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Low pH of a High Carbon Gleysol Contributes to Nitrification Inhibition Resulting in Low N_2O Soil Emissions and Limited Effectiveness of Nitrification Inhibitors

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Abstract: Nitrous oxide (N_2O) is a potent greenhouse gas, and drained tropical/subtropical wetland soils that are high in carbon (C) make a substantial contribution to global anthropogenic N_2O emissions. However, we previously reported negligible N_2O emissions from an acidic, C-rich Gleysol under aerobic rice (*Oryza sativa* L.) production in the subtropics despite ample moisture and fertiliser nitrogen (N). In a field experiment, seasonal cumulative N_2O emissions in the field following the application of 90 kg ha^{-1} N as urea were low (0.15 kg N_2O-N ha^{-1}·$season^{-1}$). An incubation study examining the effects of temperature (20 °C, 25 °C and 30 °C) and water-filled pore space (WFPS; 40% vs. 60%) on N transformations showed that incubation temperature had a larger influence on nitrification than WFPS (40% vs. 60%). There was limited nitrification at 20 °C at either WFPS over 30 days, but low concentrations of NO_3^- (<100 mg kg^{-1}) began to accumulate between 16–23 days at 30 °C and between 23–30 days at 25 °C. Liming soil resulted in nitrification after 10 days, while only minor nitrification was evident in the unlimed soil. The presence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) with urea delayed nitrification for up to 4 days in the limed soil, suggesting such inhibitors may not provide substantial benefits in high C soils. Our results suggest that a low soil pH contributes to impaired nitrification in the C-rich Gleysol examined, which is associated with low fluxes of N_2O in the field. We suggest that soil pH could potentially be manipulated to sustain low rates of nitrification and lower N losses, without compromising crop growth.

Keywords: greenhouse gas emissions; Hydrosol; lime; nitrogen fertiliser

1. Introduction

The global demand for fertiliser nitrogen (N) has grown in recent years from 110.03 Mt N in 2015 to a projected 118.76 Mt N in 2020 [1]. This rising demand for fertiliser N is closely related to the rising demand for food, fibre and biofuels [2]. It is, however, well recognised that N fertiliser application to soil can result in the production and loss to the atmosphere of nitrous oxide (N_2O), a greenhouse gas with a global warming potential of 300 times that of carbon dioxide (CO_2) on a 100 year timescale and that is of major importance for stratospheric ozone depletion [3]. Cropland soil N_2O emissions increased from 0.3 Tg N_2O-N $year^{-1}$ in the 1860s to 3.3 Tg N_2O-N $year^{-1}$ in the period 2007–2016 [4], driven mainly by the increasing application of N fertiliser.

Peat soils cover over 450 Mha of land worldwide, with an estimated 50.9 Mha drained for agricultural or forestry purposes [5]. In their native state, these soils emit methane (CH_4) but are a net sink for carbon dioxide (CO_2) due to their low rates of organic matter decomposition. However, when drained for agricultural purposes, peat soils become a source of both CO_2 and nitrous oxide (N_2O) and make a substantial contribution to agricultural greenhouse gas emissions [6].

While they represent only a small proportion of the total global area of peat soils, tropical peat soils are particularly vulnerable to high greenhouse gas (GHG) emissions when drained [6]. Couwenberg et al. [7] estimated the mean N_2O-N emissions from drained, fertilised peat soils in South East Asia to be approximately 90 kg N_2O-N ha^{-1} $year^{-1}$ compared to 6 kg N_2O-N ha^{-1} $year^{-1}$ in drained, fertilised peat soils in Europe. Carbon-rich wetland soils, or Gleysols [8]—referred to as Hydrosols in the Australian Soil classification System [9]—that are drained and used for agricultural production in the Australian subtropics also exhibit high N_2O emissions. For example, N_2O emissions from a Gleysol under a second ratoon sugarcane crop fertilised with 160 kg N ha^{-1} amounted to 46 kg N_2O-N ha^{-1} over a 342 day measurement period [10]. This can be compared to 4.7 kg N_2O-N ha^{-1} over a similar sampling period in sugarcane grown on a non-calcic brown soil with a similar N-fertiliser dose [10]. Similarly, Wang et al. [11] reported emissions of 28.2 N_2O-N ha^{-1} over a 343 day period from a sugarcane crop grown on a high organic C content (98 g kg^{-1}) Gleysol fertilised with 150 kg N ha^{-1}, while a paired experiment on a Lixisol [8] also fertilised with 150 kg N ha^{-1} showed emissions of 3.6 N_2O-N ha^{-1} over a 328 day period.

In contrast to these studies, we recently reported near-negligible N_2O emissions from high-C Gleysol in the Australian subtropics when cultivated with rice (*Oryza sativa* L.) fertilised with 90 kg N ha^{-1}, regardless of whether the N was supplied as urea or the product Entec™, which contains the chemical nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) with urea [12]. Whether the negligible emissions were a seasonal anomaly or were due to very low soil pH, which may have suppressed nitrification in the soil, leading to a lack of substrate for N_2O production, is not known. In-field measurements of soil nitrate (NO_3^-) in the topsoil (0–10 cm) indicated low NO_3^- concentrations in the month after fertiliser application, but interpreting these data as an indicator of inhibition of nitrification is challenging, because NO_3^- may also have been leached into the subsoil following rainfall or may have been taken up by the roots of the actively growing rice plants [12]. To better understand N dynamics in high-C content Gleysols, we quantified seasonal N_2O emissions under aerobic rice cultivation to verify our previous results [12]. We hypothesised that naturally low soil pH inhibited the conversion of ammonium (NH_4^+) to NO_3^-, thus limiting the substrate needed for denitrification and lowering soil N_2O emissions. This was supported mechanistically through the use of an incubation study in which we alleviated the low pH of the soil through the application of lime and determined soil NH_4^+ and NO_3^- concentrations.

2. Materials and Methods

2.1. Nitrogen Dose Experiment (2012–2013 Rice Season)

A field trial was established on an acidic peat soil classified as a Hydrosol in the Australian soil classification scheme [9] and a Gleysol in the FAO classification [8] near Coraki, NSW, Australia. Chemical properties of the soil were assessed using the methods of Lyons and Rayment [13], and key properties are shown in Table 1. Rice (cv. Tachiminori) was sown with commercial disc seeding equipment in December 2012 (Table 2). Small plots were established (3 m wide × 8 m long) five weeks after sowing in a randomised block design with five N fertiliser rates (0, 50, 100, 150 and 200 kg N ha^{-1} as urea) with three replicate plots per treatment to investigate aerobic rice yield responses to N fertiliser. Nitrogen (as urea, 46% N) was then weighed out on a per plot basis and broadcast by hand onto plots on 15 January 2013 prior to 10 mm of rain falling. The crop was managed as per the rest of the field with key activities listed in Table 2.

Table 1. Selected physiochemical chemical properties of the 0–100 mm, 100–300 mm, 300–600 mm and 600–900 mm horizons of the acidic Gleysol. EC: electrical conductivity.

Property	Soil Depth (mm)			
	0–100	**100–300**	**300–600**	**600–900**
Basic texture	Loam	Loam	Clay Loam	Clay
Total carbon (%)	6.78	7.40	4.48	2.06
Total nitrogen (%)	0.60	0.67	0.42	0.16
pH (1:5 water)	4.76	4.8	5.09	5.08
EC (dS m^{-1})	0.27	0.12	0.10	0.10
Bray 1 P (mg kg^{-1})	10.4	6.3	12.1	9.9
Total acid extractable sulfur (mg kg^{-1})	61.6	64.1	71.3	110
Cation exchange capacity ($cmol^{+}$ kg^{-1})	15.5	13.9	15.9	15.8
Base cations (%)				
Calcium	38.8	31.3	38.2	38.8
Magnesium	21.5	19.0	32.3	38.6
Potassium	3.8	1.3	0.8	0.7
Sodium	3.6	2.3	2.5	2.2
Aluminium	28.3	43.2	24.2	16.1
DPTA-extractable micronutrients				
Zinc (mg kg^{-1})	61	55	39	22
Manganese (mg kg^{-1})	111	55	34	33
Iron (mg kg^{-1})	17344	18854	15634	20744
Copper (mg kg^{-1})	26	23	20	15

Table 2. Crop management calendar for rice field trials in 2012–2013 and 2013–2014.

Crop Management		**2012–2013**	**2013–2014**
Previous crop		Sugarcane	Rice
Land preparation			
	Discing	22 November 2012	15 January 2014
	Power harrowing	2 December 2012	23 January 2014
Rice sown			
	Date	3 December 2012	24 January 2014
	Cultivar	Tachiminori	Langi
	Seeding rate	120 kg ha^{-1}	120 kg ha^{-1}
	Row spacing	200 mm	200 mm
Fertiliser applied			
	Broadcast nitrogen	8 January 2013	25 February 2014
Herbicides			
	480 g L^{-1} Clomazone at 600 mL ha^{-1}	3 December 2012	24 January 2014
	480 g L^{-1} Propanil at 8 L ha^{-1}		12 February 2014
Harvest		24 April 2013	15 May 2014

At harvest on 24 April 2013, the aboveground biomass was harvested to approximately 10 mm above the soil level by cutting 2 × 2 m lengths of row from two separate areas of each plot (i.e., 4 m of row in total). All samples were subsequently threshed by hand to separate grain from straw, and all tissue was then dried in an air-forced oven at 60 °C for 6 days. Grain yields were expressed at 14% moisture, and the harvest index (HI) was calculated by expressing the weight of grain as a proportion of the total aboveground biomass.

2.2. Seasonal N_2O Emissions from Acidic Gleysol Soil Following Application of N Fertiliser with and without the Nitrification Inhibitor DMPP (2013–2014 Rice Season)

An experiment was established in the same field in the subsequent rice season (2013–2014) to investigate seasonal N_2O emissions and the role of the nitrification inhibitor DMPP when used with urea-N fertiliser.

The trial was established with three N fertiliser treatments: urea, urea + DMPP and a 50:50 blend of urea and DMPP–urea (50:50 mix). The DMPP–urea was applied as the product Entec®® which contains 1.6 kg DMPP t^{-1} urea. Following the results of the first field experiment, all plots received 90 kg N ha^{-1} and the trial was established in a randomised block design with four replicates. Plots were 8 m long × 3 m wide, and the crop was sown with the same commercial disc seeding equipment as the preceding year. Details of crop management are given in Table 2. Nitrogen fertilisers were weighed out individually for each plot and were applied by hand on 25 February 2014. The soil was already moist as 15 mm rain fell in the period 22–24 February, and a further 4 mm fell in the afternoon of the 25 February after the fertiliser was applied.

A cold-weather event in March led to grain sterility, and no grain formed in any N treatment. Biomass cuts were taken from 2 × 1 m lengths of row from two separate areas of each plot (as per the previous study) in May 2014 prior to the crop being baled for hay using commercial equipment.

2.3. Soil N_2O Fluxes

Three 150 mm-diameter manual static chambers were deployed in each plot following the establishment of the field trial. Intensive sampling (minimum twice per week) followed key trigger events (fertiliser application, rainfall greater than 20 mm). Actual sampling dates are indicated in Figure 1. Samples were taken between 08:00 and 11:00 on each sampling event to minimise the diurnal variation of emissions.

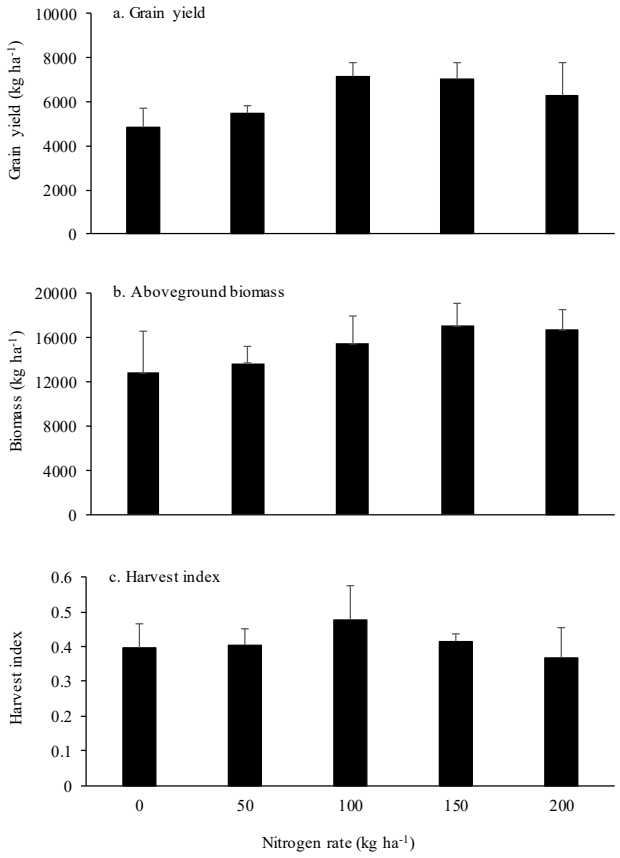

Figure 1. Rice grain yield (**a**), aboveground biomass (**b**) and harvest index (**c**) response to nitrogen fertiliser rate on an acidic Gleysol. Error bars depict SEM (n = 3).

At sampling, chambers were closed and sampled immediately (T0) and exactly 60 min later (T60). Gas samples were taken using a 25 mL gas-tight syringe (SGE, 25MDR-LL-GT) and stored in pre-evacuated 12-mL Exetainer®® vials (Labco, Lampeter, UK) as described in Van Zwieten et al. [14]. The concentration of N_2O in each sample was determined using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) in an ISO 9001 certified laboratory. Conversion of flux data to emission units was carried out as per Van Zwieten et al. [14].

2.4. Effect of Liming on Nitrification (Incubation Study)

Two closed-lid incubation experiments were established to investigate N dynamics in the C-rich Gleysol. In the first experiment, soil NH_4^+ and NO_3^- concentrations following the application of urea or DMPP–urea were investigated over time under different incubation temperatures and soil moisture contents. Soil from the above field trial was collected from the 0–10 cm horizon, oven dried and passed through a 2 mm sieve, and 10 g soil was weighed into each of 504 plastic 50 mL screw cap containers. The 504 containers comprised three replicates for each sampling time (days 2, 4, 6, 10, 15, 25 and 30 after N fertiliser addition) × fertiliser treatment (urea or urea-DMPP) × soil moisture content (40% or 60% water-filled pore space (WFPS)) × temperature treatment (20, 25 or 30 °C). Granular urea and DMPP–urea were passed through a 2 mm sieve to obtain uniformly sized granules, and single granules were placed 5 mm below the soil surface in the appropriate containers. Water was added by weight to attain either 40% or 60% WFPS. The treatment blocks were then split and placed into three separate Thermoline laboratory incubators at temperatures of 20, 25 and 30 °C. Moisture was replenished every 2 days by weight, at which point vessels were aerated by opening the lid for 30 s. At each sampling point, nominated containers were removed from the incubators and extracted with 30 mL 2 M KCl by shaking end-over-end for 1 h before being centrifuged at 3000 rpm for 10 min and filtered through a 0.45 µM syringe-filter. The extract was analysed for NH_4^+ and NO_3^- concentration by flow injection analysis (FIA).

In the second incubation experiment, the effect of liming on soil mineral N fluxes and pH following the application of urea or DMPP–urea was investigated. Lime was added to moist soil at a rate of 30g kg^{-1} in a 10 L bucket and left to incubate at room temperature for 1 month. Soil was then oven dried and passed through a 2 mm sieve. The experiment was then established as per the first incubation experiment with the exception that only one incubation temperature of 25 °C was utilised and that sampling points were extended to day 65.

Soil pH was also measured in the second incubation experiment up to day 58. At each sampling point, pH was measured on the filtered KCl extract prior to FIA analysis. Three extra containers per treatment were also established and pH was measured in the filtered KCl extract immediately after addition of the fertilisers (day 0).

2.5. Statistical Analyses and Data Presentation

Yield data from the N fertiliser response field trial and all data from incubation studies are presented as means with standard error of the mean. Mean N_2O fluxes for each fertiliser treatment in the second field experiment were plotted over time together with 95% confidence levels calculated using the package "lsmeans" [15] in the R software environment [16]. Cumulative emissions were calculated using the "auc" function from the package "MESS" [17] via linear interpolation. An ANOVA was used to determine the significance of treatment effects for cumulative emissions, and means were separated using "lsmeans".

3. Results

3.1. Response of Aerobic Rice to N Fertiliser in 2012–2013 Season

At maturity, leaves under the nil N treatment condition were visibly yellower in colour than other treatments, and no lodging was observed under any N treatment. Grain yields increased from

around 5 t ha^{-1} without N fertiliser to around 7 t ha^{-1} with 100 kg N ha^{-1} (Figure 1a). There was a trend of increasing crop biomass with N fertiliser rates up to 150 kg N ha^{-1} to a maximum of around 17 t ha^{-1}, while there was a trend of decreasing HI from 0.48 at 100 kg N ha^{-1} to 0.37 at 200 kg N ha^{-1} (Figure 1b,c).

3.2. Seasonal N_2O Emissions in the 2013–2014 Season Following Nitrogen Fertiliser with and without the Nitrification Inhibitor DMPP

A total of 508 mm of rain fell during the measurement period, with one event of >100 mm occurring on the 28 March. Fluxes of N_2O from all treatments were low overall, with no flux events exceeding 15 µg N_2O m^{-2} h^{-1} in any treatment (Figure 2). Cumulative N_2O-N emissions over the 120 day measurement period were in the order of urea (0.14 kg N_2O-N ha^{-1} season^{-1}) > DMPP–urea (0.08 kg N_2O-N ha^{-1} season^{-1}) = 50:50 mix (0.05 kg N_2O-N ha^{-1} season^{-1}) ($p < 0.05$).

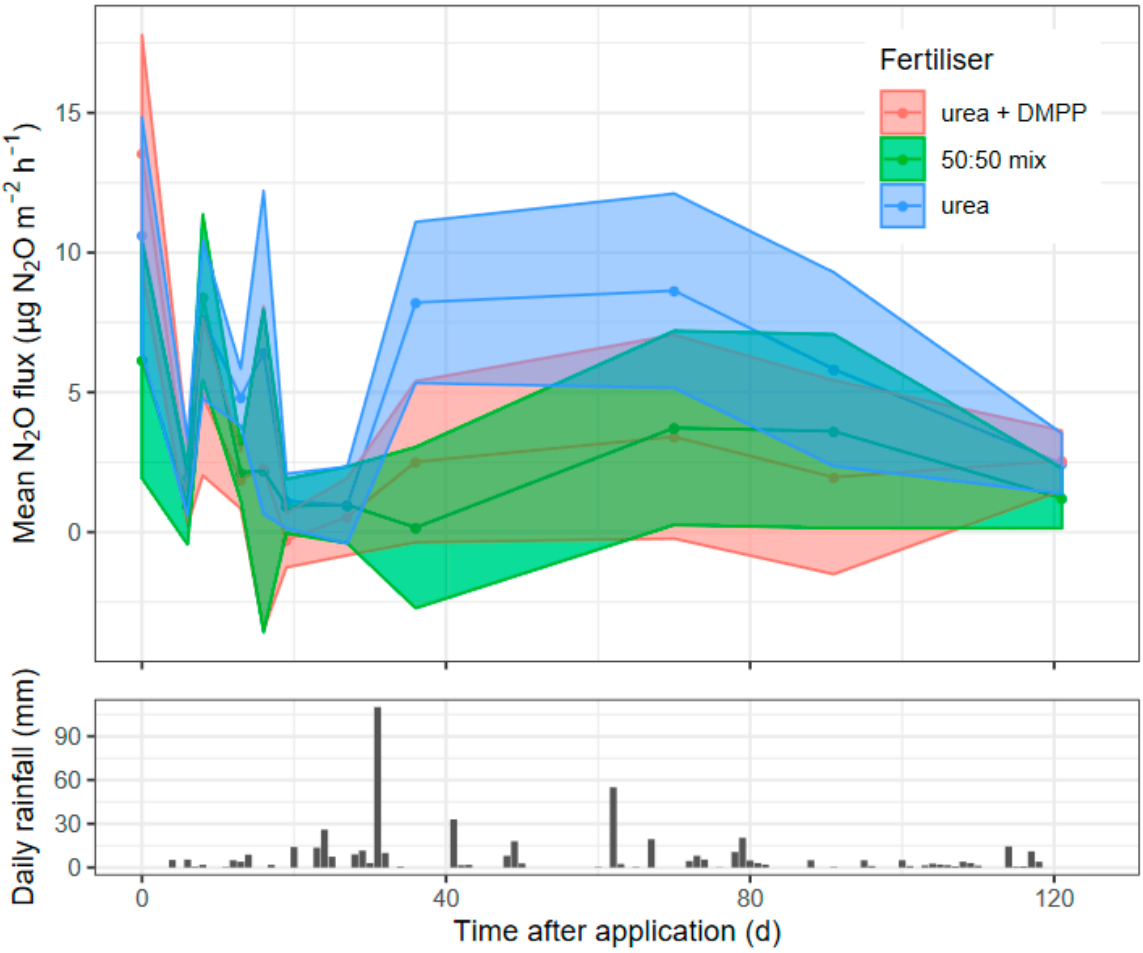

Figure 2. Nitrous oxide fluxes from an acidic Gleysol following application of 90 kg N ha^{-1} as urea, urea plus 3,4-dimethylpyrazole phosphate (DMPP) or a 50/50 blend of urea and DMPP–urea (50:50 mix). The shaded error on all the plots represents 95% confidence limits.

3.3. Effect of Soil Moisture and Temperature on Mineral N Transformations in the Gleysol

Soil NH_4^+-N concentrations were around 500 mg kg^{-1} 2 days after the addition of fertiliser, and remained high (above 350 mg kg^{-1}) until day 30 regardless of incubation temperature and soil moisture (Figure 3). There was a general trend towards lower NH_4^+ concentrations in the urea treatment beyond 6 days after fertiliser addition, with the exception of 30 °C and 60% WFPS, where the trend was only observed beyond 10 days.

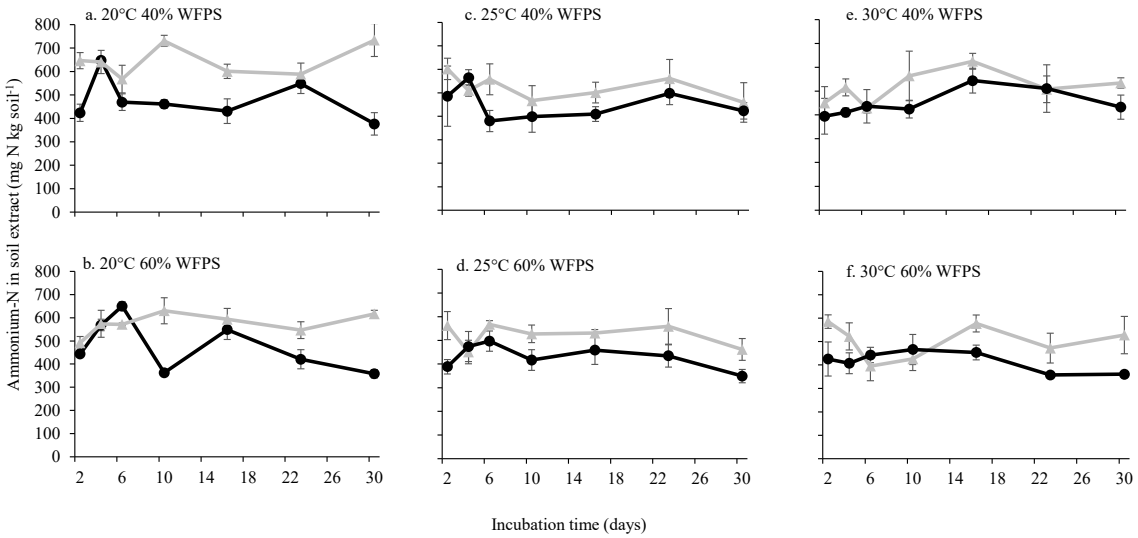

Figure 3. Effect of temperature and water-filled pore space (WFPS) on soil ammonium concentrations following the application of urea (black circles) or DMPP–urea (grey triangles) to an acidic Gleysol soil incubated for 30 days. Error bars depict SEM (n = 3).

The effect of incubation temperature was more evident on soil NO_3^- concentrations. At 20 °C, NO_3^- concentrations remained below 40 mg N kg soil^{-1} over the 30 day incubation period, while at 25 °C, NO_3^--N concentrations increased beyond 23 days to around 70 mg kg^{-1} at 40% WFPS by 30 days and to >90 mg kg^{-1} by 30 days at 60% WFPS (Figure 4). At 30 °C, NO_3^--N concentrations increased substantially beyond 16 days to around 40 mg kg^{-1} by day 30 at 40% WFPS and around 60 mg kg^{-1} by day 30 at 60% WFPS (Figure 4). The general trend for lower soil NH_4^+ concentrations in the urea treatment beyond day 6 (Figure 3) did not correspond to a reciprocal trend for higher NO_3^- concentrations in the urea treatment beyond day 6 compared to the DMPP treatment (as can be seen by comparing Figures 3 and 4).

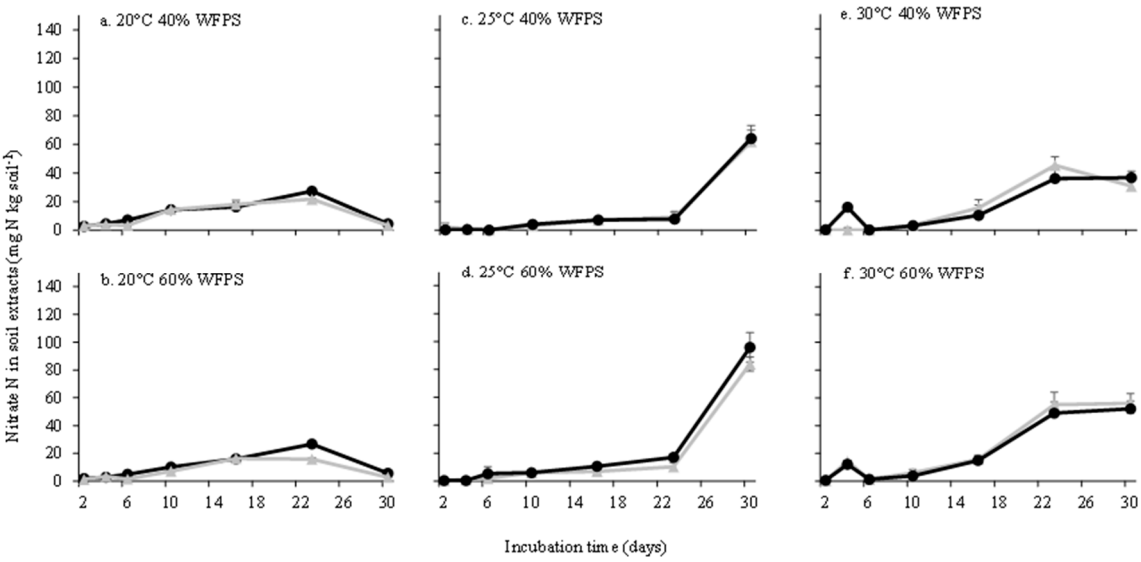

Figure 4. Effect of temperature and water-filled pore space (WFPS) on soil nitrate concentrations following application of urea (black circles) or DMPP–urea (grey triangles) to an acid peat soil incubated for 30 days. Error bars depict SEM (n = 3).

3.4. Effect of Liming on Mineral N Transformations and pH in the Peat Soil

The hydrolysis of urea (with and without DMPP) to NH_4^+ occurred within 1 day regardless of lime application (Figure 5a,b). In the unlimed treatment, there was no sharp decline in NH_4^+-N concentrations over the 65 day period, with NH_4^+-N concentrations of around 400 mg kg^{-1} at 65 day in the urea and DMPP–urea treatments (Figure 5a). In contrast, NH_4^+-N concentrations in the limed soil declined from around 500 mg kg^{-1} at 6 days to <50 mg kg^{-1} at 16 days in the urea treatment. Nitrification was delayed by up to 4 days in the limed soil in the DMPP–urea treatment compared to the urea treatment, with NH_4^+-N concentrations in the DMPP–urea treatment remaining at around 500 mg kg^{-1} until the 10 day sampling point before declining sharply (Figure 5b).

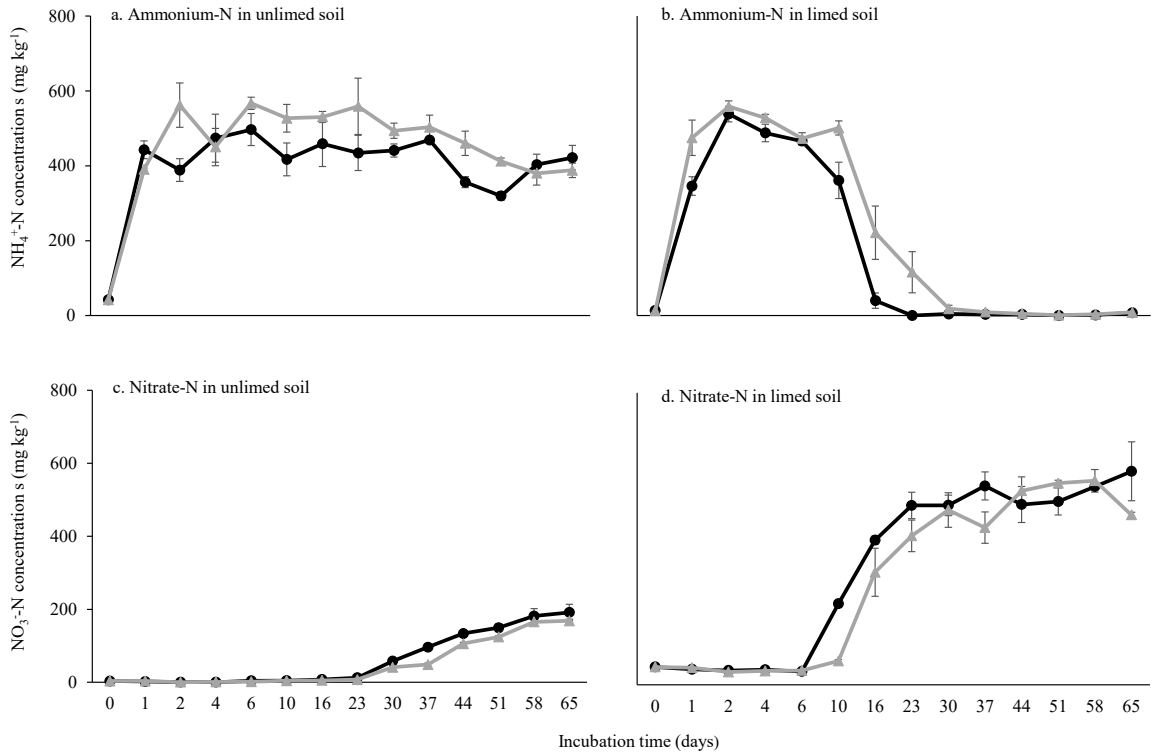

Figure 5. Effect of lime on soil ammonium (**a**,**b**) and nitrate (**c**,**d**) concentrations following the application of urea (black circles) or DMPP–urea (grey triangles) to an acidic Gleysol incubated for 65 days at 25 °C and 60% WFPS. Error bars depict SEM (n = 3).

Increases in soil NO_3^--N concentrations largely mirrored decreases in NH_4^+-N concentrations in both limed and unlimed soils (Figure 5c,d). In the unlimed soil, there was no evidence of nitrification until 23 days, after which NO_3^--N concentrations gradually increased to around 200 mg kg^{-1} at 65 days (Figure 5c). There was a general trend towards slightly lower NO_3^--N concentrations in the DMPP–urea treatment compared to the urea treatment beyond 23 days. In the limed soil, nitrification was apparent after 6 days in the urea treatment, with NO_3^--N concentrations increasing from <10 mg kg^{-1} at 6 days to around 500 mg kg^{-1} by 23 days (Figure 5d). Nitrification was again delayed by up to 4 days in the urea + DMPP treatment compared to the urea treatment.

In the unlimed soil, pH in the KCl extract increased from 3.7 ± 0.02 at 0 days to 4.0 ± 0.03 at day 1 in the urea treatment following the hydrolysis of urea and remained above 3.8 for the duration of the experiment (Figure 6). In the limed soil, pH increased from 5.9 ± 0.01 at 0 days to 6.2 ± 0.03 at day 1 in the urea treatment following the hydrolysis of urea and remained above 6.0 for the duration of the experiment (Figure 6). In both the limed and unlimed soils, pH in the DMPP–urea treatment followed the same trend as the urea treatment.

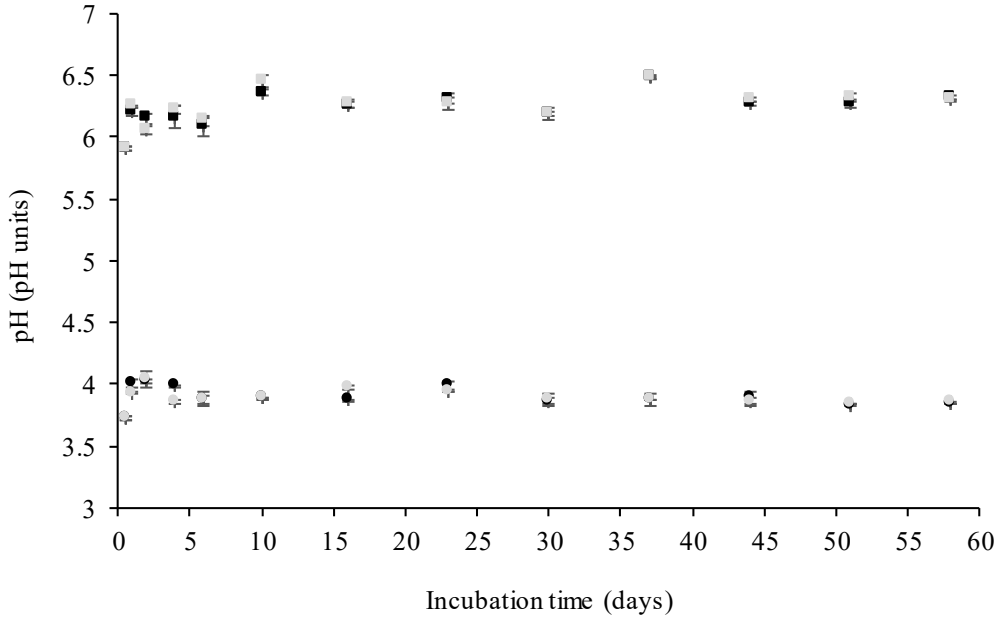

Figure 6. Effect of lime on soil pH following the application of urea (black) or DMPP–urea (grey) to an acidic Gleysol incubated for 65 days at 25 °C and 60% WFPS. Squares indicate limed soil, circles indicate unlimed soil. Error bars depict SEM (n = 3).

4. Discussion

Soils in the tropics and subtropics that are high in organic C can emit large quantities of N_2O when drained for agriculture, resulting in an unacceptably high GHG footprint [5,7]. However, consistent with our earlier study on N_2O emissions from a neighbouring high-C Gleysol [12], low fluxes of N_2O were observed after 90 kg of fertiliser N ha^{-1} was applied to a rice crop (<15 μg N_2O m^{-2} h^{-1}) and seasonal cumulative N_2O emissions were negligible (<0.15 kg N_2O-N ha^{-1} $season^{-1}$). This is in contrast to seasonal emissions for rice crops grown on clay soils in the district where cumulative seasonal emissions ranged from 0.4 kg N_2O-N ha^{-1} $season^{-1}$ [18] to 2.3 kg N_2O-N ha^{-1} $season^{-1}$ [12] or other crops such as sugarcane growing on nearby high-C Gleysols where cumulative seasonal emissions of 46 kg N_2O-N ha^{-1} $season^{-1}$ have been reported [10]. Low fluxes of N_2O can be caused by a range of factors, including moisture limitations in tropical soils [19], the lack of substrates needed for denitrification, including NO_3^- and labile C [20], and the availability of O_2 as a competing electron acceptor [21]. These factors also control the product ratio of N_2O to N_2 during denitrification.

Given that N fertiliser was broadcast onto wet soil immediately before a 4 mm rain event and the crop received a further 94 mm of rain over the following month (Figure 2), the conditions were favourable for denitrification. We also suggest that the potential complete denitrification to N_2 was unlikely as the N_2O:N_2 product ratio of denitrification was shown to negatively correlate with soil pH, meaning that less N_2 was detected in moderately acidic soils [22]. Soil C levels in the Gleysol were >5% (Table 1), and it is therefore unlikely that C substrate limited N transformation or denitrification. We therefore hypothesised that the lack of NO_3^- substrate was the probable cause for low N_2O fluxes on the acid soil and thus examined N transformations in the soil following the addition of urea-based fertilisers.

