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Pathways of atmospherically deposited nitrogen in two mountain ecosystems in central Switzerland: An experimental and model-based study using the ^{15}N isotope

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Summary

During the last few decades, human activities have increased the production of biologically reactive nitrogen (N) through intensive agriculture and fossil fuel combustion. Given the N-limited nature of most temperate forests and natural grasslands, there is a large potential for reactive nitrogen accumulation in those ecosystems with an N residence time of years to centuries, thus causing a slow eutrophication. Therefore, the overall aim of this thesis was to investigate how mountain ecosystems are affected by elevated N deposition. The main goals were (1) to understand the mechanisms regulating N retention by the identification of single ecosystem pools to which deposited N is incorporated, (2) the quantification of the N pathways through the ecosystem and (3) the dynamics of N in the short and in the longer-term. The pathways of deposited N were followed by means of the ^{15}N isotope ($^{15}NO_3^-$ and $^{15}NH_4^+$). The experiments were conducted at the Alptal valley research site, at the northern edge of the Alps of central Switzerland, at 1200 m a.s.l. in a landscape dominated by Norway spruce (*Picea abies*) forest and (nowadays often abandoned) litter meadows. We studied the N pathways at different spatial and temporal scales, ranging from the plot scale of 2.25 m^2 up to the catchment scale of 1600 m^2 and from short term (hours, days or weeks) to longer-term intervals (one year), respectively. A special focus was set on the soil pool by measuring the partitioning of ^{15}N into different biochemical soil fractions. In addition, the uptake of deposited N by moss species was assessed. To gain insight into the role of ^{15}N retention of a forest ecosystem in the longer-term, we used a model of the N, C and water cycles, TRACE. This model was adapted, calibrated and validated for the Alptal site. We used the model for assessing some implications of future deposition scenarios at the Alptal site over the coming 45 years.

At the plot scale (Chapter 2), pulses of $^{15}NH_4^+$ and NO_3^- were applied separately as a single pulse to a mountain forest or a nearby meadow ecosystem, and several ecosystem pools were sampled at short to longer-term intervals (from a few hours to one year). Shortly after the tracer application, both ecosystems had the largest recovery in total extractable N and microbial N. Later on, most of the tracer was retained in the soil pool as immobilised soil N, and was no longer available for plant N. While the extractable and microbial pools lost ^{15}N over time, a long-term increase in ^{15}N was measured in the roots.

At the catchment scale (Chapter 3), the flow of deposited inorganic N was traced in two Gleysol-dominated mountain catchments, in a mountain forest and a nearby meadow (each 1600 m^2) with $^{15}NO_3^-$ and $^{15}NH_4^+$. Both ecosystems had a high capacity to retain more than 50% of the added tracers. More NO_3^- than NH_4^+ tracer was retained, especially in the forest. Similar to the plot scale, the highest recovery was in the soil, particularly as immobilised soil N. Event-based runoff analyses showed an immediate response of ^{15}N in the runoff of both ecosystems, with sharp $^{15}NO_3^-$ concentration peaks corresponding to discharge peaks. After the cessation of the tracer application, the $^{15}NO_3^-$ leaching stopped, indicating that the tracer was either leached out of the system or immobilised in the soil or the biomass within a few weeks or month. However, the total NO_3^- leaching was still going on and showed a clear seasonality, with highest fluxes in late winter and in the spring, i.e. at snowmelt events.

At the plot scale and in the catchment experiment, the above-ground vegetation had a strong ^{15}N uptake, and especially the mosses played an important role. Therefore, a further experiment was conducted to investigate the N uptake of three moss species (*Sphagnum quinquefarium*, *Dicranum scoparium*, *Hylocomium splendens*) under different N deposition scenarios in a combined N and ^{15}N addition experiment (Chapter 4). The N addition resulted in a higher N concentration in the mosses and accordingly in a lower C:N ratio. The nitrogen uptake efficiency, especially the nitrate reductase, was reduced with increasing N deposition. Thus, with higher N deposition, nitrate reductase activity and tracer uptake were reduced. We conclude that with increasing N deposition, mosses reach an assimilation limit where the deposited N is increasingly leached through the moss layer into the soil rather than being taken up.

The ^{15}N experiments showed a high N retention over at least one year in both ecosystems. This leads to the conclusion that the increasing N concentration in the soil is likely to lead to a lower C:N ratio in the longer-term. To understand the interactions of different N retention pools in the longer-term, the model TRACE was used (Chapter 5). The attempt to use the model at the Alptal site showed how complex and difficult the adaptation of such models to other sites can be. Although the model application requires further improvements, it already contributed to a better understanding of the N cycling processes in the Alptal forest. It was especially possible to show that the increasing N deposition has an influence on the internal N status, as the N mineralization and the NO_3^- leaching increased and the C:N ratio decreased.

To conclude, the application of ^{15}N isotopes is a powerful tool for gaining insight into the N fluxes and transformations in a forest and in a meadow ecosystem. The combined approach of field experiments and modelling proved to be valuable to assess how mountain ecosystems might retain increasing N deposition. The results of this thesis help to improve our understanding about the N retention of mountain ecosystems with hindered soil permeability in the temperate climate zone.

Zusammenfassung

In den letzten Jahrzehnten ist die Produktion von biologisch reaktivem Stickstoff (N) durch menschliche Aktivitäten wie intensiver Landwirtschaft und Verbrennung fossiler Brennstoffe stark angestiegen. Der reaktive Stickstoff besitzt ein grosses Potential sich in N-limitierten temperaten Wäldern und Wiesen zu akkumulieren und weist eine Residenzzeit von Jahren bis Jahrhunderten auf. Diese Akkumulation führt zu einer langsamen Eutrophierung der Ökosysteme. Das Ziel dieser Dissertation war es zu untersuchen, ob Gebirgs-Ökosysteme durch erhöhte Stickstoff-Deposition beeinträchtigt werden. Die Themenschwerpunkte waren (1) die Retention des abgelagerten Stickstoffes in einzelnen Ökosystem-Kompartimenten zu bestimmen, (2) die Fliesswege des Stickstoffes im Ökosystem zu quantifizieren und (3) die kurzzeitige und längerfristige Dynamik des Stickstoffes zu erfassen.

Mittels dem ^{15}N Isotop ($^{15}NO_3^-$ und $^{15}NH_4^+$) wurden die Verlagerungswege des eingetragenen Stickstoffes verfolgt. Der Versuch wurde im Untersuchungsgebiet Alptal, in den Voralpen der Zentralschweiz, auf 1200 m.ü.M., in einem Fichtenwald (*Picea abies*) und einer nicht mehr genutzten Streuwiese durchgeführt. Die Stickstoffflüsse wurden auf verschiedenen räumlichen und zeitlichen Skalen untersucht, kurzzeitig (während Stunden, Tagen oder Wochen) in kleinen Flächen von 2.25 m^2 und längerfristig (ein Jahr) in kleinen Einzugsgebieten von 1600 m^2. Ein spezieller Schwerpunkt wurde auf das Boden-Kompartiment gelegt, welches in verschiedene biochemische Bodenfraktionen unterteilt wurde. Im Weiteren wurde die Retention von eingetragenem Stickstoff anhand von Moosen untersucht. Um Einblick in die längerfristige ^{15}N Aufnahme von Waldökosystemen zu erhalten, wurde das Modell TRACE, welches die N, C und die Wasserkreisläufe modelliert, angewendet. Das Modell wurde für das Untersuchungsgebiet angepasst, kalibriert und validiert. Ferner wurden mit dem Modell einige zukünftige Depositions-Szenarien im Alptal für die kommenden 45 Jahre durchgespielt.

In einem Fichtenwald und einer Streuwiese wurden auf kleinen Flächen (Kapitel 2) einzelne Gaben von $^{15}NH_4^+$ und NO_3^- getrennt ausgebracht und mehrere Ökosystem-Kompartimente in kurz- bis längerfristigen Zeitintervallen (von wenigen Stunden bis zu einem Jahr) beprobt. Kurz nach der Tracer-Anwendung hatten beide Ökosysteme die grösste Wiederfindung im

total extrahierbaren und im mikrobiellen N. Zu einem späteren Zeitpunkt war die grösste Wiederfindung im Boden-Kompartiment, der Stickstoff war im Boden immobilisiert. Während die extrahierbaren und mikrobiellen Kompartimente mit der Zeit eine Abnahme aufwiesen, war in den Wurzeln eine längerfristige Zunahme von ^{15}N zu verzeichnen.

Auf der Einzugsgebiets-Skala (Kapitel 3) wurde der Fluss vom abgelagerten inorganischem N in zwei Gleyboden dominierten Einzugsgebieten (je 1600 m^2), in einem Gebirgs-Fichtenwald und in einer Streuwiese mit $^{15}NO_3^-$ und $^{15}NH_4^+$ verfolgt. Beide Ökosysteme hatten die Kapazität mehr als 50% des zugegebenen Tracers aufzunehmen, wobei speziell im Wald mehr NO_3^- als NH_4^+ Tracer zurückgehalten wurde. Die höchste Wiederfindung war, wie auf den kleinen Flächen, im Boden, der Stickstoff war speziell im Boden immobilisiert. Die ereignisbezogenen Abfluss-Messungen zeigten eine sofortige Reaktion in den ^{15}N Messungen durch ausgeprägte $^{15}NO_3^-$ Konzentrations-Spitzen in beiden Ökosystemen, die den Abfluss-Spitzen entsprachen. Nach der Einstellung der Tracer-Anwendung stoppte die $^{15}NO_3^-$ Auswaschung. Diese Tatsache wies darauf hin, dass der Tracer entweder direkt ausgewaschen oder innerhalb weniger Wochen oder Monaten direkt im Boden oder in der Biomasse immobilisiert wurde. Im Gegensatz dazu war die totale NO_3^- Auswaschung durchgehend messbar und wies eine klare Saisonalität auf, mit den höchsten Spitzen bei der Schneeschmelze, d.h. im Spätwinter und im Frühling.

Im Experiment auf den kleinen Flächen und in den Einzugsgebieten hatte die oberflächliche Bodenvegetation eine starke ^{15}N Aufnahme, vor allem die Moose spielten eine wichtige Rolle. Deshalb wurde ein weiterer Versuch, eine kombiniertes N und ^{15}N Zugabe durchgeführt, um die N-Aufnahme von drei Moosarten (*Sphagnum quinquefarium*, *Dicranum scoparium*, *Hylocomium splendens*) bei unterschiedlicher N-Deposition zu untersuchen (Kapitel 4). Die N-Zugabe führte zu einer höheren N-Konzentration in den Moosen und entsprechend zu einem tieferen C:N Verhältnis. Die Stickstoff-Aufnahmefähigkeit, vor allem die Funktion der Nitrat-Reduktase, wurde mit steigender N-Deposition reduziert. Deshalb war auch die Nitrat-Reduktase-Aktivität und die Tracer-Aufnahme bei höherer N-Deposition reduziert. Wir schliessen daraus, dass die Moose bei steigender N-Deposition eine Assimilations-Grenze erreichen, bei welcher der eingetragene Stickstoff nicht mehr aufgenommen wird, sondern aus der Moosschicht in den Boden ausgewaschen wird.

Die ^{15}N Experimente zeigten für beide Ökosysteme während mindestens einem Jahr eine hohe N-Retention. Es lässt sich folgern, dass die steigende N-Konzentration im Boden längerfristig zu einem tieferen C:N Verhältnis führt. Um die Interaktion zwischen den verschiedenen N-Retentions-Kompartimenten über längere Zeitintervalle zu verstehen, wurde das Modell TRACE angewandt. Der Versuch dieses Modell im Untersuchungsgebiet Alptal anzuwenden hat gezeigt, wie komplex und problematisch die Implementierung von solchen Modellen aus anderen Gebieten sein kann. Obwohl der Einsatz des Modells noch weiter verbessert werden muss, hat die Anwendung bereits zu einem besseren Verständnis des N-Kreislaufes im Wald im Alptal beigetragen. Es konnte u.a. gezeigt werden, dass die steigende Stickstoff-Deposition einen Einfluss auf den N-Status des Ökosystems haben kann. Die N Mineralisierung und die NO_3^- Auswaschung waren zunehmend und das C:N Verhältnis wies sinkende Werte auf.

Abschliessend lässt sich feststellen, dass die Anwendung von ^{15}N Isotope methodisch geeignet ist, um einen vertieften Einblick in die N-Flüsse und Transformationen in einem Wald- und einem Wiesen-Ökosystem zu gewinnen. Der Ansatz von Feldversuchen in Kombination mit Modellierung hat sich als sehr wertvoll erwiesen um das Rückhaltevermögen von Stickstoff unter steigender N-Deposition von Gebirgs-Ökosystemen abzuschätzen. Die Ergebnisse dieser Dissertation tragen zum Verständnis von Stickstoff-Retention in Gebirgs-Ökosystemen temperater Zonen mit schlechter Bodendurchlässigkeit bei.

Chapter 1

1 General introduction

During the last decades, human activities have strongly altered the global nitrogen (N) cycle (Galloway et al. 1995, 2003). In general, N can be divided into biologically unreactive atmospheric nitrogen and in reactive nitrogen, which is essential for all forms of life and thus plays an important role in most environmental processes. In preindustrial times, the transfer of N between unreactive and reactive forms and vice versa was approximately balanced. Nowadays, the balance is disequilibrated by human activities. The conversion of reactive nitrogen back to N_2 by denitrification does not keep pace with the creation of new reactive nitrogen. The excess reactive nitrogen that is circulating in the atmosphere and which is deposited to ecosystems, is accumulating in various environmental reservoirs (e.g. atmosphere, soils, water) (Galloway & Cowling 2002). Not all ecosystems respond to N deposition similarly, and the responses of aquatic and terrestrial ecosystems are likely to vary also geographically (Matson et al. 2002). In the temperate zone, anthropogenic N emissions have led to increasing acidification and eutrophication of terrestrial and aquatic ecosystems, causing widespread damage to previously N-limited ecosystems (Rodhe et al. 1995; Wright & Schindler 1995; Galloway et al. 1995; Vitousek et al. 1997). In forest ecosystems, excess nitrogen may lead to changes in tree growth and species composition, acidification of soils, and increased NO_3^- leaching, which impairs groundwater and surface water quality (Wright & Rasmussen 1998; Aber et al. 1998). Not only forests, but also oligotrophic meadows are affected by N deposition. The ecosystem response to N deposition depends on factors such as ecosystem type, successional state, N demand and retention capacity, land-use history, soils, topography and climate (Matson et al. 2002).

However, in spite of numerous studies on N deposition in forest and oligotrophic meadow ecosystems (Bobbink et al. 1998; Emmett et al. 1998; Tietema et al. 1998a; Wright & Rasmussen 1998; Nadelhoffer et al. 1999a, b; Lamontagne et al. 2000; Stevens et al. 2004) some aspects of the N cycle and the relative changes in N-cycling processes are not well understood, as reviewed below.

1.1 State of knowledge

1.1.1 The history of the nitrogen cycle

Nitrogen is a structural constituent of proteins and nucleic acids and therefore strongly involved in biogeochemical cycles. It was discovered in the late 18th century. The general division in unreactive atmospheric (N_2) and in reactive forms is shown in Figure 1.

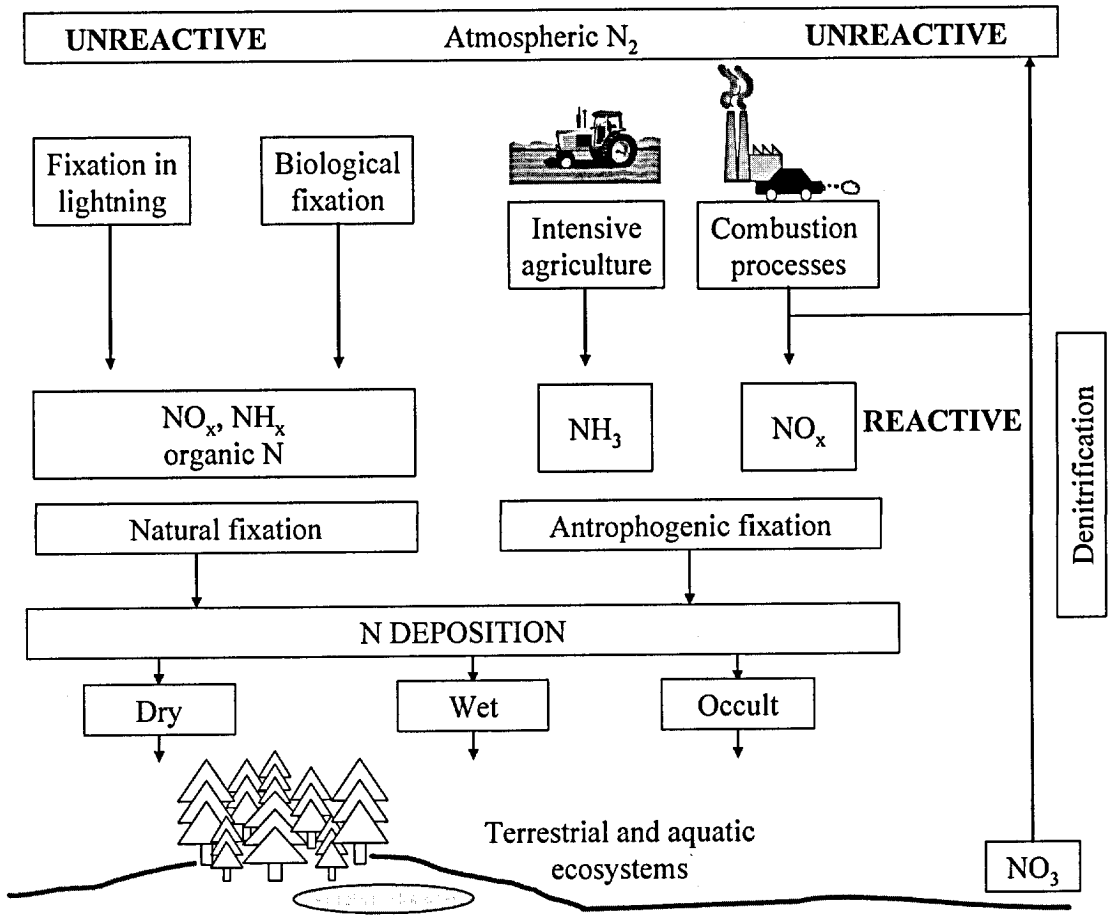

Figure 1: Unreactive and reactive nitrogen forms in the atmosphere (simplified pattern).

Nitrogen makes up nearly 80% of the total mass of the earth's atmosphere as a triple-bonded gas (N_2). To most organisms, this huge reservoir of N is not biologically available, as the triple-bound has to be broken to reduced forms of nitrogen (Vitousek et al. 2002). In its biologically active forms, a single N atom is thereby either bound to oxygen (O) and / or hydrogen (H) through N-fixation processes (NO_x, NH_x) or to carbon (C) through assimilation processes (organic N). The former reaction is called biological N fixation, and it is

accomplished mainly by certain specialised organisms or caused by lightning (oxidative fixation processes). The biological N fixation was discovered at the end of the 19th century; it was the dominant pathway through which reactive nitrogen was made available to living organisms in the preindustrialised world. During the last two centuries, human interference with the natural N cycle started to increase, and an important milestone was the invention of the Haber-Bosch process in 1913. By this industrial process, atmospheric N_2 is converted to NH_3, which is further processed to fertiliser used in food and forage production (Smil 2001). Not only food production, but also energy production from fossil fuels creates reactive nitrogen. However, in contrast to food production, the reactive nitrogen is in this case not produced on purpose but as a consequence of fossil-fuel combustion by the conversion of atmospheric N_2 and of fossil organic N in the fuel to NO_x (Scolow 1999). Thus, the two key sources releasing reactive nitrogen are intensive agriculture (NH_3) and combustion processes (NO_x). Today, about five times more reactive nitrogen comes from losses from food production compared to energy use (Vitousek et al. 1997; Galloway & Cowling 2002). The antrophogenic creation of reactive nitrogen increased between 1860 and 2000 from approximately 15 Tg N a^{-1} to 165 Tg N a^{-1} (Galloway & Cowling 2002). Reactive N is emitted, physically and chemically transformed, widely dispersed by hydrological and atmospheric transport processes and deposited after a few days over short to long distances to terrestrial and aquatic ecosystems, reemitted, transported, etc. Total deposition is the sum of dry (gases, aerosols), wet (rain, snow) and occult (fog, dew) deposition (Erisman 1993a). Once deposited, reactive N enters the biosphere and accumulates in different ecosystem compartments over several months to years and centuries (Emmett et al. 1998; Galloway et al. 1995; Galloway et al. 2002). Nitrogen is released back to the atmosphere via the process of denitrification. The pathway of reactive nitrogen moving from one environmental system to another is called the nitrogen cascade (Galloway 1998).

1.1.2 N deposition to temperate forest and oligotrophic meadow ecosystems

1.1.2.1 Forests

Temperate forests are typically N-limited (Vitousek & Howarth 1991). Forests across Europe receive a wide range of nitrogen inputs from the atmosphere as wet and dry deposition, ranging from less than 1 kg N ha^{-1} a^{-1} in northern Norway and Finland to more than 60 kg N ha^{-1} a^{-1} in the Netherlands and the Czech Republic (MacDonald et al. 2002). During dry weather conditions, gaseous N-species and N-containing aerosols are intercepted by forest

canopies (Draaijers 1993). During and after rainfall, these substances can reach the soil by throughfall and stemflow. Deposition rates vary strongly due to local conditions and canopy structures (Erisman 1993b; Ivens 1990). Forest ecosystem responses to chronically elevated N addition involve complex interactions among the major processes affecting nitrogen cycling. The ability of temperate forest ecosystems to absorb deposited nitrogen is limited and depends on the internal N status of the forest, ranging from N-limited to N-saturated (Aber et al. 1989; Gundersen et al. 1998). The theory of the N status was described by Gundersen et al. (1998) and is important in determining the N retention capacity of a forest stand as a consequence of N deposition, site conditions and management history. Nitrogen-limited forests typically have a significant capacity to retain N deposition in plants and soil microbes competing for available N. In forests with high N status, the ecosystem is N saturated, i.e. N availability exceeds the combined demand of plants and microbes (Aber et al. 1989). Possible effects of chronic N deposition are: an increase in nitrogen concentration in foliage and litter and an improved tree growth. Mineralisation and subsequently nitrification and denitrification will increase. The internal N cycling will be accelerated, and at some stage nitrate will start to leach, causing soil and stream acidification (Gundersen et al. 1998). Nitrate leaching will occur at high N status, but at low N status the N deposition may still be retained by the system, at least for several years (Gundersen et al. 1998).

1.1.2.2 Meadows

Not only forests, but also oligotrophic meadows are affected by a higher N input. Deposition loads in meadows are typically lower than in forests, due to the missing interception by a tall canopy (Wyers et al. 1992). A study in UK on acid grassland over a deposition range of 5 to 35 kg N ha^{-1} a^{-1} by Stevens et al. (2004) indicated that the chronic N deposition had significantly reduced plant species richness. Similarly, Bobbink et al. (1998) showed that increasing N input in meadows leads to changes in biodiversity, soil acidification and increased N leaching.

1.1.3 The power of ^{15}N studies

For gaining insight into the N fluxes and transformations, the application and recovery of ^{15}N isotopes in forests has proven to be a powerful tool for measuring the fate and redistribution of N at the ecosystem scale (Nadelhoffer & Fry 1994; Nadelhoffer et al. 1999a). In an isotope addition experiment, the identification of the pools in which the isotope may reside and possible transfers that may occur have to be determined in a first step (Schimel 1993).

Afterwards, the isotopes are applied as tracers and are introduced into plant, soil or aquatic systems. The tracers can be followed with great sensitivity and precision, thus allowing us to study their fate in the system.

1.1.3.1 ^{15}N recovery in different ecosystem pools

To elucidate the impact of N deposition by means of ^{15}N, the ecosystems under study have to be split into several ecosystem compartments, such as litter layer, soil (surface organic horizon and mineral horizon), trees and ground vegetation (Fig. 2).

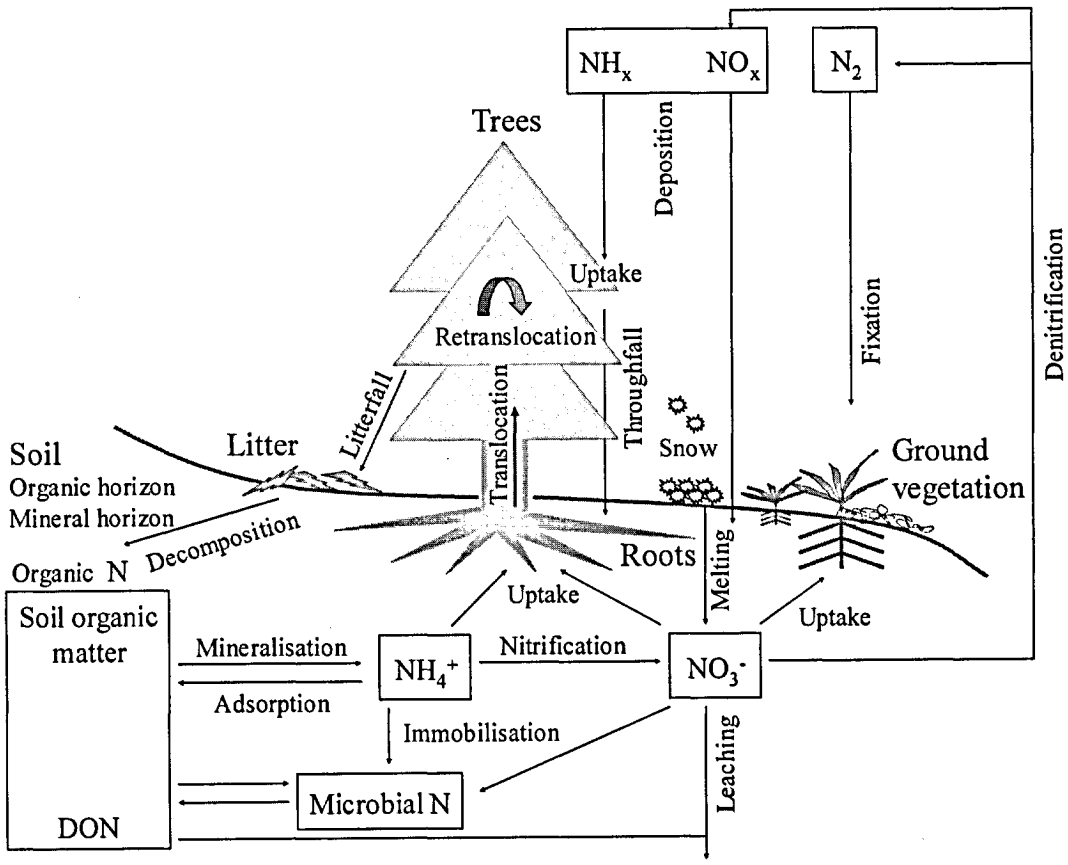

Figure 2: Schematic N cycle of terrestrial ecosystems (adapted after P. Schleppi).

1.1.3.2 ^{15}N recovery in ground vegetation and trees

In the ground vegetation, the mosses play an important role for ^{15}N uptake. The mosses act as a filter and absorb nutrients that impinge on their surface in rainfall and throughfall (Oechel & Van Cleve 1986). Thus, mosses have access to incoming nutrients before the roots of vascular plants. The uptake of nitrogen of the vascular plants occurs mainly by roots, and only a small part occurs by the foliage via the stomata (Fangmeier et al. 1994). In N tracer studies, root

biomass, especially fine roots, are initially a major sink for nitrogen (Zogg et al. 2000). However, also in the longer-term roots can be an important tracer sink.

1.1.3.3 ^{15}N recovery in soil

In many ^{15}N studies, the soil was found to be the strongest sink (Nadelhoffer et al. 1999a, b; Gebauer et al. 2000; Buchmann et al. 1996). The N retention dynamics in forest soils are probably related to immobilisation mechanisms, either biotic or abiotic, which are often poorly understood (Davidson et al. 2003). In this section an excursus about the N cycling processes in the soil is made. The N content in rocks is quite low. N reaches the soil mainly in inorganic forms (NH_x, NO_x) by atmospheric deposition, through decomposition of organic material or biological N fixation (Fig. 2). In the soil, nitrogen follows a continuous cycle of organic compounds (90 – 99 %), inorganic solutes (NO_3^-, NH_4^+) and gaseous forms. The two key processes involved are mineralisation and immobilisation. During mineralisation, organic N is decomposed by microorganisms and transformed into ammonium (NH_4^+). N immobilisation is the opposite process, where microorganisms synthesise organic N (cell content) from inorganic N. NO_3^- is easily soluble and leachable due to its weak binding to soil particles, whereas NH_4^+ adsorbs easily on soil particles or both inorganic forms are taken up by plants. Through the process of nitrification, NH_4^+ is oxidised under aerobic conditions to nitrate (NO_3^-), which can be further transformed to NO_x and N_2 by denitrification under anaerobic conditions (Fig. 2). Losses of N from the ecosystem occur by leaching of dissolved organic N (DON) and inorganic N (NH_4^+, NO_3^-).

1.1.3.4 ^{15}N addition experiments

Many N-additions combined with ^{15}N, or single ^{15}N-experiments have been carried out to assess the effects of increasing N deposition and to better understand the internal N cycle (e.g. Hart et al. 1993; Tietema 1998; Tietema et al. 1998a, b; Nadelhoffer et al. 1999a; Schleppi et al. 1999; Berntson & Aber 2000; Zogg et al. 2000). In the European NITREX project, nitrogen deposition was manipulated for plots or entire catchments to simulate modified deposition rates to coniferous forests so as to compare N fluxes, N concentrations and N pool sizes in vegetation and soil (Wright & van Breemen 1995; Emmett et al. 1998; Tietema et al. 1998a). On the different study sites, most of the deposited N was retained in the soil (Emmett et al. 1998). At the Alptal site in Switzerland, more than 60% of the added $^{15}NH_4{}^{15}NO_3$ tracer was recovered in the soil pool (Schleppi et al. 1999). Similar patterns were found in other ^{15}N tracer experiments (e.g. Nadelhoffer et al. 1999a, b; Gebauer et al. 2000; Buchmann et al.

1996; May et al. 1996). The N retention dynamics in forest soils are probably related to immobilisation mechanisms, either biotic or abiotic, which are still poorly understood (Davidson et al. 2003). Possible processes of soil N retention include the fixation of N at exchange complexes in the forest floor and mineral soil, biotic (root uptake, microbial assimilation) and abiotic immobilisation, i.e. direct incorporation into the soil organic matter (Hart et al. 1993; Aber et al. 1998; Zogg et al. 2000). Recent studies have shown that the abiotic immobilisation of N compounds may be an important process (Berntson & Aber 2000; Johnson et al. 2000; Dail et al. 2001; Perakis & Hedin 2001). These findings contrast with the classical view that biotic immobilisation (uptake by plants and microbes) is the dominant pathway.

1.2 Objectives

The ability of ecosystems to retain deposited nitrogen is limited and depends on the internal N status of each ecosystem. To understand the mechanisms regulating the N retention of a mountain forest or a nearby meadow ecosystem subjected to increasing N deposition, there is a need for an approach, which combines (1) the identification of single ecosystem pools to which deposited N is incorporated, (2) the quantification of the N pathways through the system, (3) and the dynamics of N in the short and in the longer-term. These objectives were pursued by ^{15}N field experiments and by a model approach, as listed below.

- To differentiate the effects of combustion processes ($NO_x \rightarrow NO_3^-$) and intensive agriculture ($NH_3 \rightarrow NH_4^+$), the two N species were followed separately with $^{15}NO_3^-$ and $^{15}NH_4^+$, respectively, in two contrasting ecosystems, a mountain forest and a nearby meadow at different scales, at different time steps and in different ecosystem pools.

  (1) on small plots at short term (hours, days or weeks) and longer term intervals (one year) with a special focus on the partitioning of ^{15}N in different biochemical soil fractions.

  (2) on catchments at a longer term interval (after one year) with a special focus on the partitioning of ^{15}N in different biochemical soil fractions.

  (3) the N uptake of three different moss species under different N deposition scenarios.

- to better understand the nitrogen cycle at the Alptal site in the longer term, the model TRACE (Tracer redistribution among compartments in ecosystems) was used (Currie et al. 1999). TRACE is a biogeochemical process model of C, N and water fluxes in forest ecosystems. It predicts redistributions of ^{15}N and ^{14}N isotopes through time by simulating ^{15}N : ^{14}N ratios of individual N pools and of N transferred between pools. The main goals were to adapt TRACE to the Alptal site; to calibrate and validate the model; and to use the model for an attempt for assessing the ecosystem impacts of scenarios of atmospheric CO_2 concentration and N deposition according to the IPCC.

