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Soil greenhouse gas dynamics under biosolid amendments based on laboratory, field, and modeling approaches

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The rise in human population and the advent of biological wastewater treatment has led to increased biosolid production, which requires sustainable solutions to mitigate potential negative impacts associated with the disposal of biosolids. Biosolid land application has the potential to decrease reliance on synthetic fertilizers and improve soil fertility; however, the microbial activity and associated greenhouse gas (GHG) emissions need to be evaluated to ensure there are no negative externalities of this approach. To address these issues, this study aimed to (i) assess the potential of a biosolid-amended soil system to emit nitrous oxide (N_2O), (ii) quantify actual field GHG emissions from biosolid-amended soils, and (iii) evaluate a process-based model to predict these soil GHG emissions. This study performed a comprehensive analysis, including laboratory (potential assays and gene abundances), field (static chamber GHG measurements), and modeling (process-based) approaches, to understand the effect of biosolids on soil GHG emissions. We found that biosolid application increased soil nitrate and organic matter, and decreased soil pH in the short-term. Together, the changes in soil conditions promoted more denitrification, which became more complete with laboratory potential dinitrogen higher than nitrous oxide as the end-product over time. In the field, GHG emissions were generally higher in biosolid-amended soils, particularly just after biosolid application. While the predictive model was able to simulate general trends for field GHG emissions, it often underpredicted the magnitude of these emissions. Overall, despite initial increases in GHG, biosolids have the potential as a sustainable amendment to improve soil health and mitigate GHG emissions in agricultural practices over the long term. This research contributes to understanding biosolid use in promoting environmental sustainability and offers insights for future agricultural management strategies.

KEYWORDS

agriculture, carbon, DNDC, gene abundance, methane, nitrous oxide

Introduction

The last 6 decades have witnessed a significant increase in greenhouse gas (GHG) emissions, with global net anthropogenic GHG emission of 59 ± 6.6 Gt CO_2-eq in 2019, more than 50% higher than 3 decades earlier (IPCC et al., 2023). Total GHG emissions (in CO_2-eq) are calculated by summing contributions from three principles GHG–carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) – using their Global Warming Potentials of 1, 25 and 273, respectively, on a 100-year time horizon (IPCC, 2023). In 2022, U.S. agriculture contributed 9.4% of the total GHG emissions (in CO_2-eq) compared to other economic sectors, and agricultural soils were the largest contributor of N_2O emissions (EPA, 1990). Furthermore, approximately 70% of N_2O emissions are due to the excessive use of nitrogen fertilizer and soil management practices, driven by population growth and increased demand for crop production (Syakila and Kroeze, 2011). Population growth has also led to an increase in wastewater treatment and associated treated sewer sludge (i.e., biosolids) that are regulated under EPA's 40 CFR Part 503 (O'Dette, 1998).

Biosolids must be properly disposed of to avoid detrimental environmental effects, including groundwater contamination and soil degradation (Pappu et al., 2007). In 2022, 16% of biosolids were disposed of through incineration, 27% through landfilling, and 56% through land application (EPA, 2022). Incineration generates CO_2 emissions, with 12.8 MMT CO_2-eq generated in 2021 from incineration of waste in the U.S. (EPA, 2023). Landfilling was the third largest contributor to CH_4 emissions in the U.S. in 2017, accounting for 16.4% of the total emissions (EPA, 2022). Furthermore, the transportation of biosolids to landfills or incineration facilities will consume fuel (e.g., diesel) and generate additional GHG emissions. To avoid these emissions, land application could be a sustainable alternative for disposing of biosolids, especially for land used for animal feeding and energy production, such as corn, hay, or grass, which are the largest consumers of biosolids application (NBDP and NEBRA, 2022).

Biosolids are organic materials rich in nutrients that can improve soil health and fertility, sequester carbon in the soil (Xue et al., 2015), and decreasing demand for synthetic fertilizers due to their high nutrient content (Khan and Mitall, 2023; Marchuk et al., 2023; Sharma et al., 2017). Organic soil amendments are known to modify soil properties and affect GHG emissions, although the influence is highly dependent on the amendment's chemical composition (Nguyen et al., 2014). Moreover, soil organic matter and total soil carbon storage have been found to be significantly increased in lands treated with biosolids (Torri et al., 2014), particularly with alkaline-treated biosolids (Lin et al., 2024). However, there is concern about biosolids application with the potential accumulation of contaminants (e.g., heavy metals, microplastics, and per- and polyfluorinated alkyl substances) (Popoola et al., 2023). A recent study found that heavy metals immobilization increased with biosolid application, suggesting the risk of plant uptake is minimal (Sinha et al., 2023). Soil amendments that enhance fertility may also increase soil microbial activity and associated GHG emissions, depending on the method of biosolid application, whether through incorporation or surface spread (Gutiérrez-Ginés et al., 2023). GHG emissions can be influenced by the type of biosolids, their ages, and the environmental conditions (Lu et al., 2020; Majumder et al., 2014; Roman-Perez et al., 2021). Biosolids account for 40% of wastewater treatment plant emissions, primarily due to the treatment process and the management of biosolids through landfilling or incineration (Ritter and Chitikela, 2012). Therefore, it is essential to balance potential soil emissions induced by land application compared to overall GHG from transportation, landfilling, and incineration.

