Denitrification Losses in Response to N Fertilizer Rates—Integrating High Temporal Resolution $\text{N}_2\text{O}$, In Situ $^{15}\text{N}_2\text{O}$ and $^{15}\text{N}$ Measurements and Fertilizer $^{15}\text{N}$ Recoveries in Intensive Sugarcane Systems

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Abstract   Denitrification is a key process in the global nitrogen (N) cycle, causing both nitrous oxide ($\text{N}_2\text{O}$) and dinitrogen ($\text{N}_2$) emissions. However, estimates of seasonal denitrification losses ($\text{N}_2\text{O} + \text{N}_2$) are scarce, reflecting methodological difficulties in measuring soil-borne $\text{N}_2$ emissions against the high atmospheric $\text{N}_2$ background and challenges regarding their spatio-temporal upscaling. This study investigated $\text{N}_2\text{O} + \text{N}_2$ losses in response to N fertilizer rates (0, 100, 150, 200, and 250 kg N ha$^{-1}$) on two intensively managed tropical sugarcane farms in Australia, by combining automated $\text{N}_2\text{O}$ monitoring, in situ $\text{N}_2\text{O}$ and $\text{N}_2$ measurements using the $^{15}\text{N}$ gas flux method and fertilizer $^{15}\text{N}$ recoveries at harvest. Dynamic changes in the $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio (<0.01 to 0.768) were explained by fitting generalized additive mixed models (GAMMs) with soil factors to upscale high temporal-resolution $\text{N}_2\text{O}$ data to daily $\text{N}_2$ emissions over the season. Cumulative $\text{N}_2\text{O} + \text{N}_2$ losses ranged from 12 to 87 kg N ha$^{-1}$, increasing non-linearly with increasing N fertilizer rates. Emissions of $\text{N}_2\text{O} + \text{N}_2$ accounted for 31%–78% of fertilizer $^{15}\text{N}$ losses and were dominated by environmentally benign $\text{N}_2$ emissions. The contribution of denitrification to N fertilizer loss decreased with increasing N rates, suggesting increasing significance of other N loss pathways including leaching and runoff at higher N rates. This study delivers a blueprint approach to extrapolate denitrification measurements at both temporal and spatial scales, which can be applied in fertilized agroecosystems. Robust estimates of denitrification losses determined using this method will help to improve cropping system modeling approaches, advancing our understanding of the N cycle across scales.

Plain Language Summary  Denitrification is a soil nitrogen (N) transformation process, producing the potent greenhouse gas (GHG) nitrous oxide ($\text{N}_2\text{O}$), while turning reactive N into environmentally benign dinitrogen ($\text{N}_2$). The response of these N losses to N fertilizer inputs is critical to reduce environmental impacts while maintaining crop productivity in agriculture. However, difficulties in measuring soil-borne $\text{N}_2$ against atmospheric $\text{N}_2$ and upscaling of these emissions to the farm scale hinder estimation of denitrification losses, leaving denitrification as a major uncertainty for N budgets. This study quantified denitrification losses in response to N fertilizer rates on sugarcane farms in Australia, by combining automated GHG monitoring systems, $N$ isotope techniques and statistical models. This unique approach demonstrated denitrification as a major N loss pathway, increasing non-linearly with increasing N rates. Fertilizer N budgets showed that environmentally harmful N losses increased more than proportionally with N inputs. These findings emphasize that excessive N fertilizer use leads to agronomic inefficiency with severe adverse effects on the surrounding ecosystems such as the Great Barrier Reef. The novel approach presented here will advance our understanding of N cycling across scales and thus aid in reducing the environmental footprint of global agricultural production.

1. Introduction  Denitrification is a key process in the global nitrogen (N) cycle, reducing nitrate ($\text{NO}_3^-$) to gaseous N emissions in the form of nitric oxide (NO), nitrous oxide ($\text{N}_2\text{O}$) and dinitrogen ($\text{N}_2$). Emissions of $\text{N}_2\text{O}$ contribute to climate
change, as N$_2$O is a long-lived atmospheric trace gas with a global warming potential 273 times higher than that of carbon dioxide (CO$_2$) over a 100-year period (IPCC, 2021) and the largest remaining threat to the stratospheric ozone layer (Portmann et al., 2012; Ravishankara et al., 2009). Emissions of N$_2$, while environmentally benign, still represent a loss of N from the system, with potential detrimental effects on crop growth and productivity in agricultural systems. Despite a growing body of denitrification research delivering both N$_2$O and N$_2$ data from different agroecosystems, the ratio between reactive N$_2$O and N$_2$ remains a major uncertainty for N budgets across scales (Friedl et al., 2020a; Scheer et al., 2020). Growing evidence of non-linear responses of N$_2$O emissions to N fertilizer rates (Shcherbak et al., 2014; Takeda et al., 2021a) together with increasing fertilizer 15N loss with increasing N rates (Rowlings et al., 2022; Schwenke & Haigh, 2016; Takeda et al., 2021b) in intensive cropping systems suggests excessive N inputs promote denitrification losses and lead to inefficiency of N use and adverse environmental impacts. Constraining the response of denitrification losses to N fertilizer rates is therefore critical for sustainable N management strategies to reduce N losses while maintaining crop productivity.

