



Application of liquid fraction of digestates and biochar in winter wheat: nitrous oxide emissions and yield response

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Abstract Digestate from anaerobic digestion is used for fertilizer application, but the efficiency is not always very high, and it may lead to significant emissions of nitrous oxide. In this two-year field study, we tested differently treated liquid fraction of digestate (LF) to investigate N₂O emissions and crop yield. The treatments included biochar amendment, acidification and sediment separation followed by post-digestion (SLF). The response of the treatments was tested as second fertilizer application in winter wheat (*Triticum aestivum* L.) and compared with mineral fertilizer. The N₂O emissions were measured using static chambers from April to June in 2023 and 2024. The bulk of N₂O emissions occurred within two weeks after application in both years. Biochar-amended and acidified LF and SLF did not have a significant effect on cumulative N₂O emissions in either of the two years. Biochar resulted in significantly lower cumulative and yield-scaled emissions in 2023 (but not in 2024) in the treatment with mineral N likely due to dry soil conditions in the first year. Yield was not significantly different among treatments receiving 150 kg N ha⁻¹.

The fertilizer value of treated and untreated liquid fractions of digestates ranged between 56–77% of total N in both years. In conclusion, N₂O emissions could not be reduced with acidification, biochar amendment, or SLF and were all similar to mineral N application. In addition, biochar N₂O mitigation in treatments with mineral N fertilizer in dry years may be outweighed by increased emissions in years with high rainfall.

Keywords Nitrogen fertilizer replacement value · Nitrogen use efficiency · Acidification · Anaerobic digestion · Solid–liquid separation

Introduction

Digestates, which are biproducts from biogas production by anaerobic digestion, can function as an important source of organic fertilizer for agricultural production. In Denmark more than 30% of all livestock manures are digested in biogas plants (Blicher-Mathiesen 2023). The mechanical separation of digestates into a liquid and solid fraction at the biogas plants improves the usability (Guilayn et al. 2019). The solid fraction contains large amounts of phosphorus, while the liquid fraction of digestates (LF) retains the larger share of nitrogen (N), mostly as ammonium (NH₄⁺). The LFs can then be used as organic N fertilizer with a lower risk of surpassing nutrient application limits for susceptible soils (Chuda et al. 2021)

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However, application of LF can also emit substantial amounts of nitrous oxide (N_2O), which can be as high as with cattle slurry or untreated digestate application (Meng et al. 2023). N_2O is a harmful greenhouse gas, with a global warming potential approx. 273 times higher than CO_2 , and the bulk of anthropogenic emissions originate from agricultural production (Canadell et al. 2023; IPCC 2023). Therefore, mitigation options that can reduce N_2O emissions are urgently needed. These mitigation options should not compromise yields or lead to any other environmentally problematic emissions of digestates, such as nitrate leaching, NH_3 emissions, and gaseous nitrogen losses as N_2 (Nkoa 2014; Buchen-Tschiskale et al. 2020).

For example, in Denmark, acidification of slurry can be used as an alternative to mandatory injection in connection with application of slurry to grassland to mitigate NH_3 volatilization (Pedersen et al. 2022), and acidification has been shown to reduce NH_3 emissions from digestates as well (Pedersen et al. 2023). However, acidification may also increase N_2O emissions (Dietrich et al. 2020). Another way to reduce NH_3 emissions is to further reduce the solids in digestates. This results in better infiltration after surface application in a growing crop like winter wheat (Nyang'au et al. 2023). Further, reduction of the solids in LF can be achieved with additional post-processing steps such as centrifugation with combined separation in a decanter (Scholey et al. 2019). The further removal of solids through post-processing may reduce the oxygen consumption around the manure application site and this may reduce emissions. However, the impact of different separation techniques on N_2O emissions needs further quantification.

While the LFs can be used directly as N fertilizer, the solid fraction can be converted to biochar. Biochar is char produced from biomass via pyrolysis, i.e., the thermal decomposition at high temperature at low or no oxygen content (Sohi et al. 2010). Pyrolysis of digestates is an economic technique of biochar production and can serve to recover nutrients for crop production (Maroušek et al. 2023; Pedersen et al. 2025). Biochar has reportedly increased N uptake in plants and yields, however, the latter depending on climate and soil fertility, among others (Jeffery et al. 2017; Zhang et al. 2021a, b). In addition, biochar has reportedly the potential to increase carbon

sequestration and reduce N_2O emissions (Woolf et al. 2010; Zhang et al. 2021a, b). However, the mitigation potential depends on climate and soil conditions, crop management and systems, and biochar properties, among others (He et al. 2017; Verhoeven et al. 2017; Liu et al. 2019). The biochar could potentially be applied either separately or mixed with the manure before application; these options thus require further investigation.

While it is important to reduce N_2O emissions from agriculture, mitigation measures cannot compromise yields; otherwise, farmers may decline to adopt the new measures. Organic N fertilizers and amendments are an important N source in agricultural production and contribute to the recycling of nutrients but can vary substantially in efficiency as compared to mineral N fertilizers. The efficiency of organic fertilizers as compared to mineral fertilizers can be evaluated with the Nitrogen Fertilizer Replacement Value (NFRV) based on measurement of nitrogen use efficiency (NUE) of the organic fertilizer related to NUE of a mineral N fertilizer (Westerik et al. 2024). It is therefore relevant to observe both, N_2O mitigation and yield performance of LFs as compared to mineral N fertilizer. In this study, different N_2O mitigation options for LFs were tested under field condition, and winter wheat yields quantified. The objective is to assess the efficacy of LFs as a N fertilizer and several mitigations measures that potentially can reduce N_2O emissions including biochar application, acidification and post-processing. The null hypothesis of this study was that the application of treated LFs has no significant effect on N_2O emissions and yield in winter wheat.

Material and methods

The LF was obtained from the same batch each year from a commercial biogas plant, Nature Energy at Videbæk, Denmark. The biogas plant mostly uses cattle slurry and deep litter, and a minor input of pig slurry. The digestate was separated into a liquid and a solid fraction by screw-pressing and subsequent centrifugation in a decanter centrifuge, and only the liquid fraction was used in the experiments. The screw press and decanter centrifuge used for separation was a Bioselect RC75 (Börger GmbH, Germany) and a HAUS DDE 4742 (HAUS Centrifuge

Technologies, Turkey) and a Flottweg decanter centrifuge (Flottweg SE, Germany) in 2023 and 2024, respectively. The mean total N content of the LF in 2023 and 2024 was 0.42% in both years, of which 59% and 65% was NH_4^+ , respectively, while the remainder was organic N. In 2024, an additional treatment included LF from a second separation step with a Sedicanter® centrifuge (Sedicanter, Flottweg SE, Germany) followed by a second digestion step to further reduce the dry matter concentration in the liquid fraction. A sedicanter is a combination of a separator and decanter and has been used for the separation of suspensions in different industries (Manganaro et al. 2015; Scholey et al. 2019). This post-processed LF had a total N content of 0.35% of which 76% was NH_4^+ , and a lower amount of dry matter (DM) of 3.4% compared with the LF containing 3.7% DM (Table 1).

ULF: untreated liquid fraction of digestates, $\text{LF}_{\text{pH}5-6-7}$ =liquid fraction of digestate acidified to pH5, 6, and 7, respectively; SLF=post-digested Sedicanter LF; NA: data not available; DM=dry matter; N=Nitrogen; $\text{NH}_4^+\text{-N}$ =Ammonium N; C=Carbon; P=Phosphorous; K=Potassium.

Pelleted biochar produced by pyrolysis of digestate fibres was obtained from the Stiesdal SkyClean facility at GreenLab in Skive, Denmark, using a pyrolysis temperature of 600 °C. Note that the biochar was produced from a different solid fraction of digestates. The same batch of biochar was used in both years, but with slightly varying water content of 43.4% in 2023 and 35.7% in 2024. After pyrolysis, water was added to the biochar to avoid self-ignition. The P content in the dry matter of the

biochar was 22.3 g P kg^{-1} . The Total C content in dry matter was 63% and the H to organic C ratio was 0.29 (see also Table 4).

Experimental setup

Winter wheat (*Triticum aestivum* L.) was seeded at a rate of 300 plants m^{-2} on September 10, 2022, and September 20, 2023, for the 2023 and 2024 season, respectively, in different fields each year at the Experimental farm Foulumgaard, Aarhus University, Denmark (56° 30' N, 9° 35' E, 54 masl). The annual rainfall is 678 mm and mean annual temperature is 8.2 °C for 1991–2020 (Jørgensen et al. 2024). Both experimental fields were grown with either spring barley (*Hordeum vulgare* L.) or winter wheat for the two years before the experiment. The winter wheat variety in 2023 was Wheat Mix 221 with 'Heerup', 'Kvium' and 'Rembrandt', and in 2024 it was 'Champion'. The soil is a sandy loam Orthic Acrisol with the topsoil (0–25 cm) containing 78% sand, 13% silt, and 9% clay at 0–25 cm soil depth (Nielsen et al. 1984; Petersen et al. 2023a, b). The planting row width was 12 cm. There was an initial fertilizer application for the whole experimental area of 50 kg N, 16 kg P, and 84 kg K ha^{-1} on April 1, 2023, and March 21, 2024, respectively. The experiment tested mineral N fertilizer, the abovementioned LF and biochar in different treatments for the second N fertilization. The reference mineral N fertilizer was Nutramon (N-S 27-14) with an equal share of NO_3^- and NH_4^+ .

