A Novel Method Combining FTIR-ATR Spectroscopy and Stable Isotopes to Investigate the Kinetics of Nitrogen Transformations in Soils

Understanding and quantifying N transformations in soil is critical for sustainable use of this important plant nutrient and for understanding the mechanisms through which polluting N species are discharged to the environment. Advanced methods such as the “isotope dilution technique”, which uses stable N-isotopes to estimate gross mineralization and nitrification rates, answer this need. In this study, the use of Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy for measuring isotopic N species concentrations directly in soil pastes was tested as a complementary technique to the commonly used isotope ratio mass spectrometry (IRMS). It is shown that, with proper chemometric tools (e.g., partial least squares [PLS]), FTIR-ATR enables simple tracking of changes in the concentrations of the isotopic species of nitrate and ammonium and allows estimation of the gross reaction rates of N transformations in soil. Soil incubations were performed by adding either $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$ to the soils. The incubations with added $^{15}$NH$_4^+$ yielded a gross mineralization rate of 6.1 mg N kg$^{-1}$ dry soil d$^{-1}$ compared with a net mineralization rate of 4.1 mg N kg$^{-1}$ dry soil d$^{-1}$ and a gross nitrification rate of 40.9 mg N kg$^{-1}$ dry soil d$^{-1}$ compared with a net nitrification rate of 29.5 to 25.3 mg N kg$^{-1}$ dry soil d$^{-1}$. The incubations with added $^{15}$NO$_3^-$ yielded a gross nitrification rate of 18.6 mg N kg$^{-1}$ dry soil d$^{-1}$ compared with a net nitrification rate of 11.9 to 18.3 mg N kg$^{-1}$ dry soil d$^{-1}$. The combined use of FTIR-ATR and $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$ enrichment appears to provide an effective tool for almost real-time quantification of N-dynamics in soils with minimal interference.

Abbreviations: ATR, attenuated total reflectance; FTIR, Fourier transform infrared; IDT, isotope dilution technique; IRMS, isotope ratio mass spectroscopy; PLS, partial least squares.
in N₂O or N₂ gases converted from \(^{14/15}\text{NO}_3^-\) and \(^{14/15}\text{NH}_4^+\) species for the IRMS measurements. However, sample preparation for IRMS measurements is both tedious and destructive, thus introducing limitations when studying the relatively fast dynamics of N transformations. Additionally, the equipment is expensive and is not commonly found in many laboratories.

The innovative method suggested in this study is based on FTIR obtained via ATR allowing direct and ultimately even real-time measurements of the N species of interest in soil pastes. This is possible due to the absorption of the N species of interest in the mid infrared range (800–4000 cm\(^{-1}\)). The absorbance bands of the labeled N species \(^{15}\text{NO}_3^-\) and \(^{15}\text{NH}_4^+\) are shifted compared with the non-labeled N species \(^{14}\text{NO}_3^-\) and \(^{14}\text{NH}_4^+\). Du et al. (2009) showed that with chemometric tools such as PLS determination of the concentrations of \(^{14}\text{NO}_3^-\) and \(^{15}\text{NO}_3^-\) typical to fertilized agricultural soils is feasible. The samples can be measured after a short process of extraction with distilled water to form a saturated paste for \(^{14/15}\text{NO}_3^-\) determination. At this stage, the determination of \(^{14/15}\text{NH}_4^+\) requires extraction with 1 M KCl solutions at a 1:1 soil solution ratio, particularly in soils where a significant portion of the ammonium is adsorbed. The \(^{14/15}\text{NO}_3^-\) concentrations can be determined in the KCl extracts as well.

There is a big challenge in determining the actual rate ("gross rate") of a specific reaction, particularly in complex agricultural soil exposed to manures and fertilization where nitrate and ammonium are intensively involved in several "source-sink" reactions occurring concomitantly. While common analytical measurements track the "net" changes in such systems, the "gross rates" define real measurements track the "net" changes in such systems, and thus requires special signal processing tools for analyzing the data. Tools such as PLS and neural networks (NN) have been successfully used for agro-ecosystems, including the prediction of N species concentrations (e.g., Du et al., 2009; Linker et al., 2005).

The main objective of this study is to explore the possibility of using FTIR-ATR spectroscopy in soil incubations where \(^{15}\text{N}\) enrichment of \(^{15}\text{NO}_3^-\) and/or \(^{15}\text{NH}_4^+\) pools is used for calculating gross rates of nitrification and mineralization.