Yet, measuring N$_2$O emissions from the soil against the high atmospheric N$_2$ background remains challenging (Friedl et al., 2020a; Groffman et al., 2006), reflected in the small number of studies quantifying both N$_2$O and N$_2$ in the field. The Helium/Oxygen atmosphere method (He/O$_2$ method) (Butterbach-Bahl et al., 2002; Scholefield et al., 1997) and the 15N gas flux method (Mosier & Schimel, 1993), are considered suitable for the direct quantification of N$_2$ and N$_2$O from soils. For the He/O$_2$ method, soil cores are incubated in the laboratory and the headspace atmosphere inside the closed incubation system is replaced with a He/O$_2$ mixture to measure soil-borne N$_2$ emissions. Field-scale seasonal/annual N$_2$ emissions can be estimated by repeated short laboratory measurements of soil cores, which are returned to the field after incubation. Uncertainty in the cumulative emissions with this approach however remains high due to disturbance of the soil, as in situ measurements are not possible with this method (Chen et al., 2019; Zistl-Schlingmann et al., 2019). The 15N gas flux method is the only method to measure N$_2$ emissions under both laboratory and field conditions. The method requires highly enriched 15N fertilizer to be applied to a designated plot. Gas samples are taken using the static chamber method and analyzed for their different isotopologues of N$_2$ and N$_2$O via isotope ratio mass spectrometry (IRMS) (Friedl et al., 2020a). As a result, evaluation of denitrification losses under field conditions is scarce and mostly limited to measurement periods of less than a month (Baily et al., 2012; Buchen et al., 2016; Friedl et al., 2017; Warner et al., 2019; Weier et al., 1998), as the sensitivity of this method declines in response to the decrease of the 15N enrichment in the soil NO$_3^-$ pool. Due to the shortcomings of available direct measurement methods, estimates of cumulative denitrification losses over the crop growing season require upscaling approaches accounting for the highly dynamic response of denitrification to its drivers.

Denitrification losses have been estimated by applying the average ratio between N$_2$O and N$_2$ emissions measured for a short period under laboratory conditions to N$_2$O emissions measured over the crop growing season under field conditions (Scheer et al., 2009). Burchill et al. (2016) measured the N$_2$:N$_2$O ratio bimonthly in the field and interpolated the ratio linearly between sampling events to apply to more frequent N$_2$O measurements. However, the ratio between N$_2$O and N$_2$ is highly variable and changes rapidly in a non-linear fashion depending on interactions between environmental drivers of denitrification such as soil water content (Cardenas et al., 2017; Friedl et al., 2016), temperature (Bizimana et al., 2021), carbon (C) availability (Qin et al., 2017) and N substrate availability (Chen et al., 2019; Warner et al., 2019; Yang et al., 2014), leading to considerable bias and large uncertainty in N$_2$ estimation if a fixed ratio is used. Wang et al. (2020) correlated the N$_2$:N$_2$O ratio measured under laboratory conditions to multiple soil factors and applied the ratio to field-measured N$_2$O to estimate field-scale seasonal N$_2$ emissions. These approaches account for the dynamic response of the N$_2$:N$_2$O ratio to key drivers. However, the absence of plants may bias the measured ratios, as plant-soil-microbe interactions are known to both affect magnitude and partitioning of N$_2$ and N$_2$O emissions (Henry et al., 2008; Malique et al., 2019). Furthermore, inevitable disturbance of soil through sampling is also of concern, while the lack of in situ measurements hinders the direct validation of the N$_2$:N$_2$O ratio calculated as a function of key drivers. These shortcomings denote a high uncertainty of field-scale seasonal N$_2$ estimates using current approaches and demand a refined method that allows for robust estimates of N$_2$ and N$_2$O emissions. Critically, accounting for the dynamic responses of the ratio between N$_2$O and N$_2$ to soil factors needs to occur under field conditions in the presence of plants. Such estimates are urgently needed to constrain N budgets in different agroecosystems and to refine N fertilizer management strategies for both agronomic and environmental benefits.

The aim of this study was to estimate seasonal denitrification losses (N$_2$O + N$_2$) in response to N fertilizer rates in intensively managed tropical sugarcane (Saccharum spp.) systems in Australia. To this end, this study
builds on the previously reported high-frequency N\textsubscript{2}O data measured with automated greenhouse gas (GHG) monitoring systems as well as fertilizer \textsuperscript{15}N recoveries (Takeda et al., 2022), and combines in situ measurements of N\textsubscript{2}O/(N\textsubscript{2}O + N\textsubscript{2}) ratio with the \textsuperscript{15}N gas flux method. The dynamic changes in the N\textsubscript{2}O/(N\textsubscript{2}O + N\textsubscript{2}) ratio observed in the field were explained by fitting generalized additive mixed models (GAMMs) with soil temperature, water-filled pore space (WFPS), soil mineral N contents and CO\textsubscript{2} emissions, enabling spatio-temporal upscaling of high-frequency N\textsubscript{2}O measurements to N\textsubscript{2} emissions. Fertilizer-derived N\textsubscript{2}O + N\textsubscript{2} losses were further calculated and compared with fertilizer \textsuperscript{15}N loss, corroborating the estimates of N\textsubscript{2}O + N\textsubscript{2} at the cumulative scale and differentiating fertilizer \textsuperscript{15}N loss pathways. Establishing the response of N\textsubscript{2}O + N\textsubscript{2} losses as well as their proportion of fertilizer \textsuperscript{15}N loss to N fertilizer application rates with this innovative approach will refine N budget estimates across scales and allow evaluation of N fertilizer management strategies accounting for N losses from agroecosystems.

2. Materials and Methods

In this study, in situ measurements of N\textsubscript{2}O and N\textsubscript{2} emissions from two sugarcane systems were combined with previously reported high temporal resolution measurements of N\textsubscript{2}O (Takeda et al., 2021a, 2022) and recovery of \textsuperscript{15}N-labeled fertilizer in the plant, soil and N\textsubscript{2}O presented in the previous studies (Takeda et al., 2021b, 2022) to quantify seasonal N\textsubscript{2}O and N\textsubscript{2} losses.

2.1. Study Site

The field experiments were conducted on commercial sugarcane farms in Burdekin, QLD (19° 37′ 4″S, 147° 20′ 4″E) from October 2018 to August 2019 and in Mackay, QLD (21° 14′ 4″S, 149° 04′ 6″E) from October 2019 to August 2020, described in details in Takeda et al. (2022). The climate is tropical in both Burdekin and Mackay. The soil is classified as Brown Dermosol in the Australian Soil Classification (Ishbell, 2016) or Luvisol in the World Reference Base Classification (IUSS Working Group, 2014) at the Burdekin site, and Brown Kandosol or Fluvisol at the Mackay site. During the experiment, the sugarcane crop was the third ratoon of varieties Q240 and Q208 planted in 2015 and 2016 at the Burdekin and Mackay sites, respectively. Furrow and overhead sprinkler irrigation was applied at the Burdekin and Mackay sites, respectively. Sugarcane is burnt before harvest to remove the leaves at the Burdekin site, while harvested green at the Mackay site, leaving the crop residue spread over the ground (“Green cane trash blanketing, GCTB”). Prior to the experiment at the Mackay site, there was 14.6 Mg ha\textsuperscript{-1} of crop residue on the ground with 45.0% and 0.6% of C and N contents. Selected climate conditions, soil properties and crop management are summarized in Table 1.

