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Optimising soil P levels reduces N₂O emissions in grazing systems under different N fertilisation

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Abstract

The effect of long-term soil phosphorus (P) on in situ nitrous oxide (N₂O) emissions from temperate grassland soil ecosystems is not well understood. Grasslands typically receive large nitrogen (N) inputs both from animal deposition and fertiliser application, with a large proportion of this N being lost to the environment. Understanding optimum nutrient stoichiometry by applying N fertilisers in a relative balance with P will help to reduce N losses by enabling maximum N-uptake by plants and microbes. This study investigates the N₂O response from soils of long-term high and low P management receiving three forms of applied N at two different rates: a nitrate-based fertiliser (KNO₃) and an ammonium-based fertiliser ([NH₄]₂SO₄) (both at 40 Kg N ha⁻¹), and a synthetic urine (750 Kg N ha⁻¹). Low soil P significantly increased N₂O emissions from KNO₃ and (NH₄)₂SO₄ fertilisers by over 50% and numerically increased N₂O from urine by over 20%, which is suggested to be representative of the lack of significant effect of N fertilisation on N-uptake observed in the low P soils. There was a significant positive effect of soil P on grass N-uptake observed in the synthetic urine and KNO₃ treatments, but not in the (NH₄)₂SO₄ treatment. Low P soils had a significantly lower pH than high P soils and responded differently to applied synthetic urine. There was also a significant effect of P level on potential nitrification which was nearly three times that of low P, but no significant difference between potential denitrification and P level. The results from this study highlight the importance of synergy between relative nutrient applications as a deficiency of one nutrient, such as P in this case, could be detrimental to the system as a whole. Optimising soil P can result in greater N uptake (over 12, 23 and 66% in (NH₄)₂SO₄, KNO₃ and synthetic urine treatments, respectively) and in reduced emissions by up to 50% representing a win-win scenario for farmers.

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Highlights

- P deficiency in grassland soils causes greater N₂O emissions.
- Insufficient soil P inhibits N-uptake, regardless of rate or form of N.
- Optimising soil P levels can reduce N₂O emissions and improve overall NUE.

KEYWORDS

carbon-nitrogen-phosphorus stoichiometry, nutrient use efficiency, optimum management, temperate grassland

1 | INTRODUCTION

The cycles of the key nutrients nitrogen (N) and phosphorus (P) have been massively altered by anthropogenic activities, the majority of which are a result of increasing agricultural intensification since the “Green Revolution” (Elser et al., 2007). The use of these nutrients in agriculture is infamously “leaky” with much loss and wastage to the environment via emissions, run-off, leaching and volatilisation (Selbie et al., 2015). This is a serious problem in Ireland where agriculture accounts for 64% of total land area and is the source of the majority of P and N pollution both to the atmosphere and to water ways (DAFM, 2020). This is largely attributable to permanent grasslands, which account for over 80% of total agricultural land and receive excess nutrient inputs through the addition of manures, fertilisers and animal excreta (Harty et al., 2016). However, only a small proportion of the N (~23% to 26%) consumed by grazing animals is converted to milk or live weight gain, meaning that more than 70% is lost to the environment, mainly through nitrate leaching and gaseous emissions particularly in the form of ammonia and nitrous oxide (N₂O), or immobilised in soil organic matter (Hoekstra et al., 2020). Although N dynamics have been extensively studied, especially in managed grasslands (Bolan et al., 2004; Saggar et al., 2007; Saggar et al., 2013), the interactions of N with contrasting P availability have been less investigated.

Soil N and P cycles interact with one another with numerous processes often occurring simultaneously (Guignard et al., 2017). The drivers of these two nutrient cycles are predominantly microbial and thus respond to varying availability of resources and conditions (Wang et al., 2014). Temperate grassland N₂O emissions are mainly associated with the application of organic or inorganic N-inputs stimulating microbial N₂O production (IPCC, 2021). Such inputs increase the size of ammonium (NH₄⁺) and nitrate (NO₃⁻) pools in the soil, which then undergo a variety of transformations resulting in greater N₂O emissions (Butterbach-Bahl et al., 2013). The majority of these transformations occur through nitrification

(the oxidation of NH₄⁺ to NO₂⁻ and NO₃⁻) and denitrification (reduction of NO₃⁻ to NO, N₂O and N₂) pathways.

Phosphorus is made biologically available mainly through weathering, dissolution, mineralisation and desorption (Prasad & Chakraborty, 2019) and is a relatively slow cycle compared to N. Phosphorus is essential for both plant and microbial N-uptake being a critical component of amino and nucleic acids as well as the energy currency adenosine triphosphate—ATP (Dunn & Grider, 2021; Raffaella et al., 2016). However, as P is required in smaller amounts relative to N by plants and microorganisms (Cleveland & Liptzin, 2007), P-limitation results in a lack of immobilisation of N into biomass as they are unable to assimilate it, and this can further exacerbate N-losses via emissions, leaching and run-off (Ågren et al., 2012).

In grassland soils, increasing levels of microbial activity stimulated by high carbon (C) and N inputs, from biomass decomposition, nutrient application, animal excreta, and especially the high N-loading rate present in urine patches, are most likely the main cause for high N₂O emission (Schaufler et al., 2010). However, often such nutrients can be distributed inefficiently. In terrestrial ecosystems, the availability and quality of C often limits heterotrophic microbial growth, and this C limitation can be further exacerbated by the co-limitation of P, especially as it is required in such small amounts relative to C and N (Cleveland & Liptzin, 2007; Griffiths et al., 2012; Mehnaz et al., 2018).

P-addition to soils has been reported to both stimulate and reduce N₂O emissions depending on the response from either increasing microbial N-cycling activity (nitrification and denitrification) due to nutrient relief (Baral et al., 2014), or else stimulating N-uptake by plants and immobilisation by microbes, thus reducing N available to be transformed to N₂O (Mori et al., 2013; Mori et al., 2014). A recent field study observed an increase in N₂O emissions with the alleviation of P limitation with the caveat that C must also be available, thus highlighting the importance of considering nutrient co-limitation (Anderson et al., 2022). Whereas other comparable field

studies observed a 42% reduction in cumulative N_2O emissions from P-rich soils ($45 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) compared to soils in index 1 ($0 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) (Gebremichael et al., 2022). A recent global meta-analysis has summarised this effect succinctly in its observations that P-enrichment significantly decreased soil N_2O emissions at ambient soil N concentrations, which was attributed to being a result of decreased soil NO_3^- -N content, due to increased N-uptake resulting from P-limitation relief (Shen & Zhu, 2022). P-addition at elevated soil N concentrations, however, was not found to significantly effect N_2O , nor did N-enrichment of soils of ambient and elevated P concentration (Shen & Zhu, 2022). This is likely a result of soil P cycling being very slow and requiring years of consistent management to maintain a stable concentration (Oelkers et al., 2008; Walker, 1991), with responses to added P being different to those of long-term P which is being investigated in this paper. Furthermore, investigating such responses under high N-addition represents a working farm scenario in order to help towards developing management mitigation practices.

Plant growth and microbial proliferation require a stoichiometric supply of C, N and P, but microbial C:N:P ratios are often smaller than those of plants, meaning that application inefficiencies can be overlooked (Cleveland & Liptzin, 2007; Mehnaz et al., 2018). Molar ratios of C:N:P tend to be homeostatic despite large changes in soil nutrient ratios, meaning that microbial communities in particular are hugely impacted by the nutrient availability of their surrounding soil, and that this has a direct effect on their species composition (Griffiths et al., 2012). This can often result in the promotion of fungi, which have the capacity to dominate under nutrient poor conditions, and often form the major component of microbial biomass in the soil (Antunes et al., 2012; Boswell et al., 2007; Nottingham et al., 2018). This has major implications for N_2O mitigation as fungi lack the gene (*nosZ*) which encodes the enzyme N_2O reductase responsible for N_2O to N_2 reduction, and are therefore major contributors to N_2O emissions (Maeda et al., 2015).

