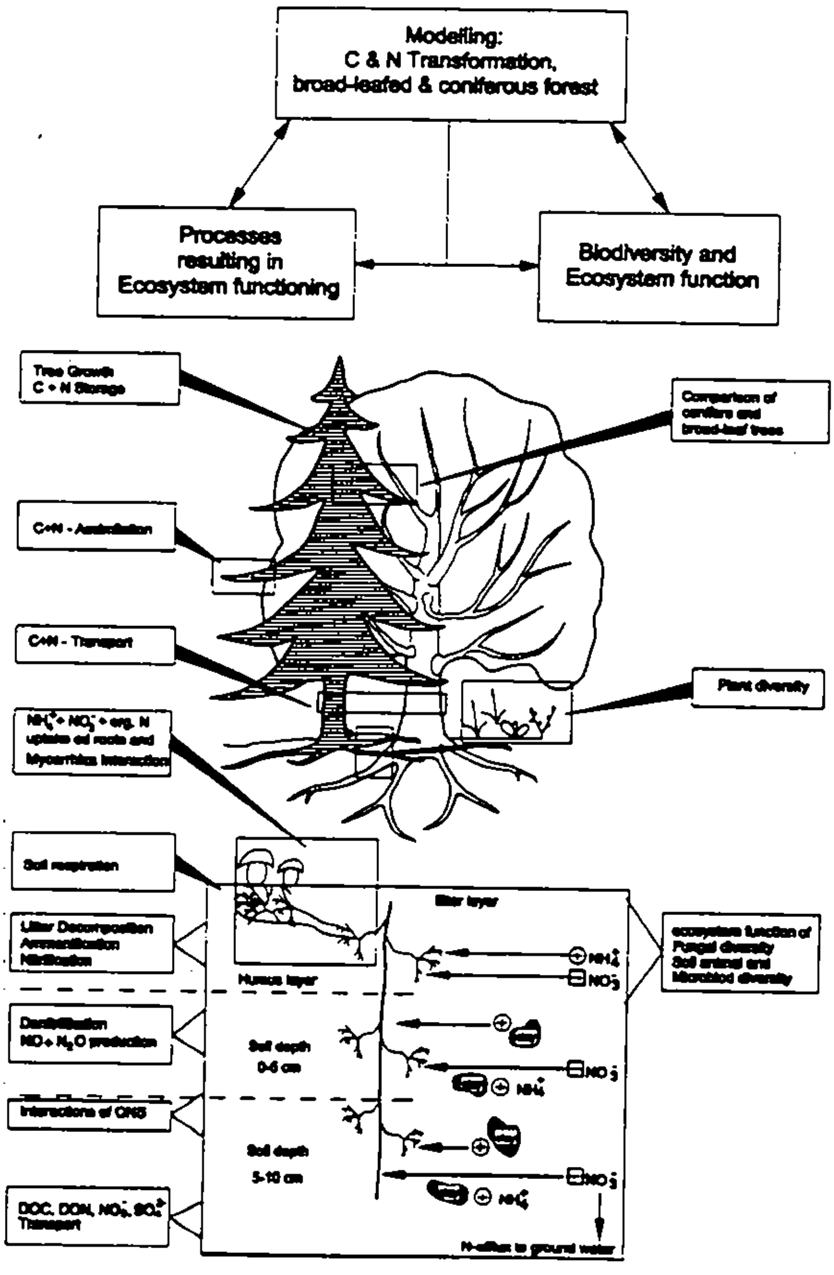
CANIF- Carbon and Nitrogen Cycling in Forest Ecosystems

Annual and Final Report

01.02.1998 to 31.01.1999

EEC contract No ENV4-CT95-0053
and
subcontract No ERB IC20 CT960024

E.-D. Schulze (coordinator)



Bayreuth/Jena, Germany
March 1999

1. **Contract No**: ENV4-CT95-005 and subcontract No ERB IC20 CT960024

2. **Title**: CANIF – Carbon and Nitrogen Cycling in Forest ecosystems

3. **Reporting period**: 01.02.1998 to 31.01.1999

4. **Scientific coordinator**: E.-D. Schulze

5. **Project participants**:
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   4. UH (DE) George: University of Hohenheim, Germany
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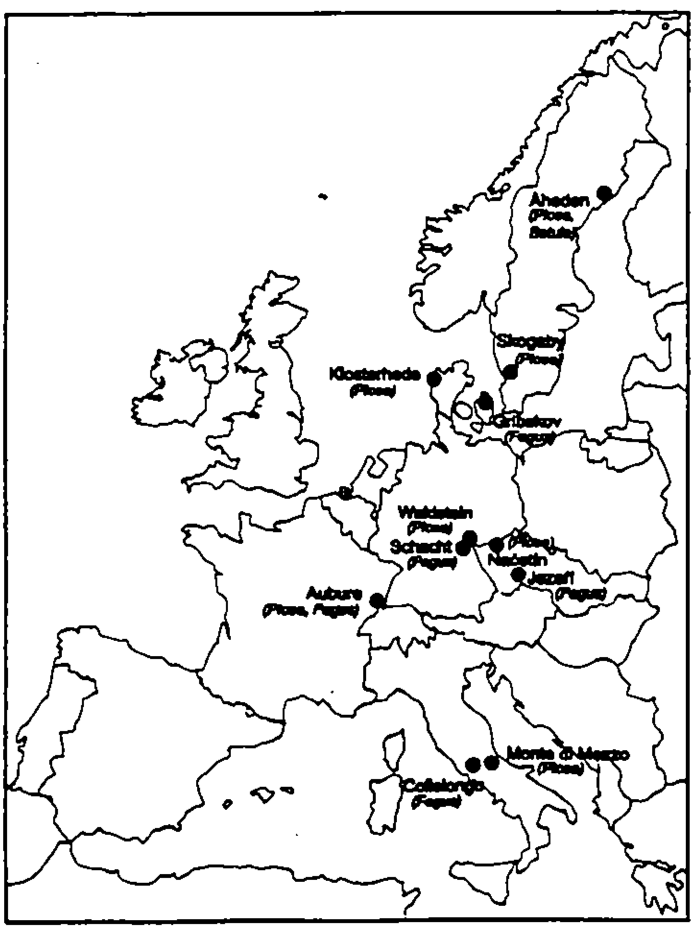
   11. DI (DK) Andersen: Danish Forest and Landscape Institute, Denmark
   12. WU (NL) Beredse: Wageningen Agricultural University, Netherlands
   13. GS (CZ) Paces: Czech Geological Survey, Czech Republic



SUMMARY PROGRESS REPORT OF THE PERIOD

I. General Objectives (from technical Annex)

CANIF investigates effects of climate, and soil-borne and deposited nitrogen on C- and N-assimilation and turnover as well as on forest organisms along a climatic transect through Europe extending from North Sweden to central Italy with following objectives:

(1) to contribute to the European and global research programs envisaged by the EEC Environment and Climate Work Program (Framework IV, 1994 – 1998) and its Terrestrial Ecosystem Research Initiative (TERI-CA), and by the IGBP-GCTE (Global Change and Terrestrial Ecosystems) – core project, which aim at

- to better understand mechanisms driving pools and fluxes of carbon, water, nitrogen, and other nutrients in forest ecosystems (TERI 3.2)
- to extrapolate this understanding to scenarios of land-use and climate change (TERI 3.2)
- to identify ways in which biological diversity regulates ecosystem function, structure and dynamics (TERI 4)

(2) to promote scientific co-operation, education and training and technology transfer within the EC by linking 12 European and 1 East European research institute via a single major multidisciplinary project.

II. Specific Objectives for the reporting period (from technical Annex)

(1) To quantify the abilities of species of different trophic levels (plants, fungi, soil animals, microbes) to use and to store different forms of C and N, and to investigate the efficiency of these assimilation processes and their contribution to open and close biogeochemical cycles (ecosystem function of biodiversity)

(2) To integrate and to model ecosystem fluxes of carbon and nitrogen, and to make predictions on the effect of atmospheric deposition, of climate and land-use changes on biogeochemical cycles in forests.

III. SPECIFIC OBJECTIVES FOR THE NEXT REPORTING PERIOD (from technical Annex)

No longer relevant

IV. MAIN ACTIVITIES UNDERTAKEN: METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS (use other sheets as necessary but preferably do not exceed 2 pages)

Main Activities: Project meetings

Thurnau:	September 1 to 4 1998: Annual project meeting
Copenhagen:	February 22, 1999: Summary of results
Frankfurt:	March 6, 1999: Summary of results

Methodology:

- CANIF operates on a N/S transect. Each participant is specialized to study certain specific ecosystem processes using its own methodology of investigation. Each group carries out comparative investigations at all sites along the transect
- In 1998 main emphasis was given to finalize the ^{15}N labeling studies, the root investigations, the analytic measurements, and to process and integrate the data.

Results:

The detailed results of the year 1998 are listed by each participant. In the following only some major findings are highlighted, a final synthesis is in preparation:

- ^{15}N-labeling indicates that a major sink for nitrogen in spruce stands is the humus layer (90%) and not the trees (10%), however the allocation depends on species. In the broadleaved beech stands, 70% of the label are found in the trees and only 30% in the humus layer. This result has consequences for the post-Kyoto discussion.
- ^{14}C-bomb measurements indicate that there are increases in residence time of organic carbon leading to carbon accumulation in soil at sites of high N deposition. This result is important in view of the Kyoto protocol and the sequestration of atmospheric CO_2.
- A reviewing of the processes of canopy uptake of atmospheric pollutant N, there is an indication suggesting that 15 to 20% of the N-requirement of trees enters the canopy directly, bypassing the root uptake. This N is metabolically incorporated and promotes tree growth. The increase in forest growth of conifers, as it appears with N-deposition, seems to be related to canopy uptake of nitrogen through stomata and through bark. Thus, generalizations about forest growth should be made in conjunction with measurements of canopy uptake of N.

- Trees and mycorrhizae are able to utilize a broad spectrum of N sources such as aminoacids, ammonium and nitrate, but the conditions vary along the transect. While mineralization is low in the North, and nitrifyers are missing, the tree cover utilizes organic nitrogen via mycorrhizal activity as main N-resource. In central Europe, mineralization on acid soils under spruce leads to surplus ammonium which becomes the main N-source for the tree cover, while under Fagus as well as on calcareous soils in Italy, nitrate is the main form of inorganic N and the source for tree growth. Thus, along the transect there is a change of the N-supply and N-use that depends on tree species and soil pH. While mycorrhizae.of Fagus can cope with eutrophication, Picea mycorrhizae suffer from high N supply. There are several important interactions between soil fauna and mycorrhizae. Apparently a number of soil animals live from grazing mycorrhizae. N-mineralization of soil animals is characterized by a change of species that maintain N mineralization independent of N-supply and climate.
- Soil respiration shows higher variability on a local than on a transect scale, which in turn points at the importance of soil biota.
- Denitrification activity is low. N_2O emission was varying according to soil factors often corresponding to less than 1 kg N ha^{-1} yr^{-1}. Higher values were only found in Aubure (3 kg N ha^{-1} $6month^{-1}$). Events such as freeze-thaw cycles may determine the total loss of N_2O.
- The beech litter decomposition rate along the transect was highest in the northern oceanic site while the rate decreased through central Europe to the Italian site. The duration of cold and dry periods seemed to be the most important limiting factor.
- The communities of microfungi was less distinctive than the higher fungal communities. The enzyme patterns support the decomposition difference in showing highest cellulolytic activity in litter decomposition in Denmark and decreasing at the sites situated at higher altitudes from Germany to Italy.
- Biodiversity appears to follow, but also drives the N-cycle according to the chemical conditions of the soil. Thus we find mycorrhizae species that only use organic N in the North, and these are replaced by species that use ammonium in central Europe, or nitrate in the South. On the other hand it remains uncertain, if trees could persist without mycorrhizae in eutrophic conditions.
- The biogeochemistry of natural stable isotopes at the catchment level indicates that sulfur is the main acidifying agent. An isotope mass balance indicates that S is stored

largely in organic form, which points in turn at the importance of the organic layer in soils.

- Dissolved organic carbon may be a significant fraction of the ecosystem net C-exchange (>10% at some sites). The same is true for organically bound N in soil solution which cannot be neglected in the N-cycle.
- The joint data processing resulted in a complete data set for the major ecosystem processes. These data were the basis for developing a process model of the biogeochemical cycles. The model has been successfully tested, and it is ready for model predictions. All data are summarized on a common data-file on CD ROM.

- **Discussion:**
- The project participants are presently summarizing and integrating the results of individual projects, and an integrated analysis of the data will be performed by May 1999.
- To describe the link between biodiversity and ecosystem functions remains a major challenge. It appears that biodiversity follows and drives the biogeochemical cycle, maybe in a way to maintain a number of key processes in fact fairly constant. It remains a task for the whole system analysis of this dataset and of the modeling project to identify those processes that were changing as a result of maintaining others constant.
- Although the results generate clear relations of C/N interactions, we are aware that the process relations are based on a limited number of study sites that werwe selected to represent acid conditions. It will be necessary in future to test these generalizations on a broader range of habitats.

Conclusions:

- The study of isotopes and mass balances together with the study of biodiversity gave insight into the mechanisms of N and C turnover as well as of C/N interactions. This becomes especially obvious in the tracer studies of ^{15}N, and the quantification of turnover rates of C and N by ^{14}C.
- At this point we can describe the interaction between ecosystem fluxes and biodiversity and we can conclude that species are important to explain certain ecosystem functions, however, we are not able to say if biodiversity follows certain physico-chemical ecosystem parameters, or if diversity determines this environment. Most likely, both components are important, but the interactive nature remains unclear.

- It becomes apparent that some of the relations that were established along the CANIF transect need to be confirmed for a broader range of habitats
- The selected sites for CANIF were mainly old stands that were in equilibrium with site conditions. It remains unclear how forest management interferes with these processes.

V. JOINT PUBLICATIONS

The CANIF participants decided to publish the results as a book, in which lead authors cover comparative aspects along the whole transect. The present outline of the book lists lead authors, co-authors will be added while writing.

