METHANE DYNAMICS IN AGRICULTURAL CROPPING SYSTEMS AND NEAR SOURCE WASTEWATER TREATMENT LAGOON AND PROCESS EVALUATION OF INCUBATION METHOD FOR METHANE OXIDATION

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Abstract

The three chapters included in this dissertation center on the dynamics of soil methane fluxes and the potential of soil as a methane sink. Soil CH_4 consumption is a natural CH_4 mitigation strategy, and understanding how it works can aid in the development of strategies to reduce CH_4 emissions from soil and support soil health. The first study quantified CH_4 fluxes at the soil surface interface near a dairy lagoon, which served as the CH_4 point source. Methane gas samples were collected using a close vented gas chamber, injected into pre-vacuumed vials, and quantified for CH_4 concentration using gas chromatograph. Net CH_4 influx were greater in chambers that were installed closest to the lagoon than in chambers further away from the lagoon. The second study evaluated a process of incubation method that uses headspace analysis of incubation chambers of CH_4 concentrations quantified by gas chromatograph (GC) using soil soils from different land management. The first phase of incubation created conditions that exposed the microbes to high levels of CH_4 and may have increased the microbes' methanotrophic potential. The derived calibration curve generated through known concentration of standard CH_4 gas can be used as a simple and practical tool in quantifying and estimating concentrations of CH_4 during the CH_4 oxidation process in a laboratory setting. The last study utilized a large data set of CH_4 that was a result of studies conducted to assess N_2O emissions. The different field experiments generated 11,837 individual measurements that were taken in 362 sampling events of which only 21% or 2,082 manifested a significant methane flux. Methane consumption is a frequent result of the CH_4 dynamics between the atmosphere and soil. The flux average value was -0.0016 mg CH_4-C m^{-2} hr^{-1} and if this flux rate accurately represents the average consumption of CH_4-C in cropland soils of the Central Great Southern Plains, it would remove 55,950.40 Metric tons CO_2 eq yr^{-1} from the 8.8 million acres of cropland in Kansas.

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CHAPTER I

INTRODUCTION

Greenhouse gases such as carbon dioxide, methane, and nitrous oxide are contributing to the increasing trends in global atmospheric warming. Methane, being one of the potent greenhouse gases, with a warming potential 34 times greater than CO_2 over a 100-yaer time horizon (Myhre et al., 2013), contributes to climate change. The necessity to reduce greenhouse gas emissions such as CH_4 are critical to mitigate climate change.

In agricultural systems, soil CH_4 consumption can play an important role in reducing emissions from different sources such as livestock production, and other activities that release CH_4 into the atmosphere. Soil CH_4 consumption is a natural process that can help to decrease CH_4 emissions. Methane consumption is carried out by methanotrophic bacteria, which consume CH_4 as their primary energy source. These bacteria are present in many soils, including those in agricultural lands. Because of this, soil has the potential to reduce atmospheric CH_4 which accounts for 10-15% in removing CH_4 from the atmosphere (Aronson et al., 2013). Additionally, soil CH_4 consumption has been linked to improvements in soil health and crop productivity.

It is in this general premise that we conducted these different studies to evaluate the phenomena with regards to soil being a potential CH_4 sink. The objectives of this study were to (1) evaluate the CH_4 dynamics in proximity to the lagoon wastewater treatment

being the point source of CH_4 (Chapter 3), (2) develop a laboratory technique which can screen for methanotrophic activities using incubation method and soils from different land-uses/management (Chapter 4), and (3) utilized a large data set across Kansas and Oklahoma to determine significant fluxes, evaluate the dynamics, frequency, and magnitude of CH_4 consumption and emission in field experiments conducted to monitor N_2O in the Central Great Southern Plains (Chapter 5).

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CHAPTER II

REVIEW OF RELATED LITERATURE

Methane (CH_4) is the second most powerful greenhouse gas, with a global warming potential 34 times greater than carbon dioxide during a 100-year estimated atmospheric residence period (Myhre et al., 2013). Even though global CH_4 emissions are significantly lower than CO_2 emissions and that CH_4 concentrations in the atmosphere are roughly 200 times lower than CO_2, CH_4 is responsible for approximately 20% of global warming (Wuebbles and Hayhoe, 2002). Natural and man-made wetlands are the world's largest CH_4 sources, accounting for one-third of total annual emissions (IPCC, 2007). Moreover, under the right environmental conditions, soil can both generate and consume CH_4 (Gentry et al., 2021). CH_4 is generated in soil by anaerobic methanogenesis and consumed by methanotrophic bacteria that utilize O_2 and CH_4 for their metabolism under aerobic conditions (Dutaur & Verchot, 2007).

Methanogenesis

Methane-producing bacteria are among the oldest microbes on the planet CH_4. Methanogenesis as a metabolic is an ancient pathway carried out by methanogenic bacteria or methanogens under the domain Archaea (Gentry et al., 2021). Methanogens are highly sensitive to oxygen and reactive oxygen species, and CH_4 generation in soils

happens only under anaerobic, strongly reducing conditions in the absence of nitrate, sulfate, or ferric iron (Topp and Pattey, 1997).

In natural systems, landfills, and agriculture, methanogens are the principal biological source of CH_4 (Aronson et al., 2013). Methanogenesis is typically active in anaerobic environments and produce CH_4 as a metabolic byproduct and is a prevalent activity in wetland soils and rice paddies. Generation of CH_4 from soil are highest when soils are saturated due to the slow diffusion of oxygen in the water or presence of rich labile organic matter that can drive biological oxygen demand through heterotrophic respiration (Gentry et al., 2021). This was supported by Minamikawa and Sakai (2005) who mentioned that water management strategies and the amount of organic material available for decomposition determine the amount of CH_4 generated. Methanogenesis may also happen in upland soils when anaerobic microsites in soil aggregates exist (Dutaur and Verchot, 2007). Aggregates as small as 250 μm has its core significantly reduced, allowing methanogenesis (Brewer et al., 2018).

Changes in temperature and precipitation due to climate change would modify soil CH_4 production. High temperatures may increase CH_4 production by increasing the activity of bacteria that offer substrates for methanogens (Feng et al., 2021). Increase in precipitation and soil moisture led to increased methane-producing microbes. Also, according to Blankinship et al., (2010), increasing precipitation lowered CH_4 uptake by 61%. The CH_4-cycling microbial population is influenced by environmental and climatic factors, which impacts the net CH_4 flux (Aronson et al., 2013).

Methanotrophic activity

Methanotrophic bacteria also known as CH_4 oxidizing bacteria or MOB are aerobic bacteria that get their energy and carbon by oxidizing CH_4 with O_2 to CO_2 (Hanson and Hanson, 1996) making them the only biological sink for CH_4. The biological oxidation of CH_4 is critical for global CH_4 balance (Serrano-Silva et al., 2014). Methanotrophs may be found in both oxic and anoxic soils, as well as microsites within anoxic soils (Aronson et al., 2013). Ecosystems with a source function of atmospheric methane are typical habitats of methane-oxidizing bacteria (Knief, 2015). Two groups of methanotrophs are dominating the soil depending on environmental conditions. Type I methanotrophs consume CH_4 at low concentrations and are found in unsaturated upland soils, whereas Type II methanotrophs oxidize CH_4 at high concentrations and are found along oxic-anoxic interfaces (Gentry et al., 2021). MOB can also be classified into two types based on cell physiology: methane assimilating bacteria (MAB) and bacteria that cooxidize methane called the autotrophic ammonia-oxidizing bacteria (AAOB) (Holmes et al., 1999).

The microbially-driven oxidation of CH_4 with O_2 to methanol, formaldehyde, formate, and eventually CO_2 via the CH_4 monooxygenase enzyme is known as methanotrophy (Bürgmann, 2011). The density and activity of the methanotrophic microbial population can be affected by a variety of environmental conditions, including humidity, temperature, soil composition, and accessible substrates (Kallistova et al., 2005), as well as pH, type and concentration of N sources, and variations in concentration of CH_4 and O_2 (Hanson and Hanson, 1996). Increased populations of methanogens and

activity occur wherever there is an increase in methane concentrations, whether from biogenic or other sources, and oxic conditions are met (Bürgmann, 2011).

Dynamics of CH4 influenced by several factors

Methanotrophy in soil is influenced by temperature, soil texture, moisture, and nitrogen (N) content and the effects of these factors on CH4 oxidation rates have been extensively studied in the laboratory and in the field (Murguia-Flores et al., 2018).

Soil moisture

Soil moisture affects the diffusion of CH_4 from the atmosphere into the soil since CH_4 oxidation tends to decrease as soil moisture increases. Changes in soil moisture account for approximately 88% of the variability in oxidation rates among the abiotic factors that influence oxidation rates (Tate, 2015). CH_4 consumption decreased steadily as soil moisture increased from 60 to 100 % water-filled pore space (WFPS), and the highest CH_4 consumption was measured at low soil moistures (20 to 60 % WFPS) (Castro et al., 1995). Transport of the gas is slow with increasing soil moisture and the ability of the bacteria to access and utilize CH_4 decreases. The physiological water stress experienced by methanotrophs or the restriction of diffusive CH_4 and O_2 transport both at low and high soil moisture contents may diminish the capacity to absorb CH_4 (Van den Pol-Van Dasselaar et al., 1998) and (Bowden et al., 1998). The osmotic gradient between the soil and soil organisms is determined by the soil's water potential. Methanotrophic bacteria have total access to the pore space and the best water

supply at pF 1.8 (6 kPa), but it is too dry at pF 4.2 (1500 kPa), where CH_4 oxidation does not take place (Geck et al., 2016). If most methanotrophs live along large pores, methanotroph activity should decrease sharply as soil moisture levels fall on the other hand, methanotroph activity should be maintained until very low soil moisture levels if methanotrophs live along smaller pores deeper in the soil matrix (von Fischer et al., 2009). Optimum CH_4 absorption rates were measured under a variety of moisture conditions ranging from 20% to 60% water-holding capacity (Whalen and Reeburgh, 1996).

One of the reasons why soil moisture has a significant influence in the movement of CH_4 could be due to the emission of CH_4 from deep saturated soil layers is balanced out by the absorption of CH_4 from the top soil layers (Zhao et al., 2019). Studies indicate a weak negative correlation of soil moisture and CH_4 fluxes (Liu et al., 2021 and Luo et al., 2012).

In a lowland broadleaf mixed forest, soil temperature and moisture had no significant influence on CH_4 consumption throughout the summer and autumn seasons but had a positive significance during the spring season (Jiří et al., 2018). Tropical deciduous forest and dense open shrubland are distinguished by relatively low soil moisture and consistent temperature throughout the year, both of which promotes high rates of CH_4 uptake by soil (Murguia-Flores et al., 2018). The study of Menyailo (2003) indicated that effects of soil moisture were highly dependent on the plant species although their results had varying response. CH_4 oxidation may be inhibited in some soils after drying due to stratification and

hunger of CH_4 oxidizing microorganisms. Additionally, increasing nitrogen dynamics during drying may alter CH_4 oxidation (Boeckx et al., 1997).

Soil Temperature

Temperature also exerts a significant role in regulating CH_4 movement, oxidation, and generation. Methanogenesis is more susceptible to changes in soil temperature than methanotrophy (Koh et al., 2009 and Dunfield et al., 1993). The majority of methanotrophic bacteria are mesophilic or neutrophilic organisms, but several isolates have been obtained from more extreme environments and are specifically adapted to lower or higher temperature, and other environmental factors such as pH, salt, or oxygen concentrations (Knief, 2015). The rate of CH_4 oxidation increases as temperature increase but protein breakdown and causes the rate of oxidation to decrease when temperature exceeds 35 ^oC (Geck et al., 2016). Murguia-Flores et al., (2018) mentioned that the most efficient soil sink for atmospheric CH_4 is found in warm and semi-arid regions. However, under dry conditions, CH_4 consumption is reduced due to moisture stress on methanotrophs (Bowden et al., 1998). Two studies yield almost the same result as to the effect of temperature on CH_4 uptake capacity. Castro et al., (1995) found that soil temperature significantly regulates CH_4 uptake at temperatures between 5 and 10 ^oC, whereas Van den Pol-Van Dasselaar et al., (1998) stipulated that CH_4 uptake capacity was more pronounced between 4 and 12 ^oC.

Cold regions had the lowest CH_4 uptake rates, particularly tundra and boreal forest, which have pronounced seasonality driven by temperature, making

soil methanotrophy in such areas potentially vulnerable to future global climate change (Murguia-Flores et al., 2018). Warming in the alpine meadows of the Tibetan plateau increased the abundance of methanotrophs and significantly increased the potential of CH_4 oxidation activity, indicating that the plateau has a high potential to consume more CH_4 in the future due to warmer conditions (Zheng et al., 2012). Moreover, atmospheric warming could stimulate the activity of methanotrophs, increasing in CH_4 uptake and longer growing season under climate warming would lead to an additional increase in the overall annual CH_4 uptake (Wang et al., 2014).

Soil Texture and Bulk Density

Soil texture, bulk density, air filled pore space, and soil water content all have a different relative effect on methane diffusivity in soil, as measured by the CH_4 soil diffusion coefficient ($DsCH_4$) (De Bernardi et al., 2022).