This study focuses on the impact of >50 years P addition to a temperate grazed grassland soil under varying forms of N-additions. Research on long-term sites such as this is uncommon and is a precious resource to build on existing knowledge and to investigate long-term trends. Similar research on grassland N_2O emissions has found that replacing calcium ammonium nitrate (CAN) with urea reduced N_2O emissions on average by 80%, emphasising the importance of the form of N applied in regard to reducing emissions but retaining yield (Harty et al., 2016; Krol et al., 2020). Previous research at this

site has highlighted co-limitation of nutrients to be critical in grassland productivity either through supply deficiency or a saturation of one nutrient in particular (Griffiths et al., 2012; Massey, 2012; Randall et al., 2019). Further research at this site has also found increasing P levels to decrease arbuscular mycorrhizal fungi (AMF) colonisation, thus having a direct effect on microbial community composition (Chen, 2012; King-Salter, 2008).

Previous ex situ incubation studies carried out on another long-term cut P trial observed approximately 70 times greater N_2O emissions from low P soils compared to high P soils, with CO_2 emission unaffected by P level (O'Neill et al., 2020). Further studies using ^{15}N isotope label on the same soils demonstrated heterotrophic nitrification to be the dominant overall N-cycling pathway, again significantly greatest in low P soils (O'Neill et al., 2021). Subsequent examination of structural and functional gene abundances in these soils revealed that the only gene to be positively correlated with N_2O emissions in the low P soils was the fungal identifying “internal transcribed spacer” gene—*ITS*, with increased nitrifier abundance in low P soils while the opposite trend was found for denitrifying functional genes (O'Neill et al., 2022).

These previous results demonstrate a significant effect of P on N-cycling communities and subsequent emissions. It is vital to supplement laboratory studies with in situ research to account for field conditions (Harty et al., 2017). To date there has been very little research carried out on the effect of contrasting P concentration on N_2O emissions from temperate grassland soils. As such, this paper aims to assess the response of applying different N-inputs to varying levels of soil P on N_2O emissions from grazed grassland and elucidate the impact on various edaphic properties.

2 | MATERIALS AND METHODS

2.1 | Site description and experimental design

This experiment was carried out on an ongoing long-term grazed P trial (5.67 ha) located at Teagasc, Johnstown Castle in Wexford, Ireland ($52^\circ 18' \text{N}$, $6^\circ 30' \text{W}$). Johnstown Castle has an annual rainfall of 1037 mm and a mean annual temperature of 10.4°C (Met.Eireann, 1981–2010). The cumulative rainfall was 306 mm and the average temperature was 11.1°C for the duration of the experiment (Met.Eireann, 2020), which is very similar to the corresponding 30-year averages of 11.6°C and 95 mm over the same time period (September, October,

November) (Met.Eireann, 1981–2010). The pasture is dominated by perennial ryegrass (*Lolium perenne*) and is established on a temperate, moderately drained sandy

TABLE 1 Soil properties (mean $\pm$ SE, $n = 5$) at 0–10 cm soil depth sampling at the start of the experiment

Parameters	P0	P30
Carbon (%)	4.07 $\pm$ 0.11 ^a	4.15 $\pm$ 0.11 ^a
Organic carbon (mg g ⁻¹)	3.17 $\pm$ 0.16 ^a	3.55 $\pm$ 0.11 ^a
Nitrogen (%)	0.42 $\pm$ 0.01 ^a	0.44 $\pm$ 0.01 ^a
Phosphorus (mg g ⁻¹)	494.06 $\pm$ 19.37 ^a	1023.48 $\pm$ 37.12 ^b
Organic matter (%)	8.79 $\pm$ 0.21 ^a	9.48 $\pm$ 0.21 ^a
Morgan phosphorus (mg g ⁻¹)	1.33 $\pm$ 0.16 ^a	12.10 $\pm$ 1.09 ^b
Morgan magnesium (mg g ⁻¹)	97.40 $\pm$ 7.00 ^a	141.7 $\pm$ 7.07 ^b
Morgan potassium (mg g ⁻¹)	84.75 $\pm$ 7.53 ^a	72.10 $\pm$ 6.17 ^a
Lime requirement (mg g ⁻¹)	9.30 $\pm$ 0.33 ^a	8.13 $\pm$ 0.17 ^a
pH	5.12 $\pm$ 0.04 ^a	5.42 $\pm$ 0.02 ^b
Bulk density (g cm ⁻³)	0.95 $\pm$ 0.02 ^a	0.83 $\pm$ 0.01 ^b
Hot-water extractable carbon (mg g ⁻¹)	6.34 $\pm$ 0.23 ^a	6.86 $\pm$ 0.28 ^a
Hot-water extractable nitrogen (mg g ⁻¹)	0.42 $\pm$ 0.09 ^a	0.55 $\pm$ 0.02 ^a

Note: Different letters within each parameter indicate significant differences ($p < 0.05$) between long-term P treatments.

loam soil (for full list of soil properties please see Table 1). This trial was first set up in 1968 (hereafter referred to as the 'Cowlands' trial) (Culleton et al., 2002; Tunney et al., 2010) and was originally established to determine the optimum application rate of P fertiliser required for grazing pasture by beef cattle. This initial trial consisted of three rates of P application of 0, 15 and 30 Kg P ha⁻¹ (P0, P15 and P30) in the form of calcium superphosphate with 12 replicates of each. Each treatment plot was approximately 0.45 ha and was under a grazing regime of either a high or low stocking level (2200 and 3300 Kg stock ha⁻¹) of beef cattle, which was altered in 1999 such that all plots received the same high stocking rate of 3300 Kg stock ha⁻¹ (approximately six-eight livestock units per ha⁻¹).

The current study investigates soils receiving P0 and P30 treatments. Animals were excluded from the sites for weeks prior to the commencement of the experiment. The study design consisted of N₂O gas and soil sampling carried out over the course of 3 months on the P0 and P30 plots with five replicate plots per treatment (Figure 1). Grasses in all plots were cut to 4 cm height prior to chamber installation. Four subplots (1.2 m $\times$ 1.2 m) were marked in each of the plots, forming a total of 40 subplots (20 in each P0 and P30 plots).

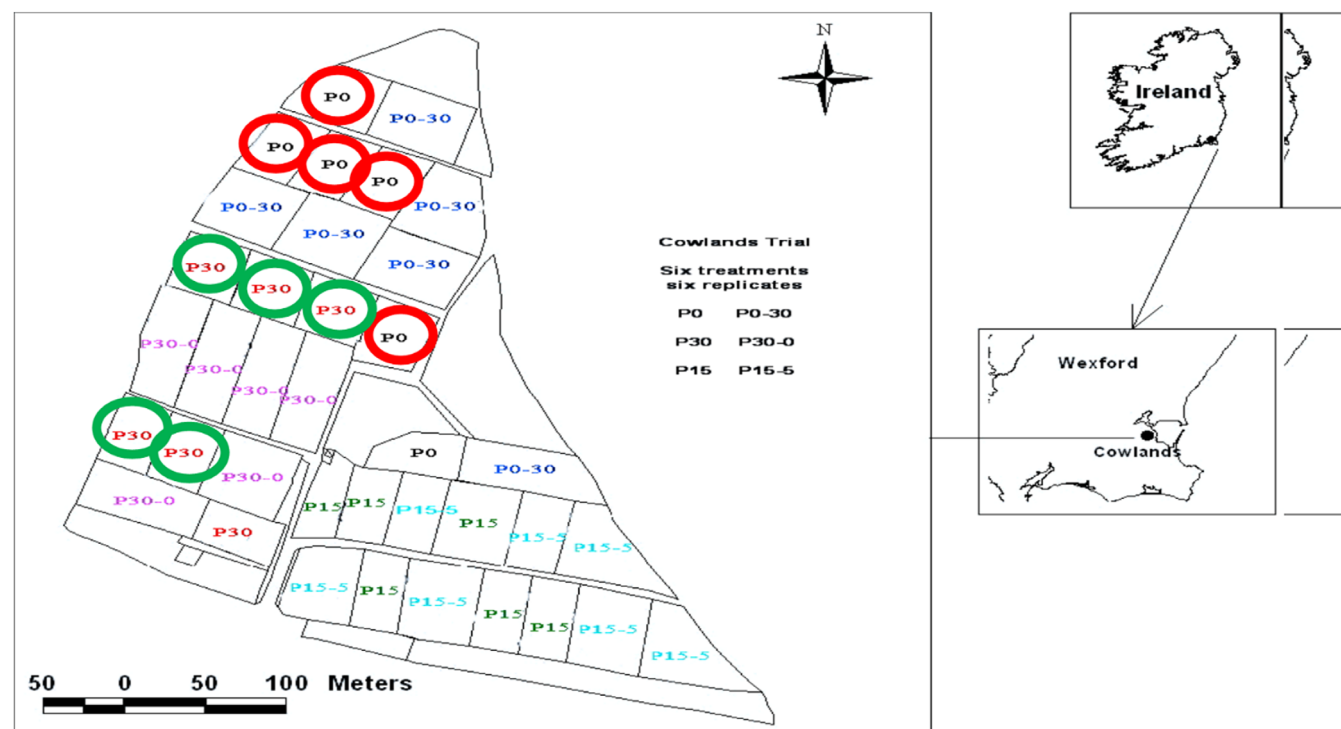


FIGURE 1 Map of the Cowlands trial, showing the layout of the six replicate plots for each of the six P treatments. P0, P15, and P30 were originally high-stock plots and have had constant P application and stocking rates since 1968. Treatments P0-30, P30-0, P15, 5 were originally low-stock plots; P application rates were changed in 1999 and stocking was increased to the high-stock level. Circles indicate sampled plots

