

QUANTIFYING THE EFFECT OF FERTILISER APPLICATION ON NITROUS OXIDE PULSES IN OIL PALM PLANTATION

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ABSTRACT

The environmental impacts of oil palm production systems have raised global concerns due to the large quantities of greenhouse gases released (including nitrous oxide, N₂O) into the atmosphere. To understand the effects and the response to the nitrogen fertiliser application on N₂O flux for mitigation planning, emissions were monitored intensively over the course of two field campaigns from 2015 to 2016. The study was conducted in an oil palm plantation cultivated on peat in Sarawak, Malaysia. N₂O flux was highly temporally variable, with large increases in N₂O flux following fertiliser application. The mean (± standard error) of N₂O flux from the first and second sampling campaigns were 129.000 ± 9 mg N m⁻² d⁻¹ and 1.021 ± 150 mg N m⁻² d⁻¹, respectively. The magnitude of the N₂O flux and the timing of the emissions peak varied for different sampling campaigns, reaching a maximum of 384.000 ± 77 mg N m⁻² d⁻¹ 30-days after the first fertiliser application event and 3.777 ± 777 mg N m⁻² d⁻¹ 7-days after the second application. During fertilisation events, environmental variables played only a weak role in modulating N₂O fluxes in this ecosystem, suggesting that N₂O flux was principally determined by fertiliser input rates.

Keywords: fertiliser, nitrous oxide, oil palm, peatland.

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INTRODUCTION

Oil palm ecosystems are globally significant environments as they take up approximately 19 million hectares of agricultural land worldwide (Murphy *et al.*, 2021). The palm oil industry has emerged as a significant player in the global food industry, driven by the remarkable versatility and high productivity of oil palm, it accounts for a substantial portion, approximately one-third, of the world's total production of vegetable oils and fats (Kushairi *et al.*, 2018). This prominence underscores

the pivotal role that palm oil plays in meeting the escalating demand for edible oils worldwide. As the availability of suitable mineral soil sites has declined in recent years, the oil palm industry has expanded cultivation into peatlands, especially in Southeast Asia, specifically Malaysia and Indonesia.

Tropical peatlands extend over an area of 25 million hectares in Southeast Asia and are estimated to contain 11%-14% (68.5 Gt) of the global peat carbon (C) reservoir (Page *et al.*, 2011). Population growth, industrialisation and economic expansion have accelerated land use change across the tropics, with heightened demand for food and natural resources encouraging small and large farmers in Southeast Asia to expand into tropical peatlands over the past two decades. For example, Miettinen *et al.* (2016) reported that both commercial and smallholder oil palm plantations cover almost 50% (7.8 million hectares) of peatlands area in insular Southeast Asia. However, cultivation of peatlands has led to negative ecological, hydrological and atmospheric impacts, which national governments, industry bodies and civil society groups now aim

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to curb or reverse through sustainability measures such as national “No Peat” conversion policies, national and industry certification schemes that prohibit growers from developing new plantations on peatland (e.g., Malaysian Sustainable Palm Oil scheme, Indonesian Sustainable Palm Oil certification scheme, Roundtable for Sustainable Palm Oil) (Carlson *et al.*, 2018; Sumarga and Hein, 2016). In addition, research has been intensified to find more climate and environmental-friendly approaches to manage existing peatland plantations, in order to reduce or mitigate the effects of past peatland conversion on greenhouse gas emissions and regional flood risk (Manning *et al.*, 2019; McCalmont *et al.*, 2021).

In order to achieve optimum plant growth and high yields, managed tropical peatlands need to be drained, which leads to enhanced decomposition of peat, compaction of soil and subsequent land subsidence (McCalmont *et al.*, 2021). Drainage often leads to enhanced carbon dioxide (CO₂) emissions to the atmosphere due to aerobic conditions in the upper peat profile and the use of fertilisers that can accelerate peat oxidation via microbial priming effects (Hirano *et al.*, 2014; Hooijer *et al.*, 2015; Jauhiainen *et al.*, 2014; McCalmont *et al.*, 2021; Sakata *et al.*, 2015). What is less well established are the impacts of drainage and fertilisation on nitrogen (N) cycling and nitrous oxide (N₂O) flux. Theoretically, these two management interventions could greatly enhance the potential of managed tropical peatlands to emit N₂O by alleviating constraints on N₂O flux imposed by redox and N substrate availability, respectively (Davidson *et al.*, 2000). These predictions are supported by evidence from managed temperate or boreal peatlands, where N₂O emissions range from 0.1 to 37.0 kg N ha⁻¹ yr⁻¹, and can exceed emissions from agricultural systems on mineral soils (Klemedtsson *et al.*, 2005; Maljanen *et al.*, 2003; Regina *et al.*, 2004). In these higher latitude systems, this increase in N₂O emissions is attributable to the mineralisation of fertiliser N combined with accelerated rates of soil organic N mineralisation (Klemedtsson *et al.*, 2005; Maljanen *et al.*, 2003; Regina *et al.*, 2004; Schrier A-Uijl *et al.*, 2013; Stichnothe and Schuchardt, 2011). However, measurements of N₂O emissions from managed tropical peatlands are fewer than those for the temperate or boreal zone (Skiba *et al.*, 2020) and show a higher degree of variability; literature estimates a range from -1.1 to 259.0 kg N ha⁻¹ yr⁻¹ (Hadi *et al.*, 2005; Inubushi *et al.*, 2003; Melling *et al.*, 2007; Takakai *et al.*, 2006; Terry *et al.*, 1981). Critically, we have relatively little data on N₂O emissions from tropical peatlands converted to oil palm (Skiba *et al.*, 2020), making it difficult to evaluate the wider regional and global consequences of oil palm production on the global N₂O budget. There is a critical shortage of information on

the temporal variability of N₂O fluxes within oil palm plantations, making it difficult to accurately predict annual emission rates (Skiba *et al.*, 2020). For example, Melling *et al.* (2007) estimated N₂O emission in the Malaysian oil palm plantation was at 566.0 kg CO₂-eq ha⁻¹ yr⁻¹; however, uncertainties were large, and data were too limited either to distinguish background emissions from event-based emissions due to fertiliser application.

This study addresses these knowledge gaps by focusing on the temporal variability in N₂O fluxes from oil palm planted on peat soils in Sarawak, Malaysia. A critical component of this study is a detailed examination of the temporal dynamics of N₂O flux after fertiliser application. Considering the substantial increases in N₂O fluxes typically observed after fertilisation, neglecting these events may result in significant underestimations of the emission potential, impacting the accurate estimation of annual N₂O budgets in these systems, as highlighted by previous studies (Skiba *et al.*, 2020; Groffman *et al.*, 2009). Our hypothesis anticipates that N₂O emissions are strongly influenced by temporal heterogeneity and would increase significantly after N fertiliser application.

MATERIALS AND METHODS

Site Description

The study was conducted in an oil palm plantation located in tropical peatland near Bintulu, Sarawak, Malaysia (3° 12.691' N, 113° 30.008' E) planted with *Elaeis guineensis* Jacq. (oil palm) in 2007. Soils are classified as histosols on deep peat, reaching a depth of 3 to 4 m (Manning *et al.*, 2019; McCalmont *et al.*, 2021). Mean annual precipitation is approximately 3200 mm yr⁻¹. The northeast monsoon from October to January has the highest rainfall, with a slightly drier southwest monsoon occurring between May and August while the two principal dry seasons fall between February to April and in September. The mean annual temperature is approximately 26.5°C (Manning *et al.*, 2019; McCalmont *et al.*, 2021).

Sampling Design and Fertiliser Application Method

This experiment was designed to quantify the temporal response of soil N₂O flux to fertiliser application. A spatially stratified sampling design was used to account for spatial heterogeneity in the landscape, with flux data collected from landscape management units (hereafter referred to as “microforms”). Sampled microforms included: Field drains (FD), frond piles (FP), harvest paths (HP),

inter-row/avenues (IR) and vegetation paths (VP). These microforms are regular repeating landscape components managed for a specific purpose and experience different levels of disturbance (Figure 1). FD are small drains in each planting block, used to drain water from the soil profile to a collecting drain; FP are the locations where the pruned fronds are stacked on the ground for nutrient recycling; HP are 50-75 cm wide area, present on every other palm row and completely free of weeds to allow access for workers; IR are lines of palms parallel to harvest paths and VP are pathways leading to the bushy vegetation.

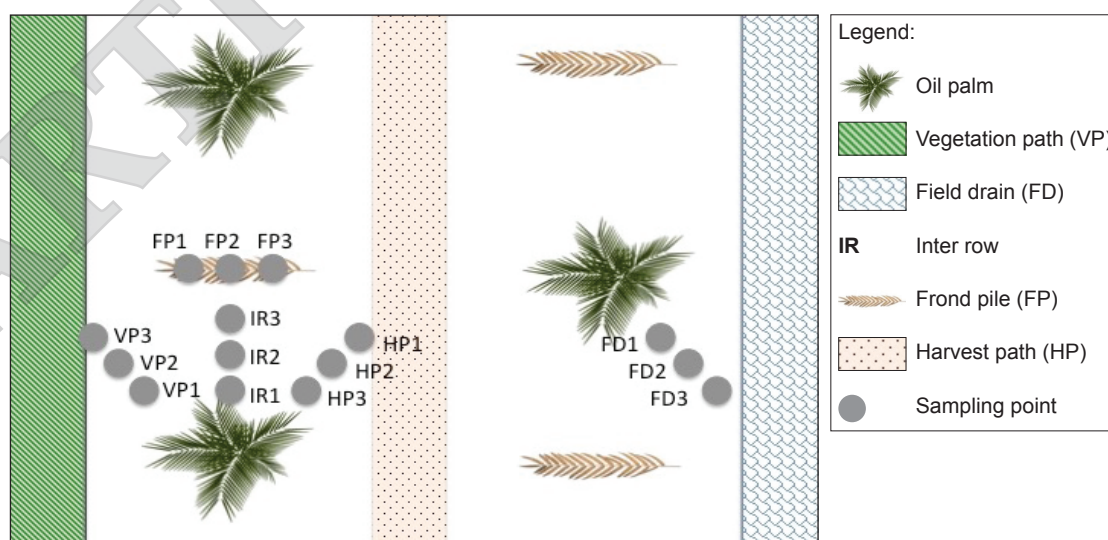
Urea was used as the N input and was applied by the broadcasting method, ensuring homogenous distribution within the circular footprint ($r = 0.0505$ m) of each flux chamber, split into two even applications over the course of the year, mimicking the estate practice. The first application of 0.925 kg urea m^{-2} was made on 8 August 2015 (first campaign), with gas fluxes quantified over 212 days until 15 March 2016. The second application of 0.925 kg urea m^{-2} was made on 26 April 2016 and gas fluxes were quantified for 121 days until 24 August 2016 (second campaign), thus completing a whole annual cycle of measurements.