Moisture availability is influenced by soil texture and structure. Low CH_4 oxidation rates are consistently linked to fine-textured soils with low porosity and high-water retention, all constantly associated to limit CH_4 diffusion into the soil profile. Soils with a coarse texture drain water quickly and have a high porosity, resulting in high CH_4 diffusion rates into the soil profile (Dutaur and Verchot, 2007). However, the study of Castro et al. (1995) had a different result since the coarser-textured soils in their study consumed methane at rates that were 2 to 3 times lower than in the finer-textured soils. They account the high consumption

rate in fine-textured soil to its higher soil fertility because it either has a population of CH_4 oxidizing bacteria that is more active or has a higher overall activity of CH_4 oxidizing bacteria compared to a region with lower fertility.

Methane emission was observed in fine-textured grassland soils, which may have been caused by the soils' high moisture content, leading to anaerobic conditions in the soil micropores (Boeckx et al., 1997). Clay soils have more micropores hence can hold water more efficiently. Compared to rice produced on clay soil, optimal N-fertilized rice grown on silt-loam soil had large CH_4 fluxes, while clay soil from N-fertilized rice had less than 23% of the seasonal emission (Kristofor et al., 2013).

The gas diffusion coefficient of soil increased with bulk density at the same air-filled porosity in both soil types because the proportion of effective pore space for gas diffusion increased relatively during the compaction procedure (Fujikawa and Miyazaki, 2005). An increase in net CH_4 oxidation is observed when the soil is more aerated, low bulk density, and high porosity (De Bernardi et al., 2022). Additionally, high bulk density values in agricultural land reduce gas diffusion into the soil, affecting CH_4 oxidation, whereas the afforested sector had a positive effect with soil bulk density like natural grassland. However, the study of Drewer et al., (2021) showed that even though the forest site had a lower bulk density than an oil palm plantation, methane fluxes were low with very high variability and showed no clear trend, with the highest range of fluxes measured in a logged forest. Consistent use of a tractor compacted a well-drained sandy loam, lowering

CH_4 absorption by half due to more restricted diffusion (Hansen, Maehlum, & Bakken, 1993).

Soil pH

Methanogens thrive in neutral or slightly alkaline environments, whereas methanotrophs are more tolerant of pH variations (Le Mer and Roger, 2001). Methane production is significantly reduced when pH decreases due to addition of acidic materials but production increases when there was a slight increase in pH in flooded soil (Wang et al., 1993). Acidic soils exhibited the highest CH_4 consumption although this is not always the case and in certain circumneutral soils, little acidity resulted in the halt of CH_4 consumption (Amaral et al., 1997). Soil on an upland ridge had suppressed gross CH_4 production due to a decrease in soil pH coupled with the occurrence drought (Sihi et al., 2021). Agricultural sites with pH > 5 demonstrated much more activity than natural systems under circumstances of increasing atmospheric CH_4 (Chan and Parking, 2001).

Nitrogen Fertilizer

Nitrogen is a basic nutrient that a plant requires in a variety of physiological processes. Often, the amount of nitrogen present in the soil is limited, hence it is supplemented by adding nitrogen fertilizers. Nitrogen fertilizers are primarily absorbed through fine roots as ammonium or nitrate. According to studies, presence of nitrogen fertilizers affects the fluxes of methane, but its influence is

far more complicated. Methane consumption was affected by nitrogen fertilization with lower CH_4 consumption in fertilized soils (Castro et al., 1995 and Wang et al., 2015) or with increased nitrogen levels (Aronson et al., 2013; Bodelier, 2011; Kim et al., 2016). Ammonium fertilization, for example, significantly reduced the methane consumption in a forest soil (King and Schnell, 1994). According to Wang et al., (2015) the activity of methanotrophs and the capacity for methane oxidation do not always increase with fertilization, since they decline after reaching high fertilization levels for instance in dry land soil. Wang et al., (2014) also emphasized that the low N addition decreased the soil water content, which promoted CH_4 uptake in the soil, while medium and high nitrogen deposition inhibited CH_4 uptake and decreased the atmospheric CH_4 sink. Plant growth stimulation with ammonium or nitrate-based fertilizers can boost methane production by increasing organic carbon availability for fermenting microbes delivering methanogenic substrates (Bodelier, 2011). Nitrogen fertilization increases CH_4 oxidation in densely rooted soils, according to studies with rice plants, because rhizosphere methanotrophs compete for nitrogen with both plants and microbes (Macalady et al. 2002 and Eller et al. 2005 as cited by Pandey et al., 2014). Madigan et al., (2003) also mentioned that ammonium is a competing substrate for methane monooxygenase that is not linked to energy generation and, at higher concentrations, acts as a poison to methanotrophs.

Some studies appear that nitrogen additions had no effect on the consumption of ambient CH_4 (Chan and Parkin, 2001 and Costa & Groffman, 2013)

indicating the presence of nitrogen does not immediately inhibit uptake. Nitrogen fertilization typically promotes the growth and activity of methanotrophs in the rhizosphere, which in turn promotes CH_4 oxidation (Kim et al., 2016). The study of Zheng et al., (2012) had a contrasting result since the addition of fertilizers (NPK) promotes growth of methanotrophs but reduces its activity thereby negatively affecting CH_4 consumption. Methanotrophs and ammonia oxidizers can switch substrates, which is thought to be responsible for the inhibition of CH_4 uptake by soil exposed to high ammonia concentrations (Hanson & Hanson, 1996). The immediate ammonium effect on soil CH_4 oxidation is due to competition for the enzyme methane monooxygenase by methanotrophs and nitrifiers (Bayer et al., 2012 and Bodelier, 2011). High ammonium can drive methane away from the monooxygenase enzyme active site, reducing the methane oxidation capacity (Mohant et al., 2006). Also, Schimel and Gulledge (1998) mentioned that depending on the nature of the methane oxidizer population, the response of CH_4 oxidation to nitrogen inputs varies from system to system. According to the model simulations of Murguia-Flores et al., (2018), global soil uptake of atmospheric CH_4 is reduced by 4%, and up to 60% in regions that receives high rates of atmospheric nitrogen deposition and nitrogen input from fertilizers.

Methane uptake in different land-use

Several studies have suggested that land use has an impact on CH_4 dynamics. Aerobic forest, grassland, agricultural, and pastoral soils consume CH_4 at varying rates,

with forest soils consuming the most and agricultural soils the least (Dalal et al., 2008 and Le Mer and Roger, 2001). Methanotrophs in agricultural soil had the lowest relative absorption (%), whereas methanotrophs in rain forest soil had the highest relative absorption (%) (Roslev and Iversen, 1999). Cultivated soil absorbed a quarter less CH_4 than semideciduous tropical forest soils (Keller et al. 1991).

The largest biological sink is upland forest soils (Dlugokencky et al., 2011 as cited by Feng et al., 2020). Forests consumed 93% of total CH_4 oxidized in the top 10 cm of the soil, and CH_4 diffusivity was substantially higher in forests than in plantations in tropical areas (Lang et al., 2020). A model developed by Murguia-Flores et al., (2018) indicated that soil CH_4 uptake by ecosystem type reveals tropical deciduous forests to have the highest georeferenced mean rates of CH_4 oxidation which was 602 mg CH_4 m^{-2} yr^{-1}. Using an empirical model, global CH_4 absorption in forest soils was expected to be 9.16 (±3.84) Tg yr^{-1} from 1981 to 2010 and has grown at a rate of 1.11 g ha^{-1} yr^{-1} during a 30-year period owing to climate change (Yu et al., 2017). On a broadleaf mixed woodland environment, CH_4 emissions from the soil is negligible, while CH_4 uptake was significantly high (Jiří et al., 2018). A low diversity methanotrophic community exists in native forest soil, with a high abundance of putative uncultured methanotrophs that most likely consume atmospheric CH_4 (Kravchenko and Sukhacheva, 2017).

Forest thinning, on the other hand, reduces CH_4 uptake owing to changes in soil moisture and litterfall (Yang et al., 2022). Reduced litterfall decreases soil nutrients because of reduced litter decomposition, impacting CH_4 oxidation, while increased soil

water content restricts oxygen and inhibits methanotrophic activity, delaying CH_4 absorption (Yang et al., 2022).

Grassland soils under well-aerated conditions are also recognized as a major contributor to the global soil CH_4 uptake. CH_4 absorption by temperate grasslands was calculated to be 2.7 Tg CH_4 yr^{-1} based on direct extrapolation of several years of observations (Mosier et al., 1997). Tropical grasslands take up 3.73 (±1.41) Tg CH_4 yr^{-1} using empirical models over a 30-year period (Yu et al., 2017). CH_4 absorption from grasslands is influenced by natural variables as well as intensive management measures. The effects of livestock grazing on CH_4 absorption in grasslands were affected by the changes in grazing density and patterns, as well as weather and vegetation conditions (Wang et al., 2014). Heavy grazing reduced soil CH_4 absorption by 36.47% in China's steppe ecosystem, which is likely due to an increase in soil temperature as well as a reduction in aboveground biomass and soil moisture, all of which are important regulators of soil CH_4 absorption (Tang et al., 2018). However, forest and grasslands are not permanent CH_4 sinks, as climate change and human activity can lead them to become sources of CH_4 (Wang et al., 2014).

The experiment of Bayer et al., (2016) showed that soil under no-till and legume cover crops acts as a greenhouse gas sink. Additionally, they showed that when no-till was used in combination with a legume cover crop, emissions per unit of crop production were reduced allowing for sustainable food production while simultaneously reducing global warming. The methanotrophic activity values in a no-tillage soil were nearly twice as high as in the cultivated soils (Szafranek-Nakonieczna et al., 2019). Crop rotation had little

effect on cumulative CO_2 and CH_4 emissions and even resulted in higher total N_2O fluxes compared to monocropping (Abagandura et al., 2019). Winter cover cropping has a greater potential for greenhouse gas reduction than conservation tillage, and organic inputs such as cover crops plus manure offers the greatest potential for greenhouse gas reduction (De Gryze et al., 2010). When land-use shifted to perennial-grain, CH_4 sink was enhanced and perennial-grain exhibited deeper, denser roots with smaller water-filled pore space implying an improved gas exchange in soil profile (Kim, et al., 2021). On the other hand, the use of plastic film mulching enhances soil organic matter breakdown and results in increased emissions of CH_4 and N_2O. The study of Skinner et al., (2014) stipulates that organic cropping systems exhibited a little higher net CH_4 uptake than non-organic cropping systems.

CH_4 oxidation rates are most likely regulated by nitrogen turnover rates in various conditions (Boeckx et al., 1997). In agricultural systems, CH_4 oxidation is lower compared to natural systems which is due in part to fertilizer nitrogen inhibition since CH_4 oxidation has been shown to be competitively inhibited by ammonium (Chan & Parking, 2001). Varying farming strategies provide different results in terms of CH_4 consumption. The study of Bosco et al., (2019) found that increased tillage intensity and mineral fertilizer rate dispersed in integrated farming systems and organic systems did not appear to impair soil CH_4 oxidation capacity; specifically, the methanotrophic activity of soil microorganisms. They argued that the different cropping techniques may not have had an impact on soil stability, gas diffusion, or methanotrophic activity in the soil, all of which can affect CH_4 absorption. The methanotrophic population in agricultural soils appeared

to be more diverse in terms of methane-oxidizing bacteria than in unmanaged soils, however, the managed soils showed lower methane oxidation rates in both in situ and laboratory experiments (Kizilova, Yurkov, & Kravchenko, 2013). Another study found that the presence of native uncultured methanotroph-like bacteria was detected less in soil with a long agricultural history and a relatively low CH_4 uptake rate (Kravchenko and Sukhacheva, 2017). This indicates that, agricultural soils have a significantly reduced ability to convert methane. For instance, in a wheat-cropped area, CH_4 consumption was lower than in a fallow area (Mosier et al, 1991).

Methanotrophs have been investigated as a bioremediation potential for polluted and altered land-use (Pandey et al., 2014). However, it is still in its infancy and needs to be validated by numerous credible studies.

Methane Incubation Procedures

Methane oxidation is a microbial metabolic process carried out methanotrophs for energy generation and carbon assimilation from methane (Bürgmann, 2011). Incubation is typically used to determine methane oxidation in soil by placing soil samples in airtight jars or bottles, injecting it with a known concentration of methane, and measuring the concentration at regular time intervals. There is no known standard procedure when doing experiment on methane consumption and oxidation. As a result, different studies use different soil weight, container volume, number of days of incubation, and whether the soil that is used is pre-incubated or not. Gas concentrations in the vials or bottles are analyzed by gas chromatography.

For instance, the study of the Lima et al., (2014) used ten grams of freshly sieved soil and placed in a 120 ml serum vial closed with butyl rubber stoppers. Different concentrations of CH_4 were injected into the vial's headspace. Soil microcosms were incubated at 25°C in the dark for up to 19 days with shaking at 150 rpm. Their results showed that oxidation of CH_4 was immediate at low concentrations (10 and 100ppmv), but there was a lag phase of 6-10 days for soils injected with high concentrations of CH4 (1000 and 10,000ppmv).

Another study by Chan and Parkin (2001) used one gram field-moist soils placed in a 40 ml screw cap vials with moisture contents adjusted to 50% gravimetric water content. The incubation was performed at laboratory temperature (24 ºC) in the dark. Vials were sampled roughly every 2 days and monitored for about 3 weeks.

Wnuk et al., (2017) studied the effects of heavy metal on methane consumed and they used ten-gram samples of air-dried soil moistened with $CaCl_2$ and $PbCl_2$ solutions in a vial. Each vial's headspace was enriched with 1% CH_4 v/v and incubated for 21 days at 25 °C which is an ideal temperature for the oxidation of CH_4.

