^{15}N immobilization in forest soil: a sterilization experiment coupled with ^{15}CPMAS NMR spectroscopy

w metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Digital.CSIC

I. Morier[a], P. Schleppi[b], R. Siegwolf[c], H. Knicker[d] & C. Guenat[a]

[a]*Swiss Federal Institute of Technology (EPFL), ISTE Institute, ECOS-WSL, Station 2, GR, CH-1015, Lausanne, Switzerland,* [b]*Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Forest Ecosystem Process Unit, CH-8903, Birmensdorf, Switzerland,* [c]*Paul Scherrer Institut (PSI), Laboratory of Atmospheric Chemistry, CH-5332, Villigen, Switzerland, and* [d]*Lehrstuhl für Bodenkunde, Technische Universität München, 85350 Freising, Germany*

Summary

In temperate forests, soils are the main sink for atmospheric N deposition. The main processes proposed for N retention are microbial and abiotic immobilization in soil organic matter. The relative importance of these processes as well as the kind of resulting chemical compounds are not totally understood. We carried out a laboratory incubation of Hg-sterilized and non-sterilized organic and organo-mineral soil horizons, labelled with either $^{15}NO_3^-$ or $^{15}NH_4^+$. The labelled samples were incubated for 1 hour, 1 day, or 6 days, then subjected to K_2SO_4 extraction and analysed with ^{15}N CPMAS NMR spectroscopy. N immobilization was already effective in all samples and treatments after 1 hour. The corresponding NMR spectra showed that part of the immobilized ^{15}N was already incorporated into an amide structure. In the sterilized soils labelled with $^{15}NH_4^+$, the tracer was rapidly and largely immobilized by an unknown process related to the presence of Hg. In the sterilized soils labelled with $^{15}NO_3^-$, between one-third and one-half of the added tracer was immobilized during the first hour and only 10% more over the 6 days. These results suggest that the sterilization was incomplete at first, allowing relatively great microbial immobilization during the first hour. By contrast, over a longer time, NO_3^- immobilization was significantly reduced to a level corresponding to an abiotic process as Hg sterilization became more effective. Even if the low signal-to-noise ratio precluded quantitative ^{15}N NMR measurements, we showed that the amide-peptide signal, considered as a biotic signature, was dominant in all cases.

Résumé

Dans les forêts tempérées, les sols constituent le puits principal vis-à-vis des apports atmosphériques azotés. Deux processus sont à l'origine de la rétention de l'azote dans le sol, l'immobilisation microbienne et l'immobilisation abiotique sur la matière organique. L'importance relative de ces deux processus ainsi que les formes chimiques qui en résultent ne sont pas élucidées. Nous avons mené au laboratoire une expérience d'incubation qui porte sur des horizons organiques, organo-minéraux, stérilisés ou non à Hg et marqués ou non à $^{15}NO_3^-$ ou $^{15}NH_4^+$. Suite à une incubation d'une heure, d'un jour et de 6 jours, des extractions chimiques à K_2SO_4 et de la N CPMAS spectroscopie de Résonance Magnétique Nucléaire ont été réalisées sur les échantillons marqués $^{15}NO_3^-$, $^{15}NH_4^+$. Après une heure, on note une immobilisation de l'azote dans tous les échantillons de sol, quelque soit le traitement effectué. Les spectres RMN correspondant indiquent qu'une partie du ^{15}N immobilisé est sous forme d'amides. Dans les échantillons stérilisés marqués à $^{15}NH_4^+$, le traceur est rapidement et massivement immobilisé par un processus non identifié et lié à la présence de Hg. Dans les sols stérilisés marqués à $^{15}NO_3^-$, entre un tiers et la moitié de la quantité du traceur sont immobilisés au cours de la première heure. Au cours des 6 jours suivants cette quantité immobilisée n'augmente que de 10%. Ces résultats suggèrent que la stérilisation est incomplète au départ et qu'une importante immobilisation microbienne se produit lors de la première heure d'incubation. Par contre, lorsque la stérilisation devient efficace, c'est-à-dire lors d'une période d'incubation plus longue, l'immobilisation de $^{15}NO_3^-$est limitée et atteint un niveau qui pourrait correspondre à celui d'une immobilisation abiotique. Malgré un bruit de fond important dans les spectres de RMN qui exclut toute quantification des composants azotés, nous avons démontré la prédominance des amides, formes considérées comme d'origine biologique, dans tous les cas étudiés.