Carbon and nitrogen cycling in European forest ecosystems (Schulze ed.)
Springer Verlag, Ecological Studies Series

A. Introduction

1. Introduction to CANIF — Schulze
    - Conceptual model
    - Hypotheses/Questions to be asked
    - Experimental design (transect vs. network)
    - Interaction/objectives with other EEC projects

B. Experimental conditions

2. Experimental sites in the NIPHYS/CANIF project — Persson/Van Oene
2.1 Site description
2.2 Soil characteristics
2.3 Ecosystem C and N pools
2.4 Database
    - Tables on
        - climate
        - soils
        - stand characteristics (age, d1.3, h, n)
        - vegetation
        - deposition
        - site history
        - COLOR plate for each site

2.5 Conclusions

C. Plant related Processes

3. Tree biomass and growth — Scarascia-Mugnozza
3.1 Introduction
3.2 Methodologies
3.3 Growth (NPP and N)
    - Aboveground
    - Roots

Herblayer
3.4 Biomass (C and N)
aboveground
belowground
Herblayer
litterfall
Allometric relations
3.5 Relations to habitat conditions
climate
nutrient
soil
forest management
3.6 Conclusions

4. Carbon and nitrogen cycling in forest trees — Bauer
4.1 Introduction
4.2 Methods
4.3 Nutrient concentrations
(N, K, Mg, Ca, P, S)
(trees and understory)
4.4 Nutrient contents
4.5 Nutrient partitioning
4.6 Conclusions

5. Fine Root rowth — Stober/George
5.1 Introduction
5.2 Methods
5.3 Spatial variation in fine roots
range of estimates by diff. methods
transect, soil depth, distance to tree
5.4 Temporal variation
5.5 Site conditions that affect root growth
5.6 Conclusions

6. Root nutrient uptake processes — Read/Hoegberg
6.1 Introduction
Hypotheses
6.2 Approaches to testing the hypotheses
6.3 Non-Mycorrhizal roots
NH4/NO3 uptake
Nitrate reductase activity
downregulation with supply
6.4 Mycorrhizal roots
NH4/NO3/organicN uptake
6.5 N-uptake under field conditions
6.6 Conclusions

7. Fate of 15N applications — Gebauer
7.1 Introduction (Hypotheses)
Mineral N from soil is dominant source
Forest herb layer interacts significantly

COLOR PLATE of organisms
17.3 Effect of environmental variables on soil invertebrates
correlation analysis
abiotic parameters
microbiological parameters
17.4 Contribution of decomposers on C and n turnover
foodweb structure
food biomass
feeding rates
17.5 Synthesis and Conclusions
Effects of environment ON diversity
Effect of diversity on flux (???)

18. Diversity and role of microorganisms — Struwe
18.1 Introduction
18.2 Diversity of saprophytic fungal populations
18.3 Fungal enzymes
18.4 BIOLOG analysis

F. Integration

19. Long-term trends in mass balance of N and C in Central European forested catchments — Dambrine
19.1 Introduction
19.2 Czech catchments
19.3 Strengbach catchment
19.4 Comparison of accumulation and release of N and C
19.5 Other nutrients
19.6 Conclusions

20. Model experiments — van Oene
20.1 Introduction
20.2 Model description (Refer to chapter 13)
20.3 Simulations for sites and tree species
current climate C/N balance
20.4 Scenario runs
Predictions for Picea
Predictions for Fagus
(Biodiversity, N-input/output)
20.5 Conclusions

21. Integration of N-uptake and supply processes — Schulze
21.1 plant-uptake
21.2 soil supply

VI. CHANGES IN STATUS

Not relevant

VII. Is the project on schedule?

The project is on schedule. Only minor remnants of analytical work are left which do not affect the final integration. The book summary will be finished early summer 1999.

VIII. SUMMARY OF PROGRESS ACHIEVED

The CANIF project has identified and quantified the main processes that drive the C and N cycle on acidic soils, in coniferous and deciduous forest. The data were integrated in a biogeochemical model that is capable of reproducing these processes, and which is now ready to make predictions about how conditions change, e.g. with climate or with management. Thus CANIF has established the basis for answering major questions that arise e.g. with the Kyoto protocol. However, in order to do so with certainty, the CANIF predictions should be verified on an additional set of habitats, including disturbances by forest management (logging and reforestation).

Bayreuth 26.4.99 E.-D. Schulze

PART B

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 01.02.98 to 31.01.99

Partner: 01 UB (DE) University of Bayreuth

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I. OBJECTIVES FOR THE REPORTING PERIOD

(1) Quantification of storage amino-acids and starch as measure for carbon/nitrogen imbalance at all sites
(2) Pulse labeling of spruce and beech stands with ^{15}N-ammonium and nitrate at the Bayreuth site (coordinated experiment of Bayreuth, Umeaa, Nancy and Viterbo)
(3) Determine growth rates of spruce and beech in a chronosequence and reconstruct growth history on the basis of tree rings at the Bayreuth sites.

II. OBJECTIVES FOR THE NEXT PERIOD

No longer relevant

III. Are there any problems? Is your part of the project on schedule?
There are no problems,
The fast labeling of soil humus has been carried out but the mass spectrometry measurements have not been completed due to the move from Bayreuth to Jena. Some of the results were also delayed in publication, but will be published in 1999.

IV. MAIN RESULTS OBTAINED: METHODOLOGY; RESULTS; DISCUSSION; CONCLUSIONS (use other pages as necessary but preferably no more than 2).

(1) Quantification of storage amino-acids and starch as measure for carbon/nitrogen imbalance at all sites

Methodology: leaf material was sampled at one time in summer, and during the season at the Bayreuth site, and measured for starch after enzymatic digestion and photometric determination of glucose and for amino acids by HPLC
Results: The data confirm a N-limitation at the N-Swedish site and a N surplus at the Bayreuth site. Aubure remains N-limited, maybe due to a loss in humus during raking earlier this century.

Discussion: The amino acids allow a distinction between storage N and metabolic N that is necessary for growth and maintenance. Thus the method is more appropriate than the investigation of total N. However, the seasonal course contains turnover of C and N which results from frost hardiness in autumn and from reactivation in spring. The method does not include protein storage, which can be important in conifers.

Conclusion: The data are needed in the overall integration of results, which require for modeling purpose a quantification of the potential N-requirements at all sites.

(2) Pulse labeling of spruce and beech stands with ^{15}N-ammonium and nitrate at the Bayreuth site (coordinated experiment of Bayreuth, Umeaa, Nancy and Viterbo)

Methodology: Two labeling approaches were followed:

(i) a long-term repeated pulse labeling throughout the growing season in Beech complementary to the labeling in spruce

(ii) a short term labeling in humus cores

Results: The long-term pulse labeling experiment showed that beech used considerably more of the nitrate and ammonium tracer than spruce. The total amount of label entering into the trees was less than 10% in spruce but more than 70% in beech. In spruce, the uptake followed the concentration ratio indicating a low nitrification rate in the root horizon, In contrast, in the broadleaved beech a greater proportion of the ^{15}N-ammonium deposition was taken up as nitrate due to a faster rate of nitrification. This is being confirmed by the short-term labeling experiments which shows in spruce a major fast reaction of humus with the label, and a second slower reaction with soil organisms. It remains unclear if the first fast reaction is a purely physico-chemical reaction.

Conclusion: The extrapolation from N-deposition on tree growth is neither linear nor direct. In spruce stands major compartment for N-storage is the field layer (ground vegetation) and the humus layer, and only over time (more than a year) this N may be available for growth. The situation is obviously entirely different for broadleaved stands. There are indications that the turnover time in the humus increases with N-deposition.

(3) Determine growth rates of spruce and beech in a chronosequence and reconstruct growth history on the basis of tree rings at the Bayreuth sites

Methodology: Trees biomass inventories were made on a plot level. Selected trees representing the range of diameters on a plot were harvested, dissected and tree volumes were reconstructed from tree rings. Assuming a similarity in the tree density, as measured at one time along the chronosequence to represent also the change of tree number with age, the change in stand growth was reconstructed.

Results: Beech responds similar to spruce, a major growth increment can be observed in old stands, which do not level of in NPP, but grow at a rate as young stands.

Conclusion: The interpretation of the increased growth rate is, that N-deposition resulted in increased growth. However, the mechanism is not that straight forward, considering the observation that N is immobilized in the humus layer. The high growth rate appears to result from a combination of effects. Leaf and twig growth of beech seems to be strongly dependent on canopy uptake of N via leaves and bark. This process may contribute as much as 60% to the actual N requirement of leaf growth. This results mainly in larger leaves and more litter, which in turn results in mire mineralization. Stem wood growth depends largely on N availability in the soil, which is in turn a result of mineralization as well as of throughfall-N. This observation may also explain that cation deficiencies are observed in the foliage despite high stem growth.

V. List of Publications arising from the project (include copies)

The results are documented mainly in diplom and doctoral theses. The publication in journals is behind schedule, due to my change in position from Bayreuth to Jena. However, the main results will summarized in the joint book volume (Ecological Studies). The manuscripts are under review at this stage, the book volume will go into print in May 99:

Scaracia-Mugnozza G, Bauer G (1999) Tree biomass and growth. In: Schulze (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, Springer Verlag Heidelberg (in preparation)

Bauer G, Schulze ED (1999) Tree nutrient relations. In: Schulze (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, Springer Verlag Heidelberg (in preparation)

Gebauer G, Zeller B (1999) The fate of ^{15}N tracers in beech and spruce ecosystems. . In: Schulze (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, Springer Verlag Heidelberg (in preparation)

Gebauer G, Taylor AFS (1999) ^{15}N natural abundance in fruit bodies of different functional groups of fungi in relation to substrate utilization. New Phytologist (in press)

Harrison AF, Schulze ED, Gebauer G, Bruckner G (1999) Canopy uptake and utilization of atmospheric pollutant N. . In: Schulze (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, Springer Verlag Heidelberg (in preparation)

Bauer G, Gebauer G, Harrison AF (1999) Stable isotopes and natural abundance. . In: Schulze (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies, Springer Verlag Heidelberg (in preparation)

Signature of the Partner:

Date:

28.2.99

PART B

DETAILED REPORT OF INDIVIDUAL PARTNERS

Reporting period: 980201-990131

Partner: Dept. of Forest Ecology, SUAS (now SLU), Sweden

Principal Investigator: Prof. Peter Högberg

Scientific Staff: Dr. Alf Ekblad, Dr. Reiner Giesler, Dr. Anders Ohlsson, Dr. Maud Quist, MSc. Mona Högberg

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I. OBJECTIVES FOR THE REPORTING PERIOD:

We have continued to analyse results of our N-15 pool dilution study of the natural N-supply gradient at Betsele in northern Sweden (Giesler et al. 1988). These studies will demonstrate rates of turnover of inorganic N pools, ammonium and nitrate, in soils in the field. We have also measured the microbial immobilization of N-15 labelled ammonium and nitrate added to soils along the gradient. Also, we have worked to further develop the possibility to separate root respiration from heterotrophic microbial respiration in forest soils.

II. OBJECTIVES FOR THE NEXT PERIOD:

The project will very soon be completed, as we just have to do some final analyses and writing up of the last results.

III. Are there any particular problems? Is your part of the project on schedule?

We have had a minor delay of our analyses due to some technical problems with our mass spectrometers. Nevertheless, the larger part of the analyses have been done within the period.

IV. MAIN RESULTS OBTAINED: METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS:

We have been studying how much rates of turnover of inorganic N vary in relation to other soil factors, e.g., pH and C/N ratio, in the same climatic region. These studies have been possible to perform along the unique 90-m-long natural N supply gradient at Betsele (Giesler et al. 1998). Along the gradient we have now conducted a detailed N-15 pool dilution study, comprising three major campaigns during a single vegetation period. At the same time, microbial immobilization of N-15 labelled ammonium and nitrate was measured by the chloroform fumigation technique. Results of these studies will be reported shortly. During this reporting period we have, lastly, been able to demonstrate that the N-15 natural abundance of ectomycorrhizal fungi is largely determined by fungal physiology (Högberg et al. 1999). From our model experiments on ectomycorrhizal plants one may conclude that the delta N-15 of plants reflects their N sources, which is of importance in the context of using natural N-15 abundance of plants to make assumptions about which forms of N they use.

V. LIST OF PUBLICATIONS ARISING FROM THE PROJECT AND PARTICIPATION IN CONFERENCES (during the reporting period):

Giesler R, Högberg M, Högberg P 1998 Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. *Ecology* 79: 119-137.
Högberg P, Högbom L, Schinkel H 1998 Nitrogen-related root variables of trees along an N-deposition gradient in Europe. *Tree Physiology* 18: 823-828.
Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P 1998 Boreal forest plants take up organic nitrogen. *Nature* 392: 914-916.
Högberg P, Högberg M, Quist M, Ekblad A, Näsholm T 1999 Nitrogen isotope fractionation during uptake of N by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* L. *New Phytologist*, in press.

Peter Högberg has given the following invited oral presentations:

1998 Barcelona, Spain, GCTE-LUCC Open Science Conference "Earth's Changing Land". Invited presentation: *Effects of nitrogen deposition on ecosystem functioning of temperate forests.*
1998 Jena, Germany, MPI Conference on "Global Biogeochemical Cycles". Invited presentation: *Interactions between hillslope hydrochemistry, nitrogen dynamics and plants in Fennoscandian boreal forests.*

Signature of Partner: Peter Högberg

Date 990215

Part B:

Reporting period: February 01st 1998 - January 31st 1999.
Partner: 03
DISAFRI - University of Tuscia

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I. Objectives for the reporting period:

1. Biomass production:
   - to continue to monitor tree growth by means of dendrometer at both sites, with larger sampling in the beech forest.
2. Plant nutrient budget and cycling:
   - to study nitrogen absorption as ammonium vs. nitrate in conifer and broadleaf forests (beech and spruce) by means of ^{15}N enrichment experiments, in contrasting seasons of the year;
   - to investigate nitrogen distribution in plant compartments by means of classical analytical techniques.
3. Soil respiration:
   - Soil respiration measurements will be continued at beech and spruce sites. At the beech site, in co-operation with the EC project EUROFLUX soil respiration will be estimated at a larger scale by means of eddy flux measurements (test and daily campaigns).
4. Soil processes:
   - Together with Prof. Guido Sanesi from Florence and Dr. Björn R. Andersen from DFLRI (Denmark) soil solution will be collected by means of gravity and suction-cup lysimeters. Microlysimeters will be also installed adjacent to suction lysimeters, in order to assess their performance and comparability. Soil water will be then analysed for the major chemicals and for DOC and DON. Due to their limited space requirement, microlysimeters will be installed at various location within the beech forest, in order to sample spatial variability of soil solution. Soil processes will be mainly investigated in the beech site.
5. Decomposition processes

- Together with Dr. Francesca Cotrufo leaf and twig litter decomposition will continue to be monitored. Leaf and twig samples will be collected and analysed after 1.5 and 2 year from their incubation in mesh bags (leaf litter) and nylon lines (twigs). The experiment is running in 4 different beech forests (Denmark, France, Germany, Italy) and conclusive results will be available at the end of the period. In the next year of work, second year mass loss data, coupled with data on chemical composition of the decomposing litter, will provide useful information towards the complete understanding of C and N cycling in these European beech forests.