Studies have investigated the impact of biosolids on the environment using field, lab, and/or modeling approaches. A biosolids field study found that environmental conditions have more impact on the mitigation of GHG than nutrient addition (Lu et al., 2020; Roman-Perez et al., 2021). A life cycle modeling approach to address the end use of biosolids revealed that transportation is the critical factor in determining biosolids' end use, aimed at reducing environmental impact (Peters and Rowley, 2009). To our knowledge, no studies have simultaneously considered field, lab, and modeling approaches to understand the influence of biosolid amendments and soil properties on GHG emissions and microbial nutrient cycling. Therefore, in this study, we measured and modeled direct GHG emissions, denitrification potential, and gene abundance from soils with and without the application of biosolids for the month following application. The specific objectives of this study were to (i) assess the potential of biosolid-amended systems to emit N_2O, (ii) quantify actual field GHG emissions from biosolid-amended soils, and (iii) evaluate predictive potential of a process-based model to predict these soil GHG emissions.

Materials and methods

Site description

The Stillwater Wastewater Treatment Plant in Stillwater, Oklahoma, recently shifted its biosolid waste management model to promote system sustainability and decrease its contribution to the local landfill. They began land applying the class B biosolids generated from the anaerobic digestors to the fields directly surrounding the treatment plant. Biosolids are classified under the U.S. EPA's 40 CFR Part 503 regulations based on pathogen reduction, metal concentration, and vector attraction reduction (O'Dette, 1998; EPA, 1994). Class A biosolids must meet strict pathogen limits, requiring fecal coliform levels below 1,000 Most Probable Number (MPN) per gram dry weight, whereas Class B biosolids, while treated, may contain higher pathogen levels and require additional land application restrictions to mitigate public exposure risks (EPA, 1994). The biosolids in this study fall under Class B but are close to meeting Class A requirements, given their relatively low fecal coliform concentrations (Supplementary Table S1). All fields were planted with hay that was sold for animal feed. The field location for this study was located on the north end of the property (36.10136, −97.02123; Supplementary Figure S1). The soil was mainly Coyle loam (fine-loamy, siliceous, active, thermic Udic Argiustolls). The experimental field first had biosolids applied in June 2019 and were disked and sprigged in May/June 2020. In the sampling year, biosolids were freshly applied to only the test field on 11 May 2022, and tilled into the soil. Sampling occurred directly following the 2022 biosolids application. A control area was selected

TABLE 1 Soil properties for biosolid-amended soil and control soil on two sampling dates.

Soil[a] collection date	Treatment	OM (%)	TN (%)	pH	NO_3^- (ppm)	NH_4^+ (ppm)
5/13/2022	Biosolid	4.86	0.28	6.7	227.5	9.15
5/13/2022	Control	3.44	0.19	7.7	11.5	4.6
6/23/2022	Biosolid	4.51	0.22	7.5	10	4.7
6/23/2022	Control	2.95	0.16	7.7	4.5	3.4

[a]The tested soils are Loam in texture.

adjacent to the experimental field, but without any historic biosolid application. The control area was not tilled and allowed natural vegetation growth with periodic mowing to provide access to field gas sampling chambers (described in Field Measurements). The different managements of the control and treatment areas can also influence differences in microbial activity and nitrogen cycling patterns, which is a limitation of the current study.

Soil sampling

Soils were sampled from both the experimental field (with biosolid application) and control (no biosolid application). The soil was collected randomly at a depth of 20 cm from each location using an auger (2 3/4 in diameter) and excluded from the vegetation layer. The soil samples used for soil assays were stored in plastic bags and kept in the laboratory fridge at 4°C for less than a month until analysis. The soil for DNA extraction was stored separately in an ice box and then transferred to the biology laboratory, where it was kept at −78°C until analysis. Soils were sampled on two dates in 2022: May 13 (2 days after biosolid application) and June 23 (more than a month after biosolid application). A subset of soil samples from each date and treatment were sent directly after sampling to an external laboratory, the Soil, Water, and Forage Analytical Laboratory (SWFAL) in Stillwater, Oklahoma, to be tested for texture, organic matter (OM), total nitrogen (TN), pH, nitrate (NO_3^-), and ammonium (NH_4^+) (Table 1).

Denitrification Assays

Soil samples collected as described above were used in denitrification enzyme activity assays (DEA) following methods previously described (Tiedje et al., 1989; Khalifah and Foltz, 2024). Briefly, two sets of triplicate samples were prepared by adding 25 g of soil into 125 mL Wheaton jars. A nutrient solution was prepared by adding 5,000 mg D-glucose, as a source of available carbon (C), and 720 mg of potassium nitrate (KNO_3), as a source of nitrogen (N), to 1,000 mL of deionized water. The nutrient solution was added to the Wheaton jars at a final concentration of 2 mg-C and 0.1 mg-N per gram of soil. To test the effects of available C on denitrification and N_2O production, additional solutions were prepared with 720 mg of KNO_3 and varying concentrations of D-glucose to achieve 0C (no D-glucose added, 0 mg-C g^{-1} soil), 0.5C (2,500 mg of D-glucose, 1 mg-C g^{-1} soil), and 2C (10,000 mg of D-glucose added, 4 mg-C g^{-1} soil). All assays were initiated by adding 25 mL of the solution to the Wheaton bottles. The Wheaton bottles were sealed well, flushed with N_2 for 2 minutes, and over-pressurized for 10 s. Acetylene gas was injected with 20 mL volume one set of triplicate samples to measure the total denitrification by blocking N_2O converted to N_2. N_2O production was measured with the second set of triplicate samples by injecting 20 mL of N_2. The incubation start time was recorded after shaking the samples for 30 s. Gas samples were collected after 2, 3, 4, and 5 h of incubation time from the jar headspace using a syringe and needle and transferred along with 10 mL N_2 to a 10 mL glass vial sealed with gray butyl rubber septa. The gas samples were analyzed using an Agilent 8890 Ga Chromatograph (GC) with an electron capture detector and PAL3 autosampler. Standards and blanks were analyzed at the start and end of each run, and the standard curves were generated using all standards. Standard curves for each GC run were used to determine N_2O concentration in samples from that GC run. Potential denitrification and potential N_2O production rates were determined using the change in N_2O concentrations over time, the ideal gas law, and soil moisture, while considering the effects of dilution and dissolved N_2O. The N_2O production potential was divided by the denitrification potential to calculate the N_2O ratio [as previously described in (Khalifah and Foltz, 2024)]. The N_2O ratio is an indicator of the completion of the denitrification process, where a value near 1 means a more incomplete process (more N_2O is emitted), while a value near 0 means a more complete process (more N_2 is emitted). Soil moisture tests via oven drying were simultaneously completed to allow assay results to be normalized based on dry weight of soil.

