Legacy effects override soil properties for CO2 and N2O but not CH4 emissions following digestate application to soil

by Rosace, M.C., Veronesi, F., Briggs, S., Cardenas, L.M. and Jeffery, S.

Copyright, publisher and additional information: Publishers’ version distributed under the terms of the Creative Commons Attribution License: http://creativecommons.org/licenses/by/4.0/

DOI: https://doi.org/10.1111/gcbb.12688
Legacy effects override soil properties for CO$_2$ and N$_2$O but not CH$_4$ emissions following digestate application to soil

Maria Chiara Rosace$^1$ | Fabio Veronesi$^1$ | Stephen Briggs$^2$ | Laura M. Cardenas$^3$ | Simon Jeffery$^1$

Abstract

The application of organic materials to soil can recycle nutrients and increase organic matter in agricultural lands. Digestate can be used as a nutrient source for crop production but it has also been shown to stimulate greenhouse gas (GHG) emissions from amended soils. While edaphic factors, such as soil texture and pH, have been shown to be strong determinants of soil GHG fluxes, the impact of the legacy of previous management practices is less well understood. Here we aim to investigate the impact of such legacy effects and to contrast them against soil properties to identify the key determinants of soil GHG fluxes following digestate application. Soil from an already established field experiment was used to set up a pot experiment, to evaluate N$_2$O, CH$_4$ and CO$_2$ fluxes from cattle-slurry-digestate amended soils. The soil had been treated with farmyard manure, green manure or synthetic N-fertilizer, 18 months before the pot experiment was set up. Following homogenization and a preincubation stage, digestate was added at a concentration of 250 kg total N/ha eq. Soil GHG fluxes were then sampled over a 64 day period. The digestate stimulated emissions of the three GHGs compared to controls. The legacy of previous soil management was found to be a key determinant of CO$_2$ and N$_2$O flux while edaphic variables did not have a significant effect across the range of variables included in this experiment. Conversely, edaphic variables, in particular texture, were the main determinant of CH$_4$ flux from soil following digestate application. Results demonstrate that edaphic factors and current soil management regime alone are not effective predictors of soil GHG flux response following digestate application. Knowledge of the site management in terms of organic amendments is required to make robust predictions of the likely soil GHG flux response following digestate application to soil.

KEYWORDS

carbon dioxide, digestate, legacy effects, methane, nitrous oxide, soil gas flux
1 | INTRODUCTION

Anaerobic digestion (AD) has potential benefits for the environment since it supports waste management, nutrient recycling and climate change mitigation though the substitution of fossil fuels (Møller, Boldrin, & Christensen, 2009; Möller & Stinner, 2009; Stinner, Möller, & Leithold, 2008). The main products of AD are biogas, which consists mainly of methane (CH$_4$; Nkoa, 2014) and digestate, a high water content mixture composed of partially degraded organic matter (OM), microbial biomass and inorganic compounds (Appels et al., 2011).

Digestate application can reduce synthetic fertilizer use by recycling nutrients and improving the OM content in agricultural lands (Pezzolla et al., 2012). It has been shown to be an effective fertilizer for crop production (Alburquerque, Fuente, Campoy, et al., 2012; Alburquerque, Fuente, Ferrer-Costa, et al., 2012; Barbosa, Nabel, & Jablonowski, 2014; Koszel & Lorencowicz, 2015; Möller & Müller, 2012) as it can increase the content of both macro and micronutrients in soil and plants (Chiew, Spångberg, Baky, Hansson, & Jönsson, 2015; Koszel & Lorencowicz, 2015). Nutrients in digestate, such as N, K and Ca are also provided in more plant available forms compared to the feedstock from which it was produced (Barbosa et al., 2014; Walsh, Jones, Williams, & Edwards-Jones, 2012). It also provides labile OM that can function as a substrate for the soil biota (Alburquerque, Fuente, Campoy, et al., 2012; Möller & Müller, 2012). Digestate use in agriculture can therefore lead to a reduction in use of fertilizers, with associated reductions in the energy usage, and hence carbon footprint, required for fertilizer manufacture (Alburquerque, Fuente, Campoy, et al., 2012).

While digestate provides a valuable source of nutrients for crop production, its environmental impact in terms of interactions with soil GHG flux remain poorly understood (Verdi et al., 2019). Nitrous oxide (N$_2$O) can be emitted from soils following digestate application, affecting air quality and contributing to climate change (Nkoa, 2014). However, N$_2$O emissions are generally lower when digestate is applied to soils compared to the application of undigested feedstock (Möller & Müller, 2012). Carbon dioxide (CO$_2$) and CH$_4$ soil fluxes are also generally reduced when applying digestate compared to the undigested feedstock (Maucieri, Nicoletto, Caruso, Sambo, & Borin, 2017; Möller & Stinner, 2009). This is likely because most of the labile carbon is turned into biogas through the AD process, resulting in a digestate with less substrate present and so a lower potential for formation and emission of these gases (Clemens, Trimborn, Weiland, & Amon, 2006). Nevertheless, an increase in CO$_2$ emissions after fertilization has been observed probably because of intensive decomposition of organic carbon after the application of easily degradable OM (Pezzolla et al., 2012) but emissions of GHG (such as CO$_2$ and CH$_4$) from fields fertilized with digestate on total emission from agriculture remain relatively low (Czubaszek & Wysocka-Czubaszek, 2018).

