Denitrification losses in response to N fertiliser rates – a synthesis of high temporal resolution N₂O, in-situ ¹⁵N₂O and ¹⁵N₂ measurements and fertiliser ¹⁵N recoveries in intensive sugarcane systems

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S1 Materials and methods

S1.1. Automated chamber system

Acrylic static chambers (0.5 m × 0.5 m × 0.15 m) were mounted on stainless steel frames inserted 10 cm into the soil. The lids of the chambers were opened and closed automatically with pneumatic pistons, and four chambers were closed at one time. Air samples were taken sequentially from each closed chamber, followed by a single-point known standard (Air Liquide, Dallas, TX, USA) of 0.5 ppm N_2O and 800 ppm CO_2 for calibration and drift correction (i.e., after every fourth sample). Changes in headspace N_2O and CO_2 concentration after chamber closure were measured with a gas chromatograph (SRI 8610C, SRI Instruments, Inc., Las Vegas, NV, USA) equipped with a ^{63}Ni electron capture detector (ECD) for N_2O analysis while an infrared gas analyser (LI-820, LI-COR Biosciences, Lincoln, NE, USA) was used for measurements of CO_2. In total, each chamber was sampled four times (every 15 min) over 60 min. This enabled up to eight single flux rates to be determined per chamber and day. The detection limit of the system was 2.0 µg N_2O-N m^{-2} h^{-1} for N_2O. Hourly N_2O and CO_2 fluxes were calculated from the slope of the linear change in gas concentration during the closure period (60 min) and corrected for air temperature, atmospheric

pressure and the ratio of chamber volume to surface area as described in detail by Grace et al. (2020). The coefficient of determination (R^2) for the linear regression was calculated and used as a quality check for the measurement. Flux rates were discarded if R^2 was < 0.80.

S1.2. Manual chamber system

Manual gas sampling was conducted with sealed polyethylene chambers (0.5 m × 0.5 m × 0.15 m). Headspace gas samples were taken between 0900 and 1200 H analogous to the sampling regime of the GHG system, connecting a syringe to a 2-way luer-lock tap installed in the lid of the chamber. Gas samples were injected into pre-evacuated 12 mL glass vials with a double wadded Teflon/silicone septa cap (Labco Exetainer ®, UK) and analysed for N_2O and CO_2 using a Shimadzu GC-2014 Gas Chromatograph (Shimadzu, Kyoto, Japan) at the Central Analytical Research Facility of the Queensland University of Technology, Australia.

S1.3. Gas sampling procedure in the micro plots

Gas samples were taken 1–3 times a week between 0900-1200H with higher frequency after N fertiliser application and irrigation or rainfall events. At the Burdekin site, polyethylene chambers with a headspace height of 19.93 cm were placed on the steel frames, ensuring airtight conditions. Headspace gas samples (20 ml) were taken by connecting a syringe to a 2-way luer-lock tap installed in the lid of the chamber. Gas samples were then injected into a pre-evacuated 12 ml glass vial with a double wadded Teflon/silicone septa cap (Labco, UK). Headspace gas samples were collected at 0, 60 and 180 min after closure. At the Mackay site, airtight polyethylene chambers with a headspace height of 15 cm were placed on the base and connected to a battery-powered sampling unit. The chambers were automatically closed at sampling events according to the pre-programmed schedule and gas samples were collected at 0, 60 and 180 min after the chamber closure. Headspace gas samples (20 ml) were automatically taken and injected into the pre-evacuated 12 ml glass vials sequentially installed on a belt in the sampling unit.

S1.4. The ^{15}N gas flux method

The ^{15}N enrichment of the NO_3^- pool undergoing denitrification ($a_p N_2$ and $a_p N_2O$) and the fraction of N_2 and N_2O emitted from this pool (f_p) were calculated following the equations given by Spott et al. (2006)

$$f_p = \frac{a_m - a_{bgd}}{a_p - a_{bgd}}$$ [S1]

where a_{bgd} is the ^{15}N abundance of the atmospheric background and a_m is the measured ^{15}N abundance of N_2 from headspace gas samples taken 0 and 180 minutes after closure, respectively. Both a_{bgd} and a_m are calculated as

$$a_i = \frac{^{29}R + 2 * {^{30}R}}{2 * (1 + {^{29}R} + {^{30}R})} \qquad \text{[S2]}$$

