



# Nitrous oxide and methane fluxes from plasma-treated pig slurry applied to winter wheat

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**Abstract** The use of livestock waste as an organic fertiliser releases significant greenhouse gas emissions, exacerbating climate change. Innovative fertiliser management practices, such as treating slurry with plasma induction, have the potential to reduce losses of carbon and nitrogen to the environment. The existing research on the effectiveness of plasma-treated slurry at reducing nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) emissions, however, is not comprehensive, although must be understood if this technology is to be utilised on a large scale. A randomised block experiment was conducted to

measure soil fluxes of N<sub>2</sub>O and CH<sub>4</sub> from winter wheat every two hours over an 83-day period using automated chambers. Three treatments receiving a similar amount of plant-available N were used: (1) inorganic fertiliser (IF); (2) pig slurry combined with inorganic fertiliser (PS); (3) plasma-treated pig slurry combined with inorganic fertiliser (TPS). Cumulative N<sub>2</sub>O fluxes from TPS (1.14 g N m<sup>-2</sup>) were greater than those from PS (0.32 g N m<sup>-2</sup>) and IF (0.13 g N m<sup>-2</sup>). A diurnal pattern in N<sub>2</sub>O fluxes was observed towards the end of the experiment for all treatments, and was driven by increases in water-filled pore space and photosynthetically active radiation and decreases in air temperature. Cumulative CH<sub>4</sub> fluxes from PS (3.2 g C m<sup>-2</sup>) were considerably greater than those from IF (-1.4 g C m<sup>-2</sup>) and TPS (-1.4 g C m<sup>-2</sup>). The greenhouse gas intensity of TPS (0.2 g CO<sub>2</sub>-eq kg grain<sup>-1</sup>) was over twice that of PS (0.07 g CO<sub>2</sub>-eq kg grain<sup>-1</sup>) and around six times that of IF (0.03 g CO<sub>2</sub>-eq kg grain<sup>-1</sup>). Although treating pig slurry with plasma induction considerably reduced CH<sub>4</sub> fluxes from soil, it increased N<sub>2</sub>O emissions, resulting in higher non-CO<sub>2</sub> emissions from this treatment. Life-cycle analysis will be required to evaluate whether the upstream manufacturing and transport emissions associated with inorganic fertiliser usage are outweighed by the emissions observed following the application of treated pig slurry to soil.

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## Introduction

Nitrogen (N) is one of the most limiting nutrients for crop growth in agricultural soils, so organic (i.e., animal manure and slurry) and inorganic (i.e., synthetic) N fertilisers are applied to provide a supply of N to support crop growth and achieve high yields (Lu et al. 2021). Organic fertilisers also provide a source of other plant nutrients, enhance soil carbon (C) content, and are increasingly being seen as part of an on-farm circular economy within the agricultural sector. The use of fertilisers in agriculture results in significant emissions of greenhouse gases (GHGs) to the atmosphere. Agriculture is responsible for 13% global carbon dioxide (CO<sub>2</sub>) emissions, 50% global methane (CH<sub>4</sub>) emissions, and 60% global nitrous oxide (N<sub>2</sub>O) emissions (Macharia et al. 2020). Nitrous oxide and CH<sub>4</sub> are of particular concern, as they have global warming potentials 273 and 27.9 times greater than CO<sub>2</sub> respectively (Smith et al. 2021) and continue to exacerbate climate change (Mikhaylov et al. 2020). Agricultural N<sub>2</sub>O emissions primarily originate from the use of inorganic and organic N fertilisers, which has increased markedly over the last 60 years (Rudaz et al. 1999; Cameron et al. 2013; Lu et al. 2021). Between 2016 and 2019, animal farming in the European Union produced more than 1.4 billion tonnes of manure annually, and over 90% of this was directly re-applied to soils (Koninger et al. 2021). Fertiliser application, particularly organic fertiliser, can also increase CH<sub>4</sub> emissions; CH<sub>4</sub> is often produced during organic fertiliser storage, as the C supply and storage conditions facilitate methanogenesis, dissolving CH<sub>4</sub> into the fertiliser and releasing it upon application to soil (Rochette and Cote 2000; Bastami et al. 2016).

There is an urgent need to minimise the negative impacts of agriculture on the environment, with the aim to achieve net zero GHG emissions becoming increasingly critical (Sakrabani et al. 2023). Despite the implementation of strategies which aim to reduce environmental N pollution (i.e., Nitrate Vulnerable Zones (UK Government 2021) and 4R

Nutrient Stewardship — right source, rate, time and place (Nutrient Stewardship 2017)), GHG emissions from agriculture, particularly N<sub>2</sub>O, remain high (Tian et al. 2020). To reduce GHG emissions from fertiliser use, crop N use efficiency (NUE) — the efficiency at which applied N is assimilated by plants (Sharma and Bali 2018) — must be improved. Given the push to increase the use of livestock waste as fertiliser and build soil C, a range of practices and innovative technologies are promoted to reduce GHG emissions from fertiliser use and improve NUE. One such example of this is the treatment of organic fertilisers, such as pig slurry, with plasma induction. This treatment primarily aims to reduce losses of the non-GHG ammonia (NH<sub>3</sub>) by ionising air to form reactive nitrogen gas which is absorbed into the slurry, creating an N-rich slurry (Nyang'au et al. 2024). This process lowers the pH of the slurry and reduces the potential for NH<sub>3</sub> emissions (Nyang'au et al. 2024). An increase in the N content of the plasma-treated slurry means the product has the potential to replace synthetic inorganic fertiliser and has been shown to increase yields compared to untreated slurry (Mousavi et al. 2022; Cottis et al. 2023), as well as reducing both CH<sub>4</sub> and NH<sub>3</sub> emissions during storage (Graves et al. 2018). Whether the beneficial gains of increasing the amount of inorganic N available for immediate plant uptake are counterbalanced by other N losses upon application to the soil, such as N<sub>2</sub>O to the atmosphere, however, are unknown. Numerous studies have investigated the impacts of fertiliser application on GHG fluxes, mainly N<sub>2</sub>O, from agricultural soils (Inselsbacher et al. 2010; Mateo-Marin et al. 2020; Adekun et al. 2021). The overarching consensus is that soils amended with organic fertiliser have higher N<sub>2</sub>O and CH<sub>4</sub> emissions than those amended with inorganic fertiliser (Thangarajan et al. 2013; Walling and Vaneeckhaute 2020; He et al. 2023). The effects of using plasma-treated slurry as an organic fertiliser on soil N<sub>2</sub>O and CH<sub>4</sub> emissions is relatively unknown, however, and most of the existing research on plasma-treated organic waste has focused on the effects of plasma-treated cattle slurry on crop yield, soil biota and NH<sub>3</sub> emissions (Mousavi et al. 2022; 2023; Cottis et al. 2023). If plasma-treated pig slurry is to become a potential solution to reduce non-CO<sub>2</sub> GHG emissions, it will be necessary to explore the extent to which it can achieve this relative to non-treated pig slurry and inorganic fertiliser.