Correspondence: C. Guenat. E-mail: claire.guenat@epfl.ch

Introduction

Atmospheric N deposition has strongly increased worldwide during the last decades due to human activities, especially agriculture and fossil fuel combustion. Therefore, increasing amounts of N are deposited over natural or other unfertilized terrestrial ecosystems as inorganic compounds, mainly as NO_3^- (from NO_X emitted by combustion processes) and NH_4^+ (from NH_3 emitted by agricultural activities). 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). On a terrestrial scale, amounts of N deposition are double those of naturally biotically fixed N. Previous studies concerning the impacts of N deposition on terrestrial temperate ecosystems have shown that the soil acts as a main sink, with its organic layer immobilizing 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). However, the processes involved in N immobilization in the soil, as well as their resulting chemical forms, are not completely understood. Previous studies (He *et al*., 1991; Hart *et al*., 1993; Dail *et al*., 2001; Fitzhugh *et al*., 2003; Morier, 2006; Providoli *et al*., 2006) show that the two main processes that seem to be involved in N retention in the soil in the case of atmospheric deposition are: (i) microbial immobilization, and (ii) direct and abiotic fixation on the soil organic matter. Such processes take place within a short time, from hours to days, following N deposition events (Berntson & Aber, 2000; Perakis & Hedin, 2001; Providoli *et al*., 2006). An efficient method to assess the relative importance of the biotic and abiotic processes is the comparison of N immobilization in sterilized and non-sterilized soils. Possible 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 a sterilizing agent seems to be the best method 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). With respect to the chemical characterization of N forms, ^{15}N CPMAS NMR (Cross Polarisation Magic Angle Spinning Nuclear Magnetic Resonance) spectroscopy is a powerful tool to assess chemical N-organic bonds in the soil (Knicker *et al*., 1993; Knicker & Lüdemann, 1995; Mathers *et al*., 2000; Knicker, 2004; Diekow *et al*., 2005). According to Mathers *et al*. (2000), by combining ^{15}N CPMAS NMR with quantitative analysis of total N and ^{15}N (and their recoveries as conventional forms of N), with pyrolysis, hydrolysis and incubation experiments, a much-needed insight into the nature and availability of organic N in soils should be gained. To improve our knowledge of the processes and the chemical forms involved in N immobilization in the soil, we coupled both: (i) ^{15}N labelling (as $^{15}NH4^+$ and $^{15}NO_3^-$) of sterilized (Hg-treated) or non-sterilized soils, and (ii) NMR spectroscopy measurements. We tracked the ^{15}N tracer after 1 hour, 1 day and 6 days. We were more specifically 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 nature of the added chemicals ($^{15}NH_4^+$ or $^{15}NO_3^-$) on this immobilization, and (iv) the main chemical forms in which ^{15}N is immobilized. To our knowledge this is the first attempt to apply ^{15}N labelling CPMAS NMR and soil sterilization, to the very short-term to short-term dynamics of N deposition in soil.

Materials and methods

Site description

Soil samples were collected within the riparian zone of the Sarine river at Grandvillard, Switzerland (46°32′N/7°04′E). The vegetation of the site is a beech forest (*Fagus sylvatica* L.) mixed with planted spruce (*Picea abies* (L.) Karst.). The parent material consists of alluvial deposits and the water table is very deep, at approximately 4 m. The soil is a well-drained Calcareous Fluvisol according to the FAO-UNESCO classification (Driessen & Dudal, 1991). Its profile consists of an OL layer (a few millimetres), a loamy calcareous A layer (0–25 cm) and a sandy loam calcareous B layer (26–50 cm), which lies above a sandy bed and the coarse alluvial deposits. The humus is of a calcareous mull type with a rapid organic matter turnover. Additional details about the site are given by Bureau (1995).

Experimental design

The experiment consisted of a randomized block design with four factors and two replications. The first factor was the soil layers, either litter (OL), the organic soil composed of the first 1–2 cms under the litter and containing partly decomposed litter and soil aggregates (OL-Aca), or the organo-mineral soil collected between 4 and 10 cm depth (Aca). The second experimental factor was soil sterilization with Hg, or non-sterilized. The third factor was the tracers, either $^{15}NH_4^+$, $^{15}NO_3^-$ or none. Finally, the fourth factor was the time between tracer application and sampling, 1 hour, 1 day, and 6 days. For practical reasons and because of the amount of work involved, the whole experiment could not be performed at a single time. It was thus done in 10 batches. The first five batches corresponded to the first replication of the experiment while the five remaining batches corresponded to the second one. Within each replication, the combination of soil layers, soil sterilization and tracers was assigned randomly (Figure 1).

Samples preparation and treatments

Soil samples were collected in the field from October 2004 to March 2005, prepared on the sampling day and incubated the