2.2 | Treatments

Four treatments; synthetic urine, ammonium sulphate ((NH₄)₂SO₄), potassium nitrate (KNO₃) and an untreated control were applied in August 2020, to the marked subplot areas in each P0 and P30 plots.

Synthetic urine was prepared in the laboratory according to the method in de Klein et al. (2014). Synthetic urine was applied at a rate of 750 Kg N ha⁻¹ to each subplot, to represent a typical N-loading rate found in natural animal urine deposition (Krol et al., 2016; Murphy et al., 2022). Ammonium sulphate ((NH₄)₂SO₄) and KNO₃ were applied to the subplots at a rate of 40 Kg N ha⁻¹. All treatments were applied to the soil surface approximately 6 h before the first gas sampling event commenced on the 14th September 2020.

2.3 | N₂O sampling and analysis

N₂O sampling was carried out as per the technique outlined by de Klein and Harvey (2012). Stainless steel collars (0.40 m × 0.40 m) were inserted into the ground in the middle of each subplot at a depth of approximately 10 cm one week prior to treatment application. Airtight lids were placed on to each collar base during sampling events and gas samples were taken by inserting a 12 ml syringe into rubber septa at the top of the lid and withdrawing a 10 ml gas sample injected into a pre-evacuated 7 ml glass vial (Labco Ltd, UK). Sampling of each chamber headspace was carried out over the course of 50 min, at T0, T25 and T50 min, between the hours of 10 am and 12 pm for the duration of the sampling campaign which ran from September to November. Background gas samples were taken on two occasions before treatment application. Intensive measurements were taken for the first month after treatment application. Initial gas samples were taken 6 h post treatment application, then daily for the next 2 weeks, then 2–3 times per week for the next 8 weeks, and once a week for the concluding 2 weeks of the experiment. N₂O was sampled a total of 36 times including the two background sampling events and was concluded when emissions returned to background levels. N₂O concentrations were analysed using a gas chromatograph (Bruker Scion 456, USA) connected to a Combi-Pal auto-sampler (CTC Analysis, Switzerland). N₂O fluxes were calculated assuming the linear accumulation of headspace gases and according to the ideal gas law (de Klein and Harvey, 2012) using the Equation below. Daily fluxes (F [daily] in g N₂O-N Kg⁻¹ ha⁻¹ d⁻¹) were calculated for each treatment from the increase in headspace concentration from the initial N₂O chamber concentration (T0) to the final N₂O

chamber concentration (taken at T50 min after enclosure) using:

$$F(\text{daily}) = (\Delta C / \Delta t) \times ((M \times P) / (R \times T)) \times (V / A) \quad (1)$$

where $\Delta C / \Delta t$ is the slope of the line of the change in N₂O concentration from T0 to T50 (Saggar et al., 2007), ΔC is the change in gas concentration in the chamber headspace during the enclosure period in ppbv, Δt is the enclosure period expressed in minutes, M the molar mass of N₂O-N (28 g mol⁻¹), P and T the atmospheric pressure (Pa) and temperature (K) (maintained in the growth chamber at 15°C), R the ideal gas constant (8.314 J K⁻¹ mol⁻¹), V the headspace volume of the closed chamber (m³) and A the area covered by the base of the chamber (m²). This flux per chamber area was extrapolated to flux on a Kg⁻¹ N ha⁻¹ d⁻¹ basis. Although, chamber flux methods can be associated with large uncertainties due to artefacts that de-couple the chamber microclimate from external conditions (Rochette et al., 2008), the chamber technique is still the most commonly used method (accounting for 95% of total field data) to quantify N₂O emissions and investigate treatment effects on soil N₂O fluxes at small spatial scales (Chadwick et al., 2014; Clough et al., 2020; Krol et al., 2017).

2.4 | Soil property analyses

Physicochemical properties were characterised from samples taken from 10 cm soil depth of random areas of each plot. Soil water content (GWC) and water holding capacity (WHC) percentage were determined gravimetrically by overnight drying of fresh and water-saturated soils at 105°C (Jones et al., 2014).

Dried soil was ball-milled and 0.2 g was used to determine the organic matter (OM), C and N content as the mass lost after ignition for 7 h at 550°C. The pH was measured in a 1:5 ratio soil to de-ionised water solution using a WTW pH 526 pH meter (Labsource, US). Total carbon (TC) and total nitrogen (TN) were measured using a TrueSpec C/N analyser (LECO, USA).

Hot water extractable organic carbon (HWC) and nitrogen (HWN), indicating labile pools, were extracted with 30 ml deionised water from 6 g dried soil incubated for 16 h in a water-bath (85°C). Samples were shaken for one hour, centrifuged, filtered (Whatman No. 42), before total organic carbon (TOC) and nitrogen (TN) were measured using a Shimadzu TOC-V analyser (Ghani et al., 2003).

Plant available P was measured using the Morgan's P method. P was extracted from 5 g dry soil using 15 ml 0.62 M NaOH and 1.25 M CH₃COOH, adjusted to pH 4.8. The extract was shaken for 30 min, filtered, and quantified using a spectrophotometer (Camspec UK).

Total soil P and metal availability (Copper, Iron, Magnesium, Manganese and Aluminium) was measured using the Mehlich 3 soil extraction method, using 0.2 M CH_3COOH , 0.25 M NH_4NO_3 , 0.015 M NH_4F , 0.013 M HNO_3 and 0.001 M EDTA from 3 g of dried soil and analysed via Inductively Coupled Plasma Optical Emission Spectrometry ICP-OES (Mehlich, 1984).

Soil bulk density (0–10 cm) was measured at the end of the sampling period using the core method (USDA, 1998) in order to calculate the volumetric moisture content and water-filled pore space (WFPS).

Soil samples for mineral nitrogen analysis were taken outside of the collar areas within the subplots prior to treatment application, Day 2, Day 4 and Day 6 post treatment application, then weekly until the completion of the experiment. Mineral nitrogen was extracted with 50 ml of 2 M KCl from 10 g fresh soil. Samples were shaken for 1 hour, and then filtered through a filter paper (Whatman No. 42 mm). Ammonium (NH_4^+), TON (total oxidised nitrogen) and combined NO_2^- NO_3^- (analysed as NO_2^-) concentrations were determined via colorimetric analysis (Aquakem 600, Thermo Electron OY, Vantaa, Finland). NO_3^- was calculated as TON minus NO_2^- concentration (Mulvaney, 1996).

2.5 | Microbial biomass analyses

Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were determined from a fresh sample using the chloroform fumigation method (Brookes et al., 1982; Vance et al., 1987). MBC and MBN were determined as the difference in TOC and TN from 10 g soil fumigated for 24 h, versus un-fumigated soils. Soils were extracted using 40 ml 0.5 M K_2SO_4 and shaken for 30 min. TOC and TN were measured using a Shimadzu TOC-V analyser. MBC was calculated using the equation of Vance et al. (1987), using a correction factor of 0.45. Similarly, MBN was determined using a correction factor of 0.56 (Brookes et al., 1982).