The aim of this study was therefore to determine the effects of treating pig slurry with plasma induction on  $N_2O$  and  $CH_4$  fluxes and crop yield when applied as an organic fertiliser. This was achieved by carrying out the following objectives: (1) measure and analyse the response of  $N_2O$  and  $CH_4$  fluxes to the application of inorganic and organic fertilisers, including plasma-treated and non-treated pig slurry; (2) compare winter wheat yield and its GHG intensity as a result of the fertiliser treatment used; and (3) quantify and explain the controls on the diurnal variation of  $N_2O$  and  $CH_4$  fluxes during the main winter wheat growth phase. Treating pig slurry with plasma-induction has been proven to reduce  $NH_3$  emissions as a result of acidification, creating an N-enriched product which has a higher content of inorganic N. Furthermore, a reduction in the pH of the slurry may prevent methanogenesis and thus  $CH_4$  formation during slurry storage, and thus potentially following application. Therefore, our first hypothesis is that non- $CO_2$  GHG emissions will be lower from the plasma-treated pig slurry compared to the non-treated pig slurry. Based on the existing research on GHG emissions and the impact of fertiliser type, our second hypothesis is that  $N_2O$  and  $CH_4$  emissions will be higher from winter wheat treated with organic fertilisers (i.e., plasma-treated and non-treated pig slurry treatments) compared to inorganic fertiliser, as a result of increasing C and N availability to soil microorganisms, thus increasing their activity.

## Materials and methods

### Field site and experimental design

The University of Leeds Research Farm is a commercial mixed arable and livestock farm near Tadcaster, UK. It has a temperate climate with mild winters and warm summers (Beck et al. 2018). The soil is a well-drained, loamy calcareous Cambisol (Cranfield University 2018), with a depth of 0.5–0.9 m (Holden et al. 2019). Soil properties of the study site are summarised in Table S1. Between 1992 and 2021 mean annual temperature  $\pm$  standard deviation was  $9.5 \pm 1$  °C (Met Office 2019) and mean annual precipitation was  $639 \pm 142$  mm (Met Office 2006). During the study period (20/03/2022–13/06/2022), drought conditions and record maximum temperatures were

experienced in the UK (Turner 2022) (Figure S1); total precipitation was 112 mm and average daily air temperature was 10.7 °C (527 mm lower and 1.2 °C higher than the annual average). On 21/10/2021, winter wheat (WW) (*Triticum aestivum*), Extase variety, was sown at a density of 440 seeds  $m^{-2}$  in an arable field (53° 51' 56.26" N 1° 19' 28.22" W; 10.4 ha; elevation 49 m). In February 2022, prior to the application of any fertiliser, a randomised block experiment was set up consisting of nine plots (2×0.5 m) and neighbouring areas for the placement of nine GHG measurement chambers. Circular collars (0.5 m diameter) were inserted into the soil to a depth of 0.1 m and Eosense eosAC-LT chambers (Eosense Canada no date) with an internal volume of 0.072  $m^{-3}$  were attached one month prior to fertiliser application. This allowed the soil to return to steady state conditions prior to the commencement of GHG measurements (Charteris et al. 2020).

Three fertiliser treatments (each with three replicates) were compared (Table S2): three applications of inorganic fertiliser (IF); two applications of pig slurry followed by two applications of inorganic fertiliser (PS); and two applications of plasma-treated pig slurry followed by two applications of inorganic fertiliser (TPS). Each plot and its neighbouring GHG chamber received the same fertiliser treatment; fertiliser was applied to the plots and chambers in split applications, the rates based on recommendations from MANNER-NPK (ADAS 2013). All fertiliser treatments were applied by hand; granular fertiliser was evenly distributed onto the soil surface and slurry was applied with a watering can, taking care to apply slurry only to the soil surface and not on WW leaves. The treatments were applied with the intention of all plots receiving a total of 220 kg available N  $ha^{-1}$ . Following analysis of the fertilisers, it was confirmed that the IF and PS treatments received a total of 220 kg available N  $ha^{-1}$ , whereas the TPS treatment received 253 kg available N  $ha^{-1}$ . More detail on application types, rates and dates are shown in Table S2. For PS and TPS, pig slurry was collected from an on-farm indoor pig facility and for TPS the pig slurry was then treated using plasma induction. The plasma treatment process uses electricity to ionise air and create nitrogen oxide gas, which combines with free  $NH_3$  to form involatile ammonium nitrate, thus reducing  $NH_3$  emissions and increasing the amount of inorganic N potentially available for immediate plant uptake upon

application to the crop (Graves et al. 2018; Nyang'au et al. 2024). This may in turn reduce the amount of N available for conversion to  $N_2O$ , thus reducing  $N_2O$  emissions, however this is highly dependent on the environmental conditions and the crop type and growth stage. The plasma induction process also prevents the conditions which facilitate methanogenesis and reduces the pH of the slurry, reducing  $CH_4$  production in storage and thus  $CH_4$  emissions upon application (Tooth et al. 2021). The nutrient composition of the organic fertiliser treatments is shown in Table S3. The IF treatment received no inputs of phosphorous or potassium, whereas the PS and TPS treatments did (Table S3), however this is unlikely to have limited the growth of wheat as the soil has a phosphorus index of 3 in the top 10 cm, and thus is not limited in the soil (Table S1).

Soil moisture and temperature were measured in each plot at a depth of 0.05 m using TEROS 11 moisture and temperature sensors (METER Group Inc. USA no date), with measurements logged at 15-min intervals. Soil moisture and bulk density were used to calculate water-filled pore space (WFPS) according to Eq. (1), adapted from De and Toor (2015):

$$WFPS(\%) = ((\theta g \times Bd) \div (1 - (Bd - Pd))) \times 100 \quad (1)$$

where  $\theta$  g is soil moisture (%), Bd is bulk density ( $g\ cm^{-3}$ ) and Pd is particle density ( $g\ cm^{-3}$ ) (assumed to be  $2.65\ g\ cm^{-3}$  for arable soils (Schjonning et al. 2017)).

#### GHG sampling and crop yield measurements

Fluxes of  $N_2O$ ,  $CH_4$  and  $CO_2$  were measured from each chamber every 120-min between 20/03/2022 and 13/06/2022 using a Picarro G2508 GHG analyser (Picarro USA no date), resulting in 9288 discrete sampling points over 83-days. The analyser uses cavity ring-down spectroscopy to measure GHG fluxes; the measurement range of  $N_2O$  is 0.3–200 ppm, of  $CH_4$  is 1.5–12 ppm and of  $CO_2$  is 180–5000 ppm (Picarro USA no date). Chamber measurements were planned to continue until harvest, however extreme temperatures caused instrument failure, so GHG measurements ceased ~6 weeks before harvest. An Eosense eosMX-P multiplexer (Eosense Canada no date) and eosLink-AC software (Eosense Canada no date) allowed each chamber to be sampled in turn.