2.2. Experimental Design

A detailed description of the experimental design and setup at the Burdekin and Mackay sites can be found in Takeda et al. (2021a, 2022), respectively. Briefly, treatments at the Burdekin site were arranged in a randomized strip design with four plots across two strips for each N treatment. The experiment at the Mackay site had a completely randomized block design with three replicates per treatment, accompanied by an unfertilized control (0 N) with three subplots. Fertilizer N rate treatments were 0 N, 150 kg N ha\textsuperscript{-1} (150 N), 200 kg N ha\textsuperscript{-1} (200 N), and 250 kg N ha\textsuperscript{-1} (250 N) at both sites, plus 100 kg N ha\textsuperscript{-1} (100 N) at the Mackay site only. The recommended N application rate was based on the district yield potential and soil C content as outlined in the SIX EASY STEPS protocol of the Australian sugar industry (Schroeder et al., 2010) and was 150 N at the Mackay site and 200 N at the Burdekin site. Urea fertilizer was banded to 0.1 m deep at 0.3 m from the bed center on both sides of the cane row at the Burdekin site and to 0.1 m deep at the bed center of the cane row (“stool splitting”) at the Mackay site. For the \textsuperscript{15}N recovery measurements, a 2.0 m section was excluded from the application of unlabeled N fertilizer in each plot and \textsuperscript{15}N enriched urea fertilizer (5 atom%) in solution was manually applied at the corresponding rate, matching the N fertilizer placement at the respective site.

Alongside the main plots, micro plots were established for \textsuperscript{15}N-labeled N\textsubscript{2}O and N\textsubscript{2} analyses with N fertilizer rates of 150, 200 and 250 kg N ha\textsuperscript{-1} at the Burdekin site and with 100, 150, 200, and 250 kg N ha\textsuperscript{-1} at the Mackay site. The micro plots were arranged in a completely randomized block design with four replicates. The designs of these main and micro plots at each site are shown in Figure S1 in Supporting Information S2.
2.3. Measurement of N\textsubscript{2}O Emissions Using an Automated Chamber System in the Main Plots

High-frequency measurements of soil-borne N\textsubscript{2}O and CO\textsubscript{2} emissions were conducted using automated GHG monitoring systems (Grace et al., 2020) from 17 October 2018 to 15 August 2019 at the Burdekin site and from 3 October 2019 to 24 August 2020 at the Mackay site. Details of the automated GHG monitoring system are given in Text S1.1 in Supporting Information S1. For the unfertilized control plots at the Mackay site, gas samples were manually taken by the static closed chamber method (Friedl et al., 2017), detailed in Text S1.2 in Supporting Information S1. The chambers were placed accounting for N fertilizer application and irrigation practices at each site: At the Burdekin site, chambers covered (a) the area from the fertilizer band to the bed center (bed chamber) and (b) the area from the fertilizer band to the furrow center (furrow chamber). At the Mackay site, bed chambers (a) were placed at the bed center (i.e., on the fertilizer band) and furrow chamber measurements (b) were substituted with those from the unfertilized control. Daily N\textsubscript{2}O and CO\textsubscript{2} emissions were calculated by averaging the measured hourly fluxes over a 24-hr period from each chamber and multiplying by 24. Missing daily N\textsubscript{2}O and CO\textsubscript{2} emissions between measurements were imputed by linear interpolation.

2.4. \textsuperscript{15}N-Labeled N\textsubscript{2} and N\textsubscript{2}O Sampling and Analysis in the Micro Plots

The application of highly enriched \textsuperscript{15}N urea fertilizer enabled us to quantify N\textsubscript{2} and N\textsubscript{2}O emissions and their respective ratio, as well as the contribution of N fertilizer to N\textsubscript{2} and N\textsubscript{2}O emissions. A steel base (0.22 m × 0.22 m × 0.22 m) was placed at the bed center (i.e., on the fertilizer band) and furrow chamber measurements (b) were substituted with those from the unfertilized control. Daily N\textsubscript{2}O and CO\textsubscript{2} emissions were calculated by averaging the measured hourly fluxes over a 24-hr period from each chamber and multiplying by 24. Missing daily N\textsubscript{2}O and CO\textsubscript{2} emissions between measurements were imputed by linear interpolation.
and 0.2 m × 0.4 m at the Burdekin and Mackay sites, respectively) was installed in each micro plot and the corresponding rate of $^{15}$N enriched urea fertilizer (70 atom%) was applied inside the base. The fertilizer band was covered with the soil after applying $^{15}$N fertilizer at both sites, and the approximately same amount of sugarcane residue was put back on the ground in the chamber area at the Mackay site. Static closed chambers were used for gas sampling at the Burdekin site from November 2018 to February 2019 and semi-automated chambers were used at the Mackay site from October 2019 to January 2020 (Takeda et al., 2022, Text S1.3 in Supporting Information S1). The gas samples were analyzed for the concentration of $\mathrm{N}_2\mathrm{O}$ and $\mathrm{CO}_2$ using a Shimadzu GC-2014 Gas Chromatograph (Shimadzu, Kyoto, Japan) and for different isotopologues of $\mathrm{N}_2$ and $\mathrm{N}_2\mathrm{O}$ using an Isotope Ratio Mass Spectrometer (IRMS) (20–22 Sercon Limited, UK).

