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Biochar-mediated reductions in greenhouse gas emissions from soil amended with anaerobic digestates

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ABSTRACT

This investigation examines nitrous oxide (N_2O) fluxes from soil with simultaneous amendments of anaerobic digestates and biochar. The main source of anthropogenic emissions of N_2O is agriculture and in particular, manure and slurry application to fields. Anaerobic digestates are increasingly used as a fertiliser and interest is growing in their potential as sources of N_2O *via* nitrification and denitrification. Biochar is a stable product of pyrolysis and may affect soil properties such as cation exchange capacity and water holding capacity. Whilst work has been conducted on the effects of biochar amendment on N_2O emissions in soils fertilised with mineral fertilisers and raw animal manures, little work to date has focused on the effects of biochar on nitrogen transformations within soil amended with anaerobic digestates. The aim of the current investigation was to quantify the effects of biochar application on ammonification, nitrification and N_2O fluxes within soil amended with three anaerobic digestates derived from different feedstocks. A factorial experiment was undertaken in which a sandy loam soil (Dunnington Heath series) was either left untreated, or amended with three different anaerobic digestates and one of three biochar treatments; 0%, 1% or 3%. Nitrous oxide emissions were greatest from soil amended with anaerobic digestate originating from a maize feedstock. Biochar amendment reduced N_2O emissions from all treatments, with the greatest effect observed in treatments with maximum emissions. The degree of N_2O production and efficacy of biochar amelioration of gas emissions is discussed in context of soil microbial biomass and soil available carbon.

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1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas estimated to have 298 times the global warming potential of CO_2 over a 100-year period [1]. Agricultural activities contribute up to 60% of the global annual anthropogenic emissions; this value is predicted to increase by 35–60% in the next 15 years because of increasing use of nitrogen fertilisers and enhanced production of animal manure [1]. In terms of N_2O emissions from soil, nitrifier-nitrification, nitrifier-denitrification and denitrification are the principal sources and may occur simultaneously at different microsites within the soil ecosystem [2]. Denitrification is affected by soil temperature, nitrate concentrations, organic matter availability, redox potential and pH [3].

Residues derived from anaerobic digestion can be used as fertilisers and soil conditioners with nutrients in digestates more readily available than those within slurry or fresh manure [4,5], although variability is associated with the feedstock used, the retention time and conditions within the AD unit [6].

Biochar is a black carbon generated by pyrolysis of biological materials such as wood, crop residues, poultry litter, cattle manure and municipal wastes [7]. Biochar is not a uniform product since it can be formed from a variety of feedstocks at different temperatures (e.g. 350–1000 °C). It has been proposed that biochar application to soil can sequestrate carbon, adsorb inorganic and organic contaminants, improve soil fertility and quality through increases in pH, macronutrients and improved soil water holding capacity [8–12]. It has been estimated that the C-residence time of biochar in soils is hundreds to thousands of years, compared to decades for that of crop residues [8].

Recently, attention has focused on the effect of biochar amendment on soil gas fluxes and in particular on N_2O. Several studies showed that biochar amendment decreased N_2O emissions [13–15] whilst others showed no effect [16] or increased emissions [17]. The impact of biochar on soil N_2O fluxes is variable and depends on factors such as soil type, soil water content, additional fertiliser application, biochar feedstock and pyrolysis temperature [15,18–20]. N_2O emissions have been measured from soil amended with anaerobic digestates [21] and from soil amended with biochar [22,23], but few have quantified the effects of N_2O fluxes associated with AD and pyrolysis residues simultaneously [24].

The effects of biochar on CO_2 fluxes are also varied, with numerous observations of increased emissions [25,26], some of decreased emissions [27] and others showing little consistent effect, with some types of biochar promoting CO_2 production whilst others inhibit it [28]. A consistent observation is that gas emissions are dependent on pyrolysis temperature and amendment rates [23].

Recent studies have investigated the effects on GHG emissions from soils amended with biochar and other fertilisers such as wastewater sludge, urea, ammonium chloride and potassium nitrate [15,22], however to date only one study has compared GHG emissions from soil simultaneously amended with anaerobic digestate and biochar [24]. Use of anaerobic digestate as an organic amendment will become more prevalent since there is an increasing drive to produce energy from waste in the UK [29] and elsewhere and to farm sustainably, meaning potentially greater use of organic fertilisers. Therefore, the aim of the current investigation was to quantify N_2O emissions from soil after simultaneous amendment with one type of biochar and three anaerobic digestates derived from different feedstock material. Carbon dioxide fluxes were also measured. Most of the emphasis in the literature has been on effectiveness of biochar produced under different pyrolysis conditions; the current study quantified the effects of modifying the digests, whilst maintaining a consistent biochar and soil type.

2. Materials and methods

2.1. Characteristics of the anaerobic digestates, biochar and soil

Anaerobic digestates (ADFs) were obtained from three different anaerobic digestion (AD) facilities in the UK and consisted of the separated fibre component. The AD plants were fed with different feedstocks; (i) cattle dung and potato waste (designated ADF 1), (ii) cattle slurry and maize silage (ADF 2) and (iii) maize silage (ADF 3). All digesters were mesophilic and sizes were (i) 265 m^3, (ii) 1.48 dam^3 and (iii) 6.60 dam^3 respectively. The feeding rates ranged from 55 Mg to 100 Mg day^{-1} on a fresh weight basis and varied according to the dry matter content of the feedstock. AD samples were all stored at 4 °C prior to analysis and soil amendment. The pH of all three ADFs was 8.2; the moisture content was 83.2%, 92.0% and 82.1% and the organic matter content based on loss on ignition was 84.0, 91.7 and 88.5% for ADF 1, ADF 2 and ADF 3 respectively. The atomic C:N ratios were 20:1, 29:1 and 21:1 respectively; the majority of extractable N was in the form of NH_4^+–N (2260 ± 120, 4309 ± 231, 3250 ± 126 mg kg^{-1} for ADFs 1–3).

Biochar was commercially sourced from BioRegional HomeGrown® (BioRegional Charcoal Company Ltd, Wallington, Surrey, UK). Mechanically chipped trunks and large branches of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. were pyrolysed at 450 °C for 48 h [11]. The C:N ratio of the biochar was 116:1; pH 9.