Forming definitive conclusions on soil N transformations by monitoring topsoil NO_3^- concentrations in situ is challenging, since NO_3^- may move below the 0–100 mm zone following rainfall events. Further, rice plants take up both NH_4^+ and NO_3^- [23]; thus, a lowering of topsoil NH_4^+ and NO_3^- concentrations over time may simply reflect plant uptake as opposed to N transformation processes. In light of these issues, we undertook incubation studies to investigate N transformations under temperature and moisture controlled conditions, evaluating the development of NO_3^- which is required as a substrate for denitrification. Consistent with data from Chen et al. [24], the incubation

temperature had a larger influence on nitrification than WFPS (40% vs. 60%), with little evidence of nitrification at 20 °C at either WFPS over 30 days, while low concentrations of NO_3^- (<100 mg kg^{-1}) began to accumulate between 16–23 days at 30 °C and between 23–30 days at 25 °C. The temperature of 25 °C was chosen for the second incubation study because, while temperatures during the summer cropping period are regularly >30 °C in the subtropics, in the 2013–2014 rice season, the maximum daily temperatures immediately after N fertiliser application ranged between 22–29 °C.

The second incubation study clearly demonstrated that nitrification was inhibited in the unlimed soil vs the limed soil, which was consistent with our hypothesis that low N_2O fluxes from soils are due to the lack of NO_3^- substrate associated with low soil pH. Interestingly, however, nitrification appeared to occur after 23 days in the unlimed soil in the second incubation study, albeit at a lower level than in the limed soil. While urea hydrolysis results in an increase in soil pH, the pH of the KCl extracts indicated that urea hydrolysis only increased the pH from 3.7 to 4.0 in the bulk unlimed soil (Figure 6). It was previously thought that nitrification was low in soils with a pH < 5.5 due to the lack of NH_3 substrate for the ammonia monoxygenase (AMO) enzyme of ammonia oxidisers and lack of growth of ammonia-oxidising bacteria (AoB) at a pH < 5.5 [25]. However, a review of more recent studies indicates that nitrification can occur at a pH as low as 3.0, with ammonia oxidising archaea—the dominant nitrifiers in acid soils [26]. Thus, while it is possible that such a subtle shift in pH was sufficient to stimulate nitrification, it is also possible that the pH of soil immediately surrounding the fertiliser granules increased more dramatically than our measurement of bulk soil pH suggests. For example, Janke et al. [27] reported an increase in soil pH around urea granules from around 5 to above 9 within 10 days of application. However, the high pH and high NH_3 concentrations subsequently inhibited nitrification over the 80 day incubation period [27]. In our incubation study, any alkalinity and NH_3 would have diffused away from the fertiliser granule over time, potentially creating pH conditions amenable to nitrification after 23 days.

In the field study, while N_2O fluxes were low (<15 μg N_2O m^{-2} h^{-1}; Figure 2) in the acid peat soil compared to other soils in the district, the cumulative N_2O flux from 25 days to 75 days after N fertiliser application in the field trial was statistically significantly higher in the urea treatment than the other two treatments, in which DMPP–urea or a 50/50 split were applied, which would be consistent with the trend of lower NO_3^--N concentrations in the DMPP–urea treatment in the unlimed soil from 23–65 days after fertiliser application (Figure 5c). However, while statistically significant, the magnitude of the reduction from 0.15 kg N_2O-N ha^{-1} $season^{-1}$ in the urea treatment to 0.05 kg N_2O-N ha^{-1} $season^{-1}$ in the 50:50 mix treatment was low due to the inherently low N_2O emissions from the system and was therefore of little practical relevance.

In the limed soil, nitrification was only inhibited for up to 4 days in the urea + DMPP treatment compared to urea at 60% WFPS at 25 °C (Figure 5d). Under the same temperature and WFPS conditions using soil collected from the 0–10 cm layer of a brown Vertosol (1.3% organic C), Chen et al. [24] found impaired nitrification in a DMPP–urea treatment up to 28 days after fertiliser addition, where nitrification was observed in the urea treatment after 7 days. While the reason for the discrepancy is not known, this would suggest that, even under limed conditions in the field, DMPP may have limited efficacy in peat soil. This is consistent with the limited efficacy of DMPP in lowering seasonal N_2O emissions that we have reported in other soils in the wet subtropics [12,18]. The reason for the low efficacy of DMPP in our studies on high organic matter soils in the wet subtropics is not known but is the subject of ongoing studies given that the mean reduction in N_2O emissions from field studies using DMPP-treated fertilisers compared to standard N fertilisers is around 40% [28].

5. Conclusions

Earlier studies have reported decreased N_2O emissions with increased soil acidity (e.g., Yamulki et al. [29]), and our results suggest that low soil pH contributes to limited nitrification in the high-C, subtropical Gleysol examined in our study, which is associated with low fluxes of N_2O in the field. Given that these high-C Gleysols can produce rice yields of 5–7 t/ha, which is equivalent to

rice yields on other soils in the region [12,30], the soil pH may be sufficiently low to inhibit nitrification without limiting the yields of crops that are moderately tolerant to low pH. There may therefore be an opportunity to exploit this phenomenon to cultivate acid-tolerant crops on peat soils while minimising N_2O emissions, which are currently a serious threat to sustainable farming on drained wetland soils in the tropics and subtropics that are high in C. Future research should aim to determine threshold pH values for nitrification in high-C wetland soils and to quantify trade-offs between N_2O emissions and potential yield losses for a range of crops.

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