MBP was determined following a separate fumigation and extraction (Brookes et al., 1982). Phosphorus was extracted from 3 g fumigated and un-fumigated samples using 39 ml 0.5 M NaHCO_3 (pH 8.5) and 1 ml deionised water. A third sub-sample was used to correct for P adsorption following fumigation. This soil was spiked using 1 ml 250 $\mu\text{g P ml}^{-1}$ (KH_2PO_4) and extracted as above. All samples were mixed for 30 min, filtered and acidified using 2.2 ml 2 M CH_3COOH . Orthophosphate concentrations were then quantified using the ammonium molybdate-ascorbic acid method. MBP was calculated following the P spike correction equation (Massey, 2012).

2.6 | Potential denitrification and nitrification and total respiration

2.6.1 | Potential denitrification

Soil denitrification potential was measured using the acetylene (C_2H_2) inhibition method (Kou et al., 2019). Soils were brought to 70% WHC using de-ionised water, 20 g of soil was placed into a flask and headspace was flushed and then filled with helium three times to maintain anoxic conditions. Background samples (T0) were taken before replacing 10% of the flask's headspace with C_2H_2 , followed by the injection of 2 ml from a 100 ml stock solution made up of 75 mM potassium nitrate (KNO_3), 37.5 mM Na-succinate ($\text{C}_4\text{H}_5\text{NaO}_4$), 25 mM glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and 75 mM Na-acetate ($\text{C}_2\text{H}_3\text{NaO}_2$). Flasks were then incubated at 15°C for 5 h, with gas samples taken once every hour, by inserting a syringe through the septa, flushing the headspace three times before withdrawing a 10 ml sample to be inserted at over-pressure into a 7 ml exetainers. Gas samples were analysed for N_2O using a gas chromatography (GC - 2 Bruker Scion 456, USA) connected to a Combi-Pal auto-sampler (CTC Analysis, Switzerland). N_2O was measured on an electron capture detector (ECD). N_2O fluxes were calculated from the slope of the linear regression line of N_2O concentration versus time as shown in Equation (1). N_2O measurements received in parts per million (ppm) were converted to $\text{ng N}_2\text{O-N g}^{-1}$ dry soil min^{-1} .

2.6.2 | Potential nitrification

Potential nitrification was determined on the basis of the long-term NO_3^- production rate from soils under optimal nitrification conditions (Hart et al., 1994). A nitrifying liquid medium was made up of 0.2 M potassium monobasic phosphate (KH_2PO_4), 0.2 M potassium dibasic phosphate (K_2HPO_4), 50 mM ammonium chloride (NH_4Cl) and distilled water. 100 ml of this medium was added to a flask containing 15 g of fresh soil. Once all contents were added, flasks were shaken at 175 rpm (Orbital Shaker OS20, Biosan) at 15°C for 24 h. The flasks were sampled four times during this 24 h period at 2 h, 6 h, 20 h and 24 h with a 15 min soil settling time between sampling. Between 15–20 ml of filtrate was collected at each sampling time (Whatman filter paper no. 2) and was analysed for NH_4^+ , NO_2^- and NO_3^- (Kony auto-analyser). The rate of N production ($\text{mg N kg}^{-1} \text{ day}^{-1}$) was calculated as:

$$\frac{\text{slope (mg NL}^{-1} \text{ h}^{-1}) \times 100 \text{ ml} + V_w) \times 24 \text{ h day}^{-1} \times 1 \text{ L } 1000 \text{ ml}^{-1} \times 1000 \text{ g Kg}^{-1}}{M_s} \quad (2)$$

Ms dry mass (g) soil ($M_s = 15 \text{ g} / (1 + \text{gwc}) / 100\%$;

Vw is the volume of water (mL contained in the 15 g of moist soil ($V_w = M_w / D_w$)).

The density of water (D_w) @20°C is 0.9982 g ml⁻¹.

$M_w = M_s * (\text{gwc} / 100\%)$.

2.6.3 | Substrate induced respiration (SIR)

Total respiration was measured using the substrate induced respiration technique to stimulate microbial activity using a readily available carbon source (glucose) resulting in enhanced respiration (Anderson & Domsch, 1973). Soils were incubated at 20°C overnight with an initial (T0) gas sample taken prior to glucose addition. Gas samples were taken at 2 and 4 h post substrate application to calculate the rate of CO₂ evolution in response to added substrate, that is, SIR rate. The rate of CO₂ evolved is calculated at T0, T2 and T4 according to the following equation:

$$\text{RCO}_2 - C (\mu\text{g CO}_2 - \text{C g}^{-1} \text{ h}^{-1}) = (V_0 \times 12 / (22.4 \times t \times \text{msd})) \times 1000 \quad (3)$$

where RCO₂-C represents rate of CO₂-C formation on a soil dry mass basis ($\mu\text{g CO}_2 - \text{C g}^{-1} \text{ h}^{-1}$), V₀ is enrichment volume of CO₂ under standard temperature and pressure conditions (ml), 12 is molar mass of CO₂-C (g mol⁻¹), 22.4 is molar volume of CO₂ under standard conditions, t is the incubation time (hours) and msd is the mass of dry soil (g). The average background CO₂ concentration is subtracted to take atmospheric input into account. The final induced rate is calculated by subtracting the RCO₂-C value obtained at T2 from that obtained at T4.

2.7 | Grass yield and nitrogen uptake

Grass was harvested from each plot to a height of 4 cm at 4 weeks after treatment application using grass shears (Art. 8885, Gardena Accu Shears, Gardena, Ulm, Germany). The fresh grass cut from each plot was weighed and oven dried at 70°C for dry matter determination (by dividing dry weight by the fresh weight). Grass dry matter yield (DMY) was expressed as g ha⁻¹ and was computed using fresh weight from the collar area and the dry matter content

(Gebremichael et al., 2021). N-uptake was calculated by multiplying the dry matter yield by the N content of the dry matter sample:

$$\text{N uptake} = \text{dry matter yield} \times \text{N content (\%)} \quad (4)$$

Nitrogen use efficiency (NUE %) was calculated by dividing the difference between N-uptake of treatment and control by the applied rate of N and expressing as a percentage:

$$\text{NUE (\%)} = \left(\frac{N_{\text{uptake treatment}} - N_{\text{uptake control}}}{(N \text{ fertiliser rate})} \right) \times 100$$

2.8 | Statistical analysis

A repeated measure ANOVA using lme4 package was used to test for the main effects of fertiliser addition (synthetic urine, (NH₄)₂ SO₄, KNO₃ and control), P addition (P0 and P30), time of measurement (days), and their interactions on the cumulative N₂O emissions and mineral N. Plots were entered as a random effect in the model. One-way ANOVA was used to test the effect of P treatments on the soil biophysicochemical characteristics such as microbial C, N and P, potential denitrification and nitrification, SIR, soil C, N, P, K, Mg, soil pH. Pair-wise comparisons of the significant differences were conducted using Tukey's HSD tests. Significant differences were identified when $p < 0.05$. Data were log-transformed when deviations of normality and homoscedasticity were detected. All statistical analyses were conducted using R, version 4.0.2 (R Core Team, 2021).

3 | RESULTS

3.1 | N₂O emissions

3.2 | Soil physicochemical properties

Both Total P and Morgan's test P were significantly influenced by P level ($p < 0.001$). Total nitrogen and Morgan's

test magnesium (Mg) were also significantly influenced by P level ($p < 0.001$, $p < 0.05$). P level significantly affect pH ($p = 0.0017$) being most acidic in P0 soils at an average pH of 5.12, while P30 soils averaged 5.42.

Bulk density also showed a significant effect with both treatment and phosphorus level but no interaction (Treatment; $p = 0.0045$, P; $p = 0.035$, and P*Treatment; $p = 0.14$), respectively. P0 was on average a BD of 0.95 g cm^{-3} while P30 soils were 0.835 g cm^{-3} .

Soil total carbon, organic carbon, hot water extractable carbon and nitrogen, Morgan's test potassium, and organic matter were not significantly influenced by P level ($p > 0.05$).

3.3 | Soil mineral N dynamics

There was a significant interaction between the synthetic urine treatment and P level (Treatment $\times$ P, $p < 0.05$) on NH_4^+ concentration, with significantly higher NH_4^+ concentrations than in all other treatments in both P levels, being highest overall in the P0 soils. Soil NH_4^+ concentrations under $(\text{NH}_4)_2\text{SO}_4$ treatments were significantly higher in P0 soils than in P30 ($p = 0.0076$). There was no significant effect of soil P on soil NH_4^+ within the KNO_3 treatment which did not differ significantly from control.

All treatments significantly affected NO_3^- concentrations although there was no observed interaction between treatment and P level. There was a significant interaction between P level and time (Time $\times$ P level, $p < 0.001$) under the synthetic urine treatment. Synthetic urine resulted in the highest NO_3^- levels compared to any other treatment. NO_3^- was significantly higher in P30 plots receiving synthetic urine treatment until the 8th October (Day 25) when NO_3^- in P0 peaked from Days 30–40 ($p < 0.05$). Soil NO_3^- was significantly higher in KNO_3 treatments than in $(\text{NH}_4)_2\text{SO}_4$ but there was no significant difference between P0 and P30.

3.4 | Grass yield and N-uptake

There was a significant interaction between treatment and P level on DM yield (DMY) and N-uptake ($p < 0.0001$) with no significant effect of treatment on DMY and N-uptake in the P0 soils, while in the P30 soils, DMY and N-uptake were significantly highest in the synthetic urine compared to the other treatments, which were not significantly different from one another. The effect of treatment on NUE was consistent with the P0 DMY and N-uptake results with no significant effects observed between treatments. In the P30 soils there was a significant effect of soil P on NUE observed in the KNO_3 (greatest efficiency) ($p = 0.033$) and synthetic urine (lowest efficiency) ($p = 0.001$) treatments, while NUE

TABLE 2 Soil biological properties (mean $\pm$ standard error, $n = 5$) at 0–10 cm soil depth sampling at the start of the experiment

Parameters	P0	P30
Microbial biomass carbon (mg kg^{-1})	856 ± 70.20^a	994 ± 57.8^a
Microbial biomass nitrogen (mg kg^{-1})	155.3 ± 15.7^a	178.5 ± 11.1^a
Microbial biomass phosphorus (mg kg^{-1})	20.9 ± 2.97^a	$26.895.93^a$
Potential denitrification ($\text{ng N g}^{-1} \text{ day}^{-1}$)	4.03 ± 0.28^a	4.37 ± 0.31^a
Potential nitrification (mg N kg day^{-1})	5.40 ± 1.18^a	13.4 ± 2.37^b
Substrate induced respiration ($\text{CO}_2\text{-C mg kg}^{-1}$)	9.55 ± 09.46^a	11.32 ± 1.49^a

Note: Different letters within each parameter indicate significant differences ($p < 0.05$) between long-term P treatments.

under $(\text{NH}_4)_2\text{SO}_4$ did not differ significantly to P0. NUE under synthetic urine treatments was nearly three times lower ($p < 0.001$) than that under KNO_3 and $(\text{NH}_4)_2\text{SO}_4$ treatments, with these treatments not differing significantly from each other under either P level ($p > 0.05$).

3.5 | Microbial analyses

There was no significant effect of P level on PDA ($p = 0.440$), however there was a significant effect of P level on PNR ($p = 0.017$), where nitrification potential was $5.4 \text{ N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ in P0 soils compared to an average of $13.4 \text{ N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ in P30 soils.

Neither MBC, MBN, nor MBP was significantly affected by P level ($p > 0.05$) (Table 2). Substrate induced respiration was not significantly affected by P level ($p = > 0.05$).

4 | DISCUSSION

4.1 | N_2O emissions pathways

4.1.1 | KNO_3 and $(\text{NH}_4)_2\text{SO}_4$

Cumulative N_2O was significantly greater in P0 soils receiving KNO_3 and $(\text{NH}_4)_2\text{SO}_4$ treatments than in P30 soils. N_2O emissions have frequently been reported to be higher from nitrate-containing fertilisers compared to urea or ammonium fertilisers due to the immediate availability of the nitrate substrate for denitrification in wet temperate grassland soils (Cowan et al., 2020;

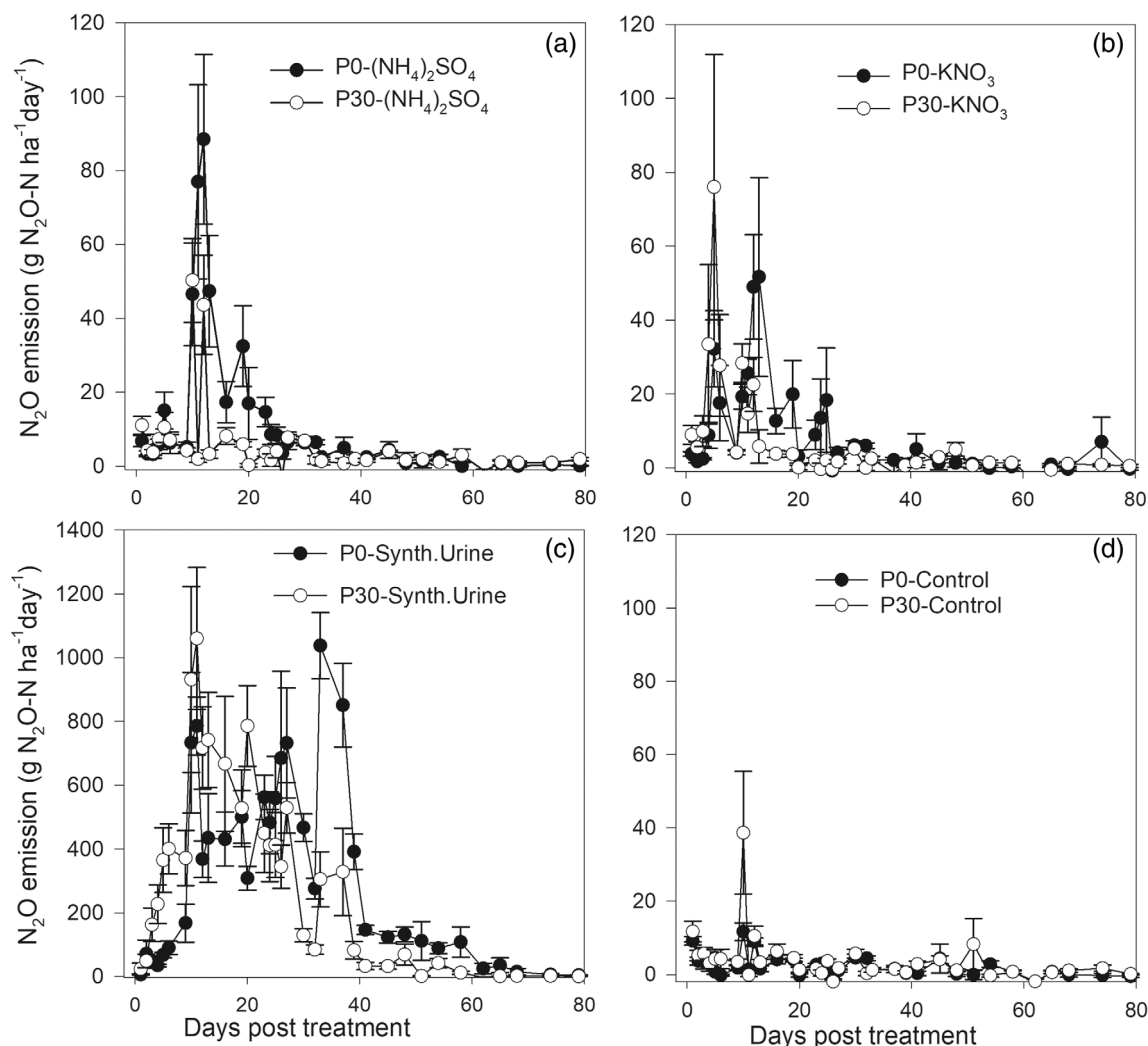


FIGURE 2 Temporal N_2O emissions ($\text{g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$) from (a) $(\text{NH}_4)_2\text{SO}_4$, (b) KNO_3 and (d) control (c) synthetic urine treatment in low P (P0 = Black) and high P (P30 = white) soils. Soil N_2O emissions increased following treatment application on Day 0. Emission peaks under the KNO_3 treatment were observed as soon as Day 4 in the P30 soils and Day 12 in the P0 soils and were initially 30% greater in the P30 soils but ultimately produced greater cumulative emissions in P0. Peaks under the $(\text{NH}_4)_2\text{SO}_4$ treatments were seen on days 10 and 11 in both P levels but were significantly highest in P0 soils. Emissions in the synthetic urine treatment *increased and peaked on day 10 in P30 soils but not until Day 32 in the P0 soils*.

