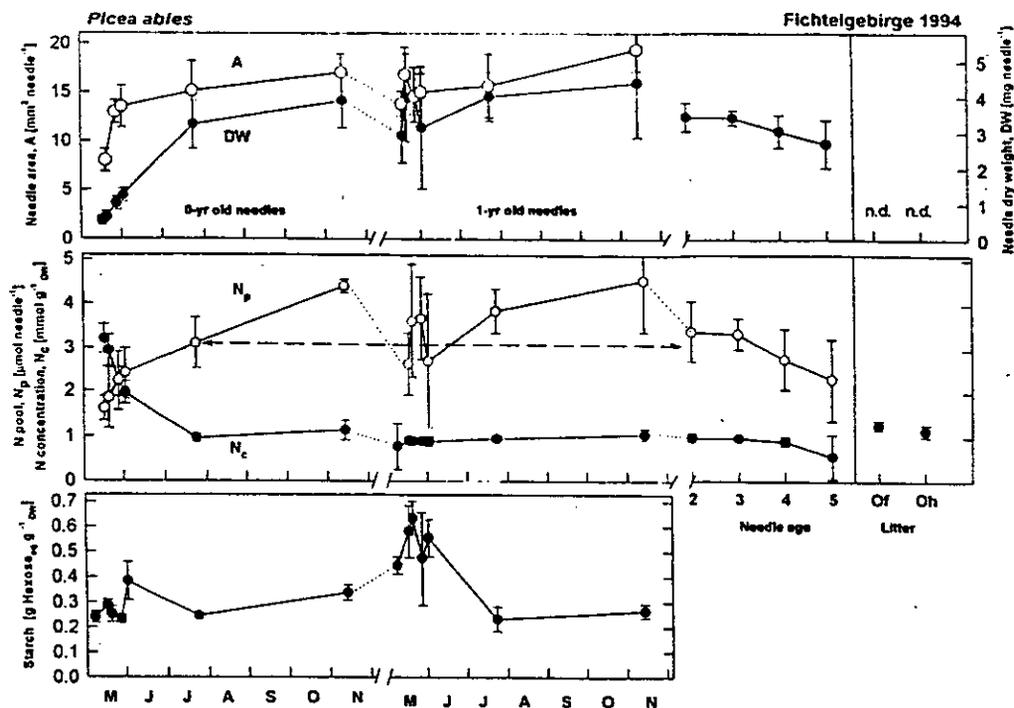
Fig. 1: Seasonal course of (A) needle dry weight, needle area, and specific leaf area; (B) N-concentration per dry weight and N content per needle, (C) Starch concentration per dry weight for 0-year and 1-year old needles of <u>Picea abies</u> sampled in the year 1994 at the Waldstein site in Germany.

Needle dry weight decreased until spring but increased again during the next season. Also the N-content decreased to 70% of the autumn maximum at the time, when the next generation of needles start to expand. The N-content which was reached at the time of new bud break was the same as in 0-year needles when they reached final dry weight. We think that this N-pool represented a "constitutional" level of N being characteristic for a given needle size (Fig. 1 dashed line). Thus N-concentration resulted from two processes, namely N and C gain in the initial phases of growth, and a N-accumulation after needle growth had terminated. Each phase had its own seasonality.

The increase of the total N-pool in 0-year and 1-year old needles from June to November, was an N-accumulation as defined by Chapin et al. (1990). In this period N supply exceeded actual demand and the accumulated N can be remobilized in later periods of increased demand. In the example shown in Fig. 1, the constitutional N-pool was 3 µmol N per needle. Additional 1.5 µmol N per needle were accumulated over summer, which supported growth in the next season. The amount of N-accumulation was the same for 0-year and 1-year needles but decreased in older needles. In the case of the Fichtelgebirge, the N-accumulation took place at the time of wood growth. Thus, the nitrogen pool at the end of the growing season consisted of a constitutional and an accumulated quantity of N at a ratio of 2:1. It is worth noting, that dead needles contained a N-concentration which were equivalent to the constitutional level in
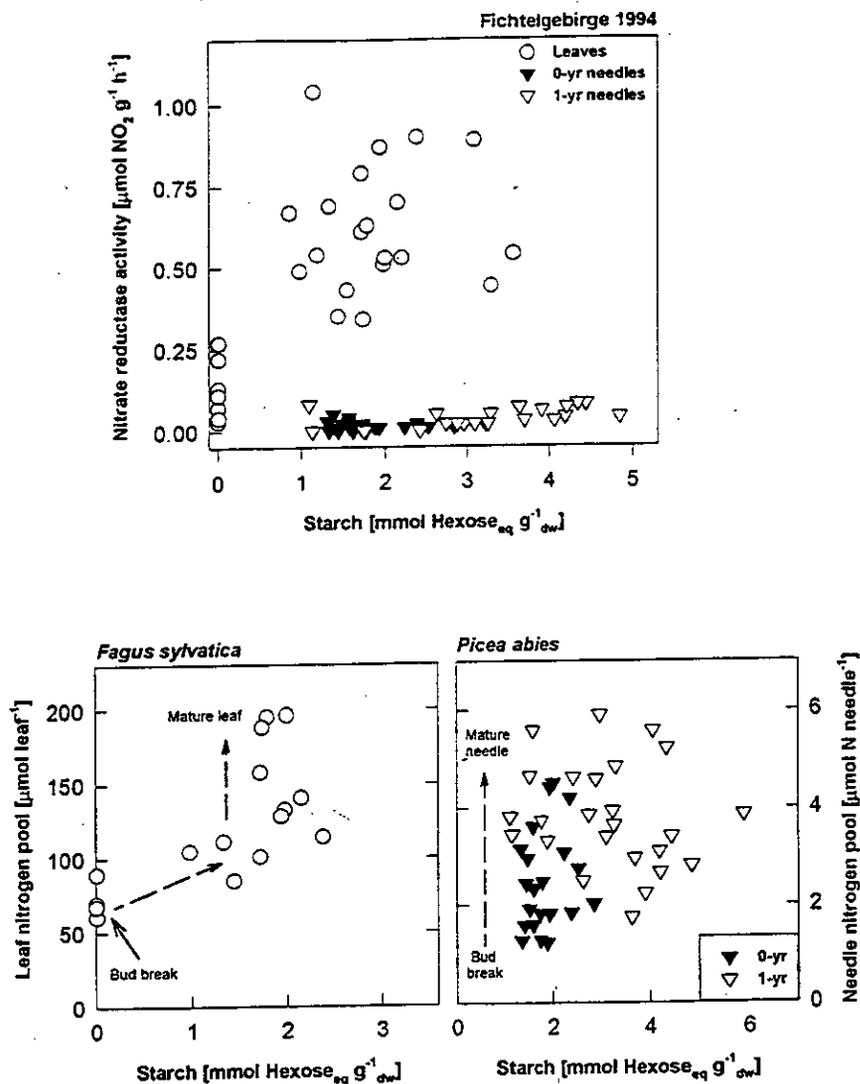
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Fig. 1. Also N-concentrations and pools in North Sweden were at a level which represented the constitutional level in Germany.

In contrast to the N-surplus in summer trees exhibited symptoms of N-deficiency at the time of new needle growth (demand exceeded the supply) as indicated by the accumulation of starch in 1-yr needles at the time when they reach the early spring N-minimum (Stitt and Schulze , 1994). However, in contrast to the response in herbaceous plants, there was no inverse relation between NRA and starch for adult assimilation organs. There is only an intrinsic difference between deciduous trees and conifers (Fig. 3). While *Fagus sylvatica* shows an increase of the leaf nitrogen pool with the leaf starch concentration (which might be interpreted as a sign of nitrigen reduction in leaves), the conifer shows no such relation (which points at nitrogen reduction in roots, or uptake of nitrogen in a reduced form).

Fig. 2: Conceptual model of interactions betwen C- and N-assimilation in determining N-concentration. The interactions between growth of new leaves and of wood are indicated by the "switch-points" (I) for nitrogen, and (II) for carbohydrates. Arrows represent fluxes of N and C.

Fig.3 top: Relations between Nitrate reductase activity and starch,
Bottom: relations between leaf nitrogen pool and starch of Fagus sylvatica leaves and of Picea abies needles

The seasonal course of dry weight and N pools indicated that the phases of needle development were independent of each other at any one time, but fed back upon each other at other times (Fig. 2). The constitutional-N-pool of a needle which was needed for the functioning of the biochemical processes of that organ seemed to originate from reallocation of N from old needles, rather than from root uptake (Hein, 1996). It determined the needle size. The dry weight per needle was correlated to the N-content per needle but not to its N-concentration (Bauer and Schulze, 1986). 80% of this "constitutional" N-pool were reached within 2 to 3 weeks after bud break even before needles reached their final area. Following this initial phase of needle or leaf development, N concentration may increase further, and the amount of N, which could be mobilized early in the next season was a function of needle size and supply in the previous season. The constitutional N-concentration determined the photosynthetic capacity of needles, because the CO_2 fixing enzyme Rubisco contains a major

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fraction of the needle N content (Stitt and Schulze, 1994). The photosynthetic capacity will then determine the needle starch pool which served the C-demand of needle and wood growth. If N-supply was high, then wood cambium acted as a stronger sink for C than needles, and SLA remained at a high area per dry weight (e.g. in Central Europe). In contrast, if the N-supply was low (e.g. in Sweden) then the sink activity of the wood cambium remained low, and starch from photosynthesis was available for further dry weight gain in the needle (SLA decreased to a low area per dry weight). Depending on the supply/demand ratio of the C- and N-pathways, the N-concentration in needles may be independent of wood growth at low supply, and only increase with wood growth under conditions of N-surplus. It was shown by Mund (1996) that N-deposition stimulated wood growth in Central Europe, and this acted in a way to maintaining N-concentration at a constant level.

2. Nitrate reductase activity in forest floor vegetation

It was shown already in NIPHYS report II that on a dry weight basis herbaceous species have 10 times higher NRA than in woody species and these again habe 10 times higher NRA than conifers (Gebauer and Schulze, 1996). The same principle was observed also along the NIPHYS transectfor the forest floor vegetation (Table 2).

SPRING	Italy	S-France	N-France	Germany	Denmark	N-Sweden
Fagus sylvatica (Natural regrowth)		o.r.	0.12±0.08	0.19±0.09	0.54±0.25	o.r.
Picea abies (Natural regrowth)		o.r.	0.011±0.005	0.058±0.049		0.02±0.008
Vaccinium myrtillus			0.028±0.021	0.02±0.016		0.026±0.012
Calluna vulgaris		0.008±0.005			0.068±0.019	
Oxalis acetosella			0.034±0.039	0.28±0.10	0.26±0.22	
Deschampsia flexuosa		o.r.	1.52±0.64	2.21±0.82	1.35±0.45	0.10±0.096
SUMMER	Italy	S-France	N-France	Germany	Denmark	N-Sweden
Fagus sylvatica (Natural regrowth)	1.25±0.19	o.r.	0.61±0.07	0.29±0.08	0.30±0.14	0.35±0.11
Picea abies (Natural regrowth)		o.r.	0.04±0	0		
Vaccinium myrtillus	o.r.		0.08±0.02	0.05±0.09		0
Calluna vulgaris		0.02±0.02				
Deschampsia flexuosa	o.r	o.r.	1.26±0.35	0.31±0.18	0	0.17±0.08

Table 2: Nitratereductase activity (μmol $NO_2 \, g^{-1} h^{-1}$) in understory vegetation (o.r. outside the range of distribution)

3. ^{15}N-labelling of ammonium and nitrate in an old spruce stand.

There is an interaction between ammonium and nitrate uptake in spruce (Berger, 1995). Ammonium uptake increased with supply even at very high rates (Fig. 3), while nitrate uptake in presence of ammonium showed a decreased uptake rate at high supply, and the maximum rate of nitrate uptake was inhibited with an increasing ammonium to nitrate ratio. At at molar ratio of ammonium/nitrate of 4:1 and at high supply of total N, nitrate uptake decreased almost to zero. This laboratory experiment was the basis for a field trial using ^{15}N - labelled ammonium and nitrate.

An initial ^{15}N-labelling experiment was carried out in a young spruce stand (Buchmann et al., 1996 a, b). This experiment showed (1) that the detected label from ammonium and nitrate ^{15}N was similar in both treatments. Only if the dilution of label in soil solution is considered than it becomes apparent, that the actual rate of uptake of ammonim was about 7 times higher than the uptake of nitrate. (2) In this first experiment the label was applied early in the season. Following an initial rapid phase of ^{15}N incorporation into the trees, there was relatively little uptake of label through the rest of the season. In this study, 80% of total N-uptake from soil originated from ammonium and 20% from nitrate (Gebauer and Schulze, 1996).

The results clearly indicated two problems in ^{15}N labelling studies, namely that the concentration of ammonium and nitrate in soil water must be known for calculations of uptake rates, and a single labelling is not sufficient to study the seasonal dynamics, because most of the label is being immobilized in the soil. These problems were considered when planning the labelling experiment in the old spruce stand.

In the 140-year-old spruce stand, the ammonium to nitrate ratio ranged between 7.6 : 1 in the litter and humus layer and decresed to 1.5 : 1 in the mineral soil (May, 1995). The absolute ammonium concentration was between 3 and 4 mmol l^{-1}. These conditions would suggest that ammonium uptake exceeds also nitrate uptake in the old spruce stand.

The labelling experiment indeed showed for needles that ammonium was preferentially used over nitrate. Repeated labelling showed that the uptake of ammonium and nitrate decreased during the growing season.

4. Isotopic composition in aerosols

The isotopic composition of atmospheric trace gases and aerosols was measured by Bruckner (1995, see Table 3).

Table. 3: Seasonal average of aerosol concentration and isotopic composition (from Bruckner, 1995)

Substance	Particle size (μm)	δ^{15}N-value ($^\circ/\infty$) average	s.e.	n	concentration (μg m^{-3} air) average	s.e.	n
Ammonium	0.05-0.14	+3.48	0.74	14	5.17	0.98	14
	0.14-3.50	+1.54	0.15	14	47.19	10.42	14
	3.50-10	+0.63	0.59	14	1.17	0.20	14
Nitrate	0.05-0.14	+0.76	0.36	9	3.70	0.37	9
	0.14-3.50	+0.75	0.60	9	7.90	3.00	9
	3.50-10	-0.20	0.12	9	0.16	0.10	9

The data indicate that the small particles contained a positive isotopic signal, which increased during summer. There was very little variation with wind direction. The small particles were considered to be of industrial origin, while the large particles were most likely of agricultural origin.

Summary

The work of the plant Ecology Laboratory demonstrated:
1. Conifers use less nitrate than deciduous trees. This is important in terms of nitrate leaching from forest ecosystems
2. There is an interaction between ammonium and nitrate uptake. At high ammonium levels, nitrate uptake is inhibited. This will further enhance nitrate leaching to ground water.
3. N-concentration is not an appropriate measure for N nutrition in trees because of the interaction with wood growth. Local variations in nutrition are of similar order of magnitude as the variations along the transect, however the transect data are an essential control in order to separate anthmospheric N-inputs from N-supply from soils. N-nutrition and surplus is better quantified by growth.
4. The stable isotope ratios of aerosols indicate that industrial inputs of ammonium are higher than previously considered.

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2. Prof.Dr. P. Högberg, Dr. L. Högbom, H. Schinkel, M. Högberg, C. Johannisson

Laboratory:	Forest Ecology, Swedish University of Agricultural Sciences, Umeå
Research area:	Natural abundance of ^{15}N in roots and mycorrhizas Nitrate reductase activity of roots and mycorrhizas Nitrogen uptake capacity of roots

Objectives:

All sites:

(1) Determine ^{15}N natural abundance of roots and mycorrhizas at different soil depths

(2) Determine nitrate reductase activity (NRA) in roots and mycorrhizas

(3) Determine uptake capacity of roots for NH_4^+ and NO_3^-

Umeå sites:

(4) Develop methods to study ^{15}N abundance of available pools of N in soil

Methodology:

Fine root material was extracted from different soil horizons at all the NIPHYS sites in 1993 and 1994. Samples of soil immediately surrounding these fine roots were also taken. Some of the fresh root material was used for NRA assays within a few hours (see below), while the remainder was oven dried for measurements of °/ooN and ^{15}N abundance. In some cases (beech roots) the ^{15}N abundance of excised ectomycorrhizal (ECM) fungal sheaths and remaining root cores were analyzed separately. In other cases ECMs were separated from non-mycorrhizal roots.

To obtain a baseline, we conducted a detailed study in 1993 of ^{15}N abundance in the long-term forest fertilization experiment at Norrliden in northern Sweden. Roots of *Pinus silvestris*, ericaceous spp. and grasses were sampled by soil horizon, and additional soil samples were also taken, from the N0 (control) and N3 (N-fertilized: 106 kg N ha^{-1} yr^{-1}, from 1971 to 1990) plots.

Needle samples collected from the experiment at Norrliden and similar trials further south were used to study changes in ^{15}N abundance over time in response to N-loading. Analysis of ^{15}N abundance and °/ooN was conducted on a CF-IRMS (Europa Scientific Ltd. Mod. 20-20). Our reference material was intercalibrated with the laboratory in Bayreuth. Results are reported in per mil (°/oo) deviations (δ) from the standard atmospheric N_2.

We used the *in vivo* NRA assay described by Gebauer *et al.* (1988), in which excised plant material is exposed to a solution containing NO_3^-, and incubated in darkness at a standard temperature. This means that the supply of NO_3^- is not limiting during the assay. Hence, NRA is related to the amount of active enzyme in the tissue, which in turn reflects the balance between synthesis/degradation and activation/inactivation of the enzyme, rather than true levels of NRA in the field. Extensive testing carried out by G. Bauer (Bayreuth) and L. Högbom showed that the anaerobic assay used produced results identical to those of the aerobic assay described by Högberg *et al.* (1986) during 1-hour-long incubations.

Nitrogen uptake capacity, which is inversely related to the plant internal N status, was measured by the technique proposed by Jones *et al.* (1991). According to this method fine roots excised in the field are immersed in solutions containing NH_4^+ or NO_3^- for 2 h. Fine roots were collected at NIPHYS sites for these studies in 1995, and analysis of them are underway.

Some development work was undertaken to establish protocols for analyzing $\delta^{15}N$ of different N pools in very dilute soil solutions. This work is still in progress. As a first approximation, however, we made a model study of a natural N supply gradient at Betsele, 120 km NW of Umeå. Mor-(H)-layer soil samples were extracted in the field at every 10th m along this 90-m-long gradient, transferred to the laboratory, passed through a 5 mm mesh and mixed with acid-washed quartz sand to give a mixture of 0.6% organic matter (d.wt.), which was put in 0.3 L plastic pots in the greenhouse. Seeds of *Pinus sylvestris*, *Vaccinium macrocarpon* and *Epilobium angustifolium* were germinated in the pots. After 63 days plants were harvested and analysed for total-N and $\delta^{15}N$. Calculations of the isotopic signature of available N were made by removing the source effect of N in the seed, and a modelling exercise was conducted to study the links between soil N pools as extracted by soil centrifugation, and the $\delta^{15}N$ of available N measured by this plant bioassay.

Results

At strongly N-limited sites in northern Sweden there was a clear progression from low $\delta^{15}N$ at the soil surface to up to 8-10 δ higher values deeper down in the mor-layer (Figs. 1 and 2). The accepted explanation of this phenomenon is plant uptake of sources of N more depleted in ^{15}N than soil total-N and redistribution of this isotopically lighter N by litterfall onto the soil surface (Nadelhoffer and Fry 1994). However, processes in N-saturated ecosystems like rapid nitrification, loss of nitrate, denitrification and ammonia volatilization lead to ^{15}N enrichment of the N remaining in the soil, and hence to elevated ^{15}N abundance in the available pool (e.g. Högberg 1990, 1991). On the N3 plots at Norrliden, roots were sometimes enriched in ^{15}N relative to soil total-N (Fig. 1), which confirms the previous suggestion. Hence, this condition

can be used as an indicator of N-saturation. In the longer term the deposition of ^{15}N-enriched litter will change the profile to one, where the surface is isotopically as heavy or heavier than deeper down. Interestingly, roots more enriched in ^{15}N than soil total-N occur at the most N-saturated NIPHYS sites in middle Europe (Fig. 2). The above results are reported in detail in Högberg et al. (1996). In a separate study of Norrliden, it was shown that δ ^{15}N of needles correlated with budgeted N losses from the forest ecosystem (Högberg and Johannisson 1993).

Fig. 1. Natural abundance of ^{15}N in soils (●) and roots of *Pinus sylvestris* (△), ericaceous plants (○) and grasses (□) by horizon in soil profiles on N-limited control plots and experimentally N-saturated (N3) plots in the experiment Norrliden, northern Sweden. The horizons are: S- (the supericial layer), F- (fermentation layer), H- (humus layer and M- (upper 5 cm of mineral soil).

δ^{15}N (‰)
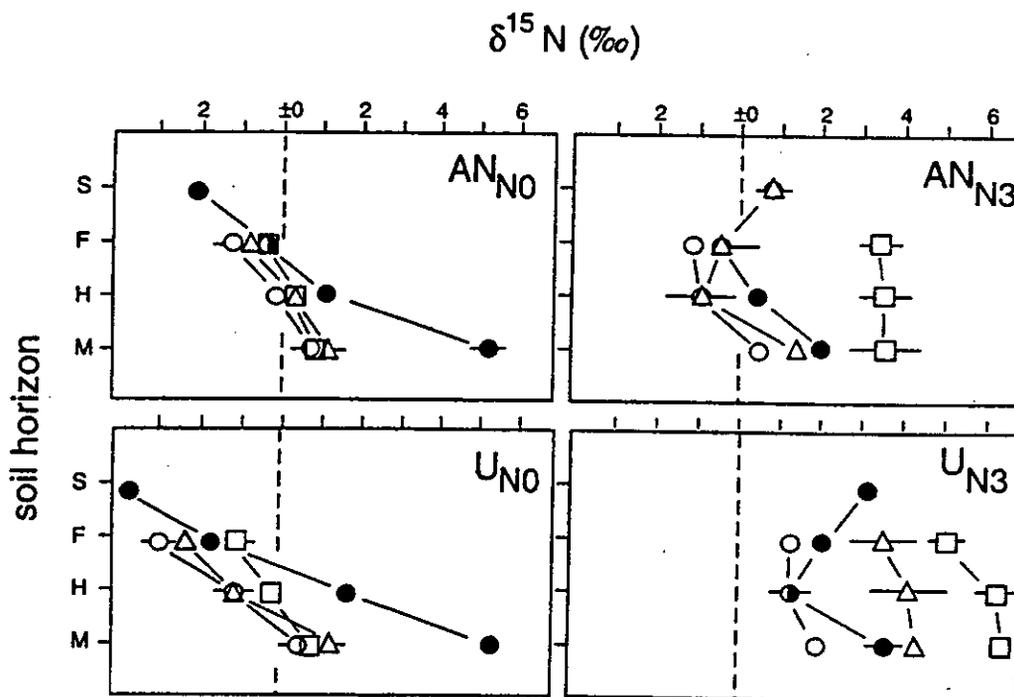
Fig. 2. Natural abundance of ^{15}N in soils (●) and non-mycorrhizal (O,△) and ECMs (□) by horizon at the NIPHYS European transect sites: a, Åheden; b, Klosterhede; c, Aubure; d, Waldstein; e, Hilleröd; f, Aubure; e, Schacht. Soil layers as in Fig. 1.

$\delta^{15}N$ (‰)
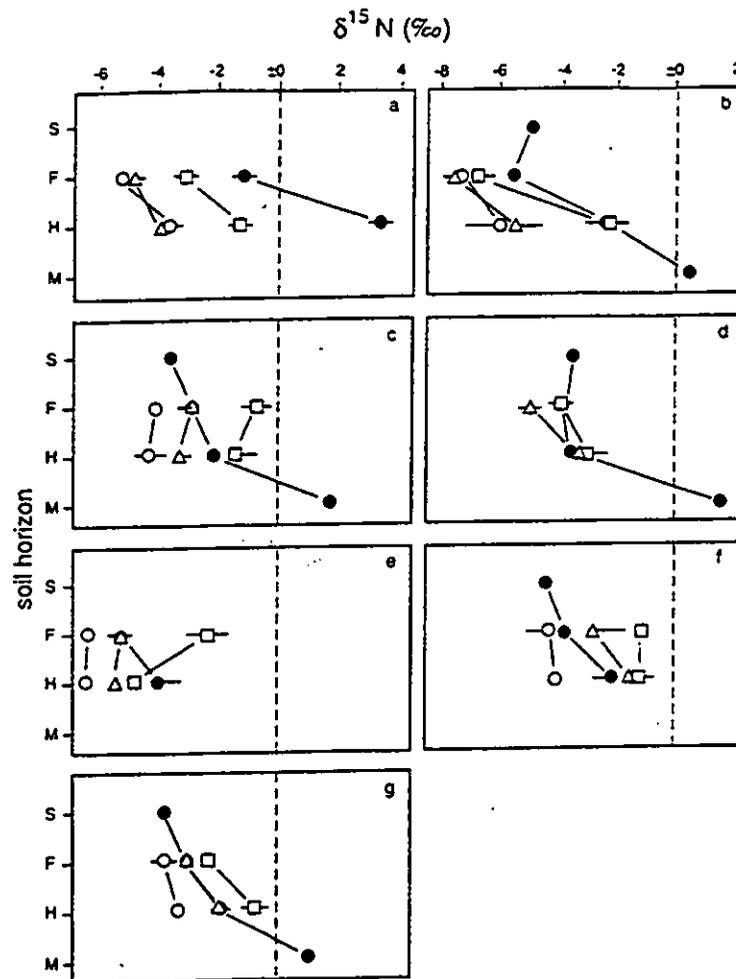
Surveys in SW Sweden, the part of the country receiving the highest N-load, showed that the difference in δ ^{15}N between soil (H-layer) and plant, ϵ, is a good predictor of N leaching. Sites with $\epsilon > -0.5$ ‰ had elevated concentrations of nitrate in soil water (> 0.1 mg N L^{-1}) at 50 cm depth ($p < 0.001$). Together with the amino acid arginine ϵ appears to be good and convenient indicator suitable for larger scale regional surveys of N saturation (Näsholm et al. in press).

Ectomycorrhizal (ECM) roots were 2 ‰ or more enriched than non-mycorrhizal roots. The fungal sheaths were 2.4-6.4 ‰ more enriched than the root core inside the sheath. A.F.S.Taylor (Sheffield) and L. Högbom demonstrated that ECM fungal sporocarps are highly enriched in ^{15}N, which confirmed a previous study by Gebauer and Dietrich (1994). At Åheden, northern Sweden Taylor and Högbom (unpubl.) showed that in 18 species of ECM fungi δ ^{15}N varied between -0.8 and 12.7 ‰, which is about 5-19 ‰ enriched relative to the

potential plant hosts. We have found that the major N compounds in fungi are all enriched relative to N in the hosts (Table 1), but also that N in free proteins and amino acids in the fungi were 9.7 °/oo more enriched (p<0.001) than N in cell walls (which includes chitin). We suspect that the variability between fungal species results from differences in internal N metabolism as well as in N sources used and soil depth levels explored. These patterns will be further explored in collaboration with F. Martin (Nancy) and D.J. Read and A.F.S. Taylor (Sheffield).

Table 1. Natural abundance of ^{15}N in per mil (δ) in different parts and N-fractions of sporocarps of ectomycorrhizal fungi collected at Åheden. The potential hosts of these fungi, *Picea abies*, *Pinus sylvestris* and *Betula pendula*, have a ^{15}N abundance of from -8 to -5 δ.

Fungal species and part	Total-N	Free proteins	Free amino acids	Cell walls
Amanita muscaria				
cap	7.2	5.7	6.4	-2.0
stipe	5.5	6.0	5.3	-3.2
Suillus bovinus				
cap	11.1	13.7	12.2	2.5
stipe	8.2	10.8	11.2	0.3
Suillus variegatus				
cap	4.6	5.7	4.7	-5.2
stipe	1.2	4.8	4.8	-4.9

Roots of broadleaves had higher NRA than roots of the conifer *Picea abies*, which however had exceptionally high NRA at the German site Waldstein (Table 2). The NRA was very low at Åheden, northern Sweden, as compared to the other sites.

Table 2. Nitrate reductase activities (μmol NO_2^- g^{-1} h^{-1}) of the different tree species at NIPHYS sites. Data are grand means ($\pm$1SE, n>16) per site including non-mycorrhizal as well as ECM roots from two horizons in the mor layer.

Site	Tree species	
	Picea abies	*Betula pendula*
Åheden	0.006$\pm$0.005	0.049$\pm$0.012
	P. abies	
Klosterhede	0.065$\pm$0.014	
		Fagus sylvatica
		0.238$\pm$0.085
	P. abies	*F. sylvatica*
Aubure	0.077$\pm$0.011	0.260$\pm$0.050
	P. abies	
Waldstein	0.272$\pm$0.043	
		F. sylvatica
Schacht		0.311$\pm$0.020

Table 3. Nitrogen concentrations (%) in roots of *Picea abies* at different NIPHYS sites. Coarse roots are thicker than 2 mm in diam. ECMs are ectomycorrhizas. Data are means (n=4-16).

Site and soil horizon	Coarse roots	Non-myc fine roots	ECMs
Åheden			
F-layer	0.51	0.79	1.25
H-layer	0.39	0.56	0.90
Klosterhede			
F-layer	0.84	1.21	2.06
H-layer	0.87	0.97	1.73
Aubure			
F-layer	1.13	1.51	2.35
H-layer	0.94	1.24	2.39
Waldstein			
F-layer		1.42	2.00
H-layer		1.60	2.11

The model study of δ ^{15}N of available N showed that a) δ of available N was low along a part of the transect where inorganic N levels are low, and where organic N is a likely source, b) δ increased significantly at the same time as available and extractable levels of ammonium increased, and c) δ decreased as ammonium was largely replaced by nitrate in the soil solution. This suggests that ammonium becomes heavily enriched with increasing nitrification until a point is reached, where nitrification is very rapid and complete and little ammonium is left.

Conclusions

The low levels of total-N and NRA in roots of trees in northern Sweden as compared to elsewhere suggest a strong N limitation in northern Sweden and a much higher N supply elsewhere. The studies of δ ^{15}N of roots and soils clearly indicated the potential of using the difference between them, the ε value, as an indicator of N saturation. Together with the amino acid arginine, ε seems most promising as an indicator suitable for larger scale surveys. The in general higher NRA in roots of broadleaves as compared to conifers should contribute to a higher retention of nitrate in forests dominated by broadleaves.

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3. Prof. Dr. Drs. h. c. H. Marschner, Dr. E. George, C. Stober

Laboratory: Institute of Plant Nutrition, Hohenheim University

Research area: Root growth and nitrogen uptake by tree roots in forest ecosystems differing in atmospheric nitrogen deposition

Objectives

All sites:

(1) To determine the dynamics of root growth of deciduous and coniferous trees in different soil depths during the growing season.

(2) To identify potential uptake of inorganic and organic nitrogen by different root zones of deciduous and coniferous trees at different sites contrasting in temperature and N deposition.

(3) To quantify the contribution of ectomycorrhiza to the N nutrition of deciduous and coniferous trees.

Hohenheim location:

(4) To seperate under controlled conditions the effects of roots and ectomycorrhiza on uptake of N provided in different forms.

(5) To investigate the effects of N on growth of roots and ectomycorrhiza.

Methodology

The dynamics of root growth had been studied *in situ* by continuous "root window" observations. Root windows are soil profiles covered with a plexiglass plate, installed near old trees in a distance of 140 cm from the trunk of a tree. Root windows were installed at all NIPHYS sites in deciduous and coniferous stands. All stands were 30 years or older. After roots had grown towards the plexiglass, their further growth was monitored non-destructively by repeated observations. The root growth was monitored throughout two growing seasons. Each visible change (elongation of existing roots, appearance of new roots, lateral branching, mycorrhizal infection of root tips) was copied to transparent foils placed on the plexiglass. The transparent foils were then analyzed for root length density per unit (m^2) root window surface.

The uptake of N supplied to trees as $^{15}NH_4^+$ or $^{15}NO_3^-$ was measured *in situ* with individual roots of Norway spruce and beech at a second set of root windows. After roots had grown towards the plexiglass plate, the plate was removed and the roots were used to carry out microfertilization experiments. For this purpose, apical zones of intact roots were exposed to agar blocks containing ^{15}N labelled ammonium or nitrate. A release of ^{15}N from the agar block to the soil was avoided by separating agar block and soil by aluminium foil. Roots were

excavated from soil after an application period of 24, 48, or 72 hours and immediately cut into two-centimeter segments. These segments were then analyzed for ^{15}N content to follow the uptake and transport of ^{15}N. It was not yet tested how much N remained in the agar after the application period. The microfertilization experiments were carried out three times during the growing season (spring, summer, and autumn) in Norway spruce and beech sites at Aubure, Waldstein/Schacht, Klosterhede/Hilleröd, and Aheden.

The contribution of ectomycorrhizal fungi to the N uptake was planned to be studied in one field experiment (Obj. 3) and two pot experiments in the greenhouse (Obj. 4 and 5). Since it was not possible to get a site-specific mycorrhization in the pot experiments, a split root experiment was set up with high N supply to only one part of the root system. Additionally, mesh bag experiments were carried out on three Norway spruce and beech sites (Aubure, Waldstein/Schacht, Klosterhede/Hilleröd).

To seperate the effect of hyphal N uptake from root N uptake, mesh bags differing in mesh size (2 mm, 30 µm, 0.45 µm) and therefore penetrable to roots and hyphae (2 mm), to hyphae only (30 µm), or neither to roots nor to hyphae (0.45 µm), were buried next to three- to five-year-old Norway spruce and beech trees. Four months later, to each mesh bag 1 mg N was supplied as (^{15}NH$_4$)$_2$SO$_4$ solution. Harvest of the aboveground biomass of the trees was carried out 40 days after application. The ^{15}N content was measured by mass spectrometry in current-year- and older needles (Norway spruce) and in leaves and buds (beech).

To test the effect of high N availability on hyphal growth, ingrowth cores (mesh size 2 mm; "roots plus hyphae") were buried next to old trees and supplied with 300 mg N (kg soil)$^{-1}$. No N was supplied in a control. Beginning three months after installation, mesh bag samples were taken in regular intervals. Hyphal length density was measured in four horizons by the method of Li et al. (Plant and Soil 136, 49-57, 1991).

Results

Root growth

Root length densities at the root windows in November 1995, two years after installation, are shown in Fig. 1.
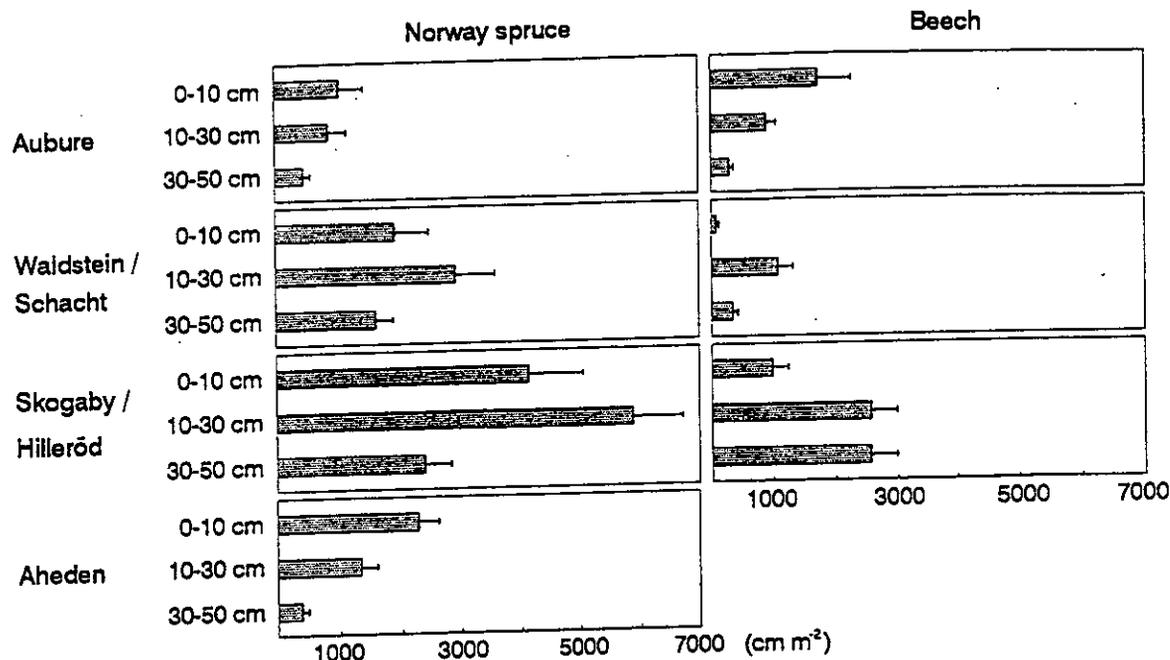
Fig. 1: Root length density (cm m^{-2}) of Norway spruce and beech at the root window surface in November 1995 at four sites in different soil depths (means and standard errors)

Root length density at the root windows was highest at the south Scandinavian sites (Skogaby and Hilleröd) for both tree species. These sites have the longest growing season of all sites studied, warm summers and continuous high soil water supply throughout the year. At most sites, root length density of beech was less when compared with Norway spruce. The highest root length densities of Norway spruce were found in the upper parts of the root windows (0-30 cm). The root length density in the subsoil (30-50 cm) was particularly low in Norway spruce at Åheden and Aubure and also for beech at Aubure. This may reflect either unfavorable conditions for growth in the nutrient-poor subsoils or relatively much more favourable conditions in the topsoil. Comparing beech and Norway spruce root length densities at the other sites, beech had relatively more roots in deeper soil layers.

The time-course of root growth is obtained by repeated monitoring during the growing season. As an example, this is shown in Fig. 2 for different root types at the Skogaby site (south Sweden). Classification into long roots and fine roots was based on their morphology. Fine roots were less than two millimeters in diameter.
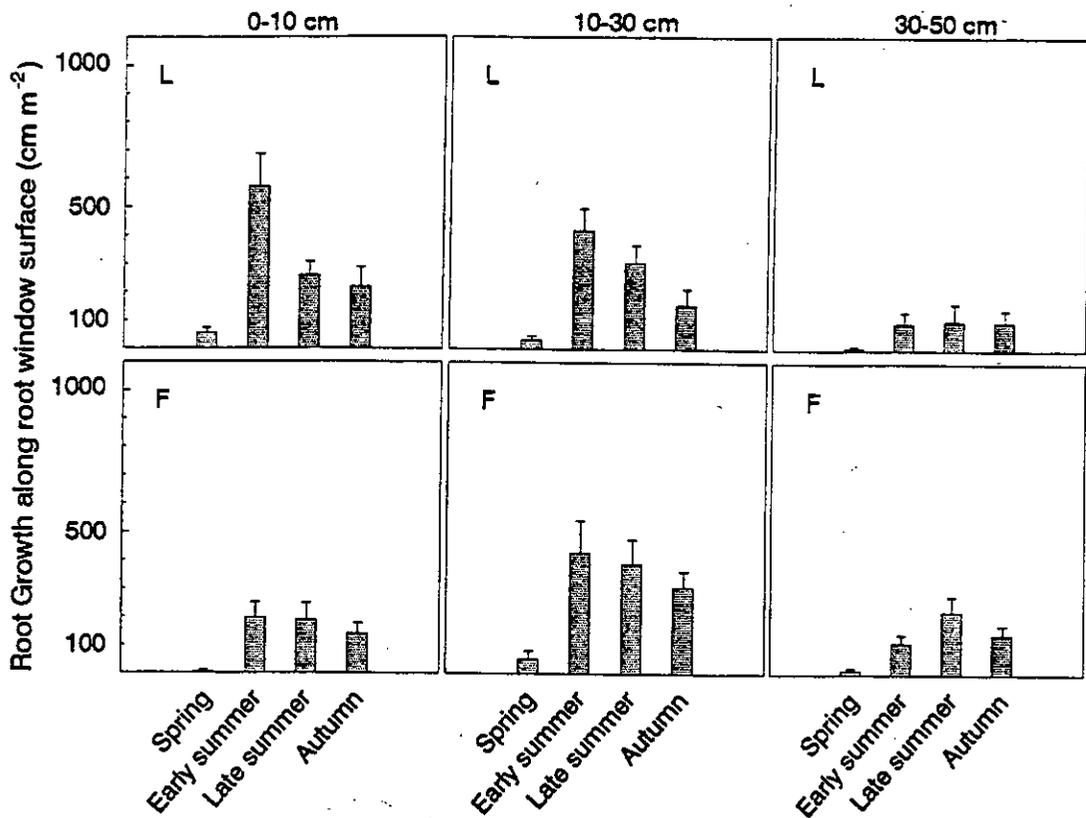
Fig. 2: Growth of longroots (L) and fine roots (F) of Norway spruce at the root window surface (cm m^{-2}) at Skogaby in different soil depths and at different times of the vegetation period (means and standard errors)

High growth rates of longroots were observed in early summer. This was most obvious in the upper 10 centimeters. In 30-50 cm depth, root growth rates were more constant throughout summer and autumn. The onset of fine root growth occured later in the season compared with longroot growth. In the upper part of the root windows, longroot growth dominated over fine root growth. Similar growth rates for both root types were observed in 10-30 cm, however, with a pronounced growth of longroots in the first half and a more pronounced growth of fine roots in the second half of the growing season. Fine root growth was higher than longroot growth in the subsoil (30-50 cm). Root turnover calculations based on the gross increase of root length density during one year in relation to the initial root length density in winter resulted in estimates of average life spans of two years and more for longroots and of one year or less for fine roots.

N uptake capacity

The capacity of individual roots to take up ammonium and nitrate was tested at two different levels of total N supply (1 mM, 10 mM). Ammonium and nitrate were supplied simultaneously, but with different ratios. The duration of application was also varied. Some typical patterns were observed at all sites (Fig. 3, 4).

26
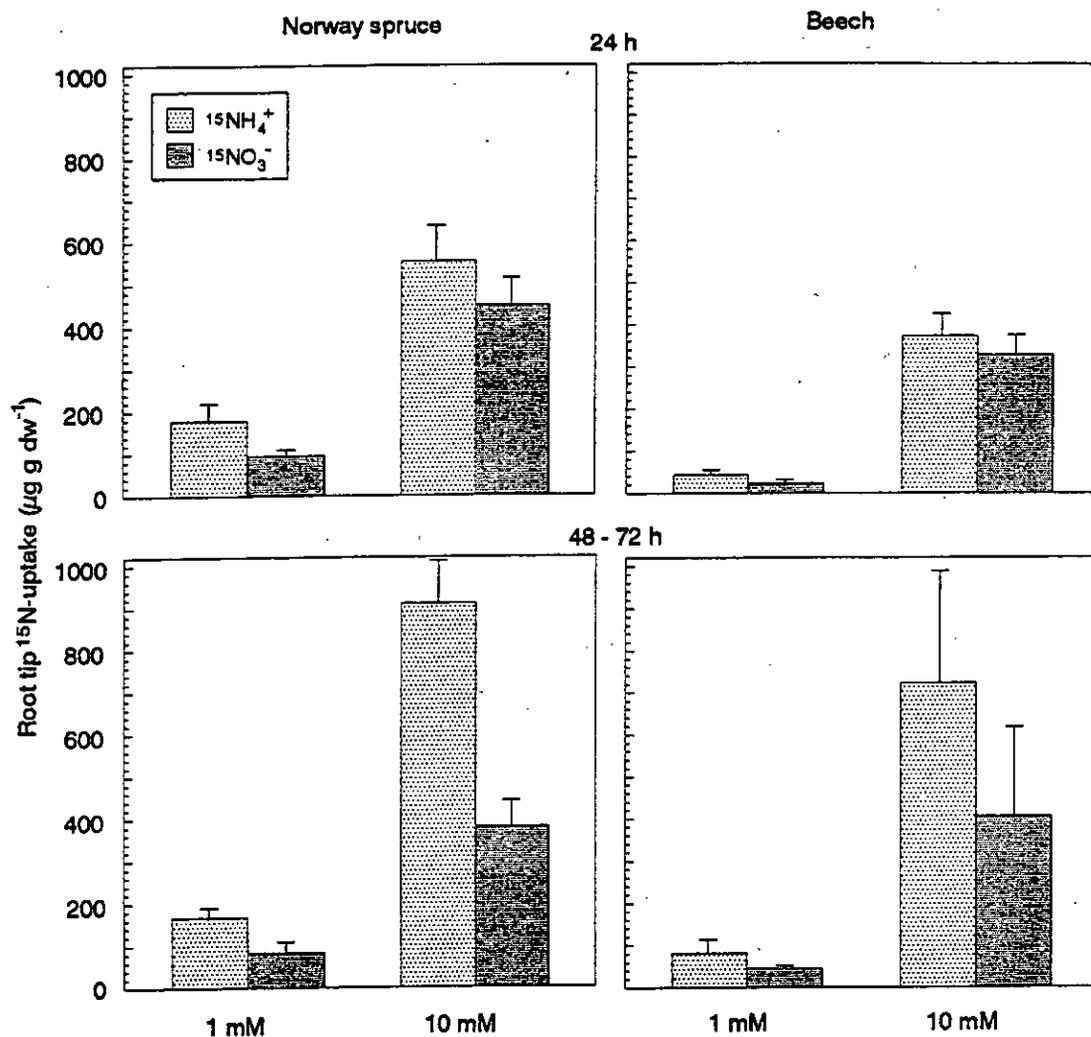
Fig. 3: Uptake of ^{15}N (μg g dw^{-1}) at apical root zones (0-2 cm) when ammonium and nitrate were supplied at 1:1 ratio (sites: Aubure, Waldstein, Klosterhede, Hilleröd; means and standard errors)

Ammonium was taken up in higher rates than nitrate at the root tip when both were supplied simultaneously in equal concentrations. With an application period of 24 hours, this preference of ammonium was more distinct in Norway spruce than in beech and clearly expressed only at the low supply concentration (1 mM). With longer application periods (48-72 h), higher uptake of ammonium compared to nitrate was also distinct at the high supply level in Norway spruce.
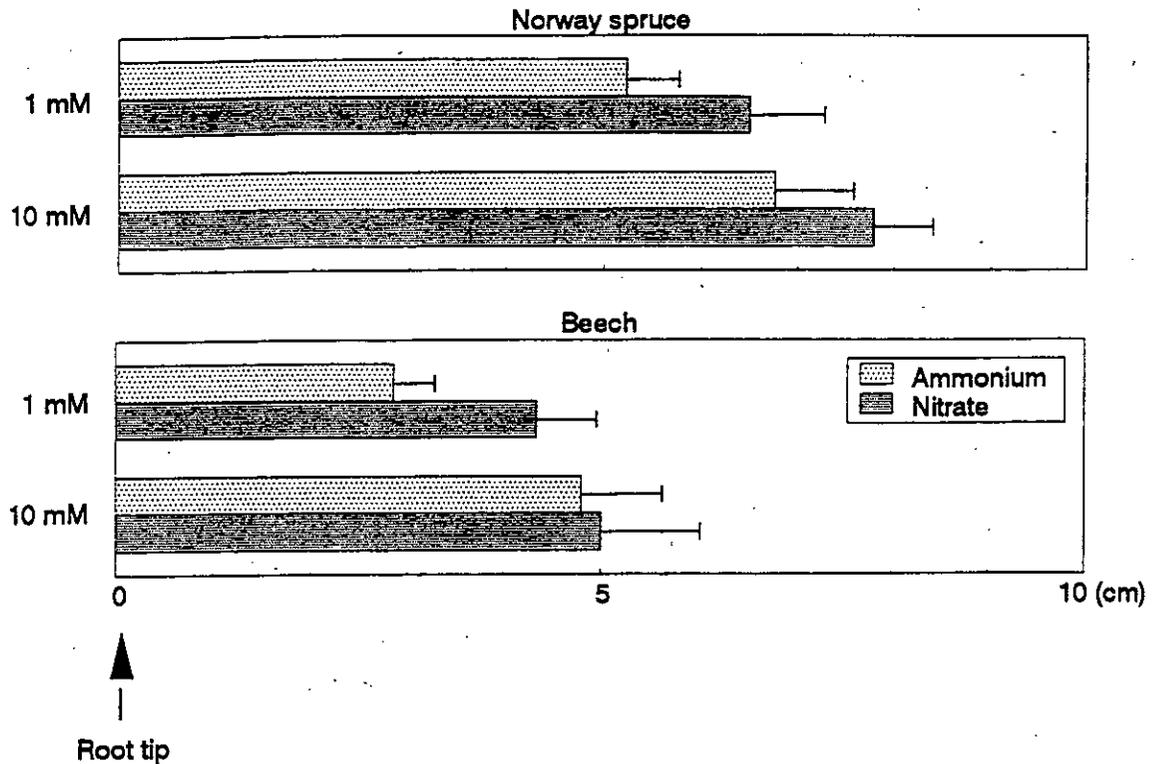
Fig. 4: Maximal basal transport distance (cm) of ^{15}N supplied to apical root zones (0-2 cm) within 24 hours (sites: Aubure, Waldstein, Klosterhede, Hilleröd; means and standard errors)

Despite of the higher uptake rate of ^{15}NH$_4^+$ (Fig.3), basal translocation of ^{15}N from the sites of supply was faster in case of nitrate than ammonium (Fig. 4). This effect was not very distinct, but the same pattern was obtained in both plant species and at both supply levels. In general, ^{15}N was transported faster in Norway spruce than in beech. A tenfold higher supply of N had only small effects on maximal transport distance.

Comparing the sites, differences in N uptake were obtained (Fig. 5). At low N supply (1 mM), at Klosterhede, Norway spruce roots took up similar amounts of ^{15}N-ammonium and ^{15}N-nitrate. At Waldstein, the site with the highest N uptake, ^{15}N-ammonium uptake was about three times the ^{15}N-nitrate uptake and at Aubure about two times higher than the ^{15}N-nitrate uptake. The high ammonium uptake rates at Waldstein may be related to the very high biomass production found at this site (Schulze et al., this report). At the high supply level (10 mM), differences between sites in total N uptake rate were less distinct, but slightly preferential ^{15}N-ammonium uptake occurred at all sites.
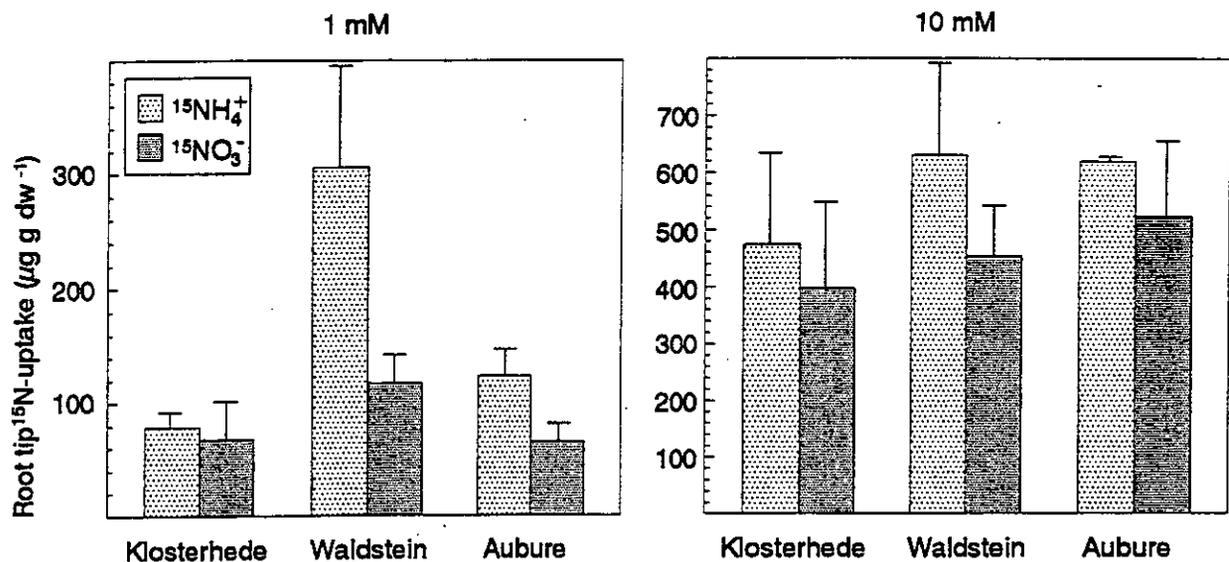
Fig. 5: Uptake of ^{15}N ammonium and ^{15}N nitrate (µg g dw^{-1}) supplied for 24 h at 1:1 ratio to apical root zones (0-2 cm) of Norway spruce at different sites (means and standard errors)

Total ^{15}N uptake was tested not only at ammonium:nitrate ratios of 1:1, but also at ratios of either 9:1 or 1:9 (Fig. 6). In Norway spruce, uptake of ^{15}N was highest when ammonium was the predominant N source. When nitrate supply was predominant, ^{15}N nitrate uptake exceeded ^{15}N ammonium uptake but total N uptake was lower. This was found at both supply concentrations (1 mM, 10 mM). In beech, the effects of the ammonium:nitrate supply ratio were less clear.

The relative uptake of ammonium and nitrate is also shown in Fig 6. When nitrogen was supplied to Norway spruce roots at a concentration of 1 mM and a 1:1 ratio of ammonium and nitrate, ammonium uptake accounted for 65% and nitrate uptake for 35% of total N uptake. A similar relation was found in beech at the same supply level. When the ammonium:nitrate ratio was 9:1 at 1 mM total N supply, uptake of ammonium accounted for 90% and nitrate uptake for 10% of total N uptake. This was true for both tree species. When nitrate was the dominant source (9:1), nitrate uptake was twice as high (Norway spruce) or as high (beech) as the ammonium uptake.
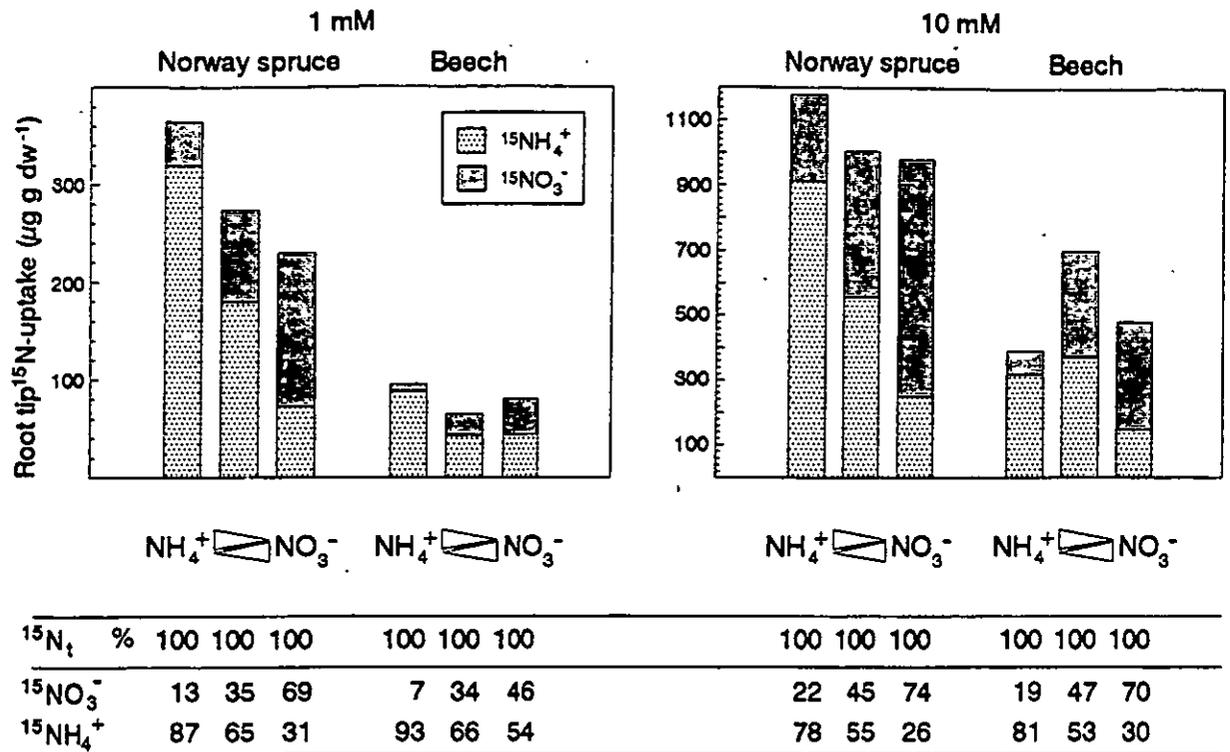
Fig. 6: Uptake of ^{15}N-ammonium and ^{15}N-nitrate (μg g dw^{-1}) supplied for 24 h to apical root zones (0-2 cm) at different concentrations of total N (1 mM, 10 mM) and different ammonium:nitrate ratios (9:1, 1:1, 1:9; sites: Aubure, Waldstein, Klosterhede, Hilleröd)

		Norway spruce (1 mM)			Beech (1 mM)			Norway spruce (10 mM)			Beech (10 mM)		
^{15}N$_t$	%	100	100	100	100	100	100	100	100	100	100	100	100
^{15}NO$_3^-$		13	35	69	7	34	46	22	45	74	19	47	70
^{15}NH$_4^+$		87	65	31	93	66	54	78	55	26	81	53	30

Contribution of ectomycorrhiza to N nutrition of trees

In mesh bag experiments, soil compartments were accessible to roots and hyphae or to hyphae only (see Methodology) to seperate hyphal N uptake from N uptake of mycorrhizal roots. The ^{15}N uptake by hyphae from root-distant soil compartments ("-R+H"-mesh bags) and delivery to young beech plants was as efficient as ^{15}N uptake by tree roots ("+R+H"-mesh bags, Fig. 7). No ^{15}N was transported from root- and hyphae-distant soil mesh bags ("-R-H") to the plant, indicating that diffusion of N out of the mesh bags was minimal. In beech, most of the labelled N occured in the buds, i.e. the youngest parts of the plants (Fig. 7). In Norway spruce, the ^{15}N taken up was mainly accumulated in the current-year-neddles (data not shown).
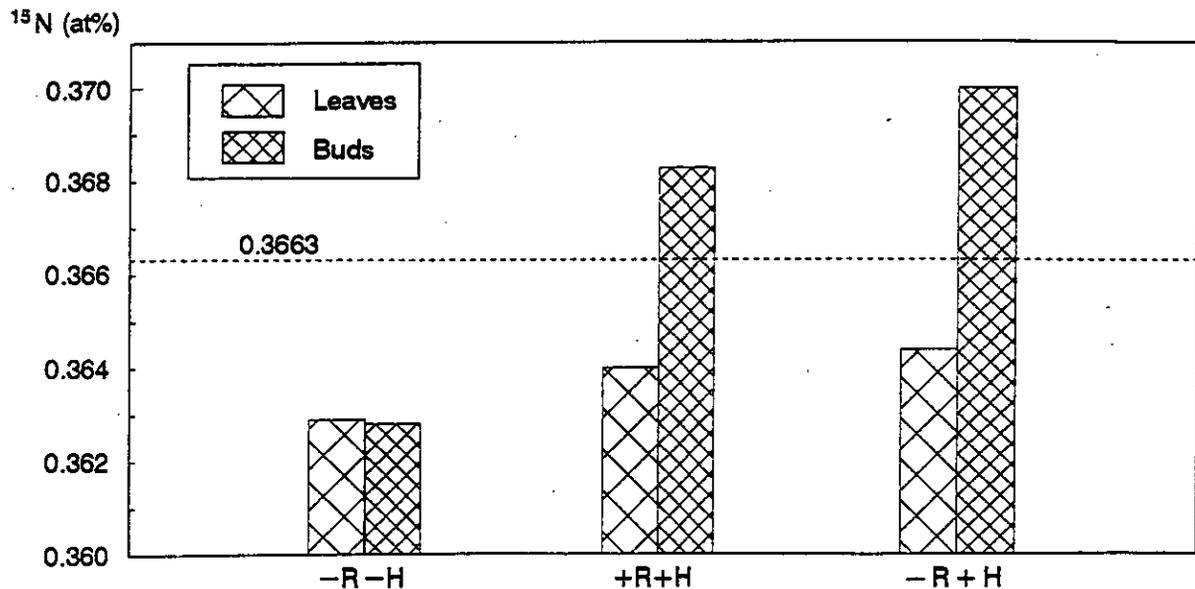
Fig. 7: Enrichment of ^{15}N in leaves and buds of 3-year-old beech plants 40 d after ^{15}N application to mesh bags near the trunk (sites: Aubure, Schacht, Hilleröd; for further explanation see Methodology)

Effect of N supply on growth of hyphae

The effect of high availability of N on hyphal growth was tested by ingrowth cores supplied with high concentrations of N. As shown in Fig. 8, N had no effect on hyphal length density in beech at Aubure. A distinct decrease of hyphal length density was found with increasing soil depth. In the Norway spruce stand at Aubure, a decrease of hyphal length density was found within the ingrowth cores supplied with N compared to the control. Highest length densities were found in the 10-20 cm horizon. In the Norway spruce stand at Aheden, increased hyphal length densities were obtained in soil supplied with N. This was most obvious in the soil depth 0-10 cm. The contrasting effects of high N supply on hyphal length densities in Norway spruce at Aubure and Åheden may be due to different levels of soil N availability (Aubure >>> Aheden) and different fungal species at these sites.
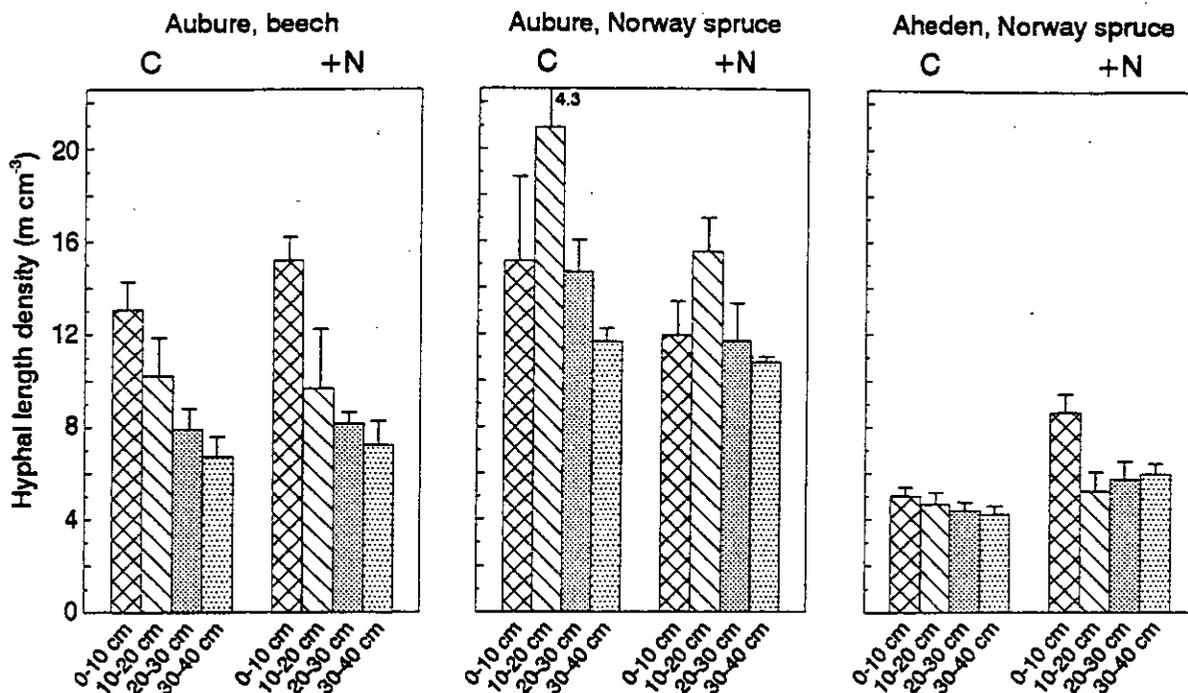
Fig 8: Length density (m cm^{-3}) of hyphal structures (<100 μm diameter) within ingrowth cores three months (Aheden: 12) after N supply (300 mg N [kg soil]$^{-1}$; control = no N supply; means and standard errors)

Conclusions

Root growth at the root windows differed between sites and between species. Highest root length densities were found for both species at the south Scandinavian sites. Climatic conditions, in particular soil temperature, and nutrient (N in particular) supply determine root growth rates. In most instances, root length densitiy was less in beech compared to Norway spruce. On the other hand, beech appeared to produce more fine roots and did not show the distinct decrease of root length density with increasing depth observed in Norway spruce. Therefore, beech may be able to take up more nitrogen from deeper soil layers than Norway spruce.