1.3 Research site and structure of the thesis

The experiments were conducted at the Alptal valley research site, at the northern edge of the Alps of central Switzerland (47°03' N, 8°43' E), at 1200 m a.s.l. (Fig. 3).

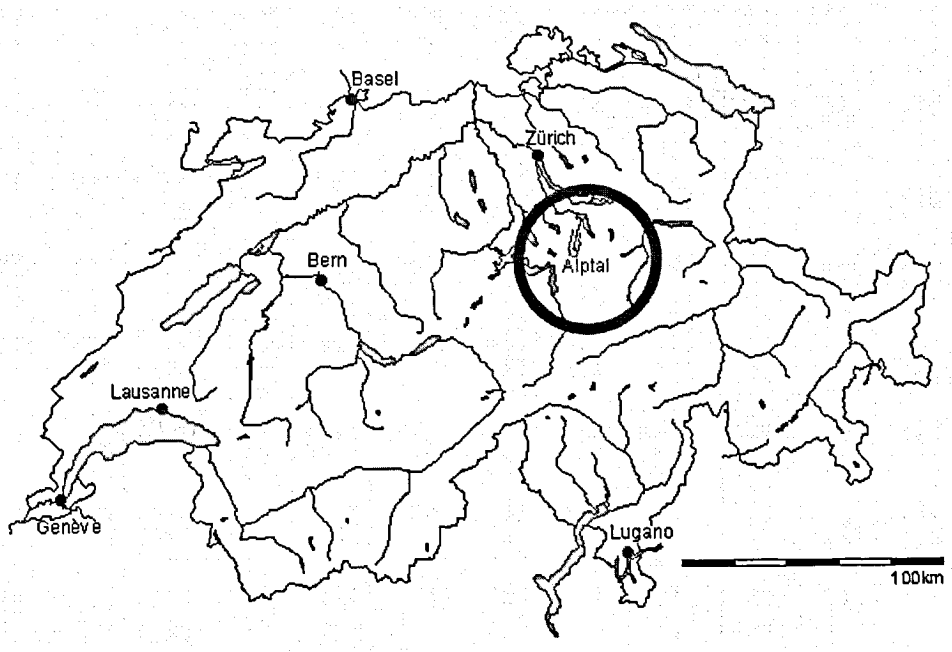

Figure 3: Map of Switzerland showing the Alptal research site.

Bulk atmospheric deposition of inorganic N at the Alptal site is 12 kg ha^{-1} a^{-1}, with equal contributions of NO_3^- and NH_4^+ (Schleppi et al. 1999). Compared to other European forest stands, this is a low-to-moderate N deposition. The Alptal site was part of the European research project NITREX, where the effects of increasing N deposition on soil, water and vegetation were studied in a mountain forest catchment (Wright & Rasmussen 1998; Emmett

et al. 1998; Schleppi et al. 1998). The climate is cool and wet, with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm.. The parent rock material is Flysch, and the major soil types are clay-rich Gleysols of low permeability with the water table close to the surface throughout the year (Hagedorn et al. 1999). The landscape consists of naturally regenerating forests (*Picea abies*) up to 250 years old and meadows that have been used for agricultural purposes until a few years to decades ago.

The four objectives were assessed separately in four sections, followed by an overall synthesis. Below, an overview of the different chapters is given.

- Chapter 2 focuses on the pathways and dynamics of $^{15}NO_3^-$ and $^{15}NH_4^+$ applied in a mountain *Picea abies* forest and in a nearby meadow at the Alptal site on small plots (each 1.5 x 1.5 m). The tracers were applied as a single pulse, and the ecosystem pools were sampled at different time steps, at short term (hours, days or weeks) and longer term intervals (one year). A special focus was on the partitioning of ^{15}N in different biochemical soil fractions.

- Chapter 3 addresses the fluxes of deposited inorganic N in two Gleysol-dominated mountain catchments (each 1600 m^2), in a mountain forest and a nearby meadow, respectively, at the Alptal site traced with $^{15}NO_3^-$ and $^{15}NH_4^+$. Both tracers were applied as two successive labellings in minimal but frequent doses during one year, weekly during the vegetation period and fortnightly in winter. The catchments were equipped for proportional sampling of runoff water. At the end of each labelling period, the ecosystem pools were sampled. Therefore, we were able to quantify after one year the ^{15}N recovery of both N forms through the ecosystem among trees, understory vegetation, litter layer, soil, roots; and to follow the flow of $^{15}NO_3^-$ in runoff (integratively and also in part event-based) continuously over one year. In this way, we were able to infer the accumulation and absorption capacity of nitrogen in both ecosystems.

- Chapter 4 sheds more light on the role of mosses for N cycling. An N addition and a $^{15}NO_3^-$ and $^{15}NH_4^+$ experiment were conducted in the Alptal forest, with three different N additions on three different moss species, *Sphagnum quinquefarium*, *Dicranum scoparium* and *Hylocomium splendens*. The main goals of this study were to investigate the N uptake of three different moss species under different N deposition scenarios; to assess if there is

an effect of N deposition on chlorophyll and carotenoid concentrations in mosses; and to compare the uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$ tracers and to relate the ^{15}N uptake to the nitrate reductase activity.

- Chapter 5 addresses the use of the TRACE model. Our main goals were to adapt the TRACE model to the Alptal site, and to validate it against short-term field data collected at the Alptal site. In a next step, we attempted to use the model for assessing some implications of scenarios of atmospheric CO_2 concentration and N deposition according to the IPCC at the Alptal site over the coming 45 years.

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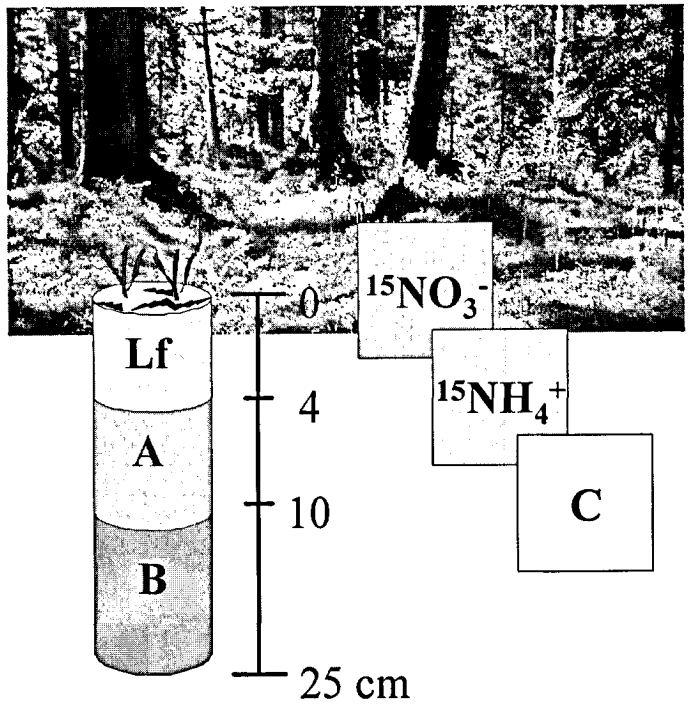
Chapter 2

Pathways and dynamics of $^{15}NO_3^-$ and $^{15}NH_4^+$ applied in a mountain *Picea abies* forest and in a nearby meadow in central Switzerland



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2.1 Abstract

To evaluate the pathways and dynamics of inorganic nitrogen (N) deposition of previously N-limited ecosystems, field additions of ^{15}N tracers were conducted in two mountain ecosystems, a forest dominated by Norway spruce (*Picea abies*) and a nearby meadow, at the Alptal research site in central Switzerland. Pulses of $^{15}NH_4^+$ and $^{15}NO_3^-$ were applied separately to trace deposited inorganic N originating from emissions from either agricultural or combustion processes. The tracers were applied on plots of 2.25 m^2, and several ecosystem pools were sampled at short to longer term intervals (from a few hours to one year), above and below-ground biomass (excluding trees), litter layer, soil LF horizon (approx. 5 - 0 cm), A horizon (approx. 0 - 5 cm) and B horizon (5 - 20 cm). Furthermore, extractable inorganic N, and microbial N pools were analysed in the LF and A horizons. Tracer recovery patterns were quite similar in both ecosystems, with most of the tracer retained in the soil pool. At the short-term (up to one week), up to 16% of both tracers remained extractable or entered the microbial biomass. However, up to 30% of the added $^{15}NO_3^-$ was immobilised just after one hour, and probably chemically bound to soil organic matter. 16% of the NH_4^+ tracer was also immobilised within hours, but it is not clear how much was bound to soil organic matter or fixed between layers of illite-type clay. While the extractable and microbial pools lost ^{15}N over time, a long-term increase in ^{15}N was measured in the roots. Otherwise, differences in recovery a few hours after labelling and one year later were surprisingly small. Overall, more NO_3^- tracer than NH_4^+ tracer was recovered in the soil. This was due to a strong above-ground uptake of the deposited NH_4^+ by the ground vegetation, especially by mosses.

Keywords: Nitrogen deposition, nitrogen cycle, nitrate, ammonium, Gleysol

2.2 Introduction

During the last few decades, human activities have dramatically increased the mobility and deposition of reactive nitrogen forms to ecosystems (Galloway et al. 2003). The two key sources of reactive nitrogen production are intensive agriculture (ammonia -> ammonium) and combustion processes (nitrogen oxides -> nitrate) with about five times more reactive nitrogen coming from former (Vitousek et al. 1997; Galloway & Cowling 2002). Since nitrogen is an essential nutrient element and therefore very involved in biogeochemical cycling, the inputs of reactive nitrogen compounds can induce eutrophication of previously N-limited systems. Temperate forests are typically N-limited (Vitousek & Howarth 1991). Forests in Europe receive inorganic N deposition ranging from less than 1 kg N ha^{-1} a^{-1} in northern Norway and Finland to more than 60 kg N ha^{-1} a^{-1} in the Netherlands and the Czech Republic (MacDonald et al. 2002). Not only forests, but also meadows are negatively affected by a higher N input. Deposition loads in meadows are typically lower than in forests, due to the missing interception of a tall canopy (Wyers et al. 1992). A study in UK on acid grassland over a deposition range of 5 to 35 kg N ha^{-1} a^{-1} by Stevens et al. (2004) indicated that the chronic N deposition had significantly reduced plant species richness. Similarly Bobbink et al. (1998) showed that increasing N input in meadows leads to changes in biodiversity and increased N leaching.

Several N-addition experiments (partly including ^{15}N) in temperate forests have shown that it is the soil, rather than the plants, that is the main long-term sink for the added N (Gundersen et al. 1998; Tietema et al. 1998; Nadelhoffer et al. 1999a, b). Similar patterns were found in ^{15}N tracer experiments where the soil was the strongest sink. Gebauer et al. (2000), Buchmann et al. (1996) and May et al. (1996) recovered more NH_4^+ than NO_3^- tracer in the soil. These N retention dynamics in forest soils are probably related to immobilisation mechanisms at work, either biotic or abiotic, which are often poorly understood (Davidson et al. 2003). Processes of soil N retention are: fixation of N at exchange complexes in the forest floor and mineral soil, biotic (root uptake, microbial assimilation) and abiotic immobilisation, i.e. direct incorporation into the soil organic matter (Hart et al. 1993; Aber et al. 1998; Zogg et al. 2000). Recent studies have shown that abiotic immobilisation of N compounds may be an important process (Berntson & Aber 2000; Johnson et al. 2000; Dail et al. 2001; Perakis & Hedin 2001). These findings contrast with the classical view that biotic immobilisation (uptake by plants and microbes) is the dominant pathway.

The present research site in the Alptal valley, central Switzerland was part of the European research project NITREX (Wright & Rasmussen 1998; Emmett et al. 1998) were the effects of increasing N deposition on soil, water and vegetation were studied in a mountain forest catchment (Schleppi et al. 1998). Over 60% of the added $^{15}NH_4^{15}NO_3$ tracer was recovered in the soil pool (Schleppi et al. 1999a). To deepen the understanding of the pathways and dynamics of the N deposition in the ecosystems (forest and meadow) a ^{15}N experiment was carried out. In this study the soil horizons were not sampled as one pool unlike in Schleppi et al. (1999a) instead the soil was further fractionated to follow the tracer in more detail.

Thus, the main goals of this study were (1) to trace the $^{15}NH_4^+$ and $^{15}NO_3^-$ pulses separately to follow deposited inorganic N in a forest and in a meadow at different time steps, at short-term (hours, days or weeks later) and longer term intervals (after one year). (2) A special focus was on the partitioning of ^{15}N in different biochemical soil fractions.

2.3 Materials and methods

2.3.1 Study area

The research site is located in the Alptal, on the northern edge of the Alps of central Switzerland (47°03' N, 8°43' E), at 1200 m a.s.l.. The climate is cool and wet, with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm, reaching a maximum in June (270 mm) and a minimum in October (135 mm). The vegetation period lasts from June to September. Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} a^{-1}, equally divided between NO_3^- and NH_4^+ (Schleppi et al. 1999a). The parent rock material is Flysch, and the major soil types are clay-rich Gleysols of low permeability, with the water table close to the surface throughout the year (Hagedorn et al. 1999). Usually, soils are covered with snow from mid-November to April. Slope is about 20% with a west aspect. The landscape consists of naturally regenerating forests and litter meadows, neither of which have ever been fertilised artificially. Trees are predominantly Norway spruce (*Picea abies*), with 15% Silver fir (*Abies alba*) and they are up to 250 years old, with a relatively low leaf area index of 3.8 (Schleppi et al. 1999b). Generally, the soil profile consists of a LF, an A and a B horizon. In the forest, vegetation and soil types form a mosaic pattern closely related to microtopography. Two different soil types are present: on mounds, with the water table at a depth below 40 cm umbric Gleysols are abundant with raw humus (LFH), Ah and oxidised or partly oxidised Bg or Br horizons. In depressions, the water table frequently reaches the

surface, leading to mollic Gleysols with a thin LF horizon, an anmoor topsoil (Aa) and an almost permanently reduced Bg or Br horizon. On the mounds, the dominant plant species are Norway spruce (*Picea abies*) and *Vaccinium myrtillus*. The waterlogged depressions are too wet for tree growth, and ground vegetation is dominated by *Caltha palustris* and *Petasites albus* in the shade of the trees, and by *Poa trivialis* and *Carex ferruginea* in open patches (Muller 1997). The meadow used for litter production has no distinct microtopography and is abandoned for 20 years. The soil has, like the depressions in the forest, an anmoor topsoil (mollic Gleysol) and an almost permanently reduced Bg or Br horizon. The vegetation consists mainly of calcareous fens (*Caricetum davallianae*) and small sedge meadows (*Molinietum*), with a few small trees (*Picea abies* and *Alnus incana*).

2.3.2 Experimental design, tracer application and sampling

In August 2001, a plot-scale field experiment with ^{15}N tracers was carried out. Four replicates were established in the forest between trees, and three replicates in the meadow. Each replicate was separated into three plots (each 1.5 x 1.5 m) at least 0.5 m apart, avoiding trees. Two plots were labelled with a single addition of either $K^{15}NO_3$ or $^{15}NH_4Cl$ (both 99 atom% ^{15}N) dissolved in deionised water (2.2 l m^{-2}). The third plot was treated as a control with deionised water only (no ^{15}N addition). The solutions were applied with a back-pack sprayer above the ground vegetation. After the treatment, all plots were flushed with 2.2 l m^{-2} deionised water to rinse the vegetation. The amount of ^{15}N added (0.12 g m^{-2}) corresponded to 10% of the yearly N-deposition rate and acted as a tracer for the nitrogen-cycling processes. Soil samples were taken with a soil corer (5 cm inner diameter, 25 cm depth) reaching into the B horizon. The labelled plots were sampled eight times over one year (after 1 h, 3 h, 8 h, 1 d, 7 d, 34 d, 91 d and 361 d). To prevent contamination during sample processing, the control plots were sampled on different dates (after 26 d, 92 d and 354 d). On the first sampling day, soil temperature was in the forest 14.1 °C and in the meadow 14.3 °C at a depth of 5 cm and 10 cm, respectively. Within each 2.25 m^2 plot, three soil cores were taken per sampling date. They were immediately put on ice in the field, transported to the laboratory and processed within one day. The three soil cores of each plot were pooled into one composite sample to obtain sufficient material for subsequent analyses. The soil cores consisted of litter layer, LF horizon (approx. 5 - 0 cm), A horizon (approx. 0 - 5 cm) and B horizon (5 - 20 cm). The above-ground vegetation (excluding trees) was sampled twice during the growing season (after 46 d and 390 d). For this purpose, the ground vegetation was clipped at two places (18.5

cm x 20 cm) on each plot and pooled into a composite sample. Each vegetation sample was kept refrigerated and processed in the laboratory within one day.

2.3.3 Laboratory analyses

The soil samples were separated into surface litter and into the soil horizons LF, A and B (Table 1).

Table 1: Summary of soil sample fractionation, analyses and the corresponding N pools.

Horizon	Pool	Pool fraction	Analyses	Measurements
L	Litter	Bulk		Total N, ^{15}N
LF	Root	> 4mm in Ø		Total N, ^{15}N
		< 4mm in Ø		Total N, ^{15}N
	Soil	Bulk		Total N, ^{15}N
		Extractable N	Extraction / Lyophilisation	Total N, ^{15}N
		NH_4-N, NO_3-N	Extraction / Ammonium-diffusion	$^{15}NH_4^+$, $^{15}NO_3^-$
		Soil microbial N and extractable N	Chloroform-fumigation / Extraction / Lyophilisation	Total N, ^{15}N
A	Root	> 4mm in Ø		Total N, ^{15}N
		< 4mm in Ø		Total N, ^{15}N
	Soil	Bulk		Total N, ^{15}N
		Extractable N	Extraction / Lyophilisation	Total N, ^{15}N
		NH_4-N, NO_3-N	Extraction / Ammonium-diffusion	$^{15}NH_4^+$, $^{15}NO_3^-$
		Soil microbial N and extractable N	Chloroform-fumigation Extraction / Lyophilisation	Total N, ^{15}N
B	Soil	Bulk		Total N, ^{15}N

From the LF and the A horizon material, the roots were picked out by hand with tweezers, rinsed free of soil with deionised water and separated into the diameter classes of > 4 mm and < 4 mm. Large roots (> 15 mm diameter) were removed. At least 200 g field-moist soil per sample was freed from roots (work time ~ 4 h) and used for total N and ^{15}N analyses as well as for extractions. Extractable inorganic N and ^{15}N (NH_4^+-N, NO_3^--N) and total extractable N and ^{15}N were determined by adding 40 g of soil material from the LF horizon or 60 g of the A horizon to 160 ml of 0.5 M K_2SO_4, then shaking the mixture for 1.5 h and filtering it through folded filters (790 ½, diameter 185 mm, Schleicher and Schuell, Dassel, Germany) into PET bottles. The extract was stored in the freezer until further processing. As a next step, half of the extract was lyophilised using a freeze dryer (Christ, BETA 1-8, Osterode am Harz, Germany) to measure total extractable N and ^{15}N through mass spectrometry. With the second

half of the extract, extractable $^{15}NO_3^-$ and $^{15}NH_4^+$ concentrations were analysed using the diffusion method (adapted from Downs et al. 1999). About 80 ml of the extract were put into 100 ml PE bottles, together with a glass microfibre filter (GF/F 25mm, Whatman, Maidstone, England; 5x12 mm, calcinated in a muffle furnace (Naber, Type No. 7, Zurich, Switzerland) for 6 h at 450 °C), wetted with 30 µl H_2SO_4 2M and enclosed in PTFE band. Then 1.5 mg l^{-1} MgO were added and the bottle was tightly closed. NH_4^+ was thus converted to NH_3, diffused through the PTFE band and finally captured on the microfibre filter. The bottles were shaken (40 min^{-1}) for a week, whereupon the filter was removed, dried in an evacuated desiccator in presence of concentrated sulphuric acid, and then unpacked from the PTFE band. The filter was stored in a glass vial and packed in a silver cup prior to mass spectrometric analysis. The remaining extract was further processed by adding a new microfibre filter and 0.5 g Devarda's alloy. Thereby, NO_3^- was reduced to NH_4^+, and the same chemical procedure was repeated as with NH_4^+.

N and ^{15}N in the microbial biomass were determined by chloroform-fumigation and extraction (Brookes et al. 1985). 20 g of the soil material from the LF horizon or 30 g from the A horizon were fumigated with $CHCl_3$ in an evacuated desiccator in the dark for 24 hours and afterwards extracted with 80 ml of 0.5 M K_2SO_4. The extract was stored in the freezer and then lyophilised entirely to determine the total N concentration and the ^{15}N / ^{14}N isotope ratio. The remaining bulk soil and all other solid samples (above and below-ground biomass, litter) were dried to constant weight at 65 °C and the dry matter content was calculated. All samples were ground, and the total N and ^{15}N concentrations were determined by an elemental analyser coupled to an isotope-ratio mass spectrometer (Delta S, Finnigan, Bremen, Germany). The isotopic values are presented in the δ notation (Eq. 1):

$$(1) \qquad \delta^{15}N = R_{sample} / R_{standard} - 1$$

where R is the molar fraction of ^{15}N / ^{14}N of the sample and standard respectively. $\delta^{15}N$ is usually expressed in ‰ (times 1000). The $\delta^{15}N$ was measured with a precision of N ± 0.1 ‰. The soil samples were ground with a vibratory mill (0.25 mm), the above and below-ground biomass materials with a centrifuge grinder (0.5 mm), and the litter layer with a coffee grinder.

The vegetation was first separated into four groups: monocotyledons, dicotyledon herbs, dicotyledon shrubs and mosses. The C:N ratio of all three soil horizons was measured with a C + N analyser (NC 2500, CE instruments Thermoquest, Milano, Italy).

2.3.4 Calculation of recoveries and statistical analyses

The N pools of the ecosystem were calculated from the dry masses and N concentrations as means of all replicates and sampling times, because these were not significantly different. The ^{15}N recovery [% of added tracer] was calculated by subtracting the natural abundance of ^{15}N measured in the control samples (after 26 d, 92 d and 354 d) from the recovered ^{15}N of the pool samples, (for equations see Chapter 3). For soil microbial N, the recovered ^{15}N was calculated as the difference between the recovered ^{15}N in the extract of fumigated soil and that in the non-fumigated soil (no correction factor was used for extraction efficiency). The extractable DO^{15}N was calculated as total extractable ^{15}N minus extractable $^{15}NO_3^-$-N and $^{15}NH_4^+$-N. The ^{15}N recovery in soil minus the recoveries in soil microbial ^{15}N and extractable ^{15}N was considered as immobilised soil N (ISN).

N concentrations and ^{15}N recoveries were subjected to an analysis of variance (ANOVA) with repeated measures. The tested effects were the tracer (NH_4^+ or NO_3^-), the time of sampling and their interaction. The time factor and the interaction were further split into polynomial contrasts (linear, quadratic, cubic, quartic) based on the logarithm of time. If necessary, tracer recoveries were themselves analysed after a log transformation in order to improve the normality of the residues, in some cases after adding a constant value to avoid negative numbers. The statistical calculations were carried out with the S-Plus Software (S-Plus 2000, Math Soft). All results were considered significant at the $P < 0.05$ level, and highly significant at the $P < 0.01$ level. Between 0.05 and 0.10, the P value was considered to indicate a tendency. Because the replications were within the forest and the meadow, the differences between these ecosystems could not be tested statistically. Comparisons between the forest and the meadow were therefore made only descriptively.

2.4 Results

2.4.1 Forest

2.4.1.1 Ecosystem N pools

The total ecosystem N content (including trees (Chapter 3) and excluding soil > 20 cm depth) was 0.7 kg m^{-2} in the forest (Table 2). Among the pools, the B horizon contained most of this

N (40% of the total N in forest), followed by the A and LF horizons, with 36% and 14% of total N, respectively. The roots had much lower values, amounting to only 0.8% in the LF and 0.6% in the A horizon. Similarly, the understory and the litter layer contained only 0.9% and 1.5% of total N in forest respectively, whereas the trees contained 5.8%.

Table 2: Masses and N concentrations in vegetation, litter layer, soil horizons and roots.

Forest Pool	Dry matter [kg/m^2]	N mass [g/m^2]	N conc.[1] [%]	C:N
Trees (above-ground) [2]	18.1	39.4	0.22	
Understory (above-ground)				
Mosses	0.09 ± 0.01	1.8 ± 0.2	1.9 ± 0.04	
Monocotyledons	0.05 ± 0.01	0.8 ± 0.1	1.6 ± 0.1	
Dicotyledon herbs	0.05 ± 0.01	0.9 ± 0.2	1.8 ± 0.1	
Dicotyledon shrubs	0.23 ± 0.2	2.7 ± 2.3	0.8 ± 0.2	
Litter layer				
Litter	0.7 ± 0.07	10.4 ± 1.0	1.4 ± 0.02	
Roots				
LF roots	0.5 ± 0.04	5.2 ± 0.4	1.0 ± 0.02	
A roots	0.5 ± 0.04	4.4 ± 0.4	0.9 ± 0.02	
Soil				
LF (5 - 0 cm)	7 ± 2	97 ± 17	1.4 ± 0.1	19.4
A (0 - 5 cm)	22 ± 5	247 ± 41	1.2 ± 0.3	15.6
B (5 - 20 cm)	45 ± 4	275 ± 11	0.7 ± 0.05	17.3
Total		685		
Meadow Pool	Dry matter [kg/m^2]	N mass [g/m^2]	N conc.[1] [%]	C:N
Vegetation (above-ground)				
Mosses	0.03 ± 0.02	0.5 ± 0.2	1.6 ± 0.1	
Monocotyledons	0.29 ± 0.03	3.6 ± 0.3	1.4 ± 0.1	
Dicotyledon herbs	0.05 ± 0.01	0.7 ± 0.1	0.7 ± 0.1	
Litter layer				
Litter	0.7 ± 0.1	9.0 ± 1.0	1.3 ± 0.02	
Roots				
LF roots	1.2 ± 0.1	10.0 ± 1.2	0.9 ± 0.03	
A roots	1.1 ± 0.1	9.1 ± 1.3	0.8 ± 0.03	
Soil				
LF (5 - 0 cm)	9 ± 1	126 ± 14	1.5 ± 0.07	16.1
A (0 - 5 cm)	30 ± 3	331 ± 37	1.2 ± 0.07	13.4
B (5 - 20 cm)	56 ± 8	290 ± 25	0.7 ± 0.10	15.6
Total		779		

[1] N concentrations are calculated as the mean and standard error over all the sampling times and replicates because these were not significantly different

[2] Results are taken from Chapter 3

2.4.1.2 Total recovery of ^{15}N

The litter layer, soil and below-ground biomass were sampled at all sampling times, whereas the understory vegetation was only sampled twice, and the trees were not sampled at all. At all sampling times in the forest, ^{15}N recovery in the litter layer, soil and below-ground biomass was higher ($P = 0.02$) for the NO_3^- tracer (60 ± 4% standard error) than for the NH_4^+ tracer (36 ± 3% SE) (Table 3, Fig. 1).

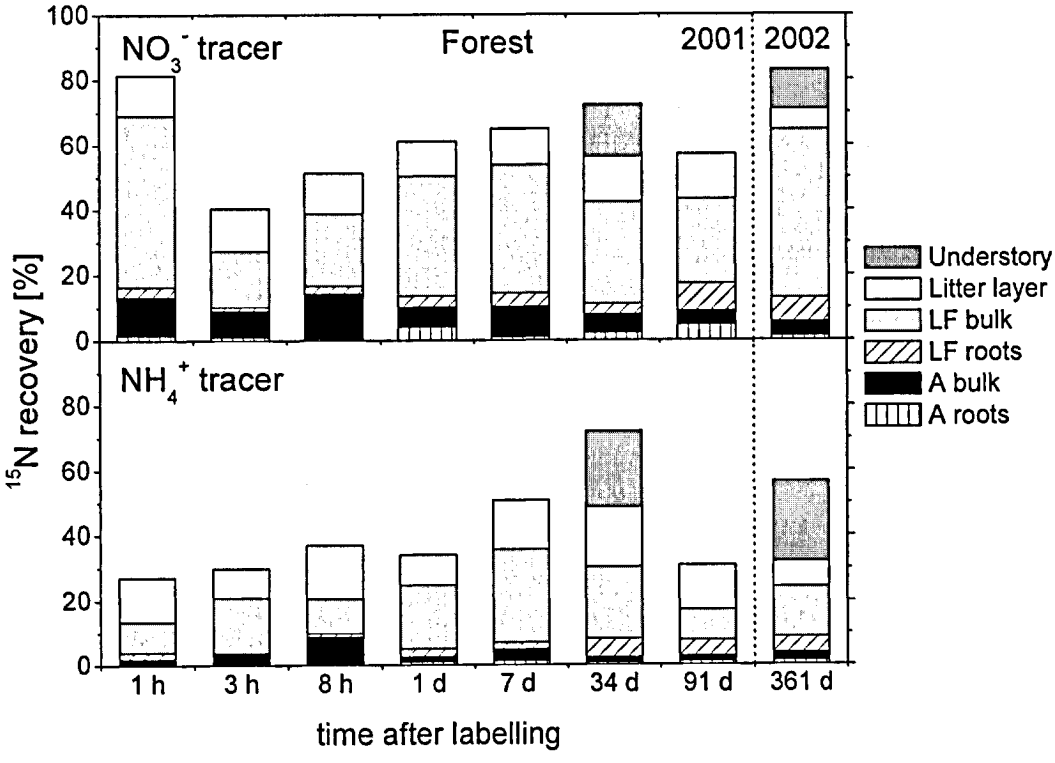

Figure 1: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in understory vegetation, litter layer, LF and A horizons, as well as in LF and A roots in the forest at eight different sampling times (1 h, 3 h, 8 h, 1 d, 7 d, 34 d, 91 d, 361 d).

After the tracer addition, there was no rain for nine hours. Between the sampling times of eight hours and one day, precipitation amounted to 11.8 mm, and between the sampling times of one day and one week it rained 1.7 mm. The sampling time of 34 days and 91 days was before snow fall in winter 2001, whereas the last sampling after one year was after winter 2001 / 2002 in August 2002. The ^{15}N tracer was already recovered in all sampled pools after one hour. The recovery showed a W-shape over time, (fourth polynomial grade, $P = 0.03$) (Table 3, Fig. 1).

Table 3: Results of ANOVA with repeated measures for the effects of the tracer (NH_4^+ or NO_3^-), the time of sampling and their interaction on ^{15}N recovery for the forest and the meadow for all sampled pools. The time factor and the interaction were further split into polynomial contrasts (linear[1], quadratic[2], cubic[3], quartic[4]).