**MATERIALS AND METHODS**

**Preliminary Soil Incubations**

Preliminary incubations were performed to determine the range and the concentrations of the inorganic N species to use for the calibration and to provide initial estimates of the expected mineralization and nitrification rates. At this stage of method development it was necessary to use a soil with known rates of mineralization and nitrification containing low concentrations of calcite to minimize the interference of carbonate with the \(^{15}\text{NO}_3^-\) and \(^{15}\text{NH}_4^+\) IR absorbance bands (e.g., Linker et al., 2004; 2005). The chosen soil-Terra Rossa (Rhodoxeralfs, USDA classification) a non-calcareous clay soil had the following properties: pH of ~7.5; electrical conductivity (1:1 extract) of 0.27 ds m\(^{-1}\); C(K\(_2\text{Cr}_2\text{O}_7\)) of 35.9 mg g\(^{-1}\); and cation-exchange capacity of 39.4 cmol (kg soil\(^{-1}\)). A solution of NH\(_4\text{Cl}\) was added to 80 g of air-dry soil to obtain an NH\(_4^+\)--N concentration of 250 mg N kg\(^{-1}\) dry soil and the gravimetric soil moisture was set to 30%, which is close to field capacity. The soil (~10 mm thickness) was kept in a glass vessel covered with a perforated lid enabling oxygen diffusion while minimizing evaporation losses. Samples were placed in an incubator at 30°C. Before each sampling event, deionized water was added to restore the water content to 30% and the soil was thoroughly mixed. Each soil sample of approximately 3 g was extracted with a 1 M KCl solution at a 1:10 ratio while stirring for 3 h. A colorimetric analyzer (auto-analyzer, LACHAT Quikchem 8500, Lachat Instruments, Loveland, CO) was used for determining NH\(_4^+\)--N, NO\(_3^-\)--N and nitrite (NO\(_2^-\)--N) concentrations in the extracts.

**Ammonium Extraction Experiments with KCl**

This experiment aimed to test the NH\(_4^+\) extraction efficiency from the clay minerals using a 1:1 soil/1 M KCl solution ratio to minimize the dilution of the extracted NH\(_4^+\) used for determination with the ATR-FTIR system. The commonly used extraction ratio of 1:10 soil/1 M KCl solution was used as reference. It is accepted as an effective ratio for extracting exchangeable soil NH\(_4^+\), but it dilutes the extracted solution, causing NH\(_4^+\) species concentrations to be too low for FTIR-ATR determination. An ammonium solution (500 mg N L\(^{-1}\)) was prepared with 1.91 g NH\(_4\text{Cl}\) (0.5 g as N) with addition of 0.02 g dicyandiamide (DCD), a known nitrification inhibitor (4% weight in respect to N), and 1 L of deionized water. Fifty grams of the air-dry Terra Rossa soil were placed in six vessels and the NH\(_4^+\) solution was added to obtain concentrations of 0, 10, 25, 50, 100, and 200 mg N kg\(^{-1}\) dry soil. The soil pastes were left for 24 h for the NH\(_4^+\) to be adsorbed onto the clay minerals.
After 24 h, two samples from each vessel were taken and KCl extractions were performed at two ratios: 1:1 and 1:10 of soil/1 M KCl solution (total of 24 samples). The samples were stirred and then filtered through a 42 Whatman filter and NH$_4^+$ concentrations were measured using a colorimetric analyzer. The obtained results were correlated and analyzed to find a “correction factor” for determination of the ammonium extraction in the 1:1 compared with the 1:10 extract.

**Calibration Experiments**

Nitrogen species in soil have only small mid infrared (MIR) band shifts (Fig. 1) and thus large determination errors may result if the spectra are not properly processed. A thorough calibration stage was performed to develop models for concomitant determination of concentrations of the isotopic species of interest. This calibration was based on 50 soil paste samples spiked with NH$_4^+$ and/or NO$_3^−$. The concentration ranges of each N species were based on the results of the preliminary incubations. The expected concentration ranges of $^{14/15}$NH$_4^+$ and $^{14/15}$NO$_3^−$ in KCl 1M extractions (1:10 ratio) were 0 to 150 mg N L$^{-1}$. Each sample was measured using both FTIR-ATR and the auto-analyzer. The FTIR measurements were performed using a Bruker Vector 22 FTIR with an ATR crystal (Pike trough plate ZnSe 45°). Dry filtered air was supplied into the FTIR sample compartment at a pressure of 0.2 MPa to minimize interference of water vapor. Each measurement consisted of 32 spectra covering the 800 to 4000 cm$^{-1}$ range, with a resolution of 2 cm$^{-1}$.

**Isotope Dilution Technique and Gross Rate Estimations**

Two types of incubations were conducted (Barraclough et al., 1985; Barraclough, 1991): with addition of $^{15}$NH$_4^+$ and $^{14}$NH$_4^+$ to the soil (denoted as type-one isotope dilution technique, IDT), and with addition of $^{15}$NO$_3^−$ and $^{14}$NH$_4^+$ (denoted as type-two IDT). Type-one incubation enables the estimation of both mineralization and nitrification gross rates ($m$ and $n$ in mg N kg$^{-1}$ dry soil d$^{-1}$):

\[
\frac{dA_{15}}{dt} = \left(0_A - m\right)\%A_{15} \quad [1]
\]

\[
\frac{dN_{15}}{dt} = n\%A_{15} + \left(0_N - n\right)\%N_{15} \quad [2]
\]

where $\%A_{15}$ is the relative abundance in the NH$_4^+$ pool during the incubation, $A_{15}$ is the $^{15}$NH$_4^+$ concentration during the incubation in mg N kg$^{-1}$ dry soil, $\%N_{15}$ is the relative abundance in the NO$_3^−$ pool during the incubation, $N_{15}$ is $^{15}$NO$_3^−$ concentration during the incubation in mg N kg$^{-1}$ dry soil, $\theta_{\text{iso}}$ is the average change rate of the relevant pool in mg N kg$^{-1}$ dry soil d$^{-1}$, $t$ is time (days). Equations 1 and 2 were developed based on Barraclough et al. (1985), and modified so that the rate equations of NH$_4^+$ and NO$_3^−$ are used instead of the rate equation for the total mineral-N. Equations 1 and 2 can be solved using the data of the abundance ratios during the incubation. A fitted curve, obtained using the abundance values measured during the incubation, describes the time dependence of the relative abundance. The rate equation is then integrated to obtain the mineralization and nitrification gross rates:

\[
A_{15}^{t_0} - A_{15}^{t_0} = \left(0_A - m\right)\int \%A_{15} \, dt \quad [3]
\]

\[
N_{15}^{t_0} - N_{15}^{t_0} = n \int \%A_{15} \, dt + \left(0_N - n\right) \int \%N_{15} \, dt \quad [4]
\]

The obtained gross rates represent the average rates during the entire incubation. When executing type-two IDT, obviously, only the gross nitrification rate can be calculated, and when neglecting the enriched NH$_4^+$ Eq. [4] reduces to:

\[
N_{15}^{t_0} - N_{15}^{t_0} = \left(0_N - n\right) \int \%N_{15} \, dt \quad [5]
\]

For comparison, the net mineralization rate is calculated from a mass balance on the total mineral N concentration, regardless of enriched species:

![Fig. 1. Fourier Transform Infrared-attenuated total reflectance (FTIR-ATR) signals showing the band shift between (a) $^{14}$NH$_4^+$ and $^{15}$NH$_4^+$, and (b) $^{14}$NO$_3^−$ and $^{15}$NO$_3^−$.](image)
where $m_{\text{net}}$ is the net mineralization rate in mg N kg$^{-1}$ dry soil d$^{-1}$, totN$_t$ is the total mineral N concentration at the end of the incubation in mg N kg$^{-1}$ dry soil, and totN$_0$ is the total mineral N concentration in mg N kg$^{-1}$ dry soil at the beginning of the incubation. The net nitrification rate can be calculated from the mass balance on the ammonium or on the nitrate:

$$n_{\text{net}} = \frac{\text{NO}_3^- \text{conc} \text{t} \text{ed} \text{at} \text{end} \text{ of} \text{incubation} - \text{NO}_3^- \text{conc} \text{t} \text{ed} \text{at} \text{begin} \text{ning} \text{of} \text{incubation}}{dt}$$

where $n_{\text{net}}$ is the net nitrification rate in mg N kg$^{-1}$ dry soil d$^{-1}$, and NO$_3^-_{t,0}$ and NH$_4^+_{t,0}$ are the total nitrate and ammonium concentrations at the end (t)/beginning (0) of the incubation in mg N (kg dry soil)$^{-1}$. The question mark in Eq. [7] emphasizes the fact that both expressions are rarely equal due to unaccounted losses or gains which occur during N-transformations in the soil.