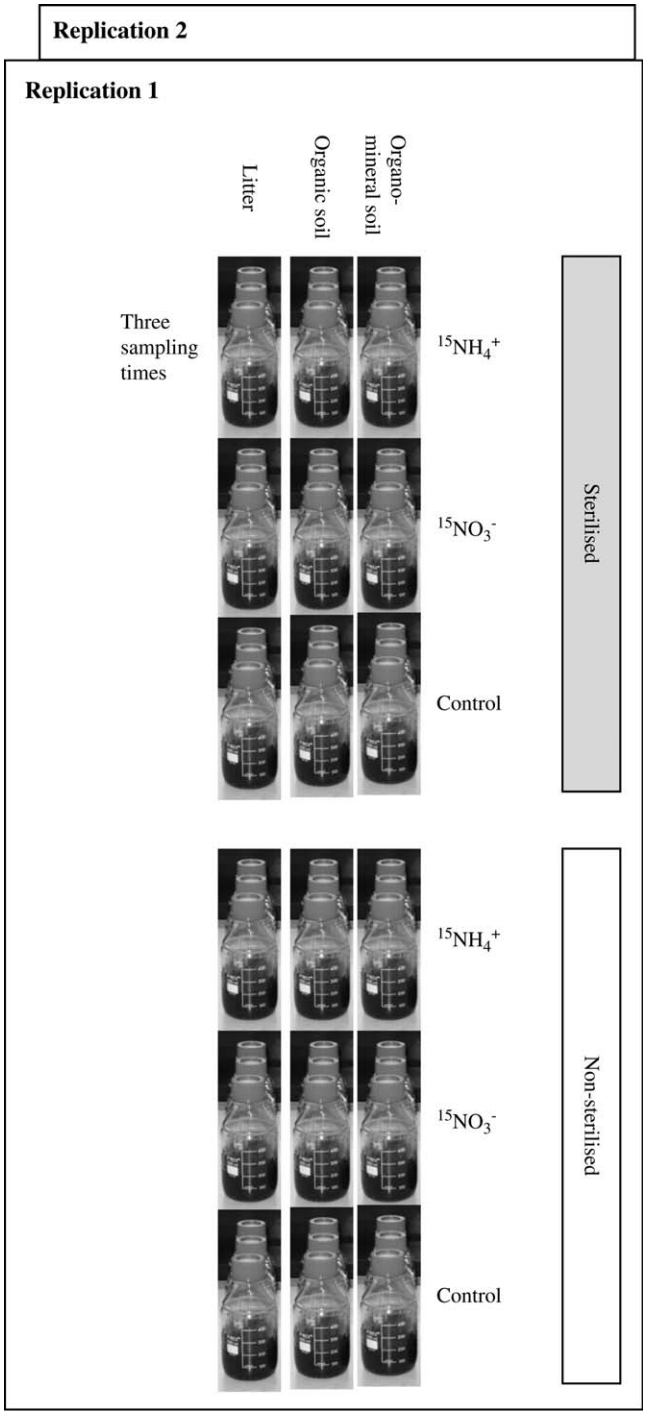


Figure 1 Experimental design. Eighteen possible combinations for each replication, randomly assigned to five batches. Three sampling times (1 hour, 1 day, 6 days) for each combination.

following morning. Small branches, fresh mosses and plant remains, as well as roots, were removed from the litter and soil samples. To homogenize the litter samples, we cut the leaves into polygons of approximately 1 cm^2 each. Aggregates larger than 5 mm in diameter were broken manually; roots from the organo-mineral samples were removed and the soil was then sieved at 5 mm. All experimental batches were incubated at standardized moisture conditions after the addition of a tracer and/or sterilization solution (gravimetric humidity relative to the dry mass: litter, 2250 g kg^{-1}; organic soil, 1500 g kg^{-1}; organo-mineral soil, 450 g kg^{-1}). The field moisture of each batch and layer was determined beforehand by drying a subsample at 105°C overnight. Samples were generally too moist compared with the target values (above). They were thus slowly dried at 30–40°C for 4 to 6 hours, and if necessary overnight at room temperature until the target moisture content was reached. Conversely, if the samples were too dry, deionised water was added. Fresh samples equivalent to the following dry masses were weighed and separately placed in 500 ml glass vials: 5–9 g for litter samples; 26–29 g for organic samples; 95–120 g for organo-mineral samples. The amount of material used varied according to the density of each soil layer in order to reach a similar volume. The ^{15}N was added to the soil samples either as $^{15}NH_4Cl$ or $K^{15}NO_3$ dissolved in deionised water (non-sterilized samples) or in a solution of mercuric chloride ($HgCl_2$, sterilized samples). Unlabelled samples were treated with deionised water or with the solution of $HgCl_2$. All solutions were added with a syringe and samples were then mixed to homogenize them. Tracers were added at a rate of 0.04 mg ^{15}N g^{-1} of dry matter. The $HgCl_2$ was added at 13.5 mg g^{-1} of dry matter in order to inhibit microbial metabolism. The $HgCl_2$ concentration applied is similar to that used by Fitzhugh *et al*. (2003), who demonstrated the efficiency of the sterilization method and we didn't test further the efficiency of the sterilization either at the beginning or at the end of the incubation.

Incubation

Immediately after adding the solutions, the vials were hermetically closed with a lid fitted with a septum so as to avoid Hg volatilization, and ^{15}N contamination of the samples by the ambient air. The samples were then incubated in the dark at room temperature (ranging between 19 and 23°C). On the fourth day, we injected between 100 and 200 ml of ambient air into the vials by means of a syringe inserted through the septum so as to prevent anaerobic conditions that could occur as a result of microbial activity during several days.

Sample preparation after incubation

After 1 hour, 1 day and 6 days, one sample of each applied treatment was processed as follows: 25 g of the organic and organo-mineral samples were weighed into a bottle and extracted immediately with 80 ml of a K_2SO_4 solution (5M). These extracts were then kept in the freezer. The parts of the soils that were not extracted were immediately frozen with liquid nitrogen and kept in the freezer.

The litter samples were immediately frozen without extraction. All samples were then freeze-dried. The litter samples were ground in a Retsch Ultra Centrifugal Mill ZM1 (0.5 mm) (Retsch, Haan) and the organic and organo-mineral samples in a Retsch Mortar Grinder RM 100. The lyophilised extracts were ground by hand in a mortar. The soil and extract samples were analysed for both their N concentration and $^{15}N/^{14}N$ ratios. For the litter, only selected samples were analysed, including both $^{15}NH_4^+$ and $^{15}NO_3^-$ labelled sterilized and non- sterilized samples. Isotopic analyses were conducted at the Paul Scherrer Institute, Laboratory of Atmospheric Chemistry, (Villigen, Switzerland) where samples were combusted in an elemental analyser (EA 1108, Finnigan, Bremen) connected to an isotope ratio mass spectrometer (DeltaS, Finnigan) to determine the $^{15}N/^{14}N$ ratio of the sample. Immobilized ^{15}N was calculated as total ^{15}N in the sample minus extractable ^{15}N.

