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## INTRODUCTION

The  $^{15}\text{N}$  tracer approaches can provide *in situ* measurements of both  $\text{N}_2$  and  $\text{N}_2\text{O}$ , but their use has been limited to fertilised arable soils due to the need for large  $^{15}\text{N}$  additions in order to detect  $^{15}\text{N}_2$  production against the high atmospheric  $\text{N}_2$ . An 'in house' laboratory designed and manufactured  $\text{N}_2$  preparation instrument interfaced to a continuous flow isotope ratio mass spectrometer (CF-IRMS) can allow the analysis of  $^{15}\text{N}-\text{N}_2$  with small injection volumes, improved precision and lower limit of detection. Such an instrumental advance could improve our ability for measuring denitrification in natural and semi-natural land use types. Therefore, we designed a study to:

- 1) Determine the precision and suitability of our preparative-IRMS instrumentation for measuring  $^{15}\text{N}-\text{N}_2$  and  $^{15}\text{N}-\text{N}_2\text{O}$  at low/trace enrichment levels
- 2) Adapt the  $^{15}\text{N}$  Gas-Flux method for application across natural and semi-natural terrestrial ecosystems
- 3) Directly compare the validity and applicability of the  $^{15}\text{N}$  Gas-Flux method with the acetylene inhibition technique (AIT) for measuring *in situ* denitrification rates.

## METHODS

For  $\text{N}_2$  gas isotopic analysis an Isoprime IRMS coupled to an 'in house' built  $\text{N}_2$  preparative interface was used (Fig 1). Headspace gas (4  $\mu\text{L}$ ) was injected and ratios for the  $m/z$  28,  $m/z$  29 and  $m/z$  30 were recorded. For  $\text{N}_2\text{O}$ , headspace gas (ca. 4mL) was injected into a TraceGas™ Preconcentrator coupled to an IRMS and ratios for  $m/z$  44,  $m/z$  45 and  $m/z$  46 were measured.

*In situ* denitrification rates in organic (OS), woodland (WL) and grassland (GL) soils were measured using static chambers (Fig 2A) according to the  $^{15}\text{N}$  Gas-Flux method<sup>1</sup>. Labelled  $\text{K}^{15}\text{NO}_3^-$  (98 at. %) was applied in each site ( $n=5$ ) via multiple injections into enclosed soils. Gas samples were collected at  $T=1\text{h}$ ,  $T=2\text{h}$  and  $T\approx 20\text{h}$  for  $\text{N}_2$  and  $\text{N}_2\text{O}$  analysis. Minimum detectable concentration (MDC) change for R29 and R30 was defined<sup>2</sup> using standards to determine if each time step sample was significantly different from ambient ( $T=0\text{hr}$ ) and if not they were excluded from the flux calculations. The flux of  $\text{N}_2$  and  $\text{N}_2\text{O}$  were determined<sup>3,4</sup>.

At the same time intact soil cores were collected and incubated *in situ* with and without the addition of  $\text{C}_2\text{H}_2$  according to the AIT approach for the determination of denitrification rates (Fig 2 B)<sup>5</sup>.

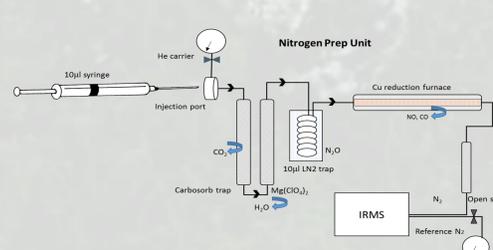


Figure 1: Schematic of the  $^{15}\text{N}-\text{N}_2$  analysis system



Figure 2:  $^{15}\text{N}$  Gas flux chamber (A) and intact soil scores with  $\text{C}_2\text{H}_2$  amendment (B)

## RESULTS AND DISCUSSION

Instrument stability checks showed standard deviation fits better than 0.05 ‰ for both gases. Precision of the instrument was better than 0.08 ‰ and 0.3 ‰ for  $\delta^{15}\text{N}-\text{N}_2$  and  $\delta^{15}\text{N}-\text{N}_2\text{O}$  gases, respectively.

The minimum detectable flux rates were 4  $\mu\text{g N m}^{-2} \text{h}^{-1}$  and 0.2  $\text{ng N m}^{-2} \text{h}^{-1}$  for  $\text{N}_2$  and  $\text{N}_2\text{O}$ , respectively, which is a significant improvement compared to earlier studies. The improved precision for both allowed us to quantify denitrification with low  $^{15}\text{N}$  enrichment under *in situ* conditions, which was not possible earlier.