A sandy loam soil (Dunnington Heath series; sand 66%, silt 18%, clay 16%, organic matter 3.7%, pH 7.35, NH_4^+–N 0.97 mg kg^{-1} and NO_3^-–N 3.5 mg kg^{-1}) was collected from the University of Nottingham farm site at Sutton Bonington, Leicestershire, UK (SK 512 267) at a depth of 10–30 cm and sieved (<2 mm) prior to use. The field was bare at the time of sampling, but is under a conventional tillage regime and the crop prior to collection was wheat.

2.2. Experimental set-up

Field-fresh soil was combined with the ADFs and biochar as outlined below:

(i) Soil only (control), (ii) ADF 1 + soil, (iii) ADF 2 + soil, (iv) ADF 3 + soil. Each ADF treatment (none, ADF 1, ADF 2 and ADF 3) also received 0%, 1% or 3% biochar (<4 mm) to give a total of 12 treatments. For the soil only treatment, 125 g dry weight

equivalent of fresh soil were added to each of 4 Duran bottles (250 ml capacity). For the ADF treatments 118.75 g dry weight equivalent of soil was mixed with 6.25 g of fibrous digestate (i.e. 5 g per 100 g soil on a dry weight basis). Biochar amendments were made on a mass fraction basis of 1% or 3% dry char and dry weight equivalent of soil or soil plus digestate as appropriate. The soil, ADFs and biochar were thoroughly mixed manually. Once mixed, the soil moisture was adjusted to 30% by mass and maintained gravimetrically over a 32-week period. Experimental units were incubated in a ventilated temperature-controlled room (20 °C) and set up in a randomised block design. The top of each Duran bottle was covered with breathable film (Parafilm, USA) to allow gas exchange and minimise evaporation. The soil/ADF/biochar mixtures were allowed to equilibrate for 1 week, after which CO_2 and N_2O fluxes were measured at 0, 1, 2, 4, 7, 16 and 32 weeks. Therefore week 0 was designated as the starting point, which was 1 week after the initial set-up. After 32 weeks, soil ammonium, nitrate, extractable-C and -N, soil microbial biomass-C and -N, pH and electrical conductivity were measured.

2.3. Gas analysis

Fluxes of CO_2 and N_2O were quantified by removing the Parafilm 30 min before sampling to ensure that the headspace in each Duran bottle equilibrated with ambient air. The Duran bottles were then sealed with modified lids that had rubber septa embedded within them to allow headspace gas sampling with a syringe. Gas sampling was performed after ensuring the headspace was mixed by gently pumping the air inside the Duran bottle three times using a syringe. Then, a 20 cm^3 sample was taken for analysis and injected into a 12 cm^3 evacuated Exetainer® vial (Labco Ltd, UK) with a butyl rubber stopper for storage pending analysis. Vials were therefore over-pressurised in order to prevent external air from diffusing in and contaminating the sample. Following removal of the first 20 cm^3 sample, gas sampling of each Duran bottle was repeated at 30, 60 and 90 min, to give a total of 4 gas samples per replicate Duran bottle on each of the sampling days over the 32 week incubation. The CO_2 and N_2O concentrations were analysed using a gas chromatograph (GC-2014, Shimadzu, Japan) fitted with an electron capture detector (ECD) and flame ionisation detector (FID). Concentrations of CO_2 and N_2O were calculated by comparing peak areas against a standard curve prepared from a certified gas standard. The linear response obtained from the time series data (i.e. time 0, 30, 60 and 90 min) was used for calculating the emission rate of each gas. The gas data were converted to mass per volume using the ideal gas equation and the molecular mass of each gas.

$$n = PV/RT \quad (1)$$

where n is the number of moles of CO_2 and N_2O, P is atmospheric pressure (≈0.101 MPa), V is the volume of head space (dm^3), R is the ideal gas constant (0.08205736 L atm K^{-1} mol^{-1}) and T is the temperature of sampling (273.15 + room temperature in °C). From this it was possible to calculate the gas flux:

$$E = (nm/at) \times 100 \quad (2)$$

where E is the flux of each gas in mg m^{-2} h^{-1}, n is the number of moles of CO_2 and N_2O, m is the molar weight of CO_2 (44.01) or N_2O (44.013), a is the soil surface area within the Duran and t is the time in hours.

2.4. Soil analysis

After 32 weeks incubation, measurements of the following soil properties were undertaken: pH and EC (Soil/water suspension equivalent to 1 kg soil in 2.5 m^3 water, probes from Hanna Instruments, UK); LOI (loss on ignition at 550 °C for 4 h after oven drying at 105 °C to constant weight); total C and total N (C, N, S Elemental Analyzer, Flash EA1112, CE Instruments, UK); NH_4^+–N (colourimetrically at 635 nm using the phenol-nitroprusside/hypochlorite method following soil extraction in 2 mol L^{-1} KCl solution (149.1 g L^{-1})); NO_3^-–N (colourimetrically at 543 nm using the spongy cadmium reduction method after extraction in 2 mol L^{-1} KCl solution). Soil microbial biomass-N and -C were determined using the fumigation-extraction method [30] and a K_{EN} factor of 0.54 [31] and a K_{EC} factor of 0.45 [32]. Soils were extracted in 0.5 mol L^{-1} K_2SO_4 (87.13 g L^{-1}) for determination of extractable organic nitrogen and carbon (EON and EOC) and of microbial biomass and analysed using a Shimadzu TOC-V/TN (Shimadzu Corporation, Koyoto, Japan).

2.5. Statistical analyses

Gas flux data were analysed by Repeated Measures Analysis of Variance and soil chemistry and microbial biomass data were analysed by two-way ANOVA using ADF and biochar as factors. GenStat Release 15.1 (Lawes Agricultural Trust/VSN International Ltd, Hemel Hempstead, UK) was used; normality was tested by plotting residuals against expected normal quantiles and post-hoc comparisons between means was based on least significant differences at the 0.05 probability level.

