New mobile, field based continuous-flow isotope ratio mass spectrometer system for automated denitrification studies

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Abstract
Terrestrial denitrification, the reduction of oxidized nitrogen (N) to nitrous oxide (N_2O) and dinitrogen (N_2), is considered the least well understood process in the global nitrogen cycle. This study introduces a novel continuous-flow isotope ratio mass spectrometer (IR-MS) system that can be deployed in the field and continuously measure N_2 and N_2O emissions. Utilizing the ^{15}N gas flux method this system can provide a better understanding of terrestrial denitrification. The system was tested over 14 days on 2 different agricultural soils (vertosol and ferrosol) which were fertilized with the equivalent of 100 kg ha^{-1} of N added in the form of KNO_3 where the N was 60 at.% ^{15}N. Total gaseous N losses over the 14 day monitoring period resulted in 14.1± 0.53 kg ha^{-1} and 5.7± 0.92 kg ha^{-1} for the ferrosol and vertosol soils, respectively. These results clearly demonstrate the ability of the field based IR-MS to measure N_2 and N_2O emissions from denitrification under field conditions. This system has the potential to improve our understanding of terrestrial denitrification and improve efforts to reduce gaseous N emissions from agricultural systems.

Key Words
Denitrification, N_2 and N_2O emissions, Isotope ratio mass spectrometer, ^{15}N gas flux method, N_2/N_2O ratios

Introduction
Terrestrial denitrification, the reduction of oxidized nitrogen (N) to nitrous oxide (N_2O) and dinitrogen (N_2), is considered the least well understood process in the global nitrogen cycle. Based on global mass balance estimates terrestrial denitrification represents the largest loss of anthropogenic N (Seitzinger et al. 2006). Efforts to reduce this loss of N are important for 2 reasons: (1) N lost as N_2O is an environmental pollutant that has a global warming potential 298 times greater than CO_2, and contributes to stratosphere ozone depletion, (2) N lost as N_2 represents a loss of applied fertilizer and is an economic cost on the agricultural sector. Current efforts to reduce denitrification losses are constrained by a lack of field data and understanding of the underlying processes.

To fully understand the denitrification process both N_2 and N_2O fluxes and the ratio between them needs to be assessed during the same denitrification event under field conditions. While N_2O as the intermediate product of denitrification has been extensively studied at high temporal resolution, similar studies of the end product N_2 are scarce. This research gap is mainly due to the fact that it is methodologically difficult to detect against atmospheric background N_2 (Groffman et al. 2006).

There are two main approaches to detect the end product of denitrification (N_2): the gas-flow soil core method and the ^{15}N gas flux method. The gas-flow soil core method requires atmosphere removal above a soil core, which cannot be done under in situ conditions. The ^{15}N gas flux method is based on the addition of $^{15}NO_3^-$ to soil undergoing denitrification and can directly detect the products of denitrification under field conditions. Following the addition of $^{15}NO_3^-$ to soil, gas samples are taken from an enclosed chamber above the soil and taken back to a laboratory to be analysed for the $^{15}N_2$ and $^{15}N_2O$ content on an isotope ratio mass spectrometer. The sole attempt to monitor denitrification in situ used a Fourier transform infrared spectrometer (FTIR) to measure $^{15}N_2O$ fluxes, unfortunately this system is not capable of detecting $^{15}N_2$ fluxes (Phillips et al. 2013).The reason samples must be transported to a laboratory are that current IR-MS are restricted to laboratory conditions and in the case of N_2O detection require a liquid N (LN) cryo-trap to isolate and focus the trace amounts of N_2O found in gas samples. Due to this need to take manual samples the logistics of getting high temporal resolution are prohibitive.

To close the research gap for high resolution ^{15}N gas flux measurements under field conditions, this study introduces a novel IR-MS system that can be deployed in the field and continuously monitor denitrification to produce a better understanding of the underlying processes and identify the main drivers.