Soil fluxes of CO$_2$, CH$_4$ and N$_2$O all result from the actions of microorganism in the soil, as well as interactions with soil micro- and mesobiota in the case of CO$_2$ in particular. These fluxes can be affected by soil properties such as bulk density, porosity and pore connectivity, water filled pore space (WFPS) and soil temperature (Bouwman, 1990; Nóbrega et al., 2015). N$_2$O and CH$_4$ emissions generally increase as WFPS increases such that soils contain more anaerobic than aerobic microsites (Bateman & Baggs, 2005; Smith et al., 2003). Some studies indicate that maximum microbial activity occurs within soil at around 60% WFPS (Fichtner, Goersmeyer, & Stefan, 2019; Linn & Doran, 1984). Furthermore, both N$_2$O and CH$_4$ emissions are generally higher in sandy soils compared with clay soils (Cai et al., 1999), likely due to the increased pore sizes of such soils facilitating gaseous diffusion meaning that such gases are emitted to the atmosphere before microbes have the chance to interact with them. However, other studies have reported higher emissions in clay soils, likely due to the generation of more anaerobic microsites in clay soil compared to sandy soils. Observed differences likely arise due to differences in management practices such as tillage leading to different soil structural impacts (Rochette, Angers, Chantigny, & Bertrand, 2008). While some agricultural management practices are well known to affect soil GHG flux, such as N fertilizer addition, the importance of legacy effects arising from management in previous growing seasons are less well understood. A previous study investigated the impact of amendments on soil nutrient turnover and GHG emissions, also comparing the application of the anaerobically digested materials together with the raw slurry (Johansen, Carter, Jensen, Hauggard-Nielsen, & Ambus, 2013). They found that digestate application increased the soil concentration of NO$_3$- and assumed that respiratory denitrification was responsible for most of the N$_2$O emitted, possibly due to anaerobic conditions. A further study by Abraha, Gelfand, Hamilton, Chen, and Robertson (2018) investigated the legacy effects of nitrogen addition on N$_2$O flux from annual crop and perennial grasslands. Nevertheless, the role and interactions between soil properties and the legacy effects of previous soil amendment practices on soil GHG fluxes following digestate application to soil remains unknown. As such, this study aims to test the hypothesis that soil fluxes of individual greenhouse gases (GHGs; i.e. CO$_2$, CH$_4$ and N$_2$O) will be impacted more by soil properties than by previous soil management.

2 | MATERIAL AND METHODS

2.1 | Field experimental set-up

In April 2017, a field experiment was set up at Norbury Park, Staffordshire, United Kingdom (52°47’43.9”N, 2°17’24.0”W). It consisted of 18 plots (200 x 6 m) of three treatments, with six replicates, set up in a completely randomized design.
Treatments were: (a) standard practice (hereafter SP), amended with 150 kg/ha of N-fertilizer; (b) farm yard manure (hereafter FYM); (c) green manure (hereafter GM) which were drilled with a 50/50 mix of fodder radish (Raphanus sativus) and vetch (Vicia sp.) seeded at 29 kg seed/ha.

The FYM was collected from indoor beef cattle fed on grass, maize and barley. The feed was amended with Mix30—high energy liquid feed. FYM was applied to the treatment plots at a rate equivalent to 40 t FYM/ha eq.; the manure was stored for less than a week prior to application in the field with a muck spreader. It was then disked in to incorporate on the same day in March 2017. Total FYM nitrogen was 2.2%; mineral nitrogen was 0.6%.

All plots, except for the GM plots, were drilled with Spring wheat (var. Mulika) at a seed rate of 150 kg/ha in April 2017, at a drill depth of 3–4 cm.

In May 2017, FYM plots were fertilized with 125 kg/ha of Nitram (34.5% N) N-fertilizer (i.e. to the same level of available N as the SP plots). GM plots did not receive any fertilizer. In October 2017, the wheat crop was harvested, and glyphosate (‘Round up Biactive GL. 360 g glyphosate’ 1.5 L/ha in 200 L water) was applied to all plots, including the GM plots, which were not harvested.

Subsequently, winter oats (var. Mascani) were drilled at 160 kg/ha eq., into all plots by direct drilling using a Weaving GD drill (Weaving Machinery) in October 2017 at a depth of 3–4 cm. This included direct drilling through the cover crop in the GM plots. Desiccated cover crops and straw residues were left on the plots as per the initial treatments. This whole process is summarized in Figure 1.

2.2 | Laboratory incubation: Experimental design

In April 2018, soil samples were collected from each of the field experiment plots to a depth of approximately 10 cm using a spade. Soil texture fractions and types are reported in Table 1. Soil was sieved to pass 4 mm, and thoroughly mixed. Polypropylene pots (0.5 L) were packed with 300 g of soil from each plot to a bulk density of 1.3 g/cm³. Pots were watered to reach 60% WFPS, to replicate microbiologically but largely aerobic soils such as soils may be when digestate is applied; in fact the heavy trailer commonly used for digestate application necessitates soils having a high load bearing capacity, which soils with a much higher WFPS do not have. Pots were maintained gravimetrically at that moisture content through the timeframe of the experiment. Duplicate pots were made for each of the 18 field plots to total 36 pots. The pots were placed in a completely randomized design and preincubated in a climate-controlled room at 20°C for 10 days to allow soil gas fluxes to equilibrate after the disturbance of soil sampling and packing into pots.

A digestate produced by a mesophilic AD process (33 ± 7°C) of a cattle slurry (a mixture of milking cow slurry and young stock slurry, which was collected postseparation. No straw was included) at Harper Adams University, was applied to the surface of half of the duplicate pots at a rate equivalent to 250 kg/ha of total N. This is representative of the maximum permissible level of N applied as part of an organic amendment to soil annually (WRAP, 2012) within a Nitrate Vulnerable Zone within the United Kingdom. The other half of duplicate pots were kept as controls with no digestate applied but with water equivalent to the amount added with the digestate.

A subsample of the digestate was analysed for pH, total solids, volatile solids (VS), ash, VFA/TA ratio (an indicator for assessing fermentation processes; Table 2).

