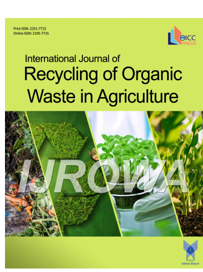

Volume 14, Issue 4, 142540 (1-11)

International Journal of Recycling of Organic Waste in Agriculture (IJROWA)

https://doi.org/10.57647/ijrowa.2025.16872

Digestate in paddy soil - methane emission and carbon sequestration

Bente Foereid[1,2,*], Maria Dietrich[2], Monica Fongen[3], Lisa Paruch[2]

[1]*Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, University of Inland Norway, Norway.*
[2]*Division of Environment and Natural Resources, Norwegian Institute of Bioeconomy Research, Norway.*
[3]*Division of Forest and Forest Resources, Norwegian Institute of Bioeconomy Research, Norway.*

[*]**Corresponding author:** bfoereid@gmail.com

Original Research

Received:
3 May 2024
Revised:
29 October 2024
Accepted:
20 April 2025
Published online:
30 May 2025

© 2025 The Author(s). Published by the OICC Press under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Abstract:

Purpose: Soils are the largest store of carbon in the biosphere, but soils can also act as the key sources of greenhouse gases. Rice cultivation in paddy soil is anoxic, thus creating an optimal condition for methane production. Digestate, a potential biofertilizer, contains a microbial consortium adapted for methane production because it comes from biogas production where methane production is optimised. In this study, we explored digestate as fertilizer in paddy soil, focussing on the effects of carbon sequestration and methane emission.
Method: A number of digestates and digestate products were incubated in the soils, both waterlogged and at field capacity. The effects of thermal treatment of the digestates were assessed to understand if the microbial community applied with the digestate played a role.
Results: Carbon dioxide emission was 53% higher in the soil with a history of mineral fertilizer application, than in the soil with digestate application history under waterlogging, at field capacity it was 13% smaller. Methane emissions came later when the digestate was heat-treated, indicating that the microbial community in the digestate could change the timing of emissions, but not the amount. Otherwise, some digestates increased methane emissions, whilst others had little effect. The supply of available carbon appeared to be an important factor to explain differences.
Conclusion: Digestate can increase carbon sequestration in paddy soil, although the interaction between waterlogging and soil history is not fully understood. The microbial community applied with the digestate can make methane emission start earlier, but it does not increase total emission.

Keywords: Rice paddy soil; Digestate; Methane; Carbon sequestration; Microbial dynamics

1. Introduction

Soils represent the largest store of carbon in the biosphere (Batjes, 1996; Jobbágy and Jackson, 2000), and moreover a source of trace gases (Smith et al., 2018). Soils are therefore important both as sources and sinks of greenhouse gases (GHG) (Oertel et al., 2016; Smith et al., 2018). Waterlogged conditions reduce decomposition and can induce peat formation and large increase in carbon storage, and a large proportion of soil carbon is stored in wetlands (Mitra et al., 2005). Soils that develop under waterlogged or periodically waterlogged conditions also develop other specific biochemical properties (Ferronato et al., 2019; Jiménez-Ballesta et al., 2025). Increased input of organic material also increases carbon storage, but can also increase the emission of methane, a powerful greenhouse gas from waterlogged soils (Oertel et al., 2016).

Rice is the most important staple food for half of the world's population, and the only grain that is almost exclusively used for human consumption (Muthayya et al., 2014; Bin Rahman and Zhang, 2023). Rice is usually cultivated under flooded conditions, which favours methane production (Wassmann et al., 2004; Gupta et al., 2021; FAO, 2023). However, the submerged condition can also lead to carbon sequestration in paddy soil (Pampolino et al., 2008), and there is a trade-off between carbon sequestration and methane emission (Das et al., 2023). Rice is estimated to contribute 5% of global anthropogenic methane emissions (Knief, 2019). Careful water management and well-balanced organic matter inputs are regarded as the most

effective ways to reduce emissions (Knief, 2019; Yagi et al., 2019; Liu et al., 2017).
Irrigated rice soil is often drained to field capacity once during the growing season, and towards harvest, to reduce methane emissions and save water (Haque et al., 2021; FAO, 2023). Rice fields can also be left dry during a dry season, sometimes there is also a crop rotation between paddy rice and dry season crops such as wheat, maize etc. This means that many rice fields will experience dry periods in between. After flooding it can take some time before methane emission starts, because other electron acceptors such as iron and sulphate need to be used up first (Frenzel et al., 1999; Hu et al., 2020). Drying-wetting cycles affected the microbial community, particularly that involved in nitrification in a wheat field (Wang et al., 2022), but less is known about how microbial activity leading to methane emission is affected by drying and re-wetting cycles in paddy soil.
Methane is primarily produced by methanogenic archaea under anoxic conditions (Buan, 2018). Methane can be consumed by methane oxidisers (methanotrophs) if oxygen is available (Bodelier and Laanbroek, 2004). Often methane is produced in deeper soil layers and a fraction is consumed in upper soils closer to the surface, where oxygen is available.
Biogas is a mixture of methane and carbon dioxide (CO_2) as well as smaller quantities of other gases, and the methane is used as a source of green energy. Biogas is produced by microbial processes in closed containers/reactors without air supply (Angelidaki et al., 2003) in conditions similar to anoxic condition in soils and sediments. There is always some organic material left that cannot be completely broken down in the reactor, which at the end, constitutes digestate (Al Seadi and Lukehurst, 2012). Digestates are currently often treated as waste products and not always optimally used, partly because high water content makes transport uneconomic (Drosg et al., 2015). However, digestates can be good fertilizers (Odlare et al., 2014; Sogn et al., 2018). The fertilizer quality is actually improved compared to the feedstock (Foereid et al., 2021; Frick et al., 2023). However, higher ammonium content can increase ammonia loss after application (Hafner et al., 2018; Lemes et al., 2023).
Partly decomposed organic matter such as digestate are thought to have smaller effects on methane emissions than fresh organic matter (Jain et al., 2004; Vu et al., 2015) as most of the methane potential is used in the digestion process. It is not known if the well adapted methanogenic microbes in a well-functioning biogas plant could introduce more efficient strains and therefore also affect methane emissions. However, Win et al. (2014) found that application of biogas slurry increased methane emission in rice paddies. Dietrich et al. (2020) investigated greenhouse gas emissions after digestate application to soil. While methane emissions were low from aerobic soil, there was a measurable effect of applied digestate for a long time. This suggests that methanogenic bacteria applied with the digestate may survive for a long time and potentially become active later. Although no long-term changes in the microbial community after digestate application could be detected in aerobic soil (Podmirseg et al., 2019), this may be different in waterlogged paddy soil.
Methane emissions from digestate composting have been shown to be high (Dietrich et al., 2021), caused by an adapted microbial community. During composting, favourable conditions for methane emissions can occur, high temperature and anaerobic microsites. These conditions are not found in aerobic soils, but similar conditions could be found in rice fields, although the highest temperatures during composting are normally not found in any soil. However, rice fields already have a microbial community adapted for methane production, so it is not clear if the addition of new methane producing microbes will have any effects.
The aim of this study is to find out how digestate application affects methane emissions and carbon accumulation in rice paddy soil, and if the microbial community applied with the digestate plays any role.

2. Materials and methods

Soil

The sampling site for the experiment was Salepur, a town in the district of Cuttack, in India. Two types of soil were used. One of the plots at this site was being treated with digestate compost for 7 years (SO). In the other plot synthetic fertilizer had been applied for at least 9 years (SI). See Table 1 for the history of each plot, and soil analysis results. Sampling was done using a soil auger. A composite sample of soil from the whole area was collected from each plot. Samples of the soils were grinded and analysed for carbon (C) and nitrogen (N) content on CHN analyser (Elementar Vario EL with TCD detector). Grinded samples (0.25 – 0.3 g) were digested in 5 mL of ultrapure nitric acid in an UltraClave from Milestone at 260 °C. Samples were diluted to 50.0 mL with DI water after digestion. Determination of inorganic elements was performed on an Agilent 8800 ICP-MS in oxygen reaction mode as a mass shift reaction 31P=>31P18O.

Digestates

Digestates were collected from two plants in Norway as well as composted digestate from a farm-scale plant in India. Romerike biogas plant (RBA) is a biogas plant to the north of Oslo, that treats food waste. This biogas plant uses THP (Thermal Hydrolysis Process) as pre-treatment and mesophilic process (38 °C). Samples were taken of unseparated (RBA) and solid fraction (RBAS) digestate. Magic factory biogas plant (MF) is another biogas plant in Norway which treats 60% mixture of manure from pigs and cattle and 40% food waste mainly from households but also some industrial food waste from commercial kitchens and food industry. This biogas plant uses a mesophilic process, and from this plant only unseparated digestate was collected. Composted digestate was collected in India (IN). The digestate comes from small-scale dome shaped bioreactors at Salepur region of India, which treats mainly cow manure. The solid is separated out by settling and composted in open piles. This was also the type of organic fertilizer used in the SO soil over 7 years (Table 1). A heat treatment was used to kill all microbes to test if the microbes imported with the digestate played any role for the emissions. RBAS (solid fraction from RBA) was heat treated at 105 °C for 24 hours

Table 1. Summary of properties and history of the two soils, one with a history of mineral fertilizer application only (SI) and one with a history of composted digestate application as organic fertilizer (SO). Measured soil properties are from topsoil (1 – 10 cm).

Soil	**SI**	**SO**
Plot Area (m^2)	400	240
Type of Soil	Sandy Loam	Sandy Loam
Amount and type of fertilizer applied before sowing (Kg/ha)	10 Gromor Potash, 10 Urea, 10 Single Super Phosphate	1200 kg/ha composted digestate
Amount and type of fertilizer applied before flowering (Kg/ha)	10 Urea	-
Duration of treatment (years)	9	7
Yield (Kg/m^2)	1.75	2.5
Soil analysis results		
pH	7.3	6.8
Total carbon (%)	0.26	0.88
Total nitrogen (%)	0.04	0.09
C:N	6.50	9.78
Phosphorus (mg/kg)	220	275
Potassium (mg/kg)	3015	2963
Magnesium (mg/kg)	2228	1942
Calcium (mg/kg)	3613	2427
Sulphur (mg/kg)	27.6	81.4
Iron (mg/kg)	17003	13612

(RBASH).
Analysis results for the digestates are given in Table 2. All organic fractions from Norway were analysed by Eurofins using their standard methods, method given in Table. The Indian digestate (IN) was analysed in the same way as the soil (because little material was available). All the samples were stored at a temperature of 4 °C until use in the incubation.

Incubation experiment

To get enough soil for the incubation from both SI and SO, soil from depths 1 – 10, 10 – 30, and > 30 cm was mixed in the ratio 3:1:6. The digestates and digestate products as described above were mixed with soil (SI and SO), but not all in both soil types. In addition, a treatment used soil only and one mineral fertilizer (urea). The treatments are summarised in Table 3. The amount of material added was chosen so that 6 mg N was applied in all. This amount was based on an application rate of 120 kg N/ha and 10 cm depth. All treatments were carried out both under waterlogged conditions and at field capacity. There were 3 replicates of each treatment.
50 mL mass of SO soil and SI soil (61 g and 62 g respectively) was transferred to 250 mL bottles at 40% water filled pore space (WFPS) and pre-incubated in a climate chamber at 30 °C and 90% humidity for 2 days. After pre-incubation, all the treatments (Table 3) were added to the soils in the bottles, and water content was adjusted to 60% WFPS in the non-waterlogged and 150% WFPS in the waterlogged. The bottles were then incubated for 21 days at 30 °C and 90% humidity. Gas samples were collected on intervals, twice a day in the beginning, then once a day. Water was added during the incubation to keep weight constant. pH (H_2O) in the mixtures was measured at the start and the end of the incubation.
Also 30 mL cups were incubated with the same treatment (proportions of soil and additions and water content). They were taken out at regular intervals and immediately stored at −80 °C for later DNA extraction.

Gas sampling and measurements

Measurements of the gas produced in the bottles were done by closing the bottles for a period of +/- 1 hour at the beginning of the incubation period and up to +/- 2 hours towards the end of the incubation. After this accumulation period 15 mL of gas was extracted through a septum with a syringe. This gas was injected into an evacuated vial and the samples were analysed by gas chromatography mass spectrometry to

Table 2. Chemical parameters measured in the digestates used. TS = total solids, TOC = total organic carbon, N_{tot} = total nitrogen.

Treatment	pH	Conductivity	TS	TOC	N_{tot}	NH_4-N
		[mS/m]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
RBA	7.6	1620	35000	11000	2700	1700
MF	7.8	2290	43000	1200	2500	2500
		[mS/m]	[%]	[%DM]	[g N_{tot}/100g DM]	[g NH_4-N/100g DM]
Method	Measured at 23/-2		Loss on ignition	TOC/NPOC	Kjeldahl	KCl extracted
RBAS	8.7	120	32.50%	43.30%	4.9	0.44
IN	7.2	129.1	28.02	32.3	1.96	

determine concentrations of CO_2 and CH_4. (Agilent Technologies 7820 A GC System gas chromatograph, coupled to a mass detector Agilent Technologies 5875 Series MSD and a Gilson 222 XL auto sampler). The sample was injected by a 5 mL sample loop, through a 0.5 m × 0.32 mm deactivated precolumn, into a 25 m × 0.32 mm CP-PoraPLOT Q-HT column (Chrompack), kept at 40 °C. Helium was used as carrier gas at 1.0 mL/min.

Microbial genomic DNA (gDNA) extraction

Soil bulk samples (stored at −80 °C) were slowly thawed and thoroughly homogenized. From each individual sample, 250 mg were taken in triplicate for subsequent gDNA extraction using DNeasy PowerLyzer PowerSoil Kit (Qiagen) following the manufacturer's instruction with minor modification for bead beating using 3000 rpm for 45 s on a Precellys®24 homogenizer. The extracted gDNA for each soil sample (in triplicate) was pooled and the yielded gDNA concentration was measured on a Qubit™ fluorometer (LifeTechnologies, Eugene, OR, USA) using Quant-IT™ dsDNA HSAssay Kit (Invitrogen, Carlsbad, CA, USA). To avoid repeated freeze-thaw cycles of the purified gDNA, small aliquots were prepared and stored at −20 °C for downstream molecular analysis.

Quantitative real-time PCR (qPCR) was performed to detect and quantify the methanogenic archaea and methanotrophic bacteria. The abundance of methanogens was measured by targeting methyl coenzyme M reductase gene (*mcrA*), using primer set ME23MF (5′ - ATGTCNGGTGGHGTMGGSTTYAC-3′) (Nunoura et al., 2008)/ME2r′ (5′- TCATBGCRTAGTTDGGRTAGT-3′) (Hales et al., 1996). The qPCR thermal cycles started with heat-up at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 52 °C for 30 s, 60 °C for 30 s, 83 °C for 10 s for plate reading at the end of each cycle. For qPCR examination of bacterial methanotrophs, methane monooxygenase gene (*pmoA*) was targeted using primer set A189f (5′-GGNGACTGGGACTTCTGG-3′) (Holmes et al., 1999)/mb661r (5′-CCGGMGCAACGTCYTTACC-3′) (Costello and Lidstrom, 1999). The established qPCR protocol included an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min and completed by plate reading at 83 °C for 10 s.

All qPCR assays were performed in duplicate on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) in 20 µL reaction mix consisting of 10 µL of SsoAdvanced™ Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 800 nM of each primer, 2 µL DNA template. Negative control (template-free) was included in each qPCR setup by substituting DNA with Nuclease-free water. To quantify the marker gene, a standard curve was established for each marker gene using serial dilutions of reference gene constructs (target gene-carrying plasmids). The verified standard curve exhibiting optimal amplification efficiency (90 – 100%) and linear regression coefficient R^2 (> 0.99)

Table 3. List of the treatments applied. All treatments were also repeated waterlogged (+W).

Treatments	SO–soil with history of organic fertilizer application	SI–soil with history of inorganic fertilizer application
Soil only	SO	SI
Unseparated RBA digestate	–	SI + RBA
Solid RBA digestate	SO + RBAS	SI + RBAS
Heat treated RBAS	SO + RBASH	SI + RBASH
Magic Factory digestate	–	SI + MF
Composted Indian digestate	SO + IN	–
Mineral fertilizer (urea)	–	SI + IF

was used for marker gene quantification of each soil sample. The collected raw qPCR data were further analysed and compiled using CFX ManagerTM Software Version 3.1 (Bio-Rad).

Volatile fatty acids

To measure the presence of volatile fatty acids (VFA) in the samples, samples from the cups were collected at the end of the incubation period. All the samples were stored at −20 °C until samples were processed for HPLC analysis. For sample preparation, to collect the liquid samples, a soil slurry was prepared, with 1.5 g of soil and 1 mL of distilled water in microcentrifuge tubes and it was mixed briefly on a vortex mixer, transferred into fresh 2.0 mL microcentrifuge tubes and then centrifuged at 10000 X g for 10 min. The supernatant was collected and filtered through a 0.2 µm membrane syringe filter. The supernatants were stored at −20 °C. Right before the analysis, 0.1 mL of conc. Sulphuric acid was added to 1 mL of supernatant. Thereafter, samples were centrifuged at 10000 X g for 1 min. Finally, the supernatant was transferred to HPLC vials. For the standard, a stock of 8 VFAs in known concentrations was prepared. From this stock, 6 dilutions of calibration standard were prepared. The concentrations of the volatile fatty acids were analysed using the Dionex UltiMate 3000 HPLC unit (Thermo Fisher Scientific) UV detection. The analytical column applied is Zorbax Eclipse Plus C18 from Agilent Technologies.

Statistics

Cumulated emissions over the whole period were calculated. The trapeze approach was applied to calculate the gas produced between the time steps before cumulating the emissions.
Minitab v19 was used for statistics. Cumulated values for gas emissions were compared using ANOVA and t-test as required. A 5% significance level was used.

3. Results and discussion

Soil carbon content was relatively low at the site, 7 years of application of digestate led to a large percentagewise increase (Table 1), but as it started from low level, the amount was relatively modest. The increase in C:N suggests that much of the increase was particulate organic matter that is not stable in soil. Other authors have also found relatively small increase after organic matter applications in paddy soil (Pampolino et al., 2008). This seems to hold true also in paddy drained part of the year as was the case here.
Soil carbon and nitrogen were higher in SO than in SI, whilst most other element contents were somewhat lower in SO than in SI (Table 1). Sulphur concentration was higher in SI than SO soil, whilst iron concentration was slightly higher in SO soil, this could affect methane production (Frenzel et al., 1999; Rath et al., 2002; Hu et al., 2020).
The total amount of nitrogen applied yearly was about the same in both fields, but higher yields were recorded with the organic fertilizer in the last year (Table 1). This reflects that organic nitrogen builds up in the soil and therefore most likely supplies more nitrogen to the crop after several years of application. The higher yield will also increase soil carbon content over time.
pH was higher in the soil with mineral fertilizer (urea) history (SI) than in the soil with a history of digestate application (SO). In most treatments, pH changed little or decreased slightly during the incubation. An exception was the mineral fertilizer treatment under waterlogging, where pH increased more than a unit (Fig. 1). This can be explained by the type of mineral fertilizer applied, urea. The initial step of urea process in the soil is dissociation with pH increase (Sigurdarson et al., 2018). There may be a pH decrease later through nitrification and/or ammonia volatilisation. Nitrification will usually not happen under waterlogging, so this can explain why the increase was only seen in the waterlogged treatment. The same effect could explain pH in the field.
CO_2 emissions during the incubation did not change very much over time during the incubation (data not shown), but emissions tended to increase and then decrease to reach a minimum somewhere between day 10 and day 15, and then sometimes have another peak after that. We do not have a good explanation for what happened between days 10 and 15, but a change is also seen in other parameters around that time. There was a significant, but not very large effect of the organic matter additions on cumulated emissions (Fig. 2), generally, higher emissions were recorded where more organic matter was applied. CO_2 emission was significantly

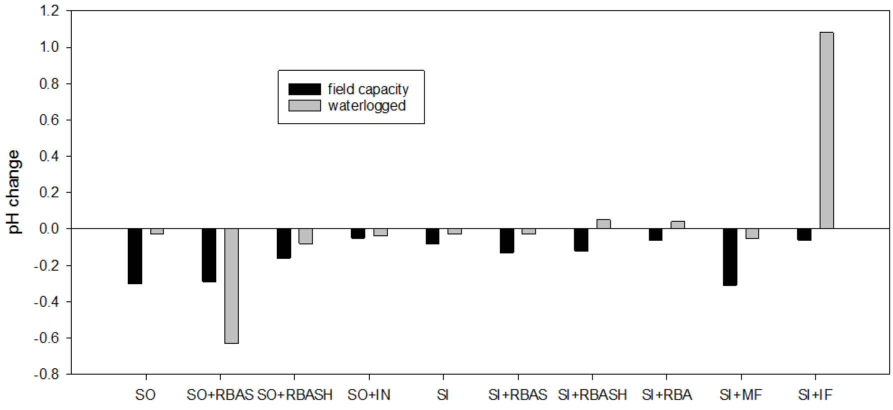

Figure 1. Change in pH during the incubation period.

Figure 2. CO_2 emission cumulated over the incubation period. Error bars are standard error (N = 3).

larger at field capacity than in waterlogged soil with a history of digestate application, whilst the opposite was true in the soil with a history of mineral fertilizer application. Liu et al. (2023) concluded that the effect of waterlogging depends on soil biochemistry, which is supported by our results. There was no significant effect of heat treatment on cumulated CO_2 emission.

In the soil with a history of mineral fertilizer use, higher CO_2 emissions were found under waterlogging than at field capacity (Fig. 2). This is surprising. Waterlogging should reduce the decomposition process. Higher CO_2 emission at waterlogging suggests that oxygen has not been limiting, and that extra water may have enhanced decomposition. Methane oxidised at the surface could contribute to this. The soil only shows the opposite, higher CO_2 at field capacity. This may suggest that the organic additions supply some easily available carbon that is not available in the soil, or nitrogen may increase decomposition rate. The latter is supported by the fact that also the mineral nitrogen treatment showed the same trend. This could, however, also be explained by the increased pH in this treatment.

Concentrations of VFA were low and below the detection limit in most cases (data not shown). Only iso-butyric acid concentrations were sometimes found above the detection limit in all replicates of a treatment. There was no significant effect of either waterlogging or organic addition, but in SO a significant interaction between them was found. Acetic acid was low in all, values above the detection limit were found most often in waterlogged samples. Methane emissions started somewhat later in the heat-treated digestate (RBASH) than in the untreated (RBAS) (Fig. 3), but then it became larger, and the cumulated emissions at the

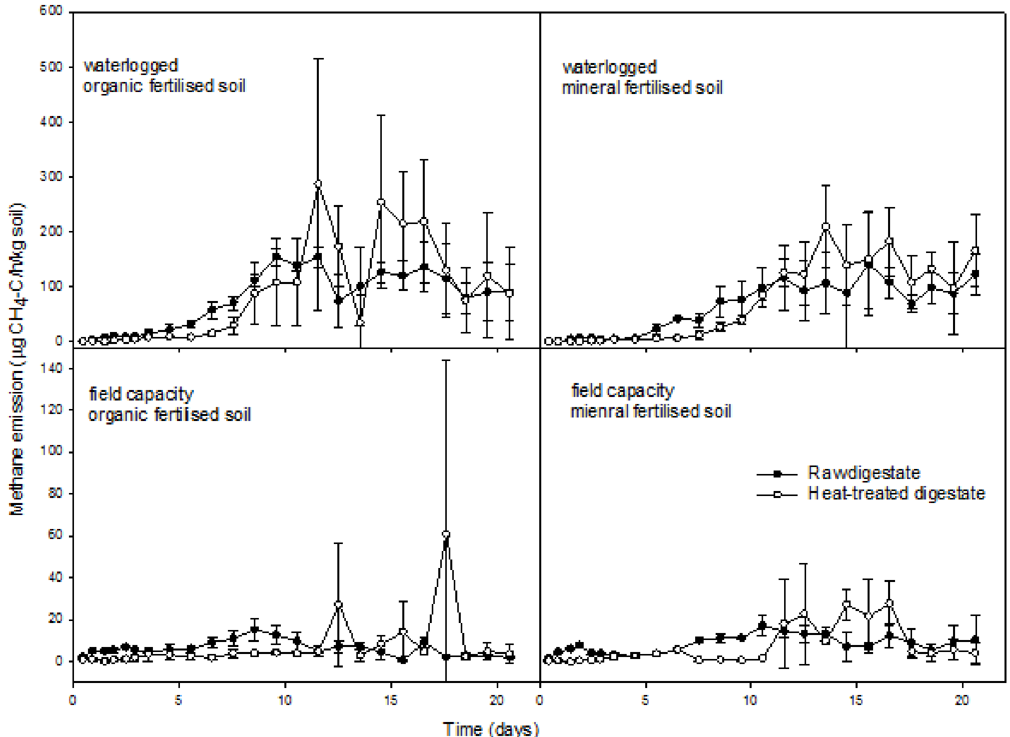

Figure 3. Methane emissions as a function of time comparing heat-treated and not heat-treated solid RBA digestate (RBAS and RBASH). Error bars are standard error (N = 3).

end of the incubation were not significantly different when heat-treated. This can be explained by methanogenic microbes imported with the digestate. However, the effect was not large as that found during composting (Dietrich et al., 2021), both because methanogenic microbes were already present in the paddy soil, and because the temperature did not reach the optimum for the methanogen in the digestate. This is supported to some extent by the DNA analysis, which showed a tendency of higher methanogen enrichment in the beginning in the untreated and towards the end in the heat-treated. This indicated that microbes applied with the digestate can have an effect in soil, but in this paddy soil there is also indigenous methanogens, so that the microbial suppression is only temporary, the population manages to revive after some time also in the heat-treated, leading to no tangible difference on overall methane production. At field capacity heat treatment had little effect because methane emissions were low overall, as expected.

Only the solid RBA digestate induced high methane emissions (Fig. 4). There was a tendency to higher emissions from liquid RBA digestate than the liquid MF digestate, although not significantly so ($p = 0.059$). Otherwise only small emissions were recorded. Methane emission from liquid RBA showed a much more distinct peak than the solid RBA digestates (RBAS(H)) (Fig. 5). Emission levelled about day 10, but it remained higher than in the beginning. The other two digestates had smaller peaks after day 10. The high emissions from RBA digestates may be because the digestion process was not run to completion in that biogas plant. In some cases the biogas process is not run to completion because shorter residence time in the reactor can give better economic return (Al Seadi and Lukehurst, 2012). Then there will still be some biogas potential in the digestate, which seems to be the case for RBA digestate. The digestate from the other plant (Magic factory MF digestate) and the composted Indian digestate (IN digestate) both induced only small emissions, not much different from soil only, suggesting that methane potential was used up in those.

Surprisingly, in soil only (both SO and SI) cumulated methane emissions were significantly higher in the soil at field capacity than in the waterlogged soil (Fig. 4). This may

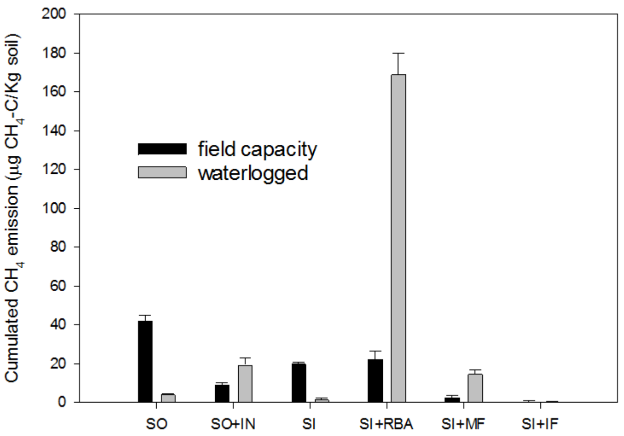

Figure 4. Methane emission cumulated over the incubation period. Error bars are standard error (N = 3).

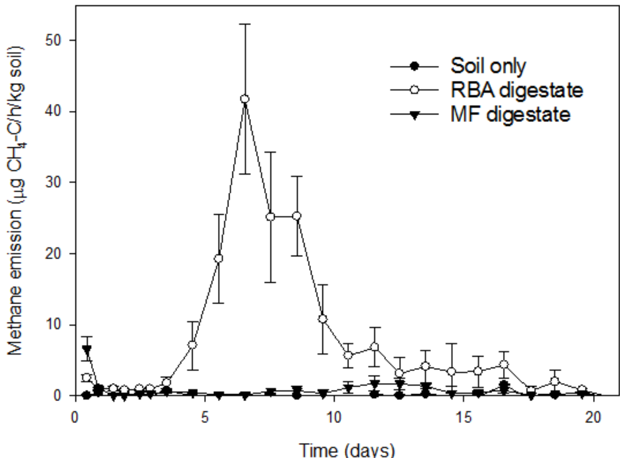

Figure 5. Methane emission for soil only and the two Norwegian liquid digestates.
Error bars are standard error (N = 3).

also suggest that oxygen was not limiting, as also suggested by the CO_2 results, but decomposition may instead be limited by supply of available carbon. The non-waterlogged soil only had significantly higher emissions than with any of the additions except the RBA digestate products, and this product had more available carbon. It was found that applications of organic matter other than RBA products (MF and IN) increased emissions much less (Fig. 4). The soil with a history of organic additions (SO) gave significantly higher emissions than the soil with history of mineral fertilizer application (SI).

Previous studies have indicated that application of organic matter (e.g. straw return) can increase carbon sequestration but also methane emission in paddy soils, careful water management could also reduce methane emission (Chen et al., 2024; Zhang et al., 2024; Zhu et al., 2024). The interaction between organic matter application and water content is not well understood. Although methanogenesis can only occur under anaerobic conditions, also aerobic soil contain anaerobic microsites that are important for carbon turnover and stabilisation (Keiluweit et al., 2017). More carbon may become available through aerobic decomposition at field capacity, to support methane emission. Abundance of methanogens also reached its peak later in the aerobic treatment rather than in the anaerobic, supporting this interpretation. Also low levels of all VFA at the end of the incubation suggest substrate limitation of methane production. The finding from our study does not agree with the widely shared knowledge on that methane production can only happen in anaerobic soil and oxidation only in aerobic soils (Knief, 2019), although methane production mostly happens in deeper soil layers, deeper than our experimental setup. There is also evidence that inhibition of methanogens by oxygen is limited and reversible (Pedizzi et al., 2016). Other authors have also found supply of available carbon to be important for methane emission particularly at high temperature (Kim et al., 2016; Wei et al., 2019). As the temperature here was 30 °C it can be expected that available carbon quickly became limiting. In the treatments with organic additions, methane emission was higher un-

der waterlogging, particularly with the RBA digestate. In this case decomposition of the abundantly available carbon quickly exhausted oxygen supply, so that methane production started.

Methanogenic population was at low level in all soils to start with, and it increased faster in SO than in SI (Fig. 6), particularly at waterlogged conditions. Waterlogging played a role in the beginning (up to day 11), the highest abundance peak was reached around day 6 – 8 in waterlogged SO and SI. Moreover, the thermal treatment suppressed the growth of methanogen population to varying extents at the start phase (up to around day 10), especially in cases of waterlogged SO and SI. However, the microbial inhibiting impact was rather time-limited, after day 14 most methanogens in both treated and non-treated samples were similar.

Methanotrophs only increased later in the incubation (after day 8 – 10) in all treatments. Soil type did not have any large effect (Fig. 7). However, whilst populations increased sharply at the end at field capacity, they stabilised under waterlogging. There were no clear effects of any of the additions or of heat treatment. This suggests that it takes time for populations to build up in response to a source of methane. This was supported by Yu et al. (2023) who found that even years were needed for stabilisation of both methanogen and methanotroph populations, so that long term and short-term effects were different. It is not possible to see any clear effect of the rise in these populations on methane emission, but it is possible that they prevent emissions to continue to increase in solid RBA (RBAS(H)) treatments. However, high numbers do not necessarily mean high activity, and methane oxidation probably did not play any major role.

Digestate applications supply organic matter, but also ammonium. Ammonium oxidation can compete with methane oxidation and therefore increase emissions, but nitrogen is also needed for methanotroph growth, and it could therefore also decrease emission (Bodelier et al., 2000; Bodelier and Laanbroek, 2004). This makes it very complicated to know what to expect when applying substances like digestate, that are rich in both ammonium and organic matter as well as a microbial population. We found lower methane emissions when urea was applied than in soil only, but the growth of methanotrophs was not much affected by any treatment.

4. Conclusion

We can conclude that digestate can safely be used as fertilisers in paddy soil, at least if the digestion process has been run to completion. The microbial community applied with digestate has a small effect on the timing of methane emissions, but it does not have any long-term effect. Digestate applications appear to increase organic matter in paddy soil, also if the soil is periodically dried. The study raises questions about the theory of anaerobic decomposition, as we sometimes found larger methane emissions and less CO_2 emission at field capacity than waterlogged, contradicting current theory. At least this suggests that both anaerobic and aerobic decomposition can occur simultaneously in different microsites of the soil. This requires further investigation.

Acknowledgement

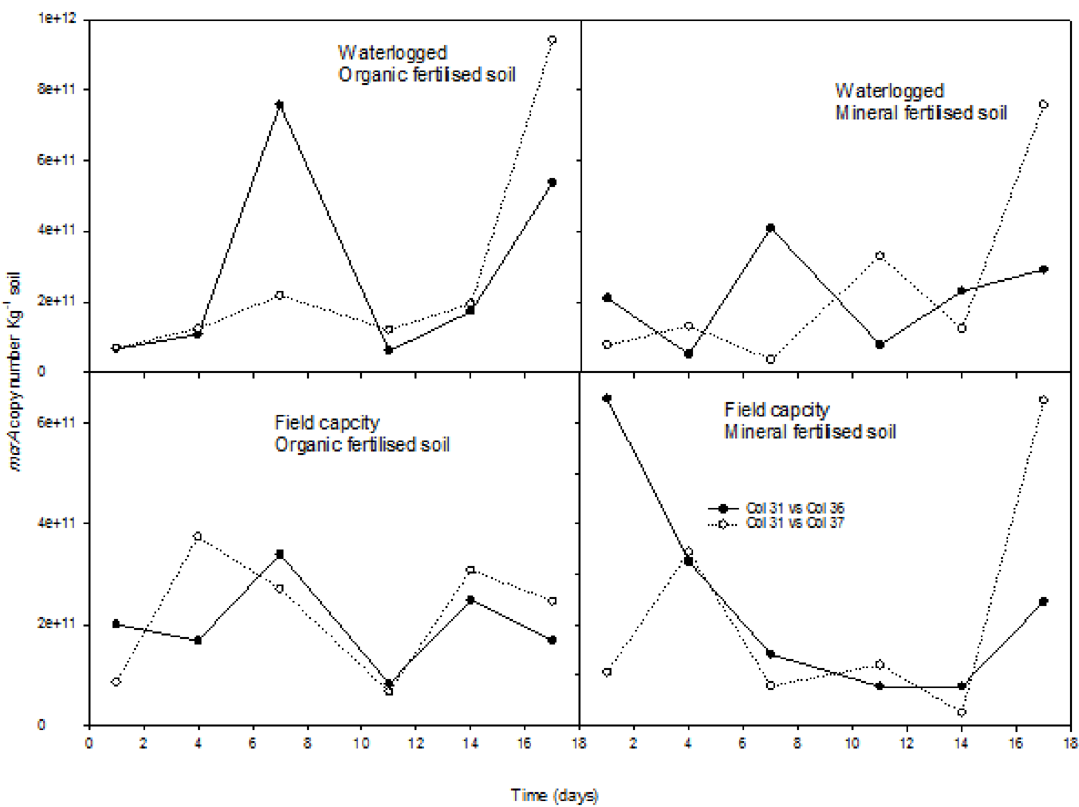

Figure 6. Copy number of methane producing genes in heat-treated and not heat-treated solid RBA digestate (RBAS and RBASH).

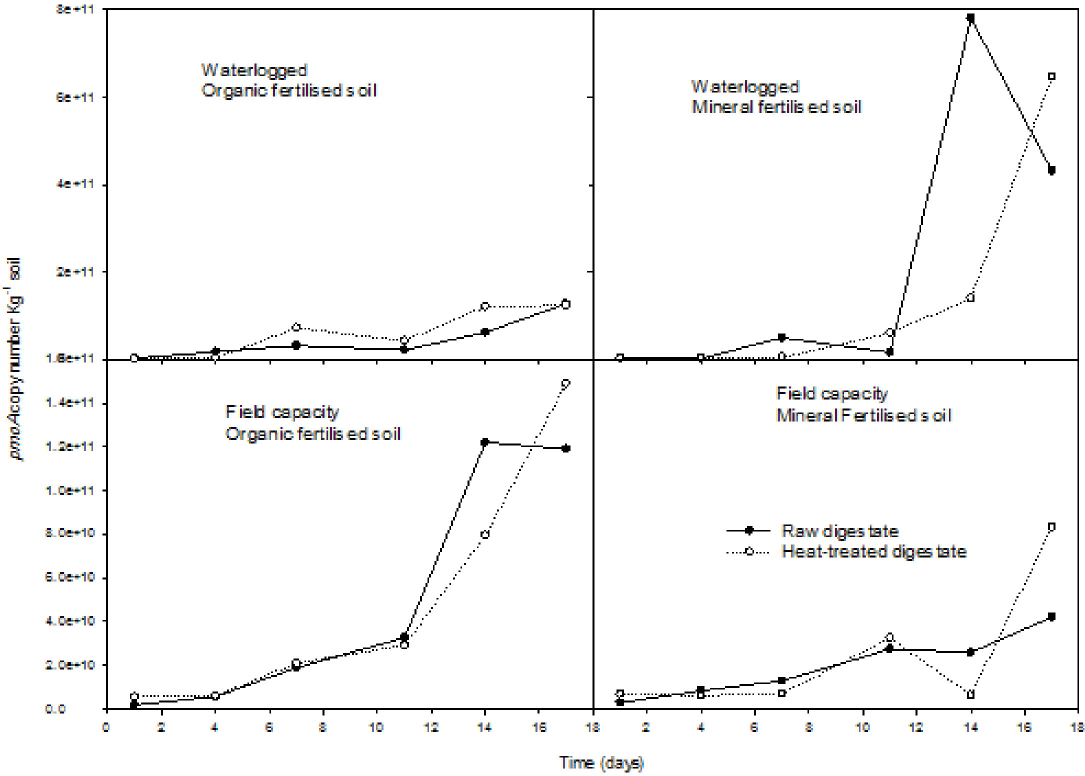

Figure 7. Copy number of methane oxidising genes in heat-treated and not heat-treated solid RBA digestate (RBAS and RBASH).

The authors wish to thank three Indian exchange students, Mitali Padhi, Atrayee Chatterjee and Debosmita Chakraborty as well as Jan Erik Jacobsen for analytical work. This study was funded by The Norwegian Research Council, grant no. 283570, RICEDIG-Greenhouse gas emissions from biogas digestate applications to rice production systems.

Authors contributions
The authors confirm the study conception and design: B. Foereid; data collection: M. Dietrich, B. Monica Fongen, Foereid, L. Paruch; analysis and interpretation of results: B. Foereid, M. Dietrich, L. Paruch; draft manuscript preparation: B. Foereid. The results were evaluated by all authors, and the final version of the manuscript was approved.

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

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Al Seadi T, Lukehurst C (2012) Quality management of digestate from biogas plant used as fertilizer. IEA Bioenergy.