6. Isotopes studies
   - Plant material will be analysed for carbon ($^{12}C/^{13}C$) and deuterium/hydrogen abundances (D/H). D/H values of plant material (leaves, branches and roots) will be compared to those of rain and of water extracted from different soil depth to investigate the rooting depth of the different forest tree species and to evaluate competitive interactions in water utilisation among trees of each forest stand.
     Analysis of leaf carbon isotopic ratio ($^{12}C/^{13}C$) of different tree species (beech/spruce) and, within a species, of different leaf layers in the canopy will be utilised to infer intrinsic water use efficiency.

II. Objectives for the next project

- No longer relevant.

III. Problems and deviations from plans

- Overall, within 1998, there were not major deviations from the stated workplan. Analyses of ^{15}N-enriched plant material have not yet been completed, but at the moment of writing (5 March 1999), the mass-spectrometry facility is working full time on that material. Due to the unusually dry and hot season, it was not possible to analyse Deuterium/Hydrogen abundances (D/H) of rain and water extracted from different soil depth.

IV. Main activities undertaken: methodology, results, discussion, conclusions

1. Biomass production:
   - Biomass growth was monitored by means of dendrometer bands, mounted on 4 dominant and 5 co-dominant trees at breast height in summer 1996.
   - At the beech site, stem growth of dominant trees initiated in third decade of May, when LAI development was at 80% of the maximum, but leaves were not mature. Co-dominant trees started growing 7-10 days later than dominant specimen. Compared to 1997, the growth trends of 1998 were much more variable.
   - The season was characterised by 3 distinct growth peaks between mid June and the beginning of August, with intervals of slower growth rates and a clear stop after the first week of August. The first growth rate peak was differentiated for dominant (mid June, 100 $\mu m\ day^{-1}$) and co-dominant trees (end June, 80 $\mu m\ day^{-1}$), while the other two occurred concurrently for both tree classes. The period with the highest growth rate was measured in the first decade of July, with 300 $\mu m\ day^{-1}$ and 70 $\mu m\ day^{-1}$, respectively for dominant and co-dominant trees. The stand showed a last significant peak at the beginning of August (200 $\mu m\ day^{-1}$ for dominant and 70 $\mu m\ day^{-1}$ for co-dominant trees).
   - Differently from 1997, the growth of the stand almost stopped after the first decade of August until mid September, when there was a slight recovery (30-50 $\mu m\ day^{-1}$). The reasons for the particular growth trend of 1998 are to be searched in the climatic conditions, with unusually warm temperature and drought. Nevertheless, the growth peaks

occurred slightly after rain events. Despite the differences between the 1997 and 1998 seasons, the overall percentage growth rate was similar.

- Leaf Area Index was monitored throughout the season with a Li-Cor PCA LAI-2000 and litter has been collected to determine both the amount of leaf production and to check the peak values obtained with the canopy analyser. Leaf unfolding started about one week earlier than in 1997, and canopy closure was already reached in the first half of June. Due to the warm and dry conditions, leaf senescence anticipated the closure of the growing season, and leaves were almost completely shed by mid October.

2. <u>Plant nutrient budget and cycling</u>:
   - Intensive ^{15}N-enrichment treatments have been performed to study nitrogen absorption as ammonium vs. nitrate in conifer and broadleaf forests (beech and spruce). Beech and spruce plants were treated at the beginning of the growing season. In the beech stand, a second treatment was performed in mid July, to investigate possible differences in N cycling in periods of water shortage.
   - For the spring treatment, in the beech forest, six groups of four trees, belonging to the 10, 20, 30 and 40 cm diameter classes, were selected. Of the six groups, three groups were treated with $^{15}NH_4Cl$ and three with $K^{15}NO_3$ diluted solutions. The two solutions were applied with two distinct shoulder pumps. In order to avoid contamination problems, a sufficient distance was left between treated trees. Two groups of four trees of the same diameter classes were treated with NO_3NH_4 and used as controls. The same scheme was applied in the spruce stands, with the difference that the trees belonged to the 10-15-20 and 25 cm diameter classes. In the beech stand, the summer treatments was performed again on six groups (three with $^{15}NH_4Cl$ and three with $K^{15}NO_3$) but on two diameter classes only (20 and 30 cm).
   - After the treatment, trees were sampled three times in spring (beech and spruce) and twice in summer (only beech). In any sampling date, the following compartment were sampled: leaves, 0-1 year-old branchlets, 2-5 years-old branches, soil down to 10 cm. Of all compartments, two samples per tree and per sampling dates were collected. In order to avoid possible damage to the sampled trees, stem wood at breast height was collected only at the end of the season. All the samples have been dried, grounded and prepared for isotopic analysis, that are currently performed.
   - Nitrogen and other nutrients budgets have been calculated for the beech and spruce stands. Analyses were performed by means of classical analytical techniques, such as CHN analyser, titration and spectrophotometric techniques. Material collection was performed concurrently with biomass sampling (see 1996 and 1997 reports). For the stem, branch and root material, wood and bark were analysed separately. Stem wood was analysed at 2-3 different height in the canopy. For each height, a stem cross-section was cut from which two wood pieces, one from the external half and one from the internal, were collected, dried and grounded together. The material has been analysed for nitrogen (N), phosphorous (P), calcium (Ca), potassium (K) and sulphur (S).
   - The separation of wood and bark for the stem, branch and root components allowed to assess their differences in the nutrient composition. In both species, for all the analysed nutrients (except P in beech, which content was comparable in wood and bark), bark showed a significantly higher concentration than woody material. As an example, in beech, [N] was from four (branches) to ten (stem) times larger in bark than in wood, while for spruce the values were even more important, with bark reaching 20 (branches) and 30 times the N concentration of stem. The differences were confirmed for all nutrients and for both species, pointing to the need of a careful separation of wood and bark when assessing nutrient composition of woody material. Hence, if the aim is to calculate accurate nutrient budgets, the two components should be treated separately and the ratio of bark over wood

must be determined. All the nutrient concentrations data have been included in the CAIN database.

- For the two Italian stands, the nutrient masses in kg ha^{-1} were:
  Beech: N=414.4; Ca=794.1; K=424.3; P=52.2; S=46.4
  Spruce: N=349.2; Ca=972.7; K=359.3; P=69.5; S=40.8

3. Soil respiration:
   - Measurements of soil respiration were performed also in 1998, at both sites. 10 measurement dates were performed at the beech site (Apr-Nov) and 4 in the spruce plantation (Mar-Oct).
     At the spruce site, soil CO_2 emission ranged between 2.5 µmol m^{-2} s^{-1} in early spring to 4 µmolCO_2 m^{-2} s^{-1}, peaking in June. In August, despite a 3 °C increase in soil temperature, soil respiration decreased to 3.3 µmol m^{-2} s^{-1}, due to a decrease in soil water content from 30 to 16%. At the beech site the soil respiration in late April - early May, just before the onset of the growing season, showed values around 2 µmolCO_2 m^{-2} s^{-1}. The peak was in early June with 5.5 µmolCO_2 m^{-2} s^{-1}. From June onwards, soil respiration decreased following a decreasing trend of soil water content, reaching values between 3 and 4 µmolCO_2 m^{-2} s^{-1} in July and August and a minimum of 2 µmolCO_2 m^{-2} s^{-1} in early September. At the end of the growing season, respiration increased again to 3.1 µmolCO_2 m^{-2} s^{-1}.
   - At the end of the three years period of soil respiration monitoring all data were quality checked, particularly for pressure correction, and analysed altogether. The data, coming from the two sites, together with data collected in Germany (Waldstein CAIN site) and in an additional site in Northern Italy, will be included in the chapter of the book that will be issued at the end of the project.
     Briefly, the summary of the results is the following:
     Seasonal trends: for all sites spring and summer peaks, winter minima. Peaks were measured after rain events. Soil water content is particularly important in summer, especially in the Mediterranean. Daily trend: almost no variation during the day, all sites (due to limited variability of temperature and moisture). Microsite variability: often larger differences between points on the same sampling date than along the day for single points. This is sometimes true at the seasonal level for sites with higher soil heterogeneity (natural forest compared to plantation). Controlling factors: temperature and moisture; the latter is more important in the south and when it is limiting. Also litter conditions and site history play a role. Ecosystem fluxes at soil level: reasonable comparison with cuvette measurements when turbulence is active. Yearly totals: the problem is how to scale from single measurements to yearly totals. Continuous records of soil temperatures are necessary. It is possible to use models with temperature and moisture.

4. Soil processes
   - Vacuum and gravimetric lysimeters for soil solution analysis have been regularly checked approximately every 20 days; if present water has been regularly collected, stored and sent, in October 1998, to the lab of DFLRI (Denmark) for analyses.
     At the beech site, vacuum lysimeters were checked 13 times (Prenart) and 9 times (University of Florence). Presence of water was checked and collected in the litter funnel and in the gravimetric lysimeters (installed at 5 and 40 cm depth) 9 times. At the spruce site, vacuum lysimeters were checked and, when needed, emptied 8 times during 1998.
     Due to the unusually dry and warm weather of summer 1998, it proved impossible to restore again vacuum in suction lysimeters for a period between July 14th and mid September in the beech forest and from August 10th and mid October in the spruce forest. The soil dryness made also difficult the assessment of the performance and comparability between classic and micro-lysimeters

5. Decomposition processes
    - Within the framework of the CAIN project, a field decomposition experiment, on leaf and twig litters, was established in Autumn 1996, and it is running since then, at the four beech sites of the CAIN project: Collelongo, Italy; Aubure, France; Schacht, Germany; Sorø, Denmark. The study consists of two experiments: i) incubation at a standard site (Collelongo) of the leaf and twig litters derived from the four beech sites; ii) incubation of the standard leaf and twig litter (derived from Collelongo) at the four beech sites.
    - Leaf and twig litters were collected in Autumn 96 as they fell, from all the four beech sites, air dried and a sub-sample was posted to the Italian site, where the leaf litter bags and the twig-lines were prepared. At the time of bag and twig-line preparation, litter water content was determined. In late Autumn 1996 litter bags and twig-lines, with the standard litter were placed in the field at Aubure, Schacht and Hilleröd, while litter bags and twig-lines, with litters derived from all the four sites, were placed in the field at Collelongo. Once collected, 7 bags and the twig-line are air dried and shipped to Italy, where they are processed for the determination of mass loss. After weighing, dried litters are milled and stored separately for later C and N concentration analysis.
    - Leaf litter decomposition in European beech forests appeared to be dependent on site-specific characteristics, with local climate being the most important rate constraining factor. In the European transect of the CAIN project, beech forests occurred along a North-South (55°58'– 41°52') latitudinal transect, at progressively higher altitude (from 45 to 1560 m a.s.l.), and the Danish site, the most Northern, was also the closest to the sea. Therefore, local climates followed site-specific seasonal patterns, which resulted in high decomposition rates at the Danish site, where winters were relatively mild and decomposition could occur all year long at high rates. After 2 year of field incubation the litter incubated at Sorø, Denmark had accumulated the highest value of mass loss (55%) whilst the litter at Collelongo showed the lowest mass loss value (21%). At both the two central European locations, Aubure and Schacht, by the end of the experiment litters had lost 36% of the original weights. Altitude was the parameter best correlated to final litter mass loss ($P<0.01$; $R^2=0.99$), but latitude was also significantly correlated to litter decomposition ($P<0.05$; $R^2=0.94$). Mean annual climatic parameters for the sites, such as temperatures, precipitation and computed Potential EvapoTranspiration and Actual EvapoTranspiration did not appear to be good predictors of decomposition in this study. Neither levels of N deposition at the different sites seemed to affect litter decay rates. Across the studied transect differences in litter elemental composition appeared to be minor, and the elemental composition of litters derived from the different sites did not affect subsequent litter decomposition rates.

6. Isotopes studies
    - Analysis of the natural abundance of ^{13}C and ^{15}N of leaf material have been performed in the beech forest.
    - Leaves from the top of the canopy (21 m) showed $\delta^{13}C$ values of -27.03±0.43 per mil, leaves growing in the mid of the crown (18 m) of -28.37±0.37 per mil and leaves growing in the lower part of the crown of -28.98±0.35 per mil. The $\delta^{13}C$ values of the leaves from top of the crown were significantly lower from those of the leaves collected at 18 and 14 m (t-test, $P<0.05$). According to the theory, this would imply a higher water use efficiency of those leaves more frequently exposed to high light and significant leaf-to-air temperature gradients.

      Even if $\delta^{15}N$ values decreased, in absolute value, from the top to the lower part of the canopy, the differences were not statistically significant. Values were -5.95±0.27, -5.7±0.22 and -5.5±0.18 per mil, respectively for leaves from the top (21 m), mid (18 m) and lower (14 m) part of the crown.

V. Publications and Communications in Workshops

Scarascia Mugnozza G., De Angelis P., **Matteucci G.**, Valentini R. (1998). FORESTE E CAMBIAMENTI CLIMATICI. *In*: Atti del *II Convegno Nazionale di Selvicoltura per la Conservazione ed il Miglioramento dei Boschi Italiani*, Venezia, 24-28 Giugno 1998, in press

Matteucci G., Valentini R., Scarascia Mugnozza G. (1998). LINKING PHYSIOLOGY TO FLUXES IN APPENNINE BEECH FOREST. *In*: EUROSILVA workshop on Tree Growth at high altitude and latitude, Obergurgl, Austria, September, 10-14, 1998.

Signature of Partner:

Date: 15 March 1999

PART B

Reporting period: February 01st 1998 - January 31st 1999.

Partner: 04, University of Hohenheim, Institute of Plant Nutrition

Principal Investigator: Dr. Eckhard George

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I. OBJECTIVES FOR THE REPORTING PERIOD:

To complete measurements and report results of:

1) investigations into the relations between nitrogen (N) uptake by, and transport of photosynthetic carbon (C) to different root zones;
2) determinations of the contribution of ectomycorrhizal hyphae to the N uptake of trees and the corresponding C supply from the trees to the hyphae, and measurements of respiration rates;
3) analysis of non-structural carbohydrates in different root parts in order to assess the current energy status of the root tissue as a measure of the corresponding C investment in the root system (together with other groups);
4) quantification of soil respiration rates and estimations of the proportion of root respiration (including mycorrhizal respiration).

II. OBJECTIVES FOR THE NEXT PERIOD:

No longer relevant.

III. ARE THERE ANY PARTICULAR PROBLEMS? IS YOUR PART OF THE PROJECT ON SCHEDULE?

No. Yes.

IV. MAIN RESULTS OBTAINED: METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS:

See attached sheet.

IV. Main results obtained

The role of the mycorrhizal association on nitrogen uptake by the root, and the corresponding allocation of photosynthetic carbon to the root and the fungus, was studied by double labelling experiments using ^{13}C and ^{15}N.

Objective 1) Investigations into the relations between nitrogen (N) uptake by, and transport of photosynthetic carbon (C) to different root zones.