2.3 | Gas flux measurement

Soil GHG fluxes (CO₂, CH₄ and N₂O) were sampled from the headspace of each of the 36 pots (six per treatment) on Day 0, 1, 2, 4, 8, 16, 32 and 64, 1 hr after closing the pot with a lid, using 30 ml gas syringe. The content of each syringe was passed through pre-evacuated 20 ml vials, with the excess gas vented to ensure samples were stored at atmospheric

**Figure 1** The timing of the application of the different treatments is reported, illustrating fertilizations, ploughings and cropping from April 2017 to April 2018.
pressure. A further six empty bottles were closed and sampled at equal times between the soil-sample collections, in order to allow correction of the sampled gases for the background values of the gases present in the air inside the laboratory during the sampling time. Gas samples were transferred to 20 ml pre-evacuated glass vials and transported to the laboratory. The concentration of GHGs was determined by gas chromatography (Perkin Elmer Clarus 500 gas chromatograph fitted with a Turbomatrix 110 automated headspace sampler, and an Electron Capture Detector [ECD] set at 300°C for N₂O analysis). The Clarus system is fitted with two parallel Perkin Elmer Elite PLOT-Q megabore capillary columns (oven temp 35°C), enabling the simultaneous analysis of methane, carbon dioxide and nitrous oxide from the air samples. Methane and carbon dioxide being detected using a Flame Ionization Detector (FID), fitted with a methanizer (350°C), and nitrous oxide via an ECD (300°C).

Daily fluxes measured in parts per million (ppm) were first converted to mg CO₂-C hr⁻¹ m⁻², mg CH₄-C hr⁻¹ m⁻² and mg N₂O-N hr⁻¹ m⁻², considering a temperature of 20°C (i.e. 293.15 K) and R (gas constant) of 0.0821 atm L⁻¹ mol K⁻¹ (R*ₜ = 24.067615) as follows:

\[
\text{mg CO}_2\text{-C hr}^{-1} \text{m}^{-2} = \left( \frac{\text{ppm} \times \text{total volume head space} \times \text{atomic weight}}{24.067615} \right) : 0.00658993 \text{m}^2 \cdot 1.000.
\]

Values were finally converted to cumulative fluxes assuming linearity of flux rate between each measurement day.

### Table 2

**Digestate properties and characteristics**

| Properties | Values |
|------------|--------|
| pH         | 8.18   |
| TS (%)     | 3.71   |
| VS (% of TS) | 65.14 |
| VFA (mg/L)     | 2,540.00 |
| TA meq CaCO₃/L | 13,952.95 |
| VFA/TA ratio | 0.18   |
| Ammonium (g/L) | 2.31   |
| Ammonium (g/kg TS) | 8.44   |

Abbreviations: TA, total alkalinity; TS, total solids; VFA, volatile fatty acids; VS, volatile solids.

### Table 1

Soil analysis showing three key soil characteristics at the end of the experiment (±SE)

| Treatment replicates (from north to south) | Soil fractions | Soil texture^a |
|-------------------------------------------|----------------|----------------|
| Green manure                              | 68.2 16.3 15.5 | Sandy loam     |
| Farm yard manure                          | 66.5 17.2 16.3 | Sandy loam     |
| Standard practice                         | 65.7 18.7 15.6 | Sandy loam     |
| Standard practice                         | 62.9 19.6 17.5 | Sandy loam     |
| Farm yard manure                          | 61.4 21.3 17.3 | Sandy loam     |
| Green manure                              | 63.3 18.9 17.8 | Sandy loam     |
| Green manure                              | 58.4 21.8 19.8 | Sandy clay loam|
| Standard practice                         | 57.7 22.2 20.1 | Sandy clay loam|
| Farm yard manure                          | 59.3 21.5 19.2 | Sandy clay loam|
| Green manure                              | 60.0 21.9 18.1 | Sandy clay loam|
| Standard practice                         | 56.5 24.1 19.4 | Sandy clay loam|
| Farm yard manure                          | 61.2 20.4 18.4 | Sandy clay loam|
| Green manure                              | 59.8 21.6 18.6 | Sandy clay loam|
| Farm yard manure                          | 61.5 21.6 16.9 | Sandy loam     |
| Standard practice                         | 61.4 21.0 17.6 | Sandy loam     |
| Farm yard manure                          | 64.2 19.0 16.8 | Sandy loam     |
| Green manure                              | 61.9 19.9 18.2 | Sandy clay loam|
| Standard practice                         | 66.9 17.1 16.0 | Sandy loam     |

^aTexture calculated using LandIS (2019) based on fractions analysed in the lab.
At the end of the experiment, soil texture was measured for each of the 36 samples by sieving and sedimentation (ADAS, 1986; Table 1).

### 2.5 Statistical analysis

Statistical analysis was undertaken by using R version 3.5.1 (R Core Team, 2018). Soil pH, electrical conductivity (EC) and OM data were first analysed using a Shapiro test and a Bartlett test. Subsequently, a two-way ANOVA, with Tukey’s HSD used post hoc, was used to assess differences between the treatments and digestate versus no digestate application.

A mixed effect model was used to compare soil texture, soil amendment legacy and digestate application, to assess which of these parameters was the most important predictor of GHG emissions.

Digestate versus no digestate application, soil amendment legacy and soil texture were included as fixed-effects variables while the sampling date (Day) was included as a random effect. The model was fitted using REML (restricted maximum likelihood estimation), a modification of maximum likelihood estimation that is more precise for mixed-effects modelling (Baayen, Davidson, & Bates, 2008). Three different data sets were built for the three different gases (CO₂, CH₄ and N₂O) and the results were interpreted separately for each. The R package ‘emmeans’ (estimated marginal means) was used for post hoc comparisons following each mixed effect model to compute contrasts (Russell, Henrik, Jonathon, Paul, & Maxime, 2018) between each combination of the factors using pairwise contrast and Tukey’s method.

### 3 RESULTS

#### 3.1 Soil chemical characteristics: pH, EC and OM

Soil pH was not significantly affected by the digestate application and no differences were found when comparing digestate-amended samples with those that did not receive digestate application ($p = .89$). GM samples had overall a higher soil pH compared with FYM ($p < .001$) and SP ($p < .001$). Digestate-amended soil treatments had a significantly higher EC than those without the digestate applied ($p < .001$) and overall SP samples exhibited lower EC values compared to GM ($p < .01$) and FYM ($p < .001$). No change was observed in the content of OM between the control pots and the digestate-amended pots ($p = .12$) but soil from the FYM-treated field plots had significantly higher OM content compared to SP-plots ($p = .01$).

