JGR Biogeosciences

RESEARCH ARTICLE
10.1029/2023JG007391

Key Points:

- A novel method to estimate $N_2O + N_2$ losses by combining high-frequency N_2O data, in situ ^{15}N gas flux measurements and fertilizer ^{15}N recoveries
- Denitrification losses of 12–87 kg N ha^{-1} were dominated by N_2 (>94%) and increased non-linearly with increasing N rates
- Denitrification accounted for 31%–78% of N fertilizer losses while the proportion of reactive N losses increased with increasing N rates

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

N. Takeda and J. Friedl,
n3.takeda@qut.edu.au;
johannes.friedl@boku.ac.at

Citation:

Takeda, N., Friedl, J., Kirkby, R., Rowlings, D., Scheer, C., De Rosa, D., & Grace, P. (2023). Denitrification losses in response to N fertilizer rates—Integrating high temporal resolution N_2O, in situ $^{15}N_2O$ and $^{15}N_2$ measurements and fertilizer ^{15}N recoveries in intensive sugarcane systems. *Journal of Geophysical Research: Biogeosciences*, *128*, e2023JG007391. https://doi.org/10.1029/2023JG007391

Received 12 JAN 2023
Accepted 10 AUG 2023

Author Contributions:

Conceptualization: Naoya Takeda
Data curation: Naoya Takeda, Johannes Friedl, Robert Kirkby
Formal analysis: Naoya Takeda, Johannes Friedl, Robert Kirkby, Daniele De Rosa
Funding acquisition: Clemens Scheer, Peter Grace

Denitrification Losses in Response to N Fertilizer Rates—Integrating High Temporal Resolution N_2O, In Situ $^{15}N_2O$ and $^{15}N_2$ Measurements and Fertilizer ^{15}N Recoveries in Intensive Sugarcane Systems

Naoya Takeda[1], Johannes Friedl[1,2], Robert Kirkby[1], David Rowlings[1], Clemens Scheer[1,3], Daniele De Rosa[4], and Peter Grace[1]

[1]Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, QLD, Australia, [2]Department of Forest and Soil Sciences, Institute of Soil Research, University of Natural Resources and Life Sciences Vienna, Vienna, Austria, [3]Institute of Meteorology and Climate Research Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology, Garmisch-Partenkirchen, Germany, [4]Land Resources Unit, Sustainable Resources Directorate, European Commission, Joint Research Centre (JRC), Ispra, Italy

Abstract Denitrification is a key process in the global nitrogen (N) cycle, causing both nitrous oxide (N_2O) and dinitrogen (N_2) emissions. However, estimates of seasonal denitrification losses ($N_2O + N_2$) are scarce, reflecting methodological difficulties in measuring soil-borne N_2 emissions against the high atmospheric N_2 background and challenges regarding their spatio-temporal upscaling. This study investigated $N_2O + N_2$ 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 N_2O monitoring, in situ N_2 and N_2O measurements using the ^{15}N gas flux method and fertilizer ^{15}N recoveries at harvest. Dynamic changes in the $N_2O/(N_2O + N_2)$ ratio (<0.01 to 0.768) were explained by fitting generalized additive mixed models (GAMMs) with soil factors to upscale high temporal-resolution N_2O data to daily N_2 emissions over the season. Cumulative $N_2O + N_2$ losses ranged from 12 to 87 kg N ha^{-1}, increasing non-linearly with increasing N fertilizer rates. Emissions of $N_2O + N_2$ accounted for 31%–78% of fertilizer ^{15}N losses and were dominated by environmentally benign 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 (N_2O), while turning reactive N into environmentally benign dinitrogen (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 N_2 against atmospheric 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 nonlinearly 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 (NO_3^-) to gaseous N emissions in the form of nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). Emissions of N_2O contribute to climate

Investigation: Naoya Takeda
Methodology: Naoya Takeda
Project Administration: Johannes Friedl
Supervision: Johannes Friedl, David Rowlings, Clemens Scheer, Peter Grace
Visualization: Naoya Takeda
Writing – original draft: Naoya Takeda
Writing – review & editing: Johannes Friedl, David Rowlings, Clemens Scheer, Daniele De Rosa, Peter Grace

change, as N_2O 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_2O and N_2 data from different agroecosystems, the ratio between reactive N_2O 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_2O emissions to N fertilizer rates (Shcherbak et al., 2014; Takeda et al., 2021a) together with increasing fertilizer ^{15}N 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 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_2O 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 ^{15}N gas flux method (Mosier & Schimel, 1993) are considered suitable for the direct quantification of N_2 and N_2O 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 ^{15}N gas flux method is the only method to measure N_2 emissions under both laboratory and field conditions. The method requires highly enriched ^{15}N 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_2O 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 ^{15}N 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_2O and N_2 emissions measured for a short period under laboratory conditions to N_2O emissions measured over the crop growing season under field conditions (Scheer et al., 2009). Burchill et al. (2016) measured the N_2:N_2O ratio bimonthly in the field and interpolated the ratio linearly between sampling events to apply to more frequent N_2O measurements. However, the ratio between N_2O 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 $N_2O/(N_2O + N_2)$ ratio measured under laboratory conditions to multiple soil factors and applied the ratio to field-measured N_2O to estimate field-scale seasonal N_2 emissions. These approaches account for the dynamic response of the N_2:N_2O 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_2O 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_2O 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_2O emissions. Critically, accounting for the dynamic responses of the ratio between N_2O 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 ($N_2O + N_2$) 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_2O data measured with automated greenhouse gas (GHG) monitoring systems as well as fertilizer ^{15}N recoveries (Takeda et al., 2022), and combines in situ measurements of $N_2O/(N_2O + N_2)$ ratio with the ^{15}N gas flux method. The dynamic changes in the $N_2O/(N_2O + N_2)$ 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_2 emissions, enabling spatio-temporal upscaling of high-frequency N_2O measurements to N_2 emissions. Fertilizer-derived $N_2O + N_2$ losses were further calculated and compared with fertilizer ^{15}N loss, corroborating the estimates of $N_2O + N_2$ at the cumulative scale and differentiating fertilizer ^{15}N loss pathways. Establishing the response of $N_2O + N_2$ losses as well as their proportion of fertilizer ^{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_2O and N_2 emissions from two sugarcane systems were combined with previously reported high temporal resolution measurements of N_2O (Takeda et al., 2021a, 2022) and recovery of ^{15}N-labeled fertilizer in the plant, soil and N_2O presented in the previous studies (Takeda et al., 2021b, 2022) to quantify seasonal N_2O and N_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 (Isbell, 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^{-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^{-1} (150 N), 200 kg N ha^{-1} (200 N), and 250 kg N ha^{-1} (250 N) at both sites, plus 100 kg N ha^{-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 ^{15}N recovery measurements, a 2.0 m section was excluded from the application of unlabeled N fertilizer in each plot and ^{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 ^{15}N-labeled N_2O and N_2 analyses with N fertilizer rates of 150, 200 and 250 kg N ha^{-1} at the Burdekin site and with 100, 150, 200, and 250 kg N ha^{-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.

