Advanced methods for tracing nitrogen transformations in soils irrigated with reclaimed wastewater

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Background and Objective
Knowledge regarding the pathways involved in N\textsubscript{2}O production is still limited despite efforts to quantify mechanisms and sources of its formation (denitrification, nitrification, nitrifier-denitrification). Even calculations of gross rates of N-mineralization or nitrification are still limited and depend on complex, time consuming and destructive methods. These are essential for developing better management tools and mitigation measures. Techniques for quantitative investigation of N transformations and N\textsubscript{2}O source partitioning in soils are based on isotopic enrichment; can be improved by dual-isotope labelling (Baggs, 2008) or alternatively by tracing changes of N\textsubscript{2}O isotopomers or isotopologues (e.g. Baggs, 2008; Sutka et al., 2006) in the gas phase. These require the use of IRMS to get quantitative results. Yet, IRMS can not be used on line and demands laborious pre-treatment of soil samples. FTIR spectroscopy with the ability to monitor changes in N-gases (using LP, Long-Path gas cells; e.g. Esler et al, 2000) and in soil N-mineral species (using ATR, Attenuated Total Reflectance, e.g. Linker et al., 2006) offers powerful tools for in-situ investigations; particularly when combining smart labelling of \textsuperscript{15}N/\textsuperscript{14}N and/or \textsuperscript{18}O/\textsuperscript{16}O allowing direct measurements in the soil phase (Du et al., 2009) or changes in N\textsubscript{2}O isotopomer concentrations in gas phase (Esler et al., 2000). A new approach used for tracing changes in heterogeneous systems of air pollutants allows in-situ investigation of changes in gas-liquid-soil phases (Segal-Rosenheimer and Dubowskii, 2007). First efforts for developing a novel method based on FTIR spectroscopy for continuous monitoring of isotopic N-species directly in moist soil and gas phase are presented emphasizing their potential to serve as efficient tools to quantify N-dynamics and N\textsubscript{2}O source partitioning in complex systems.

Alternatively, we examined the method of hot water extraction for detecting changes in soil labile organic matter, induced by different irrigation practices and soil amendments/fertilizers. Hot water extractable nitrogen was found indicative of the availability organic N in a number of researches (e.g. Ross et al., 2011). Hot water allows extraction of large concentrations of organic matter providing an advantage for spectroscopic methods used for analysing chemical components present in aqueous solutions.

EEM (excitation-emission matrix) fluorescence spectroscopy has been widely used as a method for characterizing dissolved organic matter in water and soil solutions and extracts (e.g. Saadi et al., 2006). Yet, in order to properly utilize such spectra for soil organic constituents appropriate signal processing models should be chosen for interpreting the three-dimensional, complex data obtained for soil samples. We have taken the method one step ahead hypothesizing that hot water extraction in combination with EEM may be a reliable
analytical method for characterizing changes in concentration and content of soil labile organic matter and organic or available nitrogen induced by soil types and effluent irrigation.

Materials and Methods

Direct determination of N-isotopic species during soil incubation using FTIR-ATR:

Incubation experiments were performed by adding solutions of $^{15}$NH$_4$Cl or $^{14}$NH$_4$Cl to vessels containing Terra Rossa covered with a perforated lid, and incubated at 25$^\circ$C for 8 days. Soil were sampled as follows: 10g were mixed at a ratio of 1:1 with KCl 1N solutions forming a paste that was placed on a Zinc/Se ATR crystal to obtain MIR spectra to determine $^{15}$NH$_4$, $^{14}$NH$_4$, $^{15}$NO$_3$ & $^{14}$NO$_3$ concentrations using a BRUKER Vector 22 FTIR spectrometer; Afterwards, the pastes were centrifuged, filtered and the clear solutions were placed again on the ATR crystal and MIR spectra taken again. Accordingly, 2 special calibration solutions containing mixtures of all the tested N-species were prepared with (i) soil pastes (1:1 KCl) as background or with (ii) the 1:1 KCL solution after filtration. A PLS algorithm was successfully used for the data processing and calibration of the $^{14}$NH$_4$Cl set. For the $^{15}$NH$_4$Cl set a Neural Networks based algorithm was required. Additional samples of ~2g were extracted at 1:10 ratio of soil:KCl 1N solutions, and used for determining total nitrate + nitrite and ammonium concentrations using an auto analyzer.

Tracing N$_2$O emission from soils using LP-FTIR gas cells:

Saturated soil samples of a Grumosol were placed at the bottom of an LP-FTIR cell. The LP cell was connected to a FTIR spectrometer, allowing continuous collection of MIR spectra during 22 hrs of soil incubations at different condition (aerobic and non-aerobic conditions, with and without acetylene) and 2 soil thicknesses (~ 2 and ~10 mm). Concentrations were determined using the N$_2$O peaks at the range of 2200-2250 cm$^{-1}$.