2.5. The $^{15}$N Gas Flux Method

The $^{15}$N enrichment of the soil $\mathrm{NO}_3^-$ pool undergoing denitrification ($a_p$) and the fraction of $\mathrm{N}_2$ and $\mathrm{N}_2\mathrm{O}$ emitted from this pool ($f_p$) were calculated following the equations outlined by Spott et al. (2006) and given in the Text S1.4 in Supporting Information S1. Multiplying the headspace concentrations of $\mathrm{N}_2$ by the respective $f_p$ value gave $\mathrm{N}_2$ emitted via denitrification, with fluxes expressed in g $\mathrm{N}_2$-N emitted ha$^{-1}$ d$^{-1}$. The precision of the IRMS for $\mathrm{N}_2$ based on the standard deviation of atmospheric air samples ($n = 18$) at 95% confidence intervals was 4.4 × 10$^{-7}$ and 6.0 × 10$^{-7}$ for $^{15}R$ (2$^{15}$N$^2$/2$^{15}$N$^2$) and $^{15}R$ (2$^{15}$N$^3$/2$^{15}$N$^2$), respectively. The corresponding method detection limit ranged from 0.005 g $\mathrm{N}_2$-N ha$^{-1}$ d$^{-1}$ with $a_p$ assumed at 50 atom % to 0.014 g $\mathrm{N}_2$-N ha$^{-1}$ d$^{-1}$ with $a_p$ assumed at 20 atom %. For each gas sample, the product ratio $\mathrm{RN}_2\mathrm{O}$ was calculated as $\mathrm{N}_2\mathrm{O}/(\mathrm{N}_2\mathrm{O} + \mathrm{N}_2)$.

2.6. Plant and Soil Sampling and Analyses

For the $^{15}$N recovery measurements, plant and soil samples were taken from each of the 2.0 m sections prior to harvest (on 27–28 August 2019 at the Burdekin site and 25–26 August 2020 at the Mackay site). The procedure of plant and soil sampling and analyses are detailed in Takeda et al. (2021b, 2022) as well as Text S1.5 in Supporting Information S1. Briefly, aboveground sugarcane biomass, crop residue on the ground, two green leaves at the third node from the 2.0 m section and the adjacent row, remaining stools and major sugarcane roots were harvested. Soil samples were taken at three to four points between the bed and furrow centers using a soil corer and a post-hole driver down to 1.0 m and split into 0–0.2, 0.2–0.4, 0.4–0.7, 0.7–1.0 m soil layers. The plant and soil samples were dried, finely ground and then analyzed for N and $^{15}$N contents via IRMS analysis (20–22 Sercon Limited, UK).

2.7. $^{15}$N Calculations

Fertilizer $^{15}$N recovered in the plant, soil, $\mathrm{N}_2\mathrm{O}$ and $\mathrm{N}_2$ emissions were then calculated by $^{15}$N mass balance (Friedl et al., 2017; Rowlings et al., 2016; Takeda et al., 2022) using equations detailed in the Text S1.6 in Supporting Information S1. Overall fertilizer $^{15}$N loss was calculated by the difference between the N applied and fertilizer $^{15}$N recovered in the soil and plant. The contribution of soil-derived N to plant N uptake, $\mathrm{N}_2\mathrm{O}$ and $\mathrm{N}_2$ emissions was calculated by the difference between total N and fertilizer $^{15}$N recovered in each N pool. This contribution of soil-derived N includes residue fertilizer N from the previous seasons, N in the crop residue and other sources such as N deposition or fixation.

2.8. Auxiliary Measurements

Soil samples at the 0–0.2 m depth were taken prior to fertilization at four points across the whole experimental area at each site to determine the soil properties presented in Table 1. Soil pH was analyzed in a 1:5 (w/v) water extract. Total C and total N were analyzed by CNS-2000 analyzer (LECO Corporation, St. Joseph, MI, USA). Soil particle size distribution was determined by the hydrometer method. To measure soil $\mathrm{NH}_4^+$ and $\mathrm{NO}_3^-$ contents, soil samples were taken at the 0–0.2 m depth in each plot 1 day after fertilization, every 3–7 days for the first 3 months and monthly thereafter. At each sampling event, soils were taken from the bed near the fertilizer band at the Burdekin site where N fertilizer was applied on both sides of the bed while from both bed and furrow at the Mackay site where N fertilizer was applied at the center of the bed. Extraction of soil $\mathrm{NH}_4^+$ and $\mathrm{NO}_3^-$ was conducted by adding 100 mL of 2 M KCl to 20 g of air-dried soil and shaking the solution for 1 hour. Soil $\mathrm{NH}_4^+$ and $\mathrm{NO}_3^-$ contents were then measured using a Gallery™ Discrete Analyzer (Thermo Fisher Scientific, USA). Volumetric soil water content was measured at 0.1 m depth every 30 min using a field-calibrated FDR
soil moisture probe (EnviroSCAN, Sentek, Australia) and then averaged per day. Then, WFPS was calculated from the volumetric soil water content using the measured bulk density assumed constant during the season. Soil temperature in the surface soil layer (0–0.1 m) was measured every five minutes using a PT100 probe (IMKO, Germany) and then averaged per day.

2.9. Upscaling N\textsubscript{2} Emissions and Statistical Analysis

Statistical analyses and graphical presentations in this study were conducted using R statistical software version 3.5.2 (R Core Team, 2018) with a significant level set at \( P < 0.05 \). Gap-filling of missing daily measurements of \( \text{N}_2\text{O} \) and \( \text{CO}_2 \) emissions and soil NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} contents was conducted with linear interpolation using “imputeTS” package (Moritz & Bartz-Beielstein, 2017).

Emissions of \( \text{N}_2 \) at the plot scale were calculated by fitting a statistical model trained with RN\textsubscript{N2} (=N\textsubscript{2}O/(N\textsubscript{2}O + N\textsubscript{2})) observed in the micro plots and applying the predicted RN\textsubscript{N2} to high-frequency measurements of N\textsubscript{2}O emissions in the main plots. First, daily RN\textsubscript{N2} measured in the micro plots at both sites were modeled per N rate using the following predictors: (a) soil temperature and WFPS measured at each site, (b) soil NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} contents measured near the band at the corresponding rate in the main plots, (c) CO\textsubscript{2} emissions measured in the micro plots and (d) site as a factor. Then, daily RN\textsubscript{N2} in the main plots were predicted per plot for each bed and furrow position for the whole crop growing season using soil temperature, WFPS, soil NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} contents and daily CO\textsubscript{2} emissions measured in the main plots. Daily \( \text{N}_2 \) emissions were calculated per plot for each bed and furrow position for the whole crop growing season as the product of predicted RN\textsubscript{N2} and daily N\textsubscript{2}O emissions measured in the main plots. Finally, \( \text{N}_2 \) emissions were upscaled to the plot scale by the area ratio bed:furrow = 1:1 at the Burdekin site and bed:furrow = 1:2 at the Mackay site. Cumulative \( \text{N}_2 \) emissions were calculated by the sum of daily upscaled \( \text{N}_2 \) emissions for each plot over the whole crop growing season.

Modeling of RN\textsubscript{N2} and gap-filling of the fraction of N derived from fertilizer in \( \text{N}_2 \) emissions \( (\text{N}\text{dff} \text{N}_2) \) were conducted by fitting generalized additive mixed models (GAMMs), using a package “mgcv” (Wood, 2011) and detailed in Text S1.7 in Supporting Information S1. Briefly, GAMMs can quantify non-linear relationships without specifying the functional forms (De Rosa et al., 2020; Dorich et al., 2020), which were used to analyze RN\textsubscript{N2} in response to soil variables and N\text{dff} N\textsubscript{2} in response to days after fertilization (DAF) and N rates. Furthermore, GAMMs allow the use of (a) the beta family suitable to model proportions ranging from 0 to 1 and (b) random factors to handle repeated measurements.

Effects of the sites, N fertilizer treatments and bed/furrow positions on RN\textsubscript{N2} and \( \text{N}_2 \) emissions as well as fertilizer-derived N\textsubscript{2}O + \( \text{N}_2 \) in the proportion of the N fertilizer applied and the N fertilizer lost were examined by fitting generalized linear (mixed) models, using packages “lme4” (Bates et al., 2015) and “mgcv” (Wood, 2011). The beta family was specified for RN\textsubscript{N2} and the proportions of fertilizer-derived N\textsubscript{2}O + \( \text{N}_2 \) and the gamma family for \( \text{N}_2 \) together with chamber/plot as a random factor in the case of daily variables. To establish the response of cumulative N\textsubscript{2}O + \( \text{N}_2 \) losses to N rates, (generalized) linear models were fitted for each site.

3. Results

3.1. Daily RN\textsubscript{N2} and \( \text{N}_2 \) Emissions

Daily RN\textsubscript{N2} observed ranged from <0.01 to 0.768 (Figure 1) during ~120 DAF of the measurement period, peaking at values >0.25 within 30 DAF at the Burdekin and within 60 DAF at the Mackay site. For the remainder of the measurement period, RN\textsubscript{N2} stayed below 0.1. The range of observed RN\textsubscript{N2} averaged for each N rate was 0.030–0.092 at the Burdekin site, smaller than 0.082–0.189 at the Mackay site (Table 2). Overall, the observed daily RN\textsubscript{N2} correlated positively with the N fertilizer rates (Table 2).

Fitting the RN\textsubscript{N2} observed near the fertilizer band in the micro plots using the GAMM with Site, soil temperature, WFPS, soil NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} contents and CO\textsubscript{2} emissions as predictors showed 51.7% of deviance explained and 0.151 of root mean square error. Amongst the predictors, Site, WFPS and soil NO\textsubscript{3}\textsuperscript{−} content were significant consistently across the fitted GAMMs. The predicted RN\textsubscript{N2} was larger at the Mackay site compared to the Burdekin site \( (P < 0.001) \) as well as on the bed compared to the furrow position \( (P < 0.001) \) (Table 2). The predicted RN\textsubscript{N2} increased with increasing N rates \( (P < 0.001) \) (Table 2), which was apparent within 50 DAF (Figure 1). The predicted RN\textsubscript{N2} showed larger values during the late crop growing season compared to <90 DAF (Figure S3 in Supporting Information S2).
Daily N\(_2\) emissions reached up to 5 kg N ha\(^{-1}\) d\(^{-1}\) within 50 DAF and stayed elevated for approximately 100 DAF with minor emissions for the remainder of the season (Figure 2). Daily N\(_2\) emissions increased with increasing N rates (\(P < 0.001\)) and were on average larger at the Mackay site compared to the Burdekin site (\(P < 0.001\)).

### 3.2. Cumulative Denitrification Losses (N\(_2\)O + N\(_2\))

Cumulative denitrification losses (N\(_2\)O + N\(_2\)) for the whole growing season increased exponentially from 11.9 ± 2.9 to 87.8 ± 14.4 kg N ha\(^{-1}\) with increasing N fertilizer rates from 0 to 250 kg N ha\(^{-1}\) at the Burdekin site (Figure 3). At the Mackay site, cumulative N\(_2\)O + N\(_2\) emissions increased from 29.5 ± 2.5 kg ha\(^{-1}\) in the

![Figure 1. Observed RN\(_2\)O near the band in the micro plots over the measurement period from 0 days after fertilization at N rates of 100, 150, 200, and 250 kg N ha\(^{-1}\) at the Burdekin (a) and Mackay (b) sites. Points and error bars indicate mean values and standard errors in observed data. Lines and shaded areas indicate mean values and standard errors in prediction with four-fold cross validation.](#)

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\(P\) value

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unfertilized treatment to a range from 71.7 ± 5.0 to 83.2 ± 6.5 kg N ha⁻¹ observed across N rates from 100 to 250 kg N ha⁻¹, with no differences between N fertilized treatments (Figure 3). Overall, cumulative N₂O + N₂ emissions were larger at the Mackay site compared to the Burdekin site (P = 0.027). Cumulative emissions of N₂O accounted for 2.4%–3.5% of N₂O + N₂ emissions at the Burdekin site, which was lower than 4.8%–5.8% at the Mackay site (P < 0.001) (Table 2).

3.3. Fertilizer N Contribution to Denitrification Losses (N₂O + N₂)

Contribution of N fertilizer to N₂ emissions was high within 50 DAF, accounting for >50% and 70% of N₂ emissions at the Burdekin and at the Mackay site, respectively, with a diminishing contribution for the rest of the measurement period (Figure S2 in Supporting Information S2). Of the cumulative N₂ emissions, 51.0%–57.5% and 43.1%–51.0% were derived from fertilizer N at the Burdekin and Mackay sites, respectively. Cumulative fertilizer-derived N₂O + N₂ emissions ranged from 23.9 to 45.8 and 34.2–41.7 kg N ha⁻¹ at the Burdekin and Mackay sites, respectively (Figure 4). Cumulative fertilizer-derived N₂O + N₂ emissions accounted for 30.8%–33.3% and 30.5%–77.5% of the overall fertilizer ¹⁵N loss, at the Burdekin and Mackay sites, respectively (Figure 4). The percentage of fertilizer N lost as N₂O + N₂ was larger at the Mackay site (P = 0.02) and decreased with increasing N rates at both sites (P = 0.009). Contribution of fertilizer N to N₂O + N₂ emissions accounted for 15.9%–18.3% and 16.7%–35.9% of the N applied at the Burdekin and Mackay sites, respectively.

Emissions of N₂O + N₂ derived from soil N in the fertilized treatments were 22.9–42.1 and 35.4–47.3 kg N ha⁻¹ at the Burdekin and Mackay sites, respectively.

4. Discussion

The unique combination of high-frequency N₂O and in situ N₂O/(N₂O + N₂) ratio (RN₂O) measurements using automated GHG monitoring systems...
and \(^{15}\text{N}\) gas flux method together with GAMMs enabled us to quantify field-scale \(\text{N}_2\text{O}\) and \(\text{N}_2\) emissions in response to N fertilizer rates in two sugarcane systems over the whole crop growing season. This method accounts for the dynamic nature of the \(\text{RN}_2\text{O}\) considering the overlapping effects of key drivers of \(\text{N}_2\text{O}\) and \(\text{N}_2\) production, delivering robust estimates of \(\text{N}_2\) emissions at the field scale. Furthermore, comparing fertilizer-derived \(\text{N}_2\text{O} + \text{N}_2\) emissions to fertilizer \(^{15}\text{N}\) loss allowed us to validate the estimated \(\text{N}_2\) emissions at the cumulative scale. Applying this method across two intensively managed sugarcane systems showed (a) >80 kg N ha\(^{-1}\) lost as \(\text{N}_2\text{O} + \text{N}_2\) over the growing season, with (b) emissions dominated by \(\text{N}_2\) accounting for >95% of \(\text{N}_2\text{O} + \text{N}_2\) losses, and (c) that 31%–78% of \(^{15}\text{N}\) fertilizer losses occurred in the form of \(\text{N}_2\text{O} + \text{N}_2\). The method proposed here can be used as a blueprint approach to deliver seasonal denitrification estimates, targeting a key uncertainty in N budgets of different agroecosystems.

### 4.1. Estimating \(\text{N}_2\) Emissions Over the Crop Growing Season Using \(\text{RN}_2\text{O}\)

The high temporal variability of observed \(\text{RN}_2\text{O}\) ranging from <0.01 to 0.768 (Figure 1) emphasizes the need to account for dynamic changes in \(\text{RN}_2\text{O}\) to estimate \(\text{N}_2\) emissions. The use of GAMMs in this study allowed us to express \(\text{RN}_2\text{O}\) as a function of soil water content, temperature, soil mineral N content and \(\text{CO}_2\) emissions, accounting for their effect on the \(\text{RN}_2\text{O}\) at both temporal and spatial scales (Figure 1). Banding of N fertilizer on or beside the bed creates a distinct zone in and close to the band with high N availability, decreasing toward the furrow. Direct measurements of \(\text{RN}_2\text{O}\) in the unfertilized furrow are not possible with the \(^{15}\text{N}\) gas flux method, as it requires the application of \(^{15}\text{N}\) fertilizer, highlighting the need for the GAMMs to estimate \(\text{RN}_2\text{O}\) accounting for changes in N availability. Higher values of \(\text{RN}_2\text{O}\) as a result of higher N-substrate availability are consistent with the increase in observed \(\text{RN}_2\text{O}\) from the band with increasing N fertilizer rates (Table 2). This relationship is also shown by the higher values of predicted \(\text{RN}_2\text{O}\) from the bed than the furrow at the Mackay site (Table 2), where the application of a single N fertilizer band likely increased spatial differences in N availability as compared to the Burdekin site with banding on both sides of the bed. Differences in \(\text{RN}_2\text{O}\) may be explained by preferential \(\text{NO}_3^-\) reduction over \(\text{N}_2\text{O}\) in zones of high \(\text{NO}_3^-\) availability around the fertilizer band (Friedl et al., 2020b; Senbayram et al., 2019). Since banding of N fertilizer is a common practice in intensively managed cropping systems, accounting for its effects on \(\text{RN}_2\text{O}\) as demonstrated here is of therefore of great importance to upscaling \(\text{N}_2\) emissions.
It is noteworthy that in contrast to previous studies (Bizimana et al., 2022; Wang et al., 2020), RN$_2$O data in the study presented here are based on field measurements, which removes the need for measurements of the ratio between N$_2$O and N$_2$ using laboratory assays. In situ measurements avoid a potential bias due to the disturbance of the soil and the absence of plants in the laboratory incubation. An incubation study using the soil samples from the Burdekin site without plants found much lower RN$_2$O $< 0.03$ across the whole measurement period compared to this study despite comparable ranges of soil factors (Kirkby et al., 2023). Both smaller (Bizimana et al., 2022) and larger (Wang et al., 2020) N$_2$O emissions were reported under laboratory conditions compared to in situ measurements, indicating an inconsistent discrepancy in RN$_2$O between field and laboratory measurements. This discrepancy emphasizes the need for in situ measurements as presented here. However, field measurements are likely to show a higher degree of variability, which was reflected in 52% of deviance explained on average when fitting GAMMs to the observed RN$_2$O with cross-validation. Fitting GAMMs to the entire data set without cross-validation resulted in 86% of deviance explained, comparable to the multivariate model of Wang et al. (2020) which explained 92% of the variability of RN$_2$O. In this study, the cross-validated model by replicate was used to extrapolate at both temporal and spatial scales. Setting the k-fold validation across replicates considerably minimized the potential model overfitting observed when using the entire data set for model training (Dorich et al., 2020). Comparing the fertilizer-derived N$_2$O + N$_2$ with the overall fertilizer $^{15}$N loss allowed us to constrain the RN$_2$O modeling with GAMMs. This constraint at the cumulative scale reduced the uncertainty in N$_2$ estimates, emphasizing the advantage of in situ N$_2$O and N$_2$ measurements with the $^{15}$N gas flux method combined with fertilizer $^{15}$N recovery measurements.

Applying predicted values of RN$_2$O to high temporal-resolution N$_2$O measurements gave estimates of daily N$_2$ emissions over the season (Figure 2). Similar to N$_2$O, the majority of N$_2$ emissions occurred within 100 DAF, which is consistent with peaks in soil NO$_3^{-}$ availability (Takeda et al., 2021a). High NO$_3^{-}$ substrate availability for denitrification together with limited O$_2$ in the soil following intense rainfall and/or irrigation promoted N loss in the form of N$_2$, which accounted for >95% of total N$_2$O + N$_2$ emissions over the crop growing season (Table 2). On the other hand, the average of observed RN$_2$O without temporal and spatial upscaling demonstrated up to 9% and 19% of N$_2$O + N$_2$ losses as N$_2$O (Table 2). This discrepancy indicates an underestimation of N$_2$ emissions if the average of observed RN$_2$O was directly applied to N$_2$O emissions. Using fixed RN$_2$O values from measurements with limited coverage of environmental conditions may therefore lead to a bias in estimated N$_2$ emissions. In turn, this difference emphasizes the importance to include a range of soil conditions covering the spatio-temporal variability observed within a cropping system and season when using the ratio between N$_2$O and N$_2$ to upscale N$_2$ emissions to the field scale.

4.2. Denitrification as a Major N Loss Pathway in Intensive Sugarcane Systems

Total N$_2$O + N$_2$ emissions over the season exceeded 80 kg N ha$^{-1}$ at both sites (Figure 3). Denitrification losses have been regarded as a major portion of N budgets in intensively managed sugarcane systems (Bell et al., 2014) but emissions were only measured from the fertilizer band in short-term trials (Warner et al., 2020) and larger (Wang et al., 2020) N$_2$O emissions were reported under laboratory conditions compared to in situ measurements, indicating an inconsistent discrepancy in RN$_2$O between field and laboratory measurements. This discrepancy emphasizes the need for in situ measurements as presented here. However, field measurements are likely to show a higher degree of variability, which was reflected in 52% of deviance explained on average when fitting GAMMs to the observed RN$_2$O with cross-validation. Fitting GAMMs to the entire data set without cross-validation resulted in 86% of deviance explained, comparable to the multivariate model of Wang et al. (2020) which explained 92% of the variability of RN$_2$O. In this study, the cross-validated model by replicate was used to extrapolate at both temporal and spatial scales. Setting the k-fold validation across replicates considerably minimized the potential model overfitting observed when using the entire data set for model training (Dorich et al., 2020). Comparing the fertilizer-derived N$_2$O + N$_2$ with the overall fertilizer $^{15}$N loss allowed us to constrain the RN$_2$O modeling with GAMMs. This constraint at the cumulative scale reduced the uncertainty in N$_2$ estimates, emphasizing the advantage of in situ N$_2$O and N$_2$ measurements with the $^{15}$N gas flux method combined with fertilizer $^{15}$N recovery measurements.

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Cumulative N$_2$O + N$_2$ losses responded exponentially to N fertilizer rates at the Burdekin site but did not increase across the fertilized treatments at the Mackay site (Figure 3), indicating other factors but N availability limited denitrification at the site. Mackay experienced less rainfall and received less irrigation than the Burdekin site in the critical time window 3 months after fertilization. Furthermore, irrigation was applied via overhead sprinklers.
Denitrification was dominated by N₂ emissions (Table 2) and accounted for up to 33% and 78% of the overall fertilizer ¹⁵N loss (Figure 4), showing that a large fraction of N fertilizer loss occurs in the form of environmentally benign N₂. The relative contribution of N₂O + N₂ losses to overall fertilizer ¹⁵N loss however decreased with increasing N rates (Figure 4). This suggests increasing significance of other reactive N loss pathways including NO, ammonia volatilization, leaching and runoff with increasing N rates, as denitrification may become limited by factors other than N availability. Losses of N₂O + N₂ accounted for a smaller proportion of fertilizer ¹⁵N loss at the Burdekin site compared to the Mackay site, which is consistent with furrow irrigation and severe flooding events likely causing greater losses of N fertilizer via leaching and runoff at the Burdekin site. Loss of N via runoff and leaching from Australian sugarcane systems is currently estimated to account for 46%–65% of the total dissolved inorganic N load to the Great Barrier Reef (Barlby et al., 2017). Increasing N losses via runoff and leaching with increasing N rates have been mostly demonstrated by simulation studies (Reading et al., 2019; Thorburn et al., 2017; Vilas et al., 2022). The study presented here shows that even though a large proportion of N fertilizer loss from sugarcane systems occurs as environmentally benign N₂, more N is lost via environmentally harmful pathways of N loss including ammonia volatilization, leaching and runoff as N rates increase. These findings suggest that even if N₂O + N₂ losses are not responding to increasing N rates, environmental costs of sugarcane production are likely to show a non-linear response to N fertilizer.