			Forest				Meadow		
	N pools		N tracer	Time	Tracer x Time		N tracer	Time	Tracer x Time
Understory	Mosses		0.02	0.21	0.23		n.t.	n.t.	n.t.
	Monocotyledons		0.05	0.27	0.25		0.57	0.29	0.08
	Dicotyledon herbs		0.12	0.01	0.15		0.27	0.02	0.5
	Dicotyledon shrubs		n.t.	n.t.	n.t.		n.p.	n.p.	n.p.
	Total understory		0.07	0.78	0.36		0.75	0.1	0.1
Litter layer	L		0.28	0.02 [3]	0.59	log	0.21	0.003 [1]	0.67
Below-ground	Lf bulk		0.02	0.002 [4]	0.017 [2]		0.58	0.93	0.89
	Tot extr. N	log	0.05	<0.001 [1]	0.002 [2]		0.74	<0.001 [1]	0.06
	NO_3^- fraction	log	0.08	0.02 [1,2,4]	0.008 [1]	log	0.4	<0.001 [1,2]	0.51
	NH_4^+ fraction	log	0.8	<0.001 [1,2,4]	0.01 [2,4]	log	0.33	0.004 [1]	0.4
	DON fraction	log	0.03	<0.001 [1]	0.001 [2]		0.47	<0.001 [1,2]	0.08
	Microbial biomass		0.47	<0.001 [1]	0.005 [4]		0.24	0.17	0.53
	Immobilised soil N		0.003	<0.001 [1,3,4]	0.04 [2]	log	0.02	0.13	0.33
	LF roots		0.02	<0.001 [1]	0.89		0.56	<0.001 [1,3]	0.68
	A bulk	log	0.07	0.29	0.62	log	0.67	0.03 [1,2]	0.42
	Tot extr. N	log	0.97	<0.001 [2,4]	0.003 [2]	log	0.24	<0.001 [1]	0.96
	NO_3^- fraction	log	0.34	0.04 [2,4]	0.03 [3]	log	0.67	<0.001 [1]	0.01 [1,3]
	NH_4^+ fraction	log	0.34	<0.001 [2,4]	0.008 [3]	log	0.4	<0.001 [1,2]	0.23
	DON fraction	log	0.08	0.04 [1,2,3,4]	0.29	log	0.77	0.01 [1]	0.53
	Microbial biomass	log	0.31	0.87	0.46		0.56	0.44	0.37
	Immobilised soil N	log	0.04	0.48	0.28	log	0.63	0.06	0.76
	A roots	log	0.07	0.04 [1]	0.42	log	0.23	<0.001 [1,2]	0.03 [1]
	B		n.t.	n.t.	n.t.		n.t.	n.t.	n.t.
	Total below-ground		0.02	0.03 [4]	0.02 [2]		0.75	0.02 [1]	0.62

Data used in analysis are untransformed except where noted.

log: log transformation

n.p.: not present

n.t.: not tested n < 2

2.4.1.3 ^{15}N partitioning in the litter layer, soil and roots

For both tracers, the largest sink was in the LF horizon followed by the litter layer (Fig. 1). However, for the NH_4^+ tracer, the recovery in the litter layer was higher after eight hours and 92 days than in the LF horizon. The litter layer was followed by the A horizon and the roots in the LF and A horizon. The recovery in the A horizon decreased after 34 days for the NH_4^+ tracer and after 91 days for the NO_3^- tracer, whereas the recovery in the roots in the LF horizon increased. Below a depth of 10 cm, there was almost no recovery (< 1%) for either tracer at any time within one year. The NO_3^- tracer resulted in a higher recovery than the NH_4^+ tracer in the LF horizon ($P = 0.02$) and in the roots of this horizon ($P = 0.02$) (Table 3). For the litter layer, the recovery showed an inverse N-shape over time (third polynomial grade, $P= 0.02$), for the LF horizon a W-shape (fourth polynomial grade) ($P = 0.002$) and for the LF roots a linear increase (first grade, $P < 0.001$).

2.4.1.4 ^{15}N partitioning of soil compartments

Total extractable ^{15}N - In the LF horizon, the NO_3^- tracer reached a marginally higher recovery than the NH_4^+ tracer in total extractable N (sum of NO_3^-, NH_4^+ and DON), $P = 0.05$ (Table 3, Fig. 2). Tracer recovery decreased over time for total extractable N for both tracers ($P < 0.001$), and was below 2.4% after one week. Total extractable ^{15}N was further split into $^{15}NO_3^-$, $^{15}NH_4^+$ and $DO^{15}N$ fractions. For the NO_3^- tracer, the $^{15}NO_3^-$ fraction was more than twice as high as the $^{15}NH_4^+$ fraction during the first day (Fig. 3). After seven days, the $^{15}NO_3^-$ fraction decreased and had recoveries below 7% of the extractable inorganic tracer after one month. For the NH_4^+ tracer, the proportion between the $^{15}NH_4^+$ and $^{15}NO_3^-$ fractions remained almost constant in the LF horizon during the total sampling period (Fig. 3).

The NO_3^- tracer gave a marginally higher recovery than the NH_4^+ tracer for the $^{15}NO_3^-$ fraction ($P = 0.08$), and a higher recovery for the $DO^{15}N$ fraction ($P = 0.03$), whereas the recovery decreased over time for both tracers for the $^{15}NO_3^-$ fraction ($P = 0.02$), for the $^{15}NH_4^+$ fraction ($P < 0.001$) and for the $DO^{15}N$ fraction ($P < 0.001$) (Table 3).

In the A horizon, the recovery of total extractable ^{15}N was delayed compared to the LF horizon (Fig. 2). A small peak (5%) occurred for the NH_4^+ tracer after only eight hours, and after one day (3.6%) for the NO_3^- tracer. The recovery for total extractable ^{15}N in the A horizon showed a convex shape over time (second polynomial grade, $P < 0.001$), which did

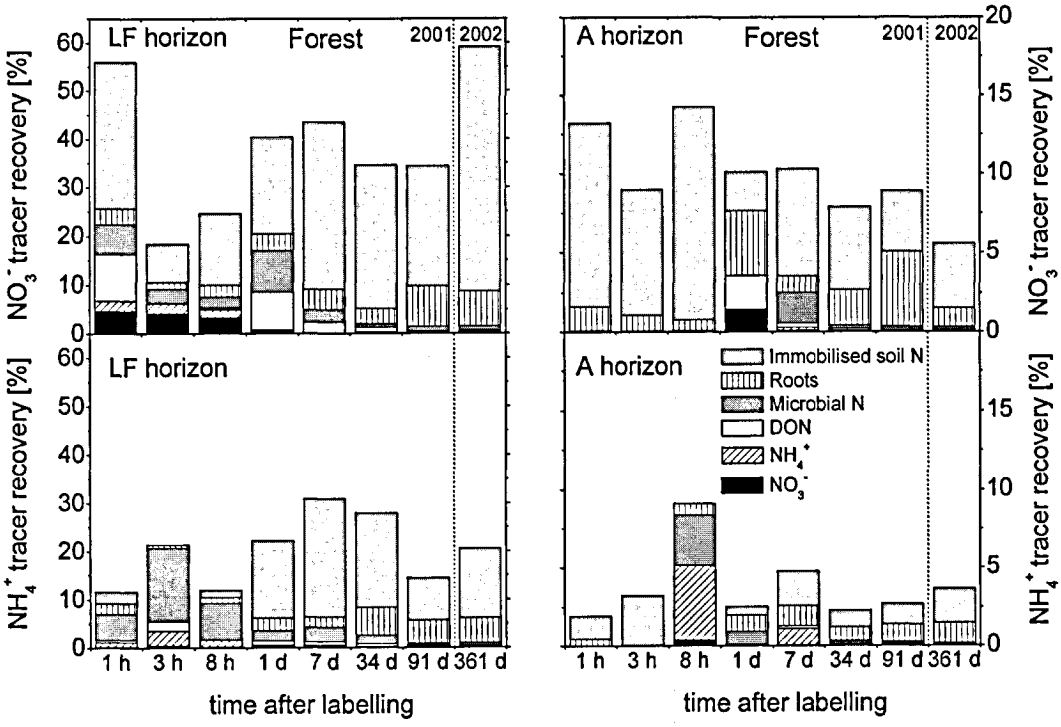

Figure 2: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in the soil pool fractions: total extractable N (sum of NO_3^-, NH_4^+, DON), microbial N, roots and the remaining soil pool fraction in the LF and A horizons in the forest at eight different sampling times (1 h, 3 h, 8 h, 1 d, 7 d, 34 d, 91 d, 361 d).

not differ between tracers (Table 3). However, the NO_3^- tracer gave a marginally higher recovery in the DO^{15}N fraction than the NH_4^+ tracer (P = 0.08). For the NO_3^- tracer, the recovery in the $^{15}NO_3^-$ fraction was higher than in the $^{15}NH_4^+$ fraction during the first day, but then, the $^{15}NH_4^+$ fraction dominated, as it did for the NH_4^+ tracer (Fig. 3).

Microbial ^{15}N - In the LF horizon, the NH_4^+ tracer had a remarkable peak in microbial ^{15}N after three hours (15%, SE = 4%), levelling off afterwards (Fig. 2). For the NO_3^- tracer, recovery was lower and a peak appeared after only one day (8%, SE = 3%). Tracer recovery decreased for both tracers after the peak ($P < 0.001$) (Table 3).

In the A horizon, a recovery delay in microbial ^{15}N was observed for both tracers. For the NH_4^+ tracer, the first peak occurred after eight hours (3%, SE = 3%), and for the NO_3^- tracer after one week (2%, SE = 1%). Neither the tracer nor sampling time had a significant effect on the recovery (Table 3).

Immobilised soil N – After one week, the recoveries of total extractable ^{15}N and microbial ^{15}N dropped and then levelled off in the LF horizon, while the recovery in the immobilised soil N (ISN) increased. In this horizon, the NO_3^- tracer had a higher recovery in ISN than the NH_4^+ tracer ($P = 0.003$) (Table 3), with high recovery rates after one hour (30%, SE = 5%), whereas the NH_4^+ tracer was detectable only after one day (16%, SE = 4%). Tracer recovery showed a complex time course for both tracers (first, third and fourth polynomial grade, $P < 0.001$). In the A horizon, the recovery of both tracers in ISN was detectable already after one hour, with higher recovery for the NO_3^- tracer than the NH_4^+ tracer ($P = 0.04$).

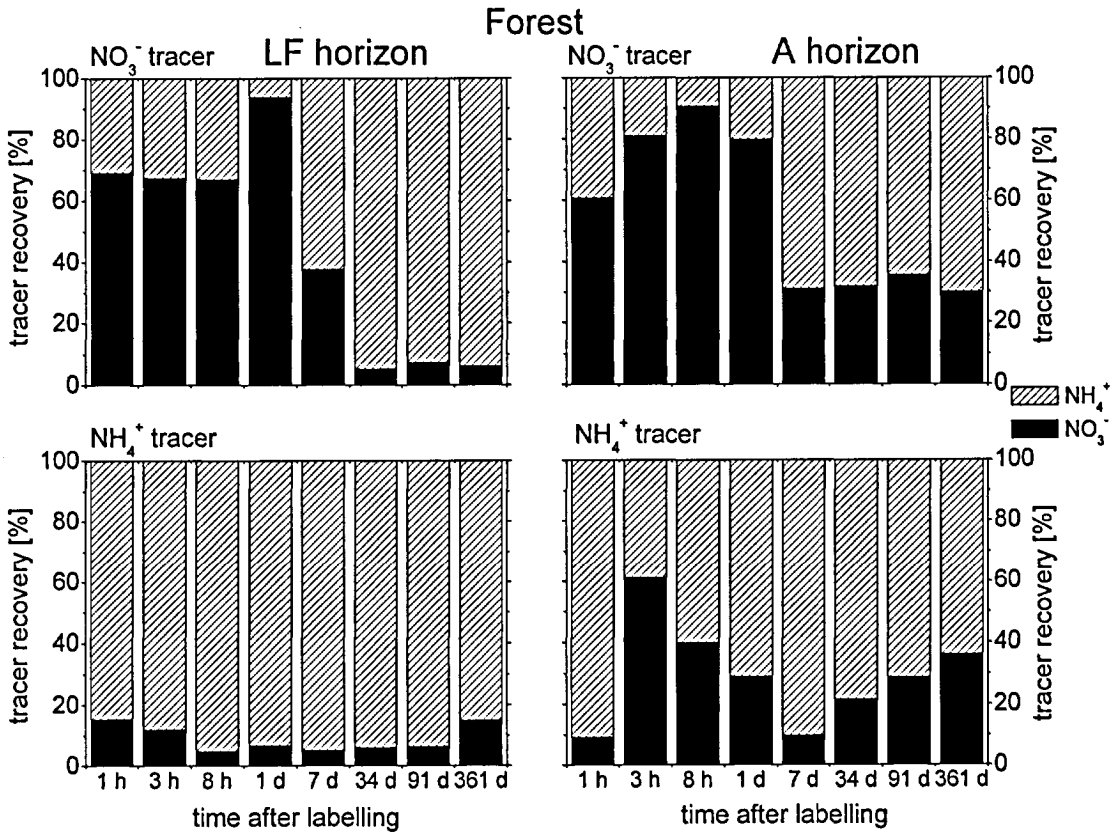

Figure 3: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in the $^{15}NO_3^-$ and $^{15}NH_4^+$ fractions of total extractable ^{15}N in the LF and A horizons in the forest at eight different sampling times (1 h, 3 h, 8 h, 1 d, 7 d, 34 d, 91 d, 361 d).

2.4.1.5 ^{15}N partitioning in understory vegetation

Recovery in the forest understory vegetation was measured after 46 days and 390 days for both tracers (Fig. 4). At both sampling times, total recovery was marginally higher for the NH_4^+ tracer than for the NO_3^- tracer ($P = 0.07$) (Table 3). Mosses showed the highest

retention overall, with the NH_4^+ tracer having a higher recovery than the NO_3^- tracer (P = 0.02). The recovery in monocotyledons and dicotyledon herbs was much lower than in the mosses. The dicotyledon herbs showed increased recovery of both tracers over time (P = 0.01), whereas the recovery in the other understory groups decreased. At both sampling times, the dicotyledon shrubs had very low recovery values under 1%, which were statistically not testable due to n < 2.

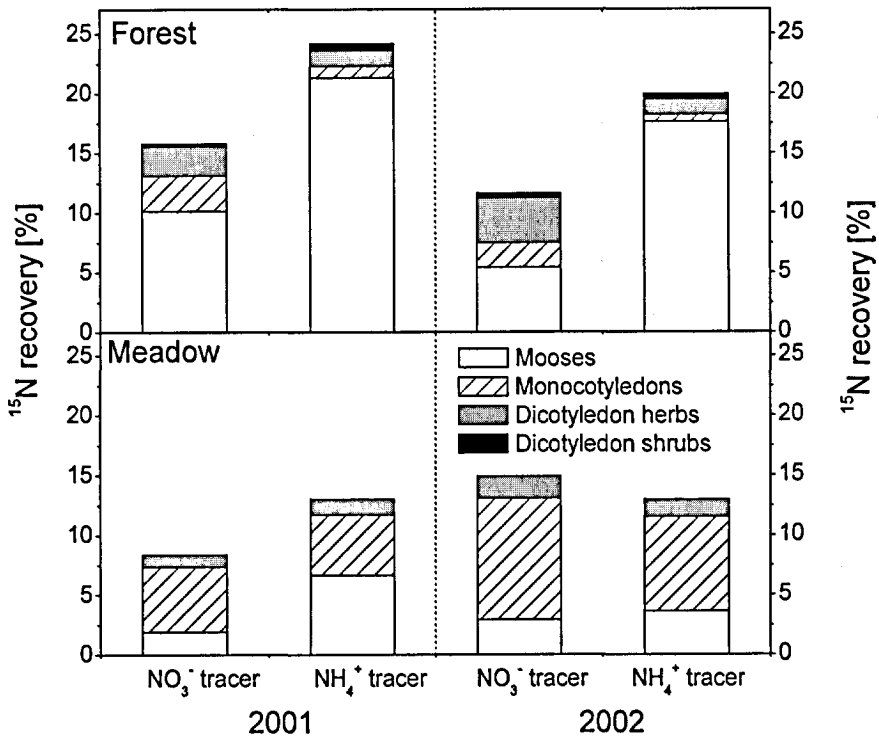

Figure 4: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in the ground vegetation divided into mosses, monocotyledons, dicotyledons herbs and dicotyledons shrubs in the forest and the meadow in September 2001 and September 2002.

2.4.2 Meadow

2.4.2.1 Ecosystem N pool

The total ecosystem N content (excluding soil > 20 cm depth) was 0.8 kg m^{-2} in the meadow (Table 2). Among the pools, the A horizon contained most of this N (42% of the total N in meadow), followed by B and LF horizons, with 37% and 16% total N, respectively. The roots had much lower values than the soil horizons, reaching only 1.3% in the LF and 1.2% in the

A horizon. Similarly, the understory and the litter layer contained only 0.6% and 1.2% of total N, respectively.

2.4.2.2 Total recovery of ^{15}N

In the meadow, the ^{15}N recovery in the litter layer, soil and below-ground biomass did not differ between the NO_3^- tracer (49% ± 5% SE) and the NH_4^+ tracer (44% ± 5% SE) (Table 3, Fig. 5). The ^{15}N tracer could be detected in all sampled pools already after one hour after labelling.

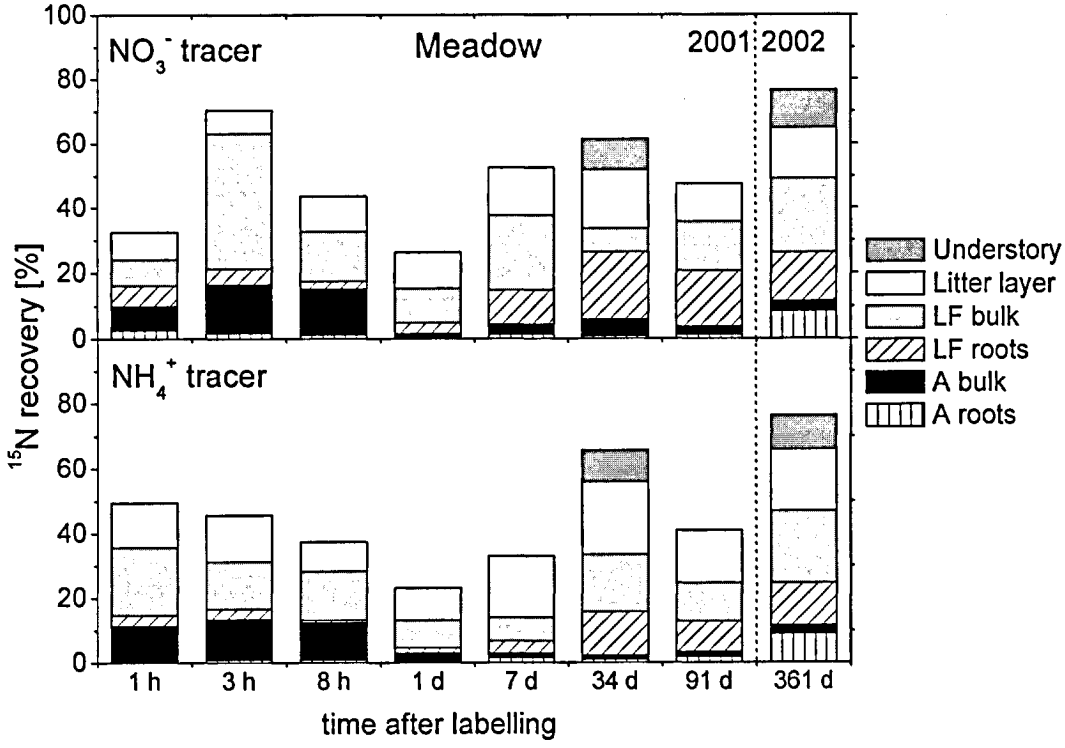

Figure 5: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in the understory vegetation, litter layer, LF and A horizons, as well as in the LF and A roots in the meadow at eight different sampling times (1 h, 3 h, 8 h, 1 d, 7 d, 34 d, 91 d, 361 d).

2.4.2.3 ^{15}N partitioning in the litter layer, soil and roots

During the first eight hours after the application, the largest sink for both tracers was the LF layer, followed by the A horizon and the litter layer for the NO_3^- tracer. For the NH_4^+ tracer, the litter layer had a higher recovery than the A horizon (Fig. 5). Both tracers had the lowest recovery in the roots. After one day, the recovery in the A horizon dropped sharply to a constant level of < 3% for both tracers. The recovery in the roots, especially in the roots of the

LF horizon, increased for both tracers. In the B horizon, there was almost no recovery (< 1%) for either tracer at any time within one year. Total tracer recovery for the different compartments was not different between tracers (Table 3). The tracer recovery over time increased for the litter layer (first grade, P= 0.003), it decreased with time for the A horizon (first and second polynomial grade, P= 0.03) and increased for the roots in the LF (first and third grade, $P < 0.001$) and A horizons (first and second polynomial grade, $P < 0.001$).

2.4.2.4 ^{15}N partitioning of soil compartments

As in the forest, the below-ground compartment in the meadow was further divided, but for logistic reasons, only for the sampling times of one hour, 34 days, 91 days and 361 days (Fig. 6).

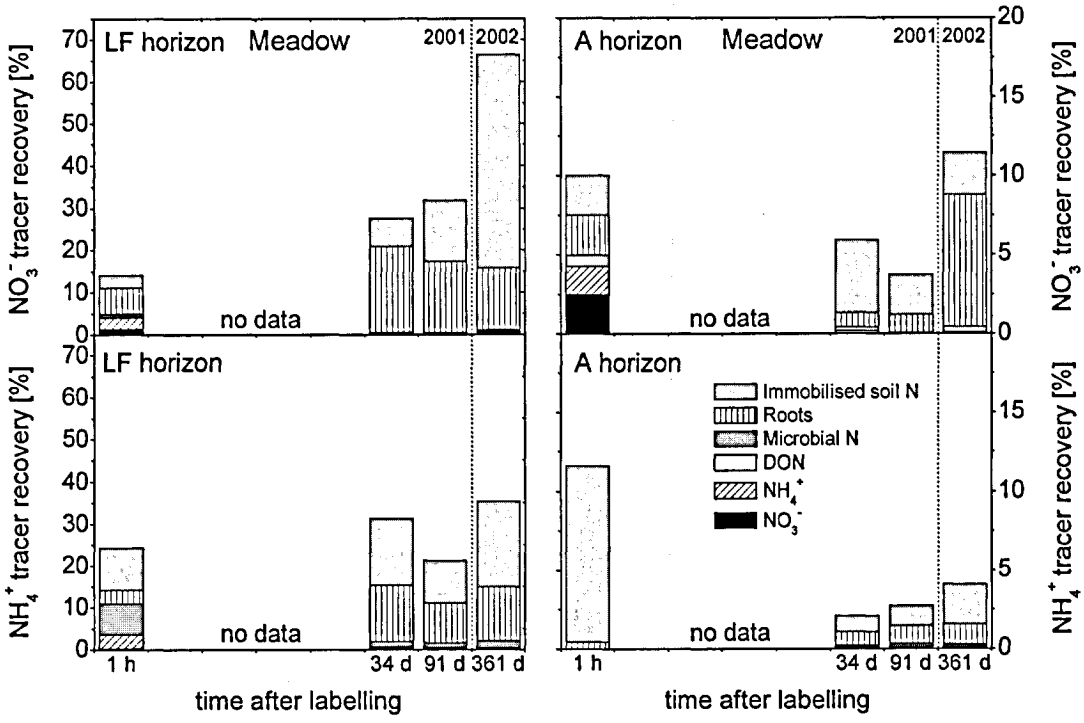

Figure 6: Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracers in the soil pool fractions: total extractable N (sum of NO_3^-, NH_4^+, DON), microbial N, roots and the remaining soil pool fraction in the LF and A horizons in the forest at four different sampling times (1 h, 34 d, 91 d, 361 d).

Total extractable ^{15}N – In the LF and A horizon, both tracers had a recovery below 3% (Fig. 6). Tracer recovery decreased over time for the total extractable N (sum of NO_3^-, NH_4^+ and DON) for both tracers (in LF horizon: $P < 0.001$, in A horizon: $P < 0.001$) (Table 3). Total extractable ^{15}N was further split into $^{15}NO_3^-$, $^{15}NH_4^+$ and $DO^{15}N$. For all three fractions, the

recovery did not differ between horizons and between tracers. However, ^{15}N recovery decreased over time for all fractions, for the $^{15}NO_3^-$ fraction (in both horizons: $P < 0.001$), the $^{15}NH_4^+$ fraction (in LF horizon: $P = 0.004$, in A horizon: $P < 0.001$) and $DO^{15}N$ (in LF horizon: $P < 0.001$, in A horizon: $P = 0.01$) (Table 3). In the LF and the A horizons, the proportion between the $^{15}NH_4^+$ and the $^{15}NO_3^-$ fraction was the same for both tracers and remained constant during the whole sampling period (Fig. 7).

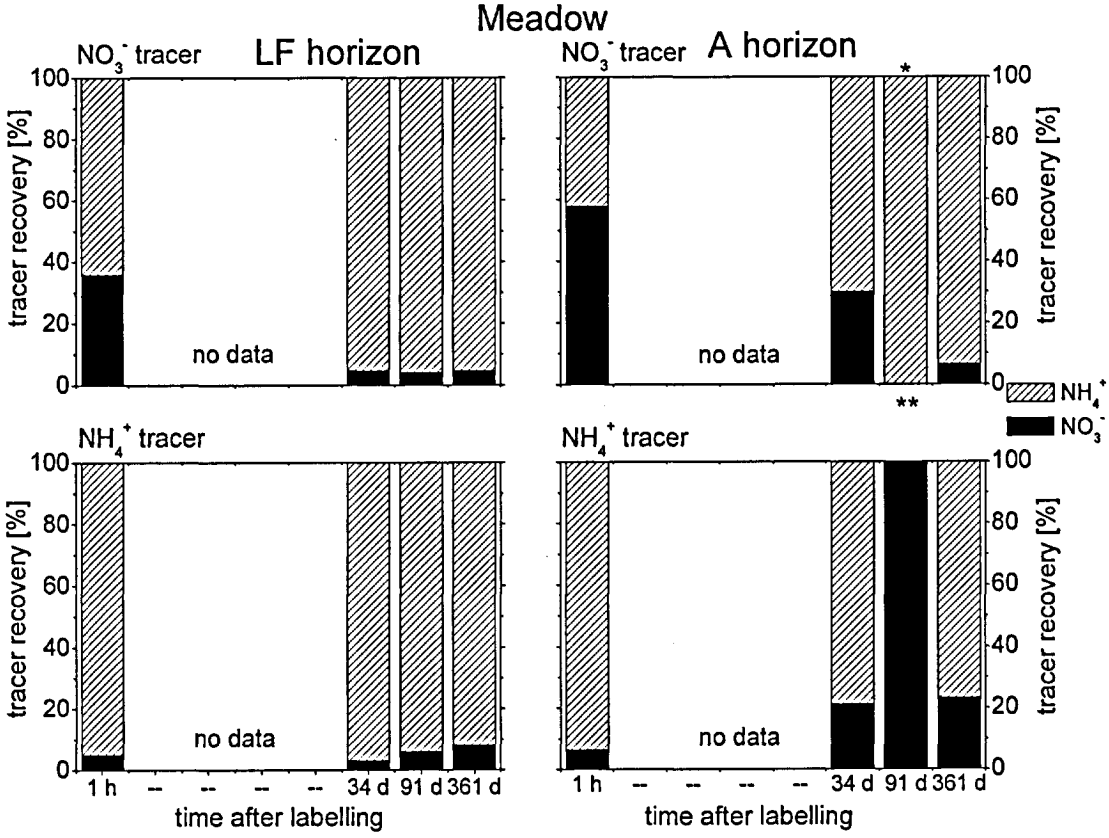

Fig. 7 Time course of total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracer in the $^{15}NO_3^-$ and $^{15}NH_4^+$ fractions of total extractable ^{15}N in the LF and in the A horizon in the meadow at four different sampling times (1 h, 34 d, 91 d, 361 d). *: nitrate below detection limit, **: ammonium below detection limit.

In the LF horizon, the $^{15}NH_4^+$ fraction was almost twice as high as the $^{15}NO_3^-$ fraction for the NO_3^- tracer after one hour (Fig. 7). In the A horizon, the $^{15}NO_3^-$ fraction was for the NO_3^- tracer after one hour almost 1.5 times higher than the $^{15}NH_4^+$ fraction. The recovery in the A horizon was below the detection limit for the $^{15}NO_3^-$ fraction of the NO_3^- tracer, and for the $^{15}NH_4^+$ fraction of the NH_4^+ tracer after 91 days.

Microbial ^{15}N - Tracer recovery in microbial ^{15}N was not different in both horizons for both tracers (Table 3, Fig. 6). Furthermore, tracer recovery over time did not decrease significantly. In the A horizon, recovery rates were below 1% at all sampling times.

Immobilised soil N - In the LF horizon, the NO_3^- tracer had a higher recovery than the NH_4^+ tracer ($P = 0.02$) (Table 3, Fig. 6). Both tracers could already be recovered after one hour. The tracer recovery over time was not significant. In the A horizon, neither the recovery of both tracers in ISN, nor the recovery over time was significant (Table 3).

2.4.2.5 ^{15}N partitioning in understory vegetation

Recovery in the ground vegetation in the meadow was measured after 46 days and 390 days (Fig. 4). The total recovery in mosses, monocotyledons and dicotyledon herbs did not differ between sampling times and tracers, neither for the total recovery of the groups nor for the single groups (Table 3). Only for the dicotyledon herbs, the recovery increased over time for both tracers ($P = 0.02$). No dicotyledon shrubs were present at all. Plant biomass was much higher for the monocotyledons than for the other two groups (Table 2). The specific labelling (labelled N / total N content of the pool) was the same for both, the monocotyledons and the dicotyledon herbs. The difference in biomass and N amount thus explained why more ^{15}N tracer was recovered in the monocotyledons.

2.5 Discussion

2.5.1 Total ^{15}N tracer recovery

The sum of tracer recovery in the measured pools ranged from 30% to 84% for both tracers and both ecosystems (Fig. 1 and 5). Total recovery of the NO_3^- tracer in the litter layer and the below-ground compartments was on average higher in the forest (60%) than in the meadow (49%), with the reverse pattern for the NH_4^+ tracer. Contrary to our expectation that the positively charged NH_4^+ would be retained better in the soil, much more NO_3^- tracer was recovered in both ecosystems. Total tracer recovery varied among the sampling times for both ecosystems. Apart from measurement uncertainties we can only speculate about a combination of factors which were not measured in this tracer experiment and which may be responsible for the incomplete tracer recovery, such as $^{15}NO_3^-$ leaching, ^{15}N uptake by trees, tracer loss due to denitrification, and ammonium volatilisation of the NH_4^+ tracer during application. However, all this factors influence only small ^{15}N tracer amounts.

2.5.2 ^{15}N tracer recovery in pools

As could have been assumed from the previous $^{15}NH_4{}^{15}NO_3$ tracer study at the same site (Schleppi et al. 1999a), the soil pool was the most important sink for both tracers and for both ecosystems for all sampling times.

However the *understory vegetation*, especially in the forest, turned out to be a major competitor for both tracers. In September 2001, the recovery in the forest understory was almost 25% of the applied NH_4^+ tracer. This high uptake was mostly due to the higher moss biomass in the forest. The moss layer acts as a very efficient filter, absorbing nutrients that arrive on its surface in rainfall or throughfall (Oechel & Van Cleve 1986; DeLuca et al. 2002). Thus, mosses have access to incoming nutrients earlier than the roots of vascular plants. Nutrients taken up by the mosses are generally not available to the vascular plants until the mosses die and undergo slow decomposition. The mosses had much higher recovery rates for the NH_4^+ than for the NO_3^- tracer for both sampling times in the forest. Mosses preferred NH_4^+ since less energy is used when NH_4^+ is directly assimilated in contrast to the higher energy requirement of nitrate reductase for the NO_3^- uptake (Chapter 4) In contrast to the mosses, the monocotyledons in the forest had a higher uptake of the NO_3^- tracer. We did not expect such a rapid ^{15}N uptake by the vegetation through the foliage, as this was the case, in particular for the mosses. Due to logistic reasons we sampled only after 46 days and 390 days, thus the samples for the understory vegetation for the first sampling times are missing. At the end of the growing season 2001, most of the above-ground vegetation (excluding trees) died after retranslocation of nutrients into the surviving below-ground organs. In both ecosystems, most species are perennial and therefore, ^{15}N recovery increased in the below-ground biomass of the LF and A horizons. In the growing season 2002, the plants partly grew from reserves in the below-ground organs, including ^{15}N. Therefore, ^{15}N could still be measured in the above-ground biomass in the second year, but was lower in the forest than in the first growing season and slightly higher in the meadow. In the meadow, the monocotyledons acted as a much stronger sink in the second year, whereas the dicotyledons had similar values to those in the first sampling. The reason for the difference between the monocotyledons and dicotyledons in tracer uptake is most likely due to plant physiology and anatomy, especially the difference in the root structure. Compared to the dicotyledons, the monocotyledons form a fibrous root system close to the soil surface, whereas the dicotyledons develop roots with a main axis, which may give them access to different N sources (Morot-Gaudry & Touraine 1997).

The *litter layer*, consisting of the tree (in the forest) and ground vegetation litter, had the second highest recovery, for both ecosystems at some sampling times. It was a strong sink through all sampling times, decreasing in the forest for both tracers only after 361 days. This decrease is probably due to (1) a dilution effect from new litter input, and (2) a transfer of ^{15}N from the litter layer into the LF horizon. After one year, the new vegetation litter had a lower ^{15}N concentration than at the previous sampling times, and the needles from adjacent trees, which received no or only low tracer input, had a low ^{15}N signal. The specific labelling in the vegetation was low and therefore the newly formed litter contained less ^{15}N than the litter directly labelled in the previous year. In the meadow, the opposite was observed, with the recovery in the litter layer increasing over one year.

