Combined effect of nitrogen fertiliser and leaf litter carbon drive nitrous oxide emissions in tropical soils

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Abstract Intensification of agriculture in the tropics is likely to increase reactive nitrogen (N) losses in the form of nitrous oxide (N₂O), however, drivers of emissions from tropical soils remain poorly understood. This study investigated the effect of leaf litter and urea fertiliser on N₂O emissions from two Australian tropical mango orchards. Treatments included urea (25 g N m⁻²), leaf litter (1500 g m⁻² dry matter), their combined application, and untreated control. Up to 80.5 ± 8.4 mg N₂O-N m⁻² were lost within two weeks of treatment application, accounting for more than 60% of annual N₂O emissions. Indirect emissions [30 mg m⁻² d⁻¹ were recorded at one site, potentially resulting from groundwater transported N. Highest annual N₂O emissions were observed from Litter + Urea with 130.4 mg N₂O-N m⁻² y⁻¹, exceeding those from Urea-Only by a factor of two and those from Litter-Only by a factor of four. This resulted in residue and fertiliser emission factors (EF) of < 0.01–0.37%, well below the IPCC and Tier 2 defaults, with whole orchard losses equivalent to 0.19–0.66 kg N₂O-N ha⁻¹. The findings suggest N₂O is N limited when no N fertiliser is applied, even when applying large amounts of litter-N, and by carbon (C) in the absence of litter. The combined effect of Litter + Urea on N₂O emissions surpassed the effect of the sole application of litter and urea, demonstrating a critical interaction effect between both substrates. This interaction effect needs to be considered when developing management strategies aimed at increasing soil C in tropical soils.

Keywords Nitrous oxide (N₂O) · Leaf litter/ residues · Mango · Fruit tree crops/orchard · Emission factor · Tropical savannah

Abbreviations
C/N Carbon/Nitrogen
CEC Cation exchange capacity
DOC Dissolved organic carbon
Introduction

Tropical soils account for an estimated two-thirds of the global nitrous oxide (N\textsubscript{2}O) production (Bai et al. 2012), a potent greenhouse gas (GHG) responsible for increased global warming (IPCC 2007) and stratospheric ozone layer depletion (Ravishankara et al. 2009). Microbial nitrification and denitrification are known to be the main production pathways of N\textsubscript{2}O, driven by alterations in mineral nitrogen (N), moisture and oxygen (O\textsubscript{2}) concentrations (Friedl et al. 2016), and organic carbon (C) availability (Huang et al. 2004) in soil. The rapid mineralisation of organic N and C, year-round high temperatures, and wet soil conditions associated with tropical soils have resulted in significant emissions of N\textsubscript{2}O being reported from humid tropical rainforests (Kiese et al. 2003; Rowlings et al. 2012), fertilised pastures (Veldkamp et al. 1998), and cultivated soils (Hatano et al. 2016; Liyanage et al. 2020).

Limited data exists, however, on N\textsubscript{2}O emissions from highly weathered agricultural soils such as those converted from tropical savannas. These soils have been reported as extremely low emitters of N\textsubscript{2}O (< 70 g N\textsubscript{2}O-N ha\textsuperscript{-1} y\textsuperscript{-1}) in their natural or unfertilised state due to their extremely low fertility and N levels (Grover et al. 2012). The temporal variability of N\textsubscript{2}O emissions from these systems can be strongly seasonal, high N\textsubscript{2}O fluxes in the wet season, and generally low emissions throughout the dry season (Rees et al. 2006). Soils typical of savanna agroecosystems, such as those found in northern Australia, are characterized by low cation exchange capacity (CEC). The low CEC tropical soils offer little chemical and physical protection to the soil organic matter (Zech et al. 1997), undergoing rapid mineralisation. The high C and N turnover are promoted by high rainfall and consistently high temperatures, leading to rapid loss of both dissolved organic N and mineral N (Matson et al. 1999) from the soil. Reported losses of N\textsubscript{2}O from these systems are small but can represent a significant loss of available N from an inherently infertile system (Rees et al. 2006).

In the tropical regions of Australia, perennial tree fruit orchards have been established on savanna soils. Despite the climatic and soil constraints, the horticultural industries are indicative of the political and demographic push for agricultural intensification in the tropics, both in Australia and globally. Mango (Mangifera indica L.), a high-value fruit is native to the south to South-East Asia and broadly cultivated in the tropical and sub-tropical world as a profitable horticultural industry (FAOSTAT 2019). The global yield from mangoes (collectively with mangosteens and guavas) has doubled, and the total production area expanded ~ 58% in the last two decades (FAOSTAT 2019). Fertiliser in mangoes is annually top-dressed on per tree basis during different growth stages based on canopy size and age, where rates widely vary between 200 and 1500 g N tree\textsuperscript{-1} (El-Motaium et al. 2019; Keshava Murthy and Kotur 1999), resulting in highly localised N around the tree canopies equivalent to 83–625 kg N ha\textsuperscript{-1}. A significant portion of applied N translocates into the leaves, with substantial amounts returning to the under-canopy soil area naturally from leaf and flower senescence (Murovhi et al. 2012), and through end-of-season pruning (Davenport 2006). The combined application of leaf litter and synthetic N fertiliser has been promoted as an effective N management strategy in tropical agriculture (Palm et al. 2001). Benefits include an increase in CEC and the supply of a slow-release nutrient source for N recycling and soil organic matter formation. Changes in nutrient availability, however, denote also a risk for tropical orchards to become a potential hot-spot for N\textsubscript{2}O emissions (Gu et al. 2019) by overcoming potential C limitations (Liyanage et al. 2020) under climatic conditions favouring N\textsubscript{2}O production.

The breakdown of leaf litter releases organic C and N, which is further mineralised to NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} providing substrate for N\textsubscript{2}O production. The processes and degrees of control that the availability of C and N have over N\textsubscript{2}O production in tropical systems are not well understood (Nobre et al. 2001), particularly under the combined applications of synthetic N fertiliser and residues as added C and N sources (Sarkodie-Addo et al. 2003). Contrasting results of adding residue and fertiliser were found across land-uses, showing a
positive interaction effect (Frimpong and Baggs 2010), but also a negative interactive effect on N$_2$O emissions compared with unamended controls (Gen tile et al. 2008). The reduction of N$_2$O emissions by adding residues has mainly been attributed to the C/N ratio of applied residues, as microbial N immobilization limits N availability in soil (Huang et al. 2004).

Few studies have investigated N$_2$O emissions from orchards in the tropics (Gu et al. 2019), despite the intensification of horticulture in the region. Furthermore, the specifics of leaf litter decomposition from orchard trees and the respective nutrient release, as well the unique climatic conditions demand in situ measurements of N$_2$O to establish the impact of leaf litter, N fertilizer and their interaction on N$_2$O emissions from mango orchards in the tropics.

This field-based study monitored annual N$_2$O emissions from two intensively managed mango orchards located in northern Australia with a single and combined application of mango leaf litter and synthetic N fertilizer as added C and N sources. The main objectives of this field study were to investigate (1) the effect of single applications of leaf litter mulch and synthetic N fertilizer on extractable soil N and C in highly weathered tropical orchard soils, (2) how N$_2$O emissions respond to the application of synthetic N fertilizer and leaf litter supplied labile N and C, and (3) the timing of N$_2$O losses in response to fertilizer N application in the tropical soils.

Methods and materials

Experimental site

The experiment was conducted on two experimental mango orchards managed by the Northern Territory Department of Primary Industry and Resources: at Katherine Research Station (KRS), 320 km south of Darwin, and Coastal Plains Research Station (CPRS), 60 km east of Darwin to cover the inland and coastal climate, respectively. The horticultural sector of this region is diverse and has been the focus for future intensive agriculture in northern Australia. The orchards inside KRS and CPRS are extended over the area of 1260 and 140-hectare facility, respectively. The climate of both sites is typical of the wet-dry tropics, where a majority of rainfall (> 90%) occurs between December and March. The soils at both sites were classified as red kandosols (Ise�l 2002), characterized as non-calcareous, deep sandy loams with lack of strong texture contrast and weakly structured B horizons, and similar to Lixisol in the WRB classification (IUSS Working Group WRB 2015). The major soil and climate characteristics of the sites are presented in Table 1. The 12-month experimental period commenced in early December 2017 and covered the summer wet season (December to March), the dry season (April to mid-October), and concluded at the end of the dry–wet transition period in December 2018. Micro-sprinkler irrigation (3.8 mm d$^{-1}$) was applied at both sites, three times every week between the dry and dry–wet transition period at CPRS, and total 19 times over the dry season at KRS.

Experimental design and treatment application

The experiment was arranged as a 2 $\times$ 2 factorial randomized complete block design (n = 3) using mango leaf litter and granular urea at both sites. Treatment combinations of Litter-Only, Urea-Only, Litter + Urea, and Control (zero litter, zero urea) were randomly assigned to the plots. Total tree canopies covered 40% and 50% of the orchard area at CPRS and KRS, respectively, with naturalized buffalo grass (Stenotaphrum secundatum) between the rows. Mature leaves (henceforth referred to as litter) for each orchard were handpicked from a mango tree in a single batch, air-dried, and well mixed. Before application, six litter subsamples (375 g) were oven-dried at 60 °C for 48 h, ground (< 1 mm) and analyzed for initial total C (44.5%±0.5) and N (1.3%±0.0) with an isotope ratio mass spectrometer (EA-IRMS, Sercon Limited, UK).

Twelve trees per site represented 12 plots, located over 3 rows (10 m apart), each having a canopy area of 24 m$^2$. The existing surface litter layer (> 5 mm) was removed, and two 50 cm by 50 cm steel frames were inserted 10 cm deep into the soil at each plot, one serving as a base for gas sampling (chamber plots) and one for soil sampling during the experiment. Litter dry matter was applied under the canopy area of respective tree at 1500 g m$^{-2}$ (equivalent to 190 kg N ha$^{-1}$ y$^{-1}$ for the under-canopy area only), accounting for end-of-season pruning (mechanised severe pruning of the lateral branches from tree crown) and tree’s annual natural leaf abscission. Urea granules were broadcast applied to the canopy area of the respective tree as a
single dose of 25 g N m\(^{-2}\) (equivalent to 250 kg N ha\(^{-1}\) y\(^{-1}\) for the under-canopy area only), the rate at the upper end of farmer’s practice in this region. Treatments were weighed at above rates according to the chamber area (0.25 m\(^2\)) and applied inside the frames of the respective plots in the second week of December following final fruit harvest and end-of-season tree pruning, with both sites receiving the same amount of leaf litter and urea. All plots were irrigated for 30 min immediately after treatment application to prevent urea volatilization losses.

N\(_2\)O flux measurement

The methods for field N\(_2\)O flux measurements using the high resolution, fully automated monitoring system utilized in this study have been described previously in detail in Scheer et al. (2014). Briefly, static closed chambers with the same dimensions (0.5 m \(\times\) 0.5 m, height 0.15 m) were utilized at both sites, with chamber closure and gas sampling conducted using an automated chamber system at KRS, and manually sampled at CPRS. The 12 chambers per site were mounted permanently on top of one set of steel bases in each plot. The chamber tops were made of stainless steel and transparent acrylic sides and lids fitted with reflective foam insulation to limit temperature increase during closure times.

Automated chamber system: During a measurement cycle, one set of four chambers was closed for 60 min, and a 300 ml headspace sample pumped sequentially from each chamber followed by a known calibration standard (\(\approx 500\) ppb for N\(_2\)O). Drawn samples were injected into the carrier stream of an in situ gas chromatograph (SRI 8610C, USA) equipped with a \(^{63}\)Ni electron capture detector (ECD) to measure N\(_2\)O concentrations. The sampling was repeated at 15-min intervals giving four headspace concentrations per event, then repeated with two additional chamber sets, allowing each of the 12 chambers to be open 2 h out of three, producing eight fluxes per chamber per day. The detection limit of the system calculated for N\(_2\)O was \(\pm 5\) \(\mu\)g N\(_2\)O-N m\(^{-2}\) h\(^{-1}\).

Manual chamber system: Gas samples (20 ml) at CPRS were extracted manually at 0, 20, 40, and 60 min after chamber closure by connecting a syringe (25 ml) to a plastic 2-way Luer-lock tap attached to the chamber’s lid (Rowlings et al. 2013). The drawn gas
was transferred into pre-evacuated Exetainer® vial (12 ml) double waded by Teflon/silicon septa cap (Labco, UK). Gas samples were analyzed by using GC (Shimadzu GC-2014) for linearity of gas diffusion inside the chamber’s headspace during the closure period. Manual gas samples were taken weekly (5 December 2017–23 January 2018), fortnightly (6 February 2018–29 May 2018; 9 October 2018–4 December 2018) and monthly (28 June 2018–18 September 2018) to record the seasonal flux dynamics after treatment application. On each sampling day, the measurements were performed between 09:00 am and 11:00 am (Rowlings et al. 2013).

Emission calculation, upscaling and emission factors

For both chamber systems, flux rates of N\textsubscript{2}O were calculated by the slope derived from a linear increase in headspace concentrations during the chamber closure period. Fluxes above the detection limit were discarded if the coefficient of determination ($r^2$) was < 0.81. Daily flux rates were corrected for air temperature, atmospheric pressure, and chamber volume and expressed as mg N\textsubscript{2}O-N m\textsuperscript{-2} d\textsuperscript{-1} (Scheer et al. 2014). Cumulative N\textsubscript{2}O emissions for the measurement period at KRS were calculated by linear interpolation between daily fluxes. Cumulative emissions were only calculated for the first 78 days at CPRS due to external influences on emissions post this period. Since measurements were not carried out across unfertilised inter-rows, the average daily and cumulative N\textsubscript{2}O emissions reported in this paper represent the fertilised tree canopy area unless otherwise stated.

The emission factors (EF) defined as N\textsubscript{2}O-N emissions per unit N input were estimated using N\textsubscript{2}O emissions from tree canopy area at KRS from the applications of litter only (EF\textsubscript{L}), urea only (EF\textsubscript{U}), and their combined application (EF\textsubscript{L+U}) using the following Eqs. (1, 2, 3):

$$EF_L(\%) = \left[\frac{\left(\sum N_2O_{N_L} - \sum N_2O_{N_C}\right)}{N_L} \right] \times 100$$

(1)

$$EF_U(\%) = \left[\frac{\left(\sum N_2O_{N_U} - \sum N_2O_{N_C}\right)}{N_U} \right] \times 100$$

(2)

$$EF_{L+U}(\%) = \left[\frac{\left(\sum N_2O_{N_{L+U}} - \sum N_2O_{N_C}\right)}{N_U} \right] \times 100$$

(3)

where $\Sigma N_2O_{N_L}$, $\Sigma N_2O_{N_U}$, $\Sigma N_2O_{N_{L+U}}$, and $\Sigma N_2O_{N_C}$ are cumulative N\textsubscript{2}O-N (mg m\textsuperscript{-2} y\textsuperscript{-1}) emitted from Litter-Only, Urea-Only, Litter + Urea and untreated Control, respectively. Furthermore, $N_L$ and $N_U$ denote N input from the litter and urea (mg N m\textsuperscript{-2} y\textsuperscript{-1}), respectively.

The orchard scale N\textsubscript{2}O emissions were estimated by adjusting emissions from respective treatments on an area-weighted basis accounting for both fertilised canopy (50%) and unfertilised inter-rows (50%) of the entire orchard area (Rowlings et al. 2013). The annual N\textsubscript{2}O emissions from the unfertilised inter-rows were assumed to be equivalent to the natural tropical savanna forest (0.02 kg N\textsubscript{2}O-N ha\textsuperscript{-1} y\textsuperscript{-1}) (Grover et al. 2012), the dominant land use in this region.

Soil sampling and analysis

Soil mineral N and DOC-DON measurements of the topsoil (0–0.15 m) were undertaken weekly for the first month following treatment application, fortnightly during the late wet season, and monthly over the dry season. For the mineral N extraction (NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+}), 20 g homogenously mixed unsieved field-moist soil and 2 M KCl (1:5 soil:extractant ratio) were mixed, rotated for an hour in a shaker (200 rev min\textsuperscript{-1}), filtered through Whatman no. 42 filter paper and analyzed colorimetrically by a Gallery\textsuperscript{TM} Discrete Analyzer (Thermo Fisher Scientific).

Soil DOC-DON was extracted by mixing 10 g of fresh soil with 50 mL of deionized (DI) water. The solution was rotated for an hour, centrifuged (2717 × g, 30 min, 4 °C), filtered through a 0.45 µm membrane filter, and analyzed by Shimadzu TOC-TN analyzer (Shimadzu Corporation, Kyoto, Japan). DON was calculated as the difference between total N and total inorganic N (NO\textsubscript{3}\textsuperscript{-} + NH\textsubscript{4}\textsuperscript{+}) (Filep and Rékási 2011). Gravimetric water content was determined by oven drying fresh soil sub-samples at 105 °C for 24 h.
105 °C for 24 h, and both soil mineral N and DOC-
DON were expressed in mg kg\(^{-1}\) dry soil.

**Auxiliary measurements**

The air temperature, soil moisture, and soil temperature dynamics were recorded during the experiment at both sites. Data loggers (Onset, HOBO) were employed to measure the air temperature inside the manual chambers at CPRS and canopy soil temperature (0.1 m soil depth) across sites. At KRS, the chamber air temperature was recorded using a PT100 probe (Temperature Controls Pty, Australia) connected to the automated GHG monitoring system. Moisture dynamics in the soil profile (down to 0.7 m canopy soil) close to the chambers across sites were assessed through Frequency Domain Reflectometers (FDR, EnviroScan probes, Sentek Sensor Technologies, Australia) and corrected against bulk density measured from paired soil cores collected from each depth to calculate the water-filled pore-space (WFPS) (Friedl et al. 2016).

**Statistical analysis**

The statistical software R (R Core Team 2019) and SigmaPlot Version 13.0, respectively were used to perform all the statistical analyses and graphical presentations of the data. The treatment effect on daily \(\text{N}_2\text{O}\) fluxes, soil variables (\(\text{NO}_3^-\), \(\text{NH}_4^+\), DOC, DON), and cumulative \(\text{N}_2\text{O}\) emissions were assessed using linear mixed-effects models with the “nlme” package after confirming the normality of the data (Pinheiro et al. 2009). For the daily \(\text{N}_2\text{O}\) fluxes and soil variables, it was included “day” nested with “rep” to account for the repeated measurement matrix. The existence of autocorrelation was not observed in the repeated measurements of daily \(\text{N}_2\text{O}\) fluxes and soil variables. Post-hoc differences between treatments were assessed using Tukey’s honest significant difference (HSD) test with the “multcomp” package (Hothorn et al. 2014). All values (numerical, tabulated and graphical) represent means ± standard error of the mean.

**Results**

**Site-specific environmental factors**

In the two months before treatment application, KRS received 210 mm of rainfall while CPRS received 218 mm. The rainfall for the entire 12-month measurement period totalled 968 mm and 1589 mm for KRS and CPRS, respectively. At KRS, over 115 mm of rain fell within the first 12 days following treatment application, including 76 mm in the first 3 days (Fig. 1). Rainfall over this same period totalled just 56 mm at CPRS, with the highest fall of 24 mm occurring 1 day after treatment application (Fig. 2). CPRS further received 1099 mm rain in January and February combined, including one heavy storm exceeding 214 mm on 28th of January. This resulted in a prolonged period of high soil water content at CPRS, with the soil becoming saturated for 4, 11 and 26 days at 0.1, 0.4 and 0.7 m soil depths, respectively, with soil WFPS rising to their maximum at 96–100% across layers. At KRS, several intense rainfall events (≥ 50 mm) led to rapid but short-lived increases in soil water content in the surface layers (Fig. 1). Soil WFPS decreased at both sites into the dry season with irrigation having little or no impact on readings at 0.1 m. Over the dry season, an additional 59 mm and 384 mm of irrigation water was supplied through sprinkler irrigation at KRS and CPRS, respectively. The daily soil temperature did not vary across sites and ranged between 17.5–31.9 °C during the year.

**Seasonal and temporal variability of \(\text{N}_2\text{O}\) fluxes**

Fluxes of \(\text{N}_2\text{O}\) followed a distinct seasonal trend, characterized by high flux rates in the wet season and lowest rates throughout the dry season at both sites (Figs. 1, 2). At KRS, pulse emissions peaked in Litter + Urea, with the highest daily emission of 9.9 mg N\(_2\)O-N m\(^{-2}\) d\(^{-1}\) occurring on the fourth day following treatment application. The Urea-Only treatment showed a similar response of N\(_2\)O pulse emissions, resulting in the highest daily emission of 4.5 mg N\(_2\)O-N m\(^{-2}\) d\(^{-1}\) on the third day after treatment application. Both treatments continued to show elevated N\(_2\)O flux rates for 2 weeks, accounting for more than 60% (80.5 ± 8.7 mg N\(_2\)O-N m\(^{-2}\)) and 45% (26.2 ± 3.1 mg N\(_2\)O-N m\(^{-2}\)) of the annual N\(_2\)O lost during this period from Litter + Urea and Urea-
Fig. 1 Daily rainfall/irrigation, soil temperature (0–0.1 m), soil water-filled pore space (WFPS%), and N₂O fluxes (mg N₂O-N m⁻² d⁻¹) during a 12-month measurement from KRS, Katherine, NT, for 2017/18. Arrows indicate treatment application time and capped vertical bars represent standard error (n = 3).
Only treatments, respectively. Flux rates in Litter-Only and Control were consistently low throughout the wet season. During the dry season, pulses of N₂O were negligible in all treatments despite the irrigation. A short-lived (2 days) N₂O pulse of 1.9–6.7 mg N₂O-N m⁻² d⁻¹ was observed in all treatments after a break-of-season rainfall of 20 mm. For KRS, the average N₂O flux rate for the whole measurement period was significantly high from Litter Urea compared to the rest of the treatments and followed the order: Litter Urea [Urea-Only * Control * Litter-Only (Table 2)].

At CPRS, initial N₂O emissions peaked only in Litter + Urea, with the highest daily emission of 10.5 mg N₂O-N m⁻² d⁻¹ occurring on the fourteenth day after treatment application. An unexpected period of substantially high N₂O flux was recorded in the late wet season (13 March to 17 April 2018), coinciding with the period of soil saturation (WFPS 96–100% down to soil depth of 0.7 m), where fluxes ranged 9.3–35.0 mg N₂O-N m⁻² d⁻¹ across all treatments (Fig. 2 shaded area). During this period, no treatment effect on N₂O flux was detected. Fluxes were low throughout the dry season, with no response from rainfall during the subsequent dry–wet transition period.

For the direct comparison of treatment effects across sites, average daily N₂O fluxes from different treatments were compared between 5 December 2017 and 20 February 2018 (first 78 days), excluding the prolonged period of soil saturation at CPRS. The daily N₂O flux rates for this period were comparable across sites, with significantly higher flux rates observed in Litter + Urea compared to other treatments (Table 2). The cumulative N₂O emissions from both plus urea treatments were higher (P < 0.05) than unfertilised Control and Litter-Only at KRS while only Litter + Urea released significantly higher N₂O emissions (P < 0.001) for this period at CPRS and followed the sequence: Litter + Urea > Urea-Only ~ Control ~ Litter-Only (Table 2).
Soil NO$_3^-$ concentrations in both Litter + Urea (98.5 ± 6.9 mg N kg$^{-1}$ soil) and Urea-Only (90.5 ± 8.2 mg N kg$^{-1}$ soil) treatments peaked one week after treatment application at KRS (Fig. 3a), however, the concentrations peaked in the second and fifth week in Litter + Urea (61.4 ± 16.2 mg N kg$^{-1}$ soil) and Urea-Only (38.5 ± 4.3 mg N kg$^{-1}$ soil) respectively at CPRS before declining rapidly (Fig. 3e). Initial NO$_3^-$ concentrations in Litter-Only were low compared to both plus urea treatments, ranged between 1–9 and 20–30 mg N kg$^{-1}$ soil at CPRS and KRS, respectively. Soil NO$_3^-$ concentrations declined gradually in Litter + Urea and Urea-Only over the rest of the wet season and were comparable to the Control and Litter-Only over the dry season at both sites. At KRS, soil NH$_4^+$ concentrations were extremely low compared to NO$_3^-$, ranging from 3.1 to 17.8 mg N kg$^{-1}$ soil throughout the measurement period (Fig. 3b). In contrast at CPRS, concentrations of NH$_4^+$ > 150 mg N kg$^{-1}$ soil were recorded one week after treatment application in Litter + Urea and Urea-Only, which decreased rapidly over the wet season and remained low similar to the rest of the treatments in the dry season (Fig. 3f). During the dry–wet transition period, soil NO$_3^-$ and NH$_4^+$ concentrations were still low in all the treatments at both sites. During the first 78 days after treatment application, the Litter-Only showed lower soil NO$_3^-$ concentrations than the Litter + Urea and Urea-Only treatments ($P < 0.05$) at both field sites. No differences were observed between Litter + Urea and Urea-Only treatments and between Litter-Only and Control.

Soil DOC concentrations were not significantly different among treatments despite an initial increase in the first week at CPRS and the fourth week at KRS in both Litter + Urea and Urea-Only ($P > 0.05$) (Fig. 3c, g). Soil DON concentrations increased within a week of treatment application in all the treatments except Control at both sites (Fig. 3d, h). DON concentrations were more than two times higher at KRS than CPRS and decreased gradually over the rest of the wet season and remained low thereafter. At CPRS, the DON concentrations decreased sharply in N applied treatments within four weeks of treatment applications and remained low until the end of the experiment.

Cumulative N$_2$O emissions and emission factors

Annual N$_2$O emissions from the canopy area varied between 35.4 and 130.4 mg N$_2$O-N m$^{-2}$ y$^{-1}$ with

| Site | Treatment     | Average daily N$_2$O flux (mg N$_2$O-N m$^{-2}$ d$^{-1}$) | Cumulative N$_2$O emission (mg N$_2$O-N m$^{-2}$ y$^{-1}$) | EF (%) |
|------|---------------|----------------------------------------------------------|-------------------------------------------------------------|--------|
|      | 78 days       | * Annual                                                 | 78 days                                                     | * Annual |
| KRS  | Control       | 0.24 ± 0.05$^{a b}$                                      | 0.11 ± 0.02$^{b}$                                          | 18.35 ± 3.68$^{c}$ | 38.86 ± 7.09$^{b}$ |
|      | Litter-only   | 0.16 ± 0.03$^{c}$                                      | 0.10 ± 0.03$^{b}$                                          | 12.38 ± 2.54$^{c}$ | 35.39 ± 10.37$^{b}$ | < 0.01 ± 0.07$^{b}$ |
|      | Urea-only     | 0.51 ± 0.05$^{b}$                                      | 0.17 ± 0.01$^{b}$                                          | 39.58 ± 4.11$^{b}$ | 58.69 ± 3.29$^{b}$ | 0.08 ± 0.04$^{b}$ |
|      | Litter + Urea 1.32 ± 0.13$^{a}$ | 0.37 ± 0.04$^{b}$                                      | 103.20 ± 9.78$^{a}$                                       | 130.40 ± 14.37$^{a}$ | 0.37 ± 0.08$^{a}$ |
| CPRS | Control       | 0.18 ± 0.06$^{b}$                                      | –                                                          | 16.43 ± 6.98$^{b}$ | – |
|      | Litter-only   | 0.13 ± 0.08$^{b}$                                      | –                                                          | 11.01 ± 6.53$^{b}$ | – |
|      | Urea-only     | 0.41 ± 0.08$^{b}$                                      | –                                                          | 28.01 ± 4.98$^{b}$ | – |
|      | Litter + Urea 1.47 ± 0.21$^{a}$ | –                                                          | 121.0 ± 17.76$^{A}$                                      | – |

The emission factor (EF) from the application of litter only (EF$_L$), urea only (EF$_U$) and their combined application (EF$_{L+U}$) reported for KRS represent canopy area only. Different superscript letters within a site and column for respective measurement indicate significant differences at $P < 0.05$

*Annual averages and total emissions were not calculated for CPRS

#Accounting for the fertiliser applied N only

Mineral N and DOC-DON dynamics in soil
emissions from Litter + Urea (P < 0.01) significantly higher than Urea-Only, Litter-Only and Control at KRS (Fig. 4, Table 2). This resulted in EFs ranging between < 0.01 ± 0.07% and 0.37 ± 0.08% of the total N input applied through litter or urea at KRS (Table 2). Since the overall effect of Litter-Only was not significant, EF L + U accounts for the fertiliser-N only precluding the amount of applied litter-N. A combined application of litter and urea increased the annual emissions by ~268% compared to the untreated Control. Sole application of urea increased (~ 64%) and litter decreased (~ 4%) the annual N₂O emissions compared to Control, however, the magnitude of the emissions were not significantly different than Control. In summary, the annual N₂O emissions generally followed the sequence of Litter + Urea > Urea-Only ~ Control ~ Litter-Only (Table 2). Accounting for the unfertilised inter-row area, the annual N₂O emissions for the whole orchard (tree canopy + inter-rows) ranged between 0.19 and 0.66 kg N₂O-N ha⁻¹ y⁻¹ at KRS (Fig. S1).

**Discussion**

This study provides critical information on N₂O emissions upon sole or in-combined application of mango leaf litter mulch and urea as added C and N sources from highly weathered, seasonally "wet-dry" tropical savanna soils of northern Australia. The combined application of litter and urea increased N₂O emission by up to 1000% compared to the untreated Control across sites within the first 78 days after treatment application. Importantly, emissions from the Litter + Urea treatment exceeded those from Urea-Only by up to 350%, while emissions from Litter-Only showed reduced N₂O emissions across sites during the same period, although did not differ from the untreated Control. Based on these results, we postulate that in tropical soils, N₂O production is limited by N availability when no N fertiliser is applied, even with leaf litter as a large N input. In the N fertilised soils, however, N₂O production appears to be limited by the lack of available C without the addition of organic matter.
of litter. The combined effect of Litter + Urea exceeded the sum of the single application of litter and urea, demonstrating a critical interaction between litter and N fertiliser resulting in the highest production of N\textsubscript{2}O from Litter + Urea across sites.

**Temporal variability of N\textsubscript{2}O fluxes**

In this study, significant pulses of N\textsubscript{2}O occurred across both sites in the wet season, followed by very low fluxes for the remainder of the year (Figs. 1, 2). The distinct seasonality of soil N\textsubscript{2}O emissions recorded in this study is consistent with previous studies carried out under tropical conditions, characterized by high fluxes in the wet season and lowest rates throughout the dry season (Kiese et al. 2003; Rees et al. 2006), linked to the variation in soil moisture and mineral N concentrations. Initial peak N\textsubscript{2}O emissions were detected when KRS and CPRS received 77 mm (within 4 days, including a single 66 mm rain event) and 56 mm (within 14 days of < 10 mm per event) rain, respectively. This shows that the initial pulses of N\textsubscript{2}O at both sites were triggered by heavy frequent rain immediately after treatment application. The highest emissions were observed in both plus urea treatments, exhibiting typical “post-fertilisation” N\textsubscript{2}O pulses (Crill et al. 2000) within the first 2 weeks after treatment application, and eventually decreasing back to soils “background” level. More than 60% of annual total N\textsubscript{2}O emissions lost in the first 2 weeks identifies this period as a critical window after N fertilisation for N\textsubscript{2}O emissions from these tropical orchard soils.

During this period of high N\textsubscript{2}O emissions, WFPS fluctuated between 60–80% and 50–60% across soil layers (down to 0.4 m) at KRS and CPRS, respectively. This indicates denitrification as the main pathway of N\textsubscript{2}O production during the wet season (Friedl et al. 2016), subject to N and C substrate availability (Davidson et al. 2000). This relationship was not confirmed across sites (except during break-of-season rainfall at KRS) in response to the dry season irrigation and subsequent wet season rainfall, where WFPS increased occasionally (> 60%) in the top 0.1 m reflecting lack of substrate to trigger denitrification in seasonal tropical soils (Werner et al. 2006). The high emissions of N\textsubscript{2}O in the initial period after treatment application coincides with high soil mineral N levels, particularly in the plus urea treatments, denoting high N substrate availability for N\textsubscript{2}O production. Despite heavy rain across sites, a large proportion of soil mineral N (> 50 mg N kg\textsuperscript{-1} soil) was still detected for the first four weeks, although decreasing over time. The mineral N retention in the soil, in particular in the form of NO\textsubscript{3}\textsuperscript{−} for an extended period is noteworthy, as leaching is generally presumed to be a significant N loss pathway immediately after heavy rainfall for these low CEC sandy soils of northern Australia (Wetselaar 1962). Similar to
mineral N, DON peaked in the first four weeks, decreasing thereafter. During the initial period of N$_2$O pulses, low concentrations of DOC indicate rapid microbial C consumption, as C becomes limiting when soil N is high (Aber 1992). The process of consumption and production may take place at the same time; therefore, the net effect is often difficult to quantify under both field or the laboratory conditions (Filep and Réka´si 2011). The low concentrations of soil NH$_4^+$ and concurrent high NO$_3^-$ concentrations at KRS throughout the study indicate either increased microbial nitrification or NH$_4^+$ immobilization. This relationship was not observed at CPRS, where both soil mineral N pools exhibited peak concentration after treatment application. The difference between sites points towards differences in microbial activity: Trees were matured, and canopy size was bigger at KRS compared to CPRS, suggesting a rhizosphere effect on stimulation of microbial activity due to a larger root system (Brzostek et al. 2013) or a larger and closed tree canopy area sheltering the soils and maintaining soil moisture (Skopp et al. 1990).

Break-of-season rain induced another pulse of N$_2$O emissions at KRS, which is consistent with previous reports from northern Australian savanna soils (Grover et al. 2012; Werner et al. 2014). The observed N$_2$O pulses were short-lived and can be explained by the cascade of N transformations associated with the wetting of dry soil (Kim et al. 2012). The magnitude of N$_2$O pulses in this study is higher (1.94–6.69 mg N$_2$O-N m$^{-2}$ d$^{-1}$) than the previously reported values (up to 0.6 mg N$_2$O-N m$^{-2}$ d$^{-1}$) from the northern Australian savanna soils (Grover et al. 2012), likely caused by the high annual N input in mango orchards through N fertilisation and historic litter nutrient inputs. Break-of-season rain induced N$_2$O emissions accounted for up to 17% of annual emissions, highlighting their significance for site-specific N$_2$O budgets.

The prolonged elevated N$_2$O pulses from CPRS following the large January/February rain events (Fig. 2 shaded area) were unexpected regarding their magnitude and the lack of treatment effect. These N$_2$O emissions were not directly associated with the earlier large rain events and occurred despite the low-intensity rain and low soil mineral N during the late wet season. The prolonged saturation of the deep soil profile (Fig. 2) suggests a groundwater table rise. The majority of N$_2$O fluxes, including those from the untreated Control may have been produced in the interface between saturated and unsaturated sub-soil layers. The lack of treatment response to N inputs further implies external sources of N (Reay et al. 2009), likely transported via groundwater movement following off-farm nutrient runoff and/or leaching (Well and Butterbach-Bahl 2010). Soil water contents and N availability suggest these N$_2$O fluxes are a result of indirect emissions, precluding the quantification of an annual EF from CPRS. The source of this N is not clear. CPRS is located on the > 2600 km$^2$ broad coastal flood plain of the Adelaide River and while intensive tree crops and horticulture are rapidly expanding in the region, the vast majority of the catchment is savanna or unfertilised pastures. Given the sparse distribution of intensive agriculture and large dilution (> 1500 mm rainfall over 5 months), the potential for elevated groundwater nitrate concentrations in this region is concerning and requires further investigation. These findings show that a rising groundwater table may lead to overestimates of N input related N$_2$O production in these soils and show the importance of nutrient transfer between farms for indirect N$_2$O emissions.

Influence of carbon and nitrogen addition

Previous studies have demonstrated increased N$_2$O emissions following manipulations of both throughfall and leaf litterfall to the soils under natural conditions (Wieder et al. 2011), and after crop residue application in agricultural soils (Velthof et al. 2002). However, such a positive effect of applying leaf litter on N$_2$O emissions was not evident in this study. In the first 78 days, Litter-Only reduced N$_2$O emissions by 26% at KRS and had no significant effect on N$_2$O emissions at CPRS compared to the untreated Control. The relatively low soil NO$_3^-$ concentrations in the Litter-Only treatment demonstrates N limitation for N$_2$O production despite the application of equivalent to 190 kg N ha$^{-1}$ through the litter. The application of mango leaves with a C/N ratio of ~ 35 is likely to promote microbial N immobilization, reducing excess mineral N in the soil matrix, subsequently reducing N$_2$O production from soils (Huang et al. 2004).

Urea-Only increased N$_2$O emissions by > 130% compared to the control at KRS over the first 78 days. This increase in N$_2$O emissions together with the high initial NO$_3^-$ levels shows the effect of added N substrate availability for N$_2$O production: Increased
NO$_3^-$ can provide more substrate for denitrification, and/or shift the N$_2$:N$_2$O ratio towards N$_2$O (Friedl et al. 2020), both resulting in higher N$_2$O emissions. However, this increase was only minor, and although it followed a similar trend at CRPS and in the annual emissions at KRS, the impact of Urea-Only was not significant, implying a limitation for N$_2$O production. Emitted N$_2$O from Litter + Urea increased compared to the sum of either input alone, showing a positive interactive effect on emissions, in line with previous studies (Abalos et al. 2013; Frimpong and Baggs 2010) from other agro-ecosystems. During the first 78 days, the interaction between litter and urea increased N$_2$O emissions compared to the untreated Control by $>500\%$ and $>1000\%$ at KRS and CPRS, respectively. This effect was confirmed at KRS over the whole experimental period, even though the increase was smaller with $>260\%$ (Fig. 4; Table 2). Comparing the drivers of N$_2$O production for Urea-Only and Litter + Urea suggests a C limitation in the Urea-Only treatment, despite the lack of differences in DOC upon single and combined application of litter and urea (Fig. 3c, g). The low CEC sandy soils in the tropics offer little protection towards the immobilisation or fixation of NH$_4^+$ and soil organic C (Zech et al. 1997), and any C mineralised from the litter, together with N from urea, would become rapidly available in the soil resulting into enhanced N$_2$O emissions from Litter + Urea treatment (Liyanage et al. 2020).

Sole versus combined application of litter and urea and the respective soil mineral N levels support the hypothesis of C limitation in the Urea-Only treatment: Soil NO$_3^-$ concentrations in Urea-Only and Litter + Urea did not differ during the period of high N$_2$O emissions (Fig. 3a), suggesting N was not limiting for N$_2$O production in the Urea-Only treatment. The high N$_2$O emissions from Litter + Urea were not associated with the litter N (Fig. 1), as low mineral N and N$_2$O emissions from treatment Litter-Only suggest a high litter C/N ratio ($\sim35$) promoting immobilization of N, and thus a reduction of N substrate availability for N$_2$O production. Soil mineral N values do not show any added effect of the urea-N on mineralisation of litter in the Litter + Urea treatment, further pointing towards a C limitation for N$_2$O production in the Urea Only treatment. These findings point towards greater C availability in the Litter + Urea treatment, where (1) labile C provides an energy source to denitrifiers, and (2) promotes microbial respiration, creating oxygen (O$_2$) deficiency in soil (Sarkodie-Addo et al. 2003) conducive for N$_2$O production. Leaf litter is known to supply DOC as a readily available C source for N$_2$O production (Wieder et al. 2011). The temporal variation of DOC levels and the lack of differences between treatments likely shows that in these highly weathered tropical soils, labile C inputs are either rapidly consumed or lost in response to high rainfall and temperature. Linking nutrient release from leaf litter, microbial respiration and N$_2$O emissions in further studies could further constrain the impact of substrate availability on N$_2$O emissions in response to leaf litter management. The proposed C limitation under non-limiting N availability is further supported by increased N$_2$O emissions when applying compost (Liyanage et al. 2020) or residues (Huang et al. 2004), highlighting that potential benefits of leaf litter retention need to be compared against increases in GHG emissions for improved farming practice in tropical mango orchards.

Cumulative N$_2$O emissions and emission factor

The annual N$_2$O emissions under the combined application of synthetic N and litter mulch were significantly higher compared to the sum of either input alone from these tropical mango orchard soils. The annual N$_2$O emission of 130.4 mg N$_2$O-N m$^{-2}$ y$^{-1}$ ($\sim1.3$ kg N$_2$O-N ha$^{-1}$) from Litter + Urea reported in this study is lower than 200 mg N$_2$O-N m$^{-2}$ y$^{-1}$ found from custard apple cultivation under synthetic N and sugarcane straw mulch in sub-tropical Australia (Huang et al. 2012), and far below 170–760 mg N$_2$O-N m$^{-2}$ y$^{-1}$ reported from subtropical lychees (Rowlings et al. 2013). The annual N$_2$O emissions of 58.7 mg N$_2$O-N m$^{-2}$ y$^{-1}$ ($\sim0.6$ kg N$_2$O-N ha$^{-1}$) from Urea-Only treatment is substantially lower than 580 mg N$_2$O-N m$^{-2}$ y$^{-1}$ reported from the N-fertilised (250 kg N ha$^{-1}$ y$^{-1}$) shaded coffee plantation in Costa Rica (Hergoualc'h et al. 2008), and similar to 60 mg N$_2$O-N m$^{-2}$ y$^{-1}$ recorded from an irrigated almond orchard in a semi-arid Mediterranean climate (Alsina et al. 2013). The treatment Litter-Only emitted 35.4 mg N$_2$O-N m$^{-2}$ y$^{-1}$ ($\sim0.4$ kg N$_2$O-N ha$^{-1}$), values substantially lower compared to the range of 400–1226 mg N$_2$O-N m$^{-2}$ y$^{-1}$ reported for litter mulched soils under longan cultivation in China (Liu et al. 2008).
Under the application of litter and urea, the estimated EFs (< 0.01 to 0.37%) was far lower than the IPCC’s Tier 1 default EF of 1% (IPCC 2007) for fertiliser and crop residue N, which overestimated emissions from N fertiliser by > 2 times and from crop residues > 100 times. The EFs in this study are substantially lower than the large EF (~ 2% of the applied N) reported from tropical fruit orchards in a meta-analysis (Gu et al. 2019) and less than twice as much as reported country-specific (Tier 2) EF of 0.85% for horticulture in Australia (Commonwealth of Australia 2017), with the values more similar to the 0.0–0.24% reported for subtropical coffee plantations in Australia (Rose et al. 2019). The low EFs reflects low fertility of these highly weathered low CEC savanna soils, where a very short residence time of labile C and N is likely to limit substrate for N₂O production, even after a combined application of leaf litter and N fertiliser.

Conclusions

The initial pulse of N₂O emissions identifies the period after N fertilisation as a critical time window for N₂O emissions from these tropical orchard soils, when high substrate availability promotes N₂O emissions, subsequently decreasing over the wet season. The contribution of N₂O pulse emissions following break-of-season rainfall highlights their importance for annual N₂O emissions from tropical orchard soils. The findings suggest that in tropical soils, N₂O production is limited by N availability when no N fertiliser is applied, even with large N input in the form of leaf litter. In the N fertilised soils, however, N₂O production appears to be limited by the lack of available C in the absence of litter. The demonstrated positive interaction between leaf litter and N fertiliser suggests that litter C overcomes this limitation, promoting N₂O emissions by either stimulating microbial activity in general, and/or providing an energy source for denitrifiers. The magnitude of N₂O losses suggests N₂O emissions as an overall minor loss from tropical orchard soils, pointing towards leaching and runoff as the main pathways of N loss from these systems. However, high potential indirect N₂O losses from groundwater transport of off-farm nutrients can overestimate the farm-scale N₂O budgets and requires further investigation. The leaf litter/fertiliser interaction, together with the rapid loss of N needs to be considered while developing litter and fertiliser management strategies aimed at increasing soil C and reducing N₂O emissions from tropical orchard soils. Targeted long-term studies linking nutrient release from leaf litter, microbial respiration, and N₂O emissions could help understand the impact of substrate availability on N₂O emissions in response to leaf litter management in highly weathered tropical soils.

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