Ammonium uptake was higher than nitrate uptake when both forms were supplied simultaneously in equal concentrations to the root tip, but roots had also the capacity to take up considerable amounts of nitrate. Total N uptake was highest when ammonium supply was higher than nitrate supply. However, when nitrate concentrations were much higher than ammonium concentrations, nitrate uptake was higher than ammonium uptake. This indicates

that the N uptake is strongly influenced by the availability of both forms and that trees may utilize also considerable amounts of nitrate in case of higher nitrate supply at a given site.

Ectomycorrhizal hyphae are able to take up ammonium and to deliver it to the host plant. In the field experiments, mycorrhizal hyphae were as efficient in N uptake as roots.

High N supply increased hyphal growth in the Norway spruce stand in northern Sweden (Aheden), but decreased it in central Europe (Aubure). No effect of N supply on hyphal growth was found in beech (Aubure). The results suggest that in forest ecosystems characterized by high N availability, additional N will depress hyphal growth, while at N limited sites, N supply will have a fertilization effect and increase hyphal growth.

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Laboratory: University of Sheffield
Research Area: Mycorrhizal uptake of organic and inorganic nitrogen and effects of high N deposition upon mycorrhizal infection

Original Objectives

NIPHYS I (1993-94)

1) To characterise the types and vertical distribution of feeding roots of spruce in particular with reference to the extent of mycorrhizal infection and the nature of the substrate (i.e. organic and inorganic) with which the roots are primarily associated.

2) Having established the nature (and where possible the taxonomic identity) of the major mycorrhizal types it is proposed to isolate as many of the predominant fungi from the roots so that their abilities to metabolise organic versus NH_4^+ vs NO_3^- can be determined in laboratory studies. Relationships will be sought between abilities to utilise the three primary N sources, and position along the North-South gradient, which is hypothesised to be essentially one of increasing potential for mineralisation, nitrification and inputs of pollutants.

NIPHYS II (1995)

1) To investigate the influence of excess ammonium and nitrate ions upon the assimilation of organic nitrogen by intact aseptically grown ectomycorrhizal tree species.

2) To determine the impact of excess mineral inputs, typical of those said to represent critical loads in forest ecosystems, upon the formation and function of mycorrhiza of boreal forest trees.

3) In the case of those major mycorrhizal types, particularly in the Russulaceae in which the fungal associate has so far proved unculturable, to collect fresh roots supporting these fungi from NIPHYS sites and to incubate them on media containing organic N sources with a view to determining their ability to mobilise the essential N.

General Hypotheses

1) That mycorrhizal fungi in the unpolluted northern part of the gradient preferentially utilise organic N sources and that this ability is susceptible to inhibition in the presence of mineral N sources.

2) That selection in the warmer and more polluted southern parts of the gradient has favoured those fungi which utilise NH_4^+ or NO_3^- preferentially.

3) That boreal forest trees, as a result of infection by some species of ectomycorrhizal fungi, have access to organic sources of nitrogen in their rooting environments.

4) That inputs of mineral nitrogen, in particular the excess mineral N loads characteristic of the many developed parts of Europe, alter physiological and biochemical pathways of N assimilation, and in so doing disrupt the nitrogen economies of the plants.

METHODOLOGY

Community surveys

Four sites were chosen along the N-S gradient for initial intensive study, namely Åheden in Northern Sweden, Klosterhede in Denmark, Waldstein in Germany, and Aubure in France. Initial sampling was restricted to the coniferous stands at each site. Root sampling was carried out in the spring of 1993. Soil cores containing root material were obtained from each site, using a 4.5cm d. soil corer. A known volume of soil was sampled from both the organic and mineral soil fractions. The fine roots (<2mm) were extracted and recorded as either mycorrhizal or non-mycorrhizal. The mycorrhizal tips were then examined in greater detail and where possible the fungal species was identified. Where the species was unknown a brief description was recorded, for future reference. When sufficient material was available, subsamples of each mycorrhizal morphotype found were treated in the following manner: representative tips were stored in gluteraldehyde for further characterisation; tips were frozen for DNA fingerprinting (in conjunction with F. Martin, Nancy); any remaining tips were surface sterilised and incubated on a range of agar media.

In the autumn of 1993, a single visit was made to each site to record and where possible obtain isolates from fruit bodies of ectomycorrhizal fungi occurring on the site. A single visit was also made to Åheden in the Autumn of 1994

Growth of isolates on organic and mineral nitrogen sources

Ten fungal isolates obtained were tested for their ability to use organic and mineral N sources when grown in liquid culture at 256mg l^{-1} N conc. The soluble protein Bovine serum albumin (BSA , MW 67,000, N content 16%) was employed as the major organic N source, while ammonium and nitrate were used as the inorganic sources. The protein was included to provide a measure of the ability of the fungi to utilise soil organic N, while ammonium and nitrate gave an indication of the likely responses of the fungi to soil environments in which ammonification, nitrification and/or pollutant deposition of these N sources were prevalent. By using combinations of organic and mineral sources, the possible impacts of pollutant mineral N deposition upon the mobilisation of organic N from the soil organic matter was determined. Fungal biomass, N utilisation and pH of the culture filtrate were determined at weekly intervals for 6 weeks.

While all ten isolates were examined for the purposes of this report, a representative data set for only two of the fungi are presented. These are a) *Suillus variegatus* and *b)* an isolate of the genus *Tylospora,* both were isolated from Åheden, N Sweden.

In addition to these quantitative determinations, a qualitative measure of the ability of all fungal isolates to mobilise N contained in the plant protein gliadin (MW 30,000, N content 14%) was obtained. Their ability to grow upon and produce clearance zones in an opaque agar medium containing gliadin as the sole N source, with and without added glucose, was examined.

Utilisation of nitrogen sources by intact ectomycorrhizal trees

Experiments were carried out in 9cm plastic Petri dishes. Each contained 20g of acid-washed perlite moistened with 25ml of Melin-Norkrans solution from which nitrogen had been omitted. Pine seedlings (*Pinus sylvestris*) were aseptically germinated and single individuals were transferred to each Petri dish. The stem was placed in a small groove cut in the wall of the dish and sealed in placed with using sterile lanolin so that the roots were grown aseptically in the perlite and the shoots were exposed. At the same time inoculum of *Suillus fluryi* was added to each dish in the vicinity of the seedling root. Uninoculated dishes provided non-mycorrhizal controls. Solutions containing ammonium, nitrate and BSA were then added to give a total of 5.12 mg N per dish. By using combinations of mineral and organic sources, the effect of the mineral N on the assimilation of organic N by the fungus was examined. Harvests were made 2 and 3 months after inoculation. At each harvest date the following seedling parameters were measured: shoot and root dry weight; length of root; number of root tips; number of mycorrhizas formed; total N content of shoot and root.

Only data for seedling total N contents and on the formation of mycorrhizas by *S. fluryi* are in this report.

Protease activity of detached ectomycorrhizas

Protease activity of detached ectomycorrhizas was assayed by incubating 2-5 tips in 0.1M citric acid: sodium phosphate buffer (pH 2.2) in which was dissolved the substrate, 20 µg ml^{-1} of fluorescein isothiocynate-labelled BSA (FITC-BSA). Assays were run for 3 hours at 37°C in a shaking water bath and were terminated by addition of 1ml of 10% trichloroacetic acid. The root tips were then removed, blotted dry and weighed. After centrifugation of the assay buffer at 3000 g for 5 mins, 0.2 ml of supernatant was mixed with 1 ml of 0.4 M Boric acid: NaOH buffer (pH 9.7) and its fluorescence measured. Excitation and emission wavelengths were 495 nm and 516 nm respectively. *Suillus* mycorrhizas from the experiment outlined above were also assayed using this procedure.

RESULTS

Ectomycorrhizal root tip and fruit body surveys

Table 1 shows the results from the spring survey of the ectomycorrhizal root tip populations on each site. A total of 6808 root tips were extracted and examined. The level of infection was high, at all sites, over 90% of all root tips examined being infected. Forty-four different ectomycorrhizal morphotypes were identified in the root samples from the four study sites (Table 2).

A total of 40 ectomycorrhizal species were recorded during the Autumn survey of the fruit bodies (Table 3) with no fungal species being found which fruited at all 4 sites and only two species, *Lactarius rufus* and *Xerocomus badius*, occurring on 3 of the 4 sites.

The two most northerly sites had the greatest number of mycorrhizal fungi both in terms of the populations identified from the mycorrhizal tips and from the fruit bodies. A better estimate of the number of species present on each site was obtained by combining the data from the mycorrhizal and the fruit body surveys and only recording a species once if it occurred in both lists. This is shown in column 6 of Table 1. The 2 more northern sites had considerably more species than the other 2 sites. Klosterhede and Åheden both had 19 types, Aubure had 18 and Waldstein had the lowest with only 12 types.

The Shannon-Wiener Diversity function was calculated using the mycorrhizal data. Waldstein had the lowest diversity and Åheden the highest.

The structure of the mycorrhizal populations, in terms of the percentage of tips infected by each morphotype, is shown in Figure 1. The high diversity index at Åheden is reflected in the community structure with no single type making up more than 20% of the population and there were 10 species which each made up between 5-20% of the population. E-type mycorrhizas

Table 1. Results from the survey of the ectomycorrhizal populations at four sites along the north-south transect (Spring, 1993).

Site	Number of root tips examined	Level of infection (%)	No. of mycorrhizal types	No. of species recorded as fruit bodies	Combined number of species*	Shannon-Wiener diversity function
Åheden	1311	99.3	19	18	33	3.55
Klosterhede	1873	90.7	19	14	35	3.34
Waldstein	1114	98.6	14	7	17	2.57
Aubure	2610	99.1	18	12	23	3.29

* The combined number of species is the total number of species from a site where species common to both the root tip and the fruit body surveys are counted only once.
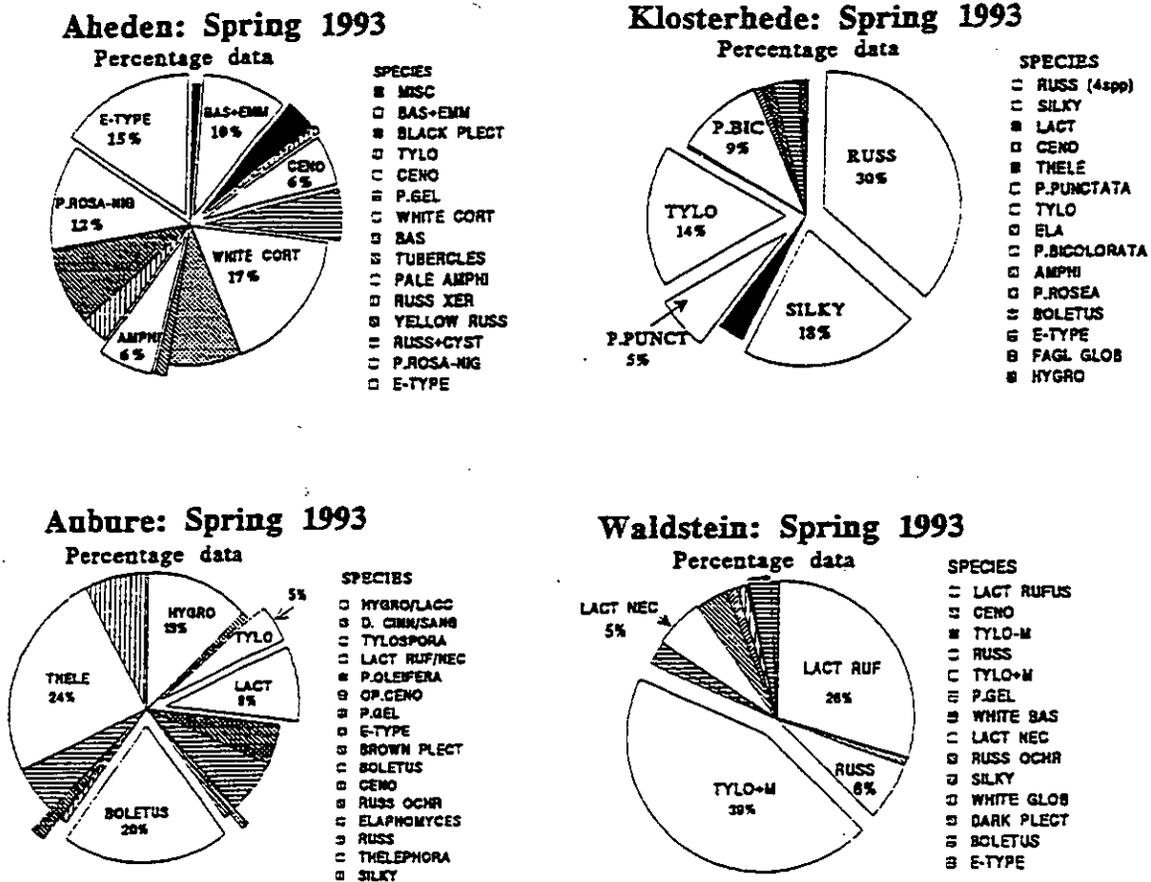
Figure 1. Species composition of the ectomycorrhizal populations at each of 4 study sites along a north-south European transect.

Key:- AMPHI-*Amphinema*, BAS-Unknown basidiomycete, CENO-*Cenococcum*, FAGI-Fagirhiza globulifera, HYGRO-*Hygrophorus*, LACT-*Lactarius*, P-Piceirhiza, PLECT-Unknown plectynchymatous, RUSS-*Russula*, SILKY-Unknown, TYLO-*Tylospora*.

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Table 2 Occurrence of ectomycorrhizal species and morphotypes in root samples collected, under Norway spruce, at four study sites along a north-south European transect. (Sites: Åheden - Northern Sweden; Klosterhede - Western Jutland, Denmark; Waldstein - Fichtelgebirge, Germany; Aubure - Vosges Mountains, NE France)

Species/type (Total number=44)	Study sites			
	Aubure (18)	Klosterhede (19)	Aheden (19)	Waldstein (14)
(cf) Amphinema		+		
(cf) Tylospora				+
Cenococcum geophilum	+	+	+	+
Cortinarius spp.			+	
Dermocybe cinnamomea	+			
D. cinnamomeobadia	+			
E-type	+	+	+	+
Elaphomyces sp.	+	+		
Fagirhiza globulifera		+		
F. setifera			+	
Hygrophorus pustulatus	+	+		
Laccaria sp.	+			
Lactarius (cf) necator				+
L. rufus	+			+
Lactarius sp.		+		
Picierhiza bicolorata		+		
P. gelatinosa	+		+	+
P. oleiferans	+			
P. punctata		+		
P. rosa-nigrescens			+	
P. rosea		+		
Piloderma croceum			+	
Russula ochroleuca	+			+
R. (cf) xerampelina			+	

Table 2 (continued)

Species/type (Total number=44)	Study sites			
	Aubure (18)	Klosterhede (19)	Aheden (19)	Waldstein (14)
Russula 1	+			
Russula 2		+		
Russula 3		+		
Russula 4		+		
Russula 5		+		
Russula 6			+	
Russula 7			+	
Russula 8			+	
Russula 9				+
Thelephora sp.	+	+		
Suillus sp.			+	
Tylospora sp.	+	+	+	+
Xerocomus spp.	+	+		+
Unknown 1			+	
Unknown 2			+	
Unknown 3			+	
Unknown 4			+	+
Unknown 5	+			
Unknown 6			+	
Unknown 7	+			
Unknown 8			+	
Unknown 9	+	+		+
Unknown 10		+		
Unknown 11				+
Unknown 12				+

Table 3 Ectomycorrhizal fungi recorded as fruit bodies in Autumn 1993 under Norway spruce in four study plots along a north-south European transect. (Sites: Åheden - Northern Sweden; Klosterhede - Western Jutland, Denmark; Waldstein - Fichtelgebirge, Germany; Aubure - Vosges Mountains, NE France)

Fungal species	Åheden	Klosterhede	Waldstein	Aubure
Amanita spissa var. excelsa	-	-	-	+
Cantharellus tubaeformis	-	+	-	-
Chroogomphus rutilus	+	-	-	-
Cortinarius anomalus	+	-	-	-
C. collinitus	-	+	-	-
C. gentilis	+	-	-	-
C. integerrimus	+	-	-	-
C. laniger	+	-	-	-
C. ochrophyllus	+	-	-	-
C. cf. paragaudis	+	-	-	-
Dermocybe cinnamomea	-	-	-	+
D. cinnamomeobadia	-	-	-	+
D. crocea	+	-	-	-
D. semisaguineus	+	-	-	-
Hebeloma (cf) claviceps	-	+	-	-
H. mesophaeum	-	-	-	+
H. (cf) pumilum	-	+	-	-
Hydnum repandum	-	+	-	-
Hygrophorus olivaceoalbus	+	-	+	-
H. piceae	+	-	-	-
H. pustulatus	-	-	+	+
Laccaria amethystina	-	+	-	-
L. (cf) bicolor	-	-	-	+
Laccaria (cf) laccata	-	+	+	+
Lactarius fuscus	+	-	-	-

Table 3 (Continued)

Fungal species	Åheden	Klosterhede	Waldstein	Aubure
L. necator	-	+	-	+
L. rufus	+	-	+	+
L. theiogalus	-	+	-	-
Paxillus involutus	-	+	+	-
Russula aeruginea	-	+	-	-
R. decolorans	+	-	-	-
R. emetica var. silvestris	-	+	-	-
R. ochroleuca	-	-	+	+
R vinosa	+	-	-	-
Tricholoma flavovirens	+	-	-	-
T. inamoenum	+	-	-	-
T. portentosum	-	+	-	-
Suillus variegatus	+	-	-	-
Xerocomus badius	-	+	+	+
X. chrysenteron	-	-	-	+
Total number (40)	18	14	7	12

were the second most commonly found mycorrhizal type colonising 15.7% of the roots. At Klosterhede, nearly one third of the tips were infected by four species of the genus *Russula* and only 6 types made up more than 5% of the community. The ectomycorrhizal community with the greatest unevenness and the lowest species richness was Waldstein, where nearly two thirds of the tips were infected by *Tylospora* species. This is in marked contrast to the other sites where *Tylospora* infected between 1-14% of the root tips. The large number of tips infected by *Thelephora* at Aubure is partially a result of a sampling artefact, as most of the *Thelephora* mycorrhizas found on the site were recovered from a single sample.

A total of 66 isolates were obtained from surface sterilised ectomycorrhizas. Over half of these came from Åheden. The types occurring here appeared to be more amenable to growth in culture than types from other sites. Out of a total of 19 morphotypes found, 13 were successfully cultured. Analysis of the recovery rates using χ^2 showed that the rates are not independent of site and that more isolates were obtained from Åheden than would be reasonably expected (χ^2=14.04, p<0.01) if all morphotypes had an equal chance of growing. In addition to the root isolates, a further 22 isolates were obtained from fruit bodies, giving a total of 88 isolates from 27 species.

Growth of isolates on Gliadin

When glucose was included with the gliadin in the medium, 93% or 14 out of the 15 species isolated from the Åheden site could utilise the gliadin (Table 4). In contrast to this, none of the isolates from the Waldstein site could use the protein and on the other two sites between 55 and 70% of the species could use the gliadin as a nitrogen source. In the absence of glucose, the numbers of species which could use the gliadin was markedly reduced but the species from Åheden still had the greatest ability to use the gliadin both as a source of nitrogen and carbon. There was some evidence to suggest that protease activity of isolates derived from ectomycorrhizas may be related to the substrate in which the ectomycorrhiza was originally found (Table 5). All of the isolates which came from mycorrhizas which were found only in the organic fractions of samples, could use the gliadin. But only about half of the isolates which came from mycorrhizas found in both the organic and mineral soil fractions showed protease activity.

Table 4 The ability of ectomycorrhizal fungal isolates to utilise gliadin, an organic nitrogen source, in relation to the substrate from which the isolates originated. Isolates obtained from ectomycorrhizas and fruit bodies from four spruce sites along a North-South European transect. (Sites: Åheden - Northern Sweden; Klosterhede - Western Jutland, Denmark; Waldstein - Fichtelgebirge, Germany; Aubure - Vosges Mountains, NE France)

Site	Isolate/species	Substrate[1]	Growth[2]	
	M= mycorrhizal isolate F= fruit body isolate		Organic N + glucose	Organic N - glucose
Åheden	Dermocybe semisanguinea (F)	n/a	+	+
	Hebeloma subsaponaceum (F)	n/a	+	-
	Suillus variegatus (F)	n/a	+++	-
	Tricholoma flavovirens (F)	n/a	+	+
	Cenococcum geophilum (M)	Org	+	-
	Cortinarius sp 1 (M)	Min	+	-
	Cortinarius sp 2 (M)	Org	+	+
	Suillus sp. (M)	Org/Min	++	-
	Tylospora fibrillosa (M)	Org/Min	(+)	-
	E-Type (M)	Org/Min	+++/-	++/-
	Unknown 1 (M)	Org/Min	+	+
	Unknown 3 (M)	Min	+	+
	Unknown 4 (M)	Org/Min	+++	+++
	Unknown 8 (M)	Org	(+)	(+)
	Unknown 12(M)	Min	+	-
Klostehede	Hebeloma sp.(F)	n/a	+++	+++
	Laccaria amethystina (F)	n/a	+	-
	Xerocomus badius (F)	n/a	+	-
	Cenococcum geophilum (M)	Min	+/-	+/-
	Picierhiza punctata (M)	Org/Min	-	-
	Russula sp (M)	Org	+	-
	Tylospora fibrillosa(M)	Org/Min	(+/-)	-

Table 4 (continued)

Site	Isolate/species	Substrate[1]	Growth[2]	
	M= mycorrhizal isolate F= fruit body isolate		Organic N + glucose	Organic N - glucose
Waldstein	Lactarius rufus (F)	n/a	(+)	-
	Paxillus involutus (F)	n/a	-	-
	Xerocomus badius (F)	n/a	(+)	-
	Tylospora fibrillosa (M)	Org/Min	(+/-)	-
Aubure	Amanita spissa (F)	n/a	+	+
	Hebeloma mesophaeum (F)	n/a	+++	++
	Xerocomus badius (F)	n/a	-	-
	Cortinarius sp (M)	Org	(+)	-
	E-Type (M)	Org	++	++
	Piceirhiza gelatinosa (M)	Org/Min	+	-
	Tylospora fibrillosa (M)	Org/Min	+	-

Key
[1] Soil fraction in which ectomycorrhizal tips were found, Org = Organic, Min = Mineral, n/a = not applicable
[2] Growth of isolate, after 1 month, on soild agar medium containing the insoluble plant protein gliadin as the sole nitrogen source, ± glucose as a carbon source. (+) slight growth; + < 3cm; ++ 3-6cm; +++ > 6cm; / intraspecific differences between isolates of the same species.

Table 5 Ability of ectomycorrhizal isolates to show growth after 1 month on agar containing gliadin as the sole N source, in relation to the soil fraction from which the isolates originated.

Substrate from which isolate originated	Number of isolates which showed growth on gliadin	Number of isolates which showed no growth on gliadin
Organic	5	0
Mineral	1	1
Organic + mineral	6	8

Utilisation of organic and mineral N sources by a) *Suillus variegatus,* **b)** *Tylospora fibrillosa* **UT1**

a) *Suillus variegatus*

Highest yields were produced on the ammonium/protein combination (Fig 2a), the NH_4/NO_3 treatment, while being less effective also gave high yields. The decline in yield at the later harvests in these two treatments is probably exhaustion of exogenous carbon and consequent loss of tissue biomass through respiratory use of substrate. Of the single sources, although all were significantly less good than the combined N treatments, NH_4 and BSA produced the greatest yields.

The profile of pH changes (Fig 2b) shows the expected rapid reduction of pH associated with the utilisation of NH_4-N and this acidification probably facilitates the proteolysis of the BSA and hence the high yields seen in the NH_4/BSA treatment. The reduction in the pH associated with the utilisation of nitrate in the NO_3 and the NO_3/BSA treatments is unexpected and may be a result of organic acid release from the mycelium.

The utilisation of ammonium preferentially over the nitrate in the NO_3/NH_4 treatment (Fig 2c) was a common feature of all the isolates tested. The utilisation of BSA, as indicated by the BSA remaining in the culture solution (Fig 2d), in NO_3/BSA and in the NH_4/BSA treatment does not appear to be significantly affected by the presence of the mineral N sources. However, the amount of BSA remaining in the NO_3/BSA treatment at the first harvest date is significantly greater than in the NH_4/BSA treatment.

b) *Tylospora* isolate

The highest yields, though considerably less than those produced by *S. variegatus*, were again produced on the NO_3/NH_4 and the NH_4/BSA treatments (Fig 3a). It is likely that the final dry weight yields of this isolate, in most of the treatments, will be greater than those shown, since the slow growth rate of this isolate meant that the stationary phase of the growth had not been reached. Yields were poorest on nitrate and of the single N sources NH_4 gave the greatest yields.

The pH profiles were predictable with decreases with NH_4-N utilisation and increases in pH with NO_3-N utilisation (Fig 3b).

In the NO_3/NH_4 treatment, ammonium was again preferentially utilised over the nitrate (Fig 3c). There was a marked decrease in the utilisation of the BSA by the Tylospora isolate when NO_3 was included with BSA in the treatment, but not when NH_4 was present (Fig 3d).
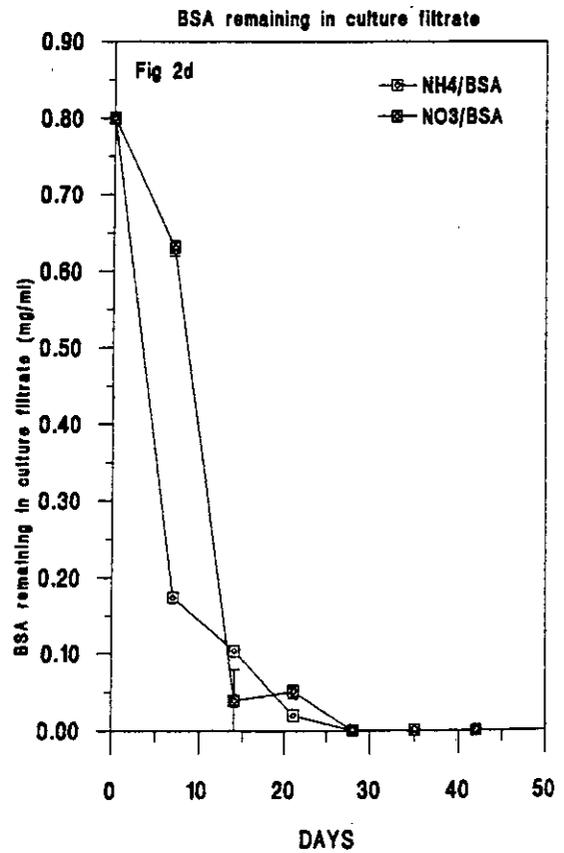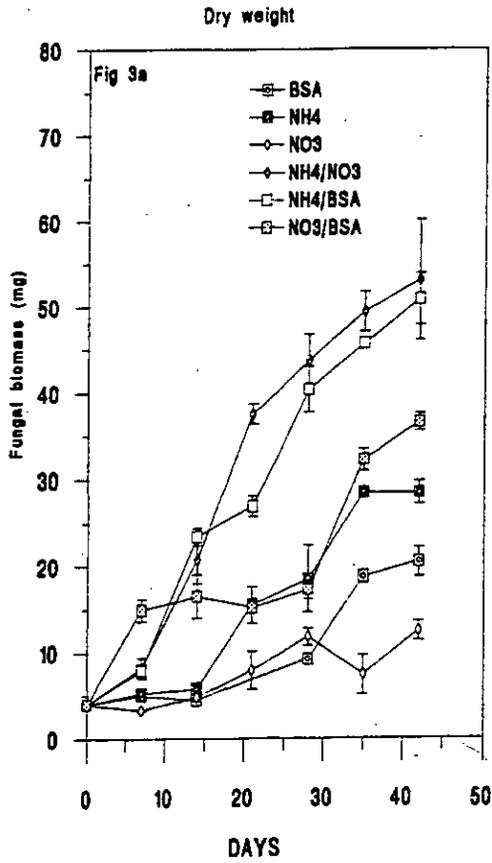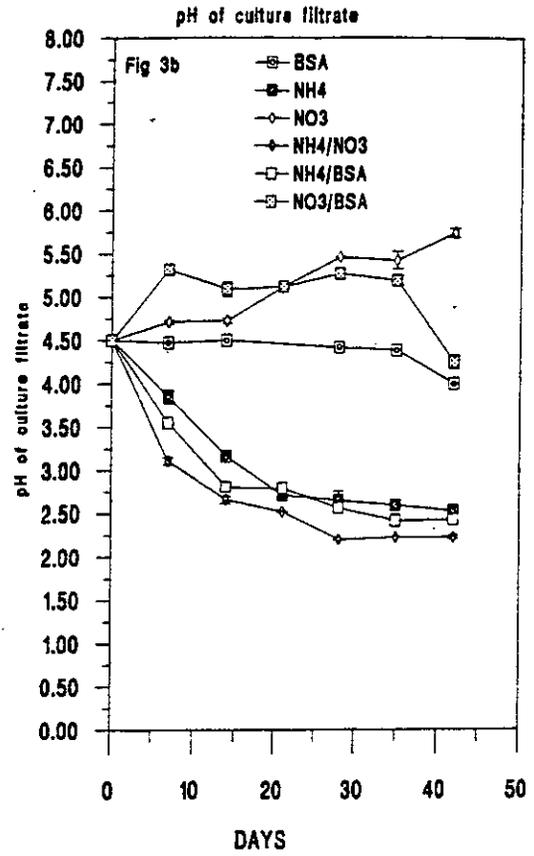
Figure 2 Growth of *Suillus variegatus* on a range of nitrogen sources.
Fig 2a - Dry weight; Fig 2b - pH of culture filtrate; Fig 2c - Nitrogen remaining in culture filtrate, when grown on nitrate and ammonium; Fig 2d - BSA remaining in culture filtrate
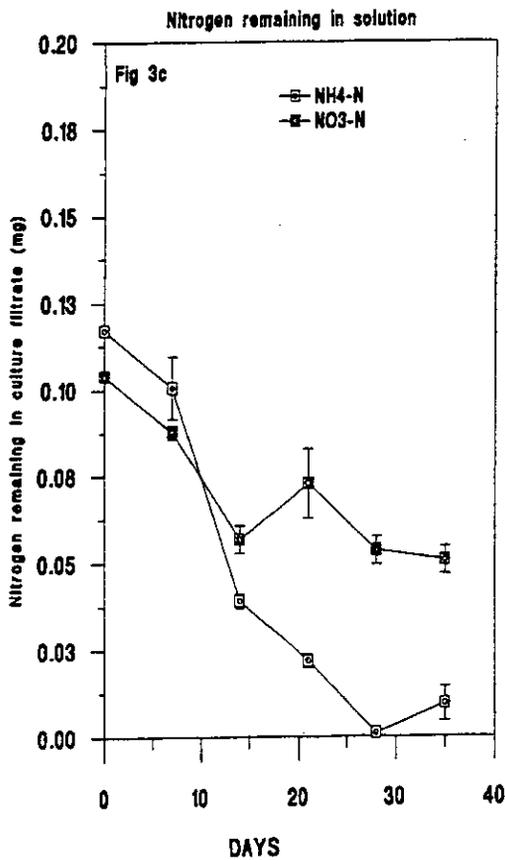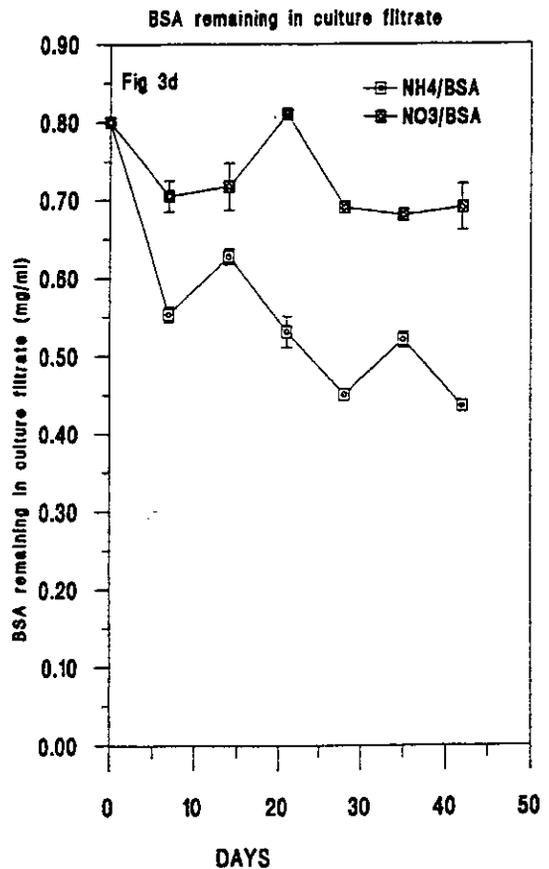
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Figure 3 Growth of Tylospora isolate UT1 on a range of nitrogen sources.
Fig 3a - Dry weight; Fig 3b - pH of culture filtrate; Fig 3c - Nitrogen remaining in culture filtrate when grown on ammonium and nitrate; Fig 3d - BSA remaining in culture filtrate

Utilisation of nitrogen sources by intact ectomycorrhizal trees

Plants of *P. sylvestris* colonised by *S. fluryi* were able to use organic N provided in the form of protein (Fig 4a). After 8 weeks their N contents were significantly greater than those of seedlings grown with NO_3 as sole N source, yet were similar to those of the NH_4 treatment. Clearly, protein or NH_4 are preferred N sources for this mycorrhizal fungus. Furthermore, addition of NO_3 to the organic N treatment (NO_3/BSA) significantly reduced the N content of the plants suggesting that NO_3 inhibits the ability of mycorrhizal plants to assimilate organic N. Ammonium, when added to the organic N source (NH_4/BSA) had no such inhibitory effect. The apparently inhibitory effect of NO_3 upon BSA utilisation was still evident after 12 weeks (Fig 4a). There was a reduction in N content of seedlings grown on organic N at this stage in relation to the 8 weeks harvest. This is attributed to competition between plant and fungus for the declining pool of N. When both NH_4 and protein were accessible to the plants, no such reduction occurred.

Interpretation of these events is further assisted by consideration of the extent of mycorrhizal colonisation in the treatments (Fig 4b). A striking feature of the results is that whereas plants in the organic N treatment (BSA) were colonised extensively by the fungus, none of the plants in any of the treatments containing NO_3 had formed mycorrhizas. Ammonium did not have this suppressive effect on the colonising ability of the fungus. This helps to explain the reduced N contents of plants in the NO_3/BSA treatment, since in the absence of colonisation the plants would not be expected to assimilate protein N.

Protease activity of detached ectomycorrhizas

It proved very difficult to detect protease activity using the field mycorrhizas. The primary reason for this appeared to be the high affinity of the surface of mycorrhizas for the FITC-BSA substrate. Measurements of the loss of substrate from the assay buffer solution (Fig 5a) showed clearly that the loss of fluorescence was significantly associated with fresh weight of root material. Surface area, which was not measured, will be more important in determining the extent of substrate binding than actual fresh weight and this will vary considerably with the degree of branching. A comparison of the loss of substrate, through binding, with mycorrhizal surface area would be a better indicator of binding than fresh weight. Attempts to elute the substrate from the surface of the mycorrhizas by switching the isoelectric point of the substrate failed to release any of the bound material. So it was not possible to determine if any proteolysis of the FITC-BSA had taken place.

Ectomycorrhizas formed by *Suillus fluryi* on pine in the BSA treatment in the experiment described above were also assayed and significant proteolytic activity was measured (Fig 5b). This clearly indicates that the mycorrhizal tips on the roots of the seedling were directly involved in the mobilisation of the organic N.

Figure 4a. Nitrogen contents (mg) of pine seedlings inoculated with Suillus fluryi and grown for 8 and 12 weeks on a range of nitrogen sources. Harvest means with different letters are significantly different at p=0.05, *- denotes greater than seed N
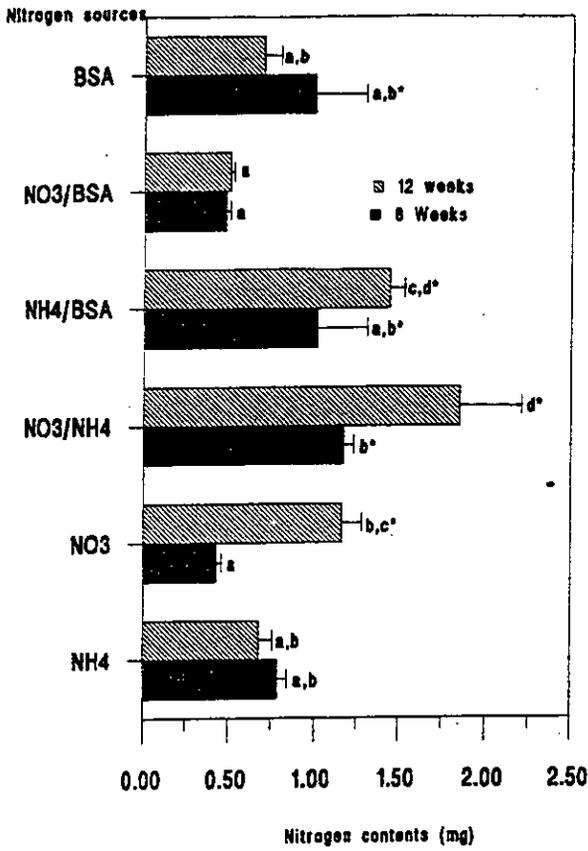

Nitrogen contents (mg)

Figure 4b. Numbers of tuberculate mycorrhizas on pine seedlings inoculated with Suillus fluryi and grown for 8 and 12 weeks on a range of nitrogen sources
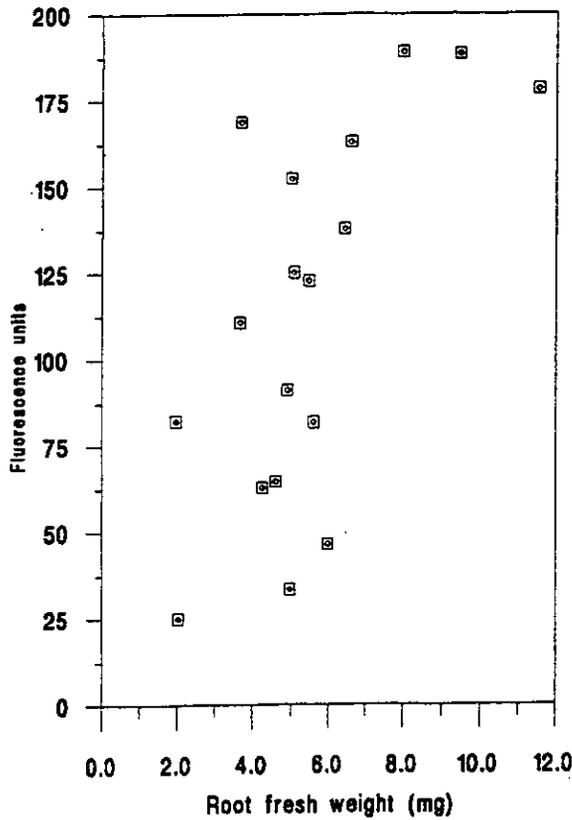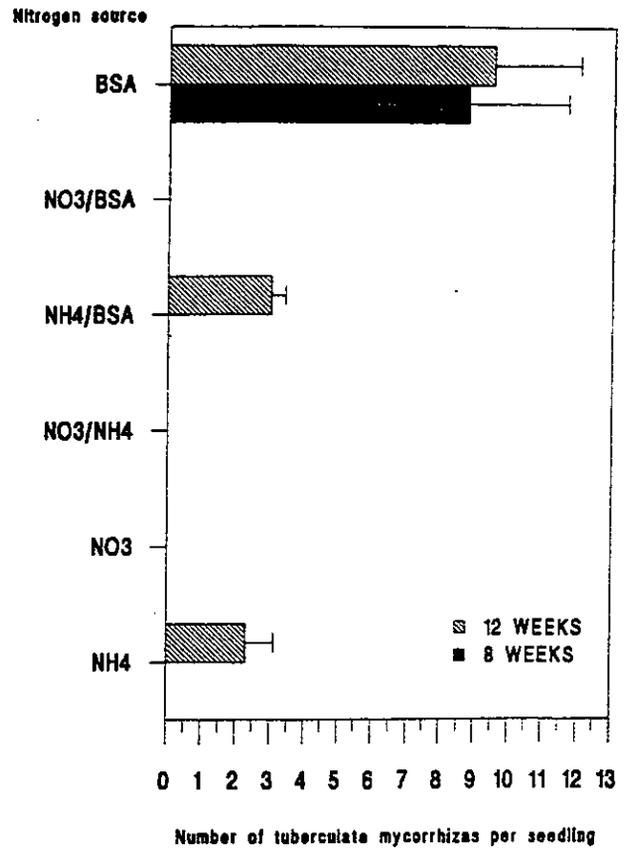

Number of tuberculate mycorrhizas per seedling

Root fresh weight (mg)

Figure 5a. Loss of fluorescence from protease assay buffer due to binding of substrate (FITC-BSA) onto ectomycorrhizal surface

Root material
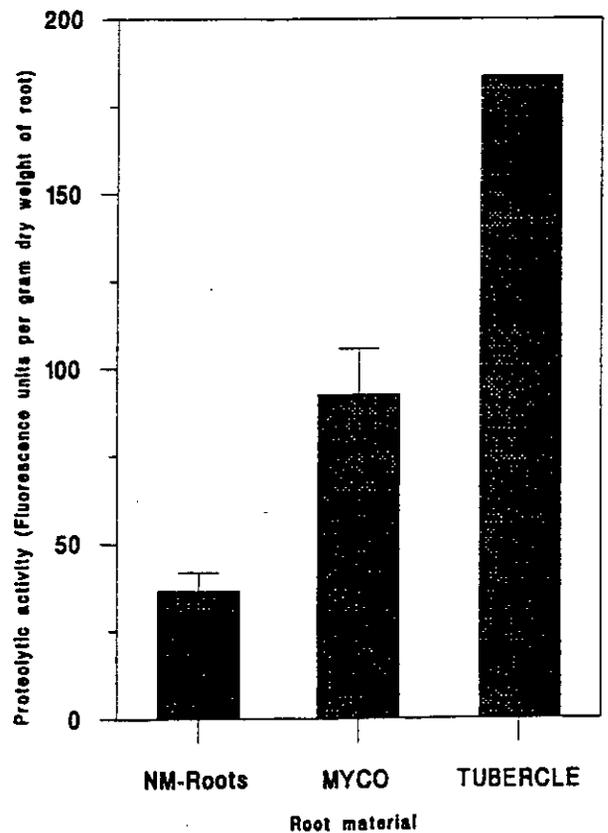
Figure 5b. Proteolytic activity of mycorrhizal (myco) and non-mycorrhizal (NM-roots) root tips from pine seedlings grown on BSA as the sole nitrogen source. (Tubercle = Tuberculate mycorrhiza)

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SUMMARY OF CONCLUSIONS

SCIENTIFIC

- Over 90% of all root tips at all sites are ectomycorrhizal.

- The plant is therefore heavily dependent upon mycorrhizal fungi for the acquisition and primary assimilation of nitrogen.

- The taxonomic diversity of fungi involved in mycorrhiza formation is large, but is significantly greater in the north than in the south of the NIPHYS gradient.

- This decline in diversity, towards the south of the gradient, is accompanied by increases in single species dominance and is paralleled by changes in the nitrogen nutrition of the fungi involved.

- Characteristic fungal species of northern sites, which are able to utilise organic N, decrease in number as availability of mineral N increases towards the south.

- Nitrate can adversely affect the ability of some fungi, typically those of the northern sites, to form mycorrhizas.

- The addition of nitrate to sources of organic N adversely affects the ability of some mycorrhizal fungi to utilise organic nitrogen.

POLITICAL

- Increasing inputs of mineral nitrogen to forest ecosystems will lead to disturbances in mycorrhizal function which are likely to have far-reaching consequences upon tree nutrition as well as on the dynamics of fungal and plant communities. Amongst these consequences, a decline in biodiversity of the symbiotic fungal community can be predicted to reduce resilience of forest ecosystems, an effect which will predispose them to the decline in vigour increasingly observed throughout Europe.

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      Research Area: Aerial Uptake and $\delta\ ^{15}N$ in Pollutant Inputs of Forests

1. Introduction

A number of studies are showing that the processes of nitrogen cycling in forest systems can be monitored sensitively and economically by investigating variations in the $\delta^{15}N$ in forest components (eg Garten, 1992, 1993; Gebauer and Dietrich, 1993; Schulze et al, 1994). They are also beginning to indicate the potential of these analyses to determine the impacts on forests of pollutant nitrogen from a variety of sources (Gebauer and Schulze, 1991; Gebauer et al, 1994; Hedin, 1994). Progress in this latter area of research is limited by the scarcity of data on the $\delta^{15}N$ of pollutant inputs in rainfall, aerosols and gases, but from the small amount available in the literature, it would seem likely that the sources might exhibit quite wide and potentially useful variations. The current studies have been undertaken

- to provide data on pollutant inputs to some European and the UK transect forests, and
- to integrate the data with $\delta^{15}N$ data of tree foliage and root systems obtained by NiPHYS project colleagues Schulze and Högberg with the aim of improving understanding of the role of pollutant inputs in tree nitrogen nutrition.

2. Objectives of the overall project are :

- To design and test suitable methods for the collection of N in rainfall and throughfall.
- To check methods for the determination of N and $\delta^{15}N$.
- To determine the $\delta^{15}N$ of N in rainfall and throughfall along European and UK transects.
- To assess the potential for the uptake of N by the tree canopy from examination of the ^{15}N isotopic mass balance.

    To determine whether or not agricultural and industrial sources of pollution can be distinguished by their $\delta^{15}N$ signatures.

3. Working Hypotheses

As a working basis for the project, two hypotheses have been developed. These are summarised as :

1. The changes in the $\delta^{15}N$ of N of ammonium-N and nitrate-N in rainfall and throughfall in relation to the $\delta^{15}N$ in foliage can be used to understand the processes of pollutant N deposition and quantify direct uptake of pollutant nitrogen into the canopy of forests,

2. The $\delta^{15}N$ signature of ammonium-N and nitrate-N in rainfall and throughfall can be used to distinguish agricultural from industrial pollutant sources.

4. Experimental strategy

4.1 Background

For both the hypotheses to hold, there has to be some consistency or identifiable patterns of the $\delta^{15}N$ signatures of the inputs of ammonium-N and nitrate-N in rainfall and throughfall, foliar $\delta^{15}N$ composition and the source origins of the N pollutants. Spatial variation in $\delta^{15}N$ of N inputs are to be expected, and seasonal variation has already been shown to occur (Freyer, 1978; 1991; Heaton 1987).

Variations in $\delta^{15}N$ in the throughfall relative to rainfall may result from a number of processes including dry deposition onto the forest canopy from the atmosphere, leaching of nitrogen from the foliage or uptake of N from the precipitation into the foliage. It could also be influenced by less predictable processes such as the dissolution of bird droppings and insect frass. Apart from some similar research currently being conducted by ITE for the UK Department of the Environment (Ineson et al, in prep) and the limited set of American data from Garten 1992, there is little information on the patterns $\delta^{15}N$ variations in rainfall and throughfall for forests. The main approach of this research has therefore been to examine the spatial and seasonal patterns of variation of $\delta^{15}N$ in rainfall and throughfall and foliage.

4.2 Use of forest transects

In keeping with the principal strategy of the NiPHYS research programme, $\delta^{15}N$ variations in rainfall and throughfall for forests along the main European transect have been investigated. However, because of the practical limitations imposed by the available technology (see methods section below) and by the funds available for travel or the return of samples, a UK forest transect and a 'within site' local study at ITE's Merlewood Research Station have been added to study seasonal variation and possible relationships between the $\delta^{15}N$ of pollutant inputs and various forest components. Details of the sites have been given in previous NiPHYS contract reports.

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4.3 Methods used

The first year's work in the UK involved the development of standardised rainfall and throughfall collectors able to collect adequate amounts of water, enough to contain about 5 mg of both NH_4-N and NO_3-N; for all but the very polluted sites, this amounted to around 5 litres per sample. Thymol was used to minimise microbial activity in the water samples during the collection period (Gillett & Ayres, 1991), which on occasions lasted for up to 3 months because of low rainfall and or low pollutant N concentrations at certain European sites. The analytical procedures were developed from method of Downs (Marine Biology Labs. Woods Hole, Mass. USA, unpublished) and extensively tested. Before processing, rain and throughfall samples were examined for contamination with bird-droppings by testing for phosphate; contaminated samples were rejected. Samples have been processed and analysed for $\delta^{15}N$ as described in an earlier report, namely by filtration, concentration on resins, elution, distillation, drying down and measurement on a Europa Roboprep/Tracermass mass spectrometer. All laboratory processes were checked for accuracy and precision by interspersing the collected samples with internal standards of ammonium nitrate of known signature.

5 European transect

5.1 Sites studied and sampling programme

The European sites covered the complete climatic transect from Aheden in Sweden to Thezan in southern France. All the forests were spruce except for Thezan which was pine. Estimates of the wet deposition of pollutant N varied from c 3 kg N ha^{-1} yr^{-1} for Aheden in northern Sweden to c 19kg N ha^{-1} yr^{-1} for the Jezeri forest in the Czech Republic. Total inputs, including dry deposition to the forest canopy and gaseous inputs, could be at least twice as high, possibly higher; dry deposition and gaseous concentrations have not been determined for the transect sites.

As the $\delta^{15}N$ has been shown elsewhere to vary with season, it was considered preferable to standardise the time of collection along the European transect. However, although attempts were made to collect rain and throughfall during the summer months, this proved to be impossible during 1995, because of an exceptionally dry year. Collection was therefore delayed and rearranged to take place between October and December. Seasonal variation patterns were studied in the UK, where on average it rained more often and where it has been practical to obtain more frequent samples. Results are given below in section 7.

5.2 Results and Discussion

The data on the $\delta^{15}N$ composition (°/oo) and the concentration of NH_4-N and NO_3-N in the rainfall and throughfall of the six studied forest sites of the European transect are given in Table 1.

The $\delta^{15}N$ data show that there appears to be a consistent pattern of results for the individual forest sites, though more samples would be necessary to confirm this. Individual forests along the European transect appear to have inputs with different $\delta^{15}N$ signatures, particularly with respect to NH_4-N inputs.

5.2.1 Ammonium-N

From a scatter plot of $\delta^{15}N$ in NH_4-N versus concentration of NH_4-N (Figure 1), it is clear that almost all throughfall samples have significantly higher concentrations than rainfall samples. The data also show that the $\delta^{15}N$ signatures have shifted signficantly negative in throughfall compared to rainfall (Figure 2). Theoretically, the physical process of deposition on the forest canopy should have resulted in more positive $\delta^{15}N$ throughfall than rainfall (Heaton, 1987) and if canopy uptake was also taking place, there should be a further $\delta^{15}N$ shift to the positive, as the lighter isotope would be preferentially taken up. Neither of these positive shifts have been detected. Foliage N in all the European forest sites was found to be $\delta^{15}N$ negative (Figure 2 - foliar $\delta^{15}N$ data provided by G Bauer, University of Bayreuth), so the more negative throughfall could be accounted for by canopy leaching, assuming any isotopic fractionation during the leaching processes would favour release of the lighter isotope. Acidic deposition onto the forest canopy could have leached sugnificant amounts of NH_4-N into the throughfall, if the pH is low enough (Turner and van Broekhuizen, 1992); the pH of throughfall in polluted sites in the UK is sufficiently low to induce this leaching (Crossley et al 1992). Further, the potential for leaching could have been enhanced as a result of cuticle erosion caused by acidic deposition; eipculticular waxes are often degenerate in polluted sites (Crossley and Fowler, 1986; Riederer, 1989). An important point is that the rainfall and throughfall samples were collected during the autumn/ winter period, when needle metabolism would be low and some catabolism of protein may be taking place to release ammonium-N within the needle cells. Nevertheless, there are suggestions from concentration data alone, that there might have been uptake by the forest canopies at Åheden and Aubure as throughfall concentrations were lower than those of the rainfall.

Table 1. Isotopic $\delta^{15}N$ composition (‰) and concentration of NH_4^+ and NO_3^- (ppm N) in rainfall and throughfall sampled between October 1994 and December 1995 at six European forest sites.