Mesh bags of different pore size (penetrable to roots and hyphae, or to hyphae only) were installed next to five-year-old trees in April/May at three spruce (Åheden, Skogaby, Aubure) and two beech (Hillerød, Aubure) sites. In July, ^{15}N-labelled ammonium, nitrate and glutamic acid were supplied to the different soil compartments. Six weeks later, the shoots of the trees were exposed to ^{13}C-labelled CO_2 for eight hours. After a further six days, shoots, roots, and mesh bags were harvested. Plants were separated into several components and were dried, weighed, and ground. One day before harvest, respiration measurements were carried out by sampling soil-emitted CO_2 in a headspace placed on one of the mesh bags or on the soil.

Table 1. Total amount of ^{15}N (ng N per plant) taken up by plants (Norway spruce or beech) at different forest sites after supply of different forms of nitrogen to soil either in a root (R) or a hyphal (H) compartment. Means and standard deviations.

		Control no N		High NH_4^+ 300 mg N		Low NH_4^+ 1 mg N		Nitrate 1 mg N		Glutamic acid 1 mg N	
Åheden spruce	R	0,03	0,02	25,38	5,79	1,99	1,99	0,02	0,02	0,01	0,01
	H	0,15	0,11	14,00	8,04	8,27	4,29	11,51	8,88	0	0
Skogaby spruce	R	0,28	0,09	130,82	49,30	7,90	4,03	6,00	0	11,80	6,04
	H	0,21	0,10	72,03	17,29	5,75	3,07	2,24	0,02	3,74	0
Aubure spruce	R	0,30	0,10	283,85	91,31	6,62	2,07	4,24	1,83	3,28	0
	H	0,01	0,01	263,13	96,71	24,22	14,13	11,29	8,43	52,12	14,73
Hillerød beech	R	0,14	0,06	212,24	52,26	4,23	1,62	6,54	2,32	5,63	2,18
	H	0,17	0,05	123,84	36,74	9,33	5,34	9,86	0	8,98	3,30
Aubure beech	R	0,20	0,11	216,90	48,48	4,79	0,45	4,80	1,82	8,23	5,98
	H	0,28	0,11	322,87	129,22	40,55	36,01	10,74	5,49	11,55	2,25

Uptake of nitrogen from the root mesh bags was much lower in Åheden compared to the other sites (Table 1). Root nitrogen uptake was increased at a high rate of ammonium supply. In a comparison of nitrogen sources, none of the sources was consistently superior. Thus, in most cases (very likely mycorrhizal) roots were able to use glutamic acid as well as ammonium and

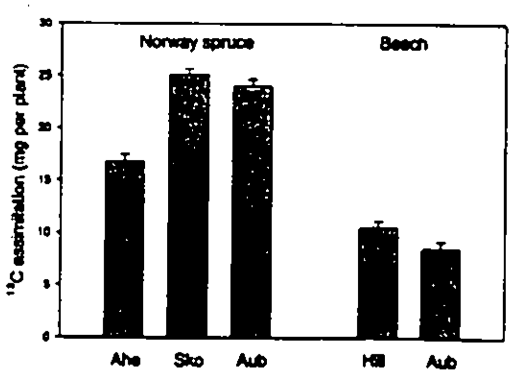

Figure 1. Total assimilation of applied ^{13}C by Norway spruce and beech plants at different forest sites. For methodology see text.

cases (very likely mycorrhizal) roots were able to use glutamic acid as well as ammonium and nitrate. There was a tendency for Norway spruce to prefer ammonium over nitrate, while this preference did not appear in beech.

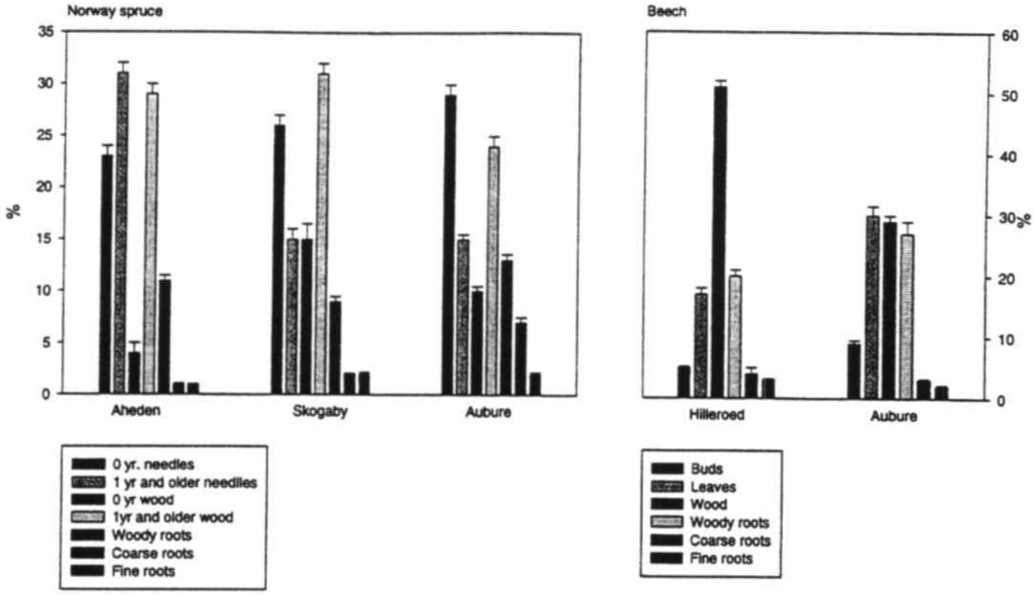

Figure 2. Relative distribution of assimilated carbon in different parts of Norway spruce and beech plants at different forest sites. For methodology see text.

Total photosynthetic carbon accumulation during the measuring period was much higher in Norway spruce than in beech (Figure 1). Carbon accumulation in Norway spruce was significantly ($P<0.001$) smaller in Åheden than in Skogaby or Aubure. Photosynthesised carbon was translocated into all parts of the plant (Figure 2). Woody roots were a much stronger sink for carbohydrates than fine roots (Figure 2).

Objective 2) Determinations of the contribution of ectomycorrhizal hyphae to the N uptake of trees and the corresponding C supply from the trees to the hyphae, and measurements of respiration rates.

Ectomycorrhizal hyphae were at least as effective as (mycorrhizal) roots in uptake of nitrogen from soil (Table 1). Hyphae were able to use ammonium, nitrate, and an amino acid (Table 1).

Objective 3) Analysis of non-structural carbohydrates in different root parts in order to assess the current energy status of the root tissue as a measure of the corresponding C investment in the root system (together with other groups).

Non-structural carbohydrates in roots did not differ distinctly in Norway spruce roots from Aubure and Schacht.

Objective 4) Quantification of soil respiration rates and estimations of the proportion of root respiration (including mycorrhizal respiration).

Respiration rates were as high or higher from soil with hyphae alone than from soil with (mycorrhizal) roots (data not shown). Thus, hyphae of ectomycorrhizal fungi make a large contribution to plant nitrogen uptake at all sites investigated and also contribute distinctly to total respiration.

V. LIST OF PUBLICATIONS ARISING FROM THE PROJECT (INCLUDE COPIES):

George, E., Stober, C. & Seith, B. (1999). The use of different soil nitrogen sources by young Norway spruce plants. *Trees* (in press).

George, E., Kircher, S., Schwarz, P., Tesar, A. & Seith, B. (1999). Effect of high soil nitrogen supply on growth and nutrient uptake of young Norway spruce plants grown in a shaded environment. *Zeitschrift für Pflanzenernährung und Bodenkunde* (in press).

Signature of Partner: Eckhard George

Date: 04/03 1999

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: February 1998 to February 1999

Partner:05 IN (FR)

Principal Investigator: Dr. Francis MARTIN

Scientific staff: Dr. M. COLIN-BELGRAND, E. DAMBRINE, B. ZELLER & Dr. F. MARTIN

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I. OBJECTIVES FOR THE REPORTING PERIOD

All sites:

(1) Analysis of ^{15}N-litter decomposition and quantitation of the size and turnover of the different soil and plant N fractions at the selected CANIF sites. Analyses of data. Writing publications.

Aubure site:

(2) Study of the leaching, accumulation and release of N in decomposing ^{15}N-labelled beech litter deposited on the site during the previous years.

(3) Identification of genotypes of ectomycorrhizal fungi by molecular diagnostics. Data analyses and publication writing.

II. OBJECTIVES FOR THE NEXT PERIOD

Not relevant

III. Are there any particular problems? Is your part of the project on schedule?

No problem to mention

Work schedule and milestones of the project for the year 1998 have been achieved.

IV. MAIN RESULTS OBTAINED

All sites

(1) Application of ^{15}N-labelled beech litter to study its decomposition rate, and size and turnover of soil N fractions at selected CANIF sites

Methodology In late summer 1998, the second sampling occurred at the Collelongo site and the third in the Steigerwald forest. ^{15}N labelled litter was collected from the soil surface of each of three experimental plots. Then, nine soil samples (0-10cm, 10-20cm and 20-30cm) underneath the labelled area were taken. At last, the trees were cut and all tree compartments were collected. At both sites the remaining trees were wrapped into a nylon net from the beginning of September until November and the leaf litter of each tree was collected separately.

Results: The results obtained are reported in detail in Chapter 10 of the Canif – Book. N concentrations in the ^{15}N-labelled litter increased at all sites, sharply during the first year, slightly during the second year and levelled off in the third year. At Aubure, in relation to the original N content, the increase was proportionally higher in the N-poor litter in comparison to the N-rich litter. The final N concentration was somewhat lower in Collelongo.

At all sites, the ^{15}N excess of the litter decreased. The slope of the decrease was higher for the N-rich litter. The combination of N concentration increase and ^{15}N excess decrease, suggested that external N was incorporated while litter N was released. At Aubure, about 16, 25 and 64% of the initial N amount of the litter poor in N had been released after 4 months, a year and three years, respectively. While N was released from the litter, external N was simultaneously incorporated into the decomposing litter. Almost all external N was incorporated during the first year. Over a two year period, the release of litter N balanced the amount of external N incorporated. Similar results were obtained at Ebrach, while a lower N release and N incorporation occurred at Collelongo during the second year.

Aubure site

(3) Seasonal variability of leaching, accumulation and release of ^{15}N from labelled decomposing litter.

Results The results obtained from this experiment are submitted for publication and the results obtained from 1994 – 1998 at the Aubure site are reported in Chapter 6 of the Canif – Book.

(4) Identification of major types of beech ectomycorrhizas by molecular diagnostics

Refinement of our understanding of the colonization and competitive strategies of species present in the beech and spruce stands will require more information about size, dissemination and persistence of genetic individuals (genotypes). In particular our understanding of the genetic structure of the identified major taxons (e.g. *Thelephora*, *Laccaria*) (see Read et al.) in the different sites depends on the differential roles of mycelial vegetative dissemination versus spores. To explore the population genetics of the major species of ectomycorrhizal basidiomycetes found on the various sites, the present study has described, as a pilot experiment, the spatial genetic structure of the ectomycorrhizal basidiomycete *Laccaria amethystina* (Bolt. ex Hooker) Murr. in the closed 150-year-old beech (*Fagus sylvatica* L.) forest in Aubure (Vosges Mountains, France). During the fall of 1994 and 1997, sporophores were collected from three 100 m^2 sampling plots located along a 120 m transect crossing the beech stand (see previous reports). The genetic variation of 676 sporophores was initially (years 1996 & 1997) estimated using heteroduplex analysis of the ribosomal DNA intergenic spacer (IGS1). Ten unique IGS1 heteroduplex/homoduplex patterns were identified, although three types represented most of the sporophores analyzed. Each group of IGS1-type was then analyzed by microsatellite-primed PCR fingerprinting (RAMS) (years 1997 & 1998). RAMS resolved about 380 different genotypes amongst the 572 sporophores analyzed from the three plots during falls of 1994 and 1997. Density as high as 5200 genets.ha^{-1} was observed. The largest clone covered 3 m^2 and comprised only 12 sporophores. Most genets occurred as patches of less than 0.5 m^2 of 2-10 sporophores. Only a dozen of genotypes identified in 1994 were found in 1997. Though *L. amethystina* has the capacity for vegetative persistence, the present study indicates that its populations maintain a genetic structure more consistent with a high frequency of sexual reproduction. This suggests that beech trees are recolonized by new genotypes each year. Alternatively, this spatial distribution may arise from erratic and differential fruiting of underground genets. These features (i.e. numerous genets of small size), typical of ruderal species, contrast with studies carried out on other ectomycorrhizal basidiomycetes occurring in mature closed forests.

V. List of publications arising from the project

1. GHERBI H, DELARUELLE C, SELOSSE MA· MARTIN F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150-year-old beech forest. Submitted to *Molecular Ecology*

2. KOERNER W., DAMBRINE E., DUPOUEY J. L., BENOIT M. (1998) δ^{15}N of forest soil and fern reflects the former agricultural land use. *Oecologia* (submitted)

3. JUSSY J. H., KOERNER W., DAMBRINE E., DUPOUEY J. L., BENOIT M. (1998) Effects of past land use on nitrification in forest soils. *J. Ecology* (submitted)

4. JUSSY J. H. , COLIN BELGRAND M., DAMBRINE E., BIENAIME S., PERSSON H. (1998) Minéralization, nitrification and N uptake in spruce and beech stands of the Strengbach catchment. (submitted)

5. JUSSY J. H. , COLIN BELGRAND M., DAMBRINE E. (1998) Comparison of N fluxes in 3 forest stands. *Proc. 16th World. Cong. Soil Sci.* Montpellier

6. MARTIN F, SELOSSE M-A, DI BATTISTA C, GHERBI H, MARTIN D, DELARUELLE C, VAIRELLES D, BOUCHARD D, LE TACON F. (1998) Molecular markers in ecology of ectomycorrhizal fungi. *Genetics Selection Evolution* 30, S333-S355.

7. ZELLER B. (1998) Contribution à l'étude de la décomposition d'une litière de hêtre, la libération de l'azote, sa minéralisation et son prélèvement par le hêtre (Fagus sylvatica L.)

dans une hêtraie de montagne du bassin versant du Strengbach (Haut-Rhin). Thesis, Université Henri Poincaré, Nancy (France), 138p

8. ZELLER B, COLIN-BELGRAND M, DAMBRINE E, MARTIN F (1998) ^{15}N partitioning and production of ^{15}N-labelled litter in beech trees following [^{15}N]urea spray. *Ann. Sci. For.* 55: 375-383

9. ZELLER B, COLIN-BELGRAND M, DAMBRINE E, MARTIN F (1999) N release and tree uptake from decomposing litter in a beech forest. Submitted.

