RETENTION OF ATMOSPHERICALLY DEPOSITED NITROGEN IN SOIL: FIELD AND LABORATORY EXPERIMENTS USING ^{15}N ISOTOPE AND ^{15}N CPMAS NMR SPECTROSCOPY

THÈSE N^{O} 3580 (2006)

PRÉSENTÉE LE 7 JUILLET 2006

À LA FACULTÉ DE L'ENVIRONNEMENT NATUREL, ARCHITECTURAL ET CONSTRUIT

Laboratoire du sol et physique environnementale

SECTION SCIENCES ET INGÉNIERIE DE L'ENVIRONNEMENT

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

POUR L'OBTENTION DU GRADE DE DOCTEUR ÈS SCIENCES

PAR

Isabelle MORIER JAQUET

Licence en biologie de l'Université de Lausanne
de nationalité suisse et originaire de Troistorrents (VS)

acceptée sur proposition du jury:

Prof. A. Mermoud, président du jury
Prof. J.C. Védy, Dr C. Guenat, directeurs de thèse
Dr P. Schleppi, rapporteur
Dr H. Knicker, rapporteur
Dr R. Siegwolf, rapporteur

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Lausanne, EPFL
2006

REMERCIEMENTS

Ce projet a été réalisé grâce à la collaboration, tant scientifique que financière, du WSL et de l'EPFL. Je remercie ces deux institutions pour la confiance qu'elles m'ont accordée.

Je tiens à exprimer ma profonde reconnaissance au Dr Claire Guenat et au Dr Patrick Schleppi pour m'avoir guidée et accompagnée tout au long de ce travail grâce à leur grande compétence scientifique et à leurs qualités humaines. Ils ont permis que cette recherche soit une riche collaboration.

Je remercie également le Prof. Jean-Claude Védy qui m'a accordé sa confiance dès le début du projet et tout au long de ma thèse.

Un grand merci aux autres membres de mon comité de pilotage et de mon jury de thèse, le Prof. Francis Andreux (Université de Bourgogne), le Prof. Alexandre Büttler (EPFL-WSL), le Dr Heike Knicker (Technische Universität München), le Dr Rolf Siegwolf (PSI), pour leur investissement et leurs judicieux conseils

L'équipe technique du LPE composée de Jean-David Teuscher, Elena Rossel, Didier Perret, Valérie Rochat et Delphine Rota, a apporté une contribution technique importante et de qualité. Ce fut également un plaisir de les cotoyer et je les en remercie chaleureusement.

Ma reconnaissance va également à mes autres collègues Pascal Boivin, Jean-Pierre Bonjour, Anne-Claude Cosandey, Nathalie Dakhel, Jean-Pascal Dubois, Fabienne Favre, Jean-Marc Froehlich, Karin Kaufman, Rodrigue Kilchenmann, Véronique Maitre, Céline Müller, Pauline Du Pasquier, Lukas Wick, Karin Zwahlen. Ils ont contribué au bonheur de ces années passées à l'EPFL. Merci à Marie Sudki qui a patiemment relut et corrigé mes textes en anglais et à Bernard Sperandio pour son efficace collaboration.

Je remercie les collaborateurs de l'Antenne Romande du WSL pour m'avoir chaleureusement accueillie dans leur équipe. Ce fut également un plaisir de travailler avec ma collègue doctorante de Birmensdorf, Isabelle Providoli et je tiens à la remercier pour tous nos fructueux échanges.

Un grand merci à l'équipe du Dr Rolf Siegwolf (PSI), en particulier au Dr Matthias Saurer, pour la qualité de son travail et sa disponibilité.

Merci au Dr Heike Knicker (TUM) pour les mesures de RMN et pour ses conseils avisés.

Merci à toutes les personnes qui m'ont aidée lors des campagnes de terrain et des expérimentations au laboratoire, Fleur Choy, Isabelle Conus, Raphaël Gallay, Christine Goy, Sarra Harbi, Michael Herger, Natacha Pasche, Samuel de Santis, Alexa Wiscott.

Je remercie tous les amis qui n'ont pas encore été cités, pour leur soutien.

Ma plus profonde reconnaissance va à ma famille, en particulier à Baptiste pour sa compréhension et son soutien, à Maximilien et Victor pour leur patience. Merci à Marblum, Michel, Danièle, Roger, Valérie et Marie pour leur précieuse aide.

ABSTRACT

Since a few decades, the balance of the nitrogen (N) cycle has been deeply disturbed by human activies. The global impact of these activities on the N cycle can be described as a doubling of the transfer from the vast and unreactive atmospheric pool to biologically available forms (N fixation). The main sources responsible for the increase of reactive N emissions are the use of artificial fertilisers (NH_3) as well as the combustion processes (NO_x). Reactive N is then transformed, transported by both the atmosphere and the hydrosphere and, finally, deposited on both the terrestrial and the aquatic ecosystems with potentially strong impacts. Amongst the terrestrial ecosystems, the temperate forests are particularly sensitive to reactive N increases for various reasons: they are located near to strongly anthropised areas and are thus subjected to strong depositions; they are naturally N-limited and their biochemical cycle can indeed be strongly influenced by additional N, which leads to eutrophication and potential impacts such as soil acidification, nitrate leaching and losses of biodiversity within microorganisms, plants and fauna communities. Previous studies carried out in temperate forest ecosystems have shown that the soil, namely the soil organic matter, acts as a main deposited N sink for the ecosystem. However, biogeochemical reactions responsible for N retention in the soil are still not fully understood. Moreover, the majority of the concerned studies were conducted in acidic or hydromorphous soils and very little is known today about the fate of N deposition under other soil physico-chemical conditions.

Consequently, the present research aims at filling some of the gaps described above and deals with two main topics concerning the retention of atmospherically deposited N in soils that are (1) the characterisation of the retention of atmospherically deposited N in the soil, in terms of both duration and quantity, more specifically, in a well drained calcareous soil and (2) the mechanisms and processes responsible for such a retention.

From a practical point of view, the retention of N deposition in the soil was characterised by means of a ^{15}N field labelling experiment simulating N atmospheric deposition and conducted at the Grandvillard research site, in the riparian zone of La Sarine River (Swiss Prealps). The stand is a beech forest mixed with planted spruces. The soil consists of a well drained calcareous fluvisol with a fast organic matter turnover. We tracked the N tracers ($^{15}NH_4^+$ or $^{15}NO_3^-$, corresponding to the main forms of deposition) from short-term (hours, days or weeks) to longer-term intervals (one year), by measuring the partitioning of ^{15}N into different biochemical soil fractions (extractable N, microbial N, roots N and N immobilised in soil). Aiming at studying the mechanisms and processes responsible for N retention, we performed two laboratory experiments. The first one was an acid hydrolysis, in order to determine the proportion of N deposition retained in the hydrolysable fraction (i.e. the more labile N forms, including the most bioavailable ones) as well as in the non-hydrolysable fraction covering the more recalcitrant N compounds. Within the framework of this experiment, we compared the results obtained in Grandvillard and in an aditional constrasting site (Alptal). The second experiment was a short-term (one hour to one week) laboratory incubation of sterilised and not sterilised soils, which purpose was to highlight the relative importance of biotic and abiotic processes in N immobilisation. Soils were labelled with either $^{15}NH_4^+$ or $^{15}NO_3^-$ and subjected to ^{15}N

CPMAS NMR spectroscopy analysis, which is a powerful technique to identify the N organic molecules in soils.

Within the framework of the field labelling experiment, more than half of the tracers (either $^{15}NO_3^-$ or $^{15}NH_4^+$) was recovered in the soil between one hour and one year after labelling. Therefore, the general assumption that non-fertilised temperate forest soils are the main sink for deposited N can be confirmed. Since previous studies showing the importance of the soil for the retention of deposited N were all conducted on either acidic or hydromorphous soils, our results allow us to extend the validity of their general conclusions to a wider range of unfertilised temperate forest soils. We further showed that the main forms of N deposition (i.e. NO_3^- and NH_4^+) were retained in the soil within the same range of magnitude, in spite of their different biochemical pathways. N retention occurred mainly in the soil organic layers and, consequently, our results confirme the importance of the organic fraction in the deposited N retention.

Since deposited N was retained in the soil in the very short term, we confirmed the presence of very fast processes and demonstrated that the very short term dynamics determined the fate of deposited N in the longer term: the main processes were i) a loss of deposited N as extractable through lixiviation (and lateral fluxes) and ii) a retention as N immobilised in soil. Furthermore, we demonstrated that N immobilisation brought deposited N both into the hydrolysable and into the recalcitrant fractions of soil N within short-time range (i.e. one week) . We also demonstrated that the hydrolysability (hydrolysable N/ total N) was constant over the year.

Within the framework of the laboratory incubation of soils subjected to the ^{15}N CPMAS NMR spectroscopy analysis, we showed that all NMR spectra were dominated by a single signal corresponding to the amide-peptide structure, whatever the soil layer concerned (organic or organo-mineral), the form added (NO_3^- or NH_4^+) and whether the soil was sterilised or not. Such dominance of proteinaceous compounds was in agreement with the results obtained in humic substances and in various soils all over the world. Further to the extractable ^{15}N dynamics during the incubation, we proposed that biotic processes were dominant in the short-term N immobilisation. However, an abiotic fixation of less importance was not excluded for $^{15}NO_3^-$.

Considering our results, we propose two mechanisms responsible for the retention of deposited N in soil in the long term. The first mechanism is a rapid and stable immobilisation in the recalcitrant pool of soil organic matter. Our results showed that biotic and abiotic processes could be involved: the afore-mentioned process of abiotic nitrate fixation could lead to the N immobilisation in heterocycles whereas N incorporated in amides and then stabilised in soil organic matter seemed to be the major pathway. The second mechanism of retention we are proposing is the biological recycling within the soil or the soil-plant system: on a short scale, microbial recycling seemed to be very rapid because deposited N was mainly present under a biosynthesised form (amides), in spite of the low amounts of tracer found in the microbes. Within the soil profile, roots recycling was probably efficient: fine roots, which are often the major contributors of soil organic matter were an important sink for N deposition all over the year.

The combination of various scales and techniques allowed the connection between the dynamics of N retention in soil organic layers observed and the mechanisms responsible for it (i.e. rapid immobilisation in a recalcitrant pool and biological recycling through micro-organisms and plants). The results of this multi-scale approach suggest further developments and impacts for the modelling of the long-term fate of deposited N.

Keywords : Atmospheric N deposition, temperate forest ecosystems, soil, ^{15}N isotope, ^{15}N CPMAS NMR

RÉSUMÉ

Depuis de nombreuses décennies, l'équilibre du cycle de l'azote (N) a été fortement perturbé par les activités anthropiques, dont l'impact se traduit par un doublement des transferts chimiques de l'azote atmosphérique non réactif en formes assimilables par les organismes vivants (processus connu sous le terme de fixation de l'azote). L'augmentation des émissions d'azote sous forme réactive est principalement due à l'utilisation de fertilisants synthétiques (NH_3) ainsi qu'à la combustion de carburants fossiles (NO_x). L'azote émis est transformé, puis transporté par l'atmosphère et l'hydrosphère pour se déposer dans les écosystèmes aquatiques et terrestres. Cette augmentation des dépôts atmosphériques azotés a une incidence importante sur les forêts tempérées, qui sont généralement très exposées car elles sont souvent proches des sources azotées d'origine anthropique. De plus, l'azote, en conditions naturelles, est un facteur limitant pour la productivité de ces forêts. Un apport additionnel de celui-ci peut se traduire par un dérèglement de leur cycle biogéochimique conduisant à l'eutrophisation et à d'autres impacts: acidification du sol, lixiviation de nitrate et diminution de la biodiversité au sein des communautés végétales, animales et de microorganismes, notamment. Lors d'études précédentes dans les écosystèmes forestiers tempérés, il a été démontré que le sol, en particulier sa fraction organique, agit comme un puits majeur face à ces dépôts. Les réactions biogéochimiques à l'origine de la rétention de ces dépôts ne sont pas encore totalement connues et la plupart des recherches menées sur des sols acides ou à caractère hydromorphe ne fournissent que peu d'informations sur leur devenir dans des sols soumis à d'autres conditions physico-chimiques.

Notre étude a pour objectif de combler ces lacunes et traite deux points essentiels: (1) la caractérisation quantitative et temporelle de la rétention de ces dépôts dans le sol qui se focalise sur les sols carbonatés et bien drainés; (2) l'étude des mécanismes et processus à l'origine de cette rétention.

Dans la pratique, une étude sur la rétention des dépôts atmosphériques azotés a été effectuée grâce à un marquage avec l'isotope ^{15}N. Une expérience, réalisée in situ et simulant ces dépositions, a été menée à Grandvillard, site alluvial d'importance nationale au bord de la Sarine (Préalpes suisses) caractérisé par une forêt composée de hêtres et d'épicéas plantés, un sol de type fluviosol carbonaté bien drainé avec turnover rapide de la matière organique. Nous avons analysé le devenir de deux traceurs: l'ammonium ($^{15}NH_4^+$) et le nitrate ($^{15}NO_3^-$) qui sont les deux formes principales des dépôts atmosphériques azotés, puis effectué un suivi temporel à court terme (heure, jour et semaine) et à plus long terme (mois, année) en mesurant le ^{15}N dans différentes fractions du sol (N extractible, N microbien, N racinaire et N immobilisé dans le sol). Les mécanismes et les processus à l'origine de la rétention d'azote dans le sol ont été étudiés par le biais de deux expériences complémentaires réalisées en laboratoire. La première, une hydrolyse acide pour quantifier les dépôts azotés retenus sous forme hydrolysable (forme la plus labile et la plus biodisponible de l'azote) et ceux retenus sous forme non hydrolysable (composés azotés les plus récalcitrants). Dans le cadre de cette expérimentation, nous avons également comparé les résultats obtenus à Grandvillard avec ceux d'un autre site aux conditions stationnelles très différentes (Aptal). La seconde expérience a consisté en une incubation à court terme (1 heure à 1 semaine), en laboratoire, de sols stérilisés ou non stérilisés, le but étant de déterminer l'importance relative des processus biotiques et abiotiques à l'origine de l'immobilisation de l'azote dans le sol. Les sols ont été marqués à $^{15}NH_4^+$ et $^{15}NO_3^-$ puis ana-

lysés par ^{15}N CPMAS NMR, technique reconnue pour l'identification des molécules organiques azotées du sol.

Les résultats de l'expérience de marquage menée in situ ont permis de démontrer que plus de la moitié du traceur ($^{15}NO_3^-$ ou $^{15}NH_4^+$) était retenu dans le sol déjà après une heure et que les quantités étaient similaires une année plus tard, d'une part et, d'autre part, de confirmer que dans le cas des écosystèmes forestiers tempérés, le sol agit comme puits essentiel face aux dépôts atmosphériques. Grâce à cette étude, nous pouvons étendre la validité des résultats précédemment acquis pour des sols acides et à caractère hydromorphe à une plus large gamme de sols forestiers des régions tempérées. Nous avons également démontré que les deux formes principales de dépôts azotés (NO_3^- et NH_4^+) sont retenues dans le sol dans un ordre de grandeur similaire malgré leur comportement biochimique différent. La rétention d'azote a eu lieu préférentiellement dans les horizons organiques du sol confirmant ainsi le rôle capital de la fraction organique dans la rétention des dépôts azotés.

Ces derniers ayant été retenues dans le sol à très court terme, nous confirmons la présence de processus très rapides déterminant le devenir de ces dépôts à plus long terme. Les principaux processus identifiés sont : 1) la perte des dépôts azotés extractibles via la lixiviation (et les écoulements latéraux); 2) la rétention d'azote dans le sol sous forme immobilisée. Nous avons également démontré que l'immobilisation de l'azote se traduit, dans un laps de temps très court (une semaine), par le transfert des dépôts azotés sous forme hydrolysable et récalcitrante. L'hydrolysabilité (N hydrolysable/N total) de l'azote natif et de l'azote marqué est constante tout à long de l'année.

Dans le cadre de l'expérience d'incubation mentionnée plus haut, la NMR a montré que la présence d'un signal unique attribué à une structure de type amide-peptide était caractéristique, quel que soit l'horizon (organique ou organo-minéral), la nature du traceur ajouté ($^{15}NO_3^-$ ou $^{15}NH_4^+$) et que le sol soit stérilisé ou non. La dominance de ces composés protéinés est en parfaite adéquation avec les résultats obtenus par d'autres chercheurs, soit dans l'étude des substances humiques, soit dans différents sols du monde. Considérant la dynamique du ^{15}N extractible au cours de l'incubation, nous pensons que l'immobilisation de l'azote est basée principalement sur un processus biologique quoiqu'une fixation abiotique ne puisse être totalement exclue dans le cas du $^{15}NO_3^-$, mais dans une moindre mesure.

En conséquence, deux mécanismes à l'origine de la rétention à long terme des dépôts atmosphériques azotés dans le sol sont proposés: le premier, une immobilisation rapide et stable de l'azote sous une forme organique récalcitrante. Par ailleurs, des processus biotiques et abiotiques pourraient être impliqués: la fixation abiotique de NO_3^- pourrait conduire à cette immobilisation de l'azote sous forme d'hétérocycle mais l'azote incorporé sous forme d'amides, puis stabilisé au sein de la matière organique, semble être le principal processus responsable de cette immobilisation. Le second mécanisme de rétention est le recyclage biologique, dans le sol proprement dit ou par le système sol-plante; à court terme, le recyclage microbien semble être très rapide puisque les dépôts azotés ont principalement été identifiés sous une forme biosynthétisée (amide) et ceci malgré un faible taux de ^{15}N dans le compartiment microbien. Dans le sol proprement dit, le recyclage par les racines semble être efficace car les racines fines qui jouent un rôle majeur dans les apports organiques dans le sol, constituent un important puits face aux dépôts azotés.

Grâce à l'utilisation de techniques variées, la combinaison de différentes échelles nous a permis de faire le lien entre la dynamique de rétention de l'azote dans le sol et les mécanismes à l'origine de cette rétention (une immobilisation rapide dans une fraction récalcitrante ou un recyclage par le système sol-plante et les microorganismes du sol). Les résultats de cette approche multiscalaire offrent des perspectives de développement pour la modélisation du devenir des dépôts atmosphériques azotés à long terme.

Mots-clés : Dépôts atmosphériques azotés, écosystèmes forestiers tempérés, sol, isotope ^{15}N, ^{15}N CPMAS NMR

TABLE OF CONTENTS

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 1

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

The global nitrogen cycle is naturally balanced by i) fixation processes (biological N fixation, lightning) leading from non-reactive nitrogen (N_2) to biologically reactive forms and ii) denitrification processes reducing nitrate (NO_3^-) to N_2 (Söderlund & Svensson, 1979; Ayers et al., 1994; Galloway et al., 1995). For several decades now, this cycle has been strongly disturbed by human activities which led to a significant increase in the amounts of fixed nitrogen (antropogenic fixation increased from approximately 15 Tg N per year in 1860 to 165 Tg N per year in 2000) at such point that at the present time, the anthropogenic fixation is greater than that recorded by all natural terrestrial ecosystems (~100 Tg N per year) (Galloway & Cowling, 2002). The main sources responsible for increasing reactive N emissions are the use of artificial fertilizers (NH_3) and combustion processes (NO_x). Reactive N is then transformed, transported into the atmosphere and the hydrosphere and finally deposited on the terrestrial and aquatic ecosystems, mainly in the form of NH_4^+ and of NO_3^-. As a consequence, reactive N compounds accumulate in the ecosystems, with potentially strong impacts. The temperate forest ecosystems are particularly sensitive to increases in reactive N for various reasons: they are located near to strongly anthropised areas and are thus subjected to strong deposition; furthermore, they are naturally N-limited and their biochemical cycle can indeed be strongly influenced by additional N, leading to eutrophication and potential impacts such as soil acidification, nitrate leaching, changes in tree growth, loss of biodiversity within microorganisms, plants and fauna communities (Fangmeier et al., 1994; Vitousek et al., 1997; Socolow, 1999; Dise et al., 2001; Sheeder et al., 2002; Aber et al., 2003; Galloway et al., 2003).

Although previous studies carried out in temperate forest ecosystems have shown that the soil, particularly the soil organic matter, acts as a main N sink for the ecosystem, biogeochemical reactions responsible for N retention in soil are still not well understood. In addition, most of the concerned studies were conducted in acidic or hydromorphous soils (Hart et al., 1993; Gundersen et al., 1997; Gebauer et al., 2000; Lamontagne et al., 2000, Providoli et al., 2006) and we know very little about the fate of N deposition under other soil physico-chemical conditions. Consequently, this research aims at filling some of the gaps described above by a multiscale study, from a field scale, by a ^{15}N labelling experiment to a molecular scale by the use of ^{15}N NMR spectroscopy.

1.2 OBJECTIVES OF THE RESEARCH

The present research deals with two main topics of the retention of atmospherically deposited N in soils: i) characterisation of its retention, in terms of both duration and quantity; more specifically, in a well drained calcareous soil; ii) mechanisms and processes responsible for such retention. Those general objectives are being approached and discussed by means of more precise questions (see above) and also by the description of the methodological steps applied to achieve them.

1) Retention of atmospherically deposited N in soil, in terms both of duration and quantity – study of a well drained calcareous soil with fast organic matter turnover

More precisely:

i) Does this soil type play the role of main sink for atmospherically deposited N, as is the case for ecosystems with acidic or/and hydromorphous soils?

ii) What is the temporal pattern of this retention?

iii) What is the relative importance of both the different layers and the different biochemical soil pools?

iv) Does such importance depends on the chemical form (NH_4^+ or NO_3^-) of the deposited N?

To answer these above questions, we simulated N deposition by applying a ^{15}N pulse on small plots in the field. Indeed the application and recovery of ^{15}N isotopes in forest soils has proven to be a powerful tool to track the fate of N in the soil layers and in the biochemical soil pools (Perakis & Hedin, 2001). We applied $^{15}NH_4^+$ and $^{15}NO_3^-$ on separate plots. We chose to work in a calcareous and well drained forest soil. We followed the ^{15}N within a timely rate, from one hour to 1 year. The tracer was followed in different soil pools: extractable N, microbial N, roots N and ISN.

2) Mechanisms and processes responsible for the retention of deposited N in soil

i) Which are the very short term (hours to days) mechanisms responsible for NH_4^+ and NO_3^- retention?

In the framework of the field labelling experiment, we were able to follow the short-term movements of ^{15}N occuring over a period of hours to days in the different soil pools (extractable N, microbial N, roots N and ISN). This very short term dynamics of deposited N in the different soil pools gave us precious information on the mechanisms responsible for its retention.

ii) In which organic fraction (labile or recalcitrant) is the deposited N immobilised? Moreover, is the distribution similar between two contrasted forest soils?

A *hot acid hydrolysis (Bremner, 1965) was performed on samples collected during the field labelling experiment, to determine the proportion of either NH_4^+ or NO_3^- deposition retained in the hydrolysable fraction (i.e. the more labile N forms, including the most bioavailable ones) and in the non-hydrolysable fraction covering the more recalcitrant N compounds. Such hydrolyses were also carried out on samples collected during a second field experiment using the same protocols in an clayed and hydromor-*

phous soil (Providoli, 2005). To our knowledge, such method has never been used within the frame of studies on deposited N in soil.

iii) Which chemical forms and which biochemical mechanisms (biotic or abiotic) are involved in the immobilisation of NH_4^+ and NO_3^- ?

We combined a laboratory incubation of sterilised and not sterilised soils, labelled with either $^{15}NH_4^+$ or $^{15}NO_3^-$ and ^{15}N CPMAS NMR spectroscopy, which is a powerful technique to describe the molecules involved in ^{15}N retention in soils (Clinton et al., 1995; Knicker et al., 1997).

iv) Which are the longer term mechanisms responsible for the retention of deposited N in the soil?

To answer this question, we synthesised observations on i) longer-term movements of both N tracers occurring over several months, up to one year, which were measured within the frame of the field labelling experiment and ii) the biochemical forms and processes involved in the N retention.

1.3 THESIS LAYOUT

This manuscript is composed of seven chapters. After the general introduction (**chapter 1**), we will point out some basic concepts concerning the natural cycle of nitrogen and the human impacts that affect it, with a special focus on soil.

Chapter 2 reviews the state of the research in the nitrogenous pollutions, namely as far as atmospheric N deposition is concerned.

Chapter 3 describes the research sites concerned.

Chapter 4, 5 and 6 will be the body of the document. Each chapter corresponds to a publication and deals with either or both subjects described above.

Finally, **chapter 7** synthesises main contributions, limits and perspectives of this research. The answers to the questions proposed above are based on the results presented in chapters 4 to 6.

REFERENCES

Aber J.F., Goodale C.L., Ollinger S.V., Smith M.-L., Magill A.H., Martin M.E., Hallett R.A. & Stoddard J.L. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests ? *Bioscience*, **53**, 375-389.

Ayers R.U., Schlesinger W.H. & Socolow R.H. 1994. Human impacts on the carbon and nitrogen cycles. In: *Industrial ecology and global change* (eds R.H. Socolow, C. Andrews, R. Berkhout & V. Thomas). pp. 121-125. Cambridge University Press, Cambridge, England.

Bremner J.M. 1965. Organic forms of soil nitrogen. In: *Methods of soil analysis, Part 2. Chemical and microbiological properties* (ed. C.A. Black), pp. 1238-1255. Agronomy 9. American Society of Agronomy, Madison, USA.

Clinton P.W., Newman R.H. & Allen R.B. 1995. Immobilization of ^{15}N in forest litter studied by ^{15}N CPMAS NMR spectroscopy. *European Journal of Soil Science*, **46**, 551-556

Dise N.B., Matzner E., Armbruster M. & MacDonald J. 2001. Aluminium output from forest ecosystems in Europe: a regional assessment. *Journal of Environmental Quality*, **30**, 1747-1756.

Fangmeier A., Hadwiger-Fangmeier A., Van der Eerden L. & Jaeger H.-J. 1994. Effects of atmospheric ammonia on vegetation - a review. *Environmental Pollution*, **86**, 53-82

Galloway J.N., Schlesinger W.H., Levy II H., Michaels A. & Schnoor J.L. 1995. Nitrogen fixation: Anthropogenic enhancement-environmental response. *Global Biogeochemical Cycles*, **9**, 235-252.

Galloway J.N. & Cowling E.B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio*, **31**, 64-71.

Galloway J.N., Aber J.D., Erisman J.W., Seitzinger S.P., Howarth R.W., Cowling E.B. & Cosby B.J. 2003. The nitrogen cascade. *Bioscience*, **53**, 341-356.

Gebauer G., Zeller B., Schmidt G., May C., Buchmann N., Colin-Belgrand M., Dambrine E., Martin F., Schulze E.-D. & Bottner P. 2000. The fate of ^{15}N-labelled nitrogen inputs to coniferous and broadleaf forest. In: *Carbon and nitrogen cycling in european forest ecosystems* (ed. E.D. Schulze). pp. 144-170. Ecological Studies 142. Springer-Verlag, Berlin, Heidelberg.

Gundersen P., Emmett B.A., Kjønaas O.J., Koopmans C.J. & Tietema A. 1997. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest and Ecology Management*, **101**, 37-55.

Hart S.C., Firestone M.K., Paul E.A. & Smith J.L. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry*, **25**, 431-442.

Knicker H., Lüdemann H.-D. & Haider K. 1997. Incorporation studies of NH_4^+ during incubation of organic residues by ^{15}N-CPMAS-NMR-spectroscopy. *European Journal of Soil Science*, **48**, 431-441.

Lamontagne S., Schiff S.L. & Elgood R.J. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. *Canadian Journal of Forest Research*, **30**, 1165-1777.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. *Ecology*, **82**, 2245-2260.

Providoli I. 2005. *Pathways of atmospherically deposited nitrogen in two ecosystems in central Switzerland: an experimental and model-based study using the ^{15}N isotope*. Doctoral dissertaion, Swiss Federal Institute of Technology, Zurich.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2006. 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. *Soil Biology and Biochemistry*, **in press**.

Sheeder S.A., Lynch J.A. & Grimm J. 2002. Modeling atmospheric nitrogen deposition and transport in the Chesapeake Bay watershed. *Journal of Environmental Quality*, **24,** 2009-226.

Socolow R. 1999. Nitrogen management and the future of food: lessons from the management of energy and carbon. *Proceedings of the National Academy of Science of the United States*

of America, **96**, 6001-6008.

Söderlund R. & Svensson B.H. 1979. The global nitrogen cycle. In: *Nitrogen, phosphorus and sulphur - global cycles* (eds R. Söderlund & B.H. Svensson). pp. 23-73. Swedish Natural Sciences Research Council, Stockolm, Sweden.

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. & Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737-750.

CHAPTER 2

2 NITROGEN IN THE ENVIRONMENT

2.1 THE NATURAL NITROGEN CYCLE

Nitrogen (N) is a very important element on Earth in terms of quantities and functions: the total amount of N in the atmosphere, lithosphere and hydrosphere is approximately 16'750 · 10^{16} kg. It is a constituant of many molecules necessary to the life, like proteins, nucleic acids or chlorophylls. The main characteristic of the N cycle is that more than 99% of N is not available to more than 99% of living organisms (Galloway et al., 2003). The main pool is indeed the enormous inert reservoir of the lithosphere (16'000 · 10^{16} kg). The second one is Earth's atmosphere (386 · 10^{16} kg) where the very stable molecule N_2 is the predominant gas (approximately 78%) and which cannot be used by most organisms. Breaking the triple bond holding the two N atoms together requires a significant amount of energy and it is a necessary stage to form biologically reactive molecules of the general formula NO_x and NH_x. The passage from stable form N_2 to reactive forms is the N fixation. Lightning is the non-biological process that is able to fix N_2, but it accounts only for a weak share (approximately 10%). The main process is the biological fixation, of which only a few micro-organisms are able. They directly use N_2 thanks to an enzymatic complex called Nitrogenase. These micro-organisms generally live in a symbiosis with a plant, which enables them to face the high energy cost required to break the triple covalent bond. Since fixation is not very high in most of the forest natural ecosystems, its productivity is generally limited by the supply of biologically available N, and the N cycle is almost closed within this system. In the opposite direction, denitrification is the primary mechanism for the return of N_2 into the atmosphere. Denitrification is mainly a biological process and consists of a reduction of NO_3^- to N_2 (or N_2O). In natural ecosystems, fixation and denitrification globally compensate and ensure the balance of the N cycle.