Date Forest	Ammonium-N in Rainfall		Throughfall		Nitrate-N in Rainfall		Throughfall	
1994	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Aheden	<0.1	-1.04	0.2	-4.19	0.1	-4.62	*Insufficient nitrogen*	
	<0.1	-1.22	*Insufficient nitrogen*		0.1	-5.36		
	<0.1	-0.03	*Insufficient nitrogen*		0.1	-5.55		
Klosterhede	0.4	+7.51	1.8	-12.69	0.4	-0.58	1.3	-0.06
	0.4	+4.33	2.0	-11.66	0.5	-	1.3	+1.43
	Collector leaked		-	-	*Collector leaked*		1.3	+1.09
Waldstein	0.3	+14.95	1.3	-10.8	0.25	+0.24	2.4	+1.4
	0.4	+11.84	1.3	-12.92	0.25	+0.82	2.8	+1.35
					Collector leaked		2.5	+1.36
Jezeri	1.1	-7.0	*Contaminated with bird droppings*		0.6	+1.7	*Contaminated with bird droppings*	
	1.1	-7.46			0.6	+1.96		
Aubure	0.2	-1.63	0.2	+1.22	0.15	-1.82	1.8	+0.4
	0.15	-1.72	*Insufficient nitrogen*		0.15	-1.19	1.5	+0.22
	0.1	-1.24	*Insufficient nitrogen*		0.15	-1.46	0.9	+1.00
Thezan	0.6	-5.44	2.5	-7.93	0.6	-2.84	3.7	-0.36
	0.6	-4.77	1.6	-3.51	0.6	-1.66	2.7	-1.53
	0.9	-1.48	2.5	-8.87	0.6	-1.47	5.5	-0.53
1995	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Aheden	*Insufficient nitrogen*				*Insufficient nitrogen*			
Klosterhede	0.9	-12.65	2.6	-16.05	0.8	+0.84	1.3	+1.41
	(3 samples bulked)		*(3 samples bulked)*		*(3 samples bulked)*		*(3 samples bulked)*	
Waldstein	*Samples unable to be collected because of adverse weather conditions*				*Samples unable to be collected because of adverse weather conditions*			
Jezeri	*Samples unable to be collected for personal reasons*				*Samples unable to be collected for personal reasons*			
Aubure	0.1	-6.02	0.15	+0.4	0.15	-1.37	0.8	+1.14
	0.15	-6.46	0.2	-3.46	0.2	-1.51	0.7	+0.82
	Insufficient nitrogen		*Insufficient nitrogen*		*Insufficient nitrogen*		0.4	+2.79
Thezan	0.2	-3.69	0.3	-7.33	0.4	-0.15	0.6	-0.37
	0.2	-0.04	0.25	-7.90	0.4	-0.88	0.6	-1.05
	0.2	-1.28	*Insufficient nitrogen*		0.4	+0.25	*Insufficient nitrogen*	

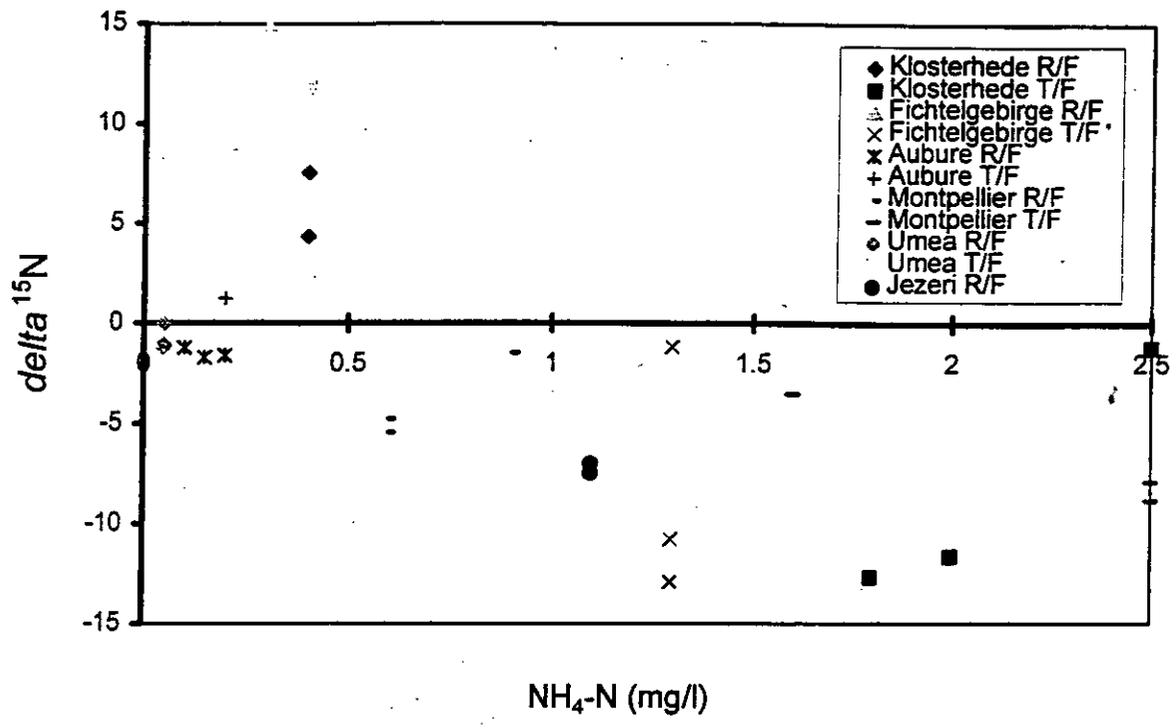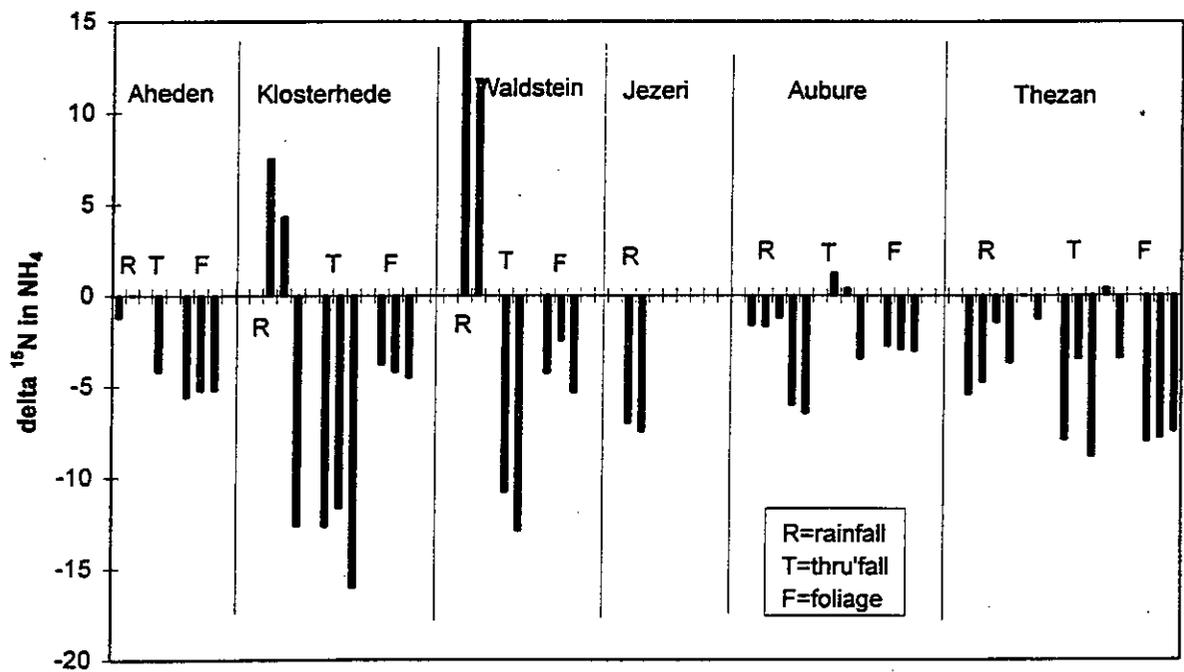Figure 1 Plot of delta^{15}N against NH_4-N concentration for the European spruce/pine forest transect

Figure 2 Plot of the delta ^{15}N in NH_4-N in rainfall and throughfall, and ^{15}N in foliage for the European transect spruce/pine forest sites.

5.2.2 Nitrate-N

The $\delta^{15}N$ values (Table 1) for NO_3-N, though varying overall less than NH_4-N, also appeared to differ between forest sites of the transect, and between rainfall and throughfall. From a scatter plot of $\delta^{15}N$ in NO_3-N versus concentration of NO_3-N (Figure 3), it is clear that the throughfall samples have higher concentrations than the rainfall samples, but in contrast to the results for NH_4-N, the $\delta^{15}N$ signatures have generally shown a signficant positive shift (Figure 4). This change would conform to expectation in relation to either of the processes of dry deposition or canopy uptake (Heaton, 1987). Acidic deposition has the potential to induce NO_3-N leaching from the canopy (Turner and van Broekhuizen, 1992), but this process may not happen with nitrate during the autumn/winter period, as there is possibly little free nitrate present in needle cells at that time. This latter point needs investigation.

6 UK transect

6.1 Sites studied and sampling programme

As indicated in previous reports, four sites were selected for study. Two were adjacent to 'industrial' sites at Knowsley Park near Liverpool and the other beside junction 39 (Shap) of the M6 motorway in Cumbria, and two were adjacent to 'agricultural' sources one at a dairy farm (Glassonby) and the other near to a large chicken farm at West Linton, near Edinburgh.

6.2 Results and discussion

The data on the $\delta^{15}N$ composition ($^o/oo$) and the concentration of NH_4-N and NO_3-N in the rainfall and throughfall of the forest sites of the UK transect are given in Table 2.

6.2.1 Ammonium-N

The variations in the $\delta^{15}N$ in NH_4-N for the UK forest sites show as large a range as found for the European transect of forest sites. This finding suggests that the $\delta^{15}N$ of N inputs is possibly governed more by local environmental conditions than by broad geographical ones. This point is further emphasised by results obtained from the 'local' transect discussed below in section 7.

Strong differences in the $\delta^{15}N$ signatures of rainfall and throughfall were found between the 'industrial site' (Knowsley Park) near to Liverpool and the 'agricultural' site (West Linton) adjacent to the chicken farm near Edinburgh. At the former site the inputs for NH_4-N were always $\delta^{15}N$ negative and for the latter site were always $\delta^{15}N$ positive. The influences of the
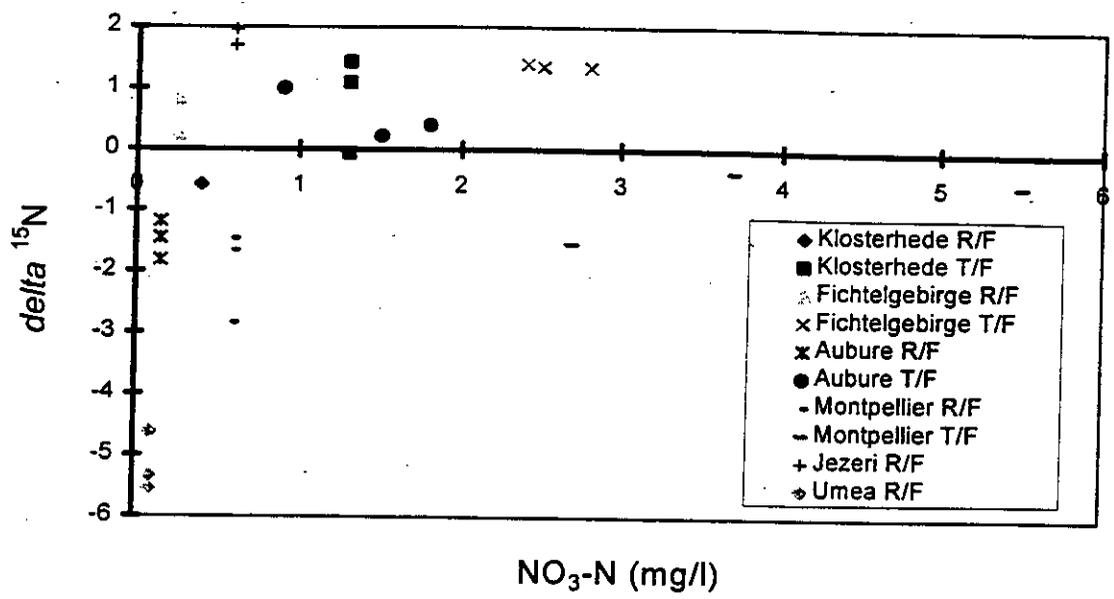
Figure 3 Plot of delta^{15}N against NO_3-N concentration for the European spruce/pine forest transect

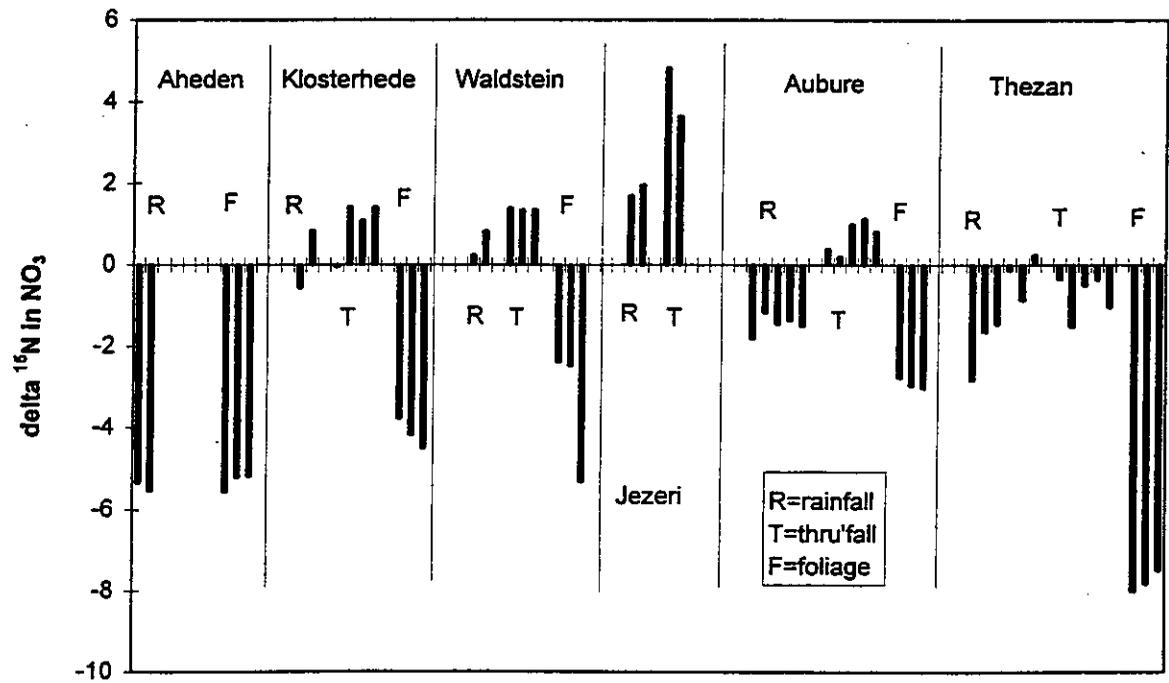Figure 4 Plot of the delta ^{15}N in NO_3-N in rainfall and throughfall, and ^{15}N in foliage for the European transect spruce/pine forest sites.

Table 2. Concentrations (ppm N) and $\delta^{15}N$ signature (‰) of ammonium and nitrate in rainfall and throughfall for the UK sites (1994-1996).

Site Collected from:	Ammonium-N				Nitrate-N			
	Rainfall		Throughfall		Rainfall		Throughfall	
Knowsley Park	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Dec-Jan 1994	0.69	-10.62	2.5	-14.97				
	0.76	-11.93	1.4	-11.23				
	0.75	-12.49	2.9	-				
July 1994	1.0	-3.38	*Contaminated*		*Not analysed for nitrate*			
	0.78	-4.02	*with*					
	1.06	-1.69	*bird droppings*					
Jan-Feb 1995	0.2	-14.27	*Insufficient sample*					
	0.2	-12.65	*collected for*					
	0.2	-15.4	*analysis*					
Feb-Mar 1995	0.2	-12.17	0.7	-13.53	0.15	+4.49	0.3	-
	0.3	-13.15	2.2	-3.23	0.2	+2.85	0.5	+3.82
	0.35	-12.49	1.4	-17.43	0.2	+3.11	0.4	+3.79
Glassonby	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Dec 93-Jan 94	0.68	-7.59	6.3	-8.10				
	0.69	-7.92	*Contaminated with*		*Not analysed for nitrate*			
	0.7	-7.44	*bird droppings*					
Jan-April 1994	1.7	-2.25	7.8	-2.68				
	0.92	-3.10	*Contamination*					
Shap	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Jan-Mar 1994	1.0	+3.99	0.96	+7.18				
	1.2	+4.23	-	-				
Apr-Jun 1994	0.21	+1.47	0.27	+5.61	*Not analysed for nitrate*			
	0.35	+0.47	*Contaminated with*					
	0.26	+1.08	*bird droppings*					
Jun-July 1994	0.65	-2.85	2.87	+6.52				
	0.55	-5.19	-	-				
Feb-Mar 1995	0.4	-3.17	*Throughfall*		0.15	+1.29	*Throughfall*	
	0.3	-6.01	*collection*		0.15	+2.18	*collection*	
	0.3	-6.57	*discontinued*		0.15	+2.20	*discontinued*	
	0.7	-0.77	*because of*		0.15	+1.48	*because of*	
	0.6	-0.16	*persistent*		0.15	+2.11	*persistent*	
	0.4	-3.43	*contamination*		0.15	+1.75	*contamination*	
West Linton	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
Jan-Apr 1994	1.0	+2.55	8.3	+15.92				
	1.0	+2.96	11	+20.37				
	Insufficient nitrogen		25	+22.71				
May-July 1994	1.77	+8.82	12.5	+14.98	*Not analysed for nitrate*			
	1.79	+7.70	*Insufficient nitrogen*					
	1.89	+6.16	10.1	+14.80				
Mar-June 1995	1.4	+4.7	15	+13.8	0.8	-0.26	5.3	+1.24
	1.4	+5.01	10.8	+12.96	0.8	-1.18	3.4	-0.08
	1.4	+5.05	15	+13.29	0.8	-0.96	5.2	+0.4

input signatures were reflected in the $\delta^{15}N$ of the foliage and the surface soil of the forests (Figures 5 and 6). It has not been possible to obtain adequate data from the other two sites to show similar patterns related to different N sources; both sites were found to be populated with large numbers of birds resulting in contamination of the water collections on many occasions. No suitable substitute sites were found to continue the work. Whilst it is tempting to suggest the results for Knowsley Park and West Linton may indicate differences in $\delta^{15}N$ signature between industrial and agricultural N inputs, the findings have not been substantiated with data from the other two UK sites. Further studies need to be carried out to demonstrate whether or not the differences in $\delta^{15}N$ signature can be found to hold elsewhere.

Although the sampling programme was arranged to collect rainfall and throughfall during the year to obtain indications of seasonal patterns in $\delta^{15}N$, many of the throughfall samples were contaminated with bird droppings as indicated by phosphate analysis, and had to be rejected. Insufficient data was therefore acquired from the UK transect foir assessing seasonal variation patterns.

What is clear however, is that the concentration of N in all throughfall samples is significantly higher than in the corresponding rainfall samples. The $\delta^{15}N$ values for NH_4-N inputs at the West Linton and Shap forest sites show a positive shift for throughfall relative to the corresponding rainfall, whereas the Glassonby site (two data sets only) and the Knowsley Park sites show no change in the $\delta^{15}N$. The positive $\delta^{15}N$ shift for the West Linton site is probably a result of a large change in the concentration indicating that the positive shift is related to high levels of dry deposition from the adjacent chicken farm. This finding conforms to the expectation that dry deposited N is $\delta^{15}N$ positive relative to its source.

6.2.2 Nitrate-N

The few data obtained for NO_3-N suggest that there may also have been a difference in the $\delta^{15}N$ signature between the Knowsley Park and the West Linton sites, supporting the conclusion from the European transect study that there are consistent between site differences, in both NH_4-N and NO_3-N. Clearly more data need to be obtained to substantiate this conclusion.
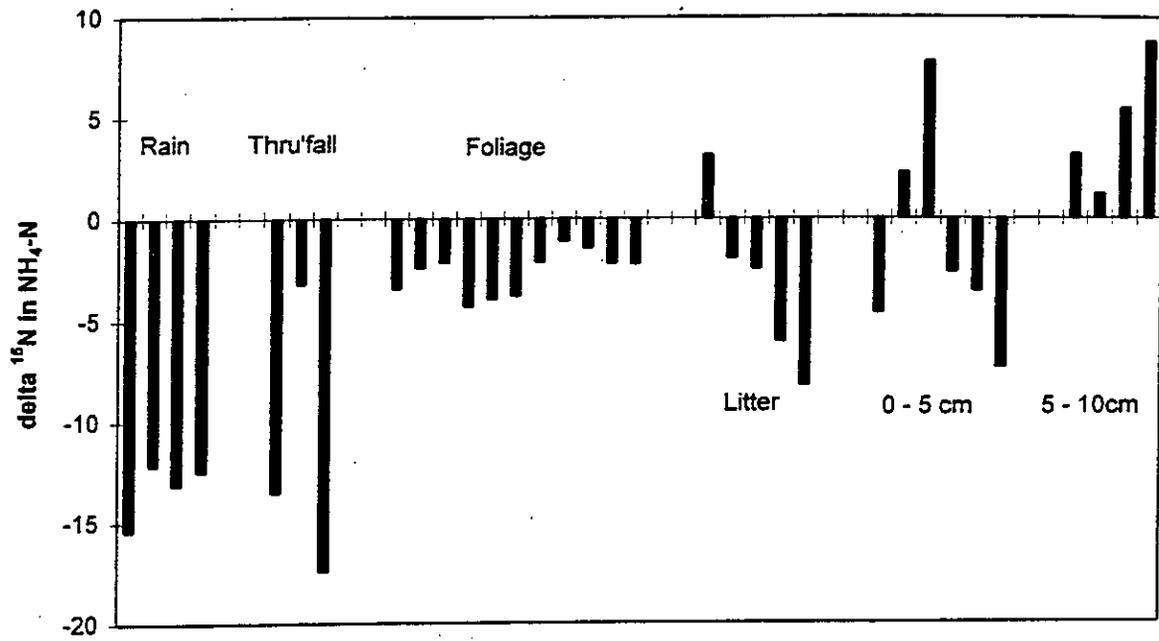
Figure 5 Plot of the delta ^{15}N in NH$_4$-N in rainfall, throughfall, foliage, litter
and 0-5 and 5-10 cm soil at Knowsley Park UK.
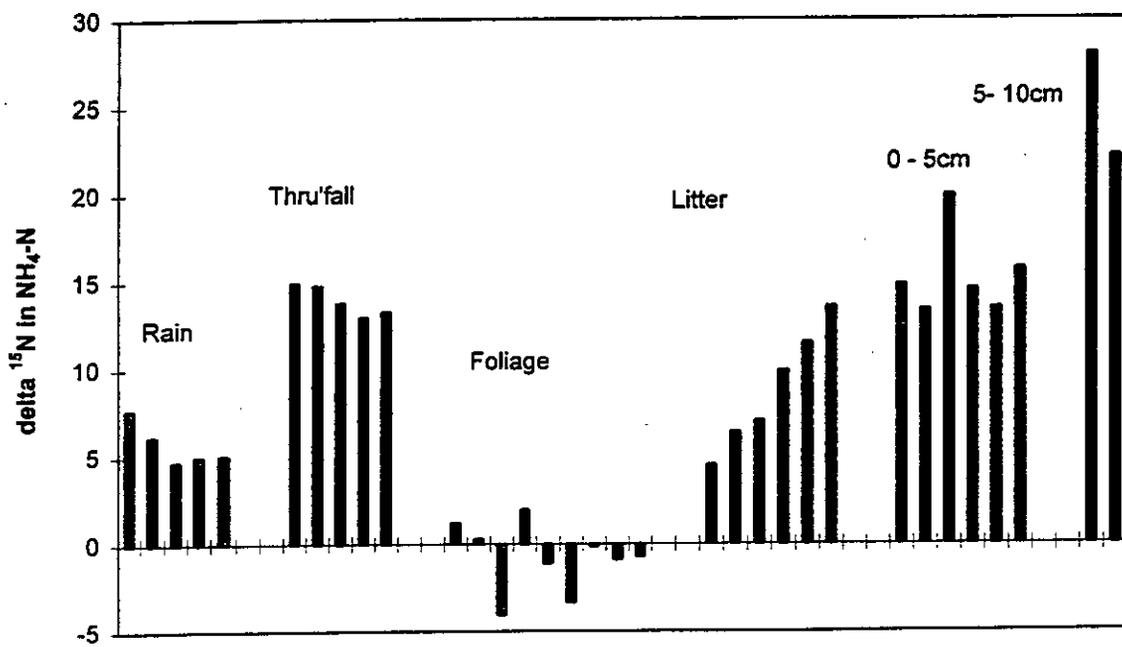

Figure 6 Plot of the delta ^{15}N in NH$_4$-N in rainfall, throughfall, foliage, litter
and 0-5 and 5-10cm soil at West Linton UK.

7 Local transect

7.1 Sites studied and sampling programme

The main objective of including a 'local transect' was to enable more frequent sampling of rainfall and throughfall than was possible with more remote sites. The transect was established in the grounds at ITE Merlewood. This area is surrounded to the west by deciduous woodland, but on its eastern boundary along the main drive to the institute there is an open grass field, which at intervals receives animal slurries. In total, six collectors were established, two in the woodland to the west on the side of a hill, two along the drive and two in between in the open of the garden. One of the two in the woodland was under a yew tree and the other under a beech. Similarly the two along the drive were under a yew and a beech. The design of the layout was to determine if tree species or position was more important in governing the $\delta^{15}N$ signature of the ammonium-N or nitrate-N. The water samples were collected at roughly monthly intervals, except for a period between July and early October 1995, when little rain occurred. Collection at frequent intervals has also allowed a study of the seasonal pattern of the $\delta^{15}N$ signature to be imnvestigated.

7.2 Results and discussion

The $\delta^{15}N$ data and the associated N concentrations for the NH_4-N and NO_3-N in rainfall and throughfall collected to Merlewood are given in Table 3. The upper row of data refer to the collectors on the drive and the lower to those situated in the woodland.

Three quite clear and general observations can be made from the 'local' data. The most striking is that the ranges of $\delta^{15}N$ values of both NH_4-N and NO_3-N are almost as large as those in the European and the UK transects. The second observation is that there are often marked changes in the $\delta^{15}N$ values between the rainfall and the throughfall, as found elsewhere. This emphasises the point that the $\delta^{15}N$ of pollutant inputs may be governed more by local than by broader geographical conditions. The third is that the throughfall N concentrations are almost always greater than the rainfall, as has been found with samples collected from the European and UK transects.

7.2.1 Ammonium-N

The $\delta^{15}N$ signature of the NH_4-N input is generally negative but there was a dramatic change in the $\delta^{15}N$ signature of the NH_4-N rainfall input in November and this co-incided with a period

Table 3. Concentrations (ppm N) and isotopic $\delta^{15}N$ composition (‰) of ammonium and nitrate in rainfall and throughfall under Yew and Beech at Merlewood.

Month of collection	Ammonium-N in				Nitrate-N in			
	Rainfall		Throughfall sampled (a) by the drive (b) in the wood		Rainfall		Throughfall sampled (a) by the drive (b) in the wood	
YEW	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
May	-	-6.78	-	-6.57	-	-1.05	-	0.49
	-	-8.56	-	-15.72	-	-0.63	-	1.65
June	0.7	-7.28	6.2	-9.57	0.4	-3.14	3.2	-0.19
	0.6	-0.57	5.5	-14.77	0.4	-1.78	4.6	-4.12
July	1.4	-6.6	4.3	-5.93	0.8	-2.82	3.5	-2.57
	1.3	-2.86	3.9	-3.07	0.7	-3.29	4.5	-1.94
July	0.3	-3.74	Contaminated		0.15	+0.77	Contaminated	
	Insufficient nitrogen		1.4	-0.38	0.15	+1.29	1.5	-6.48
October	0.5	-2.1	5.8	+2.52	0.3	+1.29	1.3	+8.28
	0.4	-6.01	3.6	-4.26	0.3	+0.77	3.6	-2.33
November	0.4	+10.35	14.2	-3.01	0.3	+2.6	4.0	+1.74
	0.4	+10.24	6.5	-5.66	0.3	+2.53	2.5	-1.44
December	0.7	-8.61	5.3	-0.23	0.5	+3.02	2.4	4.5
	0.5	-4.35	4.3	-9.73	0.4	+4.09	3.3	-1.36
BEECH	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$	ppm	$\delta^{15}N$
May	-	-6.78	-	-0.49	-	-1.05	-	+1.05
	-	-8.56	-	-11.63	-	-0.63	-	+1.64
June	0.7	-7.28	Contaminated		0.4	-3.14	Contaminated	
	0.6	-0.57	2.0	-9.23	0.4	-1.78	1.8	-0.41
July	1.4	-6.6	2.2	-7.03	0.8	-2.82	2.0	-1.13
	1.3	-2.86	2.9	-8.89	0.7	-3.29	2.4	-3.82
July	0.3	-3.74	Insufficient nitrogen		0.15	+0.77	0.4	+0.72
	Insufficient nitrogen		1.4	-7.72	0.15	+1.29	0.7	+0.50
October	0.5	-2.1	5.6	+9.73	0.3	+1.29	0.65	+9.60
	0.4	-6.01	3.2	-4.68	0.3	+0.77	2.3	+0.96
November	0.4	+10.35	2.8	-3.4	0.3	+2.6	0.2	+3.6
	0.4	+10.24	3.3	-3.71	0.3	+2.53	0.7	+7.88
December	0.7	-8.61	1.4	-1.13	0.5	+3.02	1.0	+5.41
	0.5	-4.35	4.2	-17.57	0.4	+4.09	2.1	+4.49

of active slurry spreading on the grass field adjacent to the drive. There was also a collection during a storm and this also appears to have generated a $\delta^{15}N$ positive 'pulse' of N input.

The results have been plotted against sampling period combining 'replicates' in two ways
   i) by the position in the Merlewood grounds and
   ii) by tree species.
It can be seen that when replicates are combined by position (Figure 7), there is better agreement between replicates than when combined by species (Figure 8). This suggests that the positional dimension is more important than the tree species dimension, confirming the statements made earlier about local conditions dictating the $\delta^{15}N$ signature of pollutant inputs.

It is clear from the data so far collected that there is a strong cyclic seasonal pattern in the $\delta^{15}N$ signature (Figure 6). It appears for the Merlewood site that the NH_4-N is more strongly negative in the winter period compared with the summer. The more negative $\delta^{15}N$ values may well be associated with canopy leaching of NH_4-N as discussed earlier under section 5.2.1. The 'less negative' $\delta^{15}N$ values of throughfall during the summer could be due to foliar uptake of NH_4-N, assuming the 'more reactive' lighter isotope is preferentially taken up and the NH_4-N remaining on the foliar surface becomes more positive. As found in other NiPHYS reports in this volume, spruce prefers ammonium-N to nitrate-N. Uptake of N through the foliage will be a metabolic activity and hence is likely to occur mainly in the summer months.

7.2.2 Nitrate-N

With the $\delta^{15}N$ in NO_3-N, there is also a strong 'positional effect', where the replicate collections of rainfall and throughfall on the drive are in good agreement. However the repilcate collectors situated in the woodland show marked variations.

With respect to the seasonal pattern, there is a clear and different situation from that of NH_4-N, again reinforcing the idea that NH_4-N and NO_3-N depositions behave differently in the forest canopy (see section 5). The $\delta^{15}N$ in the rainfall becomes negative in the summer, but changes to markedly positive in the winter (Figures 9 and 10). The pattern of the $\delta^{15}N$ in the throughfall on the drive also follows the same trend, but is generally more positive. More $\delta^{15}N$ positive throughfall relative to rainfall was found with the forest sites along the European transect, samples from which were collected during the winter period. It is suggested that the cyclic seasonal variations in the $\delta^{15}N$ of the NO_3-N are reflections of the N pollution source and processes of deposition on the canopy, rather than uptake, as spruce utilises nitrate only to a limited extent.
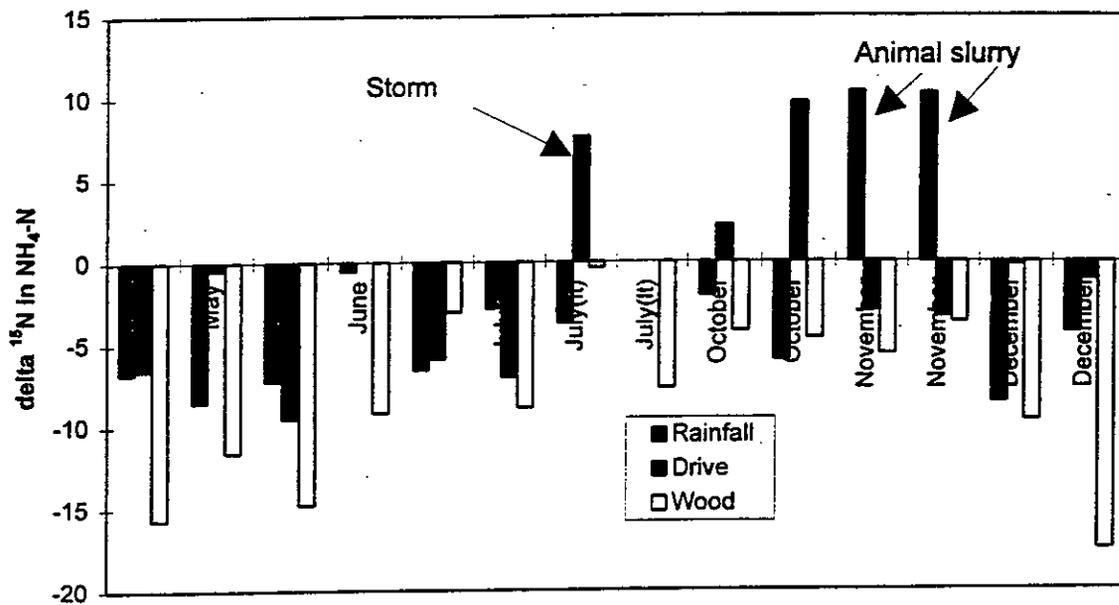
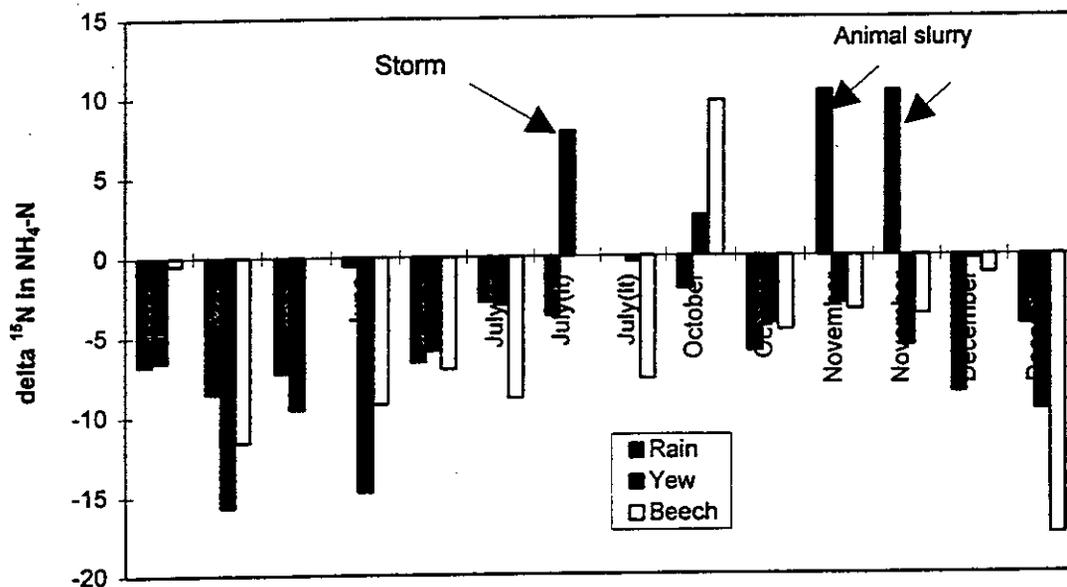Figure 7 Changes with season in delta ^{15}N in NH_4-N in rainfall, and throughfall on the drive and in the wood at ITE Merlewood.

Figure 8 Changes with season in delta ^{15}N in NH_4-N in rainfall, and throughfall under yew and beech at ITE Merlewood.

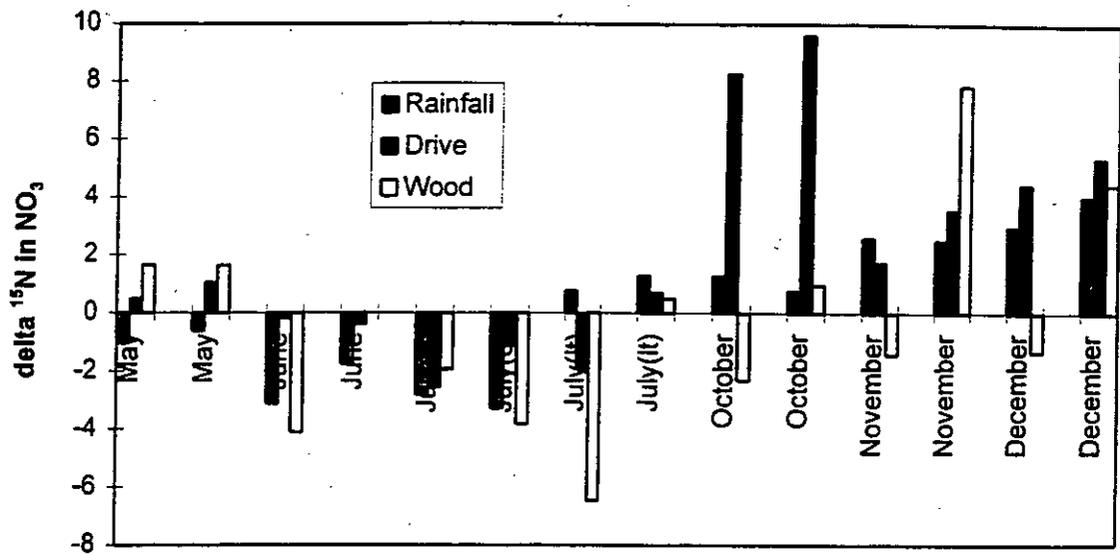Figure 9 Changes with season in delta ^{15}N in NO_3 in rainfall, and throughfall on the drive and in the wood at ITE Merlewood
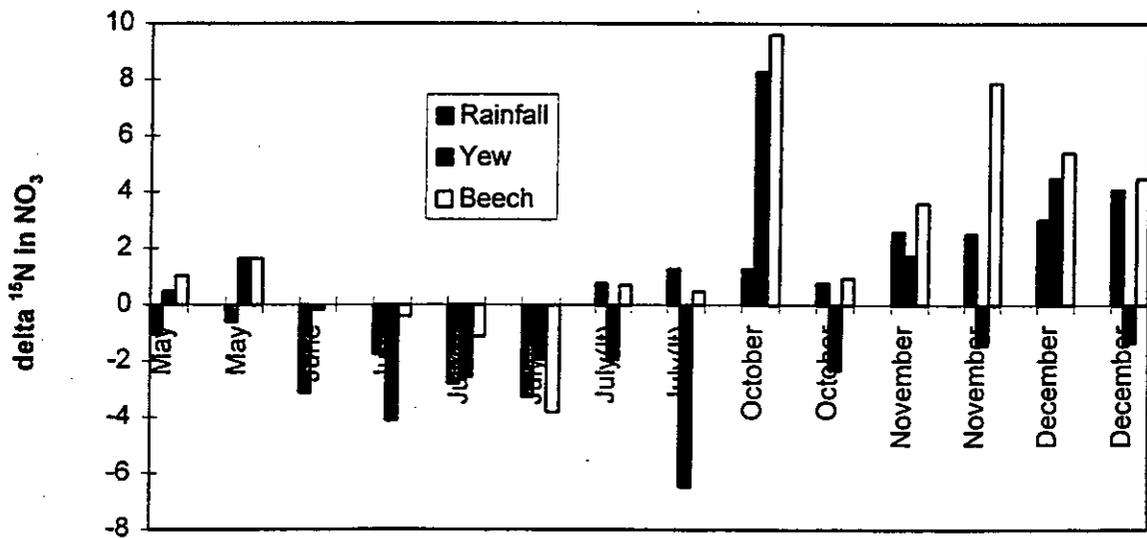

Figure 10 Changes with season in delta ^{15}N in NO_3 in rainfall, and throughfall under yew and beech at ITE Merlewood

8 Discussion

The data presented in this report may well represent the largest data set available on $\delta^{15}N$ measurements on rainfall and throughfall associated with forest ecosystems. Even since the beginning of the study, very few other data have appeared in the scientific literature. The data cover three spatial scales from the broad dimension across Europe, to UK and local transects.

Several generalisations can be made from the above results. The variations in the $\delta^{15}N$ in ammonium-N in rainfall and throughfall show much wider variations from -15 $^\circ/\circ\circ$ to + 22$^\circ/\circ\circ$ than had been expected. Those in nitrate-N were much narrower at -6.5$^\circ/\circ\circ$ to + 9.6$^\circ/\circ\circ$. These values, particularly those for NH_4-N, are much wider than the values we have found for $\delta^{15}N$ in the forest foliage. This situation thus makes it theoretically possible to utilise an isotope mass balance approach in assessing canopy uptake of pollutant N.

There appear to be reasonably consistent $\delta^{15}N$ signatures for NH_4-N and NO_3-N inputs to individual forest sites at the European and UK scale, though there is strong evidence of cyclic seasonal patterns. Inputs to individual forests can be markedly different in their $\delta^{15}N$ showing probable differences in source of the nitrogen pollution eg the forest site near to the conurbation of Liverpool and the site adjacent to the large chicken farm both in the UK. There are frequently very significant changes in the $\delta^{15}N$ between the rainfall input and the throughfall, which often accompany large changes in N concentration between rainfall and throughfall. At only two sites, Åheden and Aubure, was there an indication of a reduction in concentration in throughfall compared to rainfall. The higher concentration of N in throughfall compared to rainfall may be caused by leaching of dry-deposited N on the canopy, or leaching of N from within foliage. Both of these processes may conceal the process of foliar uptake. However, the seasonal pattern in $\delta^{15}N$, particularly the contrast between winter and summer periods, may reflect changes in the pollutant nitrogen source during the year, foliar uptake of deposited N and/or leaching of NH_4-N from the canopy.

$\delta^{15}N$ positive signatures associated with chicken farms or spreading of slurry suggest there may be differences in the signature of the N source. However, there may well be various physical fractionation processes related to deposition (Heaton, 1987), which might confuse the picture regarding $\delta^{15}N$ signature attribution to agricultural or industrial sources. In a manipulative experiment, in which ammonia gas was released onto a spruce forest canopy from a gas bottle under conditions of no rainfall and a windspeed of < 5 kmh^{-1}, the $\delta^{15}N$ of the NH_4-N in the collected throughfall varied between +5.4 and +7.0$^\circ/\circ\circ$, whereas the NH_3 gas originating from the source gas bottle had a $\delta^{15}N$ of between -0.3 and -3.6$^\circ/\circ\circ$ (Ineson et al in prep.). This result could be interpreted as indicating an isotopic fractionation during physical deposition on the

forest canopy where the heavier isotope is preferentially deposited (Heaton, 1987; Heaton, Spiro and Robertson, in prep). It could equally be interpreted as indicating that the forest canopy was preferentially taking up the lighter isotope leaving the deposited NH_4-N on the foliar surface, and later in throughfall, more enriched with ^{15}N (Schulze per. comm). It is very probable that both processes occur together. Clearly $\delta^{15}N$ data for rainfall and throughfall associated more specifically with agricultural and industrial point sources is required to clarify this area of research. It is intended to carry out these specific studies under an extension funded by the UK Department of the Environment during 1996-97.

8.1 Estimation of canopy uptake of pollutant N.

From the above results, it is clear that there is sufficient variation in the $\delta^{15}N$ in NH_4-N and NO_3-N to enable the estimation of canopy uptake of pollutant nitrogen by forest trees. In attempting to compute the amounts of nitrogen involved, a number of factors need to be taken into account. The main factors are briefly

    i) the $\delta^{15}N$ signature and concentration of N in the pollutant source(s)

    ii) the seasonal variations in the potentials for uptake and leaching from the forest canopy and

    iii) the differences in $\delta^{15}N$ in forest foliage between polluted and non-polluted forest growing in otherwise similar site conditions.

The estimation of canopy uptake will have to involve the modelling of the main processes incorporating changes in the $\delta^{15}N$ values for the various components, along the lines discussed elsewhere (Winkler and Gebauer 1993). The research carried out in this project has provided some basic information towards making the modelling possible.

To date the quantification of canopy uptake of pollutant N has been carried out using applications of enriched tracer ^{15}N techniques, rather than techniques based on changes in the natural abundance of ^{15}N. Research carried out in earlier studies by Harrison, Taylor, Chadwick and Quarmby (unpublished data) using 20% ^{15}N-labelled NH_4-N and NO_3-N applied in solution to tree seedling foliage at concentrations commonly (<10ppm) found in precipitation, showed that between 15 and 65% of nitrogen was absorbed into the plant and up to 50% was translocated to the plant root systems. Generally plants remaining wet after application absorbed more than those allowed to dry. Tree species which were able to retain larger amounts of wetness on the foliage were able to take up N in greater amounts than those tending to shed the moisture. Hence of the species studied, birch took up more than spruce, which in turn absorbed more than larch. It was also shown that spruce and larch prefers NH_4-N, whereas birch prefers NO_3-N. Studies with ^{15}N-labelled NH_4-N and NO_3-N on 10 year old

spruce trees in the field have shown a foliar uptake rate of about 9 kg N ha^{-1} yr^{-1} from wet deposition (Eilers, Brumme and Matzner, 1992). In a single experiment in which spruce and larch seedlings were exposed in chambers to ^{15}N-labelled NO$_2$ at a concentration of 10 ppb for only 1 hour, there was very fast uptake of the gas by the foliage; 66% of the gas was taken up by the foliage and circa 20% of the ^{15}N taken up was detectable in the root systems, which had been protected from exposure (Harrison, Taylor and Chadwick, 1991). A similar study with ^{15}NH$_3$ has been carried out with tree seedlings, showing similarly high rates of foliar uptake (Bruckner et al, 1993).

Both the studies with artificially-labelled ^{15}N, in wet applications and as gases, show that there is very significant potential uptake of pollutant N via the forest canopy.

Conclusions

From the limited datasets which have been obtained, some provisional observations and hypotheses can be made, though further more targetted research is required to substantiate or refute the points raised. The provisional conclusions are :

- The δ^{15}N in NH$_4$-N in rainfall and throughfall for European and UK spruce forests varies overall from c -14 to +22°/°°, whereas that for NO$_3$-N varies less from c -6.5 to +9.6°/°°.

- The δ^{15}N in both NH$_4$-N andNO$_3$-N in rainfall and throughfall for European and UK forests appear to differ between sites.

- The δ^{15}N signatures of the NH$_4$-N inputs appear to be reflected in the tree foliage and surface soil layers.

- There is generally a negative shift in the δ^{15}N of NH$_4$-N from rainfall to throughfall in autumn/winter, whereas for NO$_3$-N there is a positive δ^{15}N shift; these findings indicate differences in the behaviour of NH$_4$-H and NO$_3$-N deposition in the forest canopy.

- There appears to be cyclic seasonal changes in both the δ^{15}N in throughfall NH$_4$-N and NO$_3$-N. The NH$_4$-N in throughfall is more negative in winter possibly reflecting leaching from the forest canopy, becoming less negative during the summer possibly reflecting canopy uptake. By contrast, the NO$_3$-N in throughfall is slightly negative in summer, becoming significantly positive during the winter, the pattern possible reflecting seasonal differences in deposition on the canopy.

- There is almost as much spatial and seasonal variation in the $\delta^{15}N$ of NH_4-N and NO_3-N within a small site, as across the European forest transect; this indicates that the $\delta^{15}N$ is largely governed by local environmental pollution conditions.

- Spatial position in relation to N source appears to be more important than tree species in controlling the $\delta^{15}N$ signature of throughfall.

- Information is still required on the patterns of $\delta^{15}N$ variation in gaseous N pollutants NH_3 and NO_x.

- The estimation of direct net canopy uptake and utilisation of pollutant N is possible and requires modelling the both variations N concentration and in $\delta^{15}N$ in wet, dry and gaseous pollutant sources, throughfall composition and foliar changes in N and $\delta^{15}N$ content.

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Research Area: Decomposition of ^{15}N-labelled plant meterial in a North-south climatic sequence of coniferous forest soils

1. Objectives.

The objectives were to investigate in five main coniferous sites of the Niphys climatic transect (Åheden, Klosterhede, Waldstein, Aubure, and Thezan):

1. - the decomposition rates of ^{15}N labelled standard plant material.

2. - the ^{15}N spatial transfers through the soil organic matter profiles (upwards and downwards transfers, leaching).

3. - the ^{15}N distribution in some major labile N pools controlling N mineralisation: microbial biomass N, inorganic N (NH_4, NO_3) and labile organic N (K_2SO_4 extractable organic N).

In the selected sites, all soils are acid developed on similar parent material derived from silicates or sandstone. The soils represent from Boreal to Mediterranean climate some major humus types under European coniferous forests.

List of the sites, species and abbreviations used in this report: Åheden, pine (Ahe); Klosterhede, spruce (Klos); Waldstein, spruce (Wa); Aubure, spruce (Aub); Thezan, cluster pine (The).

2. Methodology

2.1   Design of the experiment (Figure 1)

The horizons were sampled according to pedological distribution of organic matter in the profile. The organic horizons selected for labelling (Fig. 1) were air dried before the labelled plant materiel was added.

Table 1 CHARACTERISTICS OF THE ^{15}N LABELLED WHEAT STRAW
(stems + roots)

	means	s d
C % (n = 10)	41.49	0.21
N % (n = 10)	0.618	0.0083
C/N (n = 10)	67.1	
^{15}N Isotopic Ratio (%) (n = 5)	6.786	0.0181

Unlabelled horizons

The unlabelled horizons were placed directly (moist) in plastic cylinders (inside diam. = 12cm, length = 30cm at all sites, except at Thezan where length = 25cm, because of the high stoniness in the deeper horizons). Circles cut out of polyester tissue (mesh = 1mm), were placed between the horizons. Each horizon was compacted in order to obtain a bulk density

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Figure 1   **LABELLED and UNLABELLED HORIZONS**
Characteristics of the horizons in the cylinders

CYLINDERS O horiz	A horiz	DEPTH cm	C %	N %	C:N	pH (H2O)
			AHEDEN (Pine)			
(1)		0-7	46.6	0.71	65.9	3.9
Oh		7-10	43.8	1.29	33.9	3.9
A1		10-15	2.2	0.06	34.9	4.4
A2		15-20	0.5	0.01	52.2	5.4
B		20-30	0.5	0.01	52.2	5.4
			KLOSTERHEDE (Spruce)			
LITTER		0-3	48.4	1.63	29.7	4
Oh		3-6	41.9	1.27	33	3.2
A1		6-11	2.4	0.04	61.3	3.6
A2		11-16	2.8	0.07	40.9	4
B		16-30	2.8	0.07	40.9	4
			WALDSTEIN (Spruce)			
(2)	(2)	0-3				
Oh	Oh	3-6	40.4	2.11	19.1	3.6
A1	**A1**	6-11	27.3	1.39	19.7	3.3
A2	A2	11-16	9.1	0.35	25.7	3.2
B	B	16-30	3.5	0.13	26.9	4.1
			AUBURE (Spruce)			
(2)	(2)	0-3				
Oh	Oh	3-6	41.1	1.72	23.9	3.5
A1	**A1**	6-11	11.1	0.46	24.1	3.2
A2	A2	11-16	13.1	0.61	21.5	3.2
B	B	16-30	3.4	0.19	17.8	3.5
			THEZAN (Pine)			
	LITTER	0-3	50.1	0.35	143	4.5
	O	3-5	35.3	0.91	38.9	4.2
	A1	5-9	4.8	0.15	31.8	5.2
	A2	9-14	1.4	0.03	42.9	5.7
	A2	14-25	0.9	0.04	22.8	5.8

(1): LITTER and MOSS

(2): LITTER and GRASS

Bold characters: [15]N labelled Oh or A1 horizons

similar to the initial natural bulk density. The compaction was performed with a heavy steel cylinder having the same outside diameter as the soil column.

Labelled horizons

The labelled plant material was produced by growing wheat (fast growing spring wheat, cv "Florence Aurore") from the seed to maturity during five months on a low N NPK + micro-nutrients solution containing Ca $(^{15}NO_3)_2$. $4H_2O$ as sole N source with a ^{15}N enrichment = 7%. At harvest only the stems and the roots were used for this experiment, in order to obtain labelled material with a low N content. The material was air dried, mixed (roots + stems) and milled to 0.5 - 1mm particle size. Table 1 shows the characteristics of the material. C:N ratio was high (67) and the measured ^{15}N isotopic enrichment was 6.79 ±0.02.

For each humus type, the labelling was performed in the most representative organic layers (Figure 1):

- Åheden and Klosterhede: Oh horizon,
- Waldstein and Aubure: Oh and A1 horizons
- Thezan: A1 horizon

After sampling, the soil was dried, sieved at 4mm mesh and homogenised. In order to obtain identical amount of label in each cylinder and a homogeneous distribution of the label in the samples, the material was added individually to each sample and each sample was homogenised again for 15 min in a 1 litre jar, using an overhead agitator.

Table 2   INITIAL LABELLED ^{15}N

	AHE Oh	KLOS Oh	WALDSTEIN Oh	WALDSTEIN A1	AUBURE Oh	AUBURE A1	THEZAN A1
MASS of SOIL of LABELLED HORIZON g dw	55	41	49	156	58	384	445
LABELLED PLANT MATERIAL							
Added plant material g cylinder[-1]	1.385	1.315	0.645	1.246	0.626	1.320	0.420
added C g cylinder[-1]	0.575	0.546	0.268	0.517	0.260	0.548	0.174
added C % initial soil C	1.3	1.3	0.7	1.9	0.6	4.9	3.6
added N mg cylinder[-1]	8.559	8.127	3.985	7.702	3.871	8.158	2.595
added N % initial soil N	0.7	0.6	0.2	0.6	0.2	1.8	1.7
INITIAL ^{15}N:							
Measured by initial isotopic ratio:							
Initial isotopic ^{15}N ratio (%)	0.4393	0.4618	0.3914	0.3899	0.3903	0.3957	0.405
^{15}N µg cylinder[-1] (A)	522	500	262	517	245	529	382
s d (n=4)	2	8	4	2	10	2	2
Calculated by initial plant material							
^{15}N µg cylinder[-1] (B)	550	522	256	494	249	524	402
A / B	0.95	0.96	1.02	1.05	0.98	1.01	0.95

The principle of the experimental design was to use material with low N content (C:N = 67) and with high labelling (^{15}N isotopic ratio = 6.79%) and to add it in a small proportion, i. e. without modifying the chemical and physical characteristics of the native soil organic matter. The added plant material-C, expressed in % of initial soil-native C, ranged from 4.9 to 0.6 %. (Tab. 2). The added plant material-N, expressed in % of initial soil-native N, ranged from 0.2 to 1.8%. The proportions of added material were calculated in order to obtain initial ^{15}N isotopic ratios > 0.40, (i. e. isotopic enrichment > 0.034%). The measured initial ratios ranged from 0.39 to 0.46. The measured initial amount of ^{15}N in the cylinders ranged from 245µg ±10 to 529µg ±2. The ratio between the measured amount of ^{15}N cylinder^{-1}, using the measured initial isotopic ratio (A, Tab. 2) and the calculated amount, using the mass of added plant material (B, Tab. 2) ranged from 0.95 to 1.05. For the calculations, 0.366% was used as natural ^{15}N isotopic ratio. For the data presented in this report, the initial ^{15}N was calculated using the measured initial isotopic ratio (A). The design of the experiment was based on the assumption that (1) the low amount of added plant material will not modify the physical, chemical and biological characteristics of the soil native organic matter and that (2) the variation of the added material in the range of 0.2 to 1.8 N% of initial total soil N will not affect the decomposition processes of the added N.

The labelled horizons were replaced in the cylinders over the unlabelled ones (Fig. 1) and compacted in the same way as the unlabelled horizons, in order to obtain a bulk density similar to the one in undisturbed horizons. The thickness of the labelled horizons ranged from 3 cm for the Oh horizons to 4-5cm for the A1 horizons. The labelled horizon was separated from the over- and underlying horizons by round pieces cut out from polyester tissue (mesh = 1mm). The upper horizons were covered by litter or moss according to the natural understory (Fig. 1). At Waldstein *Deschampsia flexuosa* grass rooted in a decomposing litter layer was installed in the cylinders over the labelled Oh horizon. After installation of the experiment, all the cylinders were watered with 200ml distilled water in order to humidify the laboratory dried labelled horizons.

2.2 Sampling occasions, replicates and analyses.

For each labelled horizon, 40 cylinders were installed: 40 at Åheden (Oh), Klosterhede (Oh) and Thezan (A1) and 80 at Waldstein and Aubure (Oh and A1) (Fig. 1).

Table 3   SAMPLING TIMETABLE

	TIME 0	1	2	SAMPLING OCCASIONS 3	4	5	6	7	8	9
	1993			1994				1995		
AHEDEN	16/06	31/08	01/11	14/06	25/08	26/10	13/06	30/09		
KLOSTEF	12/06	08/10	07/12	18/04	29/06	29/09	18/11	29/03	08/06	01/10
WALDST	29/05	06/10	23/12	06/04	29/06	12/09	30/12	21/04	21/06	30/08
AUBURE	30/05	18/08	15/12	06/04	24/06	23/08	30/12	06/04	13/06	30/10
THEZAN	01/07	18/10	15/12	19/04	27/06	28/09	15/12	13/03	08/06	15/11

At each sampling occasion (sampling timetable, Table 3) the cylinders were sampled in four replicates. From spring 93 to winter 95 nine samplings were regularly performed, except at Åheden where the cylinders were not sampled during the snow cover period. At sampling, the labelled and unlabelled horizons were pushed out from the cylinders and collected separately using the circles of polyester tissue to identify and separate the horizons. The unlabelled samples were air dried and stored. The labelled horizons were sent immediately to Montpellier by express delivery.

The following extractions and analyses were performed on the moist labelled soil samples:

- Extraction A: with 0.5 $\underline{M}$ K_2SO_4 for analysis of extractable NH_4-^{15}N and NO_3-^{15}N

- Extraction B: with 0.5 $\underline{M}$ K_2SO_4 for analysis of total K_2SO_4-extractable ^{15}N (inorganic + organic ^{15}N) and microbial biomass measurements (unfumigated samples)

- Chloroform fumigation and extraction C with 0.5 $\underline{M}$ K_2SO_4 for measurement of microbial biomass-^{15}N

- Analysis of total soil ^{15}N on air dried subsamples.

The extractions were performed using 10% of the mass of the moist labelled horizon (Table 2) + 150ml extraction solution and 30mn stirring. Immediately after extraction the extracts were deep-frozen and stored. NH_4-^{15}N and NO_3-^{15}N were measured on extract A, by double (NH_4, NO_3) steam distillation, flow-colorimetry for N and mass-spectrometry for ^{15}N isotopic ratio. Microbial biomass was measured using the fumigation-extraction (10% of the mass of moist labelled horizon) method (Vance *et al*, 1987) and calculated from extracts B and C. In B and C total extractable ^{15}N (inorganic + organic) was measured by the following method: 80ml extract were concentrated at 70°C with 5ml concentrated H_2SO_4 + 150mg Zn and mineralised in 100ml "Tecator" tubes at 350°C. NH_4 was separated by steam distillation in a 0.05 $\underline{N}$ H_2SO_4 solution. A subsample was analysed by flow-colorimetry for total NH_4. The remaining NH_4 solution was adjusted to pH 3, dried at 70°C and used for mass-spectrometry. Extracts A and B allowed the calculation of the K_2SO_4-extractable organic ^{15}N: K_2SO_4 extractable organic ^{15}N = total K_2SO_4-extractable ^{15}N (obtained from B) - inorganic ^{15}N (obtained from A).

The mass spectrometry was performed in the "Service Central d'Analyse du CNRS", Solaize, France. The moisture content of the samples was measured on subsamples.

The initially unlabelled layers located above and below the labelled horizons were air dried, stored and analysed for total N and ^{15}N contents and for moisture content.

3. Results

3.1 Decomposition rates: total ^{15}N remaining in the labelled horizon. (Objective 1, Fig. 2).

Since ^{15}N that was initially added to the horizons varied according to the organic matter contents (Table 2), in Figures 2 - 6, ^{15}N is expressed in % of total initial ^{15}N.

The lowest decrease of ^{15}N occurred in **Åheden**, the label remaining after two years in the horizon averaged 70% of the initial (Fig. 2). In **Klosterhede** the loss of ^{15}N reached 50 %. In both Scandinavian sites, the ^{15}N curve was still not stabilised; specially in Klos total ^{15}N
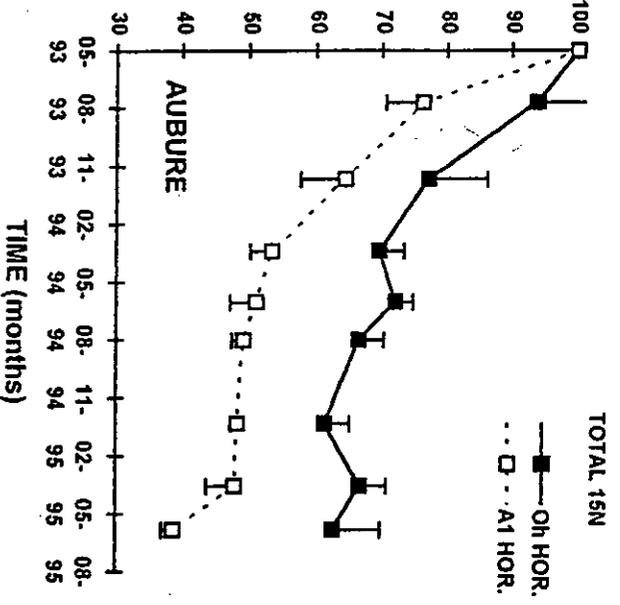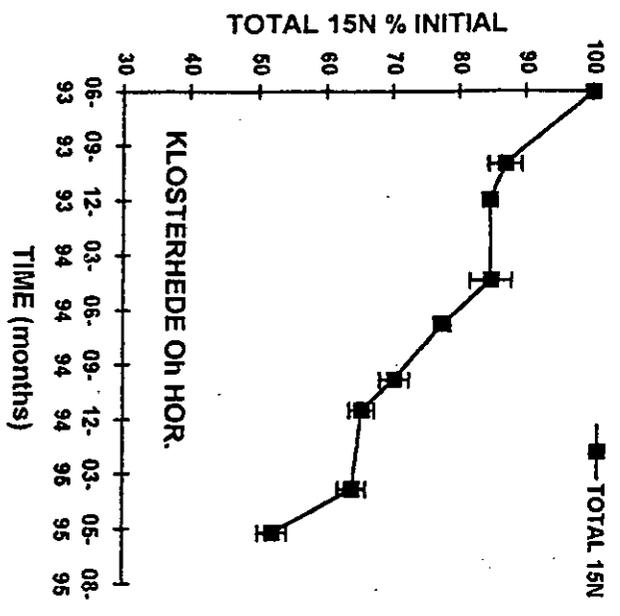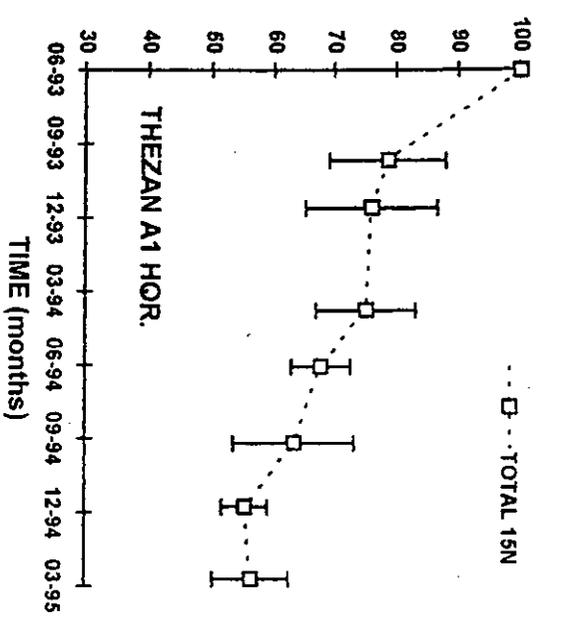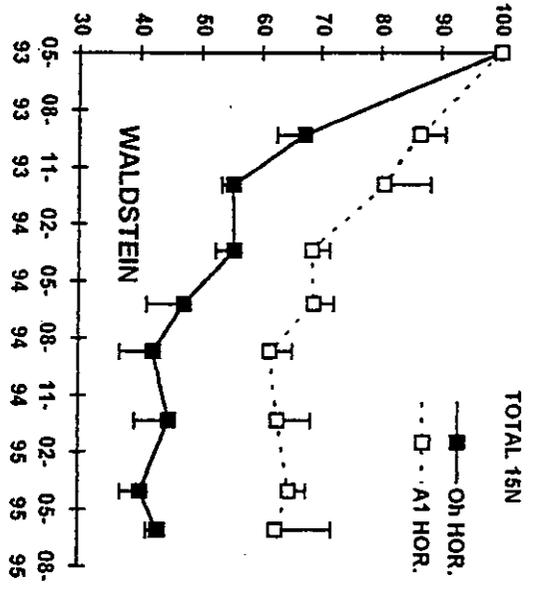
FIGURE 2

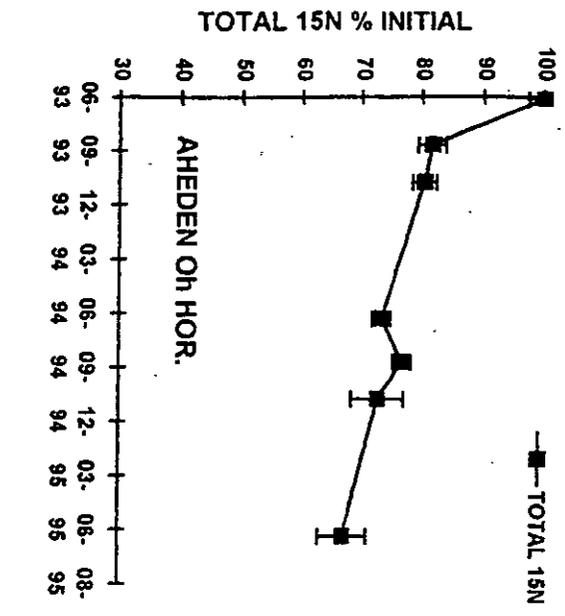TOTAL ^{15}N remaining in the labelled horizon in % of INITIAL ^{15}N

continued to decrease sharply during summer 1995. The ^{15}N loss occurred stepwise with low decreases during the winter periods. This was not observed in Åheden. A seasonal decrease was also observed in Åheden for ^{13}C (Vamos programme). In contrast with the Scandinavian soils, in the three south sites (Waldstein, Aubure and Thezan) the ^{15}N profile was stabilised beyond 1 to 1.5 years. The highest ^{15}N loss and the most rapid decrease occurred in Waldstein from the Oh horizon, reaching an asymptotic value at about 40 % ^{15}N remaining in the horizon. The ^{15}N loss from the underlying Al horizon was lower than from the Oh horizon. In both horizons, the label was stabilised beyond the first year of incubation. In Aubure the higher loss from Al horizon compared to Oh horizon was significant, indicating active transfers of material from this horizon. The soil of Thezan has only one main and thin organic horizon with low C and N content (A1). The ^{15}N loss was lower than at Waldstein. The decrease was more or less stabilised at 60 % of the initial. The high data variability at Thezan, due to the low N contents did not allow to detect a seasonal effect.

Discussion and conclusion.

(1) After a field exposure over two years, ^{15}N is still not stabilised in the Scandinavian sites. In the south sites, the remaining ^{15}N tended to reach asymptotic values at about 50-60% at Aubure and Thezan and at 40% at Waldstein.

(2) The high decrease of ^{15}N in Waldstein is explained by N uptake by the dense grass cover (*Deschampsia flexuosa*) and the root activity (see below).

(3) At Aubure ^{15}N decreased faster in A1 horizon compared to Oh horizon. The high N mobility in A1 may be explained by the low C content in A1 in this soil compared to Wa: C = 41 and 11% in Oh and A1 respectively at Aub and 40.4 and 27.3% at Waldstein (Fig. 1).

(4) The high variability of ^{15}N in Thezan is due to the low N content in the soil.

(5) At all sites, the observed decreases of ^{15}N were relatively high, almost as important as the C (Vamos programme). They are explained by mineralisation with uptake or leaching of inorganic N and by leaching of dissolved organic compounds (see below).

3.2 Down and up transfers of ^{15}N from the labelled Horizons (objective 2).
^{15}N transfers toward to horizons located above the labelled horizons.
In figure 3, the upwards transfers are illustrated by the dark symbols and the capital letters A, B, C, and D. The transfers from A1 to Oh (D, at Wa and Au) or from A1 to litter (D, at The) and the transfers from Oh to litter (B), to moss (A) or to grass layers (C) (figure 1 and 3) are explained by uptake of ^{15}N by the plants growing on the cylinders and by upwards transfers by the fungi mycelia. If the soil is covered only by litter (Klosterhede, (B), litter covering Oh and Thezan, (D), litter covering directly A1), the transfers were very low; less than 2% in Klos. and less than 1% in The of the initial ^{15}N were involved, indicating that the transfers by fungi were not really active. The transfers from Oh to the moss cover in Åheden (A) were also relatively low, involving only 2 to 3% of the initial. When the Oh horizons were rooted by grass (C) at Waldstein (*Deschampsia flexuosa*) and Aubure (*Deschampsia* and *Molinia*), the
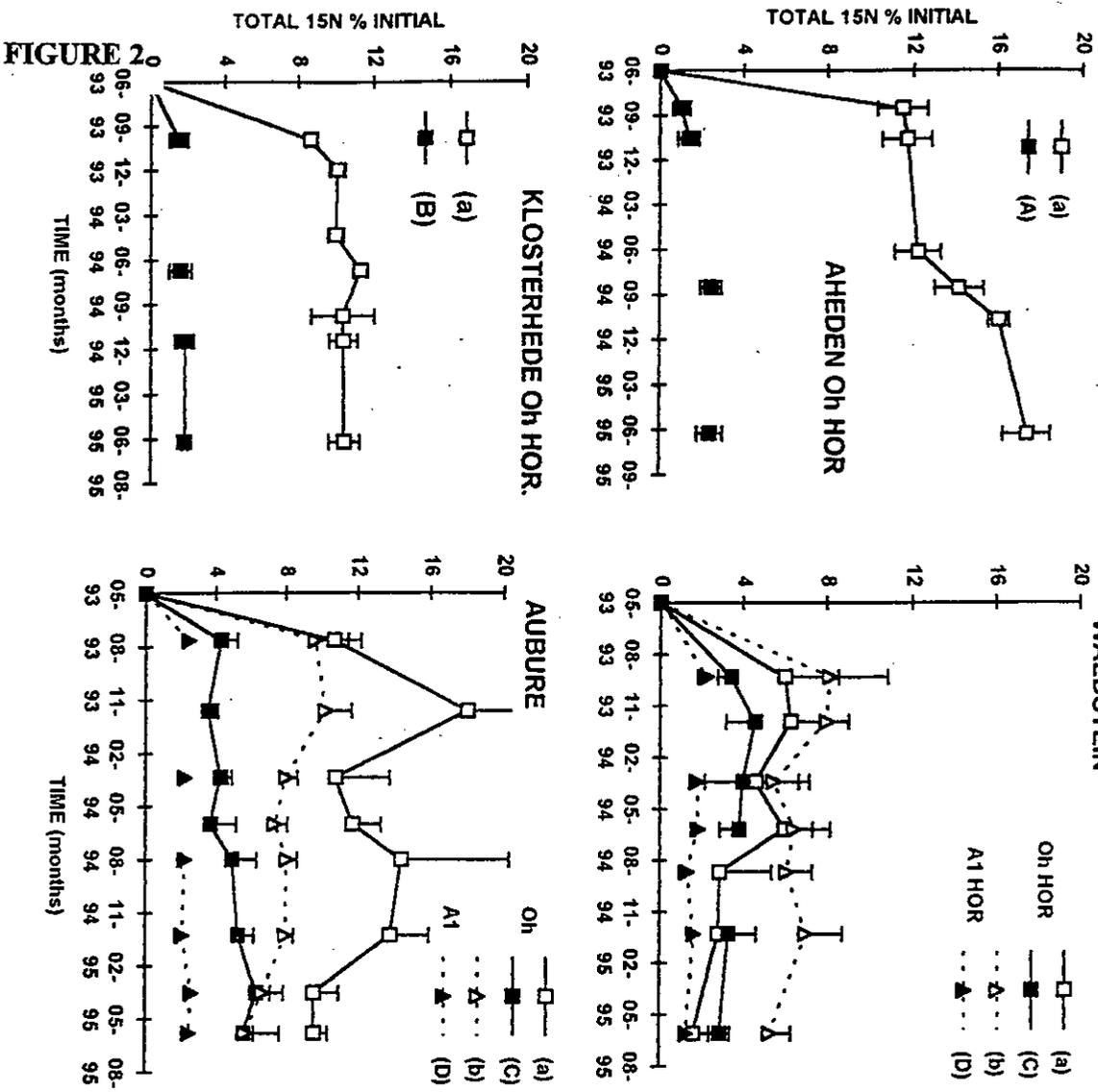
FIGURE 2

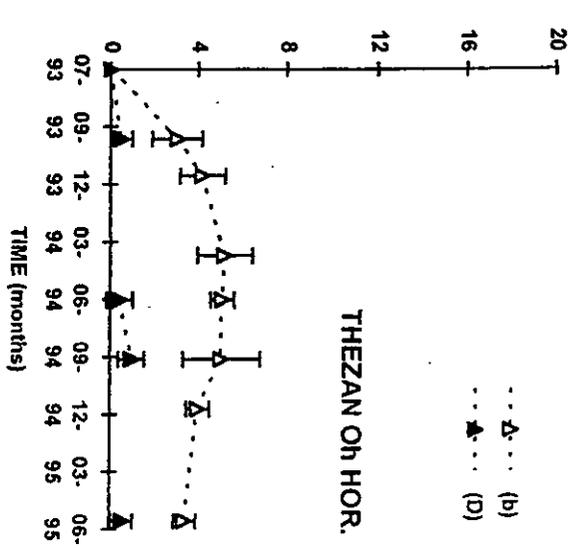TOTAL 15N % INITIAL

AHEDEN Oh HOR

(a) ■ □
(A) ■ □

TIME (months)

06-93 09-93 12-93 03-94 06-94 09-94 12-94 03-95 06-95 09-95

TOTAL 15N % INITIAL

KLOSTERHEDE Oh HOR.

(a) ■ □
(B)

TIME (months)

06-93 09-93 12-93 03-94 06-94 09-94 12-94 03-95 06-95 08-

WALDSTEIN

Oh HOR □ ■ (a) (c)
A1 HOR ·△· ·▲· (b) (d)

TIME (months)

05-93 08-93 11-93 02-94 05-94 08-94 11-94 02-95 05-95 08-95

AUBURE

Oh □ ■ (a) (c)
A1 ·△· ·▲· (b) (d)

TIME (months)

05-93 08-93 11-93 02-94 05-94 08-94 11-94 02-95 05-95 08-95

THEZAN Oh HOR.

·△· (b)
·▲· (d)

TIME (months)

07-93 09-93 12-93 03-94 06-94 09-94 12-94 03-95 06-

FIGURE 3
Down and up tranfers of 15N
In % of INITIAL 15N

(a) HOR. located directly UNDER the Oh HOR □
(b) HOR. located directly UNDER the A1 HOR ·△·

HOR located directly ABOVE the Oh HOR.
(A) LITTER and MOSS ■
(B) LITTER ■
(C) LITTER and GRASS ■
(D) HOR located directly ABOVE the A1 HOR. ·▲·

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^{15}N transfers were significantly higher, exceeding 5% at Wa and 7% at Aub. In both cases the values showed a high variability and this did not allow to detect seasonal uptake variations. The upward transfers from A1 to Oh (D) in Wa and Aub were always very low, amounting to less than 1% of the initial ^{15}N. Thus only the upwards transfers by grass and mainly through inorganic ^{15}N are of a really importance.

^{15}N leaching towards the deeper horizons.

Figure 4 presents two kinds of data:

- ^{15}N which was **measured** directly under the labelled horizons, i. e. the 5cm thick layer that is directly located under the labelled horizon (under the labelled Oh hor. = a; under the labelled A1 hor. = b, a and b are white symbols); a and b are also presented in figure 3 (white symbols), in order to compare the downwards and the upwards transfers.

- The unidentified ^{15}N fraction, that is assumed to be the part of label which was leached towards the deeper horizons (beyond the 0-5cm thick layer directly located under the labelled horizon, see Figure 1). This fraction was **calculated**: Unidentified ^{15}N = deeply leached ^{15}N = (initial ^{15}N - identified ^{15}N). It is also assumed that the loss by denitrification, volatilisation etc. was low, compared to the amount of leached ^{15}N. The leached ^{15}N from the labelled Oh hor. = (A) and from the labelled A1 hor = (B).

Confirming the data from Figure 2, at **Åheden** the leaching down to the deep horizons (A) was relatively low, compared to the other sites. Most of the leached ^{15}N remained in the layer that is directly located under the labelled Oh horizon (a, in Fig 3 and 4), representing after the two years about 15% of the initial label. Confirming the carbon data, the illuviation occurred mainly during the two summer periods and was low during the frozen winter periods. At **Klosterhede** the leaching was deeper than at Åheden but less pronounced than at Waldstein and Aubure. The process was still continuing during summer 1995, indicating that the ^{15}N profile is not yet stabilised in the deeper horizons. The downwards transfers were maximum at the **Waldstein** site. The deep ^{15}N profile was stabilised at 50% and 30% from Oh (A) and A1 (B) respectively (Fig. 4). In both cases the downwards transfers were important, since only low proportions of ^{15}N (less than 10%) remained in the layers that are located directly under the labelled horizons (a and b, Fig 3). At this site the downwards transfers are much higher than the upwards uptake by grass. At **Aubure,** the relatively lower ^{15}N leaching from Oh, compared to the one from A1 (Figure 2), is corroborated by higher proportions of label remaining in the layer directly located under Oh (a compared to b in Fig. 3). A few proportion of the leached ^{15}N coming from A1 remained in the transient layer directly located under A1. A higher proportion remained in the layer located directly under Oh. At this site, after two years of incubation, the deeply illuviated ^{15}N coming from A1 represented over 50% of the initial ^{15}N, but for Oh this proportion was only 20%. Again the leaching was more important than the uptake by grass. The downwards leaching in **Thezan** was less pronounced than in Waldstein and Aubure, but more important than in the Scandinavian sites. The deep ^{15}N profile seems to be stabilised after the two year experiment.
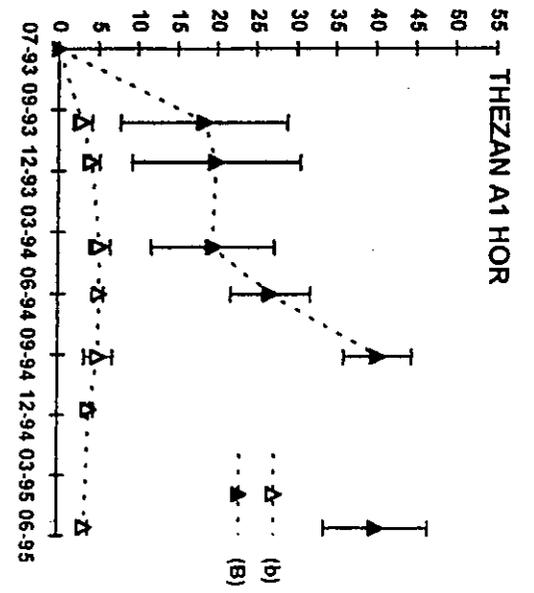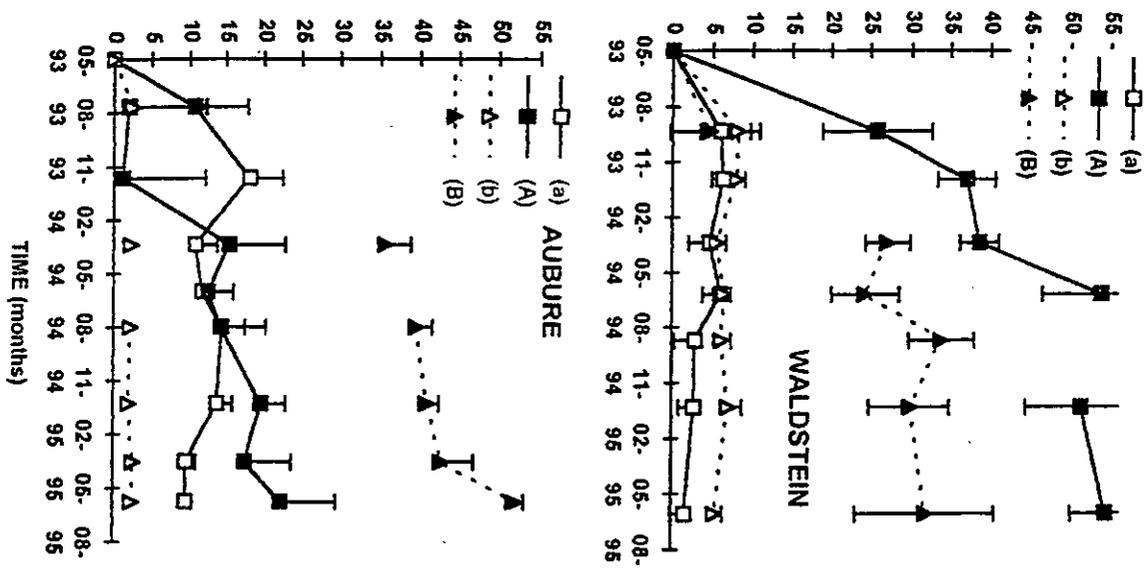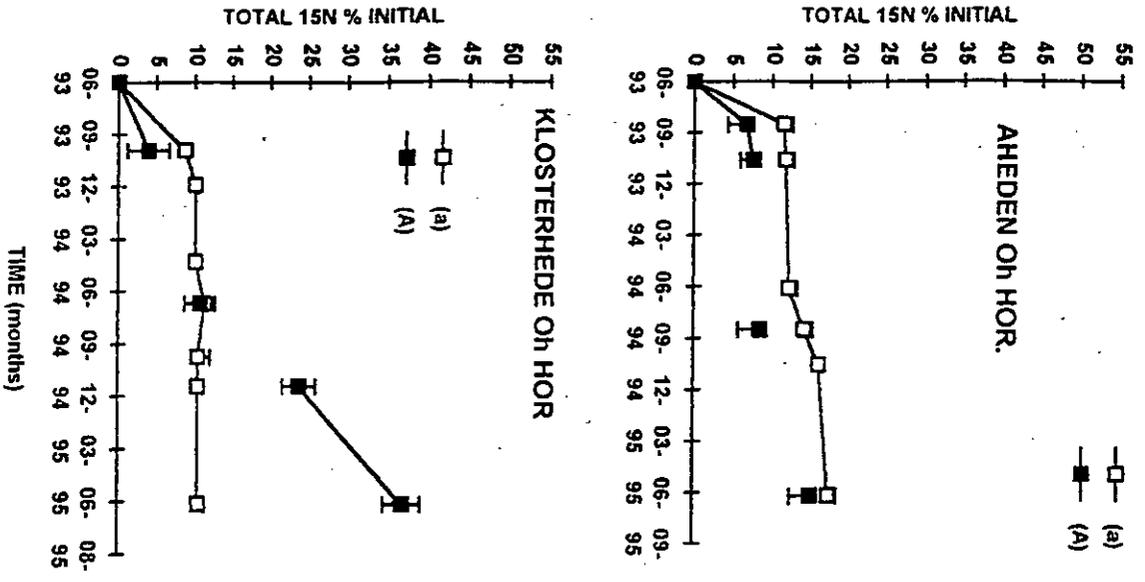
FIGURE 4

^{15}N leached down toward the deeper horizons

LEACHING from the Oh HOR.

(a) ^{15}N in the horizon directly located under the labelled Oh horizon

(A) UNRECOVERED ^{15}N (leached toward the deeper horizons)

LEACHING from the A1 HOR

(b) ^{15}N in the horizon directly located under the labelled A1 horizon

(B) UNRECOVERED ^{15}N (leached toward the deeper horizons) (^{15}N in % of initial ^{15}N)

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Discussion and conclusion

(1) In both Scandinavian soils, the upwards transfers by fungi from Oh to litter or from Oh to the moss are very low, less than 2 - 3% of the initial label are involved. This is rather surprising and may indicate that moss does not use N from the Oh layer of the raw humus horizon.

(2) At Waldstein, *Deschampsia flexuosa* was initially installed on the cylinders and at Aubure *Deschampsia* and *Molinia* recolonised the cylinders progressively during the first year and second growing season. At both sites the ^{15}N uptake exceeded 5 - 7%. Nevertheless the high variability did not allow to detect a seasonal uptake pattern. The uptake by grass from Al through Oh was very low. At Klos., Wa., Au. and The. during the second year the deep layers (below Al, i.e. below 10-15cm) were colonized by tree roots; part of the deeply leached (unrecovered ^{15}N) was probably taken up by the trees.

(3) Through the climatic sequence, the highest downwards transfers occurred under the continental (Wa) and Atlantic (Au) conditions. The low transfers observed at the Boreal site (Ahe) are explained by the low decomposition rates (see below) and by the frost remaining during long periods in the deeper horizons. The experimental design did not allow to estimate the lateral transfers. The leaching in the Mediterranean soil is relatively low, but the illuviation is deeply distributed with only few amounts remaining in the transient layers. Under these climatic conditions, a strong control of leaching by seasonally contrasted rainfall conditions was expected (Bottner *et al* 1995); but the high variability of the measured transfers and the low organic matter contents in these soils did not allow to detect the seasonal effect.

(4) The chemical forms of the leached ^{15}N is unknown, since only total ^{15}N was measured in the illuviated horizons. Nevertheless such high illuviated amounts can not be explained by an migration of exclusively inorganic N (Pansu *et al*. 1996, Bottner *et al* 1996). The essential part of this illuviation is probably in organic soluble forms.

3.3 ^{15}N distribution in some major soil N pools.

3.31 Microbial biomass-^{15}N (MB-^{15}N) in the labelled horizons (Figure 5).

For MB-^{15}N, a high variability was generally observed, due to the fact that biomass is calculated on the basis of two measured values: the extractable K_2SO_4-^{15}N from the fumigated and unfumigated samples. The five sites were installed during summer 93. The labelled MB evolution was similar in all sites (Figure 5) with (1) a phase of increasing biomass size, (2) a maximum level, followed by (3) a progressive decreasing phase. The same pattern (with different time scales) was observed in laboratory controlled experiments (Bottner *et al*, 1988, 1996) and in cropland field experiments (Ladd *et al*, 1985). At **Åheden** the increasing phase was probably not yet achieved or the maximum level occurred only during the year 1995. At **Klosterhede** the peak occurred in spring of the second year and beyond this time the MB-size decreased slowly. At **Waldstein** and **Aubure** the maximum MB development occurred in autumn and winter during of the first year. At the Waldstein site, the Oh MB-^{15}N levelled at relatively low values, compared to the other sites. As shown in Figure 2, at this site the labelled
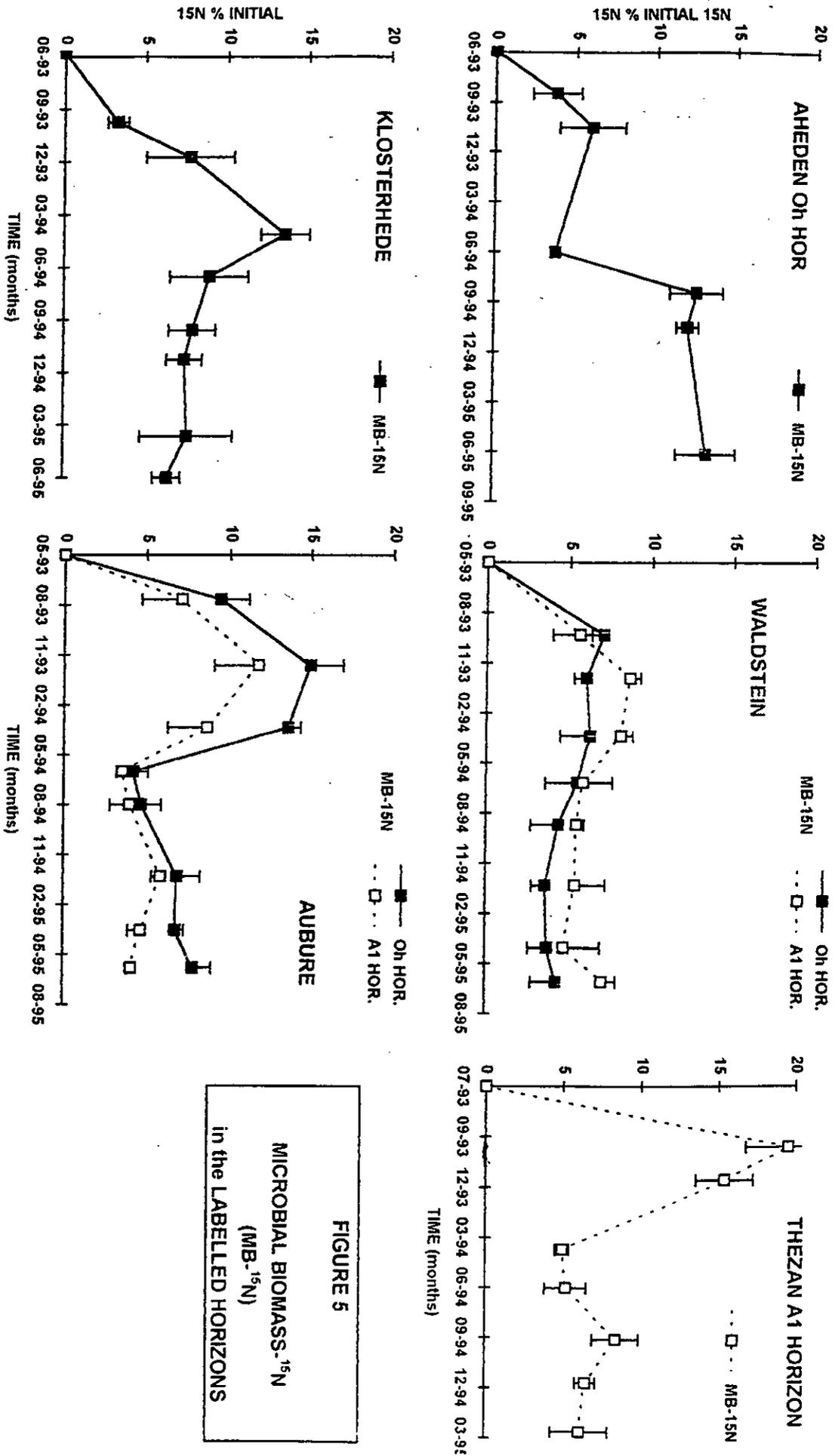
FIGURE 5

MICROBIAL BIOMASS-^{15}N
(MB-^{15}N)
in the LABELLED HORIZONS

^{15}N was also quickly leached out from Oh. The contrary was observed for Oh at **Aubure**, the leaching was relatively low and the MB reached a relatively high level. In both sites, the Al MB behaved similarly to the Oh MB. The differences were not significant. At **Thezan** the MB-^{15}N curve reached rapidly high values in autumn 1993, with MB-^{15}N amounting to 20% at the first sampling occasion. Thereafter the curve became quickly flat at low levels, indicating a fast turnover rate and a rapid exhaustion of the labile ^{15}N compounds. The pattern of MB-^{15}N did not closely reflect effects of seasonal climatic variations. These variations were better correlated with MB-total N (not presented in the Figures) than with MB-^{15}N. The measured isotopic ratio of the fumigated soil samples decreased regularly during the incubation time regardless the seasonal climatic conditions, indicating a progressive exhaustion of ^{15}N which was fast in Thezan and very slow in Ahden.

3.32 Labile N pools: organic and inorganic K_2SO_4-extractable ^{15}N (Figure 6A and B).

Labile ^{15}N fractions are defined in this report as ^{15}N that is extracted from the soil with a 0.5 $\underline{M}$ K_2SO_4 solution. Total K_2SO_4-extracted ^{15}N and inorganic K_2SO_4-extracted ^{15}N were measured, whereas organic K_2SO_4-^{15}N was calculated (see methods). At all sites, the peak values were observed at the first sampling time, including Åheden and the curve levelled at

Table 4 NH_4-^{15}N % (NH_4-^{15}N + NO_3-^{15}N)

(a) Samplings	AHEDEN Oh	KLOSTE Oh	WALDSTEIN Oh	WALDSTEIN Al	AUBURE Oh	AUBURE Al	THEZAN Al
(n = 4)							
1	100	100	96	96	80	94	97
s d	0.1	0.0	1.2	1.1	8.0	1.1	3.7
2	100	100	100	100	87	86	44
s d	0.0	0.0	0.0	0.0	7.0	2.8	5.6
3	100	100	73	40	99	97	56
s d	0.0	0.1	8.1	24.0	0.9	1.2	8.7
4	100	100	100	12	55	65	28
s d	0.0	0.0	0.0	9.3	4.6	12.2	6.3
5	100	100	49	85	86	88	71
s d	0.0	0.0	12.6	16.0	2.5	1.7	11.3
6	100	100	100	100	95	100	75
s d	0.0	0.0	0.0	0.0	5.1	0.0	9.1
7		100	100	93	97	82	62
s d		0.0	0.0	27.0	10.0	13.0	13.2
8		100	82	100	78	70	
s d		0.0	14.0	0.0	14.2	15.0	

(a): Oh=Oh horizon; Al=Al horizon

all sites at about 6% (Figure 6A, B). Beyond this time the amounts of labile ^{15}N material decreased, but the decrease was faster in the Mediterranean soil compared to the Scandinavian soils. A very fast exhaustion of the labile ^{15}N material occurred during the first autumn at **Thezan**. In contrast, the decreases were low at **Åheden** and **Klosterhede**. Again and like for MB (Figure 5), the exhaustion of labile compounds was faster at **Waldstein** than at **Aubure**. Similarly, this has to be related to a faster outleaching of ^{15}N from the Wa Oh horizons. When the ^{15}N exhaustion was low, seasonal variations were detected: at Klos and Ahe minimum values occurred during the second winter (1994-95), with a renewed activity during the following spring and summer (1995). A similar spring activity occurred at Aubure but with a very attenuated variation. Season related patterns were more pronounced for labile N compounds than for labile ^{15}N compounds. This again is explained by the progressive exhaustion of labile ^{15}N which was not yearly renewed, as it occurred for labile total N. For all sites and during the initial active phases, in total labile ^{15}N, the proportion of inorganic ^{15}N was generally higher than that of K_2SO_4 extracted organic ^{15}N. During the later phases, the variability of both components became high, due to the low values of the ^{15}N isotopic ratios.

3.33 NO_3-^{15}N and NH_4-^{15}N in inorganic ^{15}N (Table 4)

In table 4, NH_4-^{15}N is expressed in % of total inorganic ^{15}N (NH_4-^{15}N + No_3-^{15}N). At both Scandinavian sites (**Ahe** and **Klos**), NH_4-N^{15} was largely the predominant inorganic form. Nitrates were not detectable. At **Waldstein**, and **Aubure** labelled NH_4-^{15}N was generally predominant; labelled nitrate was detected at some sampling occasions in both Oh and Al horizons; nevertheless its production is apparently not related to seasonal effects. The proportion of labelled nitrate was generally measured with a high variability of the NO_3-^{15}N isotopic ratio, specially toward end of the experiment, when the values were near the natural isotopic ratio. Relatively high proportions of NO_3-^{15}N were constantly detected at the Mediterranean site of **Thezan**, with nevertheless a high variability, due to the low nitrate and low inorganic N contents.

Discussion and Conclusion.

The evolution pattern of microbial biomass was basically similar to the one observed from incubation experiments under controlled laboratory conditions, using C or N tracer technics. Under optimum conditions, the initial active phase lasts three to five weeks, corresponding to the use of the labile material (Bottner and Sallih, 1988; Sallih and Pansu, 1995; Pansu *et al*, 1996). Using similar labelled standard plant material, Bottner *et al* (1996 A) incubated, over 150 days, seven European coniferous forest soils with contrasting properties (including Åheden and Thezan) under optimal laboratory conditions. The aim was to clarify how the decomposition kinetics and C and N distribution in the organic matter pools are controlled by humus and soil properties, independently of climate conditions. They showed that for the soil coming from Åheden, the active phase lasted longer and the maximum level was lower than for
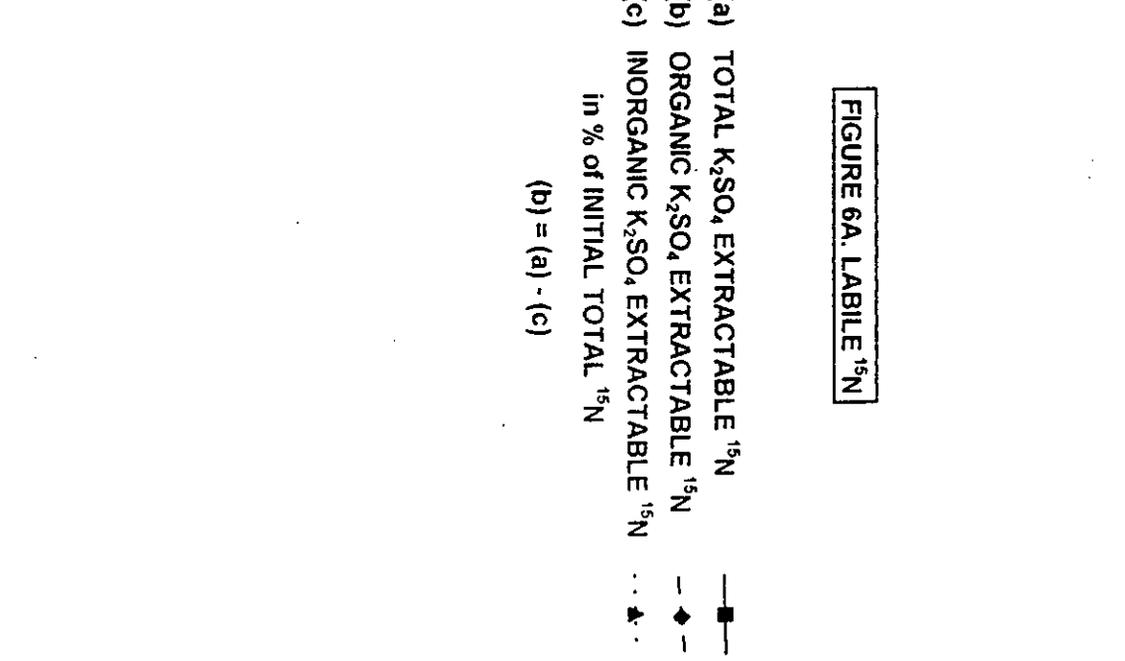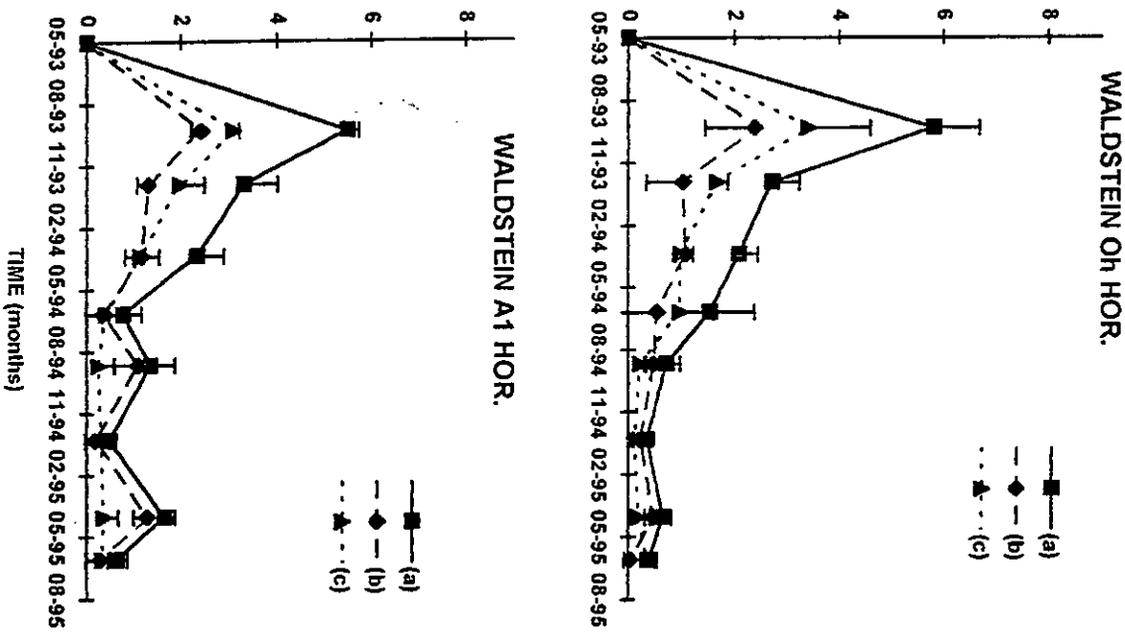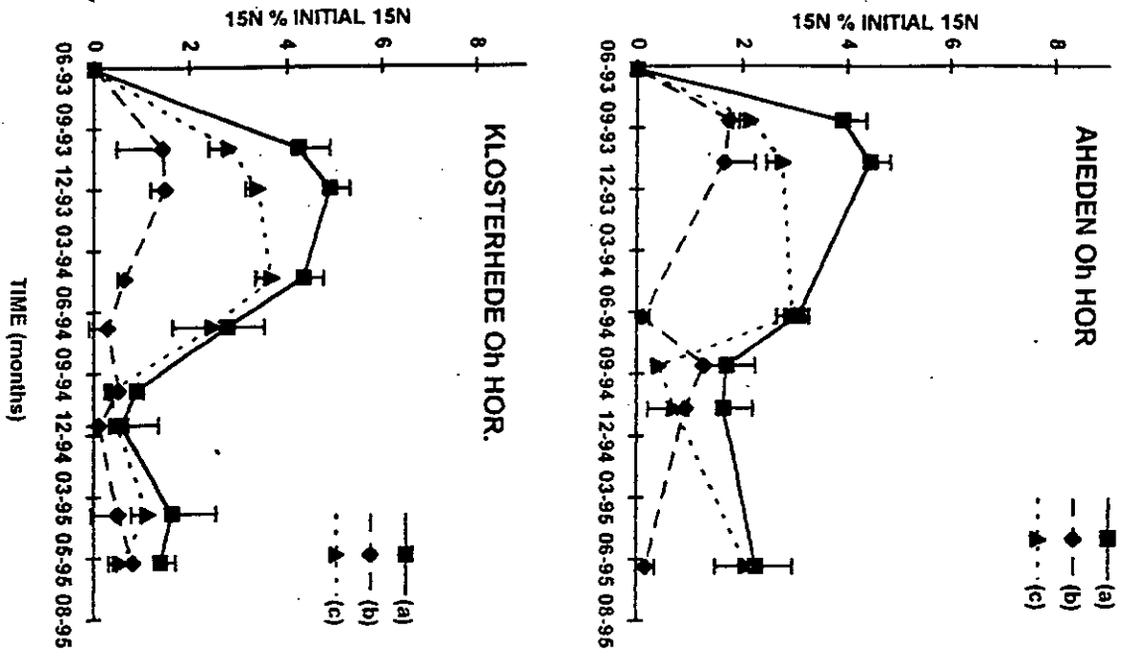
FIGURE 6A. LABILE ^{15}N

(a) TOTAL K_2SO_4 EXTRACTABLE ^{15}N
(b) ORGANIC K_2SO_4 EXTRACTABLE ^{15}N
(c) INORGANIC K_2SO_4 EXTRACTABLE ^{15}N
in % of INITIAL TOTAL ^{15}N

(b) = (a) - (c)

AUBURE Oh HOR.

AUBURE A1 HOR.

THEZAN A1 HOR.

(a) ━━■━━
(b) ━ ◆ ━
(c) ┈┈▲┈┈

15N % INITIAL 15N

TIME (months)
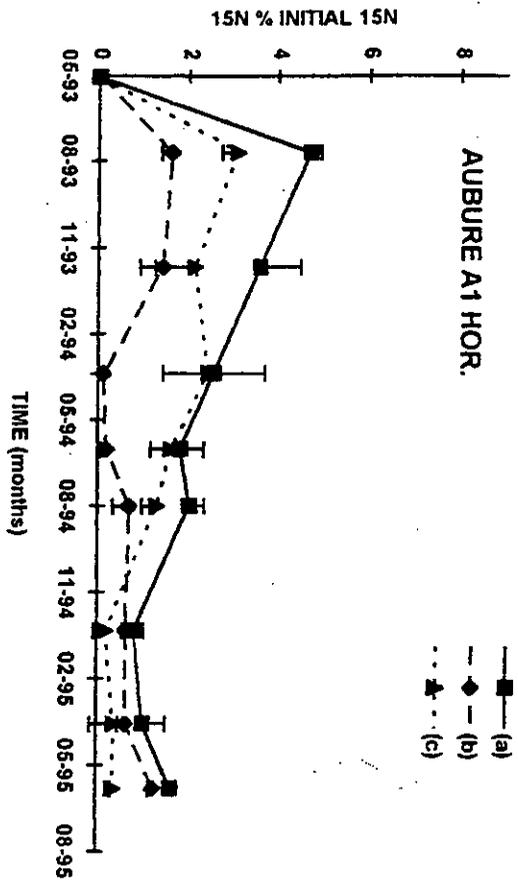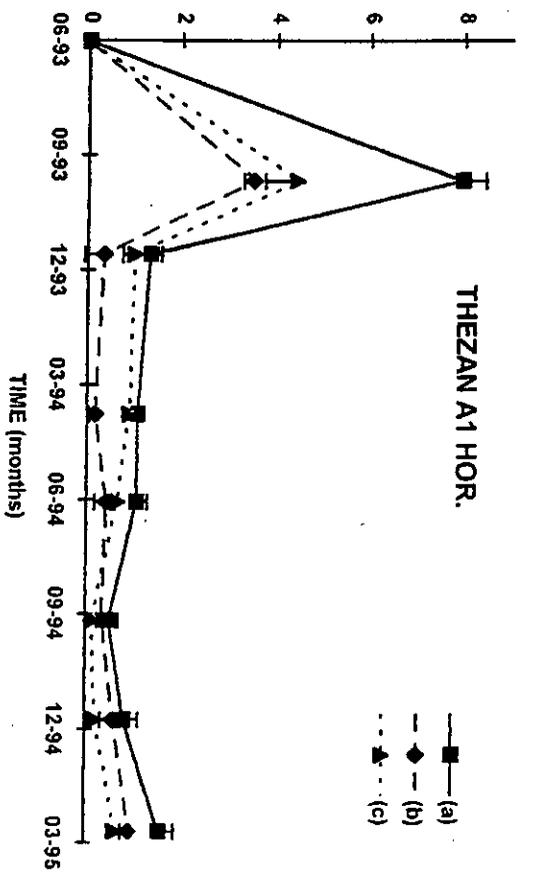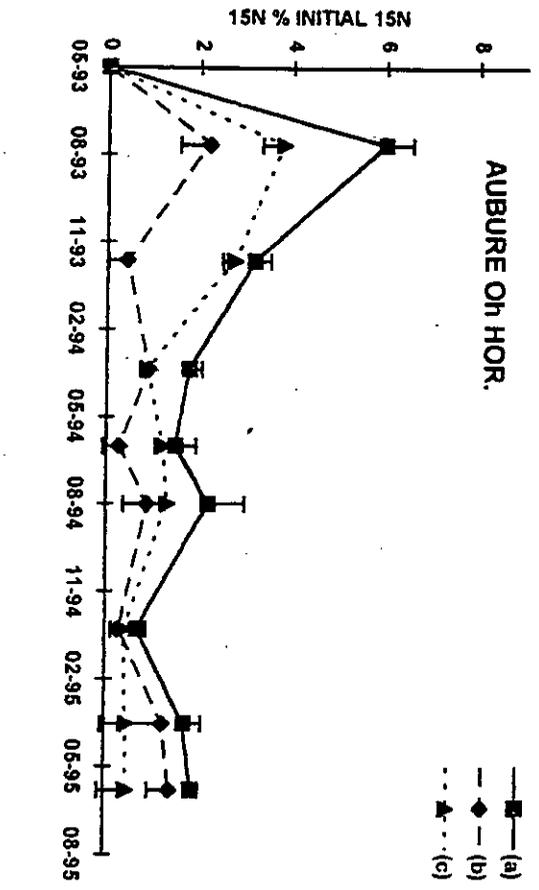
FIGURE 6B.   LABILE ¹⁵N

(a) TOTAL K₂SO₄ EXTRACTABLE ¹⁵N ━━■━━
(b) ORGANIC K₂SO₄ EXTRACTABLE ¹⁵N ━ ◆ ━
(c) INORGANIC K₂SO₄ EXTRACTABLE ¹⁵N ┈┈▲┈┈
in % of INITIAL TOTAL ¹⁵N

(b) = (a) - (c)

90

the other soils. The highest microbial biomass level was obtained in two Mediterranean calcareous soils, followed by an active neutral mull sampled in Britain under Atlantic conditions. The decreasing phase was closely controlled by the soil properties: in clay soils the microbial biomass was protected whereas in sandy soils it disappeared more quickly. Clays protect the MB under shortened resources conditions (Van Veen *et al.* 1985). Thus using both tracer field- and laboratory data, to model the soil organic matter pools, it is necessary (i) to link them to soil microclimate, using for instance climate-related coefficients controlling the decomposition rates (Parton *et al* 1987), and (ii) to integrate some essential soil related properties. Bottner *et al* (1996 B) showed that under similar incubation conditions, pH and clay content had the highest effects on decomposition rates and on microbial biomass.

In this work, the microbial biomass variations were apparently weakly related to climate variations. An essential characteristic of soil microbial populations is to survive unfavourable resource and external conditions, by modifying their activity, reflected by high variations of the metabolic quotient (Anderson and Domsch 1985, 93). In this experiment, only the size of the biomass was measured; the microbial activity or the specific respiration rates were not calculated.

(3) The K_2SO_4 extractable compounds result from initial plant material and from labelled microbial metabolites. In all sites the total extractable ^{15}N decreased during the incubation time, suggesting exhaustion of the labile ^{15}N. While in Mediterranean the exhaustion was very rapid, occurring during the first wet autumn, in the Scandinavian sites their production and use were slow, lasting over two years. Through the North-South climatic sequence comparable evolution pattern were observed for MB-^{15}N and for K_2SO_4-extractable ^{15}N. Leaching of extractable ^{15}N to the deeper horizons was evident only in the northern soils were the turnover rates of N were low, with high residence times of the labile material in the upper horizons. Leaching of labile material out from the upper horizon is another problem that has to be solved when modelling the organic matter pools.

(4) The relatively higher pH values generally observed in Mediterranean soils, even on acid parent material like at Thezan, compared to Boreal, Atlantic or Continental humus types, may explain the significant higher nitrification rates generally observed under Mediterranean forests, including conifers.

4. General discussion and conclusion.

In this work, a standard ^{15}N labelled plant material was incubated in diversified humus types from a large climatic European transect of coniferous forest soils. The aim was to investigate the transformations and transfers of ^{15}N in some major N pools and through the N profile. An increased exhaustion of labile N through the North-South soil sequence was observed, reflected by the decrease of ^{15}N in K_2SO_4-extractable N, in inorganic N and in microbial biomass N and by a decreased MB-^{15}N turnover. The upwards transfers of the label was important only through grass. Moss and fungi had little effects. The downwards transfers are

essentially controlled by (1) biological processes, regulating organic N solubilisation and mineralisation and (2) physical processes controlling the leaching. The transfers were apparently greatly lowered by frost in the deeper horizons of the Scandinavian soils. Lateral transfers chould be examined. The maximum leaching occurred at the Atlantic and continent sites. In all sites the total rainfall was comparable except at the Mediterranean site. At this later one the leaching was relatively low, despite the quick ^{15}N evolution.

There are two essential factors, acting in the same way, that may explain the North-South variations: the climatic conditions and the variations of the humus types. Exploring a large range of soils from various climatic regions, Insam *et al* (1989) and Insam (1990) demonstrated an intimate relationship between climatic conditions and the microbial biomass C pool and respiratory C flux. Nevertheless in the same way as there is a climatic distribution of vegetation and soils (on similar parent material), there is under natural vegetation a climatic distribution of humus types. The incubation experiments under controlled conditions, examining the effects of soil and humus related properties, demonstrated that in the soils from high latitudes the microbial biomass and activity are lower and the decomposition of the standard material slower than in the soils from Mediterranean regions. Similar effects of soil properties (particularly pH and exchangeable Ca) on microbial biomass and metabolic quotient for CO_2 (qCO_2) were investigated by Anderson and Domsch (1993) on the based on geographical soil distribution and by Wolters and Joergensen (1991) in response to soil acidification.

Another aim of these field and mentioned laboratory experiments was to explore the possibilities and the limits of the methods using a standard labelled material in natural ecosystem soils. The conditions were to use low N content material and to add it to the soils in small amount, i. e. without modifying the chemical, physical and biological properties of the native soil organic matter. The initial material was labelled at 7%. In the initially labelled horizons, the lowest isotopic enrichment (IE) values were measured in the total soil N, where the maximum ^{15}N dilution occurred; they ranged from 0.035 to 0.12%. The highest (IE) values were obtained from inorganic N and from fumigated samples, reaching 0.7 to 0.8%. In the ongoing programmes we use labelled material with IE = 10%. Given the high precision of the modern mass-spectrometers, this ^{15}N concentration seems to be sufficient to follow the tracer in the essential active N pools. Currently in such experiments the essential problem to overcome is the high heterogeneous distribution of the label in the soil, specially in the samples from the first samplings, when the major part of the label is still in form of undecomposed plant material. In the extracts, using high amounts of soils, the heterogeneous distribution of ^{15}N is less crucial.

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Research area: C and N mineralisation, nitrification

Objectives

All sites

(1) To quantify C and N mineralisation rates in organic and mineral soil horizons

(2) To identify nitrification types and nitrifier organisms

(3) To determine ammonium and nitrate formation rates in relation to soil pH and soil horizon

Intensive study sites

(4) To determine ammonium and nitrate formation rates in relation to different soil temperature and soil moisture conditions

(5) To compare ammonium and nitrate formation rates in undisturbed and sieved soil horizons

Materials and methods

(1) Soil samples (four replicates) were collected quantitatively from different soil horizons, plots and sites (cf Tables 1 and 2). Fresh samples were brought to the laboratory, where total C and N concentrations and pools were determined. The samples were sifted and incubated aerobically at 15°C and 50-60 % WHC (water-holding capacity at "optimum" soil moisture) for 150-200 days. CO_2 evolution rates were determined periodically over the whole incubation period on a gas chromatograph equipped with a hot wire detector. Net N mineralisation rates and net nitrification potentials were calculated based on the accumulation of ammonium and nitrate in the laboratory. The accumulation was determined after destructive samplings at certain intervals. For details see Persson & Wirén (1995).

(2) A subset of samples were treated with acetylene, sulphuric acid, calcium carbonate and urea to obtain knowledge of the nitrification types. Soils with low nitrification potential were, furthermore, inoculated with nitrifying humus.

(3) Determination of pH, CEC, base saturation and soluble aluminium was made to link the results obtained in (1) with C and N pool sizes and acidity variables.

(4) Samples of nitrifying mor humus were placed in different temperatures (-4, +0.5, +5, +15, +25°C and freezing/thawing cycles between -4 and +5°C) and soil moisture conditions (15, 30, 60 and 100 % of water-holding capacity) and incubated in the same manner as in (1). The studies were made using a nitrifying humus layer at Hassl"v, not far from Skogaby in Sweden.

(5) Intact soil columns (25 cm depth) from three sites (Klosterhede, Hilleröd, Åheden) were incubated at the same temperature and moisture conditions as in (1) to obtain a comparison with sifted soil samples as regards net N mineralisation and nitrification.

Results and discussion

(1) C and N pools and C and N mineralisation

Total C and N soil pools to a depth of 50 cm were highest for the Fichtelgebirge sites (Waldstein and Schacht) and lowest for Thezan (Tables 1 and 2). Among the individual soil layers, the 0-10 cm layer had generally high C and N contents. High C and N figures were also found for the FH layers at Waldstein and Klosterhede. High C:N ratios (can be calculated from Tables 1 and 2) were found in the litter layers at Thezan, Åheden and Skogaby (94, 45 and 37, respectively). The C:N ratios generally decreased from the litter layer to about 10 cm depth, under which the C:N ratio did not change much with depth. Åheden with low C:N ratio (12) at great depths and Klosterhede with high C:N ratio (39) in the 0-10 cm layer did not follow the general pattern. The average C:N ratio for the whole soil profile at all sites was 23 (SD=4.5) with a minimum of 16 at Aubure 1 (80-year-old spruce) and a maximum of 32 at Klosterhede.