3. Results

3.1. Ammonium, nitrate and extractable organic nitrogen

Both the 1% and the 3% biochar amendments resulted in lower ammonium concentrations in control soil (no ADF), but had no significant effect on NH_4^+ concentrations in soils amended with the three digestates. Overall, NH_4^+ levels were significantly higher in soils containing ADF 2 than in those treated with ADF 1 and ADF 3 (Fig. 1a; ADF × biochar interaction, $P < 0.001$, LSD = 0.06). At the end of the investigation NH_4^+ concentrations in the control soil were higher than those at the start of the incubation (0.001 g kg^{-1} of unamended soil). Ammonium concentrations in the ADF-free soil and the soil amended with ADFs 1–3 were 0.12, 0.13, 0.28 and 0.13 g kg^{-1} respectively at the end of the incubation (determined as a single factor). The NH_4^+ concentrations in ADF 1 and ADF 3 amended soils were similar to those at the start, which were

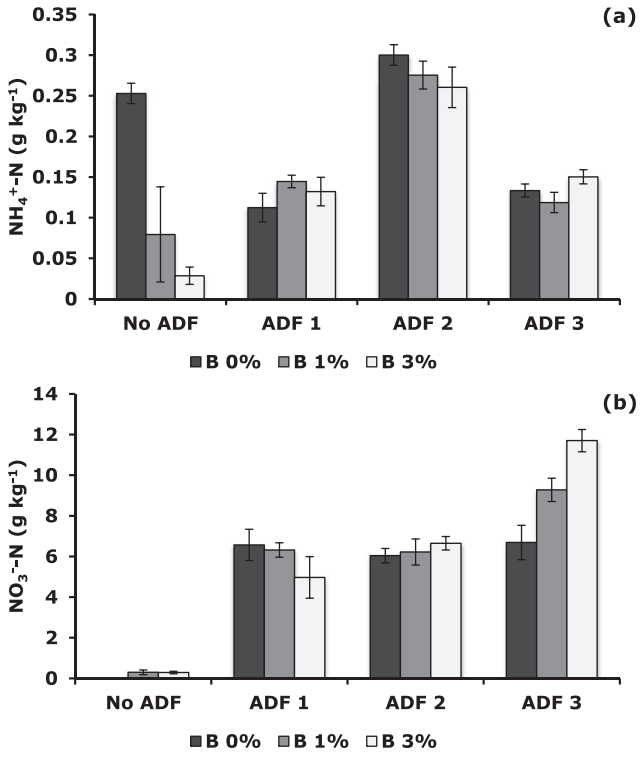

Fig. 1 – (a) Soil ammonium and (b) soil nitrate concentrations after 32 weeks of incubation. ANOVA: Anaerobic digestate (ADF) × biochar interaction, $P < 0.001$ for both soil NH_4 and NO_3. B refers to biochar amendment at 0%, 1% or 3%; ADF 1–3 are the three anaerobic digestates used as the soil amendment.

approximately 0.12 and 0.15 g kg^{-1} respectively after addition of the anaerobic digestate (calculated from the measured concentration within the digestate and the 'dilution effect' of the soil). The ADF 2 treatment resulted in a slightly higher NH_4^+ concentration at the end (0.28 g kg^{-1}) than at the start (0.22 g kg^{-1}) of the incubation.

After 32 days incubation, the NO_3^- concentration (0.003 ± 0.001 g kg^{-1}) in the control soil (no biochar or ADF) was similar to the measured starting concentration of 0.0035 g kg^{-1}. Despite ammonification having occurred in the control soils (no ADF), there was little evidence of nitrification having taken place, although the nitrate concentrations in the biochar amended soil without ADFs were higher than those of the control (no biochar), but similar to each other (0.34 ± 0.12 g kg^{-1} and 0.29 ± 0.06 g kg^{-1} for 1% and 3% biochar addition respectively). In contrast, the final concentrations of NO_3^- in ADF amended soils were considerably higher, ranging from 4.97 to 11.70 g kg^{-1} and greater than the values at the start of the incubation (0.021, 0.027 and 0.036 g kg^{-1} for soil with ADFs 1–3 respectively). Nitrate levels were similar in soils amended with ADF 1 and ADF 2 irrespective of biochar application and in soil treated with ADF 3 in the absence of biochar. Ammonium concentrations do not appear to be a limiting factor in determining the degree of nitrification that occurred in the soils containing digestates. Biochar addition to the ADF 3 amended soil resulted in a significant increase in NO_3^- concentration (Fig. 1b; ADF × biochar interaction, $P < 0.001$, LSD = 1.6).

The NO_3^-: NH_4^+ ratios of the ADF treatments (derived from the means of NO_3^- and NH_4^+ concentrations with ADF as a single factor and therefore includes the biochar treatments) indicate the degree of nitrification relative to ammonium present at the point when the experiment was terminated. The NO_3^-: NH_4^+ ratios are: No ADF, 2:1; ADF 1, 46:1; ADF 2, 23:1 and ADF 3, 69:1 on a g kg^{-1} basis, and on a molar basis, No ADF, 0.5:1; ADF 1, 13:1; ADF 2, 6:1 and ADF 3, 19:1. The pattern suggests that nitrification is limited within the ADF 2 treatments relative to soils that received the other two digestates, although the biochar additions enhanced the ratio for the ADF 3 amended soils.

Biochar amendment resulted in an increase in extractable organic N (EON) when applied with ADF 2 (at 3% biochar) relative to the 0% and 1% additions and with ADF 3 (0% < 1% ≤ 3% biochar) (Fig. 2; ADF × biochar interaction, $P = 0.013$, LSD 58.21). ADF amendment as a single factor ($P < 0.001$) increased the level of EON relative to the control, with the order of increasing EON being ADF 3 > ADF 2 > ADF 1.

3.2. *Electrical conductivity and pH*

Soil electrical conductivity (EC) mirrored the pattern observed for NO_3^- and EON (Fig. 3a; ADR × biochar interaction, $P < 0.001$, LSD = 278.2). Overall the EC values for ADF 1 and ADF 2 were similar, with ADF 3 significantly higher than that of the other treatments. The only effect of biochar was to increase the EC of the ADF 3 treated soils with both the 1% and 3% additions (Fig. 3a). The EC for soil (no ADF) was low and is indicative of a nutrient poor arable soil.