The third pool is the hydrosphere (2.30 x 10^{16} kg) where, again, 95% of the N is dissolved N_2. Under natural conditions, the N store of the ocean can be considered to be in a state of quasi-equilibrium (Stevenson, 1992).

2.1.1 IMPORTANCE OF THE SOIL IN THE N CYCLE

Despite its small size (0.024 x 10^{16}kg), the soil occupies a central place in the global N cycle. First, terrestrial biological N fixation and denitrification happen mainly within the soil. Second, it is the nutritional reservoir of the plants, which preferentially assimilate nitrate (NO_3^-) and ammonium (NH_4^+). In general, plants prefer NO_3^- but some (like, for example, conifers or mosses) preferentially take up NH_4^+. These ions are the main components of the inorganic soil N pool and are generally subjected to a strong competition between plants and micro-organisms since N is mostly a limiting element for their growth in unfertilised ecosystems.

A key feature of the soil N cycle is the microbial N turnover through mineralisation-immobilisation (Stevenson, 1992). Microbial immobilisation describes the nitrogen assimilation (mainly NH_4^+) by the micro-organisms, for the elaboration of nitrogenous molecules such as the amino acids. The common belief is that micro-organisms rather assimilate NH_4^+ because of a lower energetic cost for its metabolic assimilation. However, recent studies have shown that NO_3^- could also be rapidly immobilised by micro-organisms (Berntson and Aber, 1999; Zogg et al., 2000; Perakis et al., 2001).

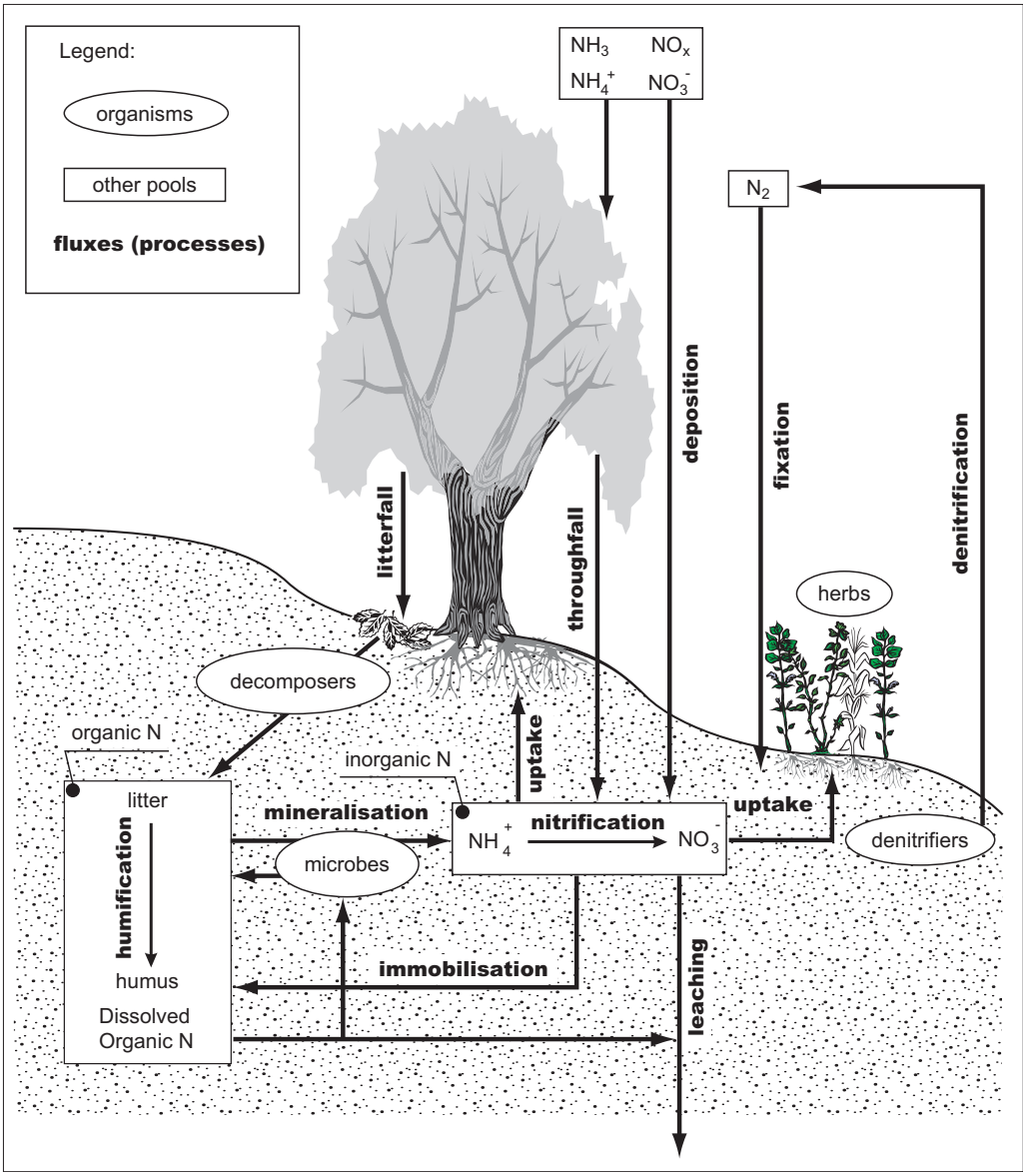

Fig. 2.1 : Schematic N cycle of terrestrial ecosystems (adapted after P. Schleppi)

The mineralisation process is the oxydation of organic molecules into mineral compounds by micro-organisms, which gives rise to their cellular respiration, coupled with energy (ATP) production. In the case of N, the mineralisation process is also called ammonification because the result of the decomposition process is NH_4^+. Microbial nitrification is also a widely spread soil process that further oxidizes NH_4^+ into NO_3^- and which happens under aerobic conditions.

At the ecosystem scale, plant litter production (aerial or underground) is the main pathway for N to get back into the soil. The N assimilated by plants is recycled in the soil either as mentionned or indirectly (through animal corpses and metabolic waste).

2.1.2 MAIN FORMS OF SOIL NITROGEN

Average amounts of total N in soil vary from one ecosystem to another. Stevenson proposes an average amount of 300 g / m^2 to a depth of 15 cm in North American forest brown soils (Stevenson, 1992).

Only a small fraction of the soil N (generally <0.1%) exists as soluble NO_3^- and exchangeable NH_4^+ which are the main forms of inorganic soil N. This pool is thus very small in size but plays a key role since it is the main form available for plants and micro-organisms.

In most soils, over 90% of the N occurs in organic forms (Kelley & Stevenson, 1996). Soil organic N consists of organic residues, plants and animals partly decomposed or not at all, and of humified soil organic matter (SOM stricto sensu). Hot acid hydrolysis (Bremner, 1965) is a method frequently used to characterise the N contained in the SOM (Stevenson, 1982). It allows to separate N compounds into a hydrolysable fraction corresponding to the more labile N forms and into a non-hydrolysable fraction covering the more recalcitrant N compounds. The labile fraction was described as consisting of amino acids (approximately 40%), amino sugars (approximately 10%) and other unknown compounds (approximately 20%) (Swoden *et al,*. 1977; Stevenson, 1982). The remaining third of organic matter is composed of non-hydrolysable compounds still poorly defined. Consequently, half of soil N remains to be more precisely identified on a chemical aspect. At the present time, several techniques are used and theories proposed for this non-identified N fraction:

Analytical pyrolysis (Py-GC/MS; Py-FIMS) is widely used in soil N studies. Researchers using such analytical techniques found a larger presence of heterocyclic compounds with values comprised between 27% and 34% of the total N (Schulten, 1994; Schulten et al., 1997; Schulten & Schnitzer, 1998) and proposed that those would represent the main part of the organic recalcitrant N pool.

^{15}N CPMAS NMR spectroscopy is a non-invasive method, widely used for the analysis of N compounds in soils. Its application to the study of various soils in the world and of humic substances has shown that the main forms of organic N are the amides functional groups (approximately 80%) (Knicker & Kögel-Knabner, 1998; Mathers et al., 2000; Knicker, 2004; Dieckow et al., 2005). The other main identified organic forms are free amino groups and indoles or pyroles (Knicker & Kögel-Knabner, 1998). Proteinaceous compounds such as amides and amines are considered as rather labile, and their presence in the recalcitrant pool of the soil organic matter seems to require some form of stabilisation. The possible mechanisms proposed by different researchers are: i) the encapsulation of proteinaceous material into the hydrophobic sites of the SOM (Knicker & Hatcher, 1997; Zang et al., 2000; Dieckow et al., 2005); ii) organomineral interactions (Leinweber & Schulten, 2000).

2.2 ANTHROPIC IMPACTS ON THE NITROGEN CYCLE

The global impact of human activities on the N cycle can be described as a doubling of the transfer from the vast and unreactive atmospheric pool to biologically available forms (N fixation) (Vitousek et al., 1997). The several processes involved are mainly the industrial fixation of N_2 for a use as fertiliser, the cultivation of crops with the capacity to fix N symbiotically and also the fixation during fossil-fuel combustion.

N fertilisation is generally accompanied by some losses. The first important loss is NO_3^- leaching, which leads to a pollution of the hydrosphere. The second is volatilisation of NH_3, which causes N deposition after transport by the atmosphere. The importance of these losses and of their negative impacts depend mainly on the type of fertiliser (artificial or manure), on the type and rate of application, on the physico-chemical properties of the soil, on the crop phenology and physiology and on the climate. In intensive cropping, yearly rates of fertilisation often exceed 100 kg N ha^{-1} and can reach 400 kg N ha^{-1}.

Atmospheric N deposition, mainly NO_x (issued by fossil-fuel combustion) and NH_x (resulting from NH_3 volatilisation) present much lower rates. In European temperate forest ecosystems, rates of N inputs through wet and dry atmospheric deposition range from less than 1 kg N ha^{-1} $year^{-1}$ (Northern Norway and Finland) to more than 60 kg N ha^{-1} $year^{-1}$ (the Netherlands and Czech Republic) (MacDonald et al., 2002). However, deposition reaches largely natural or unfertilized regions and can lead to irremediable losses of precious and fragile ecosystems. Furthermore, atmospheric deposition is a chronic process. Initially, it acts as a fertilisation of N-limited ecosystems, which can lead to an increased plant growth and productivity. Over time, however, the inputs may exceed the capacity for N uptake and retention by plants, soils and microbes. (Aber et al., 1989). A decrease of the C:N ratio is then observed in the soil. This state is called N saturation (Skeffigton & Wilson, 1988). The ability of temperate forest ecosystems to absorb deposited N depends on their internal N status, ranging from N-limited to N-saturated (Aber et al., 1989, Gundersen et al. 1998). It is further a function of how much N can be used for plant production, lost by denitrification or tolerably lost in form of NO_3^- leaching. In a mass balance, these N consumption processes determine the tolerable N imputs to an ecosystem. This limit is thus called critical load by mass balance (Downing et al., 1993). The main negative effects proposed for such a saturation are forest decline due to nutritional imbalance and physiological modifications but also acidification of soils, streams as well as lakes together with nitrate leaching and, possibly, a loss of biodiversity, while favoring the development of nitrogen-demanding species to the detriment of other species (Aerts & Berendse, 1988). Results of experiments simulating atmospheric deposition however show that impacts of enhanced N on forest ecosystems were strongly attenuated or delayed, especially because of the high capacity of soil organic matter to retain deposited N (Aber et al., 1998, Nadelhoffer et al., 1999, Boxman et al., 1998, Schleppi et al., 1999a).

2.2.1 RETENTION OF DEPOSITED N IN THE SOIL

N deposition reaches the natural terrestrial ecosystems in the form of inorganic deposition (mainly NO_3^- and NH_4^+) on the vegetation cover or the soil. During and after rainfall, N compounds intercepted by vegetation can reach the soil by throughfall and stemflow (Draaijers, 1993).

Mechanisms leading to the retention of deposited N in soil, particularly in soil organic matter, still remain partially unexplained. A fast incorporation (ranging from hours to days) has

been observed by many researchers (Bernston et Aber, 1999 ; Zogg et al., 2000 ; Perakis et al. 2001). Several authors described microbial immobilisation as a primary process in the incorporation of inorganic N into soil organic matter (Davidson et al., 1992 ; Stark & Hart, 1997 ; Zogg et al., 2000). Abiotic incorporation is another often proposed mechanism (Johnson et al., 2000; Dail et al., 2001 ; Davidson et al., 2003;. Fitzhugh et al., 2003). Finally, Aber et al. (1998) hypothesised mycorrhizal assimilation and exudation to be the dominant process involved in immobilisation of added nitrogen.

Once deposited N is retained in soil organic matter, the next issue is to know for which duration. The results obtained in the European Nitrex project showed that five years after the beginning of simulated N deposition, more than 50% were still present in the soil (Schleppi et al., 1999b; Nadelhoffer et al. 1999). Two years after ^{15}N application in an unpolluted old-growth temperate forest, Perakis & Hedin (2001) recovered nearly 75% of the added ^{15}N in the soil and they suggested that this retention was linked with the recycling of ^{15}N through the plant-soil-microbe cycle. Some thirty years after the simulation of atmospheric N deposition through a ^{15}N labelling experiment (alpine grassland in Austria), Gerzabek et al. (2004), recovered half of the deposited N remaining in the soil, almost exclusively in the upper organic layer. They also suggested that this long-term retention was due to biological recycling.

REFERENCES

Aber J.D., Nadelhoffer K.J., Steudler P. & Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience,* **39**, 378-386.

Aber J., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S., Currie W., Rustad L. & Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems - hypotheses revisited. *Bioscience*, **48**, 921-934.

Aerts R. & Berendse F. 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. *Vegetatio*, **76**, 63-69.

Berntson G.M. & Aber J.D. 1999. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry*, **32**, 151-156.

Boxman A.W., Blanck K., Brandrud T. E., Emmett B. A., Gundersen P., Hogervorst R. F., Kjønaas O. J., Persson H. & Timmermann V. 1998. Vegetation and soil biota response to experimentally-changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. *Forest Ecology and Management*, **101,** 65-79.

Bremner J.M. 1965. Organic forms of soil nitrogen. In : *Methods of soil analysis, Part 2. Chemical and microbiological properties* (ed. C.A. Black), pp. 1238-1255. Agronomy 9. American Society of Agronomy, Madison, USA.

Dail D. B., Davidson E.A. & Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry,* **54**, 131-146.

Davidson E.A., Hart S.C. & Firestone M.K. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology*, **73**, 1148-1156.

Davidson E.A, Chorover J. & Dail D.B. 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. *Global Change Biology*, **9**, 228-236.

Diekow J., Mielniczuk J., Knicker H., Bayer C., Dick D.P. & Kögel-Knabner I. 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil and Tillage Research*, **81**, 87-95.

Downing R.J., Hettelingh J.-P. & de Smet P.A.M. 1993. *Calculation and mapping of critical loads in Europe: status report 1993*. UN-ECE & RIVM Bilthoven, NL.

Draaijers G.P.J. 1993. *The variability of atmospheric deposition to forest. The effects of canopy structure and forest edges*. PhD thesis. University of Utrecht, NL.

Fitzhugh R.D., Lovett G.M. & Venterea R.T. 2003. Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biology*, **9**, 1591-1601.

Galloway J. N., Aber J. D., Erisman J. W., Seitzinger S. P., Howath R. W., Cowling E. B. & Cosby B. J. 2003. The nitrogen cascade. *Bioscience*, **53**, 341-353.

Gerzabek M.H., Haberhauer G., Stemmer M., Klepsch S. & Haunold E. 2004. Long-term behaviour of ^{15}N in an alpine grassland ecosystem. *Biogeochemistry,* **70**, 59-69.

Gundersen P., Emmett B.A., Kjønaas O.J., Koopmans C.J. & Tietema A. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management*, **101**, 37-55.

Johnson D.W., Cheng W. & Burke I.C. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of. America Journal*, **64**, 1503-1514.

Kelley K.R. & Stevenson F.J. 1996. Organic forms of N in soil. In : *Humic substances in terrestrial ecosystems* (Ed. A. Piccolo), pp. 407-427. Elsevier Science, Amsterdam., NL.

Knicker H. 2004. Stabilization of N-compounds in soil and organic matter rich sediments - What is the difference? *Marine Chemistry*, **92**,167-195.

Knicker H. & Hatcher P.G. 1997. Survival of protein in an organic-rich sediment: Possible protection by encapsulation in organic matter. *Naturwissenschaft*, **84**, 231-234.

Knicker H. & Kögel-Knabner I. 1998. Soil organic nitrogen formation examined by means of NMR spectroscopy. In: *Fate of N-containing macromolecules in the biosphere and geosphere* (eds B.A. Stankiewicz et al.), pp. 339-356. American Chemical Society Symposium Series 707. Oxford University Press, Washington, USA.

Leinweber P. & Schulten H.-R. 2000. Nonhydrolyzable forms of soil organic nitrogen: extractability and composition. *Journal of Plant Nutrition and Soil Science*, **163**, 433-439.

MacDonald J.A., Dise N.B., Matzner E., Armbruser M., Gundersen P. & Forsius M. 2002. Nitrogen imput together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Global Change Biology*, **8**, 1028-1033.

Mathers N.J., Mao X.A., Xu Z.H., Saffigna P.G., Berners-Price S.J. & Perera M.C.S. 2000. Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies. *Australian Journal of Soil Research*, **38**, 769-787.

Nadelhoffer K.J., Emmet B.A., Gundersen P., Kjønaas O.J., Koopmans C.J., Schleppi P., Tietema A. & Wright R.F. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**, 145-147.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern chile. *Ecology*, **82**, 2245-2260.

Schleppi P., Muller N., Edwards P.J. & Bucher J.B. 1999a. Three years of increased nitrogen deposition do not affect the vegetation of a montane forest ecosystem. *Phyton*, **39**, 199-204.

Schleppi P., Bucher-Wallin I., Siegwolf R., Saurer M., Muller N. & Bucher J.B. 1999b. Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N. *Water Air Soil Pollution*, **116**, 129-134.

Schulten H.-R. 1994. A chemical structure for humic acid. Pyrolysis-gaschromatography/mass spectrometry and pyrolysis-soft ionization mass spectrometry evidence. In: *Humic substances in the global environment and implications on human health* (eds N. Senesi & T.M. Milano), pp 43-56. Elsevier Science, Amsterdam, NL.

Schulten H.-R., Sorge-Lewin C. & Schnitzer M. 1997. Structure of «unknown» soil nitrogen investigated by analytical pyrolysis. *Biology and Fertility of Soils*, **24**, 249-254.

Schulten H.-R. & Schnitzer M. 1998. The chemistry of soil organic nitrogen : a review. *Biology and Fertility of Soils*, **26**, 1-15.

Skeffington R.A. & Wilson E.J. 1988. Excess nitrogen deposition : issues for consideration. *Environmental Pollution*, **54**, 159-184.

Stark J.M. & Hart S.C. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature*, **385**, 61-64

Stevenson F.J. 1982. Organic forms of soil nitrogen. In: *Nitrogen in agricultural soils* (ed. F.J. Stevenson), pp. 67-122. Agronomy 22. American Society of Agronomy, Madison, USA.

Stevenson F.J. & Cole M.A (eds). 1992. *Cycles of soil - carbon, nitrogen, phosphorus, sulfur, micronutrients*. John Wiley & Sons, New York, USA.

Sowden F.J., Chen Y. & Schnitzer M. 1977. The nitrogen distribution in soils formed under widely differing climatic conditions. *Geochimica et Cosmochimica Acta*, **41**, 1524-1526.

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. & Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737-750.

Zang X., Nguyen R.T., Harvey H.R., Knicker H. & Hatcher P.G. 2001. Preservation of proteinaceous material during the degradation of the green alga *Botryococcus braunii*: A solid-state 2D ^{15}N ^{13}C NMR spectroscopy study. *Geochimica et Cosmochimica Acta*, **65**, 3299-3305.

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology*, **81**, 1858-1866.

CHAPTER 3

3 RESEARCH SITES

3.1 GRANDVILLARD

In the frame of our research, the field experiment was conducted on the site of Grandvillard, situated in the riparian zone of La Sarine River, in the Prealps of Switzerland (46°32' N/7°04' E), 750 m a.s.l.. Mean annual precipitation is 1200 mm and mean annual air temperature is 7.1°C. We measured bulk atmospheric deposition of inorganic N rising to 16 kg ha^{-1} y^{-1}.

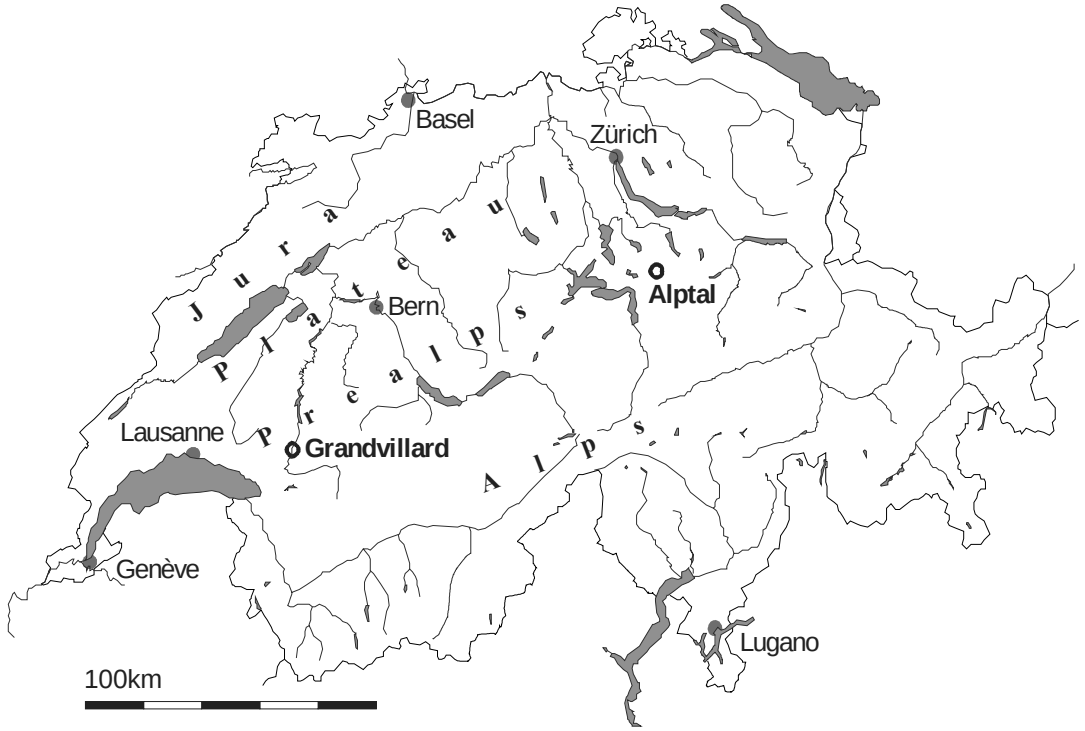

Fig. 3.1 : Location of the two study sites, Grandvillard and Alptal, on a map of Switzerland.

Fig. 3.2 : The riparian zone of La Sarine River in the Prealps of Switzerland.

The stand is a beech-grove (*Fagus sylvatica* L.) mixed with planted spruces (*Picea abies* (L.) Karst*)*. Ground vegetation is dominated by *Mercurialis perennis* L., *Polygonatum verticillatum* (L.) All., *Carex alba* Scop., *Aconitum lycoctonum* L. , *Aposeris foetida* L., *Phyteuma spicatum* L. and *Anemone nemorosa* L.. The soil is a calcareous Fluvisol, according to the FAO classification (Food and Agriculture Organization of the United Nations, 1989) and the water table is very deep, at approximately 4 m. Humus (calcareous mull) is characterized by a thin litter layer and the presence of macro-aggregates linked with a high activity of earthworms and a fast turnover of organic matter (Guenat et al., 1999). Soils have a deep organo-mineral calcareous A layer (0-25 cm) with a loamy texture, above a 20-30 cm mineral calcareous B layer with a sandy loamy texture, a sandy bed of 10 cm and the alluvial grave. Dominant mineralogical clays are illite.

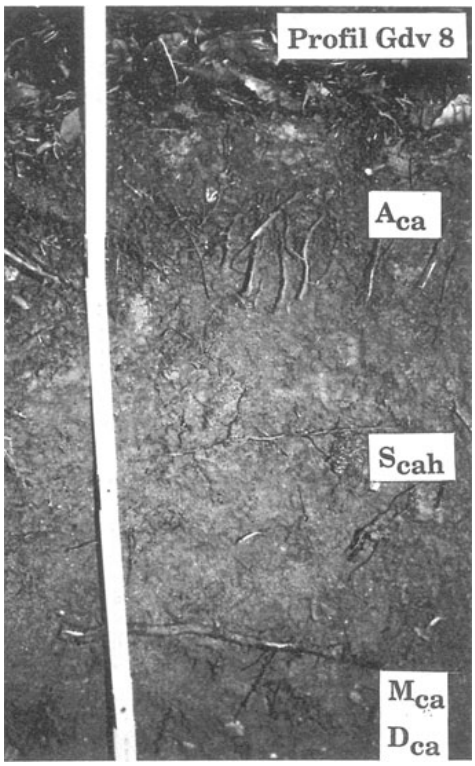

Fig. 3.3 : The calcareous Fluvisol profile of Grandvillard. Aca: calcareous A; Scah: calcareous B; Mca & Dca: calcareous parental material.

As mentioned above and in the framework of the experiment on hydrolysable ^{15}N, we used soil samples issued of a join field experiment using the same methodology on a contrasting site. Here are presented the main characteristics of this site. Further information are found in Providoli (2005).

3.2 ALPTAL

This second site, named Alptal is located in the valley of the same name within the Alps of Central Switzerland. It lies at 1200 m a.s.l. with a mean annual temperature of 6°C and a mean annual precipitation of 2300 mm. Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} y^{-1} (Schleppi et al., 1999).

Fig. 3.4 : The Alptal site within the Alps of Central Switzerland.

Vegetation and soil types form a mosaic pattern closely related to microtopography : in depressions, the water table frequently reaches the surface. Soils are mollic Gleysols with a thin fragmented litter layer (0-4 cm), a loamy clayed A layer (4-15 cm) rich in organic matter lying

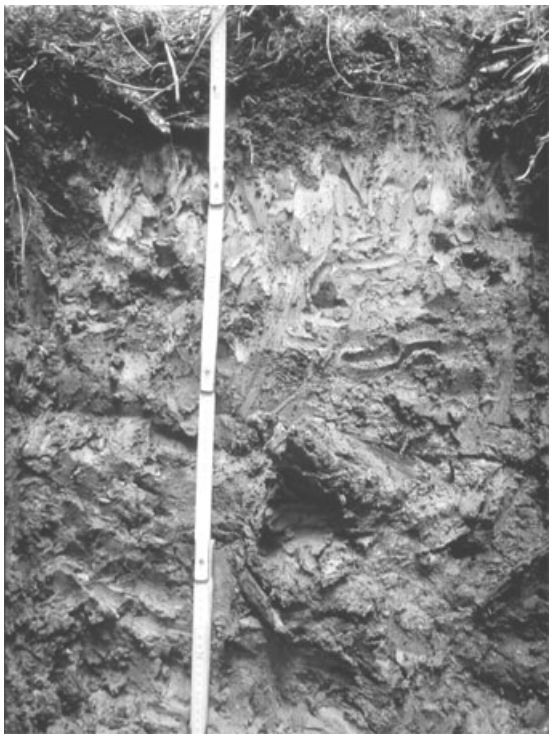

Fig. 3.5 : The Gleysol profile in Alptal.

above a loamy clayed G layer almost permanently reduced. Humus is an anmoor. Depressions are too wet for tree growth and ground vegetation is dominated by *Poa trivialis* L. and *Carex ferruginea* Scop. in open patches and by *Caltha palustris* L. and *Petasites albus* L. in the shade of the trees. On mounts, the water table is at a depth below 40 cm. Soils are umbric Gleysols with raw humus (mor), an A rich in organic matter and a Bg or Br horizon. The dominant plant species are Norway spruces (*Picea abies* L.) and *Vaccinium myrtillus* L. On the whole site ground vegetation is characterized by a moss layer with more than 30 moss species (Muller, 1997; Schleppi et al., 1998).

REFERENCES

Food and Agricultural Organization of the United Nations. 1989. Soil map of the world, revised legend. FAO-UNESCO, Rome, Italy.

Guenat C., Bureau F., Weber G. & Toutain F. 1999. Initial stages of soil formation in a riparian zone: importance of biological agents and lithogenic inheritance in the development of the soil structure. *European Journal of Soil Biology*, **35**, 153-161.

Muller N. 1997. *Short-term response of the ground vegetation in a montane forest ecosystem under increased nitrogen deposition - Influence of light and competition*. Doctoral thesis 12388, Swiss Federal Research Institute of Technology (ETH), Zurich, Switzerland.