Carbon mineralisation rates (CO_2 evolution rates) were calculated for the same soil layers and sites as mentioned above. The rates, obtained at 15°C and 50-60 % WHC, generally decreased over time in all soil materials. Estimated mean C mineralisation rates over the whole time period studied (150-200 days) in the litter and humus layers are shown in Table 3. A positive relation between the C:N ratio in the litter and humus layers and the C mineralisation rate was found when the different sites were compared (Figure 1). This relation indicates that an increase in N concentration in the upper soil layers may result in a negative feed-back on the decomposition rate and in an increase in the soil C pool. No such relation could be detected for the deeper soil layers. These findings are in agreement with earlier observations, but the mechanisms are still not obvious (Fog 1988, Nohrstedt et al. 1989, Persson & Wirén 1989).
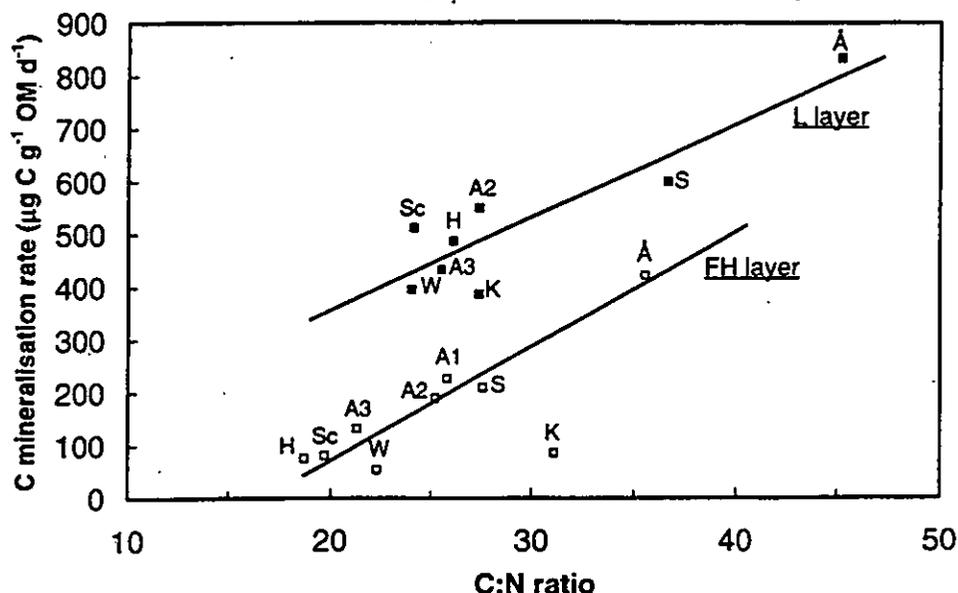

Figure 1. Mean C mineralisation rate (15°C, 50-60 % WHC) in relation to the C:N ratio in the litter (L) layers ($R^2=0.82$) and humus (FH) layers ($R^2=0.92$ when Klosterhede was omitted) at Aubure (A1, A2, A3), Fichtelgebirge (W and Sc), Klosterhede (K), Hilleröd (H), Skogaby (S) and Åheden (□).

Table 1. Carbon pools (tonnes per ha) in different soil layers in pine (Thezan), beech (Aubure 3, Schacht, Hillerød) and spruce sites in the Niphys transect through Europe. No samples were taken at 30-50 cm depth at Schacht. No FH layer was found at Thezan and Hillerød, where the 0-10 cm layer was subdivided into 0-5 and 5-10 cm layers. Aubure 1 and 2 are 80- and 40-year-old spruce stands, respectively.

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	4.3	Incl. in FH	4.0	3.5	4.3	7.0	4.9	5.2	1.9	3.4
FH	(0-5): 7.1	16.0	12.7	7.6	54.7	28.9	50.7	(0-5): 19.3	18.3	11.5
0-10 cm	(5-10): 4.4	16.0	20.9	27.7	44.8	63.7	27.5	(5-10): 12.7	25.7	21.4
10-20 cm	5.5	6.4	10.5	14.9	33.4	33.0	29.9	18.9	17.8	9.3
20-30 cm	6.2	6.0	10.5	12.0	30.0	31.1	17.9	17.1	15.0	5.7
30-50 cm	11.3	8.8	17.2	28.4	34.5	-	13.1	20.0	19.6	4.0
Total	38.9	53.2	75.7	94.0	201.7	163.6	144.0	93.1	98.3	55.4

Table 2. Nitrogen pools (tonnes per ha) in different soil layers and sites (see Table 1 for further explanation).

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	0.05	Incl. in FH	0.14	0.14	0.18	0.29	0.18	0.20	0.05	0.08
FH	(0-5): 0.25	0.62	0.50	0.36	2.46	1.47	1.65	(0-5): 1.03	0.66	0.32
0-10 cm	(5-10): 0.18	0.97	1.19	1.60	1.77	3.13	0.71	(5-10): 0.61	1.07	0.66
10-20 cm	0.21	0.49	0.58	0.84	1.31	1.52	0.90	0.84	0.76	0.39
20-30 cm	0.27	0.50	0.56	0.63	1.15	1.38	0.64	0.74	0.61	0.32
30-50 cm	0.46	0.72	0.93	1.35	1.52	-	0.49	0.92	0.83	0.33
Total	1.42	3.29	3.90	4.91	8.39	7.78	4.57	4.34	3.98	2.10

Table 3. Mean C mineralisation rate (µg C g^{-1} LOI d^{-1}) for each soil layer and site as calculated from laboratory incubations at 15°C and 50-60 % WHC. LOI=loss on ignition. n.e.=not estimated. See Table 1 for further explanations.

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	n.e.	Incl. in FH	550	433	396	512	387	487	600	833
FH	n.e.	227	190	132	54	81	75	(0-5): 76	210	423
0-10 cm	n.e.	40	83	43	25	28	63	(5-10): 33	45	57
10-20 cm	n.e.	17	23	19	20	26	22	35	17	17
20-30 cm	n.e.	9	14	12	8	15	9	21	18	11
30-50 cm	n.e.	11	12	7	4	n.e.	11	24	12	8

Table 4. Annual C mineralisation (kg C ha^{-1} yr^{-1}) for each soil layer and site as calculated from laboratory incubations, temperature and moisture corrections, and soil pools in the field (see text). LOI=loss on ignition. n.e.=not estimated. See Table 1 for further explanations.

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	n.e.	Incl. in FH	536	357	375	825	499	634	270	402
FH	n.e.	883	589	243	657	506	975	(0-5): 480	760	671
0-10 cm	n.e.	211	465	330	251	374	471	(5-10): 123	300	228
10-20 cm	n.e.	42	73	84	171	203	214	190	100	46
20-30 cm	n.e.	25	46	46	68	119	63	114	50	24
30-50 cm	n.e.	51	79	71	49	n.e.	50	170	60	30
Total		1212	1788	1132	1570	2025	2272	1710	1540	1401

The annual C mineralisation for the whole soil profile to a depth of 50 cm was estimated to be between 1100 and 2300 kg C ha^{-1} (Table 4). Most of the carbon mineralised originated from the organic layer and the uppermost (0-10 cm) mineral soil. The data are based on the mean rates obtained in the laboratory multiplied by (1) the amount of sieved soil per soil layer, (2) a temperature correction factor assuming a Q_{10} of 2.5, and a moisture correction factor (simply chosen as 75 % of the rates obtained at "optimum" moisture).

The accumulation pattern of inorganic N in the humus layers differed considerably between sites (Figure 2). Most sites had a net N mineralisation during the initial incubation period, but in the Åheden humus, almost no inorganic N accumulated during the first 50 days followed by an increased rate of N mineralisation. The data, thus, indicate that the N mineralisation was suppressed. A possible explanation is that the trees take up organic N by means of their pro-teolytic ectomycorrhiza (see Read & Taylor, this report), and the ectomycorrhizas mine the forest floor for proteins. This means that the proper decomposer microorganisms should probably be N limited and immobilise N. As a result, the decomposer organisms continued to immobilise N during the initial phase of the incubation despite the absence of roots and mycorrhizas. Later on they probably became carbon limited and started to mineralise N vigorously. There were also other differences between sites. The net N mineralisation pattern at Aubure 2 was similar to that at Åheden in the meaning that a great amount of N was mineralised during the latter phase of the incubation.
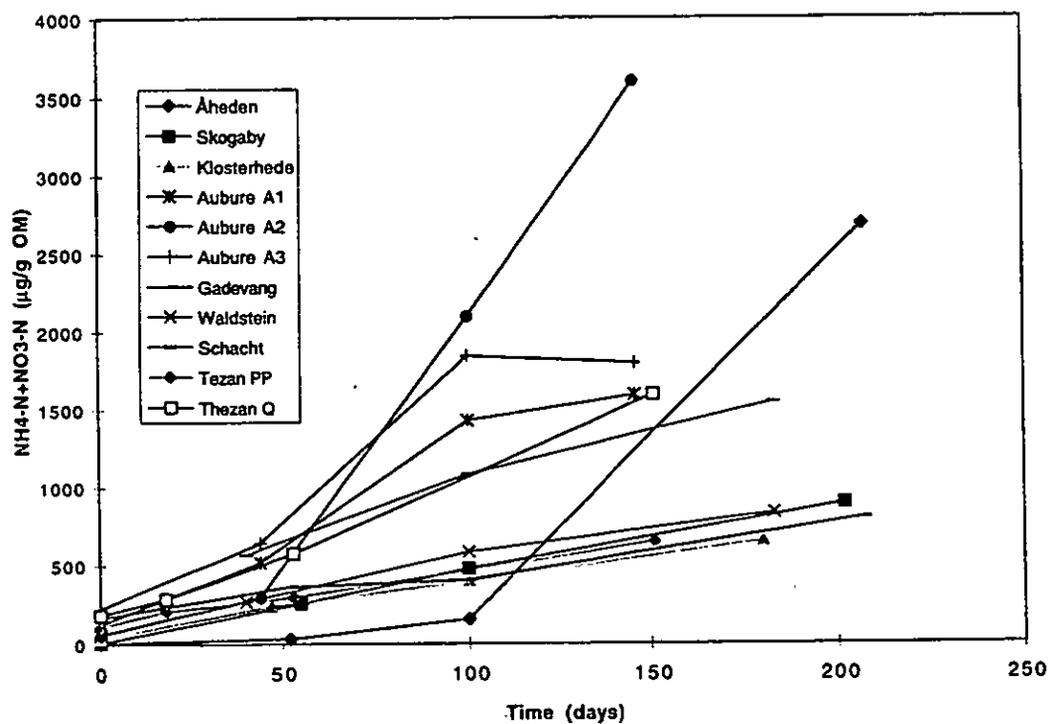
Figure 2. Accumulation of inorganic N (NH_4^+-N and NO_3^--N) in humus layers from the sites investigated. At sites where the humus layer was lacking, the uppermost 0-5 cm of the mineral soil was included in the figure. OM=organic matter.

Net N mineralisation and nitrification rates estimated per g in the laboratory are not shown here. The annual net N mineralisation and net nitrification potentials were calculated using the same assumptions as regards Q_{10} values and soil moisture as for C mineralisation. The annual estimates show that Åheden had the lowest annual N mineralisation (18 kg N ha^{-1}), and Schacht had the highest (152 kg N ha^{-1}; Table 5). Based on the first incubation period, the estimate of Åheden should be as low as 1-2 kg N ha^{-1}, whereas the plant uptake probably was in the order of 20-30 kg N ha^{-1} year^{-1}. This indicates that the uptake of organic N might be as high as 90-95 % of the total plant uptake of N at Åheden.

There are few possibilities to check the estimates independently. The best possibility occurred at Skogaby, where essential parts of the ecosystem's N budget have been estimated. N in throughfall, N in litterfall, accumulation of N in the shoot biomass and N in leaching have been estimated, and calculations of N in root litter formation have been made (Persson 1996). According to these measurements, the inflow of 62 kg N ha^{-1} year^{-1} in N mineralisation and 17 kg N ha^{-1} year^{-1} in throughfall are exactly balancing the estimate of N uptake by the roots of 79 kg N ha-1 year-1. No leaching was estimated in the control plots. The estimates based on the incubation method followed by extrapolation to the field conditions, thus, fitted independent data. Such checks are necessary but presupposes data on N in litterfall and tree fractions.

It was also possible to check whether the data on mineralised C to mineralised N were close to the data on C:N ratios in the same soil layers. The Cmin:Nmin ratios were often close to the C:N ratios in the litter and humus layers, but often a bit lower (2-5 units) than expected in the mineral soil at the sites in Central Europe and Denmark. If this discrepancy is real, the mineralised fraction consisted of a substrate rich in N, and the C:N ratio in the soil should increase. A possible explanation is that a fraction of the microbial biomass was decomposed during the incubations. This will be further tested in the CANIF project through determination of the microbial biomass.

(2) Nitrification types and nitrifier organisms

The nitrification potential varied considerably between sites. Schacht, Waldstein and Hilleröd had high nitrification potentials throughout the whole soil profile, while Klosterhede, Åheden and Skogaby had low potentials (Table 5). Differences in nitrification potentials were found between the stands at Aubure, where the 80-year-old spruce and 150-year-old beech stands had fairly high potentials and the 40-year-old spruce stand (Aubure 2) had low. Differences in nitrification potential could partly be linked to ammonium availability. Åheden, Klosterhede, Skogaby and Aubure 2 had small amounts of inorganic N, indicating that the nitrifiers were

Table 5. Annual net N mineralisation (above) and potential net nitrification (below) (kg N ha^{-1} yr^{-1}) for each soil layer and site as calculated from laboratory incubations, temperature and moisture corrections, and soil pools in the field (see text). See Table 1 for further explanations.

Net N mineralisation:

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	0.0	Incl. in FH	21.5	9.8	12.8	33.9	17.0	14.3	3.4	1.2
FH	(0-5): 9.3	40.3	40.6	18.7	47.7	41.4	43.4	(0-5): 40.0	29.0	16.9
0-10 cm	(5-10): 7.5	21.7	40.1	28.2	25.0	47.9	15.3	(5-10): 10.8	20.0	0.1
10-20 cm	8.8	5.8	5.1	11.3	15.7	17.4	8.4	12.1	6.0	0.2
20-30 cm	6.1	5.8	4.6	8.9	5.75	11.7	1.9	10.4	2.3	0.1
30-50 cm	5.4	6.1	5.7	14.4	4.02	-	0.8	6.7	1.2	-0.1
Total	37.1	79.6	117.6	91.4	111.0	152.3	86.8	94.3	61.9	18.3

Potential nitrification:

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	0.0	Incl. in FH	0.4	1.4	3.8	10.4	0.0	4.2	0.0	0.0
FH	(0-5): 3.9	10.1	1.3	6.0	10.0	14.2	0.0	(0-5): 18.3	0.0	0.0
0-10 cm	(5-10): 5.3	7.3	4.1	15.1	13.6	43.7	0.0	(5-10): 6.3	0.2	0.0
10-20 cm	6.3	6.1	1.9	8.0	19.7	24.0	0.1	8.7	4.0	0.0
20-30 cm	5.0	5.0	2.2	5.2	6.0	13.6	0.2	10.6	2.3	0.2
30-50 cm	2.6	5.6	4.0	8.1	4.6	-	0.9	6.5	1.3	0.0
Total	23.0	37.1	13.9	34.8	57.7	105.9	1.2	54.6	7.8	0.3

Table 6. pH(H$_2$O) in different soil layers in pine (Thezan), beech (Aubure 3, Schacht, Hillerød) and spruce sites in the Niphys transect through Europe. See Table 1 for further explanations.

Soil layer	Thezan	Aubure 1	Aubure 2	Aubure 3	Waldstein	Schacht	Klosterh.	Hillerød	Skogaby	Åheden
L	4.57	Incl. in FH	4.15	4.61	4.72	5.03	4.14	5.15	4.00	4.28
FH	(0-5): 5.58	3.96	3.77	4.01	3.68	4.34	3.88	(0-5): 4.29	3.93	3.93
0-10 cm	(5-10): 5.90	3.60	3.45	3.57	3.52	3.99	3.94	(5-10): 4.07	3.99	4.45
10-20 cm	5.88	3.93	3.78	3.80	3.80	4.32	4.22	4.15	4.34	5.09
20-30 cm	5.90	4.27	4.01	4.09	4.26	4.49	4.47	4.40	4.45	5.18
30-50 cm	5.75	4.48	4.24	4.43	4.44	-	4.60	4.65	4.52	5.27

suppressed by low ammonium levels. However, Skogaby, Klosterhede and Aubure 2 had high mineralisation (ammonification) potentials, showing that the ammonium concentration rather than the ammonification rate was decisive for the nitrification rate.

A higher proportion of the available ammonium was nitrified at greater soil depths than in the uppermost soil (Table 5). Consequently, net N mineralisation was high in the organic soil layers but most of the nitrate had a potential to be formed in the mineral soil.

Treatment with acetylene inhibited most of the nitrate formation at all sites. The treatment seemed to be more efficient in the conifer than in the beech stands. According to the present knowledge, this indicates that most of the nitrification was caused by autotrophic nitrifiers.

In the FH and 0-5 cm layers the nitrification process was stimulated by addition of $CaCO_3$ and reduced by addition of H_2SO_4. Thus, in these soil layers, the nitrifiers seemed to be acid sensitive and favoured by bases. At Klosterhede, the nitrate concentration was below the detection limit also with $CaCO_3$. Addition of urea had no effects on the nitrification rate at Åheden and Klosterhede, but stimulated the process in combination with $CaCO_3$. A comparison over all sites showed that the nitrification at sites with comparatively high pH (e.g. Thezan and Hilleröd) was inhibited by sulphuric acid at a higher pH value than in sites with "naturally" low pH (e.g. Aubure and Waldstein) (Table 6). Further studies will hopefully show whether the nitrifiers are different species or the same species that has adapted or evolved to the increased acidity.

In the 10-20 cm soil layer, the effect of pH on nitrification was less marked. For example, the addition of $CaCO_3$ decreased (Åheden, Skogaby, Hilleröd, Aubure 1), did not change (Waldstein, Schacht) or increased (Aubure 2 and 3) the nitrification rate. Addition of sulphuric acid had almost no effect at Skogaby, Waldstein and Schacht but decreased the nitrification rate at Aubure (all stands). We hypothesize that the nitrifiers might be protected from the acidity by having access to weatherable minerals (being absent in a proper humus layer). According to this hypothesis, addition of $CaCO_3$ and sulphuric acid should not give an effect on nitrifier activity in weatherable soils but give an effect in heavy weathered soils (for example at Aubure).

In conclusion, all sites had a nitrification potential, at least at greater depths, but the nitrification rates differed enormously, partly dependent on high or low concentrations of ammonium in the soil. There was no clear difference between beech and spruce sites as regards nitrification potential. An increase in pH in the organic soil layers stimulated nitrification and a decrease in pH reduced nitrification at the same site. This indicates that the nitrifiers were acid sensitive. However, there was no correlation between nitrification potential and soil pH seen

over all sites. Therefore, we hypothesise that acid-tolerant strains have evolved where ammonium is abundant and pH is low. Acid-tolerant forms have not evolved where ammonium is scarce or where pH is high. Results obtained from the mineral soil indicates that the nitrifiers can tolerate low pH values in soil layers with weatherable minerals.

(3) Determination of ammonium and nitrate formation rates in relation to pH and soil horizon

All data and discussion on pH relations and soil depths are given above.

(4) Effects of soil temperature and soil moisture on C and N mineralisation

Some of the results on C and N mineralisation rates at different temperature and moisture levels are presented in Table 7, where the relations are given as percent of the mean value obtained at 15°C and 60 % WHC. As expected, the C and N mineralisation rates decreased (from the reference value at 15°C and 60 % WHC) with decreasing temperature and moisture, but the C mineralisation rates decreased more than the N mineralisation rates. Thus, the ratio of mineralised C to mineralised N decreased with decreasing temperature and moisture. When the temperature was close to zero, this ratio was fairly close to 5, i.e. close to the C:N ratio of the cell contents of microorganisms. One interpretation is that the microbial exoenzymes are not functioning well at low temperature and moisture conditions, implying that the microbial energy supply should be increasingly dependent on the utilisation of cell contents.

Table 7 also shows that the C mineralisation was about the same at 60 and 100 % WHC, while the net N mineralisation was high at 60 % WHC and close to nil at 100 % WHC. Other studies showed that the denitrification rate was high at 100 % WHC, resulting in a low N_2O production and a high N_2 production. Obviously, 100 % WHC did not inhibit nitrification. Periodical freezing/thawing cycles consisting of periods with one week at -4°C and one week at +5°C resulted in a similarly high mineralisation of N as at a constant temperature of +5°C.

The study showed that our use of a Q_{10} relation of 2.5 was fairly appropriate for adjustment of net N mineralisation in the field but too low for adjustment of the C mineralisation. The study also showed that the C and N mineralisation rates are highly dependent on soil moisture. For the future development, these and other data are intended to produce better response functions for modelling, e.g. in the SOILN model.

Table 7. Mean C and N mineralisation rates at different temperature and moisture levels in relation to a reference rate (100) at 15°C and 60 % WHC. The figures are based on 150-day-incubations. Negative values indicate a reduction of N present at the start. n.e.=not estimated.

	C mineralisation				N mineralisation			
	15% WHC	30% WHC	60% WHC	100% WHC	15% WHC	30% WHC	60% WHC	100% WHC
+25°C	41	88	175	183	78	122	204	0
+15°C	24	55	*100*	86	32	67	*100*	-5
+5°C	9	21	30	33	20	31	45	-7
+0.5°C	3	9	14	13	9	16	24	-2
-4°C	1	1	1	0	4	9	11	-1
-4/+5°C	n.e.	n.e.	n.e.	n.e.	20	30	37	-7

Table 8. Net N mineralisation rates and potential nitrification rates (μg N g^{-1} LOI d^{-1}) in undisturbed soil columns and sifted soil layers from Klosterhede, Hilleröd and Åheden incubated at 15°C and 60 % WHC for 150-200 days.

Net N mineralisation rate

	Klosterhede		Hillerød		Åheden	
	Undist.	Sifted	Undist.	Sifted	Undist.	Sifted
L	8.6	13.1	6.7	16.2	1.0	1.8
FH	4.5	3.4	7.2	8.5	1.7	7.6
0-10 cm	2.9	2.2	5.8	3.6	0.4	0.0
10-20 cm	3.0	0.9	3.4	2.8	0.1	0.1

Potential nitrification rate

	Klosterhede		Hillerød		Åheden	
	Undist.	Sifted	Undist.	Sifted	Undist.	Sifted
L	0	0	3.2	3.9	0	0
FH	0	0	3.2	4.2	0	0
0-10 cm	0	0	2.2	2.2	0	0
10-20 cm	0	0	2.4	2.1	0	0

(5) Net N mineralisation and potential nitrification in undisturbed soil columns

The estimates of net N mineralisation rates in undisturbed soil columns to a depth of 20 cm on one hand and sifted soil layers incubated as separate layers on the other differed considerably (Table 8). The variation between plots and individual soil columns was very large for the Åheden soil. This variation makes the comparison uncertain. In the undisturbed soil columns at Klosterhede and Hilleröd, the net N mineralisation rates were lower in the litter layers and higher in the mineral soil in comparison with the sifted soil. A possible explanation is that the soil moisture was better controlled in the sifted soil layers that were incubated separately. In the soil columns, the (mean) soil moisture was maintained by watering, which resulted in a

drier litter layer and a wetter mineral soil due to drainage. It is also possible that inorganic N and dissolved organic N leached from the litter layer to the mineral soil in the soil columns. On the other hand, this is contradicted by the fact that the potential nitrification was very similar in the different layers in the columns and in the soil layers that were incubated separately. Therefore, most of the differences between soil columns and separate soil layers probably depended on different soil moisture resulting in different mineralisation rates.

Conclusions

(1) A positive correlation was found between the C:N ratio in the litter and humus layers and the CO_2 evolution rate, indicating that an increased N deposition can increase the pool of soil C by reduced decomposition. The lack of net N mineralisation at the northernmost site (Åheden) during the first phase of the incubation supports the ideas of an uptake of organic N via proteolytic mycorrhizas and indicates that this type of uptake can be as high as 90-95 % of the total N uptake by the conifers and dwarf-shrubs at Åheden.

(2) All sites had a nitrification potential, although in some sites a combination of $CaCO_3$ and urea additions was needed to detect any potential. There was no clear difference in nitrification potential between spruce and beech sites from the same region. Acetylene treatment inhibited most of the nitrification at all soil depths, indicating that autotrophic nitrification dominated the nitrate production. The nitrification potential were generally higher in the mineral soil than in the humus layer.

(3) Addition of $CaCO_3$ stimulated and H_2SO_4 always suppressed nitrate formation in the upper soil layers, indicating that the nitrifiers were acid sensitive, but the nitrifiers seemed to have different tolerance limits at different sites. In the mineral soil, there was no clear effect of addition of bases or acids.

(4) C and N mineralisation did not respond similarly to changes in soil temperature and soil moisture. Net N mineralisation was higher at low temperatures than expected from the estimates of C mineralisation at the same temperatures. A possible explanation is that the micro-organisms use their own cell contents with low C:N ratio as an energy source during harsh conditions. Freezing/thawing cycles resulted in more N mineralised than concluded from studies at constant temperature.

(5) Comparisons of net N mineralisation in undisturbed soil columns and sifted soil showed differences that partly could be explained by different moisture regimes. Due to the method used, the effect of sifting could not be fully evaluated.

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8. Part I

Dr. Björn R. Andersen.

Laboratory: Danish Forest and Landscape Research Institute.

Objectives

To determine the importance of soil water fluxes of dissolved organic nitrogen (DON) and carbon (DOC) under different climatic conditions in broad-leaved and coniferous forests, and to quantify organic nitrogen fluxes in both above- and below-ground compartments at the Danish spruce site. The hypotheses were 1) that the part of total N (TN) in forest soil water bound in organic compounds is significant, and 2) that this leads to a direct link between the N and C cycling in forest ecosystems, possibly most pronounced in boreal forests (northernmost part of the NIPHYS gradient).

Methodology

Soil water were sampled semi-continuously throughout the project period from all the main NIPHYS sites using chemically inert porous suction cups (PRENART(TM), Frederiksberg, Denmark) installed in different soil depths (25, 55, and 90cm). The samplers were installed with two sets of two cups in each soil depth in the beginning of the project period, i.e. a total of six samples per site per tree species. At Klosterhede existing installations were used (three triplet sets in 25 and 55cm depth); no 90cm samplers were installed here. The samplers were continuously draining into glass bottles placed in thermos boxes buried in the ground to minimize microbial conversion of the chemical compounds of interest. Suction (c. -0.6 MPa) were applied using a portable pump once every two to four weeks after collecting water obtained in the preceding period (continuous sampling at Klosterhede).

The soil water samples were stored refrigerated near the respective sites and send to our laboratory in Denmark for analysis once or a twice a year. Samples were analyzed for dissolved organic C (DOC) using a carbon analyzer (Shimadzu TOC5000), inorganic N compounds (NO_3, NH_4) using standard colorimetrical methods, and total dissolved N (TN) (see below). Detection limits for the N compounds were c. 0.04 mg NO_3-N dm^{-3}, 0.03 mg NH_4-N dm^{-3}, and 0.1 mg TN dm^{-3}, respectively. Concentrations below the detection limits were included in calculations and plots with a value of 0.5 times the detection limit. Assuming concentrations of other nitrogen compounds (e.g. NO_2) to be insignificant, dissolved organic N (DON) were found by subtraction: DON = TN - (NO_3-N + NH_4-N). A limited number of samples with concentrations of one or more N compound close to the detection limit were found to have less TN than the sum of NO_3-N and NH_4-N, yielding a nonsense estimate of a negative DON concentration; in these cases the TN concentration was set equal to the sum of NO_3-N and NH_4-N.

A new analytical method for total dissolved N determination was developed within this project to make possible the analysis of a larger number of samples. The method was based on oxidative digestion of complex compounds by OxiSolve (TM) (Merck), a persulfate-based oxidizing agent, using micro-waves as energy supply, resulting in all N being oxidized to nitrate, which was subsequently determined by standard colorimetry. The complete procedure, including the oxidative digestion step in a standard kitchen micro-wave oven, was implemented in a Flow Injection Analysis system (Perkin Elmer FIAS300) and controlled by the system software, thus eliminating the need for a labor-intensive, time-consuming separate decomposition step (Andersen & Frederiksen 1996).

Results

Analytical method for TN determination

Our novel method for determination of total dissolved N content of aqueous samples was evaluated by a ring test including four other laboratories engaged in the NITREX project. In this test a certified reference sample and composite samples from the Danish spruce stand at Klosterhede representing natural levels for throughfall and soil water samples were analyzed for the N compounds and DOC. This test showed our method to produce results comparable to other analytical methods, although methods based on reduction rather than oxidation seemed to give slightly higher values for the TN content. Further, the ring test confirmed results obtained within these major EC ENVIRONMENT program projects to be comparable for all compounds.

Significance of DON

Fractions of TN constituted by DON were very variable at most positions along the NIPHYS gradient with respect to both tree species and soil depths. Further, a substantial spatial variability was evident with a large difference between parallel samples even though the use of sets of two or three cups draining into each collection vessel yielded composite samples. Ammonium concentrations were very low at all sites throughout the project period, as should be expected with no sites situated close to large point-sources included in the subset of NIPHYS sites studied here.

At Åheden at the northernmost position of the gradient nearly all samples had very low concentrations of any N compound with the majority falling below our analytical detection limits. It is thus not possible to assess the significance of organically bound N at this site from soil water concentrations, but it may be taken as a strong indication that the stands at Åheden are N limited (both spruce and birch), and that there is practically no leaching losses of N. The percentage of TN constituted by DON were high at all other sites and in all soil depths at least during some part of the project period as shown in Figure 1.

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At the Danish beech site Hilleröd there was a distinct seasonal pattern with high DON:TN ratios (50-75%) during summer 1994 and lower ratios (0-30%) during the dormant season; the summer of 1995 was too dry to allow soil water sampling and an early on-set of soil freezing in late autumn prevented water percolation through the soil profile. Similar summer ratios were observed at the German beech site Schacht in 1993 and 1994 while the site was inaccessible due to snow during the dormant season 1993/94; samples from 1995 are being analyzed but results are not yet available (samples send to Denmark February 1996). TN concentrations were relatively low in all deciduous stands (<2.5 mg N dm^{-3}) with the following rank ing among sites: Åheden<Thezan<Schacht<Hilleröd. The presently available mean concentrations of all N compounds in all depths throughout the project period at Hilleröd and Schacht are shown stacked upon each other in Figure 2; it is seen that there was a small leaching of N from the Danish site, but the concentration levels corresponds to a N-flux of less than 0.5 kg/ha/yr using a (high) conservative water flux estimate of 100 mm/yr. At Thezan in southern France the DON:TN ratios were very high (75-100%) in all soil depths during winter while the dry Mediterranean summers prevented soil water sampling during most of the growing season (Figure 1); the concentration levels, however, were generally very low($<<1$ mg N dm^{-3}), and the N-loss to deeper soil layers with the minute percolation volumes expected at Thezan (probably $<<50$ mm/yr) was thus insignificant.

The TN concentrations found under pine at Thezan were even less than under oak, but these samples too showed high DON:TN ratios in most cases (Figure 1). At the German spruce site, Waldstein, DON:TN ratios were generally much lower ($<25\%$) than at the other sites (Figure 1), which can be explained by a pronounced nitrification resulting in relatively high NO_3-N concentrations (Figure 3). Apparently, at this site there were a shift in the relative contribution of the different N compounds in the 90cm samples in early spring 1994 from a situation with very low DON:TN ratios to a stable level around 25% accompanying a general decrease in TN concentrations from c. 5.0 to c. 3.5 mg N dm^{-3} (Figure 3). Whether this represented a functional shift or were only a temporary change must await the results covering the period from late autumn 1994 onwards. The N loss in deep percolation at Waldstein were higher than at the other sites due to the higher TN concentrations and percolation volumes expected to be among the highest in this set of sites. At the Danish spruce site Klosterhede DON:TN ratios were well above 75% in nearly all samples from the control plots (ambient conditions, Figure 1), whereas the NITREX plot which was sprayed monthly with extra N (+35 kg N/ha/yr as dissolved NH_4NO_3) showed a pattern similar to Waldstein, albeit with lower TN concentrations in the deeper soil horizons (Figure 3). It was thus indicated that the trees and/or microorganisms at Klosterhede were not able to utilize the extra N introduced even though the treatment was started well in advance of the period studied here.
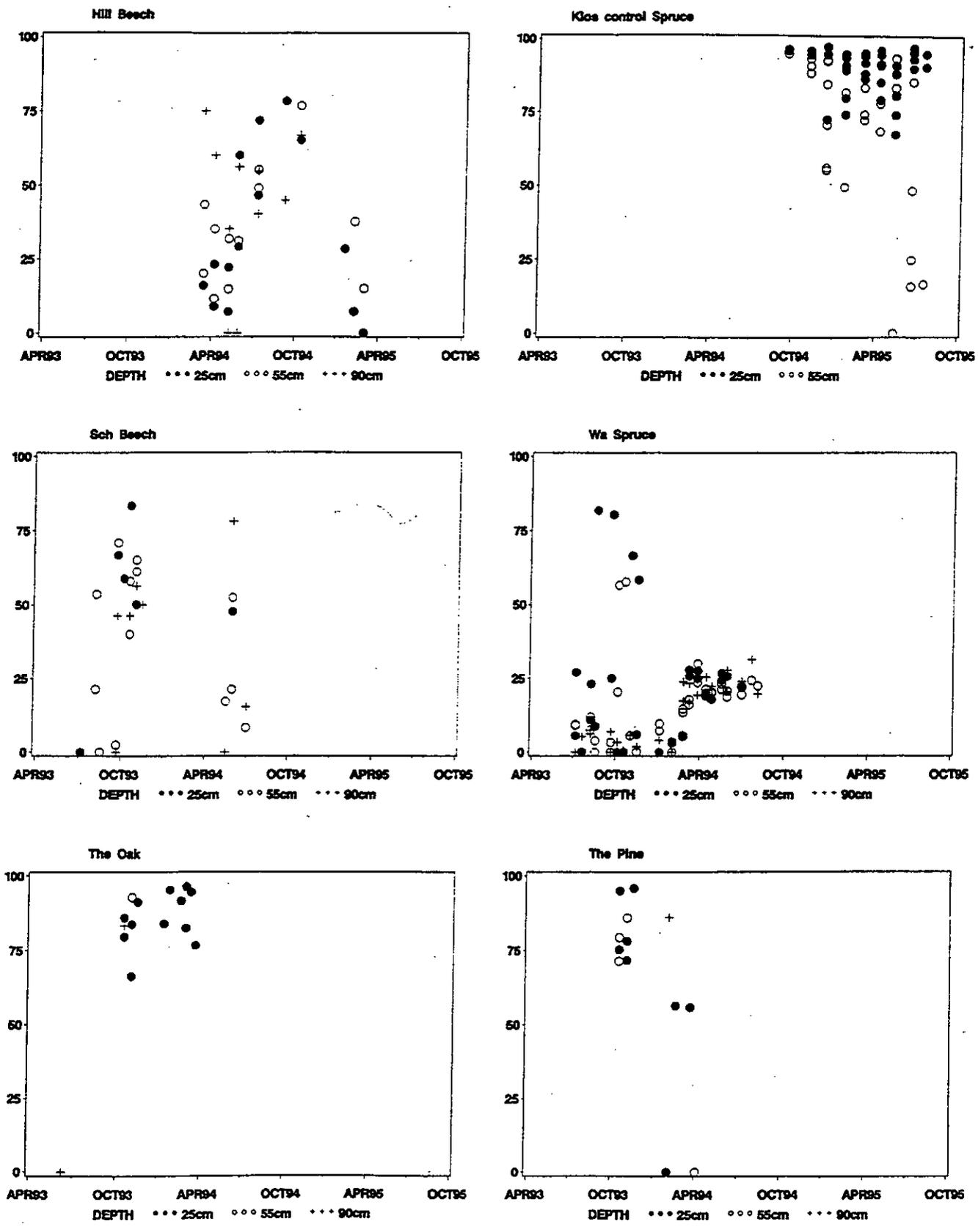
Figure 1. DON:TN ratios (in percent) plotted vs. sampling date. Symbols indicate sampling depth.
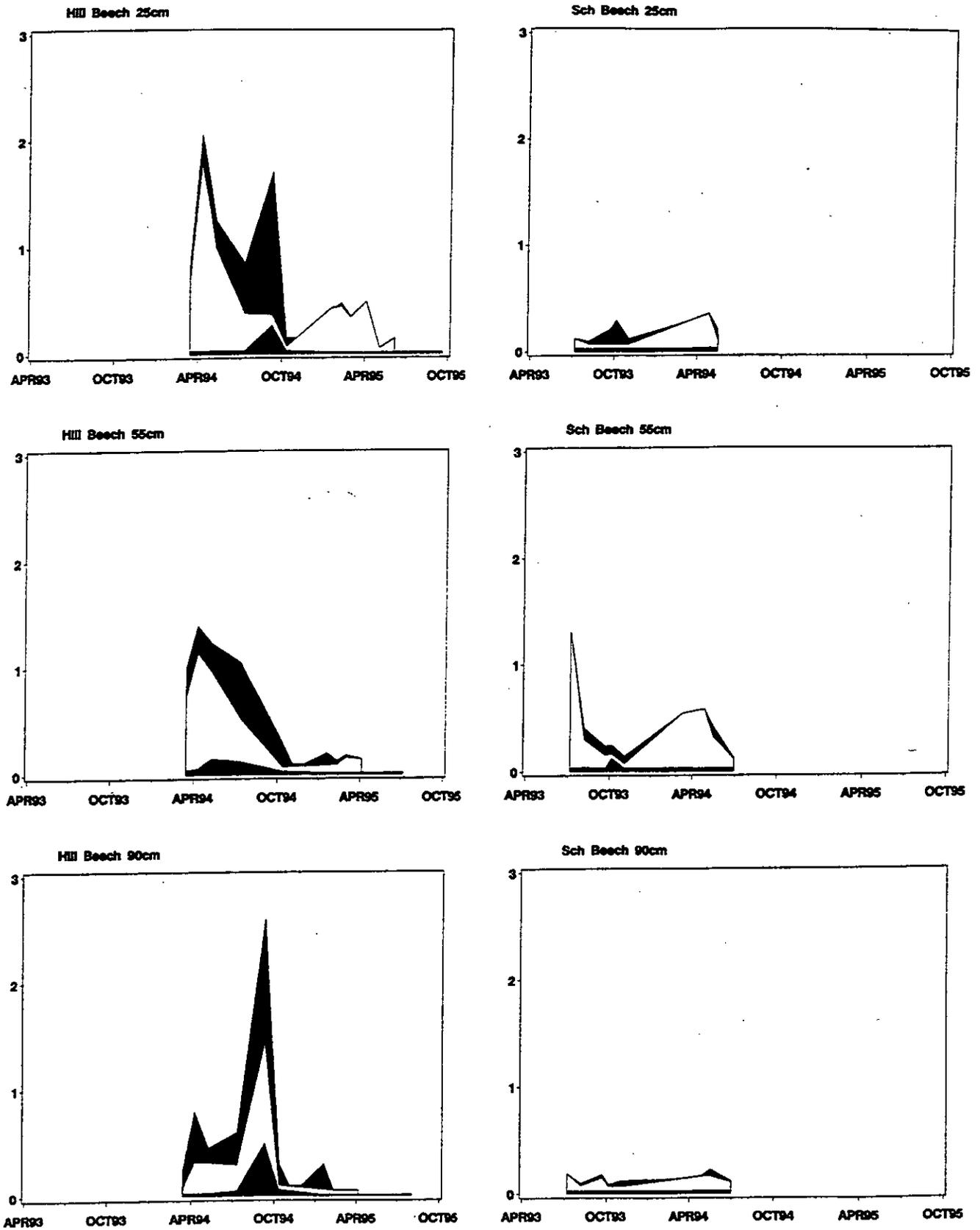
Figure 2. Mean concentrations of NH$_4$, NO$_3$, and DON (mg N dm^{-3}) for each soil depth and sampling date at the beech sites Hilleröd and Schacht plotted as stacked areas. In each subplot the lower black area is NH$_4$, the middle white area is NO$_3$, and the upper black area DON.
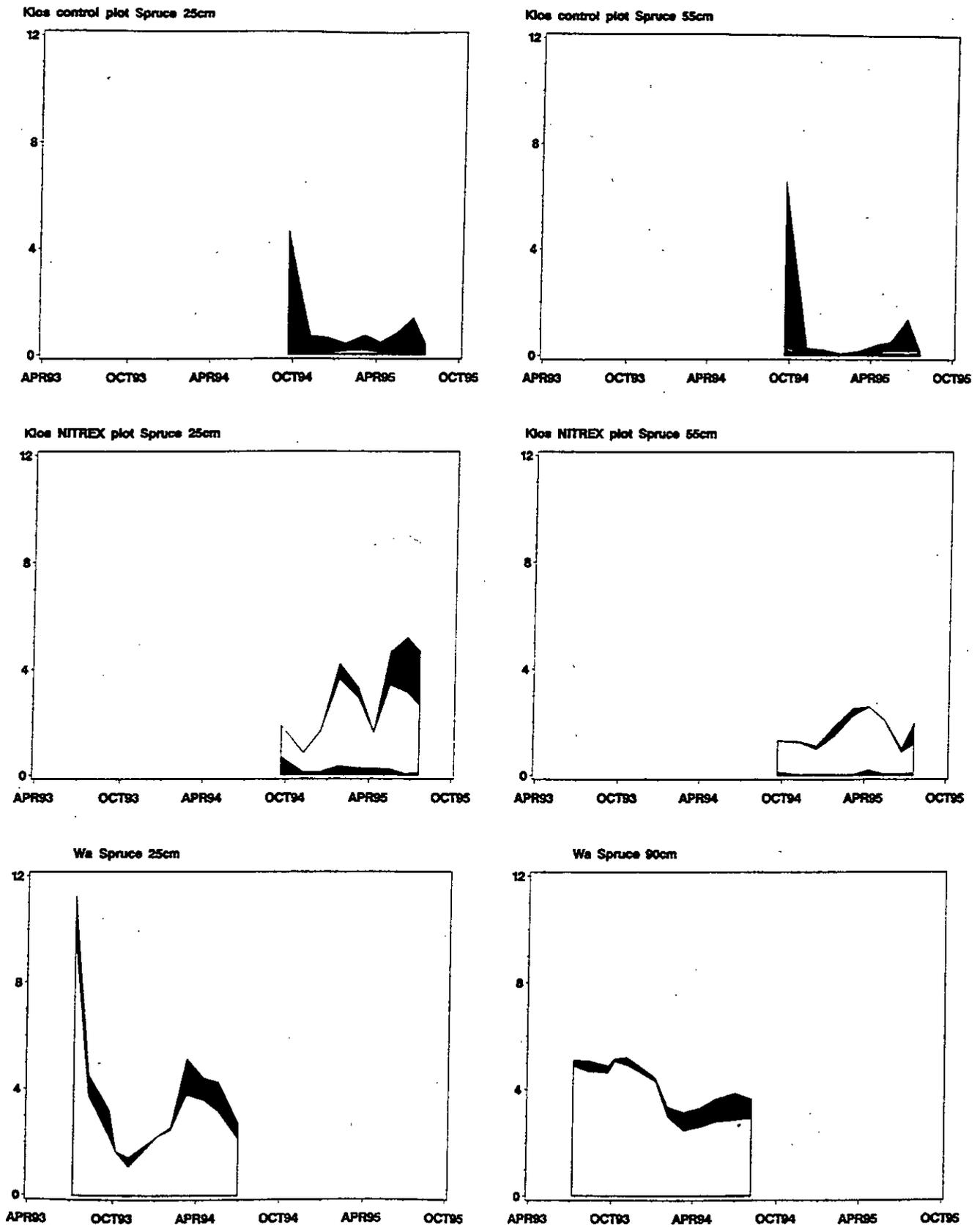
Klos control plot Spruce 25cm

Klos control plot Spruce 55cm

Klos NITREX plot Spruce 25cm

Klos NITREX plot Spruce 55cm

Wa Spruce 25cm

Wa Spruce 90cm

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Figure 3. Mean concentrations of NH_4, NO_3, and DON (mg N dm^{-3}) for the upper (right) and lower (left) soil depths vs. sampling date at the spruce sites Klosterhede and Waldstein plotted as stacked areas. In each subplot the lower black area is NH_4, the middle white area is NO_3, and the upper black area DON.
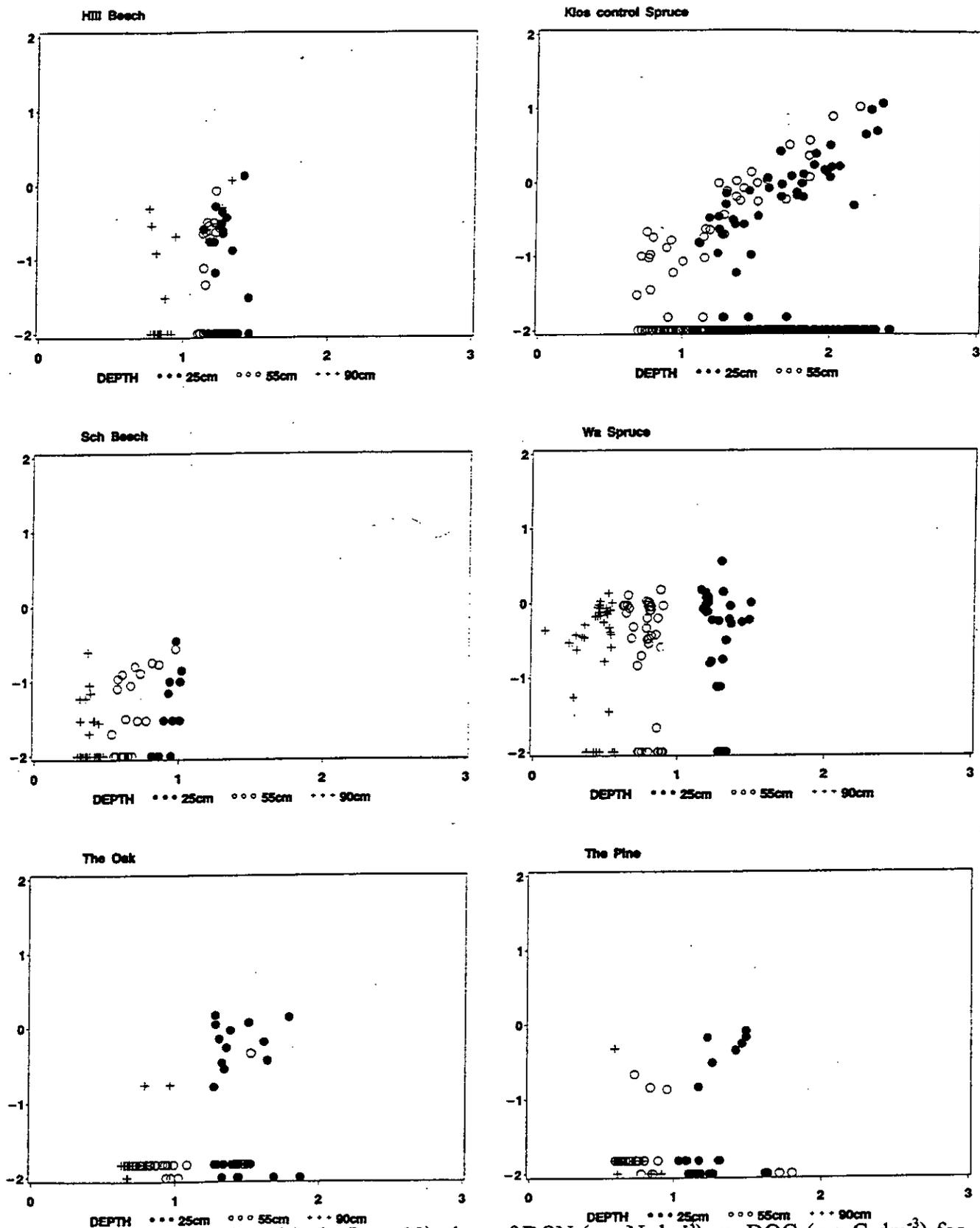
Figure 4. Double logarithmic (base 10) plots of DON (mg N dm^{-3}) vs. DOC (mg C dm^{-3}) for the Danish and German sites. Symbols indicate sampling depth. DON concentrations estimated to 0.00 mg N dm^{-3} (i.e. TN= NO$_3$-N+NH$_4$-N) are arbitrarily plotted as 0.01 mg N dm^{-3} (-2 in log units) to avoid undefined values.

DON - DOC relationship

In Figure 4 DON and DOC concentrations are plotted against each other to investigate if they were correlated (not all sites shown). The plot shows that DON and DOC were not correlated at the sites studied, except at the Danish spruce site. DOC concentrations in the different soil depths felled nicely into clusters showing the decrease in DOC concentration with increasing soil depth, as should be expected in acid soils. Also, at all sites a significant part of the samples in all soil depths had an extremely low DON concentration (<0.01 mg N dm^{-3}) even in samples with relatively high DOC concentrations.

At the control (ambient condition) plots at Klosterhede a number of samples are seen to have a positive correlation between the DON and DOC concentrations (figure 4). A test on samples having a DON concentration higher than 0.03 mg N dm^{-3} proved the correlation to be linear in both soil depth with a very high significance:

$$25 \text{ cm: DON} = -0.86 + 0.038 * \text{DOC} \quad (R^2 = 0.67***)$$
$$55 \text{ cm: DON} = -0.73 + 0.067 * \text{DOC} \quad (R^2 = 0.86***)$$

This linear relationship suggests that the composition of dissolved organic matter is fairly constant in time at some spots at Klosterhede, while at other spots only very small amounts of N is associated with DOC.

DON Fluxes at Klosterhede

The water balance is being established but is not yet available. Thus it is not possible to quantify the above- and below-ground DON fluxes. A strong positive correlation between DON and DOC in throughfall water was found (data not shown). There were practically no soil water percolation at Klosterhede following March 1995 due to an extraordinarily dry summer followed by a rapid on-set of soil freezing in late autumn. Due to these conditions it has not been possible to collect soil water.

Conclusions

"scientific"

The present study has clearly shown that the organically bound fraction of total dissolved N in forest soil water is significant in most cases, irrespective of climatic conditions and tree species, and in some cases can be the predominant fraction. This finding substantiates earlier findings and suggestions (Gundersen & Rasmussen 1990, Matzner 1988, Rasmussen et al. 1991). A direct relationship between DON and DOC in soil water was found at one site only (spruce at Klosterhede, Denmark), indicating that processes resulting in dissolved organic matter with no or very small N amounts are normally dominating.

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"general"

According to this study it may be misleading to assess N losses from forest ecosystems based on measurements of inorganic N compounds alone. This suggests that estimates of critical loads of N to forests may have to be lowered at some places, and that water supplies presently believed not to have significant N contents may be affected. However, no sites with very high atmospheric N inputs were included in the present study; at such sites it may be assumed that the inorganic N compounds will be predominant.

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Rasmussen L et al. (1991). Forest response to changes in atmospheric chemistry (mainly acid, nitrogen, and heavy metals deposition) and implications in ecosystem functioning and management. Proceedings of "First European Symposium on terrestrial Ecosystems, Forests, and Woodlands", Florence 20-24 May, 1991.

8. Part II

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Research Area: In situ denitrification in relation to abundance and distribution of physiological types of nitrate reducing bacteria in beech forest soil

Objectives:

The overall objective of the microbiological subproject was to quantify the loss of gaseous nitrogen compounds from beech forest soils and link the gas production to decomposing activity.

In order to meet this objective and to determine the limiting factors for the microbial processes experiments were established with untreated soil and with soil with different additions of the substrates, acetate and nitrate. The N_2O emissions were estimated with and without the use of acetylene as an inhibitor of nitrous oxide reduction.

Methodology:

Field measurements of N_2O and CO_2 emission:

In situ N_2O and CO_2 emission from beech forest soils were determined using a closed chamber technique: PVC tubes, 23 cm in length and 11 cm in diameter, were hammered approx. 10 cm into the soil. To ensure minimum effect on the measurements due to disturbance of the soil, the tubes were inserted one day prior to the measurement date. At the beginning of each measurement, the PVC tubes were covered with gas tight lids. Holes in the lids (1 cm in diameter) were closed with butyl rubber stoppers. Some tubes received an addition of acetylene (10 Pa to inhibit nitrification or 10 KPa to inhibit N_2O reductase in order to determine the ratio of N_2O to N_2 production) through the rubber stoppers. At regular intervals, 4.5 ml gas samples were collected from the PVC tubes through the rubber stoppers with a syringe. Five gas samples were collected during an interval of 5-6 hours from morning to mid afternoon. The gas samples were stored in vacutainers (Venojects) until they could be analysed. As soon as the Venojects arrived at the lab, gas samples were analysed for N_2O and CO_2 using a chromatograf (HP-5890) with an electron capture detector and a 3 m Porapac Q column. The chromatograf had valves and a back-flush system for shunting acetylene and water vapour past the detector. Detector temperature was 265°C and column temperature was 70°C. N_2O and CO_2 concentrations in the samples were determined by comparison with standards of known

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content. Samples from Hillerőd (DKb) were always analysed within a week from the sampling date.Rates of N_2O and CO_2 emission were determined by linear regression of the data and converted into grams of nitrogen or carbon emitted per square meter per day using the area of soil covered by a PVC tube.

Laboratory measurements:

At the end of each measurement a number of tubes were excavated and brought back to the lab for further characterisation of the soil content. The soil cores were divided into two fractions: the upper organic layer containing the fine beech roots and the mineral soil below. The soil was passed through a 2 mm sieve and carefully mixed prior to dividing it into sub samples. The following parameters were measured in these two fractions: Nitrate and ammonia content, soil moisture content, biomass of fine roots (in the organic layer) and the number of nitrate reducing, i.e. denitrifying and nitrate respiring bacteria.5 g of soil (fresh weight) was extracted for 1 hour with 0.1 M KCl. The extract was centrifuged and membrane filtered (0.4 μm). The NO_3N and NH_4N content was then determined by flow-injection technique using an autoanalyzer (Tecator, Sweden). Water content on a dry weight basis was determined gravimetrically by re-weighing soil samples of approx. 5 g after 24 hours at 90°C. Fine roots (less than 2 mm in diameter) were removed by hand and the dry weight determined after 24 hours at 90°C.The number of nitrate reducing bacteria in the soil was determined using an MPN (most probable number) method: Venojects containing 0.2 ml of growth medium under a nitrogen atmosphere were used as MPN tubes. The growth medium was nutrient broth amended with glucose, 5 mM KNO_3 and 5 mM NH_4Cl. NH_4Cl was added to inhibit assimilatory nitrate reduction. A dilution series was made from a slurry of 10 g of soil in 90 ml of Winogradsky basic mineral solution. From this dilution series MPN tubes (5 replicates per dilution) were inoculated with 0.1 ml of the slurry using a syringe and disposable needle. After 3 weeks of incubation at 22°C in the dark the concentration of nitrite were tested with Gries Reagent. Tubes positive for nitrite were considered containing nitrate respiring (nitrite accumulating) bacteria. Tubes negative for nitrite were amended with zinc powder to reduce nitrate to nitrite and tested for nitrite. Tubes negative in both tests were considered containing denitrifying bacteria. The numbers of denitrifying and nitrate respiring bacteria were determined from the combination of positive tubes using an MPN table. The formation of gaseous nitrogen in the MPN tubes were routinely monitored by gas chromatography

Experimental set-up at the different sites:

The site at Hillerőd (Dkb) was most intensively covered: the set-up at each measurement date included installation of 65 tubes: 10 tubes were amended with 10 Pa acetylene to inhibit nitrification. 10 tubes were amended with 10 KPa to inhibit N_2O reductase. The organic layer

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was removed prior to installation of 5 tubes. 20 tubes were left untreated for determination of baseline emission rates. 20 tubes were used for determining the denitrifying potential of the soil: Of these, 5 tubes were amended with 100 ml of distilled water. Another 5 tubes were amended with 100 ml of distilled water containing 1 mM KNO_3. 10 tubes were amended with 1 mM KNO_3 and 1 mM $C_2H_3NaO_2$ in 100 ml of distilled water. 5 of these tubes were additionally amended with 10 KPa acetylene. Sampling was done once a month from Marts 1995 to December 1995.At Jezeri (CRb) 25 tubes were installed in November 1994 and April 1995; at these two occasions 5 tubes remained untreated, 5 were amended with 10 KPa acetylene and 10 tubes were amended with 1 mM KNO_3 in 100 ml of distilled water. 5 of these tubes were additionally amended with 10 KPa acetylene. At June and August 1995 only 5 untreated tubes were used for measurements. At Collelongo (Is) 28 tubes were installed in October 1995: 10 remained untreated, 9 were amended with 10 Pa acetylene and 9 were amended with 10 KPa acetylene. At all sites, the untreated tubes were excavated and brought back to the lab for further characterisation of the soil, i.e. the above mentioned parameters.

Results

Hilleröd (DKb), Denmark:
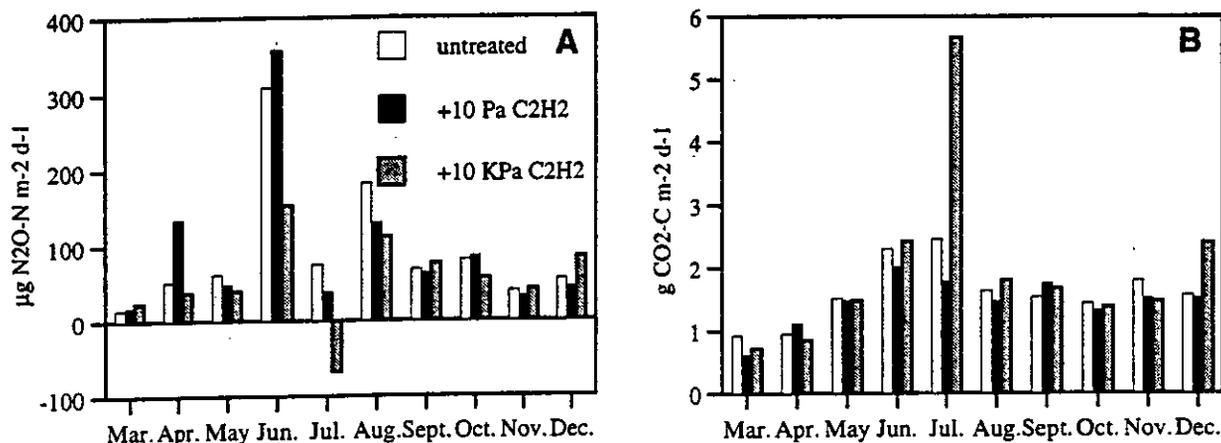
Figure 1. N_2O emission (A) and CO_2 emission (B) from Hilleröd (DKb)

In situ N_2O and CO_2 emission at Hilleröd (DKb) were monitored during 1995 (Fig. 1). A maximum N_2O emission rate of 308 µg N_2O-N m-2 d-1 was obtained from the untreated soil in June. At all other sampling dates the rates were below 200 µg N_2O-N m^{-2} d^{-1} with a minimum in Marts of 15 µg N_2O-N m^{-2} d^{-1}. Addition of low concentrations of acetylene to inhibit nitrification resulted in decreased emission rates at six sampling dates and in increased rates at four sampling dates making conclusions regarding the influence of nitrification on N_2O

emission difficult. A similar irregular pattern was seen when high concentrations of acetylene were added: N_2O emission was affected, resulting in decreased rates on six sampling dates and in increased rates at four sampling dates. In July, the soil subjected to high concentrations of acetylene had a negative emission rate, i.e., this treatment turned the soil into a sink for N_2O at that occasion. In spite of these conflicting results, the end product of denitrification at Hilleröd was mainly N_2O as acetylene added in high concentrations did not result in substantially higher N_2O emission rates at any sampling date.

