CHAPTER 6

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

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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).

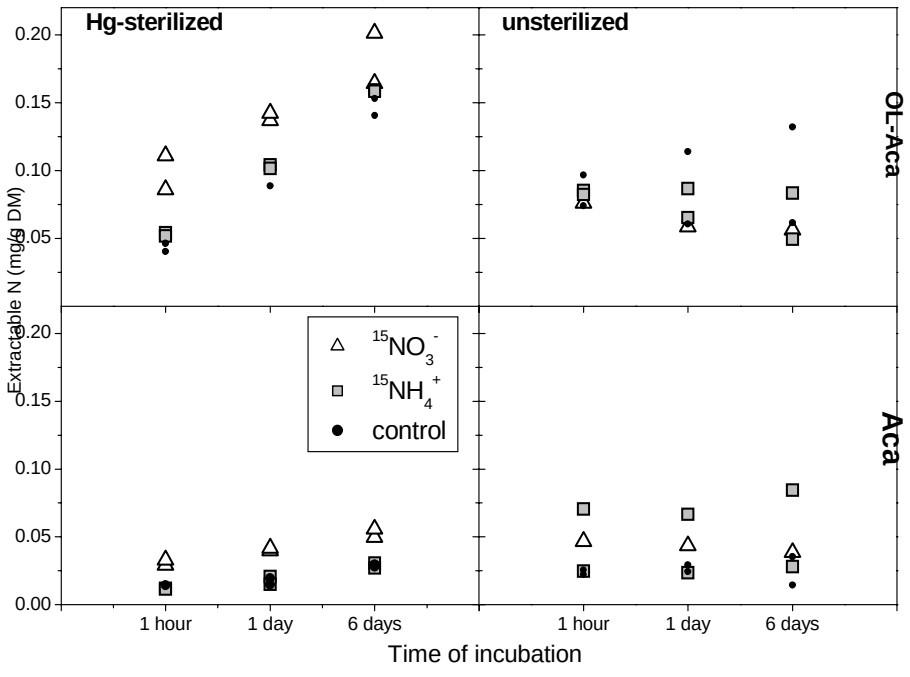

Fig. 6.1 : Total extractable N (in mg / g of dry matter) in the OL-Aca and Aca samples, Hg-sterilized and unsterilized.

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.

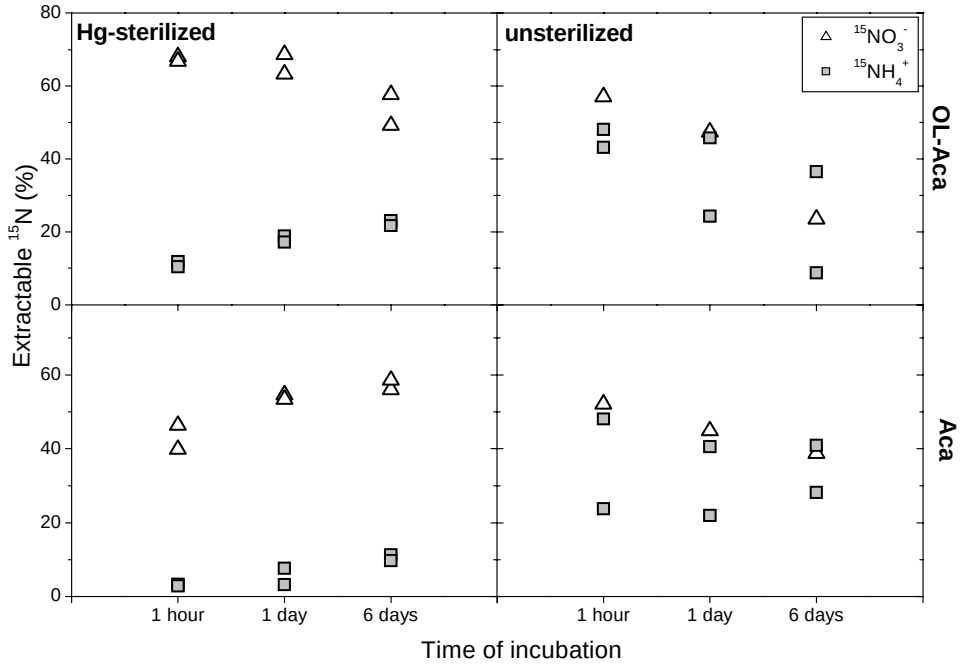

Fig. 6.2 : Recovery rates of extractable ^{15}N in the OL-Aca and Aca samples, Hg-sterilized and unsterilized.