Chambers were programmed to close (i.e., sample) for 7-min each on a continuous loop sequence. On 25/04/2022, vertical extensions (0.7 m height) were attached between the chamber collar and lid to accommodate the growing crop, increasing the internal chamber volume to  $0.209\ m^{-3}$ . The accumulation time of the chambers was then increased from 7 to 10-min in accordance with the increased chamber volume.

Winter wheat was harvested from within chamber collars and from a  $0.5\ m^2$  quadrat within each neighbouring plot on 27/07/2022. Harvesting was carried out by hand, cutting the stems 0.1 m above the soil surface. The harvested WW was weighed before and after drying at  $60\ ^\circ C$  for 24 h to determine its moisture content. At harvest the WW had an average moisture content  $\pm$  standard deviation of  $13.2 \pm 3.2\%$ . The dried WW was threshed using a HALDRUP LT-21 laboratory thresher (HALDRUP Germany no date), providing grain, chaff and stalk samples which were ground and analysed for C and N content using a Vario EL Cube elemental analyser (Elementar UK no date) according to Pella (1990a, b). Separately, filtration and digestion methods were used to calculate grain N content (Ministry of Agriculture, Fisheries and Food 1973) which was multiplied by 5.7 to calculate grain protein content (Sosulski and Imafidon 1990; Ma et al. 2019). Harvest index, or total WW biomass as grain, was calculated according to Eq. (2) (Amanullah 2016):

$$Harvest\ index(\%) = (grain\ yield \div total\ DM\ yield) \times 100 \quad (2)$$

#### Data processing

Greenhouse gas fluxes were calculated using bespoke software for the Eosense chamber system (eos-AnalyzeMX/AC V3.5.0, Eosense Canada no date); a linear fit was adjusted to the raw concentration of  $CO_2$  by identifying the start and end of each measurement, which was then used to calculate fluxes of all gases for each sampling point (Petraakis et al. 2017; Barba et al. 2019). Outliers were identified using a modified version of the method by Elbers et al. (2011) which quantifies the uncertainty of  $CO_2$  fluxes based on the threshold detection value ( $u^*$ ), statistical screening, measurement errors, and uncertainties associated with flux calculations. Measurements of  $CO_2$ , and

associated  $N_2O$  and  $CH_4$ , identified as outliers (261 sampling points) were then removed. Gaps in the data, either due to instrument failure during the measurement period or as a result of outlier removal were then gap-filled. Missing  $N_2O$  and  $CH_4$  data between 20/03/2022 and 13/06/2022 were gap-filled using linear interpolation and missing daytime and night-time  $CO_2$  data between 20/03/2022 and 13/06/2022 were gap-filled separately using linear regression (Dorich et al. 2020; Lucas-Moffat et al. 2022). Thirty-three percent of the data were gap-filled. Complete gap-filled data were analysed using The R Language and Environment for Statistical Computing V4.1.3 (R Core Team 2021). As one flux measurement was made per chamber every 2-h, measurements were converted from  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $CO_2$ ) or  $\text{nmol m}^{-2} \text{s}^{-1}$  ( $N_2O$  and  $CH_4$ ) to  $\text{g C m}^{-2}$  ( $CO_2$  and  $CH_4$ ) or  $\text{g N m}^{-2}$  ( $N_2O$ ) and daily averages were calculated. Cumulative  $CO_2$ ,  $N_2O$  and  $CH_4$  fluxes were converted to  $CO_2$ -equivalent ( $\text{g m}^{-2} \text{day}^{-1}$ ) by multiplying these gases by their GWP; 273 for  $N_2O$  and 27.9 for  $CH_4$  (Smith et al. 2021).

Greenhouse gas intensity (GHGI) was calculated according to Eq. (3) (adapted from Mosier et al. (2006) and Guo et al. (2022):

$$GHGI(\text{kg } CO_2\text{equivalent kg grain}^{-1}) = E_D \div Y \quad (3)$$

where  $E_D$  is the cumulative  $CO_2$ -equivalent emissions from each fertiliser treatment over the measurement period (i.e.,  $N_2O + CH_4$ ;  $\text{kg } CO_2\text{-equivalent ha}^{-1}$ ) and  $Y$  is grain yield from each fertiliser treatment plot ( $\text{kg ha}^{-1}$ ).

Throughout the paper, GHGIs are based on emissions recorded during the measurement period of this study; we acknowledge that these will not be GHGIs for the entire WW growing season.

Nitrogen use efficiency is the percentage of total N recovered by a plant at harvest (Scottish Government 2023); NUE of the whole crop ( $NUE_{\text{total}}$ ) and grain ( $NUE_{\text{grain}}$ ) were calculated according to Eq. (4) and (5):

$$NUE_{\text{total}}(\%) = (N \text{ output} \div N \text{ input}) \times 100 \quad (4)$$

where  $N$  output is  $N$  content of whole crop ( $\text{kg N ha}^{-1}$ ) and  $N$  input is total  $N$  added via fertiliser ( $\text{kg N ha}^{-1}$ ).

$$NUE_{\text{grain}}(\%) = (N \text{ output} \div N \text{ input}) \times 100 \quad (5)$$

where  $N$  output is  $N$  content of grain ( $\text{kg N ha}^{-1}$ ) and  $N$  input is total  $N$  added via fertiliser ( $\text{kg N ha}^{-1}$ ).

Normality tests were conducted using the Shapiro–Wilk method. Tests for statistically significant differences of mean daily and mean cumulative GHG emissions between each fertiliser treatment were conducted using Kruskal–Wallis and Wilcoxon tests as all data followed a non-normal distribution. Tests for significant differences of average WW dry matter (DM) yield, grain yield, total and grain C and N content, and grain protein content between each treatment were conducted using Kruskal–Wallis and Wilcoxon or ANOVA and Tukey tests dependent on the normality of the data. Multiple linear regression (MLR) was used to investigate the impact of environmental factors (i.e., precipitation, air temperature, soil temperature (0.05 m), WFPS and photosynthetically active radiation (PAR)) on  $N_2O$  and  $CH_4$  fluxes for each treatment. Prior to conducting MLR, a correlation matrix was used to assess for collinearity between the environmental variables. There was strong collinearity between soil temperature and air temperature (0.77); MLR showed a higher  $R^2$  value when air temperature was included compared to when soil temperature was included, so soil temperature was removed from MLR to remove the potential effects of collinearity. When considering the dataset excluding the 0–7 days after the first two fertiliser applications, the  $R^2$  value was higher when soil temperature was included compared to when air temperature was included, so for this analysis air temperature was removed from MLR.