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Champenoux, 15 February 1999

Dr. Francis MARTIN

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DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 1 January 1998 - 31 January 1999

Partner: University of Sheffield

Principal Investigator: Professor D J Read

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I. OBJECTIVES FOR THE INDIVIDUAL PARTNERS

Determination of K_M/V_{max} values for fungal isolates from CANIF sites and synthesized mycorrhizas. $^{13}C/^{15}N$ experiments in the field and with synthesized mycorrhizas to compare uptake rates with data obtained from dissected roots and to investigate to which extent and in which form C and N are transferred from the fungal symbiont to the host plant.

II. OBJECTIVES FOR THE NEXT PERIOD:

No longer necessary.

III. ARE THERE ANY PARTICULAR PROBLEMS? IS YOUR PART OF THE PROJECT ON SCHEDULE?

Our part was completed on schedule. However, because fungal isolates obtained from roots at the CANIF sites grew extremely slowly the data sets were not as comprehensive as had been planned. Greater emphasis was placed on a comparative analysis of the uptake kinetics of mycorrhizal and non-mycorrhizal roots and on the regulation of amino acid uptake. Field studies employing $^{13}C/^{15}N$ were carried out by P. Högberg.

IV. MAIN RESULTS OBTAINED: METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS

Experiments using isotopically labelled amino acids were carried out with synthesized mycorrhizas, field-collected mycorrhizas and non-mycorrhizal short roots of *Picea abies*, *Pinus* species, *Betula pubescens* and *Fagus sylvatica*.

Using large *Lactarius subdulcis/Fagus sylvatica* mycorrhizas we separated the central cylinder from fungal component. Mycorrhizal roots incubated in isotopically labelled amino acids had significantly more radioactivity in their central cylinder than those treated with the ^{14}C-labelled, NH_4^+ analogue methylammonium. This indicates that (amino acid derived carbon) is transferred from the fungal partner to the host plant. However, compared to the total uptake into these roots this transfer was of minor importance. Correspondingly, when labelled glycine was added to the mycorrhizal or non-mycorrhizal roots of Norway spruce seedlings colonised plants failed to accumulate more radioactivity in their shoots than unlabelled control plants after several hours incubation. In contrast, more labelled carbon could be detected in shoots of labelled non-mycorrhizal plants when compared to control plants, indicating the importance of amino acids as carbon sources for the fungal partner in mycorrhizal plants. Clearly this carbon is only to a minor extent provided to the host plant.
Non-mycorrhizal roots of the broad-leaved species *Fagus sylvatica* and *Betula pubescens* had lower uptake rates and V_{max} values than any of the different mycorrhizal morphotypes studied along the CANIF gradient. In contrast, non-mycorrhizal roots of the coniferous species *Picea abies* and *Pinus sylvestris* showed uptake rates in the same range as those for mycorrhizal roots of these species. For non-mycorrhizal roots of all tree species K_M values for amino acid uptake were similar to those found for the different morphotypes studied along the CANIF transect. This indicates that mycorrhization is beneficial for amino acid uptake especially for broad- leaved species.
Inhibition studies with other amino acids and inorganic N sources showed that amino acid uptake is probably mediated via a rather unspecific transport system which resembles the general amino acid permease reported for the ectomycorrhizal fungus *P. involutus* and yeasts. The availability of NH_4^+ or NO_3^- in the soil solution is unlikely to affect *in situ* uptake rates by mycorrhizal roots at the different CANIF sites, as uptake of ^{14}C-glutamine was not inhibited by these ions in the uptake solution.

IV. LIST OF PUBLICATIONS ARISING FROM THE PROJECT

Wallenda T, Read DJ (1999) Kinetics of amino acid uptake by ectomycorrhizal roots. Plant Cell and Environment, in press
Further manuscripts in preparation

Signature

Date: 22. 3. 99

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 1 February 1998 - 31 January 1999

Partner: Swedish University of Agricultural Sciences, Uppsala (part 7a)

Principal investigator: Prof. T. Persson

Scientific staff: A. Rudebeck, P. Karlsson, U Seyferth, M Sjöberg

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I. OBJECTIVES FOR THE REPORTING PERIOD (part 7a)

(1) To determine in situ C and N organic pools and C and N mineralisation rates for each soil horizon (to a depth of 50 cm) in samples from all CANIF sites.
(2) To synthesise data on field nitrification potentials based on laboratory studies with soil materials from all CANIF sites.
(3) To proceed with the identification of the nitrifier types at some CANIF sites.
(4) To publish results on response functions for temperature/moisture based on data from Skogaby.
(5) To evaluate the comparisons of soil processes in undisturbed and disturbed soil layers.
(6) To publish results on the long-term N-fertilisation experiments.
(7) To publish results on soil faunal effects on C and N mineralisation.
(8) To evaluate data on the $^{15}NO_3$ dilution study at Skogaby.

III. Are there any particular problems? Is your part of the project on schedule?

The subproject is on schedule in the sense that all field and laboratory studies are finished. The publication procedure is partly delayed. Most of the results will appear in the joint CANIF publication in Ecological Studies or have been submitted to journals (objective 4). The only exception concern objective 7, which is a joint study with another EU project (GLOBIS) to be finished in August.

IV. MAIN RESULTS OBTAINED: *METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS* (use other pages if necessary but preferably no more than 2)

(1a) C and N soil pools in the NIPHYS/CANIF gradient were low in typical boreal and mediterranean climates. The pools were higher in the areas with intermediate temperatures but varied considerably depending on earlier land-use. No differences were found between coniferous and deciduous stands. C and N pools were highly correlated except for sites with calcareous subsoil, where the N pool was comparatively high indicating a high retention of N.

(1b) Estimated soil C mineralisation C_{min}. varied between 900 (Aubure) and 3200 kg C ha^{-1} y^{-1} (Thezan) in the European gradient. There was no clear effect of latitude, tree species or pH, but C_{min} was positively correlated with mean annual temperature, organic C pool, stand age and soil C:N ratio. Comparison of control and treated plots in long-term N fertilisation experiments showed that addition of N reduced C_{min} per C pool. Thus, long-term N fertilisation or deposition can increase the soil C pool without a corresponding increase in C_{min}.

Estimated net N mineralisation varied between 60 (Aubure, old spruce) and 190 kg N ha^{-1} y^{-1} (Schacht) for all sites except Åheden, where no N seemed to mineralise. The lack of net N mineralisation at Åheden indicate that organic N uptake is important at that site. Spruce sites generally had lower net N mineralisation than beech sites, but there were several exceptions.

(2) Net nitrification did not occur or was low at coniferous sites with young stands or stands with N limitation (Åheden, Skogaby, Klosterhede, Aubure-young spruce) and Thezan. Beech stands had a higher nitrification potential than comparable spruce stands. Net nitrification was positively correlated with net N mineralisation in the mineral soil below 10 cm depth. In the litter and humus layer, net nitrification was correlated with pH. This was confirmed by laboratory tests that showed that net nitrification was always stimulated by addition of $CaCO_3$ in the humus layer but not at 10-20 cm in the mineral soil.

(3) Ammonia-oxidising bacteria were found in six out of seven sites investigated (Aubure, 3 stands, Collelongo, Monte di Mezzo and Schacht). The only site that did not contain ammonium oxidisers (according to the criterion of giving PCR products with Kowalchuk's primers) was Waldstein (two depths). Schacht lacked nitrifiers at one depth. Tests with DGGE (denaturing gradient gel electrophoresis) showed three bands for M: di Mezzo and two for Collelongo and Aubure (beech) indicating varying nitrifier diversity.

(4) A manuscript on temperature and moisture dependency for C and N mineralisation has been submitted to Soil Biology and Biochemistry (see below).

(5) Comparison of sifted and unsifted (undisturbed) soil in the laboratory showed that estimates of C and N mineralisation rates often decreased after sifting. The decrease could amount to a maximum of 30% of that in the undisturbed soil.

(6) A manuscript on effects of long-term fertilisation on C and N mineralisation is under preparation. Results will also appear in the joint volume of Ecol. Stud.

(7) The study of soil faunal impact on C and N mineralisation is still under preparation (see above).

(8) The $^{15}NO_3$ dilution study at Skogaby is finished, but more tests are needed because of problems with recovery.

V. List of publications arising from the project (include copies):

Ebenå G (1998) Diversity among ammonium-oxidizing bacteria in forest soil from seven European locations. Linköping university, IFM, Dept of Biology (Internal report).

Persson T, Breland TA, Seyferth U, Lomander A, Kätterer T, Henriksen TM, Andrén O (in press) Carbon and nitrogen turnover in forest and arable soil in relation to substrate quality, temperature and moisture. TemaNord.

Seyferth U (1998) Effects of soil temperature and moisture on carbon and nitrogen mineralisation in coniferous forests. Licentiate thesis, Dept of ecology and Environmental Research, Swedish University of Agricultural Sciences, No. 1.

Seyferth U, Persson T (subm.) Effects of soil temperature and moisture on carbon and nitrogen mineralisation in a coniferous forest soil. Soil Biology and Biochemistry.

Signature of the partner:

Tryggve Persson

Date: 22 February 1999

PART B

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period	February 1998-January 1999.
Partner:	Swedish University of Agricultural Sciences, Uppsala (part 7a)
Principal investigator:	Ass. Prof. H. Persson
Scientific staff:	K. Ahlström
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I. OBJECTIVES FOR THE REPORTING PERIOD (part 7b):

(1) To report results on root distribution (and rooting depth) in terms of dry weight, root length, carbon and mineral nutrient content in stands of Norway spruce and European beech.
(2) To relate results on the fine root distribution to fine root dynamics obtained from "root windows" (Hohenheim University Group). To carry out cross-site comparison on root distribution and growth dynamics in co-operation with other root and mycorrhizal scientists between all sites for the final report
(3) To report results on the seasonal changes in the amount of fine roots (Norway spruce and European beech) at Aubure.
(4) To finish and report results from the Waldstein and Schacht sites on the distribution of fine roots in a Norway spruce and a European beech stand.

III. Are there any particular problems? Is your part of the project on schedule?

The subproject is on schedule and all field and laboratory studies are finished. The publication procedure is partly delayed, but data has been transferred to several of the CANIF groups for joint publications. Most of the results will appear in the joint CANIF publication in Ecological Studies or have been submitted to journals, or exist in the shape of manuscripts.

IV. MAIN RESULTS OBTAINED: *METHODOLOGY, RESULTS, DISCUSSION, CONCLUSIONS* (use other pages if necessary but preferably no more than 2)

(1) Results are available on fine root distribution (and rooting depth) from the French Aubure sites, the German sites (Waldstein and Schacht) (Norway spruce and European beech sites), the Swedish Skogaby site (Norway spruce). From the results it is possible to conclude that the vertical distribution in fine root standing crops (live + dead fine roots) differs between Norway spruce and European beech sites. The total soil profile was more deeply occupied by fine roots on beech sites.

(2) Since data are available of the amount of fine roots both in terms of root dry weight and length it is possible to relate our results on the fine root distribution to the data from the root windows (the Hohenheim University Group). Thus cross-site comparisons on root distribution and growth dynamics are carried out.

No significant decreases could be detected with distance from the investigated trees (1.4, 2.5, 3.4 and 4.5 m) in the amount of fine roots (Persson, in press). Thus the fine roots were rather evenly distributed over the total area of the investigated stands.

(3) Data on the seasonal dynamics from the Aubure sites (5 sampling occasions) suggest a high fine root production and turnover in both the Norway spruce stand and European beech stands. The mean biomass was 57 and 83 gm^{-2} in the Norway spruce and European beech stand respectively and the turnover rate was 2.4 and 2.1 times the mean biomass.

(4) Data from the Aubure site have been used in a manuscript accepted and in press in Plant and Soil (see below). Another manuscript using the data from the Aubure site is also under preparation. Results have been transferred to other groups for the joint volume of Ecol. Stud.

V. List of publications arising from the project (include copies):

PERSSON H. In press. Adaptive tactics and characteristics of tree fine roots. Plant and Soil 0: 000-000.

PERSSON H. & AHLSTRÖM K: Manuscript. Fine-root growth and distribution in a Norway spruce and European beech stands in Northern France

Various manuscripts in the joint volume of Ecol. Stud.

Signature of the partner:

Hans Persson

Date: 22 February 1999

PART B

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 1.2.1998-31.1.1999

Partner: No. 8, University of Copenhagen

Principal Investigator: Sten Struwe

Scientific staff: Morten Miller, Anders Priemé, Annelise Kjøller

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I. OBJECTIVES FOR THE REPORTING PERIOD:

The aim of a joint study between the Danish and the Italian partners was to investigate the interaction of substrate quality, climate and saprophytic fungal activity in beech litter along the CANIF European transect.

Further to estimate the nitrous oxide emission and denitrification in beech forest soil in Collelongo, Auburs, Seachet and Sorø, both as field and laboratory measurements.

II. OBJECTIVES FOR THE NEXT PERIOD:

-

III. Are there any particular problems? Is your part of the project on schedule?

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IV. MAIN RESULTS OBTAINED: ***METHODOLOgY, RESULTS, DISCUSSION, CONCLUSIONS*** **(use other pages as necessary but preferably no more than 2)** -

see appendix

V. List of publications arising from the project (include copies):

Kjøller,A. & Struwe, S. 1997. Microbial diversity and its relation to decomposition processes. In Functional Implications of Biodiversity in Soil. V. Wolthers ed. Ecosystem research report no 24. European Commission. Brussels. pp 83-98.

Hui, Xu, Struwe, S. & A. Kjøller, 1997. Emission of N_2O from beech forest soil. In: Proceedings of 7th International Workshop on Nitrous Oxide Emissions. April 21-23, 1997, Cologne, Germany. pp 473-478.

Miller, M., Palojärvi, A. Rangger, A., Reeslev, M. & Kjøller, A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64, 613-17.

Møller, J., Miller, M. & Kjøller, A. 1998. Fungal-bacterial interaction on beech leaves; influence on decomposition and dissolved organic matter quality. Soil Biology and Biochemistry (in press).

Signature of Partner: [signature]

Date: 22 February 1999

Nitrous oxide emission and denitrification

Methodology

Nitrous oxide emission was measured at monthly intervals from spring 1998 to late autum. Gas was sampled from the headspace of closed chambers placed in the soil surface in the beech forest sites at Sorø, Schacht, Aubure, and Collelongo. Denitrification enzyme activity (DEA) in soil samples from the above mentioned sites was measured as nitrous oxide accumulation during anaerobic incubation of soil samples from 0-15 cm soil depth, added substrate (1 mM glucose and 1 mM KNO_3). Nitrous oxide reductase activity was inhibited by 10% acetylene (C_2H_2). Potential nitrous oxide production from denitrification was measured in a parallel set of soil samples incubated without acetylene.