Schleppi P., Muller N., Feyen H., Papritz A., Bucher J.B. & Flühler H. 1998. Nitrogen Budget of two small experimental forested catchments at Alptal, Switzerland. *Forest Ecology and Management,* **101**, 177-185.

Schleppi P., Bucher-Wallin I., Siegwolf R., Saurer M., Muller N. & Bucher J.B. 1999. Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N. *Water, Air and Soil Pollution*, **116**, 129-134.

CHAPTER 4

4 DYNAMICS OF ATMOSPHERIC N DEPOSITION IN A TEMPERATE CALCAREOUS FOREST SOIL

Submitted to the Journal of Environmental Quality

Isabelle Morier[1,4], Claire Guenat[1], Rolf Siegwolf[2], Jean-Claude Védy[3], Patrick Schleppi[4]

[1]*Swiss Federal Institute of Technology (EPFL), Laboratory of Soil and Environmental Physics, CH-1015 Lausanne, Switzerland*

[2]*Paul Scherrer Institute (PSI), Laboratory of Atmospheric Chemistry, CH-5232 Villigen-PSI, Switzerland*

[3]*Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland*

[4]*Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), CH-8903 Birmensdorf, Switzerland*

In temperate forest ecosystems, soil acts as a main sink for atmospheric N deposition, however the processes involved still have to be cleared up. A ^{15}N labeling experiment in a Swiss hardwood forest on calcareous Fluvisol was carried out within this framework. Aqueous solutions of either $^{15}NH_4^+$ or $^{15}NO_3^-$ were added in low amounts (0.125 g N/m^2) but with high isotopic concentration (99%) to small plots. Soil samples were taken after periods ranging from 1 hour to 1 year. After one day, OL layer retained approximately 28% of the $^{15}NH_4^+$ tracer and 19% of $^{15}NO_3^-$. An important fraction of deposited N went through the OL layer to reach the Aca layer within the first hours following the tracer application. During the first day, a decrease of extractable ^{15}N in the Aca layer ($^{15}NH_4^+$ tracer: 50 to 5%; $^{15}NO_3^-$ tracer: 60 to 12%) was observed and during that same time, both the amount of microbial ^{15}N remained almost constant and the ^{15}N immobilized in the soil (i.e. ISN = total ^{15}N recovered in the bulk soil less extractable ^{15}N and less microbial ^{15}N; $^{15}NH_4^+$ tracer: 11%; $^{15}NO_3^-$ tracer: 14%). Such results can therefore be understood as a net loss of ^{15}N from the Aca layer. Since $^{15}NO_3^-$ appeared within hours from $^{15}NH_4^+$ labeling, nitrification and NO_3^- leaching probably explain such N loss. We presume that the N immobilization occurs as an incorporation of deposited N into the soil organic matter and, to a lesser extent, as a fixation on clay minerals. One year after the ^{15}N tracer addition, recovery rates were similar and approximately three fourth of the deposited N was recovered in the soil. The present research concludes on the one hand that processes relevant for the fate of atmospherically deposited N do take place rapidly and, on the other, that N recycling within the microbes-plants-SOM system prevents further losses on the long term.

4.1 INTRODUCTION

Global nitrogen cycle is naturally equilibrated by fixation processes (biological N fixation, lightning) leading from non-reactive nitrogen (N_2) to biologically reactive forms and denitrification processes reducing nitrate (NO_3^-) in N_2 (Söderlund & Svensson, 1979; Ayers et al., 1994; Galloway et al., 1995).

During the past decades, the human activities significantly altered this equilibrium. At present, the amount of N converted from non-reactive atmospheric N_2 to biologically reactive forms by human activities exceeds the one converted by natural processes (Galloway et al., 1995). Moreover, anthropogenic N fixation is expected to increase even more in the coming decades (Vitousek et al., 1997; Galloway & Cowling, 2002). The main sources responsible for the increase of reactive N emission are the use of artificial fertilizers (NH_3) and combustion processes (NO_x). Reactive N is then transformed, transported by the atmosphere, the hydrosphere and finally deposited on both the terrestrial and the aquatic ecosystems. As a consequence, reactive N compounds accumulate in the ecosystems with potentially strong impacts such as eutrophication, soil acidification or loss in biodiversity within plant communities and, consequently a reduction in the diversity of fauna and microorganisms which depends on the diversity of vegetation composition (Fangmeier et al., 1994; Vitousek et al., 1997; Socolow, 1999; Dise et al., 2001; Sheeder et al., 2002; Aber et al., 2003; Galloway et al., 2003).

Anthropogenic fixed N mainly reaches the natural or other unfertilized terrestrial ecosystems in the form of inorganic deposition on the vegetation cover or on the soil. During and after rainfall, the N compounds intercepted by the vegetation can reach the soil by throughfall and stemflow (Draaijers, 1993). Furthermore, previous studies have shown that the soil is not only a cross point for N atmospheric deposition but also that it acts as a main sink for the ecosystems (Nadelhoffer et al., 1999b). Deposited N reaching the soil is temporally dissolved in soil solution or adsorbed on exchange complexes before it is absorbed by the roots or microorganisms and then recycled through soil organic matter. Another possible pathway is the abiotic fixation by organic matter, or by clays in case of NH_4^+. Previous studies demonstrated that soil organic matter accounts for most of the N retained in case of elevated N deposition (Aber et al., 1998; Nadelhoffer et al., 1999a). Incorporation within organic matter is direct (abiotic fixation) or indirect (i.e. recycling of organic compounds following root absorption or microbial assimilation). Such forms would be significant for the long-term immobilization of N in soil and as a consequence on the long-term impact of enhanced N input on the ecosystem. The relative importance of the incorporation processes can be connected with the physico-chemical properties of the soil. As most studies showing the importance of the soil in atmospheric N deposition fate, were conducted in acidic or hydromorphous soils (Hart et al., 1993; Gundersen et al., 1997; Gebauer et al., 2000; Lamontagne et al., 2000, Providoli et al., 2006), the present research focuses on a well drained calcareous soil which is a widely distributed type of soil however poorly studied.

We applied a ^{15}N pulse-chase in the field to i) study the pertinence of the processes responsible for N retention in soil and the influence of its physico-chemical properties and ii) characterize N immobilization in terms of both duration and quantity.

$^{15}NH_4^+$ and $^{15}NO_3^-$ were applied separately to take the main forms of N deposition into account and to distinguish between the fate of ammonium and nitrate deposition in soil.

Two time-scales were foreseen: short-term movements of N tracer which take place over a period from hours to days emphasizing the biological and chemical processes involved; long-

term movements which take place over months up to a year indicative of the N stabilization in soils.

4.2 MATERIALS AND METHODS

4.2.1 STUDY AREA

Our study was conducted in a hardwood alluvial forest in the Prealps of Switzerland (46°32' N/7°04' E), 750 m a.s.l. with a mean annual precipitation of 1200 mm and mean annual air temperature is 7.1°C. We measured bulk atmospheric deposition of inorganic N rising to 16 kg ha^{-1} y^{-1}. The stand is a beech-grove (*Fagus sylvatica* L.) mixed with planted spruces (*Picea abies* (L.) Karst). Ground vegetation is dominated by *Mercurialis perennis* L., *Polygonatum verticillatum* (L.) All., *Carex alba* Scop., *Aconitum lycoctonum* L. , *Aposeris foetida* L., *Phyteuma spicatum* L. and *Anemone nemorosa* L.. The soil is a calcareous Fluvisol, according to the FAO classification (Food and Agriculture Organization of the United Nations, 1989) and corresponding to a *Fluviosol carbonaté*, according to the Référentiel pédologique (A.F.E.S., 1998). The water table is very deep, at approximately 4 m. Humus (calcareous mull) is characterized by a thin OL layer and the presence of macro-aggregates linked with a high activity of earthworms and a fast turnover of organic matter (Guenat et al., 1999). Soils have a deep organo-mineral calcareous Aca layer (0-25cm) with a loamy texture, above a 20-30cm mineral calcareous Sca layer with a sandy loamy texture, a sandy bed of 10 cm and the alluvial grave *(table 4.1, p. 21)*. Dominant mineralogical clays are illite. More complete descriptions of the site and particularly of the soil are provided in Bureau (1995).

Table 4.1 : Soil parameters in ^{15}N labeled plots of our study area in the Swiss Prealps.

	Aca	Sca	Mca	Dca
Depth (cm)	0-25	25-50	50-60	>60
pH_{water}	7.5	7.8	8.0	-
C_{org} (%)	3.1	1.2	0.6	-
C/N	11.6	9	9.3	-
$CaCO_3$ (%)	32.3	39.2	44.6	-
Texture	loam	sandy loam	sand	gravel

4.2.2 ^{15}N LABELING AND FIELD SAMPLING

Nine 2.5 X 2.5 m plots were established in a 0.1 ha area of forest, as three replicates of each treatment (control, NH_4^+ and NO_3^-). In August 2001 we applied ^{15}N tracer with a backpack sprayer to simulate wet atmospheric deposition. The treatments consisted in a single addition of either $^{15}NH_4Cl$ or $K^{15}NO_3$ at a high isotopic concentration (99%) at a rate of 1.25 kg N/ha dissolved in 2.2 l m^{-2} of deionized water. Following the ^{15}N addition, we applied the same quantity of deionized water (equivalent to a rainfall) to rinse the vegetation. Control plots were only treated with deionized water.

Soil was sampled four times during the first day (t=1, 3, 8, and 24 h) and five more times over the year (t=7, 36, 99, 280 and 372 d). We used PVC cartridges with a corer of 5 cm diameter. In each plot, three soil samples were excavated (0-50 cm soil depth). PVC cartridges were placed on ice and transported to the laboratory, allowing us to begin processing samples within one hour from field collection. Because ^{15}N natural abundance is very stable over the year, we

sampled control plots only twice (t=85 and 379 d).

The herbaceous vegetation was collected once during the year of tracer application (t=57 d, i.e. Oct. 01) and once the following year (t=294 d, i.e. Apr. 02). On each plot, ground vegetation was clipped at two squares (15 cm x 15 cm) and pooled into a composite sample. Vegetation samples were kept refrigerated and processed in the laboratory within a few days.

4.2.3 LABORATORY ANALYSES

We sampled and analyzed the soil OL layer (a few millimeter depth) and the organo-mineral Aca layer (0-25 cm depth); we made some analyzes within deeper mineral S_{ca} layer (over the first day, after 280 and 372 days). For samples picked up after 1 hour and 1 year, we divided the Aca layer in two parts according to their organic matter content: Aca_{11} sublayer (0-5cm depth) and Aca_{12} sublayer (5-25cm depth).

Following layer separation, the three samples of each plot were composited.

Litter samples were sorted out by hand to remove branches and fruits, dried at 65°C and ground with a Retsch Ultra Centrifugal Mill ZM1 (0.5mm) (Retsch, Haan, Germany).

We sorted Aca and Sca soil samples to remove roots and gravels. A fraction of fresh soil was immediately used for further laboratory analyses (see below), while another fraction was dried at 105° for 2 hours then at 65°. They were passed through a 2-mm-mesh sieve. Samples were then ground with a Retsch Mortar Grinder RM 100 (Retsch). Sorted fine roots (diameter <4 mm) were rinsed free of soil and organic matter with deionized water, dried at 65°C and ground with a Retch centrifuge grinder (0.5 mm).

Vegetation samples were sorted, dried at 65°C and ground with the same centrifuge grinder.

Extractable N was determined by immediate extraction of 50 g soil (Aca and Sca layers) with 160 ml of a 0.5M solution of K_2SO_4. Extracts were filtered and frozen. One half of extract was then lyophilised to measure the total extractable N; With the second half of the extract and for the first samples series (t=1, 3, 8 and 24 h) of Aca layer, a 7-d PTFE (Teflon) tape diffusion (Stark & Hart, 1997) was performed to determine the ^{15}N in NH_4^+, NO_3^- and dissolved organic N (DON): about 80 ml of the extract were placed in a hermetically closed PE bottle, which were then gently shaken for 1 week with 1.5 mg l^{-1} MgO and a glass microfibre filter acidified with 30 µl 1M H_2SO_4 and enclosed in a Teflon band. NH_4^+ was thereby oxidized in NH_3, diffused through the Teflon band and captured on the filter. The extract was further processed with another enclosed glass microfibre filter and 0.5 g Devarda's alloy. NO_3^- was thereby reduced to NH_4^+ and the same procedure was then repeated as with NH_4^+.

$CHCl_3$-labile microbial nitrogen was determined by K_2SO_4 extraction (25 g soil with 80 ml of a 0.5M solution of K_2SO_4) following a 24 h $CHCl_3$ fumigation (Brookes et al., 1985).

Plant and soil N concentrations and $^{15}N/^{14}N$ isotopic ratio analysis were conducted at the Paul Scherrer Institute, Laboratory of Atmospheric Chemistry. Samples were combusted in an Elemental Analyser (EA 1108, Finnigan, Bremen, Germany). The evolving N_2 was led in the Heliumstream to the isotope ratio mass spectrometer (DeltaS, Finnigan, Bremen, Germany) where the $^{15}N/^{14}N$ ratio of the sample was determined relative to the international reference (N_2 in air).

4.2.4 CALCULATIONS AND STATISTICAL ANALYSIS

We chose to follow seven pools of N in the Aca organo-mineral soil layer: Soil total N (1), fine-roots N (2), extractable NH_4^+ (3), extractable NO_3^- (4), dissolved organic N (DON) (5), microbial immobilized N (6), immobilized soil N (ISN, according to Providoli et al. (2006)) (7); more details are given in *table 4.2 (p. 23)*. On each sample date, the recovered ^{15}N tracer in the seven pools was calculated by multiplying the N quantity of the pool (i.e. multiplying the N concentration of the pool by the mass of the component) by the atom % excess ^{15}N (i.e. measured percentage of ^{15}N minus natural abundance of ^{15}N). The recovered ^{15}N is expressed as a rate of the total added label (for more details, see Providoli et al., 2005).

Table 4.2 : Summary of Aca layer soil N pools and the corresponding analyses and calculations.

Pools		Analyses	Calculation
Bulk soil N			
Roots < 4mm in ØN			
Extractable N		K_2SO_4 extraction / lyophilisation	
	Extractable NH_4^+	K_2SO_4 extraction / PTFE (Teflon) tape diffusion	
	Extractable NO_3^-	K_2SO_4 extraction / PTFE (Teflon) tape diffusion	
	Dissolved Organic N (DON)		Extractable N minus extractable NH_4^+ and NO_3^-
Soil microbial N		$CHCl_3$ fumigation / K_2SO_4 extraction / lyophilisation	Extractable N after $CHCl_3$ Fumigation minus extractable N
Immobilized Soil N (ISN)			Bulk soil N minus extractable N after $CHCl_3$ fumigation

Results are in mg/g dry soil except for fine roots (mg/g dry fine roots)

We performed two Repeated Measures Analysis of Variance (RMANOVA) (S-PLUS 6.1, Mathsoft, Cambridge, MA, USA); the first one on short-term results (t=1-24 h) and the second one on one-year-term results (t=1-372 d). Statistical tests were applied on OL and Aca layers results. N concentration and ^{15}N recovery rates were tested for effects of treatment, time, and treatment versus time interactions. Profile contrasts were also calculated to test changes between successive samplings. Log transformation of the dependent variables was used to improve normality in the data when necessary. All results were considered significant at the $P<0.05$ level.

N concentration of some soil pools may show significant variations. In such a case, it is difficult to tell if the observed variations are real or merely due to irregularities in the sampling and processing of the horizons according to their depths. In those cases, we thus calculated ^{15}N recovery rates either with the N concentrations as measured for each sampling time or with an average N concentration. Statistical analyses were performed on values obtained by each of those methods.

4.3 RESULTS

4.3.1 SOIL NITROGEN POOLS

Mean concentrations and quantities of N in the OL and Aca layers over the first day are given in *table 4.3 (p. 24)* . We also present such N results in the Aca soil pools. No significant

difference between the two tracers was shown in the results *(table 4.4, p. 24)* .

Litter layer accounted for less than 3% of the soil N quantity whereas it presented high N concentration. The Aca layer covered the remaining 97%.Within Aca layer, the most important N pool was ISN (94%), followed by microbial biomass (3%) and fine roots (2,5%). Extractable N accounted approximately for 0.6% of the soil N.

In several soil pools, the N concentration did not significantly vary during the first day after labeling *(table 4.4, p. 24)*. However, some significant variations were observed in the root pools (between 3h - 8h after labeling), the microbial pool (time, the first 1h – 3h and 8h – 24h after labeling) and the DON pool (time, the first 1h - 3h after labeling; interaction time versus treatment, the first 1h – 3h after labeling).

Table 4.3 : N concentrations in OL and Aca layers like in Aca pools over the first day (t=1-24h). We also indicate the relative contribution of each compartment.

		NH_4^+ labeling			**NO_3^- labeling**		
Layers	**N pools**	N (mg/g DM)	Soil contribution (%)	Aca contribution (%)	N (mg/g DM)	Soil contribution (%)	Aca contribution (%)
OL		11.425	2.38		11.267	2.30	
Aca	NO_3^-	0.002	97.62	0.06	0.002	97.70	0.06
	NH_4^+	0.003		0.10	0.003		0.11
	DON	0.008		0.31	0.010		0.38
	Microbial N	0.062		2.24	0.060		2.20
	Fine roots N	10.981		1.50	10.967		1.66
	ISN	2.722		95.79	2.675		95.62
	Total	**13.776**	**100**	**100**	**(Aca) 13.717**	**100**	**100**

Table 4.4 : P values of RMANOVA for N% over the first day (t=1-24h).

	OL layer N	**Aca layer N**	**Aca N pool**					
N%			Fine roots N	Microbial N	NH4+	NO3-	DON#	ISN
Treatment	0.89	0.67	0.98	0.29	0.57	0.16	0.16	0.82
Replicate	0.61	0.41	0.81	0.47	0.82	0.07	0.28	0.32
Time	0.83	0.75	0.1	**0.01**	0.65	0.19	**0.01**	0.6
1h-3h	0.66	0.41	0.51	**0.01**	0.3	0.06	**0.00**	0.25
3h-8h	0.69	0.87	**0.02**	0.63	0.54	0.63	0.88	0.93
8h-24h	0.48	0.5	0.47	**0.04**	0.77	0.36	0.41	0.52
Trt*time	0.39	0.98	0.26	0.48	0.33	0.14	0.11	0.98
Trt*time 1h-3h	0.39	0.96	0.1	0.089	0.08	0.32	**0.02**	0.75
Trt*time 3h-8h	0.88	0.76	0.62	0.52	0.7	0.11	0.54	0.85
Trt*time 8h-24h	0.14	0.83	0.31	0.16	0.9	0.12	0.52	0.90

Notes: bold values P<0.05; #log transformation

Mean nitrogen concentrations over the year are given in *table 4.5 (p. 25)*. They showed no differences between the two labels, within OL and Aca layers. In contrast to the first day, almost all the N pools varied over the year *(table 4.6, p. 25)*.

Table 4.5 : N concentrations in OL and Aca layers like in Aca pools over the year (t=1-372d). We also indicate the relative contribution of each compartment.

		NH_4^+ labelling			NO_3^- labelling		
Layers	**N pools**	N (mg/g DM)	Soil contribution (%)	Aca contribution (%)	N (mg/g DM)	Soil contribution (%)	Aca contribution (%)
OL		10.87	3.28		10.750	3.29	
Aca	Extractable N	0.002	96.72	0.69	0.002	96.71	0.73
	Microbial N	0.06		3.33	0.084		3.28
	Fine roots N	10.65		3.29	10.967		3.29
	ISN	2.63		92.68	2.675		92.70
	Total	**13.33**	**100**	**100**	**(Aca) 13.728**	**100**	**100**

Table 4.6 : P values of RMANOVA for N% over the year (t=1-372d).

	OL layer N	**Aca layer N**	**Aca N pool**			
N%			Fine roots N#	Microbial N	Extractable N#	ISN
Treatment	0.90	0.25	0.32	0.34	0.27	0.33
Replicate	0.53	**0.02**	0.14	0.37	0.11	**0.02**
Time	**0.02**	0.05	**0.00**	0.14	0.20	**0.05**
1d-7d	0.14	0.10	0.07	**0.04**	0.64	0.09
7d-36d	**0.01**	0.86	0.60	0.20	**0.04**	0.96
36d-99d	0.06	**0.01**	**0.02**	0.77	0.60	**0.01**
99d-280d	0.90	0.25	**0.01**	0.34	0.46	0.23
280-372d	0.06	0.92	**0.01**	0.24	0.14	0.90
Trt*time	0.24	0.42	0.76	0.15	0.32	0.42
Trt*time 1d-7d	0.14	0.80	0.99	0.23	0.21	0.84
Trt*time 7d-36d	0.40	0.79	0.81	0.45	0.84	0.78
Trt*time 36d-99d	0.06	0.05	0.60	0.06	0.11	0.05
Trt*time 99d-280d	0.98	0.64	0.51	0.13	0.67	0.65
Trt*time 280h-372h	0.64	0.46	0.20	0.42	0.22	0.41

Notes: bold values P<0.05; #log transformation

Mean nitrogen concentrations for the two Aca sublayers are given in *table 4.7 (p. 26)*. Aca_{11} sublayer showed a higher N concentration than Aca_{12}. Values were similar between labeling.

Results of N measurements within Sca mineral layer showed a mean value of 0.8 mg/g dry matter over the year and a mean N quantity of 72 g/m^2.

Table 4.7 : N concentrations in pools of Aca_{11} and Aca_{12} sublayers : t=1d (a) and t=372d (b).

a)	N concentrations (mg/g DM)			
	NH_4^+ labeling		NO_3^- labeling	
	Aca_{11}	Aca_{12}	Aca_{11}	Aca_{12}
Extractable N	0.02±0.002	0.01±0.001	0.05±0.02	0.01±0.0004
Microbial N	0.11±0.01	0.06±0.003	0.1±0.04	0.07±0.001
ISN	3.81±0.10	2.53±0.01	3.57±0.05	2.68±0.09
Total	**3.93±0.07**	**2.60**	**3.7±0.06**	**2.77±0.09**
b)	NH_4^+ labeling		NO_3^- labeling	
	Aca_{11}	Aca_{12}	Aca_{11}	Aca_{12}
Extractable N	0.03±0.002	0.01±0.001	0.03±0.004	0.01±0.0002
Microbial N	0.13±0.01	0.05±0.002	0.10±0.02	0.07±0.004
ISN	4.85±0.20	2.34±0.1	4.39±0.31	2.29±0.12
Total	**5.00±0.36**	**2.40±0.1**	**4.52±0.32**	**2.37±0.12**

4.3.2 ^{15}N RECOVERY IN SOIL DURING THE FIRST DAY

Dynamics of the mean ^{15}N recovery rates during the first day are presented in *fig. 4.1 (p. 26)*. Those rates of approximately 1.5 were measured one hour after labeling, for both $^{15}NO_3^-$ and $^{15}NH_4^+$ tracers.

OL layer retained approximately half of the recovered $^{15}NH_4^+$ and one fifth of $^{15}NO_3^-$ during the first hour. In the same layer and over the first day, recovery rate dynamics significantly differed between the two tracers *(table 4.8, p. 27)*. We observed a decrease for $^{15}NH_4^+$ tracer (from 51% to 28%) whereas rates were smaller but more constant for $^{15}NO_3^-$ (approx. 19%) OL layer presented a low variability of the recovery rates between replicates..

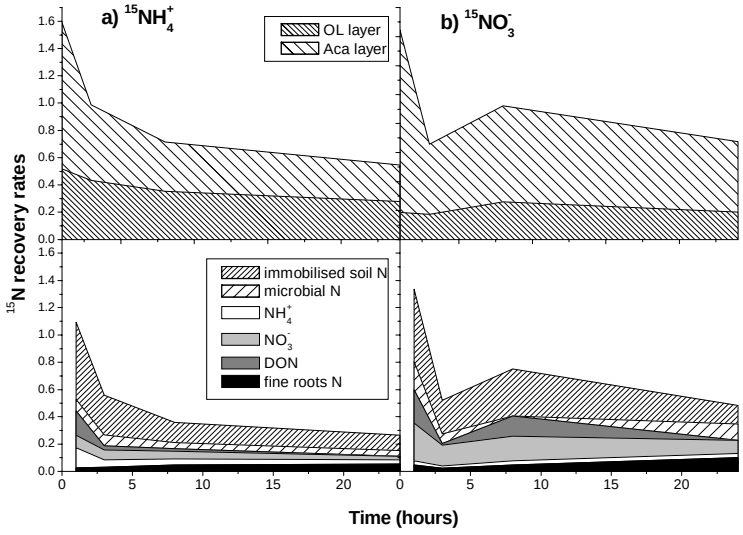

Fig. 4.1 : ^{15}N recovery rates of OL and Aca layers as well as Aca pools during the first day. Each point is the mean of three replicates.

Aca layer was a larger sink than OL layer after the first hour ($^{15}NH_4^+$ tracer: 108%; $^{15}NO_3^-$ tracer: 123%). After the first hour, 50% ($^{15}NH_4^+$ tracer) to 60% ($^{15}NO_3^-$ tracer) were still in an extractable form (NH_4^+, NO_3^- and DON) whereas 6% ($^{15}NH_4^+$ tracer) to 13% ($^{15}NO_3^-$ tracer) were immobilized by microorganisms. Fine roots already took up some ^{15}N during the first hour ($^{15}NH_4^+$ tracer: 3%; $^{15}NO_3^-$ tracer: 5%). ISN corresponded to more than half of the recovery rates ($^{15}NH_4^+$ tracer: 57%; $^{15}NO_3^-$ tracer: 53%). Within Aca layer and during the first day dynamics were quite similar for the two tracers *(table 4.8, p. 27)*. We observed a statistically significant decrease of the total extractable ^{15}N ($^{15}NH_4^+$ tracer : 50 to 5%; $^{15}NO_3^-$ tracer : 60 to 12%). Following its application, the $^{15}NH_4^+$ tracer quickly decreased (from 15% after 1 hour to 5% after 3 hours. Extractable $^{15}NO_3^-$ was also already measurable after 1 hour (9%), following $^{15}NH_4^+$ application and it decreased over the day (down to 3%). DO^{15}N showed the highest recovery rate after 1 hour (18%) but decreased down to 0 during the first day. Following $^{15}NO_3^-$ tracer application, $^{15}NO_3^-$ recovery rates were roughly 3 times higher (28% after 1 hour and 10% after 1 day), DO^{15}N slightly higher (25% after 1 hour, 0 after 1 day) and extractable $^{15}NH_4^+$ was present only in very small amounts (2-3%). Differences between recovery rates within DON pool disappeared when a mean N concentration was used to calculate them (Table 4). On the opposite, recovery in the fine roots increased slightly during the first day ($^{15}NH_4^+$ tracer: 3 to 6%; $^{15}NO_3^-$ tracer: 5 to 15%). Microbial biomass was a stable sink for ^{15}N tracer. For this pool, recovery rates which were calculated with the same mean concentration of N for each sample, showed the same tendency. ISN pool diminished during the first day ($^{15}NH_4^+$ tracer: 57 to 11%; $^{15}NO_3^-$ tracer: 53 to 14%), particularly between 8 and 24 hours.

Table 4.8 : P values of RMANOVA for ^{15}N recovery rates over the first day (t=1-24h).

	OL layer N	**Aca layer N**	**Aca N pool**					
^{15}N recovery rates			Fine roots N	Microbial N	NH_4^+	NO_3^-	DON	ISN
Treatment	**0.05**	0.29	0.72(0.68)	0.64(0.89)	0.10	0.14	0.28(0.44)	0.22
Replicate	0.57	0.82	0.86(0.86)	0.60(0.91)	0.46	0.94	0.20(0.70)	0.55
Time	**0.01**	0.05	**0.03(0.02)**	0.55(0.44)	0.14	0.05	**0.01**(0.11)	0.05
1h-3h	0.12	**0.03**	0.50(0.41)	0.88(0.76)	**0.05**	0.24	**0.01**(0.06)	0.08
3h-8h	0.45	0.3	0.30(0.37)	0.29(0.14)	0.35	0.42	0.46(0.43)	0.41
8h-24h	**0.00**	0.1	**0.01(0.00)**	0.35(0.60)	0.32	**0.01**	**0.02**(0.15)	**0.03**
Trt*time	**0.00**	0.87	0.31(0.26)	0.55(0.63)	0.16	0.92	0.45(0.97)	0.77
Trt*time 1h-3h	0.29	0.99	0.34(0.46)	0.77(0.88)	0.36	0.85	0.36(0.72)	0.71
Trt*time 3h-8h	**0.00**	0.51	0.73(0.77)	0.40(0.30)	0.14	0.59	0.38(0.87)	0.34
Trt*time 8h-24h	**0.02**	0.61	0.11(0.07)	0.27(0.47)	0.12	0.72	0.31(0.79)	0.89

Within Aca layer and one hour after labeling, a high variability of ^{15}N recovery rates between replicates was observed. Then it diminished over the first day. Such a high variability is due to recovery rates within ISN pool *(fig. 4.5, p. 30)*. On the contrary, variability between replicates is rather low even 1 hour after tracers application within OL layer.

Over the first day, no ^{15}N within the mineral Sca layer was found.