The evolved  $\text{N}_2$  and  $\text{N}_2\text{O}$  in the chamber headspace increased linearly from 1 to 20 hours (Fig 3). We calculated flux rates by applying linear regression (when  $r^2 > 0.95$ ) between 1 and 20 hours using only those time points that were above the MDC values.

The total denitrification rate measured using the  $^{15}\text{N}$  Gas flux (range: 2.4 - 416.6  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ) and the  $\text{C}_2\text{H}_2$  methods (range: 0.5 - 325.2  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ) followed a similar trend across the sites (Pearson;  $r = 0.581$ ,  $n = 25$ ,  $p < 0.01$ ) (Fig 4). However, denitrification rates measured using the  $^{15}\text{N}$  Gas flux method were between 3 and 5 times higher than the denitrification rates with the AIT method.

Bulk  $\text{N}_2\text{O}$  emission rates measured using the headspace samples from the chambers and no- $\text{C}_2\text{H}_2$  amended cores exhibited a similar trend across sites; however, the  $\text{N}_2\text{O}/\text{N}_2+\text{N}_2\text{O}$  ratios differed between the two methods (Fig 4). The  $\text{N}_2\text{O}/\text{N}_2 + \text{N}_2\text{O}$  ratio measured using the  $^{15}\text{N}$  Gas flux method was low (range: 0.03 to 13%) compared to the AIT (range: 50 to 60%). The reason for this discrepancy is that the AIT cannot discriminate  $\text{N}_2\text{O}$  sources to constrain the ratio to denitrification only and an incomplete inhibition of  $\text{N}_2\text{O}$  reduction due to diffusion constraints of  $\text{C}_2\text{H}_2$  in soil cores.

## CONCLUSION

The improved precision for both  $^{15}\text{N}-\text{N}_2$  and  $^{15}\text{N}-\text{N}_2\text{O}$  analyses allows the quantification of *in situ* denitrification rates with low  $^{15}\text{N}$  enrichment in natural and semi-natural ecosystems.

## REFERENCES

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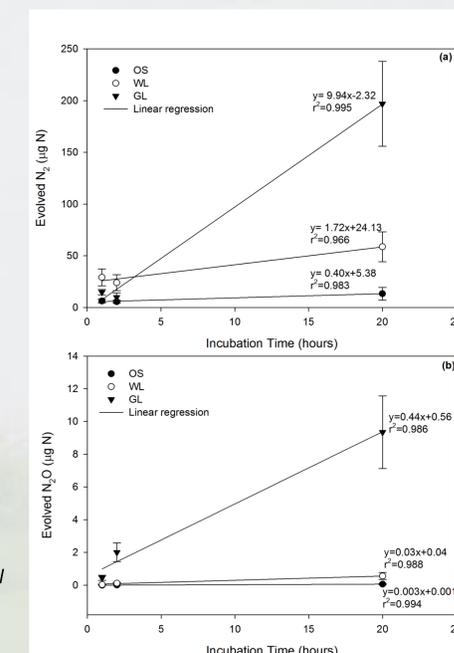


Fig. 3: Evolved gases from soils using the  $^{15}\text{N}$  Gas flux method.

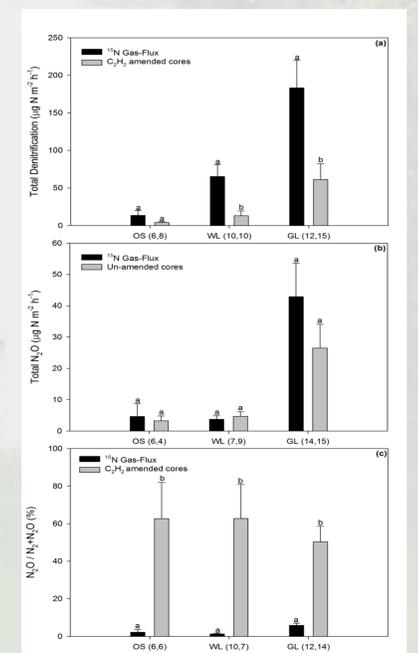


Fig. 4: Denitrification, bulk  $\text{N}_2\text{O}$  and  $\text{N}_2\text{O}/\text{N}_2+\text{N}_2\text{O}$  emission ratios.