The pH of the soil in the absence of ADF residues was similar at the end of the incubation period to that at the beginning. The pH of ADF amended soils differed (Fig. 3b). When considering the ADF treatments as single factors, ADF 1 (pH 6.73) and ADF 3 (pH 6.71) were not significantly different from one another. The pH of the soil amended with ADF 2 (pH 7.30) was significantly higher than that of both ADF 1 and ADF 3 treated soils. Of particular interest was the reduction in pH when biochar was added to the ADF 3 treatment, but which

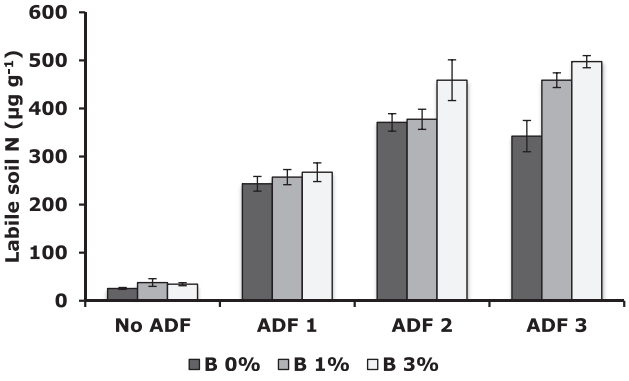

Fig. 2 – Extractable (K_2SO_4) soil nitrogen after 32 weeks of incubation. ANOVA: ADF × biochar interaction, $P = 0.013$, LSD 58.21. Data are means ± se (n = 4). Abbreviations are as described in Fig. 1.

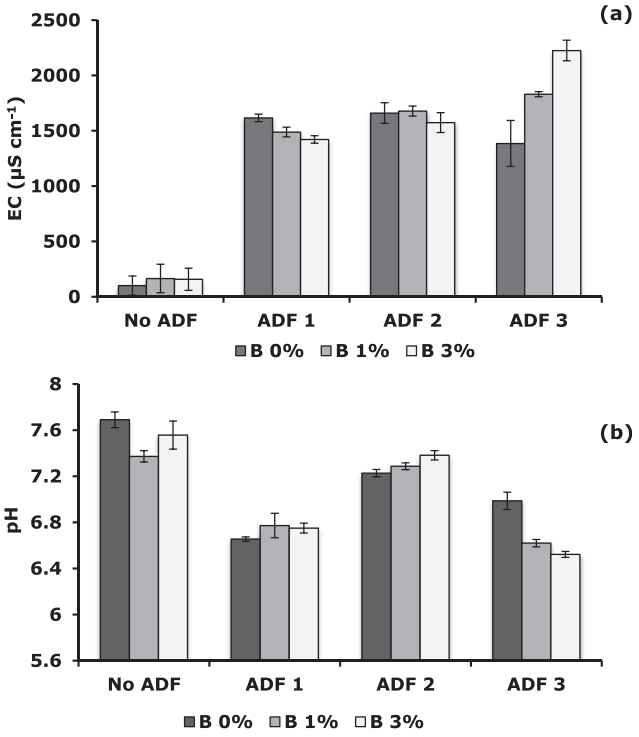

Fig. 3 – (a) Electrical conductivity of ADF and biochar amended soils after 32 weeks. ANOVA: ADF × biochar interaction, $P < 0.001$, LSD = 278.2. (b) pH of soils after 32 weeks. ANOVA: ADF × biochar interaction, $P < 0.001$, LSD = 0.18. Data are means ± se. Abbreviations are as described in Fig. 1.

did not occur when applied with the other ADF amendments (ADF × biochar interaction, $P < 0.001$, LSD = 0.18). Both the 1% and 3% additions resulted in a lower pH than the 0%, but were not different from each other. Therefore the pH profile resulting from biochar addition is different in soils amended with ADF 1 and ADF 2 (no effect) compared to ADF 3 (a significant decrease).

3.3. *Microbial biomass, soil respiration and soil carbon*

Microbial biomass-N concentrations were lower in soils treated with ADF 3 than in soils amended with the other ADF treatments (Fig. 4; ADF as a single factor, $P = 0.029$, LSD = 0.35). Biochar additions did not affect biomass-N. Microbial biomass-C was similar across all ADF treatments with the exception of ADF 2, which resulted in a significantly higher biomass than did the other ADF amendments (Fig. 4; ADF as a single factor, $P < 0.001$, LSD = 0.16). Biomass-C in the ADF 2 treated soils was 203 µg g^{-1} soil; with 99, 94 and 80 µg g^{-1} biomass-C measured in the soil amended with ADF 3, ADF 1 and no ADF respectively. Biochar did not affect biomass-C, nor was there an ADF × biochar interaction.

The increased biomass-C in the ADF 2 treatment was reflected in the soil respiration data measured as CO_2 output (Fig. 5a; ADF as a single factor, $P < 0.001$, LSD = 45.58). Respiration from the ADF amended soils followed a similar downward trajectory over time, after a flush during weeks 1–2 for

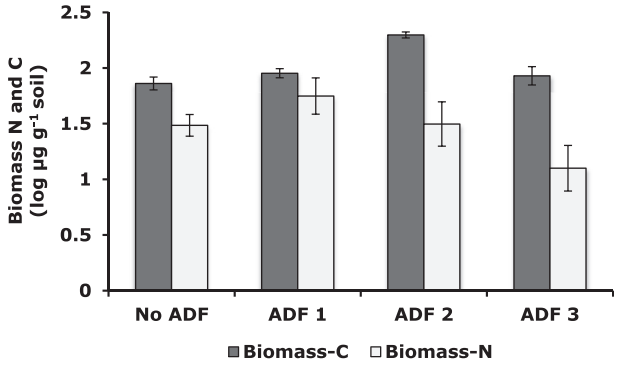

Fig. 4 – Microbial biomass-C (ANOVA: ADF as a single factor, $P < 0.001$, LSD = 0.16) and biomass-N (ANOVA: ADF as a single factor, $P = 0.029$, LSD = 0.35) after 32 weeks of incubation. Data are pooled means for ADF treatment ± se.

both the ADF 1 and ADF 2 treatments and at weeks 2–7 for ADF 3 (Fig. 5b; time × ADF interaction, $P < 0.001$, LSD = 134.9). Respiration from the control (no ADF) soils was consistent with a mean CO_2 output of 27 mg m^{-2} h^{-1} during weeks 1–16, thereafter falling to 13.5 mg m^{-2} h^{-1} by week 32. Biochar did not affect CO_2 efflux from the soil either as a single factor or as an interaction term (with time or ADF).