The soil, consisting of the LF and the A horizons, was the most important sink for both tracers and both ecosystems for all sampling times. The recovery decreased downwards through the soil profile, and below 5 cm, tracer recovery was almost negligible, even in the year after snow melt. Similar results were reported by Schleppi et al. (1999a) for the Alptal site. In other studies (Buchmann et al. 1996; May et al. 1996; Nadelhoffer et al. 1999a), the soil was also the primary tracer sink and the recovery was higher in the organic than in the mineral horizon. On our site the $^{15}NH_4^+$ recovery was much lower than the $^{15}NO_3^-$ recovery, whereas on Buchmann et al.'s (1996) site the tracer recovery was almost the same. We found the highest recovery in the LF horizon in both ecosystems and for both tracers. For both tracers, most of the ^{15}N in the LF horizon was immobilised soil N (ISN) and was no longer available for plant N uptake within one year. However, shortly after the tracer application both ecosystems had the most important recovery in total extractable N and microbial N. This recovery decreased after one week in the forest and after one hour in the meadow.

Microbial biomass had a higher NH_4^+ tracer recovery in both ecosystems, whereas the NO_3^- tracer had a delayed peak. Perakis & Hedin (2001) observed similar patterns, which indicates that microbial NH_4^+ uptake takes place rapidly. Due to the possible chemical or physical tracer translocation or transformation, we do not know whether the ^{15}N in the delayed peak for the NO_3^- tracer is a primary uptake of $^{15}NO_3^-$ or a secondary uptake of already metabolised ^{15}N. A similar transformation could have happened in the A horizon, where the peak in the NO_3^- tracer was delayed as well.

Extractable N - In the first week after tracer application, the $^{15}NO_3^-$ fraction of total extractable N, especially for the NO_3^- tracer in the forest, was almost twice as high as the $^{15}NH_4^+$ fraction. However, after one week the $^{15}NO_3^-$ fraction decreased. This leads to the hypothesis that the NO_3^- tracer was somehow rapidly reduced to NH_4^+ in a process which must involve uptake, transformation and release by biota (still living or dying). After 34 days, the $^{15}NH_4^+$ fraction was the dominant inorganic ^{15}N component and an equilibrium between the two fractions was achieved. For the NH_4^+ tracer, the $^{15}NH_4^+$ fraction in the forest were much higher than the $^{15}NO_3^-$ fraction, and it was almost constant over time. This is in agreement with the general theory that the positively charged NH_4^+ tracer was fixed to particular exchange places, whereas the NO_3^- tracer, being negatively charged, was much more mobile. For the A horizon, similar patterns were observed. There the same switch was observed for the NO_3^- tracer from a high $^{15}NO_3^-$ fraction at the beginning to a higher $^{15}NH_4^+$ fraction later on.

Immobilised soil N- As already mentioned before the ISN was the most important tracer sink in the soil. In the LF horizon in the forest, the NO_3^- tracer had immediately high immobilisation rates, whereas the NH_4^+ tracer had recovery rates only after 1 day. There was no difference between tracers in the meadow. Therefore, one important issue is how the ^{15}N became incorporated into ISN. Traditionally, the "slow" pathway of nitrogen assimilation and retention is assumed to be related to the uptake of the available nitrogen released by microbial decomposition by trees, ground vegetation, and the subsequent recycling of nitrogen through litter and humus (Aber et al. 1998). However, several recent studies have shown that other, faster processes must be responsible for the immobilisation of N in the soil by biotic or abiotic processes (Hart et al. 1993; Berntson & Aber 2000; Johnson et al. 2000; Zogg et al. 2000; Dail et al. 2001; Perakis & Hedin 2001). Also on our site, it seems that faster processes were responsible for the immobilisation processes. The very fast recovery of the NO_3^- tracer in the ISN leads us to assume that abiotic immobilisation via chemical processes played an important role. Hart et al. (1993) showed that half of the recovery in the ISN (their ISN also included surface litter and roots) may have been fixed abiotically. Similarly, several studies have found fast abiotic nitrate immobilisation in forest soils (Davidson et al. 2003; Fitzhugh et al. 2003; Dail et al. 2001, Thorn & Mikita 2000). According to these studies, we can hypothesise that the high recovery of the NO_3^- tracer in ISN is due to a possible transformation of the NO_3^- tracer into NO_2^-, which then was bound immediately to the ISN. A fraction of the NH_4^+ tracer may have been incorporated permanently in the structure of the

illite clay. This binding is strong and a release into new plant-available N compounds needs a rather long time, possibly more than a year (Gebauer et al. 2000). Also in the longer term, after 1 year the ISN had still a high recovery. However, this is in contrast to a recent study by Zak et al. (2004) on a sandy soil in a northern hardwood forest where no ^{15}N tracer was recovered in the soil organic matter after one year. They suggest that the immobilised ^{15}N in soil organic matter was released between one month and one year.

Unlike in the forest, we found in the *A horizon* of the meadow a high (the second largest) recovery rate for both tracers within the first eight hours. This is possibly due to the fact that the boundary between the LF and A horizons in the meadow is not sharp, but is densely invaded by roots. Therefore, the ^{15}N tracer is quickly transported from the root layer to the A horizon. After the rainfall of 11.8 mm between the sampling times of eight hours and one day, the recovery in the A horizon dropped to below 3%. This sharp decrease suggests that the tracer was leached out of the A horizon. In the forest, no similar pattern could be observed and the recovery in the A horizon decreased only slightly over time. This small decrease in the forest is possibly due to leaching loss by preferential and lateral flow, as they are typical on this site (Flury et al. 1994; Feyen et al. 1996). Vertical leaching through the soil matrix can be excluded because no recovery increase was measured in the B horizon.

2.6 Conclusion

The results of our ^{15}N field-experiment generally confirm the findings of previous ^{15}N tracer studies in forests that most of the tracer is retained in the soil pool (Buchmann et al. 1996; May et al. 1996; Nadelhoffer et al. 1999b; Gebauer et al. 2000) and stresses the importance of the fast N immobilisation processes (Hart et al. 1993; Berntson & Aber 2000; Johnson et al. 2000; Zogg et al. 2000; Dail et al. 2001; Perakis & Hedin 2001). It shows, on the one hand, the high capacity of the studied ecosystems to retain most of the deposited N over at least one year. But, on the other hand, this leads to the assumption that the increasing N concentration in the soil will lead to the C:N ratio decreasing in the longer term. This may lead to N saturation, and it is evident that the immobilisation capacity of the soil will be limited in the long-term. This could lead to acidification of the soil and increased NO_3^- leaching, which impairs groundwater and surface water quality.

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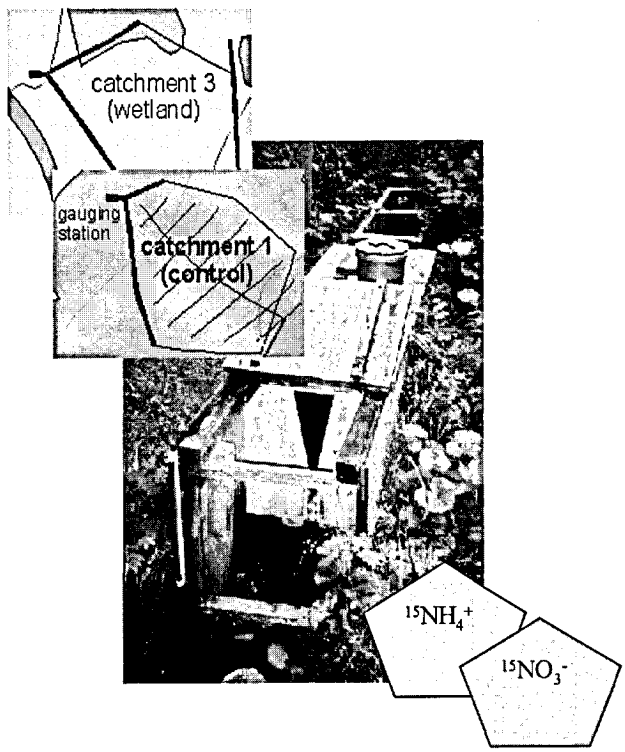
Chapter 3

Flow of deposited inorganic N in two Gleysol-dominated mountain catchments traced with $^{15}NO_3^-$ and $^{15}NH_4^+$



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Submitted to Biogeochemistry

3.1 Abstract

In two mountain ecosystems at the Alptal research site in central Switzerland, pulses of $^{15}NH_4$ and $^{15}NO_3$ were separately applied to trace deposited inorganic N. One forested and one litter meadow catchment, each approximately 1600 m^2, were delimited by trenches in the Gleysols. $K^{15}NO_3$ was applied weekly or fortnightly over one year with a backpack sprayer, thus the seasonality of the ambient atmospheric nitrate deposition was thereby mimicked approximately. After the sampling and a one-year break, $^{15}NH_4Cl$ was applied as a second one-year pulse, followed by a second sampling campaign. Trees (needles, branches and bole wood), ground vegetation, litter layer and soil (LF, A and B horizon) were sampled at the end of each labelling period. Extractable inorganic N, microbial N, and immobilised soil N were analysed in the LF and A horizons. During the whole labelling period, the runoff water was sampled as well.

Most of the added tracer remained in both ecosystems. More NO_3^- than NH_4^+ tracer was retained, especially in the forest. The highest recovery was in the soil, mainly in the organic horizon, and in the ground vegetation, especially in the mosses. Event-based runoff analyses showed an immediate response of ^{15}N in runoff, with sharp ^{15}N peaks corresponding to discharge peaks. NO_3^- leaching showed a clear seasonal pattern, being highest in spring during snowmelt. The high capacity of N retention in these ecosystems leads to the assumption that deposited N accumulates in the soil organic matter, causing a progressive decline of its C:N ratio.

Keywords: Nitrogen deposition, mountain forest, mountain meadow, ^{15}N tracer, nitrate leaching

3.2 Introduction

During the last few decades, human activities have increased the production of reactive nitrogen through intensive agriculture and fossil fuel combustion (Galloway et al. 1995; Vitousek et al. 1997). The antrophogenic creation of reactive nitrogen increased between 1860 and 2000 from approximately 15 Tg N a^{-1} to 165 Tg N a^{-1}, with about five times more reactive nitrogen coming from losses resulting from food production than from energy production (Galloway et al. 2002). These various reactive nitrogen molecules cycle by biochemical pathways and are easily distributed by hydrological and atmospheric transport processes over long distances and move from one environmental system to another (Galloway et al. 2002). This phenomenon is called the nitrogen cascade (Galloway 1998; Tietema et al. 1995). Within the nitrogen cascade, temperate forests and grasslands can be major reservoirs and a short to long-term sink for the reactive nitrogen. Given the N-limited nature of both ecosystem types there is a large potential for reactive nitrogen accumulation with a residence time of years to centuries causing a slow eutrophication (Galloway et al. 2003).

In several studies, the N deposition of forest ecosystems was manipulated to evaluate the complex interactions of processes in the N cycle and to measure N cycling within these ecosystems (Emmett et al. 1998; Tietema et al. 1998; Wright & Rasmussen 1998; Nadelhoffer et al. 1999a, b; Lamontagne et al. 2000). In the European research project NITREX, all sites showed an immediate response of nitrate leaching within the first year of either N addition or N exclusion (roof experiment). In these coniferous forests the responses of vegetation and soil were delayed (Moldan et al. 1995; Gundersen & Rasmussen 1995; Gundersen et al. 1998). The Alptal site in Switzerland was part of the NITREX project, representing N-limited mountain ecosystems. Similarly to the other NITREX sites, nitrate leaching increased at the Alptal site within a few weeks of N addition. According to Schleppi et al. (2004), NO_3^- is released at the Alptal site by three different mechanisms: (1) NO_3^- from precipitation bypassing the soil by preferential flow path, (2) flushing of older precipitation NO_3^- temporarily stored in the soil pores and (3) flushing of NO_3^- produced by nitrification. NO_3^- leaching occurred even though the trees were still slightly deficient in N (Hagedorn et al. 2001; Schleppi et al. 1999b; Schleppi et al. 2004). However, over 60% of the added $^{15}NH_4{}^{15}NO_3$ tracer was recovered in the soil pool, and only approximately 10% was leached as NO_3^- (Schleppi et al. 1999a). Despite considerable information on various aspects of the nitrogen cycling on the Alptal site, a lot about the nitrogen accumulation in the ecosystems is

still unclear. To understand the mechanisms influencing the nitrogen flow through the ecosystems, there is need for a deeper understanding of the single processes and flows, particularly in the soil pool as well as in the leaching from the catchment.

In this study, we applied, in contrast to the study of Schleppi et al. (1999a), the NH_4^+ and the NO_3^- tracers separately, on a forest and a litter meadow catchment (each 1600 m^2) to trace deposited inorganic N. Therefore, we were able (1) to quantify after one year the ^{15}N recovery of both N forms through the ecosystem among trees, understory vegetation, litter layer, soil, roots; and (2) to follow the flow of $^{15}NO_3^-$ in runoff (in part event-based) continuously over one year. This way, we were able (3) to infer about the accumulation and absorption capacity of nitrogen in both ecosystems.

3.3 Material and methods

3.3.1 Study area

The research site is located in the Alptal valley, on the northern edge of the Alps of central Switzerland (47°03' N, 8°43' E), at 1200 m a.s.l.. It lies within the Erlenbach headwater catchment, which covers 0.7 km^2 and consists of 40% naturally regenerating forest and 60% litter meadow, both never artificially fertilised. The *Picea abies* stand has a relatively low leaf area index of 3.8 (Schleppi et al. 1999b) with trees up to 250 years old. Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} a^{-1}, with equal contributions of NO_3^- and NH_4^+ (Schleppi et al. 1999a). The climate is cool and wet, with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm, reaching a maximum in June (270 mm) and a minimum in October (135 mm). Usually, soils are covered with snow from mid-November to April and the vegetation period lasts from June to September. The parent rock material is Flysch, and the major soil types are clay-rich Gleysols of low permeability with the water table close to the surface throughout the year (Hagedorn et al. 1999). Slope is about 20% with a west aspect. Generally, the soil profile consists of a LF, an A and a B horizon. In the forest, vegetation and soil types form a mosaic pattern closely related to microtopography. Trees grow on mounds, were the water table is at a depth below 40 cm and umbric Gleysols are abundant with raw humus (LFH), Ah and oxidised or partly oxidised Bg or Br horizons. On mounds, dominating plant species are *Picea abies*, with 15% *Abies alba* and *Vaccinium myrtillus*. In depressions, the water table frequently reaches the surface, leading to mollic Gleysols with a thin LF horizon, an anmoor topsoil (Aa) and an almost permanently reduced Bg or Br horizon. The waterlogged depressions are too wet for tree growth, and ground

vegetation is dominated by *Caltha palustris*, *Petasites albus*, *Poa trivialis* and *Carex ferruginea* (Muller 1997). In contrast to the forest, the meadow used for litter production has no distinct microtopography and the soil has, like the depressions in the forest, an anmoor topsoil (mollic Gleysol) and an almost permanently reduced Bg or Br horizon. The vegetation consists mainly of a small sedge meadows (*Molinietum*), with a few small trees (*Picea abies* and *Alnus incana*). It lies fallow since 20 years.

3.3.2 Experimental design

One forested and one meadow catchment, each approximately 1600 m^2, were delimited by trenches (Schleppi et al. 1998) (Fig. 1).

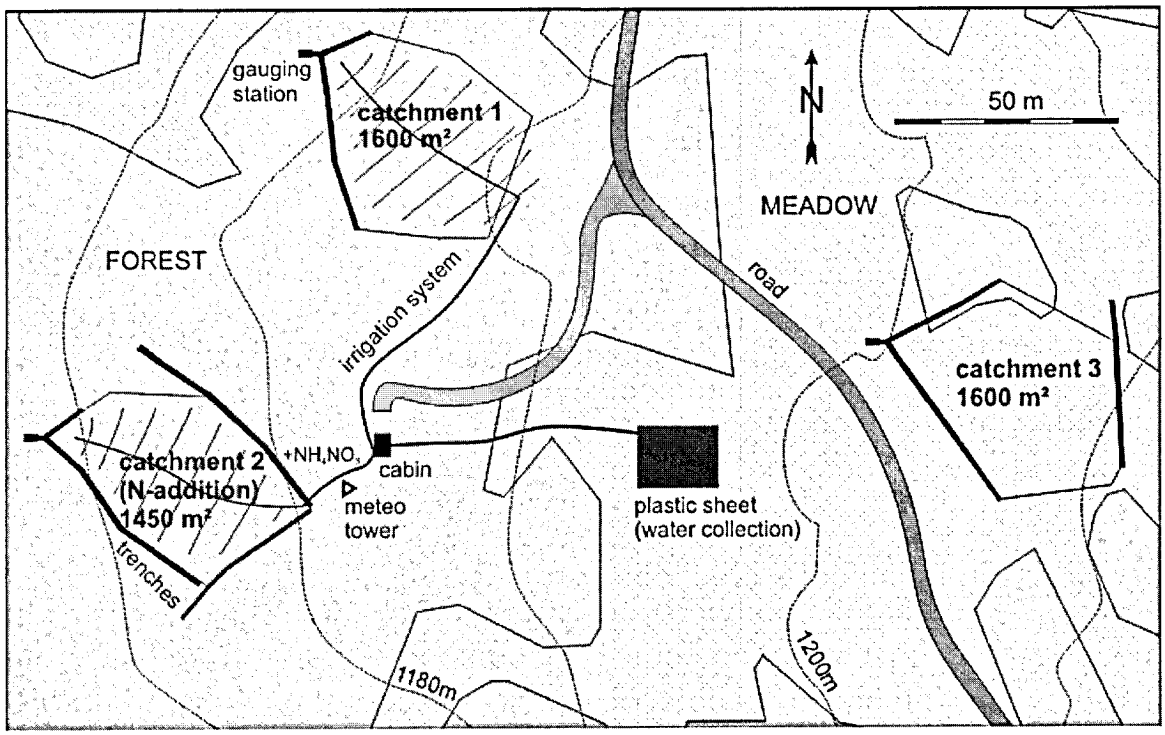

Figure 1: Experimental set-up of the forest (1) and meadow (3) catchment in Alptal, Switzerland.

$K^{15}NO_3$, resp. $^{15}NH_4Cl$ tracers (both 99 atom% ^{15}N), dissolved in deionised water (10 l / catchment), were applied as two successive labellings with a backpack sprayer on each catchments directly above the ground vegetation to label the atmospheric deposition of inorganic N. The application occurred in minimal but frequent doses during one year, weekly during the vegetation period and fortnightly in winter. The seasonality of the ambient deposition rates was thereby mimicked approximately. The first labelling ($K^{15}NO_3$) started in summer 2000, and the second labelling ($^{15}NH_4Cl$) started in summer 2002 after a one-year chase period. The second labelling was stronger to mask the residual effects of the application

of the $K^{15}NO_3$ two years earlier. Due to the high isotopic enrichment, labelling did not exceed 0.1 kg N ha^{-1}, so that the associated fertilisation effect was negligible compared to ambient deposition. The catchments were equipped for proportional sampling of runoff water. Runoff was continuously measured at a gauging station with a V-notch weir (Schleppi et al. 1998). Immediately after the first application of the NO_3^- tracer, the runoff of the first two rain events was sampled with a high temporal resolution (30 runoff samples in five days). Based on previous research (Schleppi et al. 1999a), 10 to 20% of the labelled nitrate was expected to be leached by preferential flow paths already during the first event. After this event-based analysis, the flux of ^{15}N as nitrate in runoff water was measured throughout the experiment, using samples taken weekly and pooled proportionally over two weeks, one month and later on three month periods. Additionally, soil, trees (needles, branches and bole wood), and ground vegetation were sampled in September 2001, three months after the end of the first and in 2003, three months after the end of the second labelling period. Soil samples were taken in a grid of 8 x 8 m with a soil corer (5 cm inner diameter, 25 cm depth) reaching into the B horizon. These soil cores were immediately put on ice in the field, transported to the laboratory and processed within one day. Due to the microtopography and the mosaic pattern of the soil, the soil cores were grouped according to the soil types (mollic or umbric Gleysol) to obtain sufficient soil for the analysis. Out of 21 cores in the meadow, five soil groups were formed. Each group formed one composite sample of four or five single cores. Similarly, in the forest, 24 cores were split up into four soil groups of six single soil cores. In this case, each group was further split up into two subgroups with three single soil cores from the upper or the lower part of the catchment to form one composite sample. The soil cores consisted of litter layer, LF horizon (approx. 5 - 0 cm), A horizon (approx. 0 - 5 cm) and B horizon (5 - 20 cm). The above-ground vegetation was sampled separately. For this purpose, the ground vegetation was clipped at a place (18.5 cm x 20 cm) close to the soil sampling points and pooled like the soil samples into composite samples. Each vegetation sample was kept refrigerated and processed in the laboratory within one day. The trees were sampled in winter 2000 / 2001 and in winter 2002 / 2003. Sampling was done as described previously in Schleppi et al. (1999a).

3.3.3 Laboratory analyses

The soil samples were separated into surface litter and into the soil horizons LF, A and B. From the LF and the A horizon material, the roots were picked out by hand with tweezers, rinsed free of soil with deionised water and separated into the diameter classes of > 4 mm and < 4 mm. Large roots (> 15 mm diameter) were removed. At least 200 g field-moist soil per

sample was freed from roots (work time ~4h) and used for total N and ^{15}N analyses as well as for extractions. Extractable inorganic N and ^{15}N (NH_4^+-N, NO_3^--N) and total extractable N and ^{15}N were determined by adding 40 g of soil material from the LF horizon or 60 g from the A horizon to 160 ml of 0.5 M K_2SO_4, then shaking the mixture for 1.5 h and filtering it through folded filters (790 ½, diameter 185 mm, Schleicher & Schuell, Dassel, Germany) into PET bottles. The extract was stored in the freezer until further processing. As a next step, half of the extract was lyophilised using a freeze dryer (Christ, BETA 1-8, Osterode am Harz, Germany) to measure total extractable N and ^{15}N using mass spectrometry. With the other half of the extract, extractable $^{15}NO_3^-$ and $^{15}NH_4^+$ concentrations were analysed using the diffusion method (adapted from Downs et al. 1999). About 80 ml of the extract were put into 100 ml PE bottles, together with a glass microfibre filter (GF/F 25mm, Whatman, Maidstone, England; 5 mm x 12 mm, calcinated in a muffle furnace (Naber, Type No. 7, Zurich, Switzerland) for 6 h at 450 °C), wetted with 30 µl H_2SO_4 2M and wrapped in PTFE band. Then 1.5 mg l^{-1} MgO were added and the bottle was tightly closed. NH_4^+ was thus converted to NH_3, diffused through the PTFE band and finally captured on the microfibre filter. The bottles were shaken (40 min^{-1}) for a week, whereupon the filter was removed, dried in an evacuated desiccator in the presence of concentrated sulphuric acid, and then unpacked from the PTFE band. The filter was stored in a glass vial and packed in a silver cup prior to mass spectrometric analysis. The remaining extract was further processed by adding a new microfibre filter and 0.5 g Devarda's alloy. Thereby, NO_3^- was reduced to NH_4^+, and the same chemical procedure was repeated as with NH_4^+.

N and ^{15}N in the microbial biomass were determined by chloroform fumigation and extraction (Brookes et al. 1985). 20 g of the soil material from the LF horizon or 30 g from the A horizon were fumigated with $CHCl_3$ in an evacuated desiccator in the dark for 24 hours and afterwards extracted with 80 ml of 0.5 M K_2SO_4. The extract was stored in the freezer and then lyophilised entirely to determine the total N concentration and the ^{15}N / ^{14}N isotope ratio. The remaining bulk soil and all other solid samples (above and below-ground biomass, litter) were dried to constant weight at 65 °C and the dry matter content was calculated. All samples were ground, and the total N and ^{15}N concentrations were determined by an elemental analyser coupled to an isotope-ratio mass spectrometer (Delta S, Finnigan, Bremen, Germany). The soil samples were ground with a vibratory mill (0.25 mm), the above and below-ground biomass materials with a centrifuge grinder (0.5 mm), and the litter layer with a coffee grinder. The vegetation was first separated into five groups: monocotyledons,

dicotyledon herbs, dicotyledon shrubs and mosses. The C:N ratio of all three soil horizons was measured with a C+N analyser (NC 2500, CE instruments Thermoquest, Milano, Italy).

3.3.4 Calculation of recovery rates

The N pools of the ecosystem were calculated from dry masses and N concentrations. The isotopic values are presented in the δ notation (Eq. 1):

$$(1) \qquad \delta^{15}N = R_{sample}/R_{standard} - 1$$

where R is the molar fraction ^{15}N / ^{14}N. $\delta^{15}N$ is usually expressed in ‰ (times 1000). The atmospheric N_2 is used as a standard with $R_{standard}$ = 0.0036765. The molar fraction R_{sample} is thus calculated as (Eq. 2):

$$(2) \qquad R_{sample} = \left(\delta^{15}N + 1\right) \cdot R_{standard} = {}^{15}N/{}^{14}N$$

The fractional abundance of ^{15}N in the sample is defined by (Eq. 3):

$$(3) \qquad F_{sample} = R_{sample}/\left(R_{sample} + 1\right) = {}^{15}N/\left({}^{15}N + {}^{14}N\right)$$

The tracer fraction X_{sample} defined as the molar ratio of tracer N to total N content of a pool can be calculated as (Eq. 4):

$$(4) \qquad X_{sample} = \left(F_{sample} - F_{reference}\right)/\left(F_{tracer} - F_{reference}\right)$$

where $F_{reference}$ is the fractional abundance of ^{15}N in the pre- or nonlabelled sample and F_{tracer} is the fractional abundance of the applied tracer (atom%). For the NO_3^- tracer experiment, the control (nonlabelled) samples were used as $F_{reference}$; the F_{sample} from the NO_3^- tracer (prelabelled) was then used as $F_{reference}$ for the NH_4^+ tracer. By doing this subtraction, we have to keep in mind that the ^{15}N recovery of the NO_3^- tracer is not constant over this time period and therefore it is just an approximation of the actual values. Studies by Providoli et al. (Chapter 2) showed that changes were relatively small from one month onwards.

The tracer recovery in an ecosystem pool (Z_{pool}) was calculated as a fraction of added tracer (Eq. 5):

$$(5) \qquad Z_{pool} = X_{sample} \cdot N_{pool} / N_{tracer}$$

where N_{pool} is mass of the N pool [mol] and N_{tracer} is mass of applied tracer [mol], both expressed on the same basis (per plot per unit area). Z_{pool} is usually expressed in % (times 100).

For soil microbial N, the recovered ^{15}N was calculated as the difference between the recovered ^{15}N in the extract of fumigated soil and that in the non-fumigated soil (no correction factor was used for extraction efficiency). The extractable $DO^{15}N$ was calculated as total extractable ^{15}N minus extractable $^{15}NO_3^-$-N and $^{15}NH_4^+$-N. The recovery in soil minus the recoveries in soil microbial ^{15}N and extractable ^{15}N was considered as immobilised soil N (ISN). According to Schleppi et al. (1998), the water budget of the small catchments is not influenced by unknown water ways. Therefore, quantitative N budgets were calculated from measurements of deposition inputs and leaching outputs. The tracer concentration in runoff [$\Delta\delta^{15}N$] was calculated as $\delta^{15}N$ of the sampled runoff minus $\delta^{15}N$ of the runoff before the tracer application. Background levels of denitrification were estimated based on previous studies on the manipulated forest catchment on this site (Mohn et al. 2000). These measurements were used to assess denitrification on the control catchments. For the meadow, measurements on the anmoor topsoil in the forest were used for an estimation. According to Hagedorn et al. (1999), flow paths in the soil had the highest NO_3^- supply and were the locations with the highest denitrification activity. Therefore, we assumed that the denitrification had the same $\delta^{15}N$ as the runoff, and tracer recovery was estimated. Due to logistical constraints, the experiment within the forest and the meadow was not replicated. Therefore, the differences between these ecosystems could not be tested statistically. Comparisons between the forest and the meadow were thus made only descriptively.

3.4 Results

3.4.1 Event-based runoff for NO_3^- tracer

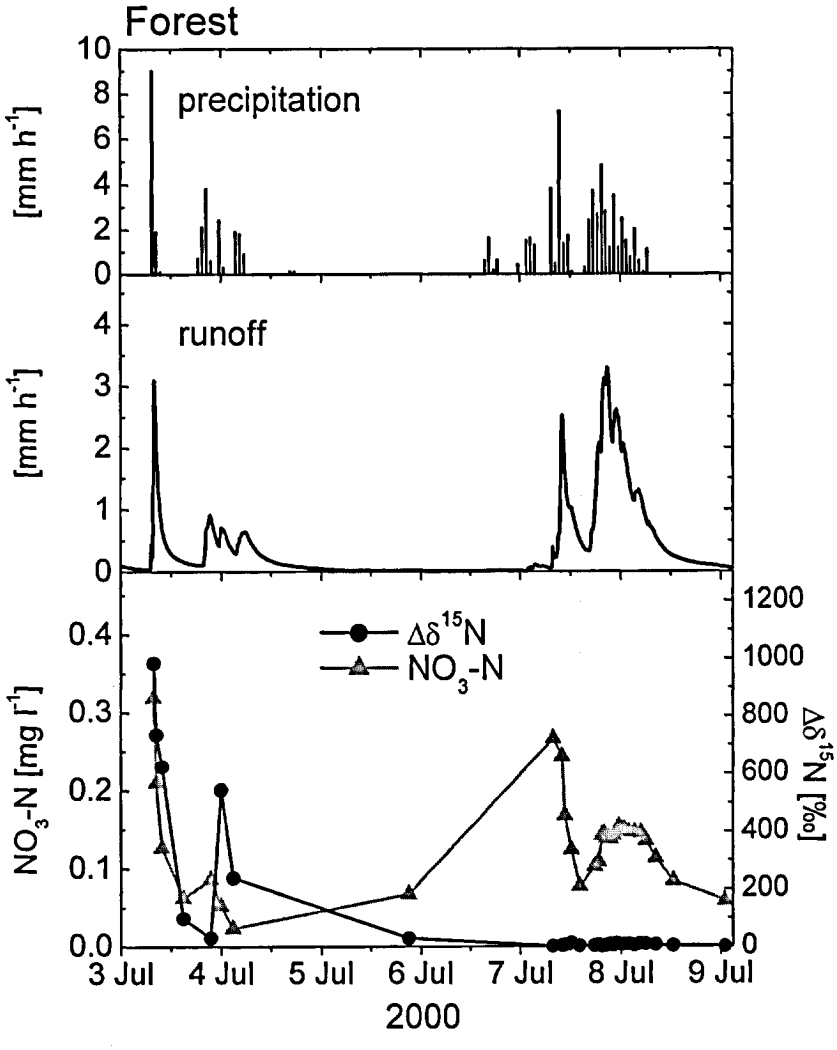

Figure 2: Runoff from the experimental forest catchment, its concentration in NO_3^- and $\Delta\delta^{15}N$ during two rainfall events in July 2000.

The first two rain events after the first NO_3^- tracer application were sampled with a high temporal resolution for both catchments starting on July 3 and July 7, 2000 (Fig. 2 and Fig. 3). During the first event, it rained 31 mm and in the second 42 mm. Discharge responded rapidly to storm events and the highest runoff peak reached in both rain events, in the forest as well as in the meadow, 3.4 mm h^{-1}. In the forest, the NO_3^- concentration was highest during the first discharge peak in both rain events. By the end of the runoff peak, the concentration declined to values close to those measured before the rain. In the meadow, the NO_3^-

concentration in runoff was lower than in the forest and the peaks were less pronounced (Fig. 2 and Fig. 3).