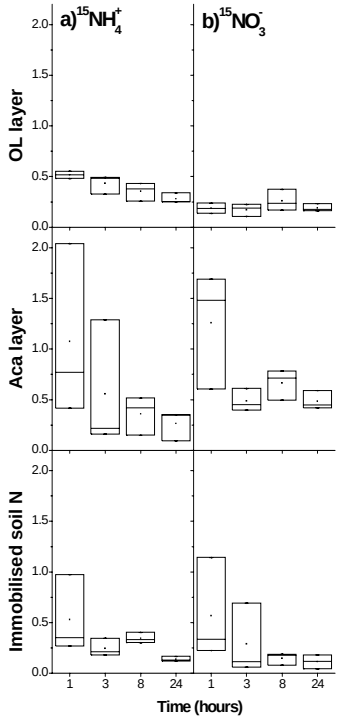

Fig. 4.2 : ^{15}N recovery rates variability during the first day for either (a) $^{15}NH_4^+$ or (b) $^{15}NO_3^-$ tracer. OL layer, Aca layer and ISN (immobilized Soil Nitrogen) within Aca layer are presented.

4.3.3 ^{15}N RECOVERY IN SOIL OVER THE YEAR

Dynamics of the mean ^{15}N recovery rates over the year are presented in *fig. 4.3 (p. 29)*. When compared, the total recovery rates after 1 and 372 days are found to be very similar, especially for $^{15}NO_3^-$ (1 day: 68%, 372 days: 70%). The situation after one day, showed to be the same after one year: no ^{15}N tracer was found within the mineral S_{ca} layer.

^{15}N recovery rates in litter layer significantly differed between the two tracers *(table 4.9, p. 29)*; the higher rates concerned $^{15}NH_4^+$ treatment. Besides, temporal dynamics showed no differences over the year for the two treatments.

^{15}N recovery rates in the Aca layer increased significantly between 1 day and 1 week-durations with regard to the two tracers ($^{15}NH_4^+$ tracer: 49 to 58%; $^{15}NO_3^-$ tracer: 26 to 47%). It then varied only little (no statistical significance). Within Aca layer, ISN was the major sink. We observed a global increase of recovery rates in this pool during the year ($^{15}NH_4^+$ tracer: 11 to 37%; $^{15}NO_3^-$ tracer: 14 to 38%) which was particularly important between 1 day and 1 week as well as between 1 month and 3 months. As far as roots N was concerned, recovery rates also increased ($^{15}NH_4^+$ tracer: 6 to 7%; $^{15}NO_3^-$ tracer: 10 to 17%), particularly between the first day and the first week. Thus they were higher for $^{15}NO_3^-$ tracer. Recovery rates within microbial biomass decreased over the year ($^{15}NH_4^+$ tracer: 4 to 3%; $^{15}NO_3^-$ tracer: 12 to 3%). Finally, extractable N showed to be the minor compartment, characterized with a decrease all over the year and with final recovery rates near to 0.

The recovery rates calculated with the same mean N concentration for each sample indicated that the significant variations in N concentration did not affect the legitimacy of ^{15}N distribution.

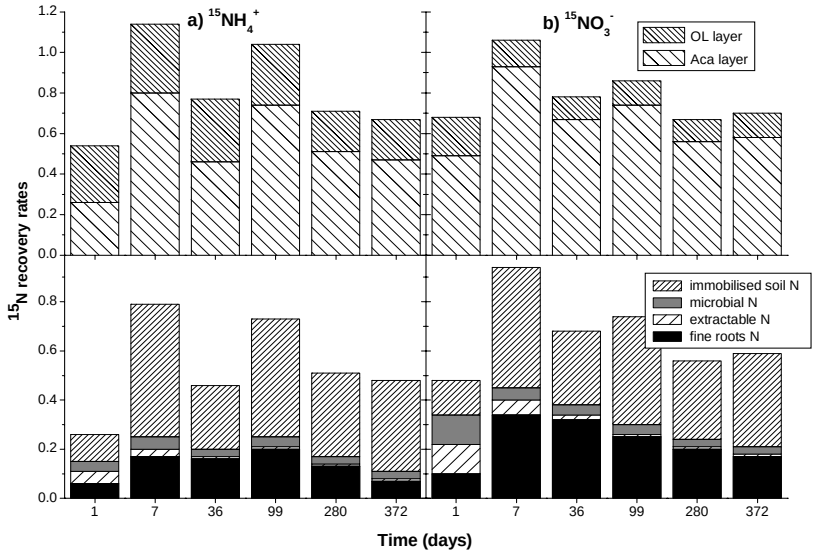

Fig. 4.3 : Recovery of ^{15}N tracers over the year either in OL and Aca layer or in Aca pools : fine roots N, extractable N, microbial N and ISN (immobilized soil N). Each point is the mean of three replicats. Note that y axes scale is different for layers and pools.

Table 4.9 : P values of RMANOVA for ^{15}N recovery rates over the year (t=1-372d). Values between brackets correspond to ^{15}N recovery rates calculated with the same mean N contration for each sample.

	OL layer N	**Aca layer N**	**Aca N pool**			
^{15}N recovery rates			Fine roots N	Microbial N	Extractable N	ISN
Treatment	**0.04** (0.08)	0.08 (0.08)	**0.05** (**0.02**)	0.16 (0.42)	0.31 (0.31)	0.34 (0.32)
Replicate	0.55 (0.57)	0.32 (0.33)	0.54 (0.67)	0.09 (0.20)	0.76 (0.68)	0.10 (0.15)
Time	0.14 (0.20)	0.14 (0.10)	**0.02** (**0.02**)	**0.03** (0.06)	**0.00** (**0.00**)	**0.00** (**0.00**)
1d-7d	0.67 (0.43)	**0.02** (**0.01**)	**0.00** (**0.00**)	0.10 (**0.03**)	**0.00** (0.05)	**0.00** (**0.00**)
7d-36d	0.38 (0.78)	0.65 (0.89)	0.15 (0.13)	0.06 (0.15)	**0.00** (**0.00**)	0.35 (0.14)
36d-99d	0.60 (0.88)	0.14 (0.42)	0.41 (0.68)	0.60 (0.90)	**0.00** (**0.00**)	**0.02** (**0.03**)
99d-280d	**0.02** (**0.02**)	0.84 (0.81)	0.43 (0.68)	0.08 (0.20)	**0.00** (**0.00**)	0.43 (0.56)
280d-372d	0.20 (0.37)	0.89 (0.62)	0.10 (0.16)	**0.04** (0.06)	**0.00** (**0.00**)	0.21 (0.28)
Trt*time	0.51 (0.53)	0.80 (0.52)	0.80 (0.78)	0.17 (0.09)	0.29 (0.24)	0.97 (0.95)
Trt*time 1d-7d	0.22 (0.39)	0.76 (0.91)	0.30 (0.29)	**0.03** (**0.01**)	0.85 (0.65)	0.97 (0.97)
Trt*time 7d-36d	0.40 (0.26)	0.46 (0.73)	0.57 (0.55)	0.29 (0.59)	0.13 (0.13)	0.62 (0.93)
Trt*time 36d-99d	0.66 (0.99)	0.30 (0.43)	0.42 (0.36)	0.31 (0.37)	0.11 (0.06)	0.66 (0.49)
Trt*time 99d-280d	0.28 (0.29)	0.52 (0.14)	0.72 (0.95)	0.60 (0.71)	0.58 (0.53)	0.60 (0.49)
Trt*time 280d-372d	0.42 (0.34)	0.73 (0.28)	0.97 (0.96)	0.44 (0.60)	0.35 (0.53)	0.81 (0.72)

4.3.4 ^{15}N RECOVERY WITHIN ACA SUBLAYERS (ACA_{11} & ACA_{12})

Recovery rates were generally much higher in Aca_{11} sublayer than in Aca_{12} *(fig. 4.4, p. 30)*, which was the case for $^{15}NO_3^-$ and $^{15}NH_4^+$ tracers applications. It was equally true after 1 hour and 1 year where 75 % to 95 % of the tracers were present in Aca_{11} sublayer.

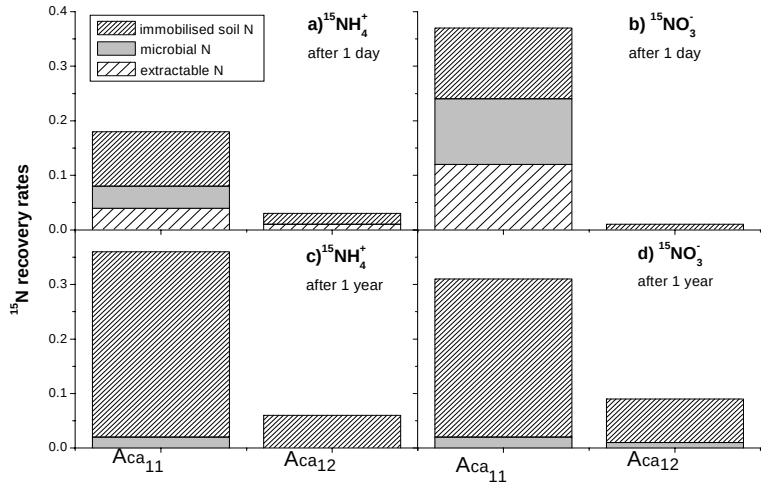

Fig. 4.4 : Recovery of ^{15}N tracer after 1 day and 1 year in the Aca sublayers (Aca_{11} & Aca_{12}).

4.3.5 NITROGEN POOL AND ^{15}N RECOVERY IN HERBACEOUS VEGETATION

^{15}N concentration within herbaceous vegetation differed over the year *(fig. 4.5, p. 30)*: after 57 days it was higher for the $^{15}NO_3^-$ treatment ($^{15}NO_3^-$: 12%; $^{15}NH_4^+$: 4%). On the contrary, both ^{15}N concentrations were similar after 294 days ($^{15}NO_3^-$: 7%; $^{15}NH_4^+$: 9%).

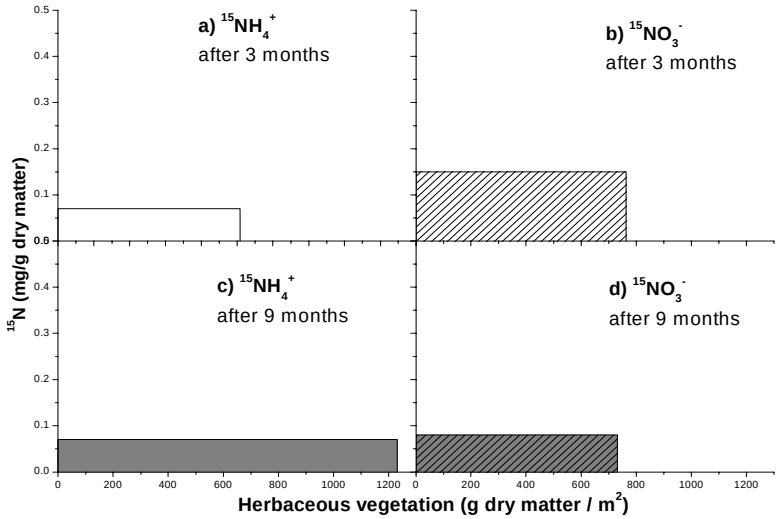

Fig. 4.5 : Concentration of added tracers ($^{15}NH_4^+$ and $^{15}NO_3^-$) recovered in herbaceous vegetation, in function of its dry matter amount (g/m^2) for two sampling periods.

4.4 DISCUSSION

4.4.1 FROM N DEPOSITION TO SOIL N RETENTION

In case of N deposition, the mechanisms responsible for the short-term N retention in the soil are the transformations of deposited inorganic N in less labile (organic N) or even immobilized forms (organic recalcitrant N or clay-fixed N). In our study, more than half of the deposited N is retained in the soil one day after tracer application. We saw that ^{15}N recovery rates after one hour were even higher than after one day but that such had to be accounted for cautiously because of the high variability between replicates. This variability is due to an uneven tracer distribution immediately following the application that may correspond to preferential flows into and within the soil because of both herbaceous vegetation and litter cover as well as heterogeneity of soil structure (Feyen et al., 1999; Hagedorn et al., 1999). Recovery rates higher than 100% have to be related to those processes. Those effects are rapidly cancelled, probably by bioturbation leading to a homogenization of tracer distribution.

One hour after tracer application, about half of the deposited NH_4^+ is retained in the litter layer. Recovery rates are only about one fifth for deposited NO_3^-. At all events, those recovery rates are twice as high as those of Providoli et al. (2006) who, in a similar experiment, recovered between 8% to 15% of $^{15}NH_4^+$ tracer and 10% to 12% of $^{15}NO_3^-$ in the litter layer during the first day. We interpret the important and immediate retention by the fact that litter forms a dense organic layer of leaves able to retain deposited N compounds. This process is particularly effective for NH_4^+ which is preferentially used by microorganisms. Furthermore, ammonia fixation by organic matter is a quite common process (Nömmik, 1965; Broadbent & Stevenson, 1966; He et al., 1990; He et al., 1991). Finally, NH_4^+ ion is less mobile than NO_3^-. During the first day, decreasing $^{15}NH_4^+$ recovery rates could be due to nitrification followed of infiltration of soluble $^{15}NO_3^-$ into the soil.

As far as the retention of deposited NO_3^- in litter layer is concerned, we think that microorganisms are also involved. Fast and important immobilization of NO_3^- by microbes has also been demonstrated in other recent studies (Zak et al., 1990; Davidson et al., 1992; Groffman et al., 1993; Stark & Hart, 1997; Berntson & Aber, 1999; Zogg et al., 2000; Perakis & Hedin, 2001). An explanation to this phenomenon is that microbial assimilation of NO_3^- can be significant in microsites where NH_4^+ is absent (Schimel & Firestone, 1989b; Davidson et al., 1990). Decomposing litter heterogeneity is in accordance with the presence of NH_4^+ depleted microsites. Abiotic fixation of NO_3^- by the soil organic matter (SOM) is another possible process which was already suggested by several studies (Perakis & Hedin, 2001; Currie et al., 1999, Berntson & Aber, 1999, Zogg et al., 2000). In our case, microbial assimilation and abiotic fixation are possibly implied in N retention.

An important fraction of deposited N directly went through the litter layer to reach the Aca organo-mineral layer within the first hours following the tracer application. Right after the tracer application, the extractable N pool is the largest sink. When the ^{15}N tracer is applied as $^{15}NH_4^+$, a rapid decrease in recovery of this ion was accompanied by a production of $^{15}NO_3^-$, showing an important nitrification. The overall decrease of tracer observed during the first day within this pool suggests a loss of extractable ^{15}N. Indeed, no other soil pool showed a concomitant increase of ^{15}N recovery rates (except fine roots but the increase is very low). The potential process involved is, either leaching or lateral fluxes. Leaching is characteristical for soluble NO_3^- ions. $DO^{15}N$ decrease is due to a decrease of this N pool size, more than to a decrease of its ^{15}N abundance, which therefore suggests that this $DO^{15}N$ corresponds to short-

lived chemical compounds. Such a fast loss of extractable ^{15}N has not been observed in similar studies: Perakis & Hedin (2001) found no significant loss after 1 day. It was neither the case in Zogg et al. (2000) nor in Providoli et al. (2006). We think that the loss of extractable ^{15}N in our site should be coupled with low microbial immobilization measured in relation to values published in other studies: Perakis & Hedin (2001) calculated recovery rates of 42% and 27% the day following the addition of $^{15}NH_4^+$, respectively NO_3^-. Their values were more then ten times higher then ours. Norton & Firestone (1996) worked with intact microcosms (27x27x2.2 cm); after two days, they found 40% of added $^{15}NH_4^+$ and 30% of $^{15}NO_3^-$ in microbial biomass. Low microbial activity has been described by Bureau (1995) on that same site where a high microbial biomass was observed but microbes were not very effective in terms of mineralization comparing to those of other ecosystems. A fast turn over of organic matter and high biological activity has to be related to other micro- and macroorganisms (like earth worms).

Extractable N was also partly taken up by plants. The process happened very quickly after tracer applications. After one hour, ^{15}N was detectable in fine roots. Recovery rates increased during the first day. This occurrence should certainly be connected to the diffusion of the deposited N within the soil. Consequently accessibility of ^{15}N to the roots is facilitated.

Deposited N incorporation into SOM and clay pools also appeared within a few hours. The possible processes involved are an abiotic fixation by SOM, a fixation on clays or an incorporation within SOM after recycling through microorganisms or root pools (indirect incorporation). During one day, indirect incorporation in SOM is certainly very small because it is too short a period for intense recycling through other organic pools. Fixation on clays is very likely the case for NH_4^+ deposition. Dominant clay type is illite, characterized by a high fixing capacity of NH_4^+. Studies about NH_4^+ fixation on clays only concern fertilization experiments related to agricultural practices and plants nutrition. Some authors compared N fixation by organic matter and by clays. He et al. (1990 and 1991) found that about 16 % of the NH_3-N injected was fixed by the soil, half of which into the soil organic fraction and the other half as clay-fixed NH_4^+. Their soil was also dominated by 2/1 clay type (montmorillonite). Trehan (1996) also compared NH_4^+ fixation by clays and by organic matter (microbial immobilisation and abiotic fixation). Among the various soils compared within the latter's study, those with similar clay content such as the one studied herein, fixed during the first 30 minutes, 1% to 5% on clays. Abiotic fixation also seemed to occur for 1% to 3%. Our results do not make it possible to differentiate between the various cited processes. However SOM seems to play a key role in soil N retention: already after one day, much of the deposited N in soil is present as ISN in Aca_{11} sublayer which corresponds to the very organic first centimetres of soil. Such a fast retention is probably and partially due to an abiotic process described in previous studies by several authors but which still remains poorly understood (Nömmik & Vahtras, 1982; Johnson et al., 2000; Dail et al., 2001 ; Fitzhugh et al., 2003). Moreover, the reduction of ^{15}N recovery rates during the first day in ISN pool suggests a mineralization process. Mobilization of NH_4^+ fixed in clays is not possible at this rate.

Some fluctuations of recovered ^{15}N tracer, due to several phenomenons were observed over the year: A significant increase appears in several pools of the organo-mineral Aca horizon between one day and one week. We therefore suggest that some ^{15}N tracer was temporarily trapped on herbaceous vegetation during the labeling and reached the soil at a later stage, during the next rain events and dew. Another significant increase of deposited ^{15}N was observed within ISN pool, between one and three months. This change in tracer recovery must certainly be linked with the seasonal dynamics of vegetation: Since litter has fallen and brought new organic N on the soil.

4.4.2 SOIL IMMOBILIZATION EFFICIENCY

One year after the tracer application, approximately three fourths of the deposited N were recovered in the soil; a similar quantity as after one day period. As hypothesized, the soil is thus clearly the most important sink for N deposition. Our results coincide with those of other recent studies carried out in different environments: Providoli et al. (2006) found 72% of the labelled $^{15}NO_3^-$. Values are lower for $^{15}NH_4^+$ (32%) because of its high absorption in mooses. One year after the tracer application, Perakis et al. (2001) obtained recovery rates near to 65% within the organic OA horizon (litter was removed during tracer application and not analyzed) for both labeling.

Deposited N is mainly recovered in the 25 first centimetres of the soil corresponding to the Aca organo-mineral layer. In this soil layer, immobilisation of ^{15}N does not differ significantly between $^{15}NH_4^+$ and $^{15}NO_3^-$ deposition. This suggests incorporation within the organic pools rather then a fixation on clays, which would differentiate both tracers recovery rates. During the year of tracer application, the herbaceous vegetation took up twice as much ^{15}N from $^{15}NO_3^-$ than from $^{15}NH_4^+$. During the following spring, however, no difference in plants vegetation uptake was observed between those tracers. It seems that transformations within the soil N cycle of the two forms of deposited N had already resulted in a similar N-bioavailability for the herbaceous plants. Thus, over the first year after deposition, a fraction of ^{15}N seems to be biologically recycled within the soil-plant system as is also the case for native N (Bringmark, 1980; Schimel & Firestone, 1989a).

Dynamics within litter layer does clearly illustrate biological recycling within the soil, or even the ecosystem. On the one hand, recovery rates are very stable over the year and on the other, in our site, litter dynamics is characterized by a fast turnover (Bureau, 1995). This means that over the year, fresh organic matter is incorporated within the soil, suggesting two non-exclusive phenomenon: firstly a recycling within litter layer through populations of microorganisms; secondly, it is likely that the tracer found in the litter one year after application does not totally correspond to the one found after a few days. A fraction of deposited ^{15}N was absorbed by the vegetation and certainly recovered in the litter layer after autumnal leaves fall.

4.5 CONCLUSION

Processes determining the fate of atmospherically deposited N within the forest soil happen surprisingly fast, over the first day. Part of deposited N is rapidly lost as extractable through lixiviation (and lateral fluxes) while another part is immobilized within stable forms (organic N or N fixed on clays). Corresponding to former studies, immobilization dominates and soil organic matter is the main sink for deposited N. It is important mentioning that the relative importance of the processes is different according to the soil conditions: we observed low microbial immobilization coupled with fast loss processes.

After one year and after on day deposited N distribution is almost similar; soil organic matter remains the main sink for deposited N. We demonstrate that further to these above-mentionned processes (i.e. N immobilization within soil organic matter and losses of extractable N), deposited N recycling within the microbes-plants-SOM system do take place and therefore prevent further losses. It corroborates the assertion that N-cycling in the temperate natural forest floor is generally tight; recycling governs dynamics of native nitrogen in unpolluted temperate forests ecosystems and appears to act in the same with atmospheric nitrogen deposition incorporated in organic pools. Therefore, N stored in microbial-plants-SOM within the first hours

after deposition remains for years within this system.

In our studies, we demonstrate that N immobilisation in a calcareous soil following atmospheric deposition shows to be similar to that of more commonly studied soils. It is however important to keep in mind that, in case of increasing amount of N deposition, N-loss would probably increase as well (because N is not a limiting factor for microbial growth) causing a nitrate leaching.

ACKNOWLEDGMENTS

We hereby thank Jean-David Teuscher (EPFL Lausanne) for his assistance both in the field and in the laboratory as well as Marie Sudki for the English revision. We further wish to thank H.R. Roth (ETH Zurich) and J. Moret (University of Neuchâtel) for their help with the statistical analyses. We also wish to mention the helpful collaboration of Isabelle Providoli (WSL Birmensdorf) which was greatly appreciated.

REFERENCES

Aber J.F., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S., Currie W., Rustad L. & Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems - hypotheses revisited. *Bioscience*, **48**, 921-934.

Aber J.F., Goodale C.L., Ollinger S.V., Smith M.-L., Magill A.H., Martin M.E., Hallett R.A. & Stoddard J.L. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? *Bioscience,* **53,** 375-389.

A.F.E.S. (eds). 1998. *A sound reference base for soils*. Collection techniques et pratiques. 322 p. INRA editions, Paris.

Ayers R.U., Schlesinger W.H. & Socolow R.H. 1994. Human impacts on the carbon and nitrogen cycles. In: *Industrial ecology and global change* (eds R.H. Socolow, C. Andrews, R. Berkhout, & V. Thomas), pp. 121-155. Cambridge University Press, Cambridge, England.

Berntson G.M. & Aber J.D. 1999. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology & Biochemistry*, **32**,151-156.

Bringmark L. 1980. Ion leaching through a podsol in a scots pine stand. *Ecological Bulletins* (Stockholm), **32**, 341-361.

Broadbent F.E. & Stevenson F.J. 1966. Organic matter reactions. In: *Agriculture Anhydrous Ammonia* (ed. M.H. McVickar), pp. 169-187. American Society of Agronomy, Madison, USA.

Brookes P.C., Landman A., Pruden G. & Jenkinson D.S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry*, **17**, 837-842.

Bureau F. 1995. *Evolution et fonctionnement des sols en milieu alluvial peu anthropisé*. PhD thesis. Swiss Federal Research Institute of Technology, Lausanne, Switzerland.

Currie W.S., Nadelhoffer K.J. & Aber J.D. 1999. Soil detrital processes controlling the movement of ^{15}N tracers to forest vegetation. *Ecological Applications*, **9,** 87-102.

Dail D. B., Davidson E.A. & Chorover, J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry*, **54**, 131-146.

Davidson E.A., Stark J.M. & Firestone M.K. 1990. Microbial production and consumption of nitrate in an annual grassland. *Ecology*, **71**, 1968-1975.

Davidson E.A., Hart S.C. & Firestone M.K. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology,* **73**, 1148-1156.

Dise N.B., Matzner E., Armbruster M. & MacDonald J. 2001. Aluminium output from forest ecosystems in Europe: a regional assessment. *Journal of Environmental Quality*, **30**, 1747-1756.

Draaijers G.P.J. 1993. *The variability of atmospheric deposition to forest. The effects of canopy structure and forest edges*. PhD thesis. University of Utrecht, Netherlands.

Fangmeier A., Hadwiger-Fangmeier A., Van der Eerden L. & Jäger H.-J. 1994. Effects of atmospheric ammonia on vegetation - a review. *Environmental Pollution*, **86**, 43-82.

Feyen H., Wunderli H., Wydler H. & Papritz A. 1999. A tracer experiment to study flow paths

of water in a forest soil. *Journal of Hydrology*, **225**, 155-167.

Fitzhugh R.D., Lovett G.M. & Venterea R.T. 2003. Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biology*, **9**, 1591-1601.

Food and Agricultural Organization of the United Nations. 1989. *Soil map of the world, revised legend*. FAO-UNESCO, Rome, Italy.

Galloway J.N., Schlesinger W.H., Levy H., Michaels A. & Schnoor J.L. 1995. Nitrogen fixation: Anthropogenic enhancement-environmental response. *Global Biogeochemical Cycles,* **9**, 235-252.

Galloway J.N. & Cowling E.B. 2002. Reactive nitrogen and the world : 200 years of change. *Ambio,* **31**, 64-119.

Galloway J.N., Aber J.D., Erisman J.W., Seitzinger S.P., Howarth R.W., Cowling E.B. & Cosby, B.J. 2003. The nitrogen cascade. *Bioscience,* **53**, 341-356.

Gebauer G., Zeller B., Schmidt G., May C., Buchmann N., Colin-Belgrand M., Dambrine E., Martin F., Schulze E.-D. & Bottner P. 2000. The fate of ^{15}N-labelled nitrogen inputs to coniferous and broadleaf forest. In: *Carbon and nitrogen cycling in european forest ecosystems* (ed. E.-D. Schulze), pp. 144-170. Ecological Studies 142. Springer-Verlag, Berlin, Heidelberg.

Groffman P. M., Zak D.R., Christensen S., Mosier S. & Tiedje J.M. 1993. Early spring nitrogen dynamics in a temperate forest landscape. *Ecology*, **74**, 1579-1585.

Guenat C., Bureau F., Weber G. & Toutain F. 1999. Initial stages of soil formation in a riparian zone: importance of biological agents and lithogenic inheritance in the development of the soil structure. *European Journal of Soil Biology*, **35**, 153-161.

Gundersen P., Emmett B.A., Kjønaas O.J., Koopmans C.J. & Tietema A. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management*, **101**, 37-55.

Hagedorn F., Mohn J., Schleppi P. & Flüher H. 1999. The role of rapid flow paths for nitrogen transformation in a forest soil: a field study with micro suction cups. *Soil Science Society of America Journal,* **63**, 1915-1923.

Hart S.C., Firestone M.K., Paul E.A. & Smith J.L. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry*, **25**, 431-442.

He, X.T., Mulvaney R.L. & Stevenson F.J. 1990. Characterization of chemically fixed liquid anhydrous ammonia in an illinois drummer soil. *Soil Science Society of America Journal,* **54**, 775-780.

He X.T., Mulvaney R.L., & Stevenson F.J. 1991. Transformations of chemically fixed liquid anhydrous ammonia by soil-microorganisms. *Biology and Fertility of Soils,* **11**, 145-150.

Johnson D.W., Cheng W. & Burke I.C. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal*, **64**, 1503-1514.

Lamontagne S., Schiff S.L. & Elgood R.J. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. *Canadian Journal of Forest Research*, **30**, 1165-1177.

Nadelhoffer K.J., Downs W.R. & Fry B. 1999a. Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. *Ecological Applications*, **9**, 72-86.

Nadelhoffer K.J., Emmet B.A., Gundersen P., Kjønaas O.J., Koopmans C.J., Schleppi P., Tietema A. & Wright R.F. 1999b. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**, 145-147.

Nömmik H. 1965. Ammonium fixation and other reactions involving a non enzymatic immobilization of mineral nitrogen in soil. In: *Soil Nitrogen* (ed. F.E. Clark), pp. 198-258.. American Society of Agronomy, Madison, USA.

Nömmik H., & Vahtras K. 1982. Retention and fixation of ammonium and ammonia in soils. p. 123-172. In: *Nitrogen in agricultural soils* (eds F.J. Stevenson et al.), pp. 123-172. Agronomy Monographs 22. American Society of Agronomy, Madison, USA.

Norton J. M. & Firestone M.K. 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. *Soil Biology & Biochemistry*, **28**, 351-362.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. *Ecology*, **82**, 2245-2260.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2005. Flow of deposited inorganic N in two Gleysol-dominated mountain catchments traced with $^{15}NO_3^-$ and $^{15}NH_4^+$. *Biogeochemistry*, **76**, 453-475.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2006. 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. *Soil Biology and Biochemistry*, in press.

Schimel J.P. & Firestone M.K. 1989a. Inorganic N incorporation by coniferous forest floor material. *Soil Biology & Biochemistry*, **21**, 41-46.

Schimel J.P. & Firestone M.K. 1989b. Nitrogen incorporation and flow through a coniferous forest soil profile. *Soil Science Society of America Journal,* **53,** 779-784.

Sheeder S.A., Lynch J.A. and Grimm J. 2002. Modeling atmospheric nitrogen deposition and transport in the Chesapeake Bay Watershed. *Journal of Environmental Quality*, **24**, 209-226.