The maximum CO_2 emission rate was 2.5 g CO_2-C m^{-2} d^{-1} from the untreated soil in July. The lowest rate was 0.9 g CO_2-C m^{-2} d^{-1} in Marts. CO_2 emission was influenced by addition of acetylene to a lesser degree than the N_2O emission rates except for one measurement in July where high concentration of acetylene resulted in a doubling of the CO_2 emission rate. The pattern of maximum CO_2 emission from the untreated soil in the summer and minimum emission rates in early spring and winter reflected to some extent the N_2O emission rates.
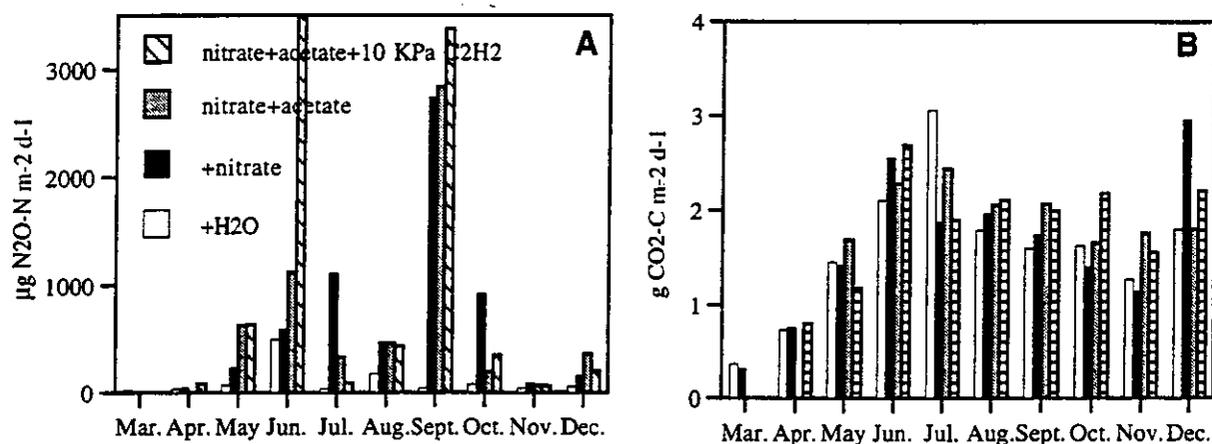
Figure 2. Effects of nitrate and acetate amendments on N_2O emission (A) and CO_2 emission (B) at Hilleröd (Dkb).

To determine the limiting factors of N_2O emission and CO_2 emission in the soil additional amendments were made (Fig. 2). At all occasions, except in March, addition of nitrate boosted N_2O emission compared to addition of distilled water indicating that the processes in Hilleröd responsible for N_2O emission were nitrate limited. In June, N_2O emission from soil treated with nitrate and acetate exceeded N_2O emission from soil treated with nitrate alone 12 times indicating that the processes at that occasion were carbon limited. At the other sampling dates, addition of acetate did not significantly increase N_2O emission indicating that the concentration of easily degradable carbon was not limiting in the soil. No major effects of addition of nitrate and acetate on CO_2 emission were detected.
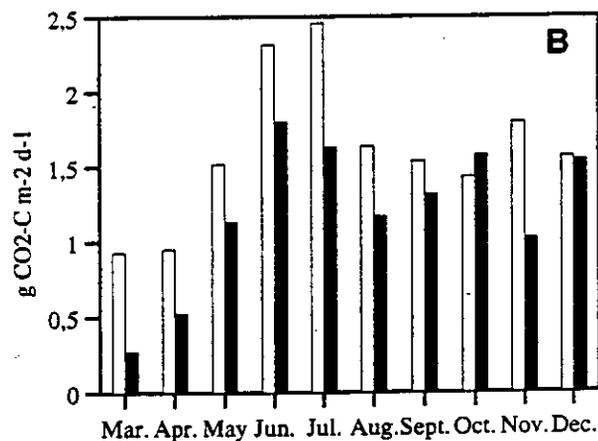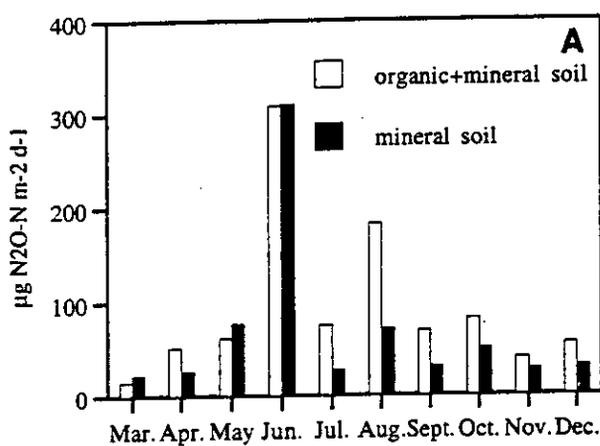
Figure 3. Contribution of organic and mineral soil layer to N_2O emission (A) and CO_2 emission (B) at Hilleröd (Dkb).

The relative contribution of the organic and mineral soil layer to N_2O and CO_2 emission was investigated (Fig. 3). Except for measurements in March, May and June the removal of the organic soil layer markedly decreased gas emission from the soil indicating that a major part of the gas emission from Hilleröd originated from the organic layer.
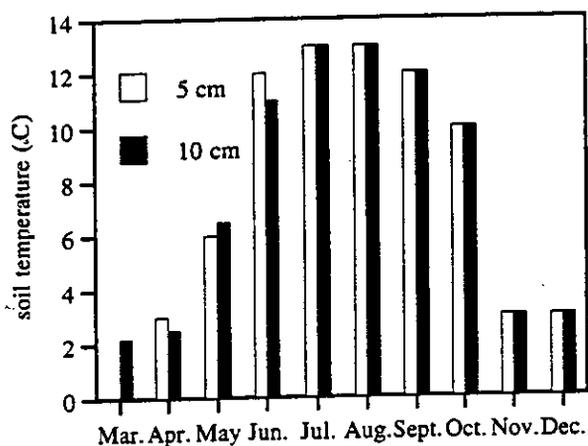

Figure 4. Soil temperature at Hilleröd.
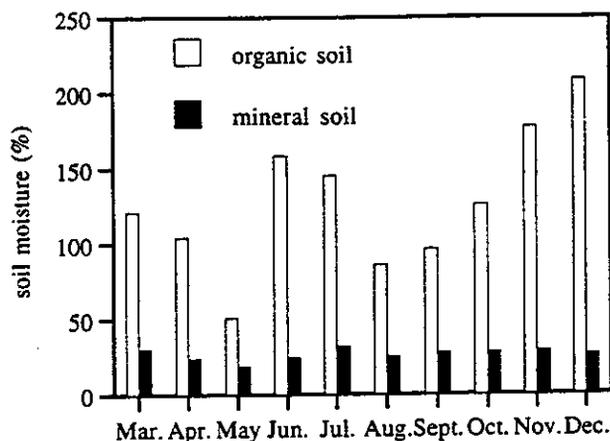
Figure 5. Soil moisture content at Hilleröd.

The soil temperature at Hilleröd (Fig. 4) increased from 2°C in March to a maximum of 13°C in July and August declining to 3°C in December. At all sampling dates, the temperature in 5 and 10 cm depth was almost equal.

Fig 5 shows the water content (on a dry weight basis) of the organic and mineral soil layer, respectively. Little fluctuations were seen in the mineral soil from 18.7 % in May to a water content of 31.5 % in July. In contrast, the water content of the organic fraction of the soil showed markedly seasonally differences ranging from 50.9 % in May to over 200 % of water in the organic soil layer in December.
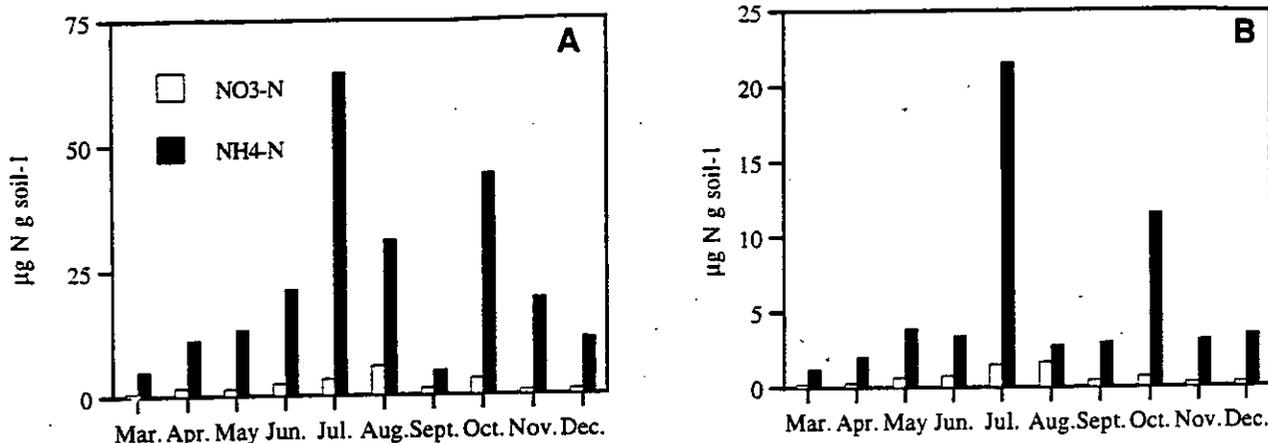
Figure 6. Soil content of extractable inorganic nitrogen from organic layer (A) and mineral layer (B) at Hilleröd (DKb).

The amount of inorganic nitrogen in the form of ammonia-N in the soil was always higher - on average eight times - than the nitrate-N fraction (Fig 6). Both nitrate and ammonia concentrations followed a seasonal pattern with highest concentrations in the soil in the summer and low concentrations in spring and winter. The concentrations of both nitrate-N and ammonia-N were on average more than three times as high in the organic fraction as in the mineral soil.
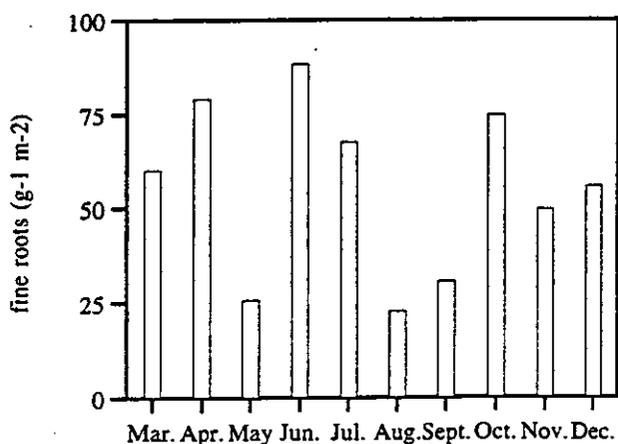
Figure 7. Biomass of fine beech roots (<2 mm in diameter) at Hilleröd (DKb).

Fig. 7 shows the biomass of fine beech roots less than 2 mm in diameter in the organic soil layer. There were marked variation in the amount of root biomass at the different sampling dates from a maximum of 88 g m-2 in June to a minimum value of 23 g m-2 in August.
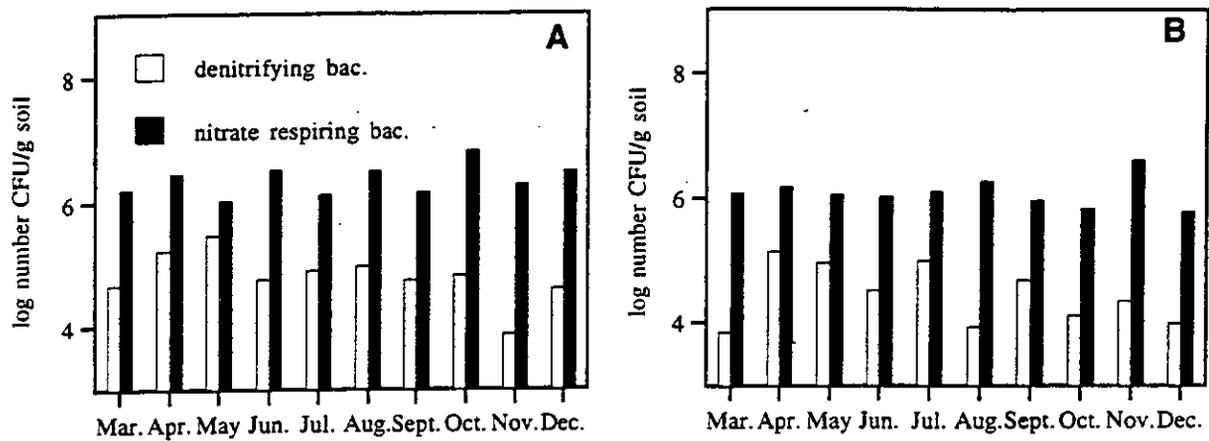
Figure 8. Nitrate reducing bacteria in organic layer (A) and mineral layer (B) at Hilleröd (DKb).

Enumeration of nitrate reducing bacteria in the soil showed larger populations of nitrate respiring bacteria (producing nitrite) than of true denitrifying bacteria capable of complete conversion of nitrate to gaseous nitrogen (Fig. 8). At most sampling dates, the denitrifying bacterial population constituted less than 10 % of the nitrate respiring bacteria in both the organic and the mineral soil layer. No clear seasonal pattern of population dynamics were discernible; the population of nitrate respiring bacteria remained remarkable constant during the year while there was a tendency to a decline of population density of denitrifying bacteria from spring on.

Table 1. Statistically significant (p<0.05) correlations between N_2O and CO_2 emission and soil parameters at Hilleröd (DKb).

	CO_2 emission	NO_3 mineral layer	NH_4 mineral layer	water content mineral layer	soil temperature
N_2O emission	0.43	0.21			0.26
CO_2 emission			0.20	0.20	

A correlation matrix of N_2O and CO_2 emission and various soil parameters is shown in Table 1. Based upon the number of replicate measurements of each parameter statistically significant correlation coefficients were determined. Significant correlations were as follow; N_2O emission was significantly correlated with CO_2 emission, nitrate content of the mineral soil layer and soil temperature. CO_2 emission was correlated with ammonia content and water content of the mineral soil layer.

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Jezeri (CRb), The Czech Republic:
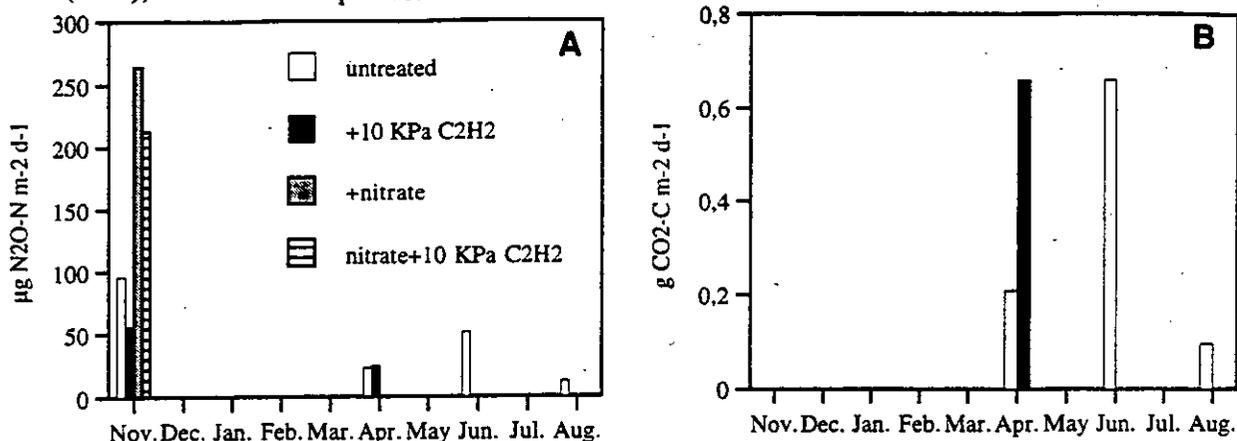
Figure 9. N₂O emission (A) and CO₂ emission (B) from Jezeri (CRb).

Fig. 9 shows N_2O and CO_2 emission from beech forest soil at Jezeri, The Czech Republic subjected to different treatments. N_2O emission rates from untreated soil were small from 96 µg N_2O-N m-2 d-1 in November to a minimum of 12 µg N_2O-N m-2 d-1 in August. Addition of high amounts of acetylene did not result in higher N_2O emission rates indicating that N_2O was the end product of denitrification in this soil. Addition of nitrate more than doubled the N_2O emission rate compared to the untreated control. CO_2 emission was also low with a maximum reached in June of 0.66 g CO_2-C m-2 d-1 and a minimum of 0.10 g CO_2-C m-2 d-1 in August.
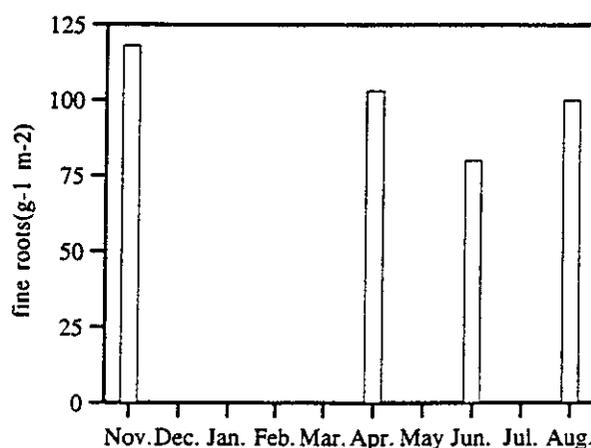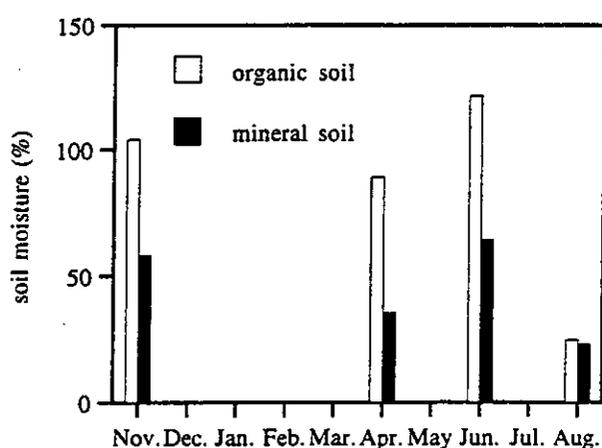
Figure 10. Soil moisture at Jezeri (CRb).   Figure 11. Biomass of fine roots at Jezeri.

The water content (on dry weigh basis) of the organic and mineral soil layer is shown in Fig 10. The water content of the organic soil layer exceeded 80 % except in August where the water content was 24.8 %. At the other measurements the water content of the organic soil was approximately twice that of the mineral soil layer. Fig. 11 shows the biomass of fine beech roots less than 2 mm in diameter in the organic soil layer.
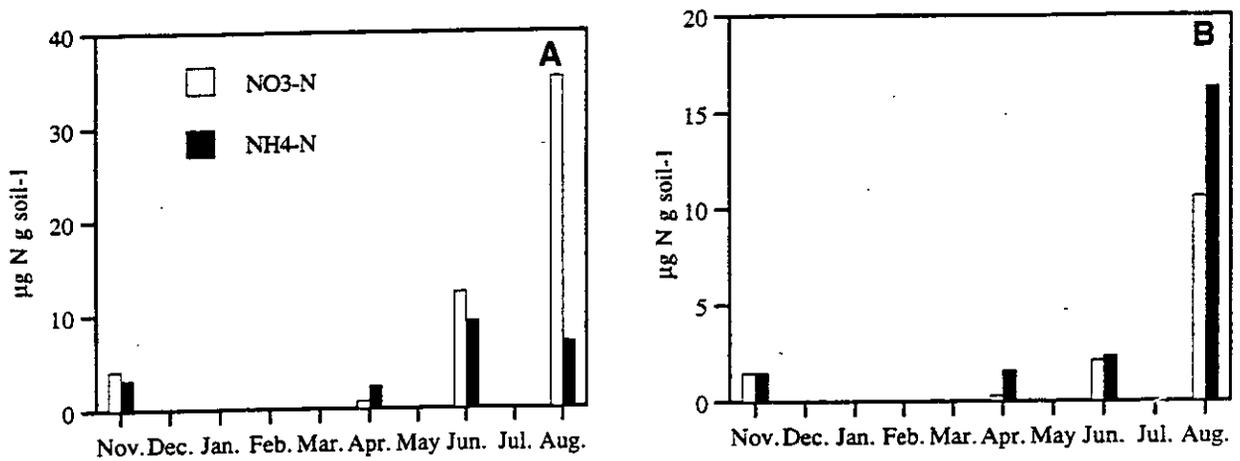
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Figure 12. Soil content of extractable inorganic nitrogen from organic layer (A) and mineral layer (B) at Jezeri (CRb).

The concentration of extractable inorganic nitrogen in the soil layers showed a seasonal pattern with maximum concentrations in the summer and lower values in fall and spring. In April, in both soil layers, the amount of nitrogen recovered as ammonia was higher than the amount found as nitrate. At the other sampling dates the concentration of nitrate-N was higher than the amount of ammonia-N in the organic layer.
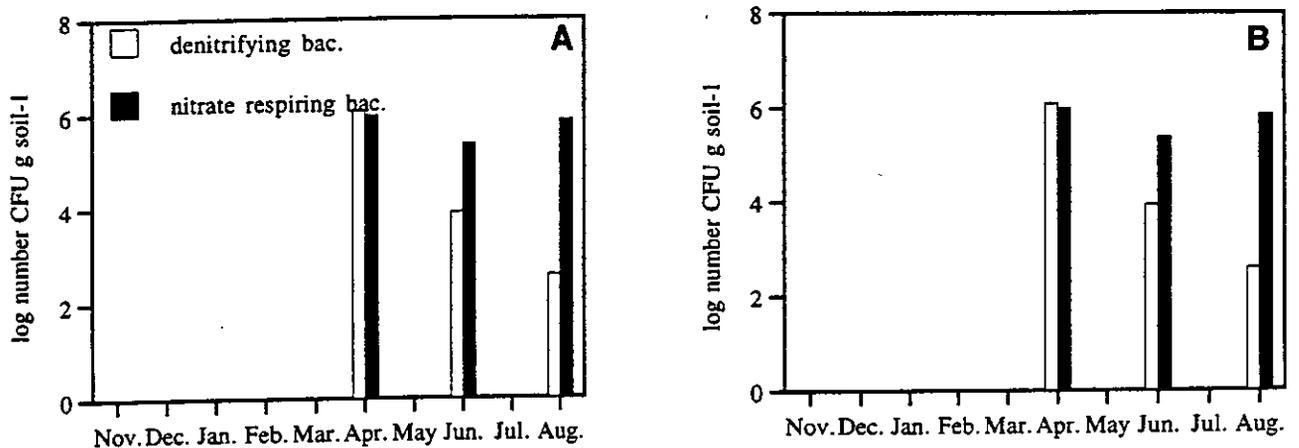
Figure 13. Nitrate reducing bacteria in organic layer (A) and mineral layer (B) at Jezeri (CRb).

Fig 13 shows the number of nitrate reducing bacteria in the organic and mineral soil. The number of nitrate respiring bacteria remained fairly constant between 100.000 and 1.000.000 per g of soil from April to August in both the organic and in the mineral soil layer. The population of denitrifying bacteria declined several orders of magnitude from 1.000.000 to below 1000 per g of soil in both soil layers during the same interval.
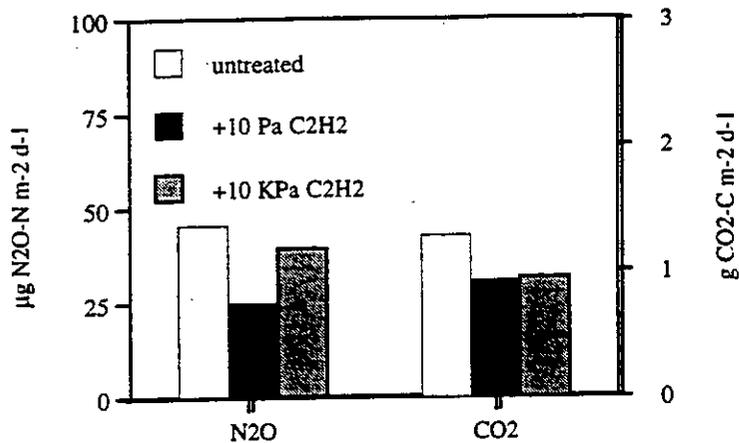
Collelongo (Is), Italy:

Figure 14. N_2O and CO_2 emission from Collelongo (Is).

In October N_2O emission and CO_2 emission were low (Fig. 14). N_2O emission was 45 μg N_2O-N m-2 d^{-1} in the untreated soil was slightly inhibited by addition of acetylene indicating that N_2O was the final product of denitrification at that sampling date. CO_2 production in the untreated soil was 1.29 g CO_2-C m^{-2} d^{-1} and was also slightly inhibited by addition of acetylene.
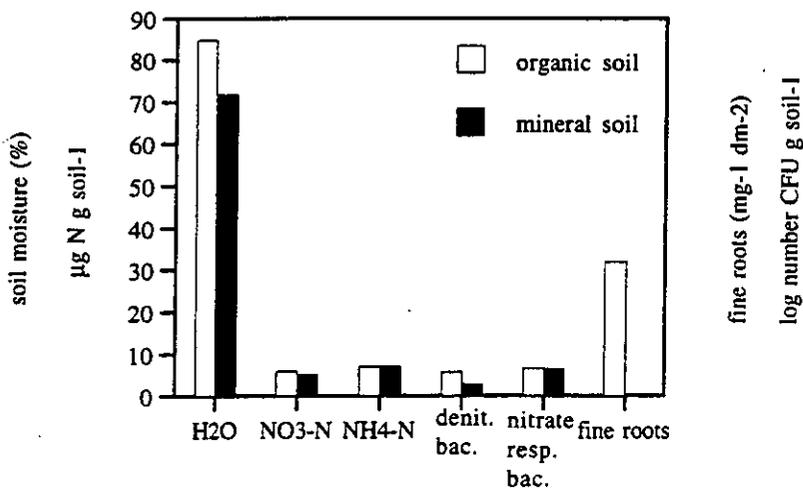
Figure 15. Soil parameters at Collelongo (Is).

Fig 15 shows water content, nitrate-N, ammonia-N, nitrate reducing bacteria and the biomass of fine roots in the soil layers at the sampling in October 1995. The water content of the mineral soil was high exceeding 80 % on a dry weight basis. The amounts of nitrate-N and ammonia-N were equally small, below 10 μg/g soil in both the organic and mineral soil layer. The number of nitrate respiring bacteria was larger than the number of denitrifying bacteria in both the organic and mineral soil layer.

Publications

N_2O emission from beech forest soil (manuscript in preparation).

The above reported results will constitute part of a Ph.D. N_2O emission from beech forest soil.

Conclusions:

Specific scientific conclusions

Due to the limited number of samplings at the CRb and Is sites, interpretation of the results should be done with caution and no statistical analyses have yet been carried out. In contrast, the data set of DKb was larger containing up to 200 data points for each parameter, thus making statistical analyses possible.

The insignificant increase in N_2O production by addition of acetylene at most sampling dates at all three sites leads to the conclusion that N_2O and not N_2 is the main gaseous product of denitrification from these soils. N_2O emission rates from untreated soil at Hilleröd (DKb), Jezeri (CRb) and Collelongo (Is) were found in an interval from a minimum value of 12.2 µg N_2O-N m^{-2} d^{-1} at CRb in August to a maximum value of 308 µg N_2O-N m^{-2} d^{-1} measured at DKb in June. By using the data from each sampling site an estimate of the yearly production of N_2O from the soil can be calculated. Average production of N_2O from the soils corresponded to 0.341 kg N_2O-N ha^{-1} yr^{-1} at DKb, 0.166 kg N_2O-N ha^{-1} yr^{-1} at CRb and 0.166 kg N_2O-N $ha^{-1}yr^{-1}$ at Is. At all three sites, these values are small compared to the amount of nitrogen transported through other parts of the nitrogen cycle. As shown for the soil at DKb and CRb, the potential for conversion of nitrate to N_2O exceeded the actual emission rates underlining the possibility of underestimation of the yearly N_2O production rates if optimal conditions for N_2O production occurred between sampling dates.

By averaging the CO_2 emission data from each sampling site an estimate of the yearly production of CO_2 from the soil at the different sites can be calculated. Average production of CO_2-C from the soils corresponded to 5.88 t ha^{-1} yr-1 at DKb, 1.17 t ha^{-1} yr^{-1} at CRb and 4.70 t ha^{-1} yr^{-1} at Is.

The processes leading to N_2O formation in forest soils, i.e. denitrification and nitrification, probably only constitutes a small part of the total number of microbial processes resulting in CO_2 formation. Assuming a 1:1 relationship between nitrate reduced and CO_2 produced during the denitrification process, the maximum denitrification (N_2O) production rate of 308 µg N_2O-N m-2 d-1 at DKb in June would only contribute 0.013 % to the total amount of CO_2 produced at that measurement date. Consequently, fluctuations in N_2O emission rate could not

influence the measurements of CO_2 emission. The statistical significant relationship between N_2O and CO_2 production at DKb may, therefore, not be trivial and could reflect a general relationship between decomposition of organic matter leading to CO_2 formation and the production of N_2O by other processes.

By using the emission data of DKb from the organic and mineral soil layer, respectively, the relative contribution of the soil layers to N_2O and CO_2 emission was calculated. N_2O emission from the mineral soil layer constituted 71.7 % of the total N_2O emission from untreated soil at DKb. By weight, the mineral soil constituted between 80 and 90 % of the total soil content of the PVC tubes used for in situ measurements (data not shown). Measured by weight, the organic soil thus produced approx. twice as much N_2O as the mineral soil.

The CO_2 emission from the mineral soil layer constituted 73.9 % of the total CO_2 emission from untreated soil at DKb. Consequently, the organic soil was as productive regarding CO_2 emission as N_2O emission. These findings indicate that the process of N_2O production in the soil may be connected to the general microbial process of CO_2 production and also supports the validity of the statistically significant correlation between N_2O emission and CO_2 emission reported above.

Two other soil parameters at DKb proved important in relation to N_2O emission: Nitrate concentration of the mineral soil layer and soil temperature. The relationship with nitrate was expected as nitrate is the substrate of denitrification leading to N_2O formation, though the fact, that there was no significant relation between nitrate concentration in the organic soil layer and N_2O production, and that the higher nitrate concentration at CRb did not result in increased N_2O emission is not readily understood. N_2O did originate in the organic soil; the organic layer was per g soil more active in N_2O production than the mineral soil. If nitrification instead of denitrification was the source of N_2O, one would expect N_2O emission to correlate with NH_4 concentration in the organic layer. This was not the case. Furthermore, the mixed reaction of the soil on acetylene addition (inhibiting nitrification) could not substantiate that nitrification was the source of N_2O emission.

It was a main hypotheses, that precipitation was the most important factor determining gas emission from the soil. This could not be supported by statistical analyses of the relationship between N_2O emission and soil water content: Small correlation coefficients, 0.06 between N_2O emission and water content of the organic layer and -0,03 between N_2O emission and water content of the mineral layer, demonstrated independence of N_2O emission of soil water content measured on dry weight basis. This was supported by the fact that addition of water at DKb did not substantially increase N_2O emission from the soil .

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Turnover of dead root biomass by microbial populations of fungi or bacteria results in release of readily degradable carbon that could be used by bacterial populations in connection with denitrifying events. Alternatively, exudates leaking from active roots may act as substrates for microbial processes in the rhizosphere. Measurements of biomass of fine roots at different sampling dates were chosen as the simplest indication of root turnover, but neither N_2O emission nor CO_2 emission were significantly correlated to root biomass. Root exudates were not quantified in this study.

The following conclusions regarding N_2O emission and CO_2 emission from beech forest soil could be made:

1. The amount of nitrogen emitted as N_2O from beech forest soils was low at all three sites, less than 400 g ha^{-1} áyr^{-1}.

2. The soil at the site at Jezeri (CRb) emitted N2O at a lower rate than at Hilleröd (DKb) even though soil nitrate content at CRb was higher.

3. Addition of acetylene did not substantially enhance N_2O emission from any of the soils suggesting that N_2O is the final product of denitrification at these sites.

4. The varying effects of addition of low concentrations of acetylene to the soil, did not allow an estimation of the role of nitrification in connection with N_2O production.

5. N_2O emission at DKb were correlated with three soil parameters: CO_2 emission, nitrate. content of the mineral soil layer and soil temperature. N_2O emission was not correlated with soil water content measured on dry weight basis.

6. Nitrate addition to the soils at DKb and CRb resulted in substantially increased N_2O emission rates indicating that the processes were nitrate limited, although extractable nitrate was present. Addition of acetate at DKb did not further increase N_2O production indicating that there was no carbon limitation.

Popular Conclusion:

It was shown that the emission of the green house gas N_2O from beech forest soils was low compared with other ecosystems.

Addition of nitrate to the soil led to an increased production and emission of N_2O. As a consequence of this finding it must be assumed that elevated nitrate concentrations in the soil from increased nitrogen deposition or use of fertilisers will result in increased N_2O emission rates.

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Objectives

<u>All Sites</u>:

2.1. To measure denitrification potentials and activities, and correlate these potentials to the geophysical and chemical properties of these sites.

2.2. To screen soils for alternative anaerobic processes that would compete with denitrification for fixed carbon.

<u>Bayreuth and 2 Other Intensive Sites</u>:

2.3. Initiate experiments to determine why denitrification potentials or activities are decreased (or different) between sites that reveal extreme differentials.

2.4. To isolate and/or characterize important mcrobial species associated with alternative redox potentials.

Methodology

<u>Field measurements</u>:

Emission of gases from soils was measured using plexiglass chambers (50 x 10 cm, 19 l volume) fitted with a septum from which to withdraw gas samples. At sites which were flooded with water, smaller chambers (1.8 l volume) glued to wooden supports were floated on the water for gas measurements. Gas samples were injected into sealed, evacuated serum vials and concentrations were determined by gas chromatography (GC) as follows: H_2, N_2 and O_2 were measured using a Molecular Sieve column and thermal conductivity detector (TCD); CO_2 with a Chromosorb column and TCD; CH_4 with a Molecular Sieve column and flame ionization detector (FID); N_2O with a Porapak Q column and electron capture detector.

<u>Isolation, characterization and enumeration of microorganisms</u>:

Enrichment cultures for bacterial isolation were prepared in an anaerobic chamber (N_2 gas phase; Mecaplex, Grenchen, Switzerland) by placing soil (5-10 g) into serum vials (120 ml) containing various anaerobic media. The serum vials were sealed with grey rubber stoppers and crimp seals, and the gas phase was exchanged from N_2 to $H_2:CO_2$ (80:20), $N_2:CO_2$ (90:10) or argon (100%). Incubation was carried out at 15-20°C. Cultures demonstrating substrate utilization were transferred to fresh media. Homogeneous cultures were obtained by streaking liquid enrichment cultures onto agar media; cultures were assumed to be homogeneous based upon colony morphology and microscopic observation.

Media were prepared using standard anaerobic techniques (Hungate, 1969; Holdeman et al., 1977) and generally contained salts, vitamins, minerals, yeast extract, bicarbonate or phosphate as buffer, and cysteine/Na_2S as reducing agent. The pH of the media was adjusted to 4, 5 or 7, and sugars, alcohols, aromatic compounds, H_2:CO_2 or CO served as carbon and energy sources. An additional anaerobic, oligotrophic medium was prepared which contained acetate (6 mM), peptone (0.0025%), and nitrate (20 mM). For characterization of methanogen populations, an anaerobic medium was prepared based on the composition of water from a stream at Waldstein and contained H_2 and CO_2 as energy and carbon sources. Nitrate utilization (and growth of denitrifiers) was studied using tryptic soy broth medium diluted 1:10 and containing 5 mM KNO_3. Nitrogen fixation was followed using an N_2-free anaerobic medium (Rennie, 1981; Limmer and Drake, 1996); acetylene and ethylene were determined by GC using a Porapak T column and FID detector.

The concentrations of all organic acids, alcohols and aromatic compounds used as substrates in media were determined by high performance liquid chromatography; gases were determined as described above. Nitrate, nitrite and ammonia were determined spectrophotometrically (Gadkari, 1984; Cataldo et al., 1975; Harrigan and McCance, 1966).

Specific microbial populations (e.g., denitrifiers, methanogens) were enumerated by most probable number analysis of diluted soil samples using the appropriate medium (Koch, 1994).

Microorganisms were identified using API analysis (BioMerieux Deutschland GmbH, Nürtingen, Germany) (Smibert and Krieg, 1994).

Microcosm studies:
Microcosm experiments were prepared in an anaerobic chamber by placing 10 g soil in an infusion bottle (150 ml) equipped with a rubber septum and aluminum screw cap. The gas phase of the bottles was H_2:CO_2 (80:20) or argon:CO_2 (80:20). Bottles were incubated at 20°C, and gas samples were analyzed during incubation.

Results

1. *Denitrification Potentials*

Bayreuth:
In situ N_2O emissions measured at Waldstein Coulissenhieb, as well as at boggy sites, were minimal. However, these acidic soils exhibited the potential to denitrify, and N_2O was the product of denitrification; only negligle amounts of N_2 were observed. Anaerobic N_2O

formation rates approximated 10 microgram N_2O-N d^{-1} g^{-1} soil (dry weight). With 1:10 soil suspensions in medium, pH had no appreciable effect on initial N_2O production rates. Most probable number (MPN) analysis indicated there were approximately 6×10^4 microorganisms per gram (dry weight) soil; several denitrifying bacteria were isolated and partially characterized. Gas samples were also collected at sites that are regularly flooded with water. Analysis of trapped gas indicated that N_2O accumulates (at up to 15X atmospheric levels) and is emitted at both acidic (pH 3.5) and non-acidic (pH 6.5) sites.

Umeå:

As observed at Waldstein, N_2O evolution was not detected in field samples collected (in late fall) at Umea. In addition, nitrate was not readily consumed by these nitrate deficient soils under anaerobic conditions, even when enriched with supplemental energy sources (10-fold electron excess). In vitro rates of N_2O evolution were higher with deeper soil horizons (i.e., below the organic horizon).

2. Alternative redox processes

Bayreuth:

The results of soil respiration studies at Waldstein and Schacht indicated there was considerable potential for anaerobic microbial activity, even at low pH. Kinetic studies indicated an immediate and ongoing activity, rather than solely in vitro capacities. At Coulissenhieb, sulfate-reducing organisms were low in number, and methanogenic potentials were essentially zero, independent of pH. In contrast, acetogenic potentials were high (Küsel and Drake, 1995); the potential to form acetate was negatively affected by pH. The process of acetate formation in these soils potentially competed with denitrification for reduced carbon, thus the effect of NO_3^- on acetate production at Waldstein and Schacht was examined. When soil from the A horizons of Waldstein and Schacht was supplemented with 50 mM NO_3^-, NO_3^- was utilized and acetate production was reduced.

At Waldstein, methane accumulated at sites which are regularly flooded, with concentrations ranging from 10 to 50% in gas samples. As with N_2O measurements, methane emission occurred independently of soil pH. N_2 levels were high when methane concentrations were low; little CO_2 and no H_2 or H_2S were detected. The source of N_2 is unclear and may have been due to diffusion rather than production; because N_2O was the product of denitrification under acidic conditions (see Section 1), it seems unlikely that N_2 production in these waterlogged soils could be significant under acidic conditions. In addition to methanogenesis, potentials for iron oxidation and reduction, and the oxidation of reduced sulfur compounds were identified.

In situ methane production was measured at an acidic site every two weeks during a three month period (August to October, 1995); rates of methane production during this period were similar and approximated 4 mmol m^{-2} d^{-1}. In microcosm experiments, methane evolution was stimulated by H_2/CO_2 and formate. Under aerobic conditions, methane was rapidly oxidized at low pH.

Umeå:

Åheden soils behaved similarly to those of Waldstein and exhibited acetogenic potentials in addition to the potential for denitrification. In contrast to N_2O production, potential methanogenic activity was only observed in the organic horizons. Trace levels of hydrogen were produced from these soils anaerobically, indicating that protons may under certain conditions serve as reductant sinks. Unlike Waldstein soils which demonstrated the capacity to produce CO_2 anaerobically, Åheden soils did not produce CO_2 under similar conditions. This indicates that either production equaled reuse (fixation) or that production was indeed zero. Similar to Waldstein, potentials for the oxidation of iron and inorganic sulfur compounds were identified.

Montpellier:

Acetogenic potentials at Thezan and Puechabon were evaluated (Küsel and Drake, 1995) and shown to be similar to those observed at the Bayreuth sites. Methanogenic potentials were essentially zero.

3. *Pure culture studies*

Acid forest soils from spruce and beech sites from Waldstein and Schacht were evaluated to characterize the anaerobic microbial populations present, and to study the potential of these organisms for denitrification. A total of 82 isolates growing at pH 4 - 7 were obtained from the organic and mineral horizons as well as from the litter layer from several sites at Waldstein and at Schacht. None of the obligately anaerobic isolates obtained at pH 7 were capable of reducing NO_3^-. Representative organisms isolated at pH 4 and 5 are shown in Table 1. As observed at pH 7, none of the obligately anaerobic bacteria isolated at pH 4 or 5 were able to reduce NO_3^-. However, all of the facultatively anaerobic bacteria (15 isolates) obtained at pH 4 or 5 reduced NO_3^-, with varying levels of NO_2^- and NH_4^+ being produced (Fig. 1). No N_2 production was detected.

Two rod-shaped bacteria (isolates 58 and 74) were isolated which exhibited growth and N_2-fixation at pH values approximating 3.4. The fermentative end-products acetate, butyrate, lactate, H_2 and CO_2 were formed during growth (Fig. 2), suggesting these organisms belonged to the genus *Clostridium*. Growth in TSB medium (diluted 1:10) at pH 7 exhibited a long lag
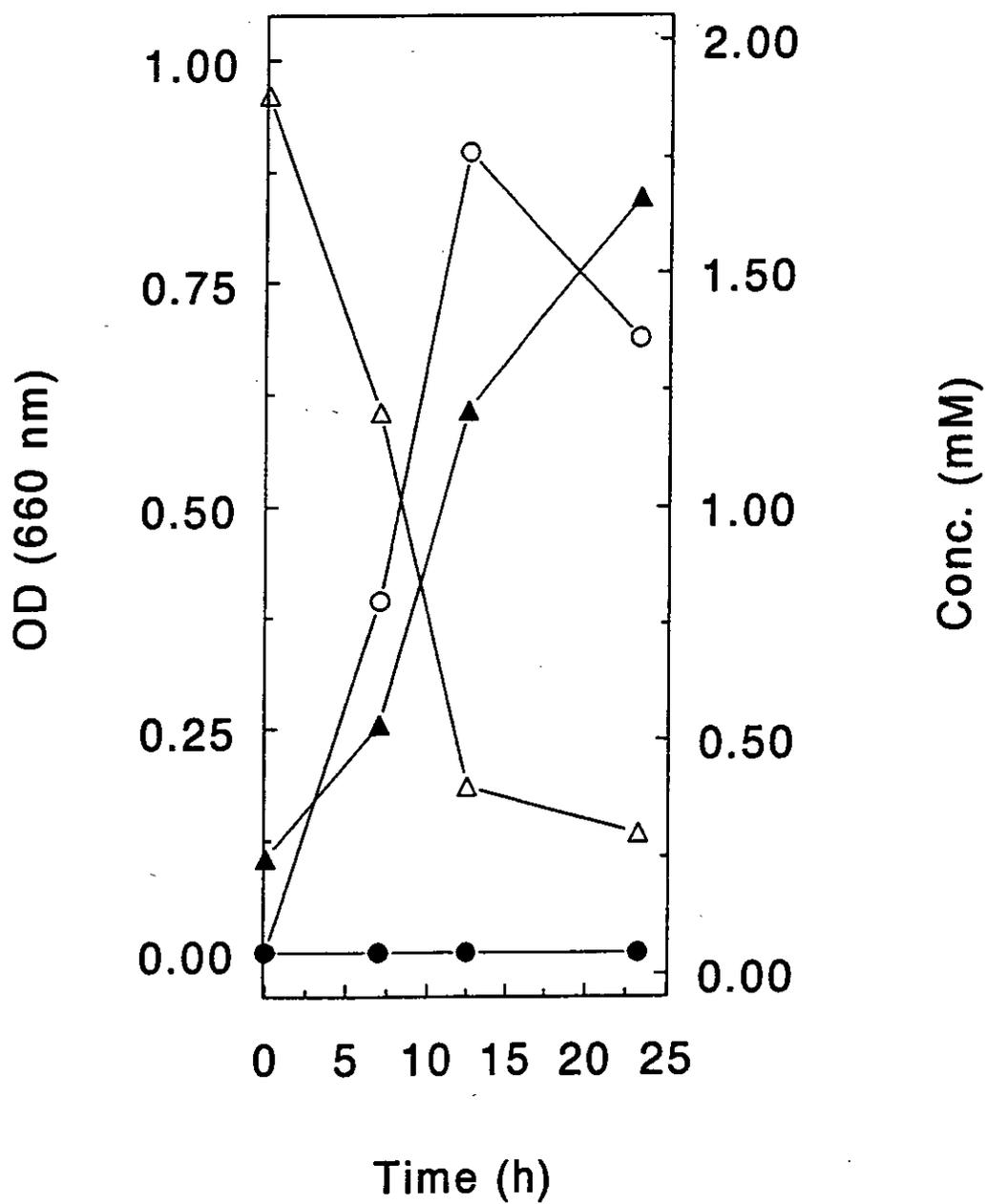
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Figure 1.   Nitrate utilization by isolate 47 during growth in TSB medium (pH 4) supplemented with glucose (10 mM). Symbols: ▲, OD; △, NO_3^-; ○, NO_2^-; ●, N_2.
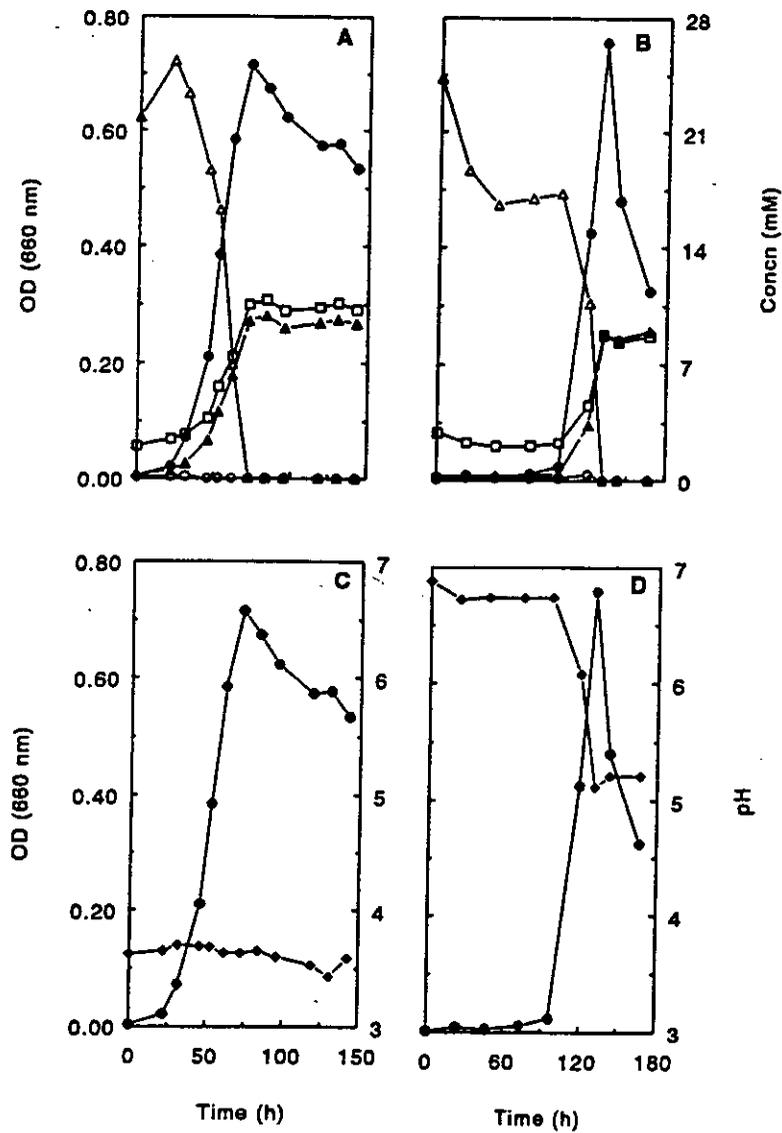
Figure 2. Growth and soluble product formation by isolate 74 in TSB medium (diluted 1:10) supplemented with glucose (25 mM). Symbols: ●, OD; △, glucose; ■, butyrate; ▲, acetate; ○, lactate; ◆, pH.

Table 1. Strictly anaerobic and facultative isolates obtained at pH 4 and 5

Soil Source[a]/ Isolation pH	Facultative/ Anaerobe	Gram Reaction	Spores[b]	Products[c]					NO$_3^-$ Reduction	Identification[d]
				F	A	L	S	B		
Moor Oh (5)	Anaerobe	+	-	++	++	+++	-	-	-	*Actinomyces israelii*
Moor Oh (4)	Anaerobe	-	+	-	+	+	-	+	-	Unknown
Schacht litter (4)	Anaerobe	+ (variable)	+	-	+	+	-	++	NG[e]	Unknown
Moor Oh (5)	Facultative	-	-	+	+	+	+	+	+	*Enterobacter cloacae*
Moor Oh (4)	Facultative	-	-	+	++	++	+	-	+	*Enterobacter sacazaki*
Schacht Ah (5)	Facultative	-	-	-	++	+	+	-	+	*Serratia liquefaciens*
Schacht Ah (5)	Facultative	-	-	-	++	+	+	-	+	*Enterobacter amnigenus 1*

[a]All isolates were obtained from the Oh horizon in media containing glucose as carbon and energy source.

[b] "+" indicates spores were observed; "-" indicates spores were not observed, even with sporulation medium (Duncan and Strong, 1968).

[c]Cells were grown anaerobically in TSB at the isolation pH; major soluble products were determined by HPLC analysis of the culture medium at the end of log phase: F, formate; A, acetate; L, lactate; S, succinate; B, butyrate. For each isolate, "+", "++", and "+++" refer to the relative amounts of detectable products measured ("-" indicates none detected).

[d]Isolates were identified using API 20E (for facultative organisms) and API 20A (for strict anaerobes); strips were incubated at 30°C and 24°C for facultative and anaerobic organisms, respectively.

[e]NG, no growth.

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phase, whereas growth at pH 3.5 did not, suggesting these organisms were adapted to growth at low pH (Fig. 3). Furthermore, during the lag phase at pH 7, cell division was impaired and cells formed highly intertwined aggregates, suggesting an attempt to establish a localized region of low pH. Growth and nitrogen fixation by these organisms at pH 3.4 appear to represent new potentials for clostridia.

Acetate was a major product of soil organic matter turnover by all forest soils examined. Additionally, H_2 was shown to be converted to acetate in stoichiometries approximating those expected for H_2-driven acetogenesis (Küsel and Drake, 1994), suggesting that acetogenic bacteria might be responsible for the conversion of soil organic carbon to acetate. Although acetogenic bacteria have been isolated from sites exposed regularly to high water levels (Wierenga, 1936; Kotsyurbenko et al., 1995; Andreesen et al., 1970; Bak et al., 1992; Wiegel et al., 1981), the presence of acetogenic bacteria in oxic, non-waterlogged soils has not yet been established. As part of studies to evaluate the organisms potentially responsible for soil acetate production, an acetogenic bacterium (DG-1) was isolated from pH neutral mineral forest soil. Strain DG-1 was a Gram positive, sporeforming rod, which grew at an optimal temperature of 25°C and pH of 6.8. The pH range for growth was pH 3.9 to 8.3. Substrates utilized for growth included fructose, formate, methanol, vanillate, ferulate, fumarate, and H_2/CO_2. Nitrogen was not fixed by this isolate, and NO_3^- was not reduced.

Conclusions

We have demonstrated potentials for both denitrification and acetate production in acidic as well as non-acidic forest soils. The laboratory supplementation of soils with nitrate reduces acetate production, suggesting that these processes compete for reduced carbon, and that increased nitrate deposition in forests affects electron flow. Although N_2 production is negatively affected by low pH, N_2O is produced under acidic conditions, as well as under more pH neutral conditions (at Waldstein).

The results of isolation experiments demonstrate that various fermentative bacteria (such as isolates 58 and 74) potentially contribute to the production of soil acetate at low pH. In addition to these organisms, however, it has been demonstrated that acetogenic bacteria are present in mineral forest soils and could also participate in the production of acetate.

The inverse relationship between N_2 and methane observed at Waldstein suggests that methanogenesis may also compete with denitrification. The addition of nitrate to an alder swamp led to a reduction in methanogenesis and stimulation of denitrification (Westermann and Ahring, 1987), suggesting that denitrifiers may divert the flow of electrons away from

methane. Whereas acetate production is inhibited in low pH soils, the reduction of carbon to methane continues at low pH. The rates of methane production at Waldstein are greater than those observed from an alpine fen or boreal marsh, but equivalent to those observed with peat or rice paddies (Kiene, 1991). The identification of other processes (such as iron oxidation and reduction) in these soils suggest that changing redox potentials (for example, due to the influx of water) play an important role in determining the direction of electron flow.

The majority of the organisms isolated from Waldstein and Schacht were facultative anaerobes, and many of the isolates were able to grow over a wide range of pH values, suggesting a remarkable ability to adapt to changing conditions of E_h and pH. We have isolated strictly anaerobic bacteria capable of growth at pH values approximating those of soil. Two of the isolates obtained may represent new strains of clostridia based on high growth rates at pH 3.4, and are believed to represent the first anaerobic isolates capable of nitrogen fixation at pH values below 4. The results obtained with pure cultures support those obtained with soils in that nitrate in acidic forest soils is reduced (by facultative anaerobes); however, the product of NO_3^- reduction is not N_2.

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10. Prof. Dr. E. Matzner, Dr. G. Matschonat (Part I), M. Stuhrmann (Part II)

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Research Area: N processes in soils, NH_4^+ sorption, NH_4^+ fixation, N storage in soils as organic N, N immobilization, humus-N-formation.

Part I: Exchangeable and nonexchangeable NH_4^+

Introduction

Exchangeable/sorbed NH_4^+ and nonexchangeable NH_4^+ constituate potential storage capacities for inorganic nitrogen. Exchangeable/sorbed NH_4^+ is plant available, the sorption is fully reversible. Nonexchangeable NH_4^+ exists in interlayer positions of layer silicate minerals; it may be inherited by the parent material of soil formation or fixed in times of high NH_4^+ solution concentrations in the soil. Nonexchangeable NH_4^+ is plant available only to a resticted degree and nonexchangeable binding is reversible, but time and concentration dependently.

Objectives

1) Quantification of the storage capacity for exchangeable/sorbed NH_4^+ in mineral soil horizons and the forest floor by means of sorption isotherms.

2) Quantification of the amount of nonexchangeable NH_4^+ existing in the field.

Methods

Exchangeable/sorbed NH_4^+: Sorption isotherms were determined in batch experiments in the laboratory using field moist soil. NH_4^+ concentrations were 0-0.6 mmol L^{-1} (forest floors) and 0-0.4 mmol L^{-1} (mineral soil horizons). Results are presented as initial mass isotherms (Nodvin et al. 1986). The slope of the isotherm gives the proportion of added NH_4^+ that is sorbed to the soil. The relation is linear.

Sites: Åheden (spruce and birch), Klosterhede, Waldstein, Schacht, Aubure (spruce and beech), Thezan + several sites of the Fichtelgebirge-region.

Nonexchangeable NH_4^+: Nonexchangeable NH_4^+ was determined after destruction of organic matter by KOBr-KOH treatment. The remaining soil minerals were dissolved in HF/HCl and NH_4^+ was measured in the diluted acid (Silva and Bremner 1966).

Sites: Waldstein, Schacht + several other sites of the Fichtelgebirge-region.
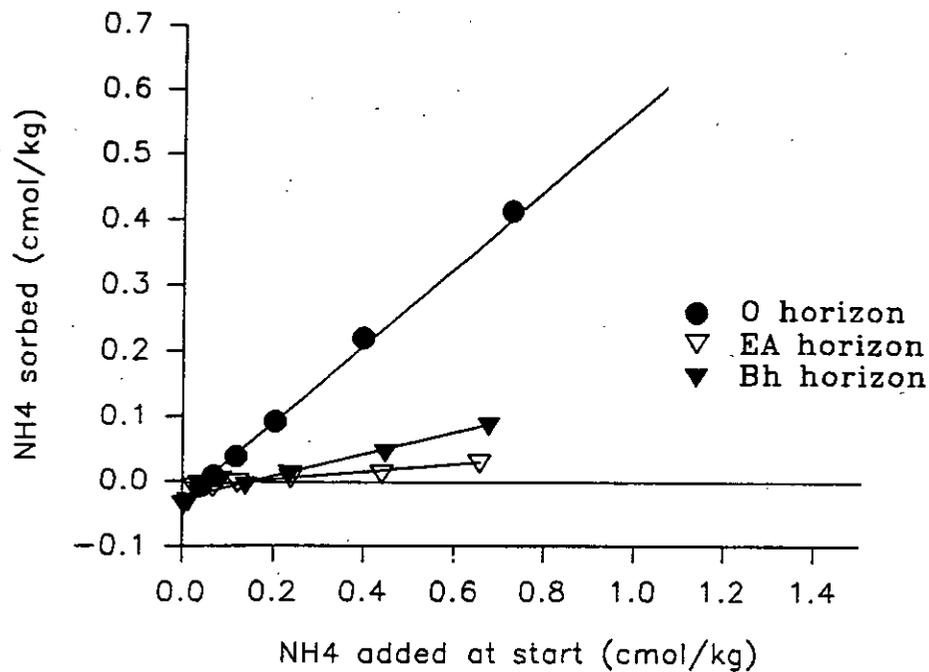
Figure 1: Initial mass isotherms describing NH4+ sorption for the "Klosterhede" soil.
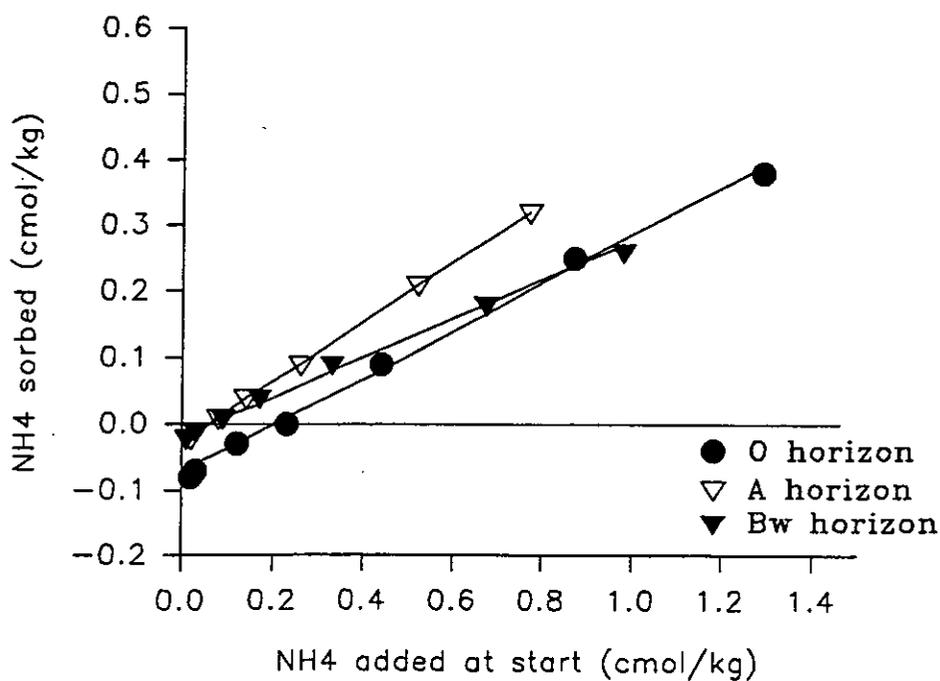

Figure 2: Initial mass isotherms describing NH4+ sorption for the "Schacht" soil.