Results and Discussion

Comparison of the results from several CANIF beech and spruce forest sites revealed that denitrification, and not nitrification, was the major source of soil nitrous oxide emission. The rates at the Aubure site was at least one order of magnitude higher compared to the other CANIF sites and represent a significant N loss from the system (Table 1). The high emission rate at this site could not be ascribed to a high N-content or a high N-turnover as the Aubure soil had lower N pools, net N mineralisation rates and potential nitrification rates (data from Tryggve Persson, Swedish Agricultural University).

Site	Nitrous oxide emission (ug $N\text{-}N_2O$ m^{-2} h^{-1})	N loss due to nitrous oxide emission (kg $N\text{-}N_2O$ ha^{-1})[a]
Sorø, wet site	7.4	0.49
Sorø, dry site	6.8	0.45
Aubure	75	3.3
Schacht	3.5	0.15
Collelongo	3.1	0.14

Table 1. Nitrous oxide emission from the CANIF beech forest sites. [a] Only during measurement period which was March to December at the Sorø sites and May to October at the remaining sites; annual N losses will be larger.

Nitrous oxide emission rates were influenced not only by potential denitrification enzyme activity but also by the activity of nitrous oxide reductase. Thus, the high emission rates from the Aubure site may in part be due to a lack of nitrous oxide reductase activity in the denitrifying community (Table 2). Nitrous oxide is more sensitive to low pH compared to the other enzymes involved in the denitrification proces and the low pH in the Aubure soil (3.57 at 0-10 cm depth) may partly explain the lacking nitrous oxide activity. Alternatively, differences among the sites in the composition of the denitrifying communities may influence nitrous oxide emission rates (we are currently investigating this possibility).

Site	Potential denitrification activity (μg N-N_2O g^{-1} h^{-1})	% activity without C_2H_2/ activity with C_2H_2
Sorø, wet	1.06±0.03	11
Sorø, dry	0.17±0.00	86
Aubure	0.63±0.01	100
Schacht	0.76±0.01	92
Collelongo	0.71±0.01	74

Table 2. Potential denitrification activity in soil samples from the CANIF beech forest sites and the ratio of potential denitrification activity without inhibition of nitrous oxide reductase to the activity with inhibition by 10% C_2H_2. Numbers are average±SE(X), n=3.

No direct relationship between N deposition and nitrous oxide emission from the CANIF beech forest sites could be indicated. However, it is possible that long-term N addition via atmospheric deposition may gradually lead to increasing emission as the plant and microbial demands for N are saturated. Soil pH may decrease concomitantly with N deposition and influence N loss through denitrification as denitrification rates are generally lower at low pH.

A literature review of nitrous oxide emission from temperate forest soils revealed that Norway spruce forest soils generally emit lower amounts of nitrous oxide (0.42 kg N-N_2O ha^{-1} yr^{-1}) compared to soils planted with beech forest (1.9 kg N-N_2O ha^{-1} yr^{-1}). Thus, we believe that nitrous oxide emission represents a fairly small N loss at the CANIF spruce forest sites compared to the beech forest sites.

Biodiversity and enzyme activity

Methodology

Analysis of beech litter has been carried out six times at different seasons during two years. Cellulase and chitinase activity and the biodiversity of the microfungal population have been determined simultaneously. Further the litter has been analysed for total C and N and cellulose/lignin content.

Two experiments were designed and set up. A transplant experiment, where beech litter samples from France (Aubure), Germany (Schacht), and Denmark (Sorø) were inoculated at the Italian site (Collelongo), and a transect experiment where Italian litter was incubated in France, Germany and Denmark.

Results and Discussion

During two years of decomposition when transplanted litter was incubated in Italy the cellulase activity was rather low, lowest in the Danish litter and highest in the indigenous Italian litter. In the transect experiment, where Italian litter was incubated at the European sites an increasing cellulase activity was observed during the two years of incubation. In this experiment the Italian litter incubated at the Danish site showed the highest cellulase activity, whereas the Italian litter in Italy the lowest. Differences in local climatic conditions could influence this result. In Denmark the cold winter period is usually very short, while snow remains at the other sites for several (6-7) months. Even if summer temperature is lower in Denmark compared with the other CANIF sites, the yearly mean temperature is higher and decomposition proceeds with the highest rate.

The preliminary results of analysis of the biodiversity of the microfungal population show the following: In the transplant experiment, (low cellulase activity), no major differences in the composition of the fungal flora were observed. Dominating fungal genera initially were Chalara and Cladosporium. Late in the decomposition period some local fungi appeared on the German and Danish litter eg. Mortierella and Trichoderma.In the transect experiments, when the Italian litter was incubated in France, Germany and Denmark the cellulolytic activity increased during the two years of decomposition, less so in the Italian litter. The composition of the fungal flora was almost identical during the first nine months of incubation; during the second year of decomposition the Danish litter was invaded by strong cellulolytic fungi as Mortierella and Trichoderma and a high cellulolytic activity was observed.

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 01.02.1998 - 31.01.1999

Partner: 09

Justus-Liebig-University Giessen

Principal Investigator: Prof. Dr. Volkmar Wolters

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I. OBJECTIVES FOR THE REPORTING PERIOD:

(i) the sampling programme was finalised with a spring sampling along the transect according to the design of the previous samplings; (ii) concluding determination of the structure of soil communities along the European transect; (iii) complementary microcosm experiments; (iv) modelling the contribution of soil organisms to C and N mineralisation; (v) final evaluation of data and report

II. OBJECTIVES FOR THE NEXT PERIOD:

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III. Are there any particular problems? Is your part of the project on schedule?

Measurement of some of the faunal and microbial parameters is currently being completed.

IV. MAIN RESULTS OBTAINED: *METHODOLOGY, RESULTS, DISCUSSION, CONCLUSION* (use other pages as necessary but preferably no more than 2)

Methodology and main results see next page (supplement).

V. List of Publications arising from the project (include copies):

Pieper B, Klein A, Ekschmitt K & Wolters V (1997): Umsatz der organischen Substanz in Waldböden: Einfluß von Abundanz und Diversität der Collembolen. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft, 85, II, 571-574.

Schröter D, Hülsmann A, Pflug A & Wolters V (1997): Die Bodenfauna in der organischen Auflage entlang eines europäischen Transekts. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft, 85, II, 599-602.

Schroeter D, Huelsmann A, Pflug A, Wolters, V (1998) Climate effects on soil biota of coniferous forests: a transect approach. The Earth's Changing Land - GCTE-LUCC OpenScience Conference on Global Change; Barcelona (Spain) 14.-18. March 1998 (Abstracts), pp. 208-209.

Pflug A, Huelsmann A, Schroeter D & Wolters V (1998): Microarthropoden als Steuergröße im Lebensraum Boden. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft, in press.

Wolters V, ed. (1997) Functional Implications of Biodiversity in Soil. Ecosystems Research Report 24, 133 pp.

Wolters V (1997) The Good, the Bad and the Ugly: Is there more to say about soil biodiversity? In: Wolters V (ed.): Functional Implications of Biodiversity in Soil. Ecosystems Research Report 24, 3-9.

Wolters V (1998) Functional aspects of animal diversity in soil - introduction and overview. Applied Soil Ecology 10, 185-190.

Wolters V (ed.) (1998) Functional aspects of animal diversity in soil. Applied Soil Ecology (Special Issue) 10, 185-288.

Wolters V (1999) Terrestrische Ökosysteme. Spektrum der Wissenschaft 2/1999 (A), 35-39.

Signature of Partner: V. Wolters

Date: 22.2.99

Supplement

IV.

The data acquisition has been continued and was extended. The following parameters were obtained: (i) species level abundance and biomass of Collembola; (ii) family level abundance and biomass of Acari; (iii) species level abundance and biomass of Testacea; (iv) feeding group level abundance and biomass of Nematoda; (v) abundance and biomass of Enchytraeidae; (vi) content of microbial carbon (C_{mic}); (vii) content of microbial nitrogen (N_{mic}); (viii) CO_2-evolution; (ix) functional diversity of soil bacteria (Biolog screening); (x) numbers and biomass of Bacteria; (xi) frequency of dividing bacterial cells; (xii) bacterial cell length, width and volume; (xiii) fungal biomass; (xiv) bulk density; (xv) water content; (xvi) thickness of soil organic layer; (xvii) pH; (xviii) content of mineral carbon; (xix) content of mineral nitrogen; (xx) C:N-ratio. Data were evaluated.

In addition to the field and laboratory experiments reported earlier another microcosm experiment on the effect of different microarthropod communities on litter decomposition was carried out.

MAIN RESULTS

Statistical analysis of the complete transect-data-set confirms that the sites host characteristic invertebrate communities: abundance of bacterivorous and root-feeding Nematodes, as well as of Testacea, Enchytraeidae, Collembola and Acari were significantly different on all sites. Furthermore bacterial and fungal biomass as well as microbial CO_2-evolution differed significantly. Trophic connectivity of mesofauna, microfauna and microflora changes along the transect, with large and small scale climatic gradients affecting the structure of the edaphon. This view is confirmed by an analysis of the taxa Collembola and Testacea on species level. For both taxa the number of species was highest on the most southern site. Diversity showed the same pattern being generally higher on the two southern sites than on the northern sites. According to the BIOLOG method, functional diversity of the microflora was particularly low at the Skogaby site (Southern Sweden). This was related to a very low decomposition rate of all carbon sources tested.

Below a certain threshold a strong correlation between water content of the organic layer and invertebrate abundance were regularly found for most taxa. Humidity often explained both within-site as well as between-site variation. No significant direct correlation of the soil fauna with temperature was found. However, temperature most probably influences the soil community via interactions with other factors.

A thick organic layer in combination with unexpectedly high CO_2-elevation and microbial biomass at the Northern Swedish site pointed to an enormous microbial potential, probably depressed by adverse environmental conditions. Alteration in climate may be of particular significance at this site.

While fungal biomass and CO_2-evolution increased from South to North abundance of Enchytraeidae showed the inverse pattern. These significant negative correlations hint to a release of grazing pressure for the more northern fungal communities. Testate amoeba, as another group of microbial grazers also showed significant negative correlations with CO_2-

evolution as well as with microbial biomass. A positive correlation of Testacea as well as Nematoda with the metabolic quotient qCO_2 (CO_2-evolution per unit microbial biomass) however indicates that these taxa enhance microbial turnover. Microarthropods also seemed to have a stimulating effect on microbial turnover (significant positive correlations with CO_2-evolution and frequency of dividing bacterial cells).

Several of these hypotheses were confirmed in an accompanying microcosm experiment. Low microarthropod abundance resulted in a strong increase in Enchytraeidae abundance. This was possibly due to reduced competition for the food source fungi. Thus assuming a strong and rather direct relationship between fungal biomass and abundance of Enchytraeidae matches the picture found in the field as well as in the laboratory. In addition, microarthropods increased bacterial performance while decreasing microbial biomass.

The transect sites have also been selected to form a gradient in terms of nitrogen supply. The mineral nitrogen content of the organic layer strongly increased from North to South, i.e. it paralleled the N-input. Increasing N availability resulted in a pronounced increase of microbial nitrogen content from North to South. In addition, Nematoda, Enchytraeidae and Testacea were positively correlated to the mineral N content of the substrate. Positive correlation of microbial N content with both Nematoda and Enchytraeidae suggests that alterations in the N-status of the microflora feed up to higher levels of the food web.

The soil biotic data of the sites were incorporated into a food web model in a co-operation with Peter de Ruiter (Utrecht, NL) (De Ruiter P, Neutel A-M & Moore JC (1994) in: TREE 9, 378-383). Preliminary calculations revealed a contribution of soil biota (fauna + microflora) to C and N mineralisation ranging from 2200 to 2600 kg C ha^{-1} yr^{-1} and from 31 to 50 kg N ha^{-1} yr^{-1}. The Waldstein site in Germany showed the highest performance. The microflora accounted for 89-95 % of total C-mineralisation and for 70-80 % of total N-mineralisation at all sites. Testate amoeba accounted for 3-6 % of C-mineralisation and up to 30 % of N mineralisation. The contribution of the mesofauna to the mineralisation of carbon and nitrogen is relatively small, and that of the Nematoda is even smaller. However, taxon-specific estimates are difficult to evaluate and have to be interpreted with great care.

Most interestingly, the N mineralisation of the soil biota does not follow the transect gradient in terms of climate and N supply. This might either point to the buffer capacity of the soil food web or might hint at a nutrient saturation of the decomposer community.

PART B

Reporting period : February 1st 1998 – January 31st 1999.

Partner : 10 Institute of Terrestrial Ecology
Principal Investigator : Dr A.F.Harrison and A.P.Rowland
Scientific Staff : Dr D.D.Harkness (NERC Radiocarbon Lab), JS Garnett
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Question I :

Objectives for the reporting period

Obtain and collate all the data for all the forest sites. Complete calculation of the soil carbon and nitrogen pools and calculate the carbon and nitrogen fluxes for all sites. Integrate data with other site data on climate, forest productivity and eddy covariance data (for the sites for which it is available). Make information available to others within the consortium, particularly the modellers for model development and validation.

Question II :

No longer relevant

Question III :

Are there any particular problems ? Is your part of the project on schedule ?

Compilation, analysis and interpretation of the data are on schedule. Basic data have been made available to the data collator and modeller. The only problem has been that data on forest productivity has not been made available, so relationships between soil C and N fluxes and forest productivity could not be determined. If the data does become available, the relationships can easily be assessed at a later stage and the information included in publications.

The project has been highly successful, for the methodology has proven to be more sensitive, and far more detailed annual soil C and N flux estimates have been derived, than expected at the outset.

Question IV:

Main results obtained :Methodology, results, discussion and conclusions.

Methodology:

All ten sites [Aheden, Skogaby, Gribskov, Waldstein, Schacht, Nacetin, Aubure (spruce), Aubure (beech), Monte-di Mezzo and Collelongo] have been sampled. Nine soil cores were taken from each and split for analysis into 4 layers (L+F, 0-5cm, 5-10 cm and 10-20 cm). Carbon and nitrogen contents were determined by a modified Tinsley oxidation and acid digestion / continuous-flow colorimetric analyses respectively. Soil carbon mean residence times (MRT) in years were derived, by modelling, from ^{14}C-bomb enrichment values determined for each soil layer by accelerator mass spectrometry. Carbon and nitrogen fluxes (annual turnover) have been computed by dividing the carbon and nitrogen pools in the soil by the soil carbon MRTs. Estimates of carbon and nitrogen pools and fluxes in the soils of the forests across the transect have been related to various site factors, such as latitude, rainfall, mean annual temperature, total pollutant N deposition and tree species affects.

Results, discussion and conclusions:

Total organic carbon pools to 20 cm depth in the mineral soil varied from 31.6 to 103.8 $t.C.ha^{-1}$, whilst the nitrogen pools varied from 1.3 to 7.6 $t.N.ha^{-1}$.