Denitrification genes

Quantitative polymerase chain reaction (qPCR) was used to assess the abundance of the microbial community using 16SrRNA and the denitrification pathway using gene targets for nitrite reductase (*nir*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*) in the soil samples. DNA extractions were performed from 0.25 g of soil material using the PowerSoil® DNA Isolation Kit (QIAGEN Sciences, Germantown, MD) in duplicate each for the control and experimental (biosolid-amended) soils. The DNA was quantified using the Qubit HS-dsDNA kit (Thermo Fisher, Waltham, MA). Targets for qPCR included *nirS*, *nirK*, *cnorB*, *qnorB*, and *nosZ*. Total reaction volume was 20 µL with forward and reverse primers (1 µL each at a final concentration of 500 nM, Integrated DNA Technologies, Coralville, IA), template DNA (5 µL), 2X SYBR Green Master Mix (10µL, Bio-Rad, Hercules, California), and PCR-grade water (3 µL).

TABLE 2 Primer and annealing information for qPCR.

Primer Name	Target	Region	Direction	Sequence 5′-3′	Annealing Temp (°C)	Source
nirS-cd3aF	Denitrifiers	nirS	Forward	GTS AAC GTS AAG GAR ACS GG	57	Michotey et al. (2000)
nirS-R3cd			Reverse	GAS TTC GGR TGS GTC TTG A		Throback et al. (2004)
nirK876	Denitrifiers	nirK	Forward	ATY GGC GGV CAY GGC GA	57	Henry et al. (2004)
nirK1040			Reverse	GCC TCG ATC AGR TTR TGG TT		
cnorB2F	Denitrifiers	cnorB	Forward	GAC AAG NNN TAC TGG TGG T	57	Braker and Tiedje (2003)
cnorB7R			Reverse	GAA NCC CCA NAC NCC NGC		
qnorB2F	Denitrifiers	qnorB	Forward	GGN CAY CAR GGN TAY GA	57	Braker and Tiedje (2003)
qnorB5R			Reverse	ACC CAN AGR TGN ACN ACC CAC CA		
nosZ1F	Denitrifiers	nosZ	Forward	ATG TCG ATC ARC TGV KCR TTY TC	62	Kim (2020)
nosZ1R			Reverse	WCS YTG TTC MTC GAC AGC CAG		
341F	16S rRNA	V3-V4	Forward	CCT ACG GGN GGC WGC AG	55	Herlemann et al. (2011), Klindworth et al. (2013)
805R			Reverse	GAC TAC HVG GGT ATC TAA TCC		

Standard curves were created with gBlocks™ (Integrated DNA Technologies). Samples were run on a CFX Connect Thermocycler (Bio-Rad). Thermocycler conditions included an initial 3-min, 98° denaturation cycle. The two-step amplification method included 40 cycles of 10 s at 98° and 45 s at the designated annealing temperature (Table 2). Melt curves were determined at the end of the 40 cycles. Primer information is provided in Table 2.

Field measurements

Field soil GHG fluxes were measured on seven dates from May to June 2022, between 8a.m. and 12p.m. Cylindrical polyvinyl chloride (PVC) chamber bases and tops with vents were constructed following published guidance (Parkin and Venterea, 2010). To test the influence of chamber dimensions on emission estimates, we constructed three different chambers (8-in, 10-in, and 12-in diameter; Supplementary Figure S2). Sampling chambers were set up across the two areas, control and experimental (biosolid-amended soil), to measure and compare GHG fluxes between the management conditions and assess the difference in chamber designs. The control was an area of soil covered by natural grass and weeds, while the experimental area was tilled soil with a recent biosolid amendment. Nine chamber bases were placed in each area with three 8-in, three 10-in, and three 12-in diameter chambers. These bases were put in place 24 h prior to sampling to allow disturbances in the soil microbiome to settle. For each sampling date, chamber tops were sealed to bases, and 15 mL gas samples were collected at 0, 30, and 60 min after chamber closure. Gas samples were immediately injected into pre-evacuated 10 mL clear glass vials with gray rubber septa. During each field visit, air and soil temperatures and relative humidity were recorded using traceable thermometers and humidity meters.