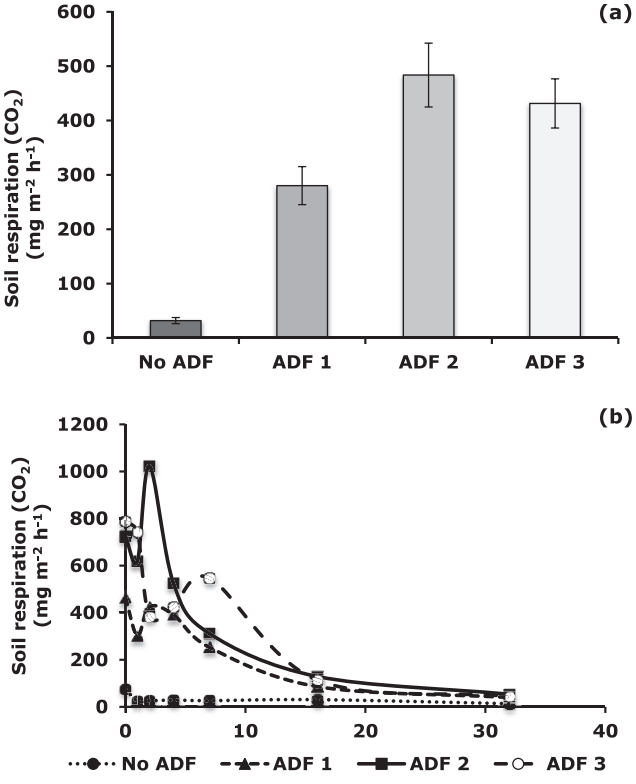

Fig. 5 – (a) Soil respiration data measured as CO_2 output (ANOVA: ADF as a single factor, $P < 0.001$, LSD = 45.58), data are pooled means ± se. (b) Respiration from the ADF amended soils over time (Repeated Measures ANOVA: time × ADF interaction, $P < 0.001$, LSD = 134.9, data are means of 4 replicates, standard errors are not shown because they are to small to be visible).

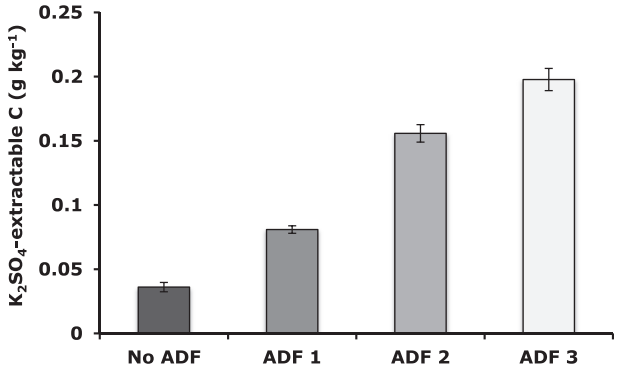

Fig. 6 – Extractable (K_2SO_4) soil carbon after 32 weeks of incubation. (ANOVA: ADF as a single factor, $P < 0.001$, LSD = 0.016). Data are pooled means ± se.

Extractable soil organic carbon (EOC) was significantly different in soils subjected to each ADF treatment (Fig. 6; ADF as a single factor, $P < 0.001$, LSD = 0.016). Biochar did not affect the EOC content of the experimental soils.

Total soil carbon was greatest in soils containing biochar (biochar as a single factor, $P = 0.005$; mean values were 2.4%, 3.5% and 4.4% C for the 0%, 1% and 3% biochar additions) and in soils containing ADFs 1 and 2 (ADF as a single factor, $P < 0.001$, LSD = 1.3). Despite the treatment-induced changes in mineral and organic nitrogen speciation, total N content of the experimental soils was similar across treatments; the total C:N ratio therefore followed a similar trend to that of the total C.

3.4. *Nitrous oxide fluxes*

Nitrous oxide emissions were affected by both ADF and biochar amendments; in general, each ADF acted as a source of N_2O whilst the unamended soil acted as both a source and a sink, although the concentrations emitted from the control soils were very low and biochar-treatment differences were not significant. Biochar addition reduced N_2O emissions from soils amended with all three ADFs with varying degrees of efficiency. The 1% and 3% biochar treatments were similarly

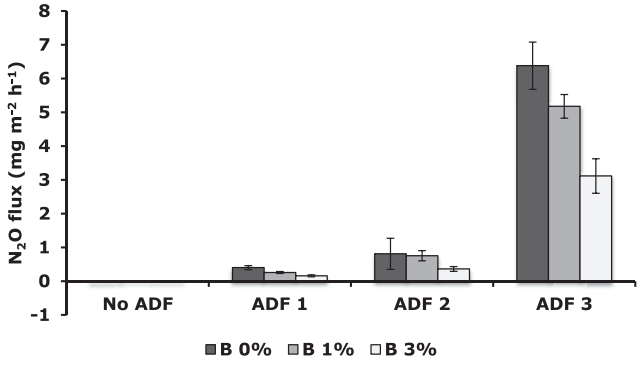

Fig. 7 – Average nitrous oxide flux over the 32 week period of incubation. Data are pooled means ± se. (ANOVA: ADF × biochar interaction, $P < 0.001$, LSD = 0.47).

efficacious in conjunction with ADF 1 and 3% was most effective with ADF 2. The soil amended with ADF 3 produced the highest concentrations of N_2O and each level of biochar significantly reduced the amount of gas emitted (Fig. 7; ADF × biochar interaction, $P < 0.001$, LSD = 0.47). Fig. 7 shows mean values combined over each sampling time during the 32-day incubation period. When data from all soil treatments were combined, the N_2O emitted from soils containing 3% biochar was 41% less than emissions from the 1% biochar amended soils, which in turn produced 18% less N_2O than soils without biochar (biochar as a single factor, $P < 0.001$).

The highest concentrations of N_2O were emitted during the first 4–7 weeks of incubation depending on the ADF treatment, although N_2O production continued for the duration of the trial (Fig. 8a–d; ADF × biochar × time interaction, $P < 0.001$, LSD = 1.97). N_2O emissions differed between ADF amendments with ADF 3 resulting in higher emissions than all other treatments irrespective of the presence or not of biochar. ANOVAs were also carried out separately for each ADF treatment (and control) and these demonstrated a significant effect of biochar for ADF 3 ($P < 0.001$, LSD = 0.69) and ADF 2 ($P = 0.001$, LSD = 0.09) where each biochar treatment was different from each other, and for ADF 1 ($P = 0.028$, LSD = 0.29) where the 3% application resulted in a reduction of N_2O compared to the 0% and 1% additions. Biochar did not significantly affect N_2O production from the control soil. However, N_2O fluxes from the control soil (no ADF) appear to be quite erratic in the biochar-free treatment during the first 4 weeks, but this was not observed in ADF-free soil with added biochar. Based on the overall difference between the ADFs with and without biochar across the 7 sampling times over the 32-week period, the 3% biochar application reduced N_2O emissions by 51%, 55% and 60% from ADF 3, ADF 2 and ADF 1 amended soils respectively.

4. Discussion

At the end of the 32-week incubation period the pH of the soils amended with ADF 1 and ADF 3 was lower than that of either the unamended soil or the ADF 2 amended soil. The reason for the reduction in pH, even though the digestate was alkaline in nature when applied, may be because of transformation of acidic compounds present in the digestate (e.g. gallic acid), which can affect soil chemical properties [33]. Since the pH of the control soil and that amended with ADF 2 was similar, a mechanism other than, or in addition to, transformation of acidic compounds may have been responsible for the reduction in pH exhibited generally and in particular with ADF 1 and ADF 3 amended soils. Nitrification is known to reduce soil pH, particularly if plants are not present to take up the NO_3^- ions [34]. Since the NO_3^-:NH_4^+ ratio within the treatments indicated less NO_3^- in ADF 2 amended soils relative to the available NH_4^+, it follows that a nitrification-related decrease in pH would be smaller in soils that received ADF 2. This is consistent with the biochar-related increase in NO_3^- concentrations and concomitant decrease in pH that occurred within the ADF 3 treated soils. It might be expected therefore that an increase in base cations would be observed in the ADF 3 + biochar treatments following their displacement [34]. However, nitrate was unable to leach from the incubation chambers and the balance of

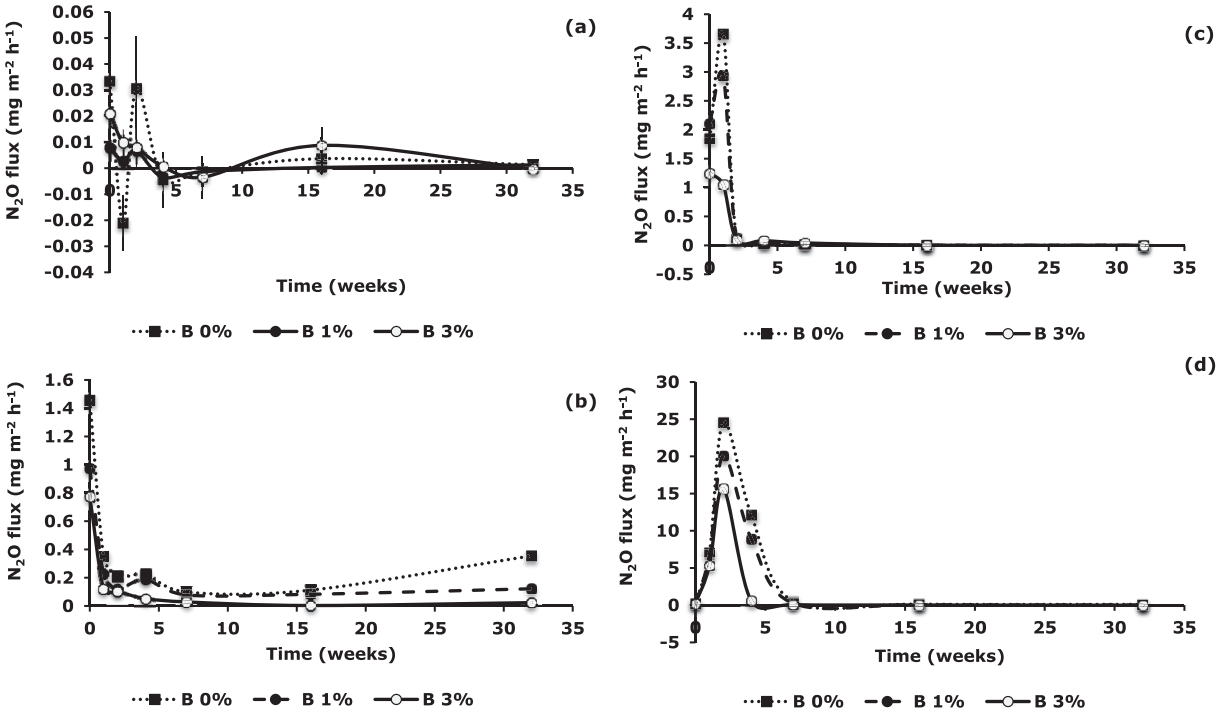

Fig. 8 – Nitrous oxide flux over the 32 week incubation period from soils simultaneously amended with anaerobic digestate and biochar: (a) soil, (b) ADF 1, (c) ADF 2, (d) ADF 3. Other abbreviations are as described in Fig. 1. Data are means presented as separate graphs for each ADF for clarity. Standard errors are presented in (a), but not in the other figures because they are too small to be visible. (Repeated Measures ANOVA for whole data set: ADF × biochar × time interaction, $P < 0.001$, LSD = 1.97).

NO_3^- and cations within the soil solution would reach an equilibrium depending on how quickly nitrification occurred within the system. Furthermore, the cations measured were exchangeable and not those within the soil solution *per se*. In an open system, the NO_3^- produced in response to simultaneous biochar and ADF 3 additions could leach and result in soil acidification [35] although within a planted system, the increase in NO_3^- is less likely to result in accumulation because plants and microorganisms would rapidly take it up since N is usually the limiting factor in the rhizosphere [36]. Biochar is widely reported to have a liming effect and has been suggested as a strategy to increase soil pH, although surface oxidation over time may reduce that effect and result in localized acidity surrounding the char particles [37]. It is possible that rapid oxidation of the biochar occurred thereby limiting the liming effect, although since microbial activity (CO_2 production) was unaffected by biochar, this is unlikely.