Spokas and Bogner (2011) did an incubation study that used a pre-incubated soil that had been exposed to 50 ml/L of methane concentration to determine soil methane oxidation potential as affected by different levels of soil moisture and temperature. A serum vial was filled with five grams of pre-incubated soil and the headspace was spiked with 5 ml of 50 ml/L CH_4 in argon bringing the headspace CH_4 concentration to around 2 ml/L which is the average CH_4 concentration observed at 10 cm in a soil. Their results

indicated that the pre-incubated soil had higher CH_4 oxidation rates than the non-pre-incubated soil. This was due to the slow growth of methanotrophs, which necessitated the relatively long pre-incubation periods required to establish a steady-state condition in the soil. Another study by Abichou et al., (2011) used 60 g of homogenized soil material and pre-incubated with 6% CH_4 for at least nine days in a 1-L flask. Yin et al., (2020) collected aliquots of four grams soil near leaky wells where methane gas escapes and exposes the soil to high concentration of methane. In the headspace of a 70-mL glass culture bottles, 0.5 mL CH_4 was added. Soils that were exposed to high concentration of CH_4 consumed 96.9% of the initial CH_4, whereas a microcosm with no soil and four grams of control soil collected farther away from the leaky wells consumed 13.3 and 14.4% of the initial CH_4, respectively.

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CHAPTER III

METHANE FLUXES IN SOIL NEAR A DAIRY WASTEWATER TREATMENT LAGOON

ABSTRACT

Methane emissions from dairy wastewater lagoons have been reported to be high, and it is expected that methanotrophs existing in the soils beside the wastewater lagoon will effectively consume the methane. The purpose of this study was to determine CH_4 fluxes at the soil surface interface near a dairy lagoon, which served as the CH_4 point source. The study was conducted at the Ferguson Dairy Center at Oklahoma State University. A closed static chamber was used to collect gas samples. Pre-vacuumed vials were injected with air samples collected at 0-, 20-, 40- and 60-minute intervals after the chamber was closed. Samples were quantified using gas chromatography Varian 450 GC. Temperatures in the air and soil, as well as fractional water indices, had no significant relationship with CH_4 fluxes. Meteorological elements were not significantly associated with CH_4 fluxes. Net CH_4 influx is greater in chambers on the berm closest to the lagoon than in chambers further away from the lagoon. At different distances from the lagoon, consumption, emission, and net 0 fluxes occurred within the same range of ambient CH_4 concentrations but consumption events occurred more frequently at the berm than at the other two locations. This shows that if soil is a net sink for CH_4 near point sources, it occurs in soils very close to the source, and that the widespread consumption of CH_4 is unlikely with the general landscape serving as a low-level net source of CH_4.

INTRODUCTION

Greenhouse gases (GHG) are atmospheric gases that absorb and reradiate infrared radiation generated by the Earth's atmosphere, therefore contributing to the greenhouse gas effect. The greenhouse gas effect occurs when GHG traps heat from the sun and warms the planet's surface, and it is necessary for life on the planet because it helps maintain a warm and stable climate in which organisms can thrive. However, since the industrial revolution, GHG emissions have increased at an unprecedented rate, altering relative abundances of atmospheric gases, resulting in substantial variations in global temperature trends. Carbon dioxide (CO_2) accounts for the majority of greenhouse gas emissions from any sector, while minor amounts of methane (CH_4), nitrous oxide (N_2O), and other trace gases are also produced. Methane has a 34-fold greater global warming potential than CO_2 because it is more efficient at absorbing and storing heat in the atmosphere (Myhre et al., 2013).

Methane sources vary in number and magnitude, and tend to be increasing, whereas CH_4 sinks are less certain (Aronson et al., 2013). Agriculture and waste emissions are estimated to have totaled 206 Tg CH_4 yr^{-1} between 2008 and 2017, accounting for 56% of gross anthropogenic emissions (Saunois et al., 2020). Enteric fermentation associated with livestock industry, treatment and decomposition of animal manures, and rice production systems are the most important anthropogenic sources of CH_4 (Ciais, 2013). Ruminant livestock produce CH_4 as a byproduct of the natural digestive process owing to the fermentation of feed reserves through enteric fermentation (Dangal et al., 2017).

Approximately 16% of the overall annual output of 550 million tons comes from livestock and manure management (De Haan et al., 1997). Net methane fluxes are the equilibrium of two microbial processes: CH_4 synthesis by methanogens in anaerobic environments and CH_4 oxidation by methanotrophs in oxic soil or microsites in anoxic environments (Knief, 2019). The amount of CH_4 released from manure is determined by the decomposition process, which is affected by climatic factors as well as the method utilized in gathering and processing manure prior to application to land (Chadwick et al., 2011). Anaerobic lagoons, which process or deposit waste, are also one of the main sources of CH_4 emissions in animal agriculture. For a one-year sample cycle, Leytem et al., (2011) recorded an average emission rate of 103 g CH_4 m^{-2} d^{-1} from a dairy wastewater storage pond. Methane emissions have been reported to be high in dairy wastewater lagoon, averaging 126 kg/ha/day (Franzluebbers, 2005; and Leytem et al., 2017).

Given the high concentration of CH_4 emitted by lagoons, oxidation by methanotrophs present in the soils adjacent to the wastewater lagoon is expected. Wherever elevated CH_4 concentrations, from biogenic or other sources, meet oxic conditions, greater populations of active methanotrophs occur. Methane emissions are believed to promote the growth of methanotrophic bacteria and enhance their activity in soils close to the source (Yin et al., 2020). Maurice and Lagerkvist (2004) also hypothesize that soils previously exposed to CH_4 emissions should already have developed methanotrophic populations. According to Bender and Conrad (1992), the consumption of CH_4 in the soil increased significantly with rising CH_4 concentrations, demonstrating methanotrophy is driven by CH_4 restricted at atmospheric levels. This might be owing to

the existence of a wide variety of methanotrophs that serves as major sinks of atmospheric CH_4 (Murrell, 2010). As the main species within the soil microbial consortium that extracts energy from the conversion of CH_4 to CO_2, methanotrophs are the only known biological sink for CH_4 (Aronson et al., 2013).

Most of the previous research about CH_4 in animal production systems has focused on exploring ways to minimize CH_4 emissions from ruminants and animal manures, but the possibility of soil close to these sources acting as a CH_4 sink has received less attention. Soil microbes are both highly adaptable to their habitats and highly susceptible to changes in soil conditions, allowing them to function as environmental markers (Zhao et al., 2015). Because CH_4 concentrations in the air near lagoons are continuously high, the soil near them may have large colonies of methane-consuming bacteria that are expected to oxidize atmospheric CH_4. Also, as the distance from the source of emission, the population of methanotrophs and the rate of CH_4 consumption is expected decrease. Moreover, it can also be deduced that physical processes such as natural diffusion of CH_4 gas regulate the amount of CH_4 fluxes in the atmosphere and into the soil. von Fischer et al., (2009) implied that high methanotroph activity is unlikely to persist in areas with low diffusivity, indicating a weak diffusive connection between the soil and the atmosphere, which would starve the methanotrophs. Also, variables other than the high ambient methane concentration may affect the consumption of CH_4. To address these uncertainties, our current study was carried out to quantify CH_4 fluxes at the soil near a dairy lagoon serving as the CH_4 point source.

METHODOLOGIES

Location of the Study

The study was conducted at the Ferguson Dairy Center at Oklahoma State University, Stillwater, Oklahoma. This facility houses over 100 heads of dairy cows and has two lagoons with the primary lagoon located directly south of the secondary lagoon (Figure 1A). The soil at the lagoon is mapped as a Zaneis -Huska complex (with Fine-loamy, siliceous, active, thermic Udic Argiustoll). Soil parameters such as pH, organic matter, and texture were analyzed (Table 1). Closed static vented gas chambers with a total of nine chambers were installed north of the lagoon, with three chambers each on the berm, 9 m north of the berm, and 45 m north of the berm. The berm is a raised strip of land or embankment between the lagoon and the area where the chambers were installed, its distance from the edge of the lagoon was approximately three meters. The chambers were approximately three meters apart at each of these sampling locations and served as replicates. The chambers were located north of the lagoon to take advantage of the prevailing winds from the south during the spring and summer months when microbial activity in the soil is expected to be most active.

Some soil properties were determined as supplemental information. The pH of the soil was determined using a 1:1 soil water (deionized) ratio. Soil organic matter was determined through combustion method using muffle furnace. Texture analysis was determined by hydrometer method. Results of the selected soil parameters are presented in table 1.

Gas Collection and Analysis:

A closed static chambers designed based on the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Greenhouse Gas Reduction through Agricultural Carbon Enhancement network (GRACEnet) Project Protocols (Parkin and Venterea, 2010) were used (Figure 1B). This technique is suitable for investigating the soil gas emission process and can measure both CH_4 emission and consumption (Mønster et al, 2019). The majority of the research on GHG emissions from soils conducted over the last three decades also used this method. The chamber consisted of a base anchor measuring 38.1 by 12.7 cm (inside dimensions) pushed flush into the ground with a lid made of steel and painted silver to reflect solar radiation. The top of the chamber lid has an airtight rubber septum through which samples were withdrawn. On each sampling date, a vented chamber lid (39.4 cm by 15.2 cm by 7 cm) was placed into a water-filled trough on the base anchor to form a gas tight seal with air exchange allowed only through the vent tube on the lid to maintain ambient air pressure within the chamber.

Vials with 30 ml capacity were capped with thick butyl rubber stopper and aluminum cap. Using a syringe, these vials were evacuated with 30 ml of gas to create a partial vacuum. Twenty (20) ml of gas samples were collected from the rubber septum of the chamber lid at 0, 20, 40, and 60 minutes using a syringe. All gas samples collected using a syringe were injected into the designated pre-vacuumed vials.

Samples were immediately transported to the lab for analysis of CH_4 and CO_2 concentration by gas chromatography using the Varian 450-GC model with a flame ionization detector and thermoconductivity detector, respectively.

Gas sampling was conducted from 9:00 am to 11:00 am as suggested by the GRACEnet Project Protocols (Parkin and Venterea, 2010). Sampling was performed almost every week from June 2020 to June 2021 except when the weather made sampling not feasible with only 45 sampling events.

Meteorological Information

Meteorological data such as air temperature, precipitation, air pressure, relative humidity, solar radiation, average wind direction, and average wind speed were collected from the Oklahoma Mesonet station, which is located approximately 1 km south of the study area, using the Mesonet website. Mesonet also provided data on soil temperature and fractional water index data. Fractional water index (FWI) is a measurement of soil moisture content at three different depths (2 inches, 10 inches, and 24 inches). Weather data specific to the time of sampling was collected and used to correlate with CH_4 fluxes.

Data Analysis

Methane fluxes were calculated from the linear change in gas concentration in the chamber headspace versus time, as described by Parkin and Venterea (2010). A T-test is conducted using Microsoft excel to determine if the slope of this regression is significantly different than zero. If it is not different from zero, the flux is considered negligible or zero. However, if a significant difference is detected, then the flux is reported as negative or positive flux. A positive flux value indicates gas emission from the soil to the atmosphere, while a negative flux value indicates gas removal from the atmosphere into the soil. The average flux is then calculated for the three chambers at the three sample locations. Carbon dioxide fluxes were also calculated as supplemental data.

RESULTS AND DISCUSSION

Meteorological conditions during the study period

The highest air and soil temperatures, which ranged between 21.8 to 30.3ºC, and 21.2 to 26.3ºC, respectively, were recorded in the summer months of June to August 2020 and May to June 2021. In contrast, the lowest air and soil temperatures, which ranged from 0 to 8.1 ºC and 2.3 to 6.2 respectively, were recorded during the winter months (December 2020 to February 2021). (Figure 2A). Precipitation was highest during summer, although most observation dates received no rain at all, however, rain fell throughout the year, ranging from 0.25 to 52.83 mm. Fractional water index (FWI) showed a substantial variation at 2-inch depth for most observation dates (Figure 1B). At the 10-inch and 24-inch depths, FWI were relatively high (>0.9) from October 2020 to May 2021 which may indicate higher evapotranspiration. During this time of the year, the meteorological conditions in Oklahoma typically become drier due to the cold and dry air. Air and soil temperature, as well as fractional water indices did not have a significant relationship with CH_4 fluxes (Appendix Table 1). Dorr et al., (1993) indicated that soil temperature has a negligible impact on the flux of methane into the soil from the atmosphere.

Other meteorological parameters such as relative humidity, air pressure, solar radiation, and wind direction were not significantly associated with CH_4 fluxes except for precipitation (0.041) and wind speed (0.044). However, the R^2 values for both precipitation (0.093) and wind speed (0.09) against CH_4 fluxes were extremely small indicating that the significant meteorological parameters have a very weak linear association with the CH_4 fluxes. Leytem et al., (2017) also found very linear regression

trends between CH_4 fluxes and meteorological conditions. The very weak linear relationship of CH_4 fluxes and meteorological condition could be because soil methane fluxes are a result of biological processes and therefore are not strongly impacted by typical weather parameters.

Previous studies have reported a link between wind speed and landfill CH_4 emissions. According to McBain et al., (2005), the primary influence of wind on CH_4 flux is caused by changes in gas pressure brought on by turbulence in the cover soil of a landfill. Wind also enhances the movement of subsurface gases and heightens vertical concentration gradients in soil, causing enhanced diffusion into the soil (Delkash et al, 2022). Poulsen and Moldrup (2006) suggested that wind-induced gas transport becomes a significant mechanism for gas transport for soil with high soil-water content because the gas diffusion coefficient in the soil rapidly decreases as soil water content increases. On the other hand, Riddick et al., (2018) found that the effects of wind speed on methane emission were insignificant.

Rainfall influences gas movement by regulating soil moisture content and soil temperature. Methane uptake significantly increased when soil moisture and temperature increased in the days that followed intense rainfall (Yue et al., 2019). Moreover, increased precipitation would reduce the soil's capacity for CH_4 uptake (Guo et al., 2020). The lack of linear correlation between CH_4 flux measured in our study and the various atmospheric and soil parameters are likely due to the complexity of interactions between those parameters which can potentially influence not only the amount of CH_4 delivered from the lagoon to the sample locations but also the diffusion

of CH_4 in the soil profile and the activity of microbes responsible for CH_4 consumption or production. Metay et al., (2007) also indicated that CH_4 fluxes did not appear to be clearly dependent on rainfall.

Soil methane flux

Soil CH_4 flux varied across different distances from the wastewater lagoon except for a peak emission observed during May 2021 (Figure 3) which could be attributed to rainfall events prior to the observation date. The average CH_4 flux for the entire duration of sample events was -0.00372, 0.00091, and -0.000051 mg m^{-2} h^{-1} at the berm, 9 meters, and 45 meters away from the lagoon was, respectively. The significant CH_4 fluxes observed in chambers located at the berm were generally negative indicating consumption of CH_4. Our findings indicate that consumption is more prevalent during warm periods when soil temperatures are higher than during colder periods. This finding was corroborated by previous studies (De Bernardi et al., (2022), D'Imperio et al., (2017), and Zheng et al., (2012)). In fact, relatively higher CH_4 consumption rates were observed at the berm when air temperatures were above 20°C which was close to the range (25 to 35°C) that was previously reported for methane oxidation to have occurred ((Borjesson and Svensson (1997) as cited by Abichou et al., (2008)). Warm regions have the most efficient soil sink for atmospheric CH_4 (Murguia-Flores et al., 2018).

Methane consumption at 9 m and 45 m sampling areas have relatively lower consumption even during periods when meteorological factors like air temperature favor

the consumption processes. The farther the soil from the source of CH_4, the less frequent are the consumption of CH_4. Methane easily dissipates in the atmosphere and after a certain distance away from the source, the concentration of CH_4 molecules in the atmosphere drops and the amount of CH_4 for consumption also decreases.

The berm is a man-made mound (ridge) constructed at the edge of the lagoon and are expected to have drier soil due to sloping contour where water buildup is less feasible. Because of this, the berm is expected to have frequent and extended periods of dry and oxic conditions. The topographic location can have a big impact on the oxidation and production of CH_4. In the mountainous tropical regions, a model developed by Sihi et al. (2021) predicted an increase in methanotrophic biomass and improved oxygen diffusion into the ridge's drier soil. Also, the berm which is closest to the wastewater treatment lagoon has a loamy texture soil (Table 1). The loamy soil texture may allow the loose soil structure in the berm for easier movement of oxygen, water, and nutrients which are key factors in stimulating microbial activity and enhance methane consumption near the source. Methane is consumed by methanotrophs during drier periods and emitted when the soil becomes anaerobic.

Highest CH_4 emissions were observed at 45 m from the lagoon. This sampling spot is situated in a valley-like location with slight depression allowing for water to accumulate during rain events. Extended durations of wet conditions could have caused prolonged anaerobic conditions which could favor reducing conditions that lead to CH_4 emissions. Wetter areas supported greater CH_4 production, according to Hanson and Hanson (1996), and waterlogged upland soils initiated methanogenesis, increasing CH_4 production.

The constant variation in CH_4 concentration above the soil (Figure 3) could be attributed to both biological and physical diffusion of gas. Despite the fact that soil CH_4 uptake has been shown to be biological due to methanotrophs, there have been evidence that not all of the uptake occurs through an oxidation pathway, which would suggest there are other mechanisms involved in CH_4 consumption (Chowdhury and Dick, 2013). Methanotrophs can oxidize CH_4 concentrations as low as 1.8 – 2 µl/L in the atmosphere (Cai et al., 2016; Smith and Murrell, 2009). High affinity CH_4 oxidation methanotrophs is only stimulated for CH_4 uptake when soil is exposed to elevated CH_4 concentrations (Chowdhury and Dick, 2013 and Cai et al., 2016). Hence, atmospheric CH_4 oxidizers may not solely depend on CH_4 for their metabolic activity (Chowdhury and Dick, 2013). Diffusion, on the other hand, is highly possible when the chamber trapped a relatively high concentration of atmospheric CH_4 while the concentration of CH_4 in the soil was low. It is also possible that the diffusion is restricted even if there is a high concentration of CH_4 trapped in the chamber, but the soil moisture is high. Diffusion of atmospheric methane into the soil is significantly impacted by soil moisture which determines the extent of methane that goes into the soil (Wang et al., 2014). High rates of methanotrophic activity are unlikely to survive in soil areas with low diffusivity because the methanotrophs would starve if the soil had a poor diffusive connection to the atmosphere (von Fischer et al., 2009). Also, if the CH_4 concentration in the atmosphere has been relatively high for a few days and was trapped in the chamber during measurement events, an influx into the soil may not be observed due to the possibility that soil CH_4 concentration is likely in equilibrium with elevated concentrations of the

atmosphere. Hence, differences in soil diffusivity, concentration gradient between the chamber headspace and soil atmosphere, and methanotrophic activity, or a combination could drive the flux of CH_4 in soil near the wastewater lagoon. Dalal et al., (2007) noted efforts to identify biological sources and control of fluxes from landscapes are hampered by the high spatial and temporal variability of CH_4 exchange between soil and the atmosphere.

Soil Carbon Dioxide flux

The average CO_2 flux was 93.75, 59.46, and -59.85 mg m^{-2} h^{-1} at the berm, 9 meters, and 45 meters away from the lagoon, respectively, for the entire duration of the sample events. Soil CO_2 flux peaked during the summer season from with highest fluxes (186.3 to 202.9 mg CO_2-C m^{-2} h^{-1}) from June to September 2020 (Figure 4). The high CO_2 emission during summer period is likely due to increased root respiration and photosynthesis of plants. Microbial activity could also be high since warmer temperatures provide the necessary energy and promote chemical reactions for microorganisms to become active. Precipitation also occurred during the summer months of 2020, and heavy precipitation can raise soil temperature and water content, which mineralizes soil organic matter, resulting in a relatively higher activity of soil microorganisms according to Sainju et al., (2021).

Among the three sampling locations, highest CO_2 emissions were more frequently observed at the berm. The high CO_2 at the berm could also be due to the relatively higher methanotrophic activity as indicated by the greater CH_4 consumption at the berm (Figure

3). If there are methanotrophic activity, the bacteria utilize CH_4 as source of energy and release CO_2 during respiration. This shows that the location relative to the lagoon is relevant in the influx of CH_4.

Ambient Methane

The average ambient CH_4 concentrations in chambers where CH_4 consumption were noted was 3.4 µl/L and 2.9 µl/L in the chambers where CH_4 emissions were observed (Table 2). The minimum ambient CH_4 concentration (2.4 µl/L) were similar when CH_4 was consumed, emitted, or had zero fluxes; however, when CH_4 consumption was observed, the maximum ambient CH_4 concentrations and coefficient of variation were higher. The number of observations when CH_4 consumption was detected was relatively the same to the number of observations when CH_4 emissions were detected. The range of values for the ambient concentration were also comparable for observation days where no significant flux was observed and days where significant consumption was observed. This implies that factors other than a high ambient concentration are influencing CH_4 consumption.

There were 73 observation events with zero fluxes of CH_4 into or out of the soil, which refers to an event at the berm, 9 meters, or 45 meters from the lagoon where none of the three chambers at each position produced a significant emission or consumption as determined by the T-stat analysis. This represents approximately 54% of all observations made in this study. These zero flux events occurred regardless of the chamber's ambient CH_4 concentration at time zero, which ranged from 2.4 to 5 µl/L. These events were observed 23 times at the berm, 24 times from 9 meters, and 26 times

from 45 meters away from the lagoon. The absence of consumption and emission indicates that the CH_4 level in the soil and the atmosphere were equal when the chamber's cover was deployed, and the equilibrium condition remained stable throughout the measurement. Methane emission, net 0 fluxes, and consumption can all occur within the same range of ambient CH_4 concentrations, but consumption events occurred more frequently at the berm than at the other two locations (Figure 3) which as explained earlier could be due to the expected longer and more frequent aerobic conditions at the berm that favors CH_4 consumption/oxidation.

Cumulative flux of methane

Chambers located at the berm had a consistent uptake of CH_4 throughout the observation period, with a cumulative consumption of 12.6 mg CH_4-C m^{-2} at the end of the experiment (Figure 5). The berm had a net CH_4 uptake or consumption could be due to its proximity to a CH_4 source, and its topographic location with sloping contour. Soils that are constantly exposed to high CH_4 concentrations increased their methanotrophic activity (Yin, et al., 2020). Methanotrophic bacteria are highly active when there is a steady supply of CH_4. A sloping topography creates an environment with less available moisture and more ambient oxygen leading to accelerated oxidation process.

On the other hand, cumulative CH_4 fluxes at the chambers located 9 m or 45 m away from the lagoon revealed a net CH_4 emission. These emissions showed consistent low level CH_4-C emission with a relatively large spike on May 27, 2021, resulting in emissions of 16.7 and 10.2 mg CH_4-C/m^2 at 9 m and 45 m, respectively. This large emission

event occurred during a period when soil moisture, as measured at the Stillwater Mesonet, was at or near field capacity on the day when 9.9 mm of rainfall was recorded. Methane emissions are typically observed in saturated soil, resulting in anaerobic conditions in soil pores. Furthermore, the ambient CH_4-C concentration was 2.6 µl/L when the chamber was deployed.

Ambient Methane vs. Methane Fluxes

There is a strong correlation between the ambient CH_4 concentration measured at time zero after the static chambers were covered and CH_4-C fluxes determined as significantly less than zero, indicating a net CH_4 consumption during the measurement event (Figure 6). The magnitude of the flux into the soil is proportional to the ambient concentration of CH_4. The static chambers operate under the assumption that a concentration gradient is responsible for the change in head space concentration.

The berm had a higher average ambient CH_4 concentration than the other two locations since it is more exposed to a relatively high concentrations of CH_4 from the lagoon. The linear regression indicates the relationship between flux and ambient concentrations was strongly influenced by one observation in the chambers located at 45 m and 9 m from the berm (Figure 6). In contrast the berm provided a much wider range influx values proportional to the ambient concentrations of CH_4. The minimum CH_4 ambient concentration is 2.5 µl/L in which it is highly possible that microbes are ineffective at consuming CH_4 at concentrations below 2.5 µl/L, but if the concentration is above 2.5 µl/L, the microbes' ability to consume CH_4 could increase. Moist soils in the

tundra biome consumed CH_4 quickly at concentrations that ranged from below to well above ambient levels (Whalen and Reeburgh, 1990). However, at sub atmospheric CH_4 concentrations, methanotrophs grow very slowly (Conrad, 1996) and this could be because availability of CH_4 and oxygen is more likely to fluctuate in natural environment settings (Roslev and King, 1995). Some methanotrophs can grow at CH_4 concentrations as low as 100 ppm; but bacterial methanotrophic activity at atmospheric CH_4 levels was not observed (Ho et al., 2013). This indicates that the atmospheric CH_4 concentration could be insufficient to sustain continuous methanotrophic activity.

Previous studies also found soil requires time and high CH_4 concentration to provide a substrate that will support the growth of a methanotrophic population capable of absorbing CH_4 from the atmosphere (Spokas & Bogner, 2011; Yin et al., 2020; and Cai et al., 2016). There is also evidence indicating that atmospheric oxidizers belonging to type II methanotrophs are considered as oligotrophic (Dunfield et al., 1999; and Ho et al., 2013), which means that these bacteria can survive on minimal resources. Even though the soil in the berm is exposed to an elevated level of CH_4, these concentrations are likely insufficient to sustain constant methanotrophic activity. The concentration gradient is greater when ambient concentrations are relatively high, and as ambient concentration gets closer to 2.5 µl/L, the gradient becomes weaker and the apparent CH_4 consumption becomes constrained (Figure 6).

The regression shows no significant relationship between CH_4 flux from observations where emissions were significant (based on T-stat analysis) and ambient CH_4 concentrations (Figure 7). Data from the berm is omitted because there were too few

observations with soil CH_4 emissions at this location. The significant emission events were generally of low magnitude with all observations except the two emissions on 27 May 2021 at 9 and 45m from the berm being less than 0.01 mg m^{-2} hr^{-1}. This implies that small emission events can occur regardless of the ambient CH_4 concentration. However, no emission events were observed when ambient concentrations exceeded 4.1 µl/L.

CONCLUSION

Net CH_4 influx is more common in chambers located on the berm which is closest to the lagoon than in chambers located further away from the lagoon. Consumption, emission, and net 0 fluxes occurred within the same range of ambient CH_4 concentration at different distance from the lagoon. The gradient concentration mechanism could simply be variations in atmospheric concentration, or it could be methanotrophic biotic consumption.

Methane fluxes into the soil are driven by diffusion occurring when the ambient atmospheric concentration is higher than the soil atmospheric concentration. This gradient could simply be caused by the variability in ambient concentrations above the soil surface which are in a state of dynamic equilibrium where CH_4 in constantly moving into and out of the soil system. In this situation we would observe a net zero cumulative flux. In our study the majority of influx events occurred at the berm and the magnitude of these flux events were proportional to the ambient CH_4 concentrations all of which were above 2.5 µl/L. This suggests that the influx of CH_4 was in part driven simply by diffusion. However, the net cumulative flux observed at this location showed a net consumption of CH_4 suggesting methanotrophic activity. However, the magnitude of methanotrophic activity is low because the CH_4 concentrations may be too low and inconsistent across time and space for a population of methanotrophs to consistently oxidize atmospheric CH_4.

The flux of CH_4 at 9 and 45 m showed a net emission that was generally equal to the consumption of the berm except for during one event where soils were apparently saturated sufficient to cause methanogenic activity. This shows that if soil serves as a net sink for CH_4 in proximity to point sources such as an animal wastewater treatment lagoon, it occurs in soils very near the source as observed at the berm and that widespread consumption of CH_4 is unlikely with the general landscape serving as a low-level net source of CH_4.

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TABLES AND FIGURES

Table 1. Soil pH, organic matter, and texture of soils at different distance near a dairy wastewater lagoon.

Location	pH	OM	Sand	Silt	Clay	Textural Class
	(1:2)	----------%----------				
Berm	5.34	10.69	46.75	30.43	22.82	Loam
9 meters	6.36	10.19	37.41	26.82	35.76	Clay Loam
45 meters	6.65	8.45	42.71	21.63	35.64	Clay Loam

Table 2. Mean, maximum, minimum ambient CH_4 concentration, coefficient of variation and the number of observation when CH_4 was consumed, had zero flux, or generated an emission from the chambers near a dairy wastewater lagoon.

	Mean	Maximum	Minimum	Coefficient of Variation	No. of Observations
	----------µl/L----------				
Consumption	3.4	5.3	2.4	24.5	33
Zero	3.2	5.0	2.4	17.9	73
Emissions	2.9	4.1	2.4	15.4	29

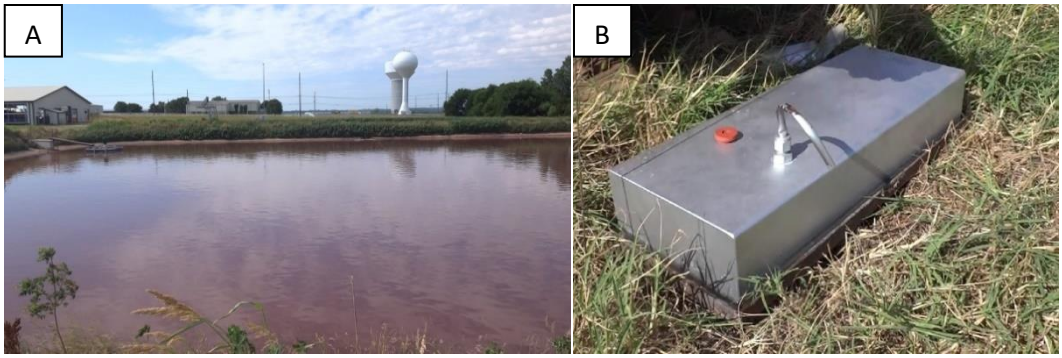

Figure 1. The wastewater treatment lagoon at the OSU Ferguson Dairy Center (A) and a close static chamber (B).

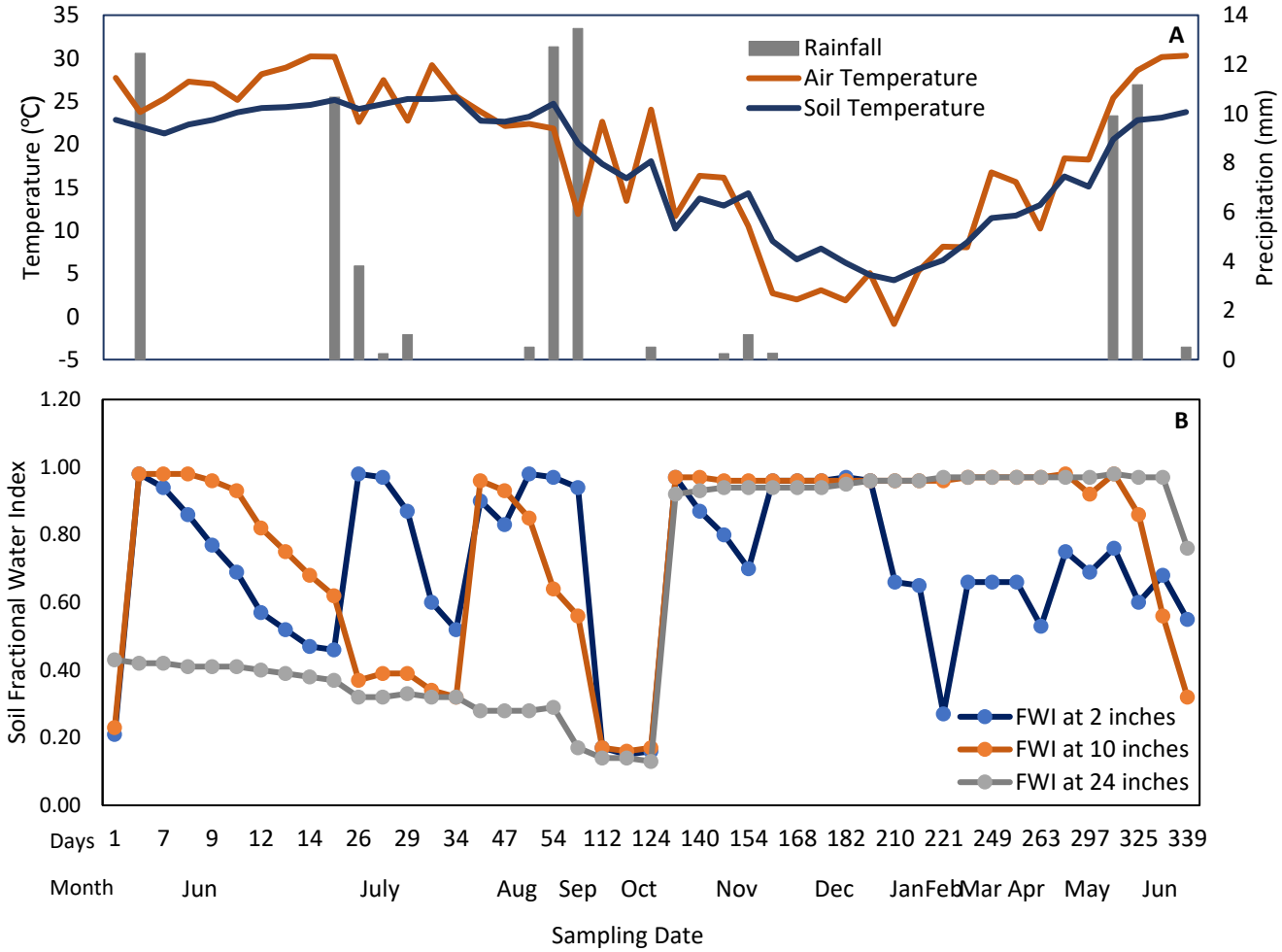

Figure 2. Air temperature, soil temperature, precipitation (A), and fractional water index (B) during observations dates from June 2020 to June 2021 as reported by the Stillwater Mesonet station.

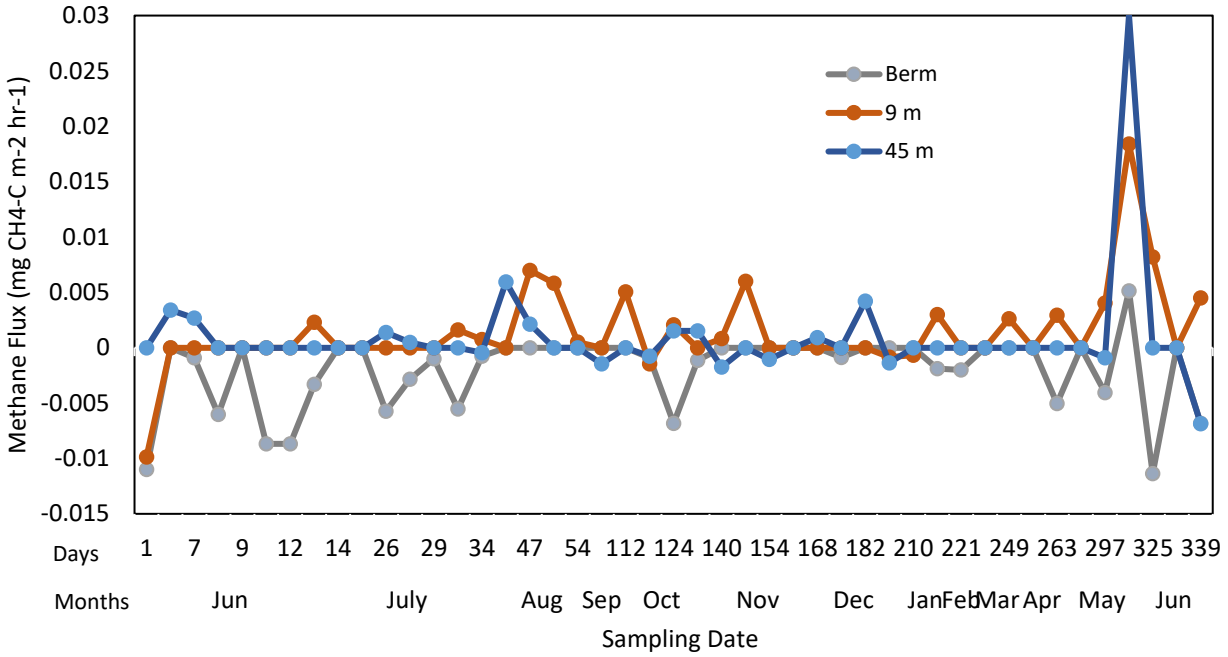

Figure 3. Soil methane flux measured at different distance near a dairy wastewater lagoon from June 2020 to June 2021.

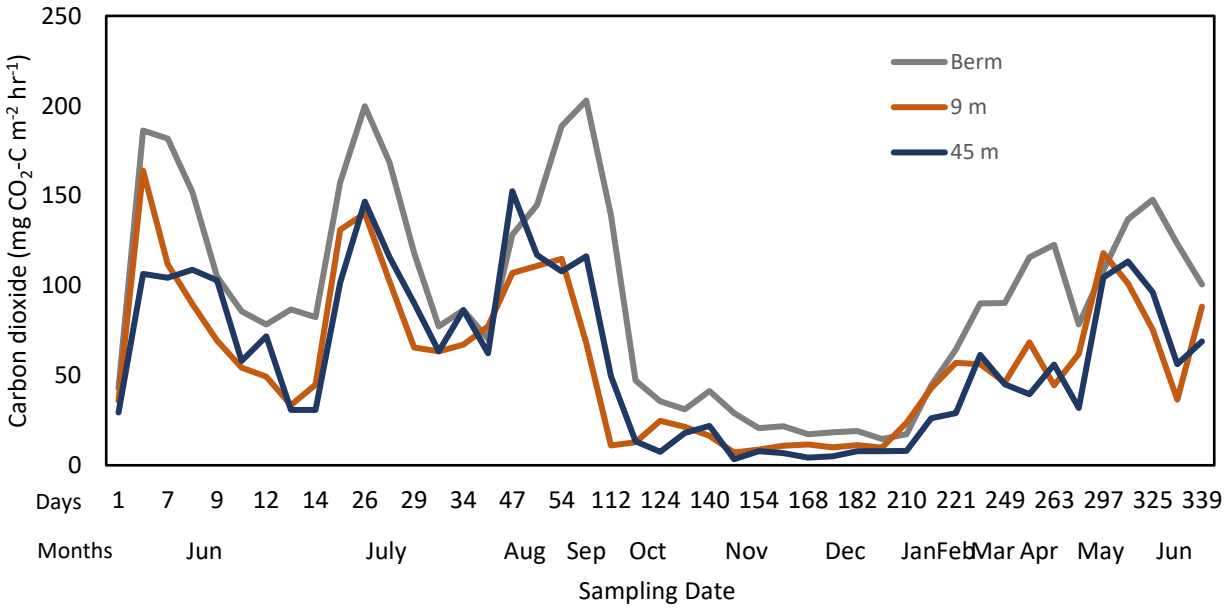

Figure 4. Soil carbon dioxide flux measured at different distance near a dairy wastewater lagoon from June 2020 to June 2021.

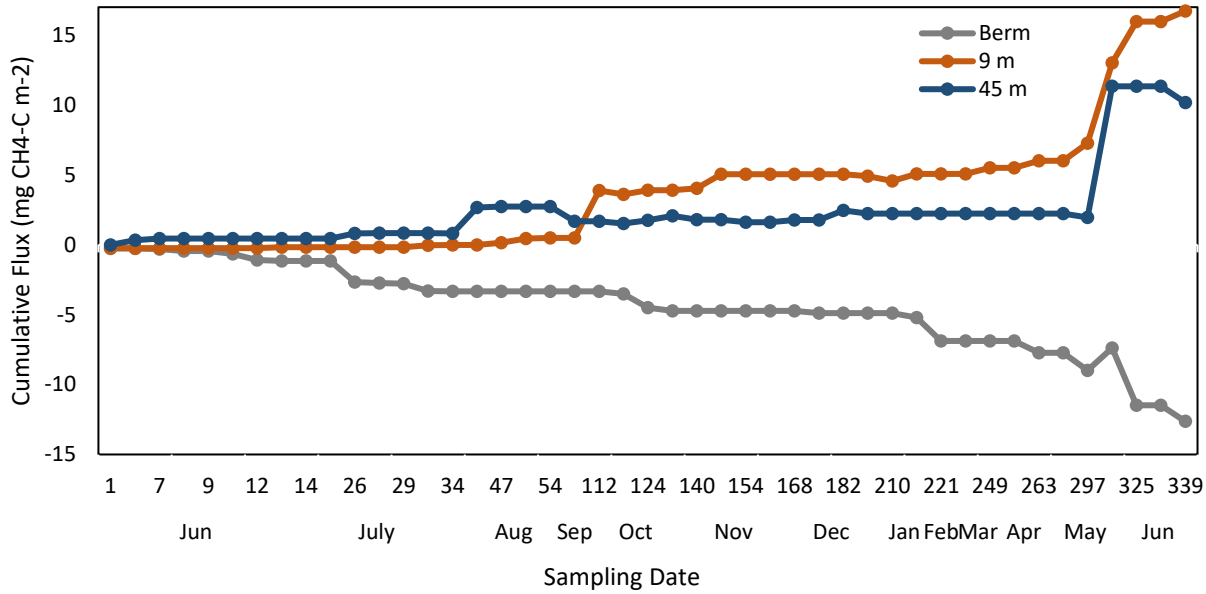

Figure 5. Cumulative CH_4-C flux measured at different distances near a dairy wastewater lagoon from June 2020 to June 2021.

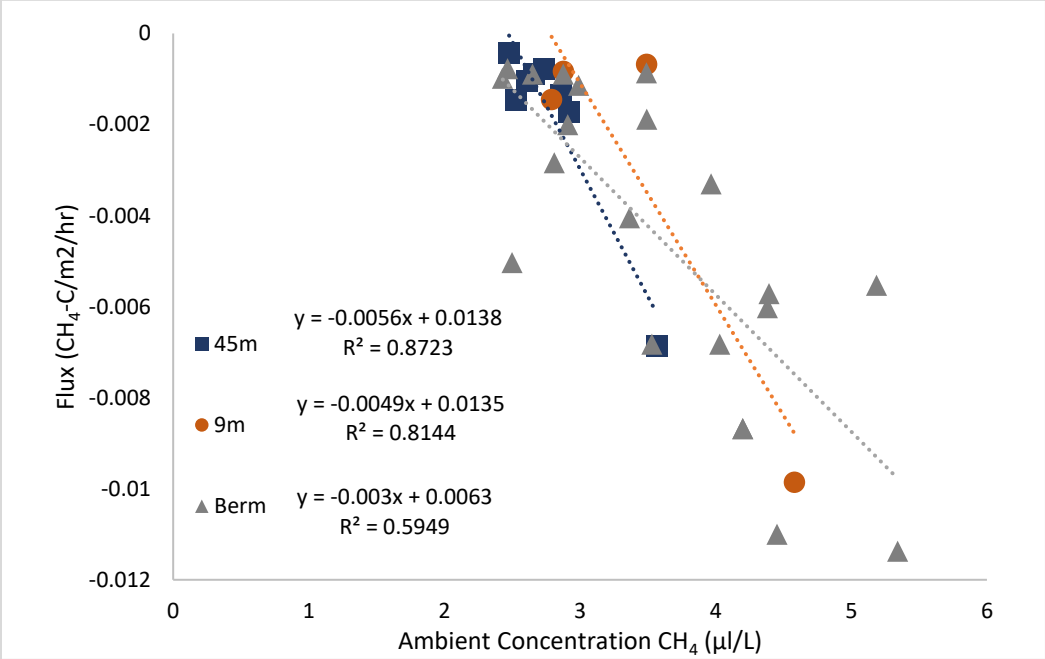

Figure 6: Ambient CH_4 concentrations measured at time 0 after chamber deployment and CH_4-C consumption observed in chambers at three different locations**.**

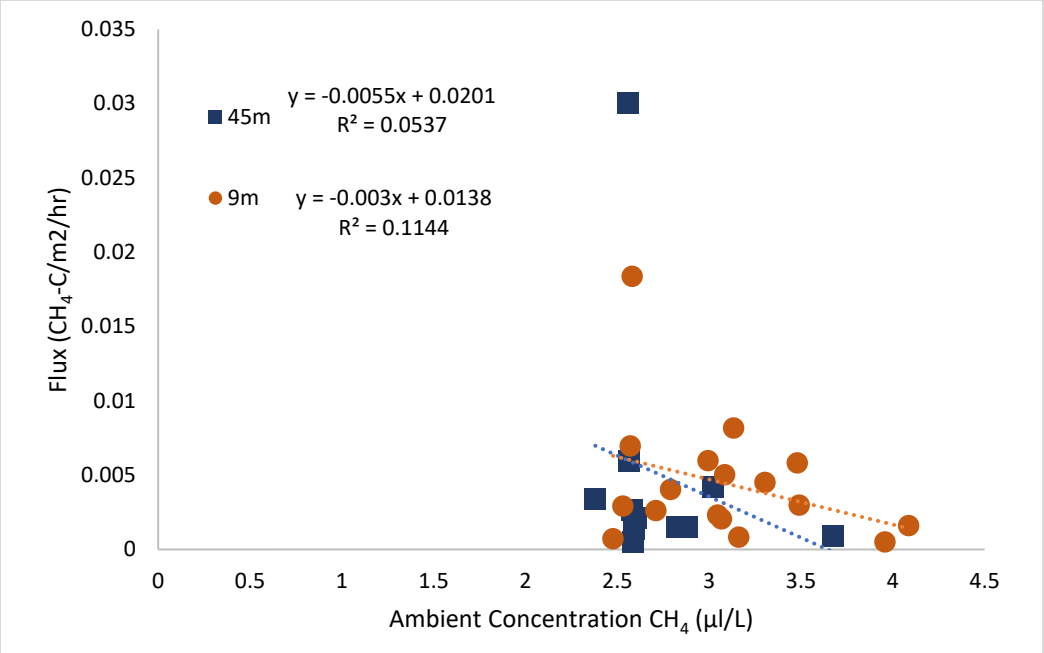

Figure 7. Ambient CH_4 concentrations measured at time 0 after chamber deployment and CH_4-C emissions observed in chambers at different locations. Note: *Data from the berm was not included since there was only one emission observation for all sampling dates.*

CHAPTER IV

PROCESS EVALUATION OF INCUBATION METHOD FOR METHANE OXIDATION USING SOIL UNDER DIFFERENT LAND USE

ABSTRACT

The ability of soil to oxidize CH_4 is typically determined through bacteriological activity of methanotrophs, however, incubation experiments to assess soil CH_4 oxidation capacity are also conducted, and the methods used in these experiments vary because the suitability of using a particular method depends on the objectives of each study. The objective of this study was to evaluate the incubation method that uses headspace analysis of incubation chambers of CH_4 concentrations with a gas chromatograph (GC) using soil from cultivated, no-till, grassland, and forest. Three ml of diluted CH_4 was injected into a sealed 120 ml amber bottle containing 25 g of air-dried and sieved soil and moistened to 25% ($^{g}/_{g}$). Headspace gas samples were periodically drawn for seven days by taking 1.5 ml and injected into a septum-sealed 20 ml nitrogen flushed vial and quantified through gas chromatograph. A decreasing trend was observed in all chambers 24 hours after the start of incubation and continued to decrease until 144 hours when soils from no-till became significantly lower compared to cultivated, grassland, forest, and sand. The minimal generation and consumption of CH_4 during the first days of incubation could possibly be due to very low bacterial populations. The first phase of incubation created conditions that exposed the microbes to high levels of CH_4 and may have

increased the microbes' methanotrophic potential. The headspace concentration in the amber bottles was sensitive to changes of the methane oxidation process occurring in a microcosm. The derived calibration curve generated by known concentrations of standard methane gas can be used as a simple and practical tool for quantifying and estimating CH_4 concentrations during the methane uptake process and methanotrophic potential of the microbial community.

INTRODUCTION

The atmospheric concentration of methane (CH_4), an important greenhouse gas, has gradually increased by 150% since the beginning of the industrial revolution in approximately 1760 (Hartmann, 2013) and a major driver of global climate change. Methane comes from both natural and anthropogenic sources. Wetlands, wild ruminants, and oceans are the principal natural sources of emissions while enteric fermentation from domestic ruminants, animal wastes, rice fields, biomass burning, landfills, and fossil fuels are examples of anthropogenic sources (Wuebbles and Hayhoe, 2002). Bottom-up CH_4 emissions are estimated to be between 542 and 852 Tg CH_4 yr^{-1} (Myhre et al., 2013), with 366 Tg CH_4 yr^{1} resulting from anthropogenic activity (Saunois et al., 2016). This means that human activities are responsible for 60 to 70 percent of emitted CH_4 (Wang et al., 2004). Levels of CH_4 in the atmosphere will continue to rise as food consumption, population growth, and land conversion increase. The CH_4 sources is determined by a variety of factors, including human population, energy demand, crop output, agricultural techniques, land use area, temperature, precipitation, and other unknown natural or anthropogenic impacts that are likely to vary dramatically in the future (Wuebbles and Hayhoe, 2002).

Methane sinks are natural and rely on abiotic and biotic processes (D'Imperio et al., 2017). Abiotic processes include tropospheric destruction and oxidation in different parts of the atmosphere and is the largest estimated CH_4 sink accounting for 80-90 percent of total production (Lelieveld et al., 1998). On the other hand, the presence of methanotrophs in soil is linked to biotic processes that result in CH_4 oxidation. Oxidation

of CH_4 in the soil is considered an important sink (Saggar et al., 2008), removing about 5-15 percent of the total CH_4 from the atmosphere on a yearly basis (Aronson et al., 2013). Microorganisms that oxidize CH_4 at oxic/anoxic interfaces play a critical role in reducing CH_4 emissions from soils. Moreover, emission rates of CH_4 from the soil are affected by soil properties and parameters such as moisture, temperature, availability of nutrients, pH (Ludwig et al., 2001) soil bulk density, diffusivity, structure, pH, and N status (Murguia-Flores et al., 2018).

The potential for soil to produce and consume CH_4 is influenced by land use and ecosystem. It has been estimated that humans have modified more than 50% of the Earth's land surface (Hooke and Martín-Duque, 2012). Land conversion from natural ecosystems to agriculture has historically been the leading cause of greenhouse gas emissions due to the loss of biomass and associated carbon found in both above and below ground biomass. Changes in land and soil management such as the conversion from native vegetation to cropland can change the physical and chemical features of the landscape, which as a result, alters soil microbial activity, negatively affecting CH_4 oxidation (Szafranek-Nakonieczna et al., 2019; Boeckx et al., 1997). However, converted lands contain methanotrophs in its soil and can potentially offset the emission of CH_4. The biotic sink strength, which is the ability of microbes to consume CH_4 and remove CH_4 from the atmosphere, is most vulnerable to alterations caused by human activity (Dunfield et al., 2007). Methane is the only source of carbon (energy) for methanotrophs. Methanotrophs vary in diversity and can be found in lands used for farming, degraded lands, and land undergoing restoration (Zheng et al., 2010). Even following the

perturbation of land, a diverse population of methanotrophs can still be found (Fjellbirkeland et al., 2001). Agricultural soils contain methanotrophs but tend to have lower CH_4 oxidation rates compared to unmanaged soils (Kizilova et al., 2013). Prior research has shown that CH_4 absorption in forest soils generally occurs at a greater rate than cultivated soils (Vanitchung et al., 2014), grassland, and arable land (Boeckx et al., 1997). This could be due to presence of organic matter and less disturbed which provides a more stable environment for microorganisms to thrive and consume CH_4 into the soil. Forest soils also have a higher microbial population of methanotrophs, more oxygen available, and a higher moisture content, which promotes a faster rate of methane oxidation than cultivated and grassland soils. Soils near sites where CH_4 is produced such as landfills and areas with natural gas seepage also contain methanotrophs (Farhan et al., 2018; Wise et al., 1999;). Unsaturated soil near or at CH_4-producing locations has the potential to biologically oxidize CH_4. This is because methanotrophic bacteria may have colonized areas with a plentiful and consistent source of CH_4 (Kallistova et al., 2005). Soils that are enriched with methanotrophs have the potential to mitigate increasing CH_4 emissions due to its ability in the assimilation and respiration and excess of CH_4 from the atmosphere by functioning as CH_4 sink. The presence of methanotrophs in soil is beneficial because they can help reduce anthropogenic greenhouse gas emissions and protect agricultural land and soils from the negative effects of greenhouse gas. Additionally, increasing activity of methanotrophs can be cost-effective, natural way of addressing climate change.

The potential of soil to oxidize CH_4 is usually measured through bacteriological activity of methanotrophs (Szafranek-Nakonieczna et al., 2019; Kravchenko & Sukhacheva, 2017; Cai et al., 2016; Amaral et al., 1998). The methanotrophic activity in the soil may be measured by determining the amount of CH_4 consumed by the soil through incubation experiments instead of the usual bacteriological analysis such as isolating methanotrophs or determining their abundance using DNA extraction, real-time polymerase chain reaction (PCR), sequencing, and phylogenetic analysis. The CH_4 oxidation studies of Yin et al., (2020); Wnuk et al., (2017); Vanitchung et al., (2014); Schroth et al., (2012); and Spokas and Bogner (2011) were conducted using incubation experiments instead of bacterial analysis.

Incubation experiments to assess soil CH_4 oxidation capacity were carried out, but the methods used in different experiments vary because the suitability of using a particular method depends on the objectives of each study. Several studies have used different procedures (Yin et al., 2020; Prajapati and Jacinthe, 2014; Czepiel et al., 1996; Nesbit & Breitenbeck, 1992) because there is no standard procedure for CH_4 oxidation incubation. This gives an opportunity to make improvements on the basic procedure for doing incubation experiments regarding CH_4 oxidation. The purpose of this current study was to evaluate the process of the incubation method that utilizes headspace analysis of incubation chambers of CH_4 concentrations with a gas chromatograph (GC) using soil from various land management systems. The process evaluation of the method can then be used to screen soils for methanotrophic activity to better understand the overall influence of various ecosystems and the influence of soil types on this important CH_4 sink.

METHODOLOGIES

Soil samples

Soil samples used in this study were collected from croplands, grassland, and forest. Croplands included no-till and cultivated systems. Soils were collected from a no-till land management system in Chickasha, Oklahoma, and soils from a cultivated management system in Altus, Oklahoma; both areas were planted with wheat. Natural systems were from an undisturbed grassland where a variety of grass species, including Bermuda grass, had established, as well as patches of forest in Lake Carl Blackwell (LCB). Composite bulk soil samples were collected from the soil surface 0-10 cm deep using a marked shovel to ensure consistent sampling depth. The samples were air-dried and grounded manually before being passed through a 2mm sieve.

Soils properties were analyzed by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory except for the mean weight diameter (MWD). Soil pH was analyzed using 1:1 soil water (deionized) ratio. Nitrogen (N), phosphorus (P), and potassium (K) was determined using the Mehlich III extraction method and quantified using inductively coupled plasma. Organic matter (OM) was determined through combustion method using muffle furnace. Texture analysis was determined through hydrometer method. The wet-sieving method of Yoder (1939), as modified by Kemper and Rosenau (1986), was used to determine MWD using the wet-sieving apparatus developed by Eijkelkamp Agriresearch Equipment (the Netherlands). Results are presented in table 3.

Methane oxidation

In our study, 25 g of air-dried and sieved soil were placed in a 120 ml amber bottle. Each soil sample from different land use was considered as treatment and had three replicates in the experiment, set up in a completely randomized design (Figure 8A). In addition, reagent grade siliceous sand was included in the incubation as a replicated treatment to observe changes in CH_4 concentrations that occur in amber bottles containing sterile media. Moisture content (mass basis) for each sample were set at 25% by adding 6.25 ml deionized water. Soil moisture levels at a range of 20% to 60% have been reported to result in the greatest CH_4 consumption measurements (Whalen and Reeburgh, 1996; Castro et al., 1995). The amber bottles were sealed with butyl rubber septa and aluminum caps to prevent gas leakage and the incubation was done at laboratory temperature (24ºC).

The CH_4 oxidation potential of soil from various land uses was evaluated by changing the headspace gas conditions in the bottles. Headspace is the air space between the soil and the top of the bottle, and this space usually contains some amount of oxygen and other gases, as well as other substances from the surrounding environment. The activity of methanotrophs and CH_4 oxidation reactions have been reported to vary when soil from various sources is exposed to changing CH_4 concentrations (Tate et al., 2012). When the bottles were sealed, 3.0 ml of diluted CH_4 was injected in each bottle using a 3-ml polyethylene syringes to elevate the concentration of CH_4. Modifying the headspace gas conditions in the bottles used for incubation allows the assessment of the potential for CH_4 production and consumption (Chan and Parkin, 2001). Monitoring of CH_4

consumption was conducted by periodically drawing out 1.5 ml of gas from the headspace using a syringe, injected into a septum-sealed 20 ml vial that had been flushed with N_2 gas (Figure 8B). Nitrogen gas flushing was needed to remove the ambient CH_4 inside the vials. Ambient air of 1.5 mL was injected back into the incubation bottle before each sample collection to maintain constant air pressure. Headspace gas samples were taken one hour after incubation began and again every 24 hours later, as well as daily for seven days. After seven days, the rubber septum was removed for an hour to allow oxygen in ambient air to be reintroduced into the amber bottles. The bottles were again sealed, and each was injected with 3.0 ml diluted CH_4. Headspace gas samples were then collected after one hour, 24 hours, and every two days for a week.

The headspace concentration of CH_4 in the vial was analyzed by gas chromatography with a flame ionization detector on a Varian 450 GC (Varian, Serial no. GCD912B060, The Netherlands (Figure 8C)). Vials for gas chromatograph (GC) calibration were prepared by initially evacuating them with N_2 gas followed by injecting standard gases at 5 and 20 ppm concentrations. Each gas sampling analysis in the GC included the two standard gases at 5 ppm and two standard gases at 20 ppm. The GC used for this study also included thermoconductivity and electron capture detectors for carbon dioxide (CO_2) and nitrous oxide (N_2O), respectively. Carbon dioxide and nitrous oxide data were also collected as supplemental information to the CH_4 oxidation process. The CO_2 data was calibrated using standard gases with concentrations of 1000, 15000, and 100000 ppmv (0.1, 1.5, 10 % CO_2), respectively and N_2O data was calibrated using a single point calibration curve utilizing a standard gas with a concentration of 2.5 ppmv. Our study

utilized the derived calibration curve equation in calculating the concentration of CH_4 from the vials. A calibration curve can be used to estimate the methane uptake and methanotrophic potential of the microbial community by establishing a relationship between methane consumption and methanotrophic activity. All calibration curves used in the calculation that were generated by linear regression had an R^2 of 0.99 or better.

Data Analysis

PROC GLM in the SAS statistical software package was used to analyze the data for analysis of variance (ANOVA) (SAS 9.4). The ANOVA was used to compare the effects of land use on CH_4 consumption at various sampling times. Means of the different land uses were compared for the different sampling time using least significant difference test (LSD) at 0.05 alpha level of significance.

RESULTS AND DISCUSSION

Methane dynamics

The methane concentration in the headspace of the bottles were calculated using the derived calibration curve equation generated by analyzing known concentrations of methane and measuring methanotrophic potential. This generates a range of calibration standards that can be compared to the concentration of methane consumed by the microbial community. By measuring methane consumption in the different samples and applying the calibration curve, we were able to estimate the level of methane uptake.

One hour after injecting the diluted CH_4, headspace CH_4 concentration was uniform at 21 ppmv in all chambers and were all observed to decrease after 24 hours, including the vials containing the siliceous sand (Figure 9A). This decrease could be attributed to diffusion of the applied gas into the soil matrix during this period. Following this initial decrease, a very minimal change in CH_4 concentrations occurred until 120 hours after the incubation began. At 144 hours, CH_4 concentration in no-till soil became significantly lower than the other treatments and continued to be the trend when the last sample was collected at 168 hours. This implies that soil microbes such as the methanotrophs may still be acclimating, and therefore growth is relatively slow, necessitating the relatively long incubation time required. Microbes could be present but may not be sufficiently active to consume a detectable level of CH_4. Similar research found a four to five-day lag time before the initial headspace CH_4 concentration decreased in soils collected from a cultivated soil (Chan and Parkin, 2001). Additionally, forest soils and

prairie soils oxidized headspace CH_4 14 days after the start of incubation and soils treated with a high rate of nitrogen fertilizer and swine manure exhibited little activity 20 days after of incubation (Chan and Parkin, 2001).

During the first days of incubation in our study, the generation and consumption of CH_4 could be quite minimal, possibly due to very small bacterial populations. Methane generation process requires a sufficient population of methanogenic microbes in the soil to occur and their population may take some time to increase in the initial days of incubation because of competition with other microorganisms for limited nutrients and other resources. Methane consumption by methanotrophic microbes also requires sufficient population which can take time to establish in the soil. After adding CH_4 to the soil and exposing it to an elevated concentration for a week, microorganisms, especially methanotrophs may have been triggered to become more metabolically active and reproduce faster causing a higher rate of CH_4 consumption by the methanotrophs. Increased microbial activity is expected to initiate methanotrophic consumption of CH_4 causing the concentration of CH_4 to decrease. The study of Yin et al. (2020) found that soils exposed to high concentrations of CH_4 due to proximity of leaking CH_4 gas wells consumed majority of the CH_4 six days later in their microcosm experiments, suggesting high methanotrophic consumption of CH_4 in the said soil.

For five days during the first week of incubation, there were no significant differences in CH_4 concentration across the different soils, indicating that CH_4 generation and consumption may have occurred concurrently. The concurrent generation and

consumption of CH_4 could be attributed to the soil's aggregates, which may contain methanotrophs on the surface but may also contain methanogens inside the aggregates due to the reduced state. Despite not being significantly different, it is noteworthy to observe that during the first 7-day incubation, the CH_4 concentrations in all treatments were similar to the CH_4 concentrations of the sand, except soil from no-till management which started to show a decline after 144 hours (six days). The no-till soil sample had a clay loam texture that is characterized as having a moderately fine texture and has moderate levels of organic matter (2.8%) and nitrogen (Table 3). The moderate levels of organic matter and nitrogen may have contributed to the oxidation of CH_4 six days after incubation. Agricultural soils that are rich in soil minerals tend to support a more diverse microbial community including methane-oxidizing bacteria. The study of Chan and Parkin, (2001) found that soil from agricultural sites had higher CH_4 oxidation rates, which they attributed to higher soil mineral concentrations.

The secondary incubation period showed the CH_4 concentration at one hour was 16.2 µl/L and averaged at 16.7 µl/L in the sand treatment for the remaining sample periods (Figure 9B). The forest soil and the no till soil started to show signs of CH_4 consumption at 241 hours however the decrease in CH_4 concentrations at 241 hours were not significant. Forest soil and no-till soil treatments showed significant decrease in CH_4 concentrations at 288 and 336 hours with concentrations dropping to 7.5 µl/L in the forest and 12.6 µl/L in no-till soils, as compared to 17.4 µl/L in the sand control. The first phase of incubation created conditions that exposed the microbes to elevated levels of CH_4 and may have increased the microbes' methanotrophic potential. Prior research has found

that forest soil absorbs more CH_4 compared to other land-uses (Feng et al., 2020; Lang et al., 2020; and Jiří, 2018). Abiotic features of the soil, such as bulk density, porosity, and moisture, aid in the diffusion of CH_4 and oxygen to microbial populations (Vanitchung et al., 2014). It should be noted that the soils from the forest and the no-till fields have the highest organic matter content. According to Bowden et al. (1998) a significant amount of organic matter when moistened, expands into nearby pore spaces, and absorbs moisture and moisture typically has a can increase CH_4 uptake rates. The increase in CH_4 uptake could be due to the priming effect of moistened organic matter that serves as a substrate for microbial growth and activity of methanotrophs. Also, soil organic matter is essential for the formation of aggregates, which is also important for the retention of water which could be one of the reasons why the forest soil, which had the highest organic matter content, has higher CH_4 consumption rates than other soils.

No-till soils consumed CH_4 second only to forest soil (Figure 9B) and were significantly different from the sand, while the CH_4 consumption by the soils from the grassland and cultivated area were not significantly different from the sand. The no till management of the soil used in this study has been applied for approximately 10 years. The capacity of agricultural soils to oxidize CH_4 had been reported to increase with the duration of no till, with significantly higher CH_4 uptake at long-term no-till fields. This is due to the more favorable soil environment for microorganisms in no-till fields than in cultivated sites (Jacinthe et al., 2013). Moreover, the high availability of organic carbon in no-till soils encourages the growth of soil microbes, including methanotrophs (Prajapati and Jacinthe, 2014). Although the decrease of CH_4 concentration in the bottle headspace

is prominent for forest soil and no-till soils, a slight decrease can also be observed in the grassland and cultivated soil.

Carbon dioxide and Nitrous Oxide

The CO_2 concentrations one hour after commencing incubation were significantly higher in all soil treatments than the headspace CO_2 concentration in the sand treatment which remained at approximately 2000 µl/L (+/- 10%) throughout the incubation period. The concentration of CO_2 increased at 24-hour sampling in all soil treatments and continued to increase throughout the 168 hour incubation period with concentration reaching approximately 146,000; 108,000; 45,000; and 16,000 µl/L in the forest, grassland, no-till, and cultivated soil treatments, respectively.

The N_2O concentration in the sand treatment was initially 1.26 µl/L and averaged 1.32 µl/L throughout the experiment with less than 10% variation. 24 hours after the onset of incubation, the N_2O concentration had increased to 2.6, 4.0, 18.6 and 20.7 µl/L in the cultivated, forest, grassland, and no-till soils, respectively indicating the rapid onset of microbial activity in these soils. While the N_2O concentration in the cultivated soil treatment continued to increase, the gap between the N_2O concentration in the cultivated soil and the rest of the treatment continued to increase until the end of the 168 hour period. In fact, the N_2O concentration in the cultivated soil was only 5.3 µl/L after 168 hours while the no-till, grassland and forested soil treatments has far higher concentrations of 38, 46, and 77 µl/L, respectively. The gap between the N2O concentration in the cultivated soil with other treatments could be attributed to the

relative amount of organic matter. Organic matter in forest, grassland, and no-till soils is relatively higher, which when organic materials decompose, releases N_2O. It must be noted that the detected concentrations for CO_2 and N_2O that were greater than 100,000 and 2.5 µl/L, respectively, were well beyond the calibration range used which increases the uncertainty of the calculated values. However, they are included in these data to provide some perspectives on the degree of microbial activity and/or substrate availability in the soils.

The CO_2 concentrations increased over time during the second incubation period showing a similar trend as was found during the first incubation period (Figure 9B). However, the CO_2 concentrations was much reduced with a maximum of 62,000 µl/L in the forest soil at 336 hours. The reduced CO_2 concentration could be due to the dissipation of CO_2 into the atmosphere leaving the bottle with less CO_2. The increase in the nitrous oxide concentrations was also dramatically reduced in the second incubation period, especially in the forest, grassland and no-till soils.

Since forest soil has the highest amount of organic matter compared to other soils (Table 3), it produced the most CO_2, followed by grassland, no-till, and cultivated soils (Figure 9C) which has the same trend with N_2O (Figure 9E). The availability of organic carbon enhances the consumption of O_2 due to increased respiration by the microbes resulting in the production of CO_2 and enhancing anaerobic conditions that favors denitrification and produce N_2O. Carbon dioxide and N_2O increases exponentially at the initial stage during the first phase of incubation but levelled off after a while. The increase of CO_2 and N_2O of the initial stage during the first phase of incubation could be due to

organic matter broken down by microbial activity releasing CO_2 and N_2O as by products. The levelling off could be due to the slow anaerobic microbial decomposition and/or reduction in substrate availability. Anaerobic decomposition requires food to continue, and decreasing substrate will also reduce the microbial population.

Analysis of CH_4 concentrations shows that there are no changes in the concentration of CH_4 in the headspace for 144 hours, indicating zero or equal CH_4 generation and consumption at the time. When bottles were opened and reaerated for the secondary phase of the incubation, CO_2 and N_2O decreased, oxygen levels were expected to increase, which may have triggered the onset of CH_4 oxidation. The opening of the bottle may have allowed the influx of oxygen into the closed environment and trigger changes in gas composition and lead to the gradual reduction in the concentration of CO_2 and N_2O. After a while, the consumption of CH_4 coincided with the increasing concentration of CO_2 in different land-use which could be due to methanotrophs producing CO_2 when decomposing CH_4 as their primary source of carbon and energy.

CONCLUSION

The headspace concentration in the amber bottles was sensitive to the changes of the methane oxidation process occurring in the microcosm. Methane concentrations in the headspace decreased after more than a week of incubation, indicating that methanotrophic microbes required a considerable amount of time to become sufficiently active to begin consuming CH_4 from its environment. Forest soil showed higher CH_4 uptake compared to other soil from different land use although no till soil also had a consistent decline of CH_4 concentration.

The method of soil CH_4 incubation can be widely employed for evaluation of CH_4 uptake in a laboratory setting. The process for CH_4 oxidation procedure using the calibration curve generated through known concentration of standard methane gas every greenhouse gas analysis run in the GC provided an estimate of the CH_4 uptake and methanotrophic potential of the microbial community under controlled conditions. The quantification of headspace and the derived calibration curve can be used as a simple and practical tool in quantifying and estimating concentrations of CH_4 during the methane oxidation process.

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TABLES AND FIGURES

Table 3. Chemical properties, texture, and land-use of soil used in the incubation experiment.

Land-use	Location	pH	Nitrogen	Phosphorus	Potassium	Organic Matter	Sand	Silt	Clay	Texture Class	Mean Weight Diameter
		(1:1)	----------(lb/A)----------			----------(%)----------					
Forest	Lake Carl Blackwell	7.4	11	51	334	4.3	50	37	13	Loam	1.15
Grassland	Lake Carl Blackwell	5.8	5	194	444	2.1	52	34	14	Sandy Loam	0.67
No-Till	Chickasha	7.5	25	149	805	2.8	25	47	28	Clay Loam	0.75
Cultivated	Altus	7.9	25	38	600	1.2	35	36	29	Clay Loam	0.07

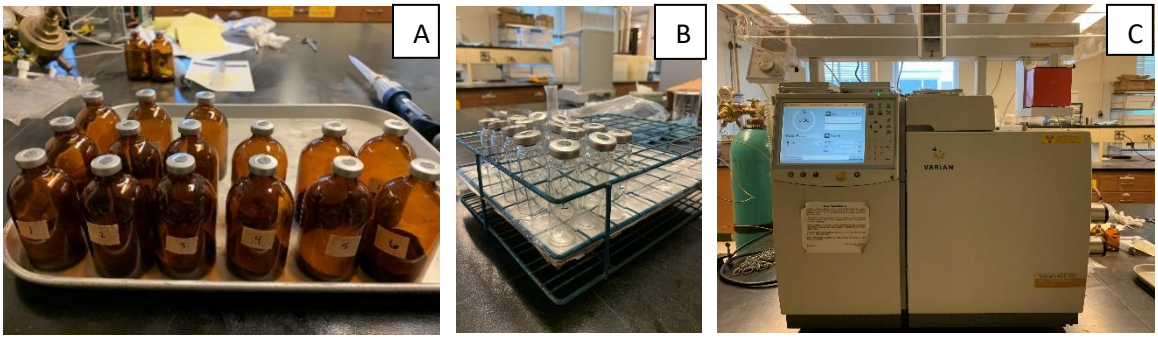

Figure 8. Amber bottles in complete randomized design (A), vials where gas samples are injected (B), and Varian gas chromatograph where gas samples are quantified (C).

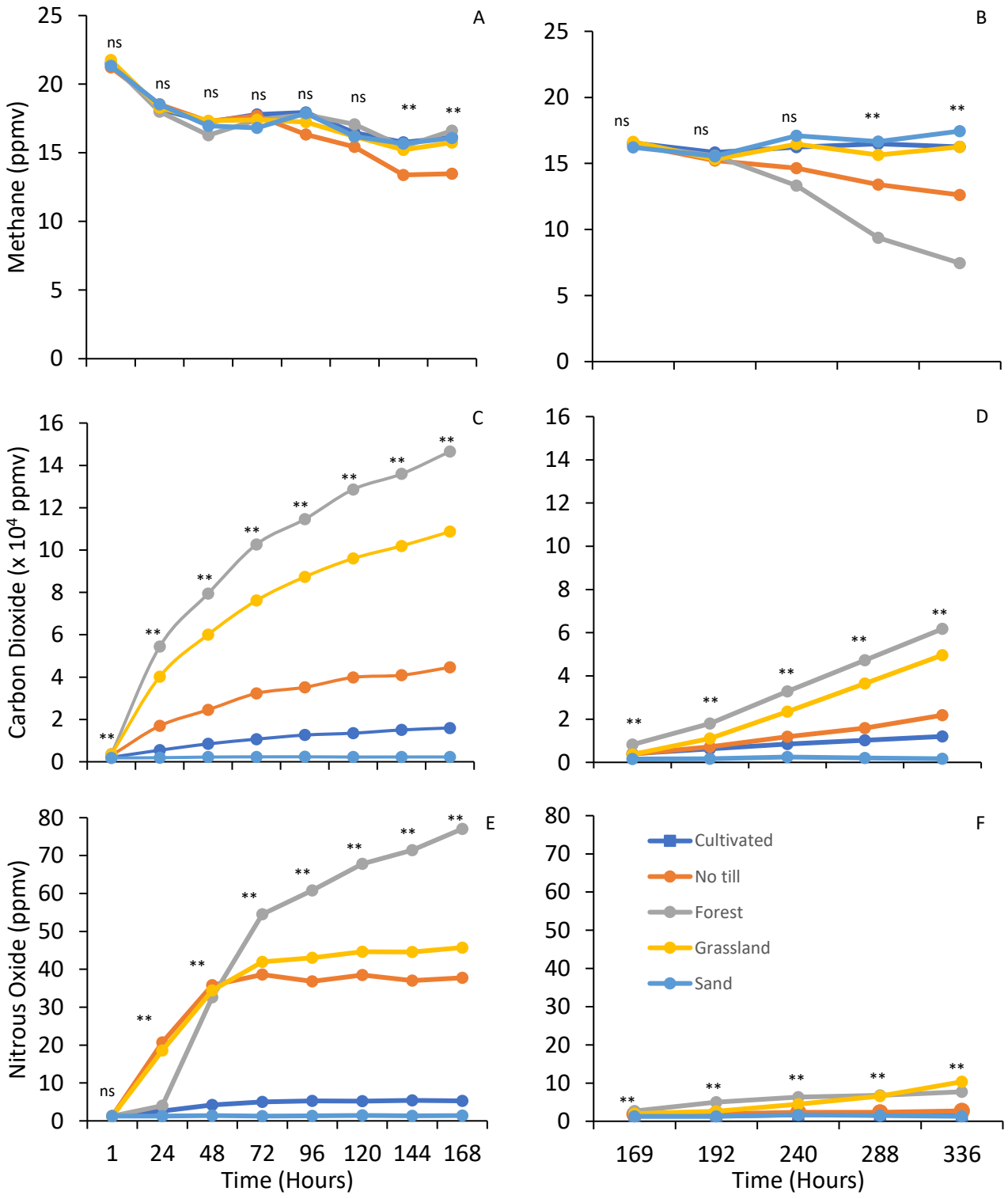

Figure 9. Greenhouse gas concentration over time in the air headspace during first phase of incubation (A, C, E) and secondary incubation (B, D, F).

CHAPTER V

METHANE DYNAMICS IN AGRICULTURAL CROPPING SYSTEMS IN THE CENTRAL GREAT SOUTHERN PLAINS IN UNITED STATES

ABSTRACT

There currently is a data gap about CH_4 fluxes from cropland soils in the Central Great Southern Plains. There are however, numerous available data sets generated by research conducted primarily to assess N_2O emissions which also generated unpublished CH_4 fluxes that were quantified by the gas chromatography. The objectives of this study were to compile and analyze the CH_4 data and evaluate the frequency, and magnitude of CH_4 fluxes. The CH_4 data presented in this paper were collected from field experiments located in five locations across Kansas and Oklahoma. Greenhouse gas samples were collected using a closed vented chamber at 0, 15, 30, and 45 minutes after the closed static gas chamber was covered and CH_4 concentrations were quantified using gas chromatography Varian 450 GC. Seven years of field experiments generated 11,837 individual measurements that were taken in 362 sampling events of which only 21% or 2,082 manifested a significant methane flux. Methane consumption, as indicated by negative fluxes, appears to be a frequent result of the CH_4 dynamics between the atmosphere and soil. Although there were periodic positive fluxes including one extremely large CH_4 emission, these were less frequent than negative fluxes. Sampling

events where more than 60% of the chambers showed significant fluxes were subject to analysis of treatment effects and revealed no significant effects. When flux was averaged across all locations and treatments, the average values was -0.0016 mg CH_4-C m^{-2} hr^{-1}. If this flux rate truly represents the average consumption of CH_4-C in cropland soils of the Central Great Southern Plains, it would represent an annual, removal of 55,950.4 Metric ton CO_2 eq yr^{-1} on the 8.8 million acres of croplands in Kansas alone regardless of cropping systems management.

INTRODUCTION

Greenhouse gases are naturally occurring in the atmosphere and are essential to all living things on the earth. Greenhouse gases are essential because they play a crucial role in regulating the Earth's temperature, which is necessary for sustaining life. Natural greenhouse gases include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and water vapor. While greenhouse gases are important for regulating the Earth's temperature, an excess of these gases can be harmful to the environment through global warming and climate change. Because of anthropogenic activities, the concentrations of these atmospheric gases have been increasing over the last two centuries. One of the important and powerful greenhouse gases is CH_4. On a mass basis, CH_4 is 34 times more potent in causing atmospheric warming than CO_2 (Myhre, 2013). Methane emissions occur within the fossil fuel industry during the extraction of coal or oil, or as leaks during the extraction, storage, or transportation of natural gas. Another source of CH_4 is the waste treatment sector, where microorganisms decompose organic materials under anaerobic conditions and produce CH_4. The bulk of worldwide anthropogenic CH_4 emissions are thought to come from agricultural management operations (IPCC, 2007). According to USEPA, (2021) agriculture is the single greatest source of anthropogenic CH_4 emission, primarily from animal production, agricultural soils, and rice cultivation.

Soils can act as either a source or a sink of CH_4, with unsaturated soil typically removing CH_4 from the atmosphere. However, this is dependent on land and soil management because it can alter the physical and chemical features of natural

ecosystems (Boeckx et al., 1997). Human induced land use change has resulted in the transformation of 12% of the Earth's land surface to cropland (Hooke and Martín-Duque, 2012). Croplands are influenced by different agricultural practices such as tillage, agrochemical inputs, crop rotation, cover cropping, integrated livestock, and integrated pest management. Several studies have found that soil temperature, moisture, and nitrate (NO_3^-) concentration influence CH_4 emission and consumption (Abagandura et al., 2019; Tian et al., 2013, Ludwig et al., 2001), all of which are affected by agricultural practices. Soil pH, organic carbon, and plant biomass are also factors that affect movement of CH_4 (Liu et al., 2021).

Nitrogen (N) fertilizer application is a common practice in agricultural ecosystems, and N can have varying effects on CH_4 fluxes; some studies emphasize that N fertilizer stimulates soil CH_4 emissions (Chang et al., 2021), others say that N fertilizer inhibits soil CH_4 fluxes (Venterea et al., 2005), and still others say that N fertilizer