Gas sample vials were analyzed for GHG concentrations using the GC method described under Denitrification Assays but with one amendment. Rather than running the GC program with an electron capture detector alone (for N_2O measurements only), the GC program for all three GHG was used. This utilized the electron capture, flame ion, and thermal conductivity detectors to simultaneously quantify N_2O, CH_4, and CO_2. Soil fluxes were calculated using the change in GHG concentration over time, the ideal gas law, and chamber dimensions following standard methods (Parkin and Venterea, 2010).

DNDC modeling

The Denitrification-Decomposition (DNDC) model (Canada version, DNDCvCAN, downloaded January 2024 from https://github.com/BrianBGrant/DNDCv.CAN) uses inputs of climate, soil, and cropping to give outputs of GHG fluxes (Li et al., 2000). As an improvement on the US version, DNDCvCAN has additional microbial input parameters which allows for more fine tuning related to nitrification and denitrification (Smith et al., 2020). Recommended values were used for all additional input parameters (Supplementary Table S2). A summary of model inputs is provided in Table 3. For this study, climate data was obtained from the Stillwater station of the Oklahoma Mesonet, a network of environmental monitoring stations throughout Oklahoma (Oklahoma Climatological Survey, 2024). Soil properties were obtained from a combination of lab testing, the Web Soil Survey, and the MesoSoil database (NRCS. Web Soil Survey, 2024; Scott et al., 2013). Model runs were completed for the years 2011–2022 with the years 2011–2021 used for spin-up time to stabilize the nitrogen and carbon pools within the model. Annual grass planted at the beginning of the year was used for the control

TABLE 3 Model fit parameters.

DNDC input parameters	Values and units		Source
	Control	Biosolids	
Max potential yield	Default kg C ha^{-1}	N/A	DNDC
Climate data (rainfall, Max and Min Temperature, Solar radiation, relative humidity, and windspeed)	Daily variation in (mm,C°)	Daily variation in (mm,C°)	Mesonet
Clay content	15%		SWFAL
Bulk density	1.36 g cm^{-3}		MesoSoil
Hydraulic conductivity	0.21 cm^3 cm^{-3}		MesoSoil
Wilting point	0.33 cm^3 cm^{-3}		MesoSoil
Slope	1.72°		Web Soil Survey
Soil Organic Carbon	0.0295 kg C kg^{-1} soil		SWFAL
Soil pH	7.7	7.1	SWFAL
Plant type	Annual grass	Fallow	Stillwater WWTP
Planting date	Jan 1	N/A	Stillwater WWTP
Harvesting date	Dec 31	N/A	Stillwater WWTP
Tillage	N/A	Ploughing with disk on May 5	Stillwater WWTP
Fertilization	N/A	Sewer sludge on May 11	Stillwater WWTP
C/N ratio for sewer sludge	N/A	5.12	DNDC

SWFAL, soil, water, and forage analytical laboratory; WWTP, wastewater treatment plant.

field while the experimental field was modeled as fallow. The site was rainfed with no additional irrigation inputs. Other field management included tilling with a disk and chisel and surface spread sewage sludge as a manure amendment in the experimental field. The model outputs of interest were daily N_2O, CH_4, and CO_2 fluxes. Daily fluxes were summed to get the cumulative fluxes for the modeled period.

Statistical analyses

Statistical analyses were completed using R version 4.2.2 and R studio (R Core Team, 2019). The data normality was tested using histograms of model residuals and the Shapiro-Wilk test. The significance of the parameters was first tested using analysis of variance (ANOVA) with a significant difference $\alpha = 0.05$. The ANOVA results were then processed using the least significant difference (LSD) tests to test the differences between the identified significant parameters. For the field measurements, the effect of treatments, chamber diameter, and date were tested for each GHG. For the laboratory measurements, the effect of treatments, nutrient addition, and date were tested for each assay. To evaluate how well the DNDC model performed the following quantitative metrics were used: root mean square error (RMSE), model efficiency, and coefficient of determination (CD) (Smith et al., 1997). Normalized RMSE was calculated by Equation 1 where a lower percent value indicates better model fit. Model efficiency was calculated using Equation 2 where positive values indicate that the simulated data describes the measured data better than the mean of the measured data. The CD was calculated using Equation 3 with the lowest value possible being zero.

$$RMSE\ (\%) = \frac{100}{|\bar{O}|}\sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \quad (1)$$

$$model\ efficiency = \frac{\sum_{i=1}^{n}(O_i - \bar{O})^2 - \sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2} \quad (2)$$

$$CD = \frac{\sum_{i=1}^{n}(O_i - \bar{O})^2}{\sum_{i=1}^{n}(P_i - \bar{O})^2} \quad (3)$$

In Equations 1–3, O_i is the ith measured value, P_i is the ith modeled value, n is the number of observations, and $\bar{O}$ is the average measured value.

Results and discussion

The GHG potential of the system

The potential of the biosolid-amended agricultural system to emit GHG (specifically N_2O) was first assessed using DEA. Biosolid-amended soil had a significantly higher denitrification potential than

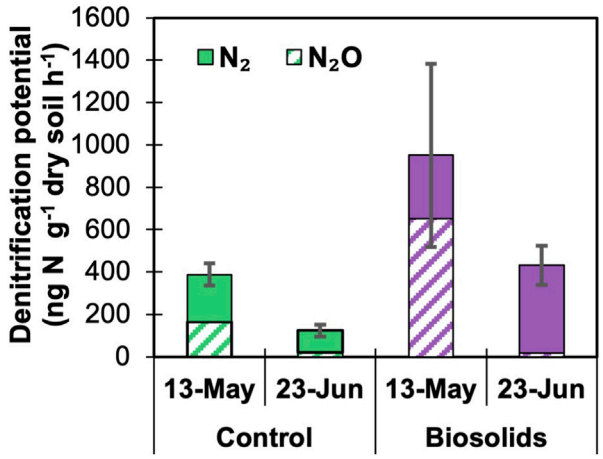

FIGURE 1
Denitrification potential (solid bar) and N_2O production potential (diagonal lined bar) for biosolid-amended (purple) and control (green) soil on two different 2022 dates – May 13 (just after biosolid application on May 11) and June 23. Biosolid-amended soil had significantly higher denitrification and N_2O production potentials than controls (ANOVA, $P < 0.001$). May 13 had significantly higher denitrification and N_2O production potentials than June 23 (ANOVA, $P < 0.004$).

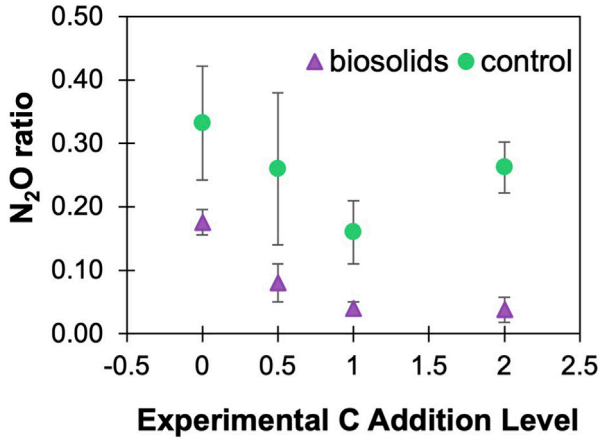

FIGURE 2
N_2O ratio under different carbon (C) addition levels for biosolid-amended and control soils. Nutrient solution carbon levels: 0 = no C added, 0.5 = 2,500 mg of D-glucose added, 1 = 5,000 mg of D-glucose added (as with the traditional DEA), and 2C = 10,000 mg of D-glucose added to 1L total nutrient solution. All nutrient solutions also had 720 mg KNO_3. Biosolids (purple) refers to the biosolid-amended soil of the experimental plot, while control (green) refers to soil from the unamended control plot adjacent to the experimental plot. Treatment (biosolid-amended vs. control) was significantly different (ANOVA, $P = 0.003$) although nutrient addition was not (ANOVA, $P = 0.27$).

the control soil ($P < 0.001$) (Figure 1; Supplementary Table S3). This outcome was likely linked to ambient soil NO_3^- concentration, which was higher in the biosolid-amended soil than control soil (Table 1). Similarly, biosolid-amended soil had higher potential to emit N_2O ($P < 0.001$), although biosolid amendment led to an overall significantly lower proportion of N_2O as the end-product (lower N_2O ratio) ($P = 0.002$) (Figure 2; Supplementary Table S4). Considering time since biosolid application, denitrification and N_2O production potentials were significantly lower ($P < 0.004$) on the second sample date (June 23) as compared to the date just after biosolid application (May 13). The May sample likely had higher denitrification rates due to higher nutrient content and rainfall (10.6 cm), which would create ideal anaerobic conditions for the denitrification process (Schwenke and Haigh, 2016).

Initially, the N_2O ratio was higher in both soils, indicating more potential for N_2O compared to N_2; however, the N_2O ratio decreased significantly ($P < 0.001$) over time, as shown in Figure 1. The findings suggest that while the application of biosolids may lead to an initial increase in N_2O production when measured directly after the application, the N_2O production over time could be lower. While the N_2O production results are expected with higher soil NO_3^-, the N_2O ratio is complicated by several factors, including soil NO_3^-, organic C, and pH (Khalifah and Foltz, 2024; Foltz et al., 2023; Pfenning and McMahon, 1997; Robinson et al., 2014; Samad et al., 2016; Sun et al., 2012; Wang et al., 2013). For NO_3^- and organic C, their availability can determine the availability of electron donors and acceptors necessary for denitrification, influencing the end-products of the process. Additionally, soil organic C can act as an energy source for microorganisms, enabling them to use available soil nutrients to generate denitrification end-products (Arango et al., 2007). Soil pH can have a major impact on microbial activity, electron donor and acceptor availability, and the enzymes involved in denitrification, all of which can ultimately affect the denitrification end-products (Khalifah and Foltz, 2024; Sun et al., 2012; Čuhel and Šimek, 2011). Acidic soils tend to emit more N_2O than alkaline soils (Khalifah and Foltz, 2024; Foltz et al., 2023; Robinson et al., 2014; Mukumbuta et al., 2018). In this study, biosolid amendments initially decreased the pH of the soil (Table 1). This could explain the high N_2O production potential from the biosolid-amended soils in May which then lowered when the pH returned to neutral in June.