Socolow R.H. 1999. Nitrogen management and the future of food: Lessons from the management of energy and carbon. *Proceedings of the National Academy of Science of the USA*, **96**, 6001-6008.

Söderlund R. & Svensson B.H. 1979. The global nitrogen cycle. In: *Nitrogen, phosphorus and sulphur - global cycles* (eds R. Söderlund and B.H. Svensson), pp. 23-73. Swedish National Science Research Council, Stockolm, Sweden.

Stark J.M. & Hart S.C. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature,* **385**, 61-64.

Trehan S.P. 1996. Immobilisation of $^{15}NH_4^+$ in three soils by chemical and biological processes. *Soil Biology & Biochemistry*, **28**, 1021-1027.

Vitousek P.M., Aber J.D., Howarth R. W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. & Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737-750.

Zak D.R., Groffman P.M., Pregitzer K.S., Christensen S. & Tiedje J.M. 1990. The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. *Ecology,* **71**, 651-656.

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology*, **81**, 1858-1866.

CHAPTER 5

5 RETENTION OF ATMOSPHERICALLY DEPOSITED N IN TWO CONTRASTING SOILS IN SWITZERLAND

Submitted to the European Journal of Soil Science

Isabelle Morier [1,2], Patrick Schleppi [2], Matthias Saurer [3], Isabelle Providoli [2], Claire Guenat [1]

[1]Swiss Federal Institute of Technology (EPFL), Laboratory of Soil and Environmental Physics, CH-1015 Lausanne, Switzerland

[2]Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), CH-8903 Birmensdorf, Switzerland

[3]Paul Scherrer Institut (PSI), Laboratory of Atmospheric Chemistry, CH-5232 Villigen-PSI, Switzerland

Atmospheric N deposition was shown to be mainly retained in the soil of temperate forest ecosystems. The soil organic layers are the main sink for deposited N but the mechanisms and the organic fractions involved are still poorly defined. We therefore performed a hot acid hydrolysis on ^{15}N labelled soil samples collected within a one-year field labelling experiment (a single application of $^{15}NO_3^-$ or $^{15}NH_4^+$) and carried out in two contrasting forests ecosystems (Grandvillard and Alptal). For $^{15}NH_4^+$ application, recovery rates in the soil were found lower in Alptal than in Grandvillard, due to a fast absorption by the mosses. The both sites organic layers retained most of the tracers within one year course. In Grandvillard, the hydrolysability (hydrolysable N / total N) of ^{15}N reached an annual average of 79% and was similar to the hydrolysability of native N. This similarity could be determined by a rapid N stabilisation into the recalcitrant N pool through organo-mineral bounds. In Alptal ^{15}N hydrolysability was higher than that of native N, particularly in case of $^{15}NH_4^+$ application (^{15}N : 84% ; native N : 72%) and could be explained by a rapid microbial turnover of hydrolysable N. On both sites, ^{15}N and native N hydrolysability was constant over the year. We propose that deposited N immobilisation within the recalcitrant pool is effective in the long term. Furthermore, the biological recycling must be involved in the stability of the hydrolysable N fraction.

5.1 INTRODUCTION

Since a few decades, the balance of the N cycle has been disturbed by human activities (combustion processes and fertilising practices), leading to an increase of N atmospheric deposition on various ecosystems. In temperate regions, the observed effects include eutrophication of aquatic and terrestrial unfertilised ecosystems (Vitousek et al., 1997) or soil acidification (Dise et al., 2001). Increased N deposition is also considered as one cause of forest decline (Aber et al., 1989; Schulze & Freer-Smith, 1991). Considering the importance of this subject, many researchers have investigated these impacts and their mechanisms more thoroughly (Hart et al., 1993; Gundersen et al., 1998; Nadelhoffer et al., 1999a; Schleppi et al., 1999; Gebauer et al., 2000). In many studies, the soil was found to be the compartment of the ecosystem where the major part of the deposited N was retained (Buchmann et al., 1996; Nadelhoffer et al., 1999b; Lamontagne et al., 2000). As a consequence, the soil is the focus of most recent researches on the subject (Berntson & Aber, 2000; Zogg et al., 2000; Perakis & Hedin, 2001; Fitzhugh et al., 2003; Providoli et al., 2006; Morier et al., submitted). However the processes involved and the chemical forms in which N is retained are not completely resolved.

Previous studies showed that the major part of deposited N was retained in the form of immobilised soil nitrogen (ISN) (Providoli et al., 2006) which represents the total soil N minus both the microbial and the extractable fractions. It was further demonstrated that most of this immobilisation happened within the organic layers of the soil (Morier et al., submitted). Despite its central role, the organic fraction is still poorly defined and it would thus be important to better characterise it. To characterise the soil N, hot acid hydrolysis (Bremner, 1965) is one frequently used method (Stevenson, 1982), since it allows the separation of N compounds on the one hand, in a hydrolysable fraction corresponding to the more labile N forms, including the most bioavailable ones (Chang et al., 1999; Johnsson et al., 1999) and on the other hand, in a non-hydrolysable fraction that covers the more recalcitrant N compounds.

So far, many studies on N deposition effects have included experimental factors like deposition rate (Gundersen et al., 1998; Perakis et al., 2005) or vegetation type (Fitzhugh et al., 2003) but one has to point out that were conducted on single sites. N dynamics and immobilisation are influenced by climatic and edaphic conditions but, because of methodological differences, comparisons across experiments are often difficult to perform. In the present study, we aimed at comparing two different sites with contrasting climate, soil and vegetation by using the same investigation.

In details, our questions were the following: i) What is the importance of the hydrolysable versus the non-hydrolysable fraction of the deposited N recovered in the soil and what is the temporal pattern of such distribution? ii) Does the distribution depend on the chemical form (NH_4^+ or NO_3^-) of the deposited N or on the site? iii) According to the distribution, what are the possible processes involved in the immobilisation of deposited N in soil? iv) Finally, what does the distribution entail for the long term dynamics of deposited N in soil?

In this context, a hot acid hydrolysis was performed on soil samples collected on one field ^{15}N labelling experiment which was carried out simultaneously in two contrasting forest ecosystems (Providoli et al., 2006; Morier et al., submitted), simulating $^{15}NO_3^-$ and $^{15}NH_4^+$ deposition. Different sampling times were selected: from short term (1 week) to long term (one year).

5.2 MATERIAL AND METHODS

5.2.1 STUDY SITES

Two sub-alpine forest ecosystems were selected to carry out a joint field ^{15}N labelling experiment *(fig. 5.1, p. 41)*.

The first site, Grandvillard, is located in the Western Prealps, within the riparian zone of La Sarine River at 650 m a.s.l.; average annual precipitation is 1200 mm and average annual air temperature is 7.1°C. In 2001-2002, bulk atmospheric deposition of inorganic N amounted to 16 kg $ha^{-1}y^{-1}$. The site's forest consists of a half half mixed of deciduous lignous species dominated by beeches (*Fagus sylvatica* L.) and of planted spruces (*Picea abies* L.). The ground vegetation is dominated by *Mercurialis perennis* L., *Polygonatum verticillatum* (L.) All., *Carex alba* Scop., *Aconitum lycoctonum* L., *Aposeris foetida* L., *Phyteuma spicatum* L. and *Anemone nemorosa* L.. The parent material is alluvial grave; the water table is very deep, approximately 4 m. Soils are well drained calcareous Fluvisol (FAO, 1989) or Fluviosols carbonatés (AFES, 1998). The soil profile consists of a OL layer (a few millimeters), a loamy Aca layer (0-25 cm) and a silty loamy Sca layer (26-50 cm) which lies above a sandy bed and the alluvial grave. Humus is a calcareous mull with a fast organic matter turnover. For further details, see Bureau (1995).

The second site, Alptal, is located in the valley of the same name within the Alps of Central Switzerland and lies at 1200 m a.s.l. The average annual temperature is 6°C and the average annual precipitation is 2300 mm. Bulk atmospheric deposition of inorganic N is 12 kg ha^{-1} y^{-1} (Schleppi et al., 1999). Vegetation and soil types form a mosaic pattern closely related to microtopography. In depressions, the water table is frequently close to the surface. Soils are

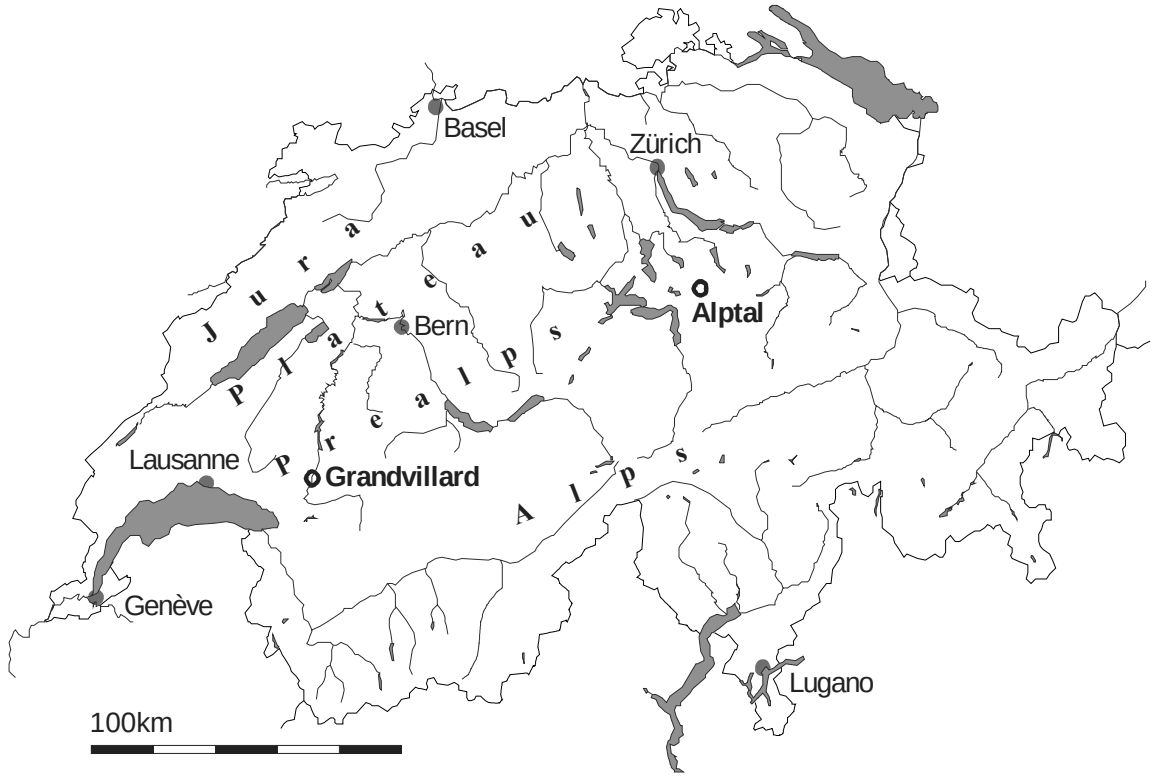

Fig. 5.1 : Location of the two study sites, Grandvillard and Alptal, on a map of Switzerland.

mollic Gleysols (FAO, 1989) or Reductisols typiques (AFES, 1998) with a thin OF layer (0-4 cm), a silty clayed A layer (4-15 cm) rich in organic matter, lying above a clay loamy G layer reduced almost permanently. Humus is an anmoor. Depressions are too wet for tree growth and the ground vegetation is dominated by *Poa trivialis* L. and *Carex ferruginea* Scop. in open patches; by *Caltha palustris* L. and *Petasites albus* L. in the shade of the trees. On mounds, the water table lies at a depth below 40 cm. Soils are umbric Gleysols (FAO, 1989) or Reductisols typiques (AFES, 1998) with raw humus (mor), an A rich in organic matter and a G horizon. The dominant plant species are Norway spruces (*Picea abies* L.) and *Vaccinium myrtillus* L. The site's ground vegetation is characterised by a moss layer with more than 30 moss species (Muller, 1997, Schleppi et al., 1998).

5.2.2 EXPERIMENTAL FIELD DESIGN

In August 2001, we performed a labelling ^{15}N experiment on both study sites simultaneously. Labelling involved of a single addition of ^{15}N at high isotopic concentration (99%). Three replicates were established in the Grandvillard forest and four in the Aptal forest because of the latter's more heterogeneous soil. Each replicate was divided into three plots (Grandvillard: 2.5 x 2.5 m each; Alptal: 1.5 x 1.5 m each). One plot was labelled with $K^{15}NO_3$ and the second with $^{15}NH_4Cl$ dissolved in deionised water (3.8 mmol l^{-1}; 2.2 l m^{-2}). The third plot was only treated with deionised water, for control. In order to simulate atmospheric N deposition, we applied the labelled solution with a back-pack sprayer.

Between one hour and one year, eight soil samples were simultaneously collected in Grandvillard and in Alptal. Each sample was taken with a soil corer (5 cm diameter and 25 cm depth). Within each plot, three soil cores were taken per sampling time. After the previous layers separation, the three soil samples per plot were pooled into one composite sample. For the analyses presented here, three samplings times were selected: 1 week, 3 months and 1 year. During the first week, a fast retention of ^{15}N in the soil (Providoli et al., 2006; Morier et al., submitted) was observed. After this first period, we recorded a relatively stable state of ^{15}N partitioning between the soil layers, with less variability between replicates than during the first day. The second sampling time, after 3 months, took place in November, after the vegetation's senescence. Finally, the one-year sampling, happened during the next vegetation period. Layers collected were i) : OL litter layer, Aca_{11} organic sublayer (0-5 cm), Aca_{12} organo-mineral sublayer (5-25 cm) in Grandvillard and ii) OL litter layer, OF organic layer (0-5 cm), A organo-mineral layer (5-10 cm) and G mineral layer (10-25 cm) in Aptal.(see layers characteristics in *table 5.1 (p. 42)*).

Table 5.1 : Characterisation of the sampled soil layers for the two study sites.

		depth	texture	C/N	bulk density
Grandvillard	OL layer	approx. 0.2 cm			
	Aca_{11} layer	0-5 cm	loam	15.9	0.62
	Aca_{12} layer	5-25 cm	loam	13.6	0.98
Alptal	OL layer	approx. 0.1 cm			
	OF layer	0-5 cm		19.4	0.12
	A layer	5-10 cm	loamy clay	15.6	0.35
	G layer	10-25 cm	loamy clay	17.3	0.30

5.2.3 LABORATORY ANALYSES

Hot acid hydrolyses (Bremner, 1965) were performed on Aca_{11} and Aca_{12} layers from Grandvillard and on OF and A layers from Alptal. Between 0.7 g (OF layer, Alptal) and 6.2 g (Aca_{12}, Grandvillard) of dry soil, according to its total N concentration, were weighted and mixed with 20 ml 6M HCl. The soil-acid mixture was heated to reach gentle boiling under reflux for 12 hours. The next step was the filtration under vacuum through a Büchner funnel fitted with a filter paper. In order to remove HCl, hydrolysates were evaporated in a microwave oven, then recuperated with deionised water, stored in the freezer and finally lyophilised to obtain dry samples for mass spectrometry N measurements.

N concentration as well as $^{15}N/^{14}N$ isotopic ratio were measured on litter, bulk soil and dried soil hydrolysates. The analyses were performed at the Paul Scherrer Institut, Laboratory of Atmospheric Chemistry, where samples were combusted in an Elemental Analyser (EA 1108, Finnigan, Bremen, Germany). The evolving N_2 was led in the Helium stream to the isotope ratio mass spectrometer (DeltaS, Finnigan, Bremen, Germany) where the $^{15}N/^{14}N$ ratio of the sample was determined relative to the international reference (N_2 in air).

5.2.4 CALCULATIONS AND STATISTICAL ANALYSES

The total N amounts in the different soil layers were calculated per unit area. The ^{15}N recovery was expressed relatively to the added label (for details, see Providoli et al., 2005). Furthermore, N hydrolysability was calculated as a ratio of hydrolysable to total N (for each native N and labelled N).

Three general linear models were performed with repeated measures (S-PLUS 6.1 – Mathsoft inc.) to test i) the amounts of total N and the recovered ^{15}N rates in the whole soil profile for effects of the site (Grandvillard, Alptal), of the tracer ($^{15}NO_3^-$, $^{15}NH_4^+$) and of the time (as logarithm); ii) the amounts of total N, the recovered ^{15}N rates, the hydrosability of total N and of ^{15}N within each soil layer, for effects of the tracers, of the time and of their interaction; iii) the hydrolysability within each soil layer, for effects of the labelling (native N, $^{15}NO_3^-$ and $^{15}NH_4^+$) of the time and of their interaction. The dependent variables Log transformation was used to improve normality in the data when necessary. When this transformation was not effective, a Kruskall-Wallis rank test was used. All results were considered significant at the $P<0.05$ level.

5.3 RESULTS

5.3.1 TOTAL N WITHIN THE TWO SOIL PROFILES

Average N amounts for both soil profiles and each soil layers are given in *table 5.1 (p. 42)*. In that same table, the factors significantly affecting them are also specified. Total N contents of both soils were similar, with average values close to 600 g/m^2, but such concentrations were much higher at Alptal. At this site, we found that 571 g/m^2 N were concentrated in 70 kg/m^2 of dry soil, while in Grandvillard 605 g/m^2 N corresponded to 227 kg/m^2 *(fig. 5.2, p. 44)*.

Table 5.2 : Mean N amounts together with the standard error for the different soil layers of Grandvillard and Alptal. Factors significantly affecting them are also indicated (P<0.05).

	Grandvillard				**Alptal**				
	Total	**OL**	**Aca$_{11}$**	**Aca$_{12}$**	**Total**	**OL**	**OF**	**A**	**G**
N (g/m^2)	605±13	13±1	130±4	461±11	571±16	6±0.5	82±8	196±11	274±11
Significant factors	-	-	-	-	Time (increase)	Replicates	Time (increase) and tracer		Interaction tracer * time

In the upper organic layers, the highest N concentration is coupled with the lowest amounts of N because of their low mass : in Grandvillard, the OL layer N concentration reached approximately 1% (for a C concentration between 30 % and 35%) against 0.4% in the Aca$_{11}$ layer (C concentration = 10-12%) and 0.2% in the Aca$_{12}$ layers (C concentration = 7%). In Alptal, N and C concentrations were higher and reached 1.4% in the OL layer, 1.4% in the OF layer (C concentration = 24-29%) and 1.2% in the A layer (C concentration = 18%)..

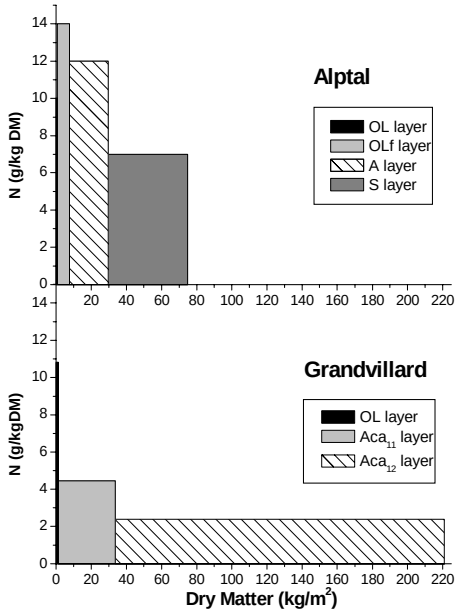

Fig. 5.2 : Soil N concentration and dry matter per soil layer in the two study sites. N amounts (g/m^2) are given by the area of the rectangles, each representing a soil layer.

5.3.2 ^{15}N TRACER RECOVERY WITHIN THE SOIL PROFILES AND WITHIN THE SOIL LAYERS

Other ^{15}N field labelling experiment results are presented in detail in Providoli et al. (2006) and in Morier et al. (submitted) which include the dynamics of extractable N during the first day. In this contribution, we only present the results in direct connection with the objectives of the present study.

Recovery rates in Alptal were found lower than in Grandvillard, especially for the $^{15}NH_4^+$ application (after 1 week, 0.6 in Grandvillard and 0.3 in Alptal) ($p = 0.02$) . On the soil layers scale, recovery rates were not statistically comparable between both sites because of the morphological differences between their respective profiles. However, on *fig. 5.3 (page 46)* we observed similarities in the partitioning of the ^{15}N tracer. The first horizon under the litter layer retained the major quantity of the tracer (Aca_{11} in Grandvillard and OF in Alptal). The litter layer generally contained less tracer despite its higher specific labelling (i.e. per unit of mass), because of its smaller N amount. The lower soil layer (Aca_{12} in Grandvillard and A in Alptal) also contained less tracer owing to a lower N concentration and a lower specific labelling.

Table 5.3 : P values of a general linear model with repeated measures for recovered ^{15}N tracers. Results are given for each soil profile, each soil layer and for the both soil profiles tested together. Corresponding mean recovered ^{15}N rates are presented in *fig. 5.3 (page 46)***.**

Grandvillard	**Soil profile**	**OL**	**#Aca_{11}**	**#Aca_{12}**
Tracer	0.30	0.07	0.16	0.07
Replicates	0.11	0.75	0.07	0.41
Log(time)	0.36	0.10	0.71	0.78
Tracer * log(time)	0.39	0.16	0.20	0.26
Alptal	**#Soil profile**	**OL**	**#OF**	**A**
Tracer	**0.02**	0.35	0.00	0.10
Replicate	0.51	0.28	0.1	0.14
Log(time)	0.14	0.04	0.72	0.87
Tracer * log(time)	0.10	0.58	0.24	0.21
Both sites	**##Soil profiles**			
Tracer	0.21			
Replicate	0.65			
Log(time)	0.58			
sites	**0.02**			

Notes : bold values correspond to $P<0.05$. # log transformed; ## Kruskall-Wallis rank test

.

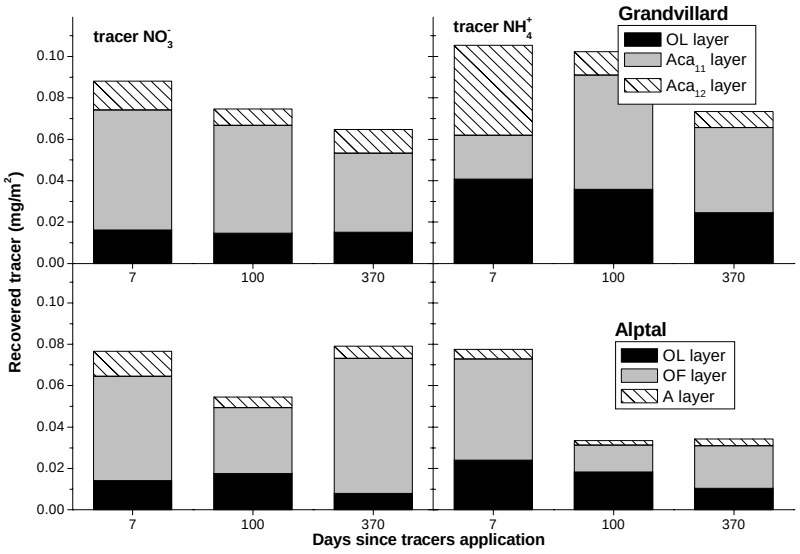

Fig. 5.3 : ^{15}N tracers recovery rates within the soil layers of the two studied sites. P values of general linear model are given in *table 5.3 (p. 45)*.

In the case of $^{15}NH_4^+$ tracer application, the OL layer was an important sink in Grandvillard. Since it retained an average of 34% of the N tracer after one week. Recovery rates were inferior for the $^{15}NO_3^-$ tracer application (13% after one week) and were even lower in Alptal (after one week, $^{15}NH_4^+$: 15%; $^{15}NO_3^-$: 11%). In Grandvillard, recovery rates were constant over the year but decreased in Alptal (after one year, $^{15}NH_4^+$: 7%; $^{15}NO_3^-$: 6%).

The organic Aca_{11} layer of Grandvillard was the main sink for $^{15}NO_3^-$ tracer application (48% recovered after one week). Recovery rates were also important after $^{15}NH_4^+$ tracer application (26% recovered after one week) and were constant within the study time frame. In Alptal, the organic OF layer retained the majority of $^{15}NO_3^-$ tracer (39% recovered after one week). For$^{15}NH_4^+$ tracer application, OF has also shown to be the main sink after one week (29%). Generally, recovery rates were constant over the year.

The organo-mineral layers, namely Aca_{12} layer in Grandvillard and layer A in Alptal, retained lower amounts of ^{15}N tracer (mean rate in Grandvillard : 8%; in Alptal : 3%). In both sites, rates did not differ within the study time frame or between the treatments. We found practically no ^{15}N tracer within layer G of Alptal (order of magnitude: 0.03%).

5.3.3 HYDROLYSABILITY OF NATIVE N AND OF ^{15}N TRACER

The proportions of soil N in hydrolysable form in the different soil layers are presented in *fig. 5.4 (page 47)*. Averages between replicates ranged from 59 to 83% while individual values showed no significant differences, neither between the tracers, nor between the sampling times. Because of the very small proportions of tracers within such N fractions, the total N hydrolysability can be considered as the hydrolysability of the native N.

The hydrolysability of the ^{15}N tracer is also presented in *fig. 5.4 (page 47)*. Again, no significant differences were found, neither between tracers, nor between sampling times. We obser-

ved that variability between replicates was higher for the tracer than for the native N, especially in the organo-mineral layers (Aca_{12} in Grandvillard and A in Alptal). For those layers, and because of the heterogeneity of variances, we used a Kruskall-Wallis rank test.

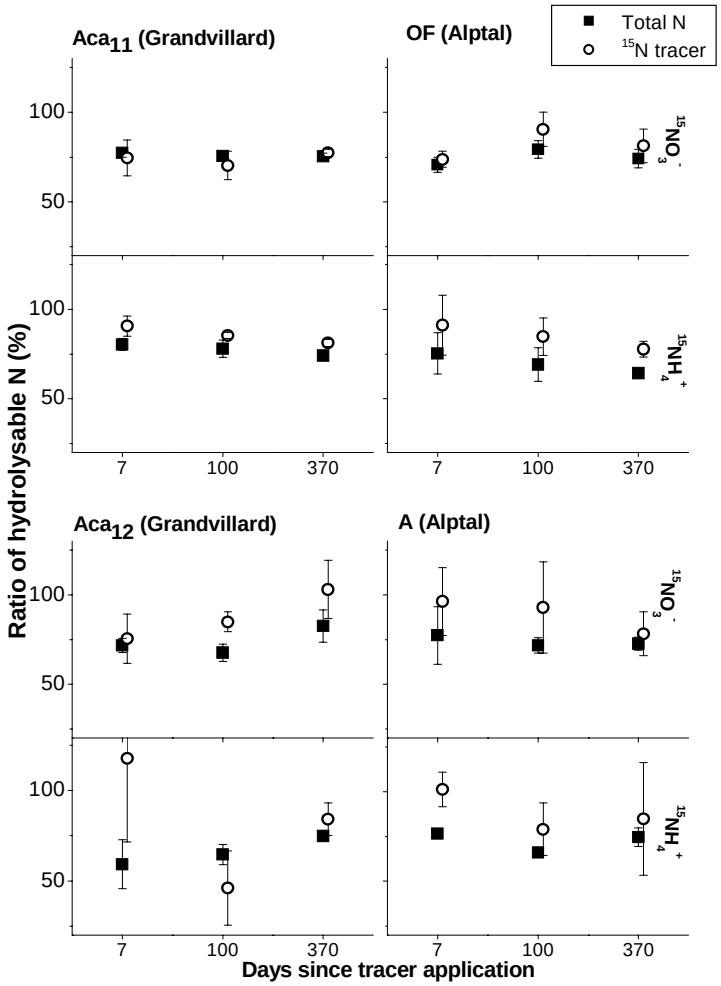

Fig. 5.4 : Ratios of hydrolysable N within total N (solid squares) and within the recovered ^{15}N tracer (empty circles).

Comparing native and ^{15}N hydrolysability, we observed that, in the Grandvillard Aca_{11} organic layer, the hydrolysability of ^{15}N was similar to that of native N (p=0.5), with an annual average of 79% (77% for native N). The ^{15}N hydrolysability was higher than the native N in the Alptal organic layer (p=0.02), particularly for the $^{15}NH_4^+$ tracer application: annual averages were of 84% for ^{15}N (72% for native N); they were of 82% for $^{15}NO_3^-$ labelling.

On both sites organo-mineral layers, no significant differences in hydrolysability were observed, neither between treatments, nor between sampling times or between ^{15}N and native N hydrolysability. For those layers and for the reason explained above, a Kruskall-Wallis rank test was used.

5.4 DISCUSSION

5.4.1 NATIVE AND DEPOSITED N: PRELIMINARY COMPARISON BETWEEN BOTH FOREST SITES

N stocks are very similar in the soil profiles of both study sites but N concentrations are very different. This is due to a large difference of the bulk densities measured. In Alptal, bulk densities are extremely low because of high contents in organic matter (OF layer) and in clay (G layer). These results significantly show that, in general, N amounts and N concentrations are two distinct parameters which must be used with precaution, especially while comparing different sites. This is even more emphasized when tracer amounts in a soil layer are compared, because they are the result from the combination of four terms: layer depth, bulk density, N concentration and isotopic enrichment. However, one has to point out that significant differences in tracer recovery can greatly vary from case to case depending on which of the previous terms is concerned the most.