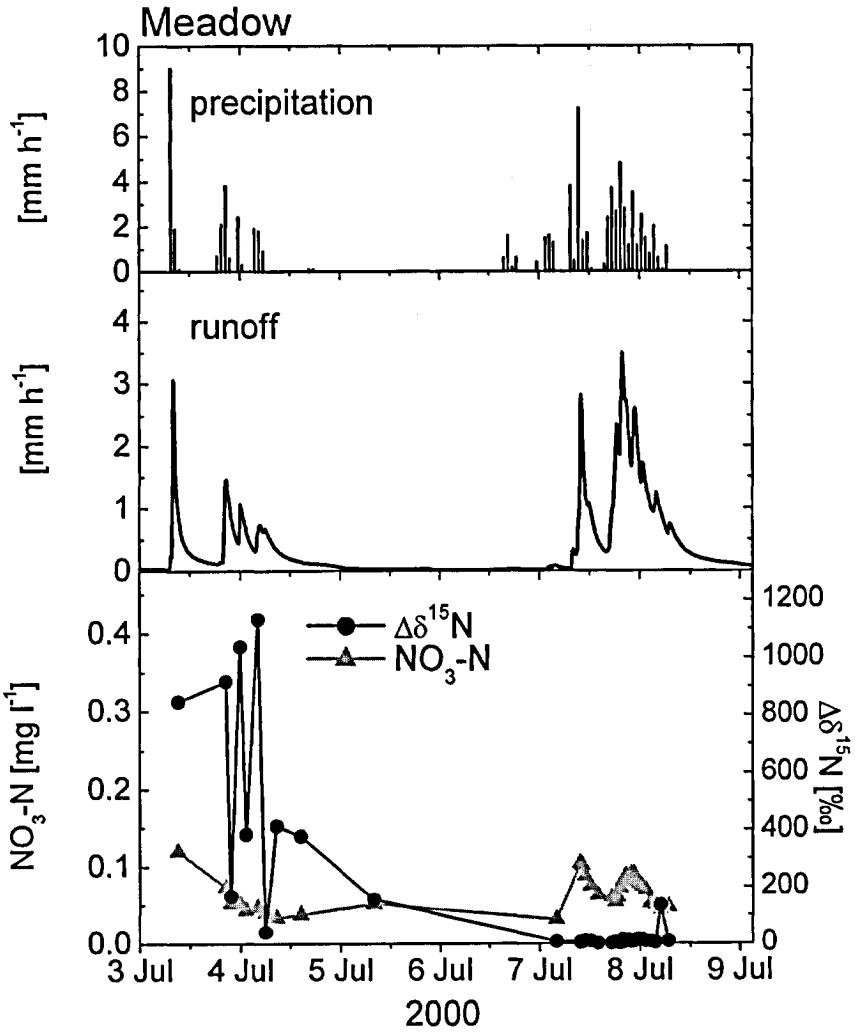

Figure 3: Runoff from the experimental meadow catchment, its concentration in NO_3^- and Δδ15N during two rainfall events in July 2000.

In the forest, $\Delta\delta^{15}N$ enrichment was highest at the first discharge peak on July 3 and had a second sharp peak at the second discharge peak on July 3 (Fig. 2). In the second rain event, on July 7, the $\Delta\delta^{15}N$ remained close to zero. In the meadow, in the first rain event, $\Delta\delta^{15}N$ showed sharp peaks coinciding with the discharge peaks (Fig. 3). As in the forest, the $\Delta\delta^{15}N$ was at a low level for the second rain event, showing only one peak at the end of the rain event. For both ecosystems, the $\Delta\delta^{15}N$ was dynamic, whereas the NO_3^- concentration was less dynamic than $\Delta\delta^{15}N$.

3.4.2 Weekly runoff for NO_3^- and NH_4^+ tracers

Over the one year of tracer application, runoff was sampled for both tracers. From nine month on, the NO_3^- leaching for the $^{15}NO_3$ labelling was higher in the meadow than in the forest (Fig. 4). For both ecosystems, a strong NO_3^- leaching started at the beginning of snowmelt in March 2001. The $^{15}NO_3^-$ leaching followed the same seasonal pattern. Especially in winter 2001 and in spring 2001, ^{15}N in runoff was two and four times higher in the meadow than in the forest, respectively. After the cessation of the $^{15}NO_3^-$ labelling in June 2001, NO_3^- leaching was still going on and had again the highest values at snowmelt in 2002, which started a bit earlier than the year before, i.e. in January. However, the $^{15}NO_3^-$ leaching stopped in both ecosystems after the cessation of the labelling.

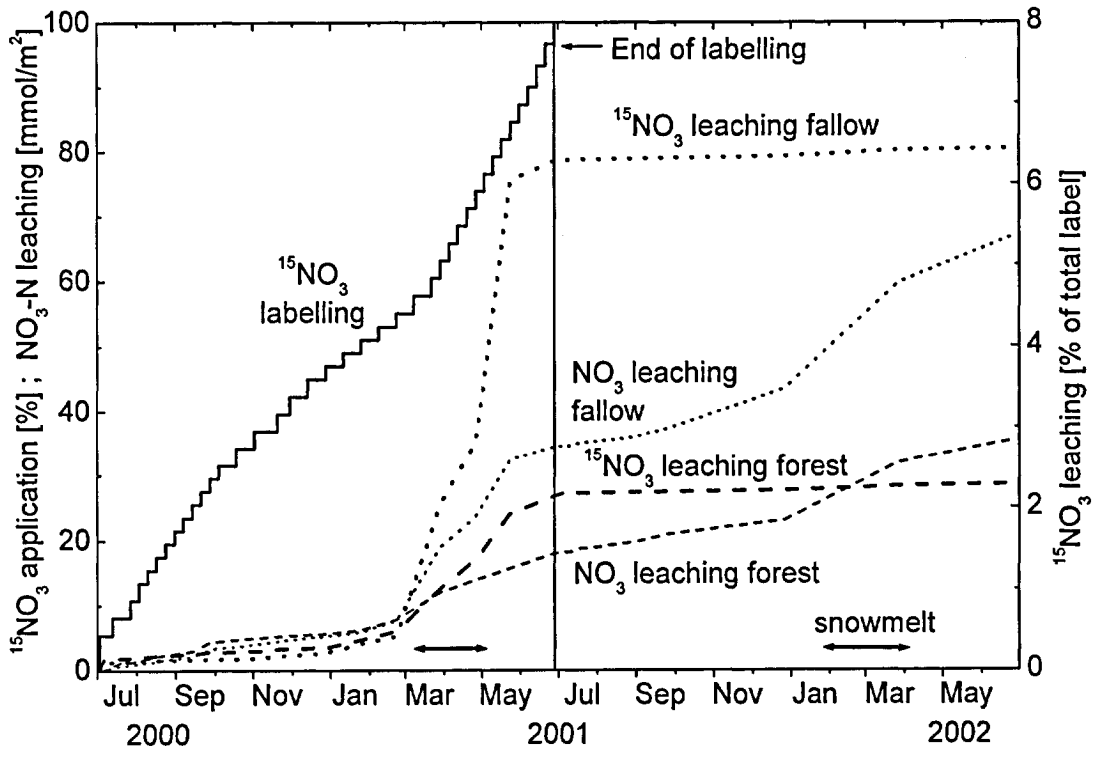

Figure 4: NO_3^- tracer addition and nitrate concentration in discharge-proportional runoff samples from the experimental forest and meadow catchment. $^{15}NO_3^-$ of total label and NO_3^- leaching concentrations from July 2000 to May 2002.

For the NH_4^+ tracer, the total ^{15}N flow in runoff was less than 0.2% in the forest over one year (June 2002 - June 2003) (Table 3). Due to the low NH_4^+ leaching an analogous figure to the figure 3 for the NO_3^- tracer is missing and we represent the results in Table 3. The recovery in the meadow was a slightly higher, reaching 0.6%. The highest ^{15}N leaching was in spring 2003 during snowmelt.

3.4.3 Total recovery of ^{15}N for the NO_3^- tracer in 2001

Total ^{15}N recovery was higher in the forest (81%) than in the meadow (67%) (Table 1).

Table 1: Partitioning of the added ^{15}N labelled nitrogen for the NO_3^- tracer in trees, ground vegetation, litter layer, soil horizons, roots and runoff in forest and meadow, as means. Pool sizes as dry matter, N concentration, N pool, tracer fraction and tracer recovery.

Forest: NO_3^- tracer		Pool size	N conc.	N pool	Tracer	
Pool		[kg/m²]	[g/kg]	[g/m²]	Fraction [µmol/mol]	Recovery [%]
Vegetation (above-ground)						
	Trees	18.1	2.2	39.4	2.3	5.6
	Mosses	0.06	17.8	1.1	321	13.6
	Monocotyledons	0.03	14.6	0.4	100	1.5
	Dicotyledon herbs	0.02	15.1	0.3	122	1.6
	Dicotyledon shrubs	0.03	11.1	0.3	87	1.1
	Other species	0.04	15.2	0.6	53	1.2
Litter layer						
	Litter	0.5	13.4	6.8	53	14.4
Roots						
	LF roots	0.8	14.2	6.5	23	5.9
	A roots	0.3	15.7	2.9	10	1.1
Soil						
	LF (5 - 0 cm)	4.9	12.2	59.7	5	11.2
	A (0 - 5 cm)	19.9	8.5	169.4	0	2.8
	B (5 - 20 cm)	68.0	2.9	195.2	2	16.8
Runoff		3276	0.00015	0.496	173	2.3
Denitrification[2]						1.7
Recovery						80.8
Meadow: NO_3^- tracer		Pool size	N conc.	N pool	Tracer	
Pool		[kg/m²]	[g/kg]	[g/m²]	Fraction [µmol/mol]	Recovery [%]
Vegetation (above-ground)						
	Trees[1]	1.3	3.1	4.1	3.2	0.4
	Mosses	0.003	23.6	0.1	181	0.4
	Monocotyledons	0.26	11.0	2.9	105	9.6
	Dicotyledon herbs	0.04	10.7	0.4	185	2.3
Litter layer						
	Litter	0.2	15.4	3.7	92	10.9
Roots						
	LF roots	0.5	11.7	4.7	30	4.5
	A roots	0.5	17.5	4.9	25	3.9
Soil						
	LF (5 - 0 cm)	8.8	10.4	91.7	7	19.1
	A (0 - 5 cm)	37.0	7.3	270.2	1	7.1
	B (5 - 20 cm)	55.5	5.5	303.6	0	0.0
Runoff		4442	0.0002	0.9	297	6.4
Denitrification[2]						2.7
Recovery						67.1

[1] Small trees growing in the meadow

[2] Calculation mentioned in material and methods

In the forest, 25% of the tracer was recovered in the above-ground biomass. The highest ^{15}N sink was the moss layer (14%), and 5% were recovered in the herbaceous ground vegetation. Both, the tracer fraction and the pool size were high for the mosses, whereas the tracer fraction as well as pool size were lower for the monocotyledons, dicotyledon herbs and dicotyledon shrubs. The trees recovered 6% of the tracer.

In the meadow, the above-ground biomass (including a few trees) recovered 13%, whereas the monocotyledons had the highest recovery (9.6%). The tracer fraction was higher for the dicotyledon herbs than for the monocotyledons, but the difference in pool size explained the higher recovery for the monocotyledons. Almost no mosses were present.

The recovery in the litter layer was higher for the forest than for the meadow. The tracer fraction of this layer was higher for the meadow, but the litter pool size in the forest was two and a half times larger leading therefore to a higher recovery.

In the LF and A horizons, the recovery in the meadow was higher, whereas the recovery in the B horizon was higher in the forest. The soil horizons were further partitioned in ^{15}N compartments (Fig. 5).

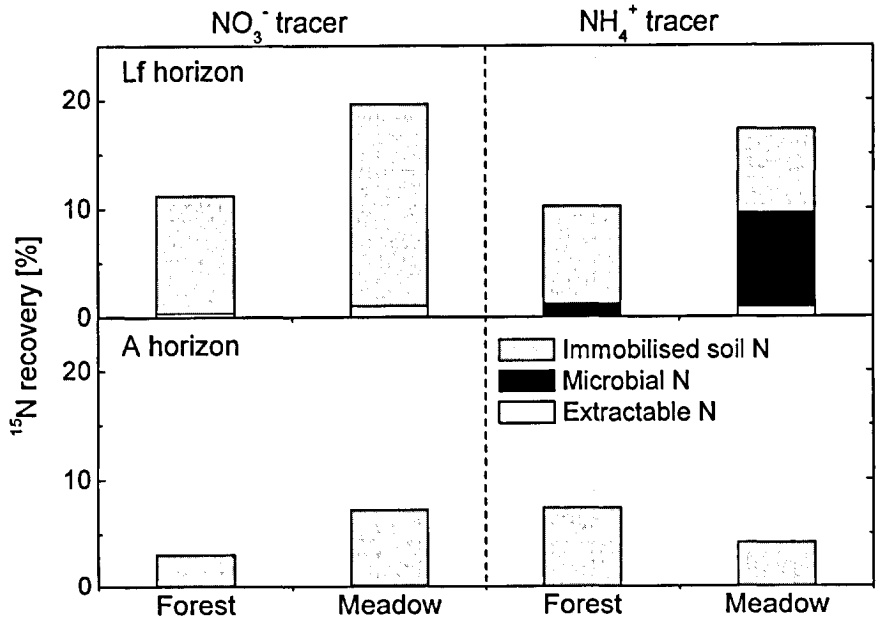

Figure 5: Total ^{15}N recovery [%] of added NO_3^- and NH_4^+ tracer in the soil pool fractions: immobilised soil N, microbial N, and extractable N in LF and A horizons in the experimental forest and meadow catchment in 2001 and in 2003. Extractable N is mainly DON with a negligible amount of extractable NO_3^- and NH_4^+.

Total extractable N and microbial N were almost not detectable in the LF and the A horizons for both ecosystems. The highest recovery within the soil was in immobilised soil N for both horizons. For all three soil horizons (LF, A and B), the C and N concentrations were measured in both ecosystems (Table 2).

Table 2: N and C concentration and C:N ratio for the soil horizons LF, A and B for the forest and the meadow in 2001, as means.

Pool		N conc. [%]	C conc. [%]	C:N
Forest	Lf (5 - 0 cm)	1.42	30.35	20.96
	A (0 - 5 cm)	1.01	17.98	17.10
	B (5 - 20 cm)	0.33	5.84	17.69
Meadow	Lf (5 - 0 cm)	1.11	18.18	16.05
	A (0 - 5 cm)	0.93	12.46	13.39
	B (5 - 20 cm)	0.65	9.87	15.09

The forest had a higher C:N ratio than the meadow, whereas especially the C concentrations were much higher in the forest. For both ecosystems, the recovery in the roots was higher for the LF roots than for the A roots. The recovery in runoff was higher for the meadow (6%) than for the forest (2%), and some tracer must have been lost due to denitrification.

3.4.4 Total recovery of ^{15}N for the NH_4^+ tracer in 2003

Total ^{15}N recovery was similar for the forest and the meadow (53%) (Table 3). In the forest, 22% of the tracer was recovered in the above-ground biomass. Again, the highest ^{15}N sink was the moss layer (16%), and the herbaceous ground vegetation recovered 5%. The trees had a recovery of 1%. In the meadow, the above-ground biomass (including a few trees) recovered 5.3%. The ground vegetation recovered 5.2%, and the monocotyledons had a higher recovery than the dicotyledon herbs. The tracer fraction was higher for the dicotyledon herbs than for the monocotyledons, but the monocotyledons had a six and a half times higher pool size, thus leading to higher ^{15}N recovery.

The recovery in the litter layer and in the LF horizon was higher for the meadow. In the A horizon, the recovery was slightly higher in the forest, whereas in the B horizon the recovery was again higher for the meadow. The soil horizons were further partitioned in ^{15}N compartments (Fig. 5). The total extractable ^{15}N was almost not detectable in the LF and the A horizon. The microbial ^{15}N could be determined for the LF horizon, and resulted in higher values in the meadow (9%) than in the forest (1%). In the forest, the highest recovery for both

Table 3: Partitioning of the added ^{15}N labelled nitrogen for the NH_4^+ tracer in trees, ground vegetation, litter layer, soil horizons, roots and runoff in forest and meadow, as means. Pool sizes as dry matter, N concentration, N pool, tracer fraction and tracer recovery.

Forest: NH_4^+ tracer		Pool size	N conc.	N pool	Tracer	
Pool		[kg/m²]	[g/kg]	[g/m²]	Fraction [µmol/mol]	Recovery [%]
Vegetation (above-ground)						
	Trees	18.1	2.2	39.4	2.2	1.0
	Mosses	0.12	13.9	1.7	915	16.1
	Monocotyledons	0.02	15.6	0.3	189	0.6
	Dicotyledon herbs	0.03	16.4	0.6	279	1.6
	Dicotyledon shrubs	0.03	11.5	0.3	352	1.2
	Other species	0.04	14.5	0.6	231	1.3
Litter layer						
	Litter	0.4	13.7	6.1	186	11.6
Roots						
	LF roots	0.6	16.5	5.3	75	4.1
	A roots	0.5	14.4	3.6	14	0.5
Soil						
	LF (5 - 0 cm)	6.1	11.7	71.0	14	10.2
	A (0 - 5 cm)	19.5	8.6	168.7	3	4.3
	B (5 - 20 cm)	61.2	3.6	219.2	0	0.0
Runoff		2140	0.0001	0.2	66	0.2
Denitrification[2]						0.0
Recovery						52.8

Meadow: NH_4^+ tracer		Pool size	N conc.	N pool	Tracer	
Pool		[kg/m²]	[g/kg]	[g/m²]	Fraction [µmol/mol]	Recovery [%]
Vegetation (above-ground)						
	Trees[1]	1.3	3.1	4.1	3.2	0.1
	Mosses	0.00	20.21	0.06	879	0.5
	Monocotyledons	0.39	12.12	4.67	85	3.2
	Dicotyledon herbs	0.06	11.69	0.66	274	1.5
Litter layer						
	Litter	0.4	13.3	4.9	413	16.7
Roots						
	LF roots	0.7	24.6	7.9	76	4.9
	A roots	0.2	14.0	1.9	22	0.3
Soil						
	LF (5 - 0 cm)	10.0	10.5	105.3	20	17.3
	A (0 - 5 cm)	22.0	8.2	181.1	2	2.3
	B (5 - 20 cm)	52.3	5.7	298.7	2	4.8
Runoff		3266	0.00017	0.571	129	0.6
Denitrification[2]						0.7
Recovery						53.1

[1] Small trees growing in the meadow

[2] Calculation mentioned in material and methods

horizons was in immobilised soil N. In the meadow, microbial ^{15}N had the highest recovery in the LF horizon, whereas in the A horizon immobilised soil N had the highest recovery. For both ecosystems, recovery in the roots was higher for the LF horizon than for the A horizon, and the recovery in runoff was slightly higher for the meadow (0.6 %) than for the forest (0.2%). An almost negligible amount of tracer was lost due to denitrification.

3.5 Discussion

3.5.1 Total ^{15}N recovery

The results showed that both ecosystems generally had a high capacity to retain more than 50% of the N added as tracer. More NO_3^- than NH_4^+ tracer was retained in general, especially in the forest. Similar to other tracer studies (Buchmann et al. 1996; Gebauer et al. 2000; Lamontagne et al. 2000, Zak et al. 2004) not all of the added tracer was recovered. Apart from measurement uncertainties, one reason for incomplete tracer recovery could be denitrification of the NO_3^- tracer or NH_3 volatilisation of the NH_4^+ tracer. However, the estimated denitrification rates are too low to be a significant loss, and likewise the NH_3 volatisation was probably low. According to Lamontagne et al. (2000), woody detritus could be an important ^{15}N sink due to its potential to immobilise N during decomposition. This N pool was not measured in our study. Taken together, all this uncertainties may explain the missing recovery.

3.5.2 Event-based and annual flow of $^{15}NO_3^-$ in runoff

The event-based runoff analyses shortly after the NO_3^- tracer application showed an immediate response of ^{15}N in runoff for both ecosystems (Fig. 2 and 3). Sharp $\Delta\delta^{15}N$ peaks and lower NO_3^- peaks were detected, indicating an immediate leaching of the added N tracer. Creed & Band (1998) showed that NO_3^- peaks indicate flushing from the soil layers, when they become saturated with water. Likewise, Schleppi et al. (2004) demonstrated on the Alptal site that NO_3^- peaks usually correspond to the rising water table during rain events, and they include "old" NO_3^-, which was already in the soil before the rain event started, as well as "new" labelled $^{15}NO_3^-$. According to Schleppi et al. (2004), the flushed NO_3^- in runoff was mainly from recently deposited N. Hagedorn et al. (2001) showed at the same site that a large proportion of the runoff had limited contact with the subsoil and originated from the precipitation and the topsoil.

In the meadow, the flushing of the ^{15}N was more dynamic than in the forest. This could be due to stronger water-table dynamics, which led to stronger flushing dynamics, or it could be an artefact of the sampling frequency, which was unevenly distributed for the two catchments. However, the total NO_3^- concentration was more dynamic in the forest than in the meadow. The annual pattern of NO_3^- in runoff showed a clear seasonal pattern (Fig. 4). For both ecosystems, the highest NO_3^- leaching occurred in late winter and in spring at snowmelt events. After the cessation of the NO_3^- tracer application the total NO_3^- leaching was still going on and had the same seasonal pattern as in the first year, showing high leaching at snowmelt events. However, the $^{15}NO_3^-$ leaching stopped. This emphasizes that the applied $^{15}NO_3^-$ was either leached out of the system or immobilised directly in the soil or the biomass within a few weeks or months as shown by Providoli et al. (Chapter 2) at the same site. Therefore, the delayed leaching of $^{15}NO_3^-$ was very minute. In the meadow, $^{15}NO_3^-$ leaching was much higher than in the forest. This could be explained by the higher N retention in the forest by trees, which are missing in the meadow, by the higher N retention of the ground vegetation in the forest, and by the much higher C content in the forest, which absorbs more N by direct immobilisation.

In the forest, tracer recovery in runoff was much lower compared to the N manipulation study by Schleppi et al. (1999a) on the same site. Schleppi et al. (1999a) recovered 10% of the $^{15}NH_4{}^{15}NO_3$ tracer in runoff, whereas we recovered in the forest only about 1% for both tracers. This large difference can be explained by the different tracer application. In the study of Schleppi et al. (1999a), the tracer was applied during rain events by rotating sprinklers, thus mimicking wet deposition, and being flushed immediately by the preferential flow paths. However, in our study, tracer application was performed not only during but also after rain events with a backpack sprayer. Thus we were mimicking wet as well as dry deposition. The tracer applied as dry deposition does not enter the preferential flow paths in the soil immediately, and is therefore stored in the system for a longer time.

3.5.3 ^{15}N recovery in pools

The sink strength of the above-ground vegetation, which consisted mostly of perennial species was higher in the forest than in the meadow for both tracers. This was especially due to the much higher moss biomass in the forest. As already described by Oechel & Van Cleve (1986) and De Luca et al. (2002) and confirmed for our site by Providoli et al. (Chapter 2), the moss layer acted as a very efficient filter, absorbing nutrients that arrive on their surface in

rainfall or throughfall. Nutrients taken up by mosses are generally not available to the vascular plants until the mosses die and undergo slow decomposition. As already shown for this site (Chapter 3), mosses take up more NH_4^+ than NO_3^-, which may be due to the higher energy requirement for NO_3^- reduction. For the NO_3^- tracer, the herb and shrub layer (especially the monocotyledons), had a two times higher recovery in the meadow than in the forest. In a previous study on the same site, Providoli et al. (Chapter 2) showed that vascular plants had a higher uptake for NO_3^- than for NH_4^+. The present study confirms those results. The recovery in the below-ground biomass was not very high for both ecosystems and both tracers (< 6%). Tracer uptake by the tree roots partly resulted in tracer recovery in the above-ground tree biomass, which was much higher for the NO_3^- tracer than for the NH_4^+ tracer. This indicates, that the trees preferred the NO_3^- tracer as did the vascular plants of the ground vegetation. This tracer recovery is comparable with a study on the same site (Schleppi et al. 1999a), trees recovered 8% of the tracer, and with a study by Buchmann et al. (1996) and May et al. (1996) in a *Picea Abies* forest, tree uptake was below 10% of the tracer.

The litter layer retained more than 10% of both tracers in both ecosystems and was therefore quite an important sink. The recovery can be due to abiotic absorption of the litter, to direct immobilisation into the litter during decomposition, but also through new litter input.

The soil was the most important sink in both ecosystems, with an exception for the NH_4^+ tracer in the forest. Other tracer studies, e.g. by Buchmann et al. (1996), May et al. (1996), Nadelhoffer et al. (1999a) or Lamontagne et al. (2000) showed that the soil was the primary tracer sink. In these studies, the recovery was decreasing downwards the soil profile and was higher in the organic than in the mineral horizon. Similar recoveries were calculated on our site and have already been reported by Schleppi et al. (1999a) and Providoli et al. (Chapter 2). However, in the actual study in the forest, the recovery in the B horizon was higher than in the upper two horizons for the NO_3^- tracer (17%), whereas the recovery in the meadow was below the detection limit. This is a remarkable difference between the two ecosystems, and it is not consistent with the studies mentioned before, showing that the recovery was decreasing downwards the soil profile. This fact could be due to a leaching process along macropores, i.e. cracks originating during the dry winter period in 2001. During snowmelt, the ^{15}N may have been leached in the B horizon, and after that the soil cracks may have closed again, so that the ^{15}N was stored in the impermeable soil layer.

On our site, in both ecosystems, both tracers were recovered particularly as immobilised soil N (ISN). This was already shown in previous short-term tracer studies on small plots on the same site (Chapter 2), where the tracer was immobilised either through biotic (by microbial biomass) or abiotic (via chemical processes) immobilisation. Also in other studies, ISN was recovered in the longer term (a few months after tracer application) (Perakis et al. 2001 and Hart et al. 1993). However, these results are in contrast to a study by Zak et al. (2004) in a sugar maple-dominated hardwood forest on a sandy soil. In this study, there was after one year no recovery in the soil organic matter (SOM), which corresponds to the ISN on our site. ^{15}N was only recovered in the SOM within hours after tracer application, and was released after time steps longer than a month and shorter than a year.

On our site, the recovery in the microbial N was only detectable for the NH_4^+ tracer in the LF horizon, especially in the meadow. These results correspond well to the previous short-term study on small plots (Chapter 2), where especially the NH_4^+ tracer was favoured by the microbial biomass. However, it is surprising that the recovery is still so high after one year. Compared to other studies the recovery in microbial N is especially known to act in the short-term (Hart et al. 1993; Perakis & Hedin 2001; Zogg et al. 2000).

3.6 Conclusion

Our tracer study showed that both ecosystems had in general a high capacity to retain most of the added N tracer. This capacity of N retention leads to the assumption that due to the increasing N deposition, soil C:N ratio will decrease in the longer term. Studies by Schleppi et al. (2004) showed a decreasing C:N ratio after 7 years of N addition at the Alptal site. The declining C:N ratio could more and more limit the ability of the soil to immobilise N from atmospheric deposition, reaching slowly a long-term N saturation. This long-term saturation would lead to higher N leaching, which would be especially high at snowmelt events, leading to an increased N-load for the ground water.

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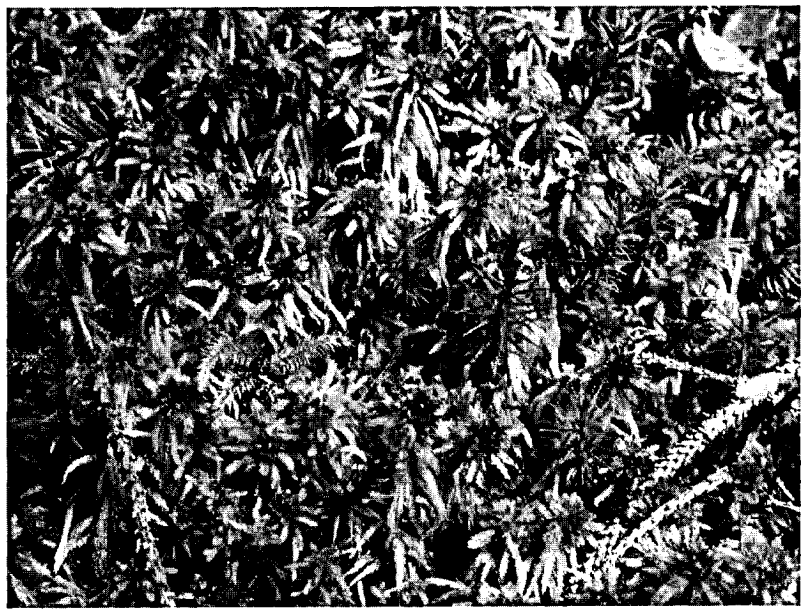
Chapter 4

Uptake of nitrate and ammonium in three moss species in a subalpine *Picea abies* forest affected by increased N deposition



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4.1 Abstract

N uptake of mosses was assessed in a subalpine forest under ambient and experimentally increased N deposition.

In a full factorial design, three moss species (*Sphagnum quinquefarium, Dicranum scoparium, Hylocomium splendens*), two ^{15}N tracers ($^{15}NO_3^-$ and $^{15}NH_4^+$) and three N additions (long-term NH_4NO_3 addition since 1995), single NH_4NO_3 addition and no N addition) were combined. Moss N concentration, C:N ratio, ^{15}N uptake, nitrate reductase activity (NRA), chlorophyll and carotenoid concentrations were measured.

The N addition resulted in a higher N concentration in the mosses and accordingly in a lower C:N ratio; chlorophyll and carotenoid contents showed no significant effect, and NRA and tracer uptake were reduced. ^{15}N recovery in moss patches was much higher for the $^{15}NH_4^+$ labelling (50 – 70%) than for $^{15}NO_3^-$ (15 – 25%) and was significantly reduced by the long-term N addition. Different moss species had different N assimilation patterns.

With increasing N deposition, mosses appear to reach N saturation, and most of the further deposited N passes through the moss layer and enters into the soil.

Keywords: Mosses, atmospheric N deposition, ^{15}N tracers, temperate forest, nitrogen uptake, nitrate reductase activity, chlorophyll, carotenoid

4.2 Introduction

In forest ecosystems, bryophytes play an important role in nutrient cycling, although they are not as important as in peatlands (Bobbink et al. 1998). They have, however, been much less extensively studied than peatland mosses, especially the *Sphagnum* species.

Most mosses in boreal and temperate climate zones are ombrotrophic, i.e. they depend on precipitation and canopy leaching for their mineral nutrition because they lack roots. Mosses develop in patches, which act as a filter and absorb nutrients that impinge on their surface in rainfall and throughfall (Oechel & Van Cleve 1986). This interception leads to moss mediated modifications of the chemistry of percolating water prior to its entry into the soil and the rhizosphere of higher plants (Woodin et al. 1985). Mosses have thus access to incoming nutrients before the roots of vascular plants. Nutrient acquisition of bryophytes is rapid and takes place through the rhizoids, through the green shoot surface or through both pathways, depending on the species. For example, *Sphagnum* species have most uptake activity in the top segment, the capitulum, with the activity decreasing down the stem (Woodin & Lee 1987; Aldous 2002a; Aldous 2002b).

Mosses generally have a high nutrient-use efficiency and represent an adaptation to low-nutrient environments (Aerts & de Caluwe 1999). At low N deposition, nitrate is rapidly and efficiently assimilated by the mosses; however, at high N deposition, nitrate in precipitation can exceed the plant requirements. In recent decades, human activities have greatly altered the global nitrogen cycle, and N concentrations in precipitation have increased (Galloway et al. 1995; Vitousek et al. 1997; Dise & Wright 1995). Because of their ombrothrophic nutrition, mosses are directly affected by air pollution and increasing N deposition. At sites with higher N inputs, the cover of vascular plants increases reducing light intensities for mosses leading to a reduced moss biomass (Woodin et al. 1985; Woodin & Lee 1987; Berendse et al. 2001; Heijmans et al. 2002).

In mosses, NH_4^+ is assimilated to glutamine, whereas NO_3^- is first reduced, as in the vascular plants, to NH_4^+ by the key enzyme nitrate reductase (NR) (Kahl et al. 1997). NR is substrate-inducible and allows a very efficient utilisation of the episodic nitrate supply (Woodin & Lee 1987). In studies with *Sphagnum* species under repeated NO_3^- treatments, a decline in the inducibility of nitrate reductase activity (NRA) was measured (Woodin & Lee 1987). This is

because the NO_3^- assimilates (NH_4^+ or amino acids) can exert a negative regulation and lead to a repression of NRA. The greater the prior NO_3^- treatment of the mosses, the lower the subsequent response to NO_3^- (Woodin et al. 1985). Similarly, the chlorophyll content is affected by changing N concentrations. Rudolph & Voigt (1986) observed that a high NO_3^- concentration in the growth medium led to a large increase in chlorophyll concentration. Conversely, a high NH_4^+ concentration led to a reduction in chlorophyll concentration due to suppressive effects of the increased NH_4^+ concentration on the glutamine synthetase (Baxter et al. 1992).