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.

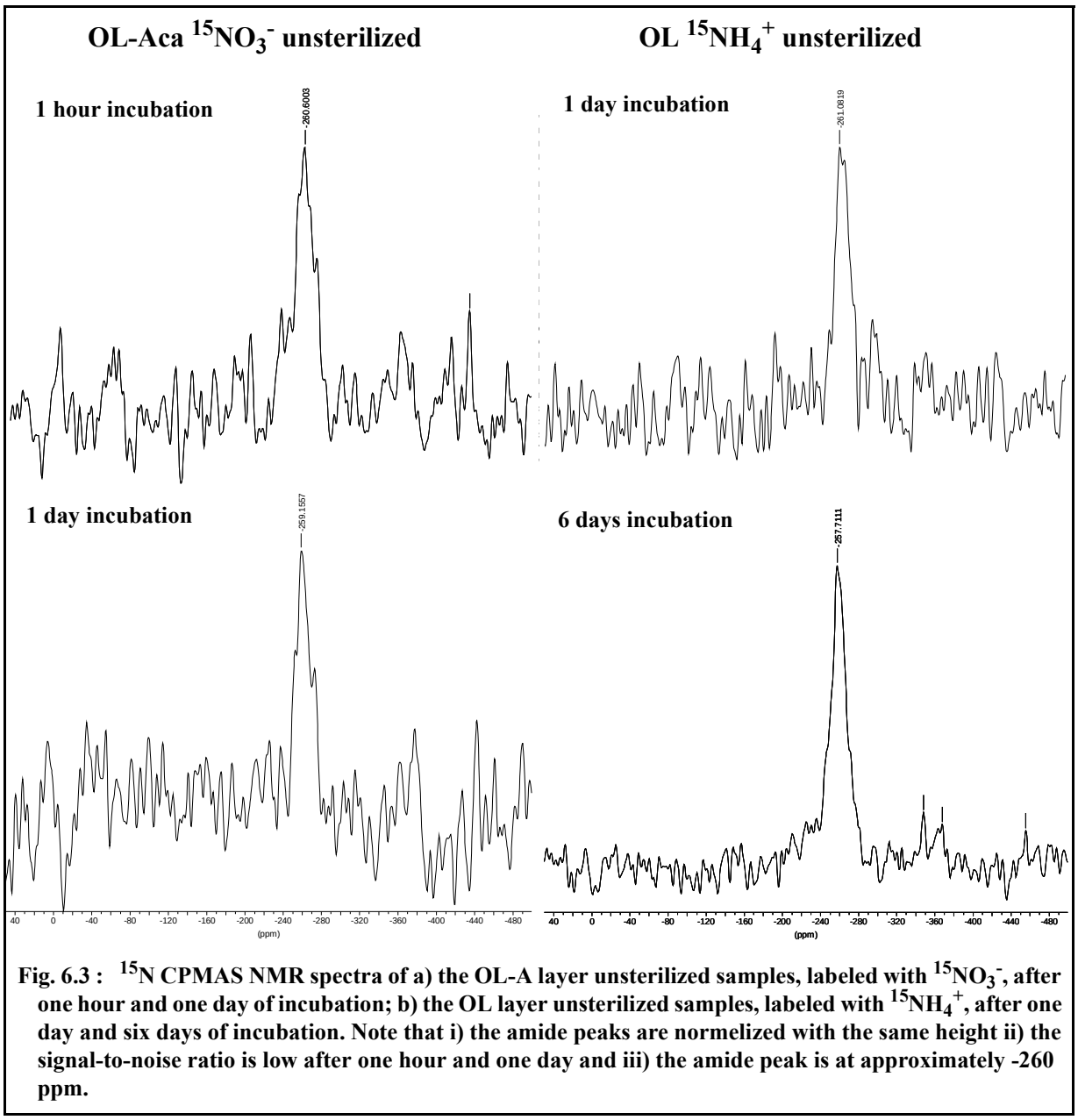

Fig. 6.3 : ^{15}N CPMAS NMR spectra of a) the OL-A layer unsterilized samples, labeled with $^{15}NO_3^-$, after one hour and one day of incubation; b) the OL layer unsterilized samples, labeled with $^{15}NH_4^+$, after one day and six days of incubation. Note that i) the amide peaks are normelized 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 $^{15}NH_4^+$.