Methods

A new IR-MS system was developed and field tested by the Queensland University of Technology (QUT) in collaboration with Sercon, UK. The IR-MS system is housed in an air-conditioned trailer and can be transported to the desired field location. This system can be directly connected to an existing automated greenhouse gas (GHG) monitoring system developed by QUT and detailed by Scheer et al. (2014). The trailer contains 3 units: A sample control unit for the GHG system, a trace gas preparation unit to isolate and focus the gas samples and an IR-MS to analyse the gas samples for concentrations and ^{15}N content.

Field IR-MS system

The IR-MS in this system is a standard Sercon continuous flow 20-22 model. The trace gas preparation unit (TGP) connected to the IR-MS was an extensively modified Sercon Cryo-Prep unit. Firstly the LN trap standard in most TGP units was replaced with a zeolite based N_2O trapping system, which allows the isolation and focusing of N_2O but removes the need for a constant supply of LN. The internal pumping of the TGP was modified to include trapping loops for N_2 (100 µL) and N_2O (200 mL), two 6 port valves (Valco) were used to introduce the trapped samples in to the helium carrier stream at precise times during the sampling cycle. After the trapped samples were introduced to the carrier stream both samples pass through individual Nafion dryers. The N_2O sample then passes through a Schultz reagent CO trap and a Carbosorb CO_2 trap before being isolated and focused on the zeolite after which it is directed to the IR-MS for ^{15}N analyses. The N_2 sample passes through a copper based reduction oven (600 ^{o}C) to remove O_2 and then through a GC column to separate N_2 from any remaining O_2 before it is directed to the IR-MS for ^{15}N analyses.

The sample control unit was used to control pneumatically operated static chambers fixed on stainless steel bases which were inserted 10 cm into the ground. It was modified from a standard GHG sampling unit to include a counter flow moisture removal system to dry the air samples and a particle filter. It was also modified so that three different reference gases could be introduced to the TGP and IR-MS at precise times during the sampling cycle. As a result of the modifications the system is capable of detecting up to 8 N_2O and N_2 fluxes per day from the 8 automatic chambers deployed over the soil in a fertilized agricultural system. The system also monitors CO_2 emissions with a single path infra-red gas analyzer (Licor, LI840, St Joseph, MI, USA), temperature and soil moisture.

Field setup

The system was tested with two typical cropping soils from SE Queensland (a vertosol collected from the Kingsthorpe research station and a ferrosol collected from the Kingaroy research station). Thirty kilograms of each soil were placed into 4 chamber bases from the automated GHG system and adjusted to field bulk density. The soil in the chambers had the equivalent of 100 kg ha^{-1} of N added in the form of KNO_3 (60 at.% ^{15}N). Water was added to each chamber so that the WFPS was 100%. On day 7 the equivalent of 360 kg ha^{-1} of carbon was added to each chamber in the form of glucose to stimulate microbial activity. At this time a further 3 L of water was added to each chamber.

Automated sampling cycle

The N_2 and N_2O concentrations were measured 4 times a day from each chamber over 2 weeks using a fully automated cycle. In the case of N_2 the flux was calculated based on the measured $^{29}N_2$ and $^{30}N_2$ detected above atmospheric background according to the procedure outline by Arah (1997). The N_2O flux was calculated based on the increase in N_2O concentration [ppm] over the chamber closure time. The ^{15}N enrichment of the N_2O was also used to calculate the ^{15}N soil pool enrichment (aD) which was then used to increase the sensitivity of the N_2 detection as described by Stevens and Laughlin (2001).

Detection limits

The detection limit (DL) of N_2 was calculated based on the standard deviation of atmospheric air samples included in each day's sampling run. The DL of the IR-MS at the 95% confidence interval (n = 25) for $\Delta^{29}R$ was 1.4 x 10^{-6} and for $\Delta^{30}R$ was 2.8 x 10^{-6}. This equates to a method detection limit (MDL) for N_2 fluxes ranging from 58 g ha^{-1} day^{-1} to 130 g ha^{-1} day^{-1} of N_2 based on a NO_3^- ^{15}N pool enrichment of 60% to 40% respectively and a closure time of 201 minutes.