The large amounts of soil N contributing to N₂O + N₂ across N rates (23–47 kg N ha⁻¹) corroborate the importance of mineralized N for N cycling in sugarcane soils (Takeda et al., 2022). These exports of soil N, together with the plant N uptake derived from soil N (67–122 kg N ha⁻¹), largely exceeded the fertilizer ¹⁵N remaining in the soil (40–60 kg N ha⁻¹) across N rates, even when accounting for N in the crop residue which can be returned (~60 kg N ha⁻¹). This negative balance demonstrates the ineffectiveness of increasing N fertilizer rates to compensate for soil N depletion. Higher rates of banded N fertilizer application with the aim of carrying surplus N into subsequent seasons (“N-bank” concept) were reported to be associated with high risks of N losses under wet conditions in sub-tropical sorghum systems (Rowlings et al., 2022). The N balance in the study here suggests long-term soil N depletion despite high N inputs in intensively managed sugarcane systems. Together with the non-linear responses of N₂O + N₂ losses and their contribution to fertilizer ¹⁵N loss, these results indicate that increasing N fertilizer rates result in lower NUE and higher environmental costs but also do not prevent soil N mining. Maintaining crop productivity while reducing environmental impacts therefore requires N fertilizer rate strategies integrated with additional measures such as the use of enhanced efficiency fertilizers (Connellan & Thompson, 2022; Friedl et al., 2023) and rotation with legume crops (Otto et al., 2020). Nevertheless, assuring the efficacy of such measures requires to extend the experimental approach demonstrated in the study here over multiple seasons. Together with the use of biogeochemical simulation models, the data obtained can expand the findings of this study to a wider range of environmental conditions and different soil types.

4.3. Extrapolating RN₂O to a Wider Range of Cropping Systems Toward the Global N Budget

Denitrification losses have been assumed to account for a significant portion of the global terrestrial N budget despite uncertainties due to limited evaluation at the plot scale (Bouwman et al., 2013; Houlton & Bai, 2009; Scheer et al., 2020). Given that measurements of N₂O emissions are relatively well established and conducted globally, the values of RN₂O play a critical role in estimating the global N budget. Nevertheless, agricultural systems or crop management practices have not been differentiated in most of the reports to date. For example, Scheer et al. (2020) showed a mean RN₂O of 0.11 for agricultural soils and 0.02 for wetlands by summarizing the previously reported RN₂O values. The values of RN₂O 0.024–0.058 (Table 2) based on the cumulative N₂ and N₂O emissions in the study presented herein are indicative of intensively managed cropping systems with high N and water inputs. Compared to the range given by Scheer et al. (2020), this would shift denitrification losses from agricultural soils toward the upper end of the current uncertainty range. The method presented in this study provides a unique tool to estimate seasonal denitrification losses accounting for spatial and temporal variability.
in intensive agroecosystems. This is therefore well suited to generate data that can close the gap in current N budgets, helping to encourage actions to mitigate N pollution.

Refinements of the global N budget require the effects of cropping systems and site conditions on RN\textsubscript{O} to be incorporated. Within this study, the larger RN\textsubscript{O} at the Mackay site (Table 2) may reflect the effect of the low pH (4.1) compared to the Burdekin site (pH 6.9) (Table 1) shifting the ratio toward N\textsubscript{2}O (Dannenmann et al., 2008; Russenes et al., 2016; Šimek & Cooper, 2002). The sandier soil texture may have led to better drainage and larger gas diffusivity at the Mackay site, contributing to the larger RN\textsubscript{O} (Friedl et al., 2017). On the other hand, GCTB management at the Mackay site possibly promoted completion of denitrification and thus reduced RN\textsubscript{O} by preventing evaporation and thus promoting anaerobic conditions (Weier et al., 1993). Accounting for these effects individually to generalize RN\textsubscript{O} estimates requires further data collection across a wide range of environmental conditions such as cropping systems, management practices, soil pH and texture. Controlling environmental factors in laboratory assays can aid in disentangling such overlapping effects, highlighting the need to integrate both laboratory and in situ measurements of N\textsubscript{2}O and N\textsubscript{2} in future research. Generalized estimation of RN\textsubscript{O} covering a wider range of cropping systems and environmental conditions, together with increasing robust in situ measurements of N\textsubscript{2}O emissions, will aid the accuracy of global N budget estimates as well as the identification of hot spots of denitrification losses.

5. Conclusions

This is the first study establishing the response of cumulative denitrification losses (N\textsubscript{2}O + N\textsubscript{2}) to N fertilizer rates over the whole crop growing season at the plot scale based on in situ measurements. We propose the integration of in situ RN\textsubscript{O} with the \textsuperscript{15}N gas flux method, high-frequency N\textsubscript{2}O with an automated GHG monitoring system and fertilizer \textsuperscript{15}N recovery measurements as a novel and robust method applicable to a wide range of cropping systems to quantify cumulative denitrification losses under field conditions. In contrast to previous approaches, this method accounts for both temporal as well as spatial variability of RN\textsubscript{O} and includes in situ data for validation of denitrification losses at the cumulative scale. The use of this method demonstrated that seasonal denitrification losses were dominated by N\textsubscript{2} emissions, and accounted for 31%–78% of total N fertilizer losses, providing critical evidence for its significance as an N loss pathway from sugarcane systems. The non-linear response of cumulative denitrification losses to increasing N rates, with >80 kg N ha\textsuperscript{-1} emitted as N\textsubscript{2} and N\textsubscript{2}O emphasizes the agronomic and environmental inefficiency of excessive N fertilizer application. Even though a large proportion of N fertilizer loss occurred as environmentally benign N\textsubscript{2}, more N was lost via environmentally harmful pathways including ammonia volatilization, leaching and runoff with increasing N rates. These findings highlight that excessive N rates not only increase agronomic inefficiencies, but also the environmental footprint of intensive sugarcane production. This research delivers critical data targeting key uncertainties in biogeochemical models and will aid parameterization and improvement of denitrification algorithms, advancing our understanding of N cycles across scales. These improvements are urgently needed to develop N fertilizer rate strategies integrated with soil fertility management and simulate their long-term impacts, to maintain crop productivity while reducing environmental impacts of intensive agroecosystems.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Summary data associated with this study are available at Environmental Data Initiative via https://doi.org/10.6073/pasta/5c34f4741f55eb4e030f22c91299ad9 (Takeda et al., 2023).

References