The incubations were performed in rectangular plastic containers (length: 25.5 cm, width: 17 cm, height: 6 cm) in which soil samples of 400 g were placed. The relatively large surface of the containers allowed the incubations to be performed with thin soil layers (10 mm thickness), thus minimizing the risk of limited oxygen diffusion into the soil during the incubations. Type one incubation included addition of $^{14}$NH$_4^+$ (60 mg N kg$^{-1}$ dry soil), and $^{15}$NH$_4^+$ (120 mg N kg$^{-1}$ dry soil). Type two incubation included the addition of $^{14}$NH$_4^+$ (80 mg N kg$^{-1}$ dry soil), and $^{15}$NO$_3^-$ (100 mg N kg$^{-1}$ dry soil). In both incubations the soil already contained small concentrations of $^{14}$NH$_4^+$ (~20 mg N kg$^{-1}$ dry soil). Each incubation lasted for 7 d in an incubator at 30°C. Before each sampling, deionized water was added to restore the initial gravimetric water content to 30% and the soil was thoroughly mixed.

Twice a day a 3-g sample was taken to determine the inorganic-N species concentrations in the soil. Each sample was extracted with a 1 M KCl solution (1:1 ratio) and stirred for 3 h. After the extraction process and during the measurements with the FTIR-ATR, whenever needed, the samples were cooled to 4°C and stored for repeated measurements. We assumed that both the cooling and the extraction with KCl limited biochemical reactivity in the system. Small subsamples of the soil pastes obtained after extraction were placed on the ATR crystal for FTIR measurements. Subsamples from the same extract were filtered and the clear liquid phase was used for colorimetric analysis as a standard reference after filtration. The NH$_4^+$ concentration in the soil was later corrected by using the "correction factor" of NH$_4^+$ extraction with the 1:1 KCl solution as compared to the 1:10 standard extraction.

DATA PROCESSING

The data was analyzed by PLS (Brereton, 2005). Before implementing the PLS procedure the spectra were subtracted from the 1 M KCl contributions. The spectral range used was restricted to 1250 to 1500 cm$^{-1}$ since this spectral interval contains the absorbance bands of all the species of interest. The number of PLS factors tested was 3 to 5.

For each calibration set (one for each type of incubation), 50 soil pastes were prepared, as if they were sampled from the incubation container, that is, addition of N species in 1 M KCl solutions (1:1 extraction ratio). Each sample was measured three times using the FTIR-ATR system. The calibration was conducted using 70% of the spectra, and the PLS model was tested on the remaining 30%. It should be noted that to prevent bias of the model, all three replicated measurements for each sample were used either as calibration or validation. The determination error was calculated on the basis of root mean square error (RMSE).

RESULTS AND DISCUSSION

Preliminary Soil Incubation

The preliminary incubation provided information regarding the change of inorganic-N species concentrations, and the proportions between them, during an aerobic incubation (Fig. 2). This information was used to design the calibration sets for the FTIR-ATR PLS analysis. As expected, the NH$_4^+$–N concentration decreased during the incubation, a small NO$_3^-$–N build-up occurred in the first 2 d and then started to decline, and the NO$_2^-$–N concentration increased throughout the incubation. Based on these results the duration of subsequent incubations was set to 7 to 8 d.

Ammonium Extraction Experiments with Potassium Chloride

The results of the NH$_4^+$ extraction experiment are presented as a percentage of the extracted ammonium %ex vs. NH$_4^+$–N concentration in the soil, Am, according to extraction with 1:1 ratio of soil/1 M KCl solution (Eq. [8]).

$$%\text{ex} = 1.97 \ln(\text{Am}) + 66.50 \quad \{R^2 = 0.79\}$$

Each predicted concentration of NH$_4^+$–N measured by the FTIR is divided by the appropriate %ex (correction factor) to obtain the actual concentration of NH$_4^+$–N in the soil.
CALIBRATION

A different calibration set was produced for each type of incubation. Species concentrations and compositions were set according to those expected during the incubation, based on the preliminary incubations. As mentioned above, the PLS models were developed using 70% of the spectra, with the remaining spectra used for validation. The results are summarized in Table 1. Figure 3 illustrates the calibration results for the four N species of interest in type-one incubation. Overall, the calibration and validation errors were <10 mg N kg\(^{-1}\) dry soil which allowed reasonable estimation of the concentrations of the species of interest in soil saturated pastes, considering that the concentration of each N specie was in the range of 0 to 150 mg N kg\(^{-1}\) dry soil.