Results

N_2O emissions over the first 6 days of the experiment were low ranging from for 0.2 – 0.8 g N_2O-N ha^{-1}

day^{-1} and 2.1–6.2 g N_2O-N ha^{-1} day^{-1} for the Kingaroy and Kingsthorpe soil, respectively. After the application of glucose at day 7 there was a sharp increase in N_2O fluxes in both treatments (Figure 1). The Kingaroy soil fluxes kept increasing over 4 days reaching a maximum value of 1008 g N_2O-N ha^{-1} day^{-1} at day 11 and declining afterwards to 88 g N_2O-N ha^{-1} day^{-1} at day 14. The Kingsthorpe soil reached its maximum N_2O flux at day 9 with 1138 g N_2O-N ha^{-1} day^{-1} and declined sharply afterwards to values below 80 g N_2O-N ha^{-1} day^{-1} at day 11.

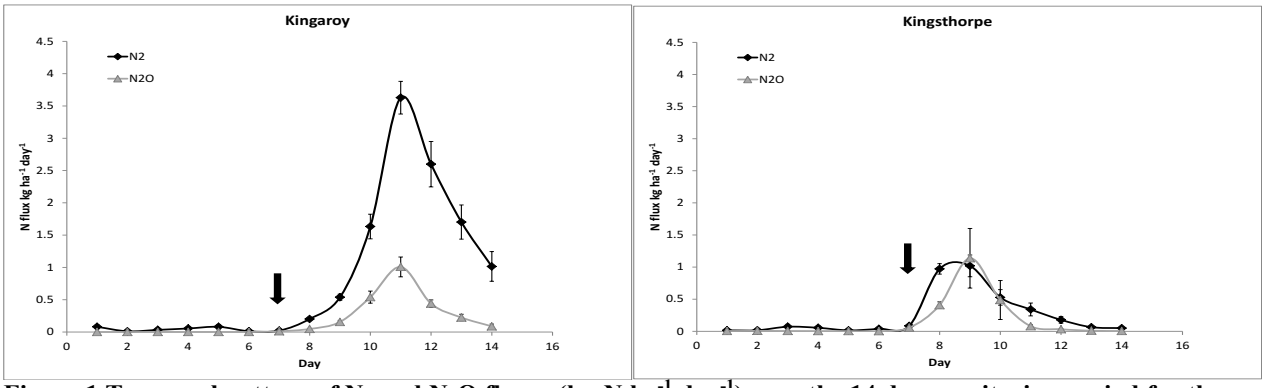

Figure 1 Temporal pattern of N_2 and N_2O fluxes (kg-N ha^{-1} day^{-1}) over the 14 day monitoring period for the Kingsthorpe (QLD) and Kingaroy (QLD) soils. The black arrow indicates the time of glucose application (360 kg ha^{-1}) to the soil.

N_2 emissions were below the detection limit of the automated field system over the first 6 days of the experiment for both soils. After the application of glucose, N_2 emissions increased strongly following a similar temporal pattern as the N_2O emissions. For the Kingaroy soil N_2 emissions were substantially higher than the N_2O fluxes reaching 3600 g N_2-N ha^{-1} day^{-1} at day 11. The Kingsthorpe soil showed significantly lower N_2 emissions reaching its maximum at day 9 with 1019 g N_2-N ha^{-1} day^{-1}.

Cumulative emissions over the 14 day monitoring period resulted in nearly 3 times higher losses from Kingaroy than Kingsthorpe (Table 1). The main product of denitrification was N_2 for both soils but the N_2/N_2O ratio was significantly higher for the Kingaroy soil.

