Interaction Between Soil and Fertiliser Nitrogen (N) Drives Plant N Uptake and Nitrous Oxide (N₂O) Emissions in Tropical Sugarcane Systems

Naoya Takeda (✉ naoya.takeda@hdr.qut.edu.au)

Queensland University of Technology <https://orcid.org/0000-0002-3977-2804>

Johannes Friedl

Queensland University of Technology <https://orcid.org/0000-0003-0468-916X>

Robert Kirkby

Queensland University of Technology

David Rowlings

Queensland University of Technology

Daniele De Rosa

Queensland University of Technology

Clemens Scheer

Queensland University of Technology

Peter Grace

Queensland University of Technology

Research Article

Keywords: Emission factor, Priming effect, N₂O priming, Added N interaction, Fertiliser 15N recovery, NUE

Posted Date: January 20th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1249386/v1>

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Abstract

Aims: High nitrogen (N) fertiliser inputs in intensive sugarcane systems drive productivity but also significant emissions of nitrous oxide (N₂O), a potent greenhouse gas. The effects of N fertiliser inputs on native soil N availability for plant N uptake as well as N₂O emissions remain unknown, hindering efficient N management. This study investigated the contribution of fertiliser and soil N and their interaction to plant N uptake and N₂O emissions in two intensively managed tropical sugarcane systems with different farming practices.

Methods: High temporal resolution N₂O measurements were combined with ¹⁵N recoveries across four N fertiliser rates, (100, 150, 200 and 250 kg N ha⁻¹) in soil, plant and N₂O emissions.

Results: Cumulative N₂O emissions ranged from 0.3 to 4.1 kg N ha⁻¹, corresponding to emission factors ranging from 0.7 to 2.4%. Native soil N accounted for > 60% of cumulative N₂O emissions and total plant N uptake. Fertiliser N addition increased N₂O emissions from native soil N compared to the unfertilised control. These findings highlight the interaction between fertiliser and soil N, with the latter being the dominant source for N uptake and loss. Overall fertiliser ¹⁵N loss responded exponentially to N rates with 50% of applied N fertiliser permanently lost even at the recommended N rate.

Conclusions: The response of soil-derived N₂O emissions to N rates largely determined the magnitude of total N₂O emissions, demonstrating the importance of site and soil specific interaction between soil and fertiliser N for N₂O emissions.

1. Introduction

Nitrogen (N) fertiliser use in agriculture has increased to supplement soil N supply to sustain yields under the pressure of global food demand. However, increasing N surpluses on a farm level have raised concerns about inefficient N fertiliser use and the associated losses of N to the environment. Dissolved N losses cause degradation of sensitive water catchments and coastal lagoons (Fowler et al. 2013). Gaseous N emissions in the form of nitrous oxide (N₂O) contribute to global climate change, as N₂O is a long-lived atmospheric trace gas with a global warming potential 273 times higher than that of carbon dioxide (CO₂) 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). Croplands are globally responsible for 66% of total anthropogenic N₂O emissions which are predicted to double by 2050 (Davidson and Kanter 2014). Fertiliser N inputs increase the magnitude of N₂O emissions and account for 54% of the increase in global N₂O emissions in the recent decade (Tian et al. 2019). Sustainable agricultural production therefore requires the adaption of N fertiliser application rates which account for soil N supply and plant N demand, reducing N₂O emissions and other N loss pathways while maintaining crop productivity (Liu et al. 2018; Mueller et al. 2014; Thompson et al. 2019).

Sugarcane (*Saccharum* spp.) often receives substantial N fertiliser inputs to fully leverage its large potential for biomass production (Roy et al. 2006). Fertiliser N inputs in sugarcane systems range from 140 to 220 kg N ha⁻¹ in Australia (Schroeder et al. 2010), 150 to 400 kg N ha⁻¹ in India (Robinson et al. 2011) and from 400 to 800 kg N ha⁻¹ in China (Li and Yang 2015). Surplus N inputs are often applied as economic insurance to protect profits against uncertainty in crop growth (Calcino et al. 2010), likely leading to applied N exceeding crop N demand. Excessive N fertiliser application leads to low N uptake efficiency and large N losses (Thorburn et al. 2017) and substantial N₂O emissions from sugarcane systems has been reported (Allen et al. 2010; Wang et al. 2016). The industry-specific emission factor (EF) of fertiliser-induced N₂O for sugarcane in Australia assumes 2% of applied N fertiliser lost as N₂O (DISER 2020), yet an exponential response of N₂O emissions to N rates has been recently reported from an intensive sugarcane system (Takeda et al. 2021a). Losses of N from intensively managed sugarcane systems not only highlight agronomic inefficiencies, but also the large environmental footprint of sugarcane production.

Both the mineralisation of soil organic N and N fertiliser inputs supply N available for plant uptake and loss. To determine the contribution of N fertiliser to plant N uptake and N₂O emissions, the difference method has been widely used, calculating the contribution of N fertiliser to plant N uptake or N₂O emissions by its difference from an unfertilised control (Antille and Moody

2021; Scheer et al. 2012). This method gives apparent plant fertiliser N uptake efficiency and N₂O EF when dividing fertiliser-derived N uptake and N₂O respectively by the N rate. This method assumes that the contribution of soil N in fertilised treatments is equal to plant N uptake or N₂O emissions in the non-fertilised control. However, N fertiliser application can affect soil N mineralisation, termed the priming effect (Kuzyakov et al. 2000), and thus the contribution of soil N, leading to a bias regarding N source partitioning by the difference method. The use of ¹⁵N labelled fertiliser can trace the fate of fertiliser N in crop and soil and provides information regarding the amount of fertiliser N lost to the environment as the balance between recovered and applied ¹⁵N fertiliser (Dourado-Neto et al. 2010). Fertiliser ¹⁵N uptake in sugarcane commonly accounts for only 20–40% of the N applied (Boschiero et al. 2018; Chapman et al. 1994; da Silva et al. 2020; Franco et al. 2011; Prasertsak et al. 2002; Takeda et al. 2021b; Vallis et al. 1996), which is lower than values typically reported from other field crops such as maize (65%), wheat (57%), and rice (46%) (Ladha et al. 2005). Subtracting the recovered fertiliser N from the total N in the respective pool enables evaluation of soil N contribution to this N pool. Soil-derived N usually dominates plant N uptake despite the high N inputs in intensive sugarcane systems (Franco et al. 2011; Oliveira et al. 2017; Prasertsak et al. 2002), and fertiliser N application has recently been shown to increase soil-derived N uptake in an intensive sugarcane system (Takeda et al. 2021b).

However, studies partitioning fertiliser and soil-derived N₂O emissions under field conditions are scarce and limited to pasture systems (Buckthought et al. 2015; Friedl et al. 2017) and short-term studies in grains systems (Xu et al. 2021). Incubation and greenhouse studies showed increases in soil-derived N₂O production in response to N fertiliser addition (Daly and Hernandez-Ramirez 2020; Roman-Perez and Hernandez-Ramirez 2021; Schleusner et al. 2018; Thilakarathna and Hernandez-Ramirez 2021), highlighting the importance of interactions between the native soil N pool and N fertiliser inputs to N losses. However, these interactions are affected by the presence and type of plants, and short-term studies from different cropping systems are therefore unlikely to be representative of intensive sugarcane systems, where ratooning, a large root system, N fertiliser banding at the beginning of the season and large amounts of irrigation and rainfall create unique conditions for N turnover.

This study investigated the contribution of fertiliser and soil N and their interaction to plant N uptake and N₂O emissions in response to different N rates in two intensive tropical sugarcane systems. Combining high temporal resolution monitoring of N₂O emissions with in-situ ¹⁵N₂O measurements and recovery of fertiliser ¹⁵N quantified contribution of fertiliser and soil N to both plant N uptake and N₂O emissions over the growing season under field conditions. The study was undertaken on two different sugarcane farms, allowing demonstration of the combined effects of fertiliser application methods (two-side banding vs stool splitting), irrigation management (furrow irrigation vs overhead sprinkler) and trash management (burnt vs green cane trash blanketing; GCTB) on sources of N for plant N uptake and N₂O emissions.

2. Materials And Methods

In the current study, we combined additional in situ ¹⁵N₂O measurements together with high temporal resolution measurements of N₂O presented in Takeda et al. (2021a) and the recovery of ¹⁵N-labelled fertiliser presented in Takeda et al. (2021b) conducted on a commercial sugarcane farm in Burdekin, QLD (19° 37' 4" S, 147° 20' 4" E) from October 2018 to August 2019 to attribute N₂O losses to fertiliser and soil (non-fertiliser) N sources. We conducted a complementary field experiment on a commercial sugarcane farm in Mackay, QLD (21° 14' 4" S, 149° 04' 6" E) from October 2019 to August 2020 and present the results together.

Study site

The climate is tropical in both Burdekin and Mackay and the average annual rainfall is 945 mm (Bureau of Meteorology, Site 033002, Ayr) and 1598 mm (Bureau of Meteorology, Site 033119, Mackay), respectively. The annual average daily minimum and maximum temperatures are 18.0 and 29.2°C in Burdekin and 19.1 and 26.5°C in Mackay. The soil is classified as Brown Dermosol and Brown Kandosol in the Australian Soil Classification (Isbell 2016), or Luvisol and Fluvisol in the World Reference Base (WRB) Classification (IUSS Working Group 2014) at the Burdekin and Mackay sites, respectively. Sugarcane varieties Q240 and Q208 were planted in 2015 and 2016 and the crop was the 3rd ratoon during the experiment at the Burdekin and Mackay sites, respectively. Irrigation was applied by furrow irrigation at the Burdekin site and overhead sprinkler at the Mackay site. Sugarcane was burnt before harvest at the Burdekin site. 'Green cane trash blanketing (GCTB)', a practice where the cane is

harvested green and the trash (crop residues) is spread over the ground, is practised at the Mackay site. Site conditions are summarised in Table 1.

Table 1
Climate conditions, soil properties at 0-0.2 m depth and crop management practices at the Burdekin and Mackay sites

Category	Variable	Burdekin	Mackay
Experiment	Coordinates	19° 37' 4" S, 147° 20' 4" E	21° 14' 4" S, 149° 04' 6" E
	Period	Oct. 2018 to Aug. 2019	Oct. 2019 to Aug. 2020
Climate	Annual mean rainfall (mm)	945	1598
	Cumulative rainfall over the experimental period (mm)	1180	1418
	Annual mean daily minimum temperature (°C)	18.0	19.1
	Annual mean daily maximum temperature (°C)	29.2	26.5
Soil (0.0-0.2 m)	BD (g cm ⁻³)	1.3	1.1
	pH (H ₂ O)	6.92	4.13
	Total C (%)	1.60	1.35
	Total N (%)	0.08	0.09
	Clay (%)	10.5	22.2
	Silt (%)	48.5	15.9
	Sand (%)	41.0	61.9
	Mineral N (kg N ha ⁻¹)	37.0	31.8
Crop management	Cultivar	Q240	Q208
	Crop	3rd ratoon	3rd ratoon
	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 fertiliser product	Urea	Urea
	Fertiliser application	Two-sided banding	Stool splitting
	Irrigation management	Furrow	Overhead
	Estimated total irrigation amount (mm)	600	180
	Trash management	Burnt	GCTB

Experimental design

The experimental design of the Burdekin site was a randomised strip design with four plots across two strips for each N treatment, detailed in (Takeda et al. 2021a). The experiment at the Mackay site had a completely randomised block design with three replicates per treatment (around 10 m in length) and three planting rows per plot with a row distance of 1.56 m, accompanied by an unfertilised control (0N) plot with three subplots (20 m of length). Fertiliser N rate treatments included 0N, 150 kg N ha⁻¹ (150N), 200 kg N ha⁻¹ (200N) and 250 kg N ha⁻¹ (250N), plus 100 kg N ha⁻¹ (100N) at the Mackay site only. The

recommended N rate in SIX EASY STEPS best management practice of the Australian sugar industry (Schroeder et al. 2010) was 150N at the Mackay site and 200N at the Burdekin site. Urea was applied by banding the fertiliser 10 cm deep and 30 cm from the bed centre on both sides of the cane row at the Burdekin site and by stool splitting 10 cm deep at the bed centre of the cane row at the Mackay site. For the ^{15}N recovery in the soil and the plant, a 2.0 m section was exempted from the application of N fertiliser in each plot and ^{15}N enriched urea fertiliser (5 atom%) in solution was manually applied at the corresponding rate (i.e. 100N, 150N, 200N and 250N) matching the N fertiliser placement at the respective site.

Measurement of N_2O and CO_2 emissions using an automated chamber system

Soil-borne N_2O and CO_2 emissions were measured at a high temporal resolution using an automated chamber system (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 chamber system are given in Supplementary materials S1.1. Manual gas sampling was conducted for the control plots of the Mackay site by the static closed chamber method (Friedl et al. 2017), detailed in Supplementary materials S1.2. The placement of the chambers accounted for N fertiliser placement and irrigation practice at each site: At the Burdekin site, chambers were installed covering the area from (a) the fertiliser band to the centre of the bed (bed chamber) and (b) the fertiliser band the centre of the furrow (furrow chamber). At the Mackay site, bed chambers (a) were placed at the centre of the bed (i.e., on the fertiliser band) and furrow chamber measurements (b) were substituted with those from the control plots.

Measurement of fertiliser and soil-derived N_2O emissions using a ^{15}N method

Emissions of fertiliser-derived N_2O were measured as described by (Friedl et al. 2017). Micro plots were established alongside the main plots with N fertiliser 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 had a completely randomised block design with four replicates. A steel base (0.22 m $\times$ 0.22 m at the Burdekin site and 0.2 m $\times$ 0.4 m at the Mackay site) was installed in each micro plot and ^{15}N enriched urea fertiliser (70 atom%) was applied inside the base at the corresponding rates. Gas sampling was conducted with static closed chambers at the Burdekin site from November 2018 to February 2019 and with semi-automated chambers at the Mackay site from October 2019 to January 2020, detailed in Supplementary materials S1.3. The gas samples were analysed for the concentration of N_2O using a Shimadzu GC-2014 Gas Chromatograph (Shimadzu, Kyoto, Japan) and for $^{15}\text{N}_2\text{O}$ using an Isotope Ratio Mass Spectrometer (IRMS) (20–22 Sercon Limited, UK).

Plant and soil sampling and analyses

Plant and soil samples were taken from each of the 2.0 m sections just before the farmer's 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 at the Burdekin site are detailed in Takeda et al. (2021b) and briefly described here. Plant sampling procedures are detailed in Supplementary materials S1.4. Soil samples were taken at three to four points between the bed and furrow centres using a soil corer and a post-hole driver down to 1.0 m. At the Burdekin site, the sampling points were 0, 0.25, 0.50, 0.75 m away from the bed centre. At the Mackay site, those were 0, 0.12, 0.40, 0.80 m away from the bed centre in 100N and 250N and the three points except for 0.12 m away from the bed centre in 150N and 200N. Each soil core sample of 1.0 m was separated into 0–0.2, 0.2–0.4, 0.4–0.7, 0.7–1.0 m soil depths and subsamples (about 100 g) were taken. The dried plant and soil samples were then finely ground and analysed for N and ^{15}N content via IRMS analysis (20–22 Sercon Limited, UK). The stalk samples were further dried with a vacuum oven at 40°C for 48 hours before fine grinding to avoid aggregation due to sugar.

^{15}N calculations

Fertiliser ^{15}N recovery (%) in the plant and soil was then calculated by ^{15}N mass balance (Friedl et al. 2017; Rowlings et al. 2016). The percentage of N in individual soil and plant material samples ('sinks') derived from ^{15}N -labelled fertiliser (N_{dff} plant or soil) was calculated from

$$Ndff(\%) = \frac{\%^{15}N_{excess\ of\ sink}}{\%^{15}N_{excess\ of\ fertiliser}} \times 100 \quad [1]$$

where the $\%^{15}N$ excess used for all sources and sinks was the ^{15}N abundance less an adjustment of $\%^{15}N$ measured for the corresponding samples in the 0N plots for background enrichment. Calculations for fertiliser ^{15}N recovery in plant and soil are detailed in Takeda et al. (2021b) and given in Supplementary materials S1.5. For the gas samples from the ^{15}N micro plots, the measured N_2O $\%^{15}N$ was corrected to account for the N_2O existing in the headspace at 0 min after chamber closure, detailed in Supplementary materials S1.6. After the correction, the proportion of N_2O emissions derived from fertiliser ($Ndff\ N_2O$) at the fertiliser band was calculated by Equation [1]. Then, $Ndff\ N_2O$ was gap-filled per N rate over the crop growing season on a daily basis at each site. The gap-filled daily $Ndff\ N_2O$ per N rate from the micro plots was then applied to daily N_2O emissions per plot measured by the automated chamber system to calculate fertiliser-derived N_2O emissions as follows:

$$Fertiliser\ derived\ N_2O\ emissions_{i,j} = area\ weighted\ N_2O\ emissions_{i,j} \times \frac{Ndff\ N_2O_{i,j}}{100} \quad [2]$$

where i and j indicate days after fertilisation and chamber position (i.e. bed or furrow). At the Burdekin site, the adjusted $Ndff\ N_2O$ at the fertiliser band in micro plots was used for both bed and furrow chambers because both chambers covered the fertiliser band and N_2O emissions did not differ between the positions. At the Mackay site, adjusted $Ndff\ N_2O$ at the furrow was assumed to be zero because the furrow chamber did not cover the fertiliser band. The rationale behind these assumptions is detailed in Supplementary materials S1.7. Soil-derived N_2O emissions (i.e. non-fertiliser-derived N_2O emissions) was calculated by the difference between total N_2O emissions and fertiliser-derived N_2O emissions per plot on a daily basis.

Auxiliary measurements

For soil ammonium (NH_4^+) and nitrate (NO_3^-) measurements, soil samples (0–20 cm depth) were taken near the fertiliser band in each plot one day after fertilisation, every 3–7 days for the first three months and then monthly thereafter. Soil NH_4^+ and NO_3^- were extracted by adding 100 mL of 2 M KCl to 20 g of air-dried soil and shaking the solution for one hour, followed by NH_4^+ and NO_3^- content measurements using a Gallery™ Discrete Analyzer (Thermo Fisher Scientific, USA).

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 $p < 0.05$. Daily N_2O emissions were calculated by averaging the measured hourly fluxes over a 24-h period from each chamber and multiplying by 24. Missing daily N_2O emissions between measurements were imputed by linear interpolation, using a package “imputeTS” (Moritz and Bartz-Beielstein 2017). Area-weighted N_2O emissions were calculated by the ratio bed:furrow = 1:1 at the Burdekin site, while bed:furrow = 1:2 at the Mackay site. Cumulative N_2O emissions were then calculated by summing the daily average of each chamber over each growing period. The 2-D interpolation of the fertiliser ^{15}N remaining in the soil from the bed centre to the furrow centre down to 1.0 m depth at harvest was conducted for each plot by fitting a thin-plate spline using a package “mgcv” (Wood 2011). Gap-filling of $Ndff\ N_2O$ values between measurements over the crop growing season was conducted by fitting generalised additive mixed models (GAMMs), using a package “mgcv” (Wood 2011) and detailed in Supplementary materials S1.8. 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 analyse $Ndff\ N_2O$ over time in response to days after fertilisation (DAF) and N rates. Two-way analysis of variance (ANOVA) was performed to assess significant differences in cumulative (fertiliser and soil-derived) N_2O emissions, plant N uptake and fertiliser ^{15}N recoveries between the N rate treatments and the sites. Subsequently, the Tukey-Kramer test was performed when the ANOVA yielded P values < 0.05 , using a package “agricolae” (de Mendiburu 2020). To evaluate the response of fertiliser ^{15}N loss to N rates across sites, (generalised) linear mixed models were fitted with the site as a random factor, using a package “lme4” (Bates et al. 2015).

3. Results

N₂O emissions and contributions from soil and fertiliser N

The response of cumulative N₂O emissions to increasing N rates differed between the two sites (Fig. 1), with larger N₂O emissions at the Mackay site compared to the Burdekin site overall across all rates ($P < 0.001$). Cumulative N₂O emissions in the control were 0.29 kg N₂O-N ha⁻¹ at the Burdekin site and 1.50 kg N₂O-N ha⁻¹ at the Mackay site. Fertiliser application increased cumulative N₂O emissions ($P < 0.001$), leading to 1.52–3.06 kg N₂O-N ha⁻¹ and 3.72–4.14 kg N₂O-N ha⁻¹ emitted from the fertilised treatments at Burdekin and Mackay sites respectively. Cumulative N₂O emissions exponentially increased with N rates at the Burdekin site ($P < 0.001$) but did not differ between fertilised treatments at the Mackay site ($P = 0.984$).

Changes in *Ndff* N₂O at the fertiliser band over the measurement period showed the peak of *Ndff* N₂O occurring at around 25 DAF at the Burdekin site while 10 DAF at the Mackay site (Fig. 1). Highest *Ndff* N₂O was about 70% at the Burdekin site and 90% at the Mackay site. At both sites, spikes of *Ndff* N₂O recurred and diminished over time. The spikes of *Ndff* N₂O occurred even after 90 DAF at the Burdekin site but diminished by 60 DAF at the Mackay site. The difference in *Ndff* N₂O among N rate treatments was smaller than the difference between the sites. Across the two sites, the period with high *Ndff* N₂O > 30% was within two months after fertilisation.

Fertiliser-derived N₂O emissions ranged from 0.39 to 0.78 kg N₂O-N ha⁻¹ at the Burdekin site and 0.97 to 1.34 kg N₂O-N ha⁻¹ at the Mackay site ($P < 0.001$), correlated positively with increasing N rates ($P = 0.043$) (Fig. 2). Fertiliser N contributed 22.8–25.40% and 23.7–35.7% to cumulative, seasonal N₂O emissions at the Burdekin and Mackay sites, respectively. The proportion of the applied N emitted as N₂O calculated by the difference from the unfertilised control was termed apparent N₂O EF, which is the standard manner to derive N₂O EF. Apparent N₂O EF ranged from 0.70–1.14 and 0.90–2.41% at the Burdekin and Mackay sites, respectively. The ¹⁵N recovery in N₂O enabled calculation of ¹⁵N N₂O EFs, which showed 0.19–0.31 and 0.53–1.02% of the N applied emitted as N₂O at the Burdekin and Mackay site, respectively. Apparent EFs exceeded ¹⁵N N₂O EFs regardless of site and N rate (Fig. 3).

Soil-derived N₂O emissions responded positively to N rates ($P < 0.001$) and were larger at the Mackay site compared to the Burdekin site ($P < 0.001$). In fertilised treatments, soil-derived N₂O emissions ranged from 1.11 to 2.28 kg N₂O-N ha⁻¹ at the Burdekin site and 2.41 to 2.88 kg N₂O-N ha⁻¹ at the Mackay site (Fig. 2). Emissions of soil-derived N₂O continued > 100 DAF while the majority of fertiliser-derived N₂O emissions occurred within two months after fertilisation (Fig. S1).

Plant N uptake and fertiliser ¹⁵N recoveries

Plant N uptake ranged from 67.2 to 177.2 kg N ha⁻¹ at the Burdekin site and 91.8 to 140.6 kg N ha⁻¹ at the Mackay site, positively correlated with N rates ($P < 0.001$) (Table 2). Fertiliser ¹⁵N uptake increased with increasing N rates ($P < 0.001$), ranging from 38.8 to 65.0 kg N ha⁻¹ at the Burdekin site and 19.8 to 45.9 kg N ha⁻¹ at the Mackay site (Table 2). Fertiliser ¹⁵N uptake accounted for 24.2–37.7% and 15.6–32.1% of the plant N uptake at the Burdekin and Mackay sites, respectively, which increased with increasing N rates ($P < 0.001$). Soil-derived N uptake in the fertilised treatments ranged from 110.9 to 121.7 kg N ha⁻¹ at the Burdekin site and 85.3 to 107.4 kg N ha⁻¹ at the Mackay site (Table 2). Fertiliser ¹⁵N uptake accounted for 25.9–26.3% and 16.2–20.8% of the N applied (¹⁵NUpE) at the Burdekin and Mackay sites respectively (Table 2). Apparent NUpE was 46.8–66.9% at the Burdekin site and 17.6–35.4% at the Mackay site (Table 2).

Table 2

Plant N uptake derived from fertiliser and soil, N uptake efficiency (^{15}N and apparent) and fertiliser ^{15}N remaining in the soil at different N rate treatments at the Burdekin and Mackay sites

		N uptake				N uptake efficiency			Fertiliser N in the soil	
	N rate	Total	Fertiliser-derived		Soil-derived		^{15}N	Apparent	Amount	% of N applied
	(kg N ha ⁻¹)	(kg N ha ⁻¹)	(kg N ha ⁻¹)	(%)	(kg N ha ⁻¹)	(%)	(%)	(%)	(kg N ha ⁻¹)	(%)
Burdekin	0	67.2 ^b ± 3.3	0	0	67.2 ^a ± 3.3	100				
	150	160.5 ^{ab} ± 14.6	38.8 ^{abc} ± 4.3	24.2 ^{ab} ± 1.4	121.7 ^a ± 11.4	75.8 ^{ab} ± 1.4	25.9 ^a ± 2.9	66.9 ^a ± 9.8	39.4 ^a ± 7.3	26.3 ^{ab} ± 4.9
	200	163.5 ^{ab} ± 31.2	52.6 ^{ab} ± 6.0	35.5 ^a ± 7.9	110.9 ^a ± 30.3	64.5 ^a ± 7.9	26.3 ^a ± 3.1	51.7 ^a ± 15.6	37.8 ^a ± 6.3	18.9 ^b ± 3.1
	250	177.2 ^a ± 23.7	65.0 ^a ± 6.7	37.7 ^a ± 3.2	112.2 ^a ± 18.6	62.3 ^a ± 3.2	26.0 ^a ± 2.7	46.8 ^a ± 9.5	36.2 ^a ± 7.5	14.5 ^b ± 3.0
Mackay	0	91.8 ^{ab} ± 12.3	0	0	91.8 ^a ± 12.3	100				
	100	127.2 ^{ab} ± 2.2	19.8 ^c ± 1.1	15.6 ^b ± 1.1	107.4 ^a ± 3.0	84.4 ^b ± 1.1	19.8 ^a ± 1.1	35.4 ^a ± 2.2	30.5 ^a ± 4.2	30.5 ^{ab} ± 4.2
	150	124.3 ^{ab} ± 22.9	24.3 ^{bc} ± 2.9	20.1 ^{ab} ± 2.3	99.9 ^a ± 20.6	79.9 ^{ab} ± 2.3	16.2 ^a ± 1.9	21.6 ^a ± 15.3	54.7 ^a ± 5.8	36.5 ^a ± 3.9
	200	126.9 ^{ab} ± 22.7	41.6 ^{abc} ± 12.6	31.5 ^{ab} ± 3.8	85.3 ^a ± 10.2	68.5 ^{ab} ± 3.8	20.8 ^a ± 6.3	17.6 ^a ± 11.4	60.8 ^a ± 3.6	30.4 ^{ab} ± 1.8
	250	140.6 ^{ab} ± 24.9	45.9 ^{abc} ± 10.2	32.1 ^{ab} ± 3.2	94.7 ^a ± 15.6	67.9 ^{ab} ± 3.2	18.4 ^a ± 4.1	19.5 ^a ± 10.0	61.1 ^a ± 5.1	24.4 ^{ab} ± 2.0
ANOVA (P value)	N rate	< 0.001***	< 0.001***	< 0.001***	0.127	< 0.001***	0.976	0.113	0.041*	0.017*
	Site	0.229	0.012*	0.176	0.592	0.176	0.007**	< 0.001***	0.005**	0.006**
Different alphabet letters indicate a significant difference between treatments and sites.										

Fertiliser ^{15}N remaining in the soil ranged from 36.2 to 39.4 kg N ha⁻¹ at the Burdekin site and 30.5 to 61.1 kg N ha⁻¹ at the Mackay site at 100–250 kg N ha⁻¹ (Table 2). The majority of fertiliser ^{15}N in the soil was recovered down to 0.3 m soil depth and within 0.2 m from the bed centre at the Mackay site (Fig. S2). There was a significant difference in fertiliser ^{15}N remaining in the soil between 100N and the higher N rates (200N and 250N) at the Mackay site but no difference among N rates ≥ 150 kg N ha⁻¹

at both sites. The fraction of applied ^{15}N fertiliser remaining in the soil decreased with increasing N rates ($P = 0.017$), ranging from 14.5 to 26.3% at the Burdekin site and 24.4 to 36.5% at the Mackay site (Table 2).

Fertiliser ^{15}N loss increased exponentially with increasing N rates ($P < 0.001$), ranging from 71.8 to 148.8 kg N ha $^{-1}$ at the Burdekin site and 49.7 to 143.0 kg N ha $^{-1}$ at the Mackay site (Fig. 4). Fertiliser ^{15}N loss accounted for 47.8–59.5% and 47.3–57.2% of the N applied at the Burdekin and Mackay sites, respectively, increasing with increasing N rates ($P = 0.023$).

Auxiliary measurements

Rainfall during the crop growing season totalled 1180 mm and 1418 mm at the Burdekin and Mackay sites (Table 1). Furrow irrigation was applied at monthly intervals during the growing season and stopped ten weeks before harvest at the Burdekin site with an estimated total amount of 600 mm. Overhead sprinkler irrigation was applied three times in the early growing season at the Mackay site, totalling 180 mm. Rainfall and irrigation water was allocated more in the early growing season at the Burdekin site, totalling 1321 mm over the first four months after fertilisation compared to 808 mm at the Mackay site (Fig. 1).

Soil NH_4^+ and NO_3^- contents increased shortly after fertiliser application and peaked at 30–60 DAF at both sites (Fig. S3). Soil NH_4^+ and NO_3^- contents at the peak were 10 and 30 mg N kg $^{-1}$ at the Burdekin site and > 200 and > 100 mg N kg $^{-1}$ at the Mackay site. Mineral N contents then decreased to below 10 mg N kg $^{-1}$ by 100 DAF and stayed for the rest of the growing season across sites.

Emissions of CO_2 were used as a relative measure for heterotrophic soil respiration enabling comparisons between treatments and sites. Cumulative emissions of CO_2 over the season were lower at the Burdekin site than at the Mackay site ($P < 0.001$), ranging from 4.1 to 5.6 Mg C ha $^{-1}$ and 10.2 to 11.2 Mg C ha $^{-1}$, respectively (Fig. S3). Cumulative CO_2 emissions did not differ between N rate treatments.

Cane yield

Cane yield ranged from 59.6 to 147.3 Mg ha $^{-1}$ at the Burdekin site and 89.1 to 135.2 Mg ha $^{-1}$ at the Mackay site (Fig. S4). The fitted yield response curves to N rates showed that 95% of the maximum yield could have been achieved at 127 and 108 kg N ha $^{-1}$ of N rates at the Burdekin and Mackay sites, respectively.

4. Discussion

This study provides further evidence that N fertiliser losses are high in intensively managed sugarcane systems with 50% of applied N fertiliser permanently lost by the end of the season even at the recommended N rate. Nitrogen fertiliser losses increased exponentially with increasing N fertiliser inputs. These high losses demonstrate that improved N rate recommendations are required, accounting for the different sources of N available for plant uptake and loss and their interaction. Combining high temporal resolution N_2O measurements with ^{15}N recoveries in soil, plant and N_2O emissions across different N fertiliser rates at two sugarcane sites demonstrated (i) higher cumulative N_2O emissions from the site with overhead irrigation under GCTB management and lower soil pH as compared to the site with furrow irrigation, pre-harvest burning and higher soil pH, (ii) that the majority of fertiliser-derived N_2O emissions occurred within two months of fertilisation (iii) that 60% of plant N uptake and N_2O emissions were derived from mineralised soil N and (iv) that added fertiliser N increased the contribution of soil N to N_2O emissions. Our findings show that the magnitude of total N_2O emissions across N rates was not driven by fertiliser N availability itself, but by the response of soil-derived N_2O emission to N inputs. The observed dominance of soil N for plant N uptake but also for N_2O emissions, highlight the importance of soil N mineralisation for N turnover in sugarcane systems. Considering the ratio between soil and fertiliser derived N in plant and N_2O , the high N fertiliser losses at both sites imply substantial soil-derived N losses from intensively managed sugarcane systems.

Response of N_2O emissions to N fertiliser rates

Cumulative N_2O emissions increased exponentially with increasing N rates at the Burdekin site. Emissions of N_2O were generally higher at the Mackay site, and fertilisation increased N_2O emissions compared to the control, but unlike in the Burdekin, N_2O emissions did not increase with N rates. This difference between sites in cumulative N_2O emissions and their response to increasing N rates is due to differences in soil water, C availability and soil pH as key drivers of N_2O production in these soils. Rainfall and irrigation totals were similar across the two sites yet 75% of rainfall and irrigation occurred in the first four months at the Burdekin site compared to 50% in Mackay. Furrow irrigation (Burdekin) causes soil water contents close to saturation (Takeda et al. 2021a), suggesting not only N_2O production but also increased reduction to N_2 (Scheer et al. 2009), while smaller amounts of irrigation supplied via overhead sprinkler (Mackay) indicate lower WFPS, potentially driving denitrification towards N_2O . Fertiliser N placement, the diffusion of the fertiliser band in response to irrigation and rainfall and resulting soil NO_3^- availability also differed between sites: Fertiliser N was banded on each side of the cane row in the Burdekin and exposed to higher amounts of rainfall and irrigation at the beginning of the season as compared to Mackay, where a single band of N fertiliser was placed by stool splitting, and subjected to smaller amounts of rainfall and irrigation. These differences suggest less diffusion of the fertiliser N from the band in Mackay, consistent with the higher soil NO_3^- concentrations near the band at the site (Fig. S3). Increasing NO_3^- concentrations $> 100 \text{ mg N kg}^{-1}$ not only increase N substrate availability for N_2O production but also limit the reduction of N_2O to N_2 (Friedl et al. 2020; Senbayram et al. 2012; Wang et al. 2013), with both mechanisms increasing N_2O emissions.

Differences between sites in N_2O emissions from the non-fertilised control point towards drivers of N_2O emissions other than N substrate availability. Unlike burnt trash management, GCTB provides C through sugarcane residues, increasing N_2O emissions (Warner et al. 2019; Weier et al. 1998) by supplying C to heterotrophic N_2O producers or stimulating microbial O_2 consumption. Larger cumulative CO_2 emissions at the Mackay site compared to the Burdekin site (Fig. S3) highlight the importance of trash management for microbial activity and resulting N_2O emissions at the site. The soil pH at the Mackay site is slightly acidic (4.1), compared to the neutral pH of 6.9 at the Burdekin site (Table 1). Similar to high NO_3^- concentrations, low soil pH inhibits the reduction of N_2O to N_2 (Dannenmann et al. 2008; Russenes et al. 2016; Šimek and Cooper 2002). The exponential increase of N_2O emissions at the Burdekin site was attributed to high NO_3^- availability (Takeda et al. 2021a), an effect that was likely masked by the low soil pH at the Mackay site, promoting high N_2O emissions even at lower N rates. Available data do not allow us to disentangle the overlapping effects of soil water, C and N substrate availability and chemical soil properties on N_2O emissions. The comparison between sites however demonstrates that the N response of N_2O emissions is highly site and soil specific, highlighting the limitation of its estimation by using a single factor or response curve.

Large fertiliser-derived N_2O emissions after fertilisation while cumulative N_2O are dominated by soil N

The majority of fertiliser-derived N_2O emissions occurred within two months after fertilisation with $> 30\%$ of N_2O derived from applied N fertiliser (Fig. 2, S1). Fertiliser N contributed to N_2O emissions shortly after fertilisation, despite the high concentration of urea in and around the fertiliser band, which can slow hydrolysis and inhibit nitrification (Janke et al. 2019; Janke et al. 2020). At the beginning of the season, the contribution of N fertiliser to N_2O emissions was greater in Mackay, reflecting a single fertiliser band as compared to two-sided banding at the Burdekin site (Fig. 1). After the initial peak, fertiliser N contribution to N_2O emissions decreased, denoting decreasing fertiliser N availability due to N immobilisation, plant N uptake and loss. Spikes in Ndff N_2O later in the season coincided with rainfall events and irrigation (Fig. 1), indicating remineralisation of N fertiliser N contributing to N_2O production. Cumulative fertiliser-derived N_2O emissions increased with increasing N rates but accounted for less than half of total N_2O emitted across N rates (Fig. 2). Comparable contributions of soil N to cumulative total N_2O emissions $> 60\%$ were reported in incubation studies using soils from pasture and grain cropping systems (Roman-Perez and Hernandez-Ramirez 2021; Thilakarathna and Hernandez-Ramirez 2021) and a modelling study (Müller et al. 2014). Our study however demonstrates the dominance of soil-derived N_2O emissions for the first time over the whole season under field conditions for a cropping system, combining high temporal resolution N_2O flux data with $^{15}\text{N}_2\text{O}$ analysis.

At both sites, N fertilisation increased the soil-derived N₂O emissions compared to the non-fertilised control (Fig. 2). At the Burdekin site, soil-derived N₂O emissions also increased with increasing N rates. This increase in the soil-derived N₂O has been attributed to priming of organic N mineralisation by added N (Schleusner et al. 2018; Xu et al. 2021), evidenced by increased CO₂ emissions in response to added N (De Rosa et al. 2018). In our study, cumulative CO₂ emissions did not respond to N fertiliser application at both sites (Fig. S3), providing no evidence of increased N mineralisation. Added N fertiliser can also undergo microbial immobilisation instead of the native inorganic N, a process termed pool substitution (Jenkinson et al. 1985; Kuzyakov et al. 2000). This increases native soil N availability for N₂O producing microorganisms, explaining the increase in the soil-derived N₂O emissions. Increased labile C availability at the Mackay site due to GCTB management likely led to the greater immobilisation and thus pool substitution, which may explain the larger soil-derived N₂O emissions at the Mackay site at the low N rates compared to the Burdekin site (Fig. 2). This assumption is further supported by the larger amount of fertiliser ¹⁵N remaining in the soil at the Mackay site (Table 2). Regardless of the underlying mechanisms, our results demonstrate that the response of total N₂O emissions to N rates was not driven by fertiliser N availability itself, as shown by the lack of correlation between total cumulative N₂O emissions and fertiliser ¹⁵N uptake or ¹⁵N loss. Instead, the response of soil-derived N₂O emissions to N rates largely determined the magnitude of total N₂O emissions, highlighting the importance of site and soil specific interaction between soil N and N fertiliser addition providing N substrate for N₂O production.

The combination of fertiliser ¹⁵N recovery in N₂O emissions and high temporal resolution N₂O measurements enabled the calculation of EF directly from the fertiliser-derived N₂O emissions in the N rate treatment. The conventional “apparent” EF method calculates fertiliser-derived N₂O emissions as the difference in N₂O emissions between fertilised treatments and control and thus includes increases in soil-derived N₂O emissions due to interaction with N fertiliser addition as the fertiliser-derived N₂O fraction. The comparison between apparent and ¹⁵N N₂O EFs demonstrates that apparent EFs consistently overestimated the contribution of fertiliser N to N₂O emissions, showing an increasing discrepancy with increasing EFs (Fig. 3). This discrepancy emphasises a potential bias in evaluating fertiliser N loss via N₂O emissions using the conventional EF method, which in the presence of N fertiliser interaction shows fertiliser induced, but not fertiliser-derived N₂O emissions. Fertiliser-induced N₂O emissions and thus the apparent EF matter for overall GHG accounting, while the use of ¹⁵N N₂O analysis can inform mitigation strategies about the N sources of N₂O emissions to target.

Exponential increase in fertiliser ¹⁵N loss and the potential of soil-derived N losses

Fertiliser ¹⁵N loss increased exponentially with increasing N rates and accounted for up to 60% of the N applied (Fig. 4), implying that these losses are driven by an increasing mismatch of N rates and plant N demand (McLellan et al. 2018). However, excess N can also be caused by the spatial and temporal allocation of N fertiliser relative to plant N uptake. Compared to the Burdekin site, more fertiliser ¹⁵N remained in the soil and less ¹⁵N fertiliser was taken up by the plant at the Mackay site (Table 2). Considering that the majority of residual fertiliser ¹⁵N was found near the fertiliser band at harvest (Fig. S2 and Takeda et al. (2021b)), two-sided fertiliser banding at the Burdekin site potentially led to a better spatial alignment between fertiliser N and the root distribution compared to the stool splitting application at the Mackay site. On a temporal scale, the high initial contribution of fertiliser N to N₂O emissions observed within two months after fertilisation (Fig. 1) indicates a large contribution of fertiliser N to other loss pathways during this period. This further implies that the majority of fertiliser ¹⁵N losses occurred earlier than the active plant N uptake period, which is typically at 2–4 months after planting or ratooning in sugarcane (Zhao et al. 2017). The mismatch between fertiliser N availability and plant N demand in space and time resulted in fertiliser ¹⁵N losses of 50% of the applied N even at the recommended N rate (Fig. 4). These findings stress the need to adapt N rate recommendations, and to improve the spatial and temporal allocation of N fertiliser to plant uptake to increase both agronomic and environmental efficiency in sugarcane systems.

Fertiliser ¹⁵N uptake increased with increasing N rates but > 60% of plant N uptake was derived from the native soil N pool even at the highest N rate (Table 2). Together with the observed large soil N contribution to N₂O emissions (Fig. 2), these results denote that N turnover is dominated by N derived from the organic N pool. Assuming N losses mostly occur from the NO₃⁻ pool

via runoff, leaching and denitrification, the soil N contribution to N₂O emissions could be used as a proxy for soil-derived N losses via these pathways relative to fertiliser N loss. The soil N contribution to both plant N uptake and N₂O emissions decreased with N rates but stayed at > 60% (Table 2 and Fig. 2), indicating that soil-derived N loss possibly exceeds fertiliser ¹⁵N loss by far. The exponential response of fertiliser ¹⁵N loss with increasing N rates (Fig. 4) as well as the interaction between soil N with N fertiliser addition observed in N₂O emissions (Fig. 2) further imply soil-derived N loss can increase with increasing N rates. The findings of this study highlight the potential use of the fraction of soil-derived N₂O emissions as an indicator for soil-derived N loss relative to fertiliser ¹⁵N loss.

5. Conclusions

This study monitored N₂O emissions at high temporal resolution together with the fate of ¹⁵N fertiliser in soil, plant and N₂O emissions in response to N rates across two intensively managed sugarcane systems with different farming practices. The majority of fertiliser-derived N₂O emissions occurred within two months after fertilisation during a growth stage of low plant N uptake, showing a temporal mismatch between plant N demand and supply. The spatial distribution of fertiliser N may further create a spatial mismatch between banded fertiliser and root N uptake, if fertiliser placement together with limited rainfall and irrigation hinder the diffusion of fertiliser N from the band across the soil profile. Future N rate recommendations should therefore not only consider the non-linear increase of N losses with increasing N rates but also integrate management practices that align N availability with plant demand at temporal and spatial scales. Soil-derived N₂O emissions accounted for > 60% of total N₂O emissions and thus largely determined the magnitude of N₂O emissions, highlighting that N₂O abatement strategies need to account for the interaction between soil and fertiliser N inputs. The comparison between apparent and ¹⁵N fertiliser recoveries show that apparent recoveries likely underestimate the contribution of soil-derived N to both plant N uptake and N₂O emissions if N fertiliser interacts with the native soil N pool. The dominance of soil N observed in plant N uptake further highlights the importance of mineralisation of organic N for N turnover in sugarcane soils and shows the dominance of this N transformation in magnitude over fertiliser N supply. Assuming a similar ratio between soil-derived and fertiliser-derived N in overall N loss from the system as observed in plant N and N₂O emissions suggests substantial soil-derived N losses > 200 kg N ha⁻¹ when applying this ratio to the ¹⁵N fertiliser loss. This further implies that the fertiliser N remaining in the soil over the season and the plant residue N returned to the soil cannot compensate the soil N exports via plant N uptake and N losses, highlighting the need to investigate in-field N budgets to avoid long-term unsustainable mining of soil N. The dataset of this study shows the potential to combine ¹⁵N₂O analysis with ¹⁵N recoveries in soil and plant to derive overall N losses from sugarcane systems, aiding modelling approaches to simulate N budgets, which will help to account for soil mineralisation in N fertiliser management and thus mitigate N loss while maintaining crop productivity in intensive sugarcane systems.

Declarations

Acknowledgements

This study is funded by Sugar Research Australia. 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 Institute for Future Environments at Queensland University of Technology.

Statements and Declarations

This study is funded by Sugar Research Australia. The authors have no relevant financial or non-financial interests to disclose.

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Figures

Figure 1

Cumulative N₂O emissions at the (a) Burdekin and (b) Mackay sites and fertiliser N contribution to N₂O (Ndff) at the fertiliser band at the (c) Burdekin and (d) Mackay sites at 0, 100 (only at the Mackay site), 150, 200 and 250 kg N ha⁻¹. The lines and error

bars in (a) and (b) indicate gap-filled mean observed values and standard errors. The points, lines and shaded areas in (c) and (d) indicate the observed values, the fitted values by the generalised additive mixed model (GAMM) and the confidence intervals of the fitted values, respectively. The columns in (c) and (d) show the amounts of rainfall (grey) and irrigation (light blue)

Figure 2

Cumulative N₂O emissions over the crop growing season derived from fertiliser (light blue) and soil (brown) at the (a) Burdekin and (b) Mackay sites at 0, 100 (only at the Mackay site), 150, 200 and 250 kg N ha⁻¹. The error bars indicate standard errors and the percentage in the columns is the proportion of cumulative total N₂O emissions

Figure 3

Apparent against ¹⁵N N₂O emission factor at the Burdekin (blue) and Mackay (red) sites across N rates at 0, 100 (only at the Mackay site), 150, 200 and 250 kg N ha⁻¹. The error bars, solid line and dashed line indicate standard error, 1:1 line and regression line, respectively

Figure 4

Fertiliser ¹⁵N loss in response to N rates across the Burdekin and Mackay sites. The points and error bars indicate mean values and standard errors. The line and shaded area show the fitted regression line and 95% confidence intervals

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