NMR methods

The solid-state ^{15}N NMR spectra were obtained with a Bruker DMX 400 operating at 40.56 MHz (Lehrstuhl für Bodenkunde, Technische Universität München, Germany), by means of a Bruker double-bearing probe of 7-mm outer diameter, phase-stabilized zirconium dioxide rotors. To increase the sensitivity, the samples containing material from the organic and organo-mineral layer were treated with hydrofluoric acid (10% w/w) for 12 hours (Knicker *et al*., 1999). After centrifugation, the supernatants were siphoned off and discarded. The procedure was repeated five times at room temperature. The remaining material was washed five times with deionised water and freeze-dried. For the NMR measurements, the cross-polarization (CP) magic-angle spinning (MAS) technique with a ramped ^1H-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 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 p.p.m.) and adjusted with ^{15}N-labelled glycine (−347.6 p.p.m.). Signal assignment was performed according to Witanowski *et al*. (1993) and Knicker (2000). Due to the low sensitivity of solid-state ^{15}N NMR spectroscopy the number of scans and thus the duration of the measurements were great. Therefore, we limited this application to a selection of samples that covered all soil layers (litter, organic soil and organo-mineral soil), incubations (1 hour, 1 day and 6 days), tracers ($^{15}NH_4^+$ and $^{15}NO_3^-$) and sterilization (Hg-sterilized or not), but not all interactions among these experimental factors. In addition, due to the low signal-to-noise ratio, the ^{15}N NMR spectra were not quantified.

Calculations and statistical analyses

The recovered ^{15}N in the soil as well as in the soil extracts was calculated by multiplying the sample mass, its N concentration and the isotopic 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 details, see Providoli *et al*., 2005). We calculated a general linear model with repeated measures (R version 2.0.1, www.r-project.org) to test the concentrations of total N and of extractable N as well as the rates of immobilized ^{15}N for effects of sterilization (Hg-sterilized or non-sterilized samples), of 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 interactions among these factors. When testing N concentrations for effects of the tracers, we also included the unlabelled samples. The residues of these statistical analyses were tested for their possible deviation from normality.

Results

N dynamics during the incubation experiment

The concentrations of total N in the dry soil were generally greater in the organic than in the organo-mineral layer (Figure 2). Some differences between $^{15}NO_3^-$-labelled, $^{15}NH_4^+$-labelled and unlabelled samples were noted after 1 hour of incubation. These differences remained throughout the experiment, showing time courses independent of the labelling. Concentrations of extractable N were mostly constant in the non-sterilized samples, whereas they increased in the Hg-sterilized ones ($P < 0.001$).

^{15}N recovery rate

Over the incubation period, the mean recovery rates of the added ^{15}N were of 96.8% (standard error 1.3) in the litter, of 97.8% (SE 1.0) in the organic soil and of 101.8% (SE 1.4) in the organo-mineral soil. The recovery rates of immobilized ^{15}N in the soil samples are presented in Figure 3.

Both tracers reacted differently to sterilization (organic soil, $P = 0.001$; organo-mineral soil, $P = 0.01$). In the organic soil, after 1 hour of incubation, less than 40% of the applied $^{15}NO_3^-$ was immobilized. We observed similar rates after 1 day and a slight decrease during the 6 days. For the $^{15}NH_4^+$application, values were much greater: in the organic soil, 90% of ^{15}N was immobilized during 1 hour of incubation. Immobilization rates then decreased to 80% during the first day and remained stable over the 6 days. In the organo-mineral soil, approximately 60% of $^{15}NO_3^-$ was recovered after 1 hour and rates decreased to 40% after 6 days. Rates of immobilized NH_4^+ evolved from more than 95% to approximately 90% during 6 days.

In the non-sterilized organic and organo-mineral soil, differences between both tracers were small, although immobilization of $^{15}NH_4^+$ was greater than $^{15}NO_3^-$ throughout the incubation period. We observed in these soil layers an increase of the recovered immobilized $^{15}NH_4^+$ over the 6 days (from 50 to 80%), whereas values for $^{15}NO_3^-$ were rather constant (40%).

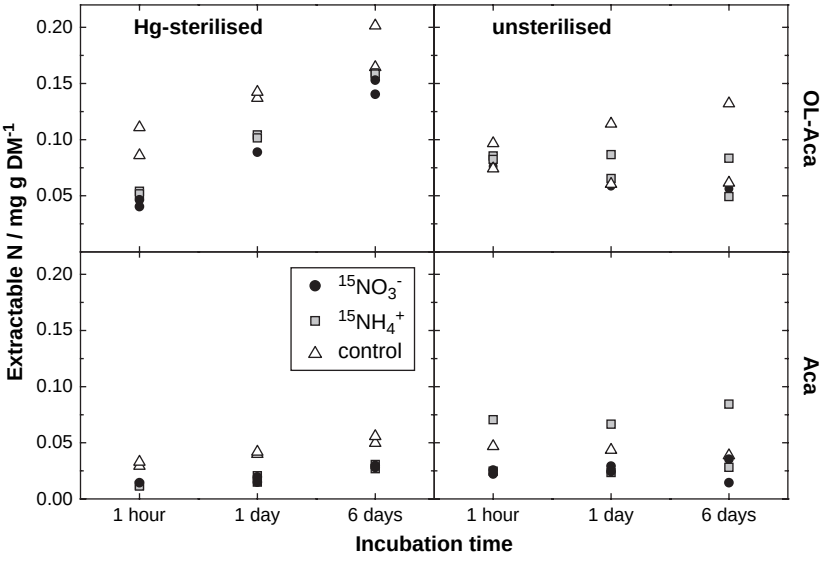


Figure 2 Total extractable N (mg/g of dry matter) in the organic (OL-Aca) and organo-mineral (Aca) samples, Hg-sterilized and non-sterilized.