Table 1: Total N losses and the resulting N_2/N_2O ratio over the 14 day monitoring period

Soil	Total N lost [kg ha^{-1}]	N_2 [kg N ha^{-1}]	N_2O [kg N ha^{-1}]	N_2/N_2O Ratio
Kingaroy (QLD)	14.1 ± 0.53	11.6 ± 0.10	2.5 ± 0.42	4.6
Kingsthorpe (QLD)	5.7 ± 0.92	3.5 ± 0.05	2.2 ± 0.87	1.6

Discussion

The field system enabled us to measure N_2 and N_2O fluxes from 8 replicate chambers continuously over a 14 days monitoring campaign. The fluxes showed a high variation over the campaign and stayed generally low over the first 6 days of the experiment. After the application of glucose to the soil we observed a significant increase in both N_2 and N_2O emission confirming our findings from other studies that carbon availability was one of the main drivers for soil denitrification. Kingaroy soil showed a greater denitrification potential than the Kingsthorpe soil with total a of 14 kg N ha^{-1} and 6 kg N ha^{-1}, respectively, lost over the 2 week period. This loss is equivalent to 14% and 6%, respectively, of the fertilizer applied at the onset of the experiment. Since fertilizer and water addition was exactly the same for the two different soils the significantly higher denitrification activity in the Kingaroy soil can be attributed to a generally higher microbial activity in this soil, in particular a more active community of denitrifiers.

The use of a field based, fully automated sampling system has the advantage to eliminate any errors associated with the manual extraction, transport and injection of gas samples. Our custom-built gas preparation unit allows for simultaneous analysis of concentration and isotopic signature of N_2 and N_2O in the sample air with high analytical precision, which resulted in a detection limit of for N_2 fluxes of 58 g ha^{-1} day^{-1}. These detection limits are at the higher end of those reported for laboratory based setups (Butterbach-Bahl et al. 2002), but offer the advantage of in situ measurement. It needs to be noted that the detection limit

of such a system depends on the sensitivity of the IR-MS, the ^{15}N enrichment of the soil NO_3^- pool, the chamber headspace volume and the chamber closure time. Thus, the detection limit can potentially be lowered by (1) reducing the chamber headspace, (2) using a higher ^{15}N enrichment of the applied tracer and (3) increasing the chamber closure time. Considering the detection limit of the current configuration and given the need to apply high levels of highly enriched $^{15}NO_3^-$ the current system is more appropriate for the detection of N_2 and N_2O losses from fertilized agricultural systems rather than natural ecosystems.

Overall, this experiment showed, that under certain conditions such as high WFPS, high nitrate content and no carbon limitation, N_2 is the main product of denitrification and that denitrification losses can reach extraordinarily high values of up to 4.6 kg N ha^{-1} day^{-1}. This might explain the high losses of 20-80 kg N ha^{-1} over a cropping cycle, which was observed from these sites during ^{15}N fertilizer recovery studies (De Antoni Migliorati et al. 2014). However, the fact that we had to add carbon to stimulate denitrification shows that it is the interaction of different parameters that trigger these high denitrification losses. We assume that under field conditions the addition of labile carbon from plants via root exudates can add the carbon required to create such conditions. On the other hand the overall N_2/N_2O ratios of 1.3 and 4.6, respectively for Kingsthorpe and Kingaroy, don't explain the high losses observed from ^{15}N recovery studies under field conditions. To explain denitrification losses of 20-80 kg N ha^{-1} over the cropping season the N_2/N_2O ratios would need to be in the range of 10 to 50. While the reasons for these low ratios are not clear, one possible explanation is that high concentrations of NO_3^- in soil inhibit the conversion of N_2O to N_2 which requires further investigation. Moreover, N_2 formed in the soil under high water content might remain trapped in the soil and although it is produced is not emitted at the surface immediately. Overall, the interaction of plants, soil and microbial community could not be represented in this short field experiment using bare soils. Ideally more field data would be required in order to verify denitrification losses and the N_2/N_2O ratios for different cropping systems.

Conclusion

The field experiment clearly demonstrated the ability of the field based IR-MS to measure N_2 and N_2O emissions from denitrification under field conditions. There is still significant room to improve this system and lower detection limits by further modification of TGP unit and altering the sampling regime. Reducing the amount of time taken to analyse each gas sample should allow improved temporal resolution. Overall this system represents a tool that may help to significantly improve our understanding of denitrification in fertilised cropping systems by closing the gap of in situ N_2 and N_2O measurements based on ^{15}N tracer methods and therefore can help to reduce gaseous N losses from agricultural systems.

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