Based on our previous meta-analysis, C concentration was found to be a major factor affecting the N_2O ratio (Foltz et al., 2023). In this study, we tested the effect in these two soils to understand how N_2O emissions could be further managed with C addition. We similarly found that as the carbon concentration increased, the N_2O ratio decreased (Figure 2), indicating more N_2 was emitted than N_2O. Although a decreasing trend in N_2O ratio was observed with increasing C addition, the difference was not significant ($P = 0.27$). Instead, the influence of biosolid amendment was more significant ($P = 0.003$) than experimental nutrient addition in the lab, suggesting that field conditions may be more critical to establish. So management that increases soil C can help to further reduce the emission of N_2O alongside other influential treatments.

The biosolid-amended soil had increased gene concentrations compared with control soil (Figure 3; Supplementary Table S5), including an approximate 1.85 increase in *16S rRNA* in the biosolid-amended soil compared to the control soil. Biosolid-amended soil had higher NO_3^-, NH_4^+, TN, and OM concentrations than the control soil (Table 1). Previous research has indicated a positive correlation between the abundance of *nirK*, *nirS*, and *norZ* (Veraart et al., 2017) as well as *nosZ* genes (Kandeler et al., 2006) with OM.

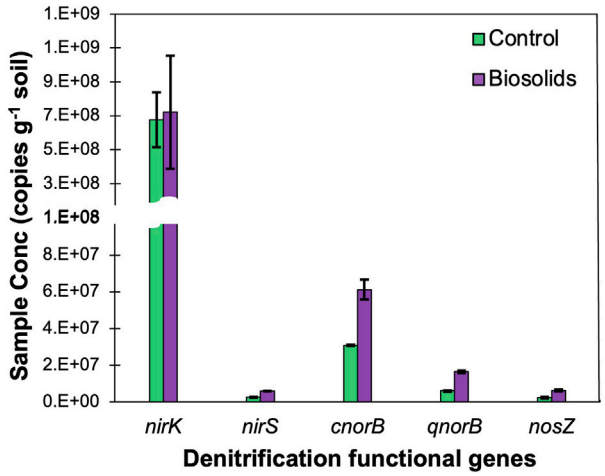

FIGURE 3
The concentration of the detected genes extracted from the samples' DNA (Mean of 2 replicates +standard deviation). Biosolids (purple) refers to the biosolid-amended soil of the experimental plot, while control (green) refers to soil from the unamended control plot adjacent to the experimental plot.

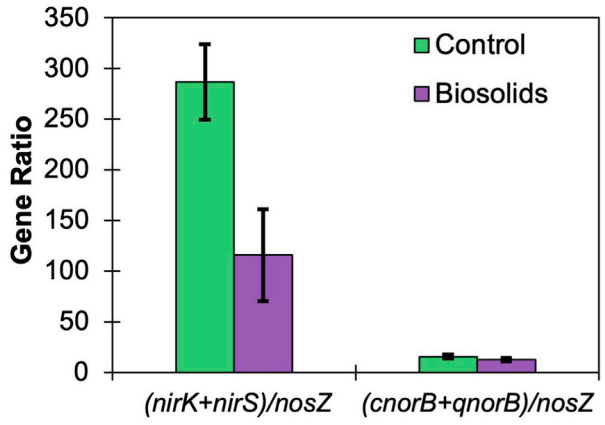

FIGURE 4
The difference in the ratio of the most dominant genes in the denitrification process (*nirK*, *nirS*, *cnorB*, *qnorB* and *nosZ*). Biosolids (purple) refers to the biosolid-amended soil of the experimental plot, while control (green) refers to soil from the unamended control plot adjacent to the experimental plot.

Correspondingly, this study's biosolid-amended soil had higher *cnorB*, *qnorB*, and *nosZ* genes concentrations than that in control soil.

It is important to consider the (*nirK* + *nirS*)/*nosZ* and (*cnorB* + *qnorB*)/*nosZ* ratios when indicating nitric oxide (NO) and N_2O production. A high (*nirK* + *nirS*)/*nosZ* ratio suggests a greater possibility of NO (and subsequently N_2O) production during denitrification, due to *nirK*/*nirS* enzymes producing NO as an intermediate product at a greater rate. A high (*cnorB* + *qnorB*)/*nosZ* ratio can indicate a greater turnover rate of NO into N_2O than can be converted to N_2 by *nosZ*. The ratio of both (*nirK* + *nirS*)/*nosZ* and (*cnorB* + *qnorB*)/*nosZ* has significantly decreased in the biosolid-amended soil (Figure 4). Therefore, the control soil had greater potential to produce NO (Vilar-Sanz et al., 2013) and N_2O (Jia et al., 2021) emissions compared with biosolid-amended soil. This finding aligns with the potential assays that found biosolid-amended soil had a lower N_2O ratio.

Field measurements of GHG emissions

To consider actual N_2O and other GHG emissions (CH_4, CO_2), field measurements were taken on seven dates in the 2 months directly following biosolid application (Figure 5; Supplementary Tables S6, S7). We first considered the influence of chamber dimensions on GHG emissions and found no significant difference in emissions between chamber sizes ($P = 0.28$, 0.79, and 0.13 for N_2O, CH_4, and CO_2, respectively). Therefore, all results discussed here are based on the averages considering all three chamber dimensions and replicates. Similar to laboratory potential tests, N_2O emissions from the biosolid-amended soil were higher than those in the control soil initially following biosolid application.