Type-One Incubation

The results of the incubation are presented in Fig. 4 and 5, and in Table 2. The concentration changes agree with the expectations: Concentrations of both NH\(_4\)^+ species decreased during the incubation, yet \(^{15}\)NH\(_4\)^+ decreased more rapidly than \(^{14}\)NH\(_4\)^+ due to the formation of the latter via mineralization. Additionally, both NO\(_3\)^− species increase during the incubation, and \(^{15}\)NO\(_3\)^− increases more rapidly than \(^{14}\)NO\(_3\)^− due to the higher initial concentration of \(^{15}\)NH\(_4\)^+. The total mineral N concentration increases during the incubation. This was due to a positive net-rate of mineralization as a result of the minimal loss of gaseous N species under the aerobic conditions of the experiment. The decrease in relative abundance of \(^{15}\)NH\(_4\)^+ throughout the incubation (Fig. 5) agrees with the expectation, where \(^{15}\)NH\(_4\)^+ decreases more rapidly than \(^{14}\)NH\(_4\)^+. The relative abundance of \(^{15}\)NO\(_3\)^− increases with time due to the \(^{15}\)NH\(_4\)^+ enrichment.

The calculated gross mineralization rate (Eq. [3]) was 6.1 mg N kg\(^{-1}\) dry soil d\(^{-1}\), compared to the net mineralization rate of 4.1 mg N kg\(^{-1}\) dry soil d\(^{-1}\) calculated on the basis of total N species mass balance during the incubation. The lower value was most probably due to losses of gaseous N species during the incubation. These may be attributed either to possible gaseous losses due to nitrification (e.g., N\(_2\)O) or denitrification which

![Fig. 3. Calibration results for type-one incubation dataset. The predicted results are presented for all 150 measurements.](image)

Table 1. Root mean square error (RMSE) and \(R^2\) values of calibration of \(^{14}\)N and \(^{15}\)N nitrate and ammonium concentrations in soil pastes using partial least squares (PLS).

<table>
<thead>
<tr>
<th>Incubation set type</th>
<th>N species</th>
<th>RMSE mg N kg(^{-1}) dry soil</th>
<th>(R^2) Validation</th>
<th>(R^2) Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(^{14})NH(_4)^+</td>
<td>8.05</td>
<td>0.88</td>
<td>0.90</td>
</tr>
<tr>
<td>Type-one</td>
<td>(^{15})NH(_4)^+</td>
<td>9.71</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Type-one</td>
<td>(^{14})NO(_3)^−</td>
<td>6.64</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>Type-one</td>
<td>(^{15})NO(_3)^−</td>
<td>6.19</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>Type-two</td>
<td>(^{14})NH(_4)^+</td>
<td>6.14</td>
<td>0.90</td>
<td>0.93</td>
</tr>
<tr>
<td>Type-two</td>
<td>(^{15})NO(_3)^−</td>
<td>9.55</td>
<td>0.88</td>
<td>0.93</td>
</tr>
</tbody>
</table>
may have occurred in anaerobic microsites in the Terra Rossa aggregates (e.g., Kremen et al., 2005). The calculated gross nitrification rate was 40.9 mg N kg⁻¹ dry soil d⁻¹, compared with the net nitrification rate of 25.3 mg N kg⁻¹ dry soil d⁻¹ based on total NH₄⁺ mass balance, and 29.5 mg N kg⁻¹ dry soil d⁻¹ when calculated according to total NO₃⁻ mass balance. Two main factors can explain the differences between these values: N species losses (e.g., gaseous emissions), and NH₄⁺ production via mineralization, which is not accounted for in the calculation of the net nitrification rate.

**Type-Two Incubation**

The results are presented in Fig. 6 and 7, and in Table 2. The concentration of ¹⁴NH₄⁺ decreases due to nitrification, and accordingly ¹⁴NO₃⁻ builds up during the incubation. The ¹⁵NO₃⁻ remains almost constant, which agrees with the fact that no ¹⁵NH₄⁺ was supplied. The total N also increases during the incubation, due to the positive net rate of mineralization and minimal loss of gaseous N species, which is supported by the fairly constant ¹⁵NO₃⁻ concentration during the incubation. The relative abundance of ¹⁵NO₃⁻ during the incubation (Fig. 7) decreases due to formation of ¹⁴NO₃⁻.