Soil Flux Measurements

Nitrous oxide (N_2O) flux was measured using a chamber-based methodology with permanently installed static flux chambers (Parkin and Venterea, 2010). High-density polyvinyl chloride (PVC) soil collars (10.1 cm diameter and 10.0 cm high) were installed at least 5.0 cm into the ground and extended no more than 5.0 cm above the surface at 15 different sampling points surrounding the palm.

Any herbaceous vegetation was cut and removed before the collars were pushed into the soil, although root in-growth inside the collars from the surrounding palms was permitted. Gas chambers with dimensions of 10.1 cm in diameter and 16.0 cm high were placed directly on the soil collars to measure gas fluxes.

For the first field campaign, flux chambers were sampled in all five microforms and at three different distances from palms (0.5, 1.0 and 1.5 m) over a period of 212 days (*i.e.*, $n = 3$ distances \times 5 microforms per distance \times 3 replicates per distance = 45 replicates). N_2O flux was quantified from August 2015 to March 2016, at 12 time points; one day prior to fertiliser application (representing the pre-fertilised condition), 1, 3, 7, 14, 30, 60, 91, 121, 152, 182, and 212 after fertiliser application. For the second field campaign, the experimental design was simplified by sampling from flux chambers only 1.5 m from each palm, rather than for all three distances from the palm over a period of 121 days (*i.e.*, $n = 1$ distance \times 5 microforms per distance \times 3 replicates per distance = 15 replicates). N_2O flux was quantified from April 2016 to August 2016, at ten time points; one day prior to fertiliser application (representing the pre-fertilised condition), 1, 3, 5, 7, 14, 30, 60, 91 and 121 after fertiliser application. The reduced duration of the second field campaign was informed by the findings from the first field campaign, which indicated that a shorter sampling period was sufficient to capture the spike in N_2O flux linked to fertiliser application. We were also forced to use different flux sampling techniques because of logistical reasons and the breakdown of our instantaneous gas analyser (INNOVA 1412; INNOVA Air Tech Instruments, Denmark) that



Note: Distance between palm/drain and each sampling points are 0.5 m.

Figure 1. The layout of each microform where N_2O fluxes were measured.

was used during the first field campaign, which meant that we were forced to adopt a more labour-intensive flux sampling technique using Exetainers (Labco Ltd., UK) and gas chromatography (GC) to collect and analyse gas samples for the second field campaign. Gas concentrations were quantified using either a portable photo-acoustic spectrophotometer (INNOVA 1412; INNOVA Air Tech Instruments, Denmark) for the first campaign or by gas chromatography (Agilent 6890, Santa Clara, USA) for the second campaign. For the photo-acoustic method, concentration measurements were collected for each chamber over a period of 3 min, with at least five concentration measurements collected over that enclosure period. A Genie Membrane Separator (Lumasense Technologies, Denmark) were used, connected externally in-line, to remove water droplets and water vapour from the sample gas, so as to prevent known interferences for water vapour in our measurements (Iqbal *et al.*, 2013; Rosenstock *et al.*, 2013). For the GC method, gas samples were collected using a polypropylene syringe at 0, 10, 20 and 30 min intervals and stored in 20 mL evacuated Exetainers (Laboco Ltd., UK), sealed with 20 mm chlorobutyl rubber/PTFE septa. Samples were analysed at the University of Aberdeen using an Agilent 6890 GC equipped with an electron capture detector (ECD) for N₂O determination and a methaniser with a flame ionisation detector (FID) for CO₂ determination. Analytes were separated on a Haysep Q column at 45°C, with the sample volume injected by an autosampler (HT29748, HTA, Italy). The GC was calibrated for CO₂ and N₂O using chemical standard of known concentrations.

Measurement of Environmental Parameters

The volumetric soil water content (θ_v) was measured by a portable moisture meter (HH2 Moisture Meter, Delta T Devices, Cambridge) and sampled close to the chamber (within 1.0-5.0 cm). Concomitant with flux measurement, soil temperature, for the top 12.5 cm soil depth, and air temperature were measured using a probe T-bar thermometer (ATP Instrumentation Ltd, Leicestershire). The depth of the water table was collected from 2.0 m piezometers that were installed in each plot.

For climate monitoring of the area, a rain gauge was installed, and daily rainfall and air temperature were measured. The water-filled pore space (WFPS) was calculated from the soil volumetric moisture measurements and using bulk density values obtained by other members of our research group (Manning *et al.*, 2019; McCalmont *et al.*, 2021).

Soil samples were taken at 10 cm depth twice, at the beginning and the end of the research study. The soil was homogenised, air-dried, ground, and

passed through a 2 mm sieve prior to laboratory analysis. The soil was analysed for soil pH, and total C and N. Several attempts were made to sample soil water for inorganic N analysis, but these were unsuccessful because termites destroyed our soil sampling apparatus (*i.e.*, mini-rhizons).

Statistical Analysis

Linear mixed-effects models were utilised to analyse the relationship between key independent variables (*e.g.*, campaign, time since fertilisation, microform) and N₂O flux. Multiple regression was used to explore the relationship between environmental variables (*e.g.*, soil temperature (°C), air temperature (°C), water table depth (m), WFPS, soil N, soil C, pH and N₂O flux). Data were tested for heteroscedasticity and homogeneity of variances and transformed where necessary to meet the assumptions of the parametric tests. Wherever treatments were found to be significant, pairwise testing with a t-test or means comparison test (*e.g.*, Tukey-Kramer HSD) was performed among the main and subplot treatments due to its effectiveness in comparing multiple treatment means while controlling the familywise error rate, providing a robust statistical approach to identify significant differences between groups following an analysis of variance (ANOVA) test. All statistical analysis was performed using JMP version 15 (SAS Institute, Inc., Cary, North Carolina, USA)

RESULTS AND DISCUSSION

The response of N₂O flux to fertilisation events was analysed using a linear mixed effects model that used campaign, time since fertilisation, and microform as fixed effects, while the plot was treated as a random effect. The instantaneous or cumulative N₂O flux was the dependent variable.

Effects of Fertiliser Application on Nitrous Oxide Flux Over Time

Campaign time since fertilisation and microform significantly influenced instantaneous and cumulative N₂O flux ($p < 0.0001$). Instantaneous and cumulative N₂O flux was both significantly lower for the first campaign as compared to the second one ($p < 0.0001$). The mean instantaneous flux was 129.0 ± 9 and 1021.0 ± 150 mg N m⁻² d⁻¹ for the first and second campaigns, respectively. This represents an increase of approximately 1.9 and 15 times, respectively, relative to the pre-fertilisation flux rate of 68.0 ± 71 mg N m⁻² d⁻¹. The mean cumulative flux, calculated over 121 days, was 14.9 ± 0.4 and 76.4 ± 0.7 g N m⁻² for the first and second campaigns, respectively.

Instantaneous and cumulative N_2O flux increased significantly over time after fertiliser application for both campaigns ($p < 0.001$; Figure 2, Figure 3). However, the temporal patterns for instantaneous and cumulative fluxes differed for the two campaigns. For the instantaneous flux, peaks in emissions differed in time for the two campaigns. In the first campaign, instantaneous flux increased gradually, reaching a maximum 30 days after fertiliser application that was almost six times higher than the pre-fertilisation flux rate (*i.e.*, $384 \pm 77 \text{ mg N m}^{-2} \text{ d}^{-1}$ *vs.* pre-fertilisation flux of $68 \pm 71 \text{ mg N m}^{-2} \text{ d}^{-1}$; Tukey-Kramer HSD, $p < 0.05$; Figure 2). Instantaneous flux declined gradually after this period only to rise again to reach a second maximum 91 days after fertiliser application ($285 \pm 52 \text{ mg N m}^{-2} \text{ d}^{-1}$; Tukey-Kramer HSD, $p < 0.05$; Figure 2). In contrast, instantaneous flux showed only one maximum in the second campaign, rising rapidly after fertiliser application to reach a peak 7 days after fertiliser application that was 56 times higher than pre-fertilisation flux rates (*i.e.*, $3777 \pm 777 \text{ mg N m}^{-2} \text{ d}^{-1}$ *vs.* pre-fertilisation flux of $68 \pm 71 \text{ mg N m}^{-2} \text{ d}^{-1}$; Tukey-Kramer HSD, $p < 0.05$; Figure 2). Instantaneous flux oscillated after this point but did not show another statistically significant increase (Tukey-Kramer HSD, $P > 0.05$). This temporal response is generally consistent with other studies of oil palm plantations on peat, where enhanced emissions are typically observed within the first month after fertiliser application (Chaddy *et al.*, 2019; Oktarita *et al.*, 2017; Sakata *et al.*, 2015).

N_2O flux was at the higher end as compared to other managed tropical peatlands in Southeast Asia, where post-fertilisation flux rates typically range from 0.02 to 235.00 $\text{mg N m}^{-2} \text{ d}^{-1}$ (Groffman *et al.*, 2009; Hadi *et al.*, 2005; Inubushi *et al.*, 2003; Melling *et al.*, 2007; Oktarita *et al.*, 2017; Sakata *et al.*, 2015; Takakai *et al.*, 2006; Toma *et al.*, 2011). This difference could have several possible explanations. One possibility is that fertiliser application rates for other studies were lower than in this experiment, leading to a proportionately lower N_2O flux (Groffman *et al.*, 2009). For example, Chaddy *et al.* (2019) applied fertiliser at a rate of 0 to 124.3 kg N ha^{-1} , Melling *et al.* (2007) at a rate of 103.0 kg N ha^{-1} and Oktarita *et al.* (2017) at a rate of 21.0 to 103.0 kg N ha^{-1} . Another potential reason is that fertiliser applied was substantially higher amount within the experimental plots as compared to other investigators due to differences in the underlying assumptions used to calculate experimental fertiliser application rates. To briefly explain, even though 158.36 kg N is applied for every 1 ha of land in the plantation, this amount of fertiliser is only applied to areas that contain palms. This means that the soil containing palms receives a substantially higher amount of fertiliser

(*i.e.*, 8510.00 $\text{kg N ha}^{-1} \text{ yr}^{-1}$) as compared to palm-free soil, which does not receive fertiliser. Here we mimicked the industry practice, applying fertiliser to the footprint of our flux chambers at the higher input rate. If other investigators used plantation-scale application rates (*e.g.*, 158.36 kg N ha^{-1}) rather than the application rate for individual palms (*e.g.*, 8510.00 $\text{kg N ha}^{-1} \text{ yr}^{-1}$), then differences in flux rates between our study and theirs could be explained by differences in N input (Groffman *et al.*, 2009). It is difficult, however, to ascertain from prior publications precisely how much fertiliser was added to experimental treatments because these details are often absent from the text (Chaddy *et al.*, 2019; Oktarita *et al.*, 2017; Sakata *et al.*, 2015). A third possibility is that the majority of the published studies, with the exception of Oktarita *et al.* (2017), did not explicitly aim to track pulses in N_2O flux linked to N fertiliser application, and consequently sampled at a lower frequency than was necessary to identify emission peaks (Chaddy *et al.*, 2019; Melling *et al.*, 2007; Oktarita *et al.*, 2017; Sakata *et al.*, 2015; Skiba *et al.*, 2020). As a consequence, these other studies may probably have underestimated the emissions from their study sites (Groffman *et al.*, 2009; Skiba *et al.*, 2020).

Given the significant effects of microforms, separate temporal analyses were conducted with disaggregated data to determine if individual microforms diverged from these general patterns. However, it was found that temporal trends for individual microforms from the palm were broadly consistent with the patterns found for the pooled data.

For the cumulative flux data, the two campaigns also showed different patterns of change over time (Figure 3). The first campaign showed a gradual and relatively linear increase over 121 days of observation (Figure 3) with 50% of total emissions occurring after ~51 days after fertiliser application. In contrast, the second campaign showed an asymptotic increase, rising rapidly over the first 20 days after fertilisation, before shifting towards a slower rate of increase for the remainder of the observation period (Figure 3). Half of (50%) to total emissions occurred in ~24 days after fertiliser application; *i.e.*, twice as quickly compared to the first campaign.

Another interesting feature of the data is that the timing and magnitude of N_2O flux differed for the two field campaigns, suggesting that patterns of soil microbial activity varied for each campaign. The slower, more gradual increase in N_2O flux (*i.e.*, over 30 days) for the first field campaign, combined with a lower mean and peak flux, suggests that microorganisms took longer to access fertiliser N pools and that N_2O production was more substrate-limited. In contrast, the more rapid rise in N_2O flux (*i.e.*, 7 days) for the second field campaign, combined with a significantly higher mean and peak flux,

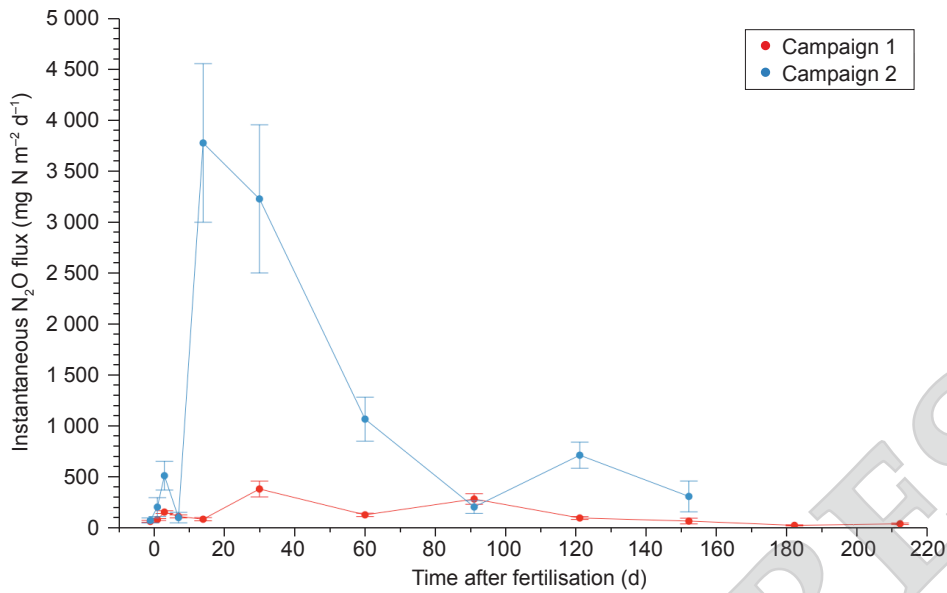


Figure 2. Instantaneous N₂O flux for the first (red) and second (blue) field campaigns. Bars indicate standard errors.

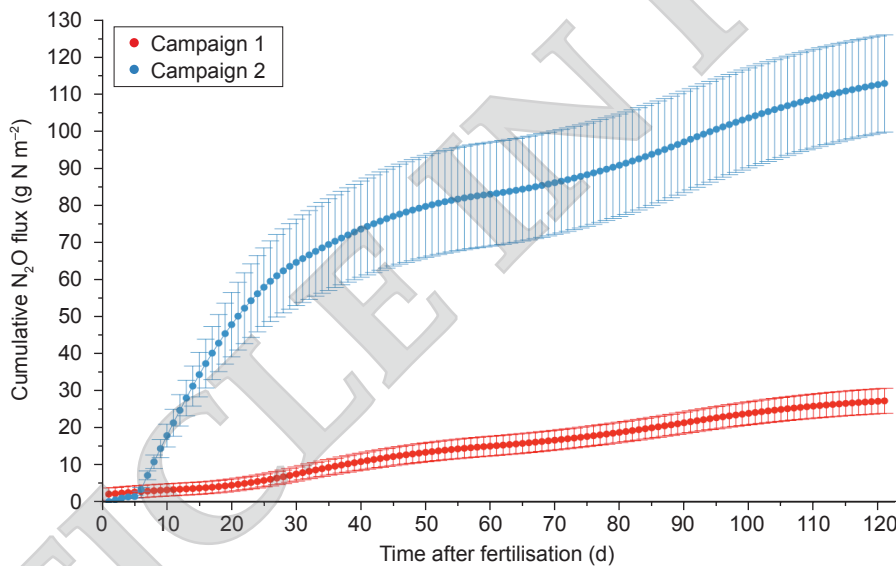


Figure 3. Cumulative N₂O flux for the first (red) and second (blue) field campaigns. Bars indicate standard errors.

suggests that microorganisms were able to access fertiliser N more rapidly and were less substrate-limited. This high variability among individual fertilisation events appears consistent with other studies (Chaddy *et al.*, 2019; Oktarita *et al.*, 2017; Sakata *et al.*, 2015), although it is unclear what causes variability among individual fertilisation events. One explanation is that differences in plant N demand could have influenced substrate availability for N₂O production between campaigns. For example, greater competition by palms for N during the first field campaign could have limited microbial access to N and suppressed N₂O production (Groffman *et al.*, 2009; Pardon *et al.*, 2016). If plant N demand was weaker during the second campaign, then more fertiliser N could

have been accessed by soil microorganisms. Plant N demand is also varied at different stages of oil palm growth, target yield, soil type, fertility and climate that might affect the variability. The greater competition by palms for N during the dry season compared to the wet season may be attributed to reduced water availability, diminished microbial activity, decreased decomposition rates, altered physiological responses, and intensified root exploration in response to limited nutrient accessibility.

A second explanation is that there were significant differences in other competing sinks for N between the two field campaigns, such as ammonia volatilisation, leaching or run-off (Pardon *et al.*, 2016). For example, volatilisation alone can

lead to >40% loss of the urea applied to oil palm soils; hence, changes in these processes, driven by environmental variables, could have significant consequences for plant uptake or downstream microbial processes such as denitrification (Pardon *et al.*, 2016). Alternatively, ammonium sulphate is a source of N and sulphur (S), however, it tends to decrease soil pH from oxidation of ammonium ion to nitrate and leaching of the nitrate ion (Sharon *et al.*, 2023).

In our study site, differences in rainfall between the two campaigns may have affected the relative partitioning of N between different pathways. The first field campaign took place during the dry season, whereas the second occurred in the wet season. It is possible that more N was lost via ammonia volatilisation during the first (dry season) campaign, whereas more N was processed via microbial pathways in the second (wet season) campaign, given that rainfall is known to significantly reduce rates of ammonia volatilisation (Black *et al.*, 1987; Bouwmeester *et al.*, 1985). However, rainwater can lead to the runoff of fertilisers, affecting fertiliser effectiveness through nutrient losses (Guo and Chen, 2022). Thus, optimising water and N management might be one of the most effective ways to reduce N₂O emissions from agricultural soils. Most studies have reported increased N₂O emissions after the application of N fertiliser, especially with high soil moisture. N₂O is generally emitted most rapidly when the soil has >60% water-filled-pore when the soil pore water displaces the amount of available oxygen in the soil pores and therefore leads to anaerobic soil moisture conditions, which are conducive to the production of N₂O. Under such conditions, the soil NO₃⁻ is reduced by facultative anaerobic bacteria (*e.g.*, *Pseudomonas citronellolis*) to NO₂⁻, N₂O and then N₂ (Wang *et al.*, 2021).

A third possibility is that ongoing peat decay and N mineralisation could strongly influence temporal patterns in N₂O flux by supplementing any fertiliser-derived N₂O (Toma *et al.*, 2011). While many investigators have assumed that fertiliser N inputs drive N₂O flux in managed tropical peatlands (Chaddy *et al.*, 2019; Melling *et al.*, 2007; Oktarita *et al.*, 2017; Sakata *et al.*, 2015; Skiba *et al.*, 2020). Toma *et al.* (2011) challenged this assumption when they found that there was no significant correlation between annual N₂O emission and fertiliser application rates. Instead, they found that long-term management plays a greater role in modulating N₂O flux, where management practices or the history of cultivation have altered the quality of the peat, characteristics of the soil microorganisms and subsequent rates of N₂O flux. This implies that N₂O derived from peat organic matter could play a significant role in trace gas exchange.

Total Cumulative Flux over 121 Days

Total cumulative flux, an estimate of the total amount of N₂O released over 121 days, was analysed using a linear mixed effects model using campaign, microform from palm as fixed effects, with plot treated as a random effect. Campaign and microform were significantly related to total cumulative flux ($p < 0.0001$). Total cumulative flux was significantly higher in the second campaign as compared to the first campaign, differing by a factor of four ($p < 0.001$; 112.7 ± 13.1 vs. 27.3 ± 3.4 g N m⁻², respectively). For the microforms, IR and HP showed the highest total cumulative flux, VP and FD showed intermediate total cumulative flux, and the FP showed the lowest total cumulative flux (Tukey-Kramer HSD, $p < 0.05$; Figure 4). The large N₂O emissions observed in this experiment suggest that a substantial proportion of the applied fertiliser could have been lost from the soil as N₂O. Total cumulative flux, calculated over 121 days, suggests that up to 27.3 ± 3.4 g N m⁻² of fertiliser could have been lost as N₂O during the first campaign, while up to 112.7 ± 13.1 g N m⁻² could have been lost during the second campaign. This equates to 6.4% and 26.5% of the applied fertiliser in the first and second field campaigns, respectively. These values are in the same order of magnitude as those found in comparable studies in oil palm plantations on peat soil (Chaddy *et al.*, 2019; Oktarita *et al.*, 2017; Sakata *et al.*, 2015). For example, Chaddy *et al.* (2019) found that total cumulative N₂O flux could account for as much as 33.4% to 80.7% of the applied fertiliser, while Sakata *et al.* (2015) found as much as 52.6% to 92.8% of the applied fertiliser could have been lost as N₂O. This suggests that these soils are behaving similarly to those in other studies, despite very high instantaneous flux rates.