After this initial spike in N_2O, the emissions returned to ambient (control) levels of near-zero for the remainder of the monitoring period. This outcome aligns with the laboratory results, where the N_2O ratio decreased with time from the application of biosolids, suggesting lower potential for N_2O emissions. This pattern of elevated N_2O emissions is consistent with prior research, which suggests that biosolids, due to their high nitrogen content, can enhance microbial denitrification activity and subsequently increase N_2O emissions (Nicholson et al., 2022). Similarly and in line with previous research, measured CO_2 emissions in biosolid-treated soils were also higher than in the control group (Donovan et al., 2011; Yang et al., 2024). Short-term biosolid land applications have been shown to temporarily increase the emission of GHG, although long-term applications have been shown to improve soil structure and health and, as a result, decrease overall GHG emissions (Buragienė et al., 2023; Obi-Njoku et al., 2022). Therefore, with time, the GHG emission at this site could decrease further in the biosolid-amended field. This prediction is supported by the laboratory potential tests, which showed increased completion of denitrification over time.

The influence of biosolid amendments on GHG can vary based on the chemical composition of biosolids and the soil conditions. Biosolids can improve soil structure by improving porosity, aeration, and soil moisture retention, which facilitate better oxygen diffusion and microbial respiration rates, ultimately affecting GHG emissions (Hu et al., 2024; Khan et al., 2022). Moreover, biosolids increase the organic matter stock and improve the microbial activity to resist decomposition and as a result, allow for greater C storage in the soil (Badewa et al., 2023; Chaker et al., 2018). Improved C storage can provide higher C levels that favor complete denitrification when NO_3^- inputs occur and stimulate denitrification.

For CH_4 emissions, the measured data shows that there is variation in the emissions. At the initial stage of the application, the biosolid-amended soil had slightly increased CH_4 emissions. Then, approximately a month after biosolid application, there were reduced emissions when compared with the control. Typically, CH_4 production is limited under aerobic conditions, and biosolid

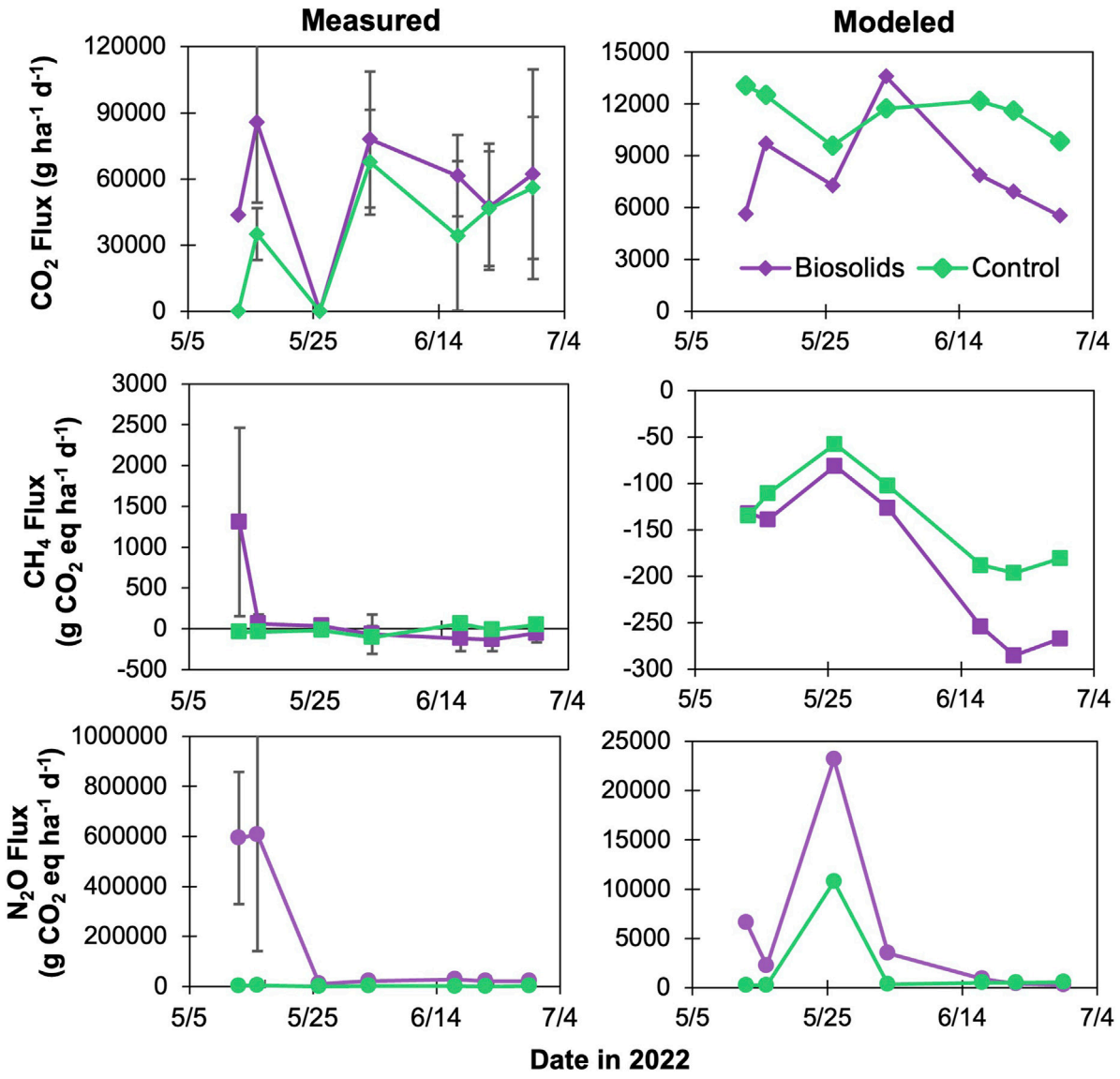

FIGURE 5
Field measurement and model prediction of GHG fluxes vs. time. Dates presented as month/day and all GHG fluxes reported in CO_2 equivalents. Field measurement values are averages of all replicates including all three chamber sizes with error bars based on standard deviation (n = 9). Biosolids (purple) refers to the biosolid-amended soil of the experimental plot, while control (green) refers to the unamended control plot adjacent to the experimental plot.

amendments can alter soil redox potential and pH directly affecting the methanogens process and supporting the reduction in CH_4 (Ali et al., 2014; Haque et al., 2021).