#### 3.2 CO₂ emissions

The legacy effects of the previous field amendments can be observed in cumulative flux differences between the controls without digestate ($p < .001$). Between Day 1 and Day 64, average soil flux rate increased across the controls between 5.5 and 23.4 mg CO₂-C hr⁻¹ m⁻² in SP, 6.2 and 17.2 mg CO₂-C hr⁻¹ m⁻² in GM and 8.9 and 27.2 mg CO₂-C hr⁻¹ m⁻² in FYM.

Following amendment of the digestate, CO₂ emissions were initially stimulated before reducing, approximately 10-fold, over the 64 days of the experiment. By treatment, fluxes increased to 310.1 mg CO₂-C hr⁻¹ m⁻² initially before reducing to 32.8 mg CO₂-C hr⁻¹ m⁻² at Day 64 in SP + digestate, to 295.7 reducing to 25.2 mg CO₂-C hr⁻¹ m⁻² in GM + digestate, and to 336 reducing to 36.7 mg CO₂-C hr⁻¹ m⁻² in FYM + digestate. The application of digestate significantly increased CO₂ emissions compared with control pots with no digestate applied ($p < .001$). No significant impact of soil texture on CO₂ flux was observed within the constraints of this experiment ($p = .21$).

Farm yard manure-amended pots showed significantly higher CO₂ emissions compared with GM treatment ($p < .01$) and SP treatment ($p < .001$). This was true for both digestate-amended pots and for control pots. No significant differences were found between GM and SP treatments ($p = .129$) in either the control or digestate-amended pots (Figure 2).

#### 3.3 CH₄ emissions

Digestate application stimulates CH₄ flux significantly ($p < .001$). Peak CH₄ emissions were observed immediately after digestate application, which lasted for about a week before declining to no net emission thereafter. In digestate-amended pots, CH₄ emissions ranged from 3.4 mg CH₄-C hr⁻¹ m⁻² on Day 1 to 0 mg CH₄-C hr⁻¹ m⁻² by Day 8 in SP, from 4 mg CH₄-C hr⁻¹ m⁻² on Day 1 to 0 mg CH₄-C hr⁻¹ m⁻² by Day 16 in GM and from 4.2 mg CH₄-C hr⁻¹ m⁻² on Day 1 to 0 mg CH₄-C hr⁻¹ m⁻² by Day 8 in FYM. Methane fluxes

---

**Table 3** Soil analysis showing three key soil characteristics at the end of the experiment (±SE)

|           | pH    | EC (µS/cm) | OM (%) |
|-----------|-------|------------|--------|
| SP + digestate | 6.64 ± 0.2 | 231.83 ± 9.46 | 3.55 ± 0.05 |
| GM + digestate  | 7.40 ± 0.18 | 263.67 ± 15.37 | 3.66 ± 0.12 |
| FYM + digestate | 6.77 ± 0.12 | 275.50 ± 18.63 | 3.82 ± 0.1  |
| SP control    | 6.68 ± 0.16 | 90.88 ± 8.26  | 3.42 ± 0.03  |
| GM control    | 7.42 ± 0.16 | 142.95 ± 12.66 | 3.59 ± 0.09  |
| FYM control   | 6.77 ± 0.12 | 131.48 ± 10.98 | 3.69 ± 0.11  |

Abbreviations: EC, electrical conductivity; FYM, farm yard manure; GM, green manure; OM, organic matter; SP, standard practice.
from control pots with no digestate applied were not significantly different from zero (Figure 3). Emissions from FYM + digestate were significantly higher than SP + digestate ($p = .03$), but no significant differences were observed between GM + digestate and SP + digestate ($p = .49$). Soil texture significantly influenced CH$_4$ emissions only when interacting with previous management practices (FYM) and digestate application ($p < .001$).
3.4 | N₂O emissions

During the first 4 days of incubation, N₂O emissions were not significantly different between any treatments (Figure 4) with values close to 0 net N₂O emissions. A marginally significant increase was observed after Day 8, which was largest in the GM + digestate treatment (p = .045).

The GM + digestate treatment produced significantly higher cumulative N₂O emissions (p < .001) compared with FYM + digestate and SP + digestate.

Soil texture did not significantly influence N₂O emissions (p = .92) but a significant interaction between texture, digestate application and GM treatment was observed (p < .001). As for CH₄ emissions, N₂O flux was higher in digestate-amended pots when the proportion of sand was lower (p = .06) but it was the legacy of previous amendment a year previously that was the strongest driver of increased N₂O emissions following the addition of digestate (p < .001).

4 | DISCUSSION

We hypothesized that soil GHGs fluxes following digestate application will be impacted more by soil properties than by previous soil management. This hypothesis should be accepted for CH₄. However, the hypothesis should be rejected for CO₂ or N₂O. This means that, all else being equal, knowledge of the soil variables, such as those which may be present in soil maps or global soil databases, would be sufficient to allow effective prediction of the likely soil CH₄ flux response to digestate application. However, it would not be sufficient to allow effective prediction of the likely soil CO₂ and N₂O flux response to digestate application. For these GHGs it would also be necessary to know the history of the previous season soil management at least, in terms of FYM application, or cropping versus cover cropping for GM, to more effectively predict the likely soil gas flux response to digestate application. This finding builds on work by Brenzinger, Drost, Korthals, and Bodelier (2018) who stated that 'Future research should focus on the interrelation of plants, soil and microbes and their impact on the global warming potential (GWP) in relation to applied organic amendments' to show that it is also necessary to know the history of management for at least the previous season to understand the GWP in relation to applied organic amendments.

The CO₂-equivalent emissions of the three gases in terms of their GWP’s in a 100 year time horizon (Forster et al., 2007) are shown in Figure 5.

4.1 | CO₂ fluxes

CO₂ soil fluxes occur in the absence of the amended digestate due to the ongoing decomposition of soil organic matter (SOM) in the pots (control samples, Figure 2).

Addition of the digestate stimulated CO₂ fluxes from the FYM and GM soil beyond those stimulated by SP treatment. This indicates the presence of labile carbon in the digestate (Pezzolla et al., 2012) and suggests that the microbial communities in the FYM and GM treatments were primed (Zimmerman, Gao, & Ahn, 2011) to mineralize that labile carbon more readily than that in the SP treatment.