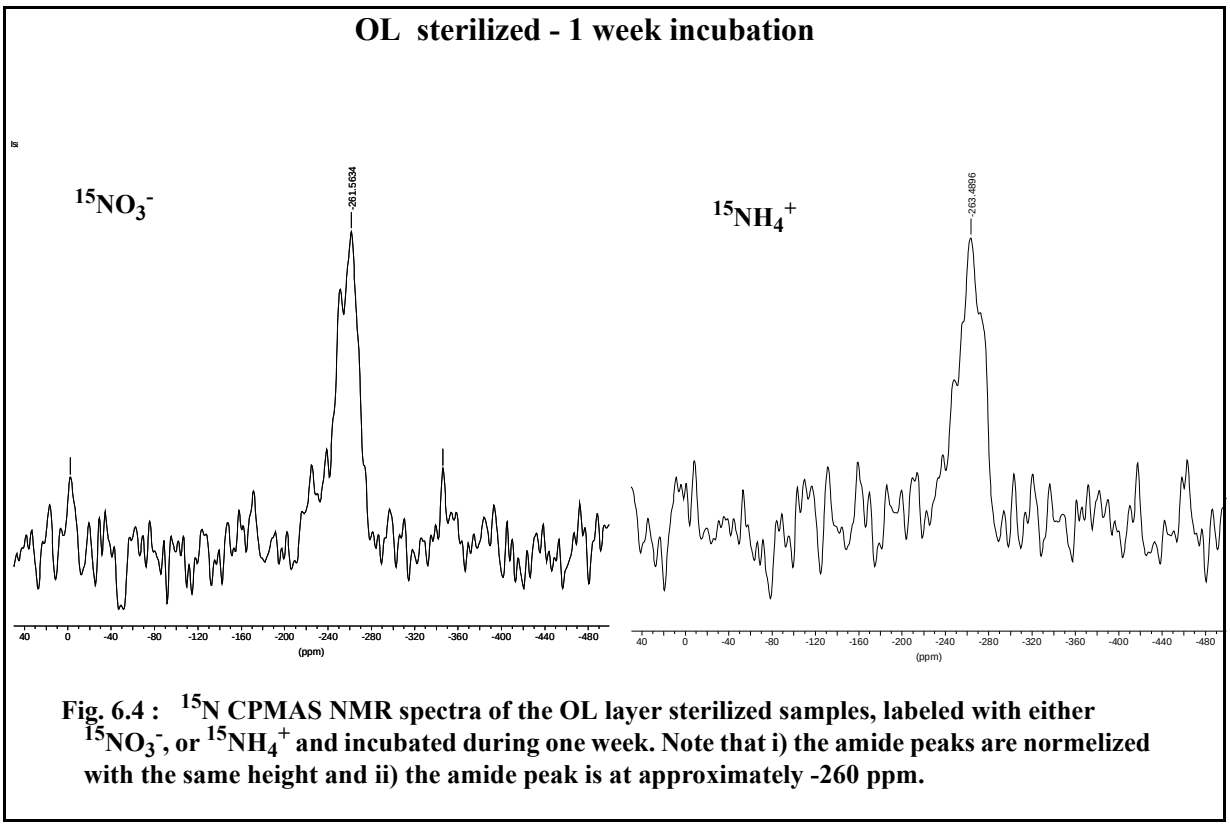

Fig. 6.4 : ^{15}N CPMAS NMR spectra of the OL layer sterilized samples, labeled with either $^{15}NO_3^-$, or $^{15}NH_4^+$ and incubated during one week. Note that i) the amide peaks are normelized with the same height and ii) the amide peak is at approximately -260 ppm.

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.