In previous studies, we applied ^{15}N tracers on the understory vegetation of a subalpine forest (Alptal, central Switzerland), both on small plots (Chapter 2) and small catchments (Chapter 3). In both studies, we found that a substantial amount of ^{15}N from either $^{15}NO_3^-$ or $^{15}NH_4^+$ was retained by the moss layer. High recovery rates (up to 21%) were measured especial, after the application of $^{15}NH_4^+$, leading us to assume that mosses preferred NH_4^+ at this study site. NR, whose synthesis is quite energy consuming, is less active in the presence of NH_4^+ (Woodin & Lee 1987). To shed more light on the role of mosses for N cycling a combined N addition and $^{15}NO_3^-$ and $^{15}NH_4^+$ experiment was conducted in the Alptal forest with three different N additions on three different moss species, *Sphagnum quinquefarium*, *Dicranum scoparium* and *Hylocomium splendens*. The main goals of this study were: (1) to investigate the N uptake of three different moss species under different N deposition scenarios; (2) to assess the effect of N deposition on chlorophyll and carotenoid concentrations in mosses; and (3) to compare the uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$ tracers and to relate the ^{15}N uptake to the NRA.

4.3 Methods

4.3.1 Study area

The study site is located in the Alptal valley, in central Switzerland (47°03' N, 8°43' E), at 1200 m a.s.l.. The climate is cool and wet, with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm, reaching a maximum in June (270 mm) and a minimum in October (135 mm). Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} a^{-1}, equally divided between NO_3^- and NH_4^+ (Schleppi et al. 1999a). The parent rock material is Flysch, and the major soil types are clay-rich Gleysols of low permeability, with a water table close to the surface throughout the year (Hagedorn et al. 1999). Usually, soils are covered

with snow from mid-November to April and the vegetation period lasts from June to September. Slope is about 20% with a west aspect. At the study site, the landscape consists of naturally regenerating forests and litter meadows, neither of which have ever been fertilised artificially. Trees are predominantly Norway spruce (*Picea abies*), with 15% Silver fir (*Abies alba*) and they are up to 250 years old, with a relatively low leaf area index of 3.8 (Schleppi et al. 1999b). In the forest, the vegetation and the humus types of the Gleysols are closely linked to microtopography and form a mosaic pattern. Three different plant associations were identified at the study site, all including a moss layer with more than 30 moss species (Muller 1997; Schleppi et al. 1998). Among the most important moss species, *Sphagnum quinquefarium* ((Braithw.) Warnst.), *Dicranum scoparium* (Hedw.) and *Hylocomium splendens* ((Hedw.) Schimp.) were chosen for this study. *Dicranum scoparium* is mainly present on the more acidic mounds, *Hylocomium splendens* is ubiquitous and *Sphagnum quinquefarium* is found on mounds and slopes.

4.3.2 Experimental field design

The Alptal site had previously been studied in the NITREX project (Wright & Rasmussen 1998; Schleppi et al. 1998). There, an increased N deposition was simulated by adding NH_4NO_3 to rain water, applied by rotating sprinklers (1.5 m above ground). Thereby, N inputs were increased by 25 kg ha^{-1} a^{-1}. This long-term N addition started in April 1995 and was replicated five times, each replication consisting of a plot (20 m^2) around a sprinkler with N addition and a plot with no N addition (control) around a sprinkler delivering unaltered rain water. In both cases, the sprinkled water corresponded to 7% of the average annual precipitation. On each long-term N addition and control plot the three moss species (*S. quinquefarium*, *D. scoparium* and *H. splendens*) were present in pure or almost pure patches. Measurements for the present study were made in 1999 and 2003 (Table 1).

Table 1: Overview of the N and ^{15}N addition and the analyses in 1999 and 2003.

	long-term NH_4NO_3 addition	no N addition (control)	single NH_4NO_3 addition	tracer addition	moss species
analyses 1999	C, N chlorophyll a+b carotenoids NRA	C, N chlorophyll a+b carotenoids NRA			*Sphagnum quinquefarium* *Dicranum scoparium* *Hylocomium splendens*
analyses 2003	$\delta^{15}N$ C, N	$\delta^{15}N$ C, N	$\delta^{15}N$ C, N	$^{15}NO_3^-$ $^{15}NH_4^+$	*Sphagnum quinquefarium* *Dicranum scoparium* *Hylocomium splendens*

On 11 August 1999, cores for nitrate reductase activity (NRA) and pigment analyses had been taken from the long-term N addition and the control plot and the C and N concentrations of the mosses were analysed. The biomass of the sampled areas was between 0.4 g and 0.6 g with a standard error of 0.02 g for all species.

Based on ^{15}N tracer studies carried out in 2001 (Chapter 2) showing a high recovery rate in the mosses, in 2003 a more focused $^{15}NH_4^+$ and $^{15}NO_3^-$ experiment was carried out. A sub-plot (5 m^2) was delimited within each control plot for a single addition of NH_4NO_3 (6 mmol m^{-1}, dissolved in deionised water). On each single-addition sub-plot the three moss species were present. This addition was done on 18 August 2003 with a hand sprayer with 6 mm water, followed by another 6 mm of deionised water to rinse the mosses. These patches were further subdivided into small areas (~100 cm^2) receiving one of three treatments, $K^{15}NO_3$, $^{15}NH_4Cl$ (both 99 atom% ^{15}N) or only deionised water. The latter tracers were applied one day after the single NH_4NO_3 addition with a hand sprayer mounted on a mobile support and adjusted to deliver 3 mmol m^{-1} of tracer in 6 mm of water. Here again, the moss patches were flushed with another 6 mm of deionised water. Two days after labelling, the moss patches were sampled (together with a few cm of soil) as cylindrical cores (5.7 cm diameter). The cores were maintained humid with deionised water and brought to the laboratory to measure the moss biomass, C and N concentrations and ^{15}N abundances. In the laboratory, the moss samples were cleaned of surface litter, vegetation and other moss species with tweezers.

4.3.3 Laboratory analyses

Nitrate reductase activity (NRA) was measured in vivo on the day of sampling according to a method adapted from Yandow & Klein (1986). The tips of four to five shoots were cut into small pieces with a razor blade and the moss material was incubated in 1.4 ml K-phosphate buffer (0.1 mM, pH = 7.5) containing 0.1 ml 180 mM KNO_3, 1 % isopropanol and 0.6 ‰ ampicillin. Incubation was carried out at 25°C under vacuum for three hours in the dark. After incubation, the sample buffer was cleared by centrifugation (1 min / 20,000 g). The supernatant was centrifugated again (15 min / 20,000 g). Activated charcoal (about 10 - 20 mg / sample) was added to 1.2 ml of the solution, which was centrifugated again (15 min / 20,000 g). For nitrite detection, 0.5 ml of the solution were mixed with 0.25 ml sulphanilamide (0.1 % in 2 N HCl) and 0.25 ml 0.02% N-Naphtyl ethylendiamine. As a blank probe, additional 0.5 ml of the solution were used, but the N-Naphtyl ethylendiamine was

replaced by distilled water. The colour reaction was measured after 30 min at 546 nm with a Photometer (UV-160, Shimadzu, Kyoto, Japan). NRA was calculated from nitrite production. To determine the chlorophyll and carotenoid concentrations, the green tips of the shoots (*S. quinquefarium*: 200 mg fresh mass, *D. scoparium*: 30 mg and *H. splendens* 80 mg) were cut and shaken for 2 min with 4 ml of an 80 % acetone solution (pH 7.5 - 8) in a dismembrator (Mikro-Dismembrator II, Braun, Melsungen, Germany). A further 6 ml of 80 % acetone were used to rinse the shaking flasks. Afterwards, the samples were centrifuged (10 min / 12,500 g) to remove the plant material. The concentrations of chlorophyll a and b and carotenoid in the extract were determined photometrically according to the method of Lichtenthaler & Wellbrun (1983).

The remaining moss samples were dried to constant weight at 65 °C and the dry matter content was calculated. All samples were ground with a centrifuge grinder (0.5 mm), and the N concentration (mg N g^{-1} moss) and percentage ^{15}N were determined by an elemental analyser coupled to an isotope-ratio mass spectrometer (delta S, Finnigan, Bremen, Germany). C and N concentrations were measured by a C+N analyser (NC 2500, CE instruments Thermoquest, Milano, Italy).

4.3.4 Calculation of recoveries and statistical analysis

For each sampled moss patch, the recovered ^{15}N tracer was determined. ^{15}N recovery (% of added tracer) was calculated by subtracting the natural abundance of ^{15}N measured in the control sample from the recovered ^{15}N of the treated sample.

The labelling experiment was a (3 x 2 x 3) full factorial design, with three moss species, two ^{15}N tracers and three N treatments. NRA and pigments were measured in a 3 x 2 full factorial design, with three moss species and two N deposition regimes. N content, C:N ratio, chlorophyll and carotenoid concentrations, NRA and ^{15}N recovery were subjected to an analysis of variance (ANOVA) followed by a Duncan test where appropriate. First-level interactions were included in the analysis. All results were considered significant at the $P < 0.05$ level. Results with P values between 0.05 and 0.10 were regarded as tendencies.

4.4 Results and Discussion

4.4.1 Rainfall 1999 and 2003

In 1999, the total rain fall amounted to 2890 mm, which was far above the annual mean of 2300 mm, whereas the year 2003 was very dry, with only 1750 mm. In 1999, sampling had taken place on August 11. Until that day, it had rained 1970 mm and it rained on the sampling day as well as on the previous days. The dry-matter content of the mosses was 4.4% for *S. quinquefarium*, 14.7% for *D. scoparium* and 9.8% for *H. splendens*. In 2003, the N addition and tracer experiment started on August 18. Until that day it had rained 1100 mm, but there was no precipitation in August prior to August 12. However, between August 12 and August 18, it rained 37 mm. Thus we can assume that the mosses were rehydrated before the experiment (dry matter contents were not measured in 2003). After the single N addition, it rained 16 mm. After the tracer addition and before the sampling two days later, it rained only 5 mm. According to Bates & Bakken (1998), moss metabolism is sensitive to rainfall volume, and the cycles of desiccation and rehydration have implications for mineral nutrition. Desiccation can notably restrict the ability of bryophytes to take up nutrients (Aldous 2002b).

4.4.2 N concentrations and C:N ratios of moss species

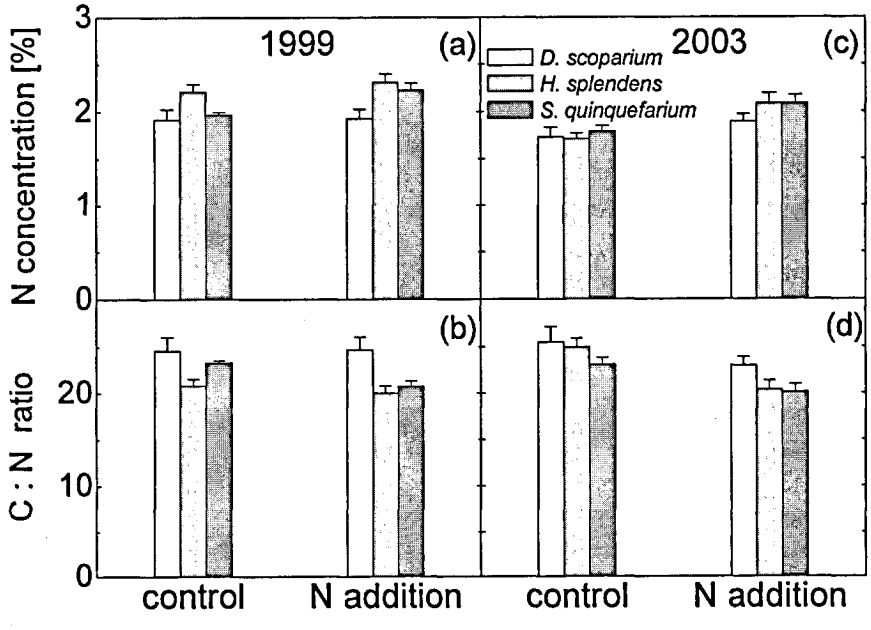

Figure 1: N concentration and C:N ratio for the three moss species on the control and the N addition plot in 1999 and 2003. Means of five replicates with standard error.

The N concentration and the C:N ratio were measured for both sampling years, 1999 and 2003 (Fig. 1). In 1999, the N concentration had a species effect. *H. splendens* had a higher N concentration than *D. scoparium*, whereas *S. quinquefarium* reached intermediate values (P = 0.002, Table 2, Fig. 1a). The C:N ratio, however, was also higher for *H. splendens* ($P < 0.001$) than for the other two species (Table 2, Fig. 1b). This can be explained by the higher C concentration in the dry matter of *H. splendens*. The long-term NH_4NO_3 treatment induced only a slight increase (P = 0.08) in the N concentration (2.15 %, SE = 0.07 %) compared to the control (2.03 %, SE = 0.03 %) (Table 2, Fig. 1a). The N treatment had no effect on the C:N ratio.

Table 2: Results (P values) of ANOVA for the effects of the three moss species, the N addition and their interactions according to the N concentration, the C:N ratio, the NRA, the chlorophyll, the carotenoids and the ^{15}N recovery.

		species	N addition	^{15}N tracer	interactions
N %	1999	0.002	0.08		ns
	2003	ns	<0.001		ns
C:N	1999	<0.001	ns		ns
	2003	0.07	<0.001		ns
NRA	1999	<0.001	<0.001		0.05
Chl a, Chl b	1999	<0.001	ns		ns
Car	1999	<0.001	ns		ns
^{15}N recovery	2003	<0.001	0.004	<0.001	ns[1]

NRA: Nitrate reductase activity
Chl: Chlorophyll
Car: Carotenoids
ns: not significant (p > 0.1)
[1]: all interactions are ns

In 2003, the differences in N concentrations between the three moss species were no longer significant (Table 2, Fig. 1c). This change can be explained by the yearly difference. However, the C:N ratio was slightly higher for *D. scoparium* than for *S. quinquefarium* (P = 0.07) (Table 2, Fig. 1d). After four more years of NH_4NO_3 addition we had a treatment effect for the N concentration as well as for the C:N ratio. The treatment resulted in a higher N concentration in the mosses than in the control ($P < 0.001$) (Table 2, Fig. 1c) and, accordingly, to a lower C:N ratio ($P < 0.001$).

Similar to our findings, Limpens & Berendse (2003) observed in *Sphagnum* an almost linear increase in total N tissue concentration with increasing N deposition. However, their N deposition was much higher (40 kg N ha^{-1} a^{-1} and 80 kg N ha^{-1} a^{-1}) than in our experiment. According to these authors, *Sphagnum* shows a luxury consumption when N is no longer limiting for growth. In *Sphagnum* mosses, Williams et al. (1999) and Van der Heijden et al. (2000) also showed an increase in total N concentration with increasing N deposition. This resulted in a slightly reduced C:N ratio. A similar increase in N concentration was measured by Mäkipää (1995) for *Dicranum polysetum* after an addition of ammonium sulphate. Similar to these studies and to Aerts et al. (1992), the C:N ratio also decreased on our site with increasing N deposition.

4.4.3 Chlorophyll and carotenoid concentrations

Chlorophyll and carotenoid concentrations were measured in 1999 on the long-term NH_4NO_3 treatment and on the control treatment (Fig. 2).

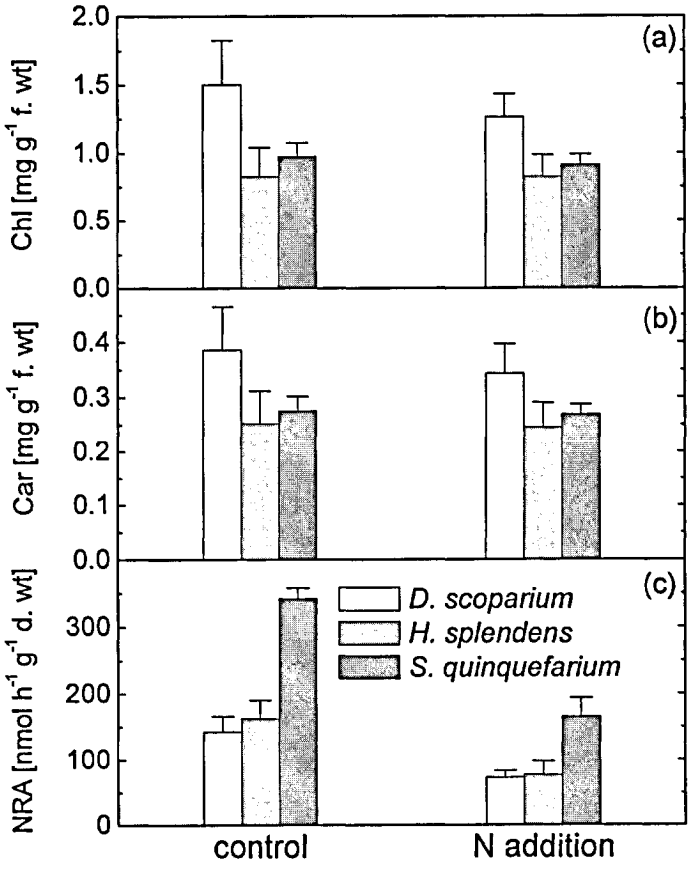

Figure 2: Chlorophyll a + b, carotenoid content and NRA for the three moss species on the control and the N-addition plot. Means of five replicates with standard errors. FW: fresh weight, DW: dry weight.

The moss species had a different chlorophyll concentration ($P < 0.001$, Table 2), with *D. scoparium* showing the highest values. Similarly, the carotenoid concentration was highest for *D. scoparium* ($P < 0.001$).

The two treatments had no effect on pigment concentrations. This finding is in contrast to that of Baxter et al. (1992). In their study, the chlorophyll content of *Sphagnum cuspidatum* decreased with increasing NH_4^+ concentrations in the growth medium. Similarly, Rudolph & Voigt (1986) observed a reduction in tissue chlorophyll concentration in *Sphagnum magellanicum* receiving NO_3^- and NH_4^+ in combination over a five-month period. In their study, the chlorophyll concentration increased with a high NO_3^- concentration in the growth medium, whereas the largest reduction occurred with the highest NH_4^+ concentration in the growth medium. According to these studies, the chlorophyll concentration should be quite sensitive to the combination of NO_3^- and NH_4^+ concentrations. However, on our site we were unable to detect a difference. In our experiment, N deposition (both ambient and elevated) contained NH_4^+ and NO_3^- in a 1:1 ratio. Since these ions generally lead to opposite effects on pigment concentrations, it is likely that they compensated for each other, which would explain the absence of an overall trend.

4.4.4 ^{15}N recovery and nitrate reductase activity (NRA)

The different moss species had different ^{15}N-tracer recovery patterns ($P < 0.001$, Table 2). *S. quinquefarium* had the highest recovery, followed by *H. splendens* and *D. scoparium* (Fig. 3). The morphology of moss species influences rainwater N capture. *S. quinquefarium* forms small but compact patches, which are hydrophilic and very efficient in capturing rainwater (Feldmeyer-Christe 1999). *H. splendens*, in contrast, is branched, flat and elongated and presumably takes up less water. Finally, *D. scoparium* has an erect shape and retains least water. All three species had a higher uptake for the $^{15}NH_4^+$ tracer (up to 60 %), whereas the $^{15}NO_3^-$ tracer reached only 20 % ($P < 0.001$, Table 2, Fig. 3). These results are in contrast to those of Williams et al. (1999), who found that *Sphagnum* moss had no preference for either NH_4^+ or NO_3^-. However, in line with previous studies on the Alptal site (Chapter 2 and chapter 3), we suggest that mosses prefer NH_4^+ uptake if sufficient NH_4^+ is present, since direct NH_4^+ uptake is less energy consuming in contrast to the higher energy requirement of NR. Compared to the previous studies on the Alptal site, the tracer recovery was in this study much higher. This can be explained by the slightly different tracer application, in the previous

studies the tracer was applied above the ground-vegetation, whereas in this study the addition was explicitly above the moss patches on a small focused area.

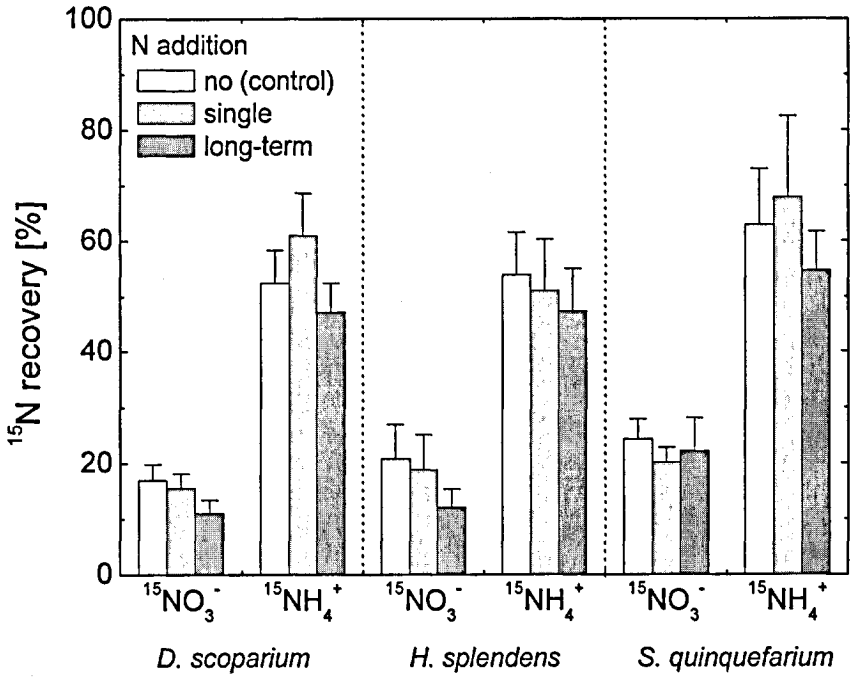

Figure 3: Total ^{15}N recovery for the control, the single and the long-term N addition for the $^{15}NO_3^-$ and the $^{15}NH_4^+$ tracer for the three moss species. Means of five replicates with standard errors.

^{15}N recovery showed different patterns depending on the three N additions (Fig. 3). The control and the single addition treatment had almost the same ^{15}N recovery and higher recovery values (P = 0.004, Table 2) than the long-term treatment. Therefore, increased N deposition reduced the uptake of both tracers. Similar results were obtained by Van der Heijden et al. (2000), who found that increasing N deposition rates decreased N recovery. This indicates that nitrogen uptake had become less efficient. However, we would have expected the short-term addition to have a higher N uptake than the control treatment due to the previous activation of the NRA by adding N, which took place two days before the ^{15}N labelling.

Since NR is substrate-inducible (Woodin et al. 1985), the availability of nitrate ions is important and NR increases immediately after NO_3^- addition. Woodin et al. (1985) and Woodin & Lee (1987) measured an NRA peak after one to two days. While NR is substrate-inducible, it is also product-inhibited (Woodin et al. 1985; Press & Lee 1982; Woodin & Lee

1987). It is thus likely that the presence of NH_4^+ in our short-term treatment prevented the induction expected from NO_3^-. In the longer-term, a clear NR inhibition was measured. In 1999, the NRA was much higher in the control treatment (216 nmol h^{-1} g DW^{-1}, SE = 16) than in the N treatment (106 nmol h^{-1} g DW^{-1}, SE = 28) ($P < 0.001$) (Table 2, Fig. 2c). In the long-term, the induction by NO_3^- strongly declines (Woodin et al. 1985; Woodin & Lee 1987) and the inhibition by reduced N (including NH_4^+) obviously prevails. This can be interpreted as an N saturation of the mosses. Thus, our findings for the NO_3^- tracer agree with the observations made in the previously mentioned studies that increased N addition leads to an NRA decline and that, on the treated plot, NRA was inhibited by the NH_4NO_3 solution. However, the decline in $^{15}NO_3^-$ uptake was much less pronounced than the NRA decline. Therefore, we assume that it was not only the NR that was responsible for the NO_3^- tracer uptake. It is possible that there was a $^{15}NO_3^-$ uptake but the NO_3^- was not reduced immediately and stored in the mosses.

We conclude that the N addition resulted in a higher N concentration in the mosses and accordingly to a lower C:N ratio. However, the increased N deposition had no effect on the chlorophyll and carotenoid concentrations. The nitrogen uptake efficiency, especially NR, was reduced with increasing N deposition. Similar to other studies, we conclude that, with increasing N deposition, mosses reach an assimilation limit where the deposited N is leached out of the moss layer into the soil rather than being taken up. With higher N deposition, NRA and tracer uptake were reduced. Different moss species had different N assimilation patterns, which can probably be related to their morphology and to their ability to capture rainwater. However, the lack of understanding in N uptake of mosses and its dependence on species and various environmental constraints makes it difficult at present to predict a general response pattern of mosses to N deposition.

Acknowledgements

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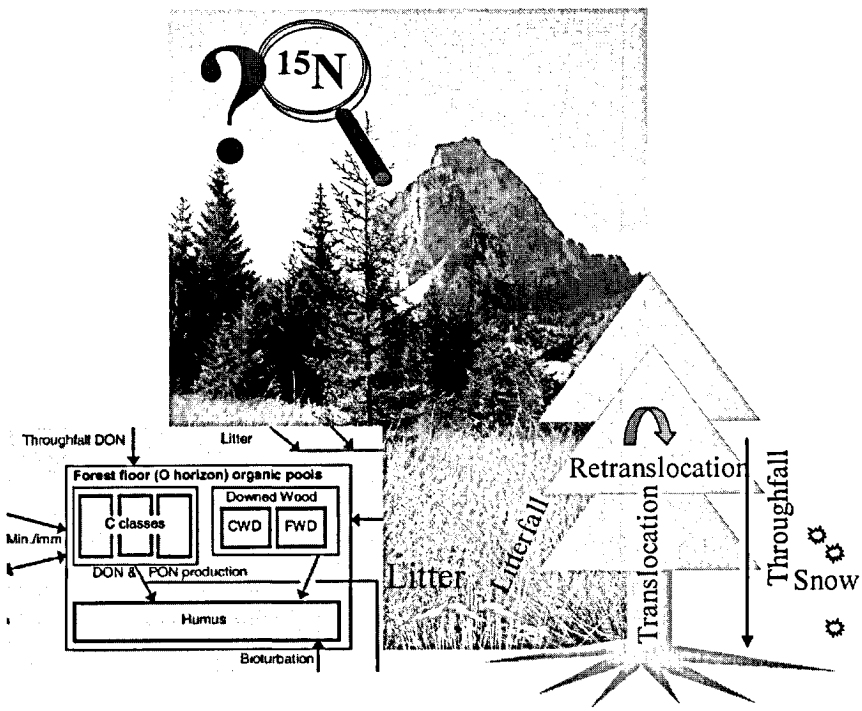
Chapter 5

A model-based evaluation of nitrogen cycling in a Norway spruce mountain forest



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5.1 Abstract

Despite numerous studies on the influence of increased nitrogen deposition on forest ecosystems, particularly on the internal nitrogen cycle in forests, many uncertainties regarding the longer-term nitrogen accumulation remain. To contribute to filling this knowledge gap, the biogeochemical process model TRACE, with the ability to simulate ^{15}N tracer redistributions in forest ecosystems was used to study N cycling processes in a mountain forest of the northern edge of the Alps in Switzerland.

First, the TRACE model had to be adapted to the conditions of the Alptal site. One new process added to the model was preferential water flow (NO_3^- leaching). The model was run with initial parameters in 1900 and calibrated with field data. Then, the modified model was validated against short-term field data of ^{15}N field experiments. Finally, the model was applied for a preliminary assessment of the implications of future scenarios of atmospheric CO_2 concentration and N deposition according to the IPCC at the Alptal site over the coming 45 years. Four sets of simulations for the A1FI scenario were performed, as a full factorial experiment.

The model validation showed satisfactory behaviour, but for certain variables large discrepancies occurred, particularly for ^{15}N recovery in the soil. The reason for this is probably that the Alptal site is characterized by Gleysol, which are not yet modelled properly in TRACE, although the preferential flow was modified in the model. Although the model calibration and the model validation showed some problems and the attempt for assessing the implications of future scenarios at the Alptal site did not fully correspond with field data from studies in the literature, the application of the TRACE model was a valuable process. The application of TRACE pointed out how difficult and how complex the adaptation of such models to other sites can be. However, it helped to better understand the N cycling processes in the forest. Therefore, we conclude that the application of such biogeochemical models, closely linked to field data, is valuable for better understanding broader-scale processes.

Keywords: Mountain forest, Norway spruce, N deposition, ^{15}N tracer, N retention, simulation model, TRACE

5.2 Introduction

During the last few decades, production of biologically reactive nitrogen (N) by human activities has increased, and reactive nitrogen is now accumulating in the environment at all spatial scales - from local to regional and global (Galloway et al. 2003). Reactive nitrogen is widely dispersed by hydrologic and atmospheric transport processes over short to long distances. The two key sources releasing reactive nitrogen are intensive agriculture (NH_x) and combustion processes (NO_x), with about five times more reactive nitrogen coming from the former (Galloway & Cowling 2002). Reactive nitrogen accumulation contributes to many contemporary environmental problems and is also affecting previously N-limited forests. Forests across Europe receive a wide range of nitrogen inputs from the atmosphere as wet and dry deposition, ranging from less than 1 kg N ha^{-1} a^{-1} in northern Norway and Finland to more than 60 kg N ha^{-1} a^{-1} in the Netherlands and the Czech Republic (MacDonald et al. 2002). In several studies in temperate forests, such as in the European research project NITREX (Wright & Rasmussen 1998; Emmett et al. 1998b), the responses of ecosystems to manipulated N input have been studied. For gaining insights into the N fluxes and transformations, the application and recovery of ^{15}N-enriched tracers in forests has proven to be a powerful tool for measuring the fate and redistribution of N at the ecosystem scale (Nadelhoffer & Fry 1994; Nadelhoffer et al. 1999a). A mountain forest catchment in the Alptal valley (Switzerland) was part of the European research project NITREX (Schleppi et al. 1998). One N-addition and several ^{15}N experiments have been carried out at the Alptal site (Schleppi et al. 1998; Schleppi et al. 1999a, b; Chapter 2 and 3). In spite of considerable knowledge on various aspects of the nitrogen cycle at the Alptal site, these field studies, especially the ^{15}N experiments, provided only short-term snapshots (up to one year). A study by Schleppi et al. (2004) showed that the ecosystem retained 90% of the added N (2/3 in the soil), but NO_3^- losses increased from 10 to 30% within seven years, indicating that the ecosystem became progressively N saturated. However, knowledge about the longer-term (several years) nitrogen accumulation in this ecosystem is lacking. Therefore, to better understand the nitrogen cycle at the Alptal site in the longer term, the model TRACE (Tracer redistribution among compartments in ecosystems) was used (Currie et al. 1999). The model predicts redistributions of ^{15}N and ^{14}N isotopes through time by simulating ^{15}N : ^{14}N ratios of individual N pools and of N transferred between pools.

By this, we hoped to gain further insights into changes in forest ecosystem processes induced by N deposition over longer time scales, and to assess the fate of N inputs and the long-term risk arising from increasing N deposition. Our main goals were (1) to adapt the TRACE model for the conditions of a Swiss mountain *Picea abies* forest (Alptal site) by incorporating additional, crucial processes and calibrating the model for the Alptal site; (2) to validate the model against short-term field data collected in the context of a nitrogen tracer experiment at the same site; and (3) to use the model for a preliminary assessment of the implications of future scenarios of atmospheric CO_2 concentration and N deposition according to the IPCC at the Alptal site over the coming 45 years.

5.3 Methods

5.3.1 Site description, N and ^{15}N-addition experiments

The research site is located in the Alptal, on the northern edge of the Alps of central Switzerland (47°03' N, 8°43' E), at 1200 m a.s.l.. The climate is cool and wet, with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm, reaching a maximum in June (270 mm) and a minimum in October (135 mm). The vegetation period lasts from June to September. Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} a^{-1}, equally divided between NO_3^- and NH_4^+ (Schleppi et al. 1999a). The parent rock material is Flysch, and the major soil types are clay-rich Gleysols of low permeability, with the water table close to the surface throughout the year (Hagedorn et al. 1999). Usually, soils are covered with snow from mid-November to April. Slope is about 20% with a west aspect. The landscape consists of naturally regenerating forests and litter meadows, neither of which have ever been fertilised artificially. Trees are predominantly Norway spruce (*Picea abies*), with 15% Silver fir (*Abies alba*). The stand at the research site has trees up to 250 years old, with a relatively low leaf area index of 3.8 (Schleppi et al. 1999b). Generally, the soil profile consists of a LF, an A and a B horizon. In the forest, vegetation and soil types form a mosaic pattern closely related to microtopography. Two different soil types are present: on mounds, with the water table at a depth below 40 cm umbric Gleysols are abundant with raw humus (LFH), Ah and oxidised or partly oxidised Bg or Br horizons. In depressions, the water table frequently reaches the surface, leading to mollic Gleysols with a thin LF horizon, an anmoor topsoil (Aa) and an almost permanently reduced Bg or Br horizon. On mounds, the dominant plant species are Norway spruce (*Picea abies*) and *Vaccinium myrtillus*. The waterlogged depressions are too wet for tree growth, and ground vegetation is dominated by *Caltha*

palustris and *Petasites albus* in the shade of the trees, and by *Poa trivialis* and *Carex ferruginea* in open patches (Muller 1997).