Apart from digestate application, two additional variables could have influenced the emission of CO₂: physical soil properties (i.e. soil texture) or previous management practices (FYM, GM or SP). Soil texture at the Norbury Park sampling site changes between plots moving from north to south.

FIGURE 5 Cumulative emissions of the three gases (CO₂, CH₄ and N₂O) as CO₂-equivalents at the end of the experiment (Day 64) on the basis of their global warming potential. Bars show standard errors. FYM, farm yard manure
(Table 1), with sandy loam soils interspersed with sandy clay loam soil. Sand percentage range varied between 56% and 69% (Table 1) and was included as a fixed effect in the mixed model. While previous studies have reported soil texture to be a predictor of soil CO₂ flux (e.g. Fiedler et al., 2017), no significant effect of soil texture was observed in this experiment ($p = .21$). Yang, Fan, and Jones (2018) reported significant impacts of texture on CO₂ flux, even between very closely related soil taxa (i.e. loam, silty loam and silty clay loam).

If greater variation in soil texture was present in the current study, it is possible that this may have influenced CO₂ flux more significantly. This contrasts with CH₄ and N₂O, as discussed below, where soil texture did significantly affect both CH₄ and N₂O fluxes when interacting with previous management practices and digestate application.

Farm yard manure-amended pots showed significantly higher CO₂ emissions compared with GM treatment ($p < .01$) and SP treatment ($p < .001$). This was true for both digestate-amended pots and for control pots. No significant differences were observed between GM and SP treatments ($p = .129$) in either the control or digestate-amended pots.

It has been demonstrated that soil microbial diversity can be affected by fertilizer and organic amendment management practices (Lupwayi, Lafond, Ziadi, & Grant, 2012; Sradnick, Murugan, Oltmanns, Raupp, & Joergensen, 2013; Yu et al., 2015). FYM application may have increased the microbial biomass (Dhull, Goyal, Kapoor, & Mundra, 2004; Ghoshal & Singh, 1995) such that it was able to more quickly utilize new source of labile carbon (i.e. digestate) following application. Dhull et al. (2004) evaluated the short-term effect of different doses of chemical fertilizers with and without organic amendment (i.e. FYM and GM). The study demonstrated that the application of organic amendments increased the microbial biomass C as well as soil respiration. There is evidence that fertilizer application and increasing inputs of crop residues increase SOM (Graham, Haynes, & Meyers, 2002) and microbial biomass carbon (Mahmood, Azam, Hussain, & Malik, 1997). Soil respiration activities display similar results as soil microbial biomass which is dependent on the organic C in the soil (Hofman, Dusek, Klanova, Bezchlebova, & Holoubek, 2004). Soil respiration can be used as a proxy for microbial activities in the soil. Ghoshal and Singh (1995) evaluated the changes in soil microbial biomass following applications of FYM in two annual cycles and demonstrated that the application of organic manure increased the microbial biomass.

Alternatively, a change in the microbial community structure may have been triggered by the application of the FYM such that the microbial community was more efficient at decomposing the applied digestate. Further studies and molecular analysis are necessary in order to investigate the importance of microbial abundance versus community structure to better understand the mechanism underlying this observed result. Nevertheless, the results show that the legacy of soil amendment 1 year before was a more important driver of differences than edaphic factors regarding CO₂ emissions from soil following digestate application (Figure 2).

### 4.2 CH₄ flux

Soil CH₄ flux can be highly variable because methane microbial production and consumption can simultaneously occur in the soil within different microsites. CH₄ is generally consumed in aerated soil, such as upland soils, when methanotrophic bacteria oxidize CH₄ to CO₂ while concurrently assimilating a large proportion of CH₄-C, often as much as half or more, into microbial biomass C (Mills et al., 2013). Conversely methanogenesis is an anaerobic process. CH₄ usually produced in wetland areas, and areas subject to flooding as methanogenic activity is higher in soils with high water content (Le Hyaric et al., 2011).

Soil amendments can stimulate CH₄ production. Chadwick-Pain and Brookman (2000) reported that over 90% of the total CH₄ emissions occur during the first 24 hr following animal manures (i.e. pig slurry and dairy cow slurry) application. Here peak CH₄ emissions were observed immediately after digestate application, which lasted for approximately 8 days before declining to no net emission thereafter (Figure 3). This result agrees with other studies that reported short-term CH₄ emissions after organic fertilizer application (e.g. Eickenscheidt, Freibauer, Heinichen, Augustin, & Drösler, 2014; Jones, Rees, Skiba, & Ball, 2005; Rees et al., 2004).

Also in agreement with previous studies (e.g. Smith et al., 2003), methane fluxes from control pots (i.e. field soil with no digestate) were not significantly different from zero.

Emissions from FYM + digestate were significantly higher than SP + digestate ($p = .03$), but no significant differences were observed between GM + digestate and SP + digestate ($p = .49$). This suggests that the GM treatment did not modify the soil environment such that it enriched or reduced the community of methanogenic microorganisms more than it did the community of methanotrophic microorganisms (Horz et al., 2002).

Higher CH₄ fluxes were emitted by FYM + digestate with a lower percentage of sand. Methanogenesis is likely to occur in more poorly drained soil because of the higher presence of anaerobic microsites, which can then be exacerbated by digestate application that can further stimulate CH₄ flux from those soils.

Applying digestate in wet or waterlogged soils can lead to higher GHG fluxes, including methane, due to the high soil moisture conditions that favour anaerobic microsites (Eickenscheidt et al., 2014). Reduced methane fluxes were observed when the sand percentage was higher, in agreement
with previous studies on marshland soils (Le Mer & Roger, 2001) as well as in semi-arid climate soils (Barton, Hoyle, Stefanova, & Murphy, 2016). This can be explained by soils with a lower proportion of sand (i.e., more clay) being less free draining, with smaller pores, and so have increased potential for more anaerobic microsites to be present where methanogenesis can occur.

### 4.3 N₂O flux

N₂O is mostly produced by microbial processes of nitrification and denitrification (Mojeremane, 2013; Wragg, Velthof, Beusichem, & Oenema, 2001) as well as other biochemical or chemical pathways (Nkoa, 2014) and processes as summarized by Butterbach-Bahl, Baggs, Dannenmann, Kiese, and Zechmeister-Boltenstern (2013). N₂O emissions can be affected by soil pH and texture, moisture content, temperature and fertilizer application (Fiedler et al., 2017; Mojeremane, 2013; Stevens & Laughlin, 1998). Both nitrification and denitrification rates can increase in nitrogen fertilized systems because nitrogen provides a substrate suitable for N₂O production. Similar to CH₄, sandier soils generally show lower N₂O emissions than clay soil due to the free draining nature of such soils meaning few anaerobic microsites are present (Skiba & Ball, 2006).

The low emissions observed initially were likely due to an initial phase of N immobilization induced by the OM application, followed by an N mineralization phase (Burger & Venterea, 2004; Morvan, Nicolardot, & Péan, 2006). It was expected that this phase would result in N₂O emissions as the pots were maintained at 60% WFPS so the diffusion of O₂ into the soil would be restricted. This would increase the proportion of anaerobic microsites compared to dry soil, thereby increasing N₂O emissions compared to soil with lower WFPS (Bateman & Baggs, 2005). However, a 90% WFPS was not used for this experiment, as it is sometimes used for experiments to stimulate N₂O emissions (e.g., Sánchez-García, Roig, Sánchez-Monedero, & Cayuela, 2014), as we aimed to mimic aerobic soils that were suitable for trafficking, such as those where digestate would more usually be applied. It is unlikely that digestate would be applied to much wetter soils as they would not well support the heavy trailer commonly used for digestate application.

The GM field treatment was drilled with a mix of fodder radish and vetch. Vetch, as a legume, can fix N and so enrich the soil's N content (Campiglia, Mancinelli, Radicetti, & Caporali, 2010; Eichner, 1990; Ranells & Wagger, 1996). As such, the presence of this legume in the GM plots, which was not present in the other field treatments, may explain why N₂O emissions from GM + digestate were significantly higher than FYM + digestate and SP + digestate. It is likely that the presence of the vetch interacted with the soil microbial community responsible for N cycling within the soil such that it was primed to cycle N more rapidly following the input of more N into the system (Lupwayi, Kennedy, & Chirwa, 2011; Sharma, Aneja, Mayer, Munch, & Schloter, 2005) — in this case the N that was present in the digestate. However, this hypothesis would require further research to test. That N₂O flux was highest in GM + digestate which shows that edaphic factors alone are not sufficient descriptors of the likely GHG response of soil but the legacy of previous soil amendments also plays a role in determining GHG emission, particularly N₂O and CO₂ fluxes, following digestate application.

### 4.4 Other variables

Soil pH did not differ significantly between the soil amended with digestate and unamended soils. Long-term studies on the impact of digestate to soil chemical properties are rather limited (Nabel, Schrey, Poorter, Koller, & Jablonowski, 2017). Due to the alkaline pH of the digestate itself (i.e., pH 8.18; Table 2) an increase in the soil pH could have been expected following application of digestate. However, digestate can also contain various acidic compounds (e.g., precipitation of carbonates as CaCO₃; e.g., Makádi, Tomócsik, & Orosz, 2012; Möller & Müller, 2012), and soil itself can have a buffering effect for pH. These factors combined such that no significant effect of digestate on pH was observed within the constraints of this experiment. Regarding soil EC, digestate-amended soil treatments had significantly higher values (p < .001), suggesting an increased concentration of ions in the soil from the digestate (Voelkner et al., 2015).

Overall, SP samples had lower EC values compared to GM (p < .01) and FYM (p < .001), which may be explained by the inputs of nutrients and salts contained in the manure and resulting from the decomposition of the GM (Lopes do Carmo, Botelho de Lima, & Silva, 2016).

Soil from the FYM-treated field plots had significantly higher OM content compared to SP plots (p = .01). This agrees with other studies (e.g., Menšík, Hlinskiovský, & Kunzová, 2019) that have also shown that SOM content can increase following the application of manure and, overall,

### Table 4 Results overview

| GHG    | Soil texture | Legacy effects | Digestate |
|--------|--------------|----------------|-----------|
| CO₂    | —            | ***            | ***       |
| CH₄    | *            | —              | ***       |
| N₂O    | *            | **             | ***       |

Note: No effects detected —, low effects*, moderate effects**, strong effects***.

Abbreviation: GHG, greenhouse gas.
the use of organic amendments such as FYM or GM may improve the availability of nutrients and soil properties (Hargreaves & Warman, 2008). This change to the edaphic environment likely had consequences for the microbial communities present, changing their structure and thus impacting on soil functions and their interactions with the applied digestate.

Table 4 reports an overview of the variables investigated in this study that potentially impacted GHG fluxes.

5 | CONCLUSIONS

This study investigated the role of legacy effects of previous management practices on GHG emissions from soil following digestate application. Digestate significantly stimulated emissions of CO₂, CH₄, and N₂O compared to controls with no digestate application. Higher CH₄ fluxes were emitted by FYM-amended soil with lower percentage of sand after digestate was applied. The legacy of previous amendments was found to not be a significant factor in determining CH₄ flux following digestate application. However, the legacy of previous soil management was found to be the key determinant of CO₂ and N₂O fluxes. Edaphic variables did not have a significant effect on CO₂ flux, but had for N₂O fluxes.

For N₂O flux, the legacy of previous soil management, particularly GM in this instance, interacted with edaphic factors to determine the soil N₂O flux rate. The presence of vetch in the GM, together with digestate application and the lower sand percentage, stimulated N₂O emissions after an initial phase of N immobilization. The critical period for N₂O emissions was between 8 and 16 days after the digestate was applied. Ensuring digestate is applied at a time such that crops are actively growing following the N mineralization phase will maximize N uptake efficiency and minimize losses through N₂O emissions and leaching.

This study demonstrates that edaphic factors and current soil management regime alone are not sufficient predictors of soil GHG flux response following digestate application. Therefore, it is essential to have knowledge of the amendment history of the site for at least the previous growing season in order to effectively predict the likely GHG emissions from soil following digestate application. Further studies and molecular analysis are necessary to investigate the microbial abundance and community structure and so better understand the mechanism underlying these observed results.

ACKNOWLEDGEMENTS

The authors would like to thank Neil Donovan from Rothamsted Research for the analysis of the gas samples (BBSRC grant BBS/E/C/00010320), Dr Marie Kirby and Dr Waseem Mirza from Harper Adams University (HAU) for the production, collection and characterization of digestate, Kevin Jones from HAU for helping with the soil texture analysis and Dr Tom Pope for access to the climate-controlled room. We would also like to thank Norbury Park for access to the field site to collect samples. This project received funding from the European Union’s Horizon 2020 Research and Innovation programme under grant agreement no. 690142 (AgroCycle project), in collaboration with the People’s Republic of China and the Hong Kong Government.

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.

ORCID

Maria Chiara Rosace https://orcid.org/0000-0002-7263-8228
Fabio Veronesi https://orcid.org/0000-0002-3105-7275
Laura M. Cardenas https://orcid.org/0000-0002-4401-9163
Simon Jeffery https://orcid.org/0000-0003-1014-9100

REFERENCES

Abraha, M., Gelfand, I., Hamilton, S. K., Chen, J., & Robertson, G. P. (2018). Legacy effects of land use on soil nitrous oxide emissions in annual crop and perennial grassland ecosystems. Ecological Applications, 28(5), 1362–1369. https://doi.org/10.1002/eap.1745
ADAS. (1986). The analysis of agricultural materials (3rd ed.). Ref. book 427. London, UK: MAFF, HMSO.
Alburquerque, J. A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., … Bernal, M. P. (2012). Agricultural use of digestate for horticultural crop production and improvement of soil properties. European Journal of Agronomy, 43, 119–128. https://doi.org/10.1016/j.eja.2012.06.001
Alburquerque, J. A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., & Bernal, M. P. (2012). Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy, 40, 181–189. https://doi.org/10.1016/J.BIOMBIOE.2012.02.018
Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., … Dewil, R. (2011). Anaerobic digestion in global bio-energy production: Potential and research challenges. Renewable and Sustainable Energy Reviews, 15, 4295–4301. https://doi.org/10.1016/J.RSER.2011.07.121
Baayen, R. H., Davidson, D. J., & Bates, D. M. (2008). Mixed-effects modelling with crossed random effects for subjects and items. Journal of Memory and Language, 59, 390–412. https://doi.org/10.1016/j.jml.2007.12.005
Barbosa, D. B. P., Nabel, M., & Jablonowski, N. D. (2014). Biogas-digestate as nutrient source for biomass production of Sida hermaphroditia, Zea mays L. and Medicago sativa L. Energy Procedia, 59, 120–126. https://doi.org/10.1016/j.egypro.2014.10.357
Barton, L., Hoyle, F. C., Stefanova, K. T., & Murphy, D. V. (2016). Incorporating organic matter alters soil greenhouse gas emissions and increases grain yield in a semi-arid climate. Agriculture,
methanogenic activity of dry mesopholic municipal solid waste digestate. *Journal of Chemical Technology & Biotechnology*, 87(7), 1032–1035. https://doi.org/10.1002/jbt.2010.08.124

Le Mer, J., & Roger, P. (2001). Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology*, 37, 25–50. https://doi.org/10.1016/S1164-5563(01)01067-6

Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Science Society of America Journal*, 48, 1267–1272. https://doi.org/10.2136/sssaj1984.03615995004800060013x

Lopes do Carmo, D., Botelho de Lima, L., & Silva, C. A. (2016). Soil fertility and electrical conductivity affected by organic waste rates and nutrient inputs. *Revista Brasileira de Ciências do Esporte*, 40, e0150152. https://doi.org/10.1590/18069657bcs20150152

Lupwayi, N. Z., Kennedy, A. C., & Chirwa, R. M. (2011). Grain legume impacts on soil biological processes in sub-Saharan Africa. *African Journal of Plant Science*, 5, 1–7.

Lupwayi, N. Z., Lafond, G. P., Ziad, N., & Grant, C. A. (2012). Soil microbial response to nitrogen fertilizer and tillage in barley and corn. *Soil and Tillage Research*, 118, 139–146. https://doi.org/10.1016/J.STILL.2011.11.006

Mahmood, T., Azam, F., Hussain, F., & Malik, K. A. (1997). Carbon availability and microbial biomass in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments. *Biology and Fertility of Soils*, 25, 63–68. https://doi.org/10.1007/s003740050281

Makadi, M., Tomócsik, A., & Orosz, V. (2012). Digestate: A new nutrient source – Review. *Biogas*, 295–310. https://doi.org/10.5772/31355

Maucieri, C., Nicoletto, C., Caruso, C., Sambo, P., & Borin, M. (2017). Effects of digestate solid fraction fertilisation on yield and soil carbon dioxide and nitrous oxide emissions. *Microbiology Ecology*, 84(3), 659–672. https://doi.org/10.1111/1574-6941.12079

Menšík, L., Hlísnikovský, L., & Kunzová, E. (2019). The state of the soil organic matter and nutrients in the long-term field experiments with application of organic and mineral fertilizers in different soil-climate conditions in the view of expecting climate change. In *Organic fertilizers-history, production and applications*. IntechOpen. https://doi.org/10.5772/intechopen.86716

Mills, C. T., Slater, G. F., Dias, R. F., Carr, S. A., Reddy, C. M., Schmidt, R., & Mandernack, K. W. (2013). The relative contribution of methanotrophs to microbial communities and carbon cycling in soil overlying a coal-bed methane seep. *FEMS Microbiology Ecology*, 84(3), 474–494. https://doi.org/10.1111/1574-6941.12079

Mojereman, W. (2013). Factors influencing methane (CH4) and nitrous oxide (N,O) emissions from soils. *International Journal of Forest, Soil and Erosion*, 3, 104–112.

Möller, J., Boldrin, A., & Christensen, T. H. (2009). Anaerobic digestion and digestate use: Accounting of greenhouse gases and global warming contribution. *Waste Management & Research*, 27, 813–824. https://doi.org/10.1108/0734242X09344876

Möller, K., & Møller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*, 12, 242–257. https://doi.org/10.1002/elsc.201100085

Möller, K., & Stinner, W. (2009). Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). *European Journal of Agronomy*, 30, 1–16. https://doi.org/10.1016/J.EJA.2008.06.003

Morvan, T., Nicolardot, B., & Péan, L. (2006). Biochemical composition and kinetics of C and N mineralization of animal wastes: A typological approach. *Biology and Fertility of Soils*, 42, 513–522. https://doi.org/10.1007/s00374-005-0045-6

Nabel, M., Schrey, S. D., Poorter, H., Koller, R., & Jablonowski, N. D. (2017). Effects of digestate fertilization on *Sida hermaphrodita*: Boosting biomass yields on marginal soils by increasing soil fertility. *Biomass and Bioenergy*, 107, 207–213. https://doi.org/10.1016/j.biombioe.2017.10.009

Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agronomy for Sustainable Development*, 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z

Nóbrega, G. N., Ferreira, T. O., Siqueira Neto, M., Queiroz, H. M., Artur, A. G., Mendonça, E. D. S., … Otero, X. L. (2015). Edaphic factors controlling summer (rainy season) greenhouse gas emissions (CO2 and CH4) from semi-arid mangrove soils (NE-Brazil). *Science of the Total Environment*, 542, 685–693. https://doi.org/10.1016/j.scitotenv.2015.10.108

Pezzolla, D., Bił, R., Gigliotti, G., Sawamoto, T., López, A. L., Cardenas, L., & Chadwick, D. (2012). Greenhouse gas (GHG) emissions from soils amended with digestate derived from anaerobic treatment of food waste. *Rapid Communications in Mass Spectrometry*, 26, 2422–2430. https://doi.org/10.1002/rcm.6362

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org

Ranells, N. N., & Wagger, M. G. (1996). Nitrogen release from grass and legume cover crop monocultures and bicultures. *Agronomy Journal*, 88, 777–882. https://doi.org/10.2134/agronj1996.00021169008800050015x

Rees, R., Jones, S., Thornam, R., McTaggart, I., Ball, T., & Skiba, U. (2004). The effect of organic and mineral nitrogen fertilisers on emissions of NO, N2O and CH4 from cut grassland. In D. J. Hatch, D. R. Chadwick, S. C. Jarvis, & J. A. Roker (Eds.), *Controlling nitrogen flows and losses* (pp. 268–276). 12th Nitrogen Workshop. Exeter, UK: University of Exeter.

Rochette, P., Angers, D. A., Chantigny, M. H., & Bertrand, N. (2008). Nitrous oxide emissions respond differently to no-till in a loam and a heavy clay. *Soil Science Society of America Journal*, 72(5), 1363–1369. https://doi.org/10.2136/sssaj2007.0371

Russell, L., Henrik, S., Jonathon, L., Paul, B., & Maxime, H. (2018). Estimated marginal means, aka least-squares means. *The American Statistician*, 74, 216–221. https://doi.org/10.1080/00031305.1980.10483031

Sánchez-García, M., Roig, A., Sánchez-Monedero, M. A., & Cayuela, M. L. (2014). Biochar increases soil N2O emissions produced by nitrification-mediated pathways. *Frontiers in Environmental Science*, 2. https://doi.org/10.3389/fenvs.2014.00025

Sharma, S., Aneja, M. K., Mayer, J., Munch, J. C., & Schloter, M. (2005). Characterization of bacterial community structure in rhizosphere soil of grain legumes. *Microbial Ecology*, 49, 407–415. https://doi.org/10.1007/s00248-004-0041-7

Skiba, U., & Ball, B. (2006). The effect of soil texture and soil drainage on emissions of nitric oxide and nitrous oxide. *Soil Use and Management*, 18, 56–60. https://doi.org/10.1111/j.1475-2743.2002.tb00500.x

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., & Rey, A. (2003). Exchange of greenhouse gases between soil and atmosphere: Interactions of soil physical factors and biological processes. *European Journal of Soil Science*, 54, 779–791. https://doi.org/10.1046/j.1351-0754.2003.0567.x
Sradnick, A., Murugan, R., Oltmanns, M., Raupp, J., & Joergensen, R. G. (2013). Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. *Applied Soil Ecology, 63*, 23–28. https://doi.org/10.1016/J.APSSOIL.2012.09.011

Stevens, R. J., & Laughlin, R. J. (1998). Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutrient Cycling in Agroecosystems, 52*, 131–139. https://doi.org/10.1023/A:1009715807023

Stinner, W., Möller, K., & Leithold, G. (2008). Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming systems. *European Journal of Agronomy, 29*, 125–134. https://doi.org/10.1016/J.JEJA.2008.04.006

Verdi, L., Kuikman, P. J., Orlandini, S., Mancini, M., Napoli, M., & Dalla Marta, A. (2019). Does the use of digestate to replace mineral fertilizers have less emissions of N₂O and NH₃? *Agricultural and Forest Meteorology, 269–270*, 112–118. https://doi.org/10.1016/J.AGRFORMET.2019.02.004

Voelkner, A., Ohl, S., Holthusen, D., Hartung, E., Dörner, J., & Horn, R. (2015). Impact of mechanically pre-treated anaerobic digestates on soil properties. *Journal of Soil Science and Plant Nutrition, 15*(4), 882–895. https://doi.org/10.4067/S0718-95162015005000061

Walsh, J. J., Jones, D. L., Williams, A. P., & Edwards-Jones, G. (2012). Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. *Journal of Plant Nutrition and Soil Science, 175*, 840–845. https://doi.org/10.1002/jpln.201200214

Wrage, N., Velthof, G. L., Van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide.