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ARTICLE

Nitrous oxide production and nitrogen transformations in a soil amended with biosolids

Carmen C. Roman-Perez and Guillermo Hernandez-Ramirez

Abstract: The application of organic amendments to agricultural soils enables the recycling of nutrients, further reducing the inputs of synthetic fertilizers for crop production. However, the production of N_2O emissions is a concern that arises from such a practice. A 35 d incubation experiment was conducted with soils receiving three contrasting types of biosolids — mesophilic anaerobic digested (BM), composted (BC), and alkaline-stabilized (BA) — at four water-filled pore spaces (WFPS): 28%, 40%, 52%, and 64%. A zero-N-addition control was also evaluated. Across all the three types of biosolids, N_2O production increased with soil moisture content, with BM and BC producing the overall highest N_2O fluxes. The most intense pulses of N_2O production were exhibited by BC at the beginning of the incubation. The highest cumulative N_2O production was found with 64% WFPS and from BC- (409 μg N_2O–N·kg^{-1} soil) or BM-amended soils (390 μg N_2O–N·kg^{-1} soil), which produced more than four and two times the emissions from the control and BA-amended soils at 64% WFPS, respectively. We also found the highest nitrification rates in the BM- and BC-amended soils. The total N_2O production was exponentially associated with the NO_3^-–N concentration present at the end of the experiment (R^2 = 0.83). Changes in the concentration of the soil available N indicated the occurrence of mineralization, nitrification, and denitrification over the incubation. These results provided insight into the interacting responses of N_2O production to soil moisture contents, biosolids treatment stabilization and properties, and soil N availability.

Key words: nitrous oxide emissions, nitrification rate, biosolids, water-filled pore space.

Résumé : L'application d'un amendement organique aux sols arables permet le recyclage des oligoéléments, ce qui réduit encore plus la quantité d'engrais chimiques employés en agriculture. Malheureusement, à cette pratique s'ajoute les préoccupations venant de l'émission de N_2O. Les auteurs ont entrepris une expérience de 35 jours durant laquelle ils ont incubé du sol bonifié avec trois sortes contrastantes de biosolides, en l'occurrence des biosolides obtenus après digestion anaérobie par des bactéries mésophiles (BM), compostage (BC) et stabilisation alcaline (BA), dans quatre espaces interstitiels remplis d'eau à 28, 40, 52 ou 64 %. S'y ajoutait un échantillon témoin, sans engrais azoté. La production de N_2O augmente avec la teneur en eau du sol pour les trois types de biosolides, BM et BC libérant les flux de gaz les plus importants, dans l'ensemble. Les biosolides BC ont donné lieu aux plus fortes bouffées de N_2O au début de l'incubation. La quantité cumulative de N_2O la plus importante a été observée dans les espaces interstitiels emplis d'eau à 64 % des sols amendés avec des biosolides BC (409 μg de N–N_2O par kg de sol) ou BM (390 μg de N–N_2O par kg de sol). Ceux-ci ont libéré respectivement plus du quadruple et du double du gaz émis par le sol témoin ou par celui bonifié avec les biosolides BA, pour la même proportion d'eau dans les espaces interstitiels (64 %). Les auteurs ont aussi observé le taux de nitrification le plus élevé dans les sols amendés avec des biosolides BM et BC. La production totale de N_2O a été associée de façon exponentielle à la concentration de N–NO_3^- relevée à la fin de l'expérience (R_2 = 0,83). Une modification de la concentration de N disponible dans le sol signalait la minéralisation, la nitrification et la dénitrification durant l'incubation. Ces résultats nous en apprennent davantage sur la manière dont la production de N_2O réagit à la teneur en eau du sol, à la stabilisation des biosolides et à leurs propriétés ainsi qu'à la quantité de N disponible dans le sol. [Traduit par la Rédaction]

Mots-clés : émissions d'oxyde nitreux, taux de nitrification, biosolides, espace interstitiel empli d'eau.

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Introduction

Agricultural soils are considered to be one of the major contributors to anthropogenic N_2O emissions, as they are the source of about 60% of the global N_2O emissions (De Rosa et al. 2016; Chai et al. 2020; Roman-Perez and Hernandez-Ramirez 2021). This figure is mainly caused by the increased application of synthetic N fertilizers to meet the increasing demand for food and biofuels (Kim et al. 2013; Smith 2017; Thilakarathna et al. 2020). Concerns around N_2O emissions arise from its high global warming potential, which is 298 times that of CO_2 (in a 100 yr timeframe), and its role as a stratospheric-ozone depleting substance (Wang et al. 2013; Charles et al. 2017).

Common pathways of N_2O production in soils are during the process of nitrification (oxidation of $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) via hydroxylamine ($NH_2OH$) oxidation ($NH_3 \rightarrow NH_2OH \rightarrow NO \rightarrow N_2O$) and denitrification (reduction of $NO_3^- \rightarrow N_2O \rightarrow N_2$) under limiting conditions of oxygen (Hernandez-Ramirez et al. 2009; T. Zhu et al. 2013; Shcherbak et al. 2014). Soil water content is considered one of the main controllers of soil N_2O production (Jäger et al. 2011; T. Zhu et al. 2013), as it regulates oxygen availability for microbes and affects gas diffusivity (Schaufler et al. 2010; Butterbach-Bahl et al. 2013; Cardoso et al. 2017).

Other factors influencing N_2O production are nitrogen input, organic carbon availability, pH, and temperature (Snyder et al. 2009; Oertel et al. 2016). Previous studies have shown an exponential increase of N_2O emissions with higher N application rates (Kim et al. 2013; Shcherbak et al. 2014). Additionally, availability of organic C increases microbial respiration, which reduces the oxygen concentration within the soil air, promoting the creation of anaerobic microsites, which further lead to N_2O production (Velthof et al. 2003; Jäger et al. 2011). Available organic C also acts as electron donor during denitrification, favoring the release of N_2O emissions (Guenet et al. 2021). Moreover, the C:N ratio of organic amendments is an indicator of the mineralization or immobilization rates of N in the soil. Organic amendments with narrower C:N ratios will mineralize the organic N faster, leading to a faster release of NH_4^+ and NO_3^-, which further serve as substrates for N_2O emissions (Jones et al. 2007; T. Zhu et al. 2013; De Rosa et al. 2016). Denitrification is also affected by the soil pH; values between 6 and 8 are considered optimum for the occurrence of denitrification (Coyne 2008). However, N_2O fluxes are higher at lower pH because the nitrous oxide reductase is inhibited while the reduction of NO_3^-, NO_2^-, and NO is favored at pH below 7 (Hu et al. 2015). The optimum temperature range for denitrification is from 28 to 37 °C (Coyne 2008), with increasing N_2O emissions with temperature up to ~37 °C (Oertel et al. 2016).

Biosolids, or treated sewage sludge, are solids, semisolids, or liquid residues that are by-products of municipal wastewater treatment plants (Wijesekara et al. 2016; Torri et al. 2017). Globally, a large amount of biosolids (1×10^8 Mg) is generated every year (Thangarajan et al. 2013). With an increasing global population, by 2050, this amount is expected to increase by 75% (17.5×10^7 Mg·yr^{-1}) for an estimated population of 9.6 billion at a production rate of 50 g·person^{-1}·day^{-1} on a dry basis (Wijesekara et al. 2016). Sustainable biosolids management is a major challenge because of the large quantities generated and their subsequent disposal (Haynes et al. 2009; Braguglia et al. 2015).

Land application of biosolids is considered to be the most economical and advantageous management method because the nutrients can be recycled and utilized by crops; concurrently, the dependence on synthetic fertilizers can be reduced (Christie et al. 2001; Rigby et al. 2009; Brown et al. 2011; Lu et al. 2012). However, there is also risk for increased field N_2O emissions when biosolids are land-applied relative to applying synthetic N fertilizers (Pu et al. 2010; Wijesekara et al. 2016; Roman-Perez et al. 2021). Although, when accounting for the elevated energy costs of industrial production of synthetic fertilizers, the lower N_2O emissions from fields receiving synthetic N fertilizers are offset (Roman-Perez et al. 2021), leading to a reduced agricultural carbon footprint when replacing synthetic fertilizers with organic amendments (Sharma et al. 2017). Thus, understanding the factors that govern N_2O emissions from the different available organic nitrogen sources is important to design management strategies to abate N_2O emissions (Pu et al. 2010; X. Zhu et al. 2013).

Most existing studies have focused on soil N_2O emissions from synthetic fertilizer use (Linzmeier et al. 2001; Barrena et al. 2017; Liu et al. 2017; Guardia et al. 2018), whereas studies assessing organic amendments have focused mostly on applications of manure (Velthof et al. 2003; Yang et al. 2003; Dalal et al. 2009) or compost (X. Zhu et al. 2013; Zhu-Barker et al. 2015), and only a few studies have assessed N_2O from biosolids applications (Inubushi et al. 2000; Pu et al. 2010; Yoshida et al. 2015, Roman-Perez et al. 2021). Therefore, there is still a knowledge gap regarding the effect of biosolids on N_2O fluxes, particularly those comparing divergent types of biosolids and stabilization methods (e.g., anaerobic digestion, alkaline stabilization, or composting) result in products with contrasting properties. These biosolid properties, as well as soil characteristics, need to be taken into account to minimize agricultural N_2O emissions while maintaining or improving crop yields. To our knowledge, controlled studies comparing N_2O emissions from a wide range of contrasting biosolids and under multiple soil water contents are not yet available in the literature. The objective of the present study was to quantify the effects of biosolids' properties, multiple soil moisture contents, and their potential interactive effect on soil N dynamics and N_2O production. We hypothesized that biosolids with an increased

availability of organic C and N, in combination with increasing soil moisture, would lead to faster N dynamics (mineralization and nitrification) and higher N_2O emissions.

Methods

Soil collection

Soil samples were taken from the 0–15-cm topsoil layer at the Ellerslie Research Station (53°25'13"N, 113°33'03"W), in Edmonton, Alberta, Canada. The soil is an Orthic Black Chernozem according to the Canadian soil classification system (the U.S. soil taxonomy equivalent is Typic Cryoboroll). The typical crop rotation in the sampled field included annual crops of wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), and barley (*Hordeum vulgare* L.) (Roman-Perez and Hernandez-Ramirez 2021). The soil texture is silty clay loam with 327 $g{\cdot}kg^{-1}$ clay, 511 $g{\cdot}kg^{-1}$ silt, and 162 $g{\cdot}kg^{-1}$ sand. The soil properties are as follows: pH 6.5, 54.8 $g{\cdot}kg^{-1}$ organic carbon, and 4.6 $g{\cdot}kg^{-1}$ total N. Soil samples were collected prior to the beginning of the growing season, in mid-May 2018. The samples were stored for 4 wks at field moisture conditions at 4 °C until the beginning of the experiment.

Biosolids collection, experimental design, and treatment preparation

Three types of biosolids were evaluated — mesophilic anaerobic digested biosolid (BM), composted biosolid (BC), and alkaline-stabilized biosolid (BA) — as they are representative of common biosolids treatments and their characteristics contrast widely. The BM and BC were provided by the Edmonton Waste Management Centre; BA was sourced from the Banff Wastewater Treatment Facility, both located within Alberta, Canada. The biosolids were obtained at each corresponding facility and transported to the Edmonton Research Station of University of Alberta (Roman-Perez et al. 2021). Composite subsamples of each type of fresh biosolids were taken from the field piles for conducting the incubation experiment. As preliminary steps, we determined the water content (as gravimetric weight loss), total carbon (TC), and total nitrogen (TN) concentrations (dry combustion with a ThermoScientific, Flash 2000 Organic Elemental Analyzer) for each of the three biosolids. The TN concentration and gravimetric water content were used to calculate the N application rates of fresh biosolids.

Prior to treatment preparation, the soil was mixed and sieved to 8 mm. The soil was then air-dried to a water content of ~28% water-filled pore space (WFPS). The experiment was a two-factor factorial design, with biosolid type (control 0 N, BM, BA, and BC) and soil moisture content (28%, 40%, 52%, and 64% WFPS) as the factors. The selected WFPS levels are common soil moisture contents over the growing season in Central Alberta. Five replicates of the 16 treatment combinations were prepared to measure N_2O production (three replicates) and soil NH_4^+–N and NO_3^-–N concentrations (two replicates) for destructive sampling on day 7 of the incubation.

The soil microcosms were prepared by placing 0.83 kg of soil (dry mass basis) into plastic containers 10 cm in height and with an 11.5 cm inner diameter. The soil microcosms were preincubated for 3 d at room temperature of 20–22 °C to create favorable conditions for microbial activity (Lin and Hernandez-Ramirez 2020, Thilakarathna and Hernandez-Ramirez 2021). After the preincubation period, the corresponding soils were mixed with each type of biosolid at an N rate of 122 mg $TN{\cdot}kg^{-1}$ soil (equivalent to a field application rate of 192 kg $N{\cdot}ha^{-1}$), whereas the controls did not receive any biosolids. Each soil microcosm was packed to a bulk density of 1.03 $g{\cdot}cm^{-3}$ and watered to the treatment WFPS. All microcosms were checked daily and maintained at their respective WFPS by adjusting the weight loss to the total weight with Milli-Q ultrapure water. Over the 35 d incubation period, the microcosms remained at room temperature (22 °C on average). To allow gas exchange, the lids of the soil microcosms were perforated (six small holes per lid).

Nitrous oxide emissions

To measure N_2O fluxes, we used a non-steady-state chamber system (12 autochambers Eosense eosAC) connected to a thermoelectrically cooled, mid-infrared quantum cascade laser absorption spectroscope system (QCLAS, Aerodyne Research Inc., Billerica, USA). The coupled systems allowed the recirculation of the gas samples between the chamber headspace (2.4 L) at ambient pressure and the QCLAS analytical cell at a pressure of 30 Torr at a flow rate of 1.61 standard $L{\cdot}min^{-1}$. Chambers were closed for 5 min, during which the N_2O concentrations were obtained at 1 Hz resolution and recorded with TDLWintel software. The TDLWintel software also controlled the QCLAS system. After the 5 min enclosure time, the chamber system was allowed to return to ambient concentrations prior to the next measurement. The QCLAS system was calibrated daily with reference gas standards as well as working standards of synthetic N_2O at 0.5 and 1.9 $\mu mol{\cdot}mol^{-1}$, and ultrahigh purity dinitrogen (N_2) for background absorption spectra subtraction. Ambient temperature and pressure were recorded with a temperature data logger (HOBO UX100, Onset, Bourne, USA) and a barometric pressure meter (Testo 511, West Chester, USA). The first N_2O measurement was conducted 3 h after the treatments had been applied, then on incubation days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 18, 21, 24, 27, 31, and 35.

Flux calculation

Nitrous oxide production rates ($\mu g\ N_2O$–$N{\cdot}kg^{-1}$ $soil{\cdot}day^{-1}$) were calculated by applying a modified ideal gas law as follows:

Table 1. Characterization of the three assessed biosolids ± standard error of the mean.

Parameter	Unit	Mesophilic anaerobic-digested	Alkaline-stabilized	Composted
TC (n = 3)	g C·kg^{-1}	283.3 ± 0.47	122.5 ± 0.48	278.4 ± 0.64
TN (n = 3)	g N·kg^{-1}	42.4 ± 0.09	7.8 ± 0.02	22.5 ± 0.048
C:N		8.29	18.7	13.2
NH_4^+–N	mg N·kg^{-1}	8230	1256.8	1402.6
pH		7.7	12.81	5.01
Electrical conductivity[a]	dS·m^{-1}	6.31	27.5	19.7
DM fraction gravimetric (mass basis)		0.26	0.71	0.76
Moisture (mass basis)		0.74	0.29	0.24

Note: TC, total carbon; TN, total nitrogen; DM, dry matter.
[a]Measured in a saturated paste of 1:2 soil:water.

$$(1) \quad PR = \frac{\text{slope} \times 2M \times V \times P \times 3600 \times 24}{R \times T \times \text{soil mass} \times 1000}$$

where slope is the linear regression coefficient during the 5 min when the chamber was enclosed (nL·L^{-1}·s^{-1}), $2M$ is the mass of two atoms of N in a mole of N_2O (28.01 g N·mol^{-1}), V is the volume of the chamber headspace (L), P is the pressure of the chamber headspace (atm), R is the universal gas constant (atm·nL·K^{-1}·nmol^{-1}), T is the temperature in the chamber headspace (K), and soil mass is on a dry weight basis (kg).

Cumulative fluxes of N_2O were calculated via linear interpolation of consecutive flux measurements over the entire incubation period (Thilakarathna and Hernandez-Ramirez 2021).

Inorganic nitrogen concentration

The patterns of NH_4^+–N and NO_3^-–N concentration in the soil throughout the incubation period were assessed by analyzing soil samples from prior to the treatment application, through destructive sampling on day 7 (two replicates); and on day 35, in all the incubated soils (three replicates). Soil available N (NO_3^-–N and NH_4^+–N) was extracted from 5 g soil samples with 50 mL of a 2M KCl solution, shaken in a reciprocal shaker for 30 min, and filtered with Whatman 42 filter paper (Fisher Scientific, Pittsburg, USA) (Chai et al. 2020; Roman-Perez and Hernandez-Ramirez 2021). The colorimetric method was applied to the filtrates with a Thermo Gallery Plus Beermaster Autoanalyzer. The colorimetric method used vanadium chloride to reduce the NO_3^-–N to nitrite (NO_2^-–N); thus, NO_3^-–N concentrations include both NO_3^- and the preexisting NO_2^-–N in the samples. The NH_4^+–N was measured using the salicylate-hypochlorite method.

Nitrification rates were calculated as the linear regression coefficients of changes in nitrate concentrations over the incubation period (Lin and Hernandez-Ramirez 2021). Net mineralization was inferred from the net changes in available N concentration (i.e., combined NO_3^-–N and NH_4^+–N) from the beginning to the end of the incubation period (i.e., net mineralization = final available N − initial soil available N).

We determine an exponential relationship between cumulative N_2O emissions over the entire incubation versus NO_3^-–N on day 35 across all the treatment combinations using the function $N_2O = ae^{b(NO_3-N)}$ with the Euler's number as a base while a and b were fitting parameters.

Statistical analyses

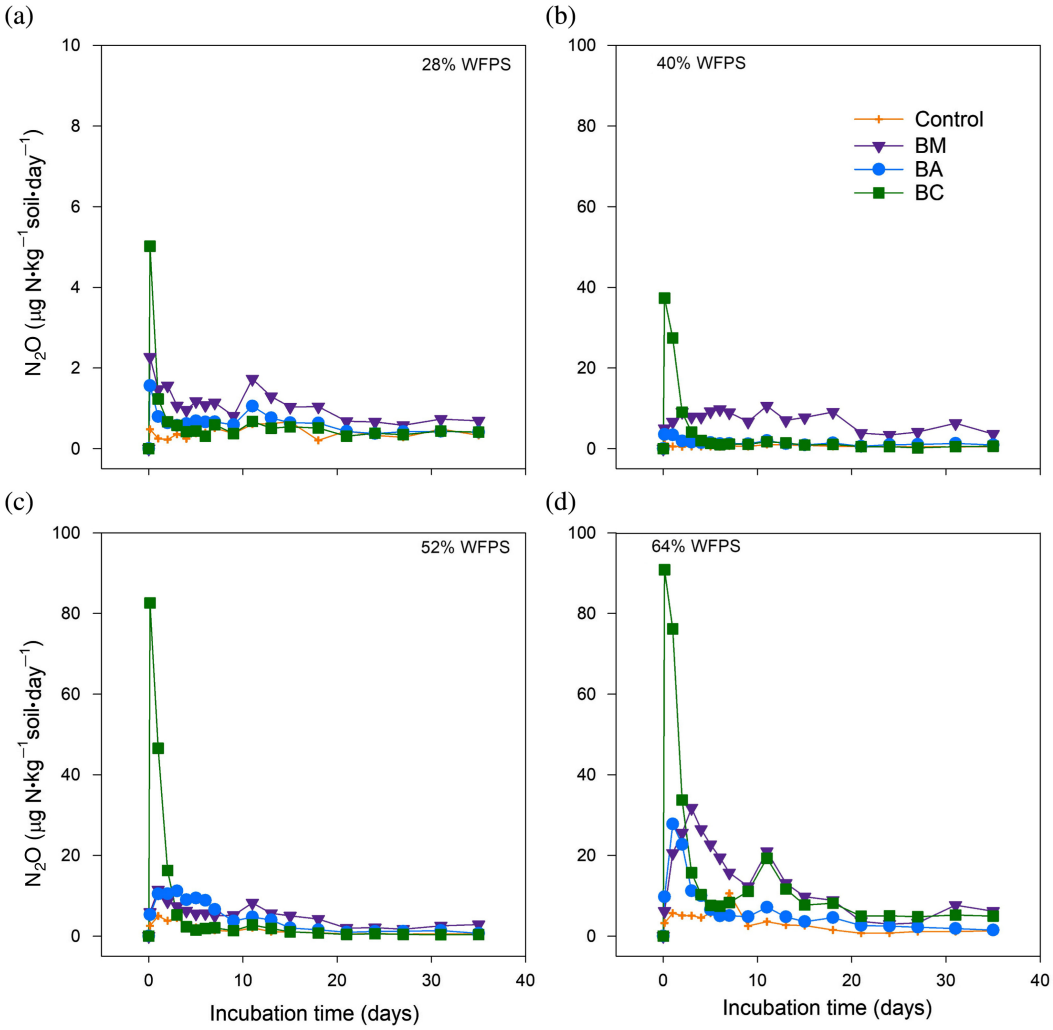
Statistical analyses were performed with R Studio software (R Core Team 2019). Regression analyses were performed to test the relationships between nitrification rates and WFPS. Biosolid type, WFPS, and their interaction (biosolid type × WFPS) were included in an ANOVA model for the N_2O fluxes and available N concentrations. Following significant ANOVAs (alpha critical value of 0.05), Tukey's honest significant difference test was performed for pairwise comparisons of the treatments. Data were Box–Cox transformed when needed to meet the assumptions of normality and homoscedasticity. The standard errors of the means are presented as error terms.

Results

Biosolids' properties

The properties of the three biosolids strongly differed between stabilization methods. The total N content of BM was five and two times the N contents of BA and BC, respectively (Table 1). The amount of TN in the form of NH_4^+–N was 19.4%, 16.1%, and 6.2% for BM, BA, and BC, respectively (Table 1). Across the biosolid types, BA had the highest pH (>12) caused by the addition of alkaline materials during its production. Similar to the results of N contents, BM also showed the highest TC concentration (283.3 g C·kg^{-1}), which was slightly higher than that of BC (278.4 g C·kg^{-1}) and more than twice the amount in BA (122.5 g C·kg^{-1}) (Table 1).

Fig. 1. N_2O–N fluxes (μg N·kg^{-1} soil·day^{-1}) at (*a*) 28%, (*b*) 40%, (*c*) 52%, and (*d*) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control over a 35 d incubation period. BM, mesophilic anaerobic-digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. Note the different *y*-scales across some of the panels. [Colour online.]

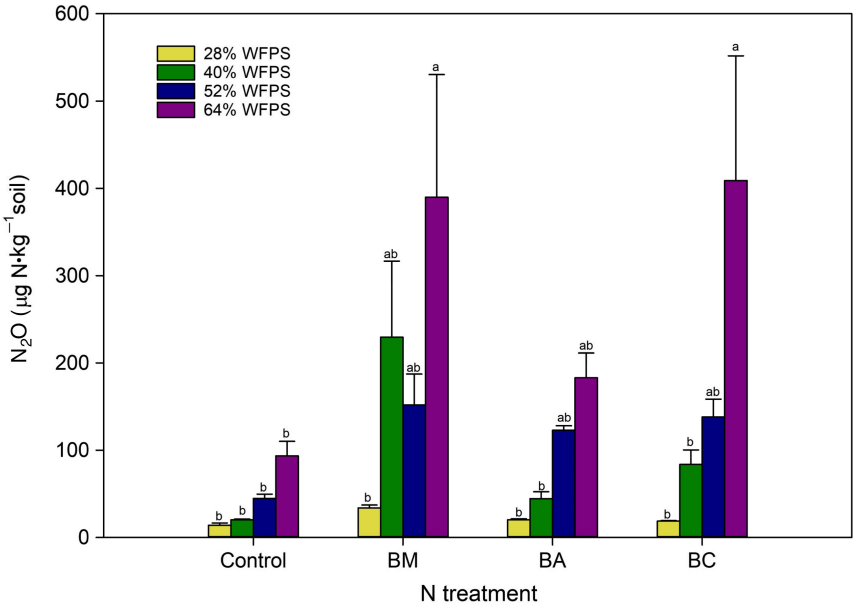


Nitrous oxide fluxes

Daily N_2O production rates increased with soil moisture and biosolids additions (Fig. 1*a*–1*d*). Overall, peak N_2O fluxes occurred shortly after the addition of biosolids (ranging from the first 3 h to day 3 of the incubation), with BC as the biosolid that presented the highest fluxes at each WFPS (Fig. 1*a*–1*d*). BC-amended soils peaked rapidly 3 h after the beginning of the incubation, but quickly dropping back to the basal fluxes by day 4. For BM- and BA-amended soils, relatively high N_2O production took place mainly within 9 d from the beginning of the incubation, particularly at 52% and 64% WFPS (Fig. 1*c*, *d*). After this initial high activity, daily N_2O fluxes remained relatively low and constant until the end of the incubation.

Cumulative N_2O emissions ranged from 14.19 to 409.01 μg N_2O–N·kg^{-1} soil for the control at 28% WFPS and the BC-amended soil at 64% WFPS, respectively. Relative to the controls, biosolids additions increased N_2O production by 4.7, 3.8, and 2.1 times on average across all moisture contents for BM-, BC-, and BA-amended soils, respectively. Statistical analysis of the cumulative N_2O showed a significant interaction between biosolid addition and WFPS ($P < 0.05$) (Fig. 2). As part of this WFPS × biosolid addition interaction, significant effects of biosolid additions were found at 64% WFPS, where BM- and BC-amended soils were fourfold higher than the control ($P < 0.05$) (Fig. 2). Moreover, in the BM- and BC-amended soils, N_2O emissions at 64% WFPS were significantly higher than the emissions at 28% WFPS by 12 and 22 times, respectively ($P < 0.05$) (Fig. 2). At 28%, 40%, and 52% WFPS, BM-amended soils had the highest cumulative N_2O fluxes; conversely, at 64% WFPS, the BC-amended soil's emissions were numerically higher than those from BM by 19 μg N_2O–N·kg^{-1} soil, but these two treatment combinations were not significantly different from each other ($P > 0.05$) (Fig. 2).

Fig. 2. Cumulative N_2O–N fluxes (μg N·kg^{-1} soil) at 28%, 40%, 52%, and 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control over a 35 d incubation period. BM, mesophilic anaerobic-digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. [Colour online.]



Ammonium and nitrate concentrations

The initial NH_4^+–N and NO_3^-–N concentrations in the soil were 4.39 and 13.61 mg N·kg^{-1}, respectively (Figs. 3 and 4). Over the incubation period, NO_3^-–N increased (Fig. 4), whereas NH_4^+–N exhibited increasing and decreasing fluctuations (Fig. 3). In general, NO_3^-–N concentrations increased with increasing soil moisture content (Fig. 4); the opposite trend was observed for NH_4^+–N concentrations (Fig. 3).

Both biosolid addition and moisture levels had separate significant effects on NH_4^+–N as well as on NO_3^-–N ($P < 0.05$) at days 7 and 35. The interaction between soil water and biosolids addition was significant only for NO_3^-–N on day 35 of the incubation ($P < 0.05$). When we compared the available N among soils receiving biosolids, BM-amended soils showed the highest NH_4^+–N and NO_3^-–N accumulation on both days 7 and 35 of the incubation (Figs. 3 and 4). In the case of NH_4^+–N, on day 7, the control soils produced significantly less than the BM-amended soils at all WFPS ($P < 0.05$), whereas on day 35, the control soils produced significantly less than the BM-amended soils at 40%, 52%, and 64% WFPS ($P < 0.05$). By the end of the incubation, NO_3^-–N in the control soils was significantly lower than from the biosolids-amended soils ($P < 0.05$), except for BC at 28% WFPS. In addition, at 40%, 52%, and 64% WFPS, BM-amended soils had significantly higher NO_3^-–N concentrations than BA- and BC-amended soils ($P < 0.05$).

Nitrification rates

Nitrification rates varied from 0.11 to 1.21 mg NO_3^-–N·kg^{-1} soil·day^{-1} for the control soil at 28% WFPS and the BM-amended soil at 52% WFPS, respectively. We observed faster nitrification rates with increasing soil moisture up to 52% WFPS in the BM- and BC-amended soils (nonlinear relationships, R^2-BM = 0.996, R^2-BC = 0.999; Fig. 5), up to 55% WFPS in the BA-amended soils (non-linear relationship, R^2-BA = 0.990; Fig. 5), and 64% in the control soils (linear relationship, R^2 = 0.998; Fig. 5).

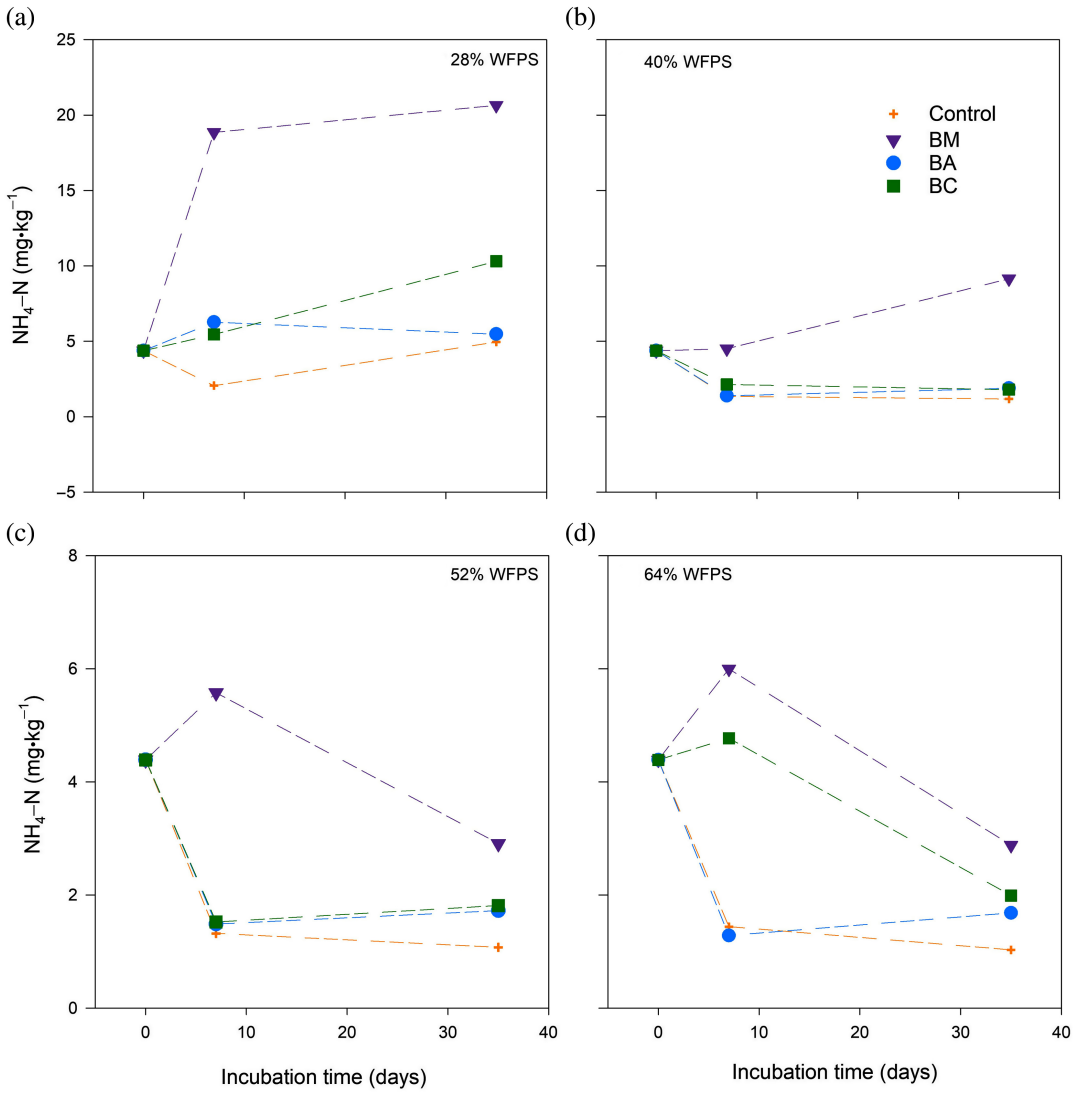
Additionally, net mineralization over the incubation period increased with soil moisture for all biosolid types and the control, reaching more than double the initial available N concentration (i.e., as indicated by combined NO_3^-–N and NH_4^+–N): an increase from 18 (4 mg N·kg^{-1} from NH_4^+–N plus 14 mg N·kg^{-1} from NO_3^-–N, day 0) to 37.6 mg N·kg^{-1} (1 plus 36.6, day 35) at 64% WFPS. Of all the biosolids-amended soils, those receiving BM showed the highest mineralization rates at each WFPS.

The concentration of available N in the BM-amended soils increased by threefold at 28% WFPS and fourfold at 40%, 52%, and 64% WFPS by the end of the incubation (on day 35) (Figs. 3 and 4). Moreover, we observed an increase in cumulative N_2O emissions with NO_3^-–N on day 35 (Fig. 6) across all the treatment combinations (exponential fit, R^2 = 0.825; Spearman's correlation ρ = 0.938, $P < 0.001$).

Discussion

The interacting effect between the biosolids type and moisture content on N_2O emissions shows that N_2O production depends on both the presence of available N as a substrate and favorable soil moisture conditions to promote the availability of the essential soluble C and

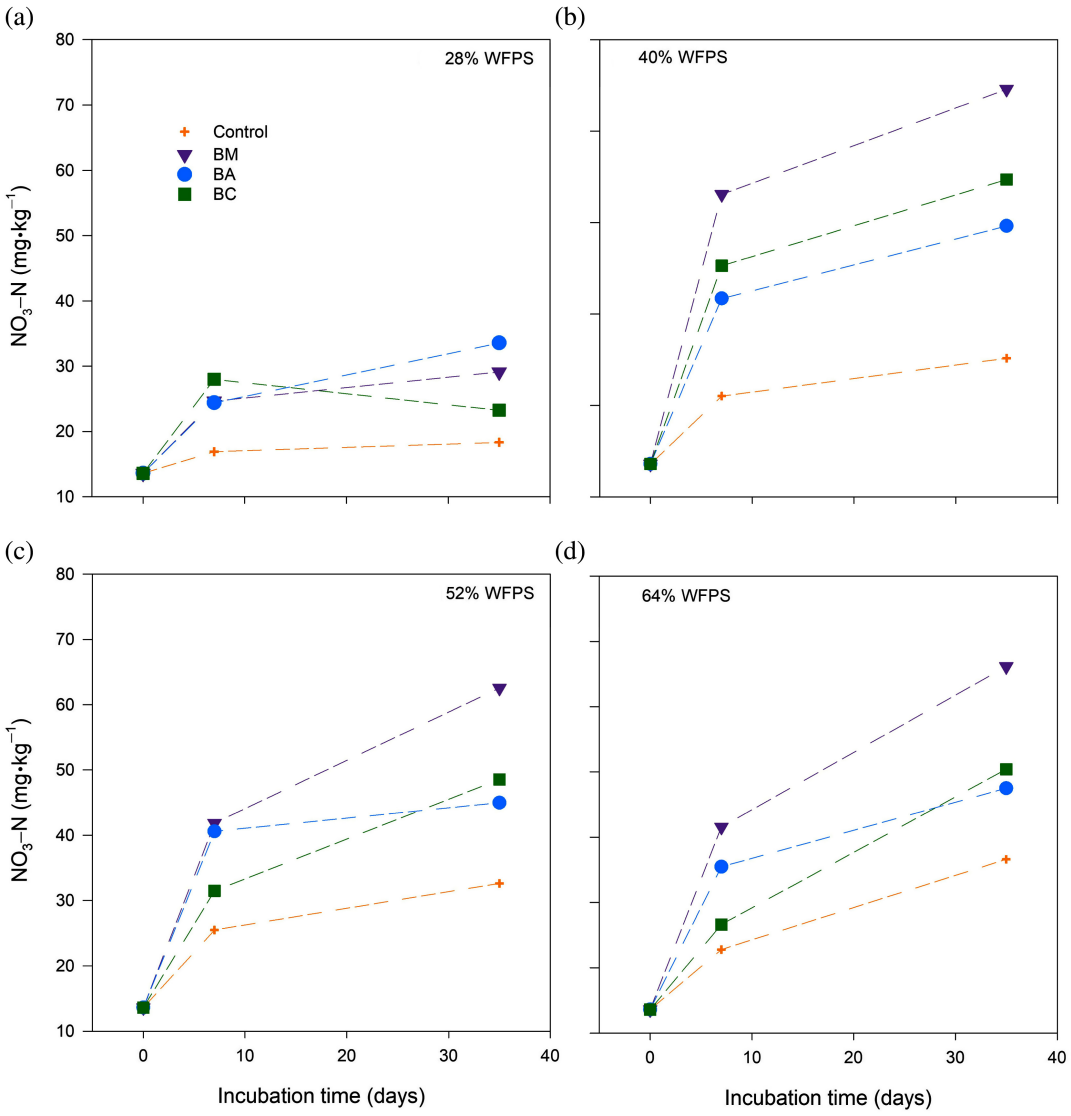
Fig. 3. Changes in NH_4^+–N concentration (mg N·kg^{-1}) during the incubation period at (*a*) 28%, (*b*) 40%, (*c*) 52%, and (*d*) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control. Vertical scales are different. BM, mesophilic anaerobic digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. [Colour online.]



N nutrients for microbial activity (Banerjee et al. 2016). In our study, a wide range of conditions for N_2O production and emission were covered by using three contrasting biosolids and four WFPS levels. In addition to contributing directly with some inorganic N, biosolids are rich sources of organic N that undergoes through mineralization to further release mainly NH_4^+, which is available for plant uptake (Rigby et al. 2016; Sharma et al. 2017). Therefore, N substrate was not likely to be a limiting factor in the biosolids-amended soils in our experiment, while the increasing soil water contents represented a range of moist, aerobic conditions (i.e., 28–64% WFPS) (Roman-Perez and Hernandez-Ramirez 2021). This range of soil moisture might have favored an increased mineralization (Curtin et al. 2012).

As expected, most of the results showed higher daily and cumulative N_2O emissions with increasing soil moisture content from 28% to 64% WFPS (Figs. 1 and 2). Similar results have been found in several studies applying either synthetic fertilizer (Bateman and Baggs 2005; Mathieu et al. 2006) or organic amendments (T. Zhu et al. 2013; X. Zhu et al. 2013). The response of N_2O emissions to increasing soil moisture can be explained by increases in the accessibility and mobility of substrates, which can be associated with solute diffusion across the soil pore network (Curtin et al. 2012; Lin and Hernandez-Ramirez 2020; Roman-Perez and Hernandez-Ramirez 2021). The range of soil moisture contents in our experiment was favorable for nitrification, as has been reported previously by Bateman and Baggs (2005) and Linn and Doran (1984). In their studies, they reported <60% WFPS as optimum soil moisture for nitrification, since the diffusion of both nutrients and O_2 is not limited at this WFPS. The occurrence of fast nitrification in our study could

Fig. 4. Changes in NO_3^-–N concentration (mg N·kg^{-1}) during the incubation period at (*a*) 28%, (*b*) 40%, (*c*) 52%, and (*d*) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control. BM, mesophilic anaerobic-digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. [Colour online.]



have increased the availability of NH_2OH and NO_2^-–N substrates for the production of N_2O emissions (Roman-Perez and Hernandez-Ramirez 2021). Mineralization of the organic N added in the biosolids might have further promoted nitrification in the aerobic domains of the soil while denitrification might have occurred in the anaerobic microsites located within the aggregates in the soil microcosms (García-Ruiz et al. 2012; T. Zhu et al. 2013; Guardia et al. 2018).

The differences in N_2O emissions across the three biosolids-amended soils could be related to the amount of nitrogen and carbon in the different biosolids (Table 1) and their availabilities. BM had a higher total N concentration than either BC and BA (Table 1); and the organic N in BM can be mineralized during the digestion process. Then BM has more available N in comparison with BA and BC, (higher proportion of NH_4^+–N concentration in BM, Table 1) (Rigby et al., 2009, 2016; Yoshida et al. 2015). In the case of BA, the addition of alkaline materials and the resulting N losses (NH_3 volatilization) during the lime stabilization treatment led to lower N contents, whereas the addition of woody bulking agents during composting led to more recalcitrant N in BC (Yoshida et al. 2015; Rigby et al. 2016; Roman-Perez et al. 2021). Addition of biosolids also provides available organic C, which increases microbial respiration and depletes the O_2; likely favoring N_2O production in the anaerobic microsites (Gentile et al. 2008; Zhu-Barker et al. 2015; Guenet et al. 2021). In our study, the C contents in BM (283.3 g C·kg^{-1}) and BC (278.4 g C·kg^{-1}) were similar, and more than double the C content of BA (122.5 g C·kg^{-1}) (Table 1). Therefore, even

Fig. 5. Nitrification rates for all biosolid additions and the untreated control as a function of moisture content expressed as water-filled pore space (WFPS). Exponential fitting and equations are provided for each biosolid treatment combination. Linear fitting and the equation are presented for the control treatment combinations. BM, mesophilic anaerobic-digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. [Colour online.]

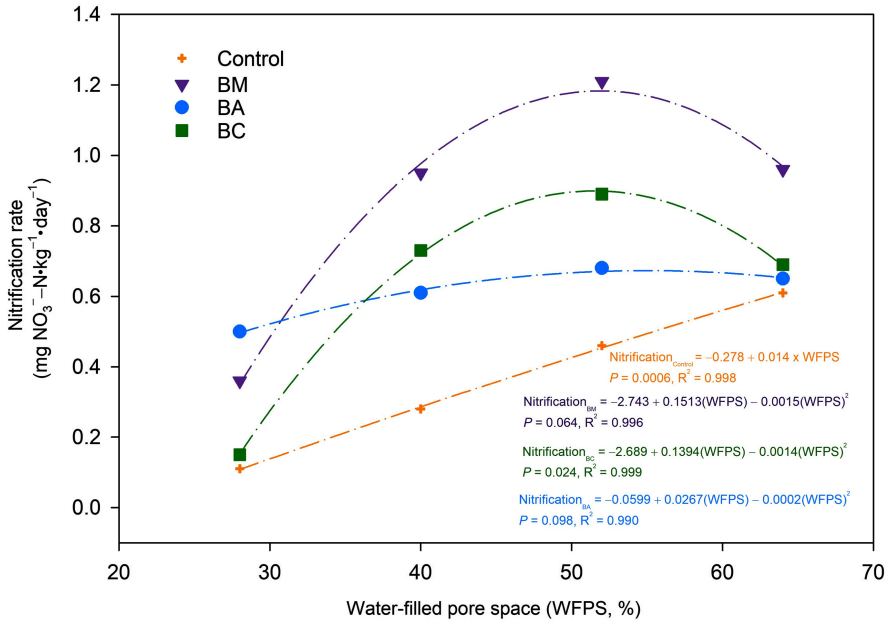
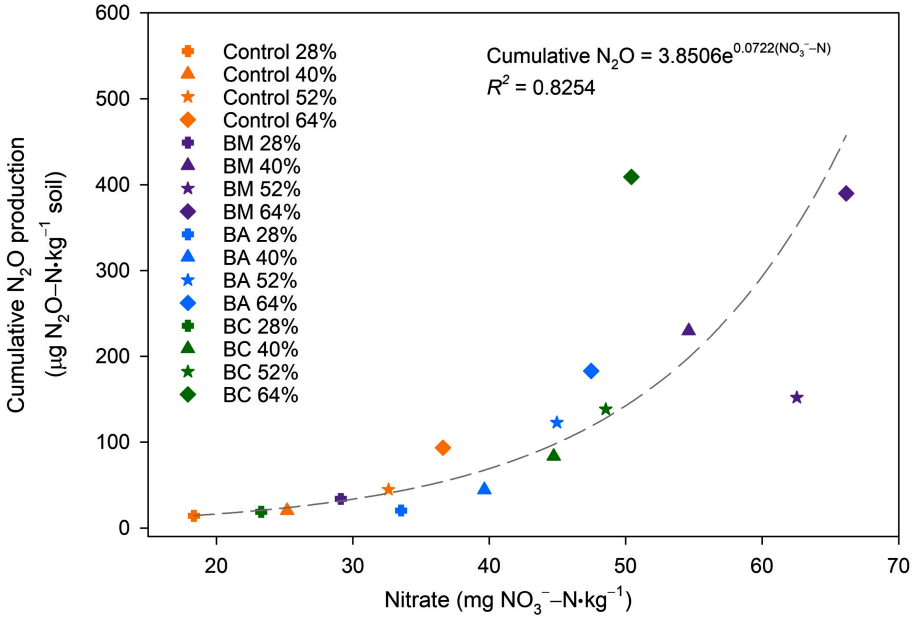


Fig. 6. Cumulative N_2O–N production (μg N·kg^{-1} soil) as a function of NO_3^-–N concentration (mg N·kg^{-1}) at the end of the incubation period across all treatment combinations (nitrogen sources × water-filled pore space). Exponential fitting and the equation are presented. BM, mesophilic anaerobic-digested biosolid; BA, alkaline-stabilized biosolid; BC, composted biosolid. [Colour online.]



though the soil moisture contents represented aerobic conditions, some anaerobic hotspots might have been created within the soil microcosms shortly after the biosolids addition. These relationships of N and C supplies across biosolids also explain the higher N_2O emissions activity, in particular, at the beginning of the incubation. In a field study, Roman-Perez et al. (2021) found how the addition of biosolids produced under mesophilic anaerobic digestion (BM) led to higher N_2O emissions than BC and BA since BM had greater N content and moisture

and narrower C:N ratio. These results are in agreement with our present incubation results; moreover, across biosolid-amended soils, Roman-Perez et al. (2021) typically found lower N_2O emissions from fields receiving BC, and these BC effects was congruently mirrored by their higher crop biomass and N use efficiency in comparison to BM and BA additions.

Additionally, we observed an exponential increase in cumulative N_2O emissions in association with the NO_3^-–N concentrations present on day 35 (R^2 = 0.825, Fig. 6). This nonlinear linkage reveals that soils with increased capacity for N_2O production also accumulate the most NO_3^-, in particular under overall aerobic moisture conditions, which probably did not favor further reduction of the produced NO_3^- to N_2O. Most of the total N_2O emissions at 52% and 64% WFPS were produced within the first 4, 9, or 13 d of the incubation for the BC-, BA-, and BM-amended soils, respectively, whereas NO_3^-–N accumulated over the entire incubation period (Fig. 4). Moreover, as the NH_4^+–N concentrations showed an overall decrease over time, this could indicate a reduction in the mineralization rate, an increase in the nitrification rate, or both effects occurring simultaneously toward the end of the incubation period. Our results for daily N_2O emissions also help to explain the dynamics of the available N. Since most of the cumulative N_2O emissions were produced within the first 4 to 13 d of the incubation as noted above, with a great increase in NO_3^-–N by day 7, it could be assumed that the NH_4^+–N produced was rapidly nitrified to NO_3^-–N, as reported by Inubushi et al. (2000) and He et al. (2017) after applying organic amendments. In the study by Inubushi et al. (2000), soils receiving sewage sludge compost (at rates of 10 and 20 Mg DM·ha^{-1}·yr^{-1}) showed a N_2O peak at the same time as the NH_4^+–N concentrations (day 30 of a 90-d incubation experiment). Moreover, He et al. (2017) reported a decrease in NH_4^+–N concentrations concurrently with an increase in NO_3^-–N in soils receiving grass clover biomass or cattle manure at a rate of 10 g DM·kg^{-1} over a 20-d incubation experiment. Therefore, in our experiment, most N_2O was probably emitted during the nitrification process because the aerobic conditions might have resulted in more N_2O coming from nitrification than from denitrification (Inubushi et al. 2000; Bateman and Baggs 2005; Liu et al. 2017; Mekala and Nambi 2017). In addition, part of the newly produced NO_3^-–N might have been reduced to N_2O in the anaerobic microsites via denitrification. This is because NO_3^-–N could have been required as an alternative electron acceptor once decomposition of the organic C provided by the biosolids caused O_2 depletion (Wrage et al. 2001; Jäger et al. 2011). After this period of high microbial activity, we observed a decline in N_2O emissions (Fig. 1) as well as in NH_4^+–N concentrations (Fig. 3), although NO_3^-–N concentrations remained high (Fig. 4). This could be explained by the fact that in the beginning of the incubation, the rapidly mineralizable C was probably depleted (Gentile et al. 2008), leading to a subsequent decrease in the mineralization rate, thus lowering the amount of NH_4^+–N produced, whereas most of the NO_3^-–N already produced remained in the soil. The reduced NH_4^+–N supply from the soil and biosolids might have caused lower nitrification rates, which explains the lower N_2O emissions after the organic matter from the biosolids became depleted, as the moisture conditions were not favorable for NO_3^-–N reduction to N_2O (Bateman and Baggs 2005; Mathieu et al. 2006; Zhu-Barker et al. 2015). The changes in microbial processes once C substrate is depleted might have induced N immobilization in the soil, as indicated by an overall decrease in NH_4^+–N concentrations (Fig. 3). Nevertheless, N_2O can also be produced from other processes such as chemodenitrification and nitrifier denitrification ($NH_3 \rightarrow NO_2^-$ followed by $NO \rightarrow N_2O \rightarrow N_2$); however, the contribution of those processes to N_2O emissions during our experiment might have been minimal since favorable conditions were not provided. For example, acidic pH (<5) and high NO_2^- concentrations are needed for chemodenitrification, which is most common in forest soils; whereas nitrifier denitrification occurs under concurrent conditions of O_2 limitations, low organic C, and high N availability (Butterbach-Bahl et al. 2013; Hu et al. 2015).

Nitrification reached optimal rates at a soil moisture content of 52% WFPS in the BM- and BC-amended soils and at 55% WFPS in the BA-amended soils (Fig. 5). Nitrification rates declined when the soil moisture was beyond these WFPS levels. As noted above, previous studies (Linn and Doran 1984; Bateman and Baggs 2005) have reported 60% WFPS to be the optimum conditions for nitrification; however, those previous studies used synthetic N fertilizers. Our results suggested a slightly different pattern for optimum conditions, with a shift toward a lower WFPS with organic amendments. The declining nitrification rates in the biosolids-amended soils while the WFPS was still lower than 60% might be related to the fact that biosolids provided readily degradable organic C, which was likely used by microbes, leading to faster consumption and depletion of soil O_2 at a lower WFPS than previously thought. This increased use of O_2 by microbial respiration can preclude or reduce the aerobic conditions that would have favored the nitrification process (Gentile et al. 2008; Zhu-Barker et al. 2015; Guenet et al. 2021). The feedback effect of adding biosolids with narrow C:N ratios would have presumably shifted the optima of nitrification rates toward less moist soil conditions. It is noted that BA-amended soils showed less variation in their nitrification rates across the soil moisture levels, probably because of the wider C:N ratio and the more recalcitrant C than in BM and BC (Table 1, Figs. 3, 4, and 5). These postulations are in line with the results of Rigby et al. (2009) from a 90-d field study in which lime-treated and mesophilic anaerobic-digested biosolids were applied. Their results showed that 20% of

the TN applied with the mesophilic anaerobic-digested biosolid was recovered as NO_3^-– N + NO_2^-–N, whereas only 10% of the TN was recovered in the lime-treated biosolids plots after 20 d of their experiment.

An additional explanation for the high N_2O fluxes observed could be the occurrence of a positive N_2O priming effect, which refers to the stimulation of soil organic matter (SOM)-derived N_2O production as triggered by the addition of labile N (Roman-Perez and Hernandez-Ramirez 2021). The underlying notion is that the labile N addition alters the decomposition and mineralization of preexisting SOM by microbes, commonly increasing (positive priming) the soil-available N derived specifically from preexisting SOM (Fiorentino et al. 2019). This positive priming effect of preexisting SOM can be also stimulated by new additions of labile organic C (Fiorentino et al. 2019). Since the addition of biosolids in our experiment provided both C and N, it is probable that positive priming of SOM occurred, which further led to a positive N_2O priming effect. The occurrence of a positive N_2O priming effect on a Black Chernozem soil was recently reported by Roman-Perez and Hernandez-Ramirez (2021). In their study, they found a positive N_2O priming effect with increasing soil moisture after adding labile N (urea), which resulted in a 19% primed N_2O flux, with even more N_2O coming from SOM than from the added urea-N (59% vs. 41%, respectively). Additional research is needed to evaluate the effects of biosolids amendments on the decomposition and mineralization of preexisting SOM and the further release of primed N_2O. A study of available N dynamics (mineralization–immobilization) in soils receiving biosolids is also needed to estimate the potential value of biosolids as an N source for crop production. Furthermore, analysis of microbial biomass N could also be meaningful, as the microbial population and dynamics play a role in biosolids N turnover (Rigby et al. 2009).

Certain study limitations can be noted. This incubation was specifically done under controlled laboratory conditions, including only one soil, packed to a single bulk density, without plants growing, and under general aerobic conditions. Furthermore, the effect of biosolids' physical properties on the soil physical properties such as bulk density, porosity, and water holding capacity was not considered. Several studies have shown that the addition of biosolids decreases the bulk density while increasing the porosity and water-holding capacity (Gardner et al. 2010; Sharma et al. 2017). However, these studies were conducted at field conditions and changes were measured after 1 yr of the application of biosolids. Therefore, we believe no major changes in the referred soil properties occurred due to the addition of biosolids in the microcosms. Nevertheless, we acknowledge that the pore size distribution of the soil microcosms might have been affected after biosolids additions. Therefore, there is a need to further evaluate the effect of applying biosolids on N_2O emissions under a wider range of soil moisture or wetting–drying cycles while taking into consideration the differences on the pore size distribution, as this controls the gas exchange between the soil and atmosphere (Balaine et al. 2013) as well as the pore continuity (van der Weerden et al. 2012). The study of van der Weerden et al. (2012) found that N_2O emissions are reduced with increased pore continuity when macropores (>60 μm diameter) become drained. Thus, soils with a greater proportion of macropores would release less N_2O. In our study, the higher moisture of added BM might have reduced the volume of aerated macropores to a greater proportion than both BA and BC, which in turn might have favored the occurrence of N_2O emissions over a longer period in the BM-amended soils when compared with BC at each water-filled pore space (Fig. 1). This hypothesis points out to the important influence of soil physical properties on N_2O emissions, which should be considered for future studies.

Conclusions

This study shows how the addition of contrasting biosolid types, a range of soil moisture contents, and their interaction significantly impacted N_2O production. As expected, N_2O emissions were enhanced by increasing soil moisture and N additions from different types of biosolids, with BM and BC amendments producing the highest N_2O emissions, pointing to the influence of the forms and availability of N and C as well as the C:N ratio on the microbial processes that generate N_2O emissions (i.e., nitrification and denitrification). As expected, soil N dynamics were enhanced by increasing soil moisture and organic-N additions, with BM and BC amendments producing the highest cumulative N_2O emissions, pointing to the influence of the forms and availability of N and C as well as the C:N ratio on nitrification and denitrification. Thus, these biosolid properties should be taken into account when designing practices for land application of biosolids in agricultural systems. For example, the addition of a biosolid with higher N, C, and NH_4^+ contents and a lower C:N ratio has the potential to increase N_2O emissions; therefore, caution should be taken when applying biosolids with such characteristics. Nevertheless, our results substantiate the need for additional research to assess the temporal variation of available N as well as the net N mineralization induced by biosolid amendments to identify the biosolid rates to be applied in fields. Land application of biosolids should aim to prevent N losses to the environment and optimize nutrient recovery by plants.

Competing Interests

The authors declare there are no competing interests.

Acknowledgements

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