The calculated net mineralization rate was 6.4 mg N kg⁻¹ dry soil d⁻¹. As mentioned earlier, in a type-two incubation the gross mineralization rate cannot be evaluated. The calculated gross nitrification rate was 18.6 mg N kg⁻¹ dry soil d⁻¹, compared with a net nitrification rate of 11.9 mg N kg⁻¹ dry soil d⁻¹ calculated based on the total NH₄⁺ mass balance, and 18.3 mg N kg⁻¹ dry soil d⁻¹ according to the total NO₃⁻ mass balance. The net nitrification rate obtained, based on the total NH₄⁺ mass balance, was ~35% smaller than the gross nitrification rate. This can be attributed to the formation of mineralized ¹⁴NH₄⁺ which is not accounted for during the incubation. The net nitrification rate obtained, based on the total NO₃⁻ mass balance, was similar (<2% difference) to the gross nitrification rate assumable due to minimal losses of NO₃⁻ during the aerobic incubation.

The gross nitrification rate of the type-two incubation was ~50% of the gross nitrification rate of type-one incubation. The total NH₄⁺ concentration of the type-two incubation, which was around 50% of the total NH₄⁺ concentration of type-one incubation, explains this decrease in the gross rate between the two incubations. In the type-one incubation, the net nitrification rate, based on the total NH₄⁺ mass balance, was ~40% smaller than the gross nitrification rate. This difference was very similar to the results obtained in the type-two incubation. However, the net nitrification rate, based on the total NO₃⁻ mass balance in type-one incubation, was ~25% smaller than the gross nitrification rate in the same incubation, unlike the negligible difference obtained in the type-two incubation. This difference can be attributed to the larger amount of NH₄⁺ in the type-one incubation. The higher concentration of NH₄⁺ may enhance oxygen depletion via nitrification (Kremen et al., 2005). Anaerobic conditions which may have developed locally due to the temporal oxygen depletion could encourage NO₃⁻ loss via coupled nitrification denitrification (CND; Kremen et al., 2005). The loss of NO₃⁻ reduced the net nitrification rate estimated via the total nitrate mass balance. This is also indicated by the mineralization rates of the type-one incubation, where the net mineralization rate was ~30% smaller than the gross mineralization rate, also possibly due to nitrate losses.

**Conclusions**

Two main objectives were set for this study. The first objective was to establish a reliable method of calibration for predicting N species with and without ¹⁵N labeling in soil saturated pastes. This was achieved successfully despite the overlapping of the absorbance bands of the N species. Yet, it should be noted that this study was conducted with a soil containing low concentration of calcium carbonate—the
main interfering compound. Overall, the use of the PLS regression enabled reasonable estimation of the N species of interest in soil saturated pastes. The RMSEs for the various NH₄⁺ and NO₃⁻ species ranged from 6.1 to 9.7 mg N kg⁻¹ dry soil, which is reasonable considering this technique enables the determination of N species almost in real time.

The final objective was the estimation of gross mineralization and nitrification rates. The analytical FTIR-ATR system tested in this research was very simple to use and with minimal handling of the tested soil. The net mineralization rate was ~30% smaller than the gross mineralization rate, possibly due to nitrate losses. The net nitrification rates, calculated using the NH₄⁺ mass balance, were around 35 to 40% smaller than the gross nitrification rates, mainly due to unaccounted for mineralized NH₄⁺. The net nitrification rates, calculated using the NO₃⁻ mass balance, showed a mixed trend, where in type-one incubation the net rate was ~25% smaller than the gross rate, presumably due to oxygen depletion, and in type two-incubation the net rate was similar to the gross rate due to minimal N losses. The deviations between net rates and gross rates, due to influences of various "sink/source" processes occurring concomitantly, emphasize the problem of tracing net changes, when estimation of real (gross) rates can provide additional important insight.

Nevertheless, there are some limitations which should be taken into account. The relatively low sensitivity of the FTIR-ATR is the main limitation, and the proposed FTIR-ATR approach cannot replace IRMS analyses. A second limitation is the need for preliminary experiments and procedures such as the 1:1 vs. 1:10 KCl extractions, and the need to utilize, at this stage, a soil specific calibration procedure. Despite these limitations, the combined use of FTIR-ATR and N enrichment appears to provide an effective tool for tracking the fast N-dynamics in soils. On-going work in our group concentrates on the ability to run soil incubations on the ATR crystal itself, which will allow repeated on-line real-time measurements of at least ¹⁴/¹⁵NO₃⁻ concentrations.

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REFERENCE