Total organic carbon pools were strongly curvilinearly related $p<0.01$) to annual rainfall to the forest, with the peak at c 950 mm.

Total nitrogen pools were significantly negatively related ($p<0.01$) to forest latitude, but the C:N ratio of the whole soil profile to 20 cm depth varying from 10.2 to 27.2 was positively related ($p<0.01$) to latitude.

Mean residence times (MRTs) of carbon in the L+F, 0-5, 5-10 and 10-20 cm soil layers in all CANIF forests can be modelled from ^{14}C-bomb concentrations in the respective layers. For each of the forest sites, one model solution only fitted its 'set' of the measured ^{14}C concentrations in the soil layers in the year of sampling, ie the model outputs were unambiguous.

MRTs for the L+F layer carbon are fairly uniform at between 4 to 6 years across all sites.

MRTs for carbon in the 0-5 cm layer range from 35 to 340 years, and show a positive correlation with forest latitude ($p<0.05$) and a negative correlation with both rainfall input ($p<0.05$) and mean annual temperature ($p<0.05$) of the forests.

MRTs for carbon in the 0-5 cm layer is strongly correlated with total N deposition ($p<0.01$) across all sites. The MRTs of 0-5 cm carbon of the sites Gribskov, Aubure P, Aubure F, Monte di Mezzo and Collelongo with N deposition of 10-15 $kg.N.ha^{-1}.yr^{-1}$ range from 35 to 80 years, whereas those for Skogaby, Nacetin, Schacht and Waldstein receiving N inputs of 15-20 $kg.N.ha^{-1}.yr^{-1}$ vary from 130-260 years. The average MRT for the latter group of sites is more than twice that of the former group, suggesting even small increases in N deposition may have large effects on carbon turnover in soils.

MRTs for carbon in 5-10 cm layer range from 10-690 years and indicate, in some forests, substantial inputs of rapidly turning over carbon from roots to soil organic matter in this layer.

These MRTs have been used to calculate annual C and N fluxes for each of the soil layers, by dividing the pool of C or N by the mean residence time of the soil carbon.

Total annual C and N fluxes for the biologically active part of the soil profiles range from c 1.4 to 5.1 $t.C.ha^{-1}.yr^{-1}$ and 46 to 416 $kg.N.ha^{-1}yr^{-1}$ respectively. The C flux of 2.1 $t.C.ha^{-1}.yr^{-1}$ for one site, Collelongo, closely agrees with the estimate of 2.03

$t.C.ha^{-1}.yr^{-1}$ derived from other studies at that site, giving validity to the flux estimates derived from the ^{14}C-bomb method.

With three out of four of the spruce/beech 'pairs' of sites at similar latitudes, the spruce forest turns over annually more carbon and nitrogen in the soil than its beech counterpart.

Total C and N fluxes for the whole active soil profile at all sites were only poorly correlated with site variables, such as latitude, altitude, mean annual temperature and rainfall.

Significant relationships with site variables were however found, when C and N fluxes were examined 'at the individual soil layer level', indicating fluxes in each of the soil layers are governed by different factors.

C and N fluxes in the L+F layer along the transect range from 0.66 to 3.07 $t.C.ha^{-1}.yr^{-1}$ and 18 to 112 $kg.N.ha^{-1}.yr^{-1}$ respectively.

Both the C and N fluxes in the L+F layer are related negatively to annual rainfall ($p<0.01$) input to the forests and N fluxes are also related negatively to the C:N ratio ($p<0.05$) of the L+F layer material.

An average of 81% of C in the annual above-ground litter input is decomposed in situ in the L+F layer and an average of 19% enters the soil mineral layers.

C and N flux in the 0-5 cm layer ranged from 0.03 to 0.49 $t.ha^{-1}.yr^{-1}$ and 1.03 to 29.8 $kg.ha^{-1}.yr^{-1}$ respectively. These C and N fluxes are related negatively and exponentially to forest latitude ($p<0.01$) and related curvilinearly to total N deposition ($p<0.01$), but N fluxes are also related negatively to the C:N ratio ($p<0.01$) of the 0-5 cm soil.

The reduction in C and N fluxes with increasing latitude and with soil C:N ratio concurs with results from other studies of latitudinal transects of forests.

The relationship of C and N fluxes in the 0-5 cm layer with total N deposition suggests that N deposition is retarding C and N cycling rate in the four sites with the highest inputs. The critical load for N deposition with respect to soil organic matter turnover appears to be between 10 - 15 $kg.N.ha^{-1}.yr^{-1}$.

Estimates of root-derived carbon inputs, mainly to the 5-10 cm layer of the mineral soil, range from 0.02 to 2.83 $t.C.ha^{-1}.yr^{-1}$. These C fluxes derived from root carbon inputs to soil are positively related to forest mean annual temperature ($p<0.01$) and the total N fluxes ($p<0.01$) in the mineral part of the soil profile.

The ^{14}C-bomb methodology has considerable potential i) for investigations of C and N fluxes, as demonstrated by the CANIF project application, in forest and other ecosystems, and ii) for enabling the development and application of much-improved dynamic process-based soil computer models or soils subroutines in ecosystem models.

Question V:
List of publications arising from the project

None at this stage. A full analysis of the research has only just been completed. However one publication is to be submitted shortly :
Harrison, AF, Harkness, DD, Rowland, AP, Garnett, JS and Bacon, PJ (1999?) Annual carbon and nitrogen fluxes in soils along the European forest transect. Chap 11. In Carbon and Nitrogen Cycling in Forests. E-D Schulze (ed). Ecological Studies series, Springer Verlag, Berlin.
Several others are planned.

Signature of Partner

Anthony Harrison

Dr A F Harrison date 20th February, 1999.

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 1. February 1998 to 31. January 1999

Partner: No. 08. Danish Forest and Landscape Research Institute, DFLRI

Principal Investigator: Dr. Bjørn R. Andersen

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I. OBJECTIVES FOR THE REPORTING PERIOD:

Completion of data base containing meteorological and stand data for the CANIF sites followed by hydrological modelling for all sites in co-operation with Dutch partner. Ending the sampling programme and analysis (N compounds, DOC, base cations) of soil water samples from the stands along the CANIF gradient. Include measurements of TON and TOC from a limited number of Danish forest stands selected due to a relatively heavy atmospheric N input (securing mutual benefits to an ongoing Nordic project). Estimation of N fluxes in the soil compartment. Prepare manuscript for a chapter in the planned book covering the entire project periods of NIPHYS and CANIF

II. OBJECTIVES FOR THE NEXT PERIOD:

(Not relevant as the period covered herein is the final project period.)

III. Are there any particular problems ? Is your part of the project on schedule ?

Yet again we had periods with prolonged dry soil conditions at several sites, including the 2 Italian sites. Consequently, the overall sampling program has yielded substantially fewer soil water samples than anticipated. This, unfortunately, decrease the strength of our conclusions as they are based on relatively small data sets. The fewer samples also resulted in less work, and we finalised our part without spending the full amount given in the original budget for the entire project period.

The completion of the common data base containing the CANIF data was too late to leave time for comparison between hydrological model estimates.
Analyses on samples from supplemental Danish stands with heavy atmospheric N deposition were delayed and can not be reported on before the co-operating projects have caught up with the delay. The CANIF project thus did not co-fund any work going on at these sites as otherwise planned for 1998.
The manuscript for a book chapter covering the entire project periods of NIPHYS and CANIF is in preparation.

IV. MAIN RESULTS OBTAINED:

RESULTS. We still observe that organically bound N is a significant part of total N in soil solutions from the investigated stands, at least during some periods. Our measurements can not demonstrate that there generally is a direct relationship between concentrations of DON (dissolved organic N) and DOC (dissolved organic C).

DISCUSSION. It is probably because many other processes than those associated with N cycling influence the concentrations of DOC that we can not demonstrate a direct link between measured C and N concentrations.

CONCLUSIONS. Our original hypothesis on the significance of DON compared to inorganic N compounds was supported by our measurements throughout the project. Even though the included forest stands were not demonstrated to leak large amounts of nitrogen (in any form) compared to reported ranges from other land-use forms, we must still recommend DON data to be included when assessing N cycling and/or leaching to systems outside forest ecosystems. Of special relevance to possible future implementations of our findings in local or regional policy-making, is the necessity to address the possibility of a significant N leaching by organically bound nitrogen into ground water sources normally thought to be better protected by forest cover than other land-use forms.

V. List of Publications arising from the project (include copies):
In prep.: Andersen, BR and Gundersen, P (1999). N and C interactions of soil water and solid phase. Manuscript for chapter in special edition of Ecological Studies (Springer Verlag) containing the final reports of the individual partners.

Signature of Partner:

Bjørn R. Andersen

Bjørn R. Andersen
Date: March 21st, 1999

DETAILED REPORT OF THE INDIVIDUAL PARTNERS

Reporting period: 01.02-98 – 31.01.99

Partner: Wageningen Agricultural University
Nature Conservation and Plant Ecology Group

Principal Investigator: Professor Dr. F. Berendse

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I. OBJECTIVES FOR THE REPORTING PERIOD:

- to finalise parameter estimation
- to finalise the data bank with data needed for the model for all sites
- to perform a sensitivity analysis to determine the relative impacts of the different
- processes and parameters
- to validate the model using data on carbon and nitrogen pools, N productivity and N mineralisation
- to validate the model using data from a ^{15}N pulse labelling experiment in a 15-year old spruce stand
- to make simulations for inter-site comparisons
- to make simulations for tree species comparisons
- to analyse the effects for climatic change on ecosystem variables

II. OBJECTIVES FOR THE NEXT PERIOD:

No longer relevant

III. Are there any particular problems? Is your part of the project on schedule?

The validation of the model by using data of a ^{15}N labelling experiment will not be done because these data did not become available.
Also the late stage at which the data of the sites became available make that the model analyses and comparisons will not be that full as first planned.

IV. MAIN RESULTS OBTAINED: *METHOLOGY, RESULTS, DISCUSSION, CONCLUSIONS* (use other pages as necessary but preferably no more than 2)

For the main sites of the CANIF project, data are collected in a databank. The collected data represent the total ecosystem and include data on the tree species, field layer vegetation, soil organic matter, soil data, soil chemistry data, hydrology as well as climatic conditions and deposition data. The databank covers in principle the years 1993 through 1998, although also information from earlier years is included when available. Since all sites are not similarly detailed investigated, the data availability varies between the sites. Data on abundance and diversity of soil fauna and soil fungi are also part of the databank. The data of the databank will become available on CD-rom as part of the end product of the project, the CANIF book.

The model analysis are in the final stage and conclusions will be reported in the modelling chapter of the book.

V. List of Publications arising from the project (include copies):

T Persson, H van Oene, T Harrison, P Karlsson 1999. Experimental sites in the NIPHYS/CANIF project. Chapter 2 in: D.-E. Schulze et al. (eds.) Carbon and nitrogen cycling in European forest ecosystems.

H van Oene, F. Berendse and others 1999. Model analyses and predictions for the CANIF sites. Chapter 21 in: D.-E. Schulze et al. (eds.) Carbon and nitrogen cycling in European forest ecosystems.

Signature of Partner:

Date: 15-3-1999

CANIF
Reporting period: Feb. 1. 1998 to Jan. 31. 1999
Partner: Czech Geological Survey
Principal Investigator: Tomas Paces
Scientific Staff: F. Buzek, M. Novak, J. Cerny, and H. Groscheova
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Question I

Objectives for the reporting period:

1) Sampling at four Czech sites: Cervena Jama (spruce), Nacetin (spruce), Jezeri (beech) and Salacova Lhota (spruce). Measurement of seasonal variation in DON and NH_4 content and ^{15}N fractionation. Carbon isotope fractionation in soil organic carbon. Determination of relationships between the humic substances and physical particles of soils.

2) Speciation of S isotopes in inorganic sulfate, ester-sulfate and carbon-bonded sulfur in the soils of CANIF sites, i.e. along the longitudinal transect across Europe. A combined $\delta^{13}C$, $\delta\,^{15}N$ and $\delta^{34}S$ analysis of vertical profiles through these soils. Monthly $\delta^{34}S$ monitoring at the two Czech study sites - Jezeri and Salacova Lhota (bulk deposition, spruce canopy throughfall and surface discharge).

Question II:

No longer relevant.

Question III:

There have been no problems met during this research. The project is on schedule. Final presentation of the results in a form of a book will be delayed by one and half month because monitoring of the nitrogen and sulfur fluxes ended on Oct. 31. 1998 (end of the hydrological year). Evaluation of the data takes about 2 months so that their final presentation will be ready for publication by March 15.

Question IV:

Main results obtained:

Methodology

Sampling at four forest plots Cervena Jama (spruce), Nacetin (spruce), Jezeri (beech) and Salacova Lhota (spruce) in the Czech Republic has proceeded monthly with the same method during the period 1996 - 1998. The samples included bulk precipitation, throughfall under spruce, beech and birch canopy stem flow under beech, soil solutions from depth of 30, 60 and 90 cm and runoff (surface discharge) from the catchments Jezeri and Salacova Lhota. Budget of nitrogen species and sulfur were evaluated in the two catchments to serve as the background for interpretation of isotopic mass balance of nitrogen and impact of acidic sulfur deposition on the nitrogen and carbon cycling in the forest ecosystems.

Soils were sampled and characterized by their physical, physicochemical and chemical properties. Nitrogen, sulfur and carbon organic and inorganic species were characterized by their isotopic composition ($\delta^{15}N\text{-}NO_3$, $\delta^{15}N\text{-}NH_4$, $\delta^{15}N$-DON, $\delta^{34}S\text{-}SO_4$ $\delta^{13}C\text{-}CO_3$ and δ^{13}DOC).

Soil samples along the transect through Europe were taken from following sites: : Aheden, Skogaby, Nacetin, Aubure and Monte de Mezzo.. They are the identical to those dated by means of ^{14}C (CANIF, Harrison et al.). Five soil pits were sampled within each stand. The samples come from depths 0-5, 5-10 and 10-20 cm. Organic forest floor was not analyzed. Three replicate samples (< 2 mm) were combined prior to isotope analysis and two more samples were analyzed separately. Carbon and nitrogen concentrations were determined on a Fisons 1108 Elemental Analyzer, while those of S on a LECO SC-132 Sulfur Analyzer. The reproducibility was better than 5 % for all three elements. Stable isotope ratios were measured on a Finnigan MAT 251 mass spectrometer coupled with the Fisons 1108 Elemental Analyzer (C and N). Sulfur was extracted from soil as $BaSO_4$ and converted to SO_2 in a vacuum line. Inorganic free plus adsorbed sulfate S was extracted from soil using 16.1 mM solution of $NaH_2PO_4.H_2O$. Ester sulfate S was extracted by HI reduction according to Johnson and Nishita (1952). Carbon bonded S was extracted by Eschka's mixture as the last step of the sequential extraction. Sulfur was precipitated from waters as $BaSO_4$. Stable isotope compositions were expressed in the delta notation (i.e., $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) as an ‰ deviation of the heavy-to-light isotope abundance ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$) in the sample from a standard. The standards used were PDB, atmospheric N_2 and CDT for C, N, and S, respectively. In each case, the reproducibility was better than 0.3 ‰.