## Results

Cumulative  $N_2O$  fluxes were highest from TPS and lowest from IF, and cumulative  $CH_4$  fluxes were highest from PS and lower from IF and TPS (Table 1; Figure S2). Despite lower  $CH_4$  fluxes from TPS compared to PS,  $N_2O$  fluxes were highest from TPS, meaning that total non- $CO_2$  fluxes were highest from TPS compared to PS, disproving our first hypothesis. Our second hypothesis is proven by the IF treatment having lower non- $CO_2$  GHG emissions than the organic fertiliser treatments (i.e., TPS and PS). The response of the non- $CO_2$  fluxes to the fertiliser

**Table 1** Mean daily and mean cumulative fluxes, and mean GHGI over the 83-day measurement period  $\pm$  standard deviation (SD) for each fertiliser treatment (*IF* inorganic fertiliser, *PS* pig slurry, *TPS* treated pig slurry)

		IF	PS	TPS
N <sub>2</sub> O	Mean daily $\pm$ SD (g N m <sup>-2</sup> day <sup>-1</sup> )	0.002 $\pm$ 0 a	0.004 $\pm$ 0 b	0.013 $\pm$ 0 a
	Mean cumulative $\pm$ SD (g N m <sup>-2</sup> )	0.13 $\pm$ 0 a	0.32 $\pm$ 0.1 a	1.14 $\pm$ 0.1 a
	Mean daily 0–7 days after first two fertiliser applications $\pm$ SD (g N m <sup>-2</sup> day <sup>-1</sup> )	0.004 $\pm$ 0 a	0.013 $\pm$ 0 b	0.068 $\pm$ 0 c
CH <sub>4</sub>	Mean daily $\pm$ SD (g C m <sup>-2</sup> day <sup>-1</sup> )	-0.0003 $\pm$ 05.8e-05 a	0.0004 $\pm$ 0.0006 a	-0.0003 $\pm$ 0.0001 a
	Mean cumulative $\pm$ SD (g C m <sup>-2</sup> )	-1.4 $\pm$ 0.3 a	3.2 $\pm$ 1.4 a	-1.4 $\pm$ 0.6 a
	Mean daily 0–7 days after first two fertiliser applications $\pm$ SD (mmol CH <sub>4</sub> m <sup>-2</sup> day <sup>-1</sup> )	-0.0002 $\pm$ 0 a	0.004 $\pm$ 0 b	-0.0001 $\pm$ 0 a
CO <sub>2</sub> -eq (N <sub>2</sub> O+CH <sub>4</sub> )	Mean cumulative $\pm$ SD (g CO <sub>2</sub> -eq m <sup>-2</sup> )	34.2 $\pm$ 7.6 a	88.8 $\pm$ 14.3 a	311.7 $\pm$ 34.9 a
	Mean GHGI $\pm$ SD (g CO <sub>2</sub> -eq kg grain <sup>-1</sup> )	0.03 $\pm$ 0.005 a	0.07 $\pm$ 0.02 a	0.2 $\pm$ 0.02 a

Across each row, different letters indicate significant differences in the variable of interest between fertiliser treatments

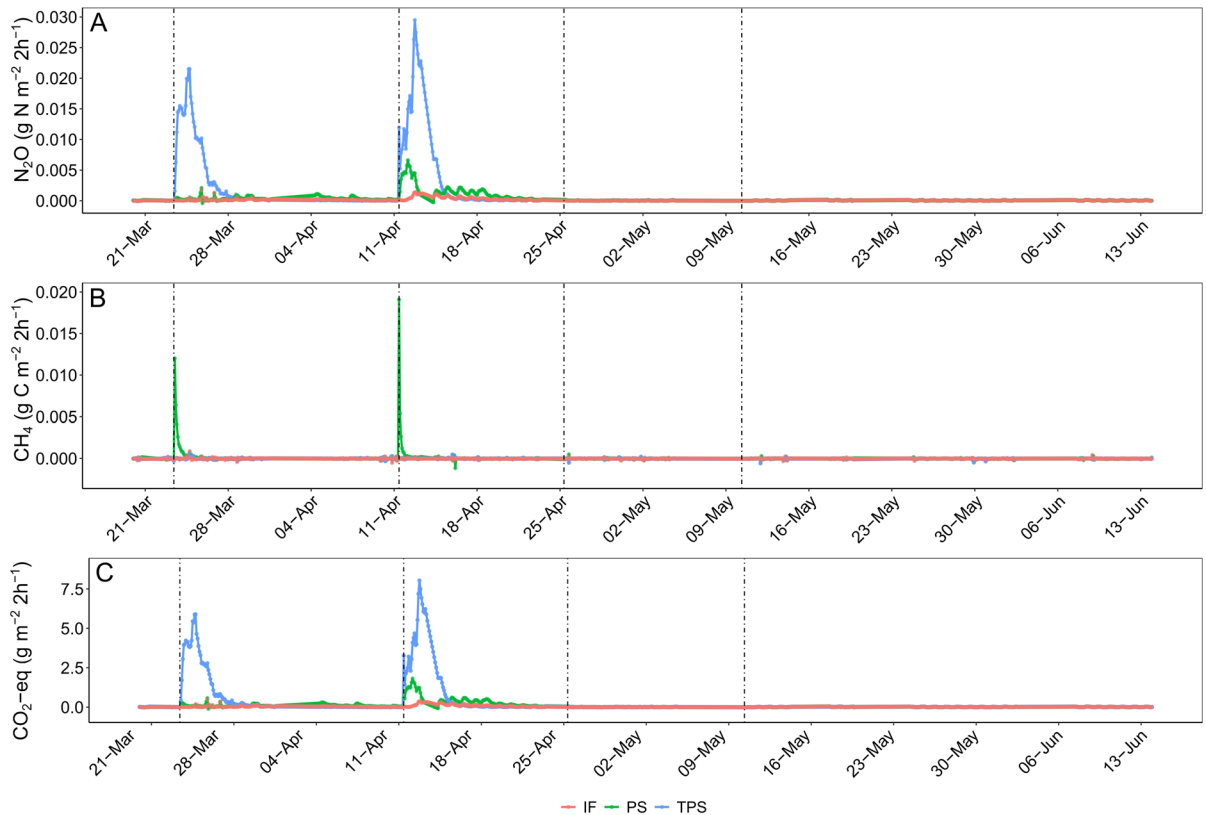
treatments is discussed in more detail below. Cumulative CO<sub>2</sub> fluxes were highest from PS and lowest from IF, and were significantly different between PS and IF but not between PS and TPS or IF and TPS (Table S4). Further results on CO<sub>2</sub> fluxes, including mean daily and cumulative CO<sub>2</sub> fluxes, and diurnal CO<sub>2</sub> fluxes for each treatment over each WW growth stage are presented in Figures S2, S3, S4 and S5. These data are not presented as main results as non-CO<sub>2</sub> GHG fluxes are the focus of this study. CO<sub>2</sub>-equivalent fluxes of N<sub>2</sub>O and CH<sub>4</sub> were highest from TPS and lowest from IF (Table 1; Figure S2).

### N<sub>2</sub>O fluxes

Cumulative N<sub>2</sub>O fluxes were highest from TPS and lowest from IF and were not significantly different between treatments (Table 1; Figure S2). Nitrous oxide fluxes increased with increasing WFPS, air temperature and the application of pig slurry and treated pig slurry ( $P < 0.05$ ), and decreased with increasing PAR ( $P < 0.05$ ) (Figures S6 and S7). When treated pig slurry was applied, significant interactions were observed between N<sub>2</sub>O fluxes, WFPS, air temperature and PAR ( $P < 0.05$ ) (Figure S7). Precipitation did not significantly influence N<sub>2</sub>O fluxes ( $P = 0.42$ ). Mean daily N<sub>2</sub>O fluxes were highest from TPS and lowest from IF and were significantly different between IF and PS ( $P = 0.004$ ) and IF and TPS ( $P = 0.03$ ) but not between PS and TPS ( $P = 0.82$ ) (Table 1). Nitrous oxide fluxes increased following the first fertiliser application to TPS and

following the second fertiliser applications to PS and TPS, peaking one day after application and decreasing over five to fourteen days before returning to pre-fertilisation levels (Figs. 1 and 2). Nitrous oxide fluxes from TPS and PS did not respond to the third and fourth fertiliser applications, which were in the form of inorganic fertiliser and contained less N than the previous two applications which were in the form of organic fertiliser (Figs. 1 and 2; Table S3). Nitrous oxide fluxes from IF did not respond to any of the fertiliser applications (Figs. 1 and 2). When considering N<sub>2</sub>O fluxes from within seven days of the first two fertiliser applications only (i.e., when organic fertilisers were added to TPS and PS) (Fig. 3), mean daily N<sub>2</sub>O fluxes were highest from TPS and lowest from IF and were significantly different between all treatments ( $P < 0.05$ ) (Table 1).

Diurnal variations in N<sub>2</sub>O fluxes were identified throughout the measurement period, apart from within 0 to 7 days of the first two fertiliser applications (i.e., when organic fertilisers were applied to PS and TPS and thus N<sub>2</sub>O flux activity was at its maximum). Therefore, to better understand the controls on the diurnal fluxes of N<sub>2</sub>O, data from days 0 to 7 after the first two fertiliser applications were excluded from further analysis. Following this removal, an increase in WFPS and PAR were found to increase N<sub>2</sub>O fluxes; however N<sub>2</sub>O fluxes decreased with increasing soil temperature (Figure S8). There was no significant effect of precipitation on N<sub>2</sub>O fluxes ( $P > 0.05$ ). Significant interactions ( $P < 0.05$ ) were identified between pig slurry application and



**Fig. 1** 2-h fluxes of **A**  $\text{N}_2\text{O}$ , **B**  $\text{CH}_4$  and **C**  $\text{CO}_2$ -equivalent fluxes of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  for each fertiliser treatment (*IF* inorganic fertiliser, *PS* pig slurry, *TPS* treated pig slurry). Each data point represents the mean of three chambers used per

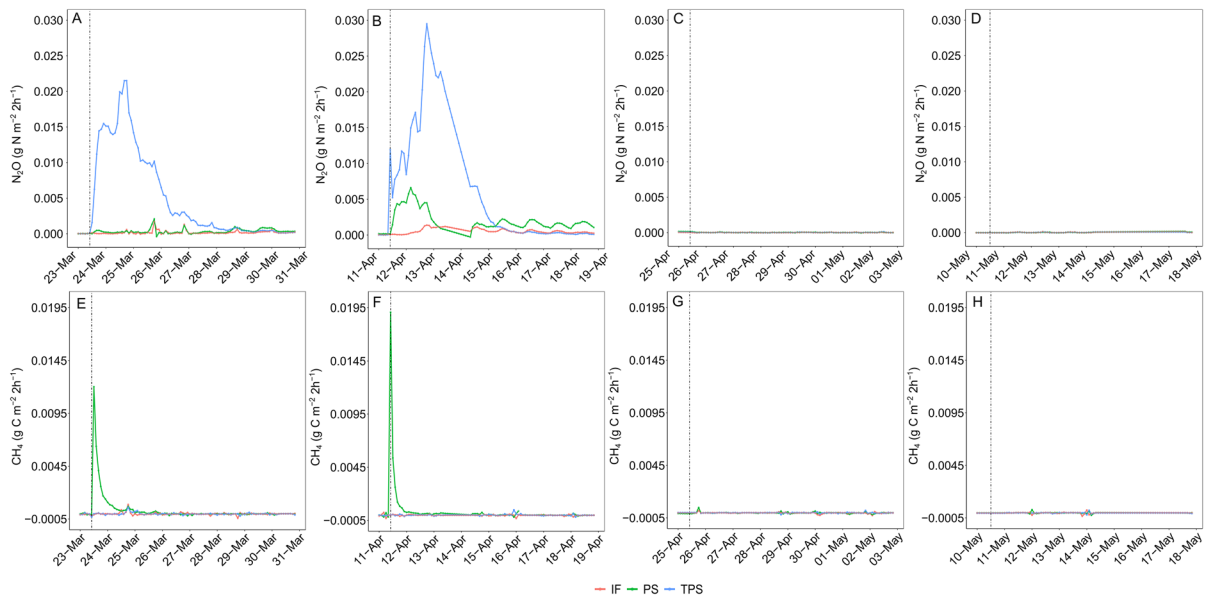
treatment and vertical dashed lines represent the split applications of fertilisers. Error bars have been removed to aid visualisation

several environmental variables and  $\text{N}_2\text{O}$  fluxes (Table S5). There was no clear diurnal trend in  $\text{N}_2\text{O}$  fluxes observed at Tillering S5 and Extension S6, although the magnitude of  $\text{N}_2\text{O}$  flux was higher from TPS compared to IF and PS at these growth stages (Fig. 3). From Extension S7 onwards a slight diurnal trend in  $\text{N}_2\text{O}$  fluxes became prevalent for all treatments and became more pronounced from Extension S10 onwards — fluxes increased during the day and decreased at night, with the highest fluxes observed between 10:00 and 12:00 (Fig. 3).

#### $\text{CH}_4$ fluxes

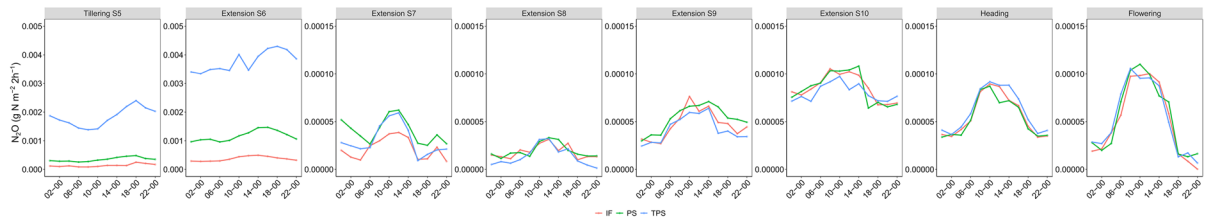
Cumulative  $\text{CH}_4$  fluxes were highest from PS and lower from IF and TPS and were not significantly different between treatments (Table 1; Figure S2). Methane fluxes increased with increasing WFPS, PAR, air

temperature and pig slurry application ( $P < 0.05$ ) (Figure S6; Figure S7). There was no significant influence of precipitation on  $\text{CH}_4$  fluxes ( $P = 0.24$ ). Mean daily  $\text{CH}_4$  fluxes were highest from PS and lower from IF and TPS but were not significantly different between treatments ( $P > 0.05$ ) (Table 1). Methane fluxes from PS peaked immediately after the first and second fertiliser applications and remained elevated for less than 24 h before returning to pre-fertilisation levels (Figs. 1 and 2). Methane fluxes did not respond to the third and fourth fertiliser applications which were in the form of inorganic fertiliser (Figs. 1 and 2; Table S5). Methane fluxes from IF and TPS remained low for the entire measurement period and did not respond to any fertiliser applications (Figs. 1 and 2). When considering  $\text{CH}_4$  fluxes from 0 to 7 days of the first two fertiliser applications only (Fig. 2), mean daily  $\text{CH}_4$  fluxes were higher from PS than IF



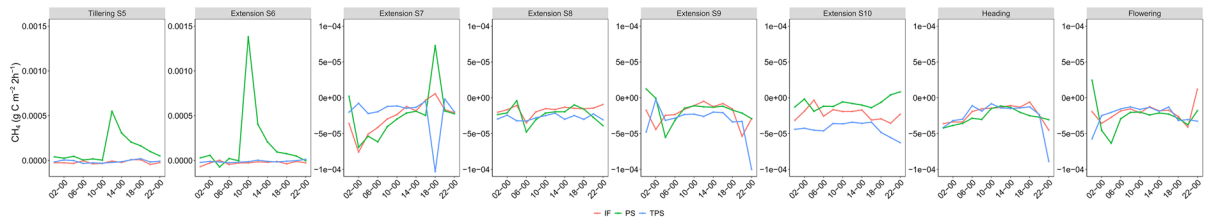
**Fig. 2** 2-h fluxes of **A–D**  $N_2O$  and **E–H**  $CH_4$  during the first 7 days of each fertiliser application for each fertiliser treatment (*IF* inorganic fertiliser, *PS* pig slurry, *TPS* treated pig slurry). Each data point represents the mean of three chambers

used per treatment and vertical dashed lines represent the split applications of fertilisers. Error bars have been removed to aid visualisation



**Fig. 3** Mean 2-h fluxes of  $N_2O$  for each fertiliser treatment (*IF* inorganic fertiliser, *PS* pig slurry, *TPS* treated pig slurry) for each winter wheat growth stage over the measurement period. Each data point represents the mean of three chambers used

per treatment. Error bars have been removed to aid visualisation. The dates of each growth stage, and the average daily air temperature and total rainfall per winter wheat growth stage are shown in Table S7



**Fig. 4** Mean 2-h fluxes of  $CH_4$  for each fertiliser treatment (*IF* inorganic fertiliser, *PS* pig slurry, *TPS* treated pig slurry) for each winter wheat growth stage over the measurement period. Each data point represents the mean of three chambers used

per treatment. Error bars have been removed to aid visualisation. The dates of each growth stage, and the average daily air temperature and total rainfall per winter wheat growth stage are shown in Table S7



and TPS but were not significantly different between treatments ( $P = > 0.05$ ) (Table 1). There was no clear diurnal trend in  $\text{CH}_4$  fluxes for any of the treatments at any of the WW growth stages (Fig. 4).

### Yield response

The average total WW DM yield did not vary significantly between treatments (Table 2) and ranged from  $22.75 \pm 1.31 \text{ t ha}^{-1}$  (PS) to  $25.21 \pm 3.68 \text{ t ha}^{-1}$  (TPS), which is slightly higher than that reported for the entire field ( $22.1 \pm 3.4 \text{ t ha}^{-1}$ ). Winter wheat grain yield ranged from  $13 \pm 1.2 \text{ t ha}^{-1}$  (PS) to  $14.5 \text{ t ha}^{-1}$  (TPS), which is slightly higher than that reported for the entire field ( $12.9 \text{ t ha}^{-1}$ ). At harvest, the harvest index was similar between treatments (Table 2). Dry matter yield, total C and N content, grain yield, grain C and N content, and grain protein content were not significantly different between any of the treatments ( $P = > 0.05$ );  $\text{NUE}_{\text{total}}$  and  $\text{NUE}_{\text{grain}}$  were highest for IF and lowest for TPS and were not significantly different between any of the treatments (Table 2). Mean GHGI was highest from TPS and lowest from

IF (Table 1) and was not significantly different between treatments ( $P = 0.1$ ).

### Discussion

#### Plasma treatment of pig slurry increased $\text{N}_2\text{O}$ emissions

The large peaks of  $\text{N}_2\text{O}$  following the two applications of treated pig slurry are responsible for TPS having the highest cumulative  $\text{N}_2\text{O}$  emissions. Similarly, the smaller  $\text{N}_2\text{O}$  peak following the second application of pig slurry to PS is responsible for this treatment having the second highest cumulative  $\text{N}_2\text{O}$  emissions relative to IF. Elevated  $\text{N}_2\text{O}$  fluxes following N fertiliser application are well-documented and are often attributed to fertiliser N becoming available for conversion to  $\text{N}_2\text{O}$  shortly after application, as there is competition between plant uptake and soil microbes for the N (Ma et al. 2013; Officer et al. 2015). Many studies have observed higher  $\text{N}_2\text{O}$  emissions from crops fertilised with organic fertiliser, or a combination of organic and inorganic fertiliser,

**Table 2** Seed planting density, total biomass and crop yield, harvest index, whole crop and grain C and N content, total C and N removed in whole crop and grain, proportion of total crop N in grain, grain protein content, nitrogen use efficiency

Fertiliser treatment	IF	PS	TPS
Planting density $\pm$ SD (seeds $\text{m}^2$ )	383.33 $\pm$ 137.7	400 $\pm$ 114.6	341.67 $\pm$ 104.1
Total biomass yield $\pm$ SD (t DM $\text{ha}^{-1}$ )	23.76 $\pm$ 1.5 a	22.75 $\pm$ 1.3 a	25.21 $\pm$ 3.7 a
Grain yield $\pm$ SD (t $\text{ha}^{-1}$ )	13.05 $\pm$ 0.9 a	12.98 $\pm$ 1.2 a	14.84 $\pm$ 2.7 a
Harvest index $\pm$ SD (%)	54.92 $\pm$ 1.1 a	57 $\pm$ 1.7 a	58.66 $\pm$ 2.3 a
Whole crop C content $\pm$ SD (%)	40.71 $\pm$ 0 a	40.58 $\pm$ 0.2 a	40.57 $\pm$ 0.1 a
Total C removed in whole crop (t $\text{ha}^{-1}$ )	9.67 $\pm$ 0.6 a	9.23 $\pm$ 0.5 a	10.23 $\pm$ 1.5 a
Grain C content $\pm$ SD (%)	39.06 $\pm$ 0.7 a	38.80 $\pm$ 0.4 a	38.84 $\pm$ 0.8 a
Whole crop N content $\pm$ SD (%)	0.78 $\pm$ 0.1 a	0.79 $\pm$ 0 a	0.78 $\pm$ 0.1 a
Total N removed in whole crop (t $\text{ha}^{-1}$ )	0.18 $\pm$ 0 a	0.18 $\pm$ 0 a	0.2 $\pm$ 0 a
Grain N content $\pm$ SD (%)	1.29 $\pm$ 0.1 a	1.23 $\pm$ 0.1 a	1.22 $\pm$ 0.1 a
Total N removed in grain (t $\text{ha}^{-1}$ )	0.17 $\pm$ 0 a	0.16 $\pm$ 0 a	0.18 $\pm$ 0 a
% of total crop N in grain	90.78 $\pm$ 1.9 a	88.93 $\pm$ 9.6 a	93.21 $\pm$ 11.6 a
Grain protein content $\pm$ SD (%)	6.17 $\pm$ 0.6 a	6.64 $\pm$ 0.8 a	5.97 $\pm$ 0.7 a
$\text{NUE}_{\text{total}}$ (%)	83.64 $\pm$ 3.7 a	81.69 $\pm$ 1 a	77.81 $\pm$ 17.4 a
$\text{NUE}_{\text{grain}}$ (%)	75.89 $\pm$ 2.4 a	72.63 $\pm$ 7.6 a	71.89 $\pm$ 15 a
% of applied N lost as $\text{N}_2\text{O}$ -N	0.6 $\pm$ 0.1 a	0.9 $\pm$ 0.1 a	4 $\pm$ 0.5 a

Note that whole crop refers to the entire harvested plant (i.e., chaff, grain and stalk). Samples taken from plots using a 0.5  $\text{m}^2$  quadrat ( $N = 3$ ). Across each row, the same letters indicate no significant difference in the variable of interest between fertiliser treatments

compared to those amended with inorganic fertiliser only (Pelster et al. 2012; Ball et al. 2014; Yang et al. 2015). Organic fertilisers have a higher labile C content which is easily decomposed by soil microorganisms and releases mineralizable N for the production of  $N_2O$  (Hangs and Schoneau 2022); this is likely to have caused the higher  $N_2O$  emissions from TPS and PS compared to IF. Furthermore, the pig slurry and treated pig slurry had a higher content of fine solids than the inorganic fertiliser; fine solids block soil pores and restrict oxygen movement through soil, which creates favourable conditions for  $N_2O$  production (Chadwick et al. 2000). We found that the plasma induction process increased the nitrate-N content of the pig slurry; the higher content of inorganic N combined with the C in the pig slurry is likely to be responsible for the higher  $N_2O$  emissions (Shurpali et al. 2016; Li et al. 2022) from TPS compared to PS. Mousavi et al. (2023) found that the nitrification potential of plasma-treated pig slurry was higher than that of other fertilisers due to its higher volatile organic C content, which reduces ammonia immobilisation, and so may also explain the higher  $N_2O$  emissions from TPS. Denitrification is highly influenced by pH, with denitrification being slowed or even inhibited at lower pH levels (Liu et al. 2010; Olaya-Abril et al. 2021). At lower pH, the transformation of  $N_2O$  to nitrogen gas is inhibited, meaning that the  $N_2O$  is available to be emitted from the soil (Liu et al. 2010; Olaya-Abril et al. 2021). The lower pH of the treated pig slurry relative to the untreated pig slurry (Table S3) may therefore also explain the higher  $N_2O$  emissions from TPS. It should be noted that the amount of available N applied to TPS was slightly higher than to PS and IF which may have contributed to its higher  $N_2O$  emission, although because the  $N_2O$  emissions from TPS are so much higher than the other two treatments, it is highly unlikely that this discrepancy is the only reason.

A higher soil moisture content can restrict aeration and reduce soil oxygen concentration, creating favourable conditions for denitrification and  $N_2O$  emission (Westphal et al. 2018; Kostyanovsky et al. 2019; Li et al. 2022). This can explain the higher  $N_2O$  emissions from TPS and PS, as the relationship between  $N_2O$  and WFPS was higher for these treatments than IF, and WFPS appeared highest at TPS. The lack of response of  $N_2O$  fluxes to the applications of inorganic fertiliser across all treatments is

explained by the drought conditions experienced during the study. The inorganic fertilisers were applied in the form of solid granules (application 1) or a small volume of liquid (subsequent applications), which did not wet the soil enough to stimulate  $N_2O$  emissions. Verdi et al. (2019) also found low  $N_2O$  emissions from a dry soil when solid inorganic fertiliser was added. The volume of liquid applied as pig slurry and treated pig slurry was greater, and thus wetted up the soil more, inducing  $N_2O$  emission.

#### Plasma treatment of pig slurry decreased $CH_4$ emissions

The immediate peaks in  $CH_4$  fluxes following the two applications of pig slurry are responsible for PS having the highest total  $CH_4$  fluxes. Methane is produced during pig slurry storage as the conditions and C content of the slurry facilitate methanogenesis; the  $CH_4$  is dissolved into the pig slurry and then volatilised and emitted to the atmosphere following slurry application (Rochette and Cote 2000; Bastami et al. 2016). Severin et al. (2015) also measured higher  $CH_4$  emissions from crops amended with pig slurry. The small  $CH_4$  uptake by IF and TPS is not unexpected, as methanotrophy occurs in well-drained agricultural soils (Serrano-Silva et al. 2014). Inorganic fertiliser does not contain a C source to facilitate methanogenesis (Moreno-Garcia et al. 2020), and thus  $CH_4$  production, and the plasma induction process prevents  $CH_4$  production during slurry storage by acidifying the slurry and reducing its pH (Tooth 2021; Petersen et al. 2012; Overmeyer et al. 2021; Ambrose et al. 2023), so no  $CH_4$  was emitted from IF and TPS upon application. There is the potential for  $CH_4$  to be produced in soil, and then emitted, following the application of slurry due to the anoxic conditions created by rapid C mineralisation after the input of C in the organic fertiliser (Le Mer and Roger 2001; Yuan et al. 2019), this accounts for the elevated  $CH_4$  emissions from PS. The lower pH of the treated pig slurry, as a result of acidification during plasma treatment, prohibiting methanogenesis during storage also appears to inhibit  $CH_4$  production on application to the field, as the C input via treated pig slurry application does not induce  $CH_4$  emissions. The plasma

induction process therefore has clear benefits in terms of reducing CH<sub>4</sub> emissions during the storage and application of pig slurry to agricultural soil.

#### CO<sub>2</sub>-equivalent emissions and GHGI highest from plasma-treated pig slurry

Nitrous oxide has a higher global warming potential (273) than CH<sub>4</sub> (27.9) (Smith et al. 2021), and, as N<sub>2</sub>O emissions were considerably higher from TPS compared to the other treatments, CO<sub>2</sub>-equivalent emissions were therefore also highest from TPS. The higher CH<sub>4</sub> fluxes from PS compared to TPS and IF were not large enough to outweigh the high N<sub>2</sub>O fluxes from TPS when converted to CO<sub>2</sub>-equivalent. Across the literature, cumulative CO<sub>2</sub>-equivalent fluxes from WW fertilised with 100–300 kg inorganic N ha<sup>-1</sup> range from 15 to 102.5 g CO<sub>2</sub>-equivalent m<sup>-2</sup> (Sainju et al. 2022; Huang et al. 2013) (Table S6); the CO<sub>2</sub>-equivalent emissions we measured from IF are within this range. There is a lack of data on CO<sub>2</sub>-equivalent emissions from pig slurry when used as an organic fertiliser, presenting a significant research gap that must be addressed to enhance the understanding of the impacts of fertiliser type on GHG emissions. As all treatments received a similar amount of plant-available N, the lack of influence of treatment type on the WW growth, including DM yield, grain yield and grain protein content is not unexpected. Cai et al. (2013) also observed no significant difference in grain yield between crops amended with a similar N rate of inorganic and organic fertilisers. Our results show that it is possible to replace over half of inorganic N fertiliser with organic N fertiliser and achieve the same yield. As yield was not significantly different between the treatments, this meant that GHGI followed the trend of cumulative CO<sub>2</sub>-equivalent emissions, with the highest fluxes from TPS. When considering WW yield, the phosphorus and potassium applied to the crop via the fertiliser treatments should be noted — the pig slurry and treated pig slurry contained phosphorus and potassium whereas the inorganic fertiliser did not. As soil potassium data is not available, it is not possible to assess whether this was a factor limiting crop production, however it is unlikely as the yield of ~12 t ha<sup>-1</sup> for all treatments is high, and the soil was not P limited (P index of 3). As we consider cumulative emissions, it is also important to note that ~6 weeks of

data are not included in this study due to an error with the GHG measurement chambers. Given the uniform and consistent flux pattern in the weeks prior to this, and the fact that there were no N fertiliser applications during this time, we propose that the addition of this missing data would have a minimal impact on the cumulative emissions.

#### Diurnal N<sub>2</sub>O emissions observed outside of N<sub>2</sub>O peaks

The diurnal pattern and peak of N<sub>2</sub>O emissions during the middle of the day (observed from Extension S10 onwards) for all treatments coincides with maximum CO<sub>2</sub> uptake. This pattern was also reported in a review by Wu et al. (2021) who found that over half of the datasets reviewed observed N<sub>2</sub>O fluxes peaking during the day. Chadwick et al. (2000) and Keane et al. (2018) hypothesise that increases in soil temperature, WFPS and PAR increased N<sub>2</sub>O fluxes. Furthermore, Keane et al. (2018) propose that, as C availability is a key driver of denitrification, higher PAR and temperature during the middle of the day would increase photosynthate exudation and microbial respiration, reducing oxygen availability, and stimulating denitrification and N<sub>2</sub>O emission. Our results support these hypotheses, as we found that, when excluding fluxes measured within 0–7 days of the first two fertiliser applications, N<sub>2</sub>O fluxes increased with WFPS and PAR. The Tillering S5 and Extension S6 growth stages coincided with the applications of pig slurry and treated pig slurry, which subsequently caused peaks of N<sub>2</sub>O emission, and so no diurnal patterns in N<sub>2</sub>O emissions were observed from any treatments during these growth stages.

#### Implications for research and policy

We show that treating pig slurry with plasma-induction does not reduce overall non-CO<sub>2</sub> GHG emissions, in fact it increases them in comparison to untreated pig slurry and inorganic fertiliser. Although soil CH<sub>4</sub> emissions were reduced by treating pig slurry with plasma induction, N<sub>2</sub>O soil emissions from plasma-treated slurry were considerably greater than non-treated slurry. Furthermore, the CO<sub>2</sub>-equivalent emissions from the organic fertiliser treatments (TPS and PS) were higher than

those from the inorganic fertiliser treatment (IF). These trade-offs between  $N_2O$  and  $CH_4$  emissions highlight the need to continue the development of innovative technologies to improve agricultural sustainability. Whilst other research has found benefits of the use of plasma-treated slurries, such as lower ammonia emissions (Gillbard 2023) and positive effects on soil fauna (Mousavi et al. 2022), the high  $N_2O$  emissions found in our study show that more research is required to determine how these emissions can be reduced. This may include de-watering slurries or using nitrification inhibitors to reduce  $N_2O$  emissions associated with the application of organic fertilisers to soils to improve on-farm waste management and farm adherence to agricultural policy (Ruser and Schulz 2015; Willen et al. 2016). Further research exploring the influence of fertiliser type on GHG emissions should also measure fluxes from a control treatment receiving no fertiliser, which would enable the calculation of emission factors, and from a range of environments to assess the influence of climate and soil variables. Whilst we show that, overall, differences in GHG emissions were considerable between treatments, the cumulative  $N_2O$  and  $CH_4$  emissions were not significantly different. This is likely to be due to the small number of replicates per treatment ( $N=3$ ). A replicated study with both an increased sample size per treatment and control treatment would strengthen the results. As this experiment only focuses on emissions from fertiliser application until ~6 weeks before harvest, future trials should be longer-term, measuring GHG emissions across a full crop season as well as across years to account for inter-annual variability. It is crucial that this research is conducted prior to the commercialisation of new technologies for organic waste management. It should be noted that the plasma induction process reduced slurry pH from ~7 to below 5 (Table S3), and that slurry acidification is known to reduce ammonia emissions by 70% (Kupper et al. 2020). Measuring ammonia emissions alongside GHGs would provide a more comprehensive understanding of the emissions associated with the use of agricultural fertilisers and ensure that all trade-offs are fully accounted for. These measurements should be integrated into dynamic biogeochemical models and life-cycle analyses to account for other significant emissions

associated with the use of agricultural fertilisers, such as those generated in fertiliser manufacturing from the Haber-Bosh process, and allow the full environmental and climatic impact of fertiliser production and application to be ascertained.

## Conclusion

The use of plasma-treated pig slurry as an organic soil amendment reduced soil  $CH_4$  emissions relative to non-treated pig slurry after application. Plasma-treated slurry increased  $N_2O$  emissions considerably, however, which outweighed the savings from  $CH_4$  reduction and so  $CO_2$ -equivalent emissions were greater from treated than non-treated pig slurry. Winter wheat yield was high for all treatments and was not affected by the fertiliser type used. Plasma-treated pig slurry is therefore not currently a suitable soil amendment should farmers wish to reduce GHG emissions from their land. Furthermore, the application of organic fertilisers (i.e., treated and non-treated pig slurries) resulted in higher GHG emissions than when inorganic fertiliser was applied. We therefore recommend that our results be integrated into a life-cycle analysis, to determine whether the use of organic fertilisers still emit more than inorganic fertilisers when the associated downstream GHG emissions are considered. In addition, future research should focus on how  $N_2O$  emissions can be reduced from plasma-treated pig slurry, conducting plot trials to assess the effect of fertiliser rate, timing and placement.

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**Data availability** The dataset generated during the current study is under embargo at <https://doi.org/10.5285/4ed0023e-da9b-45a8-86de-3a371cc7dcc1>, but can be made available by the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

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