The experimental setup consisted of nine treatments for N_2O emission measurements and winter

Table 1 Chemical composition of the liquid fraction of digestates used in the field experiments in 2023 and 2024

Digestate	DM %	Total N Kg Mg^{-1}	$\text{NH}_4^+\text{-N}$ Kg Mg^{-1}	Total C % of DM	Total P Kg Mg^{-1}	K Kg Mg^{-1}	$\text{NH}_4^+\text{-N}/\text{total N}$	C/N
<i>Application 2023</i>								
ULF	4.8	4.16	2.44	NA	0.82	4.9	0.59	NA
$\text{LF}_{\text{pH}5}$	NA	4.15	2.49	NA	NA	NA	0.60	NA
$\text{LF}_{\text{pH}6}$	NA	4.15	2.49	NA	NA	NA	0.60	NA
$\text{LF}_{\text{pH}7}$	NA	4.16	2.46	NA	NA	NA	0.59	NA
<i>Application 2024</i>								
ULF	4.0	4.21	2.81	36	0.44	3.8	0.67	3.42
$\text{LF}_{\text{pH}5}$	5.7	4.26	2.89	26	0.45	4.1	0.68	3.48
$\text{LF}_{\text{pH}7}$	4.3	4.09	2.69	32	0.45	3.8	0.66	3.36
SLF	2.8	3.41	2.58	33	0.36	3.4	0.76	2.67

wheat grain dry matter and N yield, and two additional treatments for yield only. The experimental design was a randomized block design with four blocks ($n=4$). In total there were 44 plots for yield measurements, 36 of which were for additional N_2O measurements. Each plot had a size of 2.5×1.44 m (12 rows of winter wheat), with an additional 5 and 7 rows of winter wheat on each side as buffer area. The experiment began on April 20, 2023, and April 22, 2024, respectively, with the application of LF, mineral fertilizer and biochar. The treatments for N_2O and yield measurements were: LF, either untreated LF (ULF, pH 7.9), manually acidified to pH 5.0, 6.2 and pH 6.8 using concentrated sulphuric acid (referred to as LF_{pH5} , LF_{pH6} , and LF_{pH7} , respectively), untreated LF applied with biochar, either mixed into the LF (BLFM) or applied separately (BLF), mineral N fertilizer combined with biochar (BN1), all at a rate of 150 kg N ha^{-1} , and mineral N fertilizer at a rate 0 and 150 kg N ha^{-1} as control and reference treatment (referred to as N0 and N1), respectively. Thus, total N fertilizer application (starter mineral N fertilizer and LF) corresponds to 50 and 200 kg N ha^{-1} for N0 and N1, respectively. This N fertilizer rate is the recommended amount of N for winter wheat production in Denmark, also for LF application (SEGES 2023). The biochar application rate corresponded to 30 kg P ha^{-1} . As mentioned above, one treatment was with the application of post-digested Sedicater LF (SLF) in 2024; this replaced the LF_{pH6} treatment from 2023. For details see Table 2. In addition, two treatments with 50 and $100 \text{ kg mineral N ha}^{-1}$ were

included for measurement of grain dry matter and N yield only. The LF was hand-applied on the surface between the winter wheat rows to mimic trail hose applications. Biochar, if not mixed into the digestate, and mineral N application was hand-applied, and evenly spread inside the plot.

Prior to application, 35×25 cm stainless steel frames were hammered into the soil for N_2O flux measurement with the longer side along the winter wheat row, approximately 40 cm into the plot. All fertilizers and organic amendments were hand-applied separately inside the frames to reduce the spatial variability of fertilizer application and the impact on the N_2O flux.

N_2O flux measurements

An initial measurement campaign started one and three days after the starter mineral N fertilizer application of $50 \text{ kg N } 6\text{ha}^{-1}$ on all plots in 2023 and 2024, respectively, to determine the variation of the N_2O flux across all 36 plots without any treatment effect. The N_2O concentration in the air of the chamber headspace of static (non-flow through, non-steady state) opaque chambers (L: 35 cm; W: 25 cm H: 22 cm) was determined. Each chamber had a fan to ensure proper air mixing, a vent to avoid pressure differences between inside and outside the chamber, and a septum to take air samples. When the crop grew higher than the chamber, an additional intersection placed between the soil surface and the chamber

Table 2 Treatment name and ID used for N_2O emission and yield measurements: pH of LF (mean of 2023 and 2024) and N application rate. Note that N_2O was not measure in the treatments N0.3 and N0.6

Year	Treatment name	Treatment ID	pH	N rate (kg ha^{-1})
2023, 2024	Control	N0	–	0
2023, 2024	Mineral N	N0.3	–	50
2023, 2024	Mineral N	N0.6	–	100
2023, 2024	Mineral N	N1	–	150
2023, 2024	Mineral N and biochar	BN1	–	150
2023, 2024	LF and biochar, separately applied	BLF	7.9	150
2023, 2024	LF and biochar, mixed	BLFM	7.9	150
2023, 2024	LF, untreated	ULF	7.9	150
2023, 2024	LF,	LF_{pH5}	5.0	150
2023	acidified to pH 5, 6 and 7	LF_{pH6}	6.2	150
2023, 2024		LF_{pH7}	6.8	150
2024	Post-digested Sedicater LF	SLF	8.5	150

increased the total chamber height to 55 cm. Each measurement campaign started at approx. 10 AM CEST and lasted for approx. 2 h; there was a total of 12 campaigns in each season.

The air in the chamber headspace was sampled through the septum with a 10 mL syringe and needle, and the plunger moved three times to ensure adequate mixing of the air sample. 5 mL of air was discarded and the remainder injected into a 3 mL pre-evacuated exetainer for further analysis. The first sampling was directly after deploying the chamber, and three more samples followed in approx. 30 min periods ($n=4$). All samples were analyzed for N_2O , CO_2 , and CH_4 (not used in this study) on a gas chromatograph (either a Bruker 450, Bruker Corporation, Massachusetts, United States or a SCION 456-GC, Scion Instruments, The Netherlands; %RSD=1.88).

Soil analysis

Four TMS-4 sensors (TOMST s.r.o., Czech Republic), one in each block in the mineral N treatment (N1) measured the volumetric soil water content (VWC) and soil temperature at 0–15 and 8 cm soil depth, respectively. The TMS-4 requires a calibration curve to calculate VWC, and we used a factory calibration curve with similar soil bulk density and soil texture (BD: 1.32 g cm^{-3} , 61% sand, 34% silt, 5% clay). Based on this we calculated the water filled pore space as:

$$\text{WFPS} = \text{VWC} / (1 - \text{BD}/\text{PD}) * 100 \quad (1)$$

where WFPS=percent water-filled pore space, VWC=volumetric soil water content ($\text{cm}^3 \text{ cm}^{-3}$), BD=bulk density (g cm^{-3}), and PD=particle density (here: 2.65 g cm^{-3}).

In 2023, undisturbed samples from each block in the 0–5 cm soil depth were sampled in steel rings (100 cm^3) dried at 105°C for 24 h to calculate the BD as dry soil mass divided by volume and excluding any coarse material > 2 mm. We assumed that BD in 2024 was the same as in 2023, since the cropping system and soil type were the same.

Three randomly chosen soil samples were sampled within each plot with a 2-cm soil auger in 0–15 cm soil depth at weekly to fortnightly intervals at the same time as the N_2O flux measurements. There was

a total of six soil sampling campaigns in each year for 2023 and 2024, respectively, and soil samples stayed frozen at -20°C until further processing. Mineral N analysis required to sieve the bulked soil sample to < 6 mm and to take subsamples of 10 g fresh weight for extraction with 40 ml 1 M KCl. This suspension was shaken for 30 min, centrifuged for 5 min, and was then filtrated (Filter 691, VWR Europe). The Autoanalyser AA500 (Seal Analytic, Germany) analyzed the filtrate for NH_4^+ and NO_3^- . The soil mineral N content (kg ha^{-1}) in 0–15 cm soil depth was then calculated as the soil mineral N concentration (NO_3^- -N and NH_4^+ -N; mg kg^{-1} soil) multiplied by BD and soil depth. In addition, a soil subsample from the same soil samples was analyzed for soil pH in 2024.

Plant analysis and N fertilizer replacement value

In each plot a separate area of approx. 1 m^2 was left untouched until hand-harvest on August 11, 2023, and August 12, 2024, respectively. The stubble height after harvest was approx. 10 cm. Total dry mass from the above ground biomass was derived from oven-drying the samples for 48 h at 60°C and weighing thereafter. Then the harvested material was threshed to separate wheat grains from the straw, and the grain dry mass was re-weighed. The total N concentration in grain was measured in a ball-milled subsample by elemental analysis (Vario MAX Cube, Elementar Analysensysteme GmbH). The dry matter multiplied with the N concentration gave the grain N uptake.

Data preparation and statistical analysis

The HMR package in R was used to calculate the N_2O flux from the measured N_2O concentration over time, soil surface area covered by the static chamber (m^2), total chamber volume (m^3), and hourly atmospheric pressure (mbar) and air temperature (K) from the weather station nearby the experimental site (Station No. 6069, Foulum; 56.4951°N , 9.57295°E , 54 masl) (Pedersen 2022). The concentration saturation settings of the HMR function were 90% chamber saturation earliest after 2 h, and the variance for prefiltering was set to 0.0001 (parameters SatPct, SatTimeMin, and pfvar, respectively). The HMR

function selects the regression model automatically, that is the HMR function, linear function, or no-flux due to no convergence. Approximately, 5%, 95%, and 0% of the samplings fell in the three above-mentioned categories, respectively. Four single concentrations were excluded due to poor quality (e.g. negative concentration, CO₂ concentration over time indicate faulty measurements) or were missing prior to flux calculation. R version 4.4.2 was used for any further statistical analysis and visualization (R Core Team 2019). This included the calculation of the cumulative N₂O flux for each treatment using the trapezoidal rule with the *trapz* function in the R *pracma* package (Borchers 2022). We calculated the yield-scaled N₂O emissions (YSNE), which is a measure for how N₂O mitigation measures relate to yield, and it is the quotient of the cumulative N₂O–N emissions and grain dry matter yield.

A linear relationship between the mineral N application rate (i.e., treatments with 0, 50, 100, and 150 kg N ha⁻¹, i.e., treatments N0, N0.3, N0.6, and N1, respectively) and total N uptake in grain was used to calculate the N use efficiency (NUE).

$$\text{NUE} = (\text{N uptake} - \text{N uptake}_{\text{N0}}) / \text{mineral N applied} \quad (2)$$

Where: NUE=the slope of the regression; N uptake=Grain N for each treatment (kg N uptake ha⁻¹); N uptake_{N0}=Grain N of the N0 treatment (kg N uptake ha⁻¹); mineral N applied = application rate (here: 150 kg N ha⁻¹).