Angelidaki I, Ellegaard L, Ahring BK (2003) Applications of the anaerobic digestion process, in biomethanation ii In: Ahring BK (ed) Springer, Berlin/Heidelberg, New York. 1–33. https://link.springer.com/chapter/10.1007/3-540-45838-7_1

Batjes NH (1996) Total carbon and nitrogen in the soils of the world. *Europ J Soil Sci* 47:151–163. DOI: https://doi.org/10.1111/j.1365-2389.1996.tb01386.x.

Bin Rahman ANMR, Zhang J (2023) Trends in rice research: 2030 and beyond. *Food Energy Secure* 12:e390. DOI: https://doi.org/10.1002/fes3.390.

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol Ecol* 47:265–277. DOI: https://doi.org/10.1016/S0168-6496(03)00304-0.

Bodelier PLE, Roslev P, Henckel T, Frenzel P (2000) Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* 403:421–424. DOI: https://doi.org/10.1038/35000193.

Buan NR (2018) Methanogens: pushing the boundaries of biology. *Emerg Top Life Sci* 2:629–646. DOI: https://doi.org/10.1042/ETLS20180031.

Chen Y, Guo W, Ngo HH, Wei W, Ding A, Ni B, Hoang NB, Zhang H (2024) Ways to mitigate greenhouse gas production from rice paddies. *J Environ Manag* 368:122139. DOI: https://doi.org/10.1016/j.fcr.2024.109571.

Costello AM, Lidstrom ME (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol* 65:5066–5074. DOI: https://doi.org/10.1128/AEM.65.11.5066-5074.1999.

Das SR, Nayak BK, Dey S, Sarkar S, Chatterjee D, Saha S, Sarkar D, Pradhan A, Saha S, Nayak AK (2023) Potential soil organic carbon sequestration vis-a-vis methane emission in lowland rice agroecosystem. *Environ Monit Assess* 195:1099. DOI: https://doi.org/10.1007/s10661-023-11673-0.

Dietrich M, Fongen M, Foereid B (2021) Anaerobic digestion affecting nitrous oxide and methane emissions from the composting process. *Bioresour Technol Rep* 15:100752. DOI: https://doi.org/10.1016/j.biteb.2021.100752.

——— (2020) Greenhouse gas emissions from digestate in soil. *Int J Recycl Org Waste Agric* 9:1–19. DOI: https://doi.org/10.30486/IJROWA.2020.1885341.1005.

Drosg B, Fuchs W, Al Saedi T, Madsen M, Linke B (2015) Nutrient recovery by biogas digestate processing. IEA Bioenergy.

FAO (2023) Methane emissions in livestock and rice systems-Sources, quantification, mitigation and metrics. Rome

Ferronato C, Marinari S, Francioso O, Bello D, Trasar-Cepeda C, Antisari LV (2019) Effect of waterlogging on soil biochemical properties and organic matter quality in different salt marsh systems. *Geoderma* 338:302–312. DOI: https://doi.org/10.1016/j.geoderma.2018.12.019.

Foereid B, Szocs J, Patinvoh RJ, Horváth IS (2021) Effect of anaerobic digestion of manure before application to soil – benefits for nitrogen utilisation? *Int J Recycl Org Waste Agric* 10:89–99. DOI: https://doi.org/10.30486/IJROWA.2020.1897538.1055.

Frenzel P, Bosse U, Janssen PH (1999) Rice roots and methanogenesis in a paddy soil: Ferric iron as an alternative electron acceptor in rooted soil. *Biochem Physiol Pflanz* 31:421–430. DOI: https://doi.org/10.1016/S0038-0717(98)00144-8.

Frick H, Efosa N, Oberson A, Krause H-M, Nägele H-J, Frossard E, Bünemann EK (2023) Nitrogen dynamics after slurry application as affected by anaerobic digestion, biochar and a nitrification inhibitor. *Soil Use Manage* 40:e12953. DOI: https://doi.org/10.1111/sum.12953.

Gupta K, Kumar R, Baruah KK, Hazarika S, Karmakar S, Bordoloi N (2021) Greenhouse gas emission from rice fields: a review from Indian context. *Environ Sci Pollut Res* 28:30551–30572. DOI: https://doi.org/10.1007/s11356-021-13935-1.

Hafner SD, Pacholski A, Bittman S, Burchill W, Bussink W, Chantigny M, Sommer SG (2018) The ALFAM2 database on ammonia emission from field-applied manure: description and illustrative analysis. *Agric for Meteorol* 258:66–79. DOI: https://doi.org/10.1016/j.agrformet.2017.11.027.

Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW, Saunders JR (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* 62:668–675. DOI: https://doi.org/10.1128/aem.62.2.668-675.1996.

Haque MM, Biswas JC, Maniruzzaman M, Hossain MB, Islam MR (2021) Water management and soil amendment for reducing emission factor and global warming potential but improving rice yield. *Pad Water Environ* 19:515–527. DOI: https://doi.org/10.1007/s10333-021-00851-w.

Holmes AJ, Roslev P, McDonald IR, Iversen N, Henriksen K, Murrell JC (1999) Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. *Appl Environ Microbiol* 65:3312–3318. DOI: https://doi.org/10.1128/AEM.65.8.3312-3318.1999.

Hu J, Wu H, Sun Z, Peng Q, Zhao J, Hu R (2020) Ferrous iron addition decreases methane emissions induced by rice straw in flooded paddy soils. *ACS Earth Space Chem* 4:843–853. DOI: https://doi.org/10.1021/acsearthspacechem.0c00024.

Jain N, Pathak H, Mitra S, Bhatia A (2004) Emission of methane from rice fields-A review. *J Sci Indust Res.* 63:101–115.

Jiménez-Ballesta R, San José FJ, Mongil-Manso J, Escudero-Campos L, Castellanos MP Álvarez (2025) Properties of waterlogged soils developed on arkose and aeolian sands in an agro-silvopastoral system. *Europ J Soil Sci* 1:e70055. DOI: https://doi.org/10.1111/ejss.70055.

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl* 10:423–436. DOI: https://doi.org/10.2307/2641104.

Keiluweit M, Wanzek T, Kleber M, Nico P, Fendorf S (2017) Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nat Commun* 8:1771. DOI: https://doi.org/10.1038/s41467-017-01406-6.

Kim SY, Gutierrez J, Kim PJ (2016) Unexpected stimulation of CH_4 emissions under continuous no-tillage system in mono-rice paddy soils during cultivation. *Geoderma* 267:34–40. DOI: https://doi.org/10.1016/j.geoderma.2015.12.021.

Knief C (2019) Diversity of methane cycling microorganisms in soils and their relation to oxygen. *Curr Iss Molecule Biol* 33:23–56. DOI: https://doi.org/10.21775/cimb.033.023.

Lemes LM, Nyord T, Feilberg A, Hafner SD, Pedersen J (2023) Effect of anaerobic digestion on odor and ammonia emission from land-applied cattle manure. *J Environ Manage* 338:117815. DOI: https://doi.org/10.1016/j.jenvman.2023.117815.