The dynamics of N deposition in both studied forest soils differ according to the chemical form of the deposition: the lower recovery rates in Alptal mainly concern the NH_4^+ deposition and are due to a strong absorption of NH_4^+ by the dense mosses layer (Providoli et al., 2006) which are able to assimilate incoming nutrients (particularly NH_4^+) directly from rainfall or throughfall through their aerial organs (Oechel & Van Cleve, 1986; DeLuca et al., 2002). In Grandvillard, the litter layer acts as a filter and retains an important fraction of deposited NH_4^+. In the case of $^{15}NO_3^-$ tracers application, mosses and litter play a minor role in the retention of deposited N. As proposed in our previous research (Morier et al. submitted), this can be explained by a lower assimilation by microorganisms or mosses and by the high solubility of NO_3^-. Deposited N is mainly retained in organic layers (i.e. Aca_{11} in Grandvillard and OF in Alptal), whatever the chemical form once it passed the litter or mosses and got into the soil.

5.4.2 PROCESSES INVOLVED IN THE IMMOBILISATION OF DEPOSITED N

In a previous study (Morier et al., submitted) and following other researchers results (Hart et al. 1993; Bernston & Aber, 2000; Johnson et al., 2000; Zogg et al., 2000; Dail et al., 2001; Perakis & Hedin, 2001; Providoli et al., 2006), we highlighted fast N immobilisation processes in the soil, which are active within hours or days, and largely determine the fate of deposited N in the longer term. As a main consequence, recently deposited N is rapidly no longer extractable but enters into the immobilised soil N pool. In the present study, we show that this immobilisation brings recently deposited N both into the hydrolysable as well as the recalcitrant fractions of soil N. In both study sites organic layer and, within one week after NO_3^- deposition, this partitioning is close to that of native soil N and within the range usually obtained for unfertilised soil, using the same method (according to Stevenson & Cole (1992), 65 to 80 % of total N is recovered in hydrolysates). In the case of NH_4^+ deposition, the very short-term partitioning was somewhat different to the NO_3^- one: i.e. in Alptal, the tracer had a higher hydrolysability than the native N.

Even if the importance of fast N immobilisation in the soil is clearly confirmed by our study, an important question regarding the biochemical mechanisms involved remains opened. After one week, three quarter of deposited nitrate and nine tenth of ammonium were found in the form of hydrolysable compounds. This more labile fraction was described as consisting of amino acids (approximately 60%), amino sugars (approximately 15%) and other unknown

compounds (approximately 25%) (Swoden et al. 1977; Stevenson, 1982). The two first elements are usually considered as biosynthesised molecules. Incorporation of ^{15}N into the hydrolysable pool of the soil during the first week is therefore certainly mainly due to a microbial immobilisation. We know that i) NH_4^+ is the preferential form immobilised by microorganisms and ii) that the microbial activity seems to be higher in Alptal than in Grandvillard (Morier et al. 2006). Consequently, we think that the highest hydrolysability of $^{15}NH_4^+$ (compared with native N) measured in Alptal results from an important microbial immobilisation of $^{15}NH_4^+$. However, and considering the low amounts of tracer found in the microbial fractions after one week (Providoli et al., 2006), the tracer must cycle fast through this pool, i.e. the microbial turnover seems to be rapid. Since we could show that immobilisation of new N occurs rapidly, it is most likely that N from gross mineralisation is also largely and rapidly re-immobilised.

For some authors, the immobilisation of N into the recalcitrant pool is an abiotic process by which N enters the heterocyclic organic molecules (Schulten, 1994; Schulten et al., 1997; Schulten & Schnitzer, 1998). Another mechanism proposed for the immobilisation of N into the recalcitrant pool is a stabilisation of relatively labile N compounds, such as peptides, through encapsulation in hydrophobic domains of organic matter (Knicker & Hatcher, 1997; Zang et al., 2001) or through organo-mineral interactions (Leinweber & Schulten, 2000). The incorporation of ^{15}N in the recalcitrant pool is particularly efficient in Grandvillard and we think that the earth worms high activity (Bureau, 1995) could favour the stabilisation of relatively labile compounds through such organo-mineral interactions.

5.4.3 LONG TERM RETENTION OF N DEPOSITION IN SOIL

One year after the labelled N deposition events, tracer recovery rates in the soil layers were stable. The same was observed for their distribution between the recalcitrant and hydrolysable fractions. By definition, the recalcitrant fraction is not very prone to decomposition and biological turnover. It is probable that after one year the ^{15}N present in this pool would be the same as that present after one week. Such enables us to say that the fast immobilisation in the recalcitrant pool is efficient in the mid-term whichever the soil type or the chemical form of the deposition.

However, in our forest soils, and more generally in the non fertilised forest ecosystems, the hydrolysable fraction is larger than the recalcitrant one. The ratio is thus much higher than the proportion of net N mineralisation, which typically rates between 1 to 2% in temperate forests. In other words, the hydrolysable fraction is actually very stable at a yearly time scale as well. Some of this apparent stability may currently consist of a microbial recycling involving both gross mineralisation and gross re-immobilisation. Since the hydrolysable pool includes the fraction available for plants (Chang et al., 1999; Johnsson et al., 1999), a biological recycling through the soil-plant system is certainly also implied in the longer-term, deposited N dynamics, as we suggested in our previous research (Morier et al., submitted) and which relies on two facts: first, the rates of deposited N recovered in the soil, especially in the organic layers, are similar over the year, despite the litter turnover; second, fine roots, which are often the major contributors of soil organic matter (Fitter, 1985; Aber & Melillo, 1991; Killham, 1994), were an important sink for N deposition all over the year. Biological recycling was also described by other researchers as a key mechanism. In this regard, conclusions about the long term retention of deposited N stated by Gerzabek et al. (2004) are particularly interesting. Thirty years after the simulation of atmospheric N deposition through a ^{15}N labelling experiment in an alpine grassland in Austria, half of the deposited N was still in the soil, almost exclusively in the upper organic layer. Those authors suggested that this long-term retention was due to biolo-

gical recycling. We could add that it is not only the case in alpine or sub-alpine ecosystems, such as the site of Alptal in which the cycle of N is slowed down by climate and soil conditions (Schleppi et al., 1998), but also in ecosystems with faster organic turnover, as it is the case in Grandvillard.

5.5 CONCLUSION

Deposited N retention is very similar in both contrasted forest soils, the only significant difference is the fast and important absorption of $^{15}NH_4^+$ by the mosses of the Alptal forest.

The organic soil layers are the main sink for deposited N. In the short term and in the case of NO_3^- deposition, the deposited N partitioning is rapidly similar to that of total soil N. Since deposited NO_3^- seems rapidly behave almost as native N, we can suppose that it is going to be subjected to the same soil-plant dynamics. On a yearly scale, hydrolysability of $^{15}NH_4^+$ in Alptal is higher than hydrolysability of native N and could involve a fast microbial turnover. These conclusions have impacts for the modelling of the long-term fate of deposited N (e.g. Currie and Nadelhoffer, 1999) and should thus be further tested in this regard.

Immobilisation of deposited N in a recalcitrant pool involves fast processes, active within hours to days: we suggest that this immobilisation is effective in the longer term. However, incorporation of deposited N in the hydrolysable but not exchangeable soil pool seems to be the major pathway for the incorporation of deposited N in the soil. Biological recycling, particularly microbial recycling for the short term, must be involved in the stability of this pool.

ACKNOWLEDGMENTS

We thank Jean-David Teuscher, Valérie Morier and Alexa Wiskott for their assistance in the laboratory. We further thank Marie Sudki for the English revision.

REFERENCES

Aber J.D., Nadelhoffer K.J., Steudler P. & Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience*, **39**, 378-386.

Aber J.D. & Melillo J.M. (eds). 2001 (2nd ed.). *Terrestrial ecosystems*. Harcourt Academic Press, San Diego, USA.

A.F.E.S. (eds). 1998. *A sound reference base for soils*. Collection techniques et pratiques. 322 p. INRA editions, Paris.

Berntson G.M. & Aber J.D. 2000. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry*, **32**, 151-156.

Bremner J.M. 1965. Organic forms of soil nitrogen. In : *Methods of soil analysis, Part 2. Chemical and microbiological properties* (ed. C.A. Black), pp. 1238-1255. Agronomy 9. American Society of Agronomy, Madison, USA.

Buchmann N., Gebauer G. & Schulze E.D. 1996. Partitioning of ^{15}N-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. *Biogeochemistry*, **33,** 1-23.

Bureau F. 1995. *Evolution et fonctionnement des sols en milieu alluvial peu anthropisé*. Doctoral dissertation, Swiss Federal Research Institute of Technology (EPFL), Lausanne, Switzerland.

Chang S.X., Preston C.M. & Weetman G.F. 1999. Availability of residual N-15 in a coniferous forest soil: a greenhouse bioassay and comparison with chemical extractions. *Forest Ecology and Management,* **117**, 199-209.

Currie W.S. & Nadelhoffer K.J. 1999. Dynamic redistribution of isotopically labelled cohorts of nitrogen inputs in two temperate forests. *Ecosystems*, **2**, 4-18.

Dail D. B., Davidson E.A. & Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry*, **54**, 131-146.

DeLuca T.H., Zackrisson O., Nilsson M.-C. & Sellstedt A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature, **419**, 917-920.

Dise N.B., Matzner E., Armbruster M. & MacDonald J. 2001. Aluminium output from forest ecosystems in Europe: a regional assessment. *Journal of Environmental Quality*, **30**, 1747-1756.

Fitter A.H. (Ed.). 1985. *Ecological interactions in soil. Plants, microbes and animals*. Blackwell Scientific Publication, Oxford, UK.

Fitzhugh R.D., Lovett G.M. & Venterea R.T. 2003. Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biology*, **9**, 1591-1601.

Food and Agricultural Organization of the United Nations. 1989. Soil map of the world, revised legend. FAO-UNESCO, Rome, Italy.

Gebauer G., Zeller B., Schmidt G., May C., Buchmann N., Colin-Belgrand M., Dambrine E., Martin F., Schulze E.-D. & Bottner P. 2000. The fate of ^{15}N-labelled nitrogen inputs to conif-

erous and broadleaf forest. In: *Carbon and nitrogen cycling in european forest ecosystems* (ed. E.-D. Schulze), pp. 144-170. Ecological Studies 142. Springer-Verlag, Berlin, Heidelberg, Germany.

Gerzabek M.H., Haberhauer G., Stemmer M., Klepsch S. & Haunold E. 2004. Long-term behaviour of ^{15}N in an alpine grassland ecosystem. *Biogeochemistry,* **70**, 59-69.

Gundersen P., Emmett B.A., Kjønaas O.J., Koopmans C.J. & Tietema A. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management*, **101**, 37-55.

Hart S.C., Firestone M.K., Paul E.A. & Smith J.L. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry*, **25**, 431-442.

Jokic A., Schulten H.-R., Culter J. N., Schnitzler M. & Huang P.M. 2004. A significant abiotic pathway for the formation of unknown nitrogen in nature. *Geophysical research letters*, **31**, L05502, doi: 10.1029/2003GL018520.

Johnson D.W., Cheng W. & Burke I.C. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal*, **64**, 1503-1514.

Johnsson L., Berggren D. & Kårén O. 1999. Content and bioavailability of organic forms of nitrogen in the O horizon of a podzol. *European Journal of Soil Science*, **50**, 591-600.

Killham K. (ed.). 1994. *Soil ecology.* Cambridge University Press.

Knicker H. & Hatcher P.G. 1997. Survival of protein in an organic-rich sediment: Possible protection by encapsulation in organic matter. *Naturwissenschaft*, **84**, 231-234.

Lamontagne S., Schiff S.L. & Elgood R.J. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. *Canadian Journal of Forest Research*, **30**, 1165-1177.

Leinweber P. & Schulten H.-R. 2000. Nonhydrolyzable forms of soil organic nitrogen: extractability and composition. *Journal of Plant Nutrition and Soil Science*, **163**, 433-439.

Morier I., Guenat C., Siegwolf R., Védy J.-C. & Schleppi P. Submitted. Dynamics of atmospheric N deposition in a temperate calcareous forest soil. *Journal of Environmental Quality.*

Muller N. 1997. *Short-term response of the ground vegetation in a montane forest ecosystem under increased nitrogen deposition – Influence of light and competition.* Doctoral thesis 12388, Swiss Federal Research Institute of Technology (ETH), Zurich, Switzerland.

Nadelhoffer K.J., Downs W.R. & Fry B. 1999a. Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. *Ecological Applications*, **9**, 72-86.

Nadelhoffer K.J., Emmet B.A., Gundersen P., Kjønaas O.J., Koopmans C.J., Schleppi P., Tietema A. & Wright R.F. 1999b. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**, 145-147.

Oechel W.C. & Van Cleve K. 1986. The role of bryophytes in nutrient cycling in the taiga. In: *Forest Ecosystems in the Alaskan Taiga, a synthesis of structure and function* (Eds. K. Van Cleve, F.S. Chapin, P.W. Dlanagan, L.A. Viereck & C.T. Dyrness), pp. 121-137. Springer-Verlag, Berlin, Heidelberg, Germany.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. *Ecology*, **82**, 2245-2260.

Perakis S.S., Compton J.E. & Hedin L.O. 2005. Nitrogen retention across a gradient of ^{15}N additions to an unpolluted temperate forest soil in Chile. *Ecology,* **86**, 96-105.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2005. Flow of deposited inorganic N in two Gleysol-dominated mountain catchments traced with $^{15}NO_3^-$ and $^{15}NH_4^+$. *Biogeochemistry*, **76**, 453-475.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2006. 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. *Soil Biology and Biochemistry*, in press.

Schleppi P., Muller N., Feyen H., Papritz, A., Bucher J.B. & Flühler H. 1998. Nitrogen Budget of two small experimental forested catchments at Alptal, Switzerland. *Forest Ecology and Management*, **101**, 177-185.

Schleppi P., Bucher-Wallin I., Siegwolf R. Saurer M., Muller N. & Bucher J.B. 1999. Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N. *Water, Air and Soil Pollution*, **116**, 129-134.

Schulten H.-R. 1994. A chemical structure for humic acid. Pyrolysis-gaschromatography/mass spectrometry and pyrolysis-soft ionization mass spectrometry evidence. In: *Humic Substances in the Global Environment and Implications on Human Health* (eds N. Senesi & T.M. Milano), pp. 43-56. Elsevier Science, Amsterdam.

Schulten H.-R., Sorge-Lewin C. & Schnitzer M. 1997. Structure of „unknown" soil nitrogen investigated by analytical pyrolysis. *Biology and Fertility of Soils*, **24**, 249-254.

Schulze E.-D. & Freer-Smith P.H. 1991. An evaluation of forest decline based on field observations focused on Norway spruce *Picea abies*. P*roceedings of the Royal Society of Edinburgh*, **97**, 155-168.

Stevenson F.J. 1982. Organic forms of soil nitrogen. In: *Nitrogen in agricultural soils* (ed. F.J. Stevenson), pp. 67-122. Agronomy 22. American Society of Agronomy, Madison, USA.

Stevenson F.J. & Cole M.A (Eds). 1992. *Cycles of soil - carbon, nitrogen, phosphorus, sulfur, micronutrients*. John Wiley & Sons, New York, USA.

Sowden F.J., Chen Y. & Schnitzer M. 1977. The nitrogen distribution in soils formed under widely differing climatic conditions. *Geochimica et Cosmochimica Acta*, **41**, 1524-1526.

Vitousek P.M., Aber J.D., Horwath R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. & Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737-750.

Zang X., Nguyen R.T., Harvey H.R., Knicker H. & Hatcher P.G. 2001. Preservation of proteinaceous material during the degradation of the green alga *Botryococcus braunii*: A solid-state 2D ^{15}N ^{13}C NMR spectroscopy study. *Geochimica et Cosmochimica Acta*, **65**, 3299-3305.

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology*, **81**, 1858-1866.

CHAPTER 6

6 N-15 IMMOBILIZATION IN FOREST SOIL: A STERILIZATION EXPERIMENT COUPLED WITH N-15 CPMAS NMR SPECTROSCOPY

In preparation for Soil Biology & Biochemistry

Isabelle Morier Jaquet[1,4], Claire Guenat[1], Rolf Siegwolf[2], Heike Knicker[3], Patrick Schleppi[4]

[1]*Swiss Federal Institute of Technology (EPFL), Laboratory of Soil and Environmental Physics, CH-1015 Lausanne, Switzerland*

[2]*Paul Scherrer Institute (PSI), Laboratory of Atmospheric Chemistry, CH-5232 Villigen-PSI, Switzerland*

[3]*Technische Universität München (TUM), Lehrstuhl für Bodenkunde, D-85350 Freising, Germany*

[4]*Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), CH-8903 Birmensdorf, Switzerland*

In temperate forests, soils are the main sink for atmospheric N deposition. The main processes proposed for N retention are the microbial immobilization and the abiotic fixation in soil organic matter. The relative importance of such processes as well as the kind of resulting chemical compounds are not totally resolved. In order to improve our understanding on the subject, we carried out a laboratory incubation of sterilized and unsterilized soils (organic and organo-mineral), labeled with $^{15}NO_3^-$ or $^{15}NH_4^+$, which are the main form of N deposition. Soils were incubated during one hour, one day and one week times and then subjected to a K_2SO_4 extraction and to ^{15}N CPMAS spectroscopy measurements. After one hour of incubation, immobilization was already effective within all the incubated soils. The corresponding NMR spectra were difficult to interpret since the signal-to-noise ratio was low. However, part of the immobilized ^{15}N was already incorporated as amides. In the sterilized soils labeled with $^{15}NH_4^+$, a chemical process connected with the presence of Hg, immobilized the tracer rapidly and massively (between 80% to 90% were unextractable after one hour in the organic sterilized soils against 50% in the unsterilized). However, no corresponding specific peak was observable on the NMR spectra. In the sterilized soils labeled with $^{15}NO_3^-$, between one half and one third of the added tracer was immobilized during the first hour and then, in the organic layer, 10% more during the week. We suppose, on the one hand, that the incomplete sterilization in the very short term explains the one-hour immobilization; on the other hand, an abiotic process seems to be responsible for the NO_3^- immobilization over the week. In the unsterilized soils, approximately 50% of ^{15}N was immobilized in the OL-A samples (approximately 40% in the Aca layer) during the first hour and approximately 80% during the week (approximately 60% in the Aca layer). The dynamics of immobilization were very similar for $^{15}NH_4^+$ and $^{15}NO_3^-$, mainly immobilized as amides. Within the framework of our study and because of the low signal-to-noise ratio obtained by ^{15}N NMR measurements, the rate of ^{15}N immobilized as amide could not be quantified. However, we showed that the amides-peptides signal was dominant whichever layer is concerned, or chemical form added or even whether the soil is sterilized or not. Consequently, we are able to confirm the importance of the proteinaceous compounds for the immobilization of N in the soil.

6.1 INTRODUCTION

Atmospheric N deposition strongly increased during the last decades. Such increase is mainly due to human activities and in particular, the agriculture practices as well as the combustion processes. Therefore, increasing N amounts are deposited to the natural or other unfertilized terrestrial ecosystems as inorganic compounds, mainly NO_3^- (from NO_X emitted by combustion processes) and NH_4^+ (from NH_3 emitted by agricultural activities). Previous studies concerning the impacts of N deposition on terrestrial temperate ecosystems have shown that the soil acts as a main sink and also that organic layers immobilize most of the deposited N (Buchmann et al., 1996; Nadelhoffer et al., 1999; Schleppi et al., 1999; Lamontagne et al., 2000; Providoli et al., 2006; Morier et al., submitted). However the processes involved in N immobilization in the soil, as well as their chemically resulting forms, are not completely resolved. The two main processes that seem to be involved in N retention in the soil are i) the microbial immobilization and ii) the direct and abiotic fixation on the soil organic matter (He et al., 1991; Hart et al., 1993; Dail et al., 2001; Fitzhugh et al., 2003). Such processes are active within a time framework from hours to days following the N deposition event (Bernston & Aber, 2000; Perakis et al., 2001; Providoli et al., 2006; Morier et al., submitted).

Looking forward to highlighting the relative importance of biotic and abiotic fixation of N in soil, the comparison of N immobilization in sterilized and unsterilized soil is an efficient method. Several techniques for soil sterilization are autoclaving, irradiation or use of biocides (Johnson et al., 2000; Dail et al., 2001). The use of $HgCl_2$ as the sterilizing agent seems to be the best adapted because it produces the fewest changes in the soil chemical and physical properties with no significant effects on nutrients (Wolf et al., 1989; Wolf & Skipper, 1994). In parallel, ^{15}N CPMAS NMR spectroscopy is a powerful tool used in soil organic N studies (Knicker et al., 1993; Knicker & Lüdemann, 1995; Mathers et al., 2000; Knicker, 2004; Dieckow et al., 2005) and allows the description of the chemical forms involved in the ^{15}N immobilization in organic and soil samples.

To improve our knowledge of the processes and the chemical forms involved in the N immobilization in the soil, we coupled both i) ^{15}N labeling (as $^{15}NH_4^+$ and $^{15}NO_3^-$) of soils sterilized (Hg-treated) or unsterilized and ii) NMR spectroscopy measurements. We tracked the ^{15}N tracer after one hour, one day and then six days. We were more precisely interested in i) the very short term (hours to days) dynamics of ^{15}N immobilization in the soil; ii) the identification of biotic and abiotic processes responsible for this immobilization; iii) the influence of the added chemical compounds ($^{15}NH_4^+$ or $^{15}NO_3^-$) on this immobilization; iv) the main chemical forms in which ^{15}N is immobilized.

6.2 MATERIALS AND METHODS

6.2.1 SITE DESCRIPTION

Incubated soils were collected within the riparian zone of La Sarine River. The site, named Grandvillard, is a beech-grove forest (*Fagus sylvatica* L.) mixed with planted spruces. The parent material consists of alluvial grave and the water table is very deep, at approximately 4 m. Soil is of well drained calcareous Fluvisol type (FAO, 1989) or of *Fluviosol carbonaté* one (AFES, 1998) and its profile consists of an OL layer (a few millimeters), a loamy Aca layer (0-25 cm) and a silty loamy Sca layer (26-50 cm) which lies above a sandy bed and the alluvial grave. Humus is of calcareous mull type with a fast organic matter turnover. For further details, see Bureau (1995).

6.2.2 EXPERIMENTAL DESIGN

The experiment consisted of a randomized block design with 4 factors and 2 replications. The first factor was the soil layer, either OL, OL-Aca (grouping the 1-2 first centimeters under the litter and containing partly decomposed litter and soil aggregates) or Aca (collected between 4 and 10 cm). The second experimental factor was soil sterilization, either with Hg or unsterilized. The third factor was the tracer, either $^{15}NH_4^+$, $^{15}NO_3^-$ or none. Finally, the fourth factor was the tracking-time allowed between tracer application and sampling, 1 h, 1 d or 6 d.

Because of the importance of the manipulations, the whole experiment could not be performed in one shot. It was thus done in 10 batches. The first 5 batches corresponded to replication 1 while the second corresponded to replication 2. Within these groups, the combinations of experimental factors were assigned at random.

6.2.3 SAMPLES PREPARATION AND TREATMENTS

From October 2004 to March 2005 soils were collected in the field, prepared during the same day and labeled within the next morning. The little branches and fresh vegetation were removed from the litter. For the litter samples and for homogeneity's purpose, we cut the leaves in polygons of approximately 1cm^2 each. Roots, fresh mosses and little branches were taken out of the OL-Aca samples. Aggregates larger then 5 mm in diameter were crumbled; roots from the Aca samples were removed and then, soil was passed through a 5 mm sieve.

All experimental batches were incubated at the same moisture conditions after the addition of a tracer and/or sterilisation solution (gravimetric humidity relative to the dry mass: OL: 225%; OL-Aca: 150%; Aca: 45%). The field moisture of each batch was first measured by drying overnight a subsample of each layer at 105°C. Samples were generally too moist compared to the target values. They were thus slowly dried at 30-40°C for 4-6 hours, further if necessary overnight at room temperature. Inversely, if the samples were too dry, deionized water was added.

Fresh samples equivalent to the following dry masses were weight and separately placed in a 500 ml glas vials: 5-9 g for OL samples; 26-29 g for OL-Aca samples; 95-120 g for Aca samples.

^{15}N was added to the soil samples either as $^{15}NH_4Cl$ or $K^{15}NO_3$ dissolved in deionized water (unsterilized samples) or in a solution of mercuric chloride ($HgCl_2$) (sterilized samples). Unlabelled samples were treated with deionized water or with the solution of $HgCl_2$. All solutions were added with a syringe and samples were then mixed to dispatch them. Tracers were added at a rate of 0.04 mg ^{15}N per g of dry matter. $HgCl_2$ was added at 13.5 mg per g of dry matter, as proposed by Fitzhugh et al. (2003) and in order to achieve effective inhibition of microbial metabolism.

6.2.4 INCUBATION

Immediately after the addition of the solutions, the vials were hermetically closed with a lid fitted with a septum, then incubated in the darkness at a temperature ranging between 19°C and 23°C. The forth day, we injected between 100 and 200 ml of ambient air in the vials by a means of syringe inserted to through the septum, so as to prevent anaerobic conditions.

6.2.5 SAMPLE PREPARATION AFTER INCUBATION

After 1 hour, 1 day and 6 days, a sample of each applied treatment was processed as follows: 25 g of the OL-Aca and Aca samples were weighted in a bottle and a K_2SO_4 extraction (80 ml 5M) was immediately performed. Those extracts were then kept in the freezer. The remaining soils were directly frozen through liquid nitrogen and kept in the freezer as well. We did no extraction on the OL samples and they were directly frozen. All the samples were then freeze dried.

Dry OL samples were then ground with a Retsch Ultra Centrifugal Mill ZM1 (0.5 mm) (Retsch, Haan, Germany) while soil samples (OL-Aca and Aca layers) with a Retsch Mortar Grinder RM 100 (Retsch) but the extracts were ground by hand.

All the extracts samples were analyzed to measure N concentration and $^{15}N/^{14}N$ ratio. Some chosen litter and soil samples (including both labeling of sterilized and unsterilized samples) were analyzed in order to test the rate of recovered tracers. Isotopic analyses were conducted at the Paul Scherrer Institute, Laboratory of Atmospheric Chemistry where samples were combusted in an elemental analyser (EA 1108, Finnigan, Bremen, Germany) connected to an isotope ratio mass spectrometer (DeltaS, Finnigan, Bremen, Germany) to determine the $^{15}N/^{14}N$ ratio of the sample.

6.2.6 NMR METHODS

The solid-state ^{15}N NMR spectra were obtained on a Bruker DMX 400 operating at 40.56 MHz, using a Bruker double-bearing probe with 7-mm outer diameter, phase-stabilized zirconium dioxide rotors. To increase the sensitivity, the samples containing material from the OL-Aca and Aca layer were treated with hydrofluoric acid (10% w/w) for 12 h according to Knicker et al. (1999). After centrifugation, the supernatants were siphoned off and discarded. The procedure was repeated five times at room temperature. The remaining sediment was washed five times with deionized water and freeze dried. For the NMR measurements, the cross-polarization (CP) magic-angle spinning (MAS) technique with a ramped ^{1}H-pulse was used during a contact time of 0.7 ms in order to circumvent Hartmann-Hahn mismatches (Peersen et al., 1993; Cook et al., 1996). The pulse delay was of 250 ms and line broadenings between 100 and 200 Hz were applied. Between 5 and 30×10^5 scans were accumulated at a magic-angle spinning speed of 4.5 kHz. The chemical shift was standardized to the nitromethane scale (0 ppm) and adjusted with ^{15}N-labeled glycine (-347.6 ppm). Signal assignment was performed according to Witanowski et al. (1993) and Knicker (2000). Due to the low signal-to-noise ratio of the solid-state ^{15}N NMR spectra, they were not quantified.

6.2.7 CALCULATIONS AND STATISTICAL ANALYZES

The recovered ^{15}N in the soil as well as in the soil extracts was calculated by multiplying the soil N quantity in the soil, respectively in the extract, by the atom % excess (i.e. measured abundance minus natural abundance of ^{15}N). The recovered ^{15}N was expressed as a rate of the total added label (for more details, see Providoli et al., 2005).

We calculated a general linear model with repeated measures (S-PLUS 6.1 – Mathsoft inc.) to test the concentrations of extractable N and the rates of ^{15}N recovery in the extracts for effects of the sterilization (Hg-sterilized or unsterilized samples), of the sampling time (1 hour, 1 day or 6 days, log-transformed) and of the tracers ($^{15}NO_3^-$, $^{15}NH_4^+$). We also tested the effects of the interactions between those factors. When testing N concentrations for effect of

the tracers, we also included the unlabelled samples. Log transformation of the dependent variables was used to improve the normality in the data when necessary. When this transformation was not effective, we used a Kruskall-Wallis rank test.

6.3 RESULTS

6.3.1 DYNAMICS OF EXTRACTABLE N DURING THE INCUBATION EXPERIMENT

The concentrations of extractable N evolved in a very different way in the Hg-sterilized compared to the unsterilized samples (effect of interaction between time and sterilization: p = 0.00 (OL-Aca and Aca layers)) *(fig. 6.1, p. 59)*. Within unsterilized soil samples of both layers, extractable N concentration was mostly constant within the time framework and we observed no differences between both labelings and also the unlabeled samples. Values were within the range of 0.05 to 0.10 mg/g for the OL-Aca samples and of 0.03 to 0.07 mg/g for the Aca samples. Within the sterilized soil layer, concentrations increased over the week. In the OL-A layer, such increased from 0.10 to 0.14 mg/g following $^{15}NO_3^-$ tracer application, from 0.05 to 0.16 mg/g following $^{15}NH_4^+$ and from 0.04 to 0.15 mg/g in the unlabeled samples. For the Aca layer, concentration also increased over the week ($^{15}NO_3^-$: from 0.03 to 0.05 mg/g; $^{15}NH_4^+$: from 0.01 to 0.03 mg/g; control: from 0.01 to 0.03 mg/g).