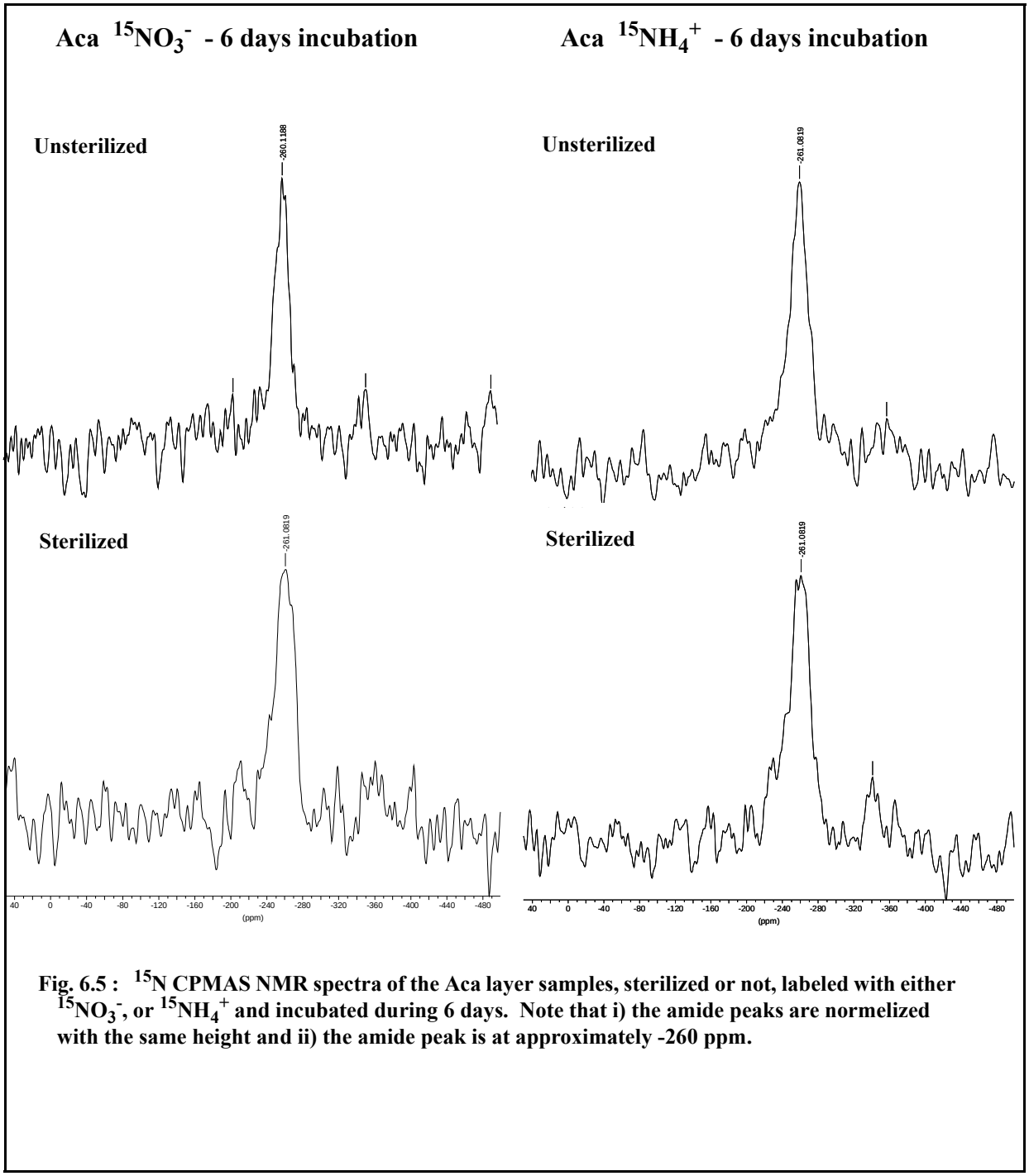

Fig. 6.5 : ^{15}N CPMAS NMR spectra of the Aca layer samples, sterilized or not, labeled with either $^{15}NO_3^-$, or $^{15}NH_4^+$ and incubated during 6 days. Note that i) the amide peaks are normelized with the same height and ii) the amide peak is at approximately -260 ppm.