Results of the objective 1:

Monitoring of fluxes of nitrogen and sulfur

The fluxes of sulfur and nitrogen in the Jezeri and Salacova Lhota catchments are given in the following table (Average for the whole CANIF period 1996 to 1998):

	kg/ha/yr			
	$N\text{-}NH_4$	$N\text{-}NO_3$	N_{total}	$S\text{-}SO_4$
		X -16 Jezeri		
Bulk precipitation	8.2	6.1	14.3	14.2
Throughfall Beech	8.3	7.3	15.6	19.5
Stemflow Beech	1.5	1.1	2.6	4.2
Throughfall Spruce	15.7	12.7	28.4	61.1
Runoff	0.04	3.8	3.8	52.3
		X - 8 Salacova Lhota		
Bulk precipitation	7.2	4.3	11.4	9.6
Throughfall Spruce	9.7	6.5	16.2	26.7
Runoff	0	0.6	0.6	7.3

The mass budget of nitrogen and sulfur during the CANIF observation period 1996-1998 is given in (kg/ha/yr)

	N-total		S-SO4	
Catchment	X -16	X - 8	X -16	X - 8
Bulk - Runoff	10.5	10.8	-38.1	2.4
Throughfall Beech - Runoff	14.4		-28.6	
Throughfall Spruce - Runoff	24.6	15.6	8.9	19.5
Weighted input* – Runoff	13		-30	

*The weighted input is atmospheric deposition calculated as the average of throughfall under spruce, beech, birch and deposition on grass weighted by the area of the biological cover in the catchment X-16 (Jezeri).

The characteristic flux ratios S/N are:

	X -16 Jezeri	X - 8 Salacova Lhota
S/N (Bulk precipitation)	0.99	0.84
S/N (Throughfall Beech)	1.3	
S/N (Throughfall Spruce)	2.2	1.6
S/N (Runoff)	13.7	12

Discussion of the flux measurements.

In spite of the differences in the sulfur fluxes, the ratios S/N in the fluxes are rather similar in acidified and less acidified catchment and in spruce and beech stand. We have tried to clarify the question whether the differences in the input and fixation or leaching of acidifying sulfur compounds in forest ecosystem influence fluxes of nitrogen species and nitrogen balance. It is assumed that the input of nitrogen species to the forest ecosystem is best characterized by bulk samples of precipitation. If this is true, than the input of total nitrogen in Jezeri is 14 kg/ha/yr and in Salacova Lhota it is 11 kg/ha/yr. Nitrogen leaching from the Jezeri catchment is elevated to 3.8 kg/ha/yr. The leaching from the less acidified catchment Salacova Lhota is very small (0.6 kg/ha/yr). Biological recycling of nitrogen is reflected by the difference between the flux due to bulk precipitation and flux due to throughfall under canopy. The difference in nitrogen (N-total) throughfall and bulk precipitation in kg/ha/yr is shown in the following table:

	X -16 Jezeri	X - 8 Salacova Lhota
Throughfall + Stemflow (Beech) - Bulk	3.8	
Throughfall (Spruce) - Bulk	14.0	4.8

The biological cycling (or production of N by needles) seems to be faster in the acidified spruce stand at Cervena Jama (14 kg/ha/yr) than in the less acidified stand at Salacova Lhota (4.8 kg/ha/yr). The cycling seems to be slower also in the beech stand (3.8 kg/ha/yr). These values, however, include not only the biologically recycled nitrogen but also the contribution by dry deposition of NO_x.

Conclusion from monitoring of fluxes and mass budgets

Extreme acidification of forest soils by atmospheric deposition of acidifying compounds of sulfur (SO_2 and H_2SO_4) affects the biogeochemical cycling of nitrogen in spruce stand in the Jezeri catchment. Sulfur acidifying compounds do not induced any observable changes in the cycling of the elements in the beech stand in the Jezeri catchment.

Our results indicate that high input of sulfur acidifying compounds increases leaching of nitrogen from spruce stand. The leaching takes place not only within the root zone (indicated by runoff) but also through stomata of needles (indicated by the difference between bulk precipitation and throughfall of nitrogen).

The fact, that the cycling of nitrogen seems to be faster in the acidified spruce stand is surprising because spruce damaged by acidification should not fix as much nitrogen as it does the undamaged spruce or beech. This phenomenon deserves a further research. Dry deposition of NO_x may explain partly the observed differences between the nitrogen flux due to bulk precipitation and throughfall.

The proportions of total nitrogen and sulfur in individual fluxes in acidified and less acidified spruce stand and in spruce and beech stand are similar. Therefore, the ratio S/N is probably not a suitable parameter to study the influence of sulfur deposition on nitrogen cycling.

Discussion of internal processes in the spruce manifested by stable isotopes ^{15}N and ^{13}C.

Soils in the spruce stands at Nacetin, Cervena Jama and Salacova Lhota and soils in the beech stand in Jezeri are characterized by their content of N, C and S as well as the physical properties and total chemical composition. The acidified soils at Nacetin exhibit a specific trend in the mobilization of organic matter. The ratio C/N in O soil horizon increases, the ratio reaches its maximum at the O/A boarder and with depth it starts to decrease. The increased mobilization of soil organic matter and the changes in the accumulation of organic C and N in soil are reflected by the isotopic composition of total soil carbon and nitrogen as well as by the isotopic composition of the individual fractions of soil organic matter. The isotopic fractionation of ^{13}C in C_{tot} of soil profiles at all studied localities is very similar. $\delta^{13}C$ starts from -27 ‰ in litter and shifts to more positive values of about -25.5 ‰ in mineral layer at B/C boundary. On the other hand, $\delta^{13}C$ values are different in humic acids and in insoluble humin at different sites. The strongly acidified soils exhibit a very constant $\delta^{13}C$ in humic acids equal to the original litter (-27 ‰). This indicates that organics in O and A soil horizons are leached and the dissolved organic matter moves to the deeper horizons of soil profile carrying with them the isotopic signature. Insoluble humin contains heavier carbon (-25.5 ‰) in A-horizon. At the A/B boundary, $\delta^{13}C$ in humin returns to the initial low value similar to the value in litter (-27 ‰). Deeper, in the B and C-horizon, carbon of humin is isotopicaly heavier again and approaches to the isotopic composition of C_{tot} (-25 ‰). Two processes can explain this fractionation. Either the initial organic carbon is not mineralized and it is transported in dissolved form to the deeper soil horizons, or the mineralization of lignin fractions has been interrupted by acidification and the isotopically more negative carbon in lignin accumulates in the upper soil horizons. We detected the accumulation of lignin in A- horizons in both the acidified spruce stands at Nacetin and Cervena Jama.

The isotopic composition of nitrogen was measured in dissolved organic nitrogen (DON), total nitrogen (N_{tot}) and in mineralized nitrogen (N_{min}). The isotopic composition of DON is derived from litter and possibly from roots of spruce. While $\delta^{15}N$ in litter is -4 ‰, it is even more negative in soil DON (-5 to -6 ‰). There are no significant differences in DON isotopic composition between our monitored sites.

Significant difference between the sites was found in the isotopic fractionation of N_{min}. N_{min} is probably more sensitive to the dynamic transformations of soil nitrogen. Simultaneous measurements of isotopic composition of atmospheric nitrogen in bulk precipitation and in throughfall with the measurements of soils solutions from lysimeters enable us to distinguish $\delta^{15}N$-NO_3 from atmosphere and $\delta^{15}N$-NO_3 produced biologically within soil. Acidified spruce stands that loose N_{min} (in nitrate form) contain higher proportion of atmospheric nitrates in runoff (up 10 to 30%). The catchment Salacova Lhota with lower leaching rate contains negligible amount of nitrate of atmospheric origin. Atmospheric ammonium ions are fixed in the mineral soil horizons (B, C). Ammonium in organic horizons (O, A) is of organic not of atmospheric origin. We have observed an increasing replacement of organic nitrogen with atmospheric nitrogen with depth. The atmospheric nitrogen prevails in C-horizon. The contribution of this nitrogen to the production of soil nitrates is, however, negligibly small.

Conclusions from isotopic research:

The acidified forest soils at Nacetin exhibit a specific trend in the mobilization of organic matter. The ratio C/N in O-horizon increases, the ratio reaches its maximum at the O/A boarder and with depth it starts to decrease. We are not able to explain this unusual trend.

The increased mobilization of soil organic matter and the changes in the accumulation of organic C and N in soil are reflected by the isotopic composition of total soil carbon and nitrogen as well as by the isotopic composition of the individual fractions of soil organic matter.

The fractionation of ^{13}C in C_{tot} of soil profiles at all studied localities is very similar. On the other hand, $\delta^{13}C$ in humic acids and insoluble humin is different. The strongly acidified soils exhibit a very constant $\delta^{13}C$ in humic acids equal to the original litter. This indicates that the organics in O and A soil horizon are leached and the dissolved organic matter moves to the deeper horizons of soil profile.

A significant difference between the sites is found in the isotopic fractionation of N_{min}.

Most of the leached nitrates are of biologic origin. However, acidified spruce stands contain higher proportion of atmospheric nitrates in runoff (up 10 to 30%). The less acidified catchment Salacova Lhota contains negligible amount of nitrate of atmospheric origin.

We have observed a replacement of organic soil nitrogen by atmospheric nitrogen with depth.

Results and Discussion of the objective 2:

The transect through Europe includes following 5 sites: Aheden, Skogaby, Nacetin, Aubure and Monte de Mezzo . All five sites showed an increase in $\delta^{13}C$ and $\delta^{15}N$ from the top to the middle level, four out of five sites exhibited a similar positive $\delta^{34}S$ shift. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were never lower in the deepest level compared to the top level, except for $\delta^{34}S$ at Monte de Mezzo where spruce was planted quite recently (1958) on agricultural land. The mean magnitude of the isotope shifts across sites was 0.9, 4.2 and 1.6 o/oo for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, respectively. The positive $\delta^{13}C$ and $\delta^{15}N$ shifts have been both known since the 1970s. Progressively higher $\delta^{13}C$ and $\delta^{15}N$ ratios at greater depth are correlated with increasing age and degree of decay of organic matter. C and N isotope fractionation related to mineralization of organic matter appears to be the ultimate cause of the observed patterns. In spite of that, a number of complementary mechanisms have been considered e.g., change in atmospheric CO_2 signature due to fossil fuel burning and plant uptake of low ^{15}N in combination with redeposition of the litterfall on the soil surface. We suggest that mineralization of soil organic matter is an isotope selective process for all three elements: C, N and S. Isotopically light products of decomposition are preferentially removed, while higher $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ residuals tend to remain *in situ*. At the five CANIF sites we found no correlation between the magnitude of the isotope shifts with mean temperature or nutrient availability. Interestingly, the vertical isotope trends existed regardless of a complex site-to-site pattern of C/N and C/S mass ratios.

Organic S constituted more than 50 % of total S throughout all profiles, except for the deepest analyzed level at Skogaby (47 %) and Aubure (11 %). With an increasing depth, the percentage of inorganic sulfate S increased at the expense of organic S. At sites where total soil S became isotopically heavier with increasing depth (i.e., has higher $\delta^{34}S$), $\delta^{34}S_{organic} > \delta^{34}S_{inorganic}$. Such sites include Aheden, Skogaby, Nacetin and Aubure. Moreover, $\delta^{34}S$ of atmospheric input at all sites was higher than $\delta^{34}S_{total}$ of the topmost analyzed soil layer. These data are consistent with the following scenario: Deeper soil layers contain older and more decomposed organic matter. As mineralization proceeds, newly formed "secondary" sulfate is enriched in low-$\delta^{34}S$ sulfur. Sulfur of organic compounds remaining *in situ* becomes isotopically heavier. In such way, soil water sulfate collected by lysimeters will contain isotopically lighter S than atmospheric deposition, while total soil sulfate will be isotopically lighter than organic S from the same depth.

Conclusions:

Mineralization of soil organic matter in spruce forests is an isotopically selective process for all three elements under study: C, N and S. Mineralization discriminates against the heavier isotopes (^{13}C, ^{15}N and ^{34}S).

Organic sulfur constitutes more than 50 % of total soil S at most sites. The vertical soil profiles are generally characterized by a $\delta^{34}S_{organic} > \delta^{34}S_{inorganic}$ pattern. Such pattern results from dynamic conversions between inorganic and organic S pools under a wide range of mean annual temperatures and pollution levels.

Elevated atmospheric S inputs result in accumulation of sulfur largely in the humus layer in an organic form.

Question V:

List of publications arising from the project:

Paces T., Cerny J., Havel M., Krejci R. and Pavcesova E. (1997) Acidification during dry, rainy and snowy events in the Czech republic. In: Acid Snow and Rain, p. 128-133, Proc. Int. Congr. of Acid Snow and Rain, Niigata University, Niigata.

Buzek F., Cerny J. and Paces T. (1998) The behavior of nitrogen isotopes in acidified forest soils in the Czech Republic. Water, Air, and Soil Pollution 105, 155-164, Kluwer Acad. Publ.

Novak M. (1998) $\delta^{34}S$ dynamics in the system bedrock - soil - runoff - atmosphere. In Proceedings of WRI-9 (eds. Arehart G.B. and Hulston J.R.), p. 67-70, Balkema, Rotterdam

Groscheova H., Novak M., Havel M. and Cerny J. (1998) Effect of altitude and tree species on $\delta^{34}S$ of deposited sulfur (Jezeri catchment, Czech Republic). *Water Air Soil Pollut.* **105**, 287-295.

Novak M., Prechova E. and Jackova I. (1998) Sulfur isotopes as a tracer in small catchment studies. - Challenges to Chemical Geology, Refereed Papers from the 10th Meeting of the Association of European Geological Societies, Carlsbad, Czech Republic, September 1997. Czech Geological Survey, Prague, pp. 187-198.

Novak M., Kirchner J.W., Groscheova H., Havel M., Cerny J., Krejci R. and Buzek F. (1999) Sulfur isotope dynamics in two Central European watersheds affected by high atmospheric deposition of SO_x (submitted for publication).

Novak M., Buzek F., Harrison T., Prechova E. and Jackova I.: $\delta^{34}S$ speciation in five European spruce forest soils in relation to vertical $\delta^{13}C$ and $\delta^{15}N$ ratio profiles (submitted for publication).