Gebremichael et al., 2021; Harty et al., 2016; Krol et al., 2020; Rahman et al., 2021). Interestingly, although the current study was carried out under comparable conditions, our results contrasted with these studies, with the ammonium treatment ($(\text{NH}_4)_2\text{SO}_4$) yielding numerically (although not statistically significant) greater levels of N_2O than the KNO_3 treatment (Figure 3).

The potential nitrification activity for these soils showed the nitrification potential to be over three times greater in the P30 soils (Table 2). The potential denitrification activity was low with no significant difference between P0 and P30, suggesting that nitrification may be an important process occurring in these soils and that P-limitation may severely impede this. This is in agreement with a previous ^{15}N tracing study carried out on another

similar long-term P trial which found that heterotrophic nitrification was the dominant process rather than denitrification (O'Neill et al., 2021). There have been mixed results from other comparable studies with some finding nitrification to dominate over denitrification in N_2O production (Ambus, 1998; Liu, Hu et al., 2016; Roche et al., 2016), whereas others found the opposite (Harty et al., 2016; Krol et al., 2020; Rahman et al., 2021). Such studies were conducted on similar soil types, soil pH and with C:N ratios in the 10:1 range. Without any further discernible differences in soil type, temperature or moisture conditions evident, processes driving this variation in nitrification and denitrification importance are most likely related to nutrient-availability, other than C and N, determining N-cycling community composition and

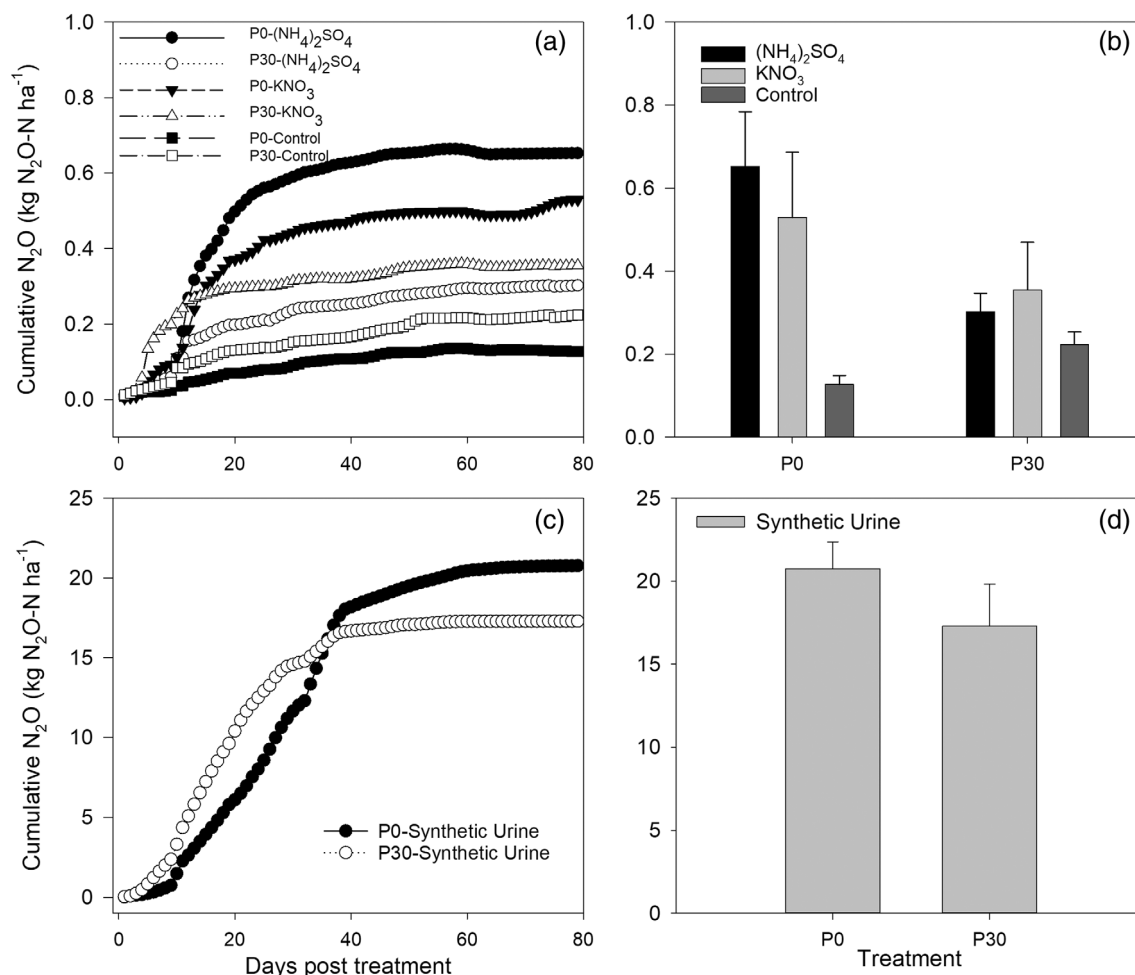


FIGURE 3 Cumulative N₂O (kg N ha⁻¹) under (a, b) control and KNO₃, and (NH₄)₂SO₄ addition; and (c, d) under synthetic urine addition ($n = 5$, means = $\pm$ SD). Cumulative N₂O emissions significantly decreased with increasing soil P level in the two fertiliser treatments (NH₄)₂SO₄ ($p = 0.0391$) and KNO₃ ($p = 0.0245$). There was no significant effect of soil P on cumulative N₂O emissions from synthetic urine ($p = 0.223$), but there was a significant interaction between P * time ($p < 0.001$). Synthetic urine N₂O emissions from P30 soils were significantly higher than those P0 soils for the first 12 days of the experiment and continued to be numerically higher until the point of crossover (Figure 3c) on day 35, where following day 35, N₂O from P0 increased and remained higher than N₂O from P30 soils for the remaining duration of the experiment.

function. This is reinforced by the effect of P on N-cycling with P-limitation found to increase N₂O emissions when not limited by carbon (O'Neill et al., 2020). P-limitation could therefore be a large cause of N-loss to the environment via lack of N-uptake from nutrient-deficient organisms as well as promoting the dominance of fungal communities more orientated to N₂O rather than N₂ production and which are more successful in nutrient poor conditions (Antunes et al., 2012; Maeda et al., 2015). The impact of added nutrients such as P and N to soils on N₂O emissions relies heavily on their pre-existing nutrient status. P-addition to a phosphorus-poor soil has been reported to enhance N-loss which was thought to be due to relieving this P-limitation such that N can be cycled (He & Dijkstra, 2015), while P-addition to an N-rich soil resulted in greater N₂O emissions especially with the

addition of glucose, which was attributed to enhanced N mineralisation and resulting denitrification by microbes (Anderson et al., 2022).

4.1.2 | Synthetic urine

In grazed pastures, urine deposition is the main source of N-cycling and loss, due to urine patches containing high inputs of N (mainly urea-N), and because urine increases the supply and turnover of labile soil organic N and C (Clough et al., 2003; Selbie et al., 2015). Urine N deposition may provide the optimal conditions for co-denitrification, i.e., the production of hybrid N₂ and N₂O products, when during sequential binding, a side reaction occurs between the initial electrophilic enzyme/N species

complex and a nucleophile (Spott et al., 2011). In a similar study, the formation of both hybrid N_2 and N_2O has been shown to be promoted by increases in pH as well as observing similar trends to this study in NO_3^- production (Selbie, Lanigan, et al., 2015). The observed delays in NO_3^- production in this study, while pH remains elevated, suggest a substantial loss of N during this period, due to inhibited uptake, either through N_2O emissions, run off or NH_3 volatilisation (Rahman et al., 2021; Saggar et al., 2013; Selbie, Buckthought, & Shepherd, 2015).

Selbie et al. (2015) also found that inhibiting nitrification chemically (dicyandiamide—DCD) resulted in a 55% reduction in hybrid N_2 emissions, but no change in “true” N_2 emissions, highlighting that nitrification can be a major source pool for co-denitrification. Several studies have found heterotrophic nitrification to be dominant, and to be driven by fungi rather than bacteria (Li et al., 2018; Zhu et al., 2015). This is further supported by work where fungal inhibition majorly reduced N_2O flux, with fungi proposed to be the principal microbial source of N-emissions arising from co-denitrification (Laughlin & Stevens, 2002; Long et al., 2013; Rex et al., 2018; Rex et al., 2019). AMF are known to be associated with nutrient poor environments and it has been found that under high P supply, root colonisation decreased, but under optimum P supply AMF was not affected (Liu, Zhang, et al., 2016). Similarly, AMF colonisation rates were found to decrease when N was limiting under P addition to nutrient poor soils but to increase when N was not limiting, showing there are interactive effects between N and P (Lin et al., 2020). As such, the high N-content in the synthetic urine may have boosted the AMF population in both P levels.

Fungi are resilient and thrive in harsher conditions than bacteria, for example, low nutrient environments, lower pH, substrates with wider C:N ratios (Antunes et al., 2012; Kawahara et al., 2016; Nottingham et al., 2018). They make up the dominant biomass in most soil systems, especially AMF, which concurrently are widely linked to soil P via their symbiotic relationship with the plant rhizosphere (Boswell et al., 2007; Laughlin & Stevens, 2002). Previous research carried out on this site has found evidence suggesting fungal abundance to be greater in the low P soils (Massey, 2012; King-Salter, 2008; Randall et al., 2019; Chen, 2012). Fungi lack N_2O reductase and can be major contributors to N_2O emissions (Maeda et al., 2015). However, they have also been reported to be the principal microbial source of N_2 emissions during co-denitrification (Laughlin & Stevens, 2002; Long et al., 2013). This study proposes fungi to be key drivers of N_2O emissions especially in the low P soils. With co-denitrification suggested to have a much greater contribution to N emissions than previously estimated, the role of fungi within this process is a key area to focus research on in the endeavour to promote N_2 production over N_2O .

4.2 | Dry matter yield and N-uptake

The lack of significant DMY and N-uptake from the P0 soils is in line with previous studies highlighting the effect that soil P-limitation has on herbage and crop yield (Sheil et al., 2016; Gebremichael et al., 2022). Both P0 and P30 soils showed over three times lower NUE from the synthetic urine treatment compared to KNO_3 and $(NH_4)_2SO_4$ treatments (Figure 6c). This highlights the

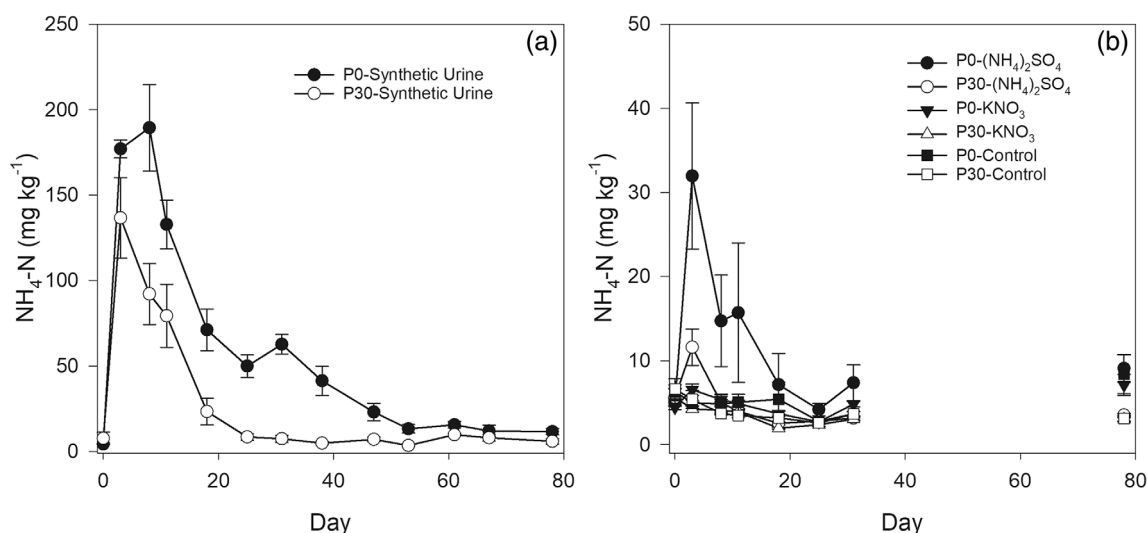


FIGURE 4 NH_4 -N levels in (a) synthetic urine plots and (b), KNO_3 , $(NH_4)_2SO_4$ and control plots in P0 (Black) and P30 (white) soils ($n = 5$, means = $\pm SD$)

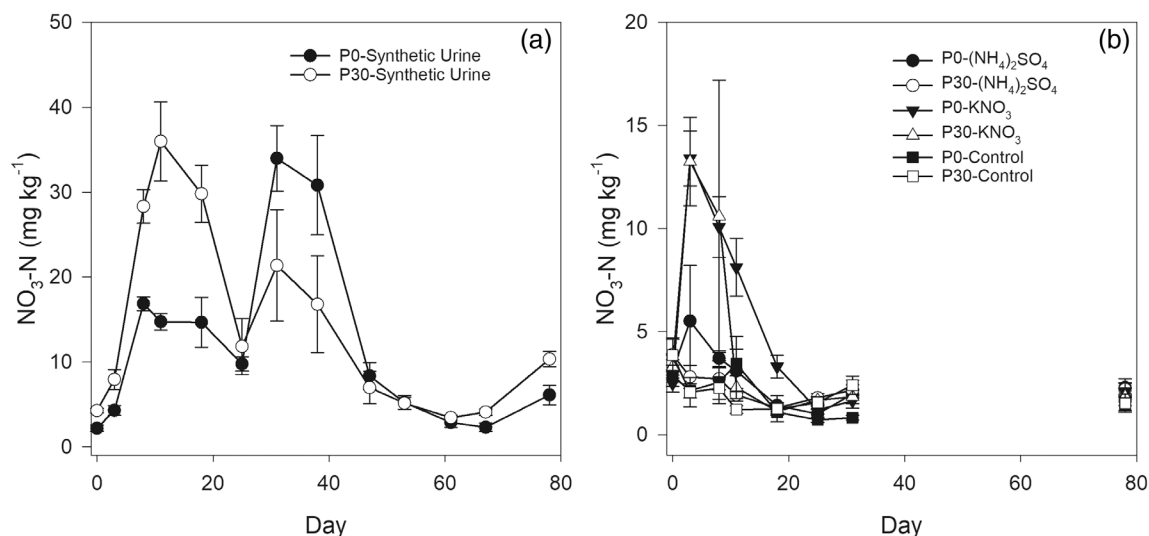


FIGURE 5 $\text{NO}_3\text{-N}$ levels in (a) synthetic urine plots, and (b) KNO_3 , $(\text{NH}_4)_2\text{SO}_4$ and control plots in P0 (Black) and P30 (white) soils ($n = 5$, means $\pm$ SD)