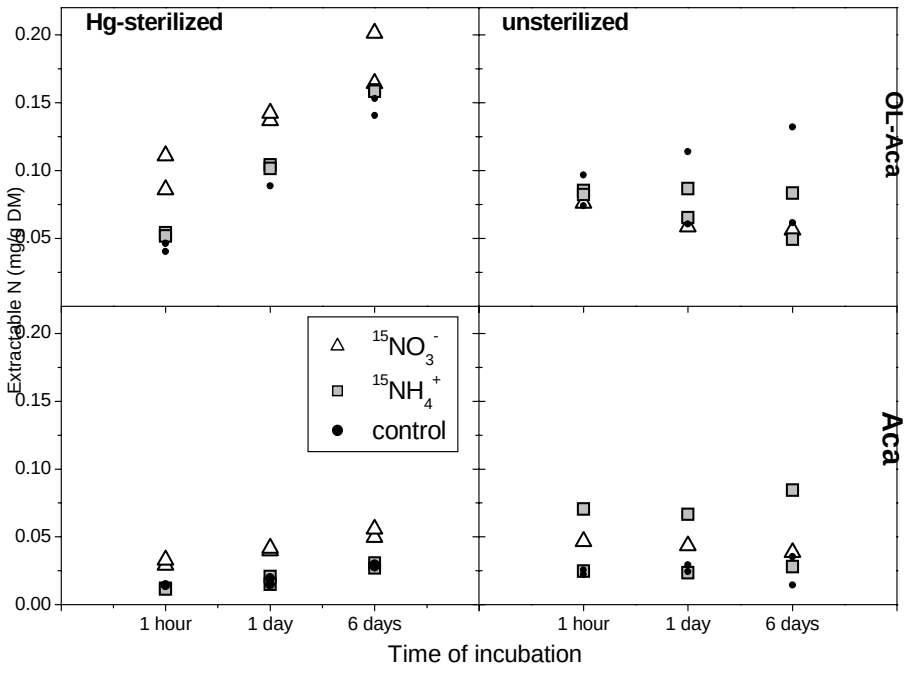

Fig. 6.1 : Total extractable N (in mg / g of dry matter) in the OL-Aca and Aca samples, Hg-sterilized and unsterilized.

6.3.2 ^{15}N RECOVERY RATES

Total ^{15}N recovery rates in the soil samples were all between 0.8 and 1.2 (average value : 0.99 ± 0.01(SE)). The recovery rates in the extractable fraction are presented in *fig. 6.2 (p. 60)*. Both tracers reacted differently to sterilization (effect of interaction between the tracers and the sterilization: p = 0.00). In the unsterilized OL-Aca layer, we did not observe a difference in the recovered extractable ^{15}N between labeling. In the framework time, recovery rates evolved from the range of 50% to the range of 20%. In the Aca samples, we did not observe differences in the recovery rates within time or labeling.

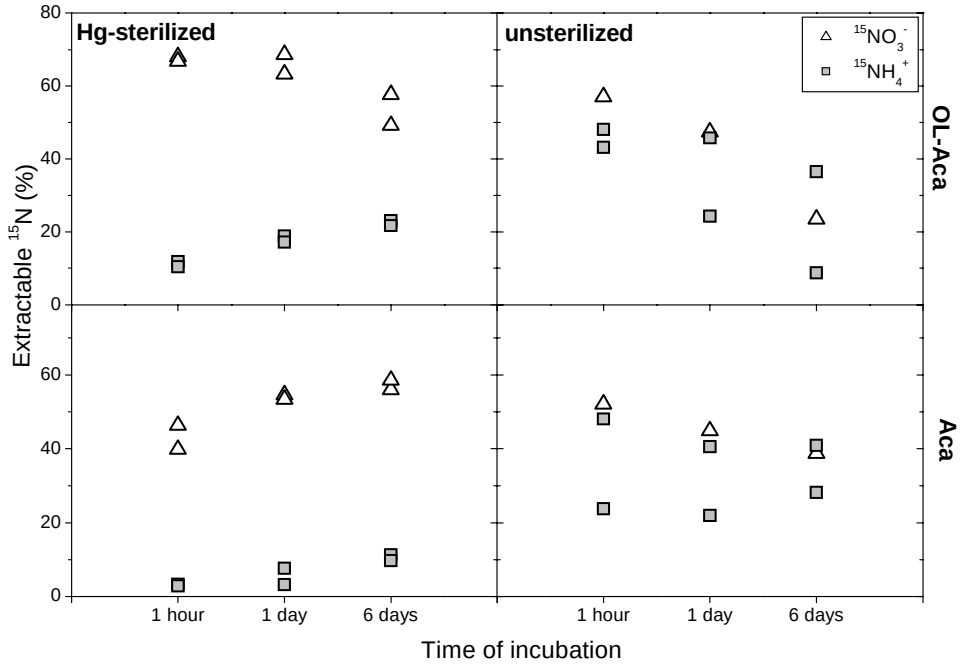

Fig. 6.2 : Recovery rates of extractable ^{15}N in the OL-Aca and Aca samples, Hg-sterilized and unsterilized.

In the Hg-sterilized samples, ^{15}N recovered as extractable N varied very much, depending on the tracer. It was the case in the OL-Aca as well as in the Aca layer. For $^{15}NO_3^-$ application and in the OL-Aca layer, more than 60% were recovered as extractable N after one hour incubation time. We observed similar rates after one day and slightly decreased during the week *(fig. 6.2, p. 60)*. In the Aca layer, approximately 40% were recovered after one hour and rates increased up to 60% after one week. For the $^{15}NH_4^+$ application, values were much lower. In the OL-Aca layer, only 10% of ^{15}N were still extractable after one hour incubation time. Rates increased up to 20% during the first day and remained stable over the week. In the Aca layer, rates were even smaller, evolving from less than 5% to approximately 10% during the week.

6.3.3 SOLID-STATE ^{15}N CPMAS NMR SPECTROSCOPY

Due to the low sensitivity of solid-state ^{15}N NMR spectroscopy, we limited this application to various representative series that also covered the whole of the soil layers (OL, OL-Aca and Aca), the incubation duration (1 hour, 1 day and 6 days), the tracers ($^{15}NH_4^+$ and $^{15}NO_3^-$) and the sterilization (Hg-sterilized or not). *Fig. 6.3 (page 61)* shows 2 time series; the first one, NMR spectra corresponding to the unsterilized OL-A layer labeled with $^{15}NO_3^-$, one hour and one day after the beginning of the incubation; the second one, NMR spectra corresponding to the unsterilized OL layer labeled with $^{15}NH_4^+$ and incubated during 1 day and 1 week.

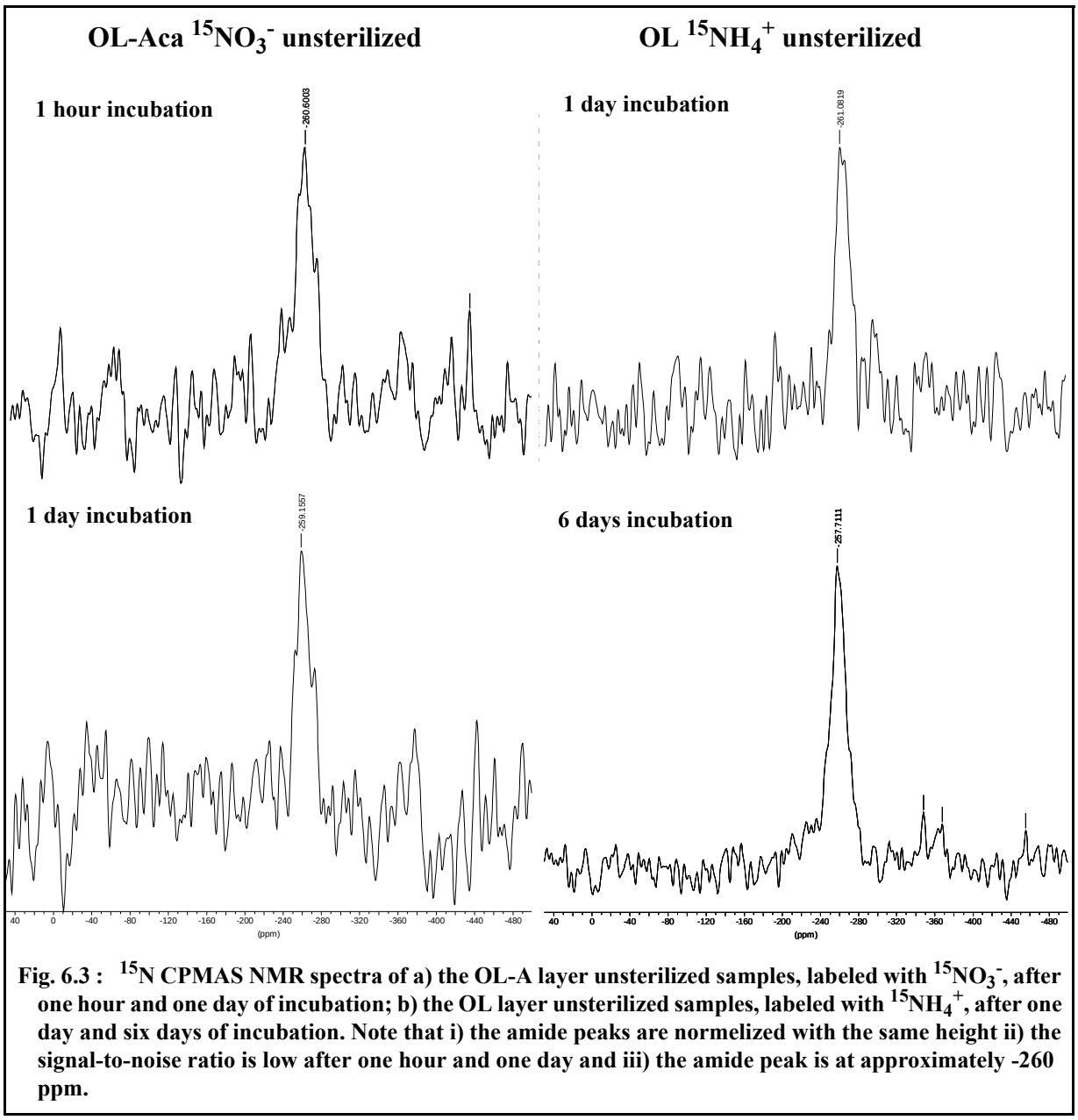

Fig. 6.3 : ^{15}N CPMAS NMR spectra of a) the OL-A layer unsterilized samples, labeled with $^{15}NO_3^-$, after one hour and one day of incubation; b) the OL layer unsterilized samples, labeled with $^{15}NH_4^+$, after one day and six days of incubation. Note that i) the amide peaks are normelized with the same height ii) the signal-to-noise ratio is low after one hour and one day and iii) the amide peak is at approximately -260 ppm.

The signal-to-noise ratio was low after one hour of incubation despite the number of scans (about three millions) or the HF pre-treatment. Here one has to consider that some of the ^{15}N intensity derived from the N fraction already occurring in the unlabeled samples. As shown in *fig. 6.6 (p. 64)*, their N-forms leads to a signal intensity in the chemical shift region that is assi-

gnable to amide/peptide N (-260 ppm). However, the spectral quality improved with increasing incubation time, showing more distinctly after one week of incubation, the largest peak located in the chemical shift region of -220 to -280 p.p.m. indicating an increase of ^{15}N in the amide-peptide structures.

fig. 6.4 (p. 62) presents NMR spectra deriving from to the sterilized OL layer after 6 days of incubation with either $^{15}NO_3^-$ or $^{15}NH_4^+$.

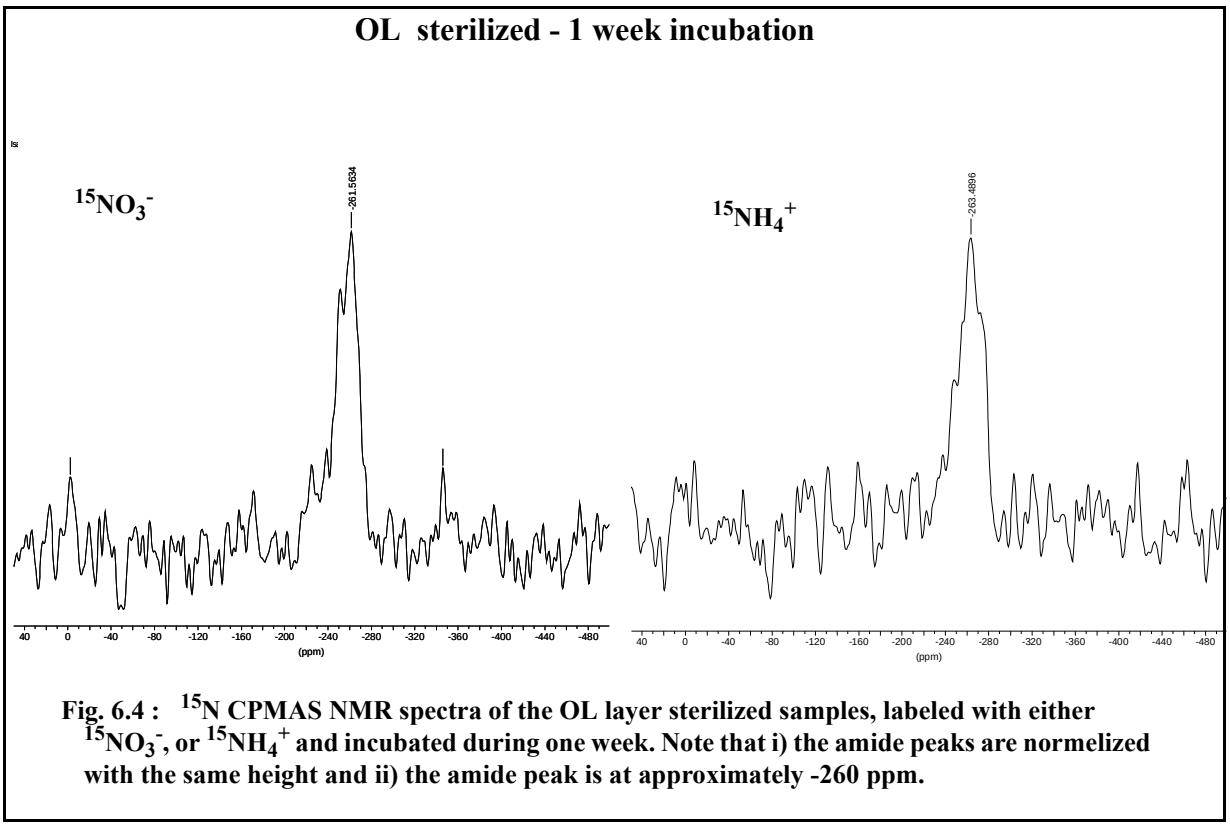

Fig. 6.4 : ^{15}N CPMAS NMR spectra of the OL layer sterilized samples, labeled with either $^{15}NO_3^-$, or $^{15}NH_4^+$ and incubated during one week. Note that i) the amide peaks are normelized with the same height and ii) the amide peak is at approximately -260 ppm.

Those spectra were dominated by the signal attributed to amide N, but showed no major qualitative differences. Comparable results were obtained from the NMR analysis of Hg-sterilized and unsterilized samples in Aca soil samples labeled with $^{15}NH_4^+$ and with $^{15}NO_3^-$ *(fig. 6.5, p. 63)*. The presented spectra were obtained after one week of incubation time. Most of the detectable ^{15}N-signals were again assigned to amide–peptide structures. No qualitative differences were observable between the sterilized and the unsterilized samples, neither for the $^{15}NH_4^+$ nor for the $^{15}NO_3^-$-labeled samples.

In all the spectra, no signal was visible in the chemical shift region assignable to $^{15}NH_4^+$ (-358 ppm) and $^{15}NO_3^-$ (25 to -25 ppm). One explanation is that the mobility properties of these ions result in inefficient cross polarization to a depression of their signal below the intensity of the noise level.

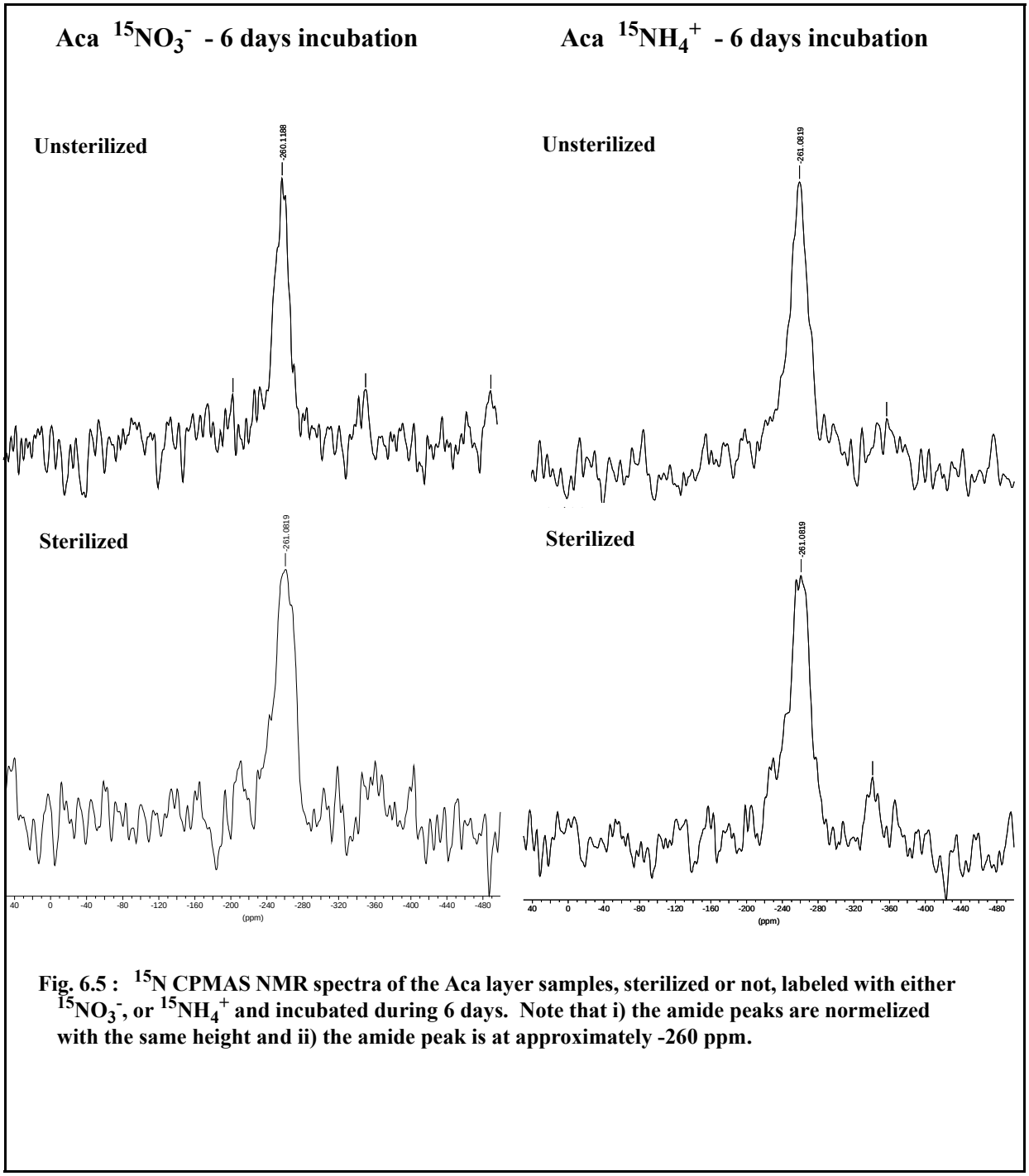

Fig. 6.5 : ^{15}N CPMAS NMR spectra of the Aca layer samples, sterilized or not, labeled with either $^{15}NO_3^-$, or $^{15}NH_4^+$ and incubated during 6 days. Note that i) the amide peaks are normelized with the same height and ii) the amide peak is at approximately -260 ppm.

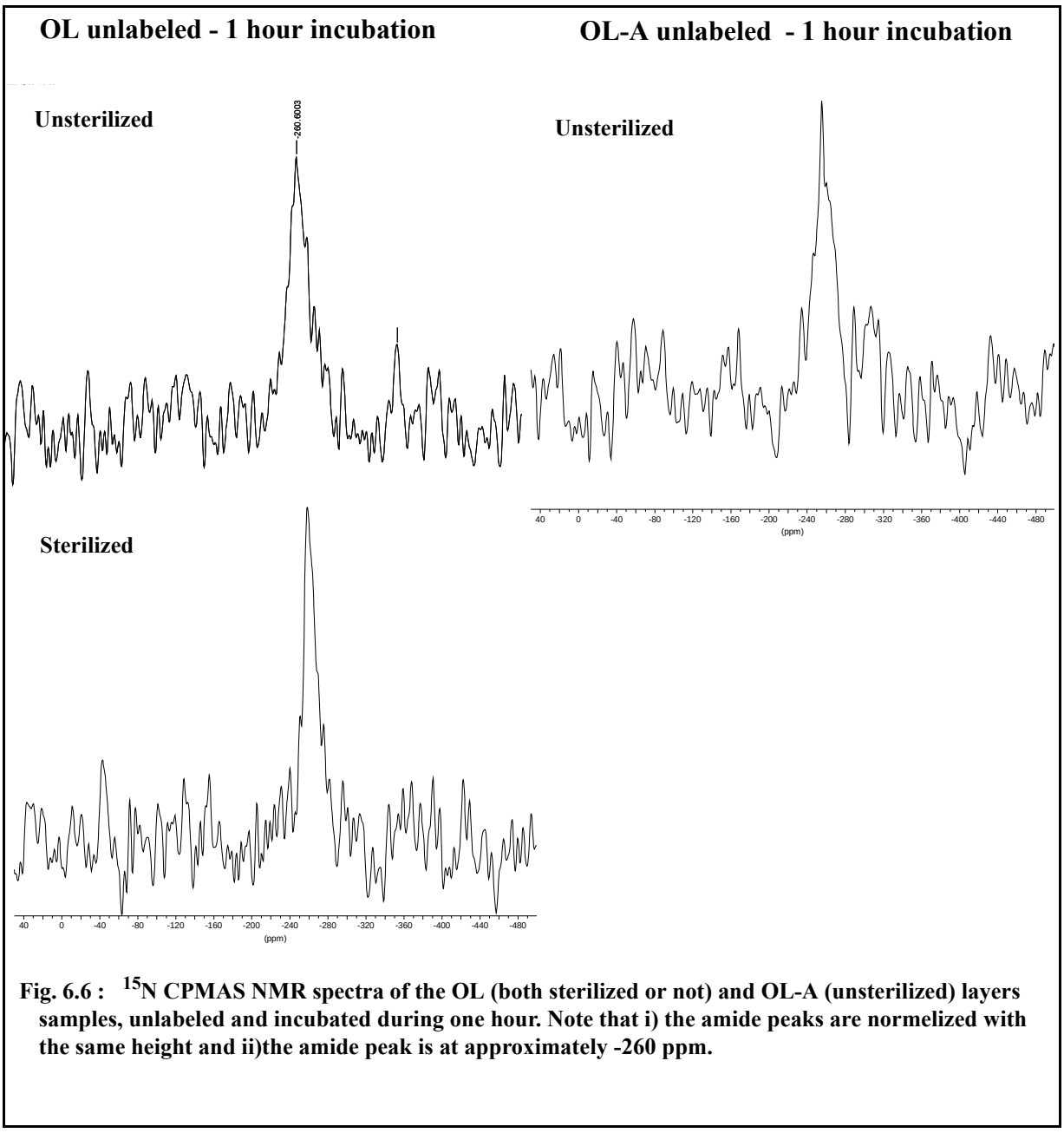

Fig. 6.6 : ^{15}N CPMAS NMR spectra of the OL (both sterilized or not) and OL-A (unsterilized) layers samples, unlabeled and incubated during one hour. Note that i) the amide peaks are normelized with the same height and ii)the amide peak is at approximately -260 ppm.

6.4 DISCUSSION

6.4.1 DYNAMICS OF THE EXTRACTABLE N IN INCUBATED SOIL SAMPLES

In the unsterilized OL-Aca layer and after one hour incubation time, the extractable N concentrations in the ^{15}N–labeled samples are already similar to that of the unlabeled ones meaning that the soil immobilized a quantity corresponding to that added as ^{15}N (0.04 mg / g of dry matter). In unfertilized forest ecosystems, N is a limiting factor for biological growth and this fast N incorporation could be due to a microbial immobilization. Furthermore, in our laboratory incubation experiments, the temperature and moisture conditions were optimal and the samples

considered highly organic. The fact that the incorporation of the same quantity of ^{15}N takes more time (i.e. one week) in the Aca samples, less organic, leads to suppose that the organic matter is strongly involved in the immobilization process.

In the sterilized samples, the increase of extractable N is important over one week incubation time and is most tentatively explained with the release of labile N compounds following the death and the lyses of the microbial cells. In the OL-Aca organic layer, this increase is similar to the quantity of N extracted following a chloroform fumigation in similar soil samples (0.1 mg N/g of dry matter (Morier et al., submitted)). Using the conversion factor (K_{EN} = 0.54) proposed by Brookes et al. (1985) and widely admitted for the estimation of the efficiency of chloroform fumigation, it would mean that, during the present incubation, half of the microbial N has become extractable in the sterilized organic samples during one week incubation time.

6.4.2 SHORT TERM DYNAMICS OF ^{15}N IMMOBILIZATION

The fast ^{15}N immobilization processes, active within hours to days and described in previous studies (Hart et al., 1993; Bernston & Aber, 2000; Johnson et al., 2000; Zogg et al., 2000; Dail et al., 2001; Perakis & Hedin, 2001; Providoli et al., 2006; Morier et al., submitted) are confirmed in the framework of this incubation experiment. We demonstrate that they are already observable during the first hour following the addition of tracer, since the extractable ^{15}N decreased in all the samples (whatever the soil layer concerned, the tracer added and whether the soil is sterilized or not). In parallel, in the corresponding NMR spectra, no additional signal to the dominant amide-N resonance could be clearly identified from the noise. Consequently, one can conclude that the major part of the ^{15}N immobilized in organic forms occurred in the form of amide N and was already incorporated in amides during the first hour.

6.4.3 *BIOTIC AND ABIOTIC PROCESSES OF ^{15}N IMMOBILIZATION IN THE SOIL*

In the sterilized samples labeled with $^{15}NH_4^+$, the low concentration of total extractable N and of extractable ^{15}N seems to imply a chemical process connected to the presence of Hg. As a matter of fact, one has to keep in mind that the whole tracer added was recovered into the bulk soil. This excludes the possibility of loss through volatilization. No specific peak is however observable on the corresponding NMR spectra and, consequently, it is not possible to identify the form within which part of the $^{15}NH_4^+$ was immobilized because of the presence of Hg. We suppose that this process implies H-bounds or other non-covalent chemical bounds that are not detectable by ^{15}N NMR spectroscopy. So far, we are not able to propose a chemical pathway since the soil is a complex system with many biogeochemical parameters that are not controlled. Further experiments on more simple systems will be necessary to suitably and efficiently bring answer to this question.

Since the Hg-sterilized soils have immobilized one third of the added $^{15}NO_3^-$ during the first hour, we could say that such activity is probably due to a rapid abiotic process. However, one has to keep in mind that efficient sterilization could require some more time and that, during this time, the surviving micro-organisms still assimilate ^{15}N. This process has been suggested by Fitzhugh et al. (2003) in a similar experiment using Hg as the sterilization agent. On the longer term, rates of $^{15}NO_3^-$ immobilization were low in the sterilized OL-Aca samples, reaching approximately 15% after one week. However, it is important to remember the fact that, during the same time, total extractable N increased. Joining both N and ^{15}N dynamics allows us to conclude that, if ^{15}N should be immobilized by micro-organisms still alive during the first

hour, their death will occur with an additional release of N. In this particular case, the gross ^{15}N immobilization is underestimated during the week. Controversially, if ^{15}N is abiotically immobilized, the processes implied are more effective on the short term (30% in one hour) than on the long term (more 15% in one week).

On the other hand and according to the results obtained by ^{15}N NMR spectroscopy, the only major organic N forms implied in ^{15}N immobilization are amides and peptides. Consequently, we think that 1 hour-immobilization is mainly due to microbial assimilation.

In addition, one-week $^{15}NO_3^-$ immobilization in sterilized soils leads us to propose that an abiotic process of less importance happens in parallel and is effective during that same week. This assertion is in agreement with the results of Dail et al., (2001), who observed an abiotic immobilization of $^{15}NO_3^-$ in organic soil samples, mainly in the form of dissolved organic nitrogen (DON). On the same subject, Davidson et al., (2003) proposed that this abiotic formation of DON is due to N incorporation in aromatic compounds. We are not able to identify the presence of such compounds on the NMR spectra but it does not completely exclude their presence in smaller, undetectable concentrations, especially considering the important background noise of the spectra.