One N-addition and several ^{15}N experiments took place at the Alptal site. The first was within the framework of the NITREX experiment, in which an artificial forest catchment was frequently sprinkled with a solution of ammonium nitrate (NH_4NO_3) to simulate an additional N input of 30 kg N ha^{-1} a^{-1} (Schleppi et al. 1998; Schleppi et al. 1999a). During the first year, the added nitrogen was labelled with $^{15}NH_4{}^{15}NO_3$. In 2000, additional ^{15}N addition experiments with NO_3^- and NH_4^+ tracers were performed on small plots (Chapter 2) and at the catchment scale (Chapter 3). The N field data were used to calibrate and the ^{15}N field data to validate the TRACE model.

5.3.2 Model overview

TRACE (Tracer Redistribution Among Compartments in Ecosystems) is a biogeochemical process model of C, N and water fluxes in forest ecosystems (Currie et al. 2004, Fig. 1). It predicts redistributions of ^{15}N and ^{14}N isotopes through time by simulating ^{15}N : ^{14}N ratios of individual N pools and of N transferred between pools, incorporating the principles of pool dilution and mass balance (Wessel & Tietema 1992; Nadelhoffer & Fry 1994). TRACE was explicitly designed for use in large-scale ^{15}N-labelled field studies. Originally, it was developed for pine and oak stands at Harvard Forest, MA, USA. It simulates the timing, atom% and forms of ^{15}N added to large plots. TRACE combines a complex soil process model (DocMod; Currie & Aber 1997) with the vegetation component of the PnET-CN ecosystem model (Aber et al. 1997). DocMod is a model of litter decomposition, humification and the production of dissolved organic C and N in the forest floor. PnET-CN model is a "lumped-parameter" model that uses generalised representations of physiological processes including photosynthesis, transpiration, respiration, allocation, phenology and litter production (Aber et al. 1996). TRACE calculates mixing and redistribution of ^{14}N and ^{15}N as NH_4^+, NO_3^- and organic N in the soluble and solid phases, while linking the fluxes of C, N and water in forest vegetation and soil. Previous publications have described the fundamental vegetation and soil processes in TRACE and PnET-CN (Currie et al. 1999; Aber et al. 1997; Currie & Nadelhoffer 1999; Currie & Aber 1997). TRACE runs on a monthly time step and can be used to project forest change over decades to centuries, including the effects of past instances of forest harvesting and other disturbances.

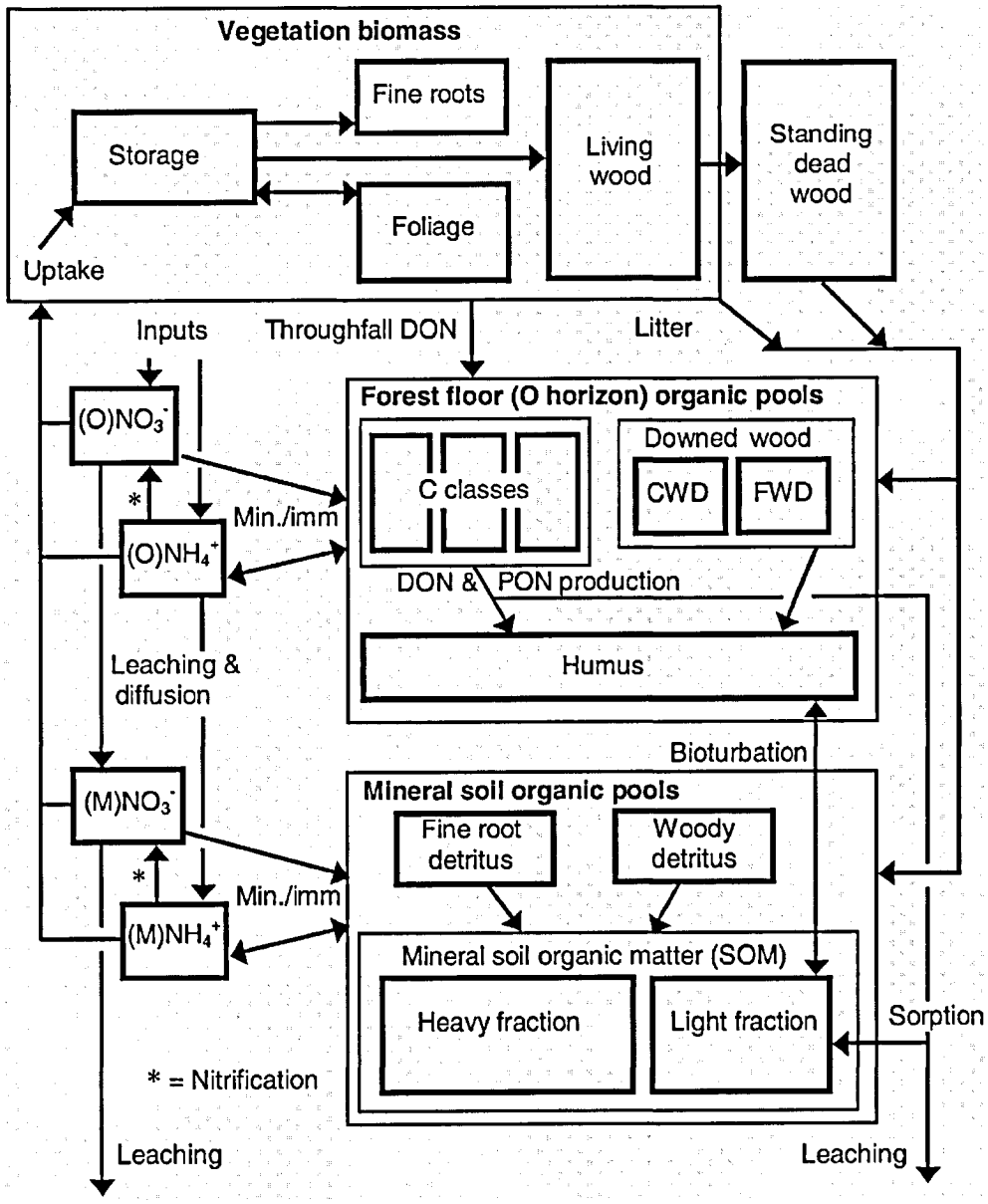

Figure 1: Schematic of pools and fluxes of N in TRACE 4.20. Plant uptake of N, detrital N dynamics, and N transformations are calculated separately in each soil layer. Pools of available N are separated by soil layer: O, O horizon; M, mineral soil; DON, PON, dissolved and particulate organic nitrogen; CWD, FWD, coarse and fine woody detritus; Min./imm., mineralisation and assimilation. Inputs: NO_3 and NH_4 in atmospheric deposition, fertiliser, and isotopic tracer additions. For clarity, not all fluxes are shown in detail (Currie et al. 2004).

5.3.3 Model adaptations for the Alptal site

We based the present analysis on version 4.20 of TRACE, which can be downloaded at http://www.al.umces.edu/currie. To use the model at the Alptal site in a Norway spruce (*Picea abies*) forest, some structural changes had to be made. Special Alptal features that are not included in TRACE are the low permeability and the reductive conditions in the gley

horizon of the soil, preferential water flow (Schleppi et al. 2004) and the presence of a well developed understory vegetation. These properties are important characteristics at our site and therefore should be incorporated into the model. However, the understory vegetation is not included in the present Alptal version, but it is planned for the next TRACE version.

In the original model, the NO_3^- concentration in the preferential water flow was zero. In the Alptal model version used here, NO_3^- concentration in the preferential water flow was added to the model, and a new variable "$NO_{3\ \text{fast flow}}$" was created. By this, NO_3^- is leached directly out of the system instead of going into the O horizon first and later into the M horizon. The O horizon in TRACE corresponds to the LF horizon at the Alptal site, and the M horizon in TRACE corresponds to the A and B horizons at the Alptal site, respectively. Thus, the NO_3^- is by-passing the soil.

The following equation for preferential water flow, "$NO_{3\ \text{fast flow}}$" was added to the water balance procedure (Eq. 1):

$$(1)\ NO_{3\ \text{fast flow}} = F_{\text{fast flow}} * (NO_{3\ \text{wet dep}} * (1 - F_{\text{snow}}) + NO_{3\ \text{snow pack}} * F_{\text{snow melt}}$$

$F_{\text{fast flow}}$: fast flow fraction, i.e. the proportion of water reaching the soil which flows through it, initialisation parameter
$NO_{3\ \text{wet dep}}$: wet NO_3 deposition [g m^{-2}], input variable
F_{snow}: proportion of precipitation as snow [no unit], state variable
$NO_{3\ \text{snow pack}}$: NO_3 present in the snow pack [g m^{-2}], state variable
$F_{\text{snow melt}}$: melting fraction of the snow pack [= water in snowmelt / water equivalent in the snow pack] [no unit], state variable

The special soil characteristics of the Alptal site, low permeability and reductive conditions in the gley horizon are difficult to include in the current model, but they can be accounted for to a certain extent by a change of the water holding capacity (WHC) parameter.

5.3.4 Model parameterisation and initialisation

TRACE is a complex model with hundreds of parameters and initial conditions for state variables, although typically only a few dozen need to be altered to apply the model to a new site or region. The data required for running the TRACE model fall into three categories, i.e.

parameter data, scenario data and climate data. The parameter data comprise information about the site's initialisation state. The parameter data for the Pine stand (*Pinus*) of the Harvard Forest (HF) were used as the basis for this. These data can be downloaded from the web site (http://www.al.umces.edu/currie), together with a detailed user guide (Currie & Helmers 2003) and the model code (Currie et al. 2004). The changes we made compared to the default parameter set are summarised in Appendix I. The parameter set is separated into parameters and initialisation data (Appendix IA and IB). The initialisation year of the model run, was in our simulation in 1900. Because the initial values of the state variables are not known with precision, we had to estimate them based on circumstantial evidence, as follows. At the Alptal site, the *Picea abies* forest was in 1900, similar as today, an open-canopy stand. Due to the proximity to a road, selective harvests (single trees) took place regularly. In contrast to today, the forest was slightly grazed until 1980 by cattle, and therefore the ground vegetation was different than today (M. Fuchs, personal communication). For the estimations of the state variables the above mentioned site specifications were kept in mind. Some parameters and initialisation data were changed based on field data and others were changed based on calibration.

The most striking changes based on field data at the Alptal site were the following (Appendix I): the average foliar retention time (FolReten) is for *Picea* longer than for *Pinus* and was changed to 6 years, based on the branch harvest by Schleppi et al. (1999a). The specific leaf weight at the canopy top (SLWmax) was changed to 440 g m^{-2}, based on yearly taken needle samples at the canopy top (Schleppi et al. 1999b). The change in specific leaf weight with canopy depth (SLW del) was increased to 0.21, based on the branch and needle harvests (Schleppi et al. 1999a, b). The initial max. foliar mass (FolMass(2)) was increased to 1000 g OM m^{-2}, based on LAI (Leaf area index) measurements and yearly taken needle samples at the canopy top (Schleppi et al. 1999b). The water holding capacity (WHC) was reduced to 4 cm. This reduction was based on estimations from hydrographs in combination with measured rooting depth in studies by Schleppi (unpublished data). The humified matter in O horizon (HOM) was increased to 3200 g organic matter (OM) m^{-2}. This increase was based on soil and C:N ratio analysis by Schleppi et al. (2004) and Schleppi (unpublished data).

The most striking changes based on calibration were the following (Appendix I): the minimum ratio of wood production to foliar production (MinWoodFolRatio) was increased to 1.25. The MinWoodFolRatio serves to adjust the relationship between foliar production and

wood production and reasonable values used in the model should range from 0.75 to 1.5. Finally, the baseline gross N mineralisation (BaseGrossNmin) was reduced to 20 g N m^{-2} a^{-1}. The BaseGrossNmin parameter is the threshold of the net N mineralisation at which foliar N concentration begins to rise above its baseline value. This threshold can be assessed from the estimated value of gross:net N mineralisation for the site.

The sites's monthly climatological input data are given in Table 1.

Table 1: Climate data for the Alptal site (ClimALP).

Day of year	Daily min. temperature [°C]	Daily max. temperature [°C]	Photosynthetically active radiation [µmoles/m^2/day]	Precipitation [mm]
15	-4.6	2.0	243	12.8
46	-4.3	2.6	296	17.5
76	-2.2	5.1	402	19.6
107	-0.5	7.3	505	19.5
137	4.6	13.7	578	24
168	7.0	15.9	597	22.4
198	9.0	18.1	608	25.7
229	9.5	17.9	523	25.2
259	5.8	13.7	428	20.3
290	3.2	10.4	321	14.2
321	-1.9	4.6	245	15.8
351	-3.7	2.6	209	18.6

Finally, the scenario data comprises model inputs that change through time, such as climate, harvest, and N amendments. We simulated a deposition of inorganic N that increased linearly from 0.5 g N m^{-2} a^{-1} in 1945 to 1.7 g N m^{-2} a^{-1} in 1980 and had constant values from 1980 onward (Fig. 2).

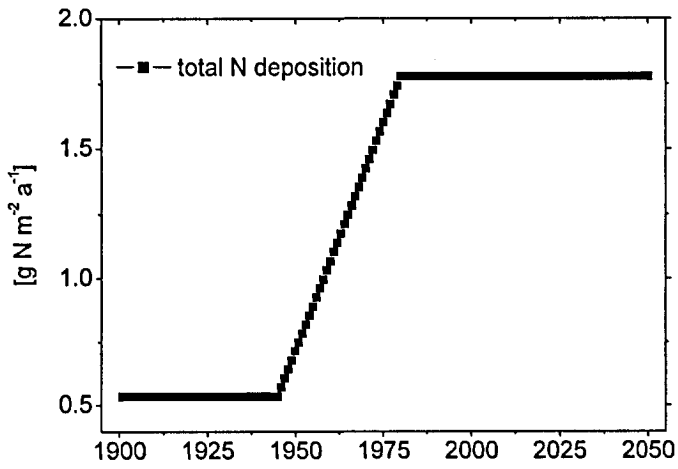

Figure 2: Total N deposition at the Alptal site for the scenario data model input from 1900 - 2050.

The CO_2 concentration was assumed to increase from 300 ppm in 1900 to 446 ppm in 2050 (Fig. 3).

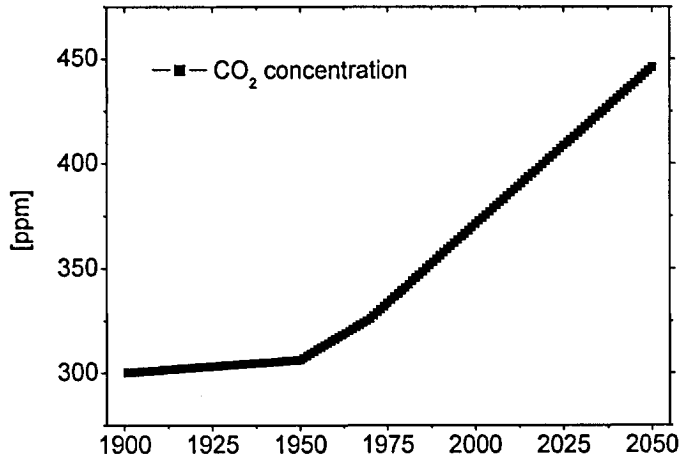

Figure 3: CO_2 concentration for the scenario data model input from 1900 - 2050.

5.3.5 Model calibration

The model was set up with the parameter and initialisation data as described above starting in 1900 and running for 150 years. The simulation was calibrated according to the TRACE user guide (Currie & Helmers 2003) for whole-system C and N cycling rates independent of ^{15}N before making model-data comparisons of ^{15}N recoveries. We calibrated the following key variables: foliar and woody production, tissue N concentrations, O horizon mass and C:N ratio, net nitrification and leaching. These key variables were tested for realism with respect to present-day data, although it was not always easy to provide the corresponding field data. The calibration for model output and field data was made for the year 2000 (Table 2).

Table 2: Calibration of N pools to assess model realism prior to analysis of ^{15}N redistribution.

	year 2000 Model	year 2000 Field data	Reference for field data
Foliar production [kg m^{-2} a^{-1}]	0.22	0.3	Muller 1997
Wood production [kg m^{-2} a^{-1}]	0.76	0.41	Schleppi (unpublished)
N concentration in green foliage [%]	0.93	1.14	Schleppi (unpublished)
N concentration in fine roots [%]	0.05	0.2	Providoli (unpublished)
Soil O horizon OM [kg m^{-2}]	3.13	2.73	Providoli (unpublished)
Soil O horizon C:N ratio [g g^{-1}]	21.5	19.7	Providoli (unpublished)
Net nitrification [g N m^{-2} a^{-1}]	-0.57	0.12 ± 0.24	Hagedorn et al. 1999
NO_3-N leaching[1]	0.31	0.28	Schleppi (unpublished)

OM: organic matter (ash-free, oven dry weight)

[1] In the original TRACE 4.20 version without the preferential flow adaption, NO_3^- leaching from the M horizon is zero.

The whole model calibration for the key variables from 1900 to 2050 can be seen in Figure 4. This system-level calibration gave an indication whether the broad-scale N cycling patterns were simulated realistically, independent of isotopic ratios.

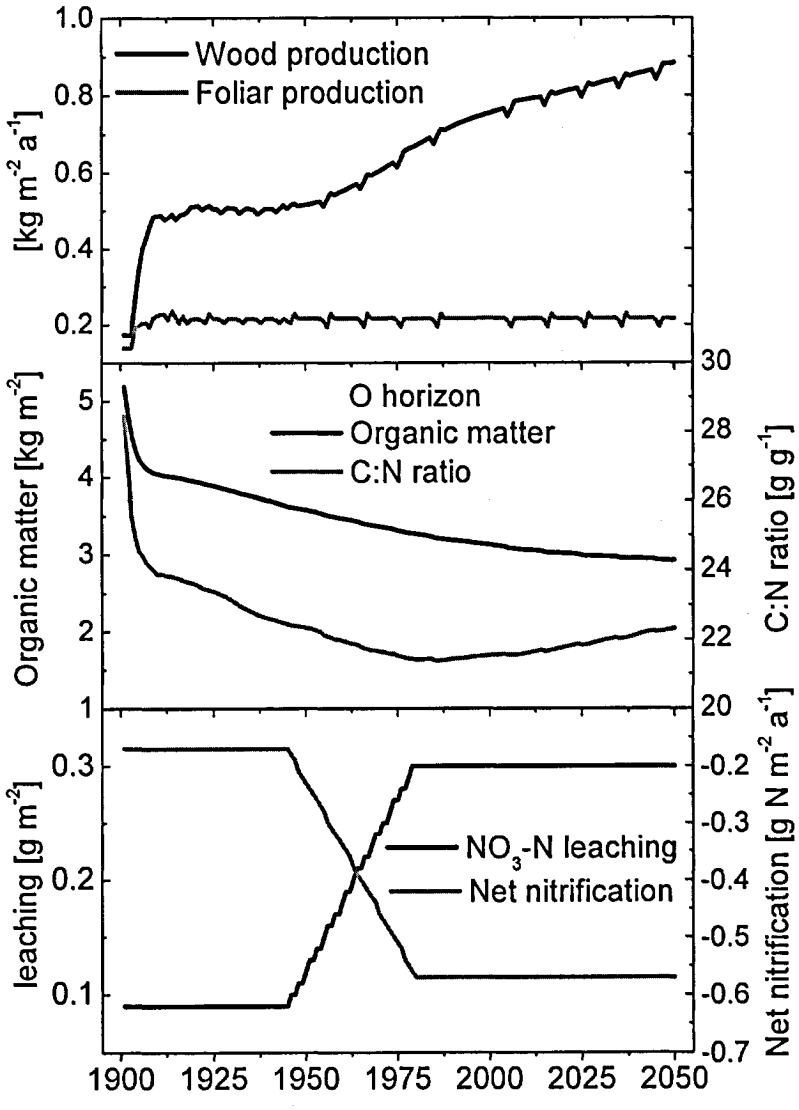

Figure 4: Simulation results for the calibration from 1900 - 2050 for the wood and foliar production, the organic matter and the C:N ratio in the O horizon, and the NO_3-N leaching and the net nitrification.

The wood production was much higher in the model than in reality (Table 2); this is probably due to the lack of an understory vegetation in the model and therefore to a higher tree N uptake, leading to a higher wood production. The wood production in the model run was even increasing over time (Fig. 4). The foliar production was quite similar for the model run and

the field data (Table 2). In the simulation, in the O horizon organic matter and the C:N ratio are both decreasing over time. This decrease in organic matter mass in the simulation can be explained by the increasing net N mineralisation. However, the simulation for the organic matter was much higher than our field data. The NO_3^- leaching had for the simulation and the field data similar values. In general, the calibration was satisfactory, taking into account that the model was created for a different forest type.

5.3.6 Model validation

As a next step, the model was validated against short-term ^{15}N field data of a forested catchment at the Alptal site with the same parameter and initialisation data set as for the calibration. In the simulation, NO_3^- (2.68 mg N m^{-2} a^{-1}) and NH_4^+ (10.4 mg N m^{-2} a^{-1}) tracers were added separately, each for one year. These amounts are identical to the additions performed at the Alptal site by Providoli et al. (Chapter 3). In TRACE, the ^{15}N recovery in the ecosystem pools is expressed as the mass of tracer recovered above background in each ecosystem compartment (for the equation, see Currie et al. 2004). For the experimental recoveries at the Alptal site, we used equivalent equations as given in Providoli et al. (Chapter 3). For the validation, the model output had to be compared against corresponding ^{15}N field data. This was not always easy to achieve due to different field sampling methods as compared with the model variables. Thus, direct model-data comparison for trees and wood mass was not possible, respectively. For the comparison of the simulation and the field ^{15}N recoveries, the recovery in foliage (first and further cohorts), fine roots (living tree roots < 2mm), and the O and M horizon contents was used. The soil horizons were further split up, for the O horizon into foliar litter N, soil organic matter N and available N (inorganic), and for the M horizon into soil organic matter N and available N (inorganic).

5.3.7 Model application

Although the model calibration and the model validation showed some problems for the key variables mentioned above, the model was used as a preliminary assessment of the implications of future scenarios of atmospheric CO_2 concentratio and N deposition at the Alptal site. For the present study, the A1 SRES[1] IPCC[2] emission scenario (IPCC report 2001) was selected. It is a global-economic scenario, which represents a world of very rapid economic growth, low population growth, and the rapid introduction of new and more

[1] Special Report on Emissions Scenarios
[2] Intergovernmental Panel on Climate Change

efficient technologies. Within A1, we chose a specific sub-scenario, A1FI, which intensively uses fossil fuel.

For the two variable types, N deposition and CO_2 concentration four sets of simulations for the A1FI scenario were performed, as a full factorial experiment. In the first simulation the N deposition as well as the CO_2 concentration were set constant. In the second and the third simulation the N deposition or the CO_2 concentration was increasing and in the last simulation, the N deposition as well as the CO_2 concentration were increasing. As climatological input data the same data were used as for the calibration and validation (Table 1). Model runs started for all four simulations in the year 1900 and were run for 150 years until the year 2050.

The N deposition scenarios used in the model run are shown in Figure 5. Until the year 1995 the N deposition corresponded to the measured "historical" values as they had been used for the calibration and the validation (Fig. 2 and 3). From 1995 to 2005, the N deposition was calculated based on the actual N deposition at the Alptal site. Afterwards, the N deposition decreased linearly until the year 2010. In the year 2010, one scenario was set constant, and the other scenario corresponded to the A1FI scenario. Because the SRES scenario focus on NO_x alone and do not include data on NH_x deposition, we used our field measurements made between 1994 and 2004, and added those data to the SRES scenario data. During this period, a relative decrease of 0.7% per year was observed, and we extrapolated this trend for all four scenarios (Fig. 5).

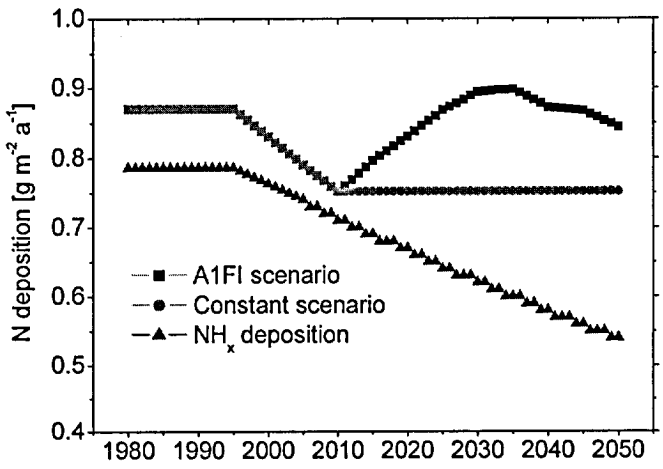

Figure 5: N deposition for the constant and the A1FI scenario. The NH_x deposition was for both scenarios the same and was added to the total N deposition.

The NH_x deposition was for all four simulations the same and was not varied at all (Fig. 5). The CO_2 concentration (Fig. 6) corresponded until 2005 to the measured "historical" values as they had been used for the calibration and the validation (Fig. 2 and 3). From 2005 onwards, one scenario was set constant and the other scenario corresponded to the A1FI scenario.

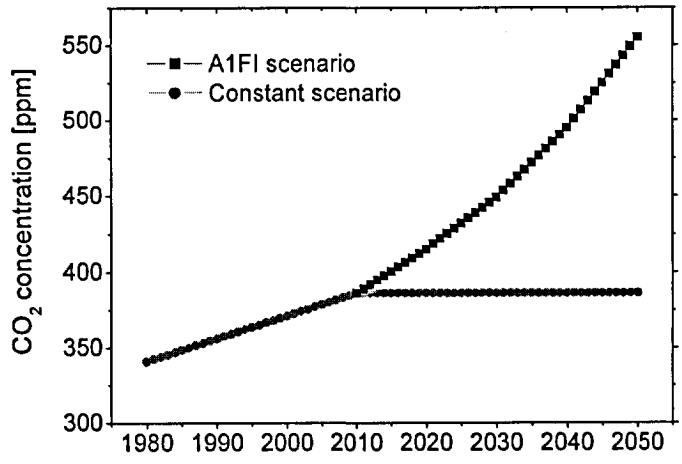

Figure 6: CO_2 concentration for the constant and the A1FI scenario.

5.4 Results

5.4.1 Model validation of simulated ^{15}N recoveries in ecosystem N pools against field data

Figure 7 and 8 present the validation variables for the $^{15}NO_3^-$ and the $^{15}NH_4^+$ additions. The broad-scale N cycling patterns were already calibrated in the calibration run mentioned above (Table 2). In the model simulation, recovery of ^{15}N in foliage was increasing over 10 years for both tracer additions (Fig. 7a and 7b). For the NH_4^+ tracer, the foliage measurements corresponded well to the simulation, whereas for the NO_3^- tracer, the field data were much higher than the simulation, especially for the further cohorts.

For fine roots (Fig. 7c and 7d), the field data were in consistency with the simulation. Our field data contained not only fine tree roots as in the TRACE model but also fine roots from the understory vegetation. However, the recovery in fine roots was predicted well for the NH_4^+ tracer, whereas the recovery for the NO_3^- tracer was underestimated by a factor of five.

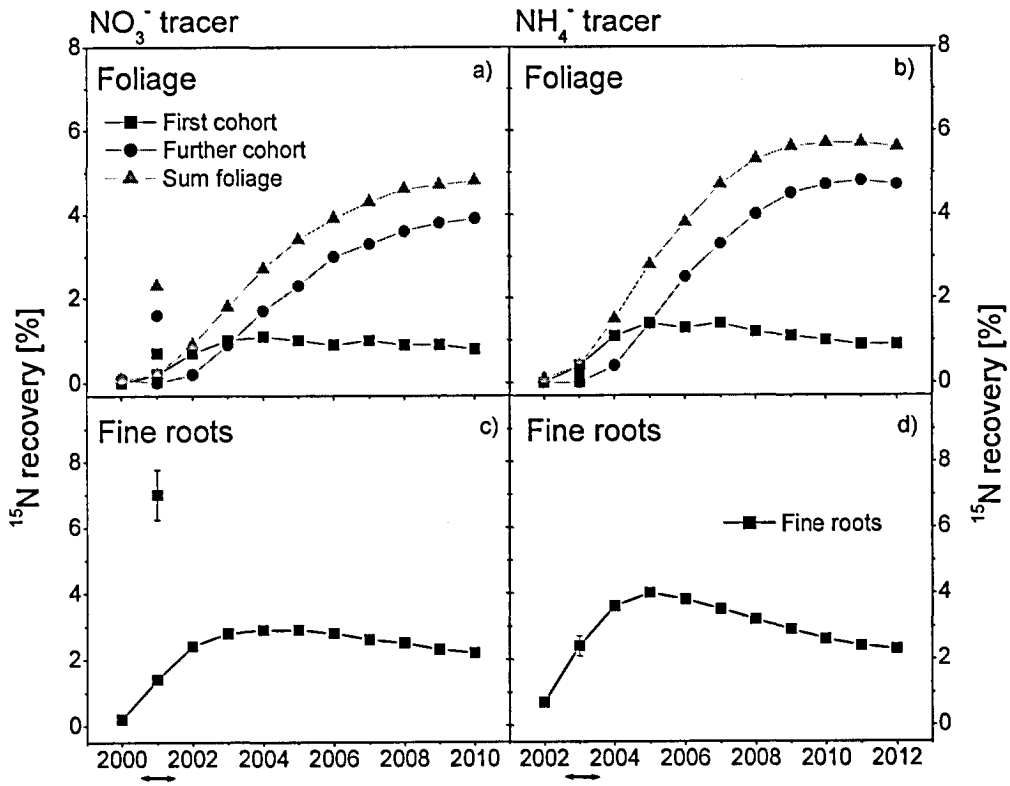

Figure 7: Predicted and field-measured recovery of NO_3^- and NH_4^+ tracers in [%] in a) and b) foliage: first cohort (current year foliage), further cohorts (foliage from all prior years); c) and d) fine roots. Trace simulation shown as a line with symbols in September of each year from 2000 to 2010 and from 2002 to 2012, respectively, field data from September 2001 and from September 2003, respectively are presented as symbols and error bars as means ± SE. Some SE values are too low to be visible. The period of tracer addition (2000 - 2001) or (2002 - 2003) is indicated on the x axis.

TRACE simulated an initial increase in ^{15}N recovery in fine roots with a stabilisation or a slight decrease after four years for both tracers.

For the soil pool, large discrepancies occurred between the model predictions and the field data (Fig. 8a and 8b). TRACE over-predicted the recovery for both tracers. For the NH_4^+ tracer, the M horizon had a much higher recovery in the simulation than the O horizon. The field results were much lower and had a slightly higher recovery in the O horizon. The NO_3^- tracer initially had a higher simulated recovery in the O horizon. This was in qualitative agreement with the field data, but measured recovery values were much lower than the simulated ones.

