Effect of the Land Application of Biosolids on Greenhouse Gas Emissions Under Atlantic Canadian Conditions

by

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Dedication

This thesis is dedicated to my mother, Gurjeet Kaur, my father, Zora Singh, and my sisters Manpreet Kaur and Navjot Kaur, whose love and support helped me to achieve everything I have today.

Thank You

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Abstract

The objective of this field study was to examine the effect of biosolids type, application rate, and application method on soil nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) emissions from a sandy loam textured soil under Atlantic Canadian conditions. Biosolids amendment to soil significantly increased cumulative N_2O and CO_2 emissions, and had no effect on CH_4 emissions as compared to urea and unamended control treatments. Cumulative N_2O and CO_2 emissions varied with biosolids type due to their different physical and chemical compositions, whereas the application rate or method did not affect N_2O and CO_2 emissions. Cumulative CH_4 fluxes did not vary with biosolids type or application rate but increased from surface spread (SS) treatments as compared to incorporated (INC) treatments. Nitrate and ammonium exposures were not correlated with N_2O emissions. Results suggest that land application of biosolids has the potential to increase soil CO_2 and N_2O emissions, thus a trade-off between improving soil health and high yield versus greenhouse gas emissions must be considered before promoting these practices.

List of Abbreviations and Symbols Used

AEM	Anion exchange membrane
ANOVA	Analysis of variance
Ar	Argon
AT	Alkaline treated
ATAD	Autothermal thermophilic aerobic digestion
B	Boron
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
CCME	Canadian Council of Ministers of the Environment
CEM	Cation exchange membrane
CEPA	Canadian Environmental Protection Act
CFIA	Canadian Food Inspection Agency
CH_4	Methane
Cl	Chlorine
cm	Centimeter
CO_2	Carbon dioxide
CP	Composted
Cu	Copper
DNDR	Dissimilatory nitrate reduction to ammonium
ECD	Electron capture detector
EF	Emission factor
Fe	Iron
FID	Flame ionization detector
g	Gram
GHG	Greenhouse gas
H_2	Hydrogen gas
ha	Hectare
HCl	Hydrochloric acid
hr	Hour
IEM	Ion exchange membrane
IER	Ion exchange resin
INC	Incorporated
IPCC	International Panel on Climate Change
K	Potassium
KCl	Potassium chloride
kg	Kilogram
m	Meter

MAD	Mesophilic anaerobically digested
Mg	Magnesium
Mg	Megagram
min	Minute
mL	Milliliter
mm	Millimeter
Mo	Molybdenum
MPN	Most probable number
N	Nitrogen
N_2	Nitrogen gas
N_2O	Nitrous oxide
Na	Sodium
NaCl	Sodium chloride
ng	Nanogram
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
L	Liter
O_2	Oxygen
P	Phosphorus
ppb	Parts per billion
ppm	Parts per million
S	Sulphur
SS	Surface spread
TCD	Thermal conductivity detector
TEQ	Toxic equivalent
U.S.EPA	United States Environmental Protection Agency
VWC	Volumetric water content
WFPS	Water-filled pore space
WWTP	Wastewater treatment plants
yr	Year
Zn	Zinc
%	Percent
µg	Microgram
µL	Microliter
^0C	Celsius

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Chapter 1: Introduction

1.1. Problem Statement

The global climate has been changing with the increase in atmospheric temperature. Greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), water vapour, ozone, chlorofluorocarbons, and hydrofluorocarbons trap some of the radiation emitted from the Earth's surface and raise the atmospheric temperature mainly near the Earth's surface and the process is known as the greenhouse effect. The increase in the concentration of GHG resulting in higher atmospheric temperature and causing more greenhouse effect which leads to global warming (Das, 2016). A number of pieces of evidence document the contribution of anthropogenic activities towards this rise in GHG levels and ultimately atmospheric temperature (IPCC, 2014a). Human activities such as fossil fuel combustion, cement production, flaring, deforestation, and other land use are cause higher CO_2 emissions. Likewise, CH_4 production has risen mainly due to the increase in area under rice cultivation, livestock, fossil fuel mining and burning, landfills and waste treatments, and biomass burning. The atmospheric N_2O concentrations are increasing as a result of an increase in the area of land under agricultural production, increased use of nitrogen (N)-fertilizers, biomass burning, fossil fuel combustion, industrial processes, human excreta, and atmospheric deposition (2007; IPCC, 2014a). As of December 2019, the atmospheric concentration of CO_2 was 412.02 parts per million (ppm) (NOAA, 2020) compared to 353 ppm in 1990 (IPCC, 2014a). In November 2019, atmospheric CH_4 and N_2O levels were recorded 1877 parts per billion (ppb) and 332.3 ppb (NOAA, 2020), whereas, in 1990, 1710 ppb and 303 ppb were reported, respectively (IPCC, 2014a).

Carbon dioxide was the highest contributor to the total global annual anthropogenic GHG emissions in 2010 accounting for 76% of the total followed by CH_4 with 16% and N_2O with more

than 6% (IPCC, 2014a). In Canada, CO_2 accounted for 79% of the total national GHG emissions compared to 14% of CH_4 and 5% of N_2O in 2015 (Environment Canada, 2017). Nitrous oxide and CH_4 contribute a small fraction to the total GHG emissions relative to CO_2. However, the global warming potentials of N_2O and CH_4 are 298 and 25 times that of CO_2 over the 100-year time horizon with a residence time of 114 and 10 years in the atmosphere, respectively (Le Mer & Roger, 2001; Signor & Cerri, 2013). Additionally, N_2O is also an ozone-depleting substance and is currently the major anthropogenic contributor to ozone depletion (Knowles, 1982).

Agriculture is an important contributor to anthropogenic GHG fluxes (Gregorich et al., 2005; IPCC, 2014a). On a global basis, almost 25% of the total GHG emissions were caused by agriculture, forestry, and other land uses in 2010 (IPCC, 2014b). In Canada, the agricultural sector was responsible only for 8% of the total national GHG emissions in 2015. The most important GHG in the agricultural ecosystem is N_2O. In 2015, N_2O accounted for 70% of Canada's total agricultural GHG emissions followed by CH_4 (27%) (Environment Canada, 2017). In Canada, the two contributors to the agricultural emissions are livestock and crop production. Livestock mainly accounts for CH_4 production from enteric fermentation and CH_4 and N_2O production from the storage and application of manures. Crop production is responsible for N_2O and CO_2 emissions from inorganic and organic N-fertilizer applications and crop residue decomposition, CO_2 emissions from the use of lime on farmlands, and N_2O and CH_4 production from burning the crop residues (Environment Canada, 2017).

Nitrogen is an essential element for plant growth and development, as a component of amino acids, protein, lipids, nucleic acids, and many other compounds (Leghari et al., 2016). In Canada, land and crop management practices have changed since 1990 and there has been an increase in the use of synthetic N-fertilizers, resulting in high total crop production (Environment

Canada, 2017). The application of synthetic N-fertilizers influences N_2O emissions by providing readily available mineral N to soil microbes, consequently promoting nitrification and denitrification, and N_2O production (Signor & Cerri, 2013). In Canada, synthetic N-fertilizers accounted for almost 19% of the total agricultural GHG emissions in 2015 (Environment Canada, 2017).

An alternative to provide required N and other essential elements to plants is the use of organic fertilizers such as crop residues, animal manures, biosolids, and other commercial products (Leghari et al., 2016). Crop residues and animal manures are responsible for a significant amount of GHG emissions, mainly as N_2O emissions (Gregorich et al., 2005; Environment Canada, 2017). In Canada, crop residue and animal manures (applied as fertilizers) contributed 13% to the total agricultural GHG emissions in 2015, lower than synthetic N-fertilizers (Environment Canada, 2017). Therefore, organic N-fertilizers can be used as a substitute for inorganic fertilizers.

Biosolids are an effective organic fertilizer in crop production and land reclamation (De Andrés et al., 2012; Sharma et al., 2017). For instance, Antolín et al. (2005) reported that land application of biosolids significantly increased barley (*Hordeum vulgare* L) grain yield compared to unamended control. Likewise, Wang et al. (2008) observed that the biosolids amendment to soil resulted in a significant increment in the biomass of native grasses (*Zoysia japonica* and *Poa annua*) when compared to unamended control. Similarly, Singh and Agrawal (2010) witnessed significantly higher yield, total plant biomass, and pod weight of mung bean (*V. radiata* L. cv. Malviya janpriya (HUM 6)) after the biosolids amendment as compared to unamended soil. Meyer et al. (2004) have seen an increase in productivity and cover of the forest vegetation from the land application of biosolids relative to unfertilized control over 4 years period after the forest fire.

Many studies have explored the beneficial effect of land application of biosolids on plant growth, crop production, and soil properties (Ros et al., 2003; Antolín et al., 2005; Singh & Agrawal, 2010; Usman et al., 2012; Dede et al., 2017; Sharma et al., 2017). There are fewer field studies that have shown the effect of land-applied biosolids on GHG emissions (Ros et al., 2006; López-Fernández et al., 2007; De Andrés et al., 2013; Camro et al., 2014; Willén et al., 2016; Wijesekara et al., 2017) and there is no study (at the time of writing) of GHG emissions resulting from the land application of biosolids in Atlantic Canada. In Canada, the International Panel on Climate Change (IPCC) 2006 Tier 1 coefficients are used to estimate GHG emissions from the land application of biosolids (CCME, 2009). These Tier 1 coefficients predict that 1% N available from the addition of organic manures, crop residues, synthetic fertilizers, and N mineralized from mineral soil is lost as N_2O (IPCC, 2006). However, this value will vary depending on the soil and environmental factors (IPCC, 2006). For instance, a field experiment conducted at three different locations (Ottawa, ON; Guelph, ON; and Saint-Valentin, QC) in Canada from 2005 to 2007 showed that 0.03 to 1.45% of total N applied was emitted as N_2O after the application of urea and urea ammonium nitrate (Ma et al., 2010). Helgason et al. (2005) generated a fertilizer-induced emission value of 1.18% in a non-manured cropping system based on 400 treatment combinations from studies conducted in various ecosystems in Canada.

There are large discrepancies among the measurements of GHG emissions found in the literature. For instance, a meta-analysis of studies examining the impact of soil amendment with various organic and inorganic materials reported that animal slurries, wastewaters, and biosolids had emission factor (EF) of 1.21±0.14%, whereas, compost, crop residues, and paper sludge had EF of 0.02±0.13% (Charles et al., 2017). Gregorich et al. (2005) reported that the application of mineral fertilizers resulted in the highest N_2O emissions followed by liquid manures, and solid

manures from Eastern Canadian studies. There are also differences associated with the type of biosolids on N_2O emissions. For example, Yoshida et al. (2015) observed 0.18, 0.37, and 0.66% of the initial N lost as N_2O-N after the application of primary, dried digested, and dewatered digested sludge, respectively. These variations in emission factors as a result of the type of biosolids and/or soil and environmental factors can lead to improper estimates of GHG emissions and carbon (C) credit allocation. This project will fill an important knowledge gap relating to the effect of land application of different types of biosolids on GHG emissions (N_2O, CO_2, and CH_4) under Atlantic Canadian conditions.

1.2. Objectives and Hypotheses

The overall objective of this study is to examine three types of biosolids (mesophilic anaerobically digested (MAD), alkaline treated (AT), and composted (CP)) based on application rate (full-rate and half-rate+urea), and application method (surface spread (SS) and incorporated (INC)) on GHG emissions under Atlantic Canadian conditions. This study includes the following objectives:

1. To measure and calculate the magnitude of GHG emissions as influenced by the land application of the three different biosolids.
2. To examine the differences among MAD, AT, and CP biosolids based on GHG emissions.
3. To determine the effect of land application of reduced application rate of biosolid (50%) in combination with inorganic N-fertilizer (urea) on GHG emissions compared to 100% of N applied as biosolids.
4. To understand the effect of the application method (SS and INC) of biosolids on GHG emissions.

5. To examine the relationship between nitrate exposure and ammonium exposure and N_2O emissions.

It was hypothesized that:

1. Biosolids will result in more GHG emissions compared to unamended control.
2. The physical, chemical, and biological properties of the biosolids will have a great influence on the magnitude of GHG emissions.
3. The application of a reduced rate of biosolids (50% of N requirement) in combination with 50% of the N requirement as inorganic N (urea) will not result in any significant differences in GHG emissions relative to soil receiving 100% of N as biosolids.
4. (a) Incorporation (INC) of biosolids or a combination of biosolids and urea will increase N_2O and CO_2 emissions compared to surface application (SS).

   (b) Methane emissions will be greater from the SS of biosolids or a combination of biosolids and urea than INC.
5. The nitrate and ammonium exposures will be positively correlated with N_2O emissions.

Chapter 2: Literature Review

2.1. Biosolids

2.1.1. Background Information on Biosolids

Biosolids, as defined by the United States Environmental Protection Agency (U.S.EPA), are "the solid organic matter produced from private or community wastewater treatment processes that can be beneficially used, especially as a soil amendment" (U.S.EPA, 1999). According to the Canadian Council of Ministers of the Environment (CCME), municipal biosolids are "organic-based products which may be solid, semi-solid or liquid and which are produced from the treatment of municipal sludge**.** Municipal biosolids are municipal sludge which has been treated to meet to jurisdictional standards, requirements or guidelines including the reduction of pathogens and vector attraction", whereas, municipal sludge is "a mixture of water and non-stabilized solids separated from various types of wastewaters as a result of natural or artificial processes" (CCME, 2012). In Nova Scotia, treated and stabilized domestic sewage and septage sludge, in order to reduce pathogens levels, is considered as biosolids (Nova Scotia Department of Environment, 2010).

Based on the pathogen and heavy metal concentrations, biosolids are categorized into two standard classes, Class A and Class B (Nova Scotia Department of Environment, 2010). Class A biosolids are highly treated and stabilized products of wastewater treatment with very low levels of pathogens, metals, and other contaminants. In Nova Scotia, the land application of Class A biosolids does not require any approval, however, the facility generating Class A biosolids must have valid approval from Nova Scotia Department of Environment (Nova Scotia Department of Environment, 2010). Class B biosolids are treated to a lesser extent than Class A biosolids. These biosolids contain reduced levels of pathogens, heavy metals, and other contaminants, which ensure

the public health and environmental safety. Class B biosolids require approval for land application in Nova Scotia (Nova Scotia Department of Environment, 2010). Depending on the jurisdiction, biosolids must be treated to meet the standard levels (Class A or Class B) in the guidelines (Hydromantis et al., 2010).

2.1.2. Production and Disposal

In Canada, most wastewater treatment plants (WWTP) are owned and operated by municipalities, however, some facilities are operated by federal or provincial governments or private entities (CCME, 2010). There are three major methods used for the disposal of the biosolids: landfill, incineration, and land application. Landfilling is the least expensive method of disposal; however, the landfilled material could cause methane (CH_4) emissions and contamination to underground water if there is a leakage. Incineration is an expensive method and releases carbon dioxide (CO_2) in the environment and contains concentrated pollutants in the ash, but it may also provide energy. Land application is one of the best options for the disposal of the biosolids as it provides organic matter and nutrients to the agricultural soils. It is important to note that land application of biosolids can cause risk to human health and environment, however, the risks can be reduced by following proper application regulations (CCME, 2012; OMAFRA, 2017).

Canada produces approximately 600,000 dry (or 2.5 million wet) tons of biosolids each year (CCME, 2012). Out of the total biosolids production in Canada, about 33% of the biosolids are land applied and the remainder are landfilled and/or incinerated (CCME, 2012). Some other developed countries apply a large percentage of biosolids to agricultural land. The United Kingdom is the leading country, using about 80% of the total biosolids production in the agricultural sector, followed by Australia (69%) and the United States of America (49%) (Rigby et al., 2016).

2.1.3. Biosolids Regulations

In Canada, federal authorities outline environmental and public safety regulations and acts for the disposal/use, sale, and import of biosolids (CCME, 2010). Environment Canada enforces the Canadian Environmental Protection Act, 1999 (CEPA, 1999), which applies to many but not all aboriginal and federal lands. Under this act, the disposal/use of pollutants (such as biosolids) on aboriginal and federal lands must be reported to Environment Canada. Canadian Food Inspection Agency (CFIA), which oversees the enforcement of the Fertilizer Act and Regulations, controls the sale and import of biosolids (CCME, 2010). The end-use and/or disposal of biosolids on provincial/territorial lands is mostly controlled by provincial/territorial governments and the acts and regulations vary among them (Table 2.1). The naming and categorization/classification of biosolids among provinces/territories is different, while the parameters used to assess the quality of biosolids are similar. Municipalities have the authority to regulate WWTP and/or land application of biosolids (CCME, 2010).

Table 2.1: Province specific regulations for land application of biosolids (CCME, 2010).

Province	Regulatory Authority & Acts and Regulations	Requirement of approval	Requirements on application rate
AB	Alberta Environment • *Environmental Protection and Enhancement Act* • *Wastewater and Storm Drainage Regulations*	Yes	Dependent on soil type and slope.
BC	Ministry of Environment • *Environmental Management Act and Health Act* • *Organic Matter Recycling Regulation*	Yes	Agronomic rate
MB	Manitoba Conservation • *The Environment Act* • *The Nutrient Management Regulation under the Water Protection Act*	Yes	Agronomic rates for N and P.
NB	Department of Environment • *Water Quality Regulation under the Clean Environment Act*	No (if composted)	No net degradation
NL	• *Environmental Protection Act* • *Reference to Part VIII of the Act on Dangerous Goods is also made*	N/A	N/A
NS	Nova Scotia Environment • *Environment Act*	Class B only	Agronomic rate
NU	Nunavut Water Board • *Water license from Nunavut Water Board*	Yes	Follows CCME guidelines
ON	Ontario Ministry of the Environment (MOE) • *Nutrient Management Act* • *Environmental Protection Act* • *Ontario Water Resources Act*	Yes	Agronomic rates for N, P, K. Maximum 22t ha^{-1} dry weight per 5 $year^{-1}$
PEI	PEI Department of Environment, Energy and Forestry • *PEI Environmental Protection Act* • *Sewage Disposal Systems Regulations*	Yes	Currently none.
QC	Environment Québec • *Environment Quality Act* • *Regulations respecting agricultural operations* • *Regulations respecting groundwater catchment*	Yes	Agronomic rate (based on N & P)
SK	Saskatchewan Environment • *Environmental Protection and Enhancement Act* • *Water Regulations*	Yes	Agronomic rate basis on N content

2.1.4. Wastewater Treatment Processes

The municipal wastewater goes through various procedures before biosolids treatment processes. These are:

1. Screening and grit removal: The coarse solids and heavy inorganic particles are taken out and landfilled.
2. Primary wastewater treatment: The suspended solids remaining after the pre-treatment are removed by gravity settling.
3. Secondary wastewater treatment: This stage involves treating the wastewater with microorganisms such as bacteria and protozoa to convert soluble solids into microbial biomass. The microbial biomass is then combined with the raw sludge from the primary stages.
4. Tertiary wastewater treatment: This stage involves filtration of the secondary treatment effluent in order to remove almost all the suspended solids which later contribute to biosolids production (U.S.EPA, 1999; Epstein, 2003; OMAFRA, 2017).

The major products of the above-mentioned treatments are sludge (solids) and wastewater effluent (liquid), further treatment (depending upon the legislation) of these solids is required for them to be considered as biosolids (CCME, 2012).

2.1.5. Biosolids Treatment Processes

The quality of biosolids is assessed basis on various parameters such as odour and quantities of heavy metals, pathogens, and trace organics. Thus, the main purposes of the treatment processes are to reduce the pathogen activity, vector attraction, volume, and odour from the biosolids (Hydromantis et al., 2010). The treatment process influences the end-use of the biosolids, as different treatments result in different concentrations of pathogens, heavy metals, available nutrients, and other contaminants in the biosolids (OMAFRA, 2017). The total solids content and

total cost are other factors in selecting a treatment process (Oleszkiewicz & Mavinic, 2002). A single process or a combination of processes are chosen to achieve the required biosolids product. The two most common methods for biosolids treatments are stabilization and dewatering (U.S.EPA, 1999).

2.1.5.1. Stabilization

Stabilization involves physical, chemical, and biological processes, which reduce the odour, pathogens, and heavy metals from biosolids (U.S.EPA, 1999). Various methods of stabilization are discussed below:

2.1.5.1.1. Aerobic Digestion

Aerobic digestion is a biological process that involves using micro-organisms to break down the organic solids in an aerated environment at mesophilic (10°C to 40°C) or thermophilic (40°C to 55°C) temperature to result in CO_2 and water (U.S.EPA, 1999; Hydromantis et al., 2010). Most of the total N in aerobically digested biosolids is present in the organic form and the remaining N is mineral N, available in the form of nitrate (NO_3^-) as aerobic conditions during the digestion favours nitrification of the organic materials (Hayens et al., 2009). Furthermore, the dewatering of the biosolids reduces the concentration of NO_3^- because of its highly soluble nature (Sommers, 1977). The product of mesophilic digestion is Class B biosolids and thermophilic digestion result in Class A biosolids (Hydromantis et al., 2010). Biosolids from both methods are quite difficult to dewater. An alternate, called autothermal thermophilic aerobic digestion (ATAD), is to use microbes to degrade organic solids by providing additional air at a temperature range of 40°C to 80°C. The ATAD results in Class A biosolids in a short time, which could be dewatered to produce sludge cakes (Hydromantis et al., 2010). Aerobically digested biosolids are used as an

organic fertilizer as they provide an appreciable amount of inorganic (mostly NO_3^-) and organic N (U.S.EPA, 1999; Hayens et al., 2009; Hydromantis et al., 2010).

2.1.5.1.2. Anaerobic Digestion

Anaerobic digestion involves microorganisms that decompose sludge in the absence of oxygen (O_2) at mesophilic (35°C) or thermophilic (50° to 57°C) temperature (Hydromantis et al., 2010). The mesophilic process produces Class B biosolids as it is not a pathogen-free product, whereas, thermophilic digestion provides Class A biosolids (Hydromantis et al., 2010). Anaerobic bacteria convert organic solids to CO_2, CH_4, and ammonia (NH_3) (CCME, 2012). Methane produced during the digestion is used as an energy source for the treatment plant or supplied to a power grid (CCME, 2012). Land application of anaerobically digested biosolids provides high organic and inorganic N (mostly ammonium (NH_4^+) as nitrification does not occur under anaerobic conditions) in the soil (U.S.EPA, 1999; Hayens et al., 2009; Hydromantis et al., 2010). Dewatering of anaerobically digested biosolids reduces NH_4^+ content by upto 60% due to the solubilization of NH_4^+ in the water. Additionally, dewatering by air-drying systems results in further loss of total N and NH_4^+ as a result of more mineralization for an extended period and NH_3 volatilization (Rigby et al., 2016).

2.1.5.1.3. Alkaline Stabilization

Alkaline Stabilization is a chemical process, where 30 to 40% alkaline substances (on the wet weight basis) are mixed with sludge to raise the pH (>12) of the mixture. The mixture of sludge and alkaline material(s) is then dried to achieve 60 to 65% solids content (Labrecque, 2010). A combination of high pH and high temperature inactivates or kills the pathogens in the mixture and lowers the odour but results in the loss of NH_3. Lime ($CaCO_3$) is not used as the alkaline material as it will not raise the pH to sufficiently alkaline level, other materials such as quicklime (CaO),

cement kiln dust, lime kiln dust, Portland cement, potassium hydroxide, and fly ash are used to achieve the required pH levels (Labrecque, 2010). Depending upon the process, the product could meet the Class A or Class B biosolids requirements. Class A biosolids are produced by raising temperature either to 70°C for 30 minutes or 52 to 62°C for 12 hours (Hydromantis et al., 2010). The additional temperature is achieved by supplementing more admixture materials and/or adding another heat source (U.S.EPA, 1999). The end product is particularly used for land application and reclamation/neutralization of acidic soils as the total and mineral N is very low (U.S.EPA, 1999; Hydromantis et al., 2010; CCME, 2012).

2.1.5.1.4. Composting

Composting is a biological process, where the decomposition of the dewatered sludge takes place under controlled aerobic conditions which result in a stable product with high organic matter (U.S.EPA, 1999; Hydromantis et al., 2010; CCME, 2012). However, if the dewatered sludge is used solely then it exceeds the optimum water content requirement for composting (50-60% water content by volume) (Wang et al., 2006). The addition of dry bulking materials such as wood chips, sawdust, and shredded yard waste to raw sludge is used to reduce water content and to achieve a solid content of about 38 to 45% (Hydromantis et al., 2010). In aerobic thermophilic digestion, solids are present in water suspension and no external materials are added (Wang et al., 2006). Compost matures in about 3 to 4 weeks, followed by 4 weeks of curing (less-active composting) (U.S.EPA, 1999). Many factors, such as the carbon (C) to N (C:N) ratio of the material, solids content, temperature, and aeration, influence the final product of composting. Although the pathogen levels in compost products are very low, some fungi may survive during the process (U.S.EPA, 1999; Hydromantis et al., 2010). Depending on the municipality, different procedures like open-air windrow, aerated static pile, in-vessel systems are adopted for composting (U.S.EPA,

1999). Composted biosolids have very low concentrations of total N which are mainly present as organic N compounds and a small fraction of mineral N. The low N is due to the dewatering of the sludge and addition of bulking materials before the composting process and immobilization of N by microbes, loss of NH_4^+ as NH_3 volatilization, and NO_3^- leaching during composting and storage (U.S.EPA, 1999; Hayens et al., 2009). Therefore, composted material is mainly used as a soil conditioner which provides N over a longer period (U.S.EPA, 1999; CCME, 2012).

2.1.5.1.5. Heat Drying/Pelletizing

Heat drying involves the use of active or passive dryers to kill pathogens and increase the solids content by removing water from the material, which results in Class A biosolids (Hydromantis et al., 2010; CCME, 2012). Two commonly used procedures for heat drying are convection dryers (such as fluidized bed and rotary drum dryers) and conduction dryers (like paddle, disc, tray, and rotary chamber dryers) (Hydromantis et al., 2010). Considering the drying system, transportation cost, and quality of the biosolids formed, heat-dried biosolids could be used as fertilizer or biofuel (U.S.EPA, 1999; Hydromantis et al., 2010; CCME, 2012). Sometimes, the dried biosolids are formed into pellets as well (U.S.EPA, 1999; CCME, 2012). The end product is very dry and low in volume; thus, the transportation costs are reduced (U.S.EPA, 1999).

2.1.5.2. Dewatering

Dewatering is a mechanical process in which water and solids are separated to produce a product with greater solids content (Hydromantis et al., 2010; CCME, 2012). Dewatering is generally done prior to the next stage of biosolids processing, other beneficial uses, or disposal (U.S.EPA, 1999; Hydromantis et al., 2010). Dewatering could be achieved by air drying systems or mechanical systems. Air drying system requires more time and area to operate, thus, they are usually used by small WWTP. Large WWTP utilize mechanical systems such as centrifuges,

vacuum filters, belt filter press, and plate and frame filter press (U.S.EPA, 1999). Dewatering increases the solids content of sludge but does not reduce pathogen or contaminant levels (Hydromantis et al., 2010).

2.1.6. Benefits of Biosolids on Soil and Plants

Land application is the most beneficial method of using biosolids. Land-applied biosolids are more suitable in terms of economic and environmental factors as compared to landfilling and/or incineration (Hargreaves et al., 2008). Biosolids are applied as organic fertilizers and soil conditioners to agricultural lands, forests, reclamation sites, public lands, golf courses, lawns, and home gardening depending upon the available nutrients, organic matter, and biosolid treatment level (U.S.EPA, 1999).

The land application of biosolids alters the physical, chemical, and biological properties of the soil (Sharma et al., 2017). The presence of a high amount of organic matter (about 40 to 70%) in biosolids results in improved soil aggregates stability because of its binding properties. The increase in soil aggregate stability enhances soil porosity and reduces soil bulk density. Also, hydraulic conductivity and infiltration rate rise with the increase in soil porosity (Haynes et al., 2009). Biosolids amendment to soil improves soil health and increases soil organic C by direct addition of organic matter to the soil and indirectly by affecting other soil properties (Haynes et al., 2009; Sharma et al., 2017). Depending on the type of biosolid applied, the pH of the soil could be affected (Sharma et al., 2017). Land-applied biosolids result in additional cation exchange sites in the soil due to the presence of negative functional groups on humic substances, which increases the soil's cation exchange capacity and help to retain and make available more nutrients for plants (Haynes et al., 2009). Biosolids can form stable complexes with heavy metals, thus reducing the solubility, leaching, and bioavailability of heavy metals (Antolín et al., 2005). Parat et al. (2007)

reported that the application of biosolids significantly affected the retention and mobility of heavy metals due to the presence of organic matter in biosolids, which formed macro-aggregates with heavy metals. Biosolids have seen to significantly improve the fertility of soils with low initial organic matter (Mattana et al., 2014). Soil enzyme activities are also enhanced after land application of biosolids mainly due to the presence of high numbers of enzymatic substrates like peptides and proteins in the biosolids (Torri et al., 2014). Biosolids help in soil microbial proliferation, as biosolids contain nutrients and a high amount of organic matter which increases the soil microbial respiration and provides energy (Sharma et al., 2017). However, the variation in organic matter can be seen with different treatments and waste sources (Haynes et al., 2009).

Nitrogen and phosphorus (P) are often the most limiting nutrients in crop production (Ågren et al., 2012). Biosolids consist of a considerable amount of nutrients in organic and inorganic forms (Rigby et al., 2016). The nutrients in biosolids include N, P, sulphur (S), potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), molybdenum (Mo), copper (Cu), and boron (B), although concentrations and type of these nutrients vary with the treatment of the biosolids (Haynes et al., 2009; Rigby et al., 2016; Sharma et al., 2017). For example, aerobically digested biosolids contain 1.6-6.1% of N and 1.8-4% of P by dry mass, whereas anaerobically digested biosolids have 2.8-9.0% of N and 1.5-6.3% of P by dry mass (OMAFRA, 2017). Mineral N in aerobically and anaerobically digested biosolids is mostly present as NH_4^+ and NO_3^-, respectively. Furthermore, composted and lime treated biosolids have a lower fraction of total and mineral N compared to digested biosolids due to the loss of NH_4^+ and NO_3^- during the process and storage due to mechanical agitation and high pH and temperature, respectively (Rigby et al., 2016). The type of biosolids treatment also affects the mineralization of organic N after biosolids amendment to the soil (Haynes et al., 2009). For instance, aerobically digested biosolids

have a greater mineralization rate than anaerobically digested biosolids due to the rapid microbial growth in an aerobic environment, whereas metabolic activities are restricted in an anaerobic environment which inhibits microbial growth. Composted biosolids have a low mineralization rate due to the immobilization of N as a result of a high C:N ratio (Haynes et al., 2009; Rigby et al., 2016). Land-applied biosolids can meet the crop requirements by providing both mineral and organic forms of nutrients (mainly N and P) over a longer period (Rigby et al., 2016).

2.1.7. Concerns Related to Biosolids

Biosolids provide many benefits to plants and soil but at the same time, biosolids can cause some negative impacts on human health and the environment. Therefore, there are concerns relating to the use of biosolids for land application by the public (Epstein, 2003; Sharma et al., 2017). The most common concerns about biosolids are the presence of odour, heavy metals, pathogens, and organic pollutants (Lu et al., 2012).

2.1.7.1. Odour

The "foul-smell" from biosolids is a nuisance and provokes fears about toxic and harmful contaminants in biosolids (U.S.EPA, 1999). However, biosolids stabilization destroys many of the odour-causing bacteria. The leftover bacteria from the stabilization or the addition of chemicals during the stabilization may also contribute to odour from the biosolids (OMAFRA, 2017). Odour hinders efforts to gain the public's acceptance of the land application of biosolids, thus elimination or mitigation of odour is a big challenge (Lu et al., 2012).

2.1.7.2. Heavy Metals

Biosolids contain some heavy metals (such as Zn, Cu, and Mo) that are required for plant growth. The repeated application of biosolids can result in more uptake of these metals by plants and accumulation in soil or leaching to surface or subsurface waters, which may affect yield and

quality of crops, soil quality, and human health through the food chain (Lu et al., 2012; OMAFRA, 2017). However, various authors have opposed or supported this theory (Lu et al., 2012). The bioavailability of heavy metals is highly affected by biosolids, soil, and plant properties (Epstein, 2003). In Canada, the CFIA under Trade Memorandum T-4-93- *Standards for metals in fertilizers and supplements* has standard for maximum acceptable cumulative heavy metal addition to soil and the application rate of the product. Thus, most provinces have strict standards for maximum heavy metal concentrations in biosolids aimed for land application (CCME, 2010).

2.1.7.3. Pathogens

Pathogens are a major concern related to biosolids because human waste is a major source of wastewater sludge. Pathogens can be categorized into primary pathogens such as bacteria, viruses, protozoa, and helminths and secondary pathogens such as fungi (Epstein, 2003; Lu et al., 2012). The main objective of stabilization is to kill or eliminate pathogens, however, they are not completely destroyed (only reduced by 90-99%) (U.S.EPA, 1999). The land application of biosolids could result in the transportation of pathogens to humans through plants and water resources (Epstein, 2003; Lu et al., 2012). Fecal coliforms, *Salmonella,* and *E.coli* are used as the selected organisms to indicate pathogen levels and treatment effectiveness of biosolids. In Nova Scotia, for the land application of Class A biosolids, *Salmonella* and fecal coliforms must be <3 most probable number (MPN) $4g^{-1}$ and <1000 MPN g^{-1}, respectively and for Class B biosolids, fecal coliforms must be <2,00,000 MPN g^{-1} (CCME, 2010).

2.1.7.4. Organic Pollutants

Wastes from industries, households, and hospitals may contain traces of some of the synthetic organic compounds such as antibiotics, steroids, dioxins, chlorinated and hormonal compounds, and personal care products. When the biosolids from these sources are land applied,

organic compounds undergo various transformations (Epstein, 2003; Lu et al., 2012). Consequently, the resulting products may be toxic or carcinogenic and have the potential to migrate from soil to water, plants, humans, and atmosphere or accumulate in soil, therefore causing harm to human health and environment (Lu et al., 2012). In Nova Scotia, dioxins and furans must not exceed 17 and 50 ng toxic equivalent (TEQ) kg^{-1} for Class A and Class B biosolids intended for land application, respectively (CCME, 2010).

2.1.7.5. Excess Nutrients

Land application of biosolids may result in excess nutrients (mainly N and P) in the soil, which can have an adverse effect on the ecosystem and human health (Lu et al., 2012). Land applied biosolids based on N requirements can increase P concentrations in surface or subsurface water and soil as biosolids have relatively low N:P ratio as compared to other inorganic and organic fertilizers (He et al., 2000). The NO_3^- present in biosolids or NO_3^- produced through the nitrification process, migrates from soil to groundwater and could pose health and environmental risks (Lu et al., 2012).

2.1.7.6. Greenhouse Gas Emissions

Land application of biosolids triggers greenhouse gas (GHG) emissions as they provide high organic matter and sufficient N for the microbes (Paramasivam et al., 2008). Many studies have reported GHG emissions after biosolids amendment of the soil (Akiyama et al., 2004; Ros et al., 2006; Paramasivam et al., 2008; De Urzedo et al., 2013; Carmo et al., 2014; Pitombo et al., 2015; Yoshida et al., 2015; Willén et al., 2016; Charles et al., 2017). Paramasivam et al. (2008) conducted an incubation study and observed an increase in GHG emissions with the increase in application rate when compared to an unamended control. Further, the authors reported that the addition of domestic sewage sludge produced more GHG emissions than industrial sewage sludge,

which they attributed to the lower C and N levels or presence of harmful compounds in industrial sewage sludge that might have inhibited microbial growth. Land application of sewage sludge compost and sewage sludge increased CO_2 emissions compared to urea and control treatments under degraded pastureland. The loss of C as CO_2-C from composted sewage sludge (0.7%) was observed lower than from sewage sludge treatment (2.7%) (Carmo et al., 2014). De Urzedo et al. (2013) found similar results after the application of sewage sludge compost that increased cumulative CO_2 (59 kg C ha^{-1}) and nitrous oxide (N_2O) (0.43 kg N ha^{-1}) emissions compared to urea treatment (35 kg C ha^{-1}; 0.05 kg N ha^{-1}) and control (31.1 kg C ha^{-1}; 0.005 kg N ha^{-1}) over 172 days of the study period. Additionally, low C loss as CO_2-C from sewage sludge compost (0.67%) was reported, which was attributed to the high C content in sewage sludge compost. A long-term study (12 years) found that the application of composted sewage sludge (previously aerobically digested) increased the soil respiration more than urea and control treatments by 58% and 43% when applied at a rate of 170 kg N ha^{-1} without urea and by 40% and 26% when applied with 80 kg N ha^{-1} of urea, respectively. The authors related this to the change in microbial community composition from the long-term exposure to composted sewage sludge, which increased the metabolic quotient and basal respiration (Ros et al., 2006). Akiyama et al. (2004) reported from their 38 days incubation study that at 80% water-filled pore space (WFPS), the application of sewage sludge pellets resulted in 200 mg N_2O-N m^{-2} compared to about 70 mg N_2O-N m^{-2} from urea and 10 mg N_2O-N m^{-2} from control treatments, respectively. Pitombo et al. (2015) observed an increase in N_2O and CO_2 emissions by 453% and 78% after seven years of sewage sludge amendment compared to the inorganic fertilized control, respectively. The authors concluded that the temperature sensitivity of CO_2 fluxes increased with the increase in sewage sludge application rate. Cumulative N_2O emissions were reported 1.31 kg N ha^{-1} after the

immediate incorporation of mesophilically digested and dewatered sewage sludge in comparison with 0.09 kg N ha^{-1} from treatments without sewage sludge (Willén et al., 2016). A laboratory study reported that the land application of anaerobic digested, composted, reed bed stabilized, and lime treated sludges decreased N_2O, CO_2, and CH_4 emissions as compared to untreated sludge treatments. The authors suggested that stabilization helped reduce N_2O and CH_4 emissions and lower the C mineralization rate (Yoshida et al., 2015). López-Fernández et al. (2007) found lower total season N_2O emissions from composted sewage sludge (3.77 kg N ha^{-1}) compared to urea applied treatments (5.89 kg N ha^{-1}) when maize plants were present while there was no difference in N_2O emissions when the soil was bare. This was related to the high C content in composted sewage sludge that favoured N immobilization, which, in combination with N uptake from the maize plants.

2.2. Nitrous Oxide

Natural and arable lands are a significant source of the total atmospheric N_2O accounting for almost 70% of the total global anthropogenic N_2O emissions. It is mainly due to the microbial processes occurring in soils (Butterbach-Bahl et al., 2013). Complete knowledge of the N_2O producing processes and soil and environmental factors that affect these processes can help in developing mitigation practices for N_2O (Ussiri & Lal, 2013).

2.2.1. Nitrous Oxide Production in Soil

Generally, denitrification and nitrification are the two major pathways that contribute to N_2O emissions from the soil, with denitrification being the dominant source (Wrage et al., 2001). Most often, both processes occur simultaneously in the soil at different microsites (Smith, 2010). Some other important processes such as chemodenitrification, chemical decomposition of hydroxylamine, coupled nitrification-denitrification, nitrifier denitrification, co-denitrification,

dissimilatory nitrate reduction to ammonium (DNRA) also contribute to N_2O production in soil (Butterbach-Bahl et al., 2013).

2.2.1.1. Nitrification

Nitrification is a two-step aerobic oxidation of NH_3 or NH_4^+ to nitrite (NO_2^-) and finally to NO_3^-. In the first step, known as NH_3-oxidation, autotrophic bacteria oxidize NH_3 to NO_2^- (Smith, 2010; Butterbach-Bahl et al., 2013). Recent findings suggest that NH_3-oxidizing archaea are also responsible for NH_3-oxidation (Ussiri & Lal, 2013; Hu et al., 2015). Ammonia monooxygenase and hydroxylamine oxidoreductase are the two enzymes that carry NH_3-oxidation. Depending upon the O_2 level and other conditions, NH_3-oxidation results in nitric oxide (NO) and N_2O as by-products (Ussiri & Lal, 2013). In the second step, called NO_2^--oxidation, autotrophic bacteria oxidize NO_2^- to NO_3^- (Smith 2010; Ussiri & Lal, 2013). In low O_2 conditions, autotrophs use NO_2^- as an electron acceptor rather than O_2, resulting in nitrogen gas (N_2) or intermediate products such as NO and N_2O, and the process is known as nitrifier denitrification (Zhu et al., 2013).

2.2.1.2. Denitrification

Denitrification is the reduction of NO_3^- to N_2 or partial reduction to NO and N_2O, which is predominately carried out by heterotrophic bacteria under anaerobic conditions. Some species of archaea and fungi are also capable of denitrification. Nitrate reductase, NO_2^- reductase, NO reductase, and N_2O reductase are the enzymes responsible for denitrification (Ussiri & Lal, 2013).

2.2.2. Factors Affecting Nitrous Oxide Fluxes from Soil

Many environmental and soil factors such as soil moisture and aeration, soil pH, soil temperature, C:N ratio of the substrate, and organic matter, influence denitrification and nitrification processes, and ultimately N_2O emissions from soil (Ussiri & Lal, 2013).

2.2.2.1. Soil Moisture and Aeration

Soil moisture influences N_2O fluxes through affecting the growth and activity of microbes, the release of N and C by wetting and drying cycles, diffusing substrates and products to microbial sites, and reducing the O_2 concentrations by filling the soil pores with water (Pathak, 1999). As denitrification occurs in anaerobic conditions, higher soil moisture (≥60% WFPS) promotes denitrification as a result of reduced O_2 availability and results in more N_2O production (Paul, 2007). However, in highly saturated soils (≥90% WFPS), the supply of O_2 is sufficiently limited that N_2O is consumed by N_2O reductase enzymes to generate N_2 (Pathak, 1999; Ussiri & Lal, 2013). Rochette et al. (2010) reported a sharp reduction in N_2O reductase activity when exposed to O_2 after an anaerobic period compared to the activity of other denitrifying enzymes. Thus, after a heavy rainfall or irrigation event, denitrification results in N_2O over N_2. When aerobic soils receive heavy rainfall or irrigation, NO_3^- and NO_2^- reductase enzymes activate sooner than N_2O reductase enzymes for one to two days after the event, resulting in more N_2O production than N_2 (Smith, 2010). Zhu et al. (2013) demonstrated that at low O_2 levels (0.5% to 3%), nitrifier denitrification was responsible for almost half of the total N_2O production depending upon the type of fertilizer applied.

2.2.2.2. Soil Mineral Nitrogen

Soil mineral N (NH_4^+ and NO_3^-) substrates for nitrification and denitrification, respectively play a very important part in N_2O production (Aguilera et al., 2013). Nitrate acts as an electron acceptor and a limiting component for denitrification in unfertilized and saturated soils, thus the availability of NO_3^- increases the N_2O production during denitrification under anaerobic conditions (Paul, 2007; Ussiri & Lal, 2013). The N_2O emissions from soil are positively correlated with soil NH_4^+ and NO_3^- concentrations in many studies (Aguilera et al., 2013), however, the magnitude

varies with the rate, type, and timing of N-fertilizers (Synder et al., 2009). Nitrogen-fertilizers are considered as the main drivers of N_2O emission from the soil (Butterbach-Bahl et al., 2013). The N_2O peaks were noticed after the application of inorganic N-fertilizers as they provide rapid mineral N to microbes (Lee et al., 2009).

2.2.2.3. Soil Temperature

Temperature is an important factor as it controls the nature and extent of soil microbial activity and indirectly influences the soil water content through evaporation (Aguilera et al., 2013). The optimum temperature range for nitrification is 25 to 35°C. Nitrifiers' activity is slow at temperatures below 15°C but continues even at 0°C. Denitrification can occur in soil temperatures ranging from 4 to 60°C (Ussiri & Lal, 2013). Dorland and Beauchamp (1991) reported that denitrification occurs even at -2°C if the organic matter is available. A significant positive relationship between soil temperature and N_2O fluxes was found in many cases (Aguilera et al., 2013; Cameron et al., 2013). Freezing and thawing change the physical environment of the soil and therefore influence microbial activities (Phillips, 2008). Christensen and Tiedje (1990) found that N_2O production was higher during thawing than at any other time of the year.

2.2.2.4. Soil pH

Soil pH controls the nitrification and denitrification in the soil, consequently influencing N_2O emissions (Cameron et al., 2013). Nitrification and denitrification occur more rapidly between pH 6 to 10 and 6 to 8, respectively (Ussiri & Lal, 2013). Some NH_3-oxidizing archaea and bacteria carry out an appreciable amount of nitrification even at pH<6 (De Boer & Kowalchuk, 2001; Wrage-Mönnig et al., 2018), but are limited below 4.5 (Ussiri & Lal, 2013). Under highly acidic conditions, NH_3 exists as NH_4^+, which is not preferred by NH_3-oxidizing bacteria. Additionally, the decomposition rate of organic matter reduces and resulting a low amount of the substrate in

the soil (Smith, 2010; Ussiri & Lal, 2013). In the long term, N_2O reduction is usually inhibited at low pH, resulting in a high N_2O: N_2 ratio (Pathak, 1999; Aguilera et al., 2013).

2.2.2.5. Carbon to Nitrogen Ratio

The C:N ratio of the organic materials is an important parameter that determines the potential immobilization or mineralization of N, thus eventually affects the amount of mineral N in the soil and the potential N losses from the soil (Paul, 2007; Ussiri & Lal, 2013). Higher C:N ratio leads to immobilization, which means lesser available NH_4^+ for plants and microbes. Nitrification accelerates when NH_4^+ exceeds the needs of plants and heterotrophic bacteria because nitrifiers are poor competitors compared to heterotrophic bacteria for C and N (Ussiri & Lal, 2013). Thus, the materials with a low C:N ratio favour mineralization over immobilization and promote N_2O emissions (Signor & Cerri, 2013).

2.2.2.6. Soil Carbon

The increase in soil C results in large N_2O production (Signor & Cerri, 2013). Soil C serves as an electron donor for NO_3^- reduction in the denitrification process and as an energy source for heterotrophic denitrifiers (Thangarajan et al., 2013). Additionally, C also influences the O_2 availability in the soil as C increases the microbial respiration, resulting in more consumption of O_2, consequently creating more anaerobic sites for denitrification (Smith, 2010). The addition of easily decomposable organic matter to the soil rapidly increases C and promotes denitrification (Smith, 2010).

2.3. Carbon Dioxide Emissions

Soil acts as a source and sink of CO_2 (Rastogi et al., 2002). Plants fix atmospheric CO_2 through photosynthesis, which can be converted to soil organic matter and can be stored in the soil (Sainju et al., 2008). A part of C after organic amendment (such as manures, composts, and cover

crops) of the soil may also be stored as soil organic C (Pitombo et al., 2015; Montiel-Rozas et al., 2016). Soil microbes decompose a part of soil organic matter and external organic residues and release C into the atmosphere as CO_2 and/or CH_4 (Rastogi et al., 2002). Thus, it is important to understand the process involved in soil respiration to sequester C into the soil.

2.3.1. Carbon Dioxide Production in Soil

Soil respiration refers to the release of the CO_2 from soil to atmosphere. It includes three biological processes: root respiration, microbial respiration, and faunal respiration. In addition to these biological processes, chemical oxidation, a non-biological process, also contributes to CO_2 production in soil (Rastogi et al., 2002). The common pathways of CO_2 production in the living tissues are the tricarboxylic acid cycle (or Krebs cycle) in aerobic conditions and fermentation in anaerobic conditions. However, some other pathways such as methanogenesis, phototrophs, and carbonic reactions, also produce or consume CO_2 (Lou & Zhou, 2006).

All plants require energy to perform essential functions of life including growth, movement, transport, repair, and reproduction. Plants utilize photosynthates as a C source to gain energy and release CO_2 in the presence of O_2, this process is known as root respiration (Parker, 2000). Root respiration is responsible for almost half of the total soil respiration, although it can vary from 10 to 90% depending upon the conditions (Lou & Zhou, 2006).

The rhizosphere is a substrate-rich zone as roots continuously release exudates into the soil. Depending upon the plant species, almost 20% of the photosynthesized C is delivered to the soil during the vegetation period (Hütsch et al., 2002). The rhizosphere is a favourable habitat for all soil microorganisms. Soil microbes decompose root exudates, plant litter, and external organic residues, resulting in CO_2 and other compounds (Lou & Zhou, 2006). There are two types of soil microbial decomposers: K-strategists and r-strategists (Weil & Brady, 2017). The r-strategists

primarily feed on the fresh organic material and have the potential for rapid growth and proliferation. The K-strategists mainly decompose soil organic matter and are known for slow growth rates. As the fresh substrate depletes, r-strategists die or become dormant, while K-strategists persist in the soil (Fontaine et al., 2003; Fontaine et al., 2011).

Soil animals also play a key role in producing CO_2 in the soil. They feed on fungi and bacteria, break down the large pieces of litter, and change the soil structure. The fragmentation of the litter by these animals produces CO_2. These animals are categorized into microfauna, mesofauna, and macrofauna. Microfauna includes the smallest animals in the soil, such as nematodes and some mites. Nematodes are abundant in soil and very important as they decompose roots, bacteria, and fungi. Mesofauna and macrofauna are important decomposers of the organic materials and release CO_2 in the atmosphere. Soil animals contribute about 5% of the total soil respiration; however, they influence decomposition by affecting microbial activities in the soil (Lou & Zhou, 2006).

2.3.2. Factors Affecting Carbon Dioxide Emissions from Soil

The most critical factors that influence CO_2 fluxes from the soil are soil temperature, soil moisture, substrate supply, soil N, soil pH, and plant growth (Rastogi et al., 2002; Lou & Zhou, 2006; Thangarajan et al., 2013).

2.3.2.1. Soil Temperature

Generally, soil respiration starts increasing exponentially between 20 to 40^0C and declines rapidly above 50^0C (Rastogi et al., 2002). The increase in soil respiration is due to the higher microbial activity at a relatively high temperature which results in more root respiration, and decomposition of the organic materials present in the soil (Thangarajan et al., 2013; Weil & Brady, 2017). Previous studies concluded that the soil temperature sensitivity is indirectly proportional to

soil respiration, indicating that soil respiration is more sensitive to temperature change at low temperatures (Lal, 2006). Soil temperature affects various aspects of soil respiration. At the biochemical level, variation in soil temperature influences the availability of soluble and membrane-attached enzymes, which are important in the production of CO_2. Soil temperature has a direct effect on plant growth and photosynthesis. Root respiration increases with a rise in temperature up to 35^0C and above that protoplasm may begin to break down. High temperatures could result in drying conditions that promote O_2 diffusion into the soil by reducing the soil moisture, thus stimulating CO_2 from the soil (Lou & Zhou, 2006).

2.3.2.2. Soil Moisture

Soil moisture plays a key role in determining the consumption or production of CO_2 by directly affecting the O_2 availability in the soil and indirectly affecting microbial and root physiological processes through water-stressed and waterlogged conditions. In general, CO_2 fluxes are low under very dry conditions, reach peak under optimum soil moisture conditions (near field capacity), and decline in anaerobic conditions (Lou & Zhou, 2006). Dry conditions cause desiccation stress for root respiration and affect microbial respiration by restricting the diffusion of the substrates (Davidson et al., 2006). Under waterlogged conditions, anaerobic or facultative organisms carry out the decomposition and consume CO_2 to produce CH_4 (Weil & Brady, 2017). Periodic drying and wetting of the soil can result in a burst of CO_2 fluxes due to the quick decomposition of the microbial biomass that died during the drying period, more interaction between microbes and organic material, and increase in the substrate through desorption from the soil matrix (Lou & Zhou, 2006).

2.3.2.3. Substrate Supply

Organic soil amendments are known to increase soil respiration (Lou & Zhou, 2006), but are influenced by the placement and quality of the organic materials as they affect the decomposition process and ultimately CO_2 flux from the soil (Weil & Brady, 2017). Decomposition of sugars, starch, and simple proteins occurs rapidly, whereas lignin and humic acids may take hundreds of years to decompose (Lou & Zhou, 2006). Organic materials with labile organic C and sufficient N are the immediate source of CO_2 under aerobic conditions. The high C:N ratio is associated with the lower decomposition rates as microbes cannot fulfill their N requirement (Paul, 2007). Incorporated materials are more prone to decomposition and result in more CO_2 than the surface spread materials due to the intimate contact of incorporated materials with soil microorganisms and more constant temperature and moisture (Weil & Brady, 2017).

2.3.2.4. Soil Nitrogen

The addition of N to the soil through organic or inorganic fertilizers enhances soil respiration directly by supplying N to plants and microbes and indirectly by affecting other soil factors such as pH (Rastogi et al., 2002). Also, materials with high N content result in more vegetative growth of the plant, producing more carbohydrates and increasing root respiration and microbial respiration (Lou & Zhou, 2006).

2.3.2.5. Soil Texture

Soil texture affects soil porosity and regulates water movement, gas diffusion, and water-holding capacity and indirectly controls soil respiration. Fine-textured soils provide a better environment for root growth than the coarse-textured soils, which ultimately affects root respiration. Soil texture is also related to decomposition rate as the fine-textured soils tend to have more organic matter and N content than coarse-textured soils (Lou & Zhou, 2006). Silver et al.

(2005) reported faster root litter decomposition in clay soil than sandy loam soil. The composition of microbial populations also affected by soil texture (Santruckova et al., 2003).

2.3.2.6. Soil pH

Soil pH is related to microbial chemical and enzymatic reactions, which help them survive and proliferate in soil. Mostly, soil microorganisms are reported to grow rapidly with the increase in pH, from very acidic to slightly alkaline conditions (Weil & Brady, 2017). Consequently, near-neutral pH conditions result in more CO_2 fluxes (Lou & Zhou, 2006). Kowalenko et al. (1978) reported rising CO_2 emissions with an increase in soil pH up to 7 and a decline afterwards.

2.3.2.7. Plant Growth

Plant growth stages have also been found to be significantly related to CO_2 flux (Schlesinger & Andrews, 2000). The increase in soil respiration with the increase in plant growth can be related to the more photosynthesis in plants and consequently more carbohydrate production, which is later oxidized to result in CO_2 in the soil by rhizosphere microbes (Xu et al., 2008).

2.4. Methane

Soils act as a source and sink of CH_4. About 70-80% of the atmospheric CH_4 is mainly produced by methanogenic bacteria that decompose organic compounds under anoxic conditions (Le Mer & Roger, 2001). Methanotrophs carry out the oxidation of CH_4 to CO_2 in the aerobic zones of the soil. Additionally, CH_4 is oxidized in the troposphere by hydroxide ion and in the stratosphere by chlorine (Cl) ion (Le Mer & Roger, 2001; Mosier et al., 2004). Most of the CH_4 produced in the soil is oxidized before it enters the atmosphere, thus most agricultural soils act as a CH_4 sink (Maljanen et al., 2003).

2.4.1. Methane Production and Consumption in Soil

The balance between the production of CH_4 through methanogenesis and oxidation through methanotrophs determines if the soil is a net source or sink of CH_4. When CH_4 production is more than the consumption, then soils act as a CH_4 source and vice-versa. In the soil environment, both these processes occur simultaneously (Le Mer & Roger, 2001).

2.4.1.1. Methanogenesis

The decomposition of the organic materials under anaerobic conditions with low levels of sulphate, Fe, and NO_3^- result in CO_2 and CH_4 (Le Mer & Roger, 2001; Hayashi et al., 2015). Acetotrophy (oxidation of acetate) and reduction of CO_2 by hydrogen gas (H_2) are two important pathways of CH_4 production in most environments with the former being dominant. Methane production requires four types of microbial populations that decompose complex compounds into simpler substrates. In the first step hydrolysis of biological polymers to monomers (such as fatty acids, glucides, and amino acids) occur which is carried out by hydrolytic microbes in either aerobic or anaerobic environments. In the second phase, acidogenesis takes place under anaerobic conditions from monomers and intermediate products, released during fermentation, to produce organic acids, alcohols, volatile fatty acids, H_2 and CO_2. In the third step, metabolites are used for acetogenesis by syntrophic or homoacetogenic microbes. In the final phase, methanogenesis occurs, in which simple compounds such as H_2+CO_2 and acetate are used to produce CH_4 and water (Le Mer & Roger, 2001).

2.4.1.2. Methanotrophy

Methanotrophs carry out CH_4 oxidation through two pathways using the CH_4-monooxygenase enzymes found in methanotrophic bacteria and NH_3-monooxygenase enzymes present in nitrifying bacteria (Le Mer & Roger, 2001). Many sequential intermediate products such

as methanol, formaldehyde, and formic acid form during the transformation of CH_4 to CO_2 (Topp & Pattey, 1997). Mostly, methanotrophs are obligate, although facultative methanotrophs were also found in recent studies. Obligate methanotrophs feed only on CH_4 or the intermediate products of the CH_4 oxidation, whereas, facultative methanotrophs thrive either on CH_4 or other multicarbon compounds (Dedysh & Dunfield, 2011). Methanotrophs are found in the oxidized soil layers, submerged leaf sheaths, inside the roots with aerenchyma and in the aerobic rhizosphere (Topp & Pattey, 1997; Le Mer & Roger, 2001).

2.4.2. Factors Affecting Methane Emissions from Soil

Methane production and consumption depend upon many soil and environmental factors (Topp & Pattey, 1997; Le Mer & Roger, 2001; Hayashi et al., 2015).

2.4.2.1. Soil Moisture

Soil moisture is the most important factor in determining CH_4 production or consumption as it directly controls the O_2 availably, diffusion of CH_4 to the atmosphere, and activity of methanogens and methanotrophs. Soil CH_4 consumption is lower under high soil moisture conditions as the methanotrophic activity reduces with the increase in water content after reaching the field capacity. The CH_4 production is high in submerged soils, however, the diffusion of the gas is restricted by more WFPS. The release of this trapped CH_4 from the soil and simultaneously consumption of CH_4 by methanotrophs increases as the soil begin to dry (Topp & Pattey, 1997; Le Mer & Roger, 2001). Methanogens are activated once the soil is exposed to heavy rainfall or irrigation event for several days (Li, 2007). Under these conditions, major soil oxidants like NO_3^-, O_2, Fe, manganese, and sulfate are used in other biological processes, favouring methanogenesis (Le Mer & Roger, 2001). This suggests that soil may act as a CH_4 source after a rainfall event and snowmelt.

2.4.2.2. Soil Temperature

The activity of methanogens and other microbes involved in fermentation decrease under low soil temperature conditions. The optimum temperature for methanogenesis is somewhere between 30 to 40^0C. However, methanotrophs are more tolerant of temperature fluctuations than methanogens. High temperature causes evaporation of the soil moisture and promotes CH_4 consumption (Le Mer & Roger, 2001).

2.4.2.3. Redox Potential

Methane production and transportation to the atmosphere are highly dependent upon the redox potential of the soil layers (Le Mer & Roger, 2001). Methane emissions take place at very low redox potential where O_2, NO_3^-, Fe, manganese, and sulphate are in a reduced state. These conditions are achieved by prolonged waterlogging of the soil as mostly found in rice fields and wetlands (Smith et al., 2003). The minimum redox potential reported was -150mV for methanogenesis and CH_4 production increases with a further drop in redox potential (Topp & Pattey, 1997). The diffusion of CH_4 to the atmosphere is affected by redox potential as aerenchyma formation increases at low redox potential (Le Mer & Roger, 2001).

2.4.2.4. Substrate Supply

Organic compounds are an important limiting factor under anaerobic conditions for methanogenesis (Dalal et al., 2008). The presence of organic materials in the soil promotes fermentation and ultimately production of CH_4 under anaerobic conditions as there is more substrate for microbes to decompose. The physical and chemical composition of the organic material significantly affects the CH_4 emissions. The application of slurries favours methanogens by providing substrates and by inhibiting O_2 diffusion into the soil (Thangarajan et al., 2013). A positive correlation was reported between C:N ratio of the organic material and CH_4 emissions (Le

Mer & Roger, 2001). De Urzedo et al. (2013) observed an increase in CH_4 emissions after the application of organic material compared to an unamended control.

2.4.2.5. Soil pH

Methanogens are very sensitive to pH changes compared to methanotrophs. Generally, high CH_4 emissions are under neutral or slightly high pH conditions (Le Mer & Roger, 2001). Low growth of many methanogen species observed at pH below 5.6 (Garcia et al., 2000). In a temperate climate, CH_4 production was found higher between the pH range of 5.5 to 7.0 (Topp & Pattey, 1997). Weslien et al. (2009) reported optimum pH was 5.5 for methanotrophs and no CH_4 oxidation occurred below pH 3.

2.4.2.5. Other Factors

Soil texture affects the diffusion of CH_4 and O_2, aerobic or anaerobic sites through influencing porosity, and decomposition rate by protecting organic matter. These factors further decide CH_4 production or consumption and diffusion into the atmosphere. Generally, clay soils are considered to emit more CH_4 because of low drainage from the soil thus creating more anoxic zones, favouring methanogenesis (Le Mer & Roger, 2001). Additionally, oxidation of CH_4 is more prominent in coarse-textured soils (Smith et al., 2003).

The presence of NO_3^-, O_2, Fe, manganese, or sulfate suppresses CH_4 production as their reduction results in more energy than methanogenesis. The three possible mechanisms that are accountable for this effect and that maybe occurring separately or at the same time are the increase in redox potential, reduction in organic matter content, and toxicity to methanogens (Segers, 1998).

2.5. Ion Exchange Membranes

Ion exchange is a reversible process that involves an interchange of ions between two phases. Ion exchange resins (IERs) are insoluble organic or inorganic polymers used to exchange

cations or anions within aqueous solutions in their vicinity. An IER resembles spherical beads with a diameter between 1 to 2 mm, usually white or yellowish. The general structure contains polystyrene chains, fixed negatively charged exchange sites, mobile positively charged exchangeable sites, divinylbenzene cross-links, and water. These are designed to accumulate ions in the soil and thus act as ion sinks (Borodkin, 1993). Two major IERs are cation and anion exchange resins respectively used to measure cations and anion fluxes in the soil (Qian & Schoenau, 2002). The use of IERs to measure nutrient availability in the soil begin in 1951 (Pratt, 1951). In the beginning, ion exchange beads were more popular but later ion exchange membranes (IEMs) were used because of their definite surface area and better handling and use (Qian & Schoenau, 2002).

The two most popular methods used for IERs in agriculture to assess nutrient concentration and rate of release in soils are batch systems and diffusion-sensitive systems. In batch systems, the IERs are mixed with a certain amount of soil for a fixed period in an excess of water. The results are affected by several factors such as the ratio of soil, resin, and solution, shaking time, type of resin, temperature, and methods to recover the ions. These systems only indicate the amount of a specific nutrient from the mixture of soil suspension, resin, and water but do not provide any information relative to the diffusion process. The nutrient movement processes are very important relative to nutrient availability in the soil. In diffusion-sensitive systems, IERs are placed with soil in situ conditions for a certain amount of time. Diffusion-sensitive systems are more efficient than the batch systems because they also determine the available forms of nutrients along with the concentrations. The diffusion of ions depends upon the resin's exchange capacity, size and type, soil moisture, and soil temperature (Qian & Schoenau, 2002).

2.5.1. Ion Exchange Membranes and Measurement of Mineral Nitrogen

Nitrogen is the most common limiting factor in crop production (Ågren et al., 2012). The land application of biosolids can provide the required N in the form of readily available mineral N and organic N through mineralization. However, estimating the amount of organic N mineralized is important to avoid various N losses (Rigby et al., 2016). Traditional soil testing methods have failed in assessing the temporal changes in mineral N in the soil, whereas IEMs provide an alternative to measure mineral N fluxes in field conditions (Hangs et al., 2004; Castro & Whalen, 2016). The IEMs measure the flux of ions in a soil solution as they (IEMs) adsorb ions to their surface that are transported through diffusion and/or mass flow in the soil solution. The IEMs constantly accumulates ions on their surface in the soil solution, acting as an ion sink, until the membranes become saturated and then their function shits to a dynamic exchanger from a sink of ions (Qian & Schoenau, 2002). The IEMs have been successfully used to determine temporal dynamics of NH_4^+ and NO_3^- fluxes released through substrate mineralization after inorganic and organic fertilization amendments (Qian & Schoenau, 1995; Harrison & Maynard, 2014; Castro & Whalen, 2016).

Nitrate exposure, an extensive variable, is the summation of the daily IEM NO_3^--N fluxes over a given sampling period (Burton et al., 2008a; Burton et al., 2008b; Zebarth et al., 2008a; Zebarth et al., 2008b; Burton & Zebarth, 2014). Similarly, ammonium exposure can be defined as the integrated daily IEM NH_4^+-N fluxes over a given sampling period. Previous studies used nitrate exposure to understand the correlation between cumulative N_2O emissions and temporal dynamics of NO_3^- over a specified time (Burton et al., 2008a; Burton et al., 2008b; Zebarth et al., 2008a; Zebarth et al., 2008b; Burton & Zebarth, 2014). Many authors reported a stronger relationship between nitrate exposure and cumulative N_2O fluxes than daily NO_3^- and N_2O fluxes (Burton et

al., 2008a; Zebarth et al., 2008b; Burton & Zebarth, 2014). This suggests that measurements of NO_3^- at the time of sampling does not capture the influence of NO_3^- on N_2O fluxes. However, these studies used soil samples to calculate nitrate exposure. The IEMs could be used to better understand the relationship between N_2O emissions and the dynamics of mineral N in the soil as they can be left in the soil for a longer period, thus measure the diffusion and availability of mineral N.

2.5.2. Factors Affecting Ion Exchange Membranes Effectiveness

Many physical and chemical characteristics of IEMs and soil strongly influence IEMs efficiency. Various factors are described below:

2.5.2.1. Selection of Counter Ions

In general, cation and anions with the lowest affinity are the most suitable for IEMs counter ions. The hydrogen ion for cation and hydroxyl ion for anion have shown great results. However, in calcareous soils, these ions are not well suitable because of the formation of CO_2 and bicarbonate after reacting with calcium carbonate. The next suitable counter ions are Na^+ and Cl^- ions due to their lower affinity (Qian & Schoenau, 2002). In some cases, bicarbonate is also used as anion counter ion, but it is only suitable for neutral to alkaline soil conditions (Kjønaas, 1999).

2.5.2.2. Residence Time in Soil

The higher residence time of IEMs in the soil gives a measure that includes ion diffusions from a great distance and nutrient release during mineralization. Several factors such as soil moisture, soil temperature, nutrients availability in the soil should be considered before deciding the residence time (Qian & Schoenau, 2002). The IEMs left in the soil for longer duration may not act as a sink of ions but a dynamic exchanger (Cooperband & Logan, 1994). Generally, IEMs are

kept in soil for 14 days and can be replaced with new IEMs if longer measurements are required (Qian & Schoenau, 1995; Qian & Schoenau, 2000).

2.5.2.3. Soil Moisture

The diffusion of the ions from soil to resins is highly dependent upon the water flow in the soil. As the soil moisture increases, the supply of the nutrients to membranes increases due to the more diffusion of nutrients (Qian & Schoenau, 2002). The measurement of nutrients under field conditions is significantly affected by soil moisture content. More nutrient uptake was seen with an increase in soil moisture levels with the use of resin in bead forms (Schaff & Skogley, 1982).

2.5.2.4. Soil Temperature

Soil temperature does not directly affect the diffusions or availability of ions but a rise in soil temperature can increase the soil microbial activity and thus result in more decomposition of organic matter and provide more inorganic nutrients. Low temperature can affect the soil moisture availability and very high temperature conditions can result in dry soil, which ultimately influences the nutrients' movement (Qian & Schoenau, 2002).

2.5.2.5. Soil Characteristics

Biological, physical, and chemical activities in soil affect the nutrient availability to plants and membranes. For instance, immobilization decreases the inorganic N concentrations whereas, mineralization increases inorganic N in the soil. Fine-textured soils have more organic material causing more water retention and higher microbial activities than coarse-textured soils, which result in more availability and diffusion of nutrients (Qian & Schoenau, 2002).

Chapter 3: Material and Methods

3.1. Site Description and Experimental Design

The study was carried out under field conditions at the Bio-Environmental Engineering Center (BEEC) located in Bible Hill, Nova Scotia, Canada at approximately 45° 23' 02" N and 63° 14' 35" W. The soil is mapped as an orthic humo-ferric podzol with sandy loam (moderately coarse) texture and highly acidic (pH=5.1 to 5.5) conditions (Webb et al., 1991). Soil samples were taken before biosolids and fertilizer applications (May 4, 2017; May 4, 2018), after the applications (July 18, 2017; July 17, 2018), during the growing seasons (September 7, 2017; August 14 & September 20, 2018), and after the crop harvesting (November 22, 2017; November 5, 2018). Soil temperature (5 cm), soil moisture (0-10 cm), relative humidity and air temperature were measured along with the greenhouse gas (GHG) sampling of the experimental plots throughout the sampling period. Additionally, various site factors including precipitation, wind velocity, and net radiation were monitored continuously using two automated weather stations located in the experimental field during the growing season. Corn (*Zea mays*) was used as the experimental crop for both years in this study.

The field was set up in a randomized block design with four blocks. The study involved three types of biosolids as mesophilic anaerobically digested (MAD), alkaline treated (AT), and composted (CP); two application methods as surface spread (SS) and incorporated (INC); and two levels of application rates of biosolids based on N requirement as half- and full-rate. The treatments with half-rates of biosolids were supplemented with urea to provide equivalent amounts of plant-available N as the full-rate plots. Urea treatments as INC and SS were included to compare the results with inorganic N-fertilizer application with biosolids. A control treatment without N-fertilizer was also included. Each treatment unit was included in each of the four replicate blocks

in a randomized complete block design. Thus, the study involved 12 biosolids treatments + 2 urea treatments + unamended control = 15 treatment combinations and 15 x 4 = 60 plots of size 6 m x 8 m each. The treatments were randomly assigned to all 60 plots (Figure 3.1).

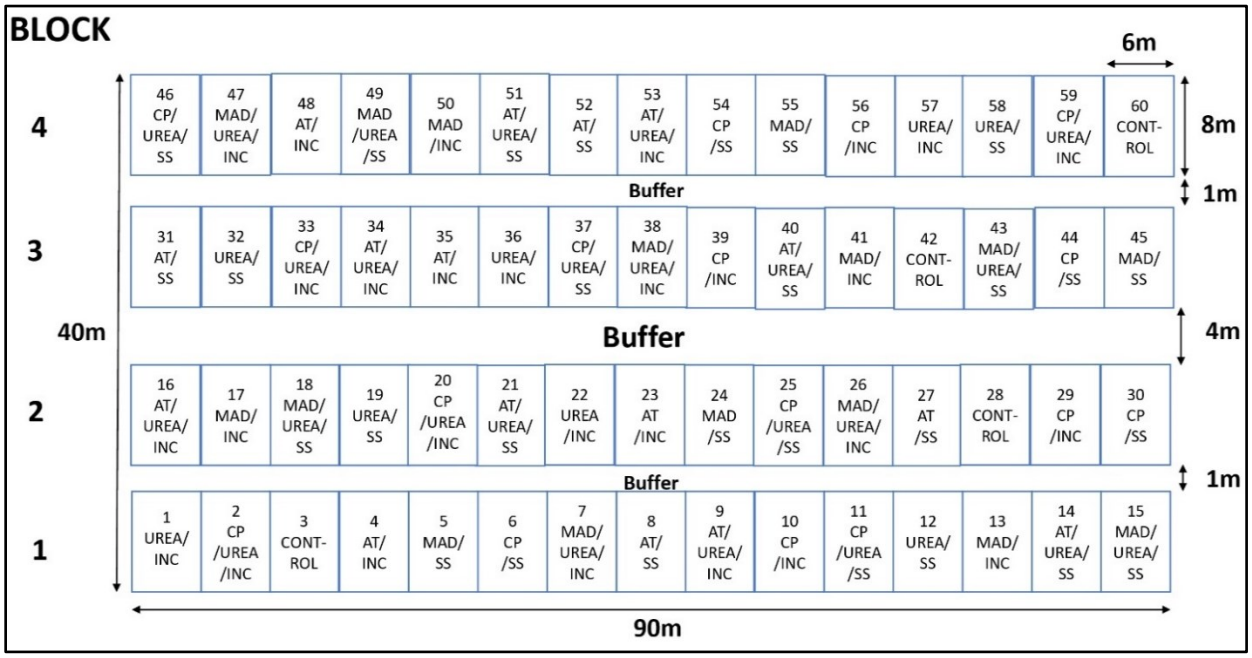

Figure 3.1: Experimental layout of the field.

3.2. Biosolids Characteristics and Application

The biosolids were collected from different locations. The AT was obtained from N-Viro Systems Canada, from their facility in Halifax, Nova Scotia; MAD was collected from the wastewater treatment plant of St. Hyacinthe, Quebec; and Fundy Compost, Brookfield, Nova Scotia provided CP biosolids. The biosolids were characterized for nitrogen (N), phosphorus (P), potassium (K), carbon (C), dry matter, and pH (Table 3.1). It is important to note that C content was not estimated in CP biosolids in 2017 as the samples were lost in a fire that occurred on the Agricultural Campus, Dalhousie University on June 20, 2018, but the materials were assumed to have similar C content in 2017 as in 2018.

The application of biosolids was based on the N requirement of the crop and estimated N availability from the biosolids. Nova Scotia recommendations for corn, 120 kg N ha^{-1}, was used as the base N application rate. Out of this, 30 kg N ha^{-1} was applied at corn planting by 5 x 5 cm bands of diammonium phosphate in each row with the corn planter. The remainder of N requirement (90 kg N ha^{-1}) was fulfilled by biosolids alone, biosolids (50%) + urea (50%), or urea alone. The required amounts of biosolids were calculated based on the total N content available of the biosolids, and assuming that 50% of total N from AT and CP and 75% of total N from MAD would become plant available through mineralization of the organic N in the year of application. Tables 3.2 and 3.3 show details of the application schedules and rates of biosolids for 2017 and 2018, respectively.

Table 3.1: Characterization of biosolids used in the experiment.

Properties	2017†			2018†		
	MAD	AT	CP	MAD	AT	CP
Dry Matter (%)	22.89	61.58	43.08	23.23	61.84	41.76
pH	7.8	10	7.4	8.1	9.8	7.3
N (%)	5.62	0.97	1.37	6.43	1.13	1.17
P (%)	2.49	0.67	0.84	3.4	0.66	0.85
K (%)	0.39	0.79	0.27	0.65	0.85	0.20
C (%)	26.41	15.91	26.24*	32.94	20.33	26.24
C:N ratio	4.7	16.4	22*	5	18	22

*The values were assumed to be the same as in 2018

†MAD- mesophilic anaerobically digested; AT- alkaline treated; and CP- composted biosolids.

Table 3.2: Application schedule of biosolids and urea in 2017 and 2018.

Date	Activity*
May 12, 2017	Biosolids applied as SS and INC, and urea incorporated
July 4, 2017	Urea applied as a side-dress
May 17, 2018	Biosolids applied as SS and INC, and urea incorporated
July 23, 2018	Urea applied as a side-dress

*SS and INC refer to surface spread and incorporated application methods, respectively.

Table 3.3: Application rates of biosolids and urea in 2017 and 2018.

Treatment†	2017		2018	
	Full-rate	Half-rate	Full-rate	Half-rate
	Mg ha^{-1} (dry basis)			
MAD	6.2	3.1	5.0	2.5
AT	6.9	3.5	6.0	3.0
CP	13.1	6.5	15.4	7.7
Urea*	195.6	97.8	195.6	97.8

*Rates are in kg ha^{-1}

†MAD- mesophilic anaerobically digested; AT- alkaline treated; and CP- composted biosolids.

3.3. Greenhouse Gas Flux Measurements

Vented static (or non-steady-state non-through-flow) chambers were used to measure carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) fluxes from the soil (Collier et al., 2014). The system consists of two parts, a collar inserted into the soil and a chamber that is placed on the collar during measurement. Rectangular collars with dimensions 75 x 15 x 15 cm were inserted into the soil to cover a corn row and the inter-row space, to a depth of approximately 5 cm into the soil and perpendicular to the cornrows in each plot after biosolids application until the harvest (Figure 3.2). The depth of the soil surface from the top of the collars was measured after the installation of the collars and periodically (monthly) and whenever the collars were disturbed or reinstalled. The rectangular chambers with the same dimensions as the collars consisted of a vent hole and a sampling port fitted with a suba-seal (Fisher Scientific ™ Cat. #470720) (Figure 3.2). A foil covered with bubble wrap (water heater blanket) was glued outside of the chambers to minimize the temperature changes inside the chambers during GHG sampling. A closed-cell foam gasket was fixed to the bottom of the chambers to form a tight seal with the collars and at the time of GHG sampling, two bricks of 19 x 9 x 5 cm and 2.7 kg (each) were placed on the top of the

chamber to help form a tight seal. The plants were removed from the inside of the collars as soon as they emerged.

The gas samples were collected weekly from May to August and once every two weeks from August to September and once or twice in a month in October or postharvest. However, gas samples were collected every two weeks from July 6, 2018, till the harvest. On the day of gas sampling, the chambers were placed on the top of the collars for 30 minutes. During these 30-minute periods, four 20 mL gas samples were collected at 5, 10, 20, and 30 minutes from each chamber headspace and injected into a pre-evacuated 12 mL Exetainers ^{TM} (Labco, UK). Collected gas samples were stored at room temperature until analyzed. It is important to note that after the corn harvest, the collars were removed, and only chambers were placed on the soil surface to take gas samples.

Figure 3.2: Installed collar in the field (left) and collar with the chamber on top during GHG sampling (right).

3.4. Greenhouse Gas Analysis

Varian CP-3800 gas chromatograph (Varian, Mississauga, ON) fitted with a COMBI PAL autosampler (Varian, Walnut Creek, California) was used to determine CO_2, N_2O, and CH_4 concentrations. The autosampler draws a 2.5 mL volume from the sample tube and injects this into

a sample valve that delivers 0.5 mL to an electron capture detector (ECD) and 0.5 mL to a flame ionization detector (FID) and thermal conductivity detector (TCD) in series which were used to detect, N_2O, CH_4, and CO_2, respectively. The ECD was operated at 300ºC, 90% Ar, 10% CH_4 carrier gas at 10 mL min^{-1}, Haysep N 80/100 pre-column (0.32 cm diameter x 50 cm length) and Haysep D 80/100 mesh analytical columns (0.32 cm diameter x 200 cm length) in a column oven operated at 70ºC. A pre-column was used along with a valve to remove water from the sample. The sample contained in the second sample loop (FID/TCD) passed through a Haysep N 80/100 mesh (0.32 cm diameter x 50 cm length) pre-column followed by a Porapak QS 80/100 mesh (0.32 cm diameter x 200 cm length) with a pre-purified helium carrier gas at 137.9 kPa maintained at 70ºC. The TCD was operated at 130ºC and the FID was operated at 250ºC. Operational conditions and data handling were performed with Varian Star™ software in 2017 and CompassCDS™ software in 2018. In each analytical run of 50 samples, five replicates of three concentrations of standard gas mixtures were run between each tray of 50 samples for quality assurance/quality control purposes. Gas samples were converted from uL L^{-1} to g ha^{-1} or kg ha^{-1} using the equations given below.

$$Internal\ area\ of\ the\ recatngular\ collar\ (A) =$$

$$75\ cm\ (length)\ x\ 15\ cm\ (width) = 1125\ cm^2 = 0.1125\ m^2 \qquad (3.1)$$

$$Internal\ volume\ of\ the\ rectangular\ collar =$$

$$75\ cm\ (length)\ x\ 15\ cm\ (width) x\ h\ cm\ (height) = 1125\ cm^2 x\ h\ cm$$

$$Internal\ volume\ of\ the\ rectangular\ chamber =$$

$$75\ cm\ (length)\ x\ 15\ cm\ (width)\ x\ 15\ cm\ (height) = 1125\ cm^2 x\ 15\ cm$$

$$Total\ volume\ (chamber + collar)(V) =$$

$$(1125\ cm^2 x\ 15\ cm) + (1125\ cm^2 x\ h\ cm) = 1125\ cm^2(15 +\ h)\ cm \qquad (3.2)$$

Where, *h* is the height of the collar top from the soil surface.

For each sampling day, the rate of the change (uL L^{-1} min^{-1}) in the gas concentrations (uL L^{-1}) over the deployment period (min) was determined using a simple linear regression. The gas fluxes then were calculated using equation 3.3 (Hutchinson & Livingston, 1993) by considering the internal area (from equation 3.1) and internal volume of the chamber and collar (from equation 3.2):

$$F = \frac{dC}{dt}\ x \frac{V}{A} \qquad (3.3)$$

Where, *dC* is the change in the gas concentration in the chamber headspace (uL L^{-1}), *dt* is the change in time (min), *V* is the total volume of the chamber and collar (cm^3) , and *A* is the area of the collar (m^2).

N_2O flux:

$$\frac{1\mu L\ N_2O}{L.hr}\ x \frac{1125cm^2(15+h)cm}{0.1125m^2} x \frac{1L}{1000cm^3} = 10(15+h)cm\ \frac{\mu L\ N_2O}{m^2.hr}$$

The gas volume (uL L^{-1}) is converted to a mass (g ha^{-1} or kg ha^{-1}) by using the ideal gas law equation, correcting for the air temperature at the time of the sampling:

$$10(15+h)cm\ \frac{\mu LN_2O}{m^2.hr} x \frac{1\mu mole\ N_2O}{0.0821.T\ (K)\mu LN_2O} x \frac{44\mu gN_2O}{1\mu moleN_2O} = 5{,}359.32 \frac{(15+h)cm.\mu gN_2O}{T(K).m^2.hr}$$

Conversion of units:

$$5{,}359.32\frac{(15+h)cm.\mu gN_2O}{T(K).m^2.hr}x\frac{1g\ N_2O}{10^6\mu gN_2O}x\frac{28gN}{44gN_2O}x\frac{24h}{1\ day}x\frac{10^4m^2}{1ha}$$

$$=818.5\frac{(15+h)cm.gN_2O-N}{T(K).ha.day}$$

CO_2 flux:

$$\frac{1\mu L\ CO_2}{L.hr}\ x\frac{1125cm^2(15+h)cm}{0.1125m^2}x\frac{1L}{1000cm^3}=10(15+h)cm\ \frac{\mu L\ CO_2}{m^2.hr}$$

$$10(15+h)cm\ \frac{\mu L\ CO_2}{m^2.hr}x\frac{1\mu mole\ CO_2}{0.0821.T\ (K)\mu L\ CO_2}x\frac{44\mu g\ CO_2}{1\mu mole\ CO_2}=5{,}359.32\frac{(15+h)cm.\mu g\ CO_2}{T\ (K).m^2.hr}$$

$$5{,}359.32\frac{(15+h)cm.\mu g\ CO_2}{T\ (K).m^2.hr}x\frac{1kg\ CO_2}{10^9\mu g\ CO_2}x\frac{12kg\ C}{44kgCO_2}x\frac{24h}{1day}x\frac{10^4\ m^2}{1ha}$$

$$=0.3508\frac{(15+h)cm.kgCO_2\ -C}{T\ (K).ha.day}$$

CH_4 flux:

$$\frac{1\mu L\ CH_4}{L.hr}\ x\frac{1125cm^2.(15+h)cm}{0.1125m^2}x\frac{1L}{1000cm^3}=10(15+h)cm\ \frac{\mu L\ CH_4}{m^2.hr}$$

$$10(15+h)cm\ \frac{\mu L\ CH_4}{m^2.hr}x\frac{1\mu mole\ CH_4}{0.0821.T\ (K)\mu L\ CH_4}x\frac{16\mu g\ CH_4}{1\mu mole\ CH_4}=1948.8\ \frac{(15+h)cm.\mu g\ CH_4}{T\ (K).m^2.hr}$$

$$1948.8\frac{(15+h)cm.\mu g\ CH_4}{T\ (K).m^2.hr}x\frac{1g\ CH_4}{10^6\mu g\ CH_4}x\frac{12g\ C}{16g\ CH_4}x\frac{24h}{1\ day}x\frac{10^4m^2}{1\ ha}$$

$$=350.8\frac{(15+h)cm.g\ CH_4-C}{T\ (K).ha.day}$$

Cumulative GHG emissions over the monitoring period were calculated from the periodic measurements of GHG flux by using a linear interpolation between the periodic GHG flux measurements for each experimental unit.

3.5. Deployment of Ion Exchange Membranes and Analysis

Anion exchange membranes (AEMs) and cation exchange membranes (CEMs) were used to monitor ion exchange membrane (IEM) NO_3^--N and IEM NH_4^+-N fluxes in the soil, respectively, over the study years. Prior to deployment each AEM strip of 6 x 5.2 cm and CEM strip of 7 x 4.5 cm were eluted in separate containers with 0.5 N HCl solution for 30 minutes by shaking on a reciprocal shaker at low speed and then rinsed three times with distilled water. After rining, IEMs were saturated with a 1N NaCl solution by shaking on a reciprocal shaker for 1 hour at low speed. IEMs were stored in distilled water until needed in the field. In each experimental plot, four AEMs and four CEMs were vertically placed into the soil at 8 cm depth, between two corn rows and within a corn row. The IEMs remained in the soil for 14-24 days in the growing season and replaced by fresh membranes at the same spot. After retrieval from the field, IEMs were rinsed with distilled water to remove the attached soil particles and then placed into a pre-labelled DigiTube containing 40 mL of 1M KCl. The extraction was done by shaking IEMs for 1 hour on a reciprocal shaker at low speed. Using a gravity filtration rack, IEMs were filtered through pre-folded KCl-rinsed Whatman No. 5 filter papers. The solution was poured into a pre-labelled 20 mL plastic scintillation vial and stored in the freezer until analyzed using the Technicon AutoAnalyzer II system (Seal analytical, UK).

After the analysis, the NO_3^--N and NH_4^+-N concentrations for each set of 4 membranes (4 membranes in each plot) were returned in units of mg NO_3^--N or NH_4^+-N L^{-1} of the total volume. The concentrations of NO_3^--N and NH_4^+-N (mg N L^{-1}) were then transferred to IEM NO_3^--N and IEM NH_4^+-N fluxes (µg N cm^{-2}) by considering the surface area of the membrane (cm^{-2}) and volume of 1M KCl used during the extraction of membranes (L) as shown in the equation 3.4 and 3.5, respectively.

$$Anion\ exchange\ membrane\ area = \ 6\ cm\ x\ 5.2\ cm\ x\ \ = 31.2\ x\ 2\ (2\ sides) = 62.4\ cm^2$$

$$Cation\ exchange\ membrane\ area = \ 7\ cm\ x\ 4.5\ cm = 31.5\ x\ 2\ (2\ sides) = 63\ cm^2$$

$$IEM\ NO_3^- - N\ flux\ (\mu g\ N\ cm^{-2}) = \frac{NO_3^- - N\ (mg\ L^{-1}) x\ 0.04\ L\ (total\ KCl\ used) x\ 1000}{62.4\ x\ 4\ cm^2 (4\ membranes\ in\ each\ plot)}$$

(3.4)

$$IEM\ NH_4^+ - N\ flux\ (\mu g\ N\ cm^{-2}) = \frac{NH_4^+ - N\ (mg\ L^{-1}) x\ 0.04\ L\ (total\ KCl\ used) x\ 1000}{63\ x\ 4\ cm^2 (4\ membranes\ in\ each\ plot)}$$

(3.5)

For each set of IEMs, the flux results were corrected by subtracting the background IEM NO_3^--N and IEM NH_4^+-N levels from the activated-unused IEMs. The sum of IEM NO_3^--N (µg N cm^{-2}) and IEM NH_4^+-N (µg N cm^{-2}) over a given period of time was presented as nitrate and ammonium exposures, respectively and reported as µg N cm^{-2}. The nitrate and ammonium exposures were calculated over the growing season for 88 days (July 17 to October 13) and 74 days (July 31 to October 13) in 2017, respectively. In 2018, nitrate and ammonium exposures were measured for 129 days (June 4 to October 11).

3.6. Statistical Analysis

Statistical analysis was performed using Minitab 18 Statistical Software (Minitab Inc., State College, PA). A three-way factorial design with four blocks was used at a 95% confidence level to test the main and interaction effects of the 3 factors (biosolids type, application rate, and application method) on cumulative GHG emissions and nitrate and ammonium exposures. Additionally, to examine the difference among 15 treatments based on cumulative GHG emissions and nitrate and ammonium exposures, one-way analysis of variance (ANOVA) as a randomized

block design was carried out with four blocks. All data were checked for normality by drawing Normal Probability Plot for each set of data using the Anderson-Darling test and the data was accepted as normal after performing the pen-test and if the $p>0.1$ (Montgomery, 2017). The constant variance was verified by plotting residuals versus fits and checked if the data points formed an impression of the horizontal band as described by Montgomery (2017). The data was transformed if either assumption was violated and then back-transformed when the results were reported. Multiple means comparisons were conducted using Fisher's LSD test when there was a significant ($p\leq0.05$) or marginally significant ($0.05<p\leq0.1$) effect. The letter groupings were generated using a 5% level of significance for both significant ($p\leq0.05$) and marginally significant ($0.05<p\leq0.1$) effects to reduce the over-inflation of Type I experiment wise error (especially in marginally significant effect). To compare different subsets among 15 treatments orthogonal contrasts were constructed using Proc GLM in SAS v.9.4 (SAS Institute, Inc. 2013). A stepwise multiple linear regression analysis was performed to determine the relationship between GHG fluxes and soil temperature and volumetric water content (VWC). Linear regression analysis was conducted to analyze the relationship between cumulative N_2O emissions and nitrate and ammonium exposures. Also, linear regression analysis was used to construct a relationship between CO_2 and CH_4.

Chapter 4: Results

4.1. Climate Data

Total rainfall and average air temperature for each month in 2017 and 2018 are displayed in Figure 4.1. Throughout the study period, rainfall intensities and occurrences differed. The months with the lowest rainfall for each growing season were September 2017 and July 2018. The fall of 2017 was the driest season with 110 mm total rainfall over 3 months (September to November), whereas a total of 696 mm rainfall was recorded in fall 2018. The total rainfall from May to December in 2018 (1207 mm) was higher than for the same months in 2017 (828 mm). The average monthly air temperature followed a similar trend in both years with the peak values in the summer seasons and a steady decline in the fall and winter seasons.

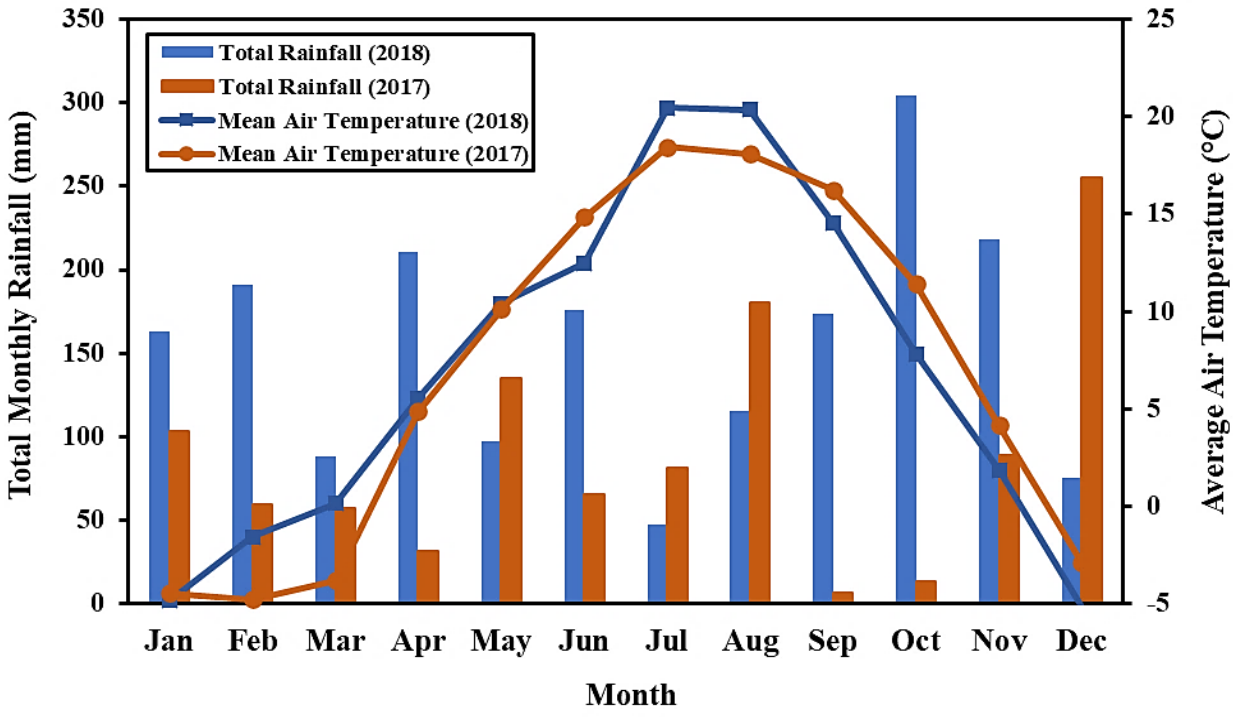

Figure 4.1: Total monthly rainfall (mm) and mean monthly air temperature (^{0}C) in 2017 and 2018.

4.2. Soil Parameters

The average volumetric water content (VWC) and soil temperature on individual sampling dates can be found in Figure 4.2 for both study years. It is important to note that both soil parameters were not measured when the soil was frozen.

The average VWC varied from 14 to 40% over the two years (Figure 4.2). In 2017, VWC was higher in the first few weeks of sampling but started decreasing at the beginning of July and the lowest value was recorded on August 2 (Figure 4.2). After this, VWC remained high throughout the growing season and winter period (Figure 4.2). A similar trend was observed in the second year as VWC was low in the summer season. Further, VWC attained zenith value on June 19 and then decreased steadily with a peak in early November (Figure 4.2). On average, the VWC over the growing season was higher in 2017 (25.7% VWC) compared to 2018 (23% VWC).

The mean soil temperature as measured during greenhouse gas (GHG) sampling ranged from 1 to 23 ^{0}C over 2017 and 2018 (Figure 4.2). Soil temperature was low (~12 ^{0}C) in the spring of 2017 but rapidly increased at the beginning of the summer season (Figure 4.2). As the season progressed, the temperature remained steady typically within a range of 16.1 to 20.6^{0}C until the end of September, except a drop on August 30 (Figure 4.2). In 2018, very low soil temperature was measured in winter and early spring seasons (Figure 4.2). After this, the temperature started rising as the summer progressed, and a steady decline was noticed after reaching the peak value (Figure 4.2).

The orthic humo-ferric podzol soil mapped in this study was highly acidic (pH≤5.6) (Webb et al., 1991). Soil pH increased after the application of biosolids and urea to the soil (Figure 4.3). However, in mesophilic anaerobically digested (MAD) amended plots, the soil pH always remained lower than unamended control (Figure 4.3). The pH was the highest in alkaline treated

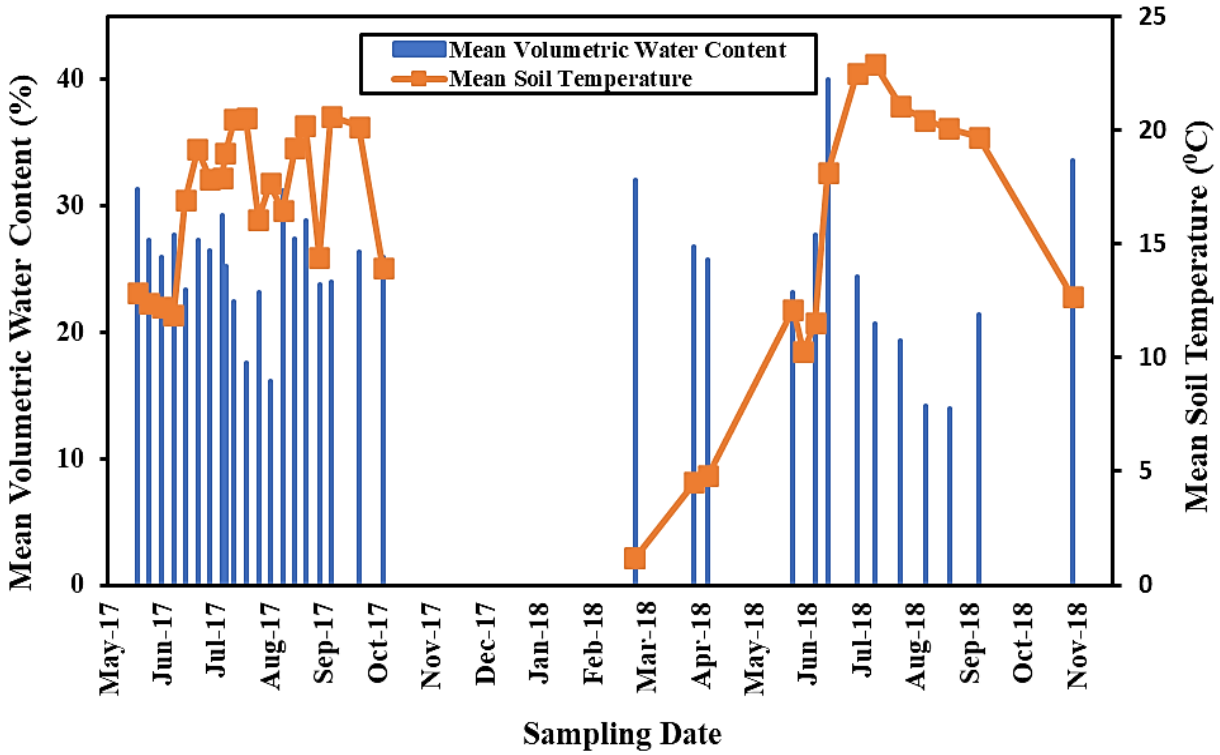

Figure 4.2: Mean volumetric water content (%) and mean soil temperature (^{0}C) in 2017 and 2018.

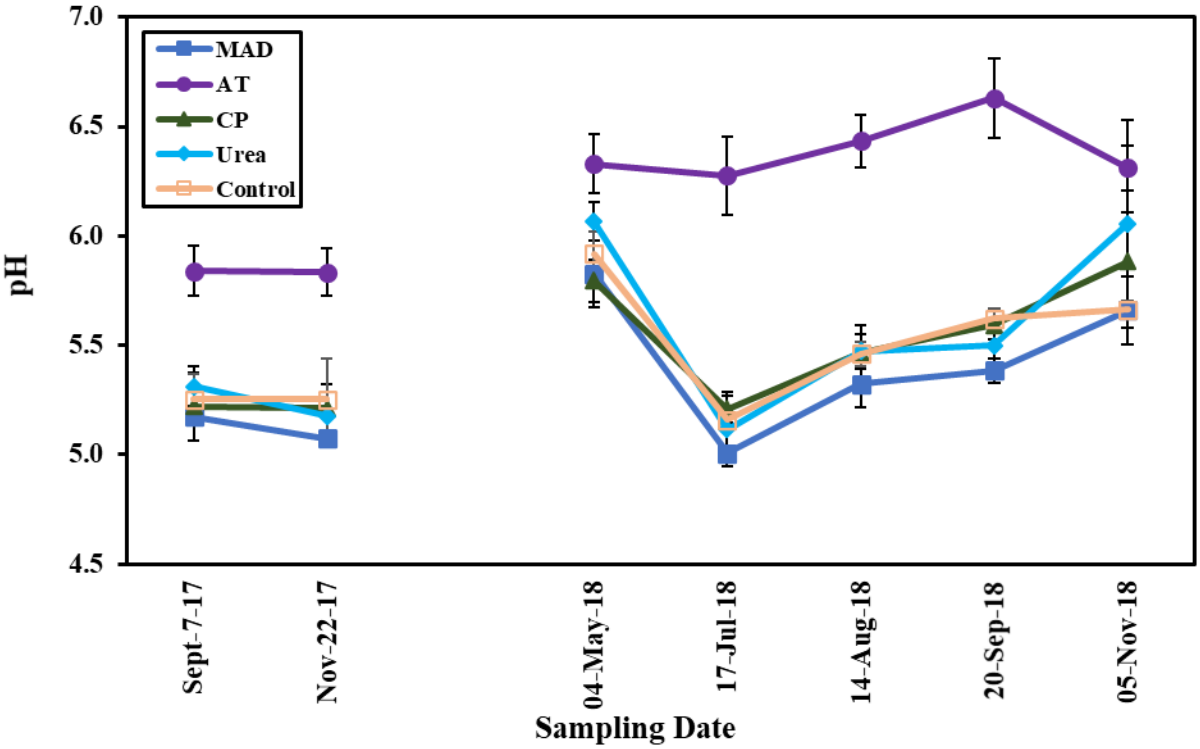

Figure 4.3: Soil pH across different treatments in 2017 and 2018. The error bars represent standard deviation.

(AT) treated plots throughout the study period (Figure 4.3). Among the biosolids, pH ranged between 5.0 to 6.6 over the two years (Figure 4.3). The graph illustrates that the repeated application of AT resulted in higher pH throughout the experimental period and increased soil pH by 0.6 units compared to the unamended control plots by the end of the examined period (Figure 4.3).

4.3. Nitrous Oxide Emissions

4.3.1. Temporal Pattern of Nitrous Oxide Fluxes

The seasonal trend of both years (2017 & 2018) showed similar patterns with a steady increase in nitrous oxide (N_2O) fluxes after the application of biosolids and urea, followed by a period of reduced emissions and then drop to almost zero emissions by the end of both years (Figure 4.4). The graph highlights the fact that most of the emissions occurred during the spring and early summer seasons after biosolids and urea addition (Figure 4.4).

Daily N_2O fluxes ranged from -4.69 to 65.33 g N ha^{-1} day^{-1} across all the treatments during the study period (15 May 2017 to 31 December 2018) with the largest emissions from MAD treatments in 2017 (65.33 g N ha^{-1} day^{-1}) and from AT treatments in 2018 (56.37 g N ha^{-1} day^{-1}) (Figure 4.4). The first peaks were observed between 13 to 40 days after the application (Figure 4.4). Additionally, in 2017, a peak was noticed after a large rainfall event (53mm) in late August (Figure 4.4). In 2017, MAD amended plots had consistently higher daily N_2O emissions from 12 to 61 days after the application (Figure 4.4). Another pulse in N_2O flux from MAD treatments was seen in late August (Figure 4.4). In 2018, the post-application daily N_2O fluxes were grater from AT treatments for almost 3 weeks and dropped to background levels afterwards (Figure 4.4).

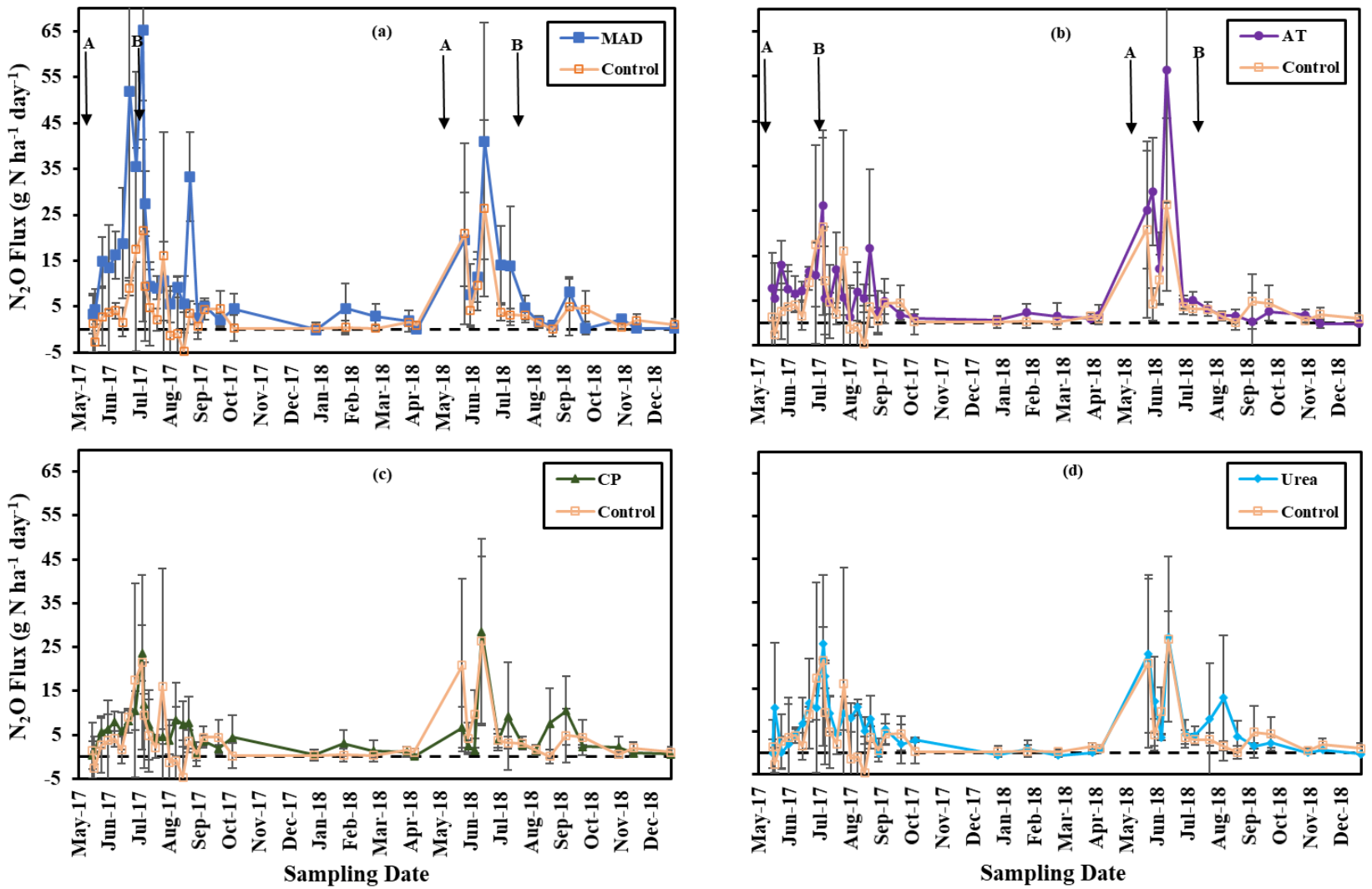

Figure 4.4: Daily mean N_2O fluxes (g N ha^{-1} day^{-1}) from (a) MAD, (b) AT, (c) CP, and (d) Urea treatments along with unamended control for 2017 and 2018. Solid arrows indicate biosolids and incorporated urea application (A), and surface applied urea (B). The error bars represent standard deviation.

4.3.2. Cumulative Nitrous Oxide Fluxes

The addition of MAD biosolids significantly ($p \leq 0.05$) increased the cumulative N_2O emissions by 65% and 105% compared to urea treatments and unamended control plots over the study period (15 May 2017 to 31 Dec 2018), respectively, suggesting that land application of MAD more than doubled the N_2O emissions from the soil (Table 4.1). ANOVA results indicated that cumulative N_2O emissions varied significantly ($p \leq 0.05$) among three biosolids types in 2017 (Table 4.2). The treatments that received MAD generated the highest cumulative N_2O emissions (2.37 kg N ha^{-1}) followed by AT (1.12 kg N ha^{-1}) and composted (CP) (1.07 kg N ha^{-1}) in 2017 (Table 4.3). The unamended control plots generated 0.68 kg N_2O-N ha^{-1} and the treatments fertilized with urea generated 1.07 kg N_2O-N ha^{-1} (Table 4.1). In 2018, no significant main effects were found. Cumulative N_2O emissions generated from AT treatments in the second year (2.15 kg N ha^{-1}) were numerically higher compared to the first year (1.12 kg N ha^{-1}) of application (Table 4.1). Similarly, in unamended control plots, N losses as N_2O were numerically greater in 2018 (1.51 kg N ha^{-1}) than in 2017 (0.68 kg N ha^{-1}) (Table 4.1). Reducing the biosolids application rate by half and replacing N with added urea had no significant effect on the cumulative N_2O emissions in 2017 (Table 4.2). In 2018, a significant ($p \leq 0.05$) interaction was noticed between biosolids type (B) and application rate (R) (Table 4.2). The AT half-rate treatments (AT+Urea) resulted in 87% higher cumulative N_2O emissions than treatments receiving full-rate of AT (Table 4.3). Different application methods did not significantly influence cumulative N_2O emissions in 2017 or 2018 (Table 4.2). Further, there was no significant three-way interaction noticed in either year (Table 4.2).

A marginally significant ($0.05 < p \leq 0.1$) treatment effect was observed in 2017 (Table 4.1). Cumulative N_2O emissions for incorporated half-rate MAD (MAD+Urea-INC) were reported

about 2.6, 2.8, 4.1, and 4.4 times higher than incorporated half-rate compost (CP+Urea-INC), incorporated urea (Urea-INC), incorporated half-rate AT (AT+Urea-INC), and unamended control treatments, respectively (Table 4.1). Further, Table 4.1 indicates that using MAD biosolids in every treatment combination except surface applied half-rate MAD (MAD+Urea-SS) resulted in significantly ($p \leq 0.05$) higher cumulative N_2O emissions than the unamended control plots. When cumulative N_2O emissions were expressed over the entire study period (15 May 2017 to 31 December 2018), a significant ($p \leq 0.05$) treatment effect was also noticed (Table 4.1).

Table 4.1: Cumulative N_2O emissions (kg N ha^{-1}) as influenced by different treatments. Cumulative emissions were calculated by linear interpolation between measurements over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment[β]	2017		Growing season-2017		2018	Growing season-2018	Total	
	kg N_2O-N ha^{-1}							
MAD+Urea-INC	2.96	a	2.83	a	2.27	1.67	5.24	a
MAD+Urea-SS	1.79	abcd	1.43	abc	1.07	0.55	3.03	bcd
MAD-INC	2.22	abc	2.15	ab	2.87	1.75	5.11	a
MAD-SS	2.50	ab	2.30	ab	2.02	1.21	4.60	ab
AT+Urea-INC	0.73	d	0.67	c	2.52	1.43	3.28	abcd
AT+Urea-SS	1.25	bcd	1.15	bc	3.10	1.87	4.42	abc
AT-INC	0.99	cd	0.90	bc	1.51	0.79	2.51	cd
AT-SS	1.50	abcd	1.42	abc	1.48	1.02	3.06	bcd
CP+Urea-INC	1.14	bcd	1.03	bc	1.84	1.31	3.06	bcd
CP+Urea-SS	1.11	bcd	0.99	bc	1.10	0.65	2.21	d
CP-INC	0.80	cd	0.72	c	1.18	0.91	2.08	d
CP-SS	1.23	bcd	0.72	c	1.30	0.86	2.55	cd
Urea-INC	1.05	bcd	0.91	bc	1.65	0.68	2.73	bcd
Urea-SS	1.09	bcd	1.01	bc	1.66	1.33	2.74	bcd
Control	0.68	d	0.65	c	1.51	0.75	2.20	d
p-value[*]	**0.095**		**0.071**		0.140	0.524	**0.016**	

[1]Values are presented as means with n=4.

[*]Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold. Treatments with the same letter in each column are not significantly different at $\alpha = 0.05$.

[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.2: ANOVA *p*-values for main and interaction effects of biosolids type (B), application rate (R), and application method (M) on cumulative N_2O emissions (kg N ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Source	2017	Growing season-2017	2018	Growing season-2018	Total
Biosolids type (B)	**0.003**	**0.002**	0.087	0.486	**0.002**
Application rate (R)	0.886	0.950	0.414	0.553	0.605
Application method (M)	0.777	0.879	0.262	0.321	0.588
B x R	0.893	0.753	**0.033**	0.263	0.251
B x M	0.469	0.324	0.248	0.229	0.128
R x M	0.331	0.378	0.760	0.566	0.352
B x R x M	0.647	0.505	0.623	0.782	0.511

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold.

Table 4.3: Effect of biosolids type (B), application rate (R), and application method (M) and their interaction on cumulative N_2O emissions (kg N ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment[β]	2017	Growing season-2017	2018	Growing season-2018	Total
	kg N_2O-N ha^{-1}				
Biosolid type (B)[1]					
MAD	2.37 a	2.18 a	2.06	1.29	4.50 a
AT	1.12 b	1.04 b	2.15	1.28	3.32 b
CP	1.07 b	0.87 b	1.35	0.93	2.48 b
Application rate (R)[2]					
Half-rate + Urea	1.49	1.35	1.98	1.25	3.54
Full-rate	1.54	1.37	1.73	1.09	3.32
Application method (M)[3]					
INC	1.47	1.38	2.03	1.31	3.55
SS	1.56	1.34	1.68	1.03	3.31
B x R interaction[4]					
MAD+Urea	2.37	2.13	1.67 bc	1.11	4.14
MAD	2.36	2.22	2.45 ab	1.48	4.85
AT+Urea	0.99	0.91	2.81 a	1.65	3.85
AT	1.24	1.16	1.50 bc	0.90	2.78
CP+Urea	1.12	1.01	1.47 bc	0.98	2.64
CP	1.02	0.72	1.24 c	0.88	2.32

[1], [2], [3], and [4] indicate that the values are presented as means with n=16, 24, 24, and, 8, respectively.
*Treatments with the same letter in each sub-column are not significantly different at $\alpha = 0.05$.
[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

4.3.3. Soil Parameters and Nitrous Oxide Fluxes

Multiple linear regression results suggested that in 2018 VWC and soil temperature accounted for 42% of the variation in N_2O emissions (R^2_{adj}=0.423; p=0.026), however, no significant relationship was found in 2017 (R^2_{adj}=0.114; p=0.139). A significant ($p \leq 0.05$) and a marginally significant ($0.05 < p \leq 0.1$) positive relationship between VWC and N_2O emissions was

observed in the growing season of 2018 (May 30 to October 1) (R^2=0.735; p=0.002) and 2018 (R^2=0.254; p=0.086), respectively (Figure 4.5). Whereas, no significant relationship between VWC and N_2O emissions was noticed in 2017 (R^2=0.084; p=0.160) (Figure 4.5). The correlation between soil temperature and N_2O fluxes was not significant in 2017 (R^2=0.041; p=0.391) and 2018 (R^2=0.046; p=0.430) (Figure 4.6).

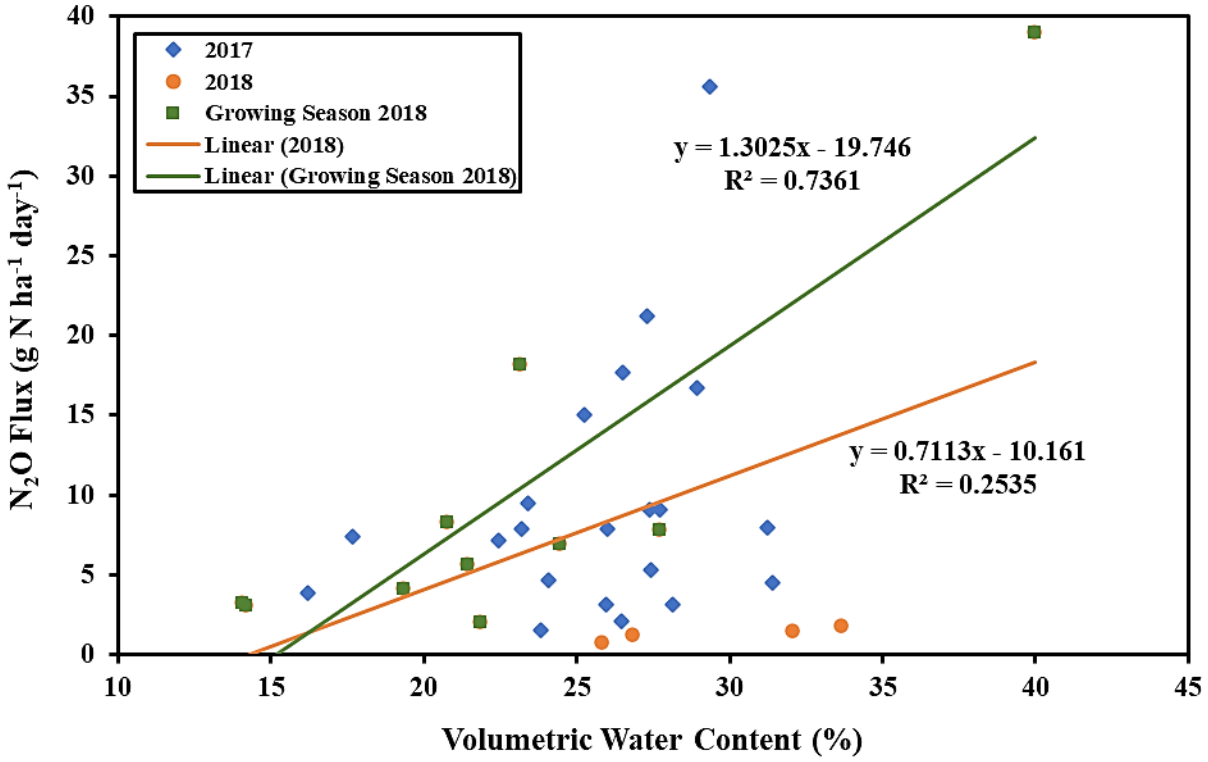

Figure 4.5: Relationship between mean volumetric water content (%) and daily mean N_2O fluxes (g N ha^{-1} day^{-1}).

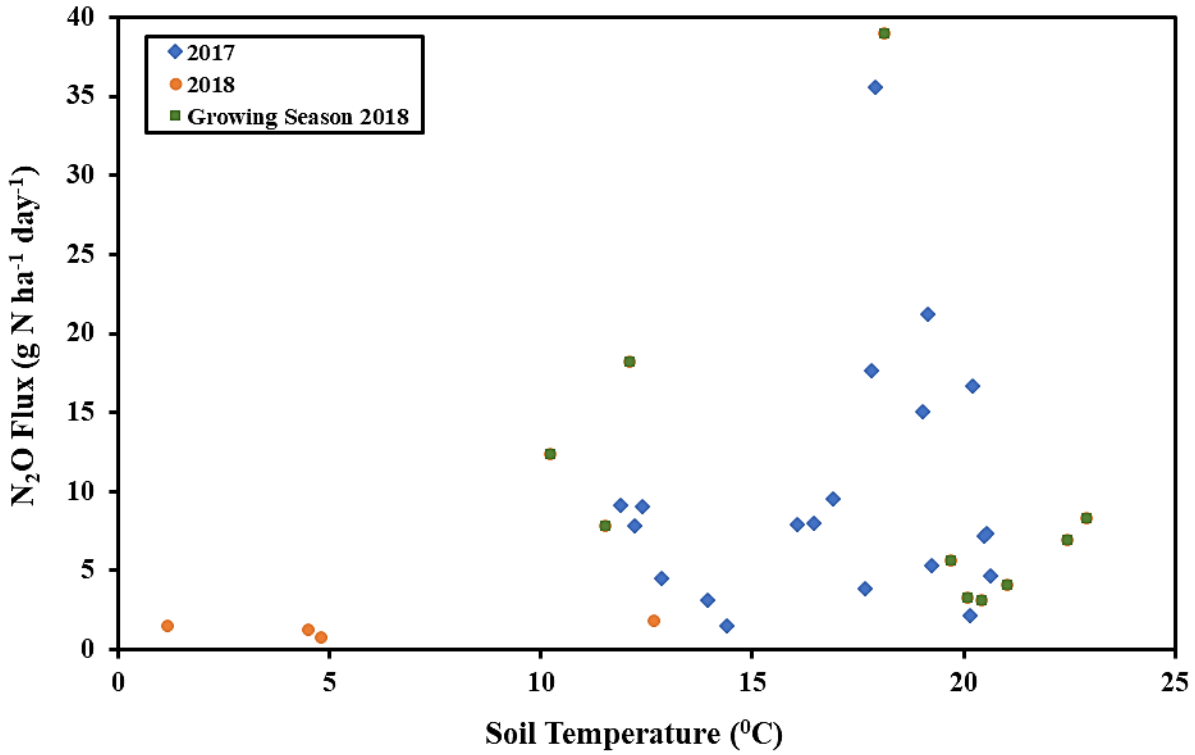

Figure 4.6: Relationship between daily mean soil temperature (^{0}C) and daily mean N_2O fluxes (g N ha^{-1} day^{-1}).

4.4. Carbon Dioxide Emissions

4.4.1. Temporal Pattern of Carbon Dioxide Fluxes

The temporal pattern of CO_2 over the two years of study is shown in Figure 4.7. In general, most of the CO_2 emissions occurred during summer and mid-fall seasons followed by a decline in the winter seasons of both years (Figure 4.7). Regardless of the treatment, the daily CO_2 fluxes ranged between 0.41 and 60.06 kg C ha^{-1} day^{-1} with a maximum flux of 60.06 kg C ha^{-1} day^{-1} from unamended control plots in 2017 and 31.95 kg C ha^{-1} day^{-1} from CP treatments in 2018, respectively. An increase in daily CO_2 fluxes was seen after the biosolids amendment to the soil (Figure 4.7). In 2017, early CO_2 peaks were noticed in urea plots (Figure 4.7). Additionally, a pulse from urea treatments was reported on July 5, 2017 after the surface application of urea followed

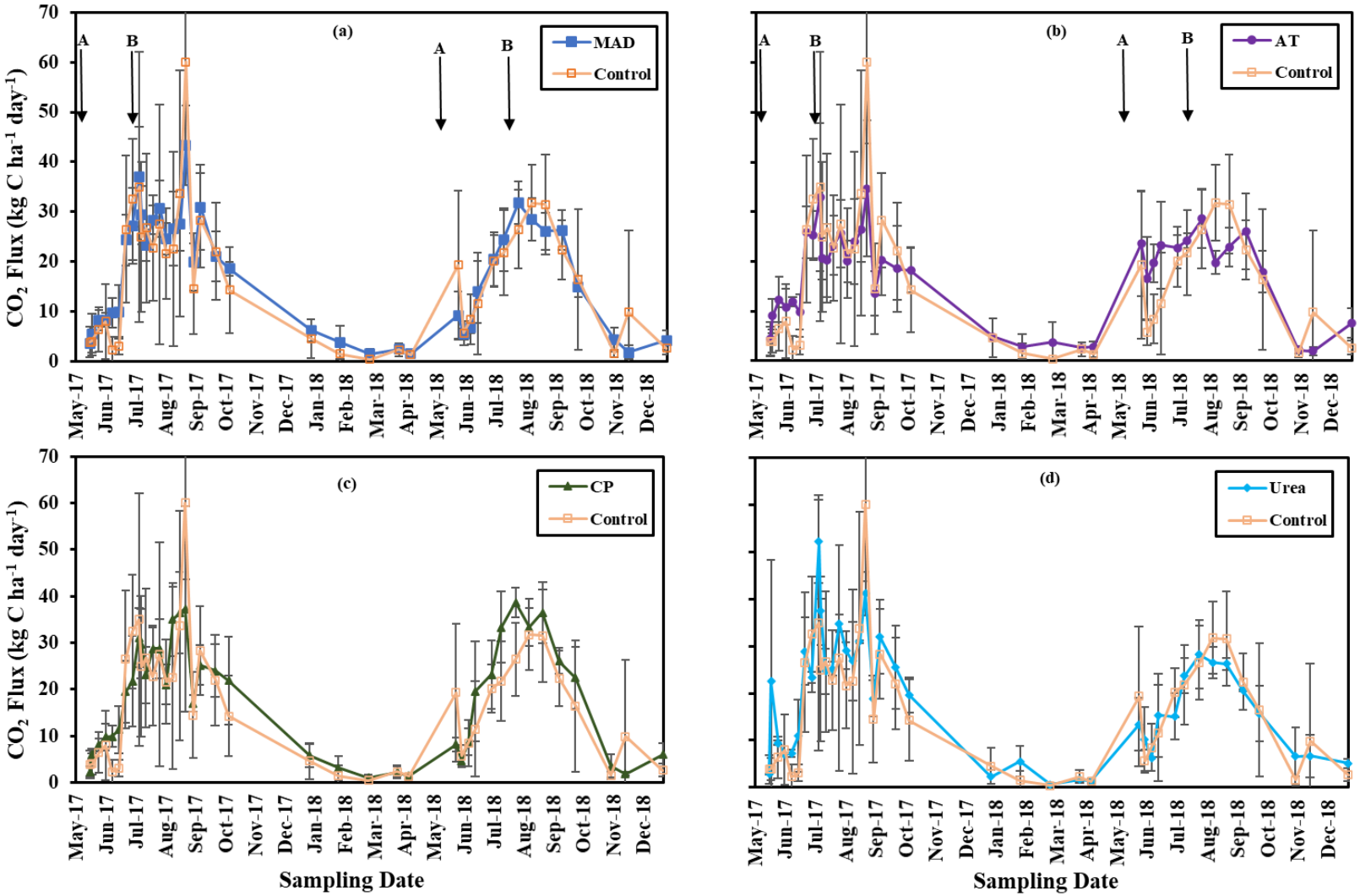

Figure 4.7: Daily mean CO_2 fluxes (kg C ha^{-1} day^{-1}) from (a) MAD, (b) AT, (c) CP, and (d) Urea treatments along with unamended control for 2017 and 2018. Solid arrows indicate biosolids and incorporated urea application (A), and surface applied urea (B). The error bars represent standard deviation.

by light rainfall event (0.4mm) (Figure 4.7). In 2018, after the application of biosolids and incorporation of urea, the highest early daily CO_2 fluxes were noticed from AT treatments (Figure 4.7). However, later in the season, the CO_2 emissions were dominated by CP treatments (Figure 4.7). Unexpectedly, some CO_2 peaks were seen from unamended control plots in both years (Figure 4.7).

4.4.2. Cumulative Carbon Dioxide Fluxes

The addition of CP biosolids significantly ($p \leq 0.05$) increased the cumulative CO_2 emissions compared to urea treatments and unamended control plots by 32% and 24% during the growing season of 2018 (May 30 to October 1), respectively (Table 4.4). Over the same period, orthogonal contrast results suggested that cumulative CO_2 emissions from the full-rate CP treatments (3.55 Mg C ha^{-1}) were larger than urea treatments (2.50 Mg C ha^{-1}) and unamended control (2.67 Mg C ha^{-1}), however, the half-rate CP (CP+Urea) combination (3.08 Mg C ha^{-1}) did not significantly differ from unamended control but was higher than urea treatments (Table 4.4). Among the biosolids, CP treatments produced significantly ($p \leq 0.05$) more CO_2 emissions than AT and MAD by 14% and 18%, respectively over the growing season of 2018 (May 30 to October 1) (Table 4.6). However, when the spring and winter emissions were included then these trends disappeared, and CP (4.36 Mg C ha^{-1}) and AT (4.32 Mg C ha^{-1}) treatments were found to produce similar and higher cumulative emissions than MAD (3.64 Mg C ha^{-1}) treatments (Table 4.6). In both years, neither application method nor application rate had any significant effect on the cumulative CO_2 emissions (Table 4.5). There was no interaction between any of the main effects (Table 4.5).

A significant ($p \leq 0.05$) treatment effect was noticed during the growing season of 2018 (May 30 to October 1). In particular, the incorporated CP (CP-INC) plots showed the highest

cumulative CO_2 emissions (Table 4.4). Orthogonal contrast results showed a significant ($p \leq 0.05$) difference between full-rate AT (2.8 Mg C ha^{-1}) and full-rate CP (3.54 Mg C ha^{-1}) treatments in the growing season of 2018 (May 30 to October 1), however, no significant difference was recorded between the half-rate AT (AT+Urea) (2.9 Mg C ha^{-1}) and half-rate CP (CP+Urea) (3.08 Mg C ha^{-1}) combinations (Table 4.4). A marginally significant ($0.05 < p \leq 0.1$) difference between full-rate CP (3.54 Mg C ha^{-1}) and half-rate CP (CP+Urea) (3.08 Mg C ha^{-1}) was found over the same period. This suggests that the application of CP biosolids at full-rate had more impact on cumulative CO_2 emissions than half-rate CP (CP+Urea) combination. There was no significant difference in the percentage of the added biosolid C lost as CO_2 among different treatments (Table 4.7). Over the study period, the loss of C relative to the total added C was the lowest in full-rate MAD treatments (1.5%) and the greatest in half-rate AT (AT+Urea) treatments (39.7%) (Table 4.8). The total % C loss from full-rate CP (16.5%) and half-rate CP (CP+Urea) (14.6%) treatments were similar over the same period (Table 4.8). The negative values in Table 4.7 represent C sequestration form different treatments.

Table 4.4: Cumulative CO_2 emissions (Mg C ha^{-1}) as influenced by different treatments. Cumulative emissions were calculated by linear interpolation between measurements over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment[β]	2017	Growing season-2017	2018	Growing season-2018		Total
	Mg CO_2 -C ha^{-1}					
MAD+Urea-INC	4.57	3.46	3.02	2.34	de	7.75
MAD+Urea-SS	4.50	3.36	3.83	2.81	bcde	8.55
MAD-INC	4.28	3.33	3.62	2.60	cde	8.03
MAD-SS	3.83	2.85	4.08	3.03	abcd	7.99
AT+Urea-INC	3.24	2.30	4.70	2.94	abcde	8.12
AT+Urea-SS	4.52	3.30	4.11	2.86	bcde	8.72
AT-INC	3.76	2.86	4.36	2.56	cde	8.21
AT-SS	3.82	3.01	4.12	3.04	abcd	8.05
CP+Urea-INC	4.96	3.89	4.26	3.27	abc	9.28
CP+Urea-SS	3.45	2.54	4.09	2.88	bcde	7.73
CP-INC	3.89	3.04	4.78	3.61	a	8.77
CP-SS	5.16	3.32	4.29	3.48	ab	9.62
Urea-INC	4.58	3.59	3.54	2.31	e	8.18
Urea-SS	4.37	3.52	3.98	2.69	cde	8.52
Control	3.94	3.14	3.93	2.67	cde	7.96
p-value[*]	0.887	0.987	0.339	**0.020**		0.987

[1]Values are presented as means with n=4.

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold. Treatments with the same letter in each column are not significantly different at $\alpha = 0.05$.

[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.5: ANOVA *p*-values for main and interaction effects of biosolids type (B), application rate (R), and application method (M) on cumulative CO_2 emissions (Mg C ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Source	2017	Growing season-2017	2018	Growing season-2018	Total
Biosolids type (B)	0.649	0.693	**0.016**	**0.007**	0.540
Application rate (R)	0.867	0.849	0.344	0.153	0.882
Application method (M)	0.849	0.835	0.873	0.555	0.888
B x R	0.809	0.897	0.450	0.244	0.762
B x M	0.715	0.497	0.145	0.172	0.863
R x M	0.698	0.866	0.925	0.321	0.821
B x R x M	0.240	0.410	0.651	0.732	0.441

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold.

Table 4.6: Effect of biosolids type (B), application rate (R), and application method (M) on cumulative CO_2 emissions (Mg C ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment[β]	2017	Growing season-2017	2018	Growing season-2018	Total
	Mg CO_2 -C ha^{-1}				
Biosolid type (B)[1]					
MAD	4.30	3.25	3.64 b	2.70 b	8.08
AT	3.84	2.87	4.32 a	2.85 b	8.28
CP	4.36	3.20	4.36 a	3.31 a	8.85
Application rate (R)[2]					
Half-rate + Urea	4.21	3.15	4.00	2.85	8.36
Full-rate	4.12	3.07	4.21	3.05	8.45
Application method (M)[3]					
INC	4.12	3.15	4.12	2.89	8.36
SS	4.21	3.07	4.09	3.02	8.44

[1], [2], and [3] indicate that the values are presented as means with n=16, 24, and 24, respectively.
*Treatments with the same letter in each sub-column are not significantly different at $\alpha = 0.05$.
[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.7: Percentage of amendment C added lost as CO_2-C obtained by subtracting the cumulative CO_2 emissions (Mg C ha^{-1}) of the treatments from the unamended control and dividing by the amount of C contained in the amendment. Values are expressed over 229 days (2017), 145 days (Growing season-2017), 216 days (2018), 125 days (Growing season-2018), and 596 days (Total).

Treatment[β]	2017	Growing season-2017	2018[z]	Growing season-2018	Total
			% CO_2 -C		
MAD+Urea-INC	77	39	-75	-40	-13
MAD+Urea-SS	69	27	7	17	36
MAD-INC	21	12	-16	-4	2
MAD-SS	-7	-18	20	22	1
AT+Urea-INC	-127	-153	43	44	14
AT+Urea-SS	105	29	8	31	66
AT-INC	-16	-25	-14	-9	11
AT-SS	-11	-12	23	30	4
CP+Urea-INC	59	44	27	30	35
CP+Urea-SS	-29	-35	14	10	-6
CP-INC	-1	-3	27	23	11
CP-SS	36	5	16	20	22
p-value*	0.935	0.929	0.124	0.475	0.993

[1]Values are presented as means with n=4.

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold.

[†]The negative values represent C sequestration.

[z]Cumulative CO_2 emissions (Mg C ha^{-1}) were calculated from May 30-Dec 31, 2018.

[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.8: Carbon input from biosolids and C lost as CO_2-C obtained by subtracting the cumulative CO_2 emissions (Mg C ha^{-1}) of the treatments from the unamended control over 596 days.

Treatment[β]	C input	CO_2 -C flux[*]	CO_2 -C loss
	(Mg C ha^{-1})		%
MAD+Urea	1.64	0.19	11.6
MAD	3.28	0.05	1.5
AT+Urea	1.16	0.46	39.7
AT	2.32	0.17	7.3
CP+Urea	3.73	0.54	14.6
CP	7.48	1.24	16.5

[*]Refer to Table 4.4 for cumulative CO_2-C emissions of the treatments and unamended control.
[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; and CP- composted biosolids.

4.4.3. Soil Parameters and Carbon Dioxide Fluxes

The soil temperature and VWC resulted in a significant ($p \leq 0.05$) effect on daily CO_2 emissions ($R^2_{adj}=0.885$; $p=0.000$) in 2018 and caused 89% of the variation in daily CO_2 fluxes. A positive exponential relationship was observed between daily mean soil temperature and daily mean CO_2 fluxes in 2017 ($R^2=0.609$), the growing season of 2018 (May 30 to October 1) ($R^2=0.839$), and 2018 ($R^2=0.872$) (Figure 4.8). A significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) negative relationship was found between VWC and daily mean CO_2 fluxes in 2018 ($R^2=0.419$; $p=0.012$) and the growing season of 2018 (May 30 to October 1) ($R^2=0.366$; $p=0.065$), respectively (Figure 4.9). However, the similar relationship was not significant in 2017 ($R^2=0.004$; $p=0.798$) (Figure 4.9).

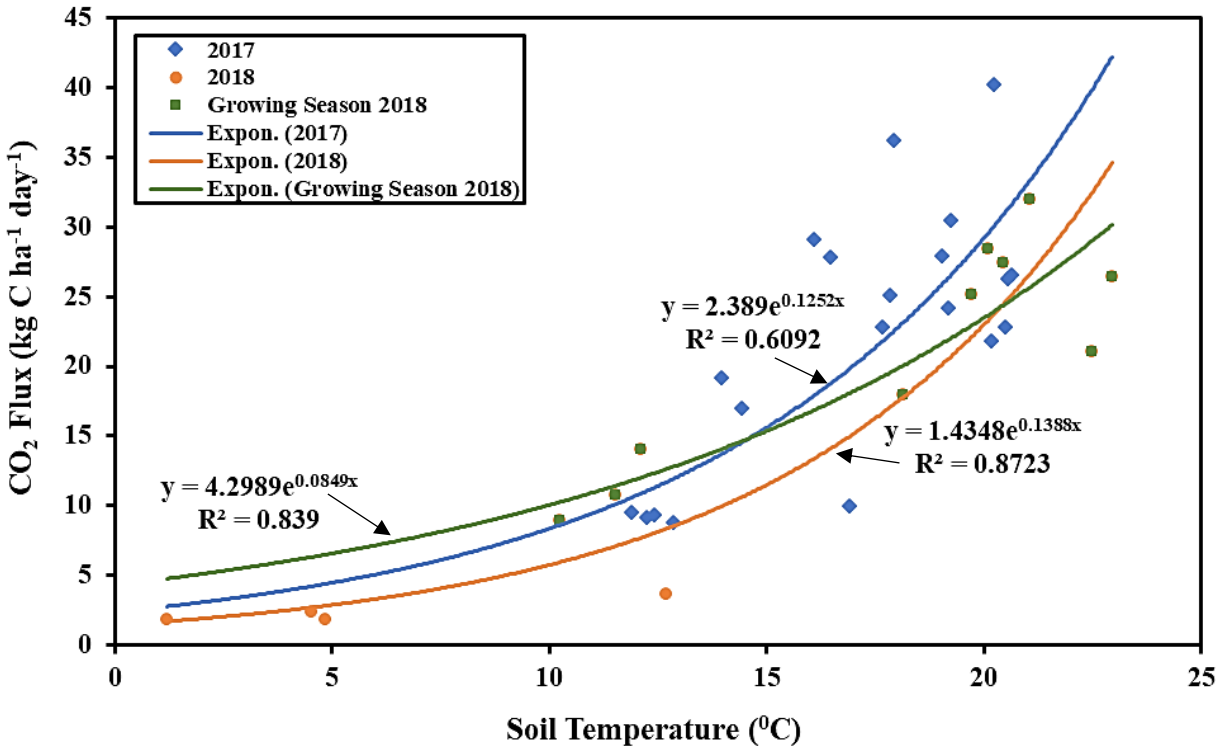

Figure 4.8: Relationship between daily mean soil temperature (^{0}C) and daily mean CO_2 flux (kg C ha^{-1} day^{-1}).

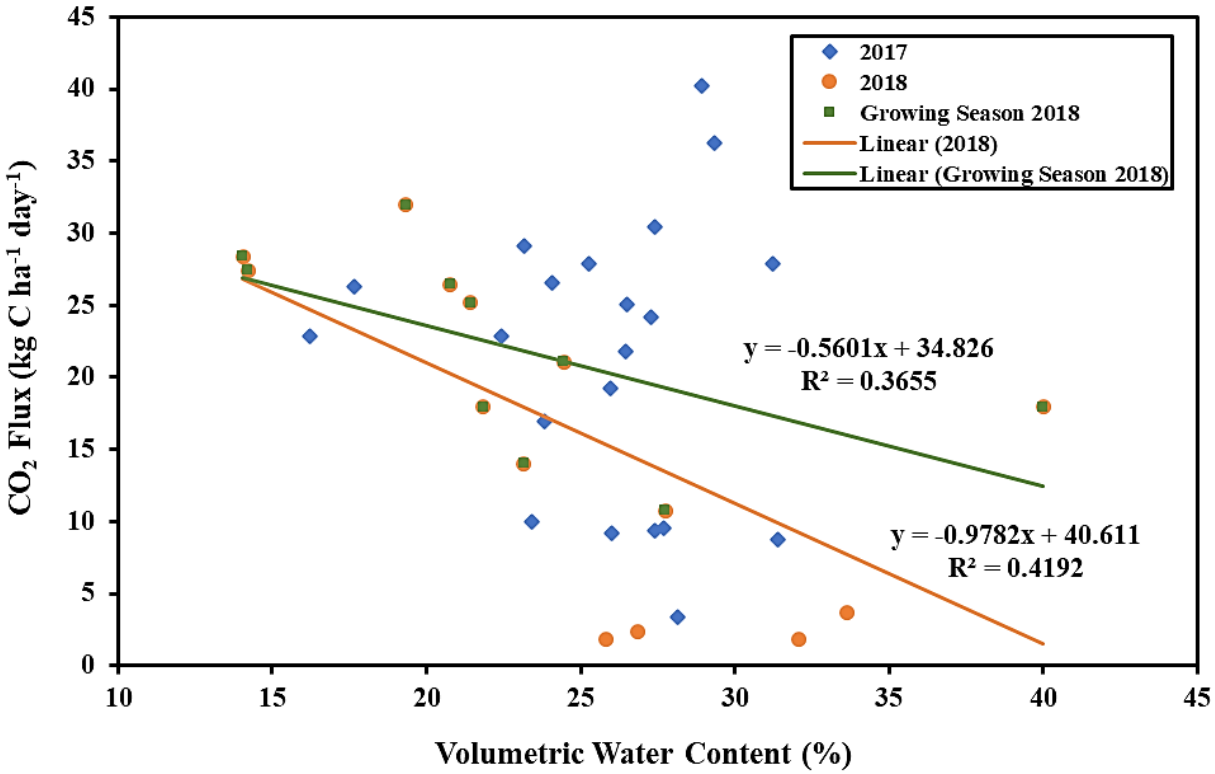

Figure 4.9: Relationship between daily mean volumetric water content (%) and daily mean CO_2 flux (kg C ha^{-1} day^{-1}).

4.5. Methane Emissions

4.5.1. Temporal Pattern of Methane Fluxes

Daily CH_4 production and consumption varied over the sampling period (Figure 4.10). Peaks of CH_4 were recorded within 3 to 19 days after the biosolids application and urea incorporation depending upon the year (Figure 4.10). Additionally, more peaks were observed during the non-growing periods within the seasons (Figure 4.10). Most of the CH_4 emissions throughout the growing season were negative suggesting that soil acted as a CH_4 sink by oxidizing CH_4 to CO_2 (Figure 4.10). Irrespective of the treatments, daily CH_4 fluxes ranged from -11.25 to 11.59 g C ha^{-1} day^{-1} with the peak values of 11.59 g C ha^{-1} day^{-1} from urea treatments in 2017 and 7.32 g C ha^{-1} day^{-1} from MAD treatments in 2018, respectively (Figure 4.10).

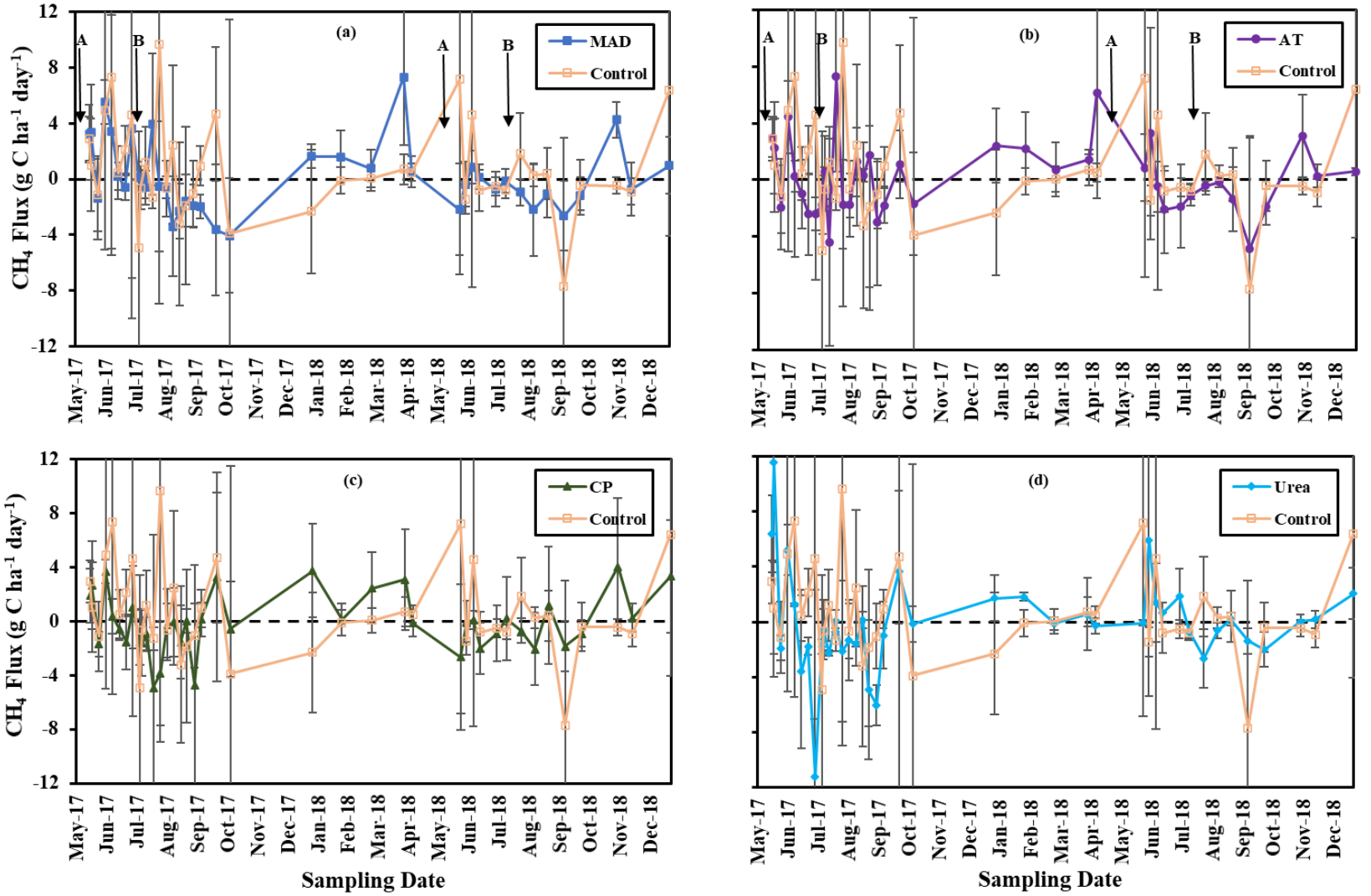

Figure 4. 10: Daily mean CH_4 fluxes (g C ha^{-1} day^{-1}) from (a) MAD, (b) AT, (c) CP, and (d) Urea treatments along with unamended control for 2017 and 2018. Solid arrows indicate biosolids and incorporated urea application (A), and surface applied urea (B). The error bars represent standard deviation.

On average, the growing season of 2018 (May 30 to October 1) (0.68 g C ha^{-1} day^{-1}) consumed more CH_4 than in 2017 (0.15 g C ha^{-1} day^{-1}), however, the trend had changed when non-growing season (after harvest and winter) CH_4 emissions were considered, then more emissions were noticed in 2018 (0.27 g C ha^{-1} day^{-1}) compared to 2017 (-0.04 g C ha^{-1} day^{-1}) (Figure 4.10).

4.5.2. Cumulative Methane Fluxes

Over the growing season of both years, soil that received biosolids acted as a CH_4 sink (Table 4.11). However, when after harvest and winter samples were considered, positive CH_4 fluxes were noticed from biosolids amended plots, except from MAD in 2017 (Table 4.11). The amendment of full-rate CP biosolids to the soil resulted in a net CH_4 sink and had lower cumulative CH_4 emissions compared to unamended control plots by more than 200% in the growing season of 2017 (May 15 to October 6), however, no significant treatment effect was observed (Table 4.9). During the growing season of 2017 (May 15 to October 6), the treatments marginally ($0.05<p\leq0.1$) differed in CH_4 fluxes due to the interactive effect between biosolids type (B) and application rate (R) (Table 4.10). In particular, the half-rate CP (CP+Urea) treatments produced higher cumulative CH_4 fluxes than full-rate CP treatments by almost 150% (Table 4.11). There was no significant difference between the two application methods (Table 4.10). However, in 2017, when only growing season (May 15 to October 6) cumulative CH_4 emissions were considered, SS treatments showed significantly ($p\leq0.05$) larger cumulative CH_4 fluxes compared to INC treatments by 133% (Table 4.10 & 4.11). There was no other 2-way, or 3-way interactions noticed between any factors (Table 4.10).

Table 4.9: Cumulative CH_4 emissions (kg C ha^{-1}) as influenced by different treatments. Cumulative emissions were calculated by linear interpolation between measurements over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment	2017	Growing season-2017	2018	Growing season-2018	Total
	kg CH_4-C ha^{-1}				
MAD+Urea-INC	-0.250	-0.132	-0.014	-0.158	-0.221
MAD+Urea-SS	-0.425	-0.105	0.310	-0.141	-0.027
MAD-INC	-0.034	-0.146	0.006	-0.045	0.031
MAD-SS	0.074	0.152	0.204	-0.165	0.285
AT+Urea-INC	0.055	-0.058	0.218	-0.296	0.360
AT+Urea-SS	0.015	-0.038	-0.145	-0.133	-0.040
AT-INC	-0.237	-0.141	0.442	-0.299	0.247
AT-SS	0.171	0.119	0.207	0.047	0.436
CP+Urea-INC	0.009	0.017	-0.058	-0.047	0.026
CP+Urea-SS	0.227	0.225	0.353	-0.147	0.602
CP-INC	-0.049	-0.407	0.020	-0.153	0.114
CP-SS	0.109	-0.070	0.224	0.062	0.324
Urea-INC	-0.399	-0.454	-0.080	-0.079	-0.447
Urea-SS	0.287	0.211	0.122	0.053	0.484
Control	-0.059	0.202	0.206	-0.080	0.111
p-value*	0.891	0.118	0.906	0.935	0.867

[1]Values are presented as means with n=4.

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold.

†The negative values represent CH_4 consumption.

βMAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.10: ANOVA *p*-values for main and interaction effects of biosolids type (B), application rate (R), and application method (M) on cumulative CH_4 emissions (kg C ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Source	2017	Growing season-2017	2018	Growing season-2018	Total
Biosolids Type (B)	0.354	0.948	0.946	0.814	0.480
Application Rate (R)	0.616	0.425	0.612	0.646	0.513
Application Method (M)	0.402	**0.027**	0.532	0.455	0.366
B x R	0.313	**0.053**	0.570	0.900	0.696
B x M	0.740	0.785	0.169	0.321	0.545
R x M	0.404	0.206	0.812	0.658	0.801
B x R x M	0.729	0.934	0.883	0.685	0.582

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold.

Table 4.11: Effect of biosolids type (B), application rate (R), and application method (M) and their interaction on cumulative CH_4 emissions (kg C ha^{-1}) over 229 days (2017), 145 days (growing season-2017), 338 days (2018), 125 days (growing season-2018), and 596 days (Total).

Treatment[β]	2017	Growing season-2017		2018	Growing season-2018	Total
	kg CH_4-C ha^{-1}					
Biosolid type (B)[1]						
MAD	-0.159	-0.058		0.127	-0.127	0.017
AT	0.001	-0.029		0.180	-0.170	0.251
CP	0.074	-0.059		0.135	-0.102	0.267
Application rate (R)[2]						
Half-rate + Urea	-0.061	-0.015		0.111	-0.154	0.117
Full-rate	0.006	-0.082		0.184	-0.113	0.240
Application method (M)[3]						
INC	-0.084	-0.144	b	0.102	-0.166	0.093
SS	0.028	0.047	a	0.192	-0.100	0.263
B x R interaction[4]						
MAD+Urea	-0.337	-0.119	ab	0.148	-0.150	-0.124
MAD	0.020	0.003	ab	0.105	-0.105	0.158
AT+Urea	0.035	-0.048	ab	0.037	-0.214	0.160
AT	-0.033	-0.011	ab	0.324	-0.126	0.342
CP+Urea	0.118	0.121	a	0.148	-0.097	0.314
CP	0.030	-0.238	b	0.122	-0.107	0.219

[1], [2], [3], and [4] indicate that the values are presented as means with n= 16, 24, 24, and 8, respectively.
*Treatments with the same letter in each sub-column are not significantly different at α = 0.05.
†The negative values represent CH_4 consumption.
[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

4.5.3. Soil Parameters and Methane Fluxes

Multiple linear regression results suggested no significant effect of the two variables together on daily CH_4 emissions in either year. A significant ($p \leq 0.05$) negative relationship was noticed between soil temperature and daily CH_4 fluxes in 2018 (R^2=0.508; p=0.004) (Figure 4.12),

whereas, no such relationship was observed in 2017 (R^2=0.094; p=0.189) (Figure 4.12). Correlations between daily CH_4 fluxes and VWC were not significant in both years (Figure 4.11).

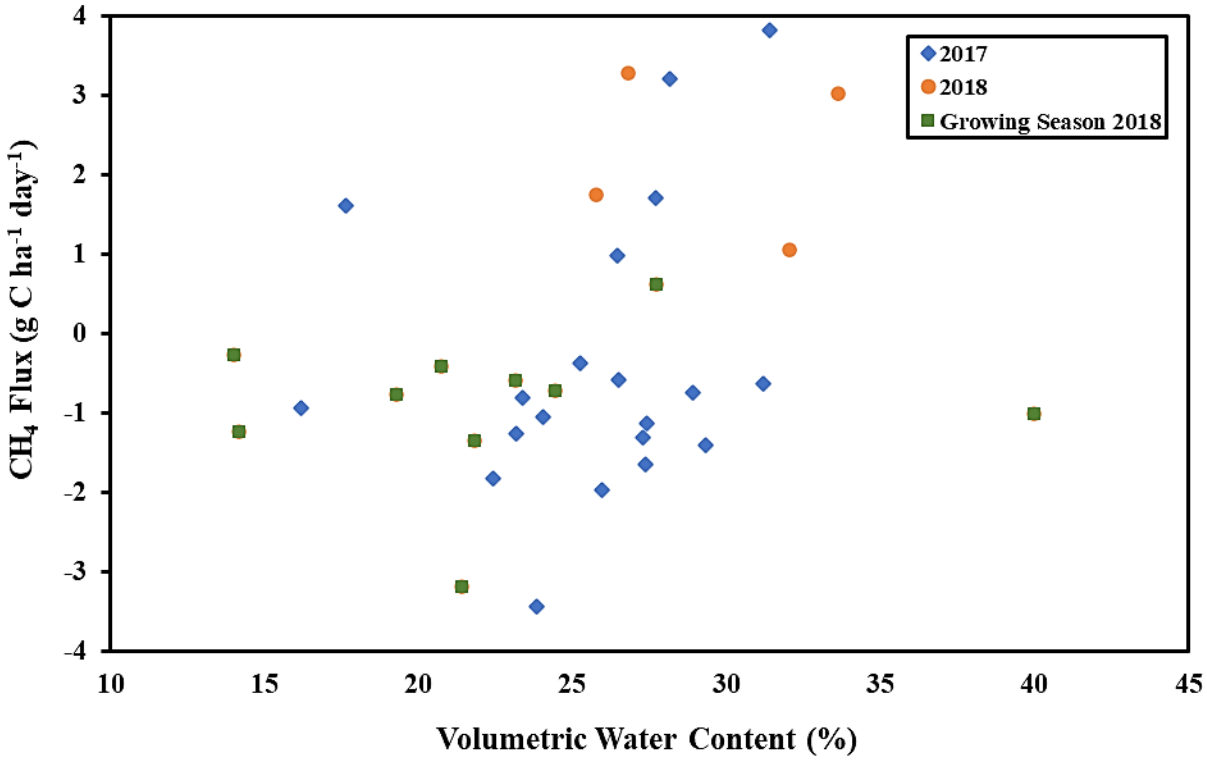

Figure 4.11: Relationship between daily mean volumetric water content (%) and daily mean CH_4 flux (g C ha^{-1} day^{-1}).

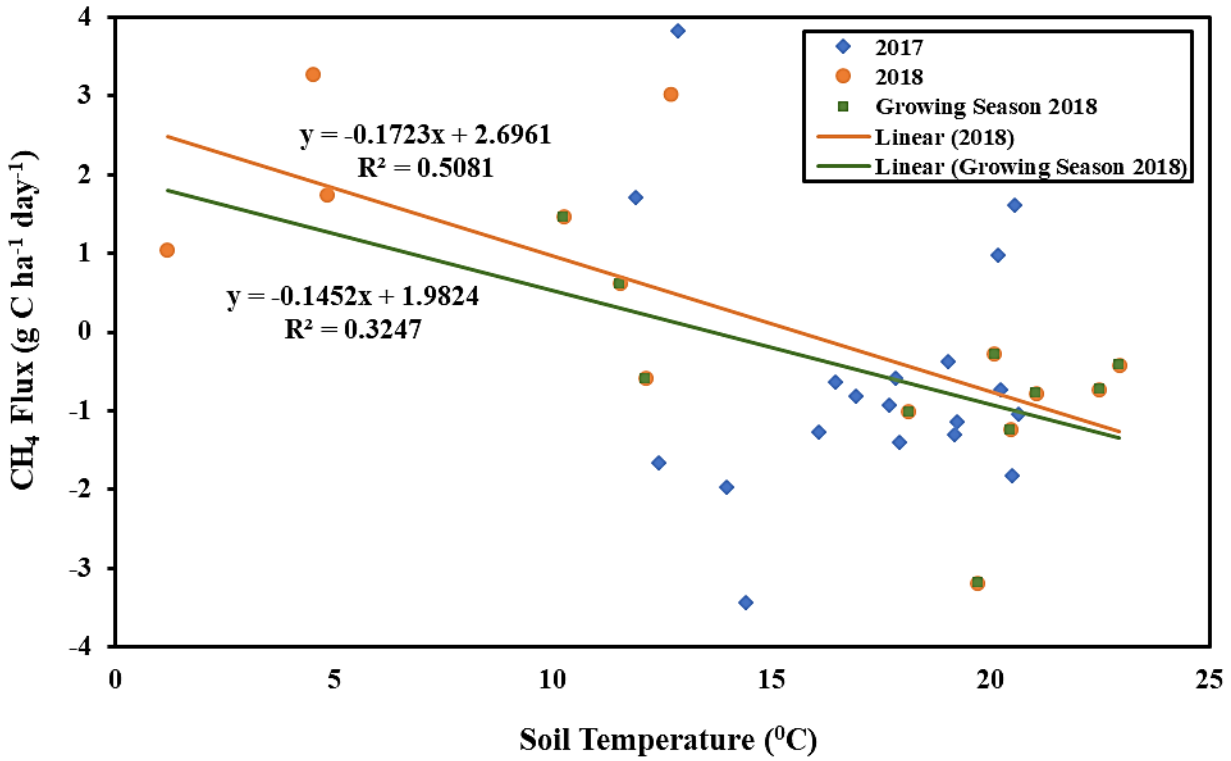

Figure 4.12: Relationship between daily mean soil temperature (^{0}C) and daily mean CH_4 flux (g C ha^{-1} day^{-1}).

4.5.4. Carbon Dioxide and Methane Fluxes

A marginally significant ($0.05<p\leq0.1$) and significant ($p\leq0.05$) negative correlation was found between CO_2 and CH_4 in 2017 (R^2=0.287; p=0.010) (Figure 4.13) and 2018 (R^2=0.585; p=0.000) (Figure 4.14), respectively. It is important to note that in 2018, GHG samples were collected over the whole year, whereas, in 2017, samples were collected from May to December. The difference between CO_2 and CH_4 emissions correlations over the two years can be attributed to the GHG sampling period and weather conditions. In both years, the correlations were weaker in the growing season as compared to the full year.

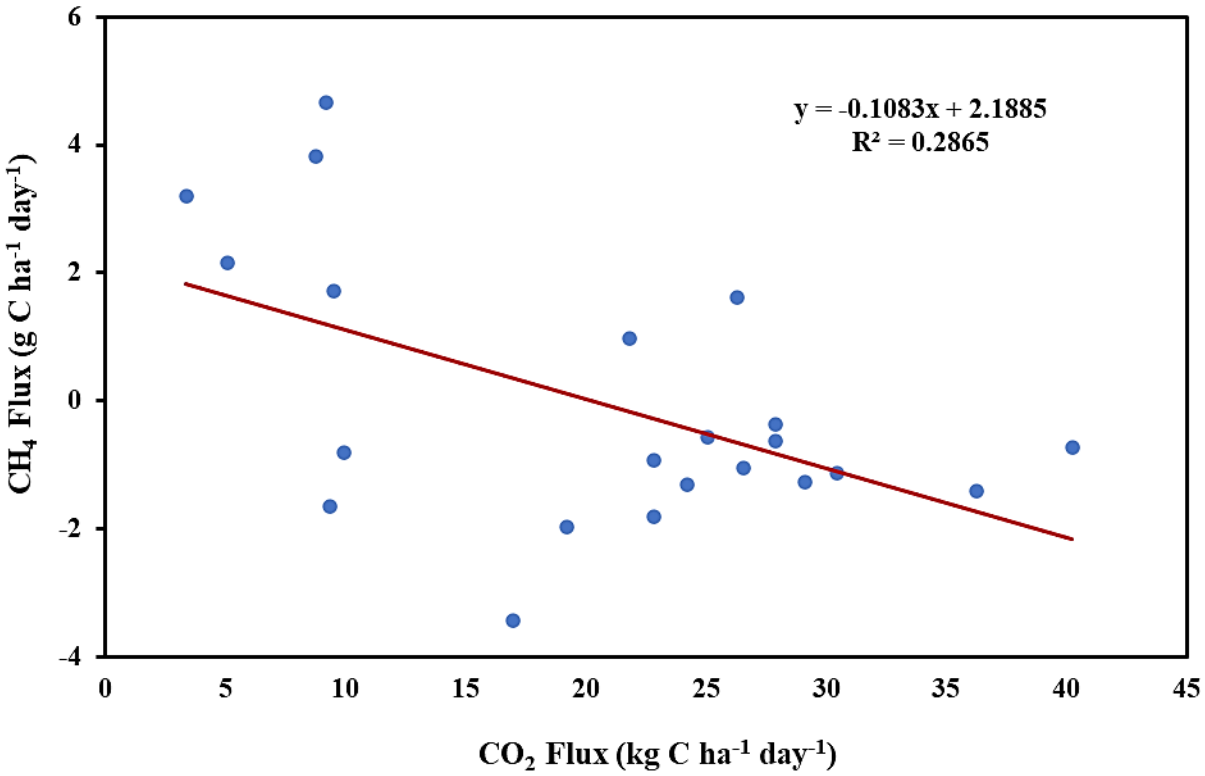

Figure 4.13: Relationship between daily mean CH_4 flux (g C ha^{-1} day^{-1}) and daily mean CO_2 flux (kg C ha^{-1} day^{-1}) in 2017.

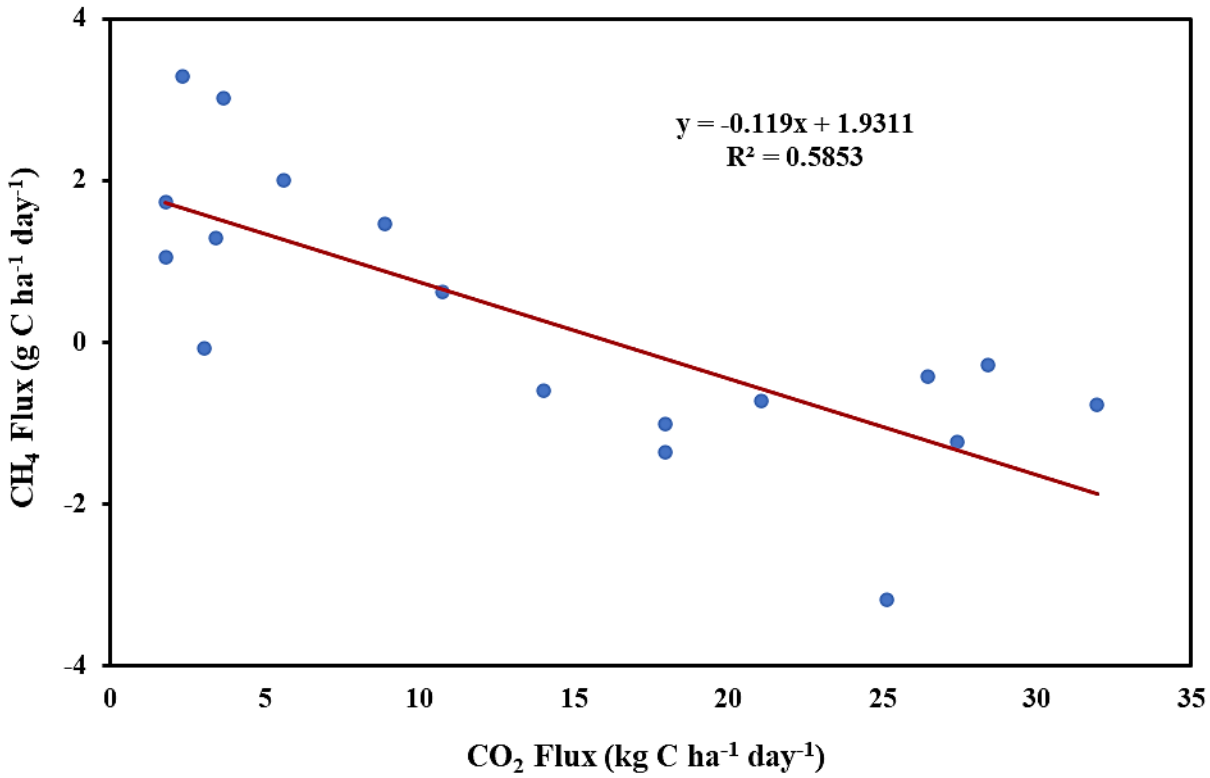

Figure 4.14: Relationship between daily mean CH_4 flux (g C ha^{-1} day^{-1}) and daily mean CO_2 flux (kg C ha^{-1} day^{-1}) in 2018.

4.6. Ion Exchange Membrane Ammonium and Nitrate Fluxes

4.6.1. Temporal Pattern of Ion Exchange Membrane Ammonium and Nitrate Fluxes

The ion exchange membrane (IEM) ammonium (NH_4^+)-N fluxes were measured over a period of 75 days in 2017 (July 31 to October 13) and 130 days in 2018 (June 13 to October 11). In 2017, very low IEM NH_4^+-N fluxes were noticed in the first sampling period (July 31 to August 14), a sharp increase was seen in the second sampling period (August 14 to August 28), and remained stable thereafter (Figure 4.15). The IEM NH_4^+-N levels remained relatively higher in unamended control plots compared to other treatments throughout the examined period (Figure 4.15). Irrespective of the treatment, IEM NH_4^+-N fluxes fluctuated from -0.15 to 1.96 µg N cm^{-2} in 2017 (Figure 4.15). In 2018, early peaks in IEM NH_4^+-N fluxes (mainly from urea and MAD

treatments) were seen after biosolids application and urea incorporation (Figure 4.16). During the third sampling period (July 12 to July 26), IEM NH_4^+-N fluxes increased from all the treatments and unamended control (Figure 4.16). Overall, IEM NH_4^+-N fluxes varied from 0.02 to 0.85 µg N cm^{-2} in 2018 (Figure 4.16). Interestingly, IEM NH_4^+-N fluxes from AT treatments always remained lower compared to MAD, CP, and urea treatments throughout the study period (Figure 4.16). Despite the shorter examined period, higher daily IEM NH_4^+-N fluxes were noticed in 2017 than in 2018 (Figure 4.15 & 4.16).

The IEM nitrate (NO_3^-)-N fluxes were calculated over a period of 89 days in 2017 (July 17 to October 13) and 130 days in 2018 (June 4 to October 11). In 2017, IEM NO_3^--N fluxes were low in the beginning, then a sudden increase was seen in the second sampling period (July 31 to August 14) (Figure 4.17). The MAD had higher initial IEM NO_3^--N fluxes which were closely followed by AT (Figure 4.17). However, later in the season, the fluxes were dominated by unamended control until the end of the sampling period (Figure 4.17). Regardless of the treatment, IEM NO_3^--N fluxes fluctuated from 3.75 to 136.88 µg N cm^{-2} in 2017 (Figure 4.17). In 2018, urea treatments resulted in early IEM NO_3^--N peak which were closely followed by MAD (Figure 4.18). During the last sampling period (September 20 to October 11), AT treatments showed an increase in IEM NO_3^--N fluxes (Figure 4.18). Overall, IEM NO_3^--N fluxes ranged from 9.44 to 87.59 µg N cm^{-2} in 2018 (Figure 4.17). The IEM NO_3^--N fluxes were recorded higher in 2017 than in 2018 (Figure 4.17 & 4.18).

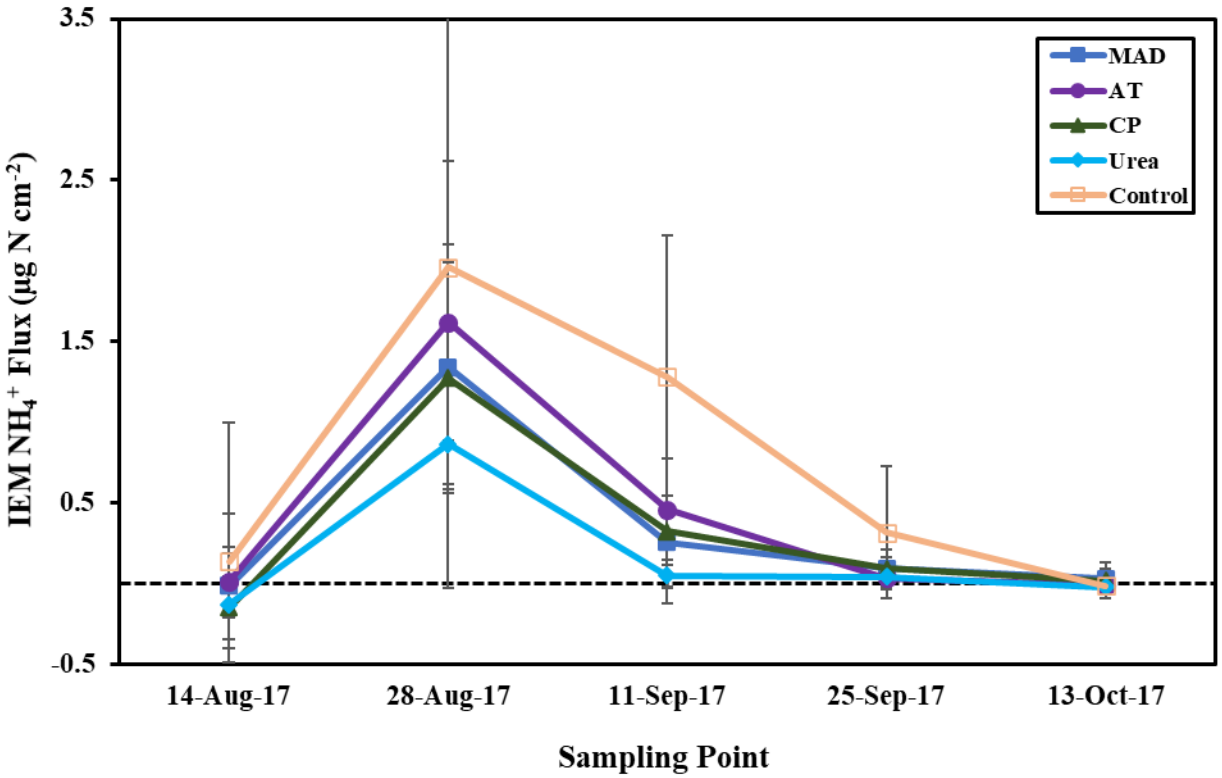

Figure 4.15: Ion exchange membrane NH_4^+–N fluxes (µg N cm^{-2}) measured over 75 days in 2017 (July 31 to October 13). The sampling points represent the day when IEMs were retrieved from the soil. The error bars represent standard deviation.

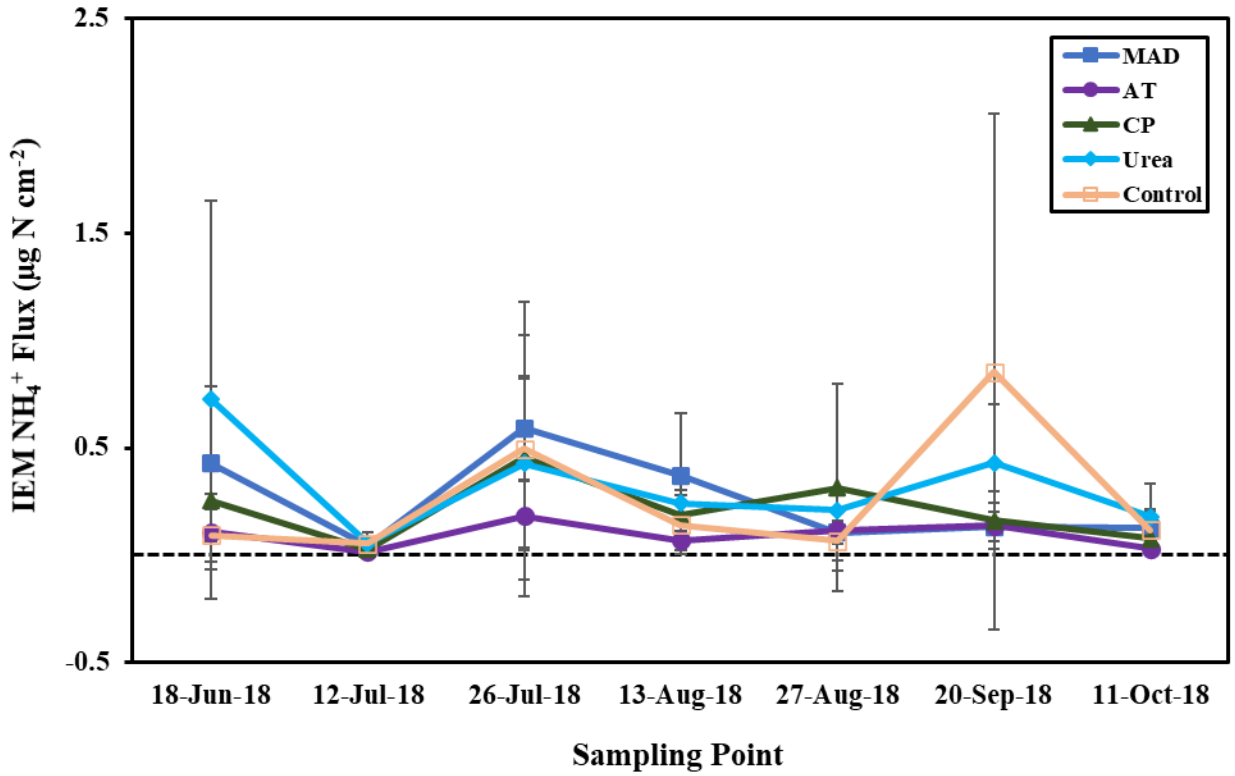

Figure 4.16: Ion exchange membrane NH_4^+–N fluxes (µg N cm^{-2}) measured over 130 days in 2018 (June 4 to October 11). The sampling points represent the day when IEMs were retrieved from the soil. The error bars represent standard deviation.

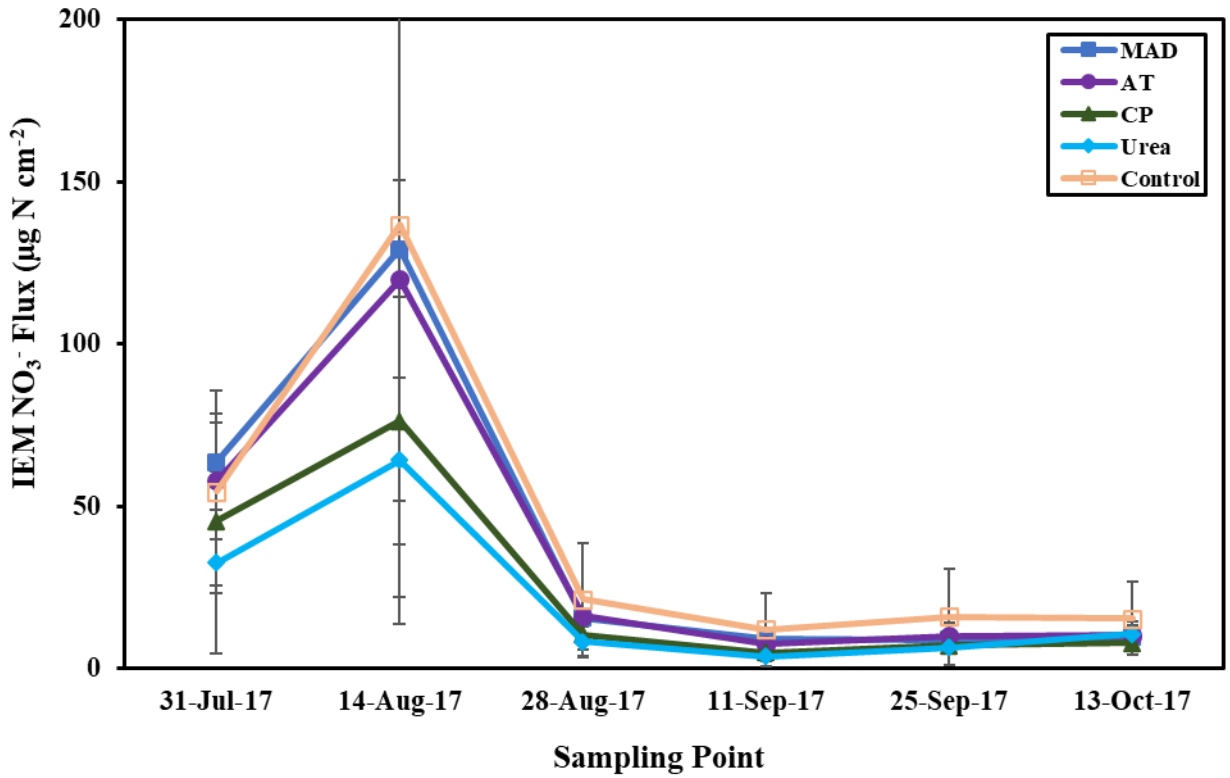

Figure 4.17: Ion exchange membrane NO_3^--N fluxes (µg N cm^{-2}) measured over 89 days in 2017 (July 17 to October 13). The sampling points represent the day when IEMs were retrieved from the soil. The error bars represent standard deviation.

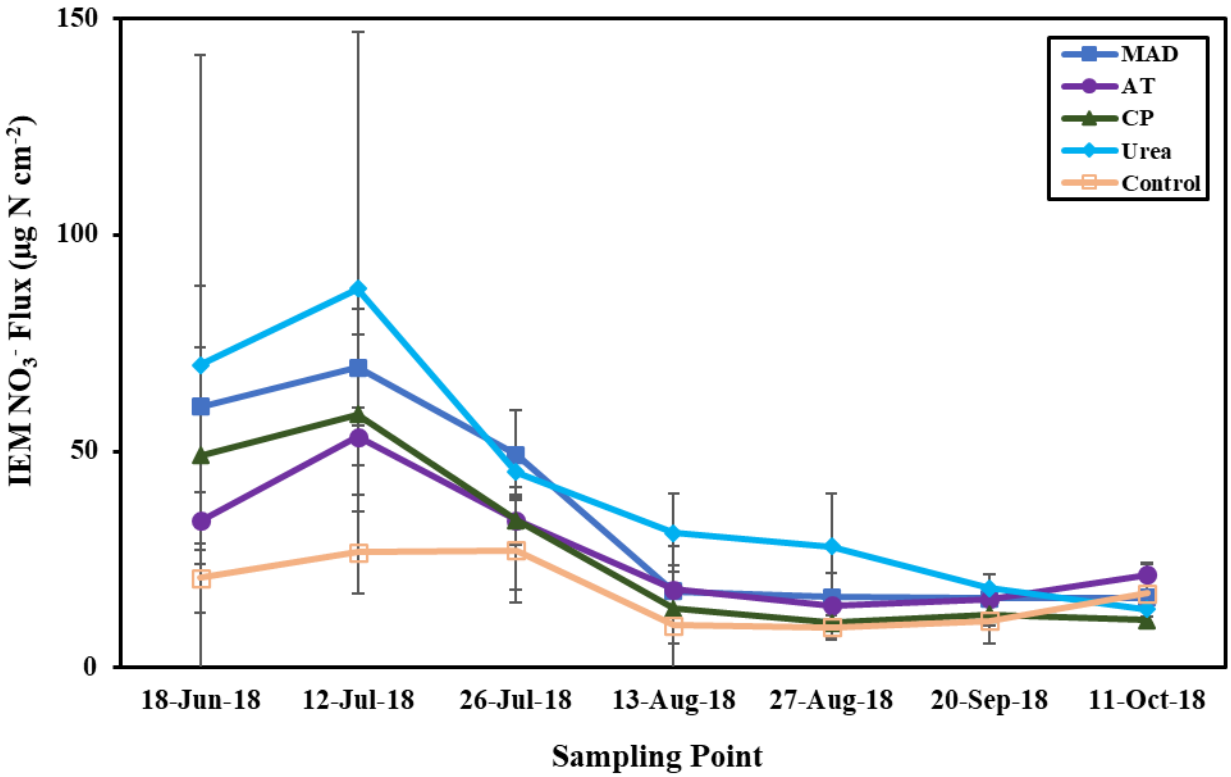

Figure 4.18: Ion exchange membrane NO_3^--N fluxes (µg N cm^{-2}) measured over 130 days in 2018 (June 4 to October 11). The sampling points represent the day when IEMs were retrieved from the soil. The error bars represent standard deviation.

4.6.2. Ammonium and Nitrate Exposures

In 2018, ammonium exposure significantly ($p \leq 0.05$) varied among biosolids with treatments receiving MAD (1.80 µg N cm^{-2}) and CP (1.48 µg N cm^{-2}) biosolids resulted in higher ammonium exposure compared to AT (0.67 µg N cm^{-2}) (Table 4.14). In 2017, the application rate showed a marginally significant ($0.05 < p \leq 0.1$) effect on ammonium exposure (Table 4.13). However, when letter grouping was generated at 95% confidence interval biosolids and urea combination (2.31 µg N cm^{-2}) and full-rate biosolids (1.28 µg N cm^{-2}) were not significantly different from each other (Table 4.14). In both years, the application method had no effect on ammonium exposure (Table 4.13). There was no significant treatment or interaction effect found in either year (Table 4.13).

The integrated application of urea and biosolids resulted in a significant ($p \leq 0.05$) effect on nitrate exposure in 2018 (Table 4.13). The application of full-rate MAD, half-rate CP (CP+Urea), and half-rate MAD (MAD+Urea) treatments had significantly ($p \leq 0.05$) higher nitrate exposure compared to full-rate CP treatments by 71%, 80%, and 92% (Table 4.14). There was no other significant interaction noticed in either year (Table 4.13). Nitrate exposure was not significantly different from the incorporation or surface spreading of biosolids (Table 4.13) but varied with the incorporation or surface spreading of urea in 2017 and 2018 (Table 4.12). In both years, a significant ($p \leq 0.05$) treatment effect was observed (Table 4.12). The surface spread half-rate CP (CP+Urea-SS) (75.47 µg IEM N cm^{-2}) and surface spread urea (Urea-SS) (67.78 µg IEM NO_3^--N cm^{-2}) showed the lowest nitrate exposure in 2017 (Table 4.12). In the same year, orthogonal contrast results showed that full-rate MAD (160.64 µg N cm^{-2}) had significantly ($p \leq 0.05$) smaller nitrate exposure than half-rate MAD (MAD+Urea) treatments (312.05 µg N cm^{-2}) (Table 4.12). In 2018, the application of MAD with and without urea significantly ($p \leq 0.05$) generated higher nitrate exposure than the unamended control plots (Table 4.12).

Table 4.12: Ammonium and nitrate exposures (μg N cm^{-2}) as influenced by different treatments.

Treatment[β]	2017[1]			2018[1]		
	IEM NO_3^--N[x]		IEM NH_4^+-N[y]	IEM NO_3^--N[z]		IEM NH_4^+-N[z]
	μg N cm^{-2}					
MAD+Urea-INC	259.99	a	2.02	289.97	ab	1.36
MAD+Urea-SS	364.11	a	2.57	229.39	bcd	2.30
MAD-INC	104.26	bc	1.48	243.33	bc	1.33
MAD-SS	217.01	ab	0.77	217.57	bcd	2.21
AT+Urea-INC	190.67	abc	2.02	204.01	cde	0.78
AT+Urea-SS	243.29	ab	3.96	176.15	cde	0.60
AT-INC	274.58	a	0.52	181.15	bcd	0.80
AT-SS	180.02	abc	1.97	203.11	bcd	0.49
CP+Urea-INC	221.17	ab	3.05	262.70	abc	0.79
CP+Urea-SS	75.47	c	0.21	223.93	bc	2.36
CP-INC	178.58	abc	0.89	143.28	de	1.92
CP-SS	133.78	abc	2.08	126.80	e	0.84
Urea-INC	184.63	ab	1.13	369.29	a	2.25
Urea-SS	67.78	c	0.48	218.12	bc	2.29
Control	256.21	ab	3.68	122.02	e	1.83
p-value*	**0.038**		0.302	**0.000**		0.124

[1]Values are presented as means with n=4.

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold. Treatments with the same letter in each column are not significantly different at $\alpha = 0.05$.

[x], [y], and, [z] indicate that nitrate exposure, ammonium exposure, and ammonium and nitrate exposures were calculated over 89 (July 17 to October 13, 2017), 75 (July 31 to October 13, 2017), and 130 days (June 4 to October 11, 2018), respectively.

[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

Table 4.13: ANOVA *p*-values for the main and interaction effects of biosolids type (B), application rate (R), and application method (M) on ammonium and nitrate exposures (µg N cm^{-2}).

Source	2017		2018	
	IEM NO_3^--N^x	IEM NH_4^+-N^y	IEM NO_3^--N^z	IEM NH_4^+-N^z
Biosolids Type (B)	0.288	0.645	0.041	**0.001**
Application Rate (R)	0.456	**0.083**	0.023	0.733
Application Method (M)	0.541	0.644	0.127	0.682
B x R	0.179	0.518	**0.024**	0.945
B x M	0.101	0.173	0.774	0.795
R x M	0.829	0.569	0.535	0.796
B x R x M	0.514	0.148	0.859	0.325

*Significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) treatment effects are shown in bold. ^{x}, ^{y}, and ^{z} indicate that nitrate exposure, ammonium exposure, and ammonium and nitrate exposures were calculated over 89 (July 17 to October 13, 2017), 75 (July 31 to October 13, 2017), and 130 days (June 4 to October 11, 2018), respectively.

Table 4.14: Effect of biosolids type (B), application rate (R), application method (M), and their interaction on ammonium and nitrate exposures (µg N cm^{-2}).

Treatment[β]	2017		2018	
	IEM NO_3^--N^x	IEM NH_4^+-N^y	IEM NO_3^--N^z	IEM NH_4^+-N^z
	µg IEM N cm^{-2}			
Biosolid type (B)[1]				
MAD	236.34	1.71	245.07	1.80 a
AT	222.14	2.12	191.10	0.67 b
CP	152.25	1.56	189.18	1.48 a
Application rate (R)[2]				
Half-rate + Urea	225.78	2.30 a	231.03	1.36
Full-rate	181.37	1.28 a	185.87	1.27
Application method (M)[3]				
INC	204.88	1.66	220.74	1.16
SS	181.37	1.93	196.16	1.47
B x R interaction[4]				
MAD+Urea	312.05	2.30	259.68 a	1.83
MAD	160.64	1.12	230.45 a	1.77
AT+Urea	216.98	2.99	190.08 ab	0.69
AT	227.30	1.24	192.13 ab	0.64
CP+Urea	148.32	1.63	243.32 a	1.57
CP	156.18	1.49	135.04 b	1.38

[1], [2], [3], and [4] indicate that the values are presented as means with n= 16, 24, 24, and 8, respectively.
*Treatments with the same letter in each sub-column are not significantly different at $\alpha = 0.05$.
[x], [y], and [z] indicate that nitrate exposure, ammonium exposure, and ammonium and nitrate exposures were calculated over 89 (July 17 to October 13, 2017), 75 (July 31 to October 13, 2017), and 130 days (June 4 to October 11, 2018), respectively.
[β]MAD- mesophilic anaerobically digested; AT- alkaline treated; CP- composted biosolids; INC- incorporated; and SS- surface spread.

4.6.3. Ammonium and Nitrate Exposures and Cumulative Nitrous Oxide Emissions

There was no significant correlation detected between cumulative N_2O fluxes and ammonium and nitrate exposures in 2017 (Figure 4.19) and 2018 (Figure 4.20). It is important to note that IEMs were deployed 23 days after the application of biosolids and urea incorporation in

2018, and 61 (AEMs) and 76 (CEMs) days in 2017. Thus, the shorter sampling period of IEMs might have caused a non-significant correlation between cumulative N_2O fluxes and ammonium and nitrate exposures.

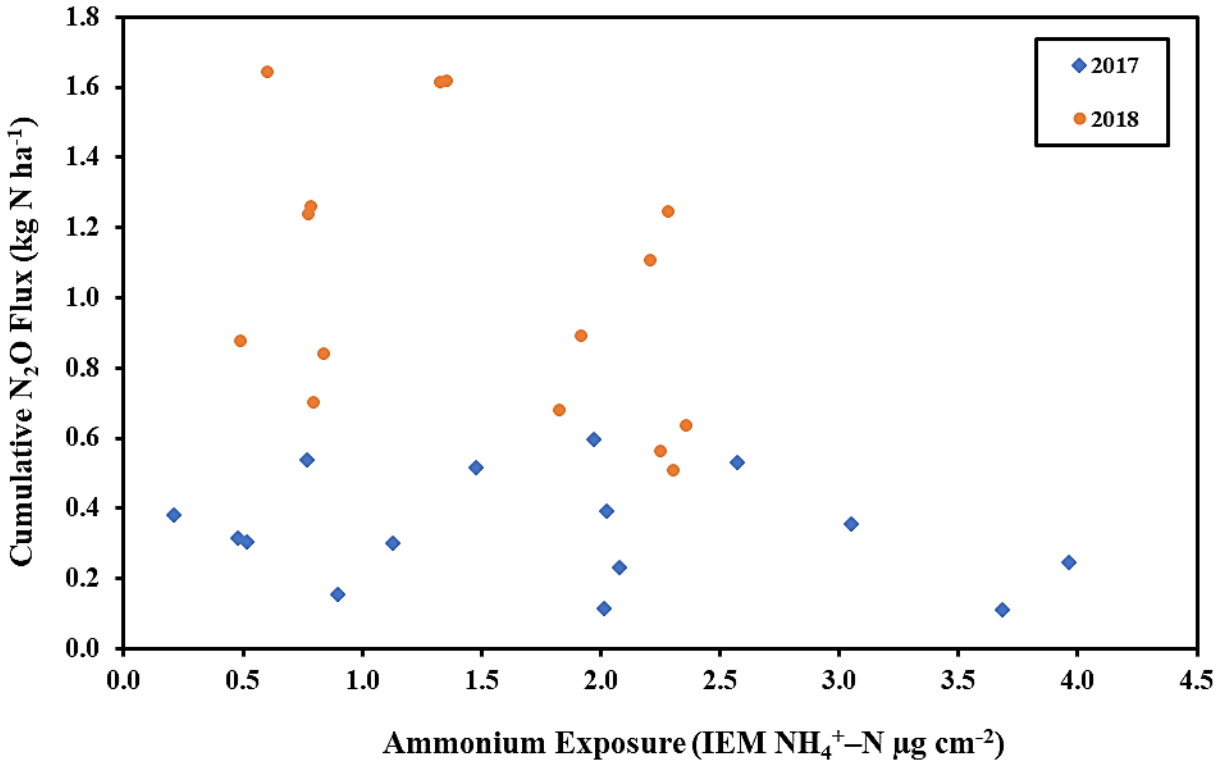

Figure 4.19: Relationship between ammonium exposure (µg IEM N cm^{-2}) and cumulative N_2O fluxes (kg N ha^{-1}).

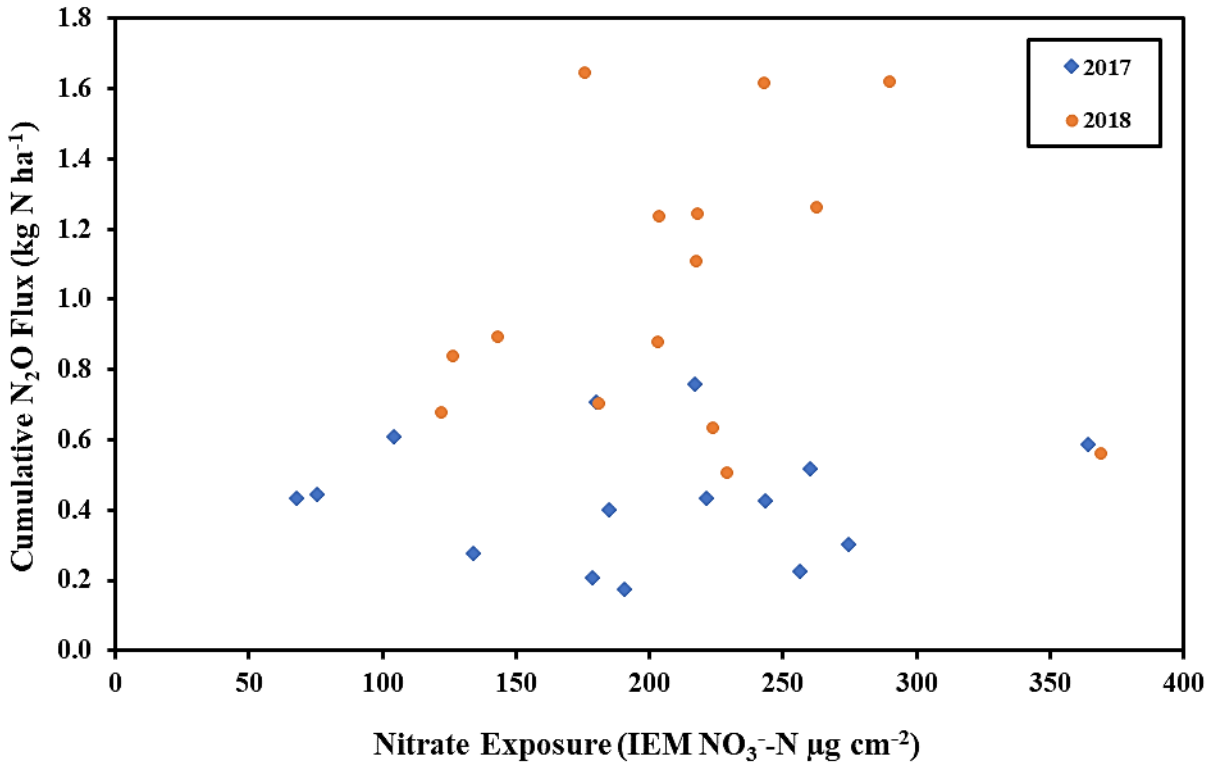

Figure 4.20: Relationship between nitrate exposure (µg IEM N cm^{-2}) and cumulative N_2O fluxes (kg N ha^{-1}).

Chapter 5: Discussion

5.1. Nitrous Oxide Emissions

Organic fertilizers contain organic carbon (C) and have an appreciable amount of nitrogen (N), thus their addition to the soil can stimulate the microbial activity and increase nitrification and denitrification rates as compared to unamended soil, and as a result nitrous oxide (N_2O) production from the soil (Jones et al., 2005). The results obtained in this study suggest that land application of biosolids increased N_2O emissions from the soil as compared to unamended control and urea treatments (Table 4.1). Daily N_2O emissions from biosolids treatments lasted longer and were larger in magnitude as compared to urea treatments where rapid peaks were seen after urea application (Figure 4.4). This can be attributed to the mineralization of the organic matter in the biosolids that provided C and N to soil microbes for an extended period which increased microbial activity and respiration, therefore influenced nitrification and denitrification processes (Ryals & Silver, 2013). Denitrification is highly dependent on available soil C because C is required as an electron donor and heterotrophs need C for energy (Thangarajan et al., 2013). Thus, the availability of C in the soil from the biosolids can increase denitrification rates and result in more N_2O emissions, whereas, in urea amended treatments, the immediate availability of ammonium (NH_4^+) might have prompted N_2O emissions as a result of nitrification (Velthof et al., 2003). The lower N_2O emissions from the urea treatments as compared to biosolids treatments suggest that the denitrification rate was low without the addition of organic material over the long term (Smith et al., 2012).

The C:N ratio of the organic materials is an important parameter that determines the potential immobilization or mineralization of N and eventually affects the amount of mineral N in the soil and N losses from the soil (Paul, 2007; Ussiri & Lal, 2013). Many authors reported a

negative correlation between C:N ratio of the organic materials and N_2O emissions (Baggs et al., 2000; Huang et al., 2004; Pilegaard et al., 2006). Among biosolids of this study, the highest cumulative N_2O emissions were noticed in mesophilic anaerobically digested (MAD) amended treatments and lower cumulative N_2O fluxes were obtained from the application of alkaline treated (AT) and composted (CP) biosolids over the two years (Table 4.3). This can be related to the C:N ratio of the materials. High C:N ratio of CP (22) and AT (~18) might have favoured immobilization or a reduced rate of mineralization, which resulted in lower nitrification and denitrification rates. The MAD had the lowest C:N ratio (~5), enhancing mineralization, nitrification, and nitrate accumulation, resulting in more N_2O emissions. Also, the rapid conversion of NH_4^+ to nitrate (NO_3^-) through nitrification result in low concentrations of NH_4^+, high NO_3^- levels accumulate, and allow for higher N_2O emissions (Baggs et al., 2000). In 2017, more N_2O fluxes from MAD can likely be related to this effect as higher ion exchange membrane (IEM) NO_3^--N fluxes and lower IEM NH_4^+-N fluxes were observed in MAD (Figure 4.14 & 4.16). It is important to note that initial IEM NH_4^+-N and IEM NO_3^--N fluxes were not recorded in 2017, but large IEM NH_4^+-N could be expected from MAD treatments due to high mineralization rate. Another possible reason to have higher N_2O emissions from MAD biosolids is that the anaerobic stored materials are reported to have more easily available C compounds, which generate more N_2O through nitrification and denitrification (Haynes et al., 2009; Lazcano et al., 2016). This can be confirmed by the bursts of carbon dioxide (CO_2) fluxes after the addition of MAD; however, the cumulative CO_2 emissions were relatively lower than CP biosolids over the study years (Figure 4.7).

Organic slurry with high mineral N content, low dry matter, and narrow C:N ratio emitted a higher amount of N_2O compared to materials with high dry matter and/or high C:N ratio (Charles et al., 2017). In 2018, MAD was applied in the form of a slurry, however, the material (MAD) was

still clumpy, and the distribution was uneven across the plot. The incorporation of MAD might have helped MAD to distribute uniformly and diffuse into microsites, become easily available to microbes and result in more anaerobic conditions by taking the air-filled pores (Bhandral et al., 2007). On the contrary, AT and CP had high dry matter content, making it hard to be accessible to soil microbes.

The application of a combination of organic and inorganic fertilizers can improve soil fertility and increase crop yield by affecting soil nutrients, organic matter, and microbial community. However, the integrated application of organic and inorganic N-fertilizers can also result in higher N_2O emissions as it provides labile C and N in the soil that stimulates microbial growth (Das & Adhya, 2014). In this study, the combination of half-rate AT and urea (AT+Urea) generated more N_2O compared to full-rate AT biosolids treatments (Table 4.3). This can be attributed to the increase in NH_4^+ content in half-rate AT (AT+Urea) treatments as a result of the hydrolysis of urea after the application, enhancing nitrification and consequently N_2O emissions in the treatments. Whereas, lower IEM NH_4^+-N flux was noticed in full-rate AT biosolids treatments (0.18 µg IEM N cm^{-2}) for the first 71 days of IEMs measurements compared to half-rate AT (AT+Urea) treatments (0.57 µg IEM N cm^{-2}). This can most likely be due to the low initial mineralization and nitrification rate in full-rate AT treatments as exemplified by low NO_3^- content in the first 39 days of IEMs measurements (80.35 µg IEM N cm^{-2}) than half-rate AT (AT+Urea) treatments (94.09 µg IEM N cm^{-2}). It is important to note that IEMs were deployed into the soil 19 days after the application of biosolids and urea incorporation in 2018. Thus, it is possible that NH_4^+ and NO_3^- from incorporated urea and biosolids were lost during that period. Frimpong and Baggs (2010) found an increase in N_2O production after the application of a combination of residue

and inorganic fertilizer (50:50 & 25:75) compared to the sole application of inorganic fertilizer or residue.

Most of the organic N is mineralized in the first season of application but there is still some un-mineralized organic N leftover from the first year referred as residual organic N, which would be mineralized in subsequent years. The availability of N in the following years of application is highly dependent on the climate conditions and management practices of the biosolids (Rigby et al., 2016). The residual N effect was reported in temperate regions for biosolids (Boyle & Paul, 1989). The dry conditions in the summer followed by winter can limit microbial activity, therefore, restricting mineralization to some extent until the favourable conditions return (Cogger et al., 1999). In this study, higher N_2O emissions were recorded in the second year compared to the first year (Figure 4.4). This can partly be related to the relatively drier growing season in 2017 (May 15 to October 6) than 2018 (May 30 to October 1) (Figure 4.1 & 4.2), which might have delayed mineralization to some extent from the biosolids and carried the conserved organic N into the following summer season. Also, the repeated application of organic materials was reported to raise soil C and N pools (Ding et al., 2013; De Rosa et al., 2018). This could also add to more N_2O emissions in 2018.

Management practices affect the pattern and magnitude of N_2O emissions by influencing N and C supply to soil microbes, altering soil moisture content, and diffusion of oxygen (O_2) into the soil (Aulakh et al., 1991). Generally, the loss of nutrients in surface spread organic materials (plant residue or organic mulch) is more by runoff or volatilization as the materials are not accessible to most of the soil microorganisms, only fungi and larger fauna decompose this, whereas, incorporated materials are more in contact with microbes, therefore, higher nutrient loss by leaching or other microbial processes (Weil & Brady, 2017). He et al. (2009) reported a greater

proportion of macroaggregates in no-till than conventional till due to the breakdown of soil aggregates by plowing in conventional till making it more exposed to decomposition. Also, materials with smaller sized particles can undergo readily decomposition as it exposes more surface area and readily digestible tissues (Weil & Brady, 2017). The difference in incorporated half-rate MAD (MAD+Urea-INC) and incorporated full-rate MAD (MAD-INC) treatments as compared to surface spread half-rate MAD (MAD+Urea-SS) can be attributed to more contact of the incorporated biosolids with soil microbes which increased the decomposition of the material and resulted in more N_2O emissions (Table 4.1). Also, incorporation using rotary tiller helped in the size reduction of the biosolids and the leftover corn residues from the first year, making it more prone to decomposition. However, in most of the studies, the changes in soil properties that influence soil N_2O emissions after tillage or no-tillage were seen over the long term (>5 years) (Lichtfouse, 2015).

Temperature is an important factor as it directly controls the soil microbial activity and indirectly influences the soil water content through evaporation (Aguilera et al., 2013). Mineralization of the organic matter during the warmer summer months can occur at a high rate, which can result in more available N in the soil and possibly higher N_2O (Rigby et al., 2016). This effect can be related to the higher N_2O emissions during the growing seasons in this study. These higher emissions may partly be explained by C supply from the root exudates, which acted as a substrate for microbes and helped them in proliferation (Bouwman, 1996). Also, the uptake of N by the corn plants is very low in the first few weeks after growing, this means more available N for nitrification, denitrification and/or leaching (Maljanen, 2003). In this study, N losses as N_2O were very low in the winter (Figure 4.4), when the temperature was very low ($<5^0$C). Low N_2O fluxes were detected in the early spring season most likely due to the limited sampling in the season

(Figure 4.4). Temperature is the key controller in freeze-thaw events for N_2O production (Oertel et al, 2016).

Rainfall events add water into the soil and increase the soil moisture content, and result in more water-filled pore space (WFPS) and low O_2 levels in the soil (Hu et al, 2015). These conditions are favourable for nitrifier denitrification and denitrification (Zhu et al., 2013; Hu et al., 2015). Generally, there is a burst in microbial activity when the soil goes from dry to wet phase (Davidson, 1992). The wetting and drying cycle (known as Birch effect or wetting pulse) can increase the mineralization rate of the easily decomposable material and result in more available mineral N (Borken & Matzner, 2009). Rabot et al. (2015) reported a peak in N_2O emissions two days after rewetting and another peak when the soil started to dry. The high emissions on 19 June 2018 can be associated with 31.2 mm rainfall, a day prior to sampling, which raised volumetric water content (VWC) to 40% (Figure 4.2 & 4.4). Similarly, in 2017, a burst in N_2O flux was noticed on 21 June 2017 led by 7 mm rain (Figure 4.4). In the same year, another peak on 22 August was recorded after a total of 53 mm rainfall in five days followed by a warm sunny day (Figure 4.4). This can partly explain the high CO_2 fluxes on the same day (Figure 4.7). More CO_2 production consumes O_2 in the soil, thus creating more favourable conditions for denitrification and N_2O emissions (Maljanen et al., 2003). The rainfall in May was lower in 2018 than in 2017 (Figure 4.1), but biosolids application was followed by heavy rainfall in 2018 that possibly resulted in an early peak in 2018 (Figure 4.4).

Higher N_2O peaks followed by thaw events were previously attributed to the burst of physically trapped N_2O (Burton & Beauchamp, 1994) and production of N_2O through denitrification led by anaerobic conditions induced by melting (Risk et al., 2013). The higher emissions in January 2018 can be explained by the production of N_2O after a rise in temperature

(4.4^0C) (Figure 4.4). The short duration increase in temperature might have resulted in some thawing (Risk et al., 2013). The death of microbes from severe frost (in the absence of snow) provided the required C for microbial respiration and reduced levels, inducing favourable conditions for denitrification (Maljanen et al., 2007; Risk et al., 2013; Ruan & Robertson., 2017). This can be confirmed from the high CO_2 emissions on the same day (Figure 4.7). Many researchers reported higher soil temperature and lower freezing degree hours with snow cover on the soil surface (Maljanen et al., 2007; Dietzel et al., 2011; Ruan & Robertson., 2017). Snow cover helps to protect the microbes below freezing temperature and lower their activities, thus reducing the availability of microbial C and mineral N in the soil (Ruan & Robertson., 2017). The lower emissions in March and April of 2018 can likely be attributed to the presence of snow on the soil surface as it snowed 47 cm and 16 cm a day before the sampling, respectively (Figure 4.4). These results were in accordance with Maljanen et al. (2007), and Ruan and Robertson (2017), who observed lower N_2O fluxes under snow-covered soils compared to bare soils.

Nitrification increases when soil conditions change from acidic to slightly alkaline due to an increase in microbial activity and a shift in equilibrium between ammonia (NH_3) and NH_4^+ towards NH_3 (Ussiri & Lal, 2013). However, high pH and/or application of alkaline materials to soil, especially surface spreading, can promote NH_3 volatilization (Weil & Brady, 2017). In this study, N losses after AT amendment (especially as surface spread) may be more as NH_3 due to volatilization than N_2O because AT consists of alkaline substances and the high pH of this material raised the soil pH levels (Figure 4.3), thereby favoured NH_3 volatilization. The hydrolysis of urea addition to the soil can increase the pH temporarily (1 to 2 weeks), which further enhances NH_3 volatilization (Rochette et al., 2009). Coarse soils are more prone to NH_3 losses as clay and organic particles can adsorb NH_3 molecules (Weil & Brady, 2017). The moderately-coarse soil at the study

site might have also added to NH_3 volatilization after AT application due to the above-mentioned effect.

5.2. Carbon Dioxide Emissions

Biosolids amendment to land improves soil health and increases soil organic C pool by direct addition of organic matter to the soil and indirectly by affecting other soil properties (Sharma et al., 2017). However, decomposition of that organic matter by soil microbes is a major threat to C sequestration, as the process results in loss of C as CO_2 and/or methane (CH_4) (Wijesekara et al., 2017). In the growing season of 2018 (May 30 to October 1), full-rate CP biosolids amended plots produced a significantly ($p \leq 0.05$) higher amount of CO_2 compared to unamended control plots and urea treatments (Table 4.4). This loss in C can be attributed to the presence of high C content in CP biosolids that stimulated the soil microbial activity and thereby increased soil respiration (Soriano-Disla et al., 2010; Carmo et al., 2014; Torri et al., 2014; Wijesekara et al., 2017). Similar results were reported in previous studies after the application of composted sewage sludge (De Urzedo et al., 2013; Carmo et al., 2014). Although microbial biomass was not measured in this study, the literature suggests that an increase in microbial biomass was observed after the application of composted sewage sludge (Ros et al., 2006). Biosolids are sources of organic substrates such as peptide and proteins that increase enzymatic activity and microbial proliferation, resulting in high soil respiration (Torri et al., 2014; Sharma et al., 2017). This effect could also be possible in this study after the application of CP biosolids, adding more to CO_2 fluxes.

The water-soluble labile organic C is very susceptible to the microbial attack (Cook & Allan, 1992). In this study, higher soil CO_2 fluxes were observed in CP biosolids compared to AT and MAD biosolids in the growing season of 2018 (May 30 to October 1) (Table 4.6). The different outcomes from three biosolids can be associated with the physio-chemical and biological

compositions of the materials (De Urzedo et al, 2013), which are affected by the treatment process (Hayens et al., 2009). The CP biosolids are considered more stable product because they have high organic C content as decomposition-resistant humic substances, but they also contain about 1-2% of labile organic C, which are readily available for decomposition (U.S.EPA, 1999; Hayens et al., 2009). Additionally, CP biosolids are known for low but persistent effects on the microbial community as compared to non-composted biosolids because of the presence of microbial population in the residues (Ros et al., 2006; Hayens et al., 2009). In this study, CP biosolids consisted of wood chips and had a C:N ratio of 22. Thus, the application of CP biosolids at the high rate provided high organic C content and external microbial biomass in the soil, which stimulated indigenous microbial activity, thereby increasing cumulative CO_2 emissions (Table 3.3). The processing of AT biosolids at high temperatures and high pH enhances microbial destruction, consequently lowering the active microbial population (U.S.EPA, 1999; CCME, 2012). The AT biosolids amendment to the soil at a high rate resulted in high organic C in the soil and the presence of carbonates formed during the processing by adding alkaline materials in AT biosolids (Table 3.3). This increase in C content in the soil triggered the microbial population and resulted in higher soil CO_2 fluxes from AT than MAD over the year 2018. Generally, digested biosolids contain 2-3% of labile organic C (Hayens et al., 2009) and the remaining C is present in a recalcitrant form (Yoshida et al., 2015). In this study, MAD biosolids had high organic C and possibly high labile C but they were added at a lower rate compared to CP biosolids (Table 3.1 & 4.8). This low availability of organic C in the soil after the MAD amendment resulted in the lowest cumulative CO_2 emissions among the biosolids over the growing season of 2018 (May 30 to October 1) (Table 4.6).

Biosolids mineralization rate increases under slightly alkaline soils (pH <8) (Rigby et al., 2016). This is due to the rapid microbial proliferation with the change in pH conditions from very acidic to slightly alkaline and this increase in microbial activity results in high soil respiration (Weil & Brady, 2017). The higher CO_2 fluxes over the second year after the AT biosolids amendment can be related to this phenomenon as pH shifted from acidic to nearly neutral (Figure 4.3). Reth et al. (2005) reported a similar outcome with the increase in pH from acidic to slightly alkaline conditions. Besides, liming materials added to AT consist of carbonates which may be converted to CO_2 after the land application of AT (Synder et al., 2009).

The biosolids+urea combination resulted in no difference in soil respiration compared to the respective full-rate biosolids treatments. However, peaks on July 5, 2017 and July 31, 2018 were noticed after the surface application of urea (Figure 4.7). This was most likely due to the hydrolysis of urea, as it rained after the urea application, which possibly resulted in the production of NH_4^+-carbonate and ultimately led to CO_2 production (Synder et al., 2009).

Soil temperature is considered as the major controller of soil respiration as it influences respiration processes directly by controlling microbial activity and decomposition rate (Thangarajan et al., 2013; Weil & Brady, 2017), and indirectly by enhancing plant growth (Kasper & Bland, 1992) and creating drying conditions (Lou & Zhou, 2006). In this study, high CO_2 fluxes were noticed with the rise in soil temperature and then a decline in CO_2 fluxes was recorded with the drop in temperature (Figure 4.8). Figure 4.8 depicts the relationship between soil temperature and daily soil CO_2 fluxes. This figure suggests that soil temperature was the main driver for temporal variation in soil respiration and the changes can be attributed to the increase in decomposition rate of the applied biosolids as well as of the soil organic C with the rise in microbial activity, which added to heterotrophic respiration. Additionally, the rise in soil temperature helped

increase the plant growth and root exudate supply, increasing microbial respiration. These observations are consistent with previous studies in which authors reported an exponential increase in CO_2 fluxes with the rise in soil temperature (Sato & Seto 1999; Reth et al., 2005).

High CO_2 fluxes occur at intermediate soil moisture levels (40-60% WFPS) under warm conditions (>10^0C) (Gritsch & Zechmeister-Boltenstern, 2014). Many authors observed a decline in CO_2 fluxes under very dry or very wet conditions and when the soil temperature was low (<10^0C) (Rastogi et al., 2002; Lou & Zhou, 2006; Gritsch & Zechmeister-Boltenstern, 2014). Soil moisture was found to have no effect on soil CO_2 emissions at low temperature (5-10^0C), (Gritsch & Zechmeister-Boltenstern, 2014). Rastogi et al. (2002) related this to the inhibition of microbial and plant activity at low temperature, directly affecting root and microbial respiration. In close accordance with the above-mentioned studies, high CO_2 fluxes were noticed at intermediate moisture levels under warm conditions, with a few exception peaks (Figure 4.8 & 4.9). This can be attributed to the optimum conditions in the soil at intermediate moisture content, which helped in the diffusion of O_2 and the allocation of substrates in the soil (Lou & Zhou, 2006). The negative relationship between soil moisture and CO_2 emission in 2018 can be explained by the rise in soil moisture content due to the rainfall events, increasing WFPS and thus reducing diffusivity of O_2 and aerobic respiration which produces CO_2 (Kim et al., 2012). However, in 2018, cumulative CO_2 fluxes in the growing season were not significantly influenced by VWC but when fluxes over the whole year were considered, VWC showed a significant ($p \leq 0.05$) impact on CO_2 emissions (Figure 4.9). As mentioned previously, the fluxes in winter are more related to soil temperature than soil moisture partially because the water content is no longer the limiting factor.

Rainfall events after a dry period (drying and wetting cycles) trigger CO_2 fluxes in various arable lands (Beare et al., 2009; Kim et al., 2012). The involved mechanisms are more available

microbial biomass accumulated over the dry period, higher availability of substrate previously attached to soil matrix, and more interaction of microbes with the substrate in soil solution (Lou & Zhou, 2006; Kim et al., 2012). The effect of rainfall was also noticed in this study. The burst in CO_2 fluxes on June 21, 2017 was led by 7 mm rainfall after a warm dry period (Figure 4.7). Similarly, the highest emissions in 2017 were recorded on August 22 can also be associated with 53 mm rainfall occurred over five days followed by a warm sunny day (Figure 4.7).

Freeze-thaw events increase CO_2 emissions from the soil (Matzner & Borken, 2008). Several mechanisms occurring simultaneously such as an increase in microbial respiration as a result of the increased availability of dead microbial biomass, breakdown of the soil aggregates and release of more nutrients and substrate in the soil, and diffusion of the trapped gas during the freezing period, cause a burst in CO_2 (Matzner & Borken, 2008; Kim et al., 2012). The emissions in January 2018 can be related to the freeze-thaw event as a short-term rise in temperature was noticed after the rainfall, which may have resulted in some thawing and burst of CO_2 (Figure 4.7). A peak in December 2018 most likely resulted from the water-soluble CO_2 that escaped from the growing ice as the temperature was reaching a freezing point (Figure 4.7). These results were in accordance with Teppe et al. (2001), who found a peak of CO_2 as the freezing started.

The fluxes are often higher in spring as the trapped CO_2 diffuses to the atmosphere and more available substrate for microbes to decompose as frost melts (Van Bochove et al., 2001; Teppe et al., 2001). However, in this study lower emissions were recorded at the thawing period (Figure 4.7). This can be related to the presence of snow cover that helped in reducing the mortality rate of microbes and breakdown of the macroaggregates as the soil was not directly exposed to freezing (Ruan & Robertson, 2017). Additionally, the presence of snow cover on the soil surface creates a physical barrier for the trapped gas to escape to the atmosphere (Van Bochove et al.,

2001; Congreves et al., 2018). Since infrequent samples were taken in the spring and winter seasons thus there are chances that CO_2 might have escaped during that period (Figure 4.7).

5.3. Methane Emissions

Over the growing season of both years, soil acted as a sink of CH_4 after the land application of biosolids (Figure 4.10). However, when postharvest samplings were considered, positive cumulative CH_4 emissions were noticed from biosolids amended plots, except for MAD in 2017 (Table 4.11). The application of organic matter provides C and N to soil microbes and stimulates their activity, which in turn increase CH_4 fluxes from the soil (Bayer et al., 2012). Methanotrophs have ability to oxidize CH_4 and NH_4^+ in the soil through CH_4-monooxygenase enzymes, which has low substrate specificity. There is a competition of substrate between NH_4^+ and CH_4 for CH_4-monooxygenase active site, resulting inhibition of CH_4 oxidation until NH_4^+ is almost nitrified. Additionally, the formation of hydroxylamine in the oxidation of NH_4^+ by CH_4-monooxygenase inhibits CH_4-monooxygenase activity (Mancinelli, 1995). Thus, after the application of urea to the soil, methanotrophs prefer oxidizing NH_3 as compared to CH_4 (Hütsch, 2001). In this study, the application of half-rate CP (CP+Urea) in the growing season of 2017 (May 15 to October 6) resulted in higher CH_4 emissions than full-rate CP biosolids (Table 4.11). This can be related to more NH_4^+ in half-rate CP (CP+Urea) treatments (1.63 IEM N µg N cm^{-2}) compared to full-rate CP treatments (1.49 IEM N µg N cm^{-2}) due to the hydrolysis of urea in the soil (Synder et al., 2009). The high concentrations of NH_4^+ favour nitrification over CH_4 oxidization, thus resulting in lower CH_4 consumption (Le Mer & Roger, 2001). Das et al. (2014) reported similar results where higher CH_4 emissions were reported after the application of manure+urea combination in flooded rice.

Tillage practices are known to increase oxidation of the organic materials by burying them into the soil layers, whereas, in no-till farming, soil disturbance and oxidation rates are low, and soil organic C was observed higher in the top 5 cm layer of the soil (Lichtfouse et al., 2015). Tillage practices damage the naturally formed capillary pores consequently resulting in lower infiltration of water and more soil aeration. On the other side, no-till practices retain more water by increasing water holding capacity and water infiltration rate (Lichtfouse et al., 2015). Many authors reported more moisture content in the top layer of the soil under the conservation tillage (Jabro et al., 2009; Zhang et al., 2009). In this study, SS treatments acted as a source of CH_4, while INC plots showed negative cumulative CH_4 emissions over the growing season in 2017 (May 15 to October 6) (Table 4.11). This can be related to the lower oxidation rate in the SS plots than INC plots. Additionally, CH_4 produced at the soil surface was lost immediately to the atmosphere, while CH_4 produced in the soil profiles might have oxidized before it lost from the soil. On average, higher soil moisture content was observed under SS treatments (27.4% VWC) compared to INC treatments (24.0% VWC), which might have helped in creating more frequent anaerobic conditions and led to higher CH_4 production and emissions (Zhang et al., 2015). The application of the organic materials not only provide the substrate for CH_4 production but also creates reduced redox potential (Le Mer & Roger, 2001). These results were in accordance with previous findings where authors reported higher CH_4 fluxes under no-till compared to conventional tillage (Plaza-Bonilla et al., 2014; Zhang et al., 2015).

In general, high CH_4 production occurs between 30-40^{0}C, although it depends on some other factors such as soil moisture, soil pH, vegetation type, and substrate availability (Le Mer & Roger, 2001). In this study, a negative correlation was seen between soil temperature and CH_4 fluxes in 2018 (Figure 4.11). This can be related to the coincidently high soil moisture on the same

days. Peaks of CH_4 fluxes were noticed under low soil temperature during late winter and spring season (February to April), when soil moisture was relatively high due to thawing as methanogenesis is higher in moist soil conditions than dry soil (Topp & Pattey, 1997; Le Mer & Roger, 2001). However, at very high soil moisture conditions, the diffusion rate of CH_4 decreases due to large WFPS. The negative CH_4 emissions on June 19, 2018 can be related to this phenomenon when VWC was about 40% (Figure 4.2 & 4.10). The CH_4 fluxes peak on May 15, 17, and 31, 2017 can be related to the high availability of fresh organic matter after the biosolids application and more anaerobic sites due to rainfall on the same day or a day before sampling (Figure 4.10). A similar peak was observed on June 5, 2018, where high CH_4 fluxes were led by 40 mm rainfall over three days prior to sampling (Figure 4.10).

The correlations between CO_2 and CH_4 were found to be significant ($p \leq 0.05$) or marginally significant ($0.05 < p \leq 0.1$) and negative in 2018 (Figure 4.14) and 2017 (Figure 4.13), respectively. This explains that the days when CH_4 emissions were the lowest (high CH_4 oxidation), accordingly CO_2 emissions were the highest on that day (Topp & Pattey, 1997; Le Mer & Roger, 2001). In general, over the growing season of both years, high CO_2 fluxes were seen and correspondingly CH_4 consumption was observed (Figure 4.7 & 4.10). However, not all the daily CO_2 and CH_4 fluxes can be related to each other as other factors are affecting CH_4 and CO_2 emissions.

5.4. Ion Exchange Membrane Ammonium and Nitrate Fluxes

In this study, high initial IEM NH_4^+-N fluxes were observed (Figure 4.15 & 4.16) and these can be related to the early release of available mineral N present in the biosolids and urea, and rapid mineralization of the easily available organic matter in the biosolids (Castro & Whalen, 2016). Later in the season, IEM NH_4^+-N remained stable (Figure 4.15) or showed a very steady increase (Figure 4.16), which can be attributed to the nitrification of the available NH_4^+ to NO_3^-

and plant uptake (Zebarth et al., 2008b). The increase in IEM NO_3^--N in the second sampling phase (July 31 to August 14, 2017 & June 18 to July 12, 2018) of both years (Figure 4.17 & 4.18) is most likely due to the nitrification (Castro & Whalen, 2016). The IEM NO_3^--N after the second phase remained stable (Figure 4.17) or showed a steady increase (Figure 4.18). This can most likely be due to the consistent plant N uptake and N losses through denitrification and/or leaching (& 4.18) (Zebarth et al., 2008b).

The land application of a combination of inorganic and organic fertilizers is known to increase soil fertility in different soil types (Yuan et al., 2017). This interactive effect also determines the N release in the soil solution and the magnitude depends upon the quantity and quality of the applied materials (Frimpong & Baggs, 2010). Composted materials are known for their low mineralization rates due to their more stable form as indicated by high C:N ratio, consequently low available mineral N (Dalal et al., 2010; Rigby et al., 2016). However, the addition of urea along with organic material(s) results in more easily available NH_4^+ due to urea hydrolysis, which can further nitrify to NO_3^- (Dobbie & Smith, 2003; Frimpong & Baggs, 2010). In this study, higher IEM NO_3^--N was noticed in half-rate CP (CP+Urea) treatments compared to full-rate CP treatments in 2018 (Table 4.14). This can be attributed to higher available mineral N in half-rate CP (CP+Urea) treatments due to urea addition and decrease in immobilization due to lower amount of CP. These results were similar to the previous finding where residue: inorganic fertilizer (50:50) resulted in more mineralization of the residue and raised the total mineral N pool (Frimpong & Baggs, 2010).

In this study, no significant correlation was detected between ammonium and nitrate exposures and cumulative N_2O emissions when expressed as mean values. These results were not in accordance with the findings of Burton et al. (2008a) and Zebarth et al. (2008b), where authors

reported significant relationships between nitrate exposure and cumulative N_2O emissions. One possible reason to not have a correlation was the shorter sampling period of IEMs (especially in 2017) and infrequent gas sampling during the growing season of 2018 (May 30 to October 1). In 2018, the lower IEM NH_4^+-N fluxes in AT treatments can be attributed to NH_3 volatilization as NH_3 volatilization is favoured in high pH conditions (Haynes et al., 2009; Weil & Brady, 2017), and pH is usually very high in AT amended soil (Rigby et al., 2016).

Chapter 6: Conclusion

This study was conducted in order to examine the effect of land application of biosolids on greenhouse gas (GHG) emissions under Atlantic Canadian conditions. Biosolids amendment to soil significantly increased carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions compared to unamended control and urea treatments. However, the results were not consistent across the years, reflecting differences in weather conditions between the two years. The soil in 2017 was much drier than in 2018. The physical, chemical, and biological properties of biosolids caused differences in GHG emissions. The C:N ratio of the biosolids was seen to be the most important factor of resulting differences in N_2O emissions as a result of differences in microbial respiration and nitrogen (N) mineralization. Therefore, mesophilic anaerobically digested (MAD) with the lowest C:N ratio produced more cumulative N_2O emissions compared to urea treatments, unamended control, and alkaline treated (AT) and composted (CP) biosolids in 2017. In the growing season of 2018 (May 30 to October 1), cumulative CO_2 emissions were significantly greater from CP biosolids treatments than AT and MAD biosolids. The amount of carbon (C) added through biosolids amendment was seen to be the leading factor affecting these emissions. The CP biosolids applied at full-rate have shown potential to produce more CO_2 emissions relative to all other treatments due to the high C content in the material, and the total C loss over 2 years was 16.5% of the total applied C. For methane (CH_4), biosolids amendment had no significant effect compared to unamended control.

Interactions between biosolids type and application rate were observed significant ($p \leq 0.05$) and marginally significant ($0.05 < p \leq 0.1$) for cumulative N_2O and CH_4 emissions, respectively. Higher cumulative N_2O fluxes in half-rate AT (AT+Urea) treatments than full-rate AT treatments can be attributed to the increase in ammonium (NH_4^+) content in half-rate AT (AT+Urea)

treatments after the addition of easily available mineral N from urea. The integrated application of half-rate CP (CP+Urea) in the growing season of 2017 (May 15 to October 6) resulted in large CH_4 emissions than full-rate CP biosolids. This can most likely be due to more NH_4^+ in half-rate CP (CP+Urea) treatments compared to full-rate CP treatments due to the hydrolysis of urea in the soil, causing an inhibitory effect on CH_4 oxidation.

Agronomical practices are very important for the efficient use of the N-fertilizers; however, results from this study suggest that application methods (surface spread (SS) and incorporated (INC)) did not have any influence on cumulative CO_2 and N_2O emissions. The surface application of biosolids and biosolids+urea combination acted as a source of CH_4 in the growing season of 2017 (May 15 to October 6). This can be attributed to the lower oxidation rate and more soil moisture content in SS treatments.

The use ion exchange membranes (IEMs) to examine the availability of NH_4^+ and nitrate (NO_3^-) in the soil and their relationship with N_2O emissions was found to be non-significant in this study. The possible reason to not have a stronger correlation was the shorter sampling period of IEMs (especially in 2017) and infrequent GHG sampling in 2018. It may be concluded from these results that NH_4^+ and NO_3^- might not be the limiting factors in N_2O production from the soil.

The interaction between soil temperature and volumetric water content (VWC) was observed to play an important role in the production and emissions of N_2O and CO_2 in the soil. Also, VWC has affected N_2O and CH_4 emissions, whereas soil temperature was a prominent factor in the production of CO_2 in the soil.

Considering observations over the two years study period, CP biosolids amendment at full-rate to soil have the potential to store more organic C into the soil and improve soil health compared to other biosolids types, providing CO_2 emissions are monitored. Further, the repeated application

of full-rate AT can be considered in soils with acidic nature as the repeated application of full-rate AT was seen to increase soil pH. Additionally, lower N_2O emissions and total % C lass as CO_2 were reported from full-rate AT treatments compared to half-rate AT (AT+Urea) treatments. The repeated soil amendment of full-rate or half-rate MAD (except MAD+Urea+SS) have the potential to produce more soil N_2O emissions. However, a trade-off between improving soil health and high yield versus GHG emissions must be considered before promoting these practices. Further research is needed to verify GHG emissions from these three biosolids under Atlantic Canadian conditions as the results were not consistent in this study. Despite the discrepancies between the two years' results, it can be implied that these findings are still representative and can be further extrapolated to other biosolids amended fields to examine GHG emissions in the region. It is also recommended to further investigate on ammonium and nitrate exposures and their relationships with N_2O emissions in the region. This would help in generating more accurate inventories of N_2O emissions.

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