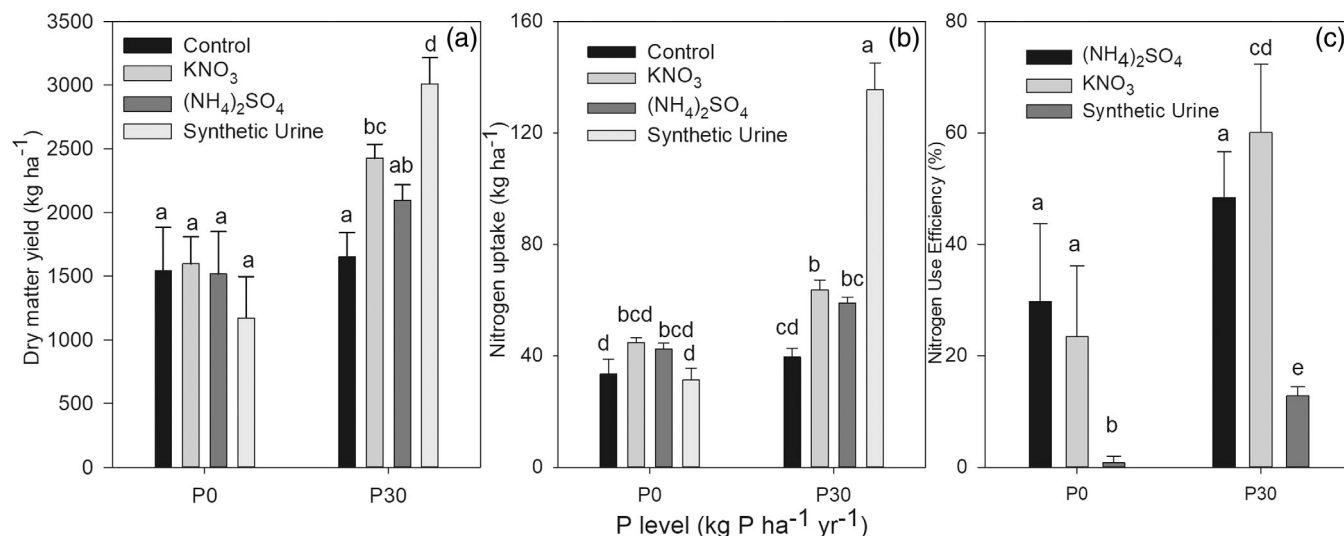


FIGURE 6 (a) Dry matter yield (kg DM ha^{-1}), (b) nitrogen uptake (kg N ha^{-1}), and (c) nitrogen use efficiency (%) (NUE) over the first 45 days after fertilisation for the control, KNO_3 , $(\text{NH}_4)_2\text{SO}_4$ and synthetic urine treatments at soil P0 and P30. Values that do not share a letter represent significant differences ($p < 0.05$) between fertiliser treatments within each P level and between P levels

disparity between yield and efficiency, showing the difference in N-uptake from soils receiving synthetic urine treatment compared to those receiving lower rates of N in the KNO_3 and $(\text{NH}_4)_2\text{SO}_4$ treatments.

This also demonstrates the variation between P and N availability, such that P0 soils from all treatments were saturated by high N availability and subsequently resulted in the greatest cumulative N_2O emissions and the lowest DMY, N-uptake and NUE. Whereas, P30 soils receiving a typical rate of 40 kg N ha^{-1} in the form of KNO_3 or $(\text{NH}_4)_2\text{SO}_4$ had the greatest NUE with no significant difference in their yield as well as the lowest cumulative N_2O losses. The high N-loading rate (750 kg N ha^{-1}) of the

synthetic urine treatment similarly demonstrates N-saturation resulting in the lowest NUE.

However, surprisingly, low productivity was also seen in P30 soils receiving $(\text{NH}_4)_2\text{SO}_4$, which were not significantly different from the control plots or from any of the P0 soils. This could be a result of greater nitrification in the P30 soils with microbes nitrifying the applied ammonium before it could be absorbed by plants, as plants are “poor competitors for available soil N relative to microbes” (Schimel & Bennett, 2004). This is reinforced by the ammonium concentration trends (rapidly depleted) observed in the P30 soils receiving $(\text{NH}_4)_2\text{SO}_4$, compared to those observed in the P0 soils under this

same treatment (Figure 4b). Furthermore, in P30 soils this drop in ammonium levels corresponds to the same time (Day 9) a peak can be observed in N_2O emissions (Figure 2a), which, with regard to the lack of nitrate being produced under this treatment (Figure 5b), most likely are arising from contributions to N_2O either from heterotrophic nitrification, or co-denitrification pathways (Selbie et al., 2014). Previous findings by O'Neill et al. (2021) reported the rate of NH_4^+ immobilisation to be over five times greater than the rate of NO_3^- immobilisation, and over 95% higher than mineralisation rates, in a similar soil type. This could be a result of positively charged NH_4^+ being easier to absorb to negatively charged soil colloids than NO_3^- , making it more available to microorganisms (Zhang & Elser, 2017), NH_4^+ is also known to be the preferred N source for the soil microbial community (Reay et al., 2019), with NO_3^- being reported to give a preferential growth response for plants (Chen et al., 2016; Liu et al., 2014). Thus, this may offer an explanation as to why plant N-uptake was greater under the KNO_3 treatment in the P30 soils, as there may have been less competition by microbes and may have been absorbed quickly by "rapid mass flow" to the plants' rhizosphere (Boudsocq et al., 2012; Hachiya and Sakakibara, 2017).

4.3 | Effect of soil pH on synthetic urine N_2O emissions

Urea deposition via urine/fertilisers has been observed to result in elevated pH (due to hydrolysis reactions) and inorganic N concentrations, which can stimulate the microbial community and drive N losses (Monaghan & Barraclough, 1993). This elevated pH and high ammonia concentrations which occur after urine deposition can inhibit oxidation of NO_2 to NO_3^- (Clough et al., 2003; Li et al., 2018; Monaghan & Barraclough, 1992), as well as disrupting N_2O reductase during the denitrification stage (Stevens et al., 1998; Wang et al., 2018; Zurovec et al., 2021). This may explain the contrasting response between P levels. P0 soils, which were already significantly more acidic prior to urine amendment than the P30 (Table 1), experienced a large significant ($\sim 20\%$) increase in pH ($p < 0.001$) immediately post application (Day 1), as opposed to the P30 soils which did not increase from the control plots in pH ($p = 0.11$) (Figure S1). The pH of both P30 and P0 soils dropped two orders of magnitude from a maximum pH of ~ 5.5 to a low of pH ~ 3 by Day 50, which was consequently 41% and 44% lower than the pre-treatment values in the P0 and P30 soils, respectively (Figure S1). The beginning of this drop in pH coincided with the point of crossover in N_2O emissions from

synthetic urine plots (Figures 3c and S1), where at this point P0 emissions overtook those arising from P30 soils on Day 32 of the trial. Thus, this contrasting response between P0 and P30 soils may be explained more by the change in pH than the pH itself, with the P0 microbial community adapted to their slightly more acidic pH levels, and experiencing more severe inhibition due to the sudden change in conditions, with activity (N_2O emissions) increasing once pH levels returned to pre-application levels.

The lack of increase in pH observed post synthetic urine amendment in the P30 soils compared to the P0 soils further suggests that nitrification was not inhibited, which is supported by the mineral N trends (Figure 4a, 5a).

Although, it was previously assumed that nitrification in acidic mineral soils was relatively low (De Boer & Kowalchuk, 2001; Robertson, 1989), many recent studies have suggested that nitrification can occur at pH values as low as 3.0 (Li et al., 2018; Norton & Stark, 2011) and that rates of nitrification based on mineralised organic nitrogen can be equal or even greater than that typically found in neutral pH soils (Booth et al., 2005). Recent work on a similar soil type has found that nitrification, if not dominant, is frequently at least an equal contributor to N_2O fluxes (Bracken et al., 2021).

The trends observed in this study were over a short period of time on one soil type. Future studies should investigate processes over long time periods and various soil types. The effect of CNP cycling on soil microbial processes and functions should be investigated. The effect of soil P on the full greenhouse gas balance and soil carbon sequestration should be further investigated to ensure a holistic assessment is conducted on the role that P fertilisation can play in achieving the target of carbon neutral farming in the future.

5 | CONCLUSION

This study found that N_2O emissions were reduced by over 50% from KNO_3 and $(\text{NH}_4)_2\text{SO}_4$ fertilisers and by over 20% from urine when soil P increased from 0 to 30 Kg P ha^{-1} . The absence of soil P also resulted in reduced plant N-uptake and potential effects on the soil microbial community. With less N being incorporated into grass growth under the low P treatments, this enables greater N-availability to N-cycling communities, and thus the subsequent increase in N_2O emissions. However, the lack of P could be altering the structure and function of such communities by inhibiting their ability to fully uptake N, or driving community composition to be more fungal dominated both of which can result in N-losses to the environment if plants themselves are too P-limited to

incorporate this N into their biomass. There was higher plant N-uptake from the nitrate fertiliser than from the ammonium fertiliser indicating lower ammonium availability to plants as a result of greater ammonium immobilisation and nitrification by soil microbes. Increasing soil P, and knowing how and where applied N is being used, is a win-win solution for farmers to balance food production with reducing greenhouse gas emissions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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