Model predictions and evaluation

DNDC model GHG emissions for the same location and time of field measurements were predicted (Figure 5). In general, predicted CO_2 emissions from the biosolid-amended soil treatment followed a similar pattern as the measured data. However, the DNDC model predicted a spike in N_2O emissions that was not replicated in the measured data. Model evaluation metrics, including RMSE and model efficiency, generally indicated poor model fit with RMSE above 87%. The DNDC model fit the CO_2 measurements the best out of the three GHG with 99% and 87% for biosolid-amended soil and control, respectively, which aligned with conclusions based on visual inspection of the data. RMSE values for N_2O and CH_4 were well over 100%, indicating poor model fit, consistent with visual inspection of the data. Model efficiency results similarly suggested the means of the measured data described the measured data better than the simulated data. Overall, DNDC was not able to simulate emissions from this system well.

It should be noted that the DNDCvCAN model was updated after the completion of this study to include a separate C pool for organic C amendments (biosolids or manure) that separates them from the soil organic C pools (Sitienei et al., 2025). It is possible that this revised model version could improve emissions predictions

from biosolid-amended systems, which is an area to be considered in future work. Emission predictions could be further improved with adjustment of the additional parameters beyond the recommended values presented in Supplementary Table S2. Additionally, it appears that the model calculates CH_4 consumption when the site is an upland crop field. Based on the field measurements in this study it is possible for an upland crop field amended with biosolids to produce some amount of CH_4, which should be considered in future model improvements. Additional longer term field measurements would be beneficial to assist with model improvement for CH_4 dynamics in upland crop fields.

Limitations and future directions

While this study primarily focused on denitrification and associated N_2O emissions, several other processes are known to contribute to N_2O, including nitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA) (Baggs, 2011; Braker and Conrad, 2011; Shan et al., 2021). Among these, nitrification and denitrification processes are typically dominant and driven by soil moisture content (Braker and Conrad, 2011; Tian et al., 2020; Kumar et al., 2020). Beyond potential increase in microbial activity and GHG emissions, the land application of biosolids raises concerns about contamination accumulation in the soil, particularly with excessive and prolonged use over approximately 15 years (Rani et al., 2024). According to information from Stillwater WWTP, the land application of biosolids was carried out biennially and adhered to the regulations and requirements set by the EPA. However, further research is needed to assess the potential accumulation of contaminants associated with the continued use of biosolid amendments to soil at this site and others like it. In general, future work should include long-term monitoring of these systems, which is a limitation of this study as it was conducted over a single season. Furthermore, future work should compare biosolid application methods, rates, and timings to provide practical implications for those interested in improving their environmental impact under this management.

Conclusion

This study provides significant insights into the effects of biosolid applications on GHG emissions, particularly focusing on N_2O dynamics in agricultural soils. The study is comprehensive by considering lab potentials, field measurements, and model predictions together. In the lab and field, the application of biosolids led to an initial increase in potential and measured N_2O emissions compared to control soils. This increase was associated with the higher concentrations of soil NO_3^- observed in the biosolid-amended soils and lower initial pH, both of which serve as critical factors influencing the denitrification process and its level of completion. The elevated denitrification potential results were similarly matched with the increased abundance of relevant functional genes. The DNDC model was able to predict the increase in GHG emissions after biosolid application, although the magnitude of emissions was inaccurate, with the model typically underpredicting GHG emissions. To make this approach more holistic, biosolids processing, transportation, and end-life usage should be incorporated. Considering life cycle GHG emissions could help make more informed decisions on the sustainability of biosolid land application. Another aspect that should be explored is the composition of biosolids and their impact on soil properties that will influence GHG emissions. Finally, the long-term impacts should be investigated as these results suggest it is possible to have lower emissions over time. These findings also carry implications for biosolid management policy. In particular, the observed short-term increases in GHG emissions following land application highlight the need for site-specific guidance that accounts for soil conditions, amendment timing, and nutrient dynamics. Incorporating field-based emissions data and life cycle assessments into regulatory frameworks, such as those established under the EPA's Part 503 rule, could help balance agronomic benefits with climate mitigation goals, improving the sustainability of biosolid land application.

Data availability statement

The original contributions presented in the study are included in the supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SK: Data curation, Formal Analysis, Investigation, Supervision, Visualization, Writing – original draft, Writing – review and editing. LB: Data curation, Formal Analysis, Investigation, Supervision, Visualization, Writing – original draft, Writing – review and editing. MW: Investigation, Writing – review and editing. SJ: Investigation, Writing – original draft. JH: Data curation, Formal Analysis, Funding acquisition, Methodology, Writing – review and editing. MF: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2025.1577071/full#supplementary-material

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