Spatial Patterns in N₂O Flux

Linear mixed effects models indicated that microform significantly influenced instantaneous and cumulative fluxes ($p < 0.0001$). For the microform data, HP and IR showed the highest instantaneous and cumulative fluxes. VP and FD showed intermediate fluxes, and FP showed the lowest fluxes (Tukey-Kramer HSD, $p < 0.05$; Figure 5, data only shown for instantaneous flux measurements).

Significant differences in N₂O flux were observed among microforms consistent with studies of trace gas exchange in other spatially stratified peatland ecosystems (Jones *et al.*, 2018; Manning *et al.*, 2019; Teh *et al.*, 2011; 2014; 2017). With respect to the role of microforms, N₂O flux was generally higher in HP and IR microforms, and significantly lower in other microforms. It is possible that differences in WFPS among the microforms influenced how much fertiliser was converted either

to N_2O or N_2 by denitrification (Wang *et al.*, 2021). HP and IR microforms were generally drier than the others, with WFPS averaging $41 \pm 1\%$ and $53 \pm 2\%$ respectively. In contrast, WFPS in FD, FP and VP microforms were significantly higher, averaging

$65 \pm 2\%$, $78 \pm 1\%$ and $56 \pm 2\%$ WFPS, respectively. Higher N_2O flux rates from HP and IR microforms may thus reflect more incomplete denitrification, while wetter microforms may experience more complete denitrification (Wang *et al.*, 2021).

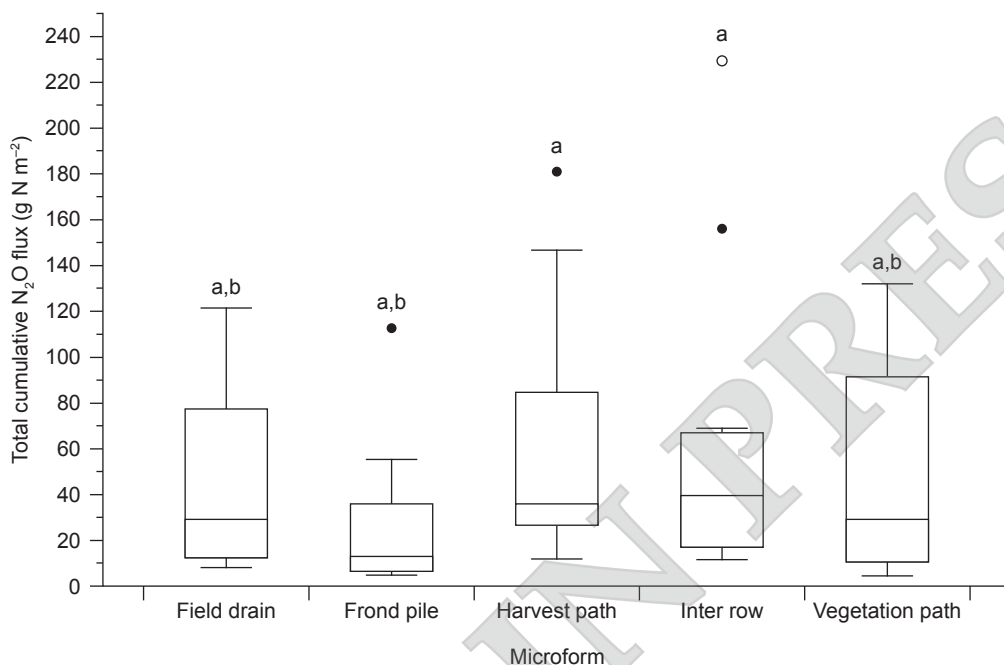


Figure 4. Total cumulative flux, calculated at 121 days, for different microforms. Data was pooled from both field campaigns. Boxes enclose the interquartile range, whiskers indicate the 90th and 10th percentiles and the solid line within each box indicates the median. Lowercase letters indicate significantly different means (Tukey-Kramer HSD, $p < 0.05$).

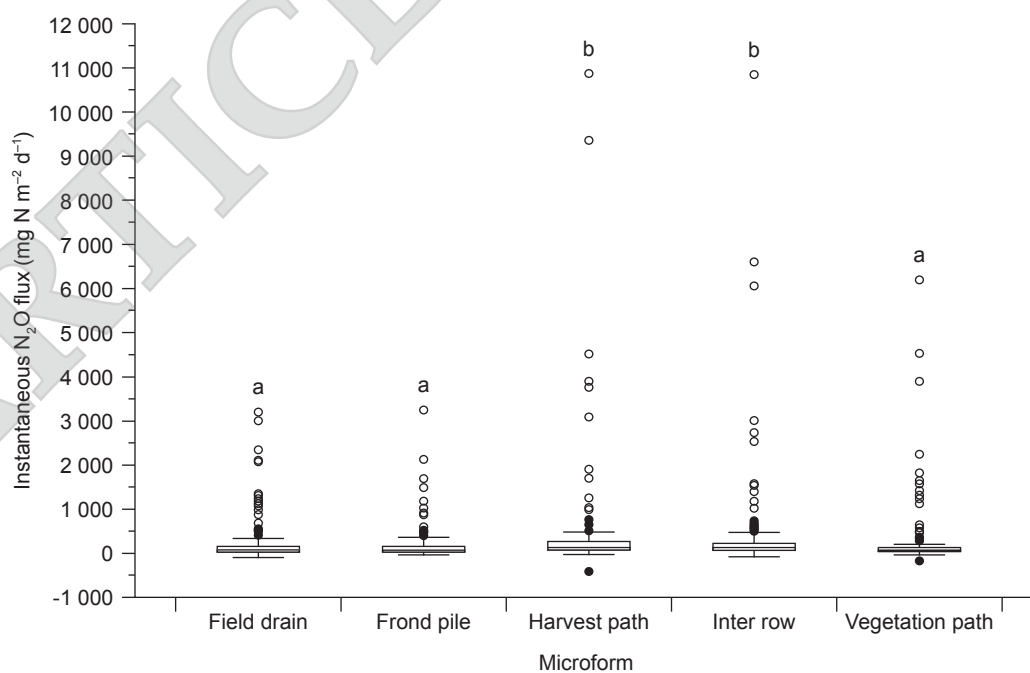


Figure 5. Instantaneous N_2O flux for different microforms. Data was pooled for both field campaigns. Boxes enclose the interquartile range, whiskers indicate the 90th and 10th percentiles and the solid line within each box indicates the median. Lowercase letters indicate significantly different means (Tukey-Kramer HSD, $p < 0.05$).

Role of Environmental Variables in Regulating N₂O Fluxes

The relationship between environmental variables (*i.e.*, water table depth, WFPS, soil temperature, air temperature, soil N, soil C and pH) and instantaneous N₂O flux was examined using multiple regression models. Two separate multiple regression analyses were conducted, one containing all the environmental variables and a second focusing only on water table depth, WFPS, soil temperature and air temperature. The second analysis was performed because soil chemical variables (*i.e.*, soil N, soil C, pH) were only measured for a subset of samples ($n = 90$), whereas soil moisture and temperature data for all flux measurements ($n = 690$). The second analysis of moisture and temperature variables would provide a more representative picture of the relationship between these drivers and N₂O flux, given the much larger sample size.

Analysis of all the environmental variables suggests that N₂O flux was negatively correlated with WFPS ($r^2 = 0.28$, $p < 0.0003$, $n = 90$), whereas the analysis of only moisture and temperature data suggests that N₂O flux was weakly predicted by water table depth (negative correlation), soil temperature (positive correlation) and air temperature (positive correlation) ($r^2 = 0.12$, $p < 0.0001$, $n = 690$).

When the data were disaggregated and data were analysed separately for each campaign, slightly different patterns emerged. For the first campaign, N₂O flux was weakly correlated with water table depth (negative correlation), WFPS (negative correlation) and air temperature (positive correlation) ($r^2 = 0.07$, $p < 0.0001$, $n = 540$). For the second campaign, analysis of the moisture and temperature data suggest that only water table depth (negative correlation) and soil temperature (positive correlation) were correlated with N₂O flux ($r^2 = 0.12$, $p < 0.0009$, $n = 150$).

In our study, patterns of N₂O emissions were affected by soil WFPS, perhaps because greater soil moisture favoured denitrification. The negative relationship between WFPS and N₂O fluxes observed in this study could be due to increasing N₂O reductase activity at higher soil moisture content, as the soils become more anaerobic. In anaerobic soil, near saturated WFPS (>60% WFPS) conditions, denitrification and nitrifier denitrification are recognised as the potential processes of N₂O consumption in soils (Wang *et al.*, 2021). Although it was negative correlation, the relationship between WFPS and N₂O fluxes in the first campaign was weak ($R = -0.2$), therefore, it is not surprising that the second fertiliser addition campaign showed no evident response. Even the occurrence of rainfall events stimulates soil N

mineralisation (Xu *et al.*, 2023; Zhang *et al.*, 2020) and wetted soils often have high carbon and N availability that is linked to high denitrification rates (Sakata *et al.*, 2015). However, a lower C:N ratio promotes increased N mineralisation and N₂O emissions (Maarit *et al.*, 2018) and soil pH has a marked effect on the products of denitrification. Denitrification rates would be slower under the strong acid conditions in the peat soils. This is commonly attributed to the sensitivity of N₂O reductase to proton activity, and it is also likely that all denitrifying enzymes are susceptible at low soil pH and produce N₂O from other intermediate products (Nägele and Conrad, 1990). Finally, numerous studies have demonstrated that the N₂O:N₂ ratio decreases with increasing pH (Žurovec *et al.*, 2021).

CONCLUSION

The high N₂O flux and N losses observed in this study site suggest that current fertiliser application rates may be higher than is necessary to sustain palm growth and yields, given that such a large proportion of fertiliser N is lost as N₂O. Further site-specific trials may be required to fine-tune fertiliser application rates for this plantation to minimise N loss and mitigate greenhouse gas emissions. Generally, adapting to better fertiliser management such as tailoring the nutrient application to crop requirements, implementing precision agriculture techniques, the use of controlled-release fertilisers, and improving soil management practices can help reduce the high N₂O flux and losses.

Large differences in N₂O flux between the first and second fertilisation events suggest major, intra-annual shifts in N dynamics that warrant further investigation, as it is unclear what proportion of the applied N is being taken up by plants or lost by other pathways such as ammonia volatilisation, nitric oxide (NO) flux, dinitrogen (N₂) flux, leaching and run-off. Future studies could exploit stable isotope tracers to more accurately track the fate of fertiliser N in these important production systems. Lastly, significant spatial heterogeneity in N₂O flux emphasises the need for more detailed, spatially explicit sampling of oil palm systems in order to accurately estimate ecosystem-scale patterns in N₂O emission and to understand the role of local management effects on N₂O flux.

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