and $^{30}x_m$ is the measured fraction of m/z 30 in N_2:

$$^{30}x_m = \frac{^{30}R}{(1 + {^{29}R} + {^{30}R})} \qquad \text{[S3]}$$

If only ^{29}R was > the detection limit (DL), f_p was calculated as

$$f_{p=} \frac{1}{1 - \dfrac{{^{29}R}(1 - a_p)^2 - 2a_p(1 - a_p)}{{^{29}R}(1 - a_{bgd})^2 - 2a_{bgd}(1 - a_{bgd})}} \qquad \text{[S4]}$$

using a_p N_2O assuming that N_2 and N_2O were derived from the same NO_3^- pool undergoing denitrification.

S1.5. Plant and soil sampling

All the sugarcane plants in the 2.0 m section were sampled by cutting at ground level and the trash on the ground in the section was also collected, followed by fresh weight measurements. Roughly six stalks from the middle of the 2.0 m section were chosen and separated into tops (above 7th node), stalk and dead leaves. Two green leaves at the 3rd node (L+3) were sampled from the plants from the 2-m section (L3_CR) and the adjacent row (L3_AR) to estimate the fertiliser ^{15}N recovery in the plant in the adjacent rows. After sampling aboveground biomass, remaining stools and major roots were sampled from a 0.5 × 0.5 m square in the middle of the 2.0 m section by digging down to 0.15 m depth. Roots were washed to remove the attached soil and separated into stool and roots. The tops, stalks, dead leaves and trash samples were coarsely ground, subsampled (about 10% on a fresh weight basis) and weighed to calculate partitioning ratios. The plant subsamples, L+3 samples, root samples and stool samples were oven-dried at 60 °C and dry weights were recorded. The stalk samples were further dried with a vacuum oven at 40 °C for 48 hours before fine grinding to avoid aggregation due to sugar.

Soil samples were taken at three to four points between the bed and furrow centres using a soil corer and a post-hole driver down to 1.0 m. At the Burdekin site, the sampling points were 0, 0.25, 0.50, 0.75 m away from the bed centre. At the Mackay site, those were 0, 0.12, 0.40, 0.80 m away from the bed centre in 100N and 250N and the three points except for 0.12 m away from the bed centre in 150N and 200N. Each soil core sample of 1.0 m was separated into 0–0.2, 0.2–0.4, 0.4–0.7, 0.7–1.0 m soil depths and subsamples (about 100 g) were taken.

S1.6. ^{15}N calculation in plant and soil

The percentage of N in the individual plant, soil, N_2O and N_2 samples ('sinks') derived from ^{15}N-labelled fertiliser was calculated from

$$Ndff\ (\%) = \frac{\%^{15}N\ excess\ of\ sink}{\%^{15}N\ excess\ of\ fertiliser} \times 100 \qquad \text{[S5]}$$

where the %^{15}N excess used for all sources and sinks was the ^{15}N abundance less an adjustment of %^{15}N measured for the corresponding plant and soil samples in the 0N plots for background enrichment or the natural abundance (0.0036765) for N_2O and N_2 samples.

Fertiliser N recovered in each plant part (*PlantFN$_i$*) was calculated from

$$PlantFN_i = Biomass_i \times N\ content_i \times \frac{Ndff_i}{100} \qquad \text{[S6]}$$

where i indicates the plant part. Fertiliser N recovered in tops, stalk, dead leaves and trash were summed to calculate the fertiliser N recovered in the aboveground biomass (*PlantFN$_{AG}$*) in the centre row in each plot. Fertiliser N recovered in the belowground biomass (*PlantFN$_{BG}$*) in the centre row was calculated by summing the fertiliser N recovered in stool and roots. Fertiliser N recovered in the adjacent rows (*PlantFN$_{AR}$*) was calculated in each plot by multiplying the total N uptake in the centre row and the Ndff calculated from L3_AR sample. Fertiliser ^{15}N uptake was then calculated as follows:

$$Fertiliser\ \ ^{15}N\ uptake = (PlantFN_{AG} + PlantFN_{BG} + PlantFN_{AR} \times 2) \qquad \text{[S7]}$$

To calculate fertiliser ^{15}N recovery in the soil, the distribution of ^{15}N fertiliser across the soil profile between bed and furrow was first analysed to account for the spatial variation caused by banding N fertiliser, following Takeda et al. (2021). (i) Fertiliser N recovered in the soil (*SoilFN$_{ij}$*) of each sample (four points from the bed centre and four depths down to 1.0 m per plot at the centre of the soil layer as the average) was calculated from

$$SoilFN_{ij} = BD_{ij} \times N\ content_{ij} \times \frac{Ndff_{ij}}{100} \qquad \text{[S8]}$$

where i and j indicate the sampling point and depth per plot. At the Mackay site, the missing *SoilFN$_{ij}$* values at the sampling point 0.12 m away from the bed centre in 150N and 200N treatments were substituted with the estimated values from regressions by N rates at each soil depth. (ii) The fertiliser ^{15}N recovered in the soil (*SoilFN$_{ij}$*) was then interpolated across one side of the sugarcane row (0.75 m width, x-axis) and down to a soil depth of 1.0 m (y-axis) for each plot by fitting a thin-plate spline using a package "mgcv" (Wood, 2011). (iii) The interpolated fertiliser N recovered in the soil

(SoilFN$_{xy}$) was integrated and divided by the area of one side of the row (*Area*, 0.75 m^2) to express fertiliser N recovered in kg N ha^{-1}. Fertiliser ^{15}N recovery in the soil as a proportion to the N rate was then calculated as follows:

$$Fertiliser \quad ^{15}N \ recovery \ in \ the \ soil \ = \frac{\sum SoilFN_{xy}}{Area}$$ [S9]

Overall fertiliser ^{15}N loss was calculated by the difference between the N applied and fertiliser ^{15}N recovered in the soil and plant.

Calculations for fertiliser ^{15}N recovered in N$_2$O emissions are detailed in Takeda et al. (2022) and those for N$_2$ emissions followed the same procedure. Briefly, the proportion of N$_2$ emissions derived from fertiliser (*Ndff* N$_2$) at the fertiliser band was first calculated by Equation [S5]. Then, *Ndff* N$_2$ was gap-filled per N rate over the crop growing season on a daily basis at each site, which were then applied to daily N$_2$ emissions per plot in the main plots to calculate fertiliser-derived N$_2$ as follows:

$$Fertiliser \ derived \ N_2 \ emissions_{i,j} = \ N_2 \ emissions_{i,j} \times \frac{Ndff \ N_{2i,j}}{100}$$ [S10]

where i and j indicate days after fertilisation and chamber position (i.e. bed or furrow). At the Burdekin site, *Ndff* N$_2$ at the fertiliser band in micro plots was used for both bed and furrow chambers because both chambers covered the fertiliser band. At the Mackay site, *Ndff* N$_2$ at the furrow was assumed to be zero because the furrow chamber did not cover the fertiliser band. Contribution of fertiliser N to N$_2$ was calculated by area-weighted sum of fertiliser-derived N$_2$ emissions over the crop growing season.

S1.7. Use of generalised additive mixed models

The use of generalised additive mixed models (GAMMs) can quantify non-linear relationships without specifying the functional forms and GAMMs further allow repeated measurements. The distribution of RN$_2$O and *Ndff* N$_2$ was assumed Beta distributed, which is suitable to fit variables taking values between 0 and 1, and the logit function was specified as the link function, respectively. A dispersion parameter can further be specified in Beta regressions, which was useful to fit the RN$_2$O densely distributed near zero. The dispersion parameter was set at 23 based on a comparison of AIC.

To model RN$_2$O, the site factor was specified as a linear term and the CO$_2$ emissions measured in the micro plots, soil NH$_4^+$ and NO$_3^-$ contents measured near the band in the main plots and WFPS and soil temperature measured across the paddock were specified as smooth terms

and the micro plots as the random variable. For RN_2O modelling, cross-validation was performed by splitting datasets into training and testing by replicates (k = 4) and the predictive performance was evaluated by the averaged deviance explained and root mean square error (RMSE). The average of RN_2O predicted across k for each plot and bed/furrow position was used to calculate N_2 emissions.

In gap-filling of *Ndff* N_2, days after fertilisation (DAF) and N rates were specified as the explanatory variables in a tensor product, allowing the changes in *Ndff* N_2 over time to differ between N rate treatments. To account for the repeated measurements of *Ndff* N_2 at the same chamber, 'chamber' was specified as the random variable nested in DAF. The estimated *Ndff* N_2 was then multiplied by 100 to show in percentage.