Solid-state ^{15}N-CPMAS NMR spectroscopy

The signal-to-noise ratio was small after 1 hour of incubation despite the HF treatment and the number of scans (about three million). In addition, it is important to keep in mind that part of the peak intensity is due to the native ^{15} N, as shown on the spectrum of unlabelled litter (Figure 4). The spectral quality improved with increasing incubation time (Figure 4), showing more distinctly, after 6 days of incubation, a largest peak in the chemical shift region of −220 to −280 p.p.m., indicating an increase of ^{15}N in the amide-peptide N structure (−260 p.p.m.).

Figure 5 presents NMR spectra deriving from the sterilized litter after 6 days of incubation with either $^{15}NO_3^-$ or $^{15}NH_4^+$. Those spectra were dominated by the signal attributed to amide N, but showed no major qualitative differences. Similar results were obtained from the NMR analysis of Hg-sterilized and non-sterilized samples in organo-mineral soil samples labelled with $^{15}NH_4^+$ and with $^{15}NO_3^-$ (Figure 6). The spectra presented were obtained after 6 days of incubation. Most of the detectable ^{15}N-signals were again assigned to the amide-peptide structure. No qualitative differences were observable between the sterilized and the non-sterilized samples, either for the $^{15}NH_4^+$ or for the $^{15}NO_3^-$ – labelled samples. In all the spectra, no signal was visible in the chemical shift region assignable to $^{15}NH_4^+$ (−358 p.p.m.) and $^{15}NO_3^-$ (25 to −25 p.p.m.). Unfortunately, due to the small signal-to-noise ratio of the solid-state ^{15}N NMR spectra, it was not possible to quantify the chemical compounds, even the amides.

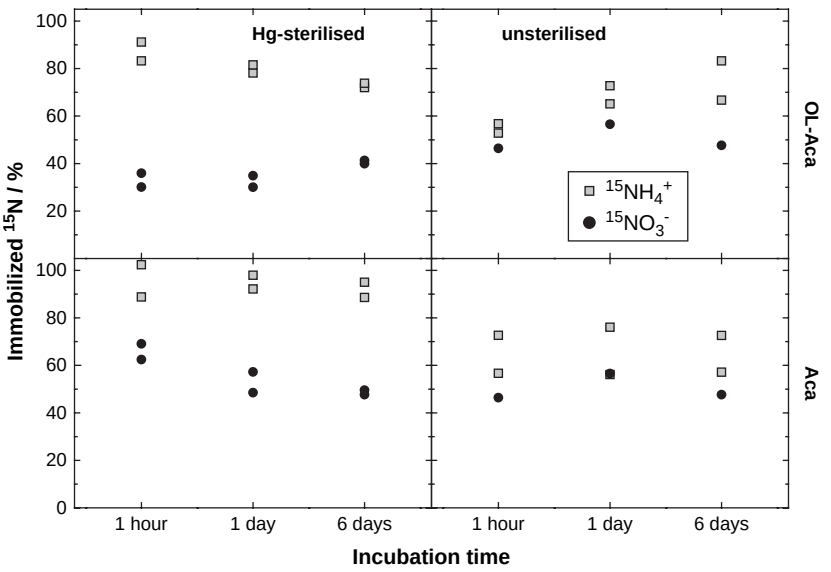
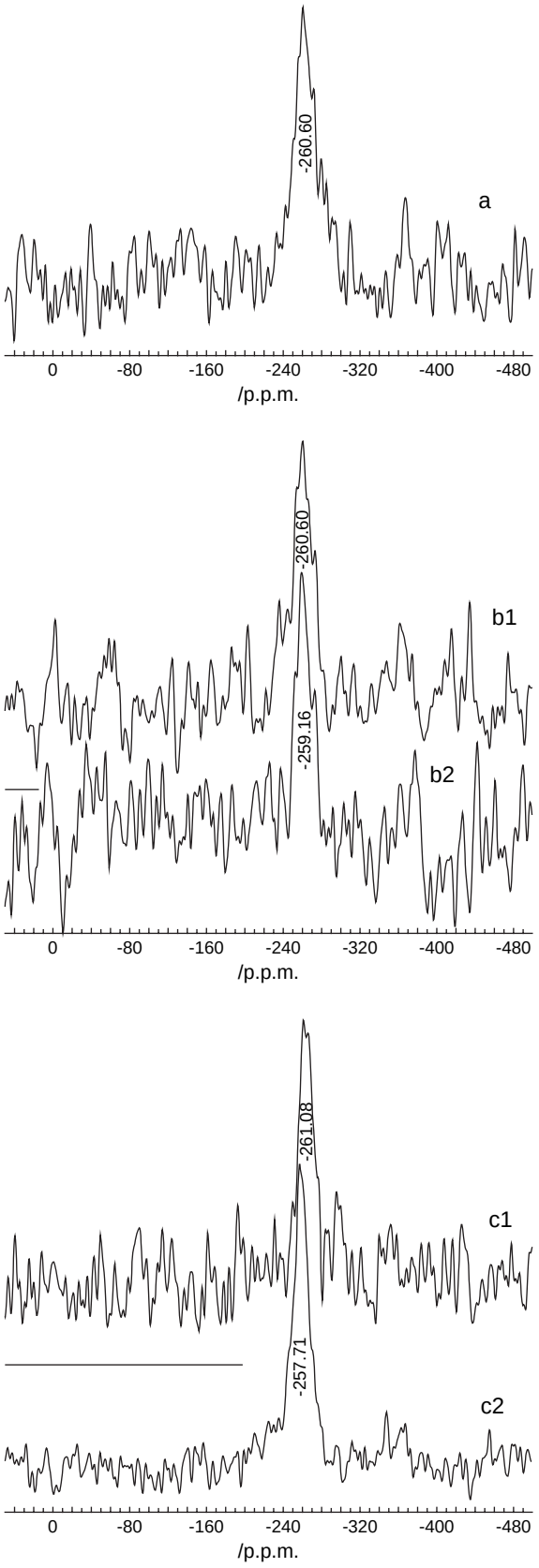


Figure 3 Recovery rates of immobilized ^{15}N in the organic (OL-Aca) and organo-mineral (Aca) samples, Hg-sterilized and non-sterilized.



Discussion

The aims of our study were to improve our knowledge of the processes and the chemical forms involved in the N immobilization in the soil in the very short term and to assess the usefulness and the limits of the NMR method in the study of these processes.

Nitrogen immobilization in the soil: a process of biological or abiotic origin?

For both $^{15}NO_3^-$ and $^{15}NH_4^+$, we confirmed that very fast N immobilization processes are active within hours to days, as described in previous studies (Hart *et al*., 1993; Berntson & Aber, 2000; Johnson *et al*., 2000; Zogg *et al*., 2000; Dail *et al*., 2001; Perakis & Hedin, 2001; Morier, 2006; Providoli *et al*., 2006). However, the relative importance of immobilization and its dynamics are different for both tracers.

In the case of $^{15}NO_3^-$ addition and during the first hour of incubation, Hg-sterilized organic soils immobilized one-third of the added tracer and more than half was immobilized in the organo-mineral soils. The 1-hour $^{15}NO_3^-$ immobilization could be an artefact from soil sterilization. In agreement with Fitzhugh *et al*. (2003), we tentatively explain our results by the fact that during the first hour, sterilization is not totally effective and the surviving micro-organisms therefore continued to assimilate ^{15}N. This hypothesis is confirmed by the early presence of biosynthesized components of the amide-peptide type (identified through NMR spectroscopy) in all sterilized soils. In the sterilized organic soil, we measured net $^{15}NO_3^-$ immobilization of approximately 15% between 1 hour and 6 days. But the gross ^{15}N immobilization is certainly greater. Extractable total N increased with time in the sterilized samples, due to the release of labile N compounds following the death and the lysis of the microbial cells. This means that ^{15}N immobilized by micro-organisms still alive during the first hour, has also been partly released in the extractable ^{15}N pool. This phenomenon is even more evident in the organo-mineral soils: over the 6 days, we observed an increase of the total extractable N and of $^{15}NO_3^-$, corresponding to tracer release. Six days of $^{15}NO_3^-$ immobilization in the sterilized soils leads us to propose that an abiotic process of less importance could be effective during this period. Abiotic fixation of $^{15}NO_3^-$ by the

Figure 4 ^{15}N CPMAS NMR spectra of: (a) litter unlabelled (OL layer) non-sterilized sample, after 1 hour of incubation, (b1) organic soil (OL-Aca layer) non-sterilized samples, labelled with $^{15}NO_3^-$, after 1 hour of incubation, (b2) organic soil (OL-Aca layer) non-sterilized samples, labelled with $^{15}NO_3^-$, after 1 day of incubation, (c1) litter (OL layer) non-sterilized samples, labelled with $^{15}NH_4^+$, after 1 day of incubation, (c2) litter (OL layer) non-sterilized samples, labelled with $^{15}NH_4^+$, after 6 days of incubation. Note that the amide peaks are normalized to the same height, the signal-to-noise ratio is small after 1 hour and 1 day and the amide peak is at approximately −260 p.p.m.

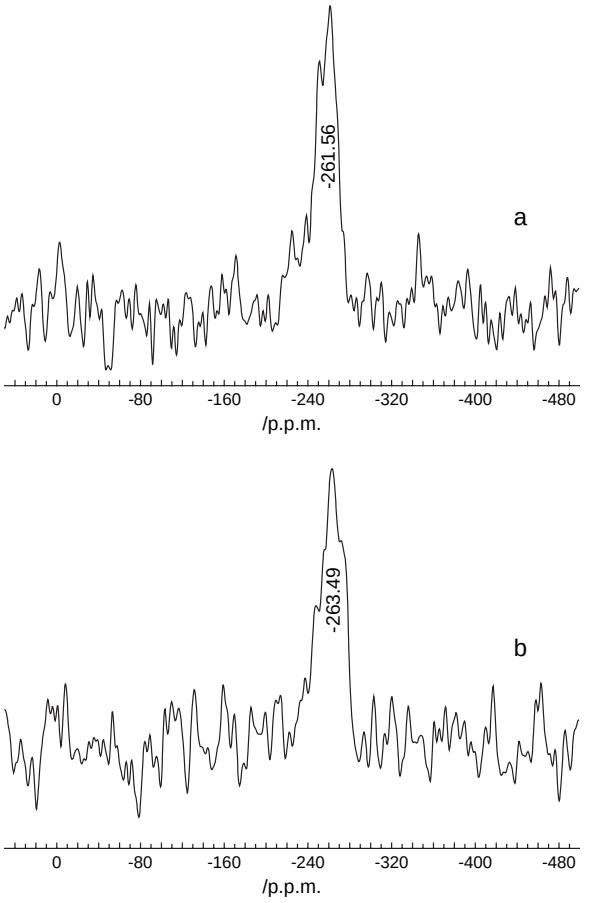


Figure 5 ^{15}N CPMAS NMR spectra of litter (OL layer) sterilized samples, (a) labelled with $^{15}NO_3^-$, and incubated for 6 days, (b) labelled with $^{15}NH_4^+$ and incubated for 6 days. Note that, (i) the amide peaks are normalized to the same height and (ii) the amide peak is at approximately −260 p.p.m.

soil organic matter was already suggested by several studies (Berntson & Aber, 2000; Zogg *et al*., 2000; Perakis & Hedin, 2001; Fitzhugh *et al*., 2003). The processes involved remain poorly understood. Fitzhugh *et al*. (2003), together with Davidson *et al*. (2003), propose a reduction of NO_3^- to NO_2^-; this reduction could be driven by iron (Fe) oxidation, or nitrification. The NO_2^- in turn would react with soil organic matter. In the frame of our study, the ^{15}N CPMAS spectroscopy did not allow us to identify the nature of those compounds of abiotic origin, because the proteinaceous molecules produced by the biotic processes dominated the NMR signal. But this does not exclude their presence in smaller, undetectable concentrations, especially considering the considerable background noise of our spectra.

In the case of $^{15}NH_4^+$, the immobilization rates in the non-sterilized soils are high after 1 hour (50%) and reach 80% after 6 days. The higher immobilization rates of $^{15}NH_4^+$ (compared with $^{15}NO_3^-$) are not a surprise because NH_4^+ is the preferential form immobilized by micro-organisms. In the

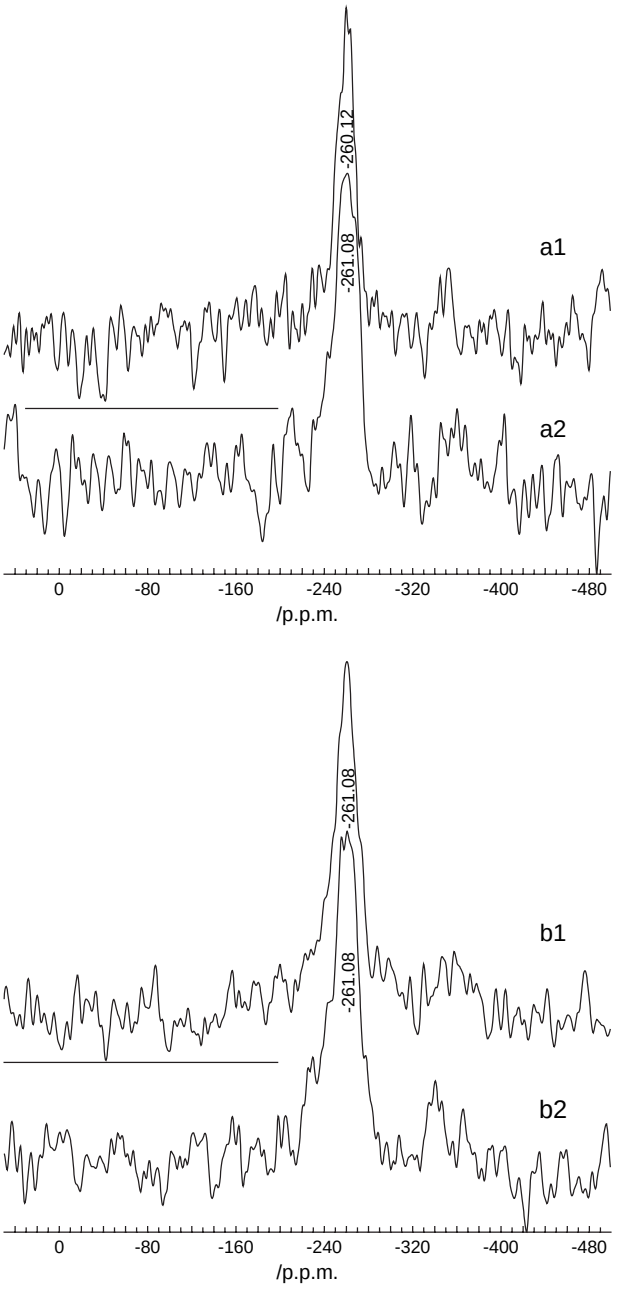


Figure 6 ^{15}N CPMAS NMR spectra of the organo-mineral samples (OL-Aca layer), (a1) non-sterilized, labelled with $^{15}NO_3^-$ and incubated for 6 days, (a2) sterilized, labelled with $^{15}NO_3^-$ and incubated for 6 days, (b1) non-sterilized, labelled with $^{15}NH_4^+$ and incubated for 6 days, (b2) sterilized, labelled with $^{15}NH_4^+$ and incubated for 6 days. Note that (i) the amide peaks are normalized to the same height and (ii) the amide peak is at approximately −260 p.p.m.

Hg-sterilized soils labelled with $^{15}NH_4^+$, we observed very considerable ^{15}N immobilization during the first hour (90%) compared with the non-sterilized ones. This ratio remained very large after 6 days. It is a very surprising result and it suggests that a chemical process involving NH_4^+ and Hg

happened right from the start of the incubation; NH_4^+ and Hg are both cations and a third negatively charged molecule is thus involved. So far, we were able to identify neither the chemical pathway involved nor the resulting chemical forms. However, no specific peak was observable on the corresponding NMR spectra and, consequently, it is probable that this process implies H-bonds or other non-covalent bonds that are not detectable by ^{15}N NMR spectroscopy. Further experiments on simplified systems (e.g. pure clay minerals or organic fractions) will be necessary to understand this process.

Role and limits of the NMR method in the study of ^{15}N retention in soil

The NMR spectra obtained in the frame of this study are characterized by the absence of a signal in the chemical shift assignable to $^{15}NH_4^+$ or $^{15}NO_3^-$. We have to bear in mind that the cross-polarization and relaxation kinetics of inorganic ^{15}N do not allow their quantitative determination with the acquisition parameters that were used to optimize the detection of organic N forms (Knicker & Lüdemann, 1995; Knicker 2000). For this reason, it is interesting to couple ^{15}N mass spectrometry measurements on soil extracts with NMR spectroscopy.

All NMR spectra are dominated by a single signal corresponding to an amide-peptide structure and consequently to biosynthesized molecules. This dominance of amide-peptide signals has been already observed in previous ^{15}N NMR experiments in various soils of the world and in humic substances, not only in ^{15}N-enriched material but also in samples with natural ^{15}N levels. Knicker *et al.* (1993) showed that, in various types of soil in Germany, the main signals found for natural ^{15}N corresponded to amide and peptide forms. The same patterns were observed in a subtropical Acrisol (Diekow *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 remained 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 residue incubation with $^{15}NH_4^+$, the major part of the detectable ^{15}N-signals was assigned to amide-peptide 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 *et al.*, 1993, 1997; Clinton *et al.*, 1995). The same researchers proposed that the remaining N was immobilized in the form of amino acids, amino sugars, or the amino groups of nucleic acids.

In the frame of this study on ^{15}N immobilization in sterilized soil, the predominance of an amide-peptide structure confirms our interpretation that the sterilization was not complete at the beginning of the experiment and part of the microorganisms were still active.

The NMR spectra do not allow exclusion of the possibility of abiotic ^{15}N immobilization. However, our data show that the abiotic pathway is not dominant. Our results agree with former studies in which the existence of ^{15}N immobilized in hetero-cyclic compounds could not be excluded either, but accounted for only a few per cent (Zhuo & Wen, 1992; Knicker *et al.*, 1997).

Conclusions

1 To our knowledge, this is the first attempt to apply ^{15}N labelling CPMAS NMR and soil sterilization to the very short-term to short-term dynamics of N in soil. The aim of combining these methods was to allow a better characterization of the N immobilization process in the soil, its temporal dynamics and the role of microbial activity in those processes.
2 We observed that the immobilization processes take place very rapidly.
3 We assume that ^{15}N immobilization results mainly from biotic processes that are dominant in the short-term. However, abiotic immobilization of less importance is not excluded for ^{15}N immobilization.
4 We cannot reject the formation of heterocyclic compounds, but assume that their contribution is very small within this time frame.
5 Our results also show it is necessary to improve all the methods used: (i) the identification and quantification of the N components by ^{15}N CPMAS spectroscopy, (ii) the chemical characterization of the non-extractable fraction, and (iii) the effectiveness of sterilization methods. Clearly each of these methods deserves more attention, and (iv) it would be useful to combine the study of natural soils with that of simpler systems (e.g. organic fractions, single species' litter, single mineral fractions) that can be understood more easily.

Acknowledgements

We hereby thank Elena Rossel and Jean-David Teuscher (EPFL Lausanne) for their assistance in the laboratory as well as Dr Edward Mitchell for the English revision.

References

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

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*. Thèse 1418, EPFL, 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. 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.

Driessen, P.M. & Dudal, R. 1991. *The Major Soils of the World*. Agricultural Univ, Wageningen, the Netherlands and Katholieke Universiteit Leuven, Belgium.

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.

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

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

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.

MacDonald, N.A., Dise, N.B., Matzner, E., Armbruster, M., Gundersen, P. & Forsius, M. 2002. Nitrogen input 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.

Morier, I. 2006. *Retention of atmospherically deposited nitrogen in soil: field and laboratory experiments using ^{15}N isotope and ^{15}N CPMAS spectroscopy*. Thèse 3580, EPFL, Lausanne, Switzerland.

Nadelhoffer, K.J., Emmett, B.A., Gundersen, P., Kjønaas, O.J., Koopmans, C.J., Schleppi, P. *et al*. 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. 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*, **38,** 1645–1657.

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* (ed. G. Webb), pp. 1–480. Academic Press, London.

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), pp. 41–51. Soil Science Society of America, Madison, WI.

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.

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.