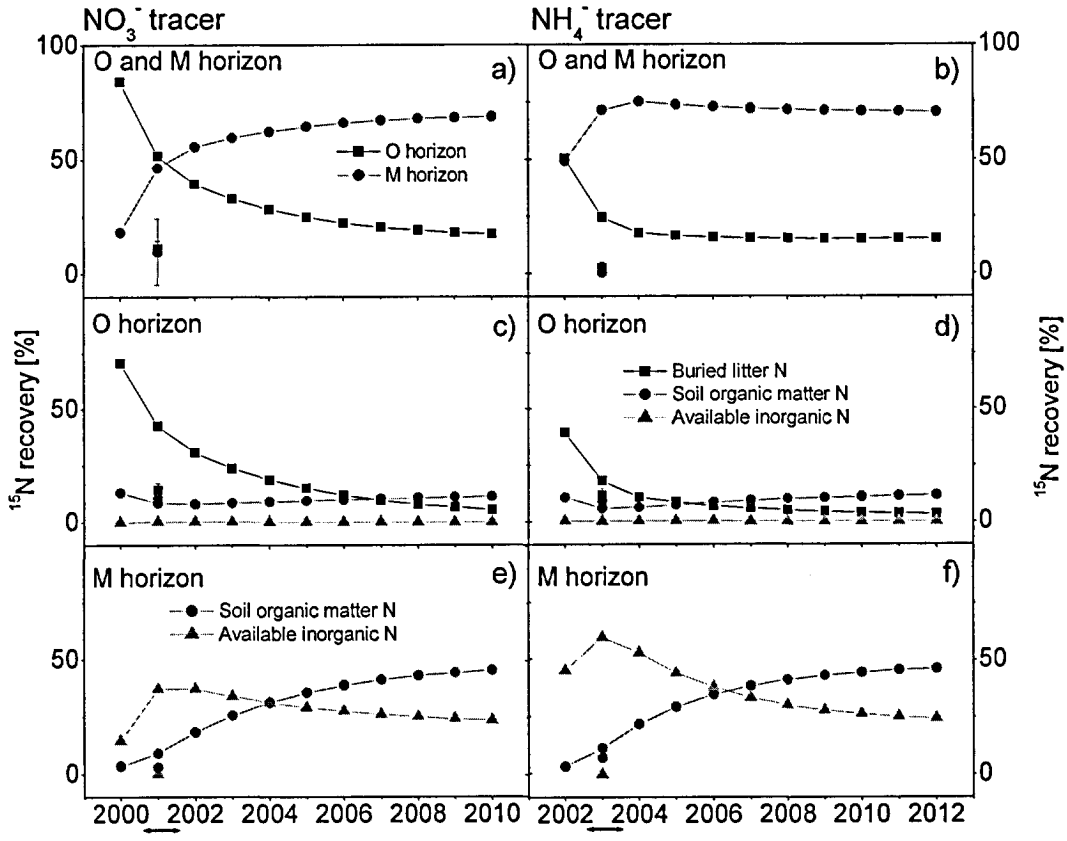

Figure 8: Predicted and field-measured recovery of NO_3^- and NH_4^+ tracers in [%] in a) and b) O and M horizons; c) and d) buried litter N, soil organic matter N and soil available N in O horizon; e) and f) soil organic matter N and soil available N in M horizon. Trace simulation shown as a line with symbols in September of each year from 2000 to 2010 and from 2002 to 2012, respectively, field data from September 2001 and from September 2003, respectively are presented as symbols and error bars as means ± SE. Some SE values are too low to be visible. The period of tracer addition (2000 - 2001) or (2002 - 2003) is indicated on the x axis.

Within the O horizon, the soil organic matter N and the soil available N were simulated fairly well for both tracers, whereas foliar litter N was overestimated strongly by the simulation (Fig. 8c and d). In the M horizon, a striking overestimation for soil available N occurred for both tracers (Fig. 8e and 8f). The soil organic matter N was also overestimated but not as much as soil available N.

5.4.2 Four simulations of a future scenario

The four sets of simulations for the A1FI scenario performed as a full factorial experiment are shown in Figure 9 and 10. As key variables wood production, N foliage concentration, soil O horizon organic matter and C:N ratio, NO_3^- leaching and net N mineralisation were selected.

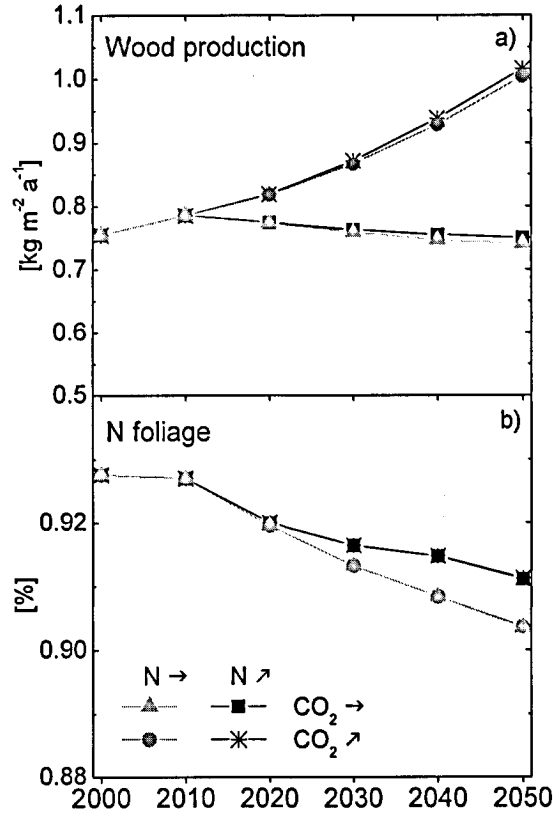

Figure 9: Four sets of simulations for the A1FI scenario with constant or increasing N deposition and constant or increasing CO_2 concentration, respectively as a full factorial experiment for the next 45 years for a) wood production and b) N concentration in foliage.

For the wood production (Fig. 9a) both scenarios with an increasing CO_2 concentration had a much higher wood production than the two scenarios with a constant CO_2 concentration. Therefore, the positive relationship between wood production and CO_2 concentration would lead to an increased C storage. The N concentration in foliage was decreasing slightly for all four simulations, whereas the two simulations with increasing N deposition had a higher N concentration than the other two scenarios (Fig. 9b).

The organic matter in the O horizon had the same pattern for all four simulations and was decreasing over time (Fig. 10a). The C:N ratio in the O horizon (Fig. 10b) was increasing over time for all four simulations, being slightly lower for the two simulations with higher N deposition. The NO_3^- leaching (Fig. 10c) did not change a lot over time, but it was higher for the two simulations with increasing N deposition. Similarly, the net N mineralisation (Fig. 10d) was highest for the two simulations with a higher N deposition.

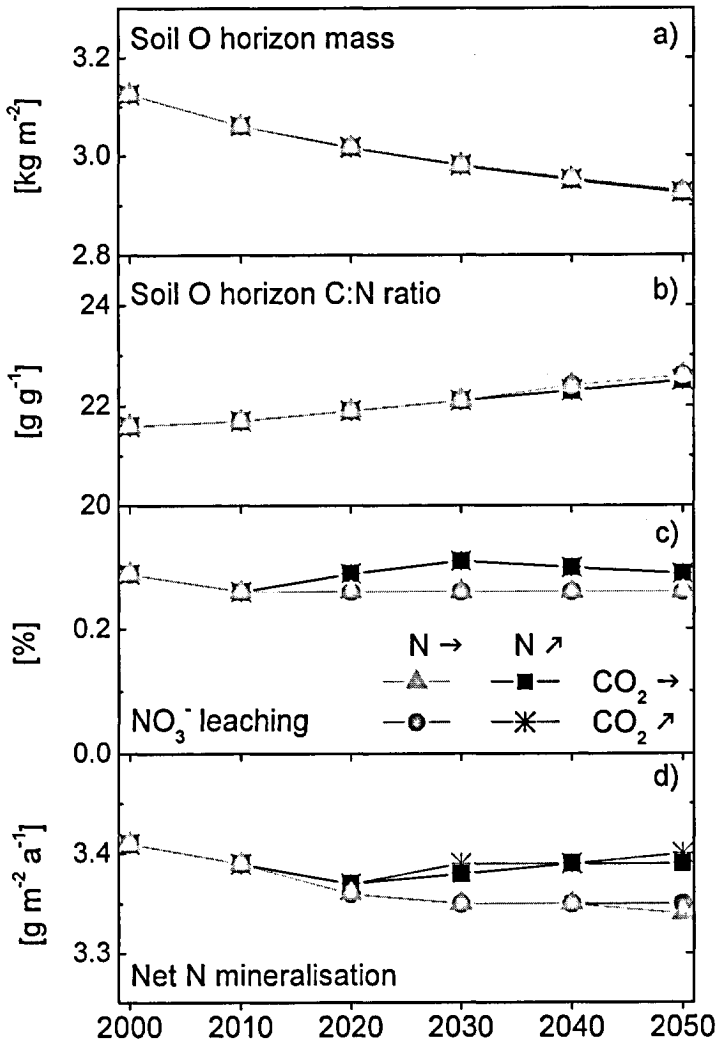

Figure 10: Four sets of simulations for the A1FI scenario with constant or increasing N deposition and constant or increasing CO_2 concentration, respectively as a full factorial experiment for the next 45 years for a) soil O horizon mass, b) soil O horizon C:N ratio c) NO_3^- leaching, and d) net N mineralisation.

5.5 Discussion

5.5.1 Model validation between simulations and field data

For certain variables, the model validation against ^{15}N field data showed large differences, and the model over-predicted total ^{15}N recovery. The simulation for the foliage was satisfactory for the NH_4^+ tracer, but it strongly underestimated the recovery for the NO_3^- tracer. A similar underestimation was found in a TRACE simulation by Currie & Nadelhoffer (1999). There, the recovery in the foliage in the field was highest for the NO_3^- tracer, whereas the simulation had similar recoveries for both tracers. In the field, a plant preference for NO_3^- is possible, whereas TRACE includes no plant preference between NO_3^- and NH_4^+ and each month, tissue is produced in the order foliage first, roots next and wood last.

For the roots, the simulation and the field data corresponded well for the NH_4^+ tracer. However, the NO_3^- tracer had a much higher recovery in the field; this may be due to a preference or due to higher availability of this tracer in the field. Similarly, Currie & Nadelhoffer (1999) found a higher recovery of the NO_3^- than the NH_4^+ tracer in the field in an oak as well as in a pine forest.

The most striking result was the large discrepancy of the simulation and the field data in the soil horizons. The model over-estimated the recovery for both tracers and both soil horizons. The soil available N had the largest discrepancy in the M horizon. In TRACE, there are four pools of plant available N, NH_4^+ in the O and M horizons and NO_3^- in the O and M horizons, which correspond well to KCl-extractable pools of inorganic N in soils. Therefore, these simulation results should compare well with soil extractions in the field. However, in TRACE, NH_4^+ is accumulating unrealistically over time and stays in the M horizon for years. Thus, a large NH_4^+ pool in the M horizon is built up. This differences may be due to the fact that in the model, soil available N is determined by inputs of NH_4^+ and NO_3^- and by consumption and outputs of NH_4^+ and NO_3^-, which are assumed to take place by microbial uptake first, then by plant uptake, prior to leaching of NO_3^- and NH_4^+ from each soil horizon.

This model sequence does not seem to be appropriate for the Alptal site, due to the much smaller ^{15}N recovery in the field data. One reason for the large differences in ^{15}N recovery in the soil horizons are probably the different soil properties at the Alptal site compared to the

Harvard forest. In the Alptal, Gleysols with a low permeability and reductive conditions are present, whereas at Harvard forest the well drained soils are present.

Another striking difference is the amount of humified matter in the O horizon, which is more than six times larger at the Alptal site than at the Harvard forest (Appendix I). Therefore, at the Alptal site the tracer is stored mostly in the O horizon and not transported further down to the M horizon. This is a clear mismatch to the Harvard forest site, where in an update TRACE version (Currie et al. 2004) the downward transport of ^{15}N tracer in the model was increased by a combination of inorganic N leaching, diffusion of isotopes, and by adding a new flux of particulate organic N transport. At the Alptal site, the small amount of ^{15}N tracer that reached the M horizon is denitrified immediately rather than being stored in the M horizon. The adaptation of the modelling of soil characteristics for the Alptal site in TRACE would need a considerable effort that is beyond the scope of this thesis.

However, the simulation of ^{15}N recoveries is quite difficult, and Currie et al. (2004) point out that the broad-scale N cycling patterns of an ecosystem can be simulated well even if the isotopic ratios and redistribution is not simulated accurately.

5.5.2 Scenario of future development

The model calibration and the model validation showed, as mentioned before, some problems. However to get an idea about the model behaviour for different N deposition and CO_2 concentration four future simulations were assessed as an attempt. This simulation results are very uncertain and they do not intend to represent accurate future scenarios.

The simulated variables of the four sets of simulations for the A1FI scenario describe the internal N status of a forest ecosystem (Fig. 9 and 10). Thus, the variables are indicators for the N status of the ecosystem, based on a theory which was described by Gundersen et al. (1998), as well as for the carbon sequestration in the forest. The internal N status is important in determining the N retention capacity of a forest stand as a consequence of N deposition, site conditions and management history.

The wood production was highest with increasing CO_2 concentration. The N deposition had no increasing effect on the wood production. Therefore, we can assume that the effect of the CO_2 concentration on the wood production is higher than the effect of the N deposition. This

result corresponds well to other studies which suggest that increasing CO_2 concentration, known as CO_2 fertilisation, enhances carbon sequestration (Beedlow et al. 2002; Goodale et al. 2002). In addition, tree growth in northern temperate regions is typically nitrogen-limited, therefore increasing nitrogen deposition could have the effect of stimulating the accumulation of forest biomass. However, a study by Nadelhoffer et al. (1999b) with ^{15}N tracers showed that elevated nitrogen deposition was unlikely a major contributor to the CO_2 sink in forested northern temperate regions. Our simulation results support this assumption.

According to our simulations results, the N concentration in the foliage was higher for the simulations with increasing N deposition.. The foliar N concentration was therefore influenced by N deposition. The CO_2 concentration showed no effect on the N concentration in the foliage.

Net N mineralisation, is also an important indicator for the N status and particularly for the N availability in an ecosystem. With increasing N deposition, net mineralisation is increasing (Gundersen et al. 1998). Our simulation results correspond well with this empirical findings. The two simulations with an increasing N deposition had a higher net mineralisation than the two simulations with a constant N deposition. The increasing CO_2 concentration led to a slightly higher net N mineralisation. The same pattern could be observed fot the NO_3^- leaching. On NITREX sites (Gundersen et al. 1998, Tietema et al. 1998), it has been shown in manipulation experiments that nitrate leaching responded promptly to changes in N input, whereby the N status of the system had to be considered. Nitrate leaching occurred at high N status even with moderate N deposition, but at low N status the deposited N might still be retained by the system. In our simulations, the NO_3^- leaching was higher for the two simulations with higher N deposition. Therefore, the model simulation represented the general field observations well. This was due to the model adaption of the NO_3^- concentration in the preferential flow path, without this addition of the new variable the NO_3^- leaching would have been zero (Table 2). The NO_3^- leaching is closely related to the C:N ratio in the O horizon (Emmett et al. 1998a). This is due to the controlling influence of the O horizon C:N ratio on the soil nitrification rate. Nitrification rates are stimulated as C:N ratios decline, thus decreasing the N retention efficiency of the O horizon (Gundersen et al. 1998). Increasing N deposition causes the C:N ratio to be lowered by N retention. The same pattern was shown in a study by Schleppi et al. (2004) at the Alptal site. A progressive increase in NO_3^- leaching within seven years of N addition was in correspondence with the decline in the C:N ratio. In

our simulations, the C:N ratio in the O horizon is for all simulations almost the same slightly increasing. The two simulations with the increasing N deposition have a slightly lower C:N ratio than the other two scenarios. This results correspond well with the empirical findings mentioned above.

In our simulations, soil O horizon organic matter mass decreased slightly for all simulations. This decrease is a model artefact and has nothing to do with the different simulation scenarios.

5.5.3 General remarks to the TRACE application at the Alptal

The application of TRACE at the Alptal site pointed out how difficult and how complex the implementation of such models to other sites can be. Although the model TRACE was created for a forest stand in the US, many changes had to be made to adapt this model to our forest stand at the Alptal. First of all, important characteristics of the Alptal site had to be adapted to the model. As a next step, the specification of the parameters and the state variables for the year 1900 was quite complex, especially because some parameters or state variables were not comparable with field measurements. Due to the initialisation in 1900 many parameters had to be estimated, based on actual field data.

Although the model calibration and the model validation showed some problems and the attempt for assessing the implications of a future scenarios at the Alptal site did not correspond with field data from studies in the literature, the application of the TRACE model was a valuable process. It helped to better understand the N cycling processes in the forest and opened the mind to the N cycling processes. This experience showed how valuable the application of such models in closed collaboration with field data can be for understanding broader-scale processes.

Acknowledgements

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Chapter 6

6 Synthesis

The aim of this study was to investigate how two mountain ecosystems at the northern edge of the Swiss Alps are affected by elevated N deposition, both as NO_3^- and as NH_4^+. The main goal was to understand the mechanisms regulating the N retention by the identification of single ecosystem pools to which deposited N is incorporated, the quantification of the N pathways through the system and the dynamics of N in the short and in the longer-term. These objectives were pursued by ^{15}N field experiments and by a model approach. We studied the N pathways at different spatial and temporal scales, ranging from the plot scale of 2.25 m^2 (Chapter 2) up to the catchment scale of 1600 m^2 (Chapter 3) and from short-term (hours, days or weeks) to longer-term intervals (one year), respectively. A special focus was set on the soil pool by measuring the partitioning of ^{15}N into different biochemical soil fractions. In addition, the uptake of deposited N by moss species was assessed (Chapter 4).

To gain insight into the role of the ^{15}N retention capacity of a forest ecosystem in the longer term, we used a model of the N, C and water cycles, TRACE. This model was adapted, calibrated and validated for the Alptal site (Chapter 5). We used the model for assessing some implications of future scenarios of atmospheric CO_2 concentrations and N deposition at the Alptal site over the coming 45 years.

6.1 Internal N status of ecosystems

The ability of ecosystems to retain deposited nitrogen is limited and depends on their internal N status, which can be viewed as the result of past N deposition, site conditions and management history (Gundersen et al. 1998). Therefore, each ecosystem has particular site characteristics which are crucial to be taken into account for studying its N retention. The present study was conducted at the northern edge of the Swiss Alps, representing a characteristic site for this region, with Flysch as parent rock material and clay-rich Gleysols of low permeability as major soil types. Due to the heavy soils, land use is limited and the landscape consists particularly of naturally regenerating forests dominated by Norway spruce (*Picea abies*) and (nowadays often abandoned) litter meadows. Although the N deposition has

been decreasing in this region since 1990 due to reduced emissions by agriculture and by traffic, it remains crucial to know how such systems react to the ongoing N deposition.

6.1.1 N Retention in the soil pool

The ^{15}N experiments showed that both mountain ecosystems retain most of the N recently deposited as NO_3^- or as NH_4^+ over the ground vegetation. Most of the tracer was recovered in the soil, especially as immobilised soil N (ISN). These results confirm the findings of previous ^{15}N tracer studies in forests, i.e. that most of the tracer is retained in the soil pool (Buchmann et al. 1996; May et al. 1996; Nadelhoffer et al. 1999; Gebauer et al. 2000) and stress the importance of N immobilisation processes. The immobilisation of both $^{15}NO_3^-$ and $^{15}NH_4^+$ was found to start within hours after the tracer application. It is, however, still not clear which processes are responsible for this fast incorporation into ISN. Several recent studies have also suggested that fast processes must be responsible for the immobilisation of N in the soil by biotic or abiotic mechanisms (Hart et al. 1993; Berntson & Aber 2000; Johnson et al. 2000; Zogg et al. 2000; Dail et al. 2001; Perakis & Hedin 2001). The very fast recovery of the NO_3^- tracer in the ISN leads us to assume that abiotic immobilisation via chemical processes played an important role. Also in the longer term, i.e. after one year, the ISN still showed a high recovery. However, this finding is in contrast to a recent study by Zak et al. (2004) in a sugar maple-dominated northern hardwood forest on a sandy soil, where no ^{15}N tracer was recovered in the soil organic matter after one year. Zak et al. (2004) suggested that the ^{15}N immobilised in soil organic matter had been released between one month and one year, but this finding was not confirmed in our study.

Despite numerous studies on the immobilisation processes in forest soils, the exact mechanisms of N retention are still poorly understood and seem to vary from site to site, depending on the soil characteristics. Therefore, the applicability of the results gained in our study may be constrained to the Flysch zone of the Alps or to other sites with corresponding soil characteristics. A promising approach for future research would be to compare the abiotic and biotic immobilisation capacity of different soils from different sites. This could be achieved by ^{15}N nuclear magnetic resonance (NMR) spectroscopy. Microbiologically active and sterilised soils labelled with ^{15}N could be incubated and the peaks corresponding to chemical bounds of N (amides, amines) measured. Clinton et al. (1995) and Knicker et al. (1997) showed that ^{15}N-NMR can be a useful technique for the study of N immobilisation in soils.

6.1.2 Relation between soil C:N ratio and NO_3^- leaching

Emmett et al. (1998) and Gundersen et al. (1998) showed that increasing N deposition causes the C:N ratio to be lowered by N retention and that NO_3^- leaching is closely related to the C:N ratio in the O horizon. Therefore, the declining C:N ratio could more and more limit the ability of the soil to immobilise N from atmospheric deposition, with the ecosystem thus progressively reaching N saturation. This long-term saturation can lead to higher N leaching, especially at snowmelt events, leading to an increased N-load for the surface and ground water. This theory was supported by Schleppi et al. (2004) at the Alptal site, where the C:N ratio decreased after 7 years of N addition concomitantly with a progressive increase in NO_3^- leaching.

Our event-based runoff analyses showed an immediate response of ^{15}N in the runoff of both ecosystems, with sharp $^{15}NO_3^-$ concentration peaks corresponding to discharge peaks. The annual pattern of NO_3^- leaching showed a clear seasonality, with highest fluxes in late winter and in the spring, i.e. at snowmelt events. This behaviour was similar for both ecosystems and was reproduced by the $^{15}NO_3^-$ leaching during the year of tracer application. This can be considered as further evidence that, at this site, leaching NO_3^- mainly originates from NO_3^- deposition (Schleppi et al. 2004). After the cessation of the NO_3^- tracer application, the $^{15}NO_3^-$ leaching stopped. This emphasizes that the applied $^{15}NO_3^-$ was either soon leached out of the system or immobilised in the soil or in the biomass within a few weeks or months.

6.1.3 Retention in the above-ground vegetation

The understory vegetation in the forest and the vegetation of the meadow, respectively, turned out to be major competitors for both tracers. Especially the mosses played an important role in the ^{15}N uptake. However, with increasing N deposition, the nitrogen uptake efficiency was reduced, when measured as either tracer uptake or as nitrate reductase activity. We conclude that, with increasing N deposition, mosses reach an assimilation limit where the deposited N is increasingly leached through the moss layer and into the soil rather than being taken up. When present, the moss layer can thus be considered as the first step of the N cascade in the ecosystem, as defined by Tietema et al. (1995). Symptoms of N saturation may therefore be measured first in the moss layer (reduced N uptake), before appearing in the soil (increased nitrification, lower C:N ratio), in water (NO_3^- leaching) or in vascular plants (increased N concentration).

6.1.4 Attempt to model the Alptal forest ecosystem

To understand the interactions of different N retention pools in the longer term, the model TRACE was used (Currie et al. 1999). The adaptation, calibration and validation of TRACE at the Alptal site and the attempt to use the model for assessing some implications of future deposition scenarios showed how complex and difficult the implementation of such models to other sites can be. Many parameter changes had to be made to adapt this model to our specific site characteristics at the Alptal. The model structure itself also had to be adapted to account for the fast leaching of NO_3^- from precipitation. At the current state, the model has still to be improved. Further changes should focus on improving the process formulations in the soil pool and in an integration of a ground vegetation layer, which plays an important role in the N uptake, as was shown in the ^{15}N field experiments.

Although the model deserves further improvements, it already contributed to a better understanding of the N cycling processes in the Alptal forest. The increasing N deposition showed an influence on the internal N status, as the N mineralisation and the NO_3^- leaching increased and the C:N ratio decreased.

6.2 Overall conclusions

The application and recovery of ^{15}N isotopes has proven to be a powerful tool for gaining insight into the mechanisms regulating the N retention of a mountain forest and a nearby meadow ecosystem subjected to atmospheric N deposition. The combined approach of field experiments and modelling proved to be valuable to assess how mountain ecosystems might retain increasing N deposition. The results of this thesis helped to improve our understanding about the N retention of mountain ecosystems. Because of specific characteristics of the Alptal site, especially its hydromorphic soil, the results obtained in this study are difficult to compare with most other sites where the N cycle of natural terrestrial ecosystems is studied intensively. They should, however, be representative of many ecosystems with hindered soil permeability in the temperate climate zone.

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Appendix

Appendix I:

Changes made compared to the default parameter set for the initialisation year 1900 separated into parameters and initialisation data.

A) PARAMETERS		Units	Harvard Forest Pine [1]	Alptal [2]	Reference
Phenology and physiology					
PsnTMin	Minimum daily temperature for photosynthesis	°C	4	0	R. Häsler (WSL, pers. comm.)
PsnTOpt	Optimum daily temperature for photosynthesis	°C	24	18	R. Häsler (WSL, pers. comm.)
AmaxFrac	Daily max. photosynthesis (Amax) as fraction	-	0.93	0.75	
FolReten	Foliar retention time	years	2.25	6	Schleppi et al. 1999a[5]
SLWMax	Specific leaf weight at top of canopy	$g\ m^{-2}$	200	440	Schleppi et al. 1999b[6]
SLWdel	Change in specific leaf weight with canopy depth	$g\ m^{-2}\ g^{-1}$	0	0.21	Schleppi et al. 1999a,b[5,6]
GDDFolStart	Degree days above 0 °C for foliar expansion to start	°C d	900	570	Hallenbarter PhD thesis 2002[3]
GDDFolEnd	Degree days above 0 °C for foliar expansion to end	°C d	1600	1400	Hallenbarter PhD thesis 2002[3]
GDDWoodStart	Degree days above 0 °C for wood growth to start	°C d	900	300	Schleppi unpublished data
GDDWoodEnd	Degree days above 0 °C for wood growth to end	°C d	1600	1400	Schleppi unpublished data
FolRelGrowMax	Max relative growth rate of canopy	-	0.3	0.2	
MinWoodFolRatio	Minimum ratio of wood production to foliar production	-	0.85	1.25	
Foliar litter					
FLPctN	N concentration in foliar litter	g N / g OM	0.0055	0.004	Schleppi et al. 1999a,b[5,6]
FolNConRange	Range over which foliar N concentration can rise	g N / g OM	0.7	0.4	
Fine root litter					
RpctACI	Acid insoluble fraction, fine root litter	100*[g / g OM]	47.3	24.5	
RpctACS	Acid soluble fraction, fine root litter	100*[g / g OM]	30.7	29	
RpctTEX	Total extractives fraction, fine root litter	100*[g / g OM]	22	46.5	
RLPctN	In fine roots, N concentration as permanent N that will reside in that tissue until it is lost	g N / g OM	0.001	0.002	
WLPctN	In wood, N concentration as permanent N that will reside in that tissue until it is lost	g N / g OM	0.0016	0.001	
Decomposition related parameters					
WooLitTrans	Annual frac rate of loss of SWD to downed woody debris	-	0.3	0.2	
WoodLitCLoss	Ratio of C loss to C transfer	-	0	0.25	
Kho	Decay rate of humus	$month^{-1}$	0.0008	0.00055	
Bioturb	Fraction of humus (Oa) mass bioturbated into mineral soil each month	$month^{-1}$	0.017	0.0008	
Klct	Humification of ACI and ACS in litter	-	0.6	0.05	
kwoodyO	Decay rate of woody litter in O horizon	$month^{-1}$	0.0019	0.003	
kwoodyM	Decay rate of woody litter in mineral soil	$month^{-1}$	0.055	0.001	

BODecayRat	Ratio of litter decay, mineral soil : forest floor	-	1.16	0.3	
CNwdw	Critical C:N for well-decayed woody detritus	-	90	60	
OGNitFrac	O horizon gross nitrification fraction	-	0.2	0.02	
MGNitFrac	M horizon gross nitrification fraction	-	0.3	0.03	
Soil parameters					
ORootFrac	Fraction of fine roots in O horizon	-	0.48	0.75	
SoilMoistFact	Soil moisture factor	-	-1	0	
Biomass					
FolMassMin	Initial minimum foliar mass	$g\ m^{-2}$	460	300	
FolMassMax	Initial maximum foliar mass	$g\ m^{-2}$	800	1600	
FolMass(1)	Initial minimum foliar mass	$g\ m^{-2}$	208	200	
FolMass(2)	Initial maximum foliar mass	$g\ m^{-2}$	52	1000	Schleppi et al. 1999b[6]
WoodCJan	Wood C storage	-	150	250	
PlantC	Plant internal C storage pool for photosynthate	-	800	1100	
Water					
WHC	Soil water holding capacity	cm	12	4	Schleppi unpublished data
PrecIntFrac	Proportion of precip. intercepted by canopy	-	0.2	0.125	Schleppi unpublished data
FastFlowFrac	Fraction of water inputs lost in first month	-	0.1	0.5	Schleppi et al. 2004[7]
Leaching					
ACIleach	Fraction of ACI mass loss that is leached	-	0.2	0.31	
ACSleach	Fraction of ACS mass loss that is leached	-	0.37	0.56	
TEXleach	Fraction of TEX mass loss that is leached	-	0.05	0.077	
DOMsorbFrac	Fraction of DOM leached in mineral soil that is retained in mineral soil solum	-	0.5	0.8	Hagedorn, PhD thesis 1999[4]
DONsorbFrac	Fraction of DON leached in mineral soil that is retained in mineral soil solum	-	0.5	0.8	Hagedorn, PhD thesis 1999[4]
MNitLeach	Fraction of available NO_3 in mineral soil that leaches from solum	-	0.05	0.3	Hagedorn, PhD thesis 1999[4]
Olscalar	Parameter to scale O horizon leaching of DOM	-	1	0.6	
Site parameters					
Lat	Latitude in degrees		42.5	47.05	
Lon	Longitude in degrees		72.2	8.72	
Elev	Elevation in m		350	1185	
Energetics parameters					
VascNWprop		-	0.5	0.25	
N fluxes					
BaseGrossNmin	Baseline (threshold) Gross N mineralisation	$g\ N\ m^{-2}\ a^{-1}$	28	20	

[1] for TRACE 4.1.5. Currie parameter set last modified 7/11/03

[2] Alptal TRACE 4.2. Providoli parameter set last modified 29/09/04

B) INITIALISATION DATA		Units	Harvard Forest Pine [1]	Alptal [2]	Reference
SnowPack	Average water equivalent stored in snowpack on Jan 1	cm	13	4.3	
Water	Average water equivalent stored in soil column on Jan 1	cm	12	4	
SWD	Initial mass of standing dead wood (snags)	g OM / m^2	10	200	
ONH4(1)	Pool size: inorganic available N in O horizon		0.4	0.05	
ONO3(1)	Pool size: inorganic available N in O horizon		0.02	0.001	
MNH4(1)	Pool size: inorganic available N in M horizon		0.5	0.1	
MNO3(1)	Pool size: inorganic available N in M horizon		0.02	0.001	
BL	Mass of nonhumified, nonwoody litter buried in mineral soil, in initial year of model runs	g OM / m^2	260	240	
BLN(1)	N pool size in nonhumified, nonwoody litter buried in mineral soil, in initial year of model runs	g N / m^2	2.45	2.9	
OFWD	Pool size (mass) of fine woody debris < 10 cm diameter in O horizon	g OM / m^2	100	365	
OFWDN(1)	N pool size in fine woody debris < 10 cm diameter in O horizon	g N / m^2	0.42	2	
OCWD	Pool size (mass) of woody debris > 10 cm diameter in O horizon	g OM / m^2	1	196	
OCWDN(1)	N pool size in fine woody debris > 10 cm diameter in O horizon	g N / m^2	0.01	1.1	
BFWD	Pool size (mass) of woody debris buried in mineral soil	g OM / m^2	1	40	
BFWDN(1)	N pool size in woody debris buried in mineral soil	g N / m^2	0.01	0.24	
HOM	Standing pool size (mass) of humified material in O horizon, in initial year of model runs	g OM / m^2	500	3200	Schleppi et al. 2004[7]
HON(1)	N pool size in humified material in O horizon, in initial year of model runs	g N / m^2	16	80	Schleppi unpublished data
SOM	Standing pool size (mass) of mineral soil organic matter, in initial year of model runs	g OM / m^2	14231	13200	Schleppi et al. 2004[7]
SON(1)	N pool size in mineral soil organic matter, in initial year of model runs	g N / m^2	370	380	Schleppi unpublished data

[1] for TRACE 4.1.5. Currie parameter set last modified 7/11/03

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Curriculum vitae

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2000	Diploma thesis (M. Sc. Geography) *"Post-fire-management and splash erosion in a chestnut coppice in southern Switzerland"*
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1992 – 1995	High school in Bern, BE, Gymnasium Neufeld (Matura Typus D)
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