6.4.4 CHEMICAL FORMS OF ^{15}N IMMOBILIZATION IN THE SOIL

We showed that all the NMR spectra obtained within the framework of this study are basically dominated by a single signal corresponding to the amide-peptide structure. The dominance of amides-peptides signals is in agreement with the observations resulting from preceding ^{15}N NMR experiments in various soils of the world and in humic substances, not only on ^{15}N-enriched material but also on samples with natural ^{15}N levels. Knicker et al., (1993) showed that, in various German soils, the main signals found for native ^{15}N corresponded to amide and peptides forms. The same patterns were observed in a subtropical Acrisol (Dieckow et al., 2005) and in Tussock Grassland soils in New Zealand (Knicker et al., 2000). In a 14-month laboratory incubation of mineral soil amended with ^{15}N-clover, DiCosty et al. (2003) observed that 85-90% of the clover-derived N was always in the amide form. In a study focused on the processes occurring during the incorporation of inorganic nitrogen into humic substances, Knicker et al. (1997) found that, over 600 days of plant residues incubation with $^{15}NH_4^+$, the major part of the detectable ^{15}N-signals was assigned to amide-peptides structures. In several ^{15}N NMR experiments, it was estimated that approximately 80% to 90% of soil ^{15}N was found in the amide form (Knicker *e*t al., 1993, 1997; Clinton et al., 1995). Those same researchers proposed that the remainder N was immobilized in the form of amino acids, amino sugars, or the amino groups of nucleic acids. The existence of ^{15}N immobilized in heterocycles was not excluded either but accounted for only some percents (Zhuo & Wen, 1992; Knicker et al., 1997). Some soils showing pyrrolic entities were shown to have suffered a burning history during which such N forms were produced by the charring process (Knicker et al., 2005). Within the framework of our study and because of the low signal-to-noise ratio, it was not possible to quantify the rate of ^{15}N immobilized as amide. We cannot completely exclude the formation of other organic molecules but, since their signals cannot be clearly identified from the noise, we can assume that their contribution is low. Furthermore, we have to bear in mind that the cross polarization and relaxation kinetics of inorganic ^{15}N does not allow their quantitative determination with the acquisition parameters that were used to optimize the detection of organic N forms (Knicker, 2000; Knicker & Lüdemann, 1995). However, we are able to show that the amides-peptides signal dominates whatever layer concerned, the chemical form added and whether the soil is sterilized or not. Consequently, we confirm the importance of the proteinaceous compounds for the immobilization of N in the soil.

REFERENCES

A.F.E.S. (eds). 1998. *A sound reference base for soils*. Collection techniques et pratiques. 322 p. INRA editions, Paris.

Berntson G.M. & Aber J.D. 2000. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry*, **32**, 151-156.

Brookes P.C., Landman A., Pruden G. & Jenkinson D.S. Chloroform fumigation and release of soil nitroger: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry*, **17**, 837-842

Buchmann N., Gebauer G. & Schulze E.D. 1996. Partitioning of ^{15}N-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. *Biogeochemistry*, **33,** 1-23.

Bureau F. 1995. *Evolution et fonctionnement des sols en milieu alluvial peu anthropisé*. Doctoral dissertation, Swiss Federal Research Institute of Technology, Lausanne, Switzerland.

Clinton P.W., Newman R.H. & Allen R.B. 1995. Immobilization of ^{15}N in forest litter studied by ^{15}N CPMAS NMR spectroscopy. *European Journal of Soil Science*, **46**, 551-556.

Cook R.L., Langford C.H., Yamdagni R. & Preston C.M. 1996. A modified cross-polarization magic angle spinning ^{13}C NMR procedure for the study of humic materials. *Analytical Chemistry*, **68**, 3979-3986.

Dail D. B., Davidson E.A. & Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry*, **54**, 131-146.

Davidson E.A., Chorover J. & Dail D.B. 2003. Global Change Biology. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. *Global Change Biology*, **9**, 228-236.

DiCosty R.J., Weliky D.P., Anderson S.J. & Paul E.A. 2003. ^{15}N-CPMAS nuclear magnetic resonance spectroscopy and biological stability of soil organic nitrogen in whole soil and particle-size fractions. *Organic Geochemistry*, **34**, 1635-1650.

Diekow J., Mielniczuk J., Knicker H., Bayer C., Dick D.P. & Kögel-Knabner I. 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil and Tillage Research*, **81**, 87-95.

Fitzhugh R.D., Lovett G.M. & Venterea R.T. 2003. Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biology*, **9**, 1591-1601.

Food and Agricultural Organization of the United Nations. 1989. Soil map of the world, revised legend. FAO-UNESCO, Rome, Italy.

Hart S.C., Firestone M.K., Paul E.A. & Smith J.L. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry*, **25**, 431-442.

He X.T., Mulvaney R.L. & Stevenson F.J. 1991. Transformations of chemically fixed liquid anhydrous ammonia by soil-microorganisms. *Biology and Fertility of Soils*, **11**, 145-150.

Johnson D.W., Cheng W. & Burke I.C. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal*, **64**, 1503-1514.

Knicker H. 2004. Stabilization of N-compounds in soil and organic matter rich sediments - What is the difference? *Marine Chemistry*, **92**,167-195.

Knicker H., Fründ R. & Lüdemann H.-D. 1993. The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften*, **80**, 219-221.

Knicker H. & Lüdemann H.-D. 1995. ^{15}N and ^{13}C CPMAS and solution NMR studies of ^{15}N enriched plant material during 600 days of microbial degradation. *Organic Geochemistry*, **23**, 329-341.

Knicker H., Lüdemann H.-D. & Haider K. 1997. Incorporation studies of NH_4^+ during incubation of organic residues by ^{15}N-CPMAS-NMR-spectroscopy. *European Journal of Soil Science*, **48**, 431-441.

Knicker H. & Kögel-Knabner I. 1998. Soil organic nitrogen formation examined by means of NMR spectroscopy. In: *Fate of N-containing macromolecules in the biosphere and geosphere* (Eds: B.A. Stankiewicz et al.), pp. 339-356. American Chemical Society Symposium Series 707. Oxford University Press, Washington, USA.

Knicker H., Saggar S., Bäumler R., McIntosh P.D. & Kögel-Knabner I. 2000. Soil organic matter transformation in tussock grassland of New Zealand by *Hieracium pilosella* L. *Biology and Fertility of Soils*, **32**, 194-201.

Knicker H., Schmidt M.W. & Kögel-Knabner I. 1999. The structure of organic nitrogen in particle size fractions determined by ^{15}N CPMAS NMR. In: *Effect of Mineral-Organic-Microorganism Interactions on Soil and Freshwater Environments* (Eds: J. Berthelin, P.-M. Huang, J.-M. Bollag & F. Andreux). Plenum Press, New York.

Knicker H. 2000. Biogenic nitrogen in soils as revealed by solid-state ^{13}C and ^{15}N nuclear magnetic resonance spectroscopy. *Journal of Environmental Quality*, **29**, 715-723.

Knicker H., González-Vila F.J., Polvillo O., González J.A. & Almendros G. 2005. Fire-induced transformation of C- and N- forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster)*. *Soil Biology and Biochemistry*, **37**, 701-718.

Lamontagne S., Schiff S.L. & Elgood R.J. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. *Canadian Journal of Forest Research*, **30**, 1165-1177.

Mathers N.J., Mao X.A., Xu Z.H., Saffigna P.G., Berners-Price S.J. & Perera M.C. 2000. Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies. *Australian Journal of Soil Research*, **38**, 769-787.

Morier I., Guenat C., Siegwolf R., Védy J.-C. & Schleppi P. Submitted. Dynamics of atmospheric N deposition in a temperate calcareous forest soil. *Journal of Environmental Quality*,

Nadelhoffer K.J., Emmet B.A., Gundersen P., Kjønaas O.J., Koopmans C.J., Schleppi P., Tietema A. & Wright R.F. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**, 145-147.

Peersen O.B., Wu X.L., Kustanovich I. & Smith S.O. 1993. Variable-amplitude cross-polarization MAS NMR. Journal of Magnetic Resonance Series A, **104**, 334-339.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. *Ecology*, **82**, 2245-2260.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2006. 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. *Soil Biology and Biochemistry*, in press.

Schleppi P., Bucher-Wallin I., Siegwolf R., Saurer M., Muller N. & Bucher J.B. 1999. Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N. *Water, Air and Soil Pollution*, **116**, 129-134.

Witanowski M., Stefaniak L. & Webb G.A. 1993. Nitrogen NMR Spectroscopy. In: *Annual Reports on NMR Spectroscopy*, *25* (ed. G. Webb), pp. 480. Academic Press, London, UK.

Wolf D.C., Dao T.H., Scott H.D. & Lavy T.L. 1989. Influence of sterilization methods on selected soil microbiological, physical and chemical properties. *Journal of Environmental Quality*, **18**, 39-44

Wolf D.C. & Skipper H.D. 1994. Soil sterilization. In: *Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties* (eds R.W. Weaver, S. Angle, P. Bottomley *et al.*), pp. 41-51. Soil Science Society of America, Madison, WI, USA.

Zhuo S.N. & Wen Q.X. 1992. Nitrogen forms in humic substances. *Pedosphere*, **2**, 307-315.

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology*, **81**, 1858-1866.

CHAPTER 7

7 SYNTHESIS

This chapter synthesises the main results obtained within the framework of this study dealing with the retention of atmospherically deposited N in the soil. The different publications (chapters 4, 5 and 6) constitute the basis of discussion for the main topics of this research, namely i) the retention of atmospherically deposited N in soil, in terms of both duration and quantity and ii) the mechanisms and processes responsible for this retention in soil. Some of the limits and perspectives are proposed as well.

1) Retention of atmospherically deposited N in soil, in terms of both duration and quantity – study of a well drained calcareous soil with fast turn over of organic matter

Within the frame of a field labelling experiment simulating N deposition, more than half of the tracers (either $^{15}NO_3^-$ or $^{15}NH_4^+$) were recovered in the soil between one hour and one year after labelling. Therefore, the general finding that non-fertilised temperate forest soils are the main sink for deposited N (Buchmann et al., 1996; Nadelhoffer et al., 1999; Hart et al., 1993; Gundersen et al., 1998; Gebauer et al., 2000; Lamontagne et al., 2000; Providoli et al., 2006) can be confirmed. Those previous studies showing the importance of the soil for the retention of deposited N were all conducted on either acidic or hydromorphous soils. Our results allow us to extend the validity of their general conclusions to a wider range of unfertilised temperate forest soils.

Furthermore, we showed that the main forms of N deposition (i.e. NO_3^- and NH_4^+) were retained in the soil within the same range of magnitude, in spite of their different biochemical pathways. Perakis & Hedin (2001) obtained similar results in a temperate forest in Chile. Inversely, in a Swiss temperate forest, Providoli et al. (2006) recovered twice less NH_4^+ than NO_3^- but considered such as being mainly an effect of a high absorption of NH_4^+ in the dense mooses layer.

N retention occured mainly in the soil organic layers (i.e. the litter layer and the above, very organic centimetres). Amounts of recovered N decreased with depth in the organo-mineral layers and was quite absent in the deeper mineral layers. In accordance with the observation of previous researchers, our results underline the importance of the organic fraction in the deposited N retention (Aber et al., 1998; Nadelhoffer et al., 1999; Schleppi et al., 1999).

We are also able to confirm that the immobilised soil N pool (ISN, as defined by Providoli et al., 2006) is the main sink for deposited N.

Tracking the deposited N over time showed that its retention in soil was effective in the very short term (i.e. one hour) and was constant in the longer term (i.e. one year) for both forms of N deposition (i.e. NO_3^- and NH_4^+).

The importance of the soil in the fate of atmospheric N deposition is demonstrated in the present study. However, one has to note that the soil may react differently to a single N tracer input or to a chronic slightly enhanced atmospheric N deposition.

2) The mechanisms and processes responsible for the retention of deposited N in soil

The very short term dynamics

Deposited N was retained in the soil in the very short term (from 1 hour to 1 day). This result allows us to confirm the presence of very fast processes already described by other searchers (Bernston & Aber, 2000; Johnson et al., 2000; Zogg et al., 2000; Dail et al., 2001; Perakis & Hedin, 2001; Providoli et al., 2006). Furthermore, we demonstrate that the very short term dynamics determine the fate of deposited N in the longer term: the main processes are i) a loss of deposited N as extractable through lixiviation (and lateral fluxes) and ii) a retention in the ISN. Such a fast loss of extractable N has not been observed in similar studies (Zog et al., 2000; Perakis & Hedin, 2001; Providoli et al., 2006). We hypothesize that the extractable ^{15}N loss on our site should be coupled with the low microbial immobilisation measured in relation to values published in other studies (Perakis & Hedin, 2001; Norton & Firestone, 1996). Low microbial activity on this site has been described by Bureau (1995). The fast turnover of organic matter and high biological activity is principally the fact of other micro- and macroorganisms (like earth worms). Our results were obtained under moderate deposition rates; under higher rates, leaching losses would probably increase, as shown in many other studies (Gundersen et al., 1998; Schleppi et al., 2004)

The relative importance of the soil organic fractions (labile or recalcitrant) in the immobilisation of N deposition. Comparison between contrasting sites.

The main sink for N deposition within the soil was the ISN pool, which basically covers N fixed in organic matter and clays. Within the framework of this study, we showed that most of the N immobilisation happened within the organic layers of the soil. Consequently and looking forward to better characterising the N immobilised in the soil, we focused our research on the soil organic matter.

Hot acid hydrolysis (Bremner, 1965) which is a method frequently used to characterise the soil organic N (Stevenson, 1982), allows the separation of N compounds in a hydrolysable fraction corresponding to the more labile N organic forms, including the most bioavailable ones (Chang *et al.*, 1999; Johnsson *et al.*, 1999) and in a non-hydrolysable fraction covering the more recalcitrant N compounds.

We demonstrate that N immobilisation brings within short-time range (i.e. one week) deposited N both into the hydrolysable and into the recalcitrant fractions of soil N. In the organic layer of both study sites and already within one week after NO_3^- deposition, this partitioning (i.e. two thirds in hydrolysates) was close to that of native soil N. Hydrolysability was within the range usually obtained for native N in unfertilised soil (according to Stevenson & Cole (1992), 65 to 80% of total N is recovered in hydrolysates).

Since deposited NO_3^- seemed to rapidly evolve towards a behaviour close to that of native N, we suggest that it would be subjected to the same soil-plant dynamics. On a yearly scale, hydrolysability of $^{15}NH_4^+$ in Alptal was higher than hydrolysability of native N and could involve a fast microbial turnover. Since incorporation of ^{15}N in the recalcitrant pool was efficient in Grandvillard, we suppose that the earth worms high activity could favour the stabilisation of relatively labile compounds through organo-mineral interactions.

Such results and conclusions suggest further developments and impacts for the modelling of the long-term fate of deposited N (e.g. Currie & Nadelhoffer, 1999) and in this regard, should thus be further tested in this regard.

In addition, the fractionation between hydrolysable and non-hydrolysable forms should be completed by investigating their chemical composition for both native and deposited N. The role of the micro- and macro-organisms in the incorporation of N in both fractions should also be improved with further experiments.

The chemical forms involved in the immobilisation of NH_4^+ and NO_3^- and the related processes (biotic or abiotic)

To improve our knowledge on the chemical forms and processes involved in the N immobilisation in the soil, we coupled, on the one hand, a laboratory incubation of soils labelled with ^{15}N, sterilised or not, and, on the other, NMR spectroscopy measurements. The comparison of N immobilisation in sterilised and unsterilised soils is an efficient method to highlight the relative importance of biotic and abiotic fixation processes. While the ^{15}N CPMAS NMR spectroscopy is a powerfull tool to describe the chemical forms involved in that immobilisation.

Within the framework of the present study, all the NMR spectra obtained were basically dominated by a single signal corresponding to the amide-peptide structure, whatever the soil layer concerned (organic or organo-mineral), the form added (NO_3^- or NH_4^+) and whether the soil was sterilised or not. Such a dominance of proteinaceous compounds is in agreement with the results obtained in humic substances and in various soils all over the world (Knicker et al., 1993; Knicker et al., 1997; DiCosty et al., 2003; Diekow et al., 2005). Within the framework of our study and because of the low signal-to-noise ratio of the spectra, we could not quantify the rate of ^{15}N immobilised as amide-peptide.

Taking into account the extractable ^{15}N dynamics during the incubation, we propose that biotic processes are dominant in the short-term N immobilisation. However, an abiotic fixation of less importance is not excluded for $^{15}NO_3^-$. Observing a similar process, Davidson et al. (2003) proposed a N incorporation in aromatic compounds. Considering the low signal-to-noise ratio of the NMR spectra, we can not totaly exclude the formation of heterocyclic compounds, but however, assume that their contribution is very low. In the presence of Hg, we observed a chemical process leading to an immobilisation of $^{15}NH_4^+$. So far, we were not able to identify the chemical pathway involved nor the resulting chemical forms, since the soil is a quite complex system with many biogeochemical parameters that are not controlled. Further experiments on simplified systems (for example, pure clay minerals or organic fractions) will be necessary to understand this process and answer this question.

All the afore-mentioned results are based on a one-week frametime. We think that it would be necessary to perform a longer term experiment to improve the quality of NMR spectra in order to quantify the rates of the immobilisation within the amide or other chemical forms.

The longer-term mechanisms responsible for the retention of deposited N retention in the soil

Rapid and stable immobilisation in the recalcitrant pool of soil organic matter seems to be one of the mechanisms responsible for the retention of deposited N in soil, since a few tens of percent of ^{15}N is immobilised in this pool within one week time and is constant over one year. For some authors, the immobilisation of N into the recalcitrant pool is an abiotic process by which N enters heterocyclic molecules (Schulten, 1994; Schulten et al., 1997; Schulten and Schnitzer, 1998). Other authors have proposed that relatively labile N compounds, such as peptides, are stabilised in soil through encapsulation in hydrophobic domains of organic matter (Knicker & Hatcher, 1997; Zang et al., 2001) or through organo-mineral interactions (Leinweber & Schulten, 2000). Therefore, N could be incorporated by the same processes as proposed above, i.e. in amino acids by micro-organisms and then, under this amide form, immobilised within the soil organic matter. Our results show that both processes could be involved: the afore-mentioned process of abiotic nitrate fixation could lead to the N immobilisation in heterocycles whereas N incorporated in amides and then stabilised in soil organic matter seems to be the major pathway.

The second mechanism of retention we are proposing, according to our results, is the biological recycling within the soil or the soil-plant system: on a short scale, microbial recycling seems to be very rapid because deposited N is mainly present under a biosynthesised form (amides), in spite of the low amounts of tracer found in the microbes. Within the soil profile, recycling through the roots is certainly efficient: fine roots, which are major contributor in soil organic matter (Fitter, 1985; Aber & Melillo, 1991; Killham, 1994), are an important sink for N deposition all over the year and show a continuous tracer uptake.

On a larger scale and in the longer term, we show that, despite vegetation and litter turnover, deposited N is constantly recovered in the organic layers, certainly due to a soil-plant system recycling. Biological recycling was also described as a key mechanism by other researchers. In this regard, conclusions about the long term retention of deposited N stated by Gerzabek et al. (2004) are particularly interesting. Thirty years after the simulation of atmospheric N deposition through a ^{15}N labelling experiment in an alpine grassland in Austria, half of the deposited N was still present in the soil and almost exclusively in the upper organic layer. Those searchers suggested that this long-term retention was due to biological recycling. We would add that it is not only the case in alpine or sub-alpine ecosystems, such as the site of Alptal in which the cycle of N is slowed down by climate and soil conditions (Schleppi et al., 1998), but also in ecosystems with faster organic turnover, i.e. Grandvillard study site.

The importance of the two proposed mechanisms (i.e. biological recycling and immobilisation in recalcitrant soil organic matter) for N retention in the soil should however be confirmed with longer-term studies (i.e. in the scale of a decade) which would widen our knowledge on biological recycling namely by following ^{15}N tracer in the soil-plant system.

OVERALL CONCLUSIONS

This study shows that a well-drained calcareous soil with organic matter fast turnover is the main sink for atmospherically deposited N, similarly to hydromorphous or acidic soils and allows to extend the validity of this assertion to a wider range of unfertilised temperate forest soils.

The combination of various scales and techniques (i.e. tracking of ^{15}N in the field, soil che-

mical extraction, soil laboratory incubations and sterilisation, ^{15}N NMR spectroscopy) allows the connection between observed mechanisms of N retention in soil organic layers and the processes responsible for it (i.e. rapid immobilisation in a recalcitrant pool and biological recycling through micro-organisms and plants). The results of this multi-scale approach suggest further developments and impacts for the modelling of the long-term fate of deposited N.

REFERENCES

Aber J.D., Nadelhoffer K.J., Steudler P. & Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience*, **39**, 378-386.

Aber J.D., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S., Currie W., Rustad L. & Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems - hypotheses revisited. *Bioscience*, **48**, 921-934.

Aber J.D. & Melillo J.M. (eds). 2001 (2nd ed.). *Terrestrial ecosystems*. 556 p. Harcourt Academic Press, San Diego, USA.

Berntson G.M. & Aber J.D. 2000. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry*, **32**, 151-156.

Bremner J.M. 1965. Organic forms of soil nitrogen. In : *Methods of soil analysis, Part 2. Chemical and microbiological properties* (ed. C.A. Black), pp. 1238-1255. Agronomy 9. American Society of Agronomy, Madison, USA.

Buchmann N., Gebauer G. & Schulze E.D. 1996. Partitioning of ^{15}N-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. *Biogeochemistry*, **33,** 1-23.

Bureau F. 1995. *Evolution et fonctionnement des sols en milieu alluvial peu anthropisé*. PhD thesis. Swiss Federal Research Institute of Technology, Lausanne, Switzerland.

Chang S.X., Preston C.M. & Weetman G.F. 1999. Availability of residual N-15 in a coniferous forest soil: a greenhouse bioassay and comparison with chemical extractions. *Forest Ecology and Management*, **117**, 199-209.

Currie W.S. & Nadelhoffer K.J. 1999. Dynamic redistribution of isotopically labelled cohorts of nitrogen inputs in two temperate forests. *Ecosystems*, **2**, 4-18.

Dail D.B., Davidson E.A. & Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry*, **54**, 131-146.

DiCosty R.J., Weliky D.P., Anderson S.J. & Paul E.A. 2003. ^{15}N-CPMAS nuclear magnetic resonance spectroscopy and biological stability of soil organic nitrogen in whole soil and particle-size fractions. *Organic Geochemistry*, **34**, 1635-1650.

Diekow J., Mielniczuk J., Knicker H., Bayer C., Dick D.P. & Kögel-Knaber I. 2005. Soil C and N stocks are affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil and Tillage Research*, **81**, 87-95.

Fitter A.H. (ed.). 1985. *Ecological interactions in soil. Plants, microbes and animals*. Blackwell Scientific Publication, Oxford, UK.

Gebauer G., Zeller B., Schmidt G., May C., Buchmann N., Colin-Belgrand M., Dambrine E.,

Martin F., Schulze E.-D. & Bottner P. 2000. The fate of ^{15}N-labelled nitrogen inputs to coniferous and broadleaf forest. In: *Carbon and nitrogen cycling in european forest ecosystems* (ed. E.-D. Schulze), pp. 144-170. Ecological Studies 142. Springer-Verlag, Berlin, Heidelberg.

Gerzabek M.H., Haberhauer G., Stemmer M., Klepsch S. & Haunold E. 2004. Long-term behaviour of ^{15}N in an alpine grassland ecosystem. *Biogeochemistry,* **70**, 59-69.

Gundersen P., Emmett B.A., Kjønaas O.J., Koopmans C.J. & Tietema A. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management*, **101**, 37-55.

Hart S.C., Firestone M.K., Paul E.A. & Smith J.L. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry*, **25**, 431-442.

Johnson D.W., Cheng W. & Burke I.C. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal*, **64**, 1503-1514.

Johnsson L., Berggren D. & Kårén O. 1999. Content and bioavailability of organic forms of nitrogen in the O horizon of a podzol. *European Journal of Soil Science*, **50**, 591-600.

Killham K. (ed.). 1994. *Soil ecology*. 242 p. Cambridge University Press, Cambridge, UK.

Knicker H., Fründ R. & Lüdemann H.-D. 1993. The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften*, **80**, 219-221.

Knicker H. & Hatcher P.G. 1997. Survival of protein in an organic-rich sediment: Possible protection by encapsulation in organic matter. *Naturwissenschaft*, **84**, 231-234.

Knicker H., Lüdemann H.-D. & Haider K.1997. Incorporation studies of NH_4^+ during incubation of organic residues by ^{15}N-CPMAS-NMR-spectroscopy. *European Journal of Soil Science*, **48**, 431-441.

Lamontagne S., Schiff S.L. & Elgood R.J. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. *Canadian Journal of Forest Research*, **30**, 1165-1177.

Leinweber P. & Schulten H.-R. 2000. Nonhydrolyzable forms of soil organic nitrogen: extractability and composition. *Journal of Plant Nutrition and Soil Science*, **163**, 433-439.

Nadelhoffer K.J., Emmet B.A., Gundersen P., Janne Kjønaas O.J., Koopmans C.J., Schleppi P., Tietema A. & Wright R.F. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**,145-147.

Norton J. M. & Firestone M.K. 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. *Soil Biology and Biochemistry*, **28**, 351-362.

Perakis S.S. & Hedin L.O. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. *Ecology*, **82**, 2245-2260.

Providoli I., Bugmann H., Siegwolf R., Buchmann N. & Schleppi P. 2006. 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. *Soil Biology and Biochemistry*, in press.

Schleppi P., Muller N., Feyen H., Papritz A., Bucher J.B. & Flühler H. 1998. Nitrogen Budget

of two small experimental forested catchments at Alptal, Switzerland. *Forest Ecology and Management,* **101**, 177-185.

Schleppi P., Bucher-Wallin I., Siegwolf R., Saurer M., Muller N. & Bucher J.B. 1999. Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N. *Water, Air and Soil Pollution*, **116**, 129-134.

Schleppi P., Hagedorn F. & Providoli I. 2004. Nitrate leaching from a mountain forest ecosystem with Gleysols subjected to experimentally increased N deposition. *Water, Air and Soil Pollution Focus*, **4**, 453-467.

Schulten H.-R. 1994. A chemical structure for humic acid. Pyrolysis-gaschromatography/mass spectrometry and pyrolysis-soft ionization mass spectrometry evidence. In: *Humic Substances in the Global Environment and Implications on Human Health* (eds N. Senesi & T.M. Milano), pp. 43-56. Elsevier Science, Amsterdam.

Schulten H.-R., Sorge-Lewin C. & Schnitzer M. 1997. Structure of «unknown» soil nitrogen investigated by analytical pyrolysis. *Biology and Fertility of Soils*, **24**, 249-254.

Schulten H.-R. & Schnitzer M. 1998. The chemistry of soil organic nitrogen : a review. *Biology and Fertility of Soils*, **26**, 1-15.

Stevenson F.J. 1982. Organic forms of soil nitrogen. In: *Nitrogen in agricultural soils* (ed. F.J. Stevenson), pp. 67-122. Agronomy 22. American Society of Agronomy, Madison, USA.

Stevenson F.J. & Cole M.A (Eds). 1992. *Cycles of soil - carbon, nitrogen, phosphorus, sulfur, micronutrients*. John Wiley & Sons, New York, USA.

Zang X., Nguyen R.T., Harvey H.R., Knicker H. & Hatcher P.G. 2001. Preservation of proteinaceous material during the degradation of the green alga *Botryococcus braunii*: A solid-state 2D ^{15}N ^{13}C NMR spectroscopy study. *Geochimica et Cosmochimica Acta*, **65**, 3299-3305.

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology*, **81**, 1858-1866.

CURRICULUMVITAE

Isabelle Morier Jaquet

née le 1er juillet 1963, de nationalité suisse

Biologiste et diplômée en Sciences Naturelles de l'Environnement

e-mail: isabelle.morier@bluewin.ch

FORMATION

- 2002-2006 Doctorat **(Ecole Polytechnique Fédérale de Lausanne - EPFL)**
- 1999-2000 Diplôme en Sciences Naturelles de l'Environnement **(Universités de Lausanne et Genève)**
- 1993-1999 Licence en Biologie **(Université de Lausanne)**

EXPÉRIENCE PROFESSIONNELLE

- 2004-2005 Chargée de cours **(Ecole Polytechnique Fédérale de Lausanne - EPFL)**
- 2000-2001 Collaboratrice scientifique **(Ecole Polytechnique Fédérale de Lausanne - EPFL)**

PUBLICATIONS

Morier I., Guenat C., Siegwolf R., Védy J.-C. & Schleppi P. Dynamics of atmospheric N deposition in a temperate calcareous forest soil. *Journal of Environmental Quality (submitted)*.

Morier I., Schleppi P., Saurer M., Providoli I. & Guenat C. Retention of atmospherically deposited N in two contrasting soils in Switzerland. *European Journal of Soil Science (submitted)*.

Morier I., Guenat C., Schleppi P., Siegwolf R. & Knicker H. ^{15}N immobilization in forest soil: a sterilization experiment coupled with ^{15}N CPMAS NMR spectroscopy. *Soil Biology & Biochemistry (in preparation)*.

CONGRÈS, CONFÉRENCES, SYMPOSIUM

Morier I., Schleppi P. & Guenat C. Retention of atmospheric N deposition in a forest soil: contribution of the ^{15}N NMR spectroscopy. 3rd European Symposium on NMR Spectroscopy in Soil, Geo and Environmental Sciences, Freising, Germany, 6-9. August, 2006. **Présentation orale en préparation**.

Morier I., Guenat C. & Schleppi P. Retention of atmospheric N deposition in soil: results from two contrasting sites in Switzerland. *General Assembly of the European Geosciences Union*, Vienna, Austria, 2-7. April, 2006. **Présentation orale.**

Morier I., Schleppi P. & Guenat C. ^{15}N labeling experiment and NMR spectroscopy : powerfull tools to understand immobilization of N deposition in soil. *Analytical chemistry and ecotoxicology workshop*, Geneva, Switzerland, 15. February, 2006. **Poster**.