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Results

Figure 1 shows initial mass isotherms for the Klosterhede soil. In the O horizon, a much higher proportion of added NH_4^+ is sorbed than in the mineral soil horizons. This is typical for the very acid forest soils included in the study. Figure 2 shows initial mass isotherms for the Schacht soil. This site is representative for forest soils that are less acid compared to the first group mentioned: Sorption of NH_4^+ in the mineral soil horizons is about equal to that of the forest floor. In multiple regression analysis, cation exchange capacity and base saturation explained up to 95% of the variability in the slope of the initial mass isotherm (m) of the mineral soil horizons. Figure 3 shows measured and calculated (according to the eqation $m = 0.0097 * CEC + 0.3 * $ base saturation $ + 0.009$) slopes of the initial mass isotherms for mineral soil horizons of the Fichtelgebirge-region. For the forest floors, no such correlation was found. Nonexchangeable amounts of NH_4^+ compared to total N content of the soils of the German sites Waldstein and Schacht are given in Table 1.
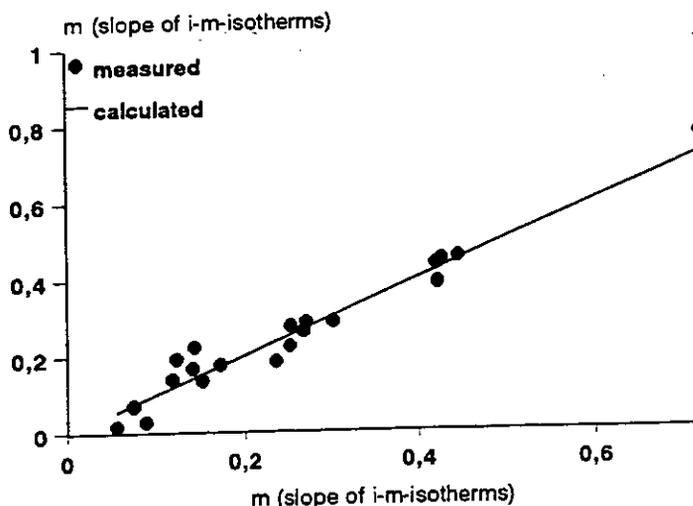
Figure 3: Measured and calculated slopes of the initial-mass (i-m)-isotherms for mineral soil horizons of several sites of the German Fichtelgebirge-region.

Table 1: Nonexchangable NH_4^+ in silicate minerals.

Site	kg ha^{-1}	% of total nitrogen
Waldstein		
AE	21.4	3.9
Bs	37.3	2.7
Bw	21.2	6.2
Schacht		
A	13.5	0.82
Bh	9.6	1.1
Bsw	34.4	0.48
CBw	21.1	3.8

Conclusions

- NH_4^+ can be stored in exchangeable/sorbed form in amounts that are in the range of the annual N depositon rates and annual uptake rates of mature forest stands. In acid soils, the major storage capacity is in the forest floor. Sorption in the mineral soil horizons in many soils may not prevent NH_4^+ from downwards transport.

- Amounts of nonexchangeable NH_4^+ present in the soils can be explained by their mineral composition alone, so NH_4^+ fixation is not assumed to be a significant mechanism for NH_4^+ storage in acid forest soils.

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Part II: Storage Mechanisms for Deposited Nitrogen in Acid Forest Soils

Objectives

1) To follow the fate of deposited N in different European forest soils with special consideration to the following processes:

        a) storage of N-depositions in the soil as Norg. by

                i) N-Immobilization

                ii) Humus-N-formation

        b) leaching of deposited nitrogen from the soil

        c) soil N-mineralization and nitrification.

2) to quantify the influence of temperature on the above mentioned processes.

3) to quantify the influence of climate on the above mentioned processes.

Methods

For this investigation only 3 of the 7 NIPHYS study sites were selected: the most northern Swedish site "Åheden" near Umeå (latitude 64° N), the German research area "Waldstein" in the Fichtelgebirge (50° N), and the most southern French site "Thezan" near Montpellier (43° N).

In order to study soil processes under quasi-natural conditions, undisturbed soil columns (soil monoliths) were taken from these sites with plexiglas cylinders (18 cm diameter) to a soil depth of about 20 cm (litter layer included). The cylinders were fixed to ceramic plates and connected with a suction pump device with a continuous pressure of -320 mbar.

To be able to follow the fate of deposited N, each soil column was labelled with 3mg of the stable isotope 15-N. The tracer ($^{15}NH_4Cl$ 99 at%) was dissolved in aqua dest. and 50 ml of this solution were sprayed on top of each soil column as one pulse.

To study the temperature dependence of soil-N processes, soil columns were incubated in the laboratory in temperature-controlled rooms at 5°, 10°, and 15°C, and artificially irrigated. The irrigation solution was adjusted in amount and concentration to the Waldstein site throughfall. Incubation time was about 6 months.

For the study of climate effects on soil-N processes, field columns were installed at their original site (5 columns per site). Additionally, soil columns from the Swedish site (Ahe) were transplanted to the Fichtelgebirge (Wa) and the Montpellier site (The) to simulate climatic change. All field columns were incubated for one vegetation period. During these incubations, percolates were being sampled each fortnight. After incubation, the soil columns were devided into 3-4 samples according to the main soil horizons.

Analyzed parameter:

Percolates were analyzed for ammonium, nitrate (Flow Injection Analyzer, QuickChem AE, Lachat) and total nitrogen (TN-05, Abimed). For 15-N analyses, samples were fractionated into ammonium and nitrate using a diffusion method according to Jensen (1991) with some modifications. 15-N enrichments were determined with an on-line-system combining an elemental analyser (Carlo Erba NA 1500) for Dumas combustion and a Finnigan MAT delta E gas isotope mass spectrometer.

For soil samples, various methods were used in order to devide the soil N in different N-fractions (see Fig. 1). For the inorganic N-fractions ammonium and nitrate, aliquots of soil samples were extracted with 0,5M K2SO4. The microbial biomass-N was determined with the chloroform-fumigation-extraction method according to Brookes et al., 1985, followed by a wet digestion with KS2O8 (Cabrera & Beare, 1993). Two fractions of organic soil-N were measured by hydrolysis with 6M HCl (Bremner, 1965): the hydrolyzable nitrogen, which consists mainly of amino acids and -sugars, and the acid insoluble fraction, which represents the more recalcitrant humus fraction. In order to be able to measure 15-N, soil extracts were diffused like percolates (Jensen, 1991). But the diffusion method did not work with digested K_2SO_4-extracts and with the hydrolyzable N-fraction. A lot of time was spent on the modification of the diffusion method for these extracts. Since all tries were not successful, the samples were at least distilled according to Bremner and Keeney (1965). Analyses of these samples are still in process and final results will not be available before summer 1996.
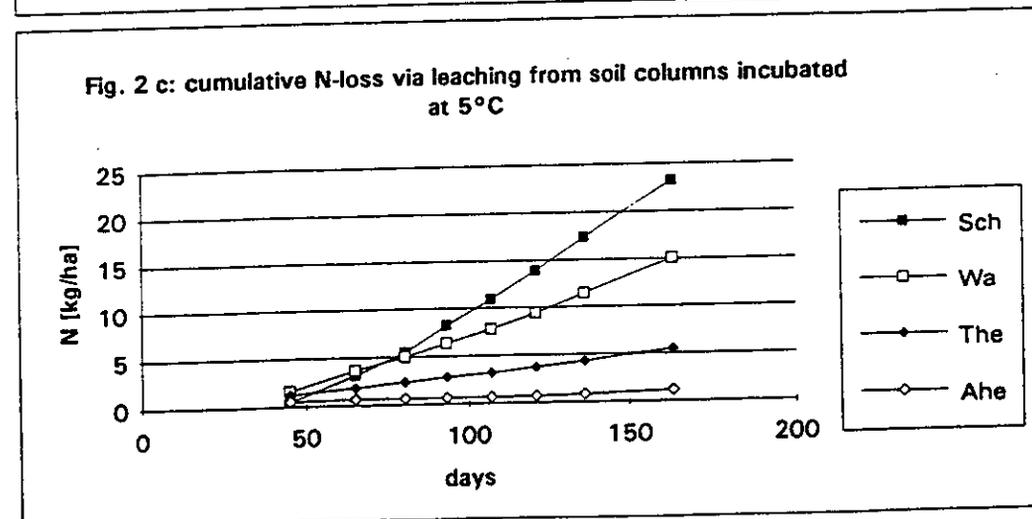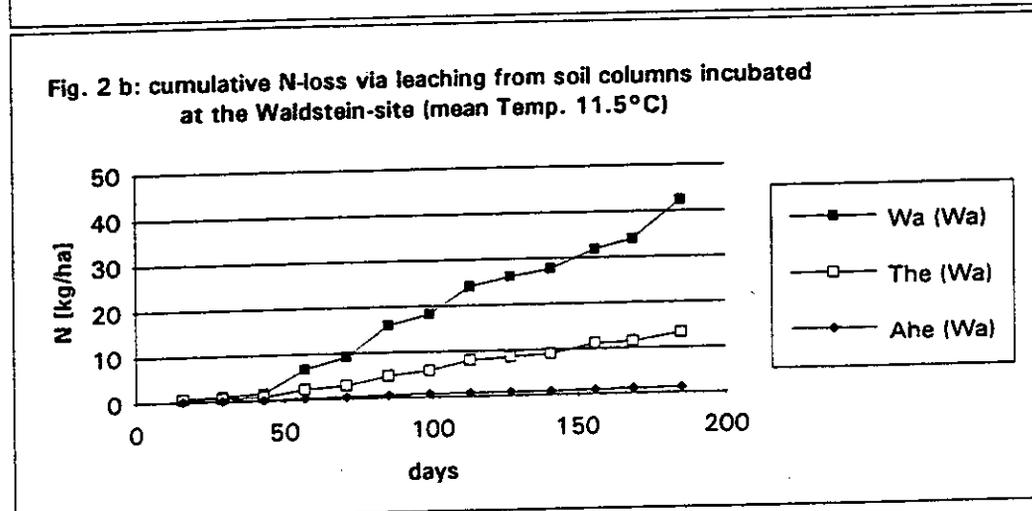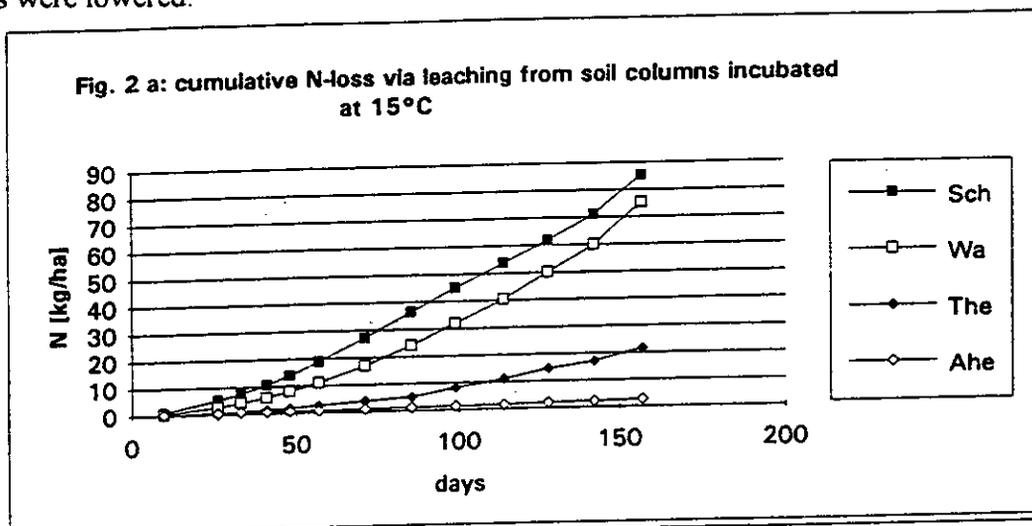
Fig. 1: N-Fractionation-scheme
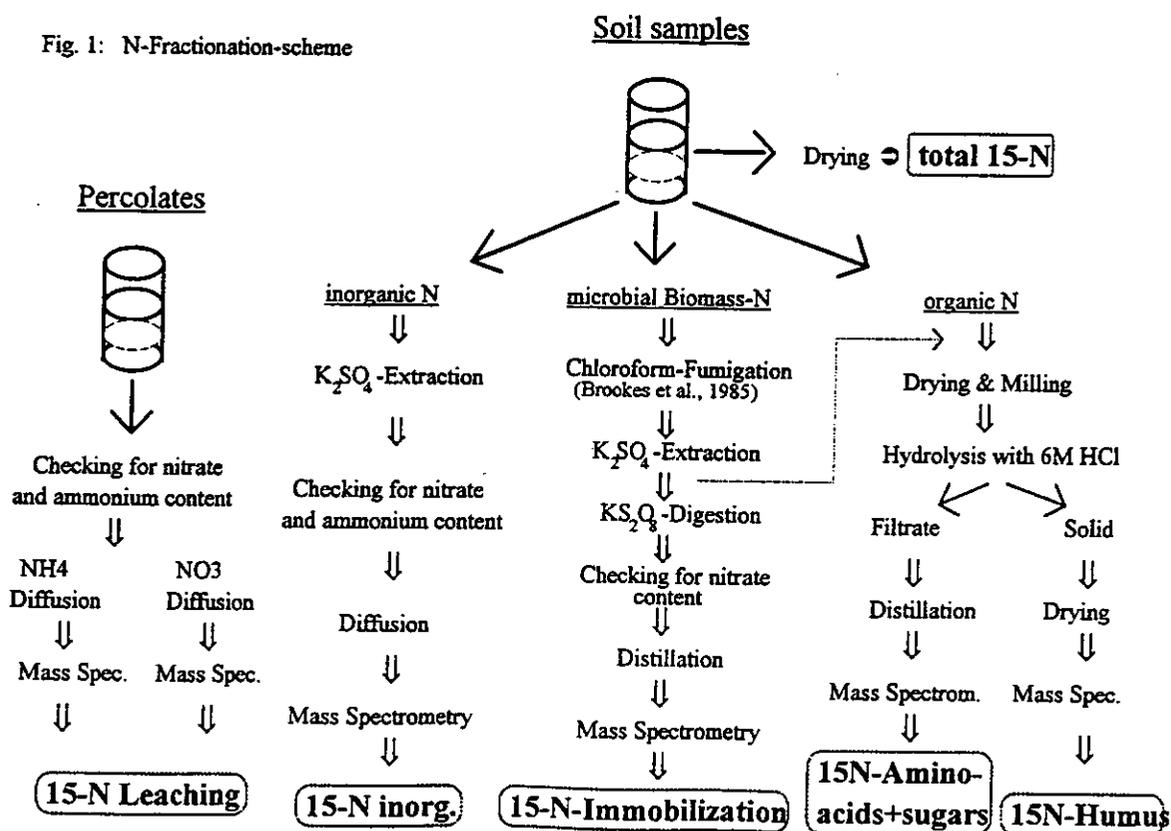
Results

1.   N-loss via leaching: "N-mineralization contra N-immobilization":

During the incubation time, soil columns lost different amounts of N via leaching. Fig. 2 a - c show the temporal course of cumulative N-leaching. At 15°C, the Fichtelgebirge columns (Wa) lost up to 85 kg N ha-1, whereas from the Swedish columns hardly any N was leached. If the N-input via irrigation is taken into account, the Swedish soils were even able to immobilize a considerable amount of N. Compared to field incubations at the Fichtelgebirge site (mean temperature during incubation: 11.5°C), and to 5°C incubations, pattern were similar but N-losses were lowered.

Fig. 2 a: cumulative N-loss via leaching from soil columns incubated at 15°C

Fig. 2 b: cumulative N-loss via leaching from soil columns incubated at the Waldstein-site (mean Temp. 11.5°C)

Fig. 2 c: cumulative N-loss via leaching from soil columns incubated at 5°C

2. Temperature dependence of N-leaching:

To clarify the differences in N-leaching at different temperatures further, total N-losses at end point of incubation at 5° and 15°C were compared. As Fig. 3 demonstrates, N-leaching-rates were clearly temperature dependent. A rise of 10°C raised N-leaching 3- to 5-fold.
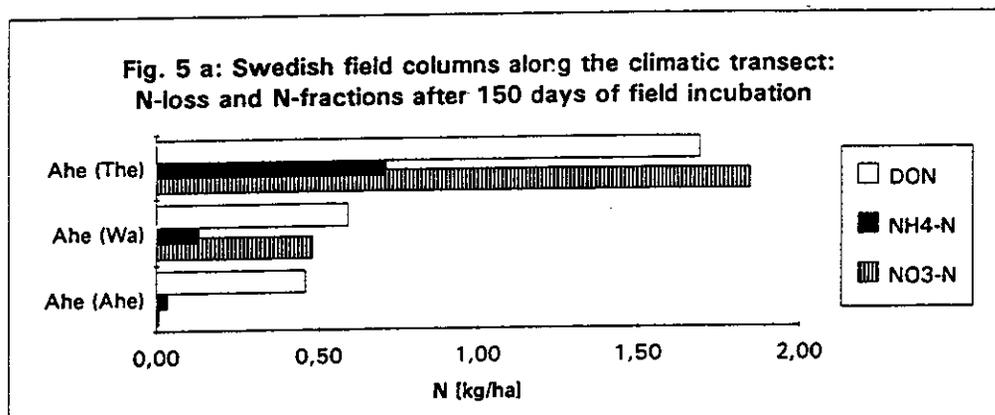

Fig. 3: N-leaching from soil columns at 5° and 15°C
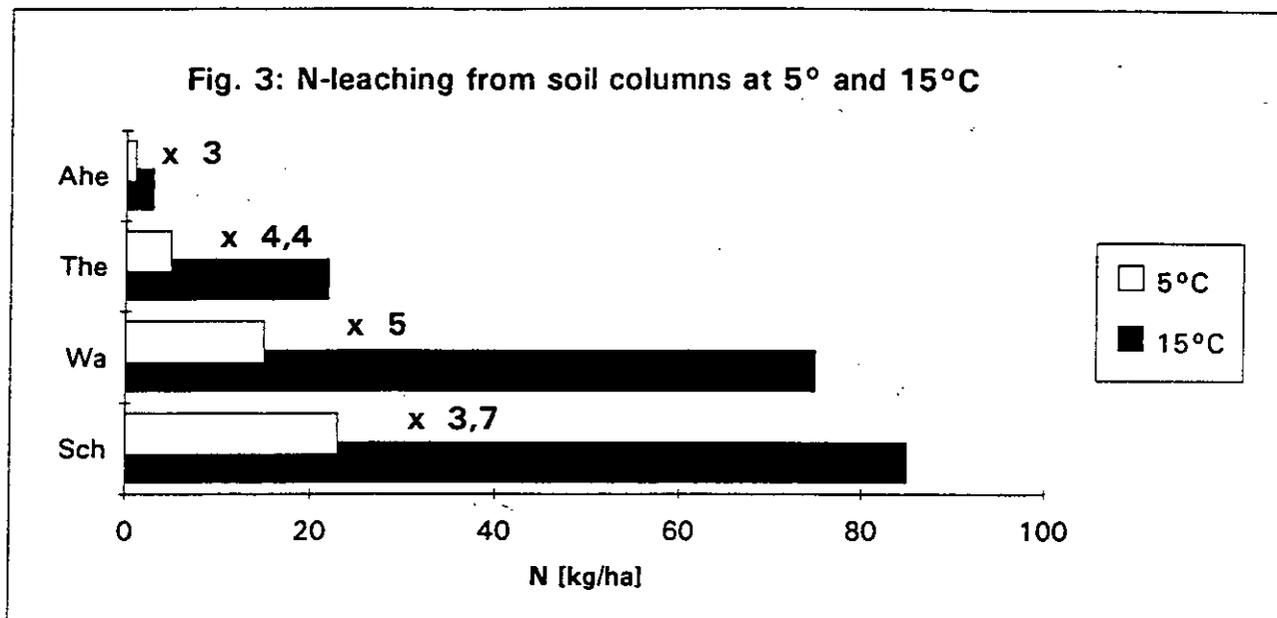
3. Temperature dependence of DON-leaching:

DON-leaching was studied in detail and was found to be temperature dependent aswell (Fig. 4), but in Wa-columns, its rise with increasing temperature was less pronounced (1.3).

Fig. 4: DON-leaching from soil columns at 5° and 15°C

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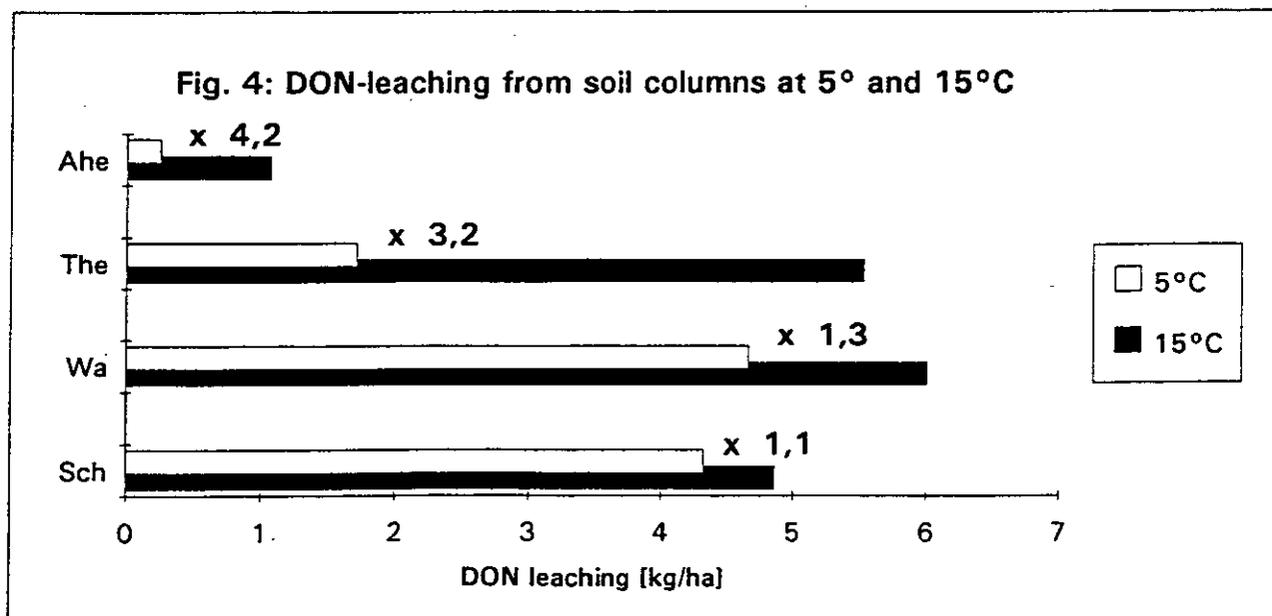
4.   Climate effects on DON-leaching and DON-fractions:

When Swedish field columns of the transplant experiment are compaired with respect to DON-leaching, DON-output was higher the more south the columns were transplanted (Fig.5 a). But the fraction of DON referring to total N-loss decreased from >90% at Åheden to <40% at Thezan (Fig 5 b).

Fig. 5 a: Swedish field columns along the climatic transect: N-loss and N-fractions after 150 days of field incubation

Ahe = Aheden;   The = Thezan;   Wa = Waldstein.

Fig. 5 b: Swedish field columns along the climatic transect: % of N-fractions in percolates
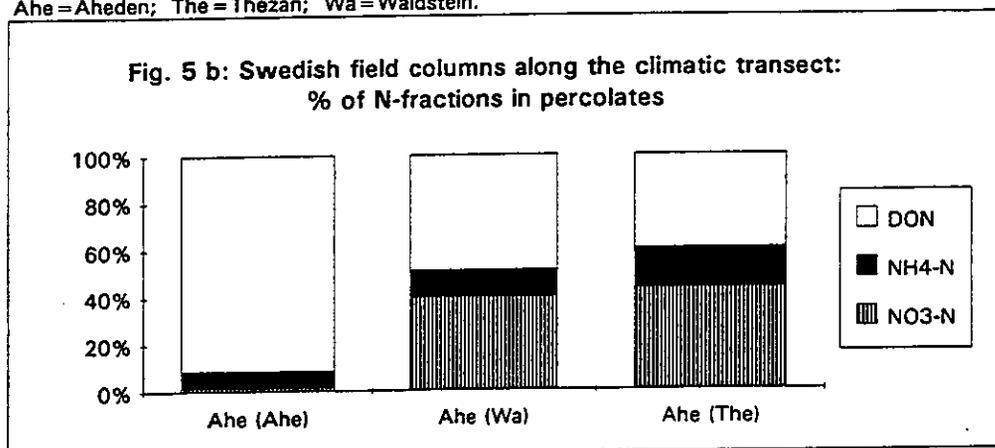
5.   N-storage in the soil compartment:

After incubation, total 15-N was measured in the column soils in order to study, in which horizon the tracer-N was stored. Fig. 6 a shows the patterns of 15-N tracer distribution for the Swedish soil columns incubated at different temperatures and sites. The predominant part of the tracer was found to be still in the uppermost moss-layer. In the 15°C incubated soils the 15-N content of the moss was lower compaired to the 5°C, and Ahe-field incubated soils. At the Thezan-site, about 50% of the tracer moved into the mineral horizon. This may be due to the extremely heavy rain falls at this site.
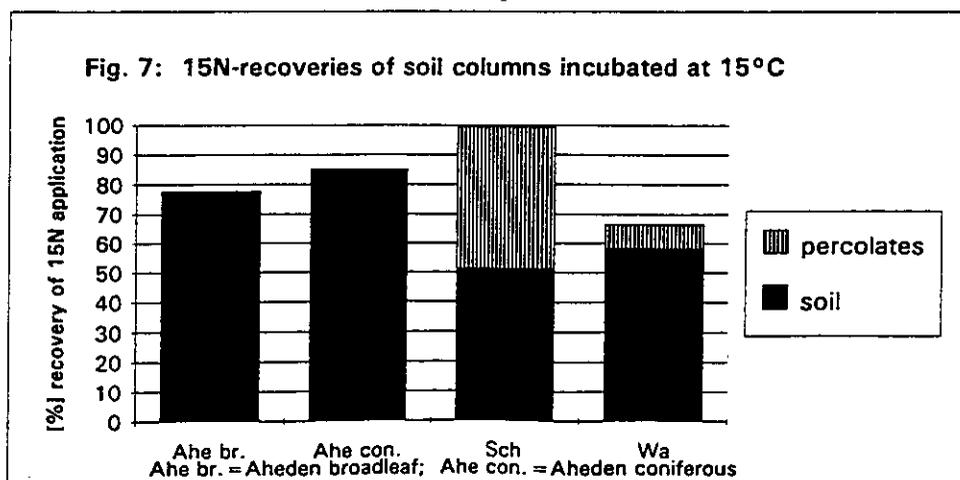
In the Fichtelgebirge soil columns, there was a more pronounced movement of the tracer to deeper horizons or even a marked loss of tracer via leaching at warmer temperatures/climates

(Fig. 6 b). In the Oh horizon only minor parts of the tracer were stored. This finding may point at low microbial activity and therefore low N-immobilization in this horizon. Results of Bottner et al. (see NIPHYS I report) confirm this assumption.

Fig. 6 a: Pattern of 15N tracer distribution in soil horizons Swedish soil columns (Ahe)

Fig. 6 b: Pattern of 15N distribution in soil horizons Fichtelgebirge soil columns (Wa)
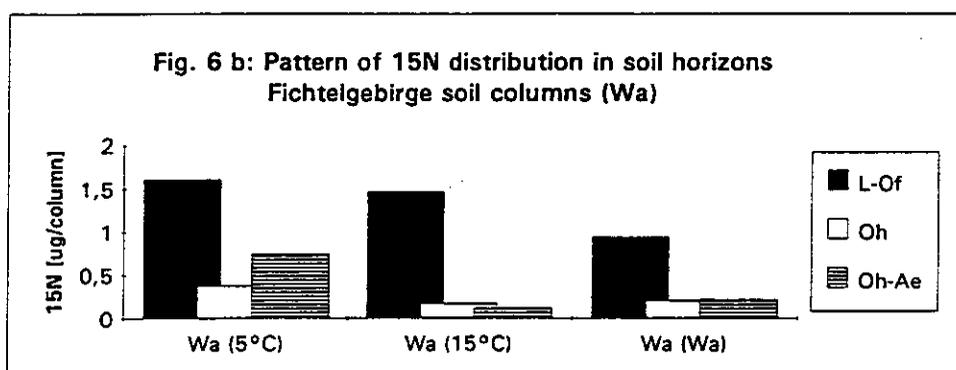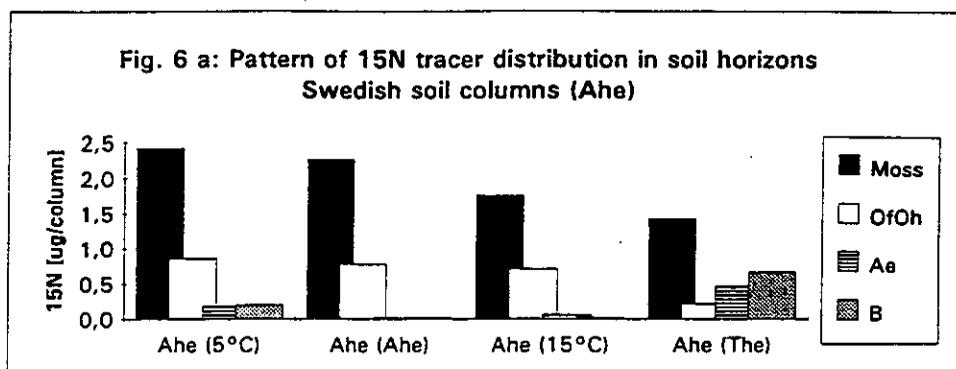
6.   Recovery of deposited N (15-N):

The recovery of the tracer, which was originally applied, was sometimes unexpectedly low (Fig. 7). For the coniferous forest soils in the Fichtelgebirge, recovery was only 65%, for Swedish soil columns about 80%. The low recovery may be partly caused by denitrification. 15-N loss via percolates was extremely high for Sch broadleaf forest soils, where up to 50% of the deposited N (applied as 15-N ammonium) was leached whereas the Swedish soils functioned as a much better sink for deposited N.

Fig. 7: 15N-recoveries of soil columns incubated at 15°C

Ahe br. = Aheden broadleaf; Ahe con. = Aheden coniferous

Conclusions

- The studied forest soils along the transect differ very much concerning soil-N processes. N-loss via leaching is highest in the Fichtelgebirge soils but negligible in North-Swedish soils. N processes in French soils always were in between these two extremes.

- The beech forest soil "Schacht" of the Fichtelgebirge lost 50% of the deposited N (15-N) via leaching after only 6 months of incubation, whereas in the Swedish soil percolates no 15-N was found.

- N- and DON-leaching were clearly temperature dependent. A rise in temperature of 10°C caused a 3 to 5 fold increase in N-/DON-loss via leaching in all studied soil types.

- Under colder temperature/climatic conditions the fraction of DON referring to total N was higher.

- Warmer climate, and especially heavy rain falls (South-France) favoured a movement of deposited N (15-N) to deeper soil horizons.

- NH_4-depositions were mainly stored in the uppermost horizons (moss, litter). Oh-horizons, especiallly of the Fichtelgebirge soils, have a low N-storage capability probably due to low microbial immobilization.

- Recoveries of 15-N (soils plus percolates) were partly low and may point at high gaseous N-losses via denitrification.

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Jensen E.S.(1991): Evaluation of automated analysis of 15N and total N in plant material and soil. Plant and Soil 133:83-92.

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Research Area: Nitrogen cycling, Microbiology, Modelling

OBJECTIVES

1) ^{15}N litter labelling: quantification of nitrogen transformations of labelled litter during the litter decomposition

2) Measurements of mineralisation, nitrification and uptake rates under field and laboratory conditions in beech and spruce stands at Aubure (Vosges)

3) Potential of plastic trees to quantify the nitrogen deposition onto real trees

4) To determine whether ectomycorrhiza influence nitrogen uptake and metabolism of roots in trees grown along a temperate climate gradient (collaboration Högberg, Univ. Umea).

RESULTS

Objective 1: ^{15}N litter labelling: quantification of nitrogen transformations of labelled litter during the litter decomposition

Introduction
The main objectives of our work was to produce a large quantity of highly enriched ^{15}N labelled beech litter and to use this litter as a substrate for large scale litter decomposition and N mineralisation experiments to follow the fate of organic litter N within beech forest ecosystems.

Materiel & Methods
see final report 1994

Results
1. ^{15}N labelled beech litter
As in a previous experiment the total N and ^{15}N concentration in the leaves increased after [^{15}N]urea spraying and decreased later. During senescence beech leaves lost about 60 % of N and ^{15}N which was translocated to other plant parts (leafs 1.6 % N, litter 0.7 % N). The obtained N retranslocation were confirmed by the results of Staaf (1982). In May 1994, leaves were highly enriched in ^{15}N (2.9 % of total leaf N) from translocation of stored ^{15}N. During spring the N and ^{15}N concentration decreased in steps from May to August. This effect is likely related to the internal N cycling (import - export) within the tree. The decrease in ^{15}N was

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stronger (- 65 %) than for total leaf N (- 40 %). More labelled N was translocated from the leaves and replaced by non-labelled N from root uptake.

Leaves from the sun-crown and leaves from the shadow-crown had different ^{15}N enrichment. Upper leaves were less enriched than shadow-crown leaves (1.7 - 7.8 at. % ^{15}N). At the end of September the differences in ^{15}N enrichment between leaves from sun - shadow crown become smaller (2.8 - 3.6 at. % ^{15}N). Leaves treated in 1993 had higher variability than leaves sprayed 1994 and 1995. The obtained effect might be due to a higher evaporation of urea from sun-crown and to a less physiological activity of the sun crown leaves. Leaves from the upper crown fall before the others and therefore the physiological activity was reduced which resulted in low foliar uptake. Covering the plot with plastic planes installed in 1994 and 1995 for 36 hours reduced the variability, but had little influence on the ^{15}N enrichment in upper leaves. With the beginning of senescence in October, a decrease in leaf N concentration was observed (1.8 % N - 1.6 %N). Foliar absorption of [^{15}N]urea in September 1993, August 1994 and August 1995, was calculated as difference between sprayed ^{15}N per tree and ^{15}N content as sum in all leaves per tree. Beech leaves absorbed 40 - 60 % of the applied [^{15}N]urea, about 20 - 40 % of the ^{15}N reached the soil as drops and a loss of 20 % mainly by evaporation occurred

The obtained litter had an average enrichment of 2.45 at. % ^{15}N (1993) (**Table 1**) and 4.7 % of the applied ^{15}N remained in the litter. In autumn 1994, a higher enrichment in the obtained litter was measured (3.24 at. % ^{15}N). For the litter produced 1995 an enrichment of 5.30 at. % ^{15}N was estimated.

Table 1: Amounts of ^{15}N applicated per tree, quantity of produced labelled beech litter and ^{15}N enrichment of the litter.

Date of Urea-Application	[^{15}N]Urea mg tree^{-1}	litter (kg dw)	(% N)	enrichment (atom % ^{15}N)	^{15}N sprayed / ^{15}N retained in litter (%)
September 1993	56.6	4.96	0.82	2.45	4.5
August 1994	26.9	7.32	0.89	3.61	7.5
August 1995	58.2	8.52	0.87	5.31*	7.7

* estimated

More than 95 % of the ^{15}N absorbed by the leaves was retranslocated and stored in different tissues of the beech trees. Results are presented as concentration (**Figure 1 A**) and as total content in the different tissues (**Figure 1 B**). Buds formed in 1993 had the highest ^{15}N concentration of all plant parts. Bark tissues from different compartments had similar ^{15}N concentrations. ^{15}N was also stored in wood and roots at similar concentrations. ^{15}N concentrations were higher in bark than in the wood. On a mass scale most ^{15}N was stored in bark, bark and wood of the branches, wood and large roots. Due to little differences in ^{15}N concentration, ^{15}N con-

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tents were related to tissue biomass. Wood is the largest compartment (> 80 %) within the beech trees.

Figure 1:[15]N concentration (A), content (B) in buds, bark, wood and roots of 10 year old beech trees sprayed with 56.6 mg ^{15}N tree[-1] at 1 st. September 1993. Sampling in February 1994 (n = 5 trees)
(br = branches, 91 + 92 = year of growth)
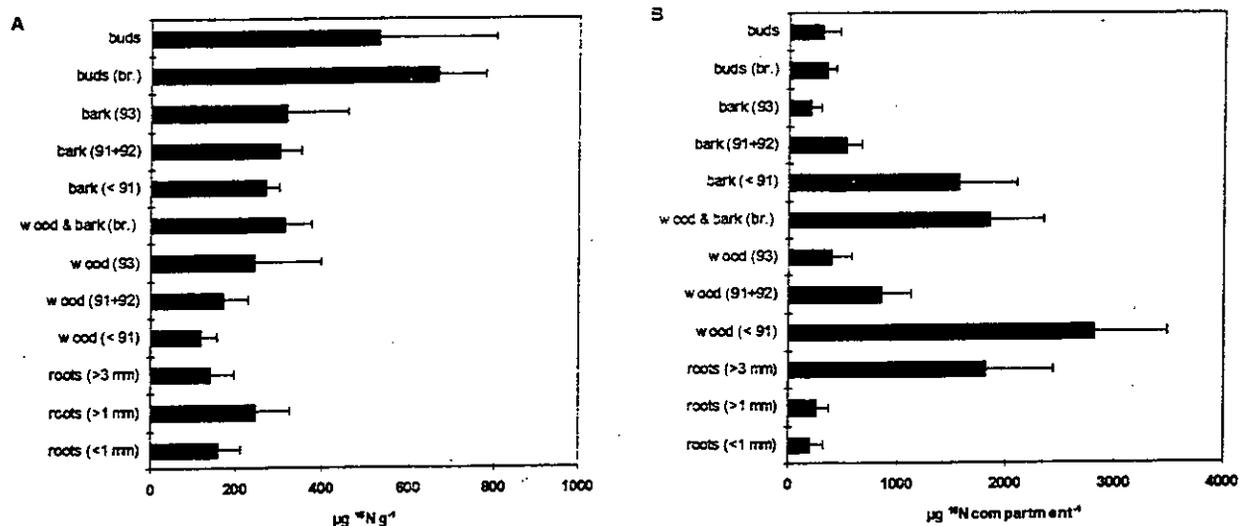
Conclusion

In conclusion [^{15}N]urea spraying of trees in late summer lead to a high retention of ^{15}N in the resulting litter. Large quantities of ^{15}N labelled litter were produced by using young trees in forests. Leaves absorbed 50 - 60 % of the applied ^{15}N, depending on spraying conditions. Use of surfactants might improve the foliar ^{15}N uptake. The third subsequent urea application enriched the trees strongly in ^{15}N, useful for further production of ^{15}N labelled litter. Due to a frost period in May 1995 and damage of new formed leaves less litter was harvested (**Figure 2**).

Figure 2:[15]N enrichment and labelled litter production after spraying leaves with [^{15}N]urea in autumn 1993 - 1995.
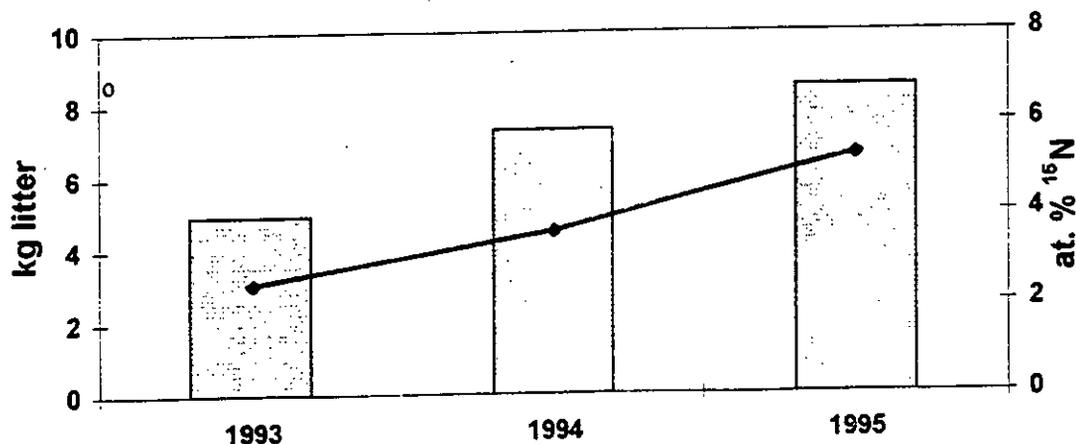
2. Decomposition and mineralisation of ¹⁵N labelled beech litter

Materiel & Methods

In summer 1994, five beech trees (30-year-old) were selected at the Aubure site for replacement of the annual litterfall by ¹⁵N-labelled litter. Selected trees were separated from surrounding trees by cutting all roots up to 0.30 m soil depth (highest root density) in a radius of 1.25 m from the selected trees and by installing a 2 mm plastic sheet as a persisting root barrier. The isolated soil surface around each tree was about 4 m². Porous cup lysimeter were horizontally installed in duplicate in - 0.15 m and - 0.30 m near 3 selected trees and two non-labelled trees. Throughfall collectors were installed for each tree and also for non-labelled trees. During the autumn 1994, annual litter was sampled fortnightly from the plots, dried at 60 °C and litter quantity was calculated for each circle. In November 1994, last year litter (fallen leaves) was removed from the plots to avoid mixing between labelled and non-labelled leaves litter. ¹⁵N labelled beech litter was layered on the soil as mass equivalent of annual litterfall. All plots were covered with a plastic net (mesh 2 cm) to avoid loss of labelled litter by wind.

Soil and litter samples were taken in: April, May, July, August and October. Soil solutions were sampled fortnightly from April to November. The following measurements were made: inorganic N(¹⁵N) in soil extracts (1 M KCl; 0.05 M K_2SO_4) and soil solutions (- 15 cm, - 30 cm), microbial biomass N(¹⁵N) (fumigation-incubation method), N(organic)(¹⁵N), N(total)(¹⁵N), roots and mycorrhizal roots N(¹⁵N), N(¹⁵N) in different tree compartments, N(¹⁵N) and nutrient elements in the labelled litter. Mass loss of labelled litter was calculated according to the mass loss models of Aber et al. (1990) and Berg and Ekbohm (1991).

Results

Mass loss during the first year of decomposition was about 30 % of initial litter mass. At 5 litter samplings during 1995 the ¹⁵N concentrations in the decomposing litter remained at the initial value (**Figure 3**). Therefore a coupling of mass loss and ¹⁵N release from decomposing litter at similar rates was assumed.

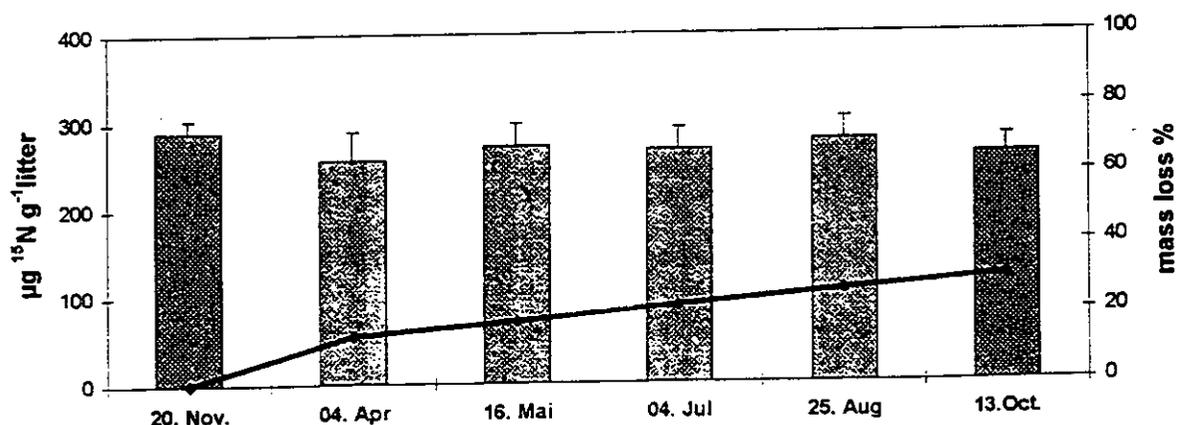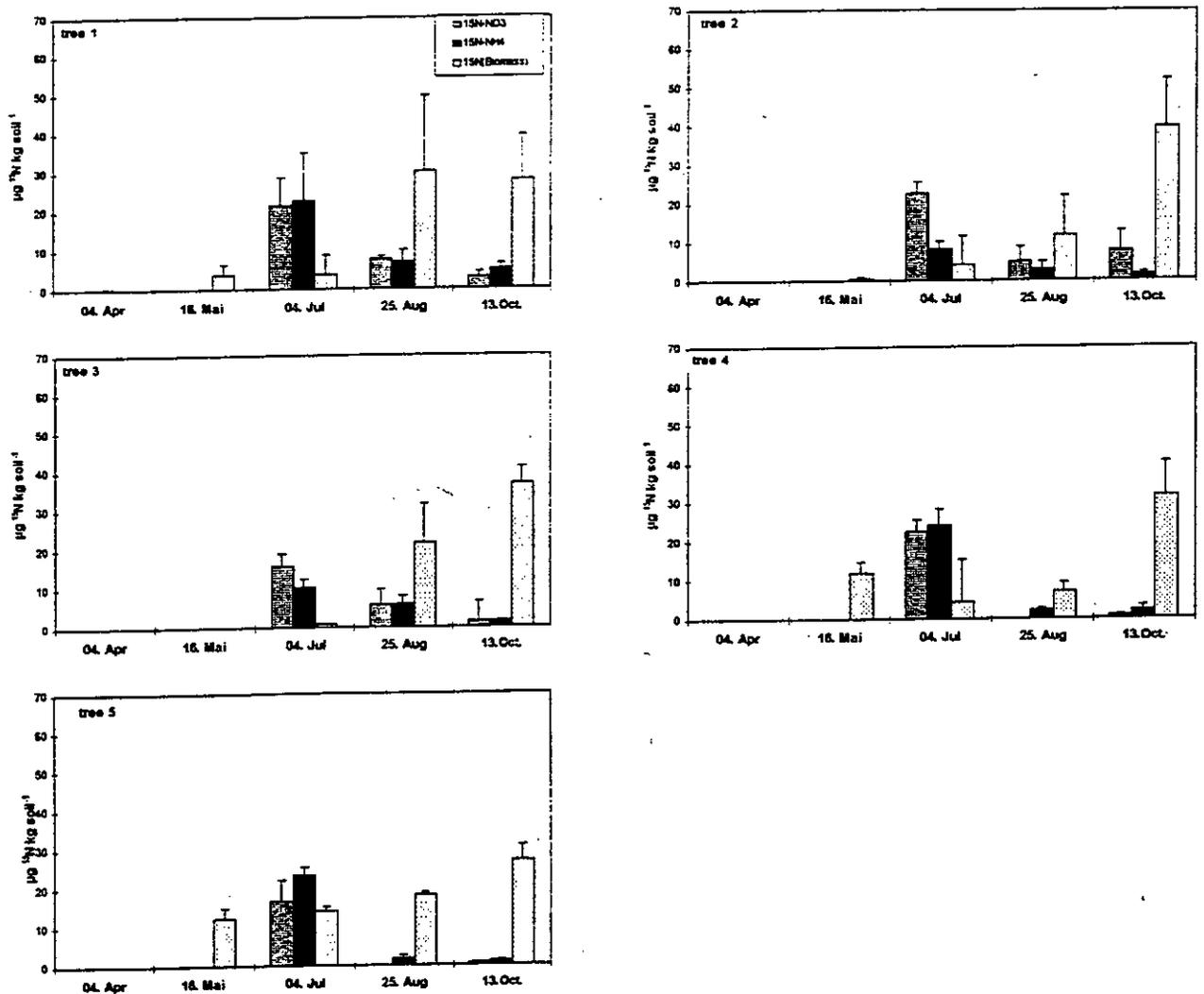**Figure 3:** Mass loss and ¹⁵N concentration in labelled litter

Figure 4: ^{15}N concentration in the soil during the first year of labelled litter decomposition. Data are given for nitrate, ammonium and microbial biomass ^{15}N for five plots (tree 1 - 5). Soil samples were taken in: April, May, July, August, October 1995.

After 5 months of litter decomposition no significant increase in ^{15}N in the different soil extracts was measured. In May, the ^{15}N concentration increased weekly in the microbial biomass N fraction and increased further by steps until October (**Figure 4**). ^{15}N enrichment of inorganic $^{15}NO_3^-$ and $^{15}NH_4^+$ was higher in July 1995 (3 % of initial litter ^{15}N) than in August and October. ^{15}N enrichment of NO_3^- and NH_4^+ in May was similar for all plot (tree 1 - tree 5), except tree 2. Variability in $^{15}NO_3^-$, $^{15}NH_4^+$ and microbial biomass ^{15}N in and between the different plots might be related to differences in soil water content. The ^{15}N partitioning obtained during ^{15}N mineralisation is in agreement with the 3 years data on N mineralisation measured in beech (Au_B). During summer (July-August) high N mineralisation occured.

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Staaf, H. (1982) Plant nutrients changes in beech leaves during senescence as influenced by site characteristics. Acta Oecologica/ Oecologia Plantarum 3 (17), 8-17

Objective 2: Measurements of N mineralisation, nitrification and uptake rates under field and laboratory conditions in beech and spruce stands at Aubure (Vosges)

Introduction

The main objective of this experiment was   i) to measure intensively the field rates of mineralisation and nitrification during a 3 year period while integrating the other fluxes of N in the ecosystem (e.g., deposition or losses by leaching) and ii) to follow the seasonal variability and to identify the factors controlling rates of mineralisation under field conditions.

Materiel & Methods

The study was carried out in three stands located at Aubure in the same climatic and in the same soil conditions: a 150 year old beech stand , a 80 year old and 40 year old spruce stands.

The method used was similar to the procedure developed by RAISON and HÜBNER (1987). It was based on a sequential soil coring and on a field incubation of undisturbed soils columns confined in steel tubes. A set of 9 stainless steel tubes were hammered in the 15 first cm of the soil. They were extracted carefully in order to set up an ion exchange resin bag at the bottom of the tubes. Anionic resins were used to trap leached nitrate. The tubes were then carefully replaced in the soil and exposed under field conditions for a 2 weeks period. Both ion exchange resins and tubes are collected fortnightly during the vegetation period and monthly during the rest period. At each sampling time, nine non-confined soils columns were also collected in the same place.

Throughfall collectors and open-lysimeters were set up in each experimental stand in order to provide data on deposition and N losses.

The RAISON's original method was improved in order to minimise the difference in soil moisture inside and outside the tubes (higher diameter of tubes, shorter incubation period). The latter choice reduced a possible over-mineralisation within the tubes due to severing fresh roots during the installation.

The principle of this experiment was based firstly on the assumption that physical, chemical and biological attributes of the soil are similar inside and outside the tubes except that there is no tree uptake within incubation tubes. This entailed obviously a higher moisture content within the tubes, but the difference found was not statistically significant.

This method allowed an estimation of N fluxes (net mineralisation, net ammonification and net nitrification, tree uptake) from a set of simple equations (*see Niphys: final report 93-94*).

Results

Yearly budgets were obtained by summation of amounts of mineralised N between two successive sampling dates. All the following data are expressed in kilogram of N per hectare at 0-10 cm depth.

•Table 2 shows that the variability of N mineralisation was relatively high between years (high standard errors, n=3). But differences between stands were never significant (Figure 5).

It was surprising to observe that N mineralisation rates were high in these acid soils (pH<4) under field conditions. Mean mineralisation was roughly comparable in the two spruce stands and 2-fold higher in beech stand (90 and 130 kg N ha^{-1} year^{-1} respectively).

•In spite of a marked variability between years, the three stands were clearly distinguished as regard to nitrification rates: as mineralisation was higher in beech stand, mean nitrification was 2-fold higher in beech stand (80 kg N ha^{-1} year^{-1}) than in 80 year-old spruce stand ($\varnothing$ 40 kg N ha^{-1} year^{-1}). Results bring into prominence a lack of nitrification under field conditions in the young spruce stand (40 yr-old): nitrification rates were of the same magnitude than the error calculated on annual or seasonal budgets. These field rates were consistent with the results of potential mineralisation and nitrification measured in laboratory incubations (*see Niphys 93-94: final report*).

Measurements of pH in the top layer were carried out in the three stands with 25 replicates. The found pH range was from 3.9 in the 80 yr-old spruce stand, to 3.8 in the 40 yr-old and to 3.7 in beech stand. But mean values were not significantly different between stands. The pH could not explain the lack of nitrification in the young spruce stands. Recent data on the microbial biomass suggested that the limiting factor could be a lack or an insufficient level of nitrifiers population in the young spruce stand. Past land use effect may explain this difference in the biodegradability of organic matter between spruce stands established in old pastures and in old forested areas. Potential nitrification in several young spruce stands located on old pastures and forested areas are in progress to confirm or not this hypothesis (Figure 5).

Annual net ammonification is highest in the young spruce stand (90 kg N ha^{-1} year^{-1}) against 45 kg N ha^{-1} year^{-1} in old spruce and beech stands.

• Annual NO_3^- and NH_4^+ budgets were detailed in two periods: the rest period was running from mid-October to mid-April and the vegetation period (growth season) from April to October. All mineral nitrogen processes were approximately 2-fold higher in the growth season than in the rest period. As concerning the species effect, results of seasonal budgets confirmed annual budgets. During the growth period, mineralisation was significantly higher in beech ($\varnothing$ 100 N ha^{-1} year^{-1}) than in old spruce (56-66 kg N ha^{-1} year^{-1}). A lack of nitrification was also

Table 2: Annual and seasonal N mineral production at Aubure (means and standard errors for 3 years studies)

Nitrification (kg N-NO$_3^-$ .ha^{-1})

	dormant season	growth season	yearly budget
80 yr-old spruce stand	9 ± 2.7	29 ± 9.3	38 ± 10.6
40 yr-old spruce stand	1 ± 1.0	1 ± 0.3	1 ± 1.5
150 yr-old beech stand	17 ± 2.7	62 ± 12.1	80 ± 1.5

Ammonification (kg N-NH$_4^+$ .ha^{-1})

	dormant season	growth season	yearly budget
80 yr-old spruce stand	21 ± 4.1	26.5 ± 4.6	47.5 ± 6.3
40 yr-old spruce stand	28 ± 10.9	65 ± 9.7	93 ± 11.7
150 yr-old beech stand	8 ± 7.9	41 ± 16.4	49 ± 9.8

Mineralisation (kg N .ha^{-1})

	dormant season	growth season	yearly budget
80 yr-old spruce stand	30 ± 4	56 ± 7.2	86 ± 11.1
40 yr-old spruce stand	28 ± 12.1	66 ± 9.6	95 ± 12.1
150 yr-old beech stand	25 ± 10.1	104 ± 28.5	129 ± 20.9

Total N deposition (kg N .ha^{-1})

	dormant season	growth season	yearly budget
80 yr-old spruce stand	5.9 ± 0.7	7.4 ± 1.7	13.3 ± 2.4
40 yr-old spruce stand	2.5 ± 0.2	3.4 ± 0.8	5.9 ± 0.9
150 yr-old beech stand	2.8 ± 0.6	5.2 ± 1.0	8.0 ± 1.6

Nitrate leaching (kg N-NO$_3^-$ .ha^{-1})

	dormant season	growth season	yearly budget
80 yr-old spruce stand	8 ± 2.9	2.8 ± 0.8	11 ± 3.3
40 yr-old spruce stand	0.13 ± 0.03	0.13 ± 0.09	0.3 ± 0.06
150 yr-old beech stand	1.8 ± 1.2	0.9 ± 0.23	2.7 ± 1.35

Total N uptake

	dormant season	growth season	yearly budget
80 yr-old spruce stand	34 ± 4.9	58 ± 5.2	92.5 ± 9.5
40 yr-old spruce stand	29 ± 10.3	71.5 ± 8.1	101 ± 14
150 yr-old beech stand	27 ± 9.4	118 ± 22.6	145 ± 16

Mean N production in the dormant season at Aubure
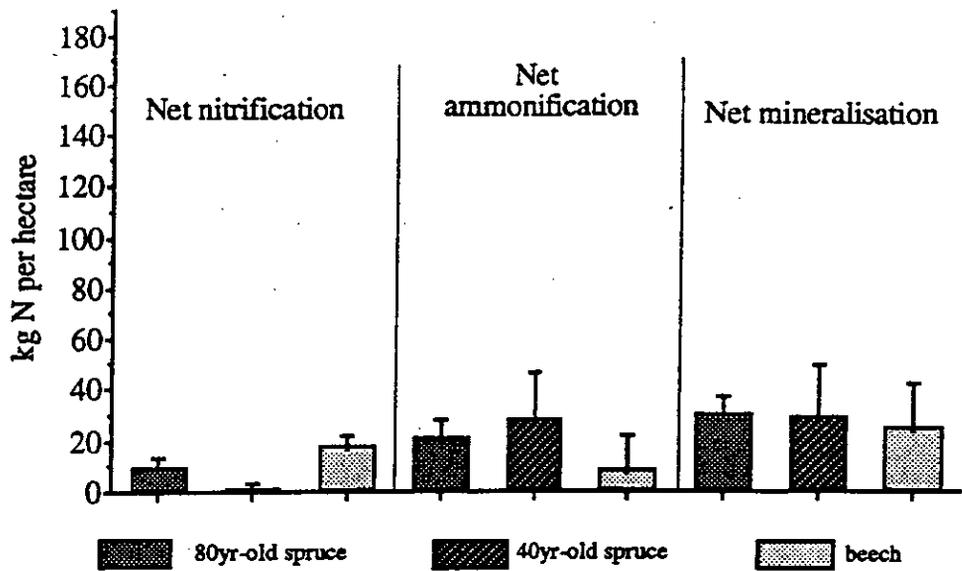
Mean N production in the growth season at Aubure
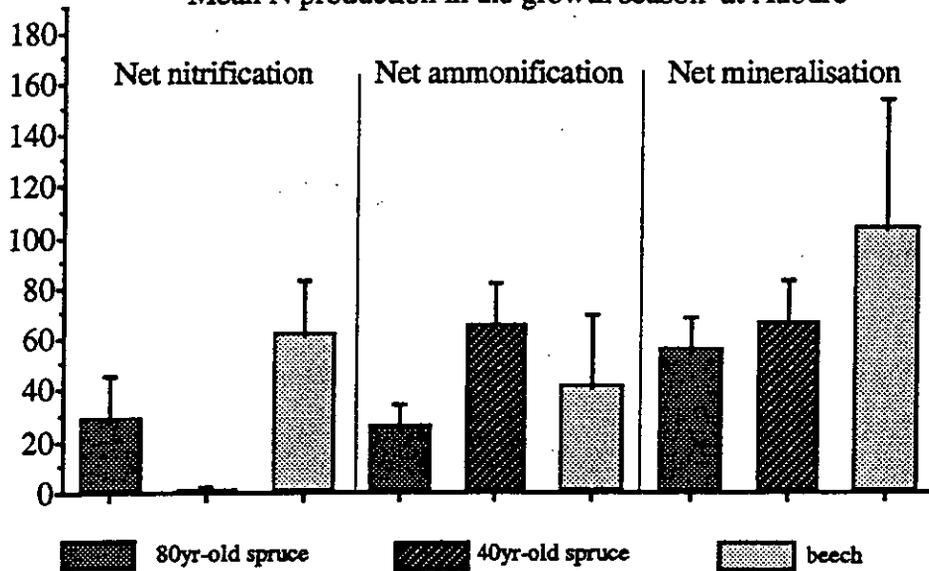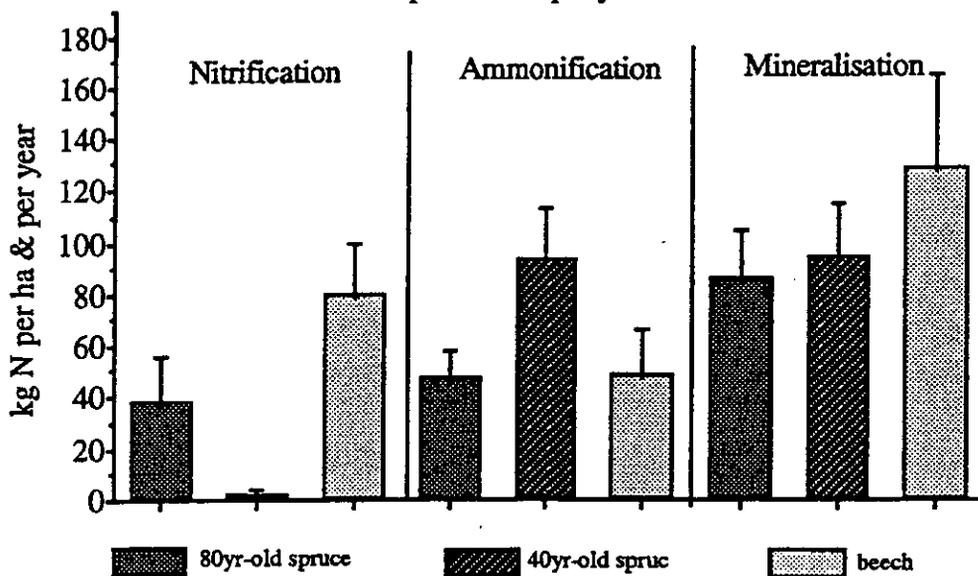
Mean N production per year at Aubure

established in the 40 yr-old spruce stand, both during the rest and the growth season (Figure 6). Nitrification was clearly increased in the growth period., It was multiplied by 3-fold and 3.6-fold in old spruce and beech stands, respectively.