10.1029/2023JG007391

Table 1
Climate Conditions, Soil Properties at 0–0.2 m Depth and Crop Management Practices at the Burdekin and Mackay Sites

Variable	Burdekin	Mackay
Climate		
Annual mean rainfall (mm)	945	1,598
Seasonal rainfall during the experiment (mm)	1,180	1,418
Annual mean daily minimum temperature (°C)	18.0	19.1
Annual mean daily maximum temperature (°C)	29.2	26.5
Soil properties (0.0–0.2 m)		
BD (g cm^{-3})	1.3	1.1
pH (H_2O)	6.92	4.13
Total C (%)	1.60	1.35
Total N (%)	0.08	0.09
Clay (%)	35.4	22.2
Silt (%)	26.0	15.9
Sand (%)	38.6	61.9
Initial mineral N (kg N ha^{-1})	37.0	31.8
Crop management		
Cultivar	Q240	Q208
Crop	Third ratoon	Third ratoon
Date of fertilization	31 October 2018	24 October 2019
N rate treatments (kg N ha^{-1})	0, 150, 200, and 250	0, 100, 150, 200, and 250
SIX EASY STEPS N rate (kg N ha^{-1})	200	150
N fertilizer product	Urea	Urea
Fertilizer application	Two-sided banding	Stool splitting
Irrigation management	Furrow	Overhead
Estimated total irrigation amount (mm)	600	180
Trash management	Burnt	Green cane trash blanket
Date of harvest sampling	27–28 August 2019	25–26 August 2020

2.3. Measurement of N_2O Emissions Using an Automated Chamber System in the Main Plots

High-frequency measurements of soil-borne N_2O and CO_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_2O and CO_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_2O and CO_2 emissions between measurements were imputed by linear interpolation.

2.4. ^{15}N-Labeled N_2 and N_2O Sampling and Analysis in the Micro Plots

The application of highly enriched ^{15}N urea fertilizer enabled us to quantify N_2 and N_2O emissions and their respective ratio, as well as the contribution of N fertilizer to N_2 and N_2O emissions. A steel base (0.22 m × 0.22

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 N_2O and CO_2 using a Shimadzu GC-2014 Gas Chromatograph (Shimadzu, Kyoto, Japan) and for different isotopologues of N_2 and N_2O 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 NO_3^- pool undergoing denitrification (a_p) and the fraction of N_2 and N_2O 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 N_2 by the respective f_p value gave N_2 emitted via denitrification, with fluxes expressed in g N_2-N emitted ha^{-1} d^{-1}. The precision of the IRMS for 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 ^{29}R ($^{29}N_2/^{28}N_2$) and ^{30}R ($^{30}N_2/^{28}N_2$), respectively. The corresponding method detection limit ranged from 0.005 g N_2-N ha^{-1} d^{-1} with a_p assumed at 50 atom % to 0.014 g N_2-N ha^{-1} d^{-1} with a_p assumed at 20 atom %. For each gas sample, the product ratio RN_2O was calculated as $N_2O/(N_2O + N_2)$.

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, N_2O and 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, N_2O and 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 NH_4^+ and 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 NH_4^+ and 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 NH_4^+ and 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_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 N_2O and CO_2 emissions and soil NH_4^+ and NO_3^- contents was conducted with linear interpolation using "imputeTS" package (Moritz & Bartz-Beielstein, 2017).

Emissions of N_2 at the plot scale were calculated by fitting a statistical model trained with RN_2O ($=N_2O/(N_2O + N_2)$) observed in the micro plots and applying the predicted RN_2O to high-frequency measurements of N_2O emissions in the main plots. First, daily RN_2O 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_4^+ and NO_3^- contents measured near the band at the corresponding rate in the main plots, (c) CO_2 emissions measured in the micro plots and (d) site as a factor. Then, daily RN_2O 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_4^+ and NO_3^- contents and daily CO_2 emissions measured in the main plots. Daily 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_2O and daily N_2O emissions measured in the main plots. Finally, 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 N_2 emissions were calculated by the sum of daily upscaled N_2 emissions for each plot over the whole crop growing season.

Modeling of RN_2O and gap-filling of the fraction of N derived from fertilizer in N_2 emissions (*Ndff* 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_2O in response to soil variables and *Ndff* N_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_2O and N_2 emissions as well as fertilizer-derived $N_2O + N_2$ 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_2O and the proportions of fertilizer-derived $N_2O + N_2$ and the gamma family for N_2 together with chamber/plot as a random factor in the case of daily variables. To establish the response of cumulative $N_2O + N_2$ losses to N rates, (generalized) linear models were fitted for each site.

3. Results

3.1. Daily RN_2O and N_2 Emissions

Daily RN_2O 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_2O stayed below 0.1. The range of observed RN_2O 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_2O correlated positively with the N fertilizer rates (Table 2).

Fitting the RN_2O observed near the fertilizer band in the micro plots using the GAMM with Site, soil temperature, WFPS, soil NH_4^+ and NO_3^- contents and CO_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_3^- content were significant consistently across the fitted GAMMs. The predicted RN_2O 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_2O increased with increasing N rates ($P < 0.001$) (Table 2), which was apparent within 50 DAF (Figure 1). The predicted RN_2O showed larger values during the late crop growing season compared to <90 DAF (Figure S3 in Supporting Information S2).

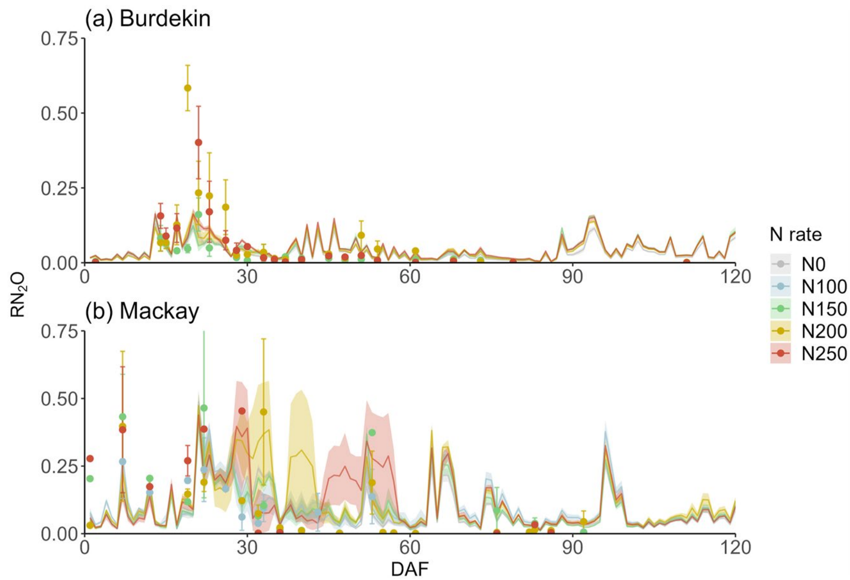

Figure 1. Observed RN_2O near the band in the micro plots over the measurement period from 0 days after fertilization at N rates of 100, 150, 200, and 250 kg N ha^{-1} at the Burdekin (a) and Mackay (b) sites. Points and error bars indicate mean values and standard errors in observed data. Lines and shaded areas indicate mean values and standard errors in prediction with four-fold cross validation.

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 ($N_2O + N_2$)

Cumulative denitrification losses ($N_2O + N_2$) 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 $N_2O + N_2$ emissions increased from 29.5 ± 2.5 kg ha^{-1} in the

Table 2
RN_2O Observed Daily, RN_2O Predicted Daily for Bed and Furrow Positions and RN_2O Calculated With Cumulative N_2O and N_2 Emissions in Response to N Rates Ranging From 0 to 250 kg N ha^{-1}, Sites and Positions

			Predicted RN_2O		
Site	N rate	Observed RN_2O	Bed	Furrow	RN_2O at cumulative
Burdekin	0		0.054 ± 0.001	0.054 ± 0.001	0.024 ± 0.002
	150	0.030 ± 0.01	0.060 ± 0.002	0.061 ± 0.002	0.032 ± 0.003
	200	0.092 ± 0.02	0.061 ± 0.001	0.063 ± 0.001	0.028 ± 0.002
	250	0.072 ± 0.02	0.061 ± 0.001	0.062 ± 0.001	0.035 ± 0.001
Mackay	0		0.091 ± 0.002	0.087 ± 0.002	0.050 ± 0.001
	100	0.082 ± 0.02	0.104 ± 0.003	0.087 ± 0.002	0.048 ± 0.007
	150	0.133 ± 0.04	0.097 ± 0.002	0.086 ± 0.002	0.051 ± 0.005
	200	0.093 ± 0.03	0.115 ± 0.003	0.087 ± 0.002	0.058 ± 0.003
	250	0.189 ± 0.06	0.109 ± 0.003	0.087 ± 0.002	0.047 ± 0.007
P value					
Site		<0.001	<0.001		<0.001
N rate		0.006	<0.001		0.121
Position			<0.001		

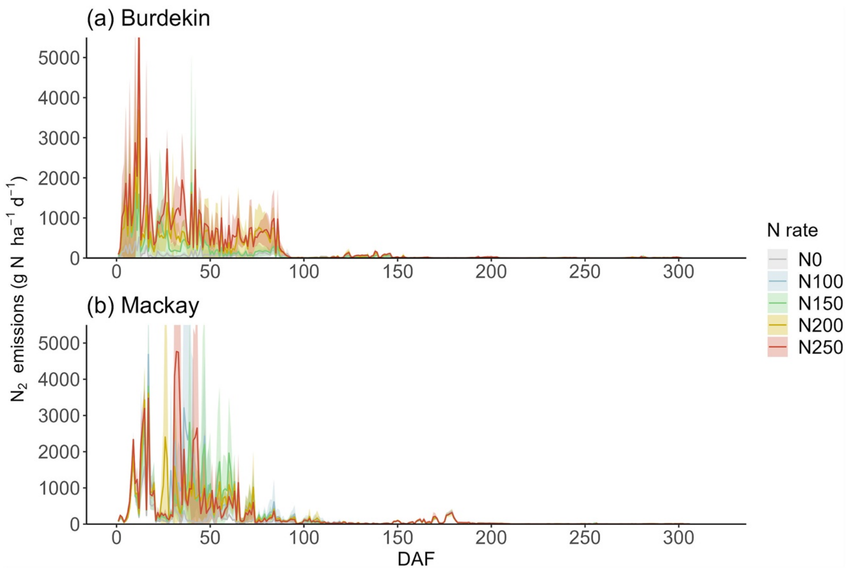

Figure 2. Daily N_2 emissions estimated over the crop growing season from 0 days after fertilization at N rates of 0, 100, 150, 200, and 250 kg N ha^{-1} at the Burdekin (a) and Mackay (b) sites. Lines and shaded areas indicate predicted mean values and 95% confidence intervals, respectively.

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 $N_2O + N_2$ emissions were larger at the Mackay site compared to the Burdekin site ($P = 0.027$). Cumulative emissions of N_2O accounted for 2.4%–3.5% of $N_2O + N_2$ emissions at the Burdekin site, which was lower than 4.8%–5.8% at the Mackay site ($P < 0.001$) (Table 2).

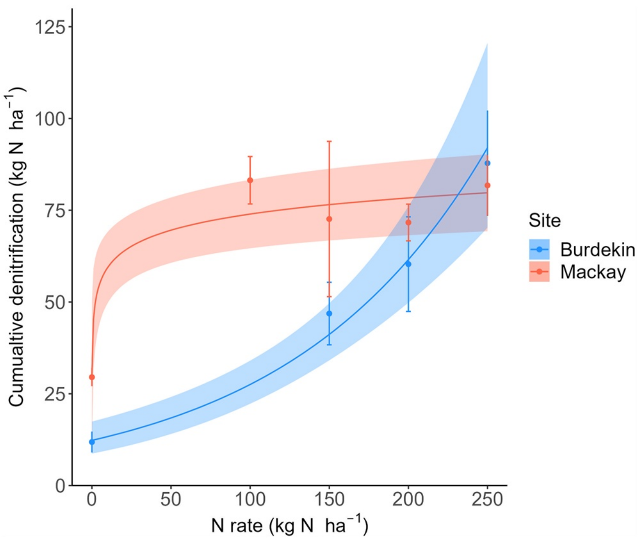

Figure 3. Cumulative denitrification losses over the crop growing season in response to N fertilizer rates at the Burdekin (blue) and Mackay (red) sites. Points and error bars indicate mean values and standard errors. Lines and shaded areas indicate fitted curves and 95% confidence intervals, respectively.

3.3. Fertilizer N Contribution to Denitrification Losses ($N_2O + N_2$)

Contribution of N fertilizer to N_2 emissions was high within 50 DAF, accounting for >50% and 70% of N_2 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 N_2 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 $N_2O + N_2$ 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 $N_2O + N_2$ emissions accounted for 30.8%–33.3% and 30.5%–77.5% of the overall fertilizer ^{15}N loss, at the Burdekin and Mackay sites, respectively (Figure 4). The percentage of fertilizer N lost as $N_2O + N_2$ 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 $N_2O + N_2$ 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 $N_2O + N_2$ 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 N_2O and in situ $N_2O/(N_2O + N_2)$ ratio (RN_2O) measurements using automated GHG monitoring systems

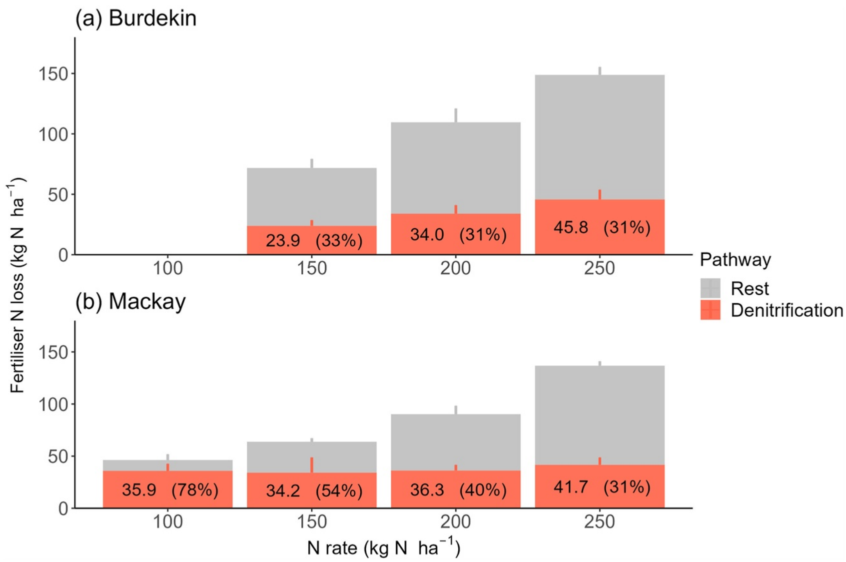

Figure 4. Cumulative fertilizer-derived denitrification ($N_2O + N_2$) losses (red) in comparison to overall fertilizer ^{15}N loss (gray) in response to N fertilizer rates 100, 150, 200, and 250 kg N ha^{-1} at the Burdekin (a) and Mackay (b) sites. The values in parenthesis show the percentage of fertilizer-derived denitrification ($N_2O + N_2$) losses. Overall fertilizer ^{15}N loss was calculated as the difference between the applied and recovered fertilizer ^{15}N in the soil and plant, including all N loss pathways such as denitrification, leaching, runoff and ammonia volatilization. Error bars show standard errors.

and ^{15}N gas flux method together with GAMMs enabled us to quantify field-scale N_2O and 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 RN_2O considering the overlapping effects of key drivers of N_2O and N_2 production, delivering robust estimates of N_2 emissions at the field scale. Furthermore, comparing fertilizer-derived $N_2O + N_2$ emissions to fertilizer ^{15}N loss allowed us to validate the estimated 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 $N_2O + N_2$ over the growing season, with (b) emissions dominated by N_2 accounting for >95% of $N_2O + N_2$ losses, and (c) that 31%–78% of ^{15}N fertilizer losses occurred in the form of $N_2O + N_2$. 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 N_2 Emissions Over the Crop Growing Season Using RN_2O

The high temporal variability of observed RN_2O ranging from <0.01 to 0.768 (Figure 1) emphasizes the need to account for dynamic changes in RN_2O to estimate N_2 emissions. The use of GAMMs in this study allowed us to express RN_2O as a function of soil water content, temperature, soil mineral N content and CO_2 emissions, accounting for their effect on the RN_2O 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 RN_2O in the unfertilized furrow are not possible with the ^{15}N gas flux method, as it requires the application of ^{15}N fertilizer, highlighting the need for the GAMMs to estimate RN_2O accounting for changes in N availability in the furrow. Higher values of RN_2O as a result of higher N-substrate availability are consistent with the increase in observed RN_2O from the band with increasing N fertilizer rates (Table 2). This relationship is also shown by the higher values of predicted RN_2O 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 RN_2O may be explained by preferential NO_3^- reduction over N_2O in zones of high 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 RN_2O as demonstrated here is of therefore of great importance to upscaling N_2 emissions.

10.1029/2023JG007391

It is noteworthy that in contrast to previous studies (Bizimana et al., 2022; Wang et al., 2020), RN_2O data in the study presented here are based on field measurements, which removes the need for measurements of the ratio between N_2O 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 $RN_2O < 0.03$ 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_2O emissions were reported under laboratory conditions compared to in situ measurements, indicating an inconsistent discrepancy in RN_2O 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_2O 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_2O. 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 $N_2O + N_2$ with the overall fertilizer ^{15}N loss allowed us to constrain the RN_2O modeling with GAMMs. This constraint at the cumulative scale reduced the uncertainty in N_2 estimates, emphasizing the advantage of in situ N_2O and N_2 measurements with the ^{15}N gas flux method combined with fertilizer ^{15}N recovery measurements.

Applying predicted values of RN_2O to high temporal-resolution N_2O measurements gave estimates of daily N_2 emissions over the season (Figure 2). Similar to N_2O, 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 $N_2O + N_2$ emissions over the crop growing season (Table 2). On the other hand, the average of observed RN_2O without temporal and spatial upscaling demonstrated up to 9% and 19% of $N_2O + N_2$ losses as N_2O (Table 2). This discrepancy indicates an underestimation of N_2 emissions if the average of observed RN_2O was directly applied to N_2O emissions. Using fixed RN_2O 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_2O 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 $N_2O + N_2$ 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., 2019; Weier et al., 1996, 1998). A recent study on a subtropical sugarcane farm in Australia reported $N_2O + N_2$ emissions equivalent to 12–36 kg N ha^{-1} on the fertilizer band at 145 kg N ha^{-1} of N rate (Friedl et al., 2023). The lack of seasonal estimates of denitrification losses in sugarcane hinders the comparison to the range of N_2+N_2O emissions observed in the study presented here. In a simulation study, Thorburn et al. (2017) predicted denitrification losses up to 50 kg N ha^{-1} with N fertilizer rates up to 200 kg N ha^{-1} from Australian sugarcane systems. This range is substantially lower than the $N_2 + N_2O$ emissions from both sites. Even though denitrification rates are subject to specific site and environmental conditions, predictions of denitrification losses in biogeochemical models rely mostly on N_2O data. The lack of N_2 data hinders the validation of overall rates, and changes in N_2O may be caused by a change in denitrification rate and/or RN_2O (Del Grosso et al., 2020). Our estimates of seasonal $N_2O + N_2$ losses not only provide experimental evidence that denitrification is a major pathway of N loss from intensively managed sugarcane systems, but also the opportunity to test and validate the representation of denitrification in biogeochemical models.

Cumulative $N_2O + N_2$ 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 in Mackay, compared to furrow (flood) irrigation in Burdekin. Considering the sandier soil texture (Table 1) at the Mackay site, the differences in management and rainfall indicate an increased frequency of aerobic conditions in the soil at the Mackay site compared to the Burdekin site (Takeda et al., 2022), limiting the response of denitrification to N rate. Regardless, relatively large $N_2O + N_2$ losses >50 kg N ha^{-1} were consistently observed at high N rates above the recommended N rate (≥200 kg N ha^{-1}) across the sites (Figure 3), suggesting increased N substrate availability for N losses via denitrification.

Denitrification was dominated by N_2 emissions (Table 2) and accounted for up to 33% and 78% of the overall fertilizer ^{15}N loss (Figure 4), showing that a large fraction of N fertilizer loss occurs in the form of environmentally benign N_2. The relative contribution of $N_2O + N_2$ losses to overall fertilizer ^{15}N 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 $N_2O + N_2$ accounted for a smaller proportion of fertilizer ^{15}N 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 (Bartley 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 N_2, 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 $N_2O + N_2$ 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 $N_2O + N_2$ 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 ^{15}N 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 $N_2O + N_2$ losses and their contribution to fertilizer ^{15}N 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 RN_2O 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 N_2O emissions are relatively well established and conducted globally, the values of RN_2O 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 RN_2O of 0.11 for agricultural soils and 0.02 for wetlands by summarizing the previously reported RN_2O values. The values of RN_2O 0.024–0.058 (Table 2) based on the cumulative N_2 and N_2O 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_2O to be incorporated. Within this study, the larger RN_2O 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_2O (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_2O (Friedl et al., 2017). On the other hand, GCTB management at the Mackay site possibly promoted completion of denitrification and thus reduced RN_2O by preventing evaporation and thus promoting anaerobic conditions (Weier et al., 1993). Accounting for these effects individually to generalize RN_2O 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_2O and N_2 in future research. Generalized estimation of RN_2O covering a wider range of cropping systems and environmental conditions, together with increasing robust in situ measurements of N_2O 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 ($N_2O + N_2$) 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_2O with the ^{15}N gas flux method, high-frequency N_2O with an automated GHG monitoring system and fertilizer ^{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_2O 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_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^{-1} emitted as N_2 and N_2O 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_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/5c34f47415f55eb4e030f22c91299ad9 (Takeda et al., 2023).

Acknowledgments
This study was funded by Sugar Research Australia (project number 2018/007). The authors would like to thank Farmacist and Sheree Biddle for their assistance as well as Richard Kelly and Mario Torrisi for the use of their property as research sites. Some of the data reported in this paper were obtained at the Central Analytical Research Facility operated by the Centre for Agriculture and the Bioeconomy at Queensland University of Technology.

References

Baily, A., Watson, C. J., Laughlin, R., Matthews, D., McGeough, K., & Jordan, P. (2012). Use of the ^{15}N gas flux method to measure the source and level of N_2O and N_2 emissions from grazed grassland. *Nutrient Cycling in Agroecosystems*, *94*(2–3), 287–298. https://doi.org/10.1007/s10705-012-9541-x

Bartley, R., Waters, D., Turner, R., Kroon, F., Wilkinson, S., Garzon-Garcia, A., et al. (2017). Scientific Consensus Statement 2017: A synthesis of the science of land-based water quality impacts on the Great Barrier Reef, Chapter 2: Sources of sediment, nutrients, pesticides and other pollutants to the Great Barrier Reef. (Scientific Consensus Statement 2017: A synthesis of the science of land-based water quality impacts on the Great Barrier Reef, issue).

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bell, M. J., Biggs, J., McKellar, L. B., Connellan, J., Di Bella, L., Dwyer, R., et al. (2014). *A review of nitrogen use efficiency in sugarcane*. Sugar Research Australia.

Bizimana, F., Luo, J., Timilsina, A., Dong, W., Gaudel, G., Ding, K., et al. (2022). Estimating field N_2 emissions based on laboratory-quantified $N_2O/(N_2O + N_2)$ ratios and field-quantified N_2O emissions. *Journal of Soils and Sediments*, *22*(8), 2196–2208. https://doi.org/10.1007/s11368-022-03212-0

Bizimana, F., Timilsina, A., Dong, W., Uwamungu, J. Y., Li, X., Wang, Y., et al. (2021). Effects of long-term nitrogen fertilization on N_2O, N_2 and their yield-scaled emissions in a temperate semi-arid agro-ecosystem. *Journal of Soils and Sediments*, *21*(4), 1659–1671. https://doi.org/10.1007/s11368-021-02903-4

Bouwman, A. F., Beusen, A. H. W., Griffioen, J., Van Groenigen, J. W., Hefting, M. M., Oenema, O., et al. (2013). Global trends and uncertainties in terrestrial denitrification and N_2O emissions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130112. https://doi.org/10.1098/rstb.2013.0112

Buchen, C., Lewicka-Szczebak, D., Fuß, R., Helfrich, M., Flessa, H., & Well, R. (2016). Fluxes of N_2 and N_2O and contributing processes in summer after grassland renewal and grassland conversion to maize cropping on a Plaggic Anthrosol and a Histic Gleysol. *Soil Biology and Biochemistry*, *101*, 6–19. https://doi.org/10.1016/j.soilbio.2016.06.028

Burchill, W., Lanigan, G. J., Li, D., Williams, M., & Humphreys, J. (2016). A system N balance for a pasture-based system of dairy production under moist maritime climatic conditions. *Agriculture, Ecosystems & Environment*, *220*, 202–210. https://doi.org/10.1016/j.agee.2015.12.022

Butterbach-Bahl, K., Willibald, G., & Papen, H. (2002). Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. *Plant and Cell Physiology*, *240*(1), 105–116. https://doi.org/10.1029/92gb02124

Cardenas, L. M., Bol, R., Lewicka-Szczebak, D., Gregory, A. S., Matthews, G. P., Whalley, W. R., et al. (2017). Effect of soil saturation on denitrification in a grassland soil. *Biogeosciences*, *14*(20), 4691–4710. https://doi.org/10.5194/bg-14-4691-2017

Chen, T., Oenema, O., Li, J., Misselbrook, T., Dong, W., Qin, S., et al. (2019). Seasonal variations in N_2 and N_2O emissions from a wheat–maize cropping system. *Biology and Fertility of Soils*, *55*(6), 539–551. https://doi.org/10.1007/s00374-019-01373-8

Connellan, J., & Thompson, M. (2022). Support of cane farmer trials of enhanced efficiency fertiliser in the catchments of the Great Barrier Reef: Final report 2016/807.

Dannenmann, M., Butterbach-Bahl, K., Gasche, R., Willibald, G., & Papen, H. (2008). Dinitrogen emissions and the N_2:N_2O emission ratio of a Rendzic Leptosol as influenced by pH and forest thinning. *Soil Biology and Biochemistry*, *40*(9), 2317–2323. https://doi.org/10.1016/j.soilbio.2008.05.009

Del Grosso, S. J., Smith, W., Kraus, D., Massad, R. S., Vogeler, I., & Fuchs, K. (2020). Approaches and concepts of modelling denitrification: Increased process understanding using observational data can reduce uncertainties. *Current Opinion in Environmental Sustainability*, *47*, 37–45. https://doi.org/10.1016/j.cosust.2020.07.003

De Rosa, D., Rowlings, D. W., Fulkerson, B., Scheer, C., Friedl, J., Labadz, M., & Grace, P. R. (2020). Field-scale management and environmental drivers of N_2O emissions from pasture-based dairy systems. *Nutrient Cycling in Agroecosystems*, *117*(3), 299–315. https://doi.org/10.1007/s10705-020-10069-7

Dorich, C. D., De Rosa, D., Barton, L., Grace, P., Rowlings, D., Migliorati, M. D. A., et al. (2020). Global Research Alliance N_2O chamber methodology guidelines: Guidelines for gap-filling missing measurements. *Journal of Environmental Quality*, *49*(5), 1186–1202. https://doi.org/10.1002/jeq2.20138

Friedl, J., Cardenas, L. M., Clough, T. J., Dannenmann, M., Hu, C., & Scheer, C. (2020a). Measuring denitrification and the N_2O:($N_2O + N_2$) emission ratio from terrestrial soils. *Current Opinion in Environmental Sustainability*, *47*, 61–71. https://doi.org/10.1016/j.cosust.2020.08.006

Friedl, J., Scheer, C., Rowlings, D. W., Deltedesco, E., Gorfer, M., De Rosa, D., et al. (2020b). Effect of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on N-turnover, the N_2O reductase-gene nosZ and N_2O:N_2 partitioning from agricultural soils. *Scientific Reports*, *10*(1), 2399. https://doi.org/10.1038/s41598-020-59249-z

Friedl, J., Scheer, C., Rowlings, D. W., McIntosh, H. V., Strazzabosco, A., Warner, D. I., & Grace, P. R. (2016). Denitrification losses from an intensively managed sub-tropical pasture—Impact of soil moisture on the partitioning of N_2 and N_2O emissions. *Soil Biology and Biochemistry*, *92*, 58–66. https://doi.org/10.1016/j.soilbio.2015.09.016

Friedl, J., Scheer, C., Rowlings, D. W., Mumford, M. T., & Grace, P. R. (2017). The nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) reduces N_2 emissions from intensively managed pastures in subtropical Australia. *Soil Biology and Biochemistry*, *108*, 55–64. https://doi.org/10.1016/j.soilbio.2017.01.016

Friedl, J., Warner, D., Wang, W., Rowlings, D. W., Grace, P. R., & Scheer, C. (2023). Strategies for mitigating N_2O and N_2 emissions from an intensive sugarcane cropping system. *Nutrient Cycling in Agroecosystems*, *125*(2), 295–308. https://doi.org/10.1007/s10705-023-10262-4

Grace, P. R., van der Weerden, T. J., Rowlings, D. W., Scheer, C., Brunk, C., Kiese, R., et al. (2020). Global Research Alliance N_2O chamber methodology guidelines: Considerations for automated flux measurement. *Journal of Environmental Quality*, *49*(5), 1126–1140. https://doi.org/10.1002/jeq2.20124

Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., et al. (2006). Methods for measuring denitrification: Diverse approaches to a difficult problem. *Ecological Applications*, *16*(6), 2091–2122. https://doi.org/10.1890/1051-0761(2006)016[2091:MFMDDA]2.0.CO;2

Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Chèneby, D., et al. (2008). Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: Insight into the role of root exudates. *Environmental Microbiology*, *10*(11), 3082–3092. https://doi.org/10.1111/j.1462-2920.2008.01599.x

Houlton, B. Z., & Bai, E. (2009). Imprint of denitrifying bacteria on the global terrestrial biosphere. *Proceedings of the National Academy of Sciences*, *106*(51), 21713–21716. https://doi.org/10.1073/pnas.0912111106

IPCC. (2021). *Climate change 2021: The physical science Basis. Contribution of working Group I to the sixth assessment report of the intergovernmental panel on climate change*. C. U. Press.

Isbell, R. (2016). *The Australian soil Classification*. CSIRO Publishing.

IUSS Working Group. (2014). World reference base for soil resources 2014. In *International soil classification system for naming soils and creating legends for soil maps (World Soil Resources Report, Issue)*.

Kirkby, R., Friedl, J., Takeda, N., De Rosa, D., Rowlings, D. W., & Grace, P. R. (2023). Nonlinear response of N_2O and N_2 emissions to increasing soil nitrate availability in a tropical sugarcane soil. *Journal of Soils and Sediments*, *23*(5), 2065–2071. https://doi.org/10.1007/s11368-023-03482-2

Malique, F., Ke, P., Boettcher, J., Dannenmann, M., & Butterbach-Bahl, K. (2019). Plant and soil effects on denitrification potential in agricultural soils. *Plant and Soil*, *439*(1–2), 459–474. https://doi.org/10.1007/s11104-019-04038-5

Moritz, S., & Bartz-Beielstein, T. (2017). imputeTS: Time series missing value Imputation in R. *The R Journal*, *9*(1), 207–218. https://doi.org/10.32614/rj-2017-009

Mosier, A., & Schimel, D. (1993). Nitrification and denitrification. In *Nitrogen isotope techniques* (pp. 181–208). Elsevier.

Otto, R., Pereira, G. L., Tenelli, S., Carvalho, J. L. N., Lavres, J., de Castro, S. A. Q., et al. (2020). Planting legume cover crop as a strategy to replace synthetic N fertilizer applied for sugarcane production. *Industrial Crops and Products*, *156*, 112853. https://doi.org/10.1016/j.indcrop.2020.112853

Portmann, R. W., Daniel, J. S., & Ravishankara, A. R. (2012). Stratospheric ozone depletion due to nitrous oxide: Influences of other gases. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1593), 1256–1264. https://doi.org/10.1098/rstb.2011.0377

Qin, S., Hu, C., Clough, T. J., Luo, J., Oenema, O., & Zhou, S. (2017). Irrigation of DOC-rich liquid promotes potential denitrification rate and decreases $N_2O/(N_2O + N_2)$ product ratio in a 0–2 m soil profile. *Soil Biology and Biochemistry*, *106*, 1–8. https://doi.org/10.1016/j.soilbio.2016.12.001

Ravishankara, A. R., Daniel, J. S., & Portmann, R. W. (2009). Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st Century. *Science*, *326*(5949), 123–125. https://doi.org/10.1126/science.1176985

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Reading, L. P., Bajracharya, K., & Wang, J. (2019). Simulating deep drainage and nitrate leaching on a regional scale: Implications for groundwater management in an intensively irrigated area. *Irrigation Science*, *37*(5), 561–581. https://doi.org/10.1007/s00271-019-00636-4

Rowlings, D. W., Lester, D. W., Grace, P. R., Scheer, C., De Rosa, D., De Antoni Migliorati, M., et al. (2022). Seasonal rainfall distribution drives nitrogen use efficiency and losses in dryland summer sorghum. *Field Crops Research*, *283*, 108527. https://doi.org/10.1016/j.fcr.2022.108527

Rowlings, D. W., Scheer, C., Liu, S., & Grace, P. R. (2016). Annual nitrogen dynamics and urea fertilizer recoveries from a dairy pasture using ^{15}N; effect of nitrification inhibitor DMPP and reduced application rates. *Agriculture, Ecosystems & Environment*, *216*, 216–225. https://doi.org/10.1016/j.agee.2015.09.025

Russenes, A. L., Korsaeth, A., Bakken, L. R., & Dörsch, P. (2016). Spatial variation in soil pH controls off-season N_2O emission in an agricultural soil. *Soil Biology and Biochemistry*, *99*, 36–46. https://doi.org/10.1016/j.soilbio.2016.04.019

Scheer, C., Fuchs, K., Pelster, D. E., & Butterbach-Bahl, K. (2020). Estimating global terrestrial denitrification from measured $N_2O:(N_2O + N_2)$ product ratios. *Current Opinion in Environmental Sustainability*, *47*, 72–80. https://doi.org/10.1016/j.cosust.2020.07.005

Scheer, C., Wassmann, R., Butterbach-Bahl, K., Lamers, J. P. A., & Martius, C. (2009). The relationship between N_2O, NO, and N_2 fluxes from fertilized and irrigated dryland soils of the Aral Sea Basin, Uzbekistan. *Plant and Soil*, *314*(1–2), 273–283. https://doi.org/10.1007/s11104-008-9728-8

Scholefield, D., Hawkins, J., & Jackson, S. (1997). Development of a helium atmosphere soil incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during denitrification. *Soil Biology and Biochemistry*, *29*(9–10), 1345–1352. https://doi.org/10.1016/S0038-0717(97)00021-7

Schroeder, B. L., Hurney, A. P., Wood, A. W., Moody, P. W., & Allsopp, P. G. (2010). Concepts and value of the nitrogen guidelines contained in the Australian sugar industry's 'Six Easy Steps' nutrient management program. In *Proceedings of the international society of sugar cane technologists*.

Schwenke, G., & Haigh, B. (2016). The interaction of seasonal rainfall and nitrogen fertiliser rate on soil N_2O emission, total N loss and crop yield of dryland sorghum and sunflower grown on sub-tropical Vertosols. *Soil Research*, *54*(5), 604–618. https://doi.org/10.1071/SR15286

Senbayram, M., Budai, A., Bol, R., Chadwick, D., Marton, L., Gündogan, R., & Wu, D. (2019). Soil NO_3^- level and O_2 availability are key factors in controlling N_2O reduction to N_2 following long-term liming of an acidic sandy soil. *Soil Biology and Biochemistry*, *132*, 165–173. https://doi.org/10.1016/j.soilbio.2019.02.009

Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences*, *111*(25), 9199–9204. https://doi.org/10.1073/pnas.1322434111

Šimek, M., & Cooper, J. E. (2002). The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, *53*(3), 345–354. https://doi.org/10.1046/j.1365-2389.2002.00461.x

Spott, O., Russow, R., Apelt, B., & Stange, C. F. (2006). A ^{15}N-aided artificial atmosphere gas flow technique for online determination of soil N_2 release using the zeolite Köstrolith SX6®. *Rapid Communications in Mass Spectrometry*, *20*(22), 3267–3274. https://doi.org/10.1002/rcm.2722

Takeda, N., Friedl, J., Kirkby, R., Rowlings, D., De Rosa, D., Scheer, C., & Grace, P. (2022). Interaction between soil and fertiliser nitrogen drives plant nitrogen uptake and nitrous oxide (N_2O) emissions in tropical sugarcane systems. *Plant and Soil*, *477*(1–2), 647–663. https://doi.org/10.1007/s11104-022-05458-6

Takeda, N., Friedl, J., Kirkby, R., Rowlings, D., De Rosa, D., Scheer, C., & Grace, P. (2023). Denitrification losses in response to N fertiliser rates—Integrating high temporal resolution N_2O, in-situ $^{15}N_2O$ and $^{15}N_2$ measurements and fertiliser ^{15}N recoveries in intensive sugarcane systems [Dataset]. Environmental Data Initiative. https://doi.org/10.6073/pasta/5c34f47415f55eb4e030f22c91299ad9

Takeda, N., Friedl, J., Rowlings, D., De Rosa, D., Scheer, C., & Grace, P. (2021a). Exponential response of nitrous oxide (N_2O) emissions to increasing nitrogen fertiliser rates in a tropical sugarcane cropping system. *Agriculture, Ecosystems & Environment*, *313*, 107376. https://doi.org/10.1016/j.agee.2021.107376

Takeda, N., Friedl, J., Rowlings, D., De Rosa, D., Scheer, C., & Grace, P. (2021b). No sugar yield gains but larger fertiliser ^{15}N loss with increasing N rates in an intensive sugarcane system. *Nutrient Cycling in Agroecosystems*, *121*(1), 99–113. https://doi.org/10.1007/s10705-021-10167-0

Thorburn, P. J., Biggs, J. S., Palmer, J., Meier, E. A., Verburg, K., & Skocaj, D. M. (2017). Prioritizing crop management to increase nitrogen use efficiency in Australian sugarcane crops. *Frontiers in Plant Science*, *8*, 1–16. https://doi.org/10.3389/fpls.2017.01504

Vilas, M. P., Shaw, M., Rohde, K., Power, B., Donaldson, S., Foley, J., & Silburn, M. (2022). Ten years of monitoring dissolved inorganic nitrogen in runoff from sugarcane informs development of a modelling algorithm to prioritise organic and inorganic nutrient management. *Science of The Total Environment*, *803*, 150019. https://doi.org/10.1016/j.scitotenv.2021.150019

Wang, R., Pan, Z., Zheng, X., Ju, X., Yao, Z., Butterbach-Bahl, K., et al. (2020). Using field-measured soil N_2O fluxes and laboratory scale parameterization of $N_2O/(N_2O+N_2)$ ratios to quantify field-scale soil N_2 emissions. *Soil Biology and Biochemistry*, *148*, 107904. https://doi.org/10.1016/j.soilbio.2020.107904

Warner, D. I., Scheer, C., Friedl, J., Rowlings, D. W., Brunk, C., & Grace, P. R. (2019). Mobile continuous-flow isotope-ratio mass spectrometer system for automated measurements of N(2) and N(2)O fluxes in fertilized cropping systems. *Scientific Reports*, *9*(1), 11097. https://doi.org/10.1038/s41598-019-47451-7

Weier, K. L., Doran, J. W., Power, J. F., & Walters, D. T. (1993). Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal*, *57*(1), 66–72. https://doi.org/10.2136/sssaj1993.03615995005700010013x
Weier, K. L., McEwan, C., Vallis, I., Catchpoole, V., & Myers, R. J. A. J. O. A. R. (1996). Potential for biological denitrification of fertilizer nitrogen in sugarcane soils. *Australian Journal of Agricultural Research*, *47*(1), 67–79. https://doi.org/10.1071/ar9960067
Weier, K. L., Rolston, D., & Thorburn, P. J. (1998). The potential of N losses via denitrification beneath a green cane trash blanket. In *Proceedings of the Australian society of sugar cane technologists*.
Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society Series B: Statistical Methodology*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x
Yang, W. H., McDowell, A. C., Brooks, P. D., & Silver, W. L. (2014). New high precision approach for measuring 15N–N2 gas fluxes from terrestrial ecosystems. *Soil Biology and Biochemistry*, *69*, 234–241. https://doi.org/10.1016/j.soilbio.2013.11.009
Zistl-Schlingmann, M., Feng, J., Kiese, R., Stephan, R., Zuazo, P., Willibald, G., et al. (2019). Dinitrogen emissions: An overlooked key component of the N balance of montane grasslands. *Biogeochemistry*, *143*(1), 15–30. https://doi.org/10.1007/s10533-019-00547-8