Addition of the digestate fibre allowed a significant capacity for N-supply over an extended period in addition to a source of carbon. Microbial immobilization of N was least in the ADF 3 soils and surprisingly, the microbial biomass-N within the control soil was similar to that of the soils treated with ADF 1 and ADF 2. The data for microbial biomass-N in the ADF-free soils suggests that N-limitation increased the microbial demand for N, which was immobilised and conserved within the biomass pool. Whether the N was immobilized as NH_4^+ or as NO_3^- is unknown but it is likely that N-immobilising heterotrophs outcompeted the nitrifiers for NH_4^+ unless NO_3^- was immobilized as soon as nitrification occurred. Tye et al. [38] demonstrated that both NH_4^+ and NO_3^- were rapidly immobilized by microbial biomass in Arctic tundra soils and cycling of N following application was very conservative. However, Rice and Tiedje [39] showed that low concentrations of NH_4^+ inhibited NO_3^- assimilation and concluded that microbial immobilisation is unlikely to be a significant determinant of NO_3^- in soil. However, where NO_3^- levels are high in comparison to NH_4^+, then NO_3^- is assimilated by heterotrophic microorganisms since competition from nitrifiers is strong [40]. Microbial biomass-N was lower in soil amended with ADF 3 than in the ADF 0, 1 and 2 treatments and the NO_3^- concentrations were enhanced by the presence of biochar, suggesting that nitrification was increased. Either the microbial biomass did not 'need' to immobilize the available nitrogen if conditions were not N-limiting, or as suggested by Burger and Jackson [40] competition for NH_4^+ by nitrifiers may have been intense; in this current context that would suggest that NH_4^+ was the preferred source of nitrogen for the heterotrophic community. Azam et al. [41] concluded that NH_4^+ is preferred in the absence of glucose, but when these authors added glucose to their soils, NO_3^- and NH_4^+ were immobilized equally. Glucose was not added to the soils in the current investigation, but other forms of labile carbon would have leached from the digestates.

The soil N:C ratios for all treatments were higher after the incubation period relative to the start because of decomposition of the organic residues present in the soil and of those added. Despite the lower N-content of the unamended soil, microbial biomass-N was similar to that of the soils receiving

ADF. The evidence of ammonification, but not of any substantial nitrification in the unamended soils is indicative of an active decomposer community, but one in which immobilization of the N occurred. It is known that N-release from decomposing litter is regulated by its initial composition and by the stoichiometric needs of the decomposers [42] and there is growing recognition that the N:C of decomposer microorganisms is generally constant [43]. The data here for the biomass-N and -C are in agreement with that premise. The decomposer community decreases its C-use efficiency when initial litter N is low [44], but this normally results in increased respiration. Respiration was low in the unamended soils within this study, although relatively consistent throughout. Respiration was higher in the soils receiving ADF and significantly higher (over the 32 week period) in the presence of ADF 2, with a peak at the start of the trial. Biomass-C was also greatest in that treatment and the high starting NH_4^+ content of the digestate would have enhanced microbial activity. Calculation of the qCO_2 for the final sampling at week 32, when both biomass-C and respiration data are available, suggested a trend towards greater C-use efficiency of the ADF 2 than that of the ADF 1 and ADF 3 treated soils since the quotient was lower, but not significantly so ($P = 0.080$; data not shown). Ideally, qCO_2 would be measured throughout the incubation period, although the usefulness of the quotient was questioned by Wardle and Ghani [45] who found it to be unpredictable relative to measures of microbial biomass and soil respiration across successional gradients. At the end of the incubation period the ADF 2 and ADF 3 treated soils respired similarly, but biomass-C was higher in the soils with ADF 2, which explains the lower qCO_2. A correlation between final biomass-C and final respiration in the ADF 2 amended soils showed a positive relationship ($r = 0.84$) between the two parameters, whilst there was no relationship between biomass and respiration at the end of the incubation period in the ADF 1 ($r = 0.13$) and ADF 3 ($r = -0.11$) treatments (data not shown). It is likely that a shift in the microbial community accounted for the lack of any correlation between biomass-C and respiration in the ADF 3 treatment, since these soils demonstrated the highest amount of nitrification and denitrification. When attributing respiratory output to C-use efficiency it should be noted that nitrifiers utilise CO_2 for biomass production and may constitute a loss of CO_2 [46]. In the current study, biochar had no observable effect on soil respiration which corroborates the findings of others [47]. Respiration should increase with increasing carbon quality or quantity [48] and since the quantity of C inputs *via* ADFs was similar, it might be concluded that the quality of ADF 1 was lower than that of ADF 2 and ADF 3 since the average respiration rate over the 32 weeks was lower.

Nitrifiers are able to function well in mildly acidic to mildly alkaline soils [49] suggesting that the pH reduction experienced in ADF 3 + biochar amended soils was not sufficient to limit nitrification. Since ammonium concentrations in the ADF-treated soils were similar to those at the start of the incubation, yet nitrification occurred, it follows that ammonification must also have taken place. Ammonium concentrations were similar irrespective of biochar treatment in the ADF 3 amended soils, although NO_3^- concentrations were highest when biochar was also present. This is either indicative of biochar-stimulated nitrification, or of accumulation of NO_3^- in the biochar + ADF 3 amended soils. Biochar-related accumulation of NO_3^- in soil has previously been reported together with an associated decrease in N_2O emissions [13,37]. Biochar may increase mineralization of organic nitrogen present in soil resulting in labile forms of organic N [50]. The concentration of extractable organic nitrogen (EON) in the ADF 3 amended soils was increased when biochar was present. However, if biochar had resulted in increased mineralization, it might be expected that ammonification would have similarly been increased and CO_2 production (as a proxy for microbial activity) would also be enhanced, yet the latter was not observed. Whether ammonification increased in tandem with nitrification resulting in an apparently limited change from the starting point is unknown. In the current study, biochar reduced N_2O losses from all three ADF amended soils, but most significantly from the soil that received ADF 3. These data suggest that in the ADF 3 amended soil, biochar may have limited the availability of NO_3^-, thereby reducing N_2O production if it occurred as a result of nitrifier-denitrification. Alternatively, if N_2O was produced by denitrifier-denitrification, improved aeration in the biochar-amended soil would limit denitrification and result in an accumulation of nitrate. It is not possible to tell whether the N_2O was produced *via* nitrification or denitrification within this investigation, but the reduction in N_2O emissions may stem from one or more of the biochar-induced mechanisms reported in the literature and collated by Ameloot et al. [23] namely: increased pH, enhanced soil aeration and surface adsorption of the gas. In the current study, unless pH increases were localised in the vicinity of the biochar particles, it seems unlikely that increased pH explains the reduced N_2O observed since biochar amendment resulted in decreased pH in soils that received ADF 3. Mørkved et al. [49] stated that low soil pH may result in an increase in the $N_2O/(NO_2^-/NO_3^-)$ ratio because of increased denitrification; in contrast, Van Zwieten et al. [13] suggested that increased pH following biochar addition led to decreased N_2O production because the product of denitrification was N_2, rather than N_2O. Production of N_2 in favour of N_2O following biochar amendment was also recently demonstrated using stable isotopes [51] although a biochar-related pH change could not be the sole reason because the same effect was not replicated by addition of calcium carbonate [37]. As in the current study, pH was discounted by others [18,47] as an explanation for biochar-related N_2O reductions; rather, Case et al. [18] proposed aeration as the cause, since that would limit activity of denitrifying enzymes [52]. In the current investigation, it is possible that different mechanisms occurred in each treatment; the N_2O produced may have resulted from nitrification, or from denitrification within anaerobic microsites that could have formed within the soil/ADF mix in the incubation vessels [52]. Nitrous oxide emissions from all the ADF/soil mixes were reduced by biochar, but NO_3^- concentrations were only significantly increased by biochar in the ADF 3 treatment. Furthermore, higher N_2O emissions arose from the ADF 3 amended soil even in the absence of biochar, where the NO_3^- concentration was similar to that of the other ADF treatments. This suggests that the ADF 3/soil mix was more biologically active than the other treatments, although the respiration data show that microbial

CO_2 production was similar in the ADF 2 and ADF 3 treatments meaning that heterotrophic microbial activity was similar. In the current study, the microbial biomass-N was lowest in the ADF 3 treated soils (irrespective of biochar) compared to the other soils, suggesting that maximum immobilization occurred in the soil with the least available EON and NO_3–N (i.e. the control soil).

The findings of the current study show increased NO_3^- concentrations and decreased N_2O emissions in soil amended with biochar and ADF 3. These findings corroborate those of others [13,51]. However, this pattern was not observed in the remaining treatments and it is interesting to note that Case et al. [18] reported a decline in NO_3^- in biochar-amended soil. Differences in the absorptive capacity of biochars have been attributed to pyrolysis temperature (see Ref. [37] and references therein), but within the current study the biochar was consistent across treatments. The interaction between the soil microbes and the biochar was different in the presence of ADF 3 compared to the other two digestate treatments. The microbiology and chemical nature of the digestates would most likely be different because the feedstocks were not the same.

The EOC content of the soil at the end of the incubation indicated greater mineralization of carbon in the order ADF 3 > ADF 2 > ADF 1 > no ADF. The total N_2O emissions followed the same order, suggesting that decomposition of the ADFs and soil organic matter lowered the redox potential and increased the reducing capacity [53] thereby enhancing N_2O emissions. Denitrification is affected by the NO_3^- content and also by the amount of dissolved organic carbon [18]. Nevertheless, this does not explain why the biochar reduced N_2O emissions and particularly why the reduction occurred in the ADF 3 treatment, which had high concentrations of EOC and EON. The increase in NO_3^- when biochar was applied with ADF 3 was mirrored by an increase in EC and EON. Enhanced nitrate concentrations might be expected to increase denitrifier activity, leading to elevated N_2O emissions, but this was not the case in the presence of biochar. It is possible that the nitrate was immobilized by biochar [51] resulting in greater soil concentrations albeit unavailable to denitrifiers, although that does not explain why the effect was only observed in the ADF 3 + biochar treatments. Alternatively, increased microbial activity in conjunction with enhanced redox potential resulting from mineralisation of the ADF 3 may have caused complete reduction of NO_3^- to N_2, resulting in lower N_2O emissions. Increased C-availability has been demonstrated to enhance N_2O reduction to N_2 [54,55] and this may be a key reason for the biochar-related decrease in N_2O emissions, particularly from the ADF 3 amended soils.

The gas fluxes described here were measured from the air above the soil (the headspace). Rarely are fluxes measured within the soil profile, yet N_2O may accumulate and be dissolved in the soil solution, particularly during periods of rapid nitrification [56]. Therefore high soil moisture contents restrict N_2O diffusion, resulting in longer residence time within the soil profile and increased likelihood of microbial reduction to N_2 [56]. Since gases diffuse through macropores, soil pore size and connectivity are important. Devereux et al. [57] demonstrated that biochar addition led to smaller, water-filled soil pores compared to biochar-free soil. This could conceivably result in accumulation of N_2O within the soil profile. These authors used powdered wood charcoal whilst the biochar in the current study consisted of a range of particle sizes up to 4 mm. Therefore in the current investigation, biochar may have been more likely to aerate the soil than to reduce pore size, although further study is required. Nevertheless, it is possible that biochar-mediated stimulation of nitrification increased N_2O production. Further studies should consider the importance of soil pore size in conjunction with increased water holding capacity and diffusion dynamics of the gases.

The current study showed that simultaneous biochar and anaerobic digestate additions to soil resulted in different processes occurring depending on the nature of the organic amendment. The soil amended with digestates formed from cattle manure (ADF 1) and cattle manure plus maize silage (ADF 2) generally did not respond to biochar as intensely as soil treated with the maize-derived digestate (ADF 3). Nitrous oxide emissions generated by ADF treatment were reduced by biochar amendment in all cases, but particularly in the case of ADF 3. The reason for the reduction is not known, but several mechanisms are likely to be responsible. One suggestion that has not been considered to date, is biochar-mediated reductions in pore size which may limit gas diffusion through the soil profile and preventing N_2O release to the atmosphere. Biochar is usually considered to enhance soil aeration which is cited as one of the reasons for reductions in N_2O emissions [25]. Whilst this is likely to be the case in many studies, the findings of Deveraux et al. [57] point to the possibility of an additional mechanism. Nevertheless, interactions between biochar and soils with different organic amendments are complex; interestingly the soils in the current study were maintained at lower water contents than might be expected for denitrification to be the main cause of N_2O production. Whilst biochar reduced N_2O emissions in all soils receiving anaerobic digestates, the intensity of the reduction was determined by the type of digestate, which in turn affected the labile C and N contents (from decomposition), the EC, pH, microbial activity (respiration) and the N_2O fluxes.

5. Conclusion

Biochar addition to soil amended with anaerobic digestates affected nitrogen dynamics of the soil/AD mix differently depending on the type of digestate present. Whether the altered responses were derived from subtle differences in digestate chemistry or microbiology are not clear, but reasons are likely to be complex. Biochar amendment reduced N_2O emissions from all AD/soil mixtures but the effect on gas emissions from unamended soil was erratic. Biochar has a potentially positive role to play in limiting gaseous emissions but a greater understanding of the mechanisms involved is required.

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