The *lm* function in R calculated the slope and intercept in Eq. 2 for each year separately. The nitrogen fertilizer replacement value (NFRV) of the different treatments including LF is a measure of efficiency as compared to mineral N fertilizer and is defined as (Jensen 2013):

$$\text{NFRV} = \text{NUE}_{\text{treatment}} / \text{NUE}_{\text{mineral N}} * 100 \quad (3)$$

where: NFRV is in %, and NUE_{treatment} is the NUE of the treatments including LF and biochar while NUE_{mineral N} is the NUE of the mineral fertilizer treatments.

Please note, that the calculated NFRV in this study was the efficiency of a second N application with LF compared to a second application of mineral N fertilizer, as all treatments received a starter fertilization with mineral N at 50 kg N ha⁻¹, thus

following a typical fertilizer application scheme in Denmark (Eller et al. 2026).

The statistical analyses included linear mixed effect models to test for differences in yield, cumulative N₂O–N emissions, the YSNE, and N uptake of winter wheat among treatments ($p < 0.05$). The fixed effects were year of the experiment, treatments, and the interaction between both, while the random effect was the block and year interaction. The initial N₂O flux prior to starting the experiment (see Section "[N₂O flux measurements](#)") was an additional fixed effect for the cumulative N₂O–N emissions to account for the variation of background N₂O emissions across treatments. The *lmer* function can run such models, including with log-transformed response variables, when normality or heterogeneity of variance was not met. A Tukey Post Hoc test was used to test for differences among means using the *emmeans* function. Information from rainfall from the abovementioned weather station was used to qualitatively assess the impact of weather conditions on N₂O fluxes. In addition, the relationship between WFPS, soil temperature (each $n=24$), soil NO₃⁻-N and NH₄⁺-N concentrations (each $n=11$) as independent variables and the mean daily N₂O flux calculated for the LF and mineral N treatments, respectively, as dependent variable was determined with cross-correlation ($p < 0.05$). The cross-correlation was performed with the *ccf*-function in the *tseries* R package with a maximum lag period of 10 days. Negative lags were not considered for WFPS and soil temperature, because this was not biological meaningful.

Results

Weather and soil conditions

Rainfall and temperature were distinct at the onset of season 2023 and 2024. Prior to the application in April 2024 the weather was cold and wet with 67 mm rainfall and daily air temperatures decreasing from 12.7 on April 7 to 3.2 °C on April 22 (days after application: 0). In contrast, the air temperature in 2023 increased from April 1 to 20 from 2.6 to 9.9 °C, and with only 25 mm rainfall. This resulted in a higher mean soil temperature of 11.3 °C, and lower mean VWC with 0.21 cm³ cm⁻³, and 43% mean WFPS at the onset of the study in 2023, as compared to 2024

with 7.1 °C mean soil temperature, 0.27 cm³ cm⁻³ mean VWC and 55% WFPS in 2024, respectively. In 2023, the soil dried out thereafter with a mean WFPS of 30% throughout, and intermittently as low as 12% WFPS, while in 2024 the lowest measured WFPS was 25% with a mean WFPS of 43%. This was due to substantial rainfall in 2024 with 165 mm as compared to 2023 with 64 mm (Fig. 1a,b,d,e).

The soil NH₄⁺ content was much higher than the NO₃⁻ content for all digestate treatments at the onset of the study with 78.5 and 59.7 kg NH₄⁺ -N ha⁻¹ soil as compared to 13.0 and 1.8 kg NO₃⁻ -N ha⁻¹ in 2023 and 2024, respectively. In contrast, the mineral N treatments (N1 and BN1) had similar NH₄⁺ and NO₃⁻ content with 68.8 and 51.6 kg NH₄⁺ -N ha⁻¹ as compared to 68.4 and 47.9 kg NO₃⁻ -N ha⁻¹ in 2023 and 2024, respectively. The control N0 treatment had low and similar mineral N contents throughout the study with 16.8 and 4.6 kg NH₄⁺ ha⁻¹ and 12.5 and 1.7 kg NO₃⁻ -N ha⁻¹ soil in 2023 and 2024, respectively. In 2023, the mean NH₄⁺ content was reduced by more than half in the LF treatments to 36 kg NH₄⁺ -N ha⁻¹ until 13 days after application (May 3), while the NO₃⁻ content doubled to 24.8 kg NO₃⁻ -N ha⁻¹. Thereafter, both soil NH₄⁺ and NO₃⁻ content decreased. This was different in 2024, where the NO₃⁻ content remained low in the LF treatments (0.9 – 3.1 kg NO₃⁻ -N ha⁻¹). For the mineral N treatments, the soil NH₄⁺ and NO₃⁻ content remained at higher levels than the LF treatments in 2023, while in 2024 soil mineral N decreased to levels comparable to the N0 treatment (Fig. 2).

N₂O flux, cumulative N₂O emissions, and yield-scaled emissions

Highest N₂O fluxes occurred in all treatments until 20 days after application with a mean flux of 6.6 and 18.5 g N₂O-N ha⁻¹ d⁻¹ across all treatments in 2023 and 2024, respectively. Another peak period occurred at 37 days after application in 2024 with 7.2 g N₂O-N ha⁻¹ d⁻¹ across all treatments. Otherwise, fluxes were low in all treatments (Fig. 1c, f).

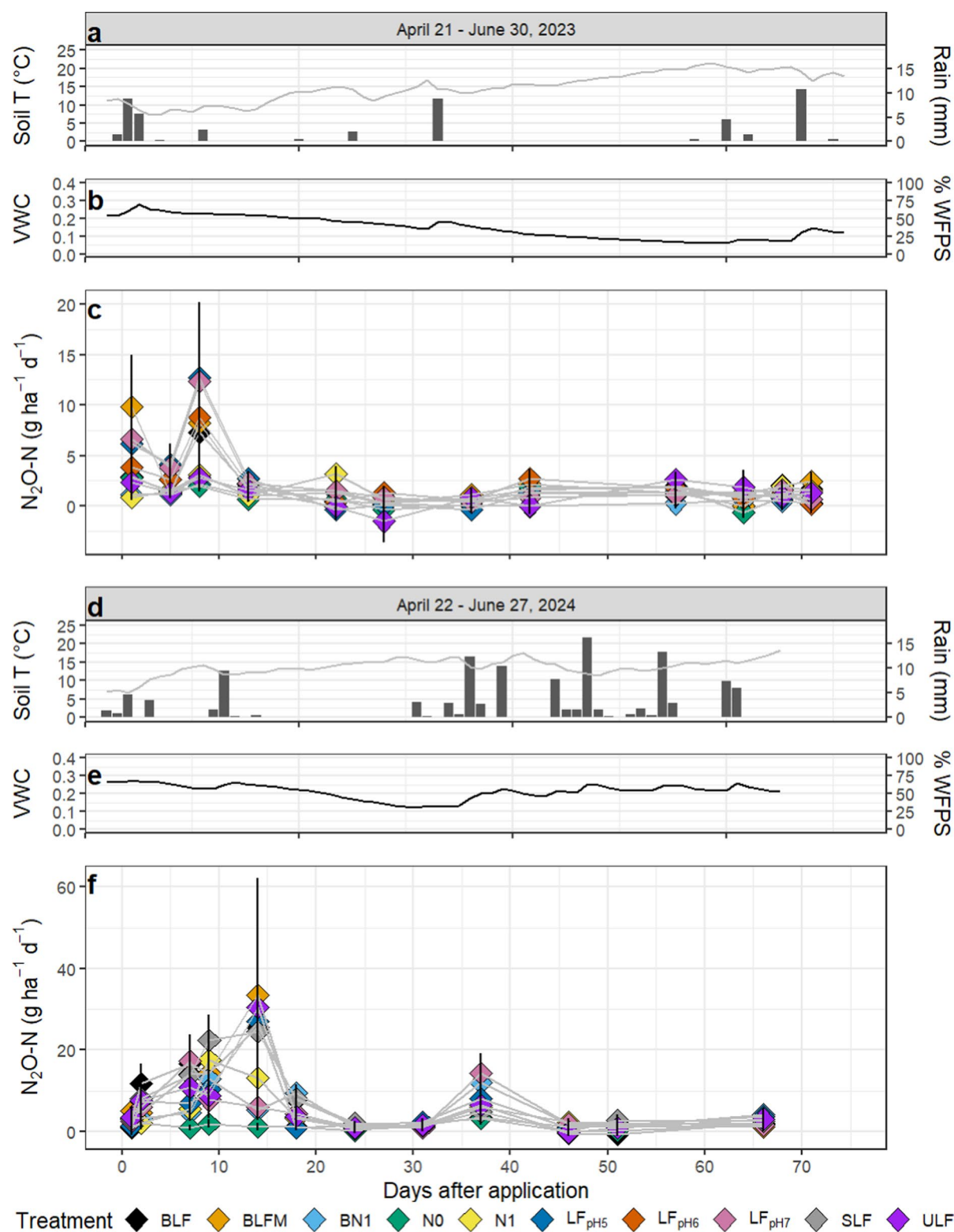
The WFPS and the N₂O flux were significantly positively correlated for LF and Mineral N treatments with a cross-correlation coefficient $r=0.53$ and 0.45 , both at concurrent time, respectively. Soil NH₄⁺-N and the N₂O flux were significantly positively

correlated with $r=0.62$ and a lag period of one day after N₂O flux increase and at concurrent time for LF, and with $r=0.78$ at a lag period of one day after N₂O flux increase for mineral N treatments, respectively. The soil NO₃⁻-N and the N₂O flux were significantly positively correlated with $r=0.71$ with a lag period of one day before N₂O flux increase in LF treatments, and with $r=0.69$ one day after N₂O flux increase for mineral N treatments, respectively. N₂O flux and soil temperature was not correlated.

In general, N₂O fluxes were lower in 2023 than in 2024, which was also reflected in the difference in initial N₂O flux measurements (prior to starting the experiment) and the cumulative emissions. The mean ($\pm$ SE) initial N₂O flux was 0.4 ± 0.3 and 5.1 ± 4.1 g N₂O-N ha⁻¹ d⁻¹, in 2023 and 2024, respectively. The higher N₂O fluxes resulted in significantly higher cumulative N₂O emissions in 2024 than in 2023 with 0.31 ± 0.17 and 0.11 ± 0.05 kg N₂O-N ha⁻¹, respectively. Also, the variation in N₂O emissions was higher in 2024 (Fig. 3a, b). In addition, the linear mixed model on cumulative N₂O emissions showed that there was a significant effect of the initial flux, a treatment-year interaction and treatment effect.

In 2023, the cumulative N₂O emission in the biochar and mineral N treatment (BN1) was significantly lower with (mean $\pm$ standard error) 0.04 ± 0.01 kg N₂O-N ha⁻¹ than mineral N (N1) with 0.10 ± 0.02 kg N₂O-N ha⁻¹, or any acidified and biochar-amended LF (LF_{pH5} = 0.12 ± 0.03 , LF_{pH6} = 0.14 ± 0.03 , LF_{pH7} = 0.15 ± 0.03 , BLF = 0.11 ± 0.03 , and BLFM = 0.14 ± 0.03 kg N₂O-N ha⁻¹). However, this was not the case in 2024, where neither of the mitigation measures had a significant impact on N₂O emissions, which were similar to the mineral N application (N1 = 0.21 ± 0.05 kg N₂O-N ha⁻¹). Neither acidified, sedicanter-treated, nor biochar-amended LF significantly reduced the cumulative N₂O emissions in either year as compared to the untreated LF (LF_{pH5} = 0.32 ± 0.07 , LF_{pH7} = 0.28 ± 0.06 , SLF = 0.40 ± 0.09 , BLF = 0.33 ± 0.07 , and BLFM = 0.27 ± 0.06 kg N₂O-N ha⁻¹). The N₂O emissions of ULF (2023: 0.07 ± 0.02 , 2024: 0.30 ± 0.07 kg N₂O-N ha⁻¹) were not significantly different to N1 in both years (Fig. 3a, b).

The yield-scaled emissions (YSNE) were significantly higher in 2024, with a significant treatment and interaction effect. The YSNE of BN1 in 2023 was significantly lower with (mean $\pm$ standard error)



◀**Fig. 1** Mean N₂O fluxes (g ha⁻¹ d⁻¹; n=4) for each treatment, rainfall (mm) and soil temperature (Soil T, in °C), volumetric water content (VWC) and water-filled pore space (WFPS) for the 2023 **a–c** and 2024 **d–f** season, respectively. BLF=biochar and LF, BLFM=biochar mixed with LF, BN1=biochar and mineral N fertilizer, N0=no additional N fertilizer, N1=mineral N fertilizer at 150 kg N ha⁻¹, LF_{pH5-6-7}=LF acidified to pH5, pH6 (2023 only), and pH7, respectively, SLF=post-Sedicanter LF (2024 only), ULF=untreated LF. Error bars show the standard error of the mean

7.0±1.7 g N₂O-N Mg⁻¹ yield than N0 or acidified or biochar-amended LF treatments, but was not significantly different, yet lower, than ULF and N1 with 15.6±3.8 and 17.6±4.2 g N₂O-N Mg⁻¹ yield, respectively. As with the cumulative N₂O emissions, BN1 YSNE was not significantly lower. Notably, the sedicanter liquid, SLF, was significantly higher than the control with an YSNE of 63.4±15.3 compared to 21.5±5.2 g N₂O-N Mg⁻¹ yield in 2024, likely due to relatively high cumulative N₂O emissions and lower yields.

Yield and nitrogen fertilizer replacement value (NFRV)

In both years, winter wheat yield (i.e., dry matter yields) was not significantly different between mineral N and digestate treatments, and only the control N0 treatment was significantly lower. The yield was significantly higher in 2024 than in 2023 with a mean (±SE) across all treatments of 6.5±0.75 and 4.8±0.44 Mg ha⁻¹, respectively (Fig. 3c, d). This resulted in a higher NUE in 2024 of 35–52% as compared to 2023 with 26–43% across LF and mineral N treatments. The grain N uptake in 2023 was significantly higher in BN1 than in BLF or ULF but was not significantly different in 2024 (Table 3). The linear regression analysis for Eq. 2 was significant at $p < 0.05$ with $R^2 = 0.95$ and 0.87 in 2023 and 2024, respectively (Fig. 4). There was no clear pattern in NFRV among digestate treatments and years, with an NFRV between 56 and 77%. The BN1 treatment with mineral N fertilizer and biochar was highest with 91% and 83% in 2023 and 2024, respectively (Table 3).

BLF=biochar and LF, BLFM=biochar mixed with LF, BN1=biochar and mineral N fertilizer, LF_{pH5-6-7}=LF acidified to pH5, pH6 (2023 only), and pH7, respectively, SLF=post-Sedicanter LF (2024

only), ULF=untreated LF, letters denote significant differences at $p < 0.05$.

Discussion

Seasonal effect on N₂O emissions

The N₂O emissions exhibited seasonal differences across all treatments, with higher N₂O emissions in 2024, likely due to an additional rainfall of 100 mm compared to 2023. Soil mineral N was similar in both years at the onset of the study (Fig. 2, Section "Weather and soil conditions"), but background N₂O emissions (i.e., without any treatments) were already higher in 2024 (Fig. 1, Section "N₂O flux, cumulative N₂O emissions, and yield-scaled emissions"). This indicates that environmental factors other than N input fostered N₂O production in 2024, of which rainfall showed the biggest difference between both years. Indeed, an increase of WFPS was concurrently related to an increase in N₂O flux, demonstrating the close and immediate relationship between soil water content and N₂O emissions. In addition, soil temperature was not correlated to the N₂O flux, further supporting that increased rainfall was the main reason for increased N₂O emissions in 2024. Seasonal variation in N₂O emissions is common in many cropping systems, and anaerobic soil conditions caused by excessive rainfall is a well-known driver of N₂O production (Dobbie et al. 1999). Higher N₂O emissions with more rainfall have been previously observed in Denmark under the same soil conditions (Petersen et al. 2023a, b). Indeed, tentatively higher N₂O emissions have been observed after moderate rain events and slurry application in Denmark, which was attributed to an increased denitrification activity (Hansen et al. 2025). However, assessing the mechanisms behind the higher N₂O fluxes was not measured and beyond the scope of this study.

While higher rainfall likely increased the magnitude of N₂O fluxes in 2024, most N₂O emissions still occurred during the first two weeks after fertilizer application, following a similar trend for both observation years at the onset of the study. In addition, soil NH₄⁺ and NO₃⁻ concentration were positively related to the N₂O flux with a 1-day lag period or concurrently, indicating an immediate impact of soil mineral N on the N₂O flux after fertilizer application. There

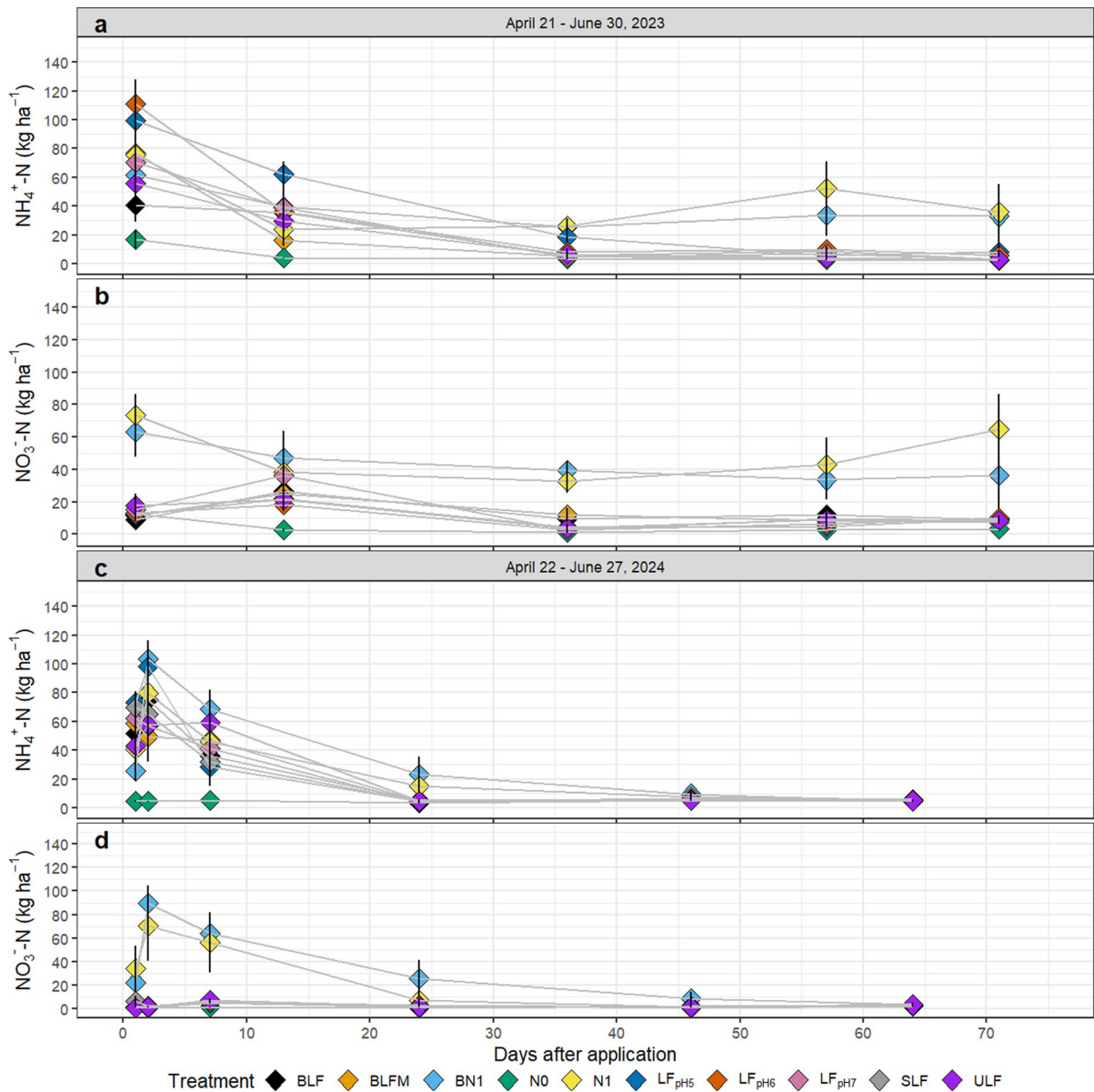


Fig. 2 Soil mineral N (NH_4^+ and NO_3^- ; $n=4$) content following the days after application for 2023 **a, b** and 2024 **c, d**. BLF=biochar and LF, BLFM=biochar mixed with LF, BN1=biochar and mineral N fertilizer, N0=no additional N fertilizer, N1=mineral N fertilizer at 150 kg N

ha⁻¹, LF_{pH5-6-7}=LF acidified to pH5, pH6 (2023 only), and pH7, respectively, SLF=post-Sedicanter LF (2024 only), ULF=untreated LF. Error bars show the standard error of the mean

was a second smaller peak period observed at 37 days after application in all treatments, which coincided with several prior rain events of up to 16 mm daily rainfall in 2024 (Fig. 1b, e, f).

These seasonal effects may have also impacted the N_2O mitigation effect of the studied treatments.

In 2023, the NH_4^+ and NO_3^- content in the mineral N treatments N1 and BN1 remained at higher levels than in the LF treatments, likely due to the dry soil conditions with almost no rainfall. In contrast, the soil NH_4^+ content decreased over time after application of LF, resulting in an increase of NO_3^- in the

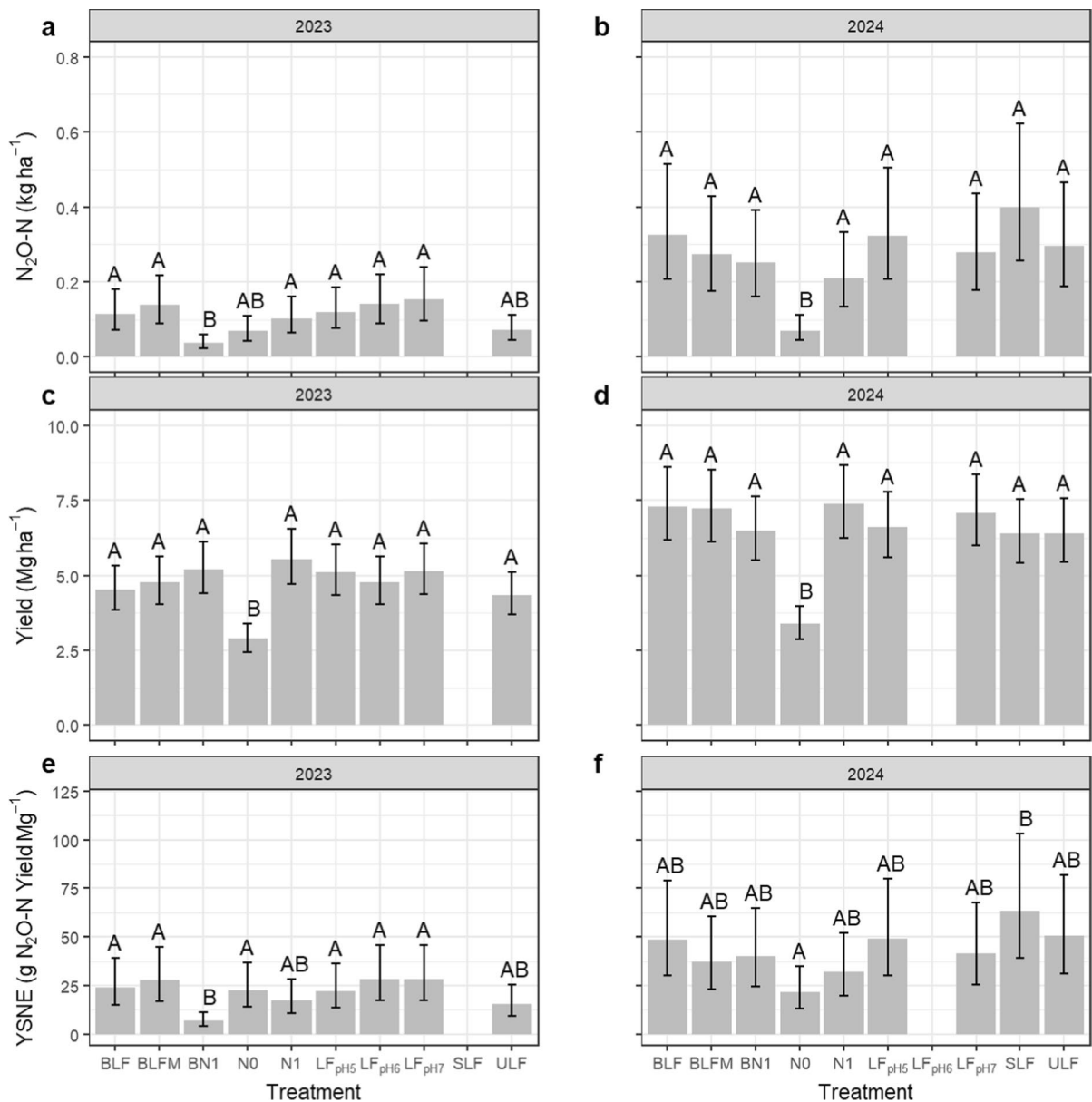


Fig. 3 Cumulative N_2O emissions ($n=4$), grain yield, and yield-scaled emissions for 2023 **a**, **c**, **e** and 2024 **b**, **d**, **f**, respectively. BLF=biochar and LF, BLFM=biochar mixed with LF, BN1=biochar and mineral N fertilizer, N0=no additional N fertilizer, N1=mineral N fertilizer at 150 kg N

soil solution in 2024, but not in 2023 (Fig. 1b, c, Fig. 2a, b). The mean N_2O peaks were much higher in the LF treatments than in the mineral N treatments (BN1, N1) in 2023 (Fig. 1f). LFs consists largely of water (here: 34–43 $Mg\ digestate\ ha^{-1}$), which likely increased WFPS, at least for a short period of time,

ha^{-1} , LF_{pH5–6–7}=LF acidified to pH5, pH6 (2023 only), and pH7, respectively, SLF=post-Sedicanter LF (2024 only), ULF=untreated LF. YSNE=yield-scaled N_2O emissions. Error bars show the standard error of the mean. Letters denote significant differences at $p < 0.05$

which could have favoured N_2O flux emissions. The drier conditions could be the reason why BN1 showed significantly lower cumulative N_2O emissions in 2023 but not in 2024. Seasonal differences in rainfall in sandy loam soils have previously impacted mitigation of nitrification inhibitors being more effective

Table 3 Mean and standard error of the Nitrogen fertilizer replacement value (NFRV) and nitrogen use efficiency (NUE) and N uptake of LF and biochar treatments in 2023 and 2024, respectively

Treatment	%NFRV		%NUE		Grain N uptake (kg N ha ⁻¹)	
	2023	2024	2023	2024	2023	2024
BN1	91 ± 8	83 ± 12	43 ± 6	52 ± 8	104.2 ± 6.5b	117.1 ± 7.3
BLF	56 ± 3	65 ± 7	27 ± 2	40 ± 2	80.0 ± 5.0a	101.5 ± 6.28
BLFM	60 ± 5	66 ± 6	29 ± 1	41 ± 6	83.0 ± 5.1ab	102.7 ± 6.4
ULF	56 ± 7	57 ± 6	26 ± 3	35 ± 5	79.5 ± 4.9a	94.4 ± 5.8
LF _{pH5}	77 ± 12	60 ± 8	37 ± 7	37 ± 7	93.8 ± 5.8ab	96.8 ± 6.0
LF _{pH6}	65 ± 3	–	31 ± 3	–	86.2 ± 5.3ab	–
LF _{pH7}	73 ± 4	67 ± 7	35 ± 3	42 ± 5	92.1 ± 5.7ab	103.7 ± 6.4
SLF	–	60 ± 7	–	38 ± 3	–	97.2 ± 6.0

in reducing N₂O emissions under wet soil conditions (Peixoto et al. 2023). This pinpoints the fact that N₂O mitigation measures are difficult to quantify and often inconsistent due to seasonality overshadowing mitigation measures.

Effect of treated LFs on N₂O emissions

LF can emit substantial amounts of N₂O compared to unseparated digestates (Meng et al. 2022), and this study tested different post-processing steps and application techniques to mitigate N₂O emission. Biochar addition to soil have been shown to decrease N₂O emissions (Cayuela et al. 2014; Borchard et al. 2019; Yang et al. 2025). Adding biochar to the solid fraction of digestates reportedly reduced N₂O emissions and the abundance of denitrifier genes, likely due to adsorption of organic compounds to biochar (Zheng et al. 2025). In addition, biochar can adsorb NH₄⁺ from LF (Zheng et al. 2024). Biochar has also been shown to reduce N₂O emissions and denitrifying activity, while nitrification was higher, when biochar was added directly to soil (Zhang et al. 2021a, b). In addition, biochar added to slurry digestate has reduced N₂O emissions by reducing nitrification processes (Liao et al. 2022). This contrasts with our study, where biochar, either mixed into the LF or applied separately had no reducing effect on N₂O emissions as compared to untreated digestate in both seasons. The N₂O mitigation potential of biochar also depends on high pyrolysis temperature, which reduces the H/C_{org} ratio, and a low concentration of carboxyl groups on the biochar surface (Cayuela et al. 2024). In this study, the H/C_{org} ratio was low with 0.29 and pyrolysis temperature was 600 °C, conditions under

which N₂O mitigation is favourable (Table 4). Note that much of the abovementioned knowledge is based on incubation studies, and it is possible that the mitigating effects of biochar on N₂O emissions from LFs are difficult to reproduce *in-situ*. For example, Efosa et al. (2023) found no effect of mixing biochar with digestate under field conditions. In addition, in this study biochar and digestate were applied on the soil surface in a growing winter crop, which could have limited the N₂O mitigation effect as compared to incorporation of biochar and LF injection into the soil, which is not possible in winter crops without causing some crop damage.

Only amending biochar to mineral fertilizer reduced N₂O emission as compared to mineral N, but not under the wet conditions in spring 2024. Biochar has previously been found to reduce N₂O emissions when added to urea, but the efficiency and mode of action depended substantially on biochar properties (Li et al. 2021). For example, N₂O increased for biochar made from chicken manure while decreased for biochars of other origin, likely due to NH₄⁺ adsorption (Grutzmacher et al. 2018). The reduction of N₂O could potentially also be an indirect effect due to higher NH₃ loss after the addition of the alkalic biochar, as indicated by the tendency of lower N uptake in the BN1 than in the N1 treatments. Biochar might also reduce N₂O production while total denitrification rates, i.e., N₂O reduction to N₂, might increase, as the alkalizing effect has been found to lead to more complete denitrification (Obia et al. 2015; Cayuela et al. 2024). Biochars can reduce N₂O emissions by changing soil pH and by adsorption of N and C compounds, which affects nitrifying and denitrifying microorganisms (Cayuela et al. 2014), but there is no indication that this was the case in our study (Fig. 5).

Note that the beneficial effect of biochar on N₂O emissions needs to be weighed against the N losses and greenhouse gas emissions during pyrolysis of the solid fraction. The solid fraction contains a significantly smaller share of N, approximately 13% of the digestate (Tambone et al. 2017). The N loss during biochar production depends on pyrolysis temperature, and at 600 °C the N loss is estimated to approximately 12% (Bruun et al. 2017). These N losses need to be compensated by mineral N fertilizer, which again creates greenhouse gas emissions of approximately 5.6 kg CO₂ eq kg⁻¹ N (Chojnacka et al. 2019). However, other methods of solid fraction treatment will likely also lead to N loss and greenhouse gas emissions (Aissaoui et al. 2025). This requires further evaluation of the carbon footprint of solid fraction treatments, and under which conditions an N₂O mitigation effect from biochar can be expected.

A previous study indicated that different methodologies of pig slurry separation can affect N₂O emissions of LF (Gómez-Muñoz et al. 2016), and similar effect could be expected for cattle slurry. In our study, the LF with post-digestion after a sedicanter separation (SLF) showed no significant reduction in N₂O emissions. Sedicanter treatment is a novel process where LF undergoes a second digestion and centrifugation to further reduce solids and phosphorus (Scholey et al. 2019). SLF was only tested in 2024 as a substitute for the LF_{pH6} treatment, since the 2023 results already indicated that acidification may not reduce N₂O emissions. Since the SLF had a lower N concentration, more liquid was applied with this fertilizer to obtain a similar N rate. Meng et al. (2023) observed high N₂O emissions after application of a diluted digestate due to the combination of a high amount of water applied with the digestate and a high content of labile C, and N which caused the denitrification of N compounds, even under lower WFPS levels. This could indicate that higher N₂O emissions can be expected from LFs with lower N content. However, more experiments are needed in different crops and across seasons to quantify and understand the N₂O dynamics on LFs with different dry matter and N contents.

The acidification of slurry and digestates is a measure to reduce NH₃ emissions after field application in Denmark (Pedersen et al. 2022). In contrast, increasing the soil pH fosters more complete denitrification and/or impacts nitrification (Feng et al. 2003;

Pfühl et al. 2025). Conversely, reducing the soil pH by applying acidified LFs may adversely affect N₂O emissions. However, the acidification of LF did not significantly increase N₂O emissions in this study in two contrasting seasons. This is in accordance with a previous study in Denmark, where slurry acidification did not affect N₂O emissions (Seidel et al. 2017). In another study, the N₂O emissions from the acidified liquid fraction of slurry was significantly higher at high water contents (Gómez-Muñoz et al. 2016). There was only a minor acidifying effect of the LF_{pH5} and LF_{pH7} treatments on soil pH in 2024 as compared to ULF (Fig. 5), suggesting that the soil might have buffered the acidic LF. Acidified slurry liquid fraction also reduced N₂O and NH₃ emissions significantly in a previous study, when applied to the surface (Fangueiro et al. 2017). However, low-dose acidification has reportedly no effect on N₂O and NH₃ emissions, while high-dose acidification reduced NH₃ but not N₂O in winter wheat (Sánchez-Rodríguez et al. 2018; Buchen-Tschiskale et al. 2023; ten Huf et al. 2023). Note that either injection or acidification of slurry and digestate is a legal requirement in Denmark for application in grassland to reduce NH₃ volatilization (Sommer et al. 2021). Acidification could potentially also be a good option for farmers for application in winter cereals, where injection of liquid organic fertilizers is uncommon. While no mitigation effect was observed in this study, the acidified digestate had comparable N₂O emissions as mineral N fertilizer in two very distinct seasons. While biochar amended to N fertilizer application can reduce N₂O emissions, the acidification of LF is an option for organic fertilizer applications, with N₂O emissions being comparable to mineral N fertilizer in winter cereals under the observed soil and environmental conditions.

Seasonal and treatment effects on yield and N uptake

Yield was significantly lower in 2023 than in 2024, indicating that water was a limiting factor for crop production in the dry year of 2023. In both years, all treatments showed no significant difference in yield as compared to mineral N1 fertilizer application. While not significant, mineral N1 treatments exhibited slightly higher yields (Fig. 3). This indicates that LF application at a similar total N rate or

biochar amendment to mineral fertilizer has no or little adverse effect on yield and could be an alternative source of N fertilizer application and C sequestration, respectively.

The Nitrogen fertilizer replacement value (NFRV) is a standard method to evaluate the crop response of organic fertilizers as compared to mineral N fertilizer, and a measure of how much organic fertilizer is needed to replace mineral N fertilizer (Jensen 2013; Westerik et al. 2024). Please note, that the calculated NFRV in this study compared directly a LF application and a mineral N application, both applied as the second N application after a similar starter mineral N application to all plots, thus following typical application methods in Denmark. Overall, the NFRV was in the expected range of LFs with 56–77% of total N applied, reflecting the amount of mineral N available for plant uptake (Table 2). In addition, the NUE was similar to NUE values for winter wheat in Denmark (Olesen et al. 2009). Also, the proportion of NH_4^+ -N to total N with 60–65% was similar to the NFRV as found in other studies (Webb et al. 2013). That indicates that NH_3 losses from the LFs were low, and that the effect of acidification on NH_3 emissions were limited. Nyang'au et al. (2023) applied unseparated digestates to winter wheat on a similar soil type and observed NFRVs of 33–44% of total N. The much lower values compared with our results indicate that in our study the fertilizer value was significantly improved in the liquid fraction after separation even without acidification. In contrast, the NFRV of digested cattle slurry applied by injection in spring barley by Nyang'au et al. (2023) and Reimer et al. (2025) was higher than NFRV by surface application of LF in this study, probably reflecting the combined benefits of reduced NH_3 loss and manure placement by injection in a spring barley crop.

In the dry 2023 season, acidification increased the NFRV compared to untreated digestates, while NFRV was unaffected by acidification in the wetter 2024 season. Meanwhile, the NFRV of ULF was similar in both years, indicating different effects of acidification on NH_3 volatilization in the two years. Measuring NH_3 was beyond the scope of the study, but it can be expected that the effect of acidification on reducing NH_3 volatilization was higher under drier soil conditions of 2023 than in 2024, leaving more mineral N available for plant uptake (Table 1). In 2024, biochar amendment of LF slightly increased

NFRV as compared to the ULF, while there was no effect in 2023. For BN1, NFRV was within the uncertainty range of the mineral N treatment in both years, indicating that biochar amendment does not or only slightly affects N from mineral fertilizer application. The yield-scaled emission sets N_2O emissions in relation to yield and can be used to quantify the effect of N_2O mitigation options on yield. There were no significant differences in YSNE between the LF treatments (excluding SLF) and mineral N fertilizer in either of the years (Fig. 3e, f). This is in accordance with Meng et al. (2023), who found no significant differences in YSNE in spring barley under similar climatic and soil conditions. This could indicate that the surface application of LFs is a feasible alternative to mineral N fertilizer without increasing N_2O emissions in winter crops. However, farmers would typically counter the lower amount of available N in digestates by increasing the application rate of LFs, which would likely also increase N_2O emissions. For SLF, the YSNE was significantly higher, owing to slightly lower yields with slightly higher cumulative N_2O emissions (Fig. 3f). SLF had a reduced N content due to additional post-processing steps, which resulted in higher liquid application rates per kg N, which could have led to higher N_2O emissions (see Section "Seasonal effect on N_2O emissions"). BN1 was the only promising mitigation option in this study that did not compromise yields in both years, which is important for the adoption of mitigation measures of farmers.

Conclusion

In this study, we found a strong seasonality with higher N_2O emissions and yields in the wetter 2024 season than in the drier 2023 season, which overshadowed possible mitigation effects. We therefore partially reject our null hypothesis, because biochar significantly reduced N_2O emissions when applied with mineral N fertilizer. However, this depends on climatic conditions with higher emission reductions in drier than in wetter years. Similar effects were not observed when biochar was applied with LFs, probably because the suspension of water, carbon and nitrogen create favourable conditions for N_2O emissions, which are not affected by biochar application. Further studies should focus on why the mechanisms behind N_2O mitigation potential of biochar observed

in incubation experiments can rarely be replicated *in-situ*, and how seasonality can affect the mitigation potential of biochar. Post-treatment of LFs had significantly higher yield-scaled emissions, but this was only tested in the wet 2024 season, and further experiments are needed for assessing its N₂O mitigation potential. Acidification of LF did not significantly reduce N₂O emissions but had no adverse effects on wheat yields. In addition, a positive effect of acidification on NH₃ emissions can be expected. Therefore, pollution swapping from NH₃ to N₂O are likely not expected from acidified LFs.

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Author contribution Conceptualization: PS, SB, AB, CD; Methodology: PS, SB, AB, CD; Software: CD, AB; Validation: PS, AB; Formal Analysis: CD, PS, AB; Investigation: CD, PS, AB, SB; Resources: PS, SB; Data Curation: CD, AB; Writing—Original draft: CD; Writing—Review and edition: CD, PS, AB, SB; Visualization: CD; Supervision: PS, SB; Project administration: PS, SB; Funding acquisition: PS, SB.

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Data availability All data supporting the findings of this study are available within the paper, and raw data will be available upon request.

Declarations

Competing interest The authors declare no conflict of interest.

Appendix

See Figs. 4, 5 and Table 4

Fig. 4 Linear regression between increasing mineral fertilizer rate and Grain N of winter wheat in 2023 (a) and 2024 (b) respectively. Coefficients are used to calculate the Nitrogen Fertilizer Replacement Value (NFRV)

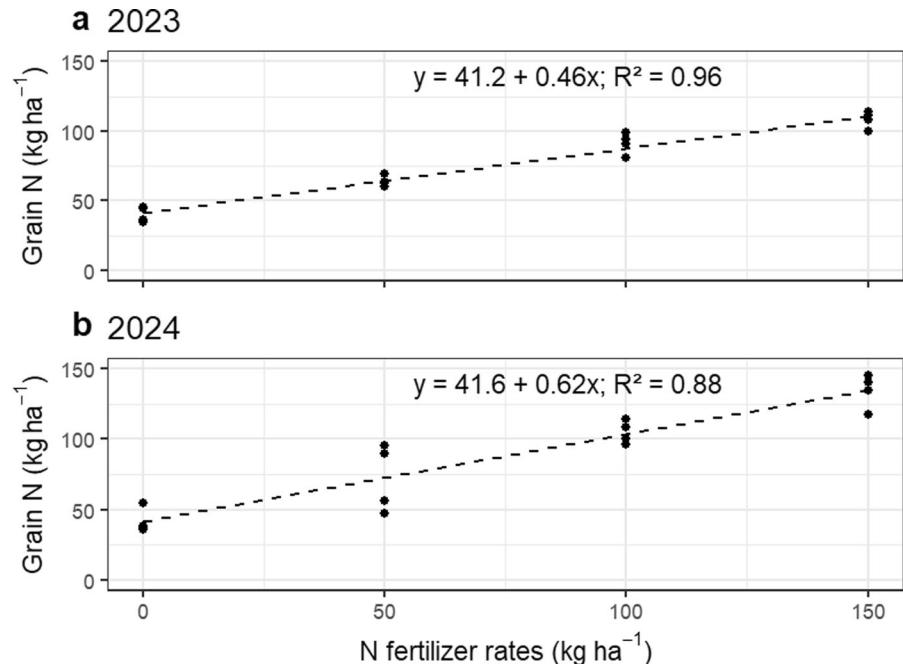


Fig. 5 Boxplot of soil pH measurements for each treatment across time periods and replicates during the 2024 season. BLF= biochar and LF, BLFM= biochar mixed with LF, BN1 = biochar and mineral N fertilizer, N0= no additional N fertilizer, N1 = mineral N fertilizer at 150 kg N/ha, LF_{pH5-6-7} = LF acidified to pH5, pH6 (2023 only), and pH7, respectively, SLF= post-Sedicanter LF (2024 only), ULF= untreated LF

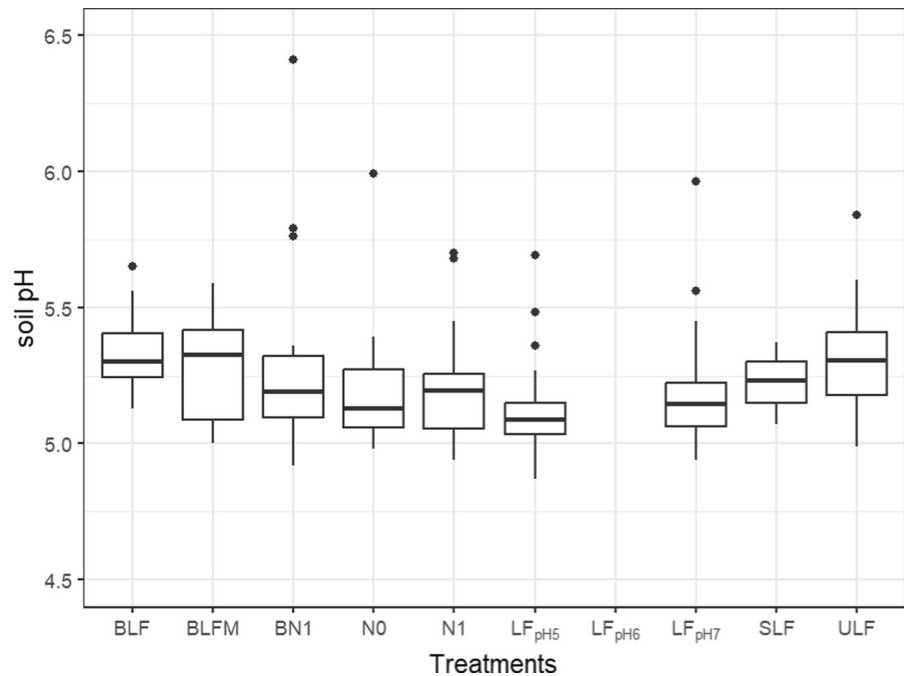


Table 4 Biochar properties used in the field experiment

Biochar properties	Value
Bulk density (g cm ⁻³)	0.43
% Ash	31.6
% Total C	63
%C _{org}	62.3
% Total N	1.56
C/N	40
H/C _{org} (molar)	0.29
pH _{CaCl2}	9.6
P (g kg ⁻¹)	22.3
K (g kg ⁻¹)	15.4

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References

- Aissaoui MH, Hertzog J, Sambusiti C, Gauthier-Maradei P, Pons M-N, Carré V, Le Brech Y, Dufour A (2025) Thermochemical conversion of solid digestates: effects of temperature and fluidizing gas on products composition. *J Anal Appl Pyrolysis* 186:106928
- Blicher-Mathiesen G, Sørensen P, Jung-Madsen S (2023) Opdatering af Baseline 2027. 159 Aarhus Universitet, DCE—Nationalt Center for Miljø og Energi
- Borchard N, Schirrmann M, Cayuela ML, Kammann C, Wrage-Mönnig N, Estavillo JM, Fuertes-Mendizábal T, Sigua G, Spokas K, Ippolito JA, Novak J (2019) Biochar, soil and land-use interactions that reduce nitrate leaching and N₂O emissions: a meta-analysis. *Sci Total Environ* 651:2354–2364
- Borchers HW (2022) pracma: Practical Numerical Math Functions. R package version 2.4.2. <https://CRAN.R-project.org/package=pracma>.
- Bruun S, Harmer SL, Bekiaris G, Christel W, Zuin L, Hu Y, Jensen LS, Lombi E (2017) The effect of different pyrolysis temperatures on the speciation and availability in soil of P in biochar produced from the solid fraction of manure. *Chemosphere* 169:377–386
- Buchen-Tschiskale C, Hagemann U, Augustin J (2020) Soil incubation study showed biogas digestate to cause higher and more variable short-term N₂O and N₂ fluxes than mineral-N. *J Plant Nutr Soil Sci* 183:208–219
- Buchen-Tschiskale C, Well R, Flessa H (2023) Tracing nitrogen transformations during spring development of winter wheat induced by ¹⁵N labeled cattle slurry applied with different techniques. *Sci Total Environ* 871:162061

- Canadell JG, Monteiro PM, Costa MH, Cotrim Da Cunha L, Cox PM, Eliseev AV, Henson S, Ishii M, Jaccard S, Koven C (2023) In climate change 2021: The physical science basis. contribution of working group i to the sixth assessment report of the intergovernmental panel on climate change, p. 673–816 Cambridge University Press
- Cayuela ML, Van Zwieten L, Singh BP, Jeffery S, Roig A, Sánchez-Monedero MA (2014) Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. *Agric Ecosyst Environ* 191:5–16
- Cayuela ML, Spott O, Pascual MB, Sánchez-García M, Sánchez-Monedero MA (2024) Key biochar properties linked to denitrification products in a calcareous soil. *Biochar* 6:90
- Chojnacka K, Kowalski Z, Kulczycka J, Dmytryk A, Górecki H, Ligas B, Gramza M (2019) Carbon footprint of fertilizer technologies. *J Environ Manage* 231:962–967
- Chuda A, Ziemniński K (2021) Digestate mechanical separation in industrial conditions: efficiency profiles and fertilising potential. *Waste Manag* 128:167–178
- Dietrich M, Fongen M, Foeroid B (2020) Greenhouse gas emissions from digestate in soil. *International Journal of Recycling of Organic Waste in Agriculture* 9.
- Dobbie KE, McTaggart IP, Smith KA (1999) Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. *J Geophys Res Atmos* 104:26891–26899
- Efosa N, Krause H-M, Hüppi R, Krauss M, Vaucher N, Zourek F, Mayer J, Six J, Bünemann EK (2023) Emissions of nitrous oxide and methane after field application of liquid organic fertilizers and biochar. *Agr Ecosyst Environ* 356:108642
- Eller F, Baggesen NS, Lykke EH, Peixoto L, Nielsen CS (2026) Contradicting default nitrous oxide emission factors: Average nitrous oxide emissions from mixed organic fertilizer application are higher than those from synthetic nitrogen fertilizers on Danish agricultural soils. *Agr Ecosyst Environ* 397:110057
- Fangueiro D, Pereira JLS, Macedo S, Trindade H, Vasconcelos E, Coutinho J (2017) Surface application of acidified cattle slurry compared to slurry injection: Impact on NH_3 , N_2O , CO_2 and CH_4 emissions and crop uptake. *Geoderma* 306:160–166
- Feng K, Yan F, Hütsch BW, Schubert S (2003) Nitrous oxide emission as affected by liming an acidic mineral soil used for arable agriculture. *Nutr Cycl Agroecosyst* 67:283–292
- Gómez-Muñoz B, Case SDC, Jensen LS (2016) Pig slurry acidification and separation techniques affect soil N and C turnover and N_2O emissions from solid, liquid and biochar fractions. *J Environ Manage* 168:236–244
- Grutmacher P, Puga AP, Bibar MPS, Coscione AR, Packer AP, De Andrade CA (2018) Carbon stability and mitigation of fertilizer induced N_2O emissions in soil amended with biochar. *Sci Total Environ* 625:1459–1466
- Guilayn F, Jimenez J, Rouez M, Crest M, Patureau D (2019) Digestate mechanical separation: Efficiency profiles based on anaerobic digestion feedstock and equipment choice. *Biores Technol* 274:180–189
- Hansen LV, Tariq A, Jensen LS, Bruun S (2025) Using weather forecasts to avoid major emission events of N_2O in connection with manure application. *Agric Water Manag* 318:109689
- He Y, Zhou X, Jiang L, Li M, Du Z, Zhou G, Shao J, Wang X, Xu Z, Hosseini Bai S, Wallace H, Xu C (2017) Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis. *GCB Bioenergy* 9:743–755
- IPCC Climate Change (2023) Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. 184
- Jeffery S, Abalos D, Prodana M, Bastos AC, Van Groenigen JW, Hungate BA, Verheijen F (2017) Biochar boosts tropical but not temperate crop yields. *Environ Res Lett* 12:053001
- Jensen LS (2013) Animal manure fertiliser value, crop utilisation and soil quality impacts. *Animal manure recycling*. pp 295–328. <https://doi.org/10.1002/9781118676677.ch15>
- Jørgensen MS, Plauborg F, Sørensen KK (2024) Climate normal for Foulum 1991–2020
- Li X, Xu S, Neupane A, Abdoulmoumine N, Debruyne JM, Walker FR, Jagadamma S (2021) Co-application of biochar and nitrogen fertilizer reduced nitrogen losses from soil. *PLoS ONE* 16:e0248100
- Liao X, Mao S, Chen Y, Zhang J, Müller C, Malghani S (2022) Combined effects of biochar and biogas slurry on soil nitrogen transformation rates and N_2O emission in a subtropical poplar plantation. *Sci Total Environ* 848:157766
- Liu X, Mao P, Li L, Ma J (2019) Impact of biochar application on yield-scaled greenhouse gas intensity: a meta-analysis. *Sci Total Environ* 656:969–976
- Manganaro JL, Lawal A, Goodall B (2015) Techno-economics of microalgae production and conversion to refinery-ready oil with co-product credits. *Biofuels Bioprod Biorefin* 9:760–777
- Maroušek J, Minofar B, Maroušková A, Strunecký O, Gavurová B (2023) Environmental and economic advantages of production and application of digestate biochar. *Environ Technol Innov* 30:103109
- Meng X, Ma C, Petersen SO (2022) Sensitive control of N_2O emissions and microbial community dynamics by organic fertilizer and soil interactions. *Biol Fertil Soils* 58:771–788
- Meng X, Sørensen P, Møller HB, Petersen SO (2023) Greenhouse gas balances and yield-scaled emissions for storage and field application of organic fertilizers derived from cattle manure. *Agric Ecosyst Environ* 345:108327
- Nielsen JD, Moberg J (1984) Klassificering af 5 jordprofiler fra forsøgsstationer i Danmark. *Tidsskrift for planteavl; Danish journal of plant and soil science*, ISSN 0040-7135.
- Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agron Sustain Dev* 34:473–492
- Nyang'au JO, Møller HB, Sørensen P (2023) Effects of electrokinetic and ultrasonication pre-treatment and two-step anaerobic digestion of biowastes on the nitrogen fertiliser value by injection or surface banding to cereal crops. *J Environ Manage* 326:116699

- Obia A, Cornelissen G, Mulder J, Dörsch P (2015) Effect of soil pH increase by biochar on NO, N₂O and N₂ production during denitrification in acid soils. *PLoS ONE* 10:e0138781
- Olesen JE, Askegaard M, Rasmussen IA (2009) Winter cereal yields as affected by animal manure and green manure in organic arable farming. *Eur J Agron* 30:119–128
- Pedersen AR (2022) HMR: Flux Estimation with Static Chamber Data. R Package Version 1:2
- Pedersen J, Nyord T (2023) Effect of low-dose acidification of slurry digestate on ammonia emissions after field application (Short communication). *Atmos Environ X* 17:100205
- Pedersen J, Feilberg A, Nyord T (2022) Effect of storage and field acidification on emissions of NH₃, NMVOC, and odour from field applied slurry in winter conditions. *J Environ Manage* 310:114756
- Pedersen IF, Müller-Stöver DS, Lemming C, Gunnarsen KC (2025) Particle size determines the short-term phosphorus availability in biochar produced from digestate solids. *Waste Manag* 191:172–181
- Peixoto L, Petersen SO (2023) Efficacy of three nitrification inhibitors to reduce nitrous oxide emissions from pig slurry and mineral fertilizers applied to spring barley and winter wheat in Denmark. *Geoderma Reg* 32:e00597
- Petersen SO, Peixoto LEK, Sørensen H, Tariq A, Braendholt A, Hansen LV, Abalos D, Christensen AT, Nielsen CS, Pullens JWM, Bruun S, Jensen LS, Olesen JE (2023) Higher N₂O emissions from organic compared to synthetic N fertilisers on sandy soils in a cool temperate climate. *Agric, Ecosyst Environ* 358
- Petersen SO, Peixoto LEK, Sørensen H, Tariq A, Braendholt A, Hansen LV, Abalos D, Christensen AT, Nielsen CS, Pullens JWM, Bruun S, Jensen LS, Olesen JE (2023) Higher N₂O emissions from organic compared to synthetic N fertilisers on sandy soils in a cool temperate climate. *Agr Ecosyst Environ* 358
- Pföhl L, Elsgaard L, Dörsch P, Fuß R, Well R (2025) Impact of liming and maize residues on N₂O and N₂ fluxes in agricultural soils: an incubation study. *Biol Fertil Soils* 61:575–594
- R (2019) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Reimer M, Möller HB, Sørensen P (2025) Utilization of cattle slurry, biogas digestates and separated digestates by injection to organically managed spring barley. *Eur J Agron* 164:127457
- Sánchez-Rodríguez AR, Carswell AM, Shaw R, Hunt J, Saunders K, Cotton J, Chadwick DR, Jones DL, Misselbrook TH (2018) Advanced processing of food waste based digestate for mitigating nitrogen losses in a winter wheat crop. *Front Sustain Food Syst* Volume 2
- Scholey D, Burton E (2019) In advanced bioprocessing for alternative fuels, biobased chemicals, and bioproducts (ed Majid Hosseini), p. 249–264 (Woodhead Publishing)
- SEGES (2023) Landsforsøgene 2023, Forsøg og undersøgelser i Dansk Landbrugsrådgivning. SEGES Innovation, Planter & Miljø, Aarhus, Denmark
- Seidel A, Pacholski A, Nyord T, Vestergaard A, Pahlmann I, Herrmann A, Kage H (2017) Effects of acidification and injection of pasture applied cattle slurry on ammonia losses, N₂O emissions and crop N uptake. *Agric Ecosyst Environ* 247:23–32
- Sohi SP, Krull E, Lopez-Capel E, Bol R (2010) Chapter 2—A review of biochar and its use and function in soil, in: advances in agronomy Vol. 105, 47–82 Academic Press
- Sommer SG & Knudsen L (2021) Impact of danish livestock and manure management regulations on nitrogen pollution, crop production, and economy. *Front Sustain* Volume 2
- Tambone F, Orzi V, D'imporzano G, Adani F (2017) Solid and liquid fractionation of digestate: Mass balance, chemical characterization, and agronomic and environmental value. *Bioresource Technol* 243:1251–1256
- Ten Huf M, Reinsch T, Zutz M, Essich C, Ruser R, Buchen-Tschiskale C, Flessa H, Olf H-W (2023) Effects of liquid manure application techniques on ammonia emission and winter wheat yield. *Agronomy Basel* 13:472
- Verhoeven E, Pereira E, Decock C, Suddick E, Angst T, Six J (2017) Toward a better assessment of biochar–nitrous oxide mitigation potential at the field scale. *J Environ Qual* 46:237–246
- Webb J, Sørensen P, Velthof G, Amon B, Pinto M, Rodhe L, Salomon E, Hutchings N, Burczyk P, Reid J (2013) In advances in agronomy Vol. 119 (ed Donald L. Sparks) 371–442 (Academic Press)
- Westerik D, Hoffland E, Hijbeek R (2024) Nitrogen fertilizer replacement values of organic amendments: determination and prediction. *Nutrient Cycl Agroecosyst* 129:445–458
- Woolf D, Amonette JE, Street-Perrott FA, Lehmann J, Joseph S (2010) Sustainable biochar to mitigate global climate change. *Nat Commun* 1:56
- Yang J, Xia L, Van Groenigen KJ, Zhao X, Ti C, Wang W, Du Z, Fan M, Zhuang M, Smith P (2025) Sustained benefits of long-term biochar application for food security and climate change mitigation. *Proc Natl Acad Sci U S A* 122:e2509237122
- Zhang L, Jing Y, Chen C, Xiang Y, Rezaei Rashti M, Li Y, Deng Q, Zhang R (2021b) Effects of biochar application on soil nitrogen transformation, microbial functional genes, enzyme activity, and plant nitrogen uptake: a meta-analysis of field studies. *GCB Bioenergy* 13:1859–1873
- Zhang QQ, Zhang X, Duan PP, Jiang XY, Shen HJ, Yan XY & Xiong ZQ (2021) The effect of long-term biochar amendment on N₂O emissions: experiments with ¹⁵N-¹⁸O isotopes combined with specific inhibition approaches. *Sci Total Environ* 769
- Zheng Y, Bolan N, Jenkins SN, Mickan BS (2024) Organic particles and high pH in food waste anaerobic digestate enhanced NH₄⁺ adsorption on wood-derived biochar. *Sci Total Environ* 946:174458
- Zheng Y, Abbott LK, Bolan N, Hu H, Jenkins SN, Mickan BS (2025) Biochar interacted with organic compounds from digestate in controlling N₂O emissions. *J Environ Manage* 385:125591

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