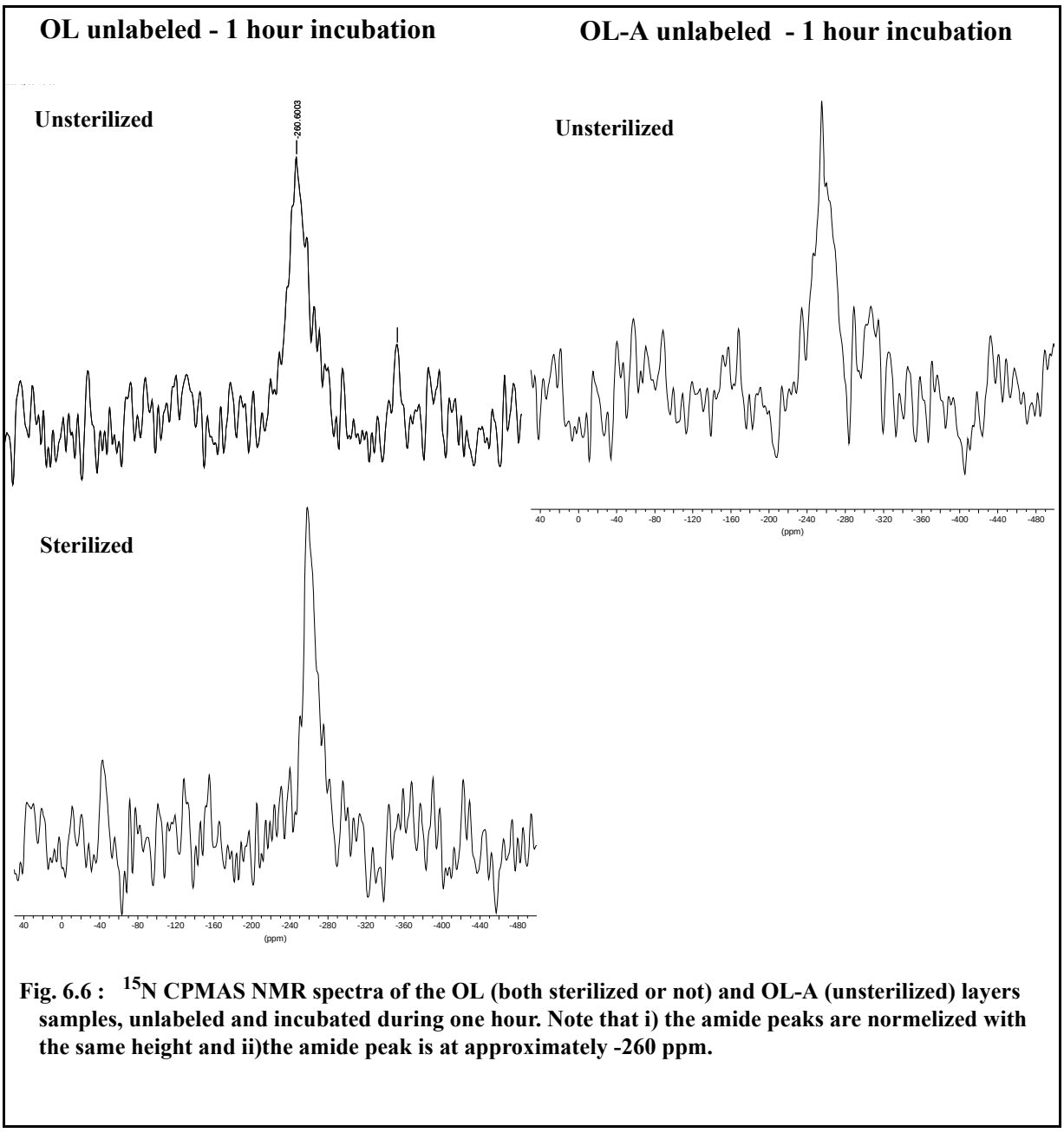

Fig. 6.6 : ^{15}N CPMAS NMR spectra of the OL (both sterilized or not) and OL-A (unsterilized) layers samples, unlabeled and incubated during one hour. Note that i) the amide peaks are normelized with the same height and ii)the amide peak is at approximately -260 ppm.

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.