Hot water Extractions combined with EEM for evaluating organic constituents and N status:

Samples from five different agricultural soil types in Israel were collected from long-term experiments in which effluent and fresh water irrigation was compared. The extraction procedure consisted of cold water extraction (CaCl$_2$ 0.01M, 20$^\circ$C, 1 hr) followed by hot water extraction (80$^\circ$C, 16 hr). TOC (Total organic Carbon), TN (Total Nitrogen) and mineral N-species ($NH_4^+$, NO$_3^-$, NO$_2^-$) concentrations were measured in the cold and hot water extractions. Organic Nitrogen availability (mineralizable N) was also estimated by aerobic incubations of soil samples for five weeks. The cold and hot water extracted solutions were characterized using a EEM (Excitation-Emission Matrix) fluorometer. PARAFAC (Parallel Factor Analysis) was used for interpreting the EEM data, by looking for the main components of the organic compounds and evaluating their relative concentration in each sample.
Results and Discussion

Using Combined systems of FTIR and N-Isotopes:

Figure 1 shows results obtained for the Terra Rosa soil enriched with $^{15}$NH$_4$ (left) or with $^{14}$NH$_4$ (right) indicating the contributions of $^{15}$NH$_4$ and $^{14}$NH$_4$ to the formation of $^{15}$NO$_3$ and $^{14}$NO$_3$, respectively, and the possibility of simple and non-destructive use of FTIR to trace N-dynamics. Gross mineralization rate estimated on the $^{15}$NH$_4$ dilution basis was $\sim$ 1.24 mg N/g soil/d, while the net rate estimates based on common measurements were negative.

Figure 1: Left: Changes with time of $^{15}$N and $^{14}$N mineral N-species concentrations obtained for incubation of Terra Rossa amended with $^{15}$NH$_4$ using FTIR spectroscopy; Right: Changes with time of $^{14}$N mineral N-species concentrations obtained for Terra Rossa incubated with $^{14}$NH$_4$ – FTIR results compared to auto-analyzer measurements.

N$_2$O emissions, measured with the LP-FTIR system, with a Grumosol were significantly affected by aeration and soil thickness. Under aerobic conditions no N$_2$O was formed, in the 2 mm saturated layer despite the saturation, presumably due to non-restricted oxygen supply; yet under non-aerobic conditions $\sim$ 60% of the initial nitrate was transformed to N$_2$O, presumably via denitrification. In the 10 mm saturated layer, exposed to aeration, $\sim$ 30% of initial mineral-N was lost as N$_2$O, possibly via nitrification and denitrification. In experiments performed with added acetylene, the losses of N$_2$O from non-aerobic saturated 2mm soil layers were about $\sim$40 to 50% larger than without acetylene addition.

Results indicate the possibility of effective and on-line tracing of concentration changes of isotopic species of mineral-N in soils with no specific sample preparation. N$_2$O emissions can be directly measured in incubated soils allowing measurement of continuous changes in the gas phase. The encouraging results observed with the separate set-ups indicate the potential for the next phase where a combined system consisting of FTIR-ATR and LP-FTIR units will be used for on-line measurements of changes in isotopic species both in soil and gas phases. Yet, special emphasis should be put on efforts of increasing accuracy. General, as expected, extracted organic matter concentrations were higher in effluent irrigated soils.
Using Hot water Extraction in Combination with EEM

Based on incubations, mineralized N was found higher in effluent irrigated soils. Moreover, hot water extractable nitrogen was found to be a good predictor for nitrogen availability. Three main components were found by PARAFAC modeling of the EEM data of the hot water extractions being related to: humic acid- 375/478nm (Ex/Em), fulvic acid- 345/423nm (Ex/Em), and protein- 290/383nm (Ex/Em). The scores calculated for the humic and fulvic components were highly correlated with hot water TOC, TN and TON concentrations. Prediction of these parameters was improved by the N-PLS method. Other spectroscopic results indicate possible differences in extractable organic matter characteristics between effluent and fresh water irrigated soils and also between different soil types. The scores calculated for the fulvic component in the PARAFAC model were well correlated with mineralizable-N in all soils. The obtained results provide encouraging indications about the potential of using hot water extractions and spectroscopy for characterizing important components of soil carbon and nitrogen in different soils.

References

5. Ros GH, MC Hanegraaf, E Holland, WH van Riemsdijk. 2011. Predicting soil N mineralization: