Agricultural Biosolids Application: Greenhouse Gas Emissions, Nitrogen Dynamics, and Crop Productivity

by

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Abstract

Agricultural activities contribute greatly to greenhouse gas (GHG) emissions, accounting for 14% of the total anthropogenic emissions of GHG such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4). Of these GHG, N_2O is the most concerning gas because of its high global warming potential (GWP), 298 times higher than that of CO_2, and its capacity for depleting stratospheric ozone. The increase of nitrogen fertilizer additions over the years has made agricultural soils responsible for around 60% of global anthropogenic N_2O emissions. Biosolids are by-products from sewage treatment processes that can be land applied to agricultural soils in order to recycle their nutrients, improve soil properties and decrease the dependence on commercial fertilizers; however, GHG can be released from this practice. Thus, there is a need to understand the effects of biosolid additions on N dynamics, nutrient use efficiency, crop productivity, and the amount of GHG emissions released by this practice. In a field study, we evaluated the fluxes of N_2O, CH_4 and CO_2, soil available N, barley (*Hordeum vulgare* L.) biomass productivity, and nitrogen use efficiency (NUE) in croplands receiving three types of biosolids (mesophilic anaerobic digested [BM], composted [BC], and alkaline-stabilized [BA]) and granular urea in a Black Chernozem soil in Central Alberta, Canada, over three experimental site-years. The combinations of each biosolid with urea was also evaluated. All N source treatments were assessed in two placements: surface (S) and incorporation (I) to 15 cm soil depth. Nitrous oxide emissions were triggered by concurrent increases of soil moisture and available N, and incorporation of the N source increased N_2O emissions compared to surface-applied N. Annual N_2O emission factor (EF_{area}) from urea-amended soils (0.62 ± 0.14%) were fivefold higher than those from soils receiving only BA or BC (0.12 ± 0.04% or 0.12 ± 0.03%, respectively, $P < 0.05$), but EF_{area} from soils amended with only BM (1.33 ± 034%) was more than double the EF_{area} from urea-amended soils ($P > 0.05$). Carbon dioxide (CO_2) fluxes generally followed similar patterns as the N_2O fluxes, while CH_4 fluxes were minimal. Overall, the mesophilic anaerobic digested under incorporation treatment (BMI) showed the highest GHG emissions. Results of a partial GHG balance showed that N_2O emissions were the main contributor (up to 96%), while urea manufacturing contribution to the GHG balance was up to 52%. This offset the comparatively low field N_2O emissions from the urea-amended fields, leading to even higher CO_2 equivalents than the BA- and BC-amended fields. Incorporating the N sources enhanced barley biomass, and in certain cases, the combinations of biosolids and urea (e.g., BMURI, BMURS, BCURS) showed even higher biomass and NUE, as well as lower N_2O emissions than biosolids-amended soils. Moreover, in an incubation study, we examined the effect of moisture (i.e., 28, 40, 52, and 64% WFPS) and the different types of biosolids aforementioned (i.e., BM, BA, and BC) on N_2O production in the referred Black Chernozem soil. We found how the different biosolids properties and soil water contents influenced soil available N dynamics to produce N_2O emissions. BM- and BC-amended soils were the higher N_2O emitters, and emissions increased with soil moisture. These biosolids-amended soils also showed higher nitrification rates than BA-amended soils and the controls. The NO_3^-–N concentration by the end of the experiment was well correlated with the total N_2O production ($r = 0.91$). In addition, we examined the sources and priming of N_2O production as a function of ^{15}N-labelled urea addition and multiple moisture contents (28, 40, 52, and 64% WFPS) in a Black Chernozem soil (high SOM: 55 g organic C kg^{-1}). More N_2O was sourced from SOM than added urea, with 59 ± 2% N_2O originating from SOM, and SOM-derived N_2O under urea was larger than that of the control, revealing a positive N_2O priming triggered by urea addition (19 ± 2% of the total N_2O from urea-amended soils). In summary, our findings will help to improve prediction ability and mitigation strategies for GHG emissions, particularly for N_2O, from agricultural soils receiving biosolids additions.

Preface

This dissertation is an original work by Carmen Cecilia Roman Perez. I was responsible for data curation, formal analysis, and manuscript writing of all the experiments included here, which were supervised by Dr. Guillermo Hernandez Ramirez. Dr. Guillermo Hernandez Ramirez was responsible for funding acquisition; methodology; project administration; resources; supervision; validation; visualization; writing-review and editing. In Chapter 2, Len Kryzanowski, Dick Puurveen and Germar Lohstraeter were responsible for field work management and guidance, and laboratory analysis.

Chapter 2 of this dissertation has been submitted as C.C. Roman-Perez, G. Hernandez-Ramirez, Len Kryzanowski, Dick Puurveen and Germar Lohstraeter "Greenhouse gas emissions, nitrogen dynamics and barley productivity as impacted by biosolids applications" to Agriculture, Environment and Ecosystems.

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1.0 General introduction

Biosolids, or treated sewage sludge, are solids, semisolids or liquid residues that are by-products from sewage treatment processes, at municipal wastewater treatment plants (Wang et al., 2008; Thangarajan et al., 2013; Torri et al., 2016; Wijesekaral et al., 2016). Although heterogeneous, biosolids can be composed of about 50% organic and 50% inorganic materials (Haynes et al., 2009). Their characteristics vary highly according to the source input (domestic, industrial, or commercial), the wastewater treatment technique, age of biosolids, and environmental conditions (Wijesekaral et al., 2016).

Globally, a large amount of biosolids (10^8 Mg) is generated every year (Thangarajan et al., 2013). This amount is expected to increase by 75% (17.5×10^7 Mg yr^{-1}) for an estimated population of 9.6 billion by 2050 (Wijesekaral et al., 2016). Biosolids production in Canada is approximately 660 000 Mg (tons; dry basis) per year (CCME, 2012a) with only 33% used for land application. This amount is lower in comparison to other countries such as United States (60%), United Kingdom (71%), and Australia (59%) (Haynes et al., 2009; McCarthy, 2015; Wijesekaral et al., 2016).

1.1 Biosolids management practices

An environmentally sustainable management of the aforementioned global quantities of biosolids is a major challenge. Currently, biosolids management includes land application to agricultural and forest soils as well as applications for land reclamation (CCME, 2012a; Thangarajan et al., 2013). Other alternatives include landfilling and incineration (Haynes et al., 2009; Sharma et al., 2017); however, those are considered disadvantageous because of land requirements and environmental pollution (Sharma et al., 2017; Öberg and Mason-Renton, 2018). Thus, land application is considered the most economical and advantageous method because the nutrients in the biosolids are recycled and utilized by vegetation, and concurrently, the dependence on commercial fertilizers is reduced (Christie et al., 2001; Pepper et al., 2006; Haynes et al., 2009; Brown et al., 2011; Lu et al., 2012; Nicholson et al., 2018; Öberg and Mason-Renton, 2018). Replacing synthetic fertilizers, totally or partially, decreases the agricultural carbon footprint, since the industrial manufacture of fertilizers requires elevated energy costs (Brown et al., 2010;

Sharma et al., 2017). Furthermore, this environmental benefit results in economic benefits to the farmers as the expenses associated with purchasing commercial fertilizers are reduced while maintaining or even improving yields in certain cases (Thangarajan et al., 2013). In addition, soil physical, chemical, and biological properties are enhanced by this practice (Haynes et al., 2009; Thangarajan et al., 2013; Sharma et al., 2017, Nicholson et al., 2018).

Considering biosolids as a resource rather than as a waste is of high importance under the current conditions where natural resources are being depleted and energy production is expensive and creates its own GHG footprint (Sharma et al., 2017). Moreover, a growing population challenges the food security supply under the ongoing environmental problems of climate change, loss of biodiversity and endangered aquatic ecosystems (Kahiluoto et al., 2015).

1.2 Effects of land application of biosolids

Several benefits can be obtained from land application of biosolids such as the enhancement of soil physical properties (e.g., improved soil structure, greater water holding capacity, soil porosity, water transport, lower bulk density) (Haynes et al., 2009; Thangarajan et al., 2013; Sharma et al., 2017). Addition of biosolids increases soil organic matter, in particular in soils with low organic matter content (Sharma et al., 2017). The enhancement of soil physical properties occurs because the binding properties of organic matter lead to a better soil aggregation, which in turn increases porosity, infiltration rate and hydraulic conductivity and decreases bulk density. Then, a decrease of the runoff and water erosion might occur (Haynes et al., 2009; Thangarajan et al., 2013; Sharma et al., 2017).

In addition, soil chemical properties such as cation exchange capacity (CEC) increases because of an increase in soil organic carbon. An improved CEC is important for the retention of available nutrients for plants (Thangarajan et al., 2013; Sharma et al., 2017). Soil pH is also affected, depending on the pH of the original soil, the pH buffering capacity of both soil and organic amendment, and the proton dissociation constants (k_d) of various functional groups in organic amendments (Thangarajan et al., 2013). Variations in pH also depend on the presence of calcium or magnesium carbonates in the biosolids, and the acid produced during organic matter decomposition (Sharma et al., 2017).

Soil biology is also enhanced as the organic substrate present in the biosolids enhances the soil microbial biomass, basal respiration, and soil enzyme activities (Haynes et al., 2009; Thangarajan et al., 2013; Sharma et al., 2017). These changes occur mainly during the rapid decomposition of the organic materials in the biosolids following land application (Haynes et al., 2009; Thangarajan et al., 2013). However, heavy metals, pharmaceuticals, or other environmental pollutants potentially contained in biosolids can cause an inhibitory effect on microbial activity, but this typically occurs after the initial phase of decomposition (Haynes et al., 2009; Sharma et al., 2017).

Several environmental concerns arise directly from land application of biosolids, such as greenhouse gas (GHG) emissions: nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) (Wijesekaral et al., 2016); accumulation of heavy metals, organic pollutants, and pathogens (Haynes et al., 2009; Lu et al., 2012; Braguglia et al., 2014; Wijesekaral et al., 2016). Moreover, when biosolids are applied at rates that exceed the crop needs, nutrient (nitrogen and phosphorus) leaching or runoff can occur, leading to water pollution (Lu et al., 2012; Thangarajan et al., 2013; Wijesekaral et al., 2016). Nevertheless, earlier studies have shown that agricultural use of biosolids is safe and beneficial (Cogger et al., 2006; Torri et al., 2016), as long as quality standards established on regulations are met (Cogger et al., 2006; Elliott et al., 2007). Still, the aforementioned concerns have led to a controversial public perception around this practice (Evans et al., 2004; Cogger et al., 2006; Elliott et al., 2007; Pritchard et al., 2010; Wijesekaral et al., 2016). For that reason, the use of synthetic fertilizers is favored and even considered advantageous because of their high solubility (which enhances nutrient plant availability), standard composition, and convenient handling and storage. However, continuous inputs of synthetic fertilizers can acidify the soil and produce nutrient imbalance (Thangarajan et al., 2013; Sharma et al., 2017), causing lower crop yields in the long term.

The N use efficiency of biosolids-amended soils depends on the treatment technology used for their production, as it affects the nutrient content and nutrient availability of the biosolids (Rigby et al., 2016). Therefore, the different biosolids characteristics from different production technologies should be considered when applying biosolids as a nutrient source. The present study evaluated several different types of biosolids with widely contrasting characteristics including mesophilic anaerobic digested (BM), alkaline-stabilized (BA) and composted (BC) biosolids as they are representative of common biosolids treatments (**Table 1-1**).

The mesophilic anaerobic digested biosolid is produced by anaerobic digestion process at mesophilic temperature (i.e., 35 °C) (Haynes et al., 2009; Braguglia et al., 2015). During the anaerobic digestion, the organic matter is decomposed in the absence of oxygen (Wang et al., 2007; CCME, 2012b), generating CH_4, CO_2, and stable organic residues as final products (Wang et al., 2007). The present study evaluated de-watered mesophilic anaerobic digested biosolid produced at the Edmonton Waste Management Centre.

The alkaline-stabilized biosolid is produced by applying the N-Viro® process, which consists on the addition of alkaline material {i.e., cement kiln dust (CKD) or lime kiln dust (LKD)} to dewatered biosolids. The alkaline material increases the temperature up to 52 °C and the pH to 12 or more, killing the pathogens. These conditions of high temperature and pH are maintained for 12 h to 72 h, then, an intense drying cycle of the mixture occurs, resulting in a soil-like material as final product (CCME, 2012b, Price et al., 2015). Walker Industries (facility located in Banff, Alberta) supplied the alkaline stabilized biosolid for the present study.

The composted biosolid is produced under aerobic conditions and thermophilic temperatures (55 °C). The organic components decompose and a stable product is obtained (Wang et al., 2007; Haynes et al., 2009; CCME, 2012b). During the composting process, a bulking agent (i.e., bark chips) is mixed with dewatered biosolids, a high temperature is reached, which decrease the presence of pathogens (Haynes et al., 2009). The composting process can be done in windrows, within-vessel or static aerated piles (Pepper et al., 2006).

The present study evaluated the composted biosolid produced at the Edmonton Waste Management Centre.

1.3 Governmental regulations

Management of biosolids (from production to end use or disposal) is regulated by a multi-faceted regulatory system, which involves both federal and provincial/territorial legislation of Canada. At the national level, the Canadian Environmental Protection Act, 1999, administrated by Environment Canada (EC), establishes that the release of substances listed in the National Pollutant Release Inventory (NPRI) is required to be reported to EC. In addition, the Fertilizers Act and Regulations, administrated by the Canadian Food Inspection Agency (CFIA), regulates the products that are sold or imported into Canada as fertilizers and supplements (CCME, 2010).

At the provincial level, in Alberta, management of biosolids is considered under the Environmental Protection and Enhancement Act; Wastewater and Storm Drainage Regulation; Activities Designation Regulation; Code of Practice for Wastewater Systems using a Wastewater Lagoon. These regulations approach the collection, treatment, and disposal of wastewater, including wastewater sludge treatment and disposal facilities, municipal wastewater collection systems, wastewater systems using wastewater lagoons and land application of biosolids. The main goals of these regulations are to support and promote the protection, enhancement, and wise use of the environment (CCME, 2010).

The government of Alberta also issued *Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Lands* in 2001 (updated in 2009), which is a resource for municipalities considering land application of biosolids coming from domestic wastewater. The guidelines ensure land application of biosolids as beneficial and environmentally acceptable disposal method, by describing the suitability of both biosolids and the site receiving them (Alberta Environment, 2009).

The guidelines establish some criteria for agricultural application of biosolids such as minimum acceptable nitrogen or phosphorus to heavy metal ratios (Cd, Cr, Cu, Hg, Ni, Pb and Zn) (Table 1-2), to prevent application of biosolids with high heavy metal concentrations and lower nitrogen or phosphorus concentrations. Additionally, minimum distances from an environmentally or socially sensitive features must be considered when applying biosolids to land (Table 1-3). A list of acceptable and unacceptable crops is also provided (Table 1-4), and re-application of biosolids should not be done with greater frequency than every three years (Alberta Environment, 2009).

1.4 Greenhouse gas emissions from agriculture

Agriculture practices are associated with the release of 14% of global anthropogenic emissions of greenhouse gases (GHG) such as N_2O, CH_4 and CO_2 (IPCC, 2014). Out of these three GHG, N_2O is of particular concern because of its global potential warming (GWP), 298 times higher than that of CO_2 and its ability to deplete stratospheric ozone (Butterbach-Bahl et al., 2013; Charles et al. 2017); while CH_4 has 34 times higher GWP than CO_2 (IPCC, 2013). The amount of GHG released by soils has been increasing since the beginning of the industrialization because of agricultural activities (Oertel et al., 2016; Smith, 2017), resulting in about 60% of anthropogenic N_2O released by agricultural soils due to nitrogen fertilizer additions (Grant et al., 2006; Hu et al., 2015; Smith, 2017).

Nitrous oxide is mainly released by nitrification (oxidation of NH_4^+ to NO_3^-) via hydroxylamine oxidation or reduction of nitrite (NO_2^-) and denitrification (reduction of NO_3^- to N_2O and N_2). Moreover, addition of labile N can cause the N_2O priming effect, which is the net change in direction and rate of SOM-derived N_2O production (Roman-Perez and Hernandez-Ramirez, 2021) through both nitrification and denitrification (Daly and Hernandez-Ramirez, 2020). Methane is produced by methanogenesis (using acetic acid as electron acceptor or CO_2 reduction), or oxidized to CO_2 via methanotrophy; while CO_2 results from root and soil microbial respiration (Le Mer and Roger, 2001; Hernandez-Ramirez et al., 2009; Ren et al., 2017; Van Zandvoort et al., 2017). Several factors influence the amount of GHG emissions from soils such as soil properties (e.g., water content, temperature, substrate availability, soil pH, and texture), weather (e.g., precipitation and temperature), land use (vegetation type) and management (e.g., fertilizer type, fertilizer rate, tillage system, use of other chemicals) (Snyder et al., 2009; Oertel et al., 2016).

Soil water content is considered an important factor regulating for the release of GHG emissions, as it controls microbial activity and regulates the oxygen availability, affected by gas diffusivity (Butterbach-Bah et al., 2013; Cardoso et al., 2017; Mekala and Nambi, 2017; Schauffler et al., 2010). Earlier studies have commonly reported 60% WFPS as a threshold for N_2O emissions mainly derived from nitrification (< 60% WFPS) versus denitrification (> 60% WFPS), respectively (Bateman and Baggs, 2005; Schaufler et al., 2010; Butterbach-Bah et al., 2013; Zhu-Barker et al., 2015; Oertel et al., 2016; Liu et al., 2017; Mekala and Nambi, 2017); with N_2O from nitrification produced even at a low water content of 20% WFPS (Oertel et al., 2016), while N_2O, from denitrification, is mainly produced under anaerobic conditions (60 to 80% WFPS) (Schauffler et al., 2010; Butterbach-Bah et al., 2013; Oertel et al., 2016; Liu et al., 2017; Mekala and Nambi, 2017). Above 80% WFPS, N_2O might be fully reduced to N_2 (Schauffler et al., 2010; Butterbach-Bah et al., 2013; Liu et al., 2017; Mekala and Nambi, 2017). Production CH_4 requires strictly anaerobic conditions (Liu and Greaver, 2009; Oertel et al., 2016; Ren et al., 2017), while under aerobic conditions, CH_4 is consumed by methanotrophs (Le Mer and Roger, 2001; Oertel et al., 2016). Methanotrophs increase their activity up to water content at field capacity, while their activity decreases at higher water content (Le Mer and Roger, 2001; Arai et al., 2014; Fang et al., 2014). Carbon dioxide is produced at low and moderate soil water content, since O_2 diffusion decreases at higher values of water content, limiting microbial respiration (Jäger et al., 2011).

Other factors influencing N_2O production are nitrogen input and organic carbon availability (Snyder et al., 2009; Oertel et al., 2016). The general effect of nitrogen on N_2O emissions is an overall increase with higher N addition rates because of the increase of N availability (Yang et al., 2003; Jäger et al. 2011; Shcherbak et al., 2014). Availability of organic carbon increases microbial respiration, which reduces the oxygen concentration, promoting the creation of anaerobic microsites, which further lead to N_2O production (Velthof et al., 2003; Jäger et al. 2011).

1.5 Purpose of the study

Most previous studies on biosolids have focused on their effects on soil physical, chemical and biological properties, as well as on crop productivity (Cogger et al., 2001; Christie et al., 2001; Lu et al., 2012; Torri et al., 2017; Nicholson et al., 2018; Kelly et al., 2011). Moreover, studies assessing the release of GHG from cropping systems have prioritized soils receiving synthetic fertilizer or organic amendments such as crop residues and manure. On the other hand, the effects of biosolid additions on N dynamics, nutrient use efficiency, crop productivity, and the amount of GHG emissions released by this practice in North America, and in particular, across the Canadian Prairies remains largely understudied.

Estimations of N_2O from agricultural activities are based on the IPCC Tier 1 emission factor (EF) of 1%, which considers that 1% of total N applied to soils is directly lost as N_2O (IPCC, 2006). However, several studies have indicated that this value could lead to either an overestimation or underestimation of the real N_2O emissions (Grant et al., 2006; Kim et al., 2013; Charles et al., 2017), since N_2O is highly variable and depends on local conditions of weather, soil properties, and land management (Grant et al., 2006; Metivier et al., 2009; Snyder et al., 2009; Oertel et al., 2016). Recently, Rochette et al. (2018) developed a methodology to calculate direct N_2O emissions, and a Tier 2 EF from agricultural soils accounting for regional conditions across Canada. Their Tier 2 EF obtained for the Canadian Prairies was 0.0019 ± 0.00064 kg N_2O–N kg^{-1} N input (equivalent to 0.19%), which is lower than the Tier 1 EF. However, this Tier 2 EF only considered the application of synthetic fertilizer, largely because of the lack of data to inform the contribution of biosolids to direct N_2O emissions. Thus, there is a need to update the Tier 2 EF under Canadian Prairies conditions in order to have a more accurate GHG emissions inventory.

In this context, a field (Chapter 2) and an incubation (Chapter 3) experiments were conducted to address the knowledge gaps regarding land application of biosolids in Central Alberta (i.e., effects on N dynamics, nutrient use efficiency, crop productivity, and GHG emissions). Because of the importance of soil water content on GHG emissions, particularly on N_2O, an incubation study (Chapter 4) was conducted to evaluate the effect of soil moisture on the sources and priming of N_2O in soils receiving synthetic N fertilizer (urea). The objectives of the present study were i) quantify the effect of several contrasting types of biosolids, both surface-applied or incorporated into the soil, on GHG emissions and ii) assess the efficacy of biosolids as a nutrient source for barley (*Hordeum vulgare* L.) for silage both of which are addressed in Chapter 2; iii) to quantify the effect of several contrasting types of biosolids, several soil moisture contents and their interaction on soil N dynamics and N_2O production (Chapter 3); iv) to determine and allocate N_2O sources from SOM and added labile N (urea), and v) to examine and quantify the priming effect of added urea on N_2O production derived from SOM at multiple soil moisture levels (both in Chapter 4). For the field experiment (Chapter 2), we hypothesized that GHG emissions from biosolids-amended soils are higher than from urea-amended soils, and that incorporation of the nutrient source increases the amount of the released GHG. In addition, application of biosolids improves or sustains plant growth relative to synthetic fertilizer. For the incubation experiment (Chapter 3), we expected that different biosolids stabilization treatments, in combination with increasing soil moisture, lead to differences on the N dynamics and the amount of N_2O released once the biosolids are applied in the soil. Finally, for the incubation experiment (Chapter 4), we hypothesized that addition of labile N leads to a positive priming effect on N_2O production, which increases with increasing soil moisture.

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1.7 List of tables

Table 1-1: Biosolids' characterization

Parameter	Mesophilic anaerobic digested	Alkaline stabilized	Composted
C[†] (g C kg^{-1})	293.0	139.3	293.8
N[†] (g N kg^{-1})	36.1	8.63	21.1
C to N ratio	6.5	15.7	13.5
pH[‡]	7.5	12.6	5.1
Conductivity[‡] (dS m^{-1})	5.4	27.5	19.7
Dry matter fraction gravimetric (mass basis)	0.23	0.73	0.68
Moisture (mass basis)	0.77	0.27	0.32

[†] Total carbon (TC) and total nitrogen (TN) were measured by dry combustion.

[‡] Measured in saturated paste 1:2

Table 1-2: Minimum acceptable ratios of nitrogen and phosphorus to metals

	Cd	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
N (organic + NO_3^- + NH_4^+)	1500	20	15	3000	100	20	10
Phosphorus (total)	600	8	6	1100	40	8	4

Alberta Environment, 2009

Table 1-3: Minimum distances required for biosolids land application

Feature	**Minimum distance[†] (m)**	
	Surface application	**Subsurface injection**
Rivers[‡], canals[‡], creeks[‡], intermittent drainage courses, lakes, sloughs, dugouts	30	10
Water wells	20	20
Areas zoned residential or devoted to urban use	500	165
Occupied dwellings	60	20
Public building perimeters	10	3
Public buildings	60	20

Feature	Minimum distance[†] (m)	
	Surface application	Subsurface injection
School yard boundaries (school in session – September to June, inclusive)	200	66
School yard boundaries (school not in session – most of July and August)	20	7
Cemeteries, playgrounds, parks, campgrounds	200	66

[†] Greater separation distances may be required based on local topographic and climatic conditions.

[‡] Distances required are from the major break in slope.

Alberta Environment, 2009

Table 1-4: Acceptable and unacceptable crops

Acceptable crops[†]	Unacceptable crops
Forages[‡]	Root crops
Oil seed crops	Fresh vegetables and fruits
Small grains[§]	Tobacco
Dried peas and beans	Dairy pasture land
Commercial sod	
Trees	

[†] Permission to apply biosolids on lands intended for growth of crops not listed may be given.

[‡] Direct grazing of biosolids treated forage land should be delayed for a minimum of three years following application

[§] Wheat is preferable to barley, and it is better to schedule these crops early in the crop rotation after biosolids application. It is better to schedule oats later in the crop rotation, preferably at least two growing seasons following biosolids application.

Alberta Environment, 2009

2.0 Greenhouse gas emissions, nitrogen dynamics and barley productivity as impacted by biosolids applications

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2.1 Abstract

Land application of biosolids is recognized as a sustainable disposal approach, as it enables the recycling of nutrients that can be used by plants. However, emissions of greenhouse gases (GHG) from such a practice is an environmental concern that needs to be addressed. We evaluated the fluxes of nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2); soil available N; barley (*Hordeum vulgare* L.) biomass productivity; and nitrogen use efficiency (NUE) as a function of the application of three contrasting types of biosolids (mesophilic anaerobic digested [BM], composted [BC], and alkaline-stabilized [BA]) and granular urea in Central Alberta, Canada, over two experimental years. The combination of each biosolid with urea was also evaluated. All N source treatments were assessed with both surface (S) and incorporation (I) placements. Concurrent increases in soil moisture and available N triggered high N_2O emissions during the growing season and spring thaw. Emissions during thawing accounted for 42% of the total annual cumulative. Incorporation of the N source increased N_2O emissions by at least 22% compared with surface-applied N. In general, CO_2 fluxes followed similar patterns to the N_2O fluxes, whereas CH_4 fluxes were minimal. Overall, BMI showed the highest N_2O, CO_2, and CH_4 emissions. On the basis of field fluxes, annual N_2O emission factor (EF_{area}) from urea-amended soils (0.62 ± 0.14%) were fivefold higher than those from soils receiving only BA or BC (0.12 ± 0.04% or 0.12 ± 0.03%, respectively, $P < 0.05$), but EF_{area} from soils amended with only BM (1.33 ± 034%) was more than double the EF_{area} from urea-amended soils ($P > 0.05$). We calculated a partial GHG balance in which field N_2O emissions were the main contributor, accounting for up to 96% of the budget. The GHG footprint of urea manufacturing also made a considerable contribution to the GHG balance (up to 49%), which offset the comparatively low field N_2O emissions from the urea-amended fields, leading to CO_2 equivalents even higher than the BA- and BC-amended fields. Incorporating the N sources enhanced barley biomass by 12% based on the 2-year means. In certain cases, the combination of biosolids and urea (e.g., BMURI, BMURS, BCURS) showed even higher biomass and NUE, as well as lower N_2O emissions. Our findings will help to improve predictions and mitigation strategies for GHG emissions, particularly for N_2O, from agricultural soils receiving biosolids applications.

Keywords: GHG emissions, biosolids, nitrous oxide emission factor

2.2 Introduction

Agricultural practices are associated with the release of 14% of global anthropogenic emissions of greenhouse gases (GHG) such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) (IPCC, 2014). Out of these three gases, N_2O is of major concern because of its high global warming potential (GWP), which is around 300 times the GWP of CO_2, and its depleting effect on the stratospheric ozone layer (Butterbach-Bahl et al., 2013; Wang et al., 2013). The amount of GHG released by agricultural soils has been increasing since the beginning of industrialization (Oertel et al., 2016), with nitrogen fertilizer additions causing about 60% of anthropogenic N_2O emissions (Grant et al., 2006; Smith, 2017; Thilakarathna et al., 2020).

The release of GHG from agricultural systems is generated by underlying soil processes that are part of the N and C cycles. Nitrification (oxidation of ammonium [NH_4^+] to nitrate [NO_3^-]) via hydroxylamine oxidation or reduction of nitrite [NO_2^-], and denitrification (reduction of NO_3^- to N_2O and N_2) are the main microbial processes that cause N_2O emissions (Hernandez-Ramirez et al., 2009; Chai et al., 2020a; Grant et al., 2020). Moreover, the addition of labile N can lead to the N_2O priming effect, which is the net change in the direction and rate of soil organic matter (SOM)-derived N_2O production (Roman-Perez and Hernandez-Ramirez, 2020). Carbon dioxide is a product of root and soil microbial respiration (Van Zandvoort et al., 2017), whereas CH_4 is produced by methanogenesis (e.g., with acetic acid as an electron acceptor or through CO_2 reduction), but CH_4 can also be oxidized to CO_2 via methanotrophy (Le Mer and Roger, 2001; Brachmann et al., 2020; Kim et al., 2021). Several factors influence the amount of GHG emissions from soils such as soil properties (e.g., water content, substrate availability, and texture), weather (precipitation and temperature), and management (fertilizer formulation, rate, and placement, and cropping system) (Snyder et al., 2009; Oertel et al., 2016; Daly and Hernandez Ramirez, 2020).

Biosolids, or treated sewage sludge, are solids, semisolids, or liquid residues that result as a by-product from wastewater treatment plants in urban centers (Wang et al., 2008; Torri et al., 2017). Globally, a large amount of biosolids (1×10^8 Mg) is generated every year. What is more, an increase of 75% (17.5×10^7 Mg yr^{-1}) is expected by 2050, at a production rate of 50 g person^{-1} day^{-1} on a dry basis (Wijesekara et al., 2016). The management of the aforementioned quantities of biosolids in an environmentally sustainable manner is a major challenge (Haynes et al., 2009; Braguglia et al., 2015). Land application of biosolids is considered the most economical and advantageous method, as the nutrients can be recycled by crops; concurrently, the dependence on commercial fertilizers can be reduced (Christie et al., 2001; Brown et al., 2011; Lu et al., 2012), which, in turn, can contribute to decreasing the agricultural GHG footprint, since industrial production of fertilizers has elevated energy costs (Sharma et al., 2017). In other words, applying biosolids to farmlands helps to transfer nutrients concentrated in cities back to the rural landscapes, fostering a circular economy (Yoshida et al., 2015; Torri et al., 2017; Sharma et al., 2017). However, there is a risk of increased GHG emissions, mainly N_2O, from soils receiving biosolids (Pu et al., 2010; Wijesekara et al., 2016). Therefore, best management practices that maximize nutrient recovery according to the crop's needs while minimizing GHG emissions are needed (Pritchard and Rigby, 2010). Site-specific climate conditions, soil characteristics, and biosolids stabilization methods should be taken into account for designing such practices, as the nutrient content and nutrient availability of the biosolids depends on the treatment technology used for their production (Rigby et al., 2016.

There is currently no information available about the N_2O emission factor (EF) of biosolids application to agricultural lands in North America. This knowledge gap is even more evident in Western Canada, where EF of applying organic amendments to croplands is lacking. In fact, the most recent Tier 2 N_2O EF in the Canadian GHG inventory of 0.33% for Black soils in the Canadian Prairies does not consider applications of any type of organic amendment (Rochette et al., 2018). Therefore, there is an urgent need to address such a knowledge gap, which will allow a more accurate GHG emissions inventory at both regional and national scales. Moreover, understanding the soil dynamics after biosolids applications is important for the predictability and development of a comprehensive strategy to assure sustainable management of biosolids under a broad range of weather conditions, soil properties, and biosolids stabilization methods. The N use efficiency of biosolids-amended soils depends on the treatment technology used for their production, as it affects the nutrient content and nutrient availability of the biosolids (Rigby et al., 2016). Therefore, the different biosolids characteristics from different production technologies should be considered when applying biosolids as a nutrient source. The objectives of the present study were (i) to quantify the effect of several contrasting types of biosolids, both surface-applied and incorporated into the soil, on GHG emissions, and (ii) to assess the efficacy of biosolids as a nutrient source for growing barley (*Hordeum vulgare* L.).

2.3 Methods

2.3.1 Site description

The experiment was conducted at the Ellerslie Research Station (53°25' 13"N, 113°33'03"W), located in Central Alberta, Canada. The site has a semiarid continental climate and the Canadian soil classification is Black Chernozem (equivalent to Typic Cryoboroll in the U.S. soil taxonomy). The soil texture is a silty clay loam with a particle size distribution of 327 g kg^{-1} clay, 511 g kg^{-1} silt, and 162 g kg^{-1} sand (hydrometer method). Other initial soil properties (including baseline pH, bulk density, organic carbon, nitrogen, and nitrate concentrations) as well as climate elements (air temperature and precipitation) at each year are shown in **Table 2-1**. A third experimental year was conducted in at the Edmonton Research Station (53°29’57″N, 113°31’51″W), which soil type is also Black Chernozem with a silty clay texture with 425 g kg^{-1} clay, 396 g kg^{-1} silt, and 180 g kg^{-1} sand (laser diffraction method; Beckman Coulter analyzer, LS 13 32) (Appendix A).

2.3.2 Experimental design and management

We used a randomized complete block design with 15 treatments and four replicates. The treatments were: a control receiving no N, three types of biosolids applied alone, urea alone, and three combinations of biosolids with urea (**Table 2-2**). The three types of biosolids applied were mesophilic anaerobic digested (BM), alkaline-stabilized (BA), and composted (BC). Furthermore, all seven treatments that received N were applied both at the soil surface and by incorporation into the soil. The blocks' dimensions were 30 × 8 m, and each treatment plot was 8 × 2 m. Blocks were separated from each other by a 4-m buffer zone. Another 2-m buffer zone was located at both ends of each block. Treatments are coded as the biosolids type (BA, BC, or BM), with UR to indicate a combination with urea, and S and I to indicate surface application and incorporation, respectively.

The TN application rates for each experimental treatment are described in **Table 2-2.** From a perspective of agronomic N rates, we assumed that just 50% of the TN present in the biosolids was available to the barley crops during the growing season. In other words, when applying 192 kg TN ha^{-1} of biosolids, only 96 kg N ha^{-1} was assumed to become released over the growing season and available for plant uptake. And hence, this 96 kg N ha^{-1} derived from the TN in the applied biosolids equates the N added through urea-N (**Table 2-2**). This assumption reflects that most of the N in biosolids is present in organic forms which would need to mineralize to become available N for plant uptake as NH_4^+ or nitrify to NO_3^-. In the case of the biosolids-urea combinations, biosolids were applied a rate of 96 kg TN ha^{-1} (assuming an availability of 48 kg N ha^{-1}) while other 48 kg N ha^{-1} were supplied by the urea, and hence, collectively making up a total N release of 96 kg N ha^{-1}. Thus, all 14 treatments receiving N in our study were considered to experience an N release and availability of 96 kg N ha^{-1}, making them comparable on this basis. All N sources were applied uniformly in the experimental plots on 31 May 2017 and 4 June 2018. On the same dates, the N sources were incorporated in the appropriate treatments with a JD5203 tractor and a Sovema RP/2 200 rotary tiller at a 15 cm depth. Subsequently, barley (*Hordeum vulgare* L.) for silage biomass was seeded at 90 kg seed ha^{-1} on 1 June 2017 and 5 June 2018. Phosphorus as triple superphosphate (0–46–0 N–P–K) was applied at 20 kg P ha^{-1} by placing it near the seeding rows through rear double disc openers.

Additionally, on 5 June 2018, barley for silage was also seeded in the plots of the first experimental year (2017) with the aim of evaluating the effects of residual N (as a legacy from the first year of biosolids application) on barley biomass productivity during a second growing season. No fertilizers were added to these plots.

The experimental design and management for the third experimental year is presented in Appendix A.

2.3.3 Composition of biosolids

The three evaluated biosolids (i.e., BM, BC, and BA) represent a broad range of common biosolids types and their characteristics are widely contrasting (**Table 2-3**). The BM and BC biosolids were collected from the Edmonton Waste Management Centre; BA was collected from the Banff Waterwaste Treatment Facility, both located within Alberta, Canada. Subsamples of each type of fresh biosolids were taken to measure the total carbon (TC) and total N (TN) concentrations by dry combustion and to determine the water content as the gravimetric weight loss (**Table 2-3**). The TN concentration and gravimetric water content were used to calculate the N application rates of fresh biosolids. Several properties of the biosolids in both experimental years are presented in **Table 2-3**. The biosolids' properties for the third experimental year are presented in Appendix A.

2.3.4 Gas sampling and flux calculation

The GHG emissions were measured by the non-steady-state chamber method (Lin et al., 2017; Chai et al., 2020a). Each chamber (64.1cm × 15.6 cm × 15cm) was placed perpendicular to the crop rows, in the middle of each half plot to a depth of 5 cm, after sowing was completed. For the gas sampling, the chambers were covered with a lid and a gas sample was taken through the chamber headspace with a 20-mL syringe inserted into the rubber septum of the lid and immediately transferred into a pre-evacuated 12-mL vial (Exetainer, Labco, UK). Gas sampling was conducted within the time frame of 10:00 AM and 2:00 PM. The frequency of gas sampling was twice a week during the weeks with potential for high fluxes (i.e., shortly after treatment application, high rainfall events, and spring thaw) (Kim et al., 2021). Otherwise, gas samples were taken once per week over the growing season until harvesting, then once every 10 days until the soil froze. Emissions during the frigid winter period were considered to be negligible (Thilakarathna et al., 2020), and hence, gas sampling was not done over the winter periods as the soil remains froze and snowpack is present, while air temperature can often drop below −20 °C (He et al., 2015, Congreves et al., 2018).

Three gas samples were collected from each chamber at 16, 32, and 48 min after chamber enclosure, and six ambient samples were collected at the beginning, in the middle, and at the end of each sampling event. Ambient samples were collected at the chamber height level adjacent to the chamber to represent GHG concentrations at Time 0. The GHG concentrations in the gas samples were analyzed with a gas chromatograph (Varian 3800, Varian Inc., Walnut Creek, CA). The N_2O, CH_4, and CO_2 concentrations were measured with an electron capture detector, a flame ionization detector, and a thermal conductivity detector, respectively (Lin and Hernandez-Ramirez, 2020). The minimum analytical detectable concentrations for this equipment are 10 parts per billion for N_2O, 0.085 parts per million (ppm) for CH_4, and 8.846 ppm for CO_2. Calibrations curves were routinely conducted with at least five standard gases. For N_2O, standard gases concentrations ranged from 0.25 to 9.77 ppm; for CH_4, from 0.83 to 7.99 ppm; and for CO_2, from to 282 to 10,099 ppm. Daily GHG fluxes were calculated by fitting a linear (88% N_2O, 94% CH_4, and 77% CO_2) or quadratic (12% N_2O, 6% CH_4, and 23% CO_2) relationship between each GHG concentration and the sampling time (i.e. the ambient sample or Time 0, 16, 32, and 48 min) (Lin et al., 2017; Kim et al., 2021). The modified ideal gas law was applied to calculate the GHG fluxes as follows:

$$PR = \frac{S \times P \times V}{R \times T \times A} \quad [1]$$

where *PR* is the production rate of the gas (μmol N_2O, mmol CO_2-C, and mmol CH_4-C $\min^{-1}$ m^{-2}), *S* is the slope from simple linear regression or the first-order derivative at Time 0 from the quadratic curve (μL $L^{-1}\min^{-1}$ for N_2O or mL $L^{-1}\min^{-1}$ for CH_4 and CO_2) (Pennock et al., 2010; Yates et al., 2006), *P* is the pressure at chamber headspace (atm), *V* is the volume of the chamber headspace (L), *R* is the universal gas constant (atm μL K^{-1} μmol^{-1}), *T* is the temperature in the chamber headspace (K), and *A* is the chamber area (m^2).

Cumulative fluxes were calculated by the area below the curve formed by consecutive daily fluxes. Cumulative fluxes were analyzed by period as follows: growing season (GS) (from field treatment application in early June to harvesting), post-harvest (from harvesting until soil freezing), and early spring (ES) (from spring thaw to late May). The annual cumulative fluxes are the sum of these three periods. As noted above, fluxes during the winter months were assumed to be negligible (Thilakarathna et al., 2020) as soil microbial activity and gas transport become minimal under cold air temperature (Lin et al., 2017).

The annual N_2O area-based EF (EF_{area}) was calculated as the difference between the annual cumulative N_2O of an N treatment and the control, divided by the N application rate (kg N ha^{-1}) (Thilakarathna et al., 2020) as follows:

$$EF_{area} = \frac{(N_2O_{treatment-}\ N_2O_{control})x100}{N\ rate} \quad [2]$$

An advantage of estimating EF_{area} is that this approach enables comparisons across treatments that even received different total N rates (e.g., urea alone with total N rate of 96 kg ha^{-1} vs. a biosolid alone with total N rate of 192 kg ha^{-1}; **Table 2-2**). This is because the N addition rate is accounted for in this Eq. [2] as the denominator (Lin et al., 2017; Chai et al., 2020a).

Partial GHG budgets were estimated, including the cumulative N_2O and CH_4 emissions measured in the fields, the fuel consumption of the field operations, the GHG footprint of the biosolids production, and the GHG footprint of urea manufacturing, expressed as kg CO_2-equivalent (CO_{2eq}) ha^{-1} yr^{-1}. The addition of these five contributors to the GHG footprint was considered to be the area-related CO_{2eq}. It is noted that plant C and CO_2 fluxes were not accounted for, partly because the plant canopy was growing inside the chamber in July and August. Therefore, these GHG budgets are considered partial, although soil C storage was probably operating at equilibrium in these fields (i.e., Σ inputs = Σ outputs on annual basis).

The yield-related CO_{2eq} (kg kg^{-1} dry mass [DM)]) was also calculated by dividing the area-related CO_{2eq} by the biomass produced by each treatment (Glab and Sowiński, 2019; Chai et al., 2020a).

2.3.5 Soil sampling and analyses of inorganic nitrogen concentrations

Composite soil samples (*n* = 3 cores) were collected from every experimental plot multiple times within both experimental years for analyses of NH_4^+–N and NO_3^-–N concentrations. Two sampling depth increments were considered in this repeated sampling scheme; 0–7.5 cm and 7.5–15 cm. These soil samples were taken 2 weeks, 1 month and 2 months after the field treatment application. Two additional soil samplings were conducted, one before soil freezing (late October) and one after the transition from winter to spring (April or May). Furthermore, deep soil samples were taken as a baseline prior to treatment application (four cores per block replicate) and after barley harvesting (to evaluate any residual N concentrations in the soil profile) at four different depth increments (0–15 cm, 15–30 cm, 30–60 cm, and 60–90 cm). After each soil sampling and before laboratory analysis, soil samples were air-dried and ground. Concentrations of NH_4^+–N and NO_3^-–N were measured by 30-min extraction with 2 M KCl in a proportion of 1:5 in a reciprocal shaker. The colorimetric method, including vanadium chloride reduction, was applied to the extract by a Thermo Gallery Plus Beermaster Autoanalyzer.

2.3.6 Plant biomass sampling and N use calculations

Aboveground biomass barley was harvested at the dough stage (from an area of 3.1 m^2, which represents 19% of the total plot area) on 17 August 2017 and 15 August 2018. The barley harvest during the second growing season in the first year (2017) was done on 16 August 2018. To determine the N concentration in the barley biomass, a composite subsample from each plot was oven-dried at 60°C and analyzed via near-infrared spectroscopy (NIRS, FOSSDS2500). The NIRS calibration protocol was established with N content data in barley biomass that had been independently measured via dry combustion. We calculated the plant N uptake (kg N ha^{-1}), N use efficiency (NUE; kg DM kg^{-1} N), and uptake efficiency (UE; kg plant N kg^{-1} N) as follows:

$$N\ uptake = DM \times \%protein \times 0.16\ [3]$$

$$NUE = \frac{DM_{treatment} - DM_{control}}{N\ rate} \quad [4]$$

$$UE = \frac{N\ uptake_{treatment} - N\ uptake_{control}}{N\ rate} \quad [5]$$

where *DM* is the aboveground dry matter biomass (kg DM ha^{-1}) in each treatment and the control.

In addition, the environmental footprint at the field scale was calculated as the yield-based EF (EF_{yield}, kg N_2O–N kg^{-1} DM) by dividing the annual cumulative N_2O emissions (kg N_2O–N ha^{-1}) by the aboveground biomass (kg DM ha^{-1}) (Chai et al., 2020a).

2.3.7 Ancillary measurements

Soil moisture and soil temperature were measured at depths of 5 and 10 cm with 5TM sensors interfaced with EM50 data loggers (Decagon Devices, Inc., Pullman, WA). Soil moisture data were only presented in this study when the soil temperature was greater or equal to 0 °C. Air temperature and precipitation data for the Ellerslie and Edmonton sites were obtained from permanent weather stations located at the Edmonton International Airport and Edmonton South Campus, respectively.

As a measurement of canopy greenness, the normalized difference vegetation index (NDVI) was quantified so we could have an indicator of the N content in the crop. The NDVI was measured weekly as the crop canopy developed from June to middle August during the second experimental year. The NDVI data were obtained by positioning the active greenseeker sensor (HCS-100 GreenSeeker, Trimble Inc., Sunnyvale, CA, USA) 0.6 m above the canopy. Nine readings within each experimental plot were averaged to obtain a representative NDVI data.

2.3.8 Statistical analyses

All statistical analyses were performed with R Studio software version 1.1 3.8.3 (RStudio Inc.) at an alpha critical value of 0.05. The effects of N source, placement, and their interaction on cumulative GHG emissions, available nitrogen, plant biomass, N uptake, NUE, UE, EF_{area}, EF_{yield}, area-related CO_{2eq}, and yield-related CO_{2eq} were analyzed with a random effect model, with the block as the random effect. Normality and homoscedasticity assumptions were checked by the Shapiro-Wilk and Barlett tests. Data were Box-Cox transformed when needed to meet those assumptions. Following significant ANOVAs, Tukey's honest significant difference test was performed for treatment comparisons.

2.4 Results

2.4.1 Weather conditions

The long-term normal (1987–2016) indicates an average annual temperature of 3.3 ˚C and total annual precipitation of 447 mm. During the two experimental years, the average annual temperature and the total annual precipitation were below the normal values, 3.0 ˚C and 398 mm in 2017, 2.2 ˚C and 378 mm in 2018 (**Table 2-1**).

2.4.2 Peak N_2O fluxes

Over the two experimental years, pulses of N_2O fluxes typically occurred at three specific timings (**Fig. 2-1D, Fig. 2-1E, Fig. 2-2D, Fig. 2-2E**) as described below:

(i) The first pulse was observed within 2 weeks and up to 1 month following the treatment application. Treatments with BM and BA (alone or combined with urea) showed the highest pulses. The highest emitters were BMI (72.1 g N_2O–N ha^{-1} day^{-1}) and BMURI (75.2 g N_2O–N ha^{-1} day^{-1}) in 2017, and BAS (19.7 g N_2O–N ha^{-1} day^{-1}) and BAI (24.3 g N_2O–N ha^{-1} day^{-1}) in 2018. The N_2O pulses in 2018 were three times lower than those in 2017, most probably because less soil NH_4^+ and NO_3^- became available in 2018 (**Fig. 2-1B, Fig. 2-1C, Fig. 2-2B, and Fig. 2-2C**). Soil N availability (**Fig 2-1B, Fig. 2-1C, Fig. 2-2B, Fig. 2-2C**) and rainfall events (**Fig. 2-1A, Fig. 2-2A)** provided favorable conditions for these N_2O pulses. For instance, 21 mm rainfall occurred from 9 to 14 June 2017 (**Fig. 2-1A**), and 36 mm on 10 and 11 June 2018 (**Fig. 2-2A**).

ii) The second pulse of N_2O fluxes was generally observed in July and August, and it was mainly related to abundant and continuous rainfall (85 mm in 2017 and 39 mm in 2018).

iii) Lastly, elevated soil moisture (>70% water filled pore space [WFPS]) and the high availability of residual soil N generated a third episode of high N_2O efflux during the spring thaws, with peak emissions on 20 April 2018 and 28 March 2019. Spring thaw N_2O pulses rose up to a maximum of 109.2 g N_2O–N ha^{-1} day^{-1} (BMS) in 2018 (**Fig. 2-1D**) and 68.6 g N_2O–N ha^{-1} day^{-1} (BMURS) in 2019 (**Fig. 2-2D**). Within the first experimental year, it was noticeable, that in general, incorporation treatments led to higher N_2O emissions over the growing season, whereas during the spring thaw, the surface-applied (and the control) treatments showed higher daily fluxes. Conversely, during the following experimental year, incorporation led to higher N_2O emissions over the early spring periods, as well as during the preceding growing seasons. Nevertheless, over the entire study, most of the surface-applied N treatments (and the unamended control) showed their highest daily N_2O production during the spring thaw.

2.4.3 Area-based EF (EF_{area}), seasonal and annual cumulative N_2O fluxes

The highest EF_{area} within each experimental year was measured in the BMI treatment with values of 1.90% in 2017 and 2.02% in 2018 (**Table 2-5**), as BMI showed the highest cumulative N_2O emissions (**Table 2-4**). During both experimental years, the EF_{area} showed positive values, with the exception of BCURS (−0.002% in 2018–2019), for which the annual cumulative N_2O emissions were lower than those of the control (**Table 2-4**). We found a significant effect of the N source during both experimental years, with BM- and BMUR-amended soils having generally greater EF_{area} than BA-, BC-, BAUR-, BCUR-, and urea-amended soils, irrespective of placement (**Table 2-5**). A significant interaction effect of N placement × N source was only found in 2018, when the EF_{area} for BMI was six times higher than that for BMS (**Table 2-5**).

Significant interaction effects between N source and N placement on N_2O emissions occurred during the post-harvest in 2018, when BCS and BCURS had lower emissions than URI, BMI, and BMURI by 6, 15, and 10 times, respectively. Nitrogen source had a significant effect on the N_2O fluxes consistently at every period during both experimental years. For instance, BMI was 30 times significantly higher than the control during GS 2018 and 69 times higher than BAI after harvest 2017 (**Table 2-4**). Placement of N sources also affected cumulative N_2O emissions, with significant incorporation-induced effects on the GS 2018 and annual cumulative 2018. In GS 2018, incorporation treatments had three times higher emissions than the surface-applied treatments (**Table 2-4**). Similarly, in 2018, N incorporation led to twice the annual cumulative N_2O emissions of the surface-applied treatments (**Table 2-4**). The application of urea in combination with biosolids resulted in numerically lower N_2O emissions up to 54% in the BM-amended soils at both placements, in both experimental years, whereas in 2018, the BA-amended soils showed a reduction between 11 and 15% (at surface and incorporated placement, respectively), and in the BC-amended soils a reduction of 25% was observed in the surface-applied treatments. (**Table 2-4**).

2.4.4 Soil nitrate and ammonium concentrations

Soil available N (NH_4^+–N plus NO_3^-–N) was high shortly after N addition and subsequently showed a sharp decrease over the GS at each soil depth increment within each experimental year (**Fig. 2-1B, Fig. 2-1C, Fig. 2-2B, Fig. 2-2C**). We found that after harvesting, the available N concentrations were similar and below 20 mg N kg^{-1} in both experimental years at 0–15 cm depth. The concentrations of available N measured after biomass harvest remained quite similar to those measured during the early spring. On average, at the depth of 0–7.5 cm, the residual available N carried over to the following spring represented 19% and 36% of the initial concentration (i.e., 2 weeks after the treatment application) in ES 2018 (13.7 mg N kg^{-1}) and ES 2019 (11.2 mg N kg^{-1}), respectively. At the depth of 7.5–15 cm, higher percentages of available N were retained in the soil, with 52% in 2018 and 39% in 2019, which accounted for 12.8 and 13.5 mg N kg^{-1}, respectively. The residual available N provided a substrate for N_2O production during the spring thaw, when high N_2O pulses were observed (**Fig. 2-1D, Fig. 2-1E, Fig. 2-2D, Fig. 2-2E**).

When we averaged each type of N source (i.e., urea, BM, BA, BC, BMUR, BAUR, and BCUR) within the first month of the treatment application, the available N from the urea-amended soils was generally higher (29.6 – 90 mg N kg^{-1}) than the biosolids-amended treatments at both depths. Significant differences were found only for BA- and urea-amended soils at the depth of 0–7.5 cm in 2017 (24.0 vs. 79.1 mg N kg^{-1}, respectively) and for all three types of biosolids at both depths in 2018, when the available N concentration in the urea-amended soils was 50% more than in the biosolids-amended treatments.

At both soil depth increments (0–7.5 and 7.5–15 cm) in 2017 and 2018, more available N was measured for the combined biosolids-urea treatments for each type of biosolid, within the first month following field N additions. Significant differences were found in 2018, when BAUR and BCUR nearly doubled the available N concentration of BA- and BC-amended soils (at the 0–7.5 cm depth), respectively.

2.4.5 Aboveground biomass productivity and EF_{yield}

During both experimental years, the amount of aboveground biomass was affected by the N source only (**Table 2-6**). The biosolids-only treatments had lower biomass than the urea-amended soils for both types of N placement. Exceptions to this pattern were observed for BAS and BCS in 2018, which were numerically higher than URS only ($P >$ 0.05). Significant differences were only observed between URI and BAI in 2017, as the biomass from URI (7282 kg DM ha^{-1}) was more than double that from BAI (3148 kg DM ha^{-1}), and between the control (4535 kg DM ha^{-1}) and BCURI (7915 kg DM ha^{-1}) in 2018 (**Table 2-6**).

Combining biosolids with urea improved the biomass production of all three types of biosolids. The exception was the surface-applied BA, as its alkaline composition and surface placement are conducive to increased ammonia volatilization. However, the amount of biomass was still numerically lower than that of the urea-only treatments, with the exception of BCURS (2017), for which production was higher than URS by 33 kg DM ha^{-1} ($P > 0.05$; **Table 2-6**). Some exceptions were also observed in 2018, when the biomass for BMURS and BCURS were numerically higher than for URS (5096 kg DM ha^{-1}) by 3 and 24%, respectively ($P > 0.05$), whereas BCURI had 9% higher biomass than URI (7915 kg DM ha^{-1}) ($P > 0.05$; **Table 2-6**).

Similar to biomass, the EF_{yield} was significantly affected only by the N source over the two experimental years. At each N placement, treatments with BM alone showed the highest EF_{yield} during both experimental years, with the highest value for BMI in 2017 (0.97 g N_2O–N kg^{-1} DM) and 2018 (0.73 g N_2O–N kg^{-1} DM). This was a consequence of the higher N_2O emissions from BM-amended soils (**Table 2-4**) and relatively low biomass production (**Table 2-6**) in both years. Significant differences were observed in 2017 when the EF_{yield} of BMI was 15 times higher than that of URI, whereas in 2018, the EF_{yield} of BMS was four times higher than that of BCURS ($P < 0.05$), and BMI was significantly higher than BAI (by fivefold), BCI (by sixfold), BAURI (by eightfold), and BCURI (by sixfold) (**Table 2-5**). Contrary to 2017, in 2018 the urea-only treatments showed higher EF_{yield} as a consequence of greater N_2O emissions (one order of magnitude higher than in 2017) (**Table 2-4**).

In general, treatments with BA (alone or combined with urea) showed slightly higher EF_{yield} than the BC treatments (alone or combined with urea), even they had lower N_2O emissions, although these differences were not statistically significant. Higher yields from the BC treatments contributed to lowering their EF_{yield}. Nevertheless, no significant differences were observed among these treatments (**Table 2-5**).

2.4.6 Nitrogen uptake, nitrogen use efficiency (NUE), and uptake efficiency (UE)

In general, the barley N uptake varied in accordance with the barley biomass in both experimental years. Higher N uptake was observed for the urea-only treatments than for the biosolids-only treatments, with the exception of BMI vs. URI in 2017 ($P > 0.05$)

(**Table 2-6**), as the barley tissue N content in BMI was 40% more than that in URI (data not shown.

The N source significantly influenced the NUE and UE during both years. As observed for biomass and N uptake, the urea-only treatments had higher NUE and UE than the rest of the treatments at both placements (**Table 2-6**). When compared across biosolids-amended soils, overall, treatments with BC showed the greatest NUE and treatments with BA showed the lowest (alone or combined with urea) over both experimental years. The BM-amended soils (alone or combined with urea) had the greatest UE in 2017 only, whereas in 2018, BC-amended soils had the highest UE, except for BMI (2018). In the same way as NUE, most of the BA-amended soils had the lowest UE over both experimental years.

2.4.7 Fluxes of CH_4 and CO_2

Most daily CH_4 fluxes were negligible and below 10 g CH_4–C ha^{-1} day^{-1} in 2017 and below 15 g CH_4–C ha^{-1} day^{-1} in 2018. Nevertheless, larger fluxes were observed for treatments with BM (alone or combined with urea) shortly after field application. For instance, in 2017, a flux of 44.4 g CH_4–C ha^{-1} day^{-1} was measured in BMI (**Fig. 2-4A**), and in 2018 the same treatment emitted 78.6 g CH_4–C ha^{-1} day^{-1}, whereas BMURI emitted 35.4 g CH_4–C ha^{-1} day^{-1} (**Fig. 2-4B**).

Annual cumulative CH_4 fluxes were positive during both experimental years, in which BMI had the highest fluxes with 265 (in 2017) and 405 g CH_4–C ha^{-1} (in 2018), whereas URI showed the lowest fluxes, with 11 (in 2017) and 63 g CH_4–C ha^{-1} (in 2018). On average, surface-applied N treatments led to higher CH_4 emissions during both experimental years, but a significant effect was observed only in 2017. However, in the case of the BM-amended soils, BMI showed larger fluxes than BMS in 2017 (three times higher; $P < 0.05$) and in 2018 (33% more).

Daily CO_2 fluxes showed similar temporal patterns to N_2O fluxes. In general, over the study period, early peaks were observed at the same time that the N_2O peaks occurred, shortly after treatment application and during the spring thaw (**Fig. 2-5**). The cumulative CO_2 flux (partial) profoundly differed as a function of the N source in 2017, when BMI and BCURI had significantly higher fluxes than the control by more than twofold (1.094 and 1.048 vs. 0.477 Mg CO_2–C ha^{-1} day^{-1}, respectively; **Table 2-7**).

2.4.8 Partial GHG footprint budget

The largest contribution to the area-related CO_{2eq} came from the field N_2O emissions. Field N_2O emissions accounted for up to 96% of the area-related CO_{2eq} in the specific case of BMI, which also showed the highest area-related CO_{2eq} during both experimental years (**Table 2-8**). It was noteworthy that the GHG footprint of urea manufacturing (457 CO_{2eq} kg ha^{-1} yr^{-1}) represented more than a half of the area-related CO_{2eq} in 2017, and more than one-third of that in 2018 for the urea-only treatments. The GHG contributions of both biosolids production and their transport to the research sites were minimal (< 8% of the GHG footprint budget), whereas the contribution of the urea manufacturing to the GHG balance clearly offset the differences in N_2O–CO_{2eq} from the urea vs. the biosolids-only treatments. For instance, in 2017, the area-related CO_{2eq} of URS and URI were more than double the area-related CO_{2eq} of the BA- and BC-amended soils for both the surface and incorporated treatments (except that URI was only 66% higher than BCI in 2017), whereas in 2018, the area-related CO_{2eq} of URI was even more than three times than that of the BAI and BCI. However, significant differences were only observed in 2018 between URS and BCS as well as between URI and both BAI and BCI (**Table 2-8**). Methane's contribution to the area-related CO_{2eq} was minimal (below 5%) within each year (**Table 2-8**). Fossil fuel consumption from field operations neither represented a difference across N management options nor contributed a large proportion to the GHG balance (**Table 2-8**).

The yield-related CO_{2eq} showed a similar pattern to the area-related CO_{2eq} between the urea-only and biosolids-only treatments (BA and BC), as long as the biomass produced for those treatments was similar. For example, in 2017, the yield-related CO_{2eq} of URS was more than double that of BAS and BCS, whereas URI was only 11 and 27% higher than BAI and BCI, respectively, since their biomasses were much lower than that of URI (**Table 2-6**). In 2018, the yield-related CO_{2eq} from the urea-only treatments was more than twice and three times higher than that of the BA- ($P > 0.05$) and BC-amended soils ($P < 0.05$) at each N placement (**Table 2-8**). The larger differences in the yield-related CO_{2eq} in 2018 than in 2017 among these treatments were because the area-related CO_{2eq} from the urea-only treatments was higher in 2018 (14% more for URS and more than double for URI).

The results for the third experimental year are presented in Appendix A.

2.5 Discussion

2.5.1 Effects of type of N source on EF_{area}

This study contributes, for the first time in the literature, a multiyear dataset of annual N_2O EF_{area} values after the application of biosolids to agricultural soils in North America. The development of a country-specific N_2O EF for Canada was approached by Rochette et al. (2008), who determined an EF of 0.8% for the Black soil zones in the Prairies. This EF_{area} was updated to 0.33% by Rochette et al. (2018). Both of these EFs were calculated for synthetic N additions only, meaning that the application of biosolids, or any other organic amendment, was not considered. This was largely because of the lack of data at that time to inform the contribution of biosolids to direct N_2O emissions. Nevertheless, inputs of organic amendments derived from pig slurry and paper sludge were included in their study for Eastern Canada only and the EF was 1.2% (Rochette et al., 2018).

In our study, the overall 2-year mean N_2O EF_{area} for the BM-only treatments was 1.33 ± 0.34%, which was 11 times higher the EF_{area} of BA- and BC-amended soils (0.12 ± 0.04 and 0.12 ± 0.03%, respectively, $P < 0.05$). Likely, Charles et al. (2017) performed a meta-analysis for global EF of soils receiving organic amendments, which were classified as high, medium, and low risks according to the organic amendments' properties (i.e., water content and C:N ratio). They found higher EF from high-risk organic amendments (higher water content and low C:N ratio) (1.21 ± 0.13%) than from medium-risk and low-risk (0.35 ± 0.13% and 0.02 ± 0.13%, respectively), as those organic amendments had lower moisture content and wider C:N ratio. Nevertheless, their global EF for organic amendments was 0.57 ± 0.3%, which is similar to the 2-year mean of EF_{area} from biosolids-only treatments in our study (0.52 ± 0.16% ranging from 0.07% for BAI to 1.96% for BMI) (**Table 2-5**). These EF_{area} values are similar to those found by Lin et al. (2017), who reported annual EFs ranging from 0.10 to 2.4% for manure-amended soils in Central Alberta. Charles et al. (2017) also reported an EF of 1.76 ± 0.42% for soils receiving synthetic fertilizers. These results are in agreement with ours as EF from urea-amended soils (0.62 ± 0.14%) were five times higher than that of BA- and BC-amended soils ($P < 0.05$).

In the case of the EF_{area} of BM-only treatments, it was more than twofold the EF_{area} from urea-amended soils (1.33 ± 0.34% versus 0.62 ± 0.14%). Similarly, Jones et al. (2007) found higher EF from soils receiving sewage sludge at a rate of 3066 TN ha^{-1} (2.8%) than from urea-amended soils applied at 300 kg N ha^{-1} (0.25%), respectively. Moreover, in a long-term modelling study, Bruun et al. (2016) also found higher EFs from soils receiving mesophilic anaerobic digested biosolids (up to 2.9%) than from ammonium nitrate-amended soils (up to 1.9%), when applied at rates increasing by 30 kg mineral N ha^{-1} from 0 to 330 kg mineral N ha^{-1}. With regards to the overall 2-year mean annual EF_{area} for the urea-only treatments, (0.62 ± 0.14%), it was lower than the Tier 1 EF of 1% (IPCC, 2006), but 88% higher than the regional value reported by Rochette et al. (2018), and twice the value reported by Thilakarathna et al. (2020) after applying a wide range of synthetic N fertilizer formulations (e.g., urea or anhydrous ammonia) at a rate of 100 kg N ha^{-1} to soils cropped to wheat (*Triticum aestivum* L.) in locations in Central Alberta. Overall, these results point to the need for a country-specific EF or even a region-specific EF, as the N_2O emissions are strongly affected not only by the N rate application but also by the soil properties (e.g., texture, pH), weather (e.g. precipitation, air temperature), the cropping system (e.g. annual vs. perennial) (Rochette et al., 2018), and the characteristics of the N source applied (organic, synthetic, or a combination) (Charles et al., 2017). Thus, the use of the Tier 1 EF might overestimate the field N_2O emissions from synthetic fertilizer. In the case of biosolids additions, the field N_2O emissions might be overestimated or underestimated by Tier 1 EF, depending on the type of biosolid applied.

2.5.2 Linkages of soil available N and moisture with N_2O emissions

Fluctuations of N_2O were driven by the co-occurrence of available N and rainfall events. The increase of available N (NO_3^- + NH_4^+) after N additions was evident within the first month of the treatment application (**Fig. 2-1B, Fig. 2-1C, Fig. 2-2B, Fig. 2-2C**). This points out that N mineralization actively occurred in all the treatments that received N additions, whereas overall the control, showed the same available N concentration across each experimental year. As more of the N applied with biosolids is in an organic form, it undergoes decomposition, mineralization, and ammonification, producing NH_4^+ (Rigby et al., 2009; Díaz-Rojas et al., 2014; Alvarez-Campos and Evanylo, 2019). In the case of the urea-amended soils, NH_4^+ is rapidly produced via urea hydrolysis (Di and Cameron, 2008). The released NH_4^+ can be further nitrified to NO_3^-, which serves as a substrate for denitrification, as noted above. Nitrification and denitrification are the main processes that release N_2O in soils (Hernandez-Ramirez et al., 2009; Zhu et al., 2013; Thangarajan et al, 2013; Hu et al., 2015).

The occurrence of rainfall events further fostered the N_2O pulses during this period. Several studies have reported increased N_2O emissions after major rainfall events shortly following the application of organic amendments (Jones et al., 2007; Pelster et al., 2012; De Rosa et al., 2016; Willén et al., 2016, Lin et al., 2017) or synthetic N fertilizer (Linzmeier et al., 2001; Pelster et al., 2012; Chai et al., 2020a; Thilakarathna et al., 2020). Rainfall increases the soil moisture and reduces aeration, which governs N_2O emissions by regulating gas diffusivity and oxygen availability for microbes (Schaufler et al., 2010; Butterbach-Bah et al., 2013; Brenzinger et al., 2018; Grant et al., 2020). In our study, recurrent rainfall events increased the soil moisture to between 60 and 73% WFPS (**Fig. 2-1A, Fig. 2-2A**). Such a high soil moisture probably favored the occurrence of denitrification, as 60% WFPS has been commonly reported as a threshold for N_2O emissions from denitrification (>60% WFPS) (Bateman and Baggs, 2005; Schaufler et al., 2010; Butterbach-Bah et al., 2013), as soil conditions gradually become anoxic at this WFPS, leading to a decrease in aerobic microbial activity (nitrification and respiration) (Linn and Doran, 1984; Grant et al., 2020; Lin and Hernandez-Ramirez, 2020). Nevertheless, when soil moisture was below 60%, N_2O fluxes coming from nitrification might have occurred (Bateman and Baggs, 2005; Schaufler et al., 2010; Butterbach-Bah et al., 2013), as nitrification increases at a WFPS between 30 and 60% (Linn and Doran, 1984; Roman-Perez and Hernandez-Ramirez, 2020). Moreover, Roman-Perez and Hernandez-Ramirez (2021) found faster nitrification rates with increasing soil moisture from 28 to 64% WFPS, which was further associated with higher N_2O production rates. The N_2O pulses during the spring thaw (**Fig. 2-1D, Fig. 2-1E, Fig. 2-2D, Fig. 2-2E**) also occurred as a consequence of higher soil moisture (between 73 and 79% WFPS) and residual available N near the soil surface from the previous growing season (**Fig. 2-1B, Fig. 2-1C, Fig. 2-2B, Fig. 2-2C, Fig. 2-6**). The higher soil moisture and available N, added to increasing temperatures might have favored the occurrence of denitrification, even at low temperatures (between 0 and 6 °C). Similarly, Nyborg et al. (1997) reported that higher spring thaw N_2O emissions from denitrification occurred when NO_3^- was present while the soil surface thawed, becoming water saturated, with temperatures that ranged from 2 to 10 °C in a Black Chernozem soil in Alberta, Canada, similar to our three study sites.

Another factor that might have exacerbated N_2O fluxes during both experimental years could have been the occurrence of the N_2O priming effect. In an incubation study of a Black Chernozem soil from Alberta, Roman-Perez and Hernandez-Ramirez (2020) showed that 59% of the total N_2O emissions were SOM-derived, with a 19% corresponding to primed N_2O. In their study, the primed N_2O resulted from a dynamic turnover of SOM that produced NH_4^+–N, which acted as a substrate for nitrification. The occurrence of this nitrification probably increased the availability of hydroxylamine and NO_2^-–N, which are substrates for primed N_2O production.

2.5.3 Effects of N placement on N_2O emissions

Incorporation of the N source led to greater emissions than surface application, as mixing N with the soil facilitates increased nutrient availability for microbial activity (Rezaei Rashti et al. 2017), via the aforementioned processes. The creation of anoxic microsites after the application of organic amendments was shown by Zhu et al. (2015). In their experiment, they found rapid oxygen consumption throughout the soil when manure was mixed homogeneously in the soil, reaching anoxic conditions (< 0.14 mg O_2 L^{-1}) after 6 h from the start of their experiment. In addition, incorporation can reduce N losses via ammonia volatilization (Abalos et al., 2016; Willén et al., 2016), which keeps more N available in the soil. In an incubation study of soils receiving a pig manure application by Velthof et al. (2003), they found 44% more N_2O emissions when the manure was incorporated into the soil than when placed on the soil surface. Moreover, in field trials, Wulf et al. (2002) and Thomsen et al. (2010) found up to three and five times higher N_2O emissions after slurry injection than with surface application, respectively.

2.5.4 Effects of N source on N_2O emissions

When comparing the effect of the different biosolids on their ability to promote N_2O emissions from the soil, we found that soils receiving either composted biosolids (BC) or alkaline-stabilized biosolids (BA) (alone or combined with urea) had one order of magnitude lower N_2O emissions than BM-amended soils over both experimental years. However, significant differences were only found in the incorporated treatments in 2018, when BMI had six times higher N_2O emissions than BAI and BCI, whereas BMURI was five times higher BAURI. These could be explained since BM had the highest total N content (36.1 g N kg^{-1}), and since digestion allows the mineralization of the organic N present in the sludge, probably more NH_4^+–N was provided with BM (Rigby et al., 2016). This available N might have been rapidly nitrified to NO_3^-–N, which further was reduced to N_2O. The reduction to N_2O was likely favored by the creation of more anaerobic microsites in the soil due to the limited oxygen diffusion caused by the high water content of BM (77%, **Table 2-3**) and the persistence of pre-existing anaerobiosis within the added BM lumps, as BM had been produced under anaerobic digestion (Yoshida et al., 2015).

The lower N_2O emissions from BA- and BC-amended soils (alone or combined with urea) are partly explained by their lower TN content (7.95 and 21.0 g N kg^{-1}, **Table 2-3**). The low N content in BA is because of the N loss via ammonia volatilization during its production (Yoshida et al., 2015) Moreover, soils receiving BA most probably increased their pH because of the high pH of BA (>12), which in turn might have decreased N_2O production. This is because complete denitrification (N_2 production) is favored at a higher soil pH (>7) by enhancing the transcription of the gene *nos*Z and post-transcriptional assembly of the corresponding periplasmic enzyme N_2O reductase (Bakken et al., 2012; Shaaban et al., 2018). In the case of BC, its lower TN concentration is because of the addition woody bulking agents during the composting process (Rigby et al., 2016). In addition, composting results in a stabilized product with more recalcitrant organic N, and thus the mineralizable N pool might have been smaller than for other biosolids stabilization methods (Rigby et al., 2016). However, more available N as NH_4^+−N and NO_3^-−N, which are substrates for nitrification and denitrification, was provided by the composted biosolids (i.e., BC, **Fig. 2-1B, Fig. 2-1C**) in 2017, whereas in 2018, the available N across biosolids-amended soils were similar (**Fig. 2-2B, Fig. 2-2C)**. Then a faster mineralization and nitrification rates might have occurred in the BC-amended soils, which led to a release of more available N at the beginning of the experiment, in 2017. It is interesting that despite the higher available N in BC-amended soils (alone or combined with urea), less N_2O was released. This indicates that the released available N might have been mainly uptaken by the crops, as shown by the better aboveground biomass as well as higher NUE in BC-amended soils (**Table 2-6**).

The C:N stoichiometric ratio in the biosolids is another factor that influences the N_2O emissions, as it affects the mineralization rate of the added amendment, with narrower C:N ratios leading to faster mineralization than wider C:N ratios (Jones et al., 2007). The C:N ratio of BA (15.7) and BC (13.5) more than doubled that of BM (6.5) (**Table 2-3**); therefore, microbial decomposition and mineralization of BM should have released more available N than both BA and BC through the growing season. However, as mentioned above, more available N was measured in the BC-amended soils, mainly in 2017. Thus, the higher N_2O emissions from BM-amended soils (alone or combined with urea) most probably were promoted by the higher water content in BM than in BC and BA. Similarly, Jones et al. (2007) found N_2O fluxes of up to 3490 g N ha^{-1} day^{-1} for sewage sludge and 2200 g N ha^{-1} day^{-1} for poultry manure, which had C:N ratios of 5.6 and 6.8, respectively. In addition, De Rosa et al. (2016) found higher cumulative N_2O emissions from their manure-amended treatments (1636 g N_2O ha^{-1}) than from their composted-amended treatments (1047 g N_2O ha^{-1}), which had C:N ratios of 3.3 and 12, respectively. Moreover, the higher N loss as N_2O emissions in the BM-amended soils was supported by their lower aboveground biomass compared to BC-amended soils (alone or combined with urea) in both experimental years ($P > 0.05$), which also showed higher NUE and (**Table 2-6**).

In the case of urea-amended soils, in general, the cumulative N_2O emissions were lower than that of BM-amended soils (alone or combined with urea) at both types of placement ($P > 0.05$), but higher than BC- and BA-amended soils (alone or combined with urea). Significant differences were observed between URI and, BAI, BCI, and BAURI in 2018. Several studies have found higher (Velthof et al., 2003; Jones et al., 2007; Díaz-Rojas et al. 2014) or lower N_2O emissions (Hernandez-Ramirez et al., 2009, Chantigny et al., 2010) from soils receiving organic amendments, when compared to those receiving synthetic fertilizer. Higher N_2O emissions from organic amended-soils can be explained by the supply of easily mineralizable N and C. Mineralization of the organic N produces NH_4^+, which is nitrified to NO_3^-, and this NO_3^- can be biochemically reduced to N_2O, whereas mineralization of C increases microbial respiration and growth, which further deplete O_2 (Gentile et al., 2008; Pelster et al., 2012; Thangarajan et al., 2013, Zhu-Barker et al., 2015; Wijesekara et al., 2017), probably favoring the occurrence of N_2O losses via denitrification as anoxic microsites are created (Jones et al., 2007, Pelster et al., 2012; Díaz-Rojas et al. 2014; De Rosa et al., 2016, Charles et al., 2017; Guenet et al., 2020). The lower N_2O emission from the BA- and BC-amended soils than from the urea-amended soils might be explained by a higher available N with urea addition than that of BA- and BC-amended soils (**Fig. 2-1B, Fig. 2-1C, Fig. 2-2B, Fig. 2-2C)**, as NH_4^+ was likely supplied faster in the urea-amended soils than in BA- and BC- amended soils.

2.5.5 Effects of combining biosolids and urea additions on N_2O emissions and barley biomass

Combining biosolids with urea resulted in lower N_2O emission than biosolids-only treatments in the case of soils receiving BM (2017 and 2018), BA (2018) at both types of placements, and BC (2018) in the surface-applied treatment ($P > 0.05$, **Table 2-4**). We infer that in such cases, better matching and synchrony of the available N with the crop demands might have occurred, as synthetic N fertilizers can provide rapidly available N, whereas most of the N provided by organic amendments needs to be mineralized first to be available for plant uptake (Rigby et al., 2016; Rezaei Rashti et al., 2017). This can be further supported by the fact that more plant biomass and higher N uptake were measured in the biosolids-urea combinations than in the biosolids-only treatments (**Table 2-6**). As a consequence, higher NUE and UE were obtained. In a modeling study that assessed the combination of chicken manure (raw or composted) with urea, De Rosa et al. (2017) found lower plant yield and N uptake in the organic amendment-only treatments than when they were combined with urea in a crop rotation including sweet corn (*Z. mays* var. *saccarina*), broccoli (*Brassica oleracea* var. *Italica*), and lettuce (*Latuca sativa* L.) within the same year. In the same way, Hernandez et al. (2014) found significantly lower tomato (*Lycopersicum esculentum* Mill. cv. “Optima”) yield and N content in leaves and fruit when applying organic amendments only (cow manure-based compost or sheep and goat manure-based compost) than when mixing the organic amendments with Hoagland’s nutrient solution. Nevertheless, the combination of organic amendments with synthetic fertilizer might or might not match the same yield obtained by adding synthetic N fertilizer alone. In the study conducted by De Rosa et al. (2017), the plant yield from the manure-urea treatments was slightly lower than their urea-only treatments, although the opposite trend was found by Hernandez et al. (2014). In our study, the barley biomass from some of the biosolids-urea combined treatments were higher than the urea-only treatments (e.g., BCURS > URS during both experimental years, BCURI > URI in 2018); however, no significant differences were found among them (**Table 2-6**).

2.5.6 Effects of biosolids placement on aboveground biomass

Interestingly, higher biomass was generally obtained when the N source was incorporated into the soil (**Table 2-6**). Castillo et al. (2011) also found significantly higher elephantgrass (*Pennisetum purpureum* Schum.) biomass production (by 33%) when incorporating municipal biosolids (at a rate of 350 kg TN ha^{-1}) than when leaving them on the surface. Their results were explained by an increase of 25% in organic N mineralization in the incorporated treatment. In the present study, the organic N mineralization rate was not calculated, but the soil available N was measured several times over the growing season. Higher available N was observed for the surface-applied treatments than for the incorporated N treatments over time, within each growing season. As higher biomass was achieved by the incorporated N treatments, it is more likely that the incorporated treatments released more available N in the soil and also that the crop was able to uptake most of this available N effectively, as N incorporation allows closer proximity and contact among the nutrient source, the soil, and roots; thus, it can be better utilized by the crop (Rezaei Rashti et al., 2017). This beneficial effect of N incorporation was also observed on the NDVI measurements (2018), as significantly higher NDVI values (canopy greenness) were observed in the incorporated N treatments than in surface-applied treatments, even in the early growth stages of the barley crop (**Fig. 2-3**). Nevertheless, the positive effect of N incorporation in biomass production should be treated carefully, as this placement method led to higher N_2O emissions, as aforementioned. This reveals the tradeoff between plant productivity and environmental outcomes as a function of N management options, such as biosolids-N placement in this case.

2.5.7 Effects of biosolids' properties on CO_2 and CH_4 emissions

The release of CO_2 emissions was promoted by the addition of biosolids, since they provided mineralizable C that increased microbial respiration (Zhu-Barker et al., 2015), which further promoted N_2O emissions by reducing the O_2 availability in the soil, as aforementioned. Furthermore, some carbonates, particularly in the BA-amended soils might have been released as CO_2 (Fangueiro et al., 2017). Moreover, CO_2 emissions might have been related to a positive priming effect, which is the acceleration of SOM decomposition and mineralization after the addition of organic materials and/or synthetic fertilizers, leading to an increase in the CO_2 fluxes (Jenkinson et al., 1985; Kuzyakov et al., 2000; Chen et al., 2014, Fiorentino et al., 2019). Several studies have reported a positive priming effect after the addition of organic amendments (alone or combined with synthetic N fertilizer) (Bell et al., 2003; Chen et al., 2014; Li et al., 2018) or easily degradable carbon sources such as glucose (Blagodatskaya et al., 2007) or artificial root exudates (Daly and Hernandez-Ramirez, 2020).

The production of CH_4 requires strongly anaerobic conditions (Willén et al., 2016; Chai et al., 2020b; Brachmann et al., 2020), which did not occur in our study, as the highest WFPS during the growing season was of 73%. Most probably, CH_4 fluxes coming from BM-amended soils (alone or combined with urea) at the beginning of the growing season might have been caused by the outgassing from the added BM (produced under anaerobic digestion) (Wulf et al., 2002) and its high water content enhancing the creation of highly anoxic hotspots in the soil, favoring the release of CH_4 (Yoshida et al., 2015; Willén et al., 2016). On the contrary, this condition was unlikely to have been created after the addition of BA or BC, since their water contents were so much lower than that of BM (**Table 2-3**), leading to numerically lower annual cumulative CH_4 emissions than the control (**Table 2-7**). Similarly, Díaz-Rojas et al. (2014) did not find differences across their treatments (urea, urea + charcoal, wastewater sludge, and sludge + charcoal) applied at 150 kg N ha^{-1}. Nevertheless, the production of CH_4 can be counteracted by its oxidation, as both processes can occur concurrently within the soil (Willén et al., 2016; Schaufler et al., 2010; Chai et al., 2020b; Kim et al., 2021). Kim et al. (2021) recently demonstrated that increasing soil aeration and root growth were jointly associated with enhanced CH_4 uptake in crop fields.

Our GHG balance showed how accounting for urea manufacturing offset the differences in N_2O emissions between urea and biosolids, as this industrial process consumes high amounts of fossil energy (Sharma et al., 2017; Glab and Sowiński, 2019). On the basis of the IPCC Guidelines for National Greenhouse Gas Inventories (IPCC, 2006), Glab and Sowiński (2019) calculated the CO_{2eq} emissions from land applications of organic amendments (i.e., solar-dried sewage sludge and beet pulp digestate) and the addition of urea (100 kg N ha^{-1}) by using the BioGrace Excel GHG calculation tool. Their results showed that less than half the CO_{2eq} emissions were produced from land applications of organic amendments compared with urea at a rate of 100 kg N ha^{-1}. In addition, Miller-Robbie et al. (2015) found that CO_{2eq} emissions from other ways of disposing biosolids such as landfilling, or combustion were 19 and 12 times higher than the CO_{2eq} emissions from land application of biosolids. Overall, these findings show the potential for the reduction of GHG emissions when biosolids are applied to the land, instead of sole addition of synthetic N fertilizers.

2.6 Conclusions

Our study shows how soil GHG emissions develop after the application of different types of biosolids, and that soil available N, soil moisture (modified by rainfall or thawing), and incorporation of the N source are the main triggers for higher soil N_2O emissions. We also described how the different methods of biosolids stabilization (i.e., anaerobic mesophilic, alkalinization, and composting) generate differences in N_2O emissions, as these methods alter the N content and availability. For example, in the case of BM, its higher N content, narrower C:N ratio, and higher water content were conducive to the highest N_2O emissions. Biosolids-only applications did not meet plant N requirements. Thus, future studies can focus on further evaluating and optimizing the release of available N from biosolids-amended soils in order to have a better understanding of the potential of biosolids for replacing synthetic fertilizers, totally or partially, while sustaining similar yields and lowering GHG emissions. Future research is also needed to evaluate how the promising option of applying nitrification inhibitors with biosolids could potentially help to decrease N_2O emissions from biosolids-amended soils. As part of an emerging circular economy, considering biosolids as a valuable nutrient resource rather than waste is of high importance since the lower field GHG emissions (as CO_{2eq}) from urea-amended soils became meaningless when we consider the high CO_{2eq} released during urea manufacturing. When managed proactively, land application of biosolids gives an overall opportunity to obtain both economic and environmental benefits. To our knowledge, our results show, for the first time in the literature, the annual N_2O EF_{area} within North America after applying a range of very contrasting biosolids. Additionally, these results will further improve the Canadian Tier 2 GHG Inventory Methodology.

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2.9 List of tables

Table 2-1: Soil properties (0–15 cm depth increment), precipitation, and air temperature during the study.

Properties	Unit	2017	2018
SOC†	g organic C kg^{-1}	55.8	54.8
TN†	g N kg^{-1}	4.8	4.6
C to N ratio		11.63	11.91
pH‡		6.1	6.5
Bulk density	g cm^{-3}	1.18	1.18
Baseline NO_3^-–N§	mg kg^{-1}	4	12.8
Baseline P	mg kg^{-1}	20	n.a.
Total annual precipitation¶	mm yr^{-1}	398.1 (446.8)	377.7 (446.8)
Total growing season precipitation (June–August) ¶	mm	155.8 (228.5)	158.4 (228.5)
Annual air temperature¶	ºC	3.0 (3.3)	2.2 (3.3)

†Soil organic carbon (SOC) and total nitrogen (TN) were measured by dry combustion.

‡Measured in a saturated paste (1:2 soil:water).

§The initial NO_3^-–N concentration was measured at depth soil increments of 0–40 cm in 2017, and 0–30 cm in 2018 at the beginning of each growing season prior to any N treatment addition and barley seeding.

¶Values inside the parentheses are the long-term normal (Alberta Climate Information Service) from 1987 to 2016. Values outside the parentheses correspond to the measurement year.

Table 2-2: List of treatments.

Treatment	Placement	Acronym	N rate (kg N ha^{-1})
Control	n/a	–	–
Urea	Surface	URS	96
Liquid mesophilic anaerobic digested biosolids (BM)		BMS	192
Alkaline-stabilized biosolids (BA)		BAS	192
Composted biosolids (BC)		BCS	192
BM + urea†		BMURS	144
BA + urea†		BAURS	144
BC + urea†		BCURS	144
Urea	Incorporation‡	URI	96
BM		BMI	192
BA		BAI	192
BC		BCI	192
BM + urea†		BMURI	144
BA + urea†		BAURI	144
BC + urea†		BCURI	144

[†] In these six treatments, urea was applied at a rate of 48 kg N ha^{-1}, whereas biosolids were applied a rate of 96 kg TN ha^{-1}.

[‡] Incorporation was done on the same day as the treatment applications at 15 cm depth.

Table 2-3: Biosolids' properties during the study.

Parameter	Unit	Mesophilic anaerobic digested (BM)			Alkaline-stabilized (BA)			Composted (BC)		
		2017	2018	Average	2017	2018	Average	2017	2018	Average
TC[†]	g C kg^{-1}	n.a.	283.3	283.0	n.a.	122.5	122.5	n.a.	278.4	278.4
TN[†]	g N kg^{-1}	17.9	42.4	30.2	8.1	7.8	7.95	19.5	22.5	21.0
C to N ratio		n.a.	6.7	6.7	n.a.	15.7	15.7	n.a.	12.4	12.4
pH		7.7[‡]	n.a.	7.7	12.8[§]	n.a.	12.8	5.01[§]	n.a.	5.01
Electrical conductivity[‡]	dS m^{-1}	4.5	n.a.	4.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Dry matter fraction, gravimetric (mass basis)		0.23	0.23	0.23	0.69	0.74	0.72	0.59	0.72	0.66
Moisture (mass basis)		0.77	0.77	0.77	0.31	0.26	0.29	0.41	0.28	0.35

[†] Total carbon (TC) and total nitrogen (TN) were measured by dry combustion.

[‡] Measured in saturated paste 1:2.

[§] Measured by the TMECC 04.11 Electrometric pH Determinations for Compost. 1:5 Slurry Method.

Table 2-4: Cumulative N_2O emissions (g N ha^{-1}) during growing season, post-harvest, and early spring periods, and total N_2O emissions (g N ha^{-1}) during the study (± standard error of the mean).

Treatment†	2017–2018				2018–2019				2-year mean			
	Growing season 2017 6-Jun to 16-Aug	Post-harvest 2017 22-Aug to 26-Oct	Early spring 2018 20-Apr to 31-May	**Cumulative N_2O 2017-2018 6-Jun-17 to 31-May-18‡**	Growing season 2018 7-Jun to 13-Aug	Post-harvest 2018 23-Aug to 25-Oct	Early spring 2019 28-Mar to 16-May	**Cumulative N_2O 2018-2019 7-Jun-18 to 16-May-19**	Growing season	Post-harvest	Early spring	**Cumulative N_2O**
Control	112.8^{a}	24.3^{ab}	271.9^{ab}	**409.0 ± 24**	83.7^{a}	95.3^{abc}	334.4^{ab}	**513.5 ± 54^{a}**	98.3^{d}	59.8^{bc}	303.1^{ab}	**461.2 ± 20^{f}**
URS	444.6^{ab}	31.9^{ab}	283.6^{ab}	**760.1 ± 186**	286.2^{abcd}	116.8^{abcd}	602.7^{ab}	**1005.8 ± 149^{abc}**	365.4^{c}	74.4^{bc}	443.2^{ab}	**882.9 ± 135^{bcde}**
BMS	1303.7^{ab}	447.6^{ab}	640.0^{b}	**2391.2 ± 923**	476.8^{bcde}	168.8^{bcde}	535.6^{ab}	**1181.2 ± 331^{abc}**	890.3^{bc}	308.2^{abc}	587.8^{a}	**1786.2 ± 624^{abc}**
BAS	442.5^{ab}	27.0^{ab}	223.6^{ab}	**693.1 ± 121**	358.9^{abcde}	194.5^{bcde}	328.9^{ab}	**882.2± 185^{ab}**	400.7^{bc}	110.7^{abc}	276.2^{ab}	**787.7 ± 136^{cdef}**
BCS	223.4^{ab}	37.5^{ab}	311.5^{ab}	**572.5 ± 83**	154.4^{ab}	88.8^{abc}	435.3^{ab}	**678.4 ± 190^{a}**	188.9^{cd}	63.2^{bc}	373.4^{ab}	**625.5 ± 79^{def}**
BMURS	524.8^{ab}	77.2^{ab}	505.6^{ab}	**1107.6 ± 310**	463.0^{bcde}	241.0^{cde}	415.8^{ab}	**1119.8 ± 146^{abc}**	493.9^{bc}	159.1^{abc}	460.7^{ab}	**1113.7 ± 178^{abcde}**
BAURS	576.8^{ab}	29.4^{ab}	163.2^{ab}	**769.5 ± 315**	196.7^{abc}	105.1^{abcd}	485.9^{ab}	**787.7 ± 70^{ab}**	386.8^{bc}	67.3^{bc}	324.6^{ab}	**778.6 ± 178^{cdef}**
BCURS	347.7^{ab}	52.2^{ab}	331.9^{ab}	**731.8 ± 121**	163.2^{ab}	73.56^{abc}	273.9^{a}	**510.6 ± 81^{a}**	255.4^{cd}	62.9^{bc}	302.9^{ab}	**621.2 ± 98^{ef}**
URI	231.9^{ab}	43.2^{ab}	162.2^{ab}	**437.3 ± 98**	999.2^{cde}	232.9^{bcde}	777.7a^{b}	**2009.9 ± 525^{bc}**	615.5^{abc}	138.1^{abc}	470.0^{ab}	**1223.6 ± 241^{abcd}**
BMI	2957.9^{b}	717.2^{b}	378.4^{ab}	**4053.5 ± 1413**	2502.9^{e}	1092.0^{e}	803.2^{b}	**4398.1 ± 1213^{c}**	2730.4^{a}	904.6^{a}	590.8^{ab}	**4225.8 ± 736^{a}**
BAI	328.1a^{b}	10.4^{ab}	76.3^{a}	**414.7 ± 79**	306.4^{abcd}	103.0^{abc}	357.4^{ab}	**766.9 ± 127^{ab}**	317.2^{bc}	56.7^{bc}	216.9^{b}	**590.8 ± 24^{ef}**
BCI	639.9a^{b}	20.5^{a}	105.4^{ab}	**765.8 ± 202**	203.3^{ab}	88.7^{abc}	462.1^{ab}	**754.1 ± 120^{ab}**	421.6^{bc}	54.6^{c}	283.8^{ab}	**760.0 ± 69^{cdef}**
BMURI	1671.8^{b}	182.3^{ab}	542.2^{ab}	**2396.3 ± 1440**	1643.4^{de}	970.9^{de}	629.8^{ab}	**3244.1 ± 1499^{c}**	1657.6^{ab}	576.6^{ab}	586.0^{ab}	**2820.2 ± 967^{ab}**
BAURI	498.8^{ab}	40.4^{ab}	336.3^{ab}	**875.5 ± 151**	245.5^{abc}	57.8^{a}	349.9^{ab}	**653.2 ± 117^{a}**	372.1^{bc}	49.1^{c}	343.1^{ab}	**764.4 ± 48^{cde}**
BCURI	658.8^{ab}	60.1^{ab}	376.6a^{b}	**1095.5 ± 223**	381.0^{abcde}	64.5^{ab}	422.7^{ab}	**868.2 ± 114^{ab}**	519.9^{bc}	62.3^{bc}	399.7^{ab}	**981.9 ± 132^{bcde}**
Overall mean ± SE	730.9 ± 132.7	120.1 ± 34.3	313.9 ± 38.2	**1164.9 ± 185.1**	564.3 ± 109.0	246.3 ± 58.7	481.0 ± 34.7	**1291.6 ± 184.5**	647.6 ± 101.7	183.2 ± 37.5	397.5 ± 26.7	**1228.2 ± 153.2**
ANOVA *P* values§												
Treatment	0.0147	0.0336	0.0252	**0.0125**	<0.0001	<0.0001	0.0509	**<0.0001**	<0.0001	0.0001	0. 0375	**<0.0001**
N source	0.0676 (0.0518)	0.0029 (0.0014)	0.021 (0.0237)	**0.0034 (0.0028)**	<0.0001 (<0.0001)	<0.0001	0.0238 (0.0185)	**<0.0001 (<0.0001)**	0.0004 (0.0007)	<0.0001 (<0.0001)	0.0039 (0.0021)	**<0.0001 (<0.0001)**
N placement	0.1296 (0.1183)	0.3359 (0.3158)	0.0653 (0.0701)	**0.8882 (0.8881)**	0.0027 (0.0041)	0.7132	0.0874 (0.0814)	**0.0015 (0.0029)**	<0.0001 (0.0001)	0.9099 (0.9138)	0.8756 (0.8708)	**0.0011 (0.0018)**
N source × N placement	0.7346	0.861	0.2561	**0.446**	0.0927	0.0028	0.6099	**0.0513**	0.1215	0.1449	0.8032	**0.1026**

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even the *P* value was below 0.05.

[§] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD. N_2O emissions during winter period were assumed to be negligible.

Table 2-5: Annual area-based N_2O emission factors (EF_{area}) (% kg N_2O–N kg^{-1} N fertilizer), yield-based emission factors (EF_{yield}) (g N_2O–N kg^{-1} grain DM) and estimated annual N_2O emission factors (EF) as a function of rainfall only (N_2O EF % = $e^{(0.00558 \times H_2O - 7.701)} \times 100$ (Rochette et al., 2018; Liang et al., 2020) (± standard error of the mean).

Treatment†	EF_{area} (% kg N_2O–N kg^{-1} fertilizer)			EF_{yield} (g N_2O–N kg^{-1} DM)		
	2017–2018	2018–2019	2-year mean	2017–2018	2018–2019	2-year mean
Control				0.19 ± 0.04^{ab}	0.12 ± 0.02^{abc}	0.15 ± 0.01^{bcd}
URS	0.37 ± 0.18^{ab}	0.51 ± 0.13^{bcde}	0.44 ± 0.14^{abcdef}	0.12 ± 0.02^{ab}	0.22 ± 0.05^{abcde}	0.17 ± 0.03^{bcd}
BMS	1.03 ± 0.48^{ab}	0.35 ± 0.17^{abcd}	0.69 ± 0.32^{abcd}	0.54 ± 0.22^{b}	0.34 ± 0.13^{bcde}	0.44 ± 0.17^{ab}
BAS	0.15 ± 0.07^{ab}	0.19 ± 0.09^{ab}	0.17 ± 0.06^{def}	0.12 ± 0.02^{ab}	0.17 ± 0.04^{abcd}	0.14 ± 0.03^{bcd}
BCS	0.09 ± 0.04^{ab}	0.09 ± 0.09^{ab}	0.09 ± 0.04^{ef}	0.10 ± 0.02^{ab}	0.13 ± 0.03^{abc}	0.11 ± 0.02^{cd}
BMURS	0.49 ± 0.22^{ab}	0.42 ± 0.07^{abcde}	0.45 ± 0.12^{abcde}	0.18 ± 0.04^{ab}	0.25 ± 0.05^{bcde}	0.21 ± 0.04^{abc}
BAURS	0.25 ± 0.23^{ab}	0.19 ± 0.05^{ab}	0.22 ± 0.11^{cdef}	0.15 ± 0.06^{ab}	0.18 ± 0.04^{abcde}	0.17 ± 0.04^{bcd}
BCURS	0.22 ± 0.09^{ab}	-0.002 ± 0.04^{a}	0.11 ± 0.06^{def}	0.11 ± 0.01^{ab}	0.08 ± 0.01^{a}	0.09 ± 0.01^{d}
URI	0.03 ± 0.11^{a}	1.56 ± 0.52^{de}	0.79 ± 0.24^{abc}	0.06 ± 0.02^{a}	0.28 ± 0.08^{cde}	0.17 ± 0.04^{bcd}
BMI	1.90 ± 0.74^{b}	2.02 ± 0.65^{e}	1.96 ± 0.39^{a}	0.97 ± 0.45^{b}	0.73 ± 0.21^{e}	0.85 ± 0.21^{a}
BAI	0.003 ± 0.04^{ab}	0.13 ± 0.09^{ab}	0.07 ± 0.02^{f}	0.16 ± 0.04^{ab}	0.16 ± 0.01^{abcd}	0.15 ± 0.02^{bcd}
BCI	0.19 ± 0.11^{ab}	0.13 ± 0.09^{ab}	0.16 ± 0.03^{def}	0.15 ± 0.05^{ab}	0.12 ± 0.01^{abcd}	0.13 ± 0.02^{bcd}
BMURI	1.38 ± 1.00^{ab}	1.90 ± 1.02^{cde}	1.64 ± 0.66^{ab}	0.37 ± 0.25^{ab}	0.46 ± 0.19^{de}	0.41 ± 0.14^{ab}
BAURI	0.32 ± 0.12^{ab}	0.10 ± 0.12^{ab}	0.21 ± 0.04^{cdef}	0.21 ± 0.07^{ab}	0.09 ± 0.01^{ab}	0.15 ± 0.03^{bcd}
BCURI	0.48 ± 0.16^{ab}	0.25 ± 0.06^{abc}	0.36 ± 0.08^{bcdef}	0.17 ± 0.04^{ab}	0.12 ± 0.02^{abc}	0.14 ± 0.03^{bcd}
Overall mean ± SE	0.49 ± 0.1	0.56 ± 0.1	0.53 ± 0.1	0.24 ± 0.05	0.23 ± 0.03	0.23 ± 0.03
ANOVA *P* values‡						
Treatment	0.0144	<0.0001	<0.0001	0.04	<0.0001	<0.0001
N source	0.0024 (0.0024)	<0.0001	<0.0001 (<0.0001)	0.0043 (0.0030)	<0.0001	<0.0001 (<0.0001)
N placement	0.9697 (0.9701)	0.0012	0.0011 (0.002)	0.8673 (0.8654)	0.1288	0.0187 (0.0166)
N source × N placement	0.3140	0.0132	0.0663	0.5719	0.0461	0.5781
$EF_{rainfall}$ from Jun to Aug	0.11	0.11	**0.11**			
$EF_{rainfall}$ from May to Oct§ (Rochette et al., 2018)	0.24	0.22	**0.23**			

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The numbers inside the parentheses are the P values after the interaction effect was removed.

[§] The $EF_{rainfall}$ for the long-term normal (1987–2016) was 0.16 from June to August, and 0.29 from May to October.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Table 2-6: Total aboveground biomass (kg dry matter (DM) ha^{-1}), N uptake (kg N ha^{-1}), N use efficiency (NUE) (kg DM kg^{-1} N) and uptake efficiency (UE) (kg plant N kg^{-1} N) during the study (± standard error of the mean).

Treatment†	Total aboveground biomass (kg DM ha^{-1})				N uptake (kg N ha^{-1})			NUE (kg DM kg^{-1} N)			UE (kg plant N kg^{-1} N)		
	2017	2017-II‡	2018	2-year mean	2017	2018	2-year mean	2017	2018	2-year mean	2017	2018	2-year mean
Control	2385 ± 371^{a}	1779 ± 455^{a}	4535 ± 455^{ab}	3460 ± 177^{d}	20 ± 4^{a}	45 ± 6^{a}	33 ± 2^{c}						
URS	6753 ± 1302^{b}	2486 ± 840^{ab}	5096 ± 840^{abc}	5925 ± 821^{abcd}	66 ± 11^{bc}	69 ± 11^{abc}	68 ± 9^{abc}	45.5 ± 16^{cd}	5.8 ± 5^{ab}	25.7 ± 8^{ab}	0.48 ± 0.1^{b}	0.24 ± 0.1^{abc}	0.36 ± 0.1^{abc}
BMS	4800 ± 523^{ab}	2947 ± 701^{ab}	4227 ± 701^{a}	4514 ± 608^{bcd}	62 ± 9^{abc}	57 ± 8^{abc}	60 ± 8^{abc}	12.6 ± 4^{ab}	−1.6 ± 3^{a}	5.5 ± 3^{c}	0.22 ± 0.1^{ab}	0.06 ± 0.0^{ab}	0.14 ± 0.0^{bcd}
BAS	6066 ± 588^{ab}	2580 ± 462^{ab}	5353 ± 462^{abc}	5710 ± 419^{abcd}	60 ± 8^{abc}	59 ± 4^{abc}	59 ± 5^{abc}	19.2 ± 5^{abc}	4.3 ± 3^{ab}	11.7 ± 2^{bc}	0.21 ± 0.1^{ab}	0.07 ± 0.0^{ab}	0.14 ± 0.0^{bcd}
BCS	6045 ± 879^{ab}	2561 ± 908^{ab}	5623 ± 908^{abc}	5834 ± 890^{abcd}	48 ± 9^{abc}	68 ± 11^{abc}	58 ± 10^{abc}	19.1 ± 6^{abc}	5.7 ± 3^{ab}	12.4 ± 4^{bc}	0.14 ± 0.1^{ab}	0.12 ± 0.1^{ab}	0.13 ± 0.0^{bcd}
BMURS	5879 ± 409^{ab}	2261 ± 1109^{ab}	5229 ± 1109^{abc}	5554 ± 567^{abcd}	65 ± 10^{bc}	78 ± 19^{abc}	71 ± 10^{ab}	24.3 ± 4^{abcd}	4.8 ± 5^{ab}	14.5 ± 3^{bc}	0.31 ± 0.0^{ab}	0.23 ± 0.1^{abc}	0.27 ± 0.1^{abcd}
BAURS	5223 ± 625^{ab}	2148 ± 1032^{ab}	4941 ± 1032^{abc}	5082 ± 825^{abcd}	50 ± 12^{abc}	57 ± 9^{abc}	53 ± 11^{abc}	19.7 ± 6^{abc}	2.8 ± 6^{a}	11.3 ± 5^{bc}	0.21 ± 0.1^{ab}	0.08 ± 0.1^{ab}	0.14 ± 0.1^{bcd}
BCURS	6786 ± 555 ^{b}	1802 ± 704^{a}	6344 ± 704^{abc}	6565 ± 374^{ab}	64 ± 10^{abc}	84 ± 8^{bc}	74 ± 7^{ab}	30.6 ± 5^{abcd}	12.6 ± 6^{abc}	21.6 ± 2^{bc}	0.30 ± 0.1^{ab}	0.27 ± 0.1^{bc}	0.28 ± 0.1^{abc}
URI	7282 ± 847^{b}	2458 ± 624^{ab}	7287 ± 624^{bc}	7284 ± 487^{a}	65 ± 9^{bc}	96 ± 9^{c}	80 ± 6^{a}	51.0 ± 13^{d}	28.7 ± 5^{c}	39.8 ± 5^{a}	0.46 ± 0.1^{b}	0.52 ± 0.1^{c}	0.49 ± 0.0^{a}
BMI	5566 ± 1107^{ab}	3221 ± 982^{b}	6106 ± 982^{abc}	5836 ± 894^{abcd}	70 ± 15^{bc}	75 ± 11^{abc}	73 ± 10^{ab}	16.6 ± 7^{ab}	8.2 ± 6^{ab}	12.4 ± 5^{bc}	0.26 ± 0.1^{ab}	0.15 ± 0.1^{ab}	0.21 ± 0.1^{bcd}
BAI	3148 ± 248^{a}	1867 ± 672^{ab}	4895 ± 672^{ab}	4022 ± 428^{cd}	29 ± 1^{ab}	50 ± 5^{ab}	39 ± 3^{bc}	4.0 ± 3^{a}	3.9 ± 4^{ab}	3.9 ± 2^{c}	0.04 ± 0.0^{a}	0.02 ± 0.0^{a}	0.03 ± 0.0^{d}
BCI	5709 ± 812^{ab}	1631 ± 483^{a}	6418 ± 483^{abc}	6064 ± 433^{abc}	48 ± 11^{abc}	61 ± 6^{abc}	55 ± 5^{abc}	17.3 ± 4^{abc}	9.8 ± 2^{abc}	13.6 ± 1^{bc}	0.15 ± 0.1^{ab}	0.08 ± 0.0^{ab}	0.11 ± 0.0^{cd}
BMURI	7215 ± 429^{b}	2907 ± 924^{ab}	6944 ± 924^{abc}	7079 ± 489^{a}	83 ± 9^{c}	90 ± 14^{bc}	87 ± 11^{a}	33.5 ± 1^{bcd}	16.7 ± 4^{abc}	25.1 ± 3^{ab}	0.44 ± 0.1^{b}	0.31 ± 0.1^{bc}	0.37 ± 0.1^{ab}
BAURI	5380 ± 1271^{ab}	2279 ± 447^{ab}	6906 ± 447^{abc}	6143 ± 834^{abc}	51 ± 15^{abc}	75 ± 8^{abc}	63 ± 11^{abc}	20.8 ± 11^{abc}	16.5 ± 2^{abc}	18.6 ± 6^{bc}	0.21 ± 0.1^{ab}	0.20 ± 0.0^{abc}	0.21 ± 0.1^{bcd}
BCURI	6861 ± 753^{b}	2809 ± 1093^{ab}	7915 ± 1093^{c}	7388 ± 853^{a}	64 ± 14^{abc}	90 ± 12^{bc}	77 ± 12^{a}	31.1 ± 6^{abcd}	23.5 ± 7^{bc}	27.3 ± 5^{ab}	0.30 ± 0.1^{ab}	0.31 ± 0.1^{bc}	0.31 ± 0.1^{abc}
Overall mean ± SE	5673 ± 249	2382 ± 89	5855 ± 226	5764 ± 204	56 ± 3.1	70 ± 3.0	63 ± 2.7	24.7 ± 2.4	10.1 ± 1.5	17.4 ± 1.6	0.27 ± 0.03	0.19 ± 0.02	0.23 ± 0.02
ANOVA *P* values§													
Treatment	0.0004	0.0029	0.001	<0.0001	0.0026	0.0004	0.0001	0.0001	0.0003	<0.0001	0.0018	<0.0001	<0.0001
N source	0.0057 (0.0092)	0.0181	0.0362 (0.0372)	0.0011	0.0254 (0.0279)	0.002 (0.0021)	0.0007 (0.0009)	<0.0001 (<0.0001)	0.0058 (0.0071)	<0.0001 (<0.0001)	0.0001 (0.0001)	<0.0001 (<0.0001)	<0.0001 (<0.0001)
N placement	0.9197 (0.9238)	0.7087	0.0001 (0.0001)	0.0178	0.7716 (0.7755)	0.0475 (0.0504)	0.2624 (0.2791)	0.9400 (0.9402)	<0.0001 (<0.0001)	0.0063 (0.0094)	0.8247 (0.8234)	0.0288 (0.0333)	0.1499 (0.1576)
N source × N placement	0.1145	0.0127	0.3422	0.0371	0.2856	0.2939	0.1786	0.4005	0.2216	0.0864	0.5053	0.1984	0.2656

†Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

‡2017-II represents the aboveground biomass obtained at the 2017 site during 2018 growing season to evaluate the effect of the residual N.

§The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Table 2-7: Cumulative partial record of CO_2 emissions (g CO_2–C ha^{-1}) and annual CH_4 emissions (Mg CH_4–C ha^{-1}) during the study (± standard error of the mean).

Treatment[†]	Cumulative annual of CH_4 emissions (g CH_4-C ha^{-1})			Cumulative partial record of CO_2 emissions[‡] (Mg CO_2-C ha^{-1})		
	2017–2018	2018-2019[§]	2-year mean	2017–2018	2018–2019	2-year mean
Control	97 ± 46^{ab}	357 ± 45	227 ± 33^{ab}	0.477 ± 0.08^{a}	1.464 ± 0.25	0.971 ± 0.10^{ab}
URS	122 ± 28^{ab}	299 ± 44	210 ± 25^{ab}	0.819 ± 0.07^{ab}	1.235 ± 0.24	1.027 ± 0.14^{ab}
BMS	78 ± 33^{a}	304 ± 69	191 ± 51^{ab}	0.961 ± 0.08^{ab}	1.278 ± 0.18	1.120 ± 0.07^{ab}
BAS	95 ± 63^{ab}	241 ± 69	168 ± 58^{ab}	0.627 ± 0.03^{ab}	1.472 ± 0.07	1.050 ± 0.03^{ab}
BCS	94 ± 36^{ab}	350 ± 39	222 ± 30^{ab}	0.674 ± 0.06^{ab}	1.667 ± 0.33	1.171 ± 0.14^{ab}
BMURS	49 ± 39^{a}	356 ± 91	202 ± 61^{ab}	0.777 ± 0.15^{ab}	1.559 ± 0.20	1.168 ± 0.10^{ab}
BAURS	79 ± 30^{a}	376 ± 84	227 ± 55^{ab}	0.700 ± 0.07^{ab}	1.087 ± 0.14	0.894 ± 0.04^{ab}
BCURS	40 ± 26^{a}	314 ± 64	177 ± 43^{ab}	0.744 ± 0.12^{ab}	0.916 ± 0.08	0.830 ± 0.07^{b}
URI	11 ± 63^{a}	63 ± 59	37 ± 49^{b}	0.911 ± 0.04^{ab}	1.323 ± 0.05	1.117 ± 0.03^{ab}
BMI	265 ± 76^{b}	405 ± 199	335 ± 115^{a}	1.094 ± 0.22^{b}	1.528 ± 0.09	1.311 ± 0.12^{ab}
BAI	24 ± 61^{a}	125 ± 83	74 ± 52^{b}	0.859 ± 0.07^{ab}	1.227 ± 0.22	1.043 ± 0.14^{ab}
BCI	12 ± 28^{a}	156 ± 83	84 ± 55^{b}	0.952 ± 0.11^{ab}	1.433 ± 0.24	1.192 ± 0.17^{ab}
BMURI	65 ± 58^{a}	244 ± 77	155 ± 57^{ab}	0.963 ± 0.11^{ab}	1.862 ± 0.16	1.412 ± 0.12^{a}
BAURI	12 ± 24^{a}	183 ± 74	97 ± 40^{b}	0.912 ± 0.09^{ab}	1.577 ± 0.16	1.244 ± 0.06^{ab}
BCURI	23 ± 21^{a}	121 ± 52	72 ± 22^{b}	1.048 ± 0.14^{b}	1.462 ± 0.17	1.255 ± 0.14^{ab}
Overall mean ± SE	70.9 ± 13.1	259.5 ± 23.3	165.2 ± 15.8	0.834 ± 0.032	1.406 ± 0.052	1.120 ± 0.031
ANOVA *P* values[¶]						
Treatment	0.0012	0.015	0.0007	0.0086	0.1199	0.0174
N source	0.01	0.2079 (0.2101)	0.0468	0.3083 (0.2549)	0.1242 (0.1517)	0.1376 (0.1457)
N placement	0.294	0.0004 (0.0004)	0.0006	0.0006 (0.0003)	0.0629 (0.0723)	0.0017 (0.0018)
N source × N placement	0.004	0.3865	0.0280	0.8925	0.159	0.3153

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The cumulative partial record for CO_2 emissions does not include the growing season period.

[§] The Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even the P value was below 0.05.

[¶] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Table 2-8: Partial greenhouse gas budget area- and yield-related kg CO_2-equivalent (CO_2eq) during the study (± standard error of the mean).

	Total flux of CO_2 equivalents						
Treatment†	N_2O emissions‡	CH_4 emissions‡	Biosolids production process§	Fuel consumption§	N manufacture¶	Area-related CO_{2eq} (kg ha^{-1} yr^{-1})	Yield-related CO_{2eq} (kg kg^{-1} DM)
	kg CO_{2eq} ha^{-1} yr^{-1}						
2017–2018							
Control	191.6	4.4	0.0	61.6	0	257.6 ± 10^{d}	0.118 ± 0.02
URS	356.0	5.5	0.0	61.6	457.6	880.7 ± 87^{abc}	0.148 ± 0.03
BMS	1120.0	3.5	12.9	61.6	0	1198.0 ± 433^{ab}	0.272 ± 0.10
BAS	324.7	4.3	41.9	61.6	0	432.4 ± 59^{abcd}	0.074 ± 0.01
BCS	268.1	4.3	13.7	61.6	0	347.7 ± 39^{bcd}	0.062 ± 0.01
BMURS	518.8	2.2	6.4	61.6	228.8	817.8 ± 146^{abc}	0.137 ± 0.02
BAURS	360.4	3.6	20.9	61.6	228.8	675.3 ± 147^{abcd}	0.135 ± 0.03
BCURS	342.8	1.8	6.9	61.6	228.8	641.8 ± 56^{abcd}	0.095 ± 0.01
URI	204.8	0.5	0.0	68.9	457.6	731.9 ± 47^{abc}	0.106 ± 0.02
BMI	1898.6	12.0	12.9	68.9	0	1992.4 ± 665^{a}	0.476 ± 0.22
BAI	194.3	1.1	41.9	68.9	0	306.1 ± 39^{cd}	0.102 ± 0.02
BCI	358.7	0.5	13.7	68.9	0	441.9 ± 95^{abcd}	0.086 ± 0.02
BMURI	1122.4	3.0	6.4	68.9	228.8	1429.5 ± 677^{ab}	0.216 ± 0.12
BAURI	410.1	0.5	20.9	68.9	228.8	729.3 ± 71^{abc}	0.181 ± 0.06
BCURI	513.1	1.0	6.9	68.9	228.8	818.7 ± 105^{abc}	0.125 ± 0.02
Overall mean ± SE	545.6 ± 86.7	3.2 ± 0.6				780.1 ± 86.1	0.156 ± 0.02
ANOVA *P* values#							
Treatment						<0.0001	0.0758
N source						<0.0001 (<0.0001)	0.0043 (0.0019)
N placement						0.6532 (0.6426)	0.4803 (0.4577)
N source × N placement						0.7690	0.9385
2018–2019							
Control	240.5	16.2	0.0	61.6	0	318.3 ± 24^{f}	0.073 ± 0.01^{c}
URS	471.1	13.6	0.0	61.6	457.6	1003.9 ± 71^{abc}	0.214 ± 0.04^{ab}
BMS	553.3	13.8	5.4	61.6	0	634.1 ± 157^{cdef}	0.182 ± 0.07^{abc}
BAS	413.2	10.9	43.5	61.6	0	529.2 ± 84^{cdef}	0.101 ± 0.02^{bc}
BCS	317.8	15.9	11.9	61.6	0	407.1 ± 88^{ef}	0.076 ± 0.02^{c}
BMURS	524.5	16.1	2.7	61.6	228.8	833.8 ± 67^{abcd}	0.183 ± 0.04^{ab}
BAURS	369.0	17.0	21.7	61.6	228.8	698.1 ± 29^{bcde}	0.166 ± 0.04^{abc}
BCURS	239.2	14.2	5.9	61.6	228.8	549.7 ± 36^{cdef}	0.089 ± 0.01^{bc}
URI	941.4	2.8	0.0	68.9	457.6	1470.8 ± 243^{ab}	0.206 ± 0.04^{ab}
BMI	2060.0	18.4	5.4	68.9	0	2152.8 ± 575^{a}	0.360 ± 0.10^{a}
BAI	359.2	5.7	43.5	68.9	0	477.3 ± 60^{cdef}	0.098 ± 0.01^{bc}
BCI	353.2	7.1	11.9	68.9	0	441.1 ± 56^{def}	0.068 ± 0.00^{c}
BMURI	1519.5	11.1	2.7	68.9	228.8	1831.0 ± 704^{ab}	0.262 ± 0.09^{ab}
BAURI	306.0	8.3	21.7	68.9	228.8	633.7 ± 52^{bcde}	0.092 ± 0.00^{bc}
BCURI	406.7	5.5	5.9	68.9	228.8	715.8 ± 53^{bcde}	0.096 ± 0.01^{bc}
Overall mean ± SE	605.0 ± 86.4	11.8 ± 1.1				846.4 ± 89.1	0.151 ± 0.01
ANOVA *P* values#							
Treatment						<0.0001	<0.0001
N source						<0.0001	<0.0001 (<0.0001)
N placement						0.0014	0.405(0.439)
N source × N placement						0.0145	0.062
2-year mean							
C	216.0	10.3	0.0	61.6	0	287.9 ± 10^{g}	0.096 ± 0.01^{bcde}
URS	413.6	9.5	0.0	61.6	457.6	942.3 ± 64^{abc}	0.181 ± 0.02^{abc}
BMS	836.7	8.7	6.0	61.6	0	916.1 ± 293^{bcde}	0.227 ± 0.08^{abc}
BAS	368.9	7.6	23.8	61.6	0	480.8 ± 64^{def}	0.088 ± 0.01^{cde}
BCS	293.0	10.1	3.7	61.6	0	377.4 ± 37^{fg}	0.069 ± 0.01^{e}
BMURS	521.7	9.2	3.0	61.6	228.8	825.8 ± 85^{bcd}	0.160 ± 0.03^{abc}
BAURS	364.7	10.3	11.9	61.6	228.8	686.7 ± 82^{cde}	0.151 ± 0.03^{abcd}
BCURS	291.0	8.0	1.8	61.6	228.8	595.8 ± 45^{cdef}	0.092 ± 0.00^{bcde}
URI	573.1	1.7	0.0	68.9	457.6	1101.3 ± 111^{abc}	0.156 ± 0.02^{abc}
BMI	1979.3	15.2	6.0	68.9	0	2072.6 ± 349^{a}	0.418 ± 0.10^{a}
BAI	276.7	3.4	23.8	68.9	0	391.7 ± 10^{fg}	0.100 ± 0.01^{bcde}
BCI	356.0	3.8	3.7	68.9	0	441.5 ± 33^{efg}	0.077 ± 0.01^{de}
BMURI	1321.0	7.0	3.0	68.9	228.8	1630.3 ± 455^{ab}	0.239 ± 0.07^{ab}
BAURI	358.0	4.4	11.9	68.9	228.8	681.5 ± 23^{cde}	0.136 ± 0.03^{bcd}
BCURI	459.9	3.3	1.8	68.9	228.8	767.3 ± 62^{bcde}	0.111 ± 0.02^{bcde}
Overall mean ± SE	575.3 ± 71.8	7.5 ± 0.7				813.3 ± 73.0	0.153 ± 0.01
ANOVA *P* values#							
Treatment						<0.0001	<0.0001

			Total flux of CO_2 equivalents				
Treatment†	N_2O emissions‡	CH_4 emissions‡	Biosolids production process§	Fuel consumption§	N manufacture¶	Area-related CO_{2eq} (kg ha^{-1} yr^{-1})	Yield-related CO_{2eq} (kg kg^{-1} DM)
			kg CO_{2eq} ha^{-1} yr^{-1}				
N source						<0.0001	<0.0001 (<0.0001)
N placement						0.0013	0.0798 (0.0829)
N source × N placement						0.0110	0.3188

†Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

‡The coefficients used to convert from N_2O and CH_4 to CO_2 equivalents were 298 and 34, respectively (IPCC, 2013). These coefficients operate on a mass basis, accounting for the full molecular weights.

§The CO_{2eq} for biosolids productions (including transport) was based on Brown et al. (2010) who applied the Biosolids Emissions Assessment Model (BEAM) to calculate the CO_{2eq} for biosolids production system. The factors used were 749.7 kg CO_{2eq} Mg^{-1} DM for BM, 1104.3 kg CO_{2eq} Mg^{-1} DM for BA, and 870.1 kg CO_{2eq} Mg^{-1} DM for BC.

¶Rotary tillage, crop seeding, and forage harvesting corresponded to 2, 3.2, and 13.6 kg C equivalent ha^{-1}. Production, packaging, storage, and distribution of urea were accounted for as 1.3 kg C equivalent kg^{-1} N (Lal, 2004).

#The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Indirect N_2O emissions caused by volatilization of ammonia and deposition were unaccounted for. Soil carbon change and nitrate leaching (and associated N_2O formation elsewhere) were assumed to be negligible. It is noted that CO_2 and plant C were not accounted in these partial greenhouse gas budgets.

2.10 List of figures

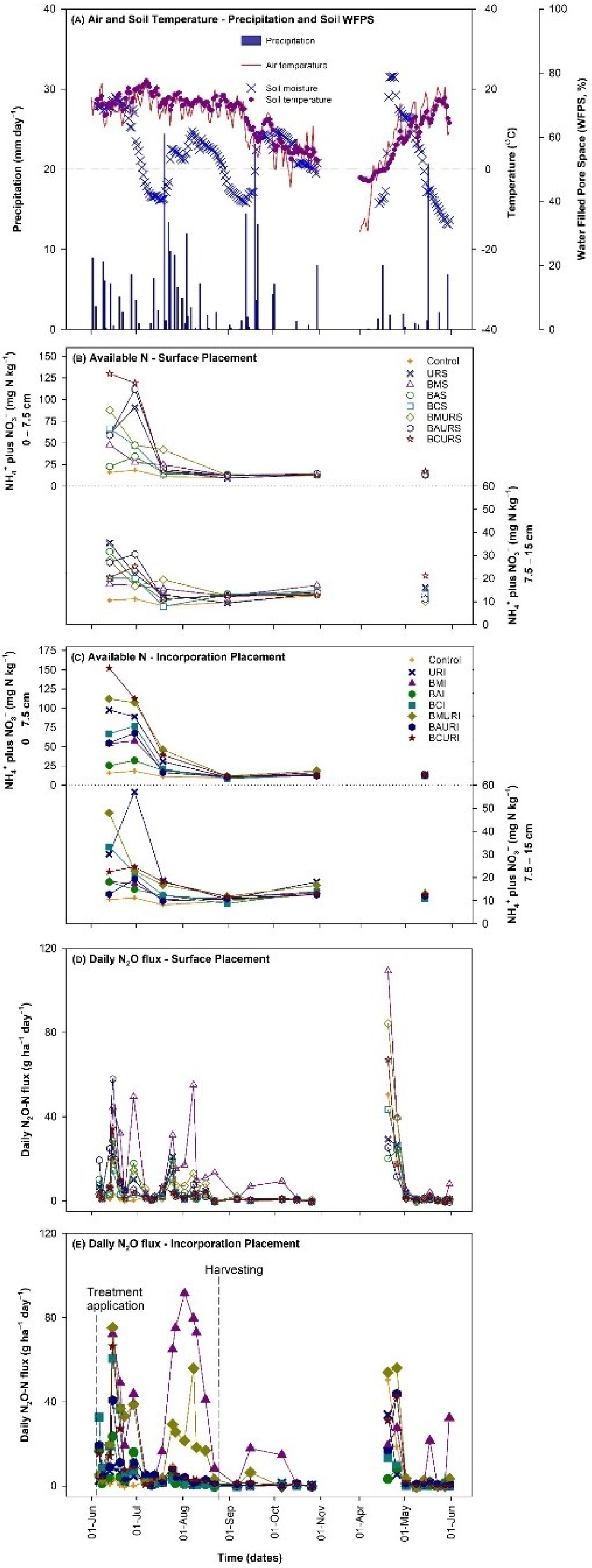

Figure 2-1: (A) Daily average air temperature, daily cumulative precipitation, daily average soil temperature (5–10 cm), and daily average soil water-filled pore space (WFPS) (5–10 cm depth); (B) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the surface-applied treatments plus the control; (C) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the incorporation treatments plus the control; (D) daily N_2O flux for the surface-applied treatments; and (E) daily N_2O flux for the incorporation treatments. Data in each panel are within the period from June 2017 to May 2018 (the interval from 1 Nov. to 1 Apr. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively.

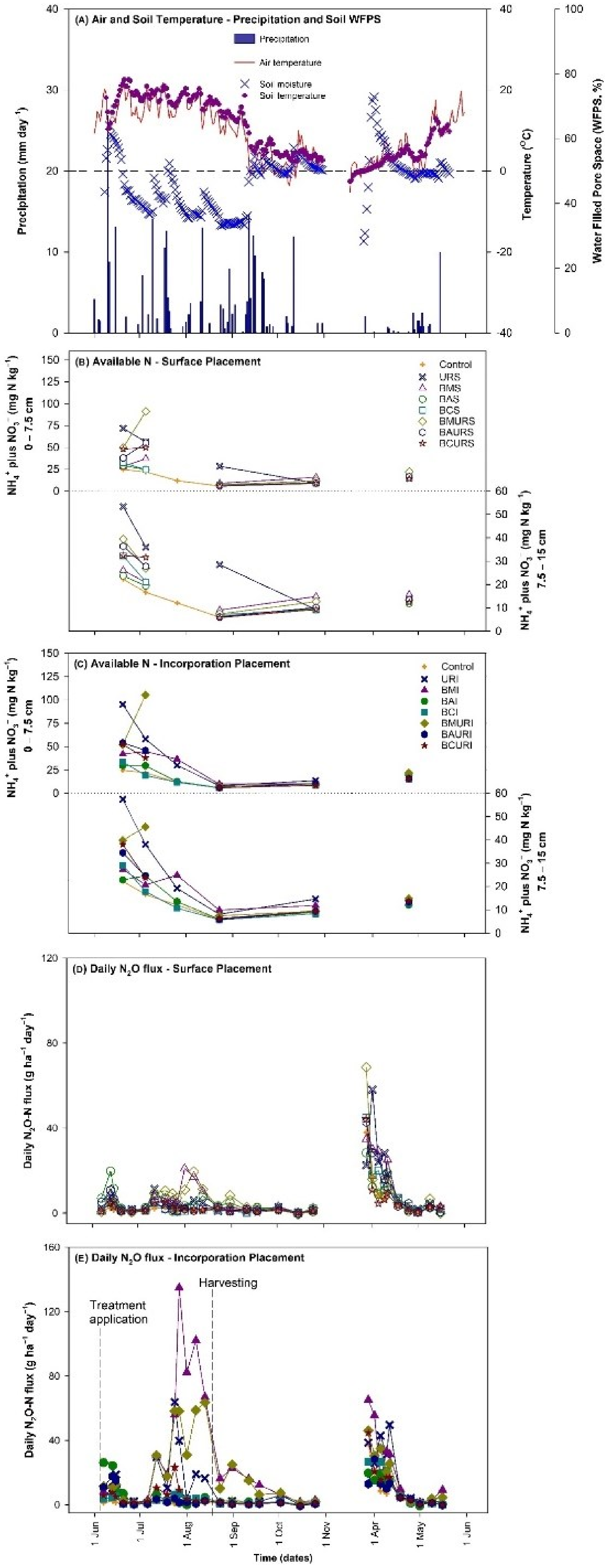

Figure 2-2: (A) Daily average air temperature, daily cumulative precipitation, daily average soil temperature (5–10 cm), and daily average soil water-filled pore space (WFPS) (5–10 cm depth); (B) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the surface-applied treatments plus the control; (C) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the incorporation treatments plus the control; (D) daily N_2O fluxes for the surface-applied treatments; and (E) daily N_2O flux for the incorporation treatments. Data in each panel are within the period from June 2018 to May 2019 (the interval from 1 Nov. to 1 March was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively.

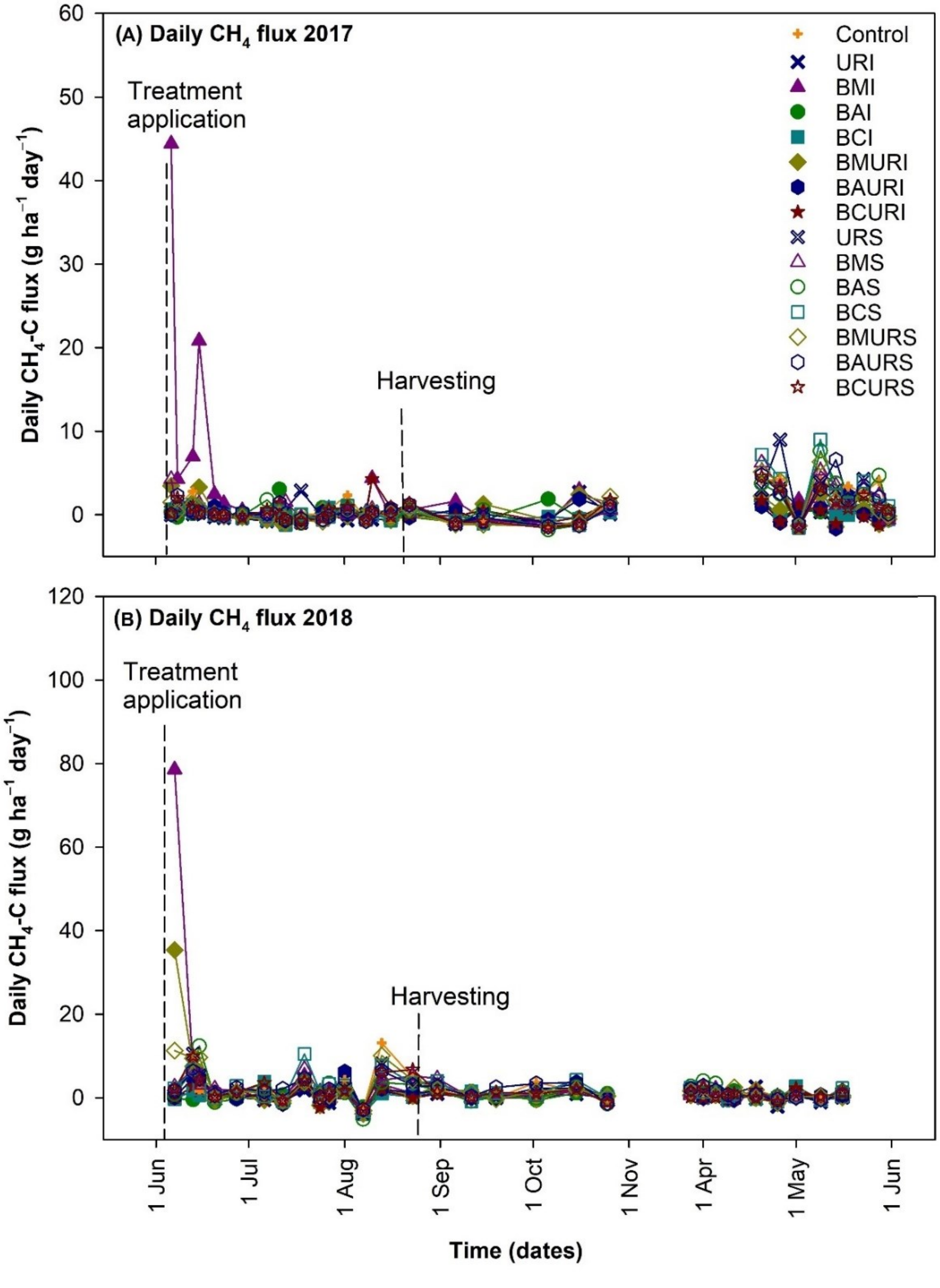

Figure 2-3: (A) Daily CH4 fluxes for the surface-applied and incorporation treatments from June 2017 to May 2018; (B) daily CH4 fluxes for the surface-applied and incorporation treatments from June 2018 to May 2019 (note that the interval from 1 Nov. to 1 March. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively.

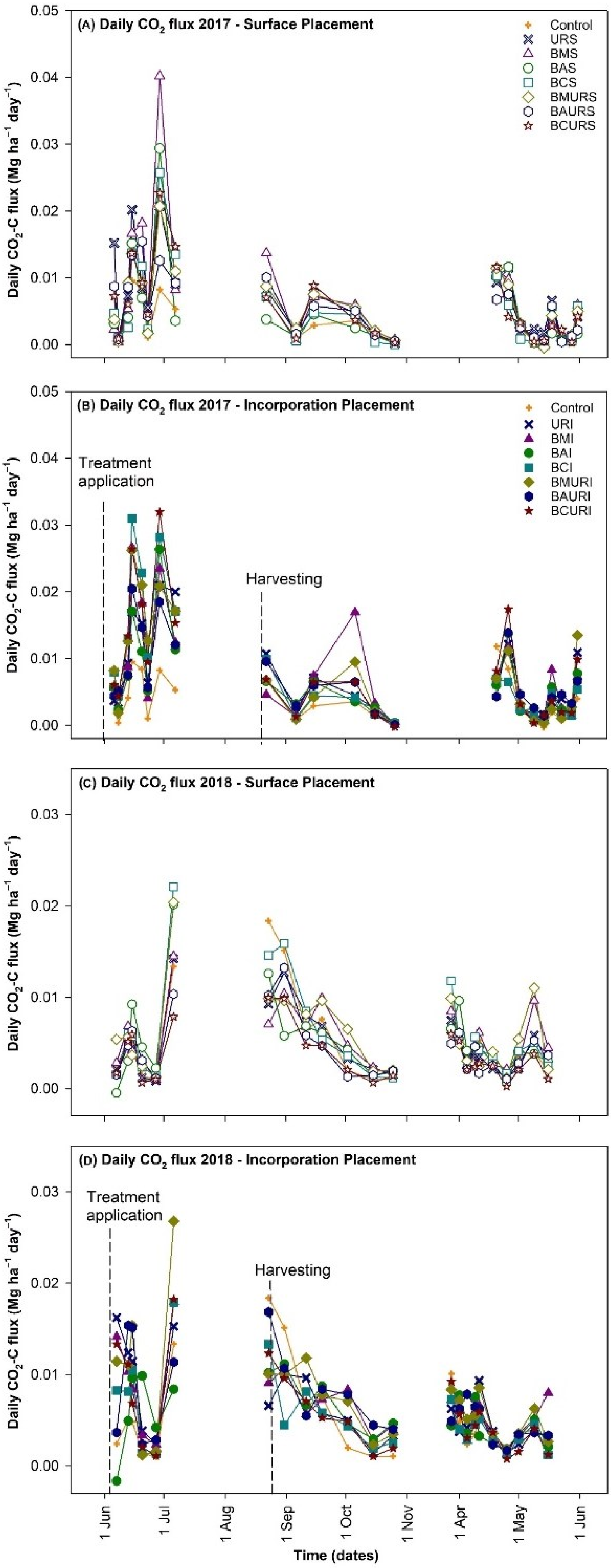

Figure 2-4: (A) Daily CO_2 fluxes for the surface-applied treatments from June 2017 to May 2018; (B) daily CO_2 fluxes for the incorporation treatments from June 2017 to May 2018; (C) daily CO_2 fluxes for the surface-applied treatments from June 2018 to May 2019; (D) daily CO_2 fluxes for the incorporation treatments from June 2018 to May 2019 (note that the interval from 1 Nov. to 1 March. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively. Data from July and August are not presented, as plant canopy was growing inside the chamber.

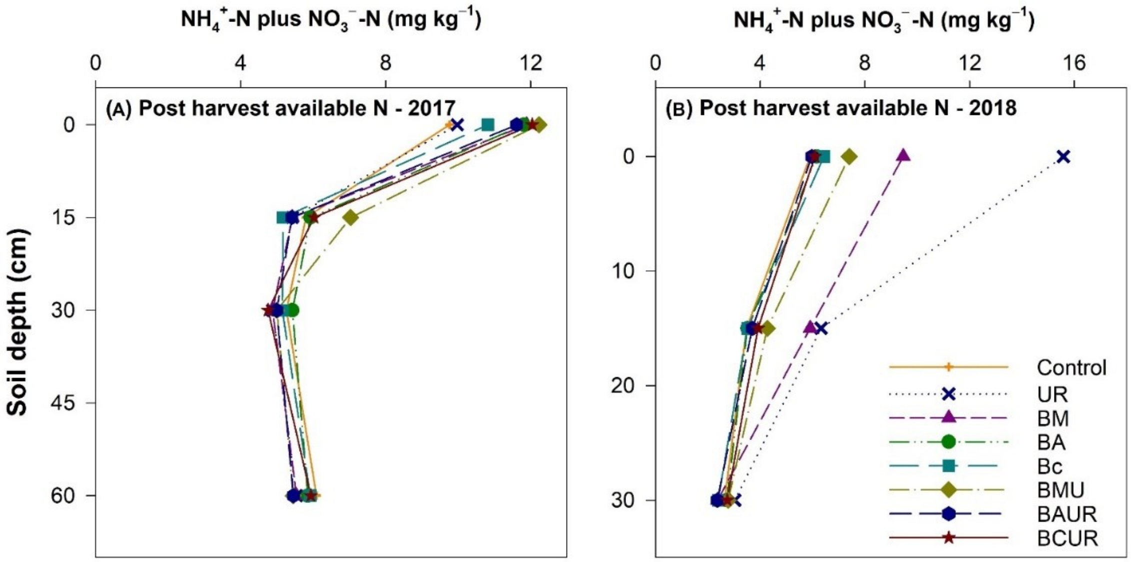

Figure 2-5: Post-harvest available N (mg kg^{-1}) with soil depth for each type of N source measured in (A) the first growing season (2017); and (B) the second growing season (2018). UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; BMUR, mesophilic anaerobic digested biosolids combined with urea; BAUR, alkaline-stabilized biosolids combined with urea; BCUR, composted biosolids combined with urea. Soil samples were collected at depth increments of 0–15, 15–30, 30–60, and 60– 90 cm in August 2017, whereas in August 2018, soil samples were collected at depth increments of 0–15, 15–30, and 30–60 cm.

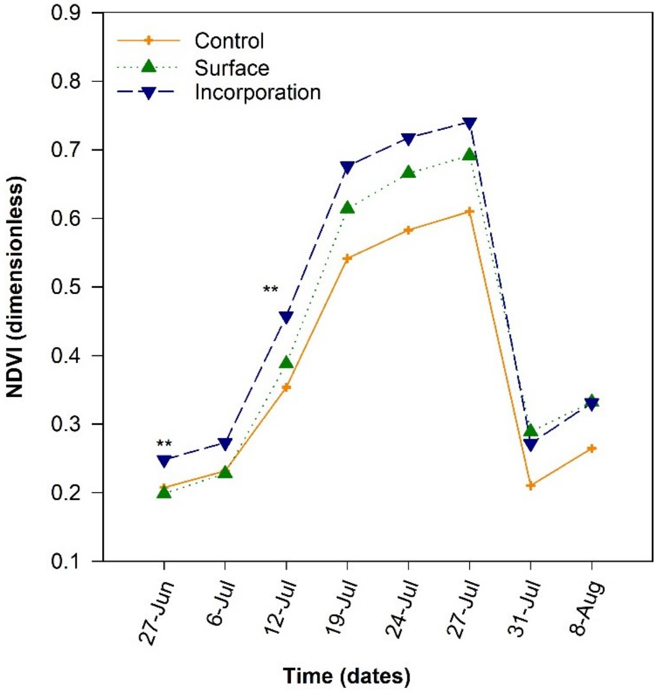

Figure 2-6: Normalized difference vegetation index (NDVI) over the growing season for the control, the average of surface-applied treatments, and the average of incorporation treatments for barley cropland in 2018. ** indicates statistically significant differences (incorporation > surface, *P*s < 0.05).

3.0 Nitrous oxide production and nitrogen transformations in a soil amended with biosolids: Effects of biosolid types and soil moisture contents

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3.1 Abstract

Application of organic materials (e.g., manure, compost, or biosolids) as soil amendments is a recommended practice, as it enables nutrient recycling for plant uptake and the reduction of synthetic fertilizers inputs to agricultural soils. However, the production of N_2O emissions is a concern that arises from such a practice. To date, little is known about how the addition of biosolids to soils can impact N_2O emissions. A 35-day 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 space (WFPS) levels: 28, 40, 52, and 64%. A zero-N-addition control was also evaluated. Across all the three types of biosolid additions, 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 soils receiving BC (409 μg N_2O–N kg^{-1} soil) or BM (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 ($R^2 = 0.83$). Soil inorganic N dynamics indicated that mineralization, nitrification, and denitrification occurred at various stages during the incubation. These results provided insight into the interacting responses of N_2O production to varying soil moisture contents, biosolids treatment stabilization and properties, and soil N availability.

Keywords: nitrous oxide emissions, nitrification rate, biosolids, water-filled pore space

3.2 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 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 around 300 times that of CO_2, and its role as a stratospheric-ozone depleting substance (Wang et al., 2013; Charles et al., 2017).

Nitrous oxide is mainly produced during the processes of nitrification (oxidation of NH_4^+ to NO_3^- via NO_2^-) and denitrification (reduction of NO_3^- to N_2O and N_2) (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).

Biosolids, or treated sewage sludge, are solids, semisolids, or liquid residue 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 management of biosolids is a major challenge because of the large quantities generated (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 vegetation; concurrently, the dependence on commercial fertilizers can be reduced (Christie et al., 2001; Rigby et al., 2009; Brown et al., 2011; Lu et al., 2012). However, there is a risk that N_2O emissions will increase when biosolids are land-applied (Pu et al., 2010; Wijesekara et al., 2016). Nevertheless, the enlarged agricultural carbon footprint that arises from the elevated energy costs of industrial fertilizer production can be reduced by 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 in order 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; Bateman and Baggs, 2005; 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 crop residue (Gentile et al., 2008; García-Ruiz et al., 2012; T. Zhu et al., 2013; X. Zhu et al., 2013), and only a few studies have assessed N_2O from application of biosolids (Inubushi et al., 2000; Pu et al., 2010; Yoshida et al., 2015). Therefore, there is still a knowledge gap regarding the effect of biosolids on N_2O fluxes, particularly those comparing divergent types of biosolids, as the different stabilization methods used for biosolids (e.g., anaerobic digestion, alkaline stabilization, or composting) result in products with contrasting properties. These biosolid properties, as well as soil characteristics, need 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 several contrasting types of biosolid, multiple soil moisture contents and their potential interactive effect on soil N dynamics and N_2O production. We hypothesized that different biosolids stabilization treatments, in combination with increasing soil moisture, would lead to differences in the N dynamics and the amount of N_2O released once the biosolids were applied to the soil.

3.3 Methods

3.3.1 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 soil texture is silty clay loam with 327 g kg^{-1} clay, 511 g kg^{-1} silt, and 162 g kg^{-1} sand. The soil properties are as follows: pH 6.5, 54.8 g kg^{-1} organic carbon, and 4.6 g kg^{-1} total N. Soil samples were collected prior to the beginning of the growing season, in mid-May 2018. The samples were stored at field moisture conditions at 4 °C until the beginning of the experiment.

3.3.2 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 Waterwaste Treatment Facility, both located within Alberta, Canada. Subsamples of each type of fresh biosolids were taken to measure the water content as the gravimetric weight loss, and to determine the total carbon (TC) and total nitrogen (TN) concentrations by dry combustion. 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, which simulates the disturbance of a typical tillage operation. 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 0N, 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 values 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 days to create favorable conditions for microbial activity. After the preincubation period, the corresponding soils were mixed with each type of biosolid at an N rate of 122 mg TN kg^{-1} soil (equivalent to a field application rate of 192 kg N ha^{-1}), whereas the controls did not receive any biosolids. Each soil microcosm was packed to a bulk density of 1.03 g 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 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).

3.3.3 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 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 µmol mol^{-1}, and ultra-high 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.

3.3.4 Flux calculation

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

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

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).

3.3.5 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.

3.3.6 Statistical analyses

Statistical analyses were performed with R Studio software (RStudio Inc.). 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.

3.4 Results

3.4.1 Biosolids' properties

The biosolids' characteristics contrasted strongly among the three types. For example, the total N content of BM was five and two times the N contents of BA and BC, respectively (**Table 3-1**). 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 3-1**). The wide difference in N contents among the three types of biosolids used are associated with differences in the sewage treatment stabilization methods. In the case of BM, anaerobic digestion provides favorable conditions for N mineralization during the process (Yoshida et al., 2015; Rigby et al., 2016), whereas the addition of wood chips during composting (BC) dilutes and decreases the concentration and availability of N during the process. Moreover, in the case of BA, a large portion of the N content in the raw material is lost via volatilization of ammonia during the alkaline stabilization treatment (Yoshida et al., 2015; Rigby et al., 2016). 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 3-1**).

3.4.2 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 (**Fig. 3-1, Fig. 3-2**). Over the incubation period, NO_3^-–N increased (**Fig. 3-2**), whereas NH_4^+–N exhibited fluctuations (**Fig. 3-1)**. In general, NO_3^-–N concentrations increased with increasing soil moisture content (**Fig. 3-2**); the opposite tendency was observed for NH_4^+–N concentrations (**Fig. 3-1**).

Overall, biosolid addition and increasing moisture had significant effects on both NH_4^+–N and NO_3^-–N ($P < 0.05$). 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 (**Fig. 3-1, Fig. 3-2**). 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 ($P < 0.05$).

3.4.3 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 higher nitrification rates with increasing soil moisture up to 52% WFPS in the BM- and BC-amended soils (non-linear relationships, R^2-BM = 0.996, R^2-BC = 0.999; **Fig. 3-3**), up to 55% WFPS in the BA-amended soils (non-linear relationship, R^2-BA = 0.990; **Fig. 3-3**), and 64% in the control soils (linear relationship, $R^2 = 0.998$; **Fig. 3-3**).

Additionally, net mineralization (as the combined accumulation of NH_4^+–N + NO_3^{-}–N) 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., combined NO_3^-–N + NH_4^+–N): an increase from 18 (Day 0) to 36.7 mg N kg^{-1} soil (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 three-fold at 28% WFPS and four-fold at 40, 52, and 64% WFPS by the end of the incubation (on Day 35) (**Fig. 3-1, Fig. 3-2**). Moreover, we observed an increase in cumulative N_2O emissions with NO_3^-–N on Day 35 (**Fig. 3-4**) across all the treatment combinations (exponential fit, $R^2 = 0.825$; Spearman's correlation $\rho = 0.938$, $P < 0.001$).

3.4.4 Nitrous oxide fluxes

Daily N_2O production rates increased with soil moisture and biosolids additions (**Fig. 3-5**). Overall, peak N_2O fluxes occurred shortly after the biosolids additions (ranging from the first 3 h to Day 3 of the incubation) for all of the three types of biosolids, with BC as the biosolid that presented the highest fluxes at each WFPS (**Fig. 3-5**). 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 days from the beginning of the incubation, particularly at 52 and 64% WFPS (**Fig. 3-5c, Fig. 3-5d**). After this initial high activity, daily N_2O fluxes remained relatively low and constant until the end of incubation.

Cumulative N_2O emissions ranged from 14.19 to 409.01µg 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. 3-6**). 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. 3-6**). 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. 3-6**). 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. 3-6**).

3.5 Discussion

The interacting effect between the biosolid N source 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 N nutrients for microbial activity (Banerjee et al., 2016). In a field experiment, Linzmeier et al. (2001) found the significant amounts of N_2O emissions were only released after rainfall events, despite a high NO_3^-–N supply, showing that both factors are concurrently needed for the development of N_2O emissions. 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 to 64% WFPS) (Roman-Perez and Hernandez-Ramirez, 2021). As expected, most of the results showed higher daily and cumulative N_2O

emissions with increasing soil moisture content from 28 to 64% WFPS (**Fig. 3-5, Fig. 3-6**). 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 nitrification as the main process of N transformation at soil moisture levels below 60% WFPS, with 60% WFPS as the optimum condition since the diffusion of both nutrients and O_2 are not limited at this WFPS. Thus, the occurrence of nitrification could have increased the availability of the hydroxylamine and nitrite (NO_2^-–N) substrates for the production of N_2O emissions (Roman-Perez and Hernandez-Ramirez, 2021). In addition, mineralization of the organic N added in the biosolids might have promoted nitrification further in the aerobic domains of the soil. Nevertheless, denitrification might have also occurred in anaerobic microsites located within aggregates present 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, and their availability within these types of biosolids. BM had a higher total N concentration than either BC and BA (**Table 3-1**); moreover, the organic N in BM can be mineralized during the digestion process, leading to more available N in comparison with BA and BC, as shown by the higher proportion of ammonium-N in BM (**Table 3-1**) (Rigby et al., 2009, 2016,). In comparison with BM, the addition of alkaline materials and the resulting N losses (NH_3 volatilization) during the lime stabilization treatment led to lower N contents within BA, whereas the addition of woody bulking agents during composting led to relatively more recalcitrant (slowly turnover), N in BC (Rigby et al., 2016). Aside from the N supply, the addition of biosolids provides available organic C, which leads to increasing microbial respiration and depletes the O_2; this is likely to favor N_2O production in the anaerobic microsites (Gentile et al., 2008; Zhu-Barker et al., 2015; Guenet et al., 2021), particularly when the biosolids are incorporated into the soil (Rigby et al., 2016). In our study, the C content in BM (283.3 g C kg^{-1}) was only slightly higher than that in BC (278.4 g C kg^{-1}), and both had more than double the C content of BA (122.5 g C kg^{-1}) (**Table 3-1**). These relationships of N and C supplies across biosolids and their C:N ratios also explain the higher N_2O emissions activity, especially within the first 9 days of the incubation, shortly after the addition of available C and N substrates from the biosolids. The driving role of the biosolid's properties on N dynamics and N_2O emissions has been observed in earlier studies, including controlled experiments (Gentile et al., 2008; T. Zhu et al., 2013).

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. 3-4**). This non-linear linkage reveals that soils with increased capacity for N_2O production also accumulate the most NO_3^-, thus indicating an overall consistency of high N dynamics in moist soils. Most of the total N_2O emissions were produced within the first nine days of the incubation, as mentioned before, whereas NO_3^-–N likely accumulated over the incubation period (**Fig. 3-2**). 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 towards 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 9 days of incubation, 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-day 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-day incubation experiment. Therefore, in our experiment, most N_2O was probably emitted during the nitrification process because the aerobic conditions in our experiment 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. 3-5**) as well as in NH_4^+–N concentrations (**Fig. 3-1**), although NO_3^-–N concentrations remained high (**Fig. 3-2**). This could be explained by the fact that in the beginning of the incubation, the rapidly-mineralizable C was 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, as it was no longer required as an alternative electron acceptor. 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 provided with the biosolids became depleted, because the moisture conditions were not favorable for NO_3^-–N reduction to N_2O via denitrification (Bateman and Baggs, 2005; Mathieu et al., 2006; Zhu-Barker et al., 2015). This change in microbial processes upon hypothetically reaching a C substrate limitation is likely to induce N immobilization in the soil as well, as perhaps indicated by an overall decrease in NH_4^+–N concentrations (**Fig. 3-1**).

According to the graphic analysis, 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. 3-3**). 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 towards a lower WFPS with organic amendments. The declining nitrification rates in the biosolids-amended soils while the WFPS was lower than 60% might be related to the fact that biosolids provided readily degradable organic C, which was 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 towards 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 3-1, Fig. 3-1, Fig. 3-2, Fig. 3-3**). These postulations are in line with the results of Rigby et al. (2009) from a 90-day 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 days 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 pre-existing 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 pre-existing 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 led to a further 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 pre-existing 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 be also meaningful, as the microbial population and dynamics play a role in N turnover in biosolids (Rigby et al., 2009). Our present study documents how N_2O emissions occur after the addition of biosolids under aerobic conditions in a controlled environment, without plants growing. Therefore, there is also a need to evaluate the effect of applying biosolids under field conditions, as such conditions are subject to weather variability and land management options (e.g., type of crop, N rate application, tillage system).

3.6 Conclusions

This study shows how the addition of contrasting biosolid-N sources, 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 substrates on the microbial processes that generate N_2O emissions (i.e., nitrification and denitrification). The organic carbon added by applying biosolids has a role in N_2O production, since it increases microbial activity, which is likely to decrease soil O_2 as a consequence. The results emphasize the need for additional research to assess the temporal variation of available N as well as the net N mineralization induced by biosolids amendments in order to identify the biosolid rates to be applied in fields, with the aims of preventing N losses to the environment and optimizing nutrient recovery by plants.

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3.9 List of tables

Table 3-1: Characterization of the three assessed biosolids.

Parameter	Unit	Mesophilic anaerobic digested (BM)	Alkaline-stabilized (BA)	Composted (BC)
TC	g C kg^{-1}	283.3 ± 0.47	122.5 ± 0.48	278.4 ± 0.64
TN	g N kg^{-1}	42.4 ± 0.09	7.8 ± 0.02	22.5 ± 0.048
TC:TN		6.69 ± 0.021	15.71 ± 0.100	12.38 ± 0.248
NH_4^+–N	mg N kg^{-1}	8230	1256.8	1402.6
pH§		7.7	12.81	5.01
Electrical conductivity†	dS m^{-1}	6.31	27.5	19.7
Dry matter fraction gravimetric (mass basis)		0.26	0.71	0.76
Moisture fraction (mass basis)		0.74	0.29	0.24

†Measured in a saturated pasta 1:2 soil:water

3.10 List of figures

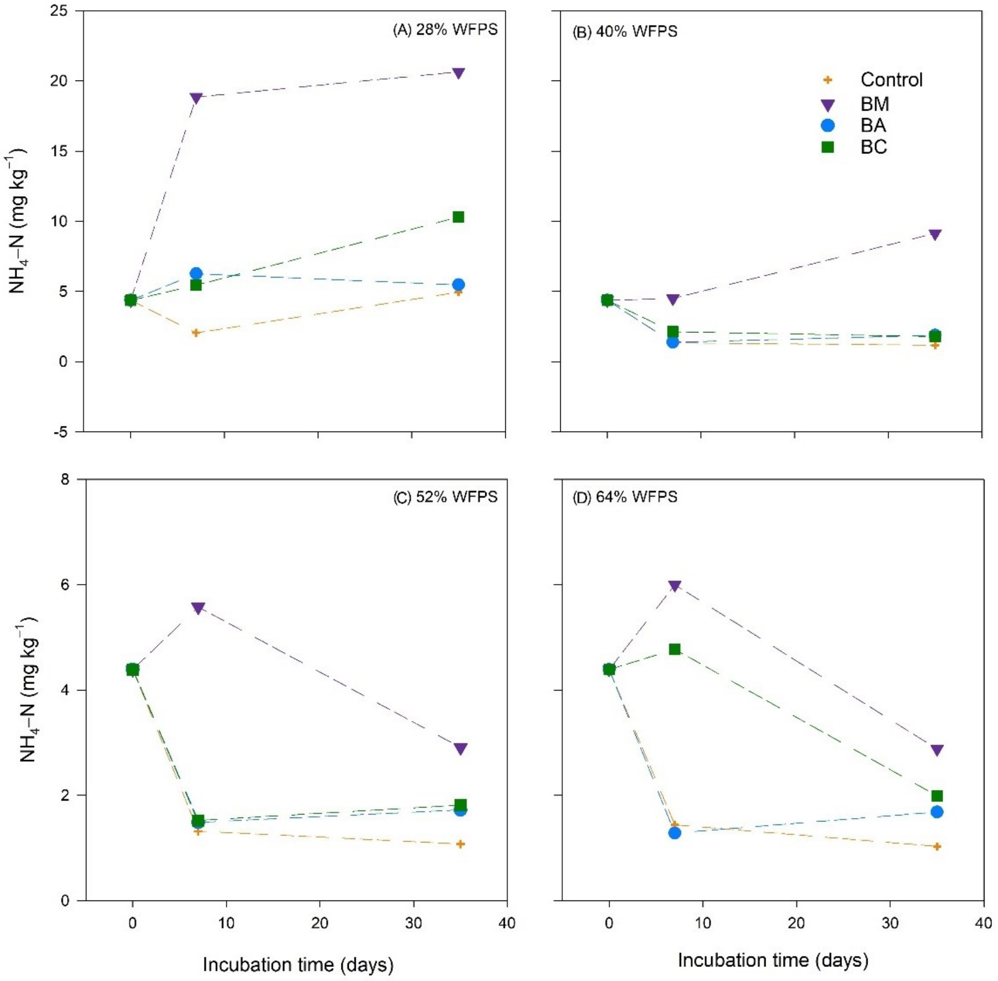

Figure 3-1. Changes in NH_4^+–N concentration (mg N kg^{-1}) during the incubation period at (A) 28, (B) 40, (C) 52, and (D) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control. Vertical scales are different. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid.

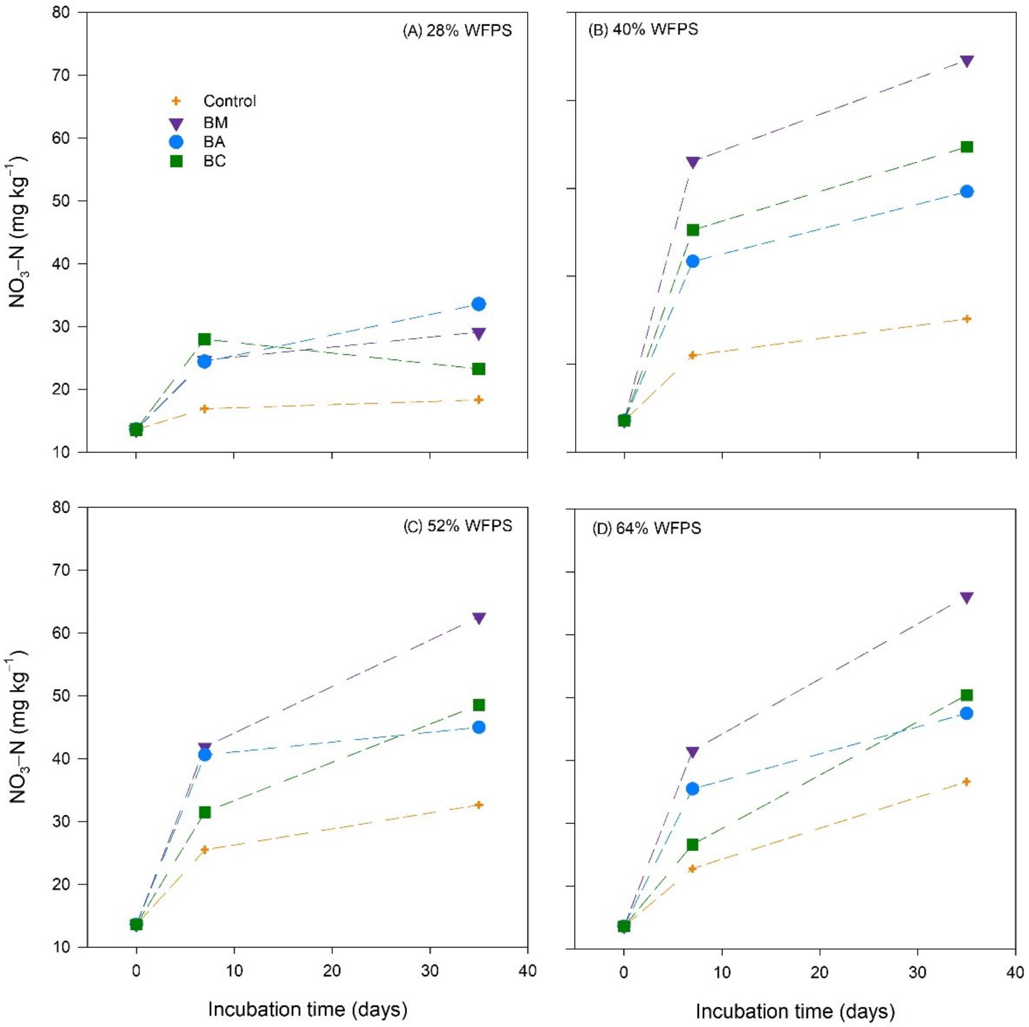

Figure 3-2. Changes in NO_3^-–N concentration (mg N kg^{-1}) during the incubation period at (A) 28, (B) 40, (C) 52, and (D) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid.

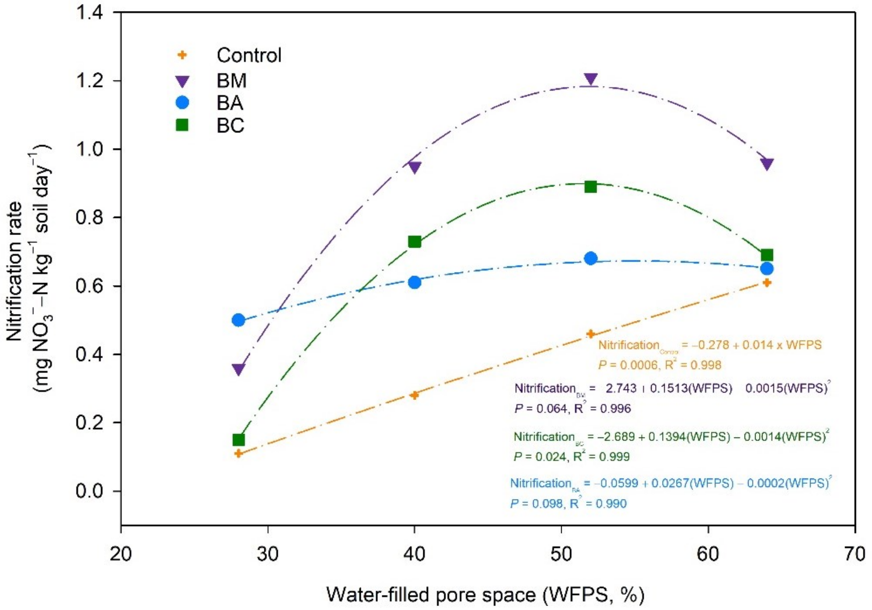

Figure 3-3. Nitrification rates for all biosolid additions and the untreated control as a function of moisture content expressed as water-filled pore space (WFPS). Exponential fitting and equations are provided for each biosolid treatment combination. Linear fitting and the equation are presented for the control treatment combinations. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid.

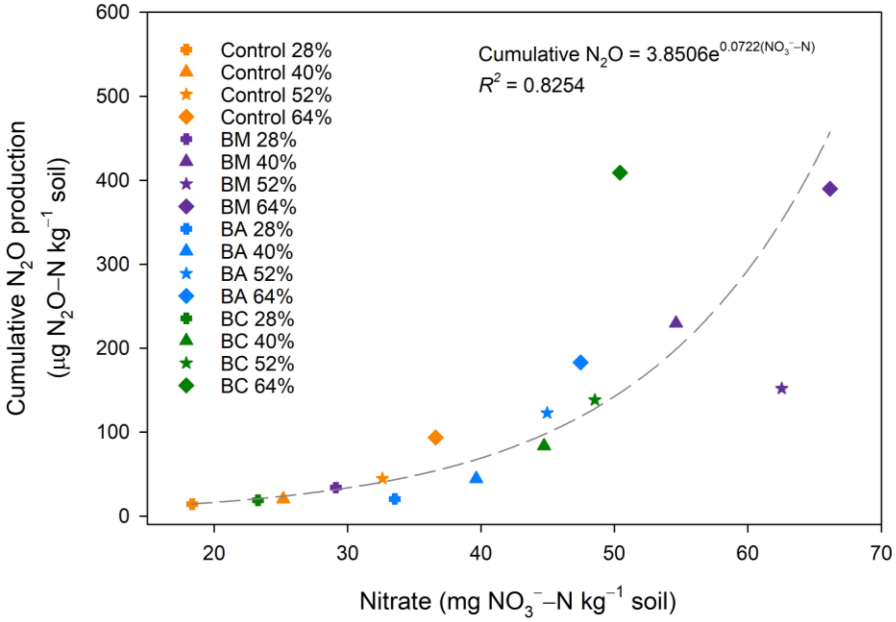

Figure 3-4. Cumulative N_2O–N production (µg N kg^{-1} soil) as a function of NO_3^-–N concentration (mg N kg^{-1} soil) at the end of the incubation period across all treatment combinations (nitrogen sources × water-filled pore space). Exponential fitting and the equation are presented. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid.

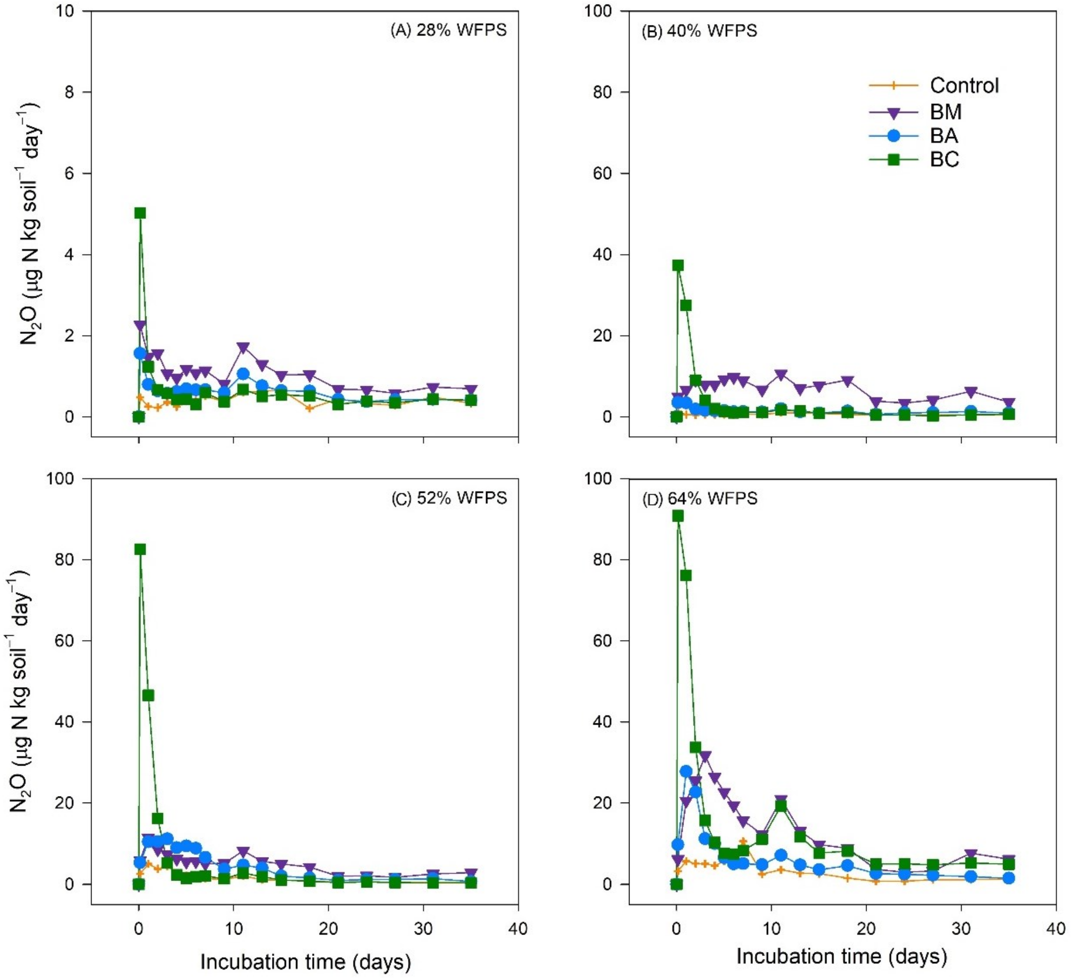

Figure 3-5. N_2O–N fluxes (μg N kg^{-1} soil day^{-1}) at (A) 28, (B) 40, (C) 52, and (D) 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control over a 35-day incubation period. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid. Note the different *y*-scales across some of the panels.

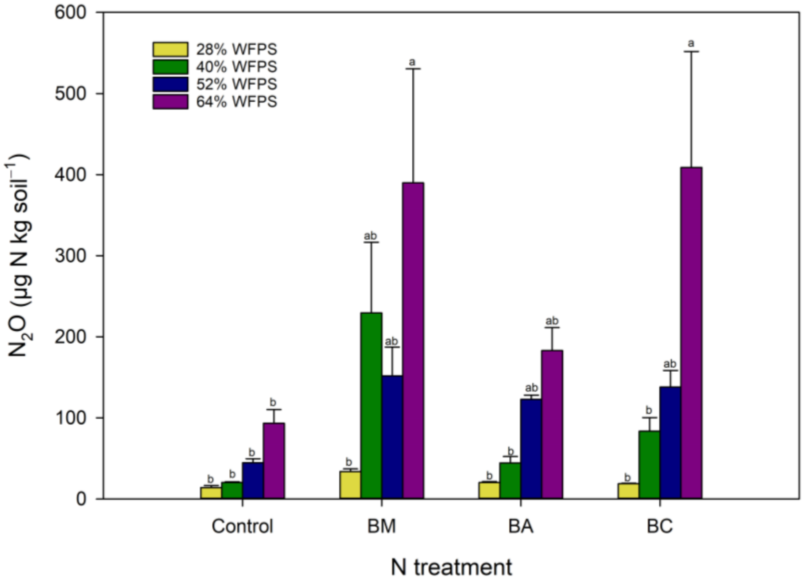

Figure 3-6. Cumulative N_2O–N fluxes (µg N kg^{-1} soil) at 28, 40, 52, and 64% water-filled pore space (WFPS) for all biosolid-amended soils and the untreated control over a 35-day incubation period. BM: mesophilic anaerobic digested biosolid; BA: alkaline-stabilized biosolid; BC: composted biosolid.

4.0 Sources and priming of nitrous oxide production across a range of moisture contents in a soil with high organic matter

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4.1 Abstract

Adding nitrogen fertilizers to agricultural soils contributes to increasing concentrations of nitrous oxide (N_2O) in the atmosphere. However, the impacts of N addition on soil organic matter (SOM) turnover, SOM availability, and the ensuing SOM-derived N_2O emissions remain elusive. Within this context, the net change in direction and rate of SOM-derived N_2O production triggered by added N is termed the N_2O priming effect. This incubation study examined the sources and priming of N_2O production as a function of urea addition and multiple moisture contents in a soil with high SOM (55 g organic C kg^{-1}). We assessed four water-filled pore space (WFPS) conditions: 28, 40, 52, and 64%. Relative to controls receiving no N, urea addition increased N_2O production by 2.6 times ($P < 0.001$). Cumulative N_2O production correlated well with nitrification rates ($r = 0.75$, $P = 0.03$). We used ^{15}N-labelled urea to trace the added urea into N_2O. Of the N added via urea, the recovery as N_2O–N shifted from 0.02 to 0.17% when WFPS increased from 28 to 64% ($P < 0.05$). We also partitioned the N_2O production into urea versus SOM sources. More N_2O was sourced from SOM than urea, with 59 ± 2% N_2O originating from SOM. The magnitude of SOM-derived N_2O under urea was larger than that of the control, revealing that positive N_2O priming was triggered by urea addition. Upon subtracting the controls, the primed N_2O was a consistent 19 ± 2% of the total N_2O produced by urea-amended soils. Nevertheless, the priming magnitude rose sharply with increasing moisture by more than one order of magnitude from 4 to 48 μg N_2O–N kg^{-1} soil, and in exponential mode ($R^2 = 0.98$). Soil moisture, SOM, and nitrification interacted to drive the sources and priming of N_2O.

Keywords: nitrous oxide sources, priming effect, soil organic matter, nitrification, soil moisture

4.2 Introduction

Agricultural soils are major sources of the potent greenhouse gas nitrous oxide (N_2O), contributing 60% of the anthropogenic N_2O emissions to the atmosphere (De Rosa et al., 2016; Van Zandvoort et al., 2017; Della Chiesa et al., 2019; Chai et al., 2020). This is primarily caused by increased application of synthetic N fertilizers (Kim et al., 2013;

Smith, 2017; Thilakarathna et al., 2020). Globally, N_2O emissions caused by N fertilizer applications in agriculture increased from 2.8 Tg N_2O–N year^{-1} in the 1990s to 4.1 Tg N_2O–N year^{-1} by 2006 (IPCC, 2013). Concerns about N_2O emissions arise from its high global warming potential, which is around 300 times that of CO_2, as well as its role as a stratospheric ozone-depleting substance (Wang et al., 2013; Charles et al., 2017).

Adding labile N to soils via synthetic N fertilization can influence soil organic matter (SOM) mineralization by stimulating microbial activity (Löhnis, 1926; Kuzyakov et al., 2000; Daly and Hernandez-Ramirez, 2020). The net change in the direction and rate of SOM decomposition and mineralization is known as the priming effect (Jenkinson et al., 1985; Chen et al., 2014; Thilakarathna and Hernandez-Ramirez, 2020). This triggered acceleration or deceleration markedly increases or decreases available soil N species such as ammonium (NH_4^+–N) and nitrate (NO_3^-–N) (Woods et al., 1987; Liu et al., 2017; Fiorentino et al., 2019), which can be used for producing N_2O (Chai et al., 2020; Lin and Hernandez-Ramirez, 2020; Rees et al., 2020). In other words, N_2O priming consists of the fertilizer-induced N_2O emissions that originate from the SOM substrate (Daly and Hernandez-Ramirez, 2020). Positive priming can exacerbate the contribution of background N_2O produced from existing SOM (Liu et al., 2017; Schleusner et al., 2018), which already represents an important fraction of gross N_2O production in agricultural soils (Della Chiesa et al., 2019; Thilakarathna et al., 2020). Gaining a better understanding of SOM priming and the associated N_2O production derived from SOM is essential for devising management strategies that abate N_2O emissions (Zhu et al., 2013; Lin et al., 2017; Thilakarathna et al., 2020) and for developing more accurate predictions of the N_2O emissions caused by N additions in croplands (Grant et al., 2020).

With the aim of explaining the underlying mechanisms of SOM priming effects on N_2O production, two competing hypotheses can be posited: stoichiometric decomposition vs. N-mining (Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). The stoichiometry hypothesis states that pulse N additions satisfy microbial N requirements for balanced nutrition and the synthesis of exoenzymes, which facilitates increased N mineralization of pre-existing SOM (Curtin et al., 2012, Chen et al., 2014).

This is expected to trigger and accelerate the positive priming effects of SOM on N_2O production. On the other hand, an N-mining mechanism is expected to produce the opposite effect. Daly and Hernandez-Ramirez (2020) recently reviewed the N-mining hypothesis within the context of N_2O production. They described how microbial mining operates by releasing N through decomposition and mineralization of recalcitrant SOM while soil microbial activity is subjected to basal N limitations (Chen et al., 2014; Mason-Jones et al., 2018). However, when the soil system receives an external N input and this N constraint is removed, N-mining declines or stops. Therefore, with the resulting cessation of microbial utilization of mined N, this mechanism generates negative or at least reduced priming of both SOM mineralization and the associated N_2O production shortly after a labile N addition (Daly and Hernandez-Ramirez, 2020). In other words, both stoichiometry and N-mining respond to changes in N availability but in opposite directions. There is still a lack of information regarding which of these two mutually exclusive hypotheses explains and drives the priming effects of N_2O production from SOM. In fact, Daly and Hernandez-Ramirez (2020) suggested the need to postulate multiple simultaneous hypotheses to comprehend the complexity of N_2O priming effects. Our study addresses this knowledge gap.

Among the synthetic N fertilizers, urea is the most commonly used formulation worldwide (Food and Agriculture Organization of the United Nations, 2020), with annual applications to a broad range of agricultural systems (Chai et al., 2020; Thilakarathna et al., 2020). Therefore, it is pertinent to examine the priming effects of SOM availability on N_2O production caused by typical urea applications. In addition to the potential priming effects described above (Daly and Hernandez-Ramirez, 2020), applying urea to soils temporally raises the pH of the soil solution (Overrein and Moe, 1967; Cabrera et al., 1991), which typically induces an increase of SOM solubility and availability for microbial activity (Curtin et al., 1998; Magdoff and Weil, 2004). This pH effect on SOM availability is specific to urea hydrolysis, which does not occur in the case of NH_4^+–N-based fertilizers (Overrein and Moe, 1967; Cabrera et al., 1991). Overall, the manifestation of either a positive or negative priming effect on N_2O emissions following urea addition would encompass the net combined effect of both explicit microbial responses (i.e., stoichiometry or N-mining) and indirect pH-mediated effects on SOM solubility.

The decomposition and mineralization of SOM can also be stimulated by increasing moisture (Curtin et al., 2012; Curtin et al., 2014). Hence, it can be hypothesized that the priming effects of N_2O derived from SOM interact and shift with differences in soil moisture; however, there is a paucity of information regarding this. To date, reports assessing the sources of N_2O production in controlled experiments have been conducted only at a single soil moisture content (Zhu-Barker et al., 2015; Schleusner et al. 2018). Therefore, to better understand and accurately predict how moisture affects N_2O priming, there is a need to evaluate a range of soil moisture contents that are relevant to real field scenarios. Furthermore, in the case of field experiments assessing N_2O sources (Linzmeier et al., 2001; Guardia et al., 2018), most studies examining moisture effects become confounded by weather variability (Chai et al., 2020; Thilakarathna et al., 2020). In fact, to our knowledge, the comparative effects of multiple moisture contents on the sources of N_2O production and the corresponding priming effects have not been comprehensively documented in the available literature. Reports focusing on N_2O priming in soils with high SOM concentrations are even scarcer. Evaluating soils with abundant SOM is important because of their large organic N reservoir that could be remobilized by the priming of exogenous N, leading to crucial ramifications for substantial additional N_2O production (Lin et al., 2017; Thilakarathna et al., 2020).

The objectives of this incubation study were: i) to determine and allocate the N_2O sources from SOM and added labile N (urea) and ii) to examine and quantify the priming effect of added urea on N_2O production derived from SOM at multiple soil moisture contents. The results will further elucidate the linkage between N transformations and the N_2O produced in soils with and without synthetic N fertilizer application. We hypothesized that i) the addition of labile N stimulates extra N_2O production from SOM (a positive priming effect on N_2O production), and that ii) the size of this priming effect increases with increasing soil moisture.

4.3 Methods

4.3.1 Soil collection

We collected composite topsoil (0–15 cm layer) from a field at the Ellerslie Research Station (53°25'13"N, 113°33'03"W) in Edmonton, Alberta, Canada. The climate is categorized as sub-humid with a normal annual precipitation of 441.5 mm yr^{-1}. The soil is classified as an Orthic Black Chernozem according to the Canadian soil classification system (the US soil taxonomy equivalent is Typic Cryoboroll). The texture of the soil is silty clay loam with 32.7% clay, 51.1% silt, and 16.2% sand. Particle size distribution was measured with a laser defraction particle size analyzer (LS 13 32, Beckman Coulter). Soil pH was 6.5 (1:2 soil:water). Soil organic C and total N concentrations were 54.8 and 4.6 g kg^{-1}, respectively, as measured by the dry combustion method with an elemental analyzer (Flash 2000, ThermoScientific). The ^{15}N isotopic composition of the soil was measured with an isotope ratio mass spectrometer (Finnigan Delta V Plus IRMS, Thermo Electron), resulting in 0.37 atom% ^{15}N.

The sampled field was previously managed under annual crop species common to the region, such as wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), and barley (*Hordeum vulgare* L.) (Lin et al., 2017; Thilakarathna et al., 2020). Soil collection was done in mid-May 2018, prior to the start of the new growing season. The field-moist soil was kept at 4 °C until the beginning of the experiment.

4.3.2 Treatment preparation and experimental design

The soil was mixed, and coarse materials were removed. Soil was passed through an 8-mm mesh sieve, which simulates the disturbance of a typical tillage operation. Subsequently, all the soil was placed on a laboratory bench for drying until reaching a target water content [~28% water-filled pore space (WFPS)]. Gravimetric water content was monitored by oven-drying soil subsamples.

The experimental design was a complete randomized design. The experimental factors were the N addition (control 0N vs. ^{15}N-labelled urea) and four soil moisture contents: 28, 40, 52, and 64% WFPS. These WFPSs represent a typical range of topsoil moisture contents found during the growing season in croplands across Central Alberta. The factorial arrangement resulted in eight treatment combinations. Five replicates were prepared: three for measuring the production rate and isotopic composition of N_2O and two for measuring soil NH_4^+–N and NO_3^-–N concentrations over the incubation period.

Soil (equivalent to 0.83 kg on an oven-dry mass basis) was placed into plastic containers (10 cm height x 11.5 cm inner diameter) and preincubated for 3 d. After the preincubation period, each microcosm was prepared by mixing the soil with 5 atom% ^{15}N-labelled urea in powdered form at an N rate of 61 mg urea-N kg^{-1} soil (equivalent to a field urea application rate of 96 kg N ha^{-1}) and then packing the microcosm to a bulk density of 1.03 g cm^{-3}. The same procedures were applied to the controls but without adding N. The selected bulk density represents the effect of a spring tillage operation as found under typical field conditions. The selected N addition rate followed a common urea application rate for croplands in the region (Thilakarathna et al., 2020).

The soil microcosms were moistened to the treatment WFPS. The WFPS of each microcosm was kept constant over the incubation period by adding Milli-Q ultrapure water on the basis of daily weight loss. The microcosms were kept at laboratory room temperature for the duration of the incubation period (average air temperature, 22 °C) and were covered with plastic lids with multiple holes to facilitate gas exchange.

4.3.3 N_2O production and isotopic composition

Concentration and isotopic measurements of N_2O were performed with the mid-infrared quantum cascade laser absorption spectroscope (QCLAS, Aerodyne Research Inc., Billerica, USA) (Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). The laser was thermoelectrically cooled in a hermetically sealed housing; operated in continuous mode; and used a 200-m pathlength, a 2-L volume, and a multiple pass absorption cell for sampling at a pressure of 30 Torr at a flow rate of 1.61 standard L min^{-1}. The laser frequency for N_2O for the QCLAS is 2188 cm^{-1}. Routine calibrations of the QCLAS were done with standard reference gases at the beginning and end of each measurement day (Mohn et al., 2014; Rees et al., 2020). The calibrations also included working standards of synthetic N_2O at concentrations of 0.5 and 1.9 µmol mol^{-1} and ultra-high purity dinitrogen (N_2) for background absorption spectra subtraction. Besides N_2O, the secondary standard gases included CO_2 at concentrations of 396 and 1000 ppm, with a balance of N_2. Nafion (Perma Pure, Lakewood, USA) was used to remove water vapor from the gas samples. Concentrations and ^{15}N:^{14}N isotopic ratios of N_2O were acquired and recorded at 1 Hz resolution with TDLWintel software, which also controlled the QCLAS system.

An automatic non-steady-state chamber system (Eosense eosAC) comprising 12 autochambers was coupled with the QCLAS system in a continuous recirculation mode (Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). This system recirculated each gas sample between the headspace of the soil microcosm and the QCLAS analytical cell. The headspace of the microcosm was created by the autochamber enclosure, generating a total volume of 2.4 L in the recirculating system. The microcosm headspace (chamber enclosure) was closed for 5 minutes to collect the continuous measurements of N_2O. Subsequently, the chamber system was opened to the room air for 1 minute with the aim of returning it to ambient concentrations and isotopic compositions prior to measuring the next chamber. The autochamber system was controlled by the eosAnalyze-AC software. Air temperature (HOBO® UX100, Onset, Bourne, USA) and ambient pressure (Testo 511, Testo, West Chester, USA) were recorded.

The N_2O measurements were conducted 3 hours after the beginning of the incubation and then on Incubation Days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 18, 21, 24, 27, 31, and 35.

4.3.4 Calculation of the N_2O production rate

Nitrous oxide production rates (µg N_2O–N kg^{-1} soil day^{-1}) were derived by a simple linear regression of the increase in concentration in the headspace over time (Mathieu et al., 2006; Chai et al., 2020), scaled to the chamber volume and soil mass, and adjusted to the pressure and temperature at the time of measurement (Savage et al., 2014). A modified ideal gas law was applied as follows:

$$Production\ rate = \frac{Slope \times 2M \times V \times P \times 3600 \times 24}{R \times T \times soil\ mass \times 1000} \quad , \qquad [1]$$

where slope is the linear regression coefficient during the 5 minutes 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).

4.3.5 Allocation of N_2O sources

The use of ^{15}N-labelled urea enabled separation of the N_2O derived into urea and SOM sources (Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). The SOM source encompasses the N mineralized from SOM during the experiment as well as the NH_4^+–N and NO_3^-–N present at the beginning of the experiment, given that the vast majority of native soil N has iteratively cycled through SOM. We calculated the urea and SOM allocations for each measuring day via a two-end member mass balance model on the basis of atom% notation. The atom% ^{15}N–N_2O was determined as the linear regression intercepts of Keeling plots (Keeling 1958; Vardag et al., 2016; Harris et al., 2017). Briefly, each atom% ^{15}N–N_2O measurement (1 Hz) was plotted as a function of the reciprocal of the corresponding N_2O concentration (i.e., $1/[N_2O]$). Subsequently, a linear regression was fitted, and the resulting regression intercept in the axis of atom% ^{15}N–N_2O became the statistical estimate of the N_2O isotopic composition of the soil microcosm (in atom% ^{15}N–N_2O). These Keeling-derived atom% ^{15}N–N_2O measurements enabled us to determine the relative contributions of the N_2O sources (urea and SOM). Under certain specific conditions, the N_2O produced in the soil can be reduced to N_2, and this shift affects the isotopic composition of the emitted N_2O (Congreves et al., 2019; Daly and Hernandez-Ramirez, 2020). Hence, as customary, the data-processing protocols examined and accounted for the isotopic effect of the conversion from N_2O to N_2 on the basis of pre-established linear relationships (Ostrom et al., 2007; Yamamoto et al., 2017; Congreves et al., 2019). This correction became applicable only in 4.9% of the entire dataset, indicating that the isotopic fractionation effect of N_2O conversion to N_2 was minimal in this experiment. This is consistent with the aerobic conditions and intermediate moisture range assessed in our study, which are known to be non-conducive for complete denitrification and the associated N_2 production (Balaine et al., 2016; Daly and Hernandez-Ramirez, 2020).

The mixing model used to determine the relative contributions of the N_2O sources (urea and SOM) is as follows:

$$Fraction\ N_2O_{urea} + Fraction\ N_2O_{SOM} = 1\ ; \qquad [2]$$

$$at\%^{15}N_{urea\ treatment} = at\%^{15}N_{urea} \times Fraction\ N_2O_{urea} + at\%^{15}N_{SOM} \times Fraction\ N_2O_{SOM} \quad , \qquad [3]$$

where $Fraction\ N_2O_{urea}$ and $Fraction\ N_2O_{SOM}$ are the contributions of the urea and the pre-existing SOM to the total N_2O in the urea-amended microcosms (as fractions), $at\%^{15}N_{urea\ treatment}$ is the atom% ^{15}N of the total N_2O produced by the urea-amended microcosms, $at\%^{15}N_{urea}$ is 5 atom%, and $at\%^{15}N_{SOM}$ is the atom% ^{15}N in the N_2O produced by the SOM. The isotopic composition of the N_2O produced by SOM source was measured in the control microcosms, with a typical mean ± standard error of 0.348 ± 0.011 atom% ^{15}N.

The daily N_2O produced by the urea and SOM sources was calculated by multiplying the total N_2O emitted from the urea-amended microcosms and the respective fraction:

$$SOM\ derived\ N_2O = Fraction\ N_2O_{SOM} \times Total\ N_2O_{urea\ treatment,} \qquad [4]$$

$$Urea\ derived\ N_2O = Fraction\ N_2O_{urea} \times Total\ N_2O_{urea\ treatment.} \qquad [5]$$

The daily magnitude of the N_2O priming effect caused by urea addition was calculated as the difference between the SOM-derived N_2O in urea-amended microcosms and the total N_2O production in the control at each WFPS:

$$Primed\ N_2O = SOM\ derived\ N_2O\ - \ Total\ N_2O_{control}\ . \qquad [6]$$

A positive priming effect of urea addition is an excess of SOM-derived N_2O above the total N_2O production of the controls (baseline without urea), expressed on a daily or cumulative basis as an absolute magnitude or as a relative quantity (%) relative to the total N_2O emitted from the urea-amended microcosms.

We further calculated the ^{15}N recovery of added urea as N_2O–N produced and expressed this recovery as a relative quantity (%).

Cumulative production, sources, and priming of N_2O were calculated via linear interpolation of consecutive measurements over the incubation period.

4.3.6 NH_4^+–N and NO_3^-–N concentrations

To assess the evolution of NH_4^+–N and NO_3^-–N concentrations in the soil throughout the duration of the experiment, soil samples were separated immediately prior to treatment application at the beginning of the incubation. Additionally, two replicates encompassing all eight treatment combinations were destructively sampled on Day 7 of incubation. Finally, at the end of the incubation study, the soils were removed from all remaining microcosms (three replicates). Nitrate and NH_4^+–N were extracted from 5-g soil samples with 50 mL of 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). Filtrates were analyzed via a colorimetric method on a Thermo Gallery Plus Beermaster Autoanalyzer. As part of this method, vanadium chloride was used to reduce the NO_3^-–N to nitrite (NO_2^-–N); hence, our NO_3^-–N concentrations include both NO_3^- and the pre-existing NO_2^-–N in the samples.

4.3.7 Statistical analyses

Statistical analyses were performed with R Studio software (version 1.1 3.8.3, RStudio Inc, 2017) at an alpha critical value of 0.05. Regression analyses were performed to test the relationships amongst the continuous variables. The analysis of variance (ANOVA) model included the fixed effects of N addition, WFPS treatment, and the two-way interaction (N × WFPS). Following significant ANOVAs, 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. Where error terms are presented, they correspond to the standard errors of the means.

4.4 Results

4.4.1 Total N_2O production

Daily N_2O production rates increased with soil moisture and urea addition (**Fig. 4-1A, Fig. 4-1B**). Shortly after the urea addition, peak N_2O production was observed during the first 7 d of the incubation. After this initial high activity, daily N_2O production rates remained relatively low and constant until the end of the incubation.

The N addition × WFPS interaction was not significant. However, the main effects of N addition and soil moisture on cumulative total N_2O production were consistently significant (Ps < 0.001) (**Table 4-1**). Relative to the controls, urea addition increased N_2O production by 2.6 times when averaged across moisture contents. More specifically, at 40% WFPS, the N_2O produced by the urea-amended soil was significantly higher than the control ($P < 0.05$) (**Table 4-1**). Moreover, cumulative total N_2O production ranged from 14.19 µg N_2O–N kg^{-1} soil in the control (without urea) at 28% WFPS to 246.85 µg N_2O–N kg^{-1} soil receiving urea at 64% WFPS (**Table 4-1, Fig. 4-2C, Fig. 4-2D**).

4.4.2 SOM source of N_2O production

After all urea-amended soils were averaged across moisture contents, the average (± SEM) fraction of N_2O derived from the SOM source corresponded to a consistent substantial 59.4 ± 2.4% ($n = 12$) (**Table 4-1**; **Fig. 4-1C,** inset and **Fig. 4-3**). In other words, numerically more N_2O was produced from SOM than from the added urea in the soils receiving urea regardless of the WFPS. Although the SOM-derived N_2O fraction did not differ across moisture contents ($P > 0.05$), the magnitude of cumulative SOM-derived N_2O was significantly different among several of the WFPSs (64% > 40 and 28%; 52% > 28%; $P < 0.05$; **Table 4-1; Fig. 4-2B**).

4.4.3 N_2O priming effect

On a cumulative basis, the magnitude of SOM-derived N_2O production was consistently larger than the total N_2O production of the control for each of the four soil moisture contents (**Table 4-1; Fig. 4-2B; Fig. 4-2C**), revealing that a strong positive priming effect was triggered by the addition of urea (**Fig. 4-2A**).

After the daily total N_2O production of the controls was subtracted from the daily SOM-derived N_2O production at each WFPS (**Fig. 4-1A** and **Fig. 4-1C**), the pattern of N_2O priming manifested clearly (**Fig. 4-1E**). In general, the daily primed N_2O production was markedly greater during the first 6 d of the incubation and then shifted to be near neutral for the rest of the experiment.

Relative to the total N_2O produced by the urea-amended soils, the cumulative primed N_2O production was consistently 19.4 ± 2.0% when averaged across moisture contents ($P > 0.05$; **Table 4-1**; **Fig. 4-1E,** inset).

Increasing the moisture intensified the magnitude of N_2O priming (**Fig. 4-2A**). The magnitude of cumulative primed N_2O production increased exponentially with WFPS ($R^2 = 0.980$; **Fig. 4-2A**). This clear exponential behavior was inherently linked to the responses of total and SOM-derived N_2O production to moisture. With increases in WFPS, we also noticed exponential increases in cumulative SOM-derived N_2O production ($R^2 = 0.999$; **Fig. 4-2B**) as well as in total N_2O production of the untreated control soils ($R^2 = 0.994$; **Fig. 4-2C**) and the urea-amended soils ($R^2 = 0.997$; **Fig. 4-2D**).

When WFPS increased from 28 to 64%, the magnitude of cumulative primed N_2O production increased numerically by 12-fold (3.82 vs. 48.47 µg N_2O–N kg^{-1} soil, respectively; **Table 4-1; Fig. 4-2A**).

4.4.4 Urea source and recovery in N_2O production

The daily N_2O production specifically derived from added urea reached a maximum on incubation Day 2. This overall peak of high urea-derived N_2O production lasted from

Day 1 to Day 3 (**Fig. 4-1D**). This peak for urea-derived N_2O was shorter and lower than the peak generated by SOM-derived N_2O production, which lasted from incubation Days 1 to 6 (**Fig. 4-1D**, **Fig. 4-1C**). In other words, following peak N_2O production, the rate of decline was much faster in the urea-derived N_2O than in the SOM-derived N_2O.

When the urea-amended soils were averaged across the four moisture contents, the fraction of N_2O production derived specifically from added urea corresponded to 40.6 ± 2.4 % (direct contribution of N added via urea). The N_2O primed after urea addition was +19% (indirect contribution of adding urea); therefore, the overall N_2O produced by urea addition is 60% of the total N_2O production in the urea-amended soils.

The recovery of N added via urea as cumulative N_2O–N varied widely by one order of magnitude from 0.015 ± 0.001 to 0.172 ± 0.048% with increasing WFPS from 28 to 64%, respectively ($P < 0.05$) (**Table 4-1**). As an equivalent to the N_2O emission factor of N fertilization in fields, we also calculated the coefficients of added urea (**Table 4-1**), which numerically changed from 0.02 to 0.25% for 28 and 64% WFPS ($P > 0.05$), respectively.

4.4.5 NO_3^-–N and NH_4^+–N concentrations and nitrification rates

The initial NO_3^-–N and NH_4^+–N concentrations in the soil were 13.61 mg N kg^{-1} and 4.39 mg N kg^{-1}, respectively (**Table 4-2**). With time, NH_4^+–N decreased but NO_3^-–N accumulated, and the combined outcome of NH_4^+–N + NO_3^-–N increased consistently in each of the eight treatment combinations (**Table 4-2**). In general, these shifts in soil N were even more pronounced during the first 7 d of the experiment. As expected, both N addition and increasing moisture had significant effects overall on increasing the available N concentration (NH_4^+–N + NO_3^-–N concentrations) ($P < 0.05$).

The transformation of NH_4^+–N into NO_3^-–N in all treatment combinations indicated rapid nitrification rates. Faster nitrification rates were seen with increasing soil moisture. This was particularly evident in the case of the untreated control soils, where a robust significant linear relationship between nitrification rates and WFPS emerged ($R^2 = 0.998$; **Fig. 4-2E, Table 4-2**). On the other hand, this relationship was not found in the urea-amended soils, where nitrification rates were one order of magnitude higher than in the control soils and were much more variable (**Fig. 4-2F, Table 4-2**). In fact, across the eight treatment combinations, nitrification rates in the control soils at 28 and 40% WFPS were significantly lower than in the urea-amended soils at 52 and 64% WFPS ($P = 0.002$; **Table 4-2**). In general, average (± SEM) nitrification rate in the control soil was 0.31 ± 0.05 mg NO_3^-–N kg^{-1} soil day^{-1}; this value was significantly different from the urea-amended soils, which had an average nitrification rate of 1.07 ± 0.23 mg NO_3^-–N kg^{-1} soil day^{-1} (two-tailed t-test $P = 0.018$) (**Fig. 4-2E**, **Fig. 4-2F**, **Table 4-2**).

Rising nitrification rates were significantly associated with an increase in total N_2O production rates for both urea-amended and control soils ($r = 0.75$; $R^2 = 0.57$, $P = 0.03$; **Fig. 4-4**). This correlation between nitrification and N_2O production was particularly evident in the case of the control soil across the four moisture contents (**Fig. 4-4**).

Similar to changes in nitrification, net mineralization shifted with increasing moisture. This was a clear reflection of the combined accumulation over time of NH_4^+–N + NO_3^-–N derived specifically from the SOM source in the controls (without added urea). For instance, the mineralized N concentration more than doubled in the control soil at 64% WFPS over the incubation period from 18 mg N kg^{-1} soil (4 mg N kg^{-1} soil from NH_4^+–N plus 14 mg N kg^{-1} from NO_3^-–N) at the beginning of the incubation period (time 0) to 38 mg N kg^{-1} soil (1 plus 37) on Day 35 (**Table 4-2**).

4.5 Discussion

A positive priming effect of N_2O production manifested as an asymmetrical interaction between SOM mineralization and a pulse availability of added urea. Although the percentage of priming was found to be consistent (+19.4 ± 2.0 %), we clearly witnessed the vigorous response of primed N_2O magnitudes to increasing soil moisture (**Fig. 4-2A**). This supports our initial hypotheses that i) adding labile N stimulates extra N_2O production from SOM, and ii) higher moisture increases the magnitude of primed N_2O production. This is the first report in the literature specifically documenting and quantifying the direct dependency of primed N_2O magnitude on a range of soil moisture contents. For instance, Schleusner et al. (2018) evaluated only one soil moisture content (i.e., 60% WFPS), reporting positive priming that ranged from 5 to 8% of the total N_2O production. Because our study represents a range of moist, aerobic conditions (i.e., 28 to 64% WFPS), the stimulating role of increasing moisture on primed N_2O production could be ascribed to greater diffusion of SOM dissolved in the soil solution. As the volume in the soil solution increased and concentration gradients of dissolved SOM rose, an increase in solute diffusion may have enhanced the supply, redistribution, and delivery of SOM substrates across the pore network within the soil (Curtin et al., 2012; Lin and Hernandez-Ramirez, 2020). In other words, with more moisture, the remobilization and diffusion of available SOM through the soil solution boost its utilization for decomposition and mineralization as mediated by microbes and their exoenzymes (Linn and Doran, 1984; Curtin et al., 2012; Chai et al., 2020).

If we compare two competing hypotheses that aim at explaining SOM priming—N-mining vs. stoichiometry (Chen et al., 2014; Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020)—the fact that positive priming was clearly dominant in the studied soil supports the relevance of the stoichiometry hypothesis. In other words, urea addition can satisfy the stoichiometric prerequisites by microbial activity for further growth and synthesis of exoenzymes (Curtin et al., 2012, Chen et al., 2014), which can collectively enable fast N mineralization from SOM. Consequently, ample availabilities of SOM-C and SOM-N in the soil system (Jenkinson et al., 1985; Xu et al., 2019) can generate a consistently positive N_2O priming effect. This inference applies instead of a microbial N-mining mechanism, which typically operates under an N constraint (Chen et al., 2014; Mason-Jones et al., 2018; Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). Beyond this dualistic approach, it is plausible that a successional shift in priming mechanisms could take place, with stoichiometry initially controlling microbial activity shortly after N addition, transitioning over time into N-mining mechanisms as the initial soil N availability gradually becomes constrained or immobilized in SOM. Prolonged priming experiments tracing N_2O–N sources and simultaneously examining microbial activity could test for this hypothetical succession or even the co-existence of multiple priming mechanisms (Daly and Hernandez-

Ramirez, 2020). Regardless of the underlying priming mechanism (e.g., stoichiometry vs. mining) and the moisture-induced diffusion effects noted above, the actual transformations of the available N substrate pool (encompassing background N derived from SOM, newly mineralized N from SOM, and added fertilizer-N) is collectively operated and facilitated by the enzymatic activity of soil microbes as an overarching mediating entity (Curtin et al., 2012).

Adding labile N to a soil rich in SOM, as in our study, exacerbated the N_2O being produced from SOM mineralization to the extent that more than half of the N_2O originated from SOM. Our soil with high organic C (54.8 g organic C kg^{-1} soil) resulted in 59% of the total N_2O produced by urea-amended soils being sourced from SOM (**Fig. 4-3**). This finding is consistent with several existing studies. Similar to our study, Müller et al. (2014) found that more than half of the total N_2O came from the SOM, according to experimental and modeling work in an old grassland soil enriched with organic C (i.e., 66 g C kg^{-1}) (**Fig. 4-3**). This is also in line with Buckthought et al. (2015), who studied a grazed pasture with a high soil organic C (i.e., 55 g C kg^{-1}), that had received double the urea addition rate of our study, in which the SOM contribution still made up the majority of the N_2O produced. As for our soil, it is reasonable to assume that a major contribution of SOM mineralization to both the total and the primed N_2O can emerge only when an abundant SOM substrate exists. Furthermore, relative to the results of our study, other available reports showed much lower SOM contributions to N_2O production as a function of their much lower soil organic C concentrations. For instance, the results of both Guardia et al. (2018) and Schleusner et al. (2018) documented low primed SOM contributions to N_2O production, which were probably constrained by the much lower organic C in their soils (**Fig. 4-3**). Guardia et al. (2018) found only a 30% SOM contribution to the total N_2O production in a soil with a very low organic C concentration (i.e., 8 g C kg^{-1}). Similarly, Schleusner et al. (2018) quantified SOM-derived N_2O being as low as 9 to 13% of the total production in soils with organic C concentrations ranging between 11 and 14 g C kg^{-1}, respectively. Overall, compared with the range of these earlier studies (Müller et al., 2014; Buckthought et al., 2015; Guardia et al., 2018; Schleusner et al., 2018), the soil in our study could be considered as a relatively high SOM contributor to N_2O production (**Fig. 4-3**).

In our study, the dynamic turnover of SOM also became self-evident through the elevated background N_2O production. The total N_2O production of the controls was typically 40% of that in the urea-amended soils (i.e., 40 ± 4% in **Fig. 4-1A**; 93 / 247 for the 64% WFPS treatment in **Table 4-1**). Background N_2O production represents an important fraction of gross N_2O production in fertilized agricultural soils (Della Chiesa et al., 2019; Chai et al., 2020; Thilakarathna et al., 2020). Earlier reports further support the notion that additional N mineralized from the native SOM pool and converted into NH_4^+–N and NO_3^-–N becomes a substrate that is available for primed N_2O production (Woods et al., 1987; Liu et al., 2017; Fiorentino et al., 2019; Daly and Hernandez-Ramirez, 2020). For instance, after assessing soils with initially low background concentrations of NH_4^+–N and NO_3^-–N, as in our study (**Table 4-2**), Woods et al. (1987) reported enhanced N mineralization from SOM following the addition of labile N. Likewise, in line with our results, Di and Cameron (2008) found that fast nitrification following intense mineralization of SOM influenced N_2O emissions. The initial NH_4^+–N and NO_3^-–N as well as the mineralized N in our study were derived from SOM as part of the iterative cycling of native soil N through SOM. Within this context, we suggest that the decomposition of SOM and subsequent ammonification are co-determining steps of the magnitude of N_2O priming. Future research could further address the effects of C availability on the primed N_2O derived from SOM (Kuzyakov et al., 2000; Thilakarathna and Hernandez-Ramirez, 2020). This is because an increased C allocation to the recalcitrant SOM pool can decrease N availability (Woods et al., 1987; Li et al., 2018). Likewise, SOM availability is influenced by temporal changes in soil pH that are induced by the rapid hydrolysis of added urea in the soils (Curtin et al., 1998; Magdoff and Weil, 2004). Early work by Overrein and Moe (1967) revealed that urea incorporated into the soil increases the pH in the immediately adjacent soil solution from 6.5 to 8.1 for over 1 week. Such an increase in pH magnitude has been found to increase the mineralization of N and C from the existing SOM pool by two-fold (Curtin et al., 1998). In other words, as the soil solution becomes alkaline through urea hydrolysis (Overrein and Moe, 1967; Cabrera et al., 1991), enhanced solubility and availability of SOM for increased microbial activity (Curtin et al., 1998; Magdoff and Weil, 2004) can contribute to increasing the primed N_2O production following urea additions. This indirect contribution to the priming effect caused by urea hydrolysis is currently uncertain. Future research can also aim at separating the priming contributions of microbial responses to exogenous N availability (i.e., stoichiometric decomposition or N-mining of SOM) vs. urea-induced pH effects on SOM availability. This investigation could be addressed via isotope labeling at several addition rates of urea vs. ammonium nitrate as well as examining CO_2 sources (Daly and Hernandez-Ramirez, 2020). In addition to the influence of N availability and pH on SOM mineralization, it is being recognized that other factors, such as soil texture (Gentile et al., 2008), plant residue incorporation (Rezaei Rashti et al., 2017; Xu et al., 2019), and nitrification inhibition (Linzmeier et al., 2001; Guardia et al., 2018; Thilakarathna and Hernandez-Ramirez, 2020), can modulate and interact with the priming effects of N addition on N_2O derived from SOM.

The primed SOM mineralization triggered by the addition of urea was closely coupled with the subsequent nitrification of the newly available NH_4^+–N derived from SOM, particularly during the first 7 d of the experiment (**Table 4-2**). In addition to increased NH_4^+–N and NO_3^-–N (**Table 4-2**), nitrification that cascaded from the priming of SOM mineralization probably augmented the availability of the hydroxylamine and NO_2^-–N substrates for primed N_2O production. This is expected because ammonification of SOM-derived N in aerobic soils is closely followed by nitrification, and both hydroxylamine and NO_2^-–N are byproducts during nitrification (Hu et al., 2015; Qiu et al., 2016; Congreves et al., 2019; Daly and Hernandez-Ramirez, 2020). As recently found by Thilakarathna and Hernandez-Ramirez (2020), the increased availability of native N substrates early in the experiment is also a reflection of daily SOM-derived N_2O production reaching a sharp peak 24 hours after N addition (**Fig. 4-1C**).

Our incubation study documented the existence of N_2O priming in soils, where the nonlinear responses of N_2O priming to moisture (**Fig. 4-2**) and also probably to soil organic C (**Fig. 4-3**) commonly manifest (Kim et al., 2013; Lin and Hernandez-Ramirez, 2020). Nevertheless, there is still a need to further examine and quantify such priming responses in soil profiles in typical fields and over entire annual cycles (Chai et al., 2020; Thilakarathna et al., 2020). A comprehensive understanding of priming effects in agricultural systems receiving exogenous N inputs can provide insights and considerations for proactively managing N additions while reducing N_2O emissions (Kim et al., 2013; Lin et al., 2017; Thilakarathna et al., 2020; Thilakarathna and Hernandez-Ramirez, 2020) as well as for mechanistically modeling and predicting N_2O emissions (Müller et al., 2014; Chai et al., 2020; Grant et al., 2020).

Beyond the insights gained from this study, more research is needed encompassing a broader diversity of soils, biophysical conditions, and long-term recurrent N inputs. We hypothesize that repeated N fertilizer applications will create inherent priming of the soils—similar to soil biological memory (Banerjee et al., 2016; Targulian and Bronnikova, 2019; Thilakarathna and Hernandez-Ramirez, 2020)—by selecting, training, and acclimating microbial activity for rapid utilization of N added in large, recurrent pulses. This notion is in line with Thilakarathna and Hernandez-Ramirez (2020) who recently reported legacy effects of annual, long-term urea additions on the positive priming of N_2O fluxes from soils continuously cropped to barley. Additionally, where biophysically relevant, examining soils at near saturation could inform whether anoxic microenvironments in the soil could decrease net N_2O priming by favoring N_2O reduction to N_2 as part of complete heterotrophic denitrification (Balaine et al., 2016; Daly and Hernandez-Ramirez, 2020), even leading to an apparent negative N_2O priming. This collectively entails that the contribution of N_2O priming would reach a maximum at an intermediate moisture range (Linn and Doran, 1984; Lin and Hernandez-Ramirez, 2020). Likewise, the priming effect of plant roots following additions of labile N also remains understudied (Liu et al., 2017; Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2020). Our incubation study did not capture the potential effects of living plant roots. For instance, root growth and exudation in conjunction with exogenous N additions could positively prime N_2O production from denitrification directly by donating electrons or indirectly by increasing microbial respiration and the associated oxygen consumption. These parallel cooperating hypotheses warrant further investigation.

4.6 Conclusion

This study documents how labile N addition and soil moisture concurrently affect N_2O production from the SOM sources. Within a moisture range of 28 to 64% WFPS, the disproportional response of the soil system to urea additions generated a positive N_2O priming and a shift towards having more N_2O produced from SOM than from the added N. Moreover, the priming partitioning fractions were consistent across typical moisture contents experienced by the evaluated SOM-rich soil under field conditions. The priming effects of N fertilizer application as well as their interactions with the native C and N availability in the soil need to be studied further to gain additional insights into their combined stimulating influences on soil N cycling and N_2O emissions to the atmosphere.

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4.9 List of tables

Table 4-1: Cumulative total N_2O production from control treatments and urea treatments; soil organic matter (SOM)-derived, urea-derived, and primed N_2O; recovery of N added via urea as cumulative N_2O–N and urea addition coefficients; SOM-derived N_2O and primed N_2O at four soil moisture contents over a 35-d incubation (± SEM).

Moisture treatments WFPS (%)	Control treatments Total N_2O	Urea treatments Total N_2O	SOM-derived N_2O[†]	Urea-derived N_2O[‡]	Primed N_2O[§]	Urea to N_2O recovery[#]	Urea addition coefficient[¶]	% SOM-derived N_2O[††]	% Primed N_2O[‡‡]
	μg N_2O–N kg^{-1} soil					%			
28	14.19 ± 2.5 ^{D}[§§]	27.17 ± 1.1 ^{CD}	18.01 ± 0.3 ^{c}[§§]	9.17 ± 0.9 ^{c}	3.82 ± 2.8	0.015 ± 0.001 ^{c}	0.02 ± 0.00	66	14
40	20.23 ± 1.0 ^{CD}	63.95 ± 5.1 ^{B}	35.33 ± 2.5 ^{bc}	28.62 ± 4.3 ^{b}	15.10 ± 1.8	0.047 ± 0.007 ^{b}	0.07 ± 0.01	55	24
52	44.68 ± 4.9 ^{BC}	115.96 ± 28.2 ^{AB}	68.08 ± 19.8 ^{ab}	47.89 ± 9.3 ^{ab}	23.39 ± 17.4	0.079 ± 0.015 ^{ab}	0.12 ± 0.05	59	20
64	93.42 ± 16.7 ^{AB}	246.85 ± 72.6 ^{A}	141.89 ± 46.6 ^{a}	104.96 ± 29.0 ^{a}	48.47 ± 60.8	0.172 ± 0.048 ^{a}	0.25 ± 0.12	58	20
Overall mean	43.13 ± 10.1	113.48 ± 30.1	65.83 ± 17.9	47.66 ± 12.6	22.70 ± 14.4	0.078 ± 0.021	0.115 ± 0.04	59.4 ± 2.4	19.4 ± 2.0

Note. Data are mean ± SEM. WFPS, water-filled pore space.

[†] Calculated via Equation [4] per experimental replicate ($n = 3$).

[‡] Calculated via Equation [5] per experimental replicate ($n = 3$).

[§] Calculated via Equation [6] per experimental replicate ($n = 3$).

[#] Urea-derived N_2O–N expressed as a fraction of added urea-N (61 mg urea-N kg^{-1} soil) for the experimental replicates ($n = 3$).

[¶] $Urea\ addition\ coefficient = [(Urea\ treatment\ total\ N_2O - Control\ treatment\ total\ N_2O)/Added\ urea\ N\ rate] \times 100$ calculated per experimental replicate ($n = 3$). These are equivalent to the emission factors calculated for field fertilizer inputs.

[††] $\%\ SOM = (SOM - derived\ N_2O/Urea\ treatment\ total\ N_2O) \times 100$ calculated from treatment means.

[‡‡] $\%\ Primed\ N_2O = (Primed\ N_2O/Urea\ treatment\ total\ N_2O) \times 100$ calculated from treatment means.

[§§] Uppercase letters correspond to significant comparisons for total N_2O across all eight treatment combinations (N addition × moisture); lowercase letters indicate significant differences among the four moisture treatments within a given column. Statistics are based on ANOVA followed by Tukey's Honest Significant Difference test ($P < 0.05$).

Table 4-2: Soil NH_4^+–N and NO_3^-–N concentrations on Days 7 and 35 of incubation as well as nitrification rates for eight treatment combinations encompassing the no-N control and urea addition across four moisture contents shown as 28, 40, 52, and 64% water-filled pore space (WFPS) (± SEM). Sample sizes were *n* = 2 on Day 7, and *n* = 3 on Day 35. NH_4^+–N and NO_3^-–N concentrations on Day 0 (initial baseline) were 4.39 and 13.61 mg N kg soil^{-1}, respectively.

N Treatment	28% WFPS		40% WFPS		52% WFPS		64% WFPS	
	Day 7	Day 35	Day 7	Day 35	Day 7	Day 35	Day 7	Day 35
NH_4^+–N concentrations (mg N kg^{-1} soil)								
Control	2.1 ± 0.01 ^{CD†}	5.0 ± 0.05 ^{b†}	1.4 ± 0.00 ^{D}	1.2 ± 0.00 ^{c}	1.3 ± 0.01 ^{D}	1.1 ± 0.00 ^{c}	1.4 ± 0.04 ^{D}	1.0 ± 0.00 ^{c}
Urea	48.3 ± 0.27 ^{A}	37.4 ± 0.09 ^{a}	17.7 ± 1.07 ^{AB}	4.5 ± 0.09 ^{b}	6.1 ± 0.04 ^{ABC}	1.3 ± 0.01 ^{c}	5.1 ± 0.04 ^{BC}	1.8 ± 0.04 ^{c}
NO_3^-–N concentrations (mg N kg^{-1} soil)								
Control	16.92 ± 0.01 ^{E}	18.35 ± 0.06 ^{e}	21.04 ± 0.04 ^{DE}	25.18 ± 0.12 ^{de}	25.48 ±0.04 ^{CD}	32.61 ± 0.06 ^{cd}	22.81 ± 0.05 ^{D}	36.60 ± 0.14 ^{c}
Urea	30.27 ± 0.08 ^{BC}	35.92 ± 0.20 ^{c}	41.31 ± 0.64 ^{AB}	71.91 ± 0.16 ^{ab}	57.27 ± 0.59 ^{A}	63.50 ± 0.45 ^{b}	57.59 ± 0.33 ^{A}	78.11 ± 0.10 ^{a}
Nitrification rate‡ (mg NO_3^-–N kg^{-1} soil day^{-1})								
Control	0.11 ± 0.01 (0.72) $^{\boldsymbol{B}\dagger}$		0.28 ± 0.03 (0.77) $^{\boldsymbol{B}}$		0.46 ± 0.07 (0.79) $^{\boldsymbol{AB}}$		0.61 ± 0.05 (0.95) $^{\boldsymbol{AB}}$	
Urea	0.51 ± 0.10 (0.67) $^{\boldsymbol{AB}}$		1.50 ± 0.25 (0.91) $^{\boldsymbol{A}}$		1.08 ± 0.36 (0.54) $^{\boldsymbol{AB}}$		1.53 ± 0.42 (0.74) $^{\boldsymbol{A}}$	

Note. Sample sizes were *n* = 2 on Day 7 and *n* = 3 on Day 35. Concentrations of NH_4^+–N and NO_3^-–N on Day 0 (initial baseline) were 4.39 and 13.61 mg N kg soil^{-1}, respectively.

[†] Grouping: uppercase letters correspond to pairwise treatment comparisons for NO_3^-–N or NH_4^+–N concentration on Day 7; lowercase letters are for Day 35. Italicized uppercase letters correspond to pairwise treatment comparisons for nitrification rates. Different letters indicate significant differences among the eight treatment combinations (N addition × moisture) based on ANOVA followed by Tukey's Honest Significant Difference test ($P < 0.05$).

[‡] Net nitrification rates were estimated from the change in the nitrate-N pool over time, derived as the regression coefficients of fitted linear regressions considering concentrations on Days 0, 7, and 35. Coefficients of determination of the linear fittings are provided in parentheses to qualify the regressions.

4.10 List of figures

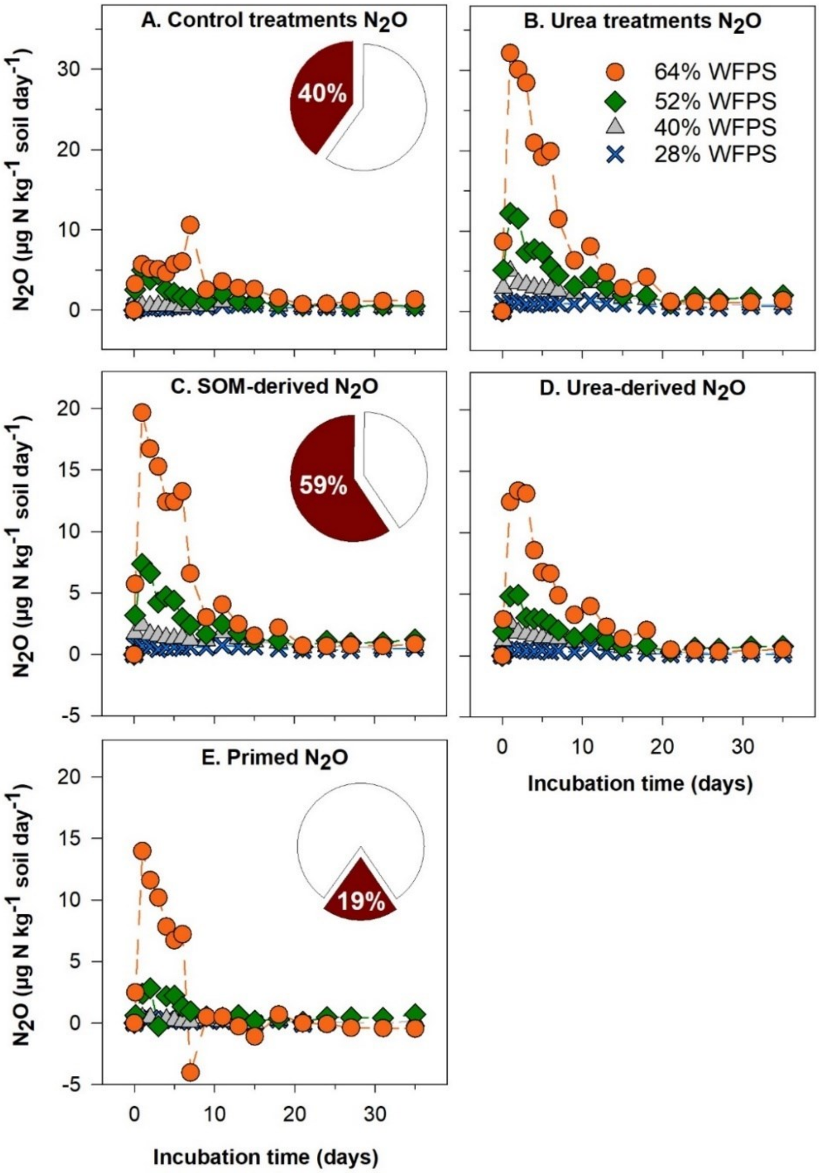

Figure 4-1: Daily total N_2O fluxes from (A) control and (B) urea treatments, (C) soil organic matter (SOM)-derived N_2O (Eq. [4]), (D) urea-derived N_2O (Eq. [5]), and (E) primed N_2O (Eq. [6]) at four water-filled pore spaces (WFPSs) over a 35-d incubation. The solid dark brown segments of the inset pie graphs in panels A, C, and E show the 35-d cumulative N_2O as fractions of the total N_2O production in panel B averaged across all four moisture contents. Panels A and B have the same y-axis scale; C, D, and E share a narrower y-axis scale. Error bars are not shown for clarity. The SEM ranged as follows: (A) (control), 0.01–6.31 µg N_2O–N kg−1; (B) (urea treatments), 0.02–12.10; (C) (SOM-derived N_2O), 0.01–7.94; (D) (urea-derived N_2O), 0.01–5.83; and (E) (primed N_2O), 0.02– 10.33.

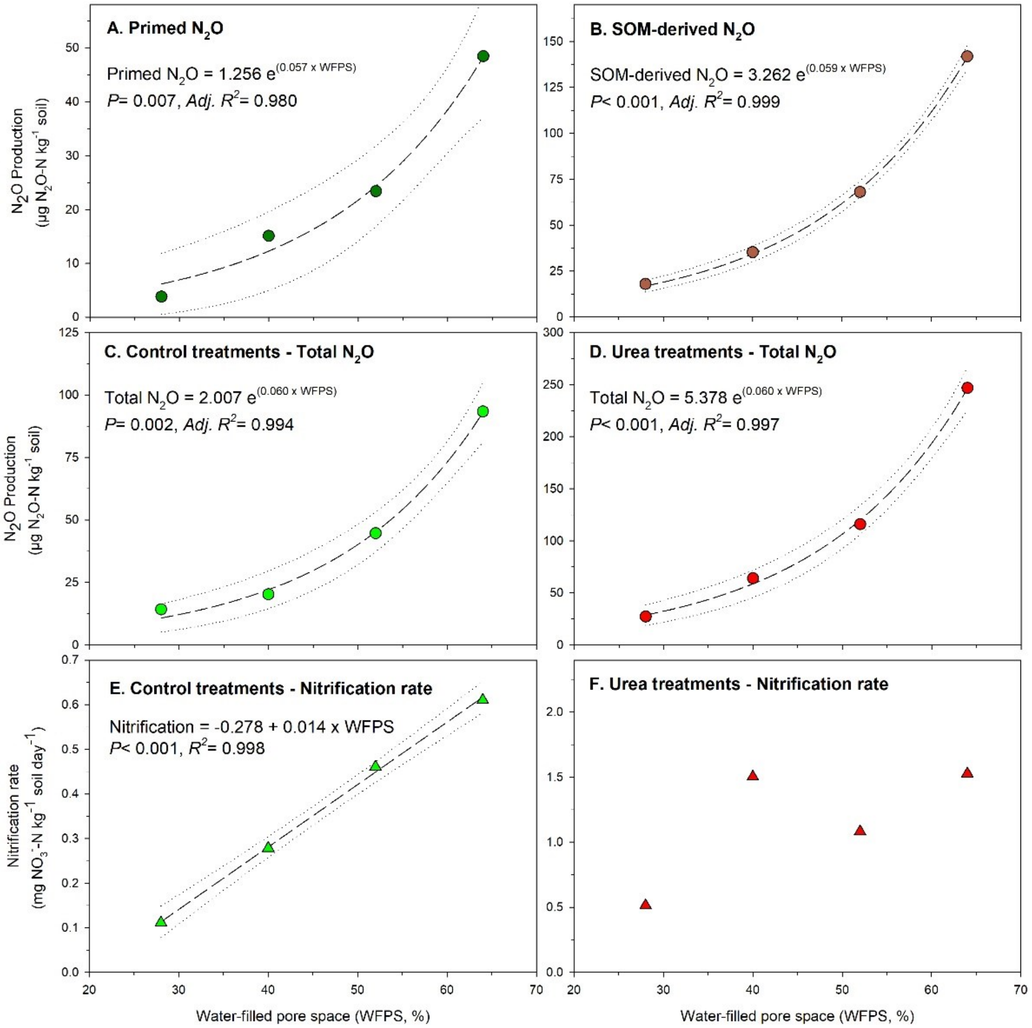

Figure 4-2: (A) Cumulative primed N_2O production, (B) N_2O production derived from soil organic matter (SOM) mineralization, (C) total N_2O production in untreated control soils, (D) total N_2O production in urea-amended soil, (E) nitrification rate in untreated control soil, and (F) nitrification rate in urea-amended soils as a function of moisture expressed as water-filled pore space (WFPS). Exponential fitting and equations are provided for panels A, B, C, and D. Linear fitting and the equation are presented for Panel E for the control treatments, which showed the clear dependency of background nitrification rate on moisture. Data points in panel F (urea-amended soils) did not yield a significant fitting. Confidence bands (95%) of the fitting in panels A, B, C, D, and E are presented. Vertical scales differ across panels.

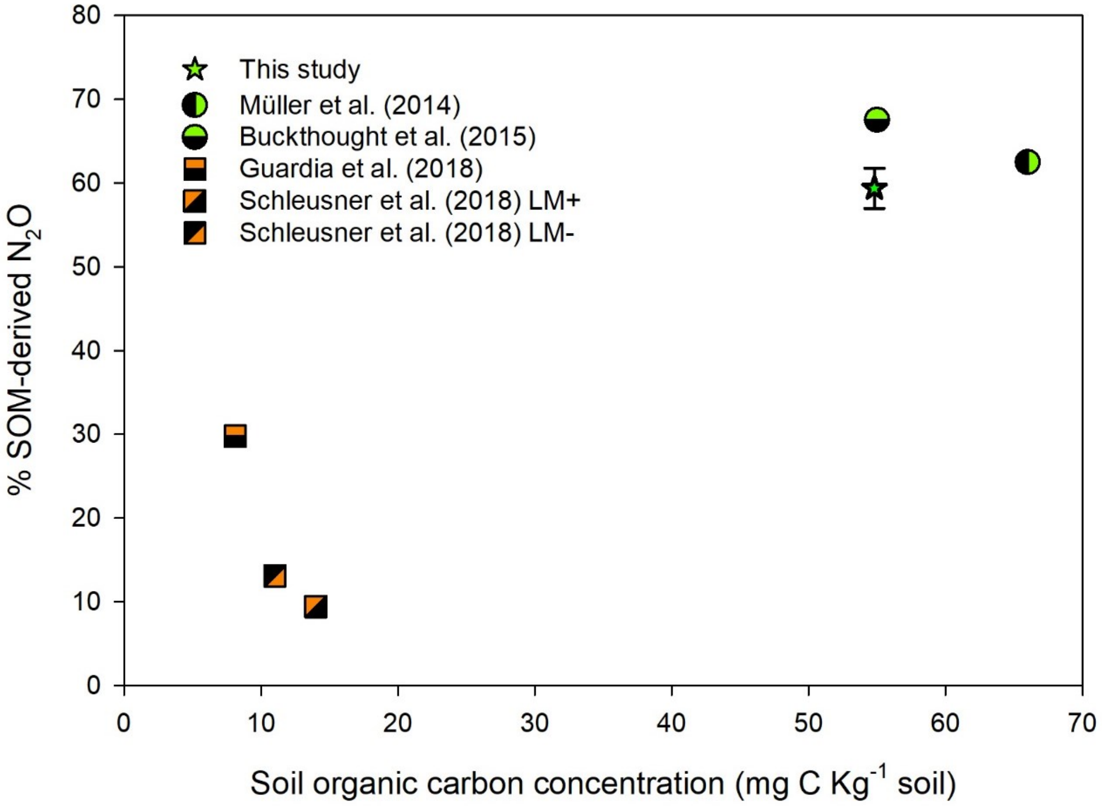

Figure 4-3: Proportion of N_2O derived from soil organic matter (SOM) vs. organic C in four published studies and the soil in our study. The methodologies differed across studies, varying in duration, biophysical conditions, and management, thus making a synthesis challenging. Buckthought et al. (2015) and Guardia et al. (2018) were field studies; the rest were incubations. All studies took ^{15}N-labelling approaches; Müller et al. (2014) also applied modelling. The N addition source and rates varied. At 100 kg N ha^{-1}, Schleusner et al. (2018) had two field treatments: with and without liquid manure (LM+ and LM–). For Buckthought et al. (2015), we only included their 200 kg urea-N ha^{-1} rate, because this N rate is closer to that in our study. The other two studies used ammonium nitrate at 180 kg N ha^{-1}.

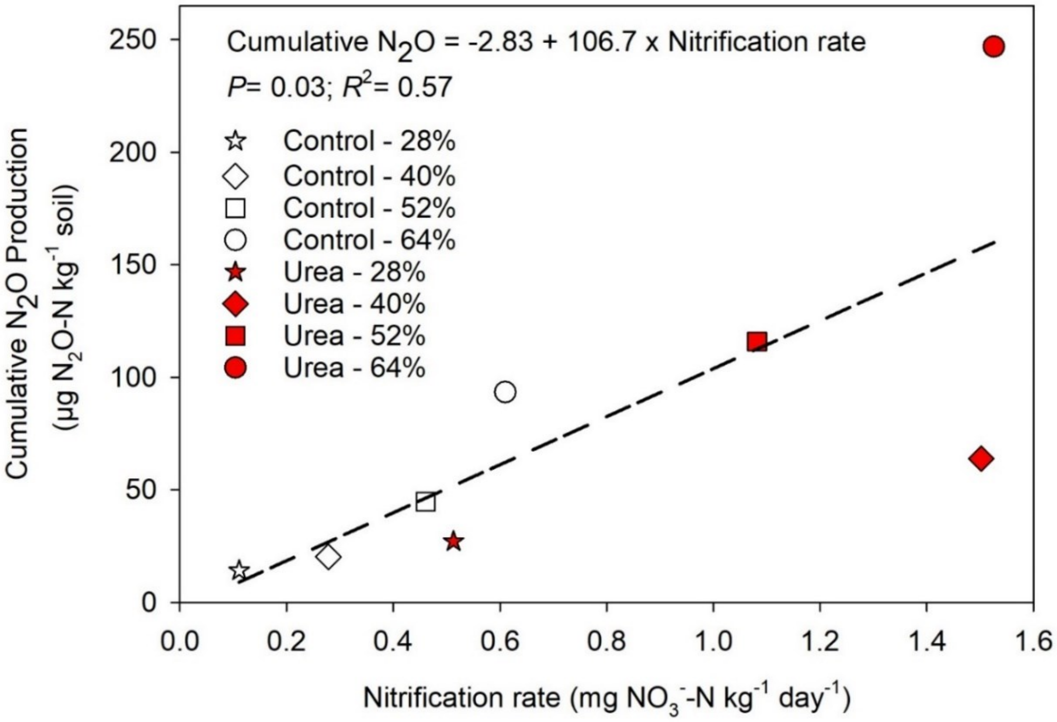

Figure 4-4: Cumulative N_2O production associated with nitrification rates across treatment combination means encompassing control, urea addition, and all four water-filled pore spaces. The linear fitting and the regression equation of the relationship are presented.

5.0 Conclusions

The compilation of studies included in the present dissertation assessed the GHG emissions and soil N dynamics in agricultural soils receiving biosolids as an N source (i.e., BM, BA, and BC). The field study showed that greater GHG (i.e. N_2O, CO_2 and CH_4) emissions are produced from biosolids-amended soils when compared against the controls (no-N additions) or urea-amended soils. Out of the three assessed biosolids, BM developed the highest GHG emissions, mainly because its particular characteristics of higher TN content and NH_4^+–N, and lower C:N ratio. We found that soil available N, soil moisture, and incorporation of the N source were the main drivers for the release of higher N_2O fluxes. Moreover, the N application rate at which biosolids were applied did not provide enough N required by the crops, whereas soils receiving a biosolids-urea combination, under incorporation, increased the biomass productivity, and even decreased N_2O emissions, most probably because of a better matching between the available N supply and plant requirements. In addition, the field study also showed that N_2O represented the highest contributor to the GHG footprint (measured in CO_{2eq}), however, in the urea-amended soils, the CO_{2eq} released during its manufacturing offset largely their low N_2O field emissions. As a result, urea-amended soils had even higher CO_{2eq} than the BA- and BC-amended soils. In the incubation study applying biosolids, we found how soil N dynamics (e.g., mineralization, nitrification, denitrification) are affected under the application of biosolids and increased soil moisture (at an aerobic range). As in our field study, higher N_2O emissions were observed with greater water content, and higher NO_3^-–N concentration. Moreover, the results from a laboratory incubation showed a lower optimum WFPS for nitrification in biosolids-amended soils (up to 55% WFPS), when compared against the reported 60% WFPS in soils receiving synthetic fertilizer, as the decomposition and mineralization of the biosolids organic C depleted the soil O_2.

Both the field and incubation studies applying biosolids showed that different biosolids stabilization methods further impact the biosolids' properties (e.g., N content, C:N ratio, pH, and water content), and that these properties directly affect the soil N cycle processes such as mineralization, nitrification, and denitrification. In that sense, biosolids properties lead to differences in the rate and amount of soil available N release as well as N losses (e.g., N_2O emissions). In the case of field conditions, biosolids properties further influenced the N use efficiency and aboveground biomass, which were lower than in the urea-amended soils. Nevertheless, biosolids should be considered as a resource instead of a waste, and best management practices should be oriented for replacing synthetic fertilizer (totally or partially) since the high amounts of CO_{2eq} released during urea manufacturing offset the lower field N_2O emissions of urea-amended soils. The best management practices should take into account the biosolids' properties, while keeping or improving yield, and minimizing GHG emissions, to concurrently achieve economic and environmental benefits.

Furthermore, in an incubation study applying urea, we found how addition of labile N led to a positive N_2O priming effect, leading to more N_2O emitted by the soil N than by the urea-N, probably because of the high soil organic C content. This positive N_2O priming effect increased its magnitude with higher water content, confirming the key role of soil moisture to produce N_2O emissions. This showed the importance of the soil C concentration on SOM-N turnover to produce primed N_2O, thus the combined effect of the soil C and N availability on the primed N_2O after additions of labile N should be further studied.

Overall, our results point to the need for future studies on the biosolids N mineralization rates, in order to have a better estimation of an optimal N application rate that can meet the plant N requirements. Additionally, the use of nitrification inhibitors to reduce soil N_2O emissions after biosolids applications should be studied. Moreover, the effects of biosolids additions on pre-existing SOM mineralization and primed N_2O, considering both C and N dynamics need to be addressed. Studying the soil microbial community variations after biosolids additions is an important factor to be considered as the microbes have a key role on the N turnover as well as N losses.

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Appendix A: Growing season 2019-2020

The total N application rates in the third experimental year evaluated case scenarios of extremely high biosolids-N inputs. The TN rate of application is shown in **Table A-1**. These extremely high application rates in the third year represent biosolids rates that may become relevant to commercial farming operations using the different types of biosolids. The N sources were applied uniformly in the experimental plots on 5 June 2019. On the same date, the N sources were incorporated in the appropriate treatments. Then, barley (*Hordeum vulgare* L.) for silage biomass was seeded at 90 kg seed ha^{-1} on 5 June 2019. Phosphorus as triple superphosphate (0–46–0 N–P–K) was applied at 20 kg P ha^{-1}. Aboveground biomass barley was harvested at the dough stage (from an area of 3.1 m^2) on 22 August 2019. Soil moisture and soil temperature were measured at depths of 5 and 10 cm with 5TM sensors interfaced with EM50 data loggers (Decagon Devices, Inc., Pullman, WA). Air temperature and precipitation data for the Edmonton site were obtained from permanent weather stations located at the Edmonton South Campus.

Table A-1: List of treatments and N rates application in the third experimental year.

Treatment[†]	Placement	N rate (kg N ha^{-1})
Control	n/a	–
Urea (URS, URI)	Surface / Incorporation[§]	96
BMI, BMS		1230
BAS, BAI		402
BCS, BCI		351
BMURS, BMURI[‡]		663
BAURS, BAURI[‡]		249
BCURS, BCURI[‡]		223.5

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] Urea was applied at a rate of 48 kg N ha^{-1}.

[§] Incorporation was done on the same day as the treatment applications at 15 cm depth.

Table A-2: Soil properties (0–15 cm depth increment), precipitation, and air temperature at the Edmonton site.

Properties	Unit	2019
SOC[†]	g organic C kg^{-1}	59.1
TN[†]	g N kg^{-1}	5.7
C to N ratio		10.37
pH[‡]		6.7
Bulk density	g cm^{-3}	1.13
Baseline NO_3^-–N[§]	mg kg^{-1}	9.9
Total annual precipitation[¶]	mm yr^{-1}	399.6 (433.8)
Total growing season precipitation (June–August)[¶]	mm	252.0 (228.7)
Air temperature[¶]	ºC	3.4 (3.7)

[†] Soil organic carbon (SOC) and total nitrogen (TN) were measured by dry combustion.

[‡] Measured in a saturated paste (1:2 soil:water).

[§] The initial NO_3^-–N concentration was measured at depth soil increments of 0–30 cm at the beginning of the growing season prior to any N treatment addition and barley seeding.

[¶] Values inside the parentheses are the long-term normal (Alberta Climate Information Service) from 1987 to 2016. Values outside the parentheses correspond to the measurement year.

Table A-3: Biosolids' properties during the third year of the study.

Parameter	Unit	Mesophilic anaerobic digested (BM)	Alkaline-stabilized (BA)	Composted (BC)
TC†	g C kg^{-1}	302.7	156.1	309.1
TN†	g N kg^{-1}	48.0	10.0	21.2
C to N ratio		6.3	15.6	14.6
Biosolids TN rate applied	kg TN ha^{-1}	1230	402	351
pH		7.2‡	12.4‡	5.1‡
Electrical conductivity[‡]	dS m^{-1}	6.3	27.5	19.7
Dry matter fraction, gravimetric (mass basis)		0.23	0.76	0.72
Moisture fraction (mass basis)		0.77	0.24	0.28

[†] Total carbon (TC) and total nitrogen (TN) were measured by dry combustion.

[‡] Measured in saturated paste 1:2.

[§] Measured by the TMECC 04.11 Electrometric pH Determinations for Compost. 1:5 Slurry Method.

Table A-4: Cumulative N_2O emissions (g N ha^{-1}) during growing season, post-harvest and early spring periods, and annual N_2O emissions (g N ha^{-1}) during the third year of the study (± standard error of the mean).

Treatment[†]	Growing season 2019 6-Jun to 21-Aug	Post-harvest 2019 27-Aug to 25-Oct	Early spring 2020 20-Apr to 29-May	**Cumulative N_2O 2019-2020 7-Jun-19 to 29-May-20**
Control	652.2^{a}	167.7^{abc}	903.7^{ab}	**1723.6 ± 414^{a}**
URS	798.6^{a}	101.5^{ab}	532.7^{ab}	**1432.8 ± 213^{a}**
BMS	35577.6^{d}	2298.2^{e}	1290.5^{ab}	**39166.4 ± 4164^{c}**
BAS	1293.5^{ab}	138.6^{ab}	91.7^{a}	**1523.8 ± 221^{a}**
BCS	1035.3^{ab}	109.4^{ab}	309.7^{ab}	**1454.4 ± 310^{a}**
BMURS	10701.8^{cd}	1124.5^{cde}	1508.9^{ab}	**13335.3 ± 4155^{bc}**
BAURS	989.6^{ab}	159.5^{bcd}	276.7^{ab}	**1425.9 ± 325^{a}**
BCURS	1443.8^{ab}	143.5^{abc}	515.6^{ab}	**2102.9 ± 417^{a}**
URI	974.3^{a}	99.8^{ab}	351.1^{ab}	**1425.2 ± 469^{a}**
BMI	42637.0^{d}	1179.3^{de}	2011.1^{b}	**45827.4 ± 5284^{c}**
BAI	2868.4^{bc}	119.5^{ab}	337.5^{ab}	**3325.4 ± 853^{ab}**
BCI	863.7^{a}	98.7^{ab}	579.8^{ab}	**1542.3 ± 221^{a}**
BMURI	20853.1^{cd}	624.9^{bcde}	854.2^{ab}	**22332.2 ± 6889^{bc}**
BAURI	1108.4^{ab}	49.6^{a}	448.4^{ab}	**1606.3 ± 185^{a}**
BCURI	691.7^{a}	92.4^{ab}	603.6^{ab}	**1387.6 ± 253^{a}**
Overall mean ± SE	8165.9 ± 1818.2	433.8 ± 89.3	707.7 ± 93.8	**9307.4 ± 1955.0**
ANOVA *P* values[‡]				
Treatment	<0.0001	<0.0001	0.0138	**<0.0001**
N source	<0.0001	<0.0001 (<0.0001)	0.0005 (0.0003)	**<0.0001 (<0.0001)**
N placement	0.584	0.0036 (0.0045)	0.2305 (0.2183)	**0.4019 (0.4171)**
N source × N placement	0.047	0.1733	0.7167	**0.1827**

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD. N_2O emissions during winter period were assumed to be negligible.

Table A-5: Annual area-based N_2O emission factors (EF_{area}) (% kg N_2O–N kg^{-1} N fertilizer), yield-based emission factors (EF_{yield}) (g N_2O–N kg^{-1} grain DM) and estimated annual N_2O emission factors (EF) as a function of rainfall only (N_2O EF % = $e^{(0.00558 \times H_2O - 7.701)} \times 100$ (Rochette et al., 2018; Liang et al., 2020) during the third year of the study (± standard error of the mean).

Treatment[†]	EF_{area} (% kg N_2O–N kg^{-1} fertilizer)	EF_{yield} (g N_2O–N kg^{-1} DM)
Control		0.25 ± 0.09^{ab}
URS	-0.30 ± 0.43^{a}	0.20 ± 0.04^{ab}
BMS	3.04 ± 0.35^{cd}	3.80 ± 0.43^{d}
BAS	-0.05 ± 0.06^{ab}	0.18 ± 0.02^{a}
BCS	-0.08 ± 0.20^{ab}	0.14 ± 0.04^{a}
BMURS	1.75 ± 0.58^{bcd}	1.44 ± 0.60^{bcd}
BAURS	-0.12 ± 0.23^{ab}	0.22 ± 0.04^{ab}
BCURS	0.17 ± 0.36^{ab}	0.26 ± 0.08^{ab}
URI	-0.31 ± 0.86^{a}	0.16 ± 0.06^{a}
BMI	3.59 ± 0.41^{d}	3.72 ± 0.47^{d}
BAI	0.40 ± 0.19^{abc}	0.40 ± 0.11^{abc}
BCI	-0.05 ± 0.17^{ab}	0.19 ± 0.01^{ab}
BMURI	3.11 ± 1.04^{cd}	1.93 ± 0.31^{cd}
BAURI	-0.05 ± 0.11^{ab}	0.23 ± 0.07^{ab}
BCURI	-0.15 ± 0.17^{ab}	0.16 ± 0.04^{a}
Overall mean ± SE	0.78 ± 0.2	0.89 ± 0.17
ANOVA *P* values[‡]		
Treatment	<0.0001	<0.0001
N source	<0.0001 (<0.0001)	<0.0001 (<0.0001)
N placement	0.5429 (0.5307)	0.8102 (0.8148)
N source × N placement	0.7468	0.2300
$EF_{rainfall}$ from Jun to Aug	**0.18**	
$EF_{rainfall}$ from May to Oct[§] (Rochette et al., 2018)	**0.28**	

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The numbers inside the parentheses are the P values after the interaction effect was removed.

[§] The $EF_{rainfall}$ for the long-term normal (1987–2016) was 0.16 from June to August, and 0.29 from May to October.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Table A-6: Total aboveground biomass (kg dry matter (DM) ha^{-1}), N uptake (kg N ha^{-1}), N use efficiency (NUE) (kg DM kg^{-1} N) and uptake efficiency (UE) (kg plant N kg^{-1} N) during the third year of the study (± standard error of the mean).

Treatment[†]	Total aboveground biomass (kg DM ha^{-1})	N uptake (kg N ha^{-1})	NUE (kg DM kg^{-1} N)	UE (kg plant N kg^{-1} N)
Control	7933 ± 1113	175 ± 31^{ab}		
URS	7488 ± 970	150 ± 34^{ab}	−4.6 ± 15	−0.26 ± 0.3
BMS	10660 ± 1557	256 ± 33^{ab}	2.2 ± 2	0.07 ± 0.0
BAS	8653 ± 1233	200 ± 24^{ab}	1.8 ± 5	0.06 ± 0.1
BCS	10754 ± 1643	259 ± 50^{ab}	8.0 ± 3	0.24 ± 0.1
BMURS	10645 ± 1244	278 ± 31^{ab}	4.1 ± 2	0.16 ± 0.1
BAURS	6502 ± 901	137 ± 20^{a}	−5.7 ± 8	−0.15 ± 0.2
BCURS	9495 ± 2222	225 ± 61^{ab}	7.0 ± 8	0.22 ± 0.2
URI	9548 ± 910	212 ± 13^{ab}	16.8 ± 20	0.38 ± 0.4
BMI	12705 ± 1846	316 ± 21^{b}	3.9 ± 2	0.11 ± 0.0
BAI	9364 ± 1601	225 ± 46^{ab}	3.6 ± 2	0.12 ± 0.1
BCI	7920 ± 940	177 ± 24^{ab}	−0.037 ± 2	0.005 ± 0.0
BMURI	11017 ± 1544	279 ± 35^{ab}	4.7 ± 3	0.16 ± 0.1
BAURI	8738 ± 2181	205 ± 64^{ab}	3.2 ± 5	0.12 ± 0.1
BCURI	9629 ± 1329	222 ± 39^{ab}	7.6 ± 5	0.21 ± 0.1
Overall mean ± SE	9403 ± 389	221 ± 11	3.7 ± 2.0	0.10 ± 0.04
ANOVA *P* values§				
Treatment	0.2486	0.0199	0.8654	0.4219
N source	0.0931 (0.0785)	0.0085 (0.0082)	0.9402 (0.9365)	0.8645 (0.8846)
N placement	0.3695 (0.3598)	0.2843 (0.2874)	0.3362 (0.3284)	0.1931 (0.2086)
N source × N placement	0.6485	0.3640	0.5884	0.1783

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Table A-7: Cumulative partial record of CO_2 emissions (g CO_2–C ha^{-1}) and annual CH_4 emissions (Mg CH_4–C ha^{-1}) during the third year of the study (± standard error of the mean).

Treatment[†]	Cumulative annual of CH_4 emissions (g CH_4-C ha^{-1})	Cumulative partial record of CO_2 emissions[‡] (Mg CO_2-C ha^{-1})
Control	−147 ± 22^{a}	1.364 ± 0.09^{ab}
URS	−191 ± 49^{a}	1.364 ± 0.16^{ab}
BMS	13134 ± 2512^{b}	1.975 ± 0.02^{cd}
BAS	−136 ± 29^{a}	1.017 ± 0.09^{a}
BCS	−158 ± 44^{a}	1.397 ± 0.08^{ab}
BMURS	4134 ± 1550^{b}	1.764 ± 0.19^{bc}
BAURS	−216 ± 35^{a}	1.111 ± 0.05^{a}
BCURS	−164 ± 48^{a}	1.468 ± 0.12^{abc}
URI	−132 ± 35^{a}	1.187 ± 0.13^{a}
BMI	9926 ± 3187^{b}	2.335 ± 0.17^{d}
BAI	−127 ± 32^{a}	1.041 ± 0.07^{a}
BCI	−179 ± 20^{a}	1.509 ± 0.10^{abc}
BMURI	2646 ± 1441^{b}	1.521 ± 0.12^{abc}
BAURI	−187 ± 33^{a}	1.161 ± 0.11^{a}
BCURI	−128 ± 40^{a}	1.265 ± 0.06^{ab}
Overall mean ± SE	1871.7 ± 587.8	1.432 ± 0.052
ANOVA *P* values[§]		
Treatment	<0.0001	<0.0001
N source	<0.0001 (<0.0001)	<0.0001 (<0.0001)
N placement	0.4370 (0.4229)	0.5989 (0.5995)
N source × N placement	0.7499	0.4135

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The cumulative partial record for CO_2 emissions does not include the growing season period.

[§] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey’s HSD.

Table A-8: Partial greenhouse gas budget area- and yield-related kg CO_2-equivalent (CO_{2eq}) during the third year of the study (± standard error of the mean).

	Total flux of CO_2 equivalents						
Treatment[†]	N_2O emissions[‡]	CH_4 emissions[‡]	Biosolids production process§	Fuel consumption[§]	N manufacture[¶]	Area-related CO_{2eq} (kg ha^{-1} yr^{-1})	Yield-related CO_{2eq} (kg kg^{-1} DM)
	kg CO_{2eq} ha^{-1} yr^{-1}						
2019–2020							
Control	807.3	−6.7			0	862.2 ± 194^{cd}	0.125 ± 0.05^{d}
URS	671.1	−8.7	0.0	61.6	457.6	1181.6 ± 98^{cd}	0.164 ± 0.02^{bcd}
BMS	18345.2	595.4	0.0	61.6	0	19005.8 ± 1913^{a}	1.845 ± 0.20^{a}
BAS	713.7	−6.2	12.9	61.6	0	793.4 ± 103^{cd}	0.095 ± 0.01^{d}
BCS	681.2	−7.2	41.9	61.6	0	739.1 ± 147^{d}	0.073 ± 0.02^{d}
BMURS	6246.2	187.4	13.7	61.6	228.8	6725.7 ± 2015^{ab}	0.724 ± 0.29^{abc}
BAURS	667.9	−9.8	6.4	61.6	228.8	960.6 ± 153^{cd}	0.151 ± 0.02^{cd}
BCURS	985.0	−7.4	20.9	61.6	228.8	1269.7 ± 197^{cd}	0.157 ± 0.04^{cd}
URI	667.5	−6.0	6.9	61.6	457.6	1188.1 ± 221^{cd}	0.131 ± 0.03^{cd}
BMI	21465.2	450.0	0.0	68.9	0	21987.7 ± 2615^{a}	1.783 ± 0.23^{a}
BAI	1557.6	−5.7	12.9	68.9	0	1645.0 ± 401^{bc}	0.197 ± 0.05^{bcd}
BCI	722.4	−8.1	41.9	68.9	0	786.6 ± 104^{cd}	0.099 ± 0.00^{d}
BMURI	10460.2	120.0	13.7	68.9	228.8	10879.7 ± 3292^{a}	0.941 ± 0.15^{cd}
BAURI	752.4	−8.5	6.4	68.9	228.8	1053.8 ± 85^{cd}	0.150 ± 0.04^{cd}
BCURI	650.0	−5.8	20.9	68.9	228.8	943.6 ± 117^{cd}	0.107 ± 0.02^{d}
Overall mean ± SE	4359.5 ± 915.7	84.9 ± 26.6	6.9	68.9		4661.9 ± 933.8	0.45 ± 0.08
ANOVA *P* values[#]							
Treatment						<0.0001	<0.0001
N source						<0.0001 (<0.0001)	<0.0001 (<0.0001)
N placement						0.1497 (0.171)	0.5406 (0.5521)
N source × N placement						0.1011	0.2050

[†] Treatment codes are as follows: BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; UR, urea, S, surface application; I, incorporated.

[‡] The coefficients used to convert from N_2O and CH_4 to CO_2 equivalents were 298 and 34, respectively (IPCC, 2013). These coefficients operate on a mass basis, accounting for the full molecular weights.

[§] The CO_{2eq} for biosolids productions (including transport) was based on Brown et al. (2010) who applied the Biosolids Emissions Assessment Model (BEAM) to calculate the CO_{2eq} for biosolids production system. The factors used were 749.7 kg CO_{2eq} Mg^{-1} DM for BM, 1104.3 kg CO_{2eq} Mg^{-1} DM for BA, and 870.1 kg CO_{2eq} Mg^{-1} DM for BC.

[¶] Rotary tillage, crop seeding, and forage harvesting corresponded to 2, 3.2, and 13.6 kg C equivalent ha^{-1}. Production, packaging, storage, and distribution of urea were accounted for as 1.3 kg C equivalent kg^{-1} N (Lal, 2004).

[#] The numbers inside the parentheses are the *P* values after the interaction effect was removed.

Different letters within a column indicate significant differences across the N treatments. The differences across N treatments were determined using Tukey's HSD.

Indirect N_2O emissions caused by volatilization of ammonia and deposition were unaccounted for. Soil carbon change and nitrate leaching (and associated N_2O formation elsewhere) were assumed to be negligible. It is noted that CO_2 and plant C were not accounted in these partial greenhouse gas budgets.

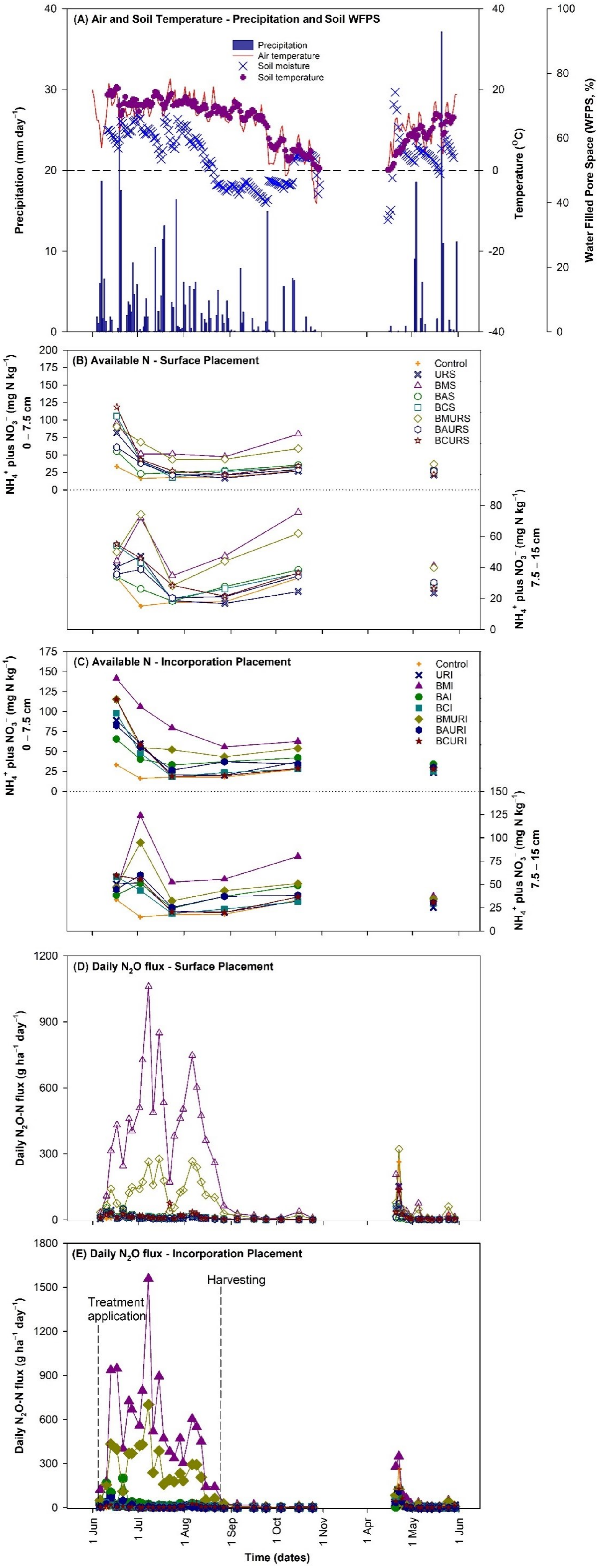

Figure A-1: (A) Daily average air temperature, daily cumulative precipitation, daily average soil temperature (5–10 cm), and daily average soil water-filled pore space (WFPS) (5–10 cm depth); (B) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the surface-applied treatments plus the control; (C) soil available N at depths of 0–7.5 cm and 7.5–15 cm for the incorporation treatments plus the control; (D) daily N_2O flux for the surface-applied treatments; and (E) daily N_2O flux for the incorporation treatments. Data in each panel are within the period from June 2019 to May 2020 (the interval from 1 Nov. to 1 Apr. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively.

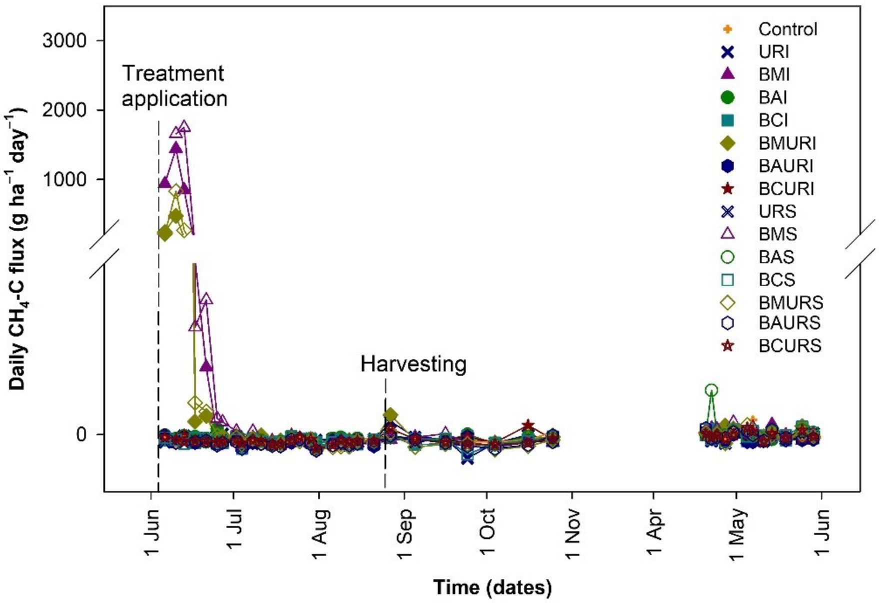

Figure A-2: Daily CH$_4$ fluxes for the surface-applied and incorporation treatments from June 2019 to May 2020 (note that the interval from 1 Nov. to 1 April. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively.

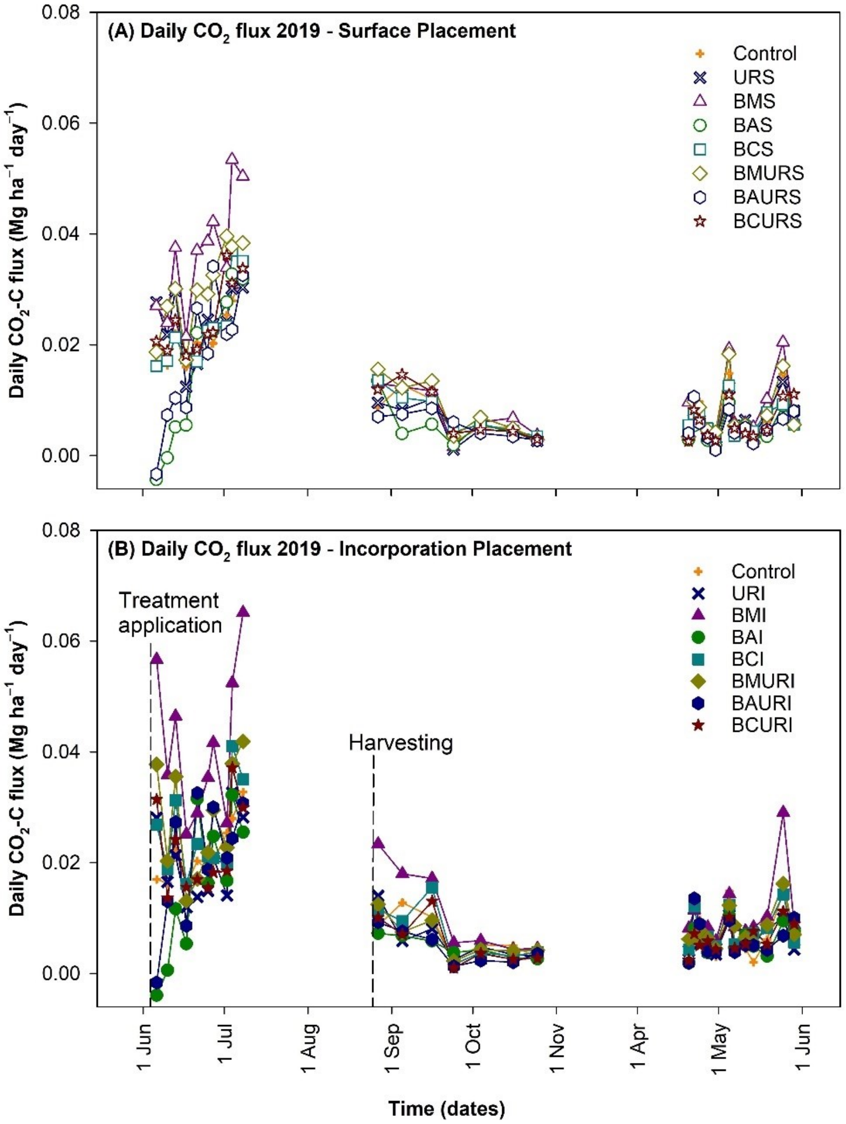

Figure A-3: (A) Daily CO_2 fluxes for the surface-applied treatments from June 2019 to May 20120 (B) daily CO_2 fluxes for the incorporation treatments from June 2019 to May 2020 (note that the interval from 1 Nov. to 1 April. was omitted). Error bars are not shown for better display of the data. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; S and I after each treatment label indicate that the treatment was surface-applied or incorporated into the soil, respectively. Data from July and August are not presented, as plant canopy was growing inside the chamber.

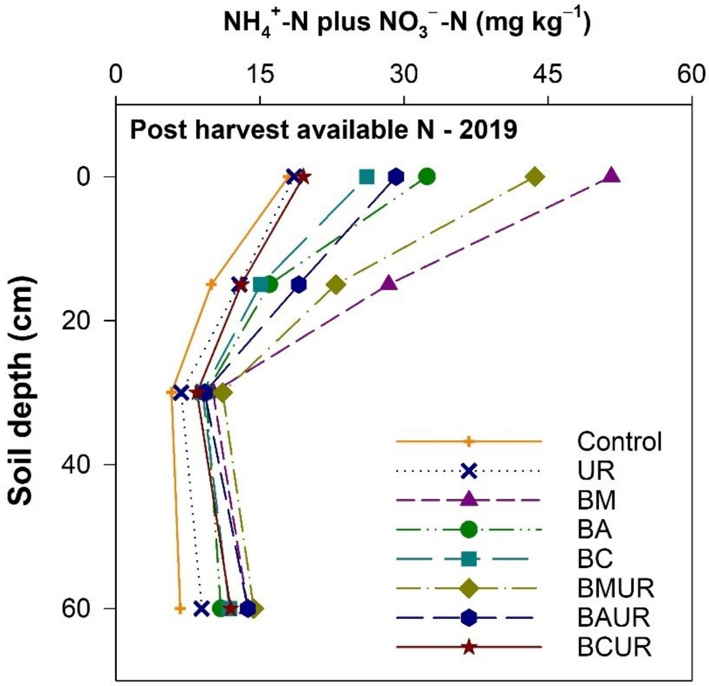

Figure A-4: Post-harvest available N (mg kg^{-1}) with soil depth for each type of N source measured in the growing season of 2019. UR, urea; BM, mesophilic anaerobic digested biosolids; BA, alkaline-stabilized biosolids; BC, composted biosolids; BMUR, mesophilic anaerobic digested biosolids combined with urea; BAUR, alkaline-stabilized biosolids combined with urea; BCUR, composted biosolids combined with urea. Soil samples were collected at depth increments of 0–15, 15–30, 30–60, and 60– 90 cm in August 2019.

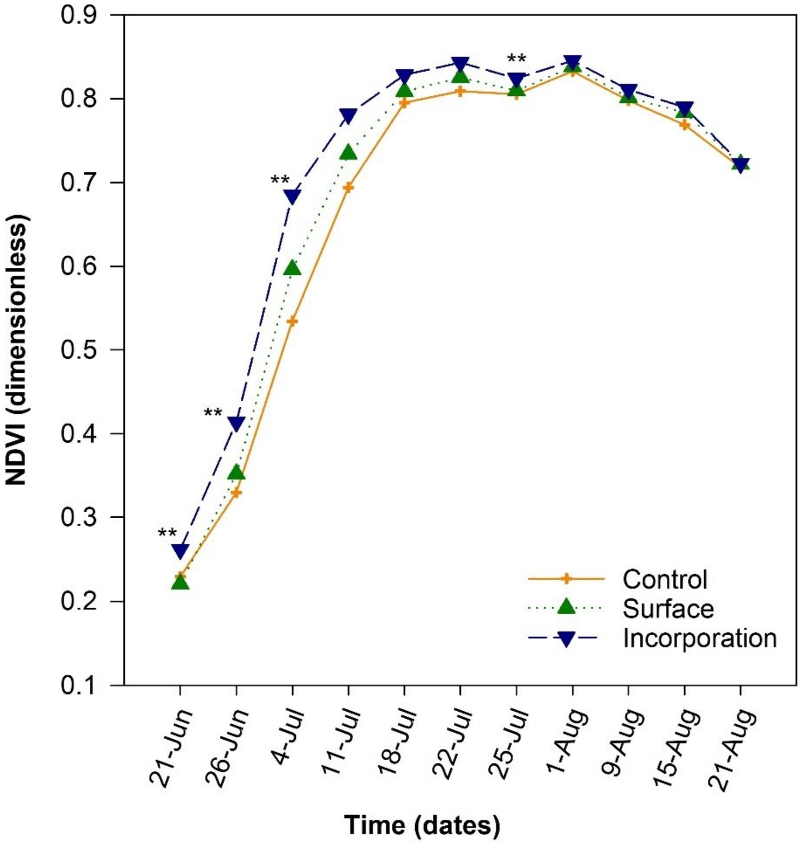

Figure A-5: Normalized difference vegetation index (NDVI) over the growing season for the control, the average of surface-applied treatments, and the average of incorporation treatments for barley cropland in 2019. ** indicates statistically significant differences (incorporation > surface, *P*s < 0.05).