Net ammonification was also strongly increased in the growth season in young spruce and beech stands, 2-fold and 5-fold higher, respectively. In contrast, net ammonification varied slightly over the year in the old spruce stand ($\varnothing$ 20-25 kg N ha^{-1} per period of 6 months).

•Nitrification varied markedly with season. Two main peaks of nitrification per year could be observed in beech stand: a first peak in summer (from mid July to mid August). Then nitrification drops sharply from the end of August to October when the soil becomes dryer: the microbial immobilisation becomes the leading process in soil. The second main peak of nitrate production occurred from the end of September with the re-wetting of the soil by fall rains. Figure 6 shows that a higher nitrification rate could happen in the beginning of spring at the melting of the snow (mid-March, or April). Seasonal variability of nitrification is less marked in the old spruce stand. High nitrification rates were observed in the end of June, also in the beginning of the summer and the end of September. A higher production of nitrate is also observed in 80 yr-old stand at the thawing (Figure 7).

• The used method allowed the assessment of the total N tree uptake. A significant N uptake take place during the rest period (specially in the fall) with approximatively 30 kg ha^{-1} year^{-1} in the 3 stands (Table 2). Tree uptake is expectedly increased by 2-fold or 3-fold during the growing season.

• All these data on nitrogen fluxes, estimated under field conditions, can be easily integrated in a model of N fluxes and validated at Aubure and would be generalized to other sites.

Conclusions

• Notwithstanding a low pH (3.7 - 3.8), the Aubure sites (Au$_B$, Au$_S$) exhibited high mineralisation rates under field conditions (with 85 to 130 kg N ha^{-1} year^{-1}).

•The mineralisation rate was clearly higher in the beech stand (Au$_B$) than in spruce stands (Au$_S$). Mineral production of total N was 86, 95 and 130 kg N ha^{-1} year^{-1} in the old and young spruce stands and in beech stand, respectively. Nitrification rate reached 40 kg N ha^{-1} year^{-1} in Au$_S$ and 80 kg N ha^{-1} year^{-1} in Au$_B$, respectively.

• A lack of nitrification in the 40 yr-old spruce stand under field conditions was strengthened by results of potential nitrification in laboratory incubations. This lack of nitrification could be an effect of past land use.

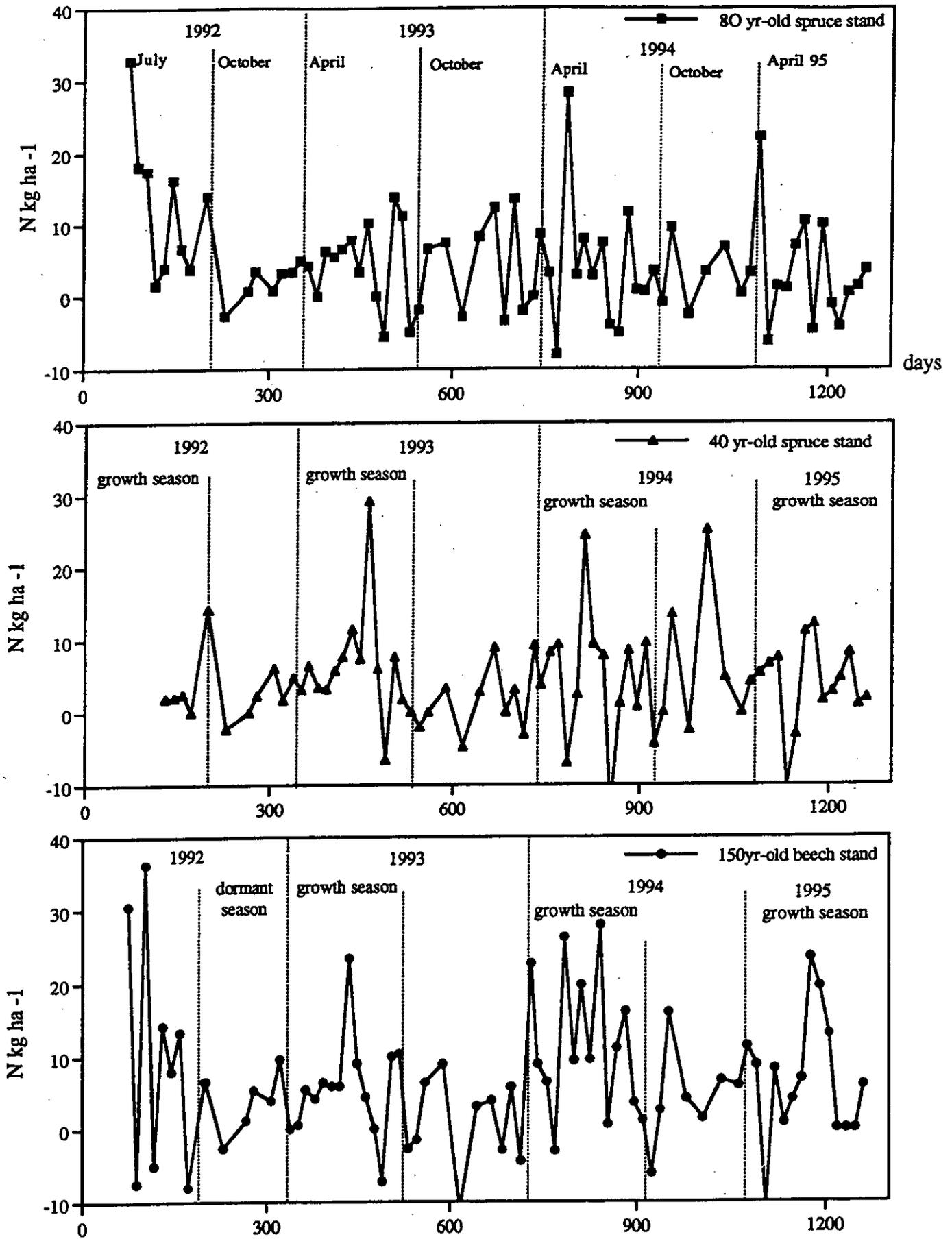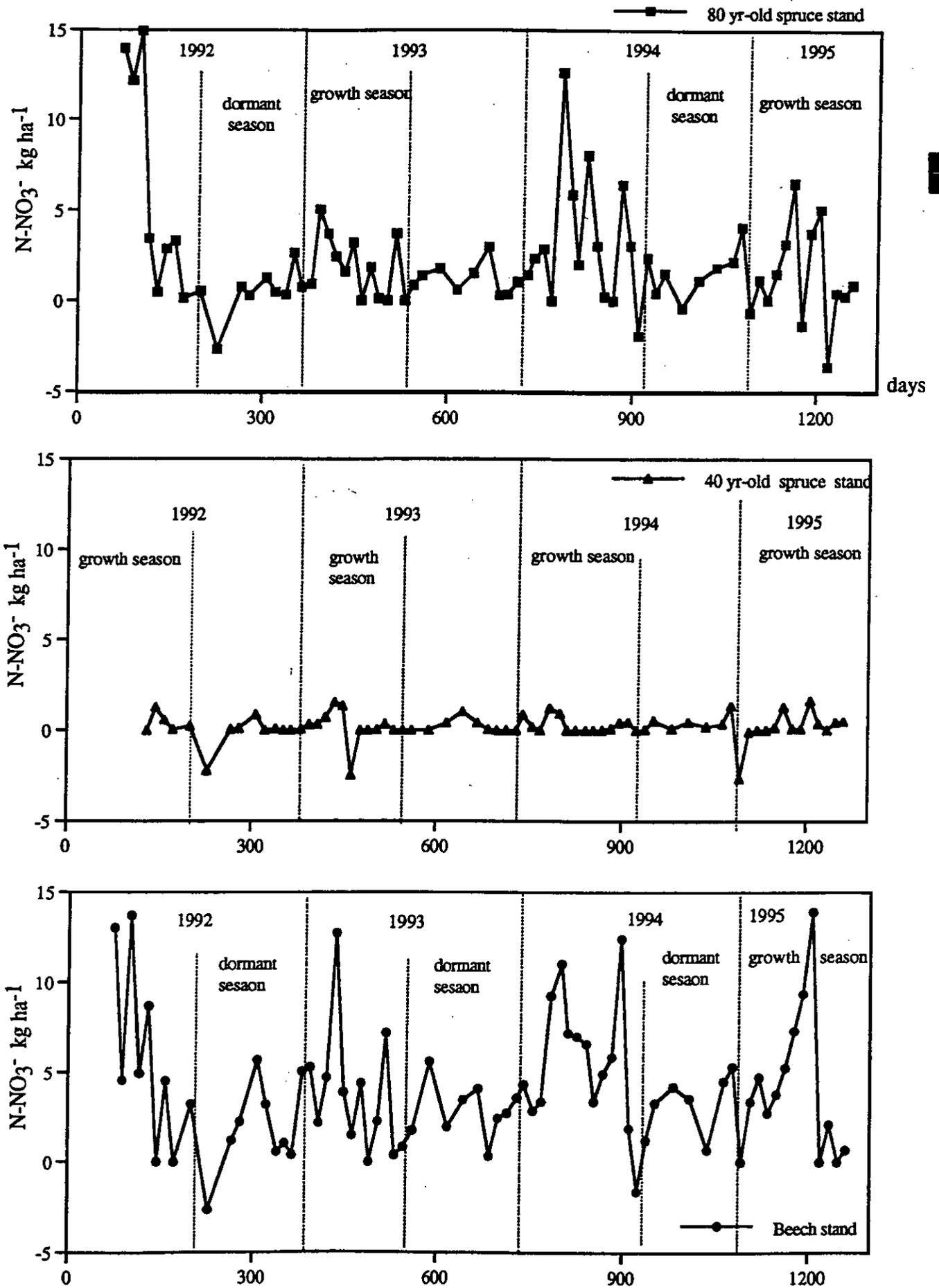# Figure 6: Total N mineralisation in 0-10 cm soil layer (kg ha-1)

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Figure 7: Nitrification in 0-10 cm soil layer (kg ha^{-1})

• A marked seasonal variability was observed. Two main peaks of nitrification in the growth period (mid summer and end of September with the re-wetting of the soils) and one peak of higher activity in the rest season at the melting of the snow were identified.

• Nitrogen uptake by trees reached up to 100 kg N ha^{-1} in Au$_S$ and 145 kg N ha^{-1} year^{-1} in Au$_B$.

• Seasonal budget revealed significant uptake by trees in the fall with an average uptake equal to 30 kg N ha^{-1} in the all studied stands.

• Data on root N uptake confimed that ammonium is the favoured form absorbed by spruce. In contrast, nitrate uptake in beech was higher than NH$_4^+$ input.

Objective 3: Potential of plastic trees to quantify the nitrogen deposition onto real trees.
E. DAMBRINE, N. IGNATOVA

Material and Methods

In a clearing of the crest of the Aubure catchment, 5 plastic trees (1.6 meter height) were set up during 9 months (february-october). Five isolated young spuce trees (S 8) of approximately the same height were selected in their immediate vicinity. Below each tree were set up 8 plastic funnels (diameter 20 cm) disposed in two circles to collect throughfall on an area basis. Four funnels were were set up in the open to collect bulk deposition. Bulk precipitation and throughfall were collected in 2 liters polyethylene flasks, sampled and analysed twice a month. Parallely, throughfall was collected by replicates of 2 meters long trouts in 3 adjacent closed spruce stands aged 15, 35 and 90. At all sites, stemflow was neglected. However, water budgets below the plastic trees showed that a significant part of throughfall was lost as stemflow. Measurements made in autumn showed that stemflow below the plastic trees amounted 10 to 20% of thoughfall water. These hypothesis were considered in the following calculations.

Results and Discussion

Concentrations

Figures 8 shows the concentration in NH$_4^+$ and NO$_3^-$ in the open (Bulk P), below the plastic trees (Pastic), the isolated young spruce trees (S 8) and the closed stands.

NH$_4^+$ Concentrations were always much higher below the plastic trees than below S 8 and much higher than in the open. Below the closed stands, concentrations were much higher in S 90 than in the younger stands. In the latters, concentrations are almost similar and slightly lower than in bulk precipitation.

NO$_3^-$ Concentrations . Exept in S 15 NO$_3^-$ was much higher in throughfall than in bulk precipitation. In the closed stands, NO$_3^-$ concentration increased with age. Concentrations

below the plastic and S 8 varyied parallely, with peaks more accused in spring and autumn below the real trees.

Fluxes

Measured NH_4^+, NO_3^- and Na fluxes over the study period are presented in table 1.

Using Na in net throughfall as an index of the fitration factor of the crowns, and the NH_4^+/Na and NO_3^-/Na ratio in net plastic tree thoughfall (Table 3) as an index of N deposition, it was possible to calculate N deposition (Table 4) and uptake (Table 5) under the living trees. Over the 9 month period of the study, 200 to 350 NH_4^+ eq.ha^{-1} and 0 to 150 NO_3^- eq.ha^{-1}, depending on the site, were taken up by the crowns. These values can be considered as an underestimation of the real value, because plastic trees are probably less efficient as collecting nitrogen than real trees. Nevertheless it shows that a significant part of spruce nitrogen requirements are met by foliage uptake, especially of NH_4^+.
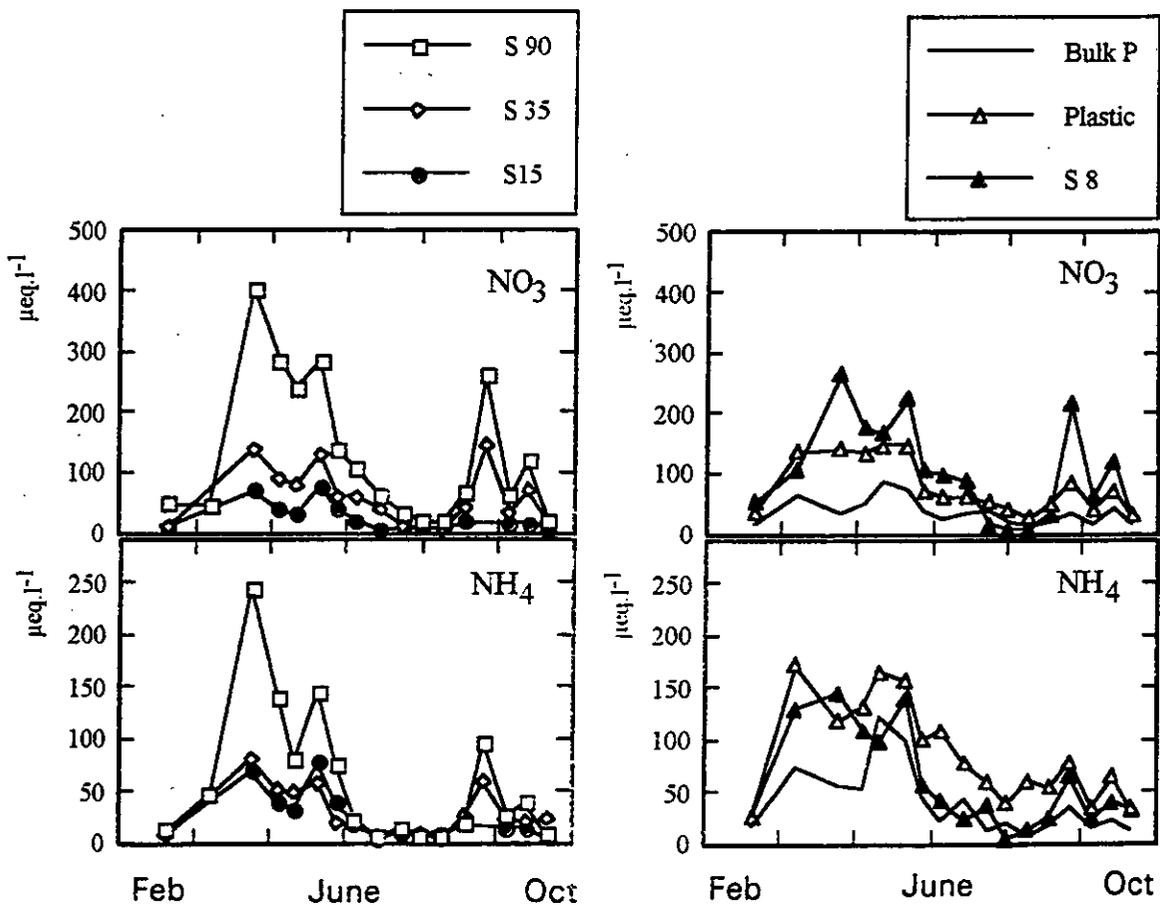

Figure 8: Concentrations of NH_4^+ and NO_3^- in bulk precipitation, and throughfall below plastic trees, isolated 8 years old spruce trees (S 8) and closed spruce stands aged 15 (S 15), 35 (S35) and 90 (S 90).

Total	Na eq.ha^{-1}	NH$_4^+$ eq.ha^{-1}	NO$_3^-$ eq.ha^{-1}
Bulk P	84	218	218
Plastic	107	341	306
S 8	146	247	428
S 15	114	106	196
S 35	106	106	190
S 80	142	177	407

Table 3: Measured Na, NH$_4^+$ and NO$_3^-$ fluxes deposited in throughfall

Net	Na eq.ha^{-1}	NH$_4^+$ eq.ha^{-1}	NO$_3^-$ eq.ha^{-1}
Plastic	22	123	88
S 8	61	29	210
S 15	30	-112	-22
S 35	22	-113	-28
S 80	58	-41	189

Table 4: Na, NH$_4^+$ and NO$_3^-$ fluxes in net throughfall

Dry dep	Na eq.ha^{-1}	NH$_4^+$ eq.ha^{-1}	NO$_3^-$ eq.ha^{-1}	Na* eq.ha^{-1}	NH$_4$* eq.ha^{-1}	NO$_3$* eq.ha^{-1}
S 8	61	338	241	61	269	210
S 15	30	165	118	30	131	102
S 35	22	122	87	22	97	75
S 80	58	317	226	58	252	197

Table 5: NO$_3^-$ and NH$_4^+$ dry deposited considering the measured amount of throughfall below the plastic trees or * an estimation of 20% stemflow

uptake.	NH$_4^+$ eq.ha^{-1}	NO$_3^-$ eq.ha^{-1}	NH$_4$* eq.ha^{-1}	NO$_3$* eq.ha^{-1}
S 8	309	32	240	0
S 15	277	140	244	124
S 35	234	115	209	103
S 80	359	38	294	8

Table 6: NO$_3^-$ and NH$_4^+$ taken up by the crowns.

12. Doc. RNDr. Tomas Paces, DrSc.

With contributions by: Frantisek Buzek, Jiri Cerny, Martin Novak & Simon Bottrell

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Introduction

In the Czech Republic ecosystem-level investigations of nitrogen fluxes have been under way since 1975 (Paces 1985). During the two years of our participation in NIPHYS (1994 -1995), we focused on catchment-scale nutrient mass balances and quantification of the dispersion pathways of pollutant N and S in forest soils. In doing so, we used our previous experience with isotope techniques applied at heavily polluted sites (Buzek et al. 1991; Novák et al. 1994; Cerny, Paces 1995; Novák, Prechova 1995). Data summarized in this final report have been interpreted by Buzek et al. (1995), Cerny (1996), Novák et al. (1996), and Bottrell & Novák (1996). The persevering high atmospheric deposition of sulfur in the Northern Czech Republic gave us an opportunity to study the disturbance of element cycling in forest ecosystems along a steep air pollution gradient. Specifically, we studied quantitative relationships between N and S along an East-West transect across Europe.

Objectives

(1) to establish sites along an East-West transect which can serve as an independent test area for NIPHYS hypotheses developed at the main North-South transect,

(2) to determine internal ecosystem fluxes of nitrogen and sulfur,

(3) to quantify the fluxes of ammonium and nitrate in forest ecosystems using the stable isotope ^{15}N,

(4) to quantify the transition of atmospheric water to soil and groundwater reservoir using ^{18}O and natural radiotracers,

(5) to model the meso-scale biogeochemical cycle and reversibility of processes under the influence of varying inputs of sulfur and nitrogen oxides, and

(6) to develop and maintain a capacity to test NIPHYS hypotheses with independent data sets.

Results

(1) The East-West transect

A simple monitoring network was established in early 1994 along a steep chemical gradient acros Europe. The easternmost of the five sites (Fig. 1) is situated near the Ocean Bog, Czech Republic, the westernmost at Connemara, Ireland. Each of the sites is equipped with bi-monthly precipitatioɪ collectors in an open area ("bulk") and underneath spruce canopy ("throughfall"). Isotopᴄ composition of sulfur (δ^{34}S) was monitored in 1994-1995. In addition, δ^{15}N monitoring started iɾ early 1995. Multiple "grab" samples of spruce forest floor and living *Sphagnum* (n=18 for each sitɪ and type of material) were analyzed for S and N concentrations and δ^{34}S ratios (Bottrell, Novak 1996).

With an increasing atmospheric pollution (in the sequence: Isle of Mull, Scotland; Connemara Ireland; Rybarenska slat, Southern Czech Republic; Thorne Moors, England; and Ocean, Northern Czech Republic) sulfur concentrations in both forest floor and living *Sphagnum* also increased. *Sphagnum* contained less S than forest floor in both Mull (mean 0.08 % vs. 0.175 %) and Oceaɴ (0.21 % vs. 0.226 %).The statistical (p < 0.05) significance of the difference in S retention betweeɴ the polluted and unpolluted sites was rather surprising (cf. Johnson, Lindberg 1992). Previously, cycling of sulfur was believed to be controlled by biogeochemical processes under low S inputs and by geochemical (i.e., inorganic) processes under high atmospheric S loads. Our findings may bᴇ related to the large between-site deposition span (less than 8 kg S ha^{-1} yr^{-1} in Mull, and more than 70 kg S ha^{-1} yr^{-1} at Ocean). The pollution gradient along the E-W transect is reflected also in S isotopᴇ composition (Fig.2). At the near-shore sites isotopically heavy sea-spray sulfate dominates, while at the inland, industrially polluted sites the δ^{34}S ratios reflect isotope composition of local coal (mean +2 ‰ CDT in the Czech Republic). Interestingly, S isotope signatures of forest floor are systematically lighter compared to *Sphagnum*. The N vs. S concentration plot (Fig. 3) shows a disturbance of element cycling at the extremely polluted site compared to the most unpolluted site: the *Sphagnum* data points at Ocean are displaced to higher N and off the common regression line typical of Mull.

(2) Internal ecosystem fluxes of nitrogen and sulfur

The input and output fluxes of nitrogen and sulfur have been measured along the steep gradient oɪ the sulfur atmospheric deposition in two catchments. The data obtained during NIPHYS in 1994 1995 (Table 1) were compared to data representing the fluxes during the period 1976-1982 (Paceʂ, 1985) when the atmospheric pollution by SO_2 was the highest in Central Europe. The catchmenᵗ Salacova Lhota represented low pollution by SO_2 with the input of sulfur equal to 24.2 kg.ha^{-1}.yr^{-1} i the period of 1976-1982. The catchment Jezeri represented the highest pollution by SO_2 with the input of sulfur equal 108.4 kg.ha^{-1}.yr^{-1} in the same period The recent input of sulfur in 1994-95 ha
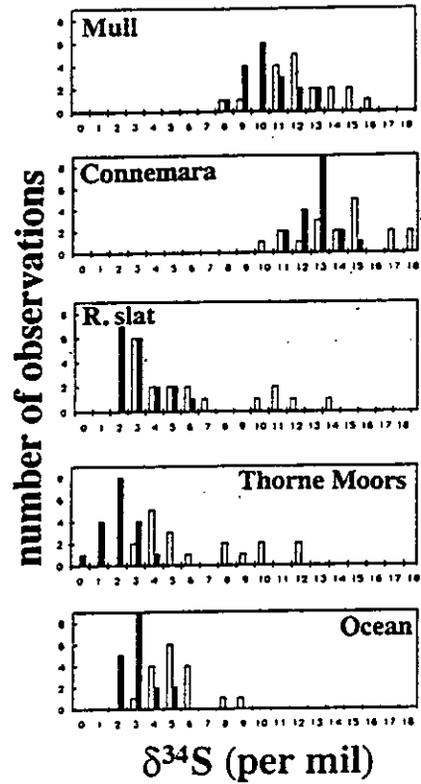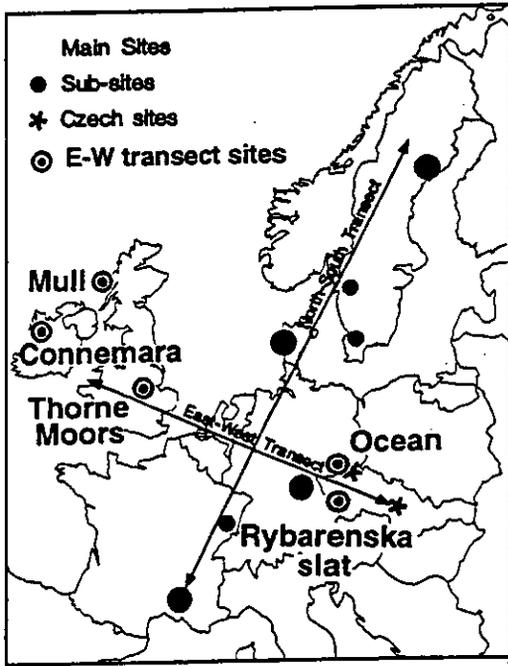
Figure 1: The East-West transect

Figure 2: Histograms of S isotope compositions of forest floor (full bars and *Sphagnum* (open bars) „grab" samples along the E-W transect
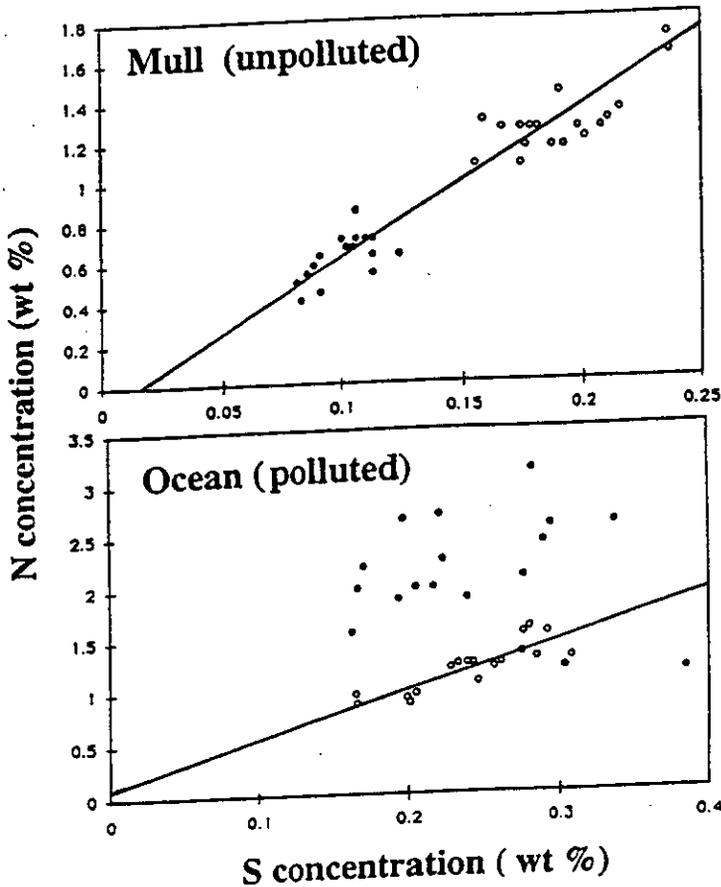

Figure 3: Relationship between S and N concentration in forest floor (open circles) and *Shpgnum* (full circles)

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Table 1   Summary of nitrogen species and sulfur mass fluxes.Units: $kg.ha^{-1}.yr^{-1}$

	Bulk	Throughfall	Runoff	Balance*
Salacova Lhota 1976-1982				
N-NH$_4$	4.9		0	
N-NO$_3$	3.7		0.58	
N-Tot.	8.6		0.58	8.02
S	10.5	24.2	9	15.2
Salacova Lhota 1994				
N-NH$_4$	5.61	8.24	0	
N-NO$_3$	3.09	6.63	0.26	
N-Tot.	8.7	14.87	0.26	8.44
S	6.44	21.32	6.11	15.21
Salacova Lhota 1995				
N-NH$_4$	6.63	7.55	0	
N-NO$_3$	4.22	5.67	0.45	
N-Tot.	10.85	13.22	0.45	10.4
S	7.61	25.87	9.54	16.33
Jezeri 1976-1982				
N-NH$_4$	7.5		0	
N-NO$_3$	5.5		12	
N-Tot.	13		12	1
S	19.6	108.4	96	12.4
Jezeri 1994				
N-NH$_4$	9.41	13.06	0.04	
N-NO$_3$	6.35	12.4	4.39	
N-Tot.	15.76	25.46	4.43	11.33
S	20.82	66.72	63.66	3.06
Jezeri 1995				
N-NH$_4$	10.1	8.95	0	
N-NO$_3$	6.9	12.4	5.3	
N-Tot.	17	21.35	5.3	11.7
S	22.5	62.72	86.2	-23.48

* Balance is calculated using bulk for N and using throughfall for S

Table 1: Summary of nitrogen species and sulfur mass fluxes. Units: $kg.ha^{-1}.yr^{-1}$

been 21.3-25.9 kg.ha^{-1}.yr^{-1} in Salacova Lhota and it has been 66.7-62.7 kg.ha^{-1}.yr^{-1} in Jezeri. This indicates that while the acidification of soils at the Czech/German boarder has substantially decreased (by 40 % since the period 1976-1982), such atmospheric pollution has not significantly changed in the interior of the Czech Republic.

The atmospheric input of nitrogen has not changed significantly in the Salacova Lhota catchment while it has increased from 13 kg ha^{-1} yr^{-1} in the period 1976-1982 to 15.8-17 kg.ha^{-1} yr^{-1} in the period 1994-95. The decrease in the atmospheric input of sulfur and the increase in the atmospheric input of nitrogen have a profound influence on the health status of spruce forest. While *Picea abies* died back in the period 1976-1982, it is now growing and fixing nitrogen species. The accumulation of ammonium and nitrate nitrogen in the biomass plus the output of N_2 and N_2O due to denitrification have increasedfrom 1.0 kg ha^{-1} yr^{-1} in 1976-1982 to 11.3/11.7 in 1994/95. The mass balance indicates that the internal fluxes of nitrogen, which are being evaluated using the stable isotope data, correspond to a period of *Picea abies* recovery due to decrease in acidic pollution by SO_2.

(3) Discussion of natural fluxes of ammonium and nitrate using ^{15}N

At the spruce stand Nacetin stable isotope composition of various N pools/fluxes ($\delta^{15}N$) was monitored throughout 1995. Both nitrate and ammonium $\delta^{15}N$ were measured in bulk precipitation and throughfall on a monthly basis, soil water in plots dominated by *Callamagrostis vilosa* and *Descampsia flexuosa* was sampled by suction lysimeters 90 cm below surface (12 months, 4 replicas each), soil N pools (total N, nitrate and ammonium) were analyzed at 5 sites at 3 depth intervals (5, 15 and 40 cm), and $\delta^{15}N$ of total nitrogen was measured in class-1 spruce needles. The objectives of the study were (1) to take an inventory of nitrogen isotope signatures in the ecosystem, and (2) to trace dispersion pathways of atmogenic nitrogen in the soil profile by calculating unmeasured isotope fluxes.

Fig. 4 shows $NO_3^- -\delta^{15}N$ ratios of the input (bulk and throughfall precipitation) and output (soil water leaving the soil profile at 90 cm depth). Although there appears to be an indistinct common annual minimum in spring (April - May), clearly these data cannot be used to distinquish output nitrate from input nitrate or to evaluate the response of soil solution to input pulses in time. This contrasts to the behaviour of sulfur at the same site, which shows distinct isotope signatures of individual sulfate fluxes (Fig. 4, Novak et al. 1996). Relationships between concentration of nitrate and ammonium and their $\delta^{15}N$ ratios in the inputs are plotted in Fig. 5. For both bulk and throughfall precipitation there was a slight negative correlation which may indicate mixing between two isotopically different nitrogen sources.

The plot of C/N mass ratio vs. $\delta^{15}N$ of total soil nitrogen (Fig. 6) exhibits an opposite trend to a number of less polluted sites (Johnson, Lidberg 1992): from topsoil to mineral soil C/N ratio
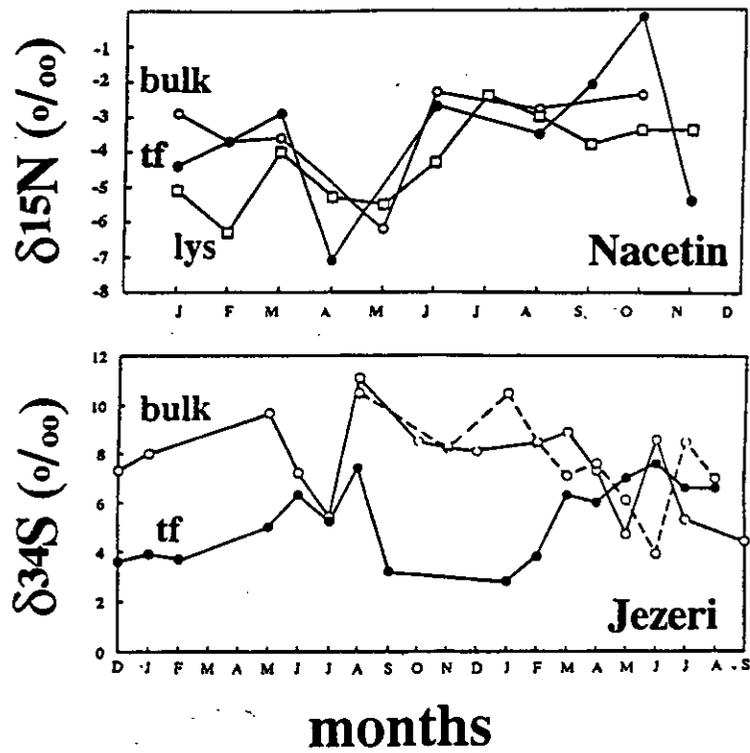
Figure 4: N and S isotope signatures in the Northern Czech Republic: Bulk - open area deposition, tf - spruce throughfall, lys - lysimeter water
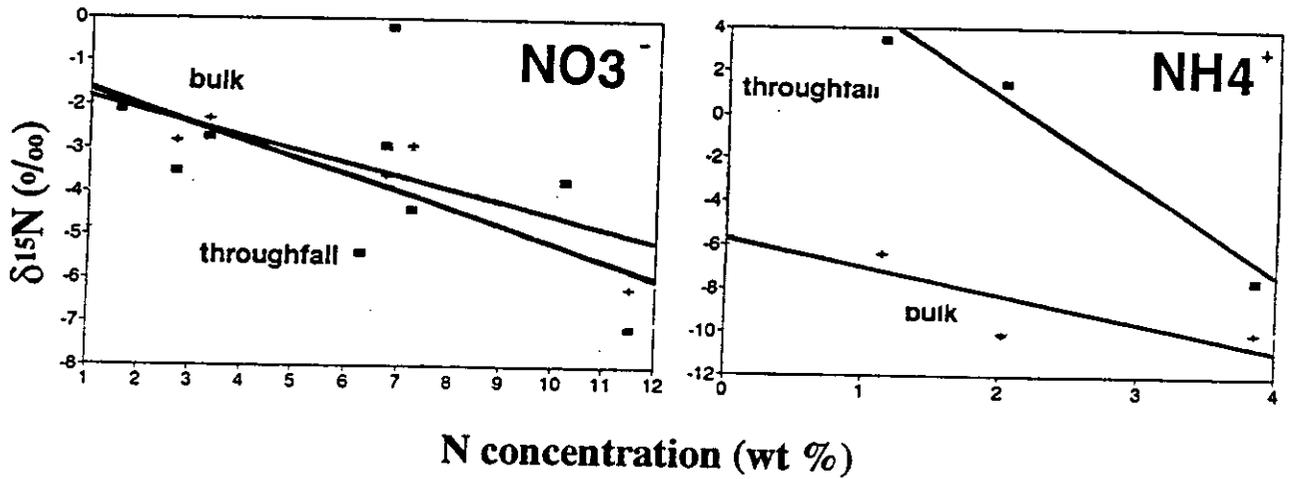

Figure 5. N concentration vx. $\delta^{15}N$ plots in inputs.

Table 2 Nitrogen isotope monitoring at Nacetin

Soils Depth (cm)	N content (w%)	C content (w%)	C/N	δ15N Ntot (‰)	N mineralized N-NH4 (mgN/100g of soil)	δ15N-NH4 (‰)	N-NO3 (mgN/100g soil)	δ15N-NO3 (‰)	calculated δ15N-Nmin (‰)	Lyzimeter cN-NO3 (mgN/L)	δ15N-NO3 (‰)
5	1.2	18.74	15.74	-1.5	1.95	3.2	0.9	1.8	2.8		
15	0.21	5.3	25.81	2.5	0.92	0.8	1.56	-0.8	-0.2		
40	0.09	2.26	24.75	5.5	0.64	-5.6	0.66	-1.8	-3.7		
90										6.7	-4.2

increases with increasing $\delta^{15}N$. While an increase in $\delta^{15}N_{tot}$ with increasing depth was reported at variety of other sites, the C/N increase is rather unusual and merits further study. No ammonium was detected in the soil solutions.

Vertical trends in $\delta^{15}N$ of total soil nitrogen, KCl-extracted nitrate and ammonium are given in Fig. 7. Both inorganic N species become progressively lighter with increasing depth. The vertical isotope shifts are a result of a combination of fractionation processes and a seepage effect and will be used to estimate isotope mass balances in the following paragraphs.

The discussion of internal N fluxes is based on soil composition and available mineralized nitrogen in the soil profile at Nacetin (Table 2). Mineralized nitrogen pool (exchangeable ammonium and nitrate ions $N_{min} = N_{NH4} + N_{NO3}$), is of primary importance for overall nitrogen uptake and N leaching to groundwater. Following the changes in concentration N and nitrogen isotope composition $\delta^{15}N$ of N_{min}, N_{NH4} and N_{NO3} in soil profile, processes that are typical for each horizon are specified.

The 1st horizon, 0-5 cm

The first horizon has unusually low C/N ratio and positive $\delta^{15}N_{min}$ which is far from the input values (with $\delta^{15}N$ from -10 to -2 $°/°°$) and organic (total) nitrogen in soil ($\delta^{15}N = -1.8°/°°$). Processes responsible for low C/N ratio - high mobilization of C and N and high N input - are both likely to occur at Nacetin. Most of the mineralized nitrogen is in the form of ammonium ions. As it is unprobable that ammonia volatilizes in such acidic environment (pH of soil varies from 3.9 to 4.5) with the exception of very dry periods, most of the ammonium ions are transferred with infiltrating water to lower horizons and isotopically fractionate during cation-exchange process to yield more positive $\delta^{15}N\text{-}NH_4$ in the upper horizon. The $\delta^{15}N$ difference between ammonium and nitrate corresponds to small extent of nitrification ($D = \delta^{15}N_{NO3} - \delta^{15}N_{NH4} = -1.8°/°°$).

The 2nd horizon, 5-15 cm

C/N ratio in the second horizon is higher than in the upper horizon and corresponds to a value common in forest soils. $\delta^{15}N$ of N_{min} ($-0.2°/°°$) is too high (i.e., less negative) for an N uptake source (which is $-4 °/°°$). Overall, N_{min} decreases in comparison with the upper horizon. Soil contains more nitrates, however, their increase does not correspond to ammonium decrease as would be expected in the case of a simple nitrification reaction. That means that part of ammonium is consumed by tree roots. Apparent fractionation between ammonium and nitrate is the same as in the first horizon ($D = -1.8°/°°$) corresponding to low extent of nitrification.

The 3rd horizon, 15-40 cm

There is no difference in C/N ratio between the second and third horizon, which has only half of its C and N content compared to the second horizon. Similarly, N_{min} is just about 50% of the second
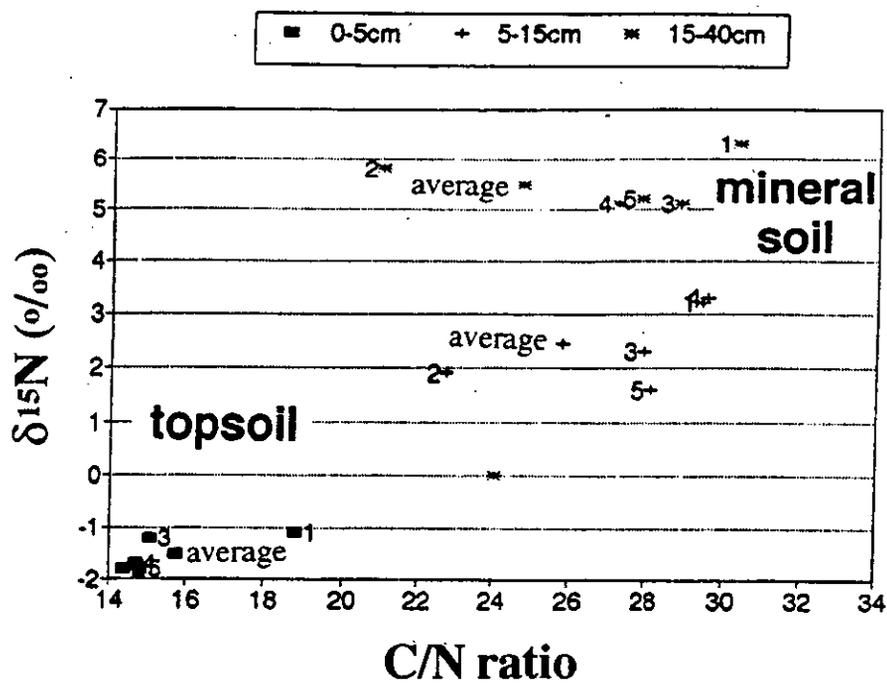
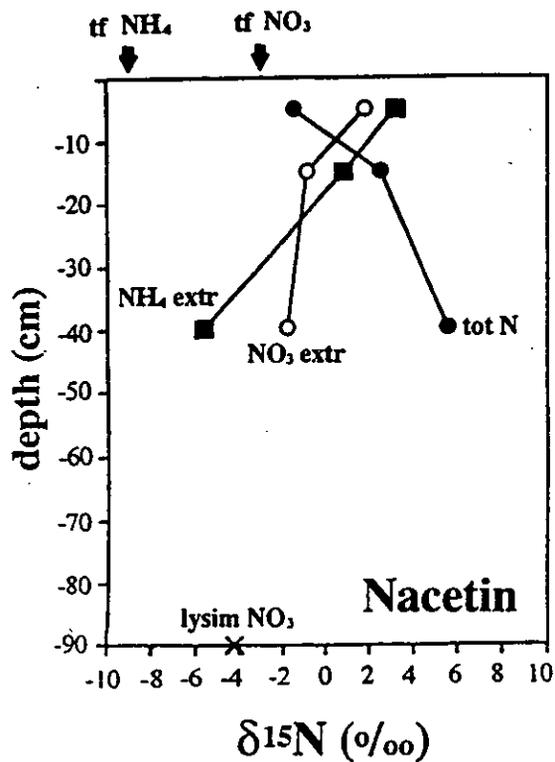Figure 6. C/N vs. $d^{15}N$ plot for three soil horizons.

Figure 7. Nitrogen isotope sinals in the vertical soil profile. tf - spruce throughfall, extr - KCl extracts, lysim - lysimeter water.

horizon. $\delta^{15}N$ of mineralized N (-3.7°/∞) is very close to average forest values. It corresponds to the amount of consumed N by vegetation. Ammonium and nitrate pools are approximately of the same size with apparent fractionation (D = 3.8°/∞) possibly corresponding to denitrification.

The 4th horizon at 90 cm

Nitrate is the only nitrogen pool in soil water at this depth. There are no detectable ammonium ions. The average $\delta^{15}N$ of nitrate (-4.2°/∞) corresponds to the consumption of the whole ammonium pool by the nitrication.

Organic nitrogen

The depth profile of organic nitrogen generally corresponds to forest soil at this latitude in content as well as in $\delta^{15}N$. Forest floor has a lower organic nitrogen content than similar soils at the German plots. This may be related to a higher mobility of extractable organic matter in the upper horizons.

Ammonium transport

Mobilized organic nitrogen and CEC (cation exchange capacity) decrease with increasing depth stimulate nitrogen transfer to the ammonium pool which is efficiently fractioned by ion-exchange during infiltration through the unsaturated zone. Positive values of $\delta^{15}N$ of exchangeable ammonium in comparison with the nitrogen input indicate effective leaching of "light ammonium N" by infiltrating water.

Nitrification

The ammonium ions are readily available in the upper part of soil profile. Therefore, they cannot limit the nitrate production. Consequently, the low apparent fractionation between ammonium and nitrate (D = - 1.8°/∞) may result from a low internal production of nitrate in comparison with the input and output of additional nitrogen within this open system. Changes in ammonium and nitrate contents can be explained by preferential uptake of ammonium in a shallow horizon and nitrate in deeper horizons. Remaining ammonium ions are completely nitrified at the depth of 90 cm (lysimeter level).

Denitrification

The only evidence on denitrification in the soil profiles at Nacetin is the change of fractionation between ammonium and nitrate ions in the third horizon (D changes from -1.8 to 3.8°/∞). Denitrifiction products (N_2O) measured at Jezeri, one of the NIPHYS sites (Kjöller, this volume) does not exceed 0.2-1 kg N ha^{-1} yr^{-1}. Availability of DOC (dissolved organic carbon) probably controls the extent of the denitrification reaction. DOC was not detected in the lysimeter soil water.

(4) Quantification of the transition of atmospheric water to soil and groundwater

Seepage patterns were monitored using $\delta^{18}O$. For this study we selected the forested Lysina catchment which is situated in the heavily polluted Northern Czech Republic west of Nacetin. The streamwater draining the catchment was characterized by extremely high concentrations of total dissolved Al (volume weighed mean of 66 mmol L^{-1}). There was a strong positive correlation between the stream acidity and stream discharge. Temporal variation in $\delta^{18}O$ for precipitation, soil water and streamwater is shown in Fig. 8. $\delta^{18}O$ in soilwater and precipitation followed similar trends, their maxima and minima coincided. Imortantly, streamwater was characterized by an extremely low variability in $\delta^{18}O$. This indicates that the direct component of precipitation in discharge water was very low. Therefore a three-component model of runoff generation was developed using the $\delta^{18}O$ time series and an apparent anion deficit (Buzek et al. 1995). In this model the indirect components in runoff is represented by soilwater and groundwater. The apparent anion deficit in runoff was attributed to leaching of organic acids from the upper soil horizons. Fig. 9 quantifies the soil water contribution to total runoff based on the apparent anion deficit in streamwater.

In the model, the direct surface, or overland, component had a residence time on the scale of hours. Its contribution to streamwater runoff was low, averaging 4 %. During the greatest flood event this component made up 20 % of the runoff. The contribution of the indirect component derived from shallow soil water averaged 40 %. During the flood this component dominated. The third component originated in the groundwater reservoir. This component contributed on average 55 % of the runoff. Its residence time was about one year.

(5) Meso-scale modelling of the biogeochemical cycle in the soil under the influence of varying inputs of sulfur and nitrogen oxides

We modelled the behaviour of pollutant sulfur in a soil profile from the NIPHYS study site Jezeri using isotope mass balance equations. The data were derived from a laboratory soil column experiment in which isotopically extremely heavy sulfate S was used in the wetting solution. Our experiment complemented similar incubation experiments with ^{15}N carried out at BITOK, Bayreuth also as part of NIPHYS. The question we specifically asked was whether revolatilization of the incoming sulfur can be detected in such experiments. We were also interested in the isotope signature of the biogenic reemissions of S with respect to the isotope compositions of the remaining soil S.

A total of 25 soil columns (horizons 0+A, AE and Bvs; 6.5 cm in diameter, 30 cm deep) were incubated under two temperature regimes for 0, 13 and 18 weeks. "Summer" temperatures were represented by 26° C at daytime and 17 °C at night, "winter" temperatures by 3°C. There were 5 replicas for each treatment. The surface of the soil was wetted three times a week (90 mm per month) and eluent was collected at the 30 cm depth. Natural throughfall precipitation (TOC = 13
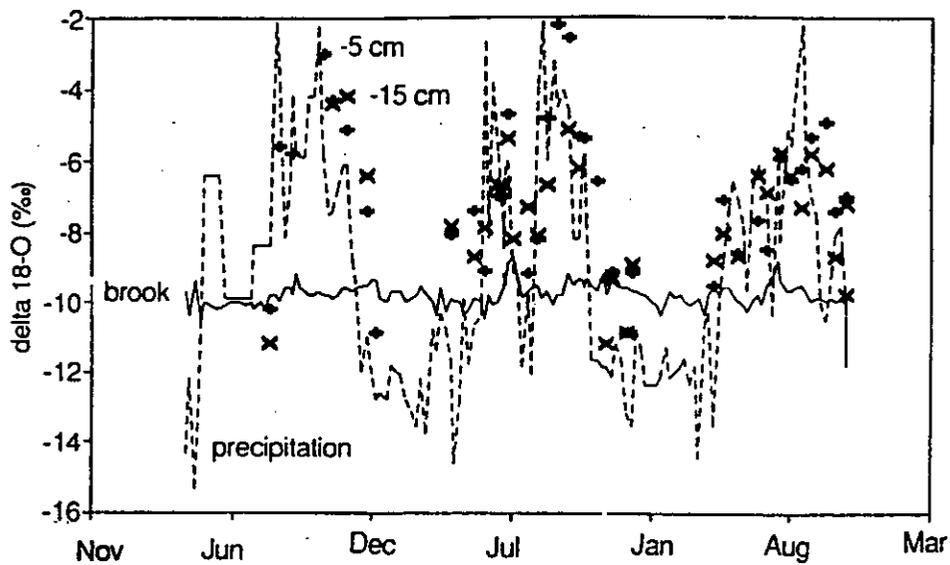
Figure 8. δ^{18}O seasonality at Lysina. solid line - streamwater discharge, dashed line - preicipitation, + - soil water at 5 cm depth, x - soil water at 15 cm depth.
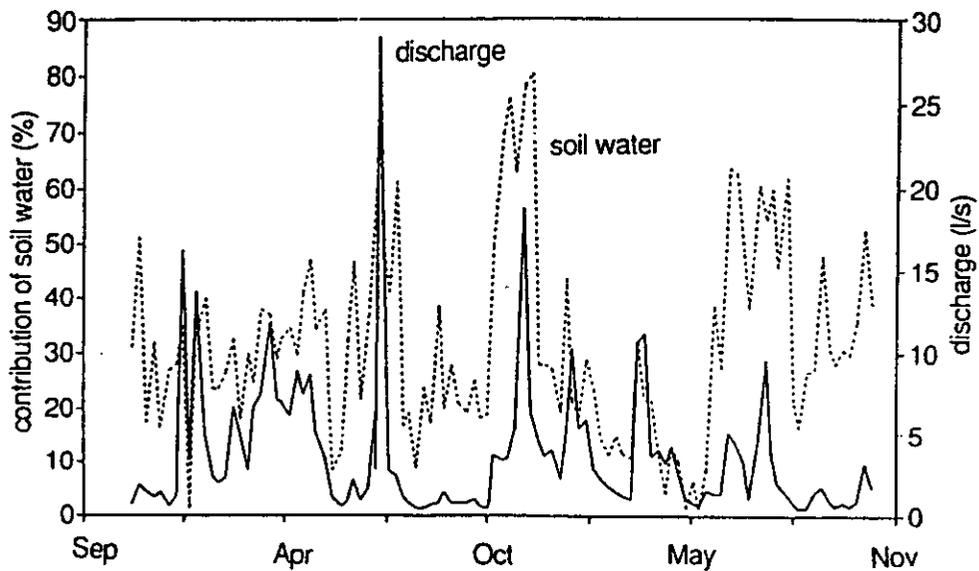

Figure 9. Contribution of soil water to streamwater discharge from the Lysina catchment.

mg l^{-1}) was artificially enriched in the heavier sulfur isotope ($\delta^{34}S$ = +33.4‰). The initial $\delta^{34}S$ ratios in the soil were between +1 and +5‰. The concentration of sulfate in the wetting solution was 106 mg l^{-1}.

Table 3 gives mean $\delta^{34}S$ ratios in the soil and eluents at the end of each treatment. During the experiment $\delta^{34}S$ increased with time and increasing depth. $\delta^{34}S$ of the eluent were higher (i.e., closer to the isotopically heavy S of the wetting solution) at the lower temperature. Consequently, the retention of sulfur decreased with decreasing temperature.

Table 3 Soil column incubations using isotopically extremely heavy S in the
wetting solution. $\delta^{34}S$ ratios in per mil CDT.

	26/17 °C			3 °C		
	0 weeks	13 weeks	18 weeks	0 weeks	13 weeks	18 weeks
0+A	1.6	4.5	4.8	1.6	3.8	2.9
AE	5.1	7.3	6.9	5.1	8.3	6.5
Bvs	5.2	8.4	11.7	5.2	11.8	12.7
Eluent	-	4.3	7.8	-	11.5	15.0

For each treatment an isotope mass balance was constructed using the equation:

$$\delta_{rain}.m_{rain} + \delta_A.m_A + \delta_B.m_B + \delta_C.m_C = \delta A^*.m_A^* + \delta B^*.m_B^* + \delta C^*.m_C^* + d_{EL}.m_{EL}, \quad (1)$$

where δ denotes $\delta^{34}S$ in ‰, m number of moles of a given S pool, the rain subscripts denote the wetting solution, A, B, C are the individual soil horizons 0+A, AE and Bvs, respectively, and EL is eluent at the 30 cm depth (Novak, Prechova, in prep.). The number of moles can be substituted with the total amount of S in the particular soil horizon in mg. The left side of the equation represents the initial state, i.e., prior to the experiment, the right side the final state after the experiment has been completed. Values measured at the end of the experiment are marked with an asterisk. When measured values are used in the equation, it turns out that the sum on the left side becomes systematically higher compared to the right side. To preserve the balance, on the right side an unnknown member must be added. This member is a product of $\delta^{34}S$ and mass of the revolatilized sulfur x:

$$\delta_{rain}.m_{rain} + \delta_A.m_A + \delta_B.m_B + \delta_C.m_C =$$
$$\delta A^*.m_A^* + \delta B^*.m_B^* + \delta C^*.m_C^* + \delta_{EL}.m_E + \delta_X.m_X \quad (2)$$

Mass of the escaping S was calculated from a deficit of the measured S masses in the individual S pools at the end of the experiment compared to S masses at the beginning.

Mass of the escaping S was calculated from a deficit of the measured S masses in the individual S pools at the end of the experiment compared to S masses at the beginning.

$$\Sigma\, m_{init} = \Sigma\, m_{end} + mX.$$

(3)

The mass deficit was larger for the "summer" temperatures. Substituting mX values from eq. (3) into eq. (2) we calculated S isotope signature of the biogenic reemission from the soil. δX for the 13-week experiment was +16.0 °/oo and dX for the 18-week experiment δX was +15.9 °/oo (26/17° C regime). In summary, our data indicated (i) that sulfur reemisson from forest soils is isotopically lighter than S of the atmospheric input (+16 vs +33 °/oo), and (ii) that with an increasing temperature the retention of S in the soil column increases. This contrasts with the behaviour of nitrogen, whose retention decreases with increasing temperature (see the BITOK data, this report).

(6) The capacity to test NIPHYS hypotheses with independent data sets

Our research deals with an ecosystem which has developed under environmental conditions of the Communist Central Europe. The ecosystems in this region were substantially damaged by industrial emissions of SO_2. Since 1989, the political and related economic changes in the Czech Republic and the neighboring eastern lands of Germany curbed on this type of pollution by 40% in the studied border region represented by the catchment Jezeri. Thus, the evaluation of the external and internal fluxes of nitrogen yields data which represent a different environmental development with respect to catchments and plots investigated by other participants of NIPHYS program. These differences may be caused by different methodological approaches. To better understand the influence of such methodological differences on the obtained data, we have studied the catchment Salacova Lhote in the least polluted central part of the Czech Republic. The ecological conditions of the catchment are comparable to the conditions in Germany and southern Sweden as follows from the assesment of the mass balance. At present, the internal fluxes using stable isotopes of nitrogen and sulfur have not been evaluated in this catchment. Therefore, we wish to continue the isotopic study of the Salacova Lhota catchment during the next research project.

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