Liu X, Shi Z, Bai H, Zhang J, Sun D, Chen Y (2023) Soil carbon sequestration in paddy field and its simultaneous mineralization to supply available nutrients for the crops are affected by no-tillage with straw management: A meta-analysis. *Appl Soil Ecol* 188:104850. DOI: https://doi.org/10.1016/j.apsoil.2023.104850.

Liu Y, Jia B, Zhang Y, Cui H, Li XG (2017) The effect of waterlogging on soil organic carbon decomposition is dependent on its biochemistry. *J Soil Sci Plant Nutr* 23:4609–4619. DOI: https://doi.org/10.1007/s42729-023-01377-2.

Mitra S, Wassmann R, Vlek PLG (2005) An appraisal of global wetland area and its organic carbon stock. *Curr Sci* 88:25–35. DOI: https://doi.org/10.000226364700011.

Muthayya S, Sugimoto JD, Montgomery S, Maberly GF (2014) An overview of global rice production, supply, trade, and consumption. *Ann NY Acad Sci* 1324:7–14. DOI: https://doi.org/10.1111/nyas.12540.

Nunoura T, Oida H, Miyazaki J, Miyashita A, Imachi H, Takai K (2008) Quantification of mcrA by fluorescent PCR in methanogenic and methanotrophic microbial communities. *FEMS Microbiol Ecol* 64:240–7. DOI: https://doi.org/10.1111/j.1574-6941.2008.00451.x.

Odlare M, Pell M, Arthurson JV, Abubaker J, Nehrenheim E (2014) Combined mineral N and organic waste fertilization-effects on crop growth and soil properties. *J Agric Sci* 152:134–145. DOI: https://doi.org/10.1017/S0021859612001050.

Oertel C, Matschullat J, Zurba K, Zimmermann F, Erasmi S (2016) Greenhouse gas emissions from soils-A review *Geochem* 76:327–352. DOI: https://doi.org/10.1016/j.chemer.2016.04.002.

Pampolino MF, Laureles EV, Gines HC, Buresh RJ (2008) Soil carbon and nitrogen changes in long-term continuous lowland rice cropping. *Soil Sci Soc Am J* 72:798–807. DOI: https://doi.org/10.2136/sssaj2006.0334.

Pedizzi C, Regueiro L, Rodriguez-Verde I, Lema JM, Carballa M (2016) Effect of oxygen on the microbial activities of thermophilic anaerobic biomass. *Bioresour Technol* 211:765–768. DOI: https://doi.org/10.1016/j.biortech.2016.03.085.

Podmirseg SM, Waldhuber S, Knapp BA, Insam H, Goberna M (2019) Robustness of the autochthonous microbial soil community after amendment of cattle manure or its digestate. *Biol Fertil Soils* 55:565–576. DOI: https://doi.org/10.1007/s00374-019-01371-w.

Rath AK, Ramakrishnan B, Sethunathan N (2002) Effect of application of ammonium thiosulphate on production and emission of methane in a tropical rice soil. *Agric Ecosyst Environ* 90:319–325. DOI: https://doi.org/10.1016/S0167-8809(02)00031-2.

Sigurdarson JJ, Svane S, Karring H (2018) The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. *Rev Environ Sci Biotechnol* 17:241–258. DOI: https://doi.org/10.1007/s11157-018-9466-1.

Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Rey A (2018) Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *Europ J Soil Sci* 69:10–20. DOI: https://doi.org/10.1111/ejss.12539.

Sogn TA, Dragicevic I, Linjordet R, Krogstad T, Eijsink VGH, Eich-Greatorex S (2018) Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching. *Int J Recycl Org Waste Agric* 7:49–58.
DOI: https://doi.org/10.1007/s40093-017-0188-0.

Vu QD, Neergaard A de, Tran ATD, Hoang QQ, Ly P, Tran TM, Jensen LS (2015) Manure, biogas digestate and crop residue management affects methane gas emissions from rice paddy fields on Vietnamese smallholder livestock farms. *Nutr Cycl Agroecosyst* 103:329–346.
DOI: https://doi.org/10.1007/s10705-015-9746-x.

Wang X, Azarbad H, Leclerc L, Dozois J, Mukula E, Yergeau É (2022) A drying-rewetting cycle imposes more important shifts on soil microbial communities than does reduced precipitation. *Systems* 7:e00247–22. DOI: https://doi.org/10.1128/msystems.00247-22.

Wassmann R, Neue HU, Ladha JK, Aulakh MS (2004) Mitigating greenhouse gas emissions from rice-wheat cropping systems in Asia. *Environ Develop Sustain* 6:65–90.
DOI: https://doi.org/10.1023/B:ENVI.0000003630.54494.a7.

Wei L, Razavi BS, Wang W, Zhu Z, Liu S, Wu J, Kuzyakov Y, Ge T (2019) Labile carbon matters more than temperature for enzyme activity in paddy soil. *Soil Biol Biochem* 135:134–143.
DOI: https://doi.org/10.1016/j.soilbio.2019.04.016.

Win AT, Toyota K, Win KT, Motobayashi T, Ookawa T, Hirasawa T, Chen D, Lu J (2014) Effect of biogas slurry application on CH_4 and N_2O emissions, Cu and Zn uptakes by whole crop rice in a paddy field in Japan. *Soil Sci Plant Nutr* 60:411–422.
DOI: https://doi.org/0.1080/00380768.2014.899886.

Yagi K, Sriphirom P, N Cha un, Fusuwankaya K, Chidthaisong A, Damen B, Towprayoon S (2019) Potential and promisingness of technical options for mitigating greenhouse gas emissions from rice cultivation in Southeast Asian countries. *Soil Sci Plant Nutr* 66:37–49.
DOI: https://doi.org/10.1080/00380768.2019.1683890.

Yu H, Zhang X, Meng X, Luo D, Liu X, Zhang G, Zhu C, Li Y, Yu Y, Yao H (2023) Methanogenic and methanotrophic communities determine lower CH_4 fluxes in a subtropical paddy field under long-term elevated CO_2. *Sci Tot Environ* 904:166904.
DOI: https://doi.org/10.1016/j.scitotenv.2023.166904.

Zhang Z, Macedo I, Linquist BA, Sander BO, Pittelkow CM (2024) Opportunities for mitigating net system greenhouse gas emissions in Southeast Asian rice production: A systematic review. *Agric Ecosyst Environ* 361:108812.
DOI: https://doi.org/10.1016/j.agee.2023.108812.

Zhu K, Zhou T, Li Z, Zhang W, Wang Z, Gu J, Yang J (2024) Controlled irrigation can mitigate the greenhouse effects of rice paddy fields with long-term straw return and stimulate microbial necromass carbon accumulation. *Field Crop Res* 317:109571.
DOI: https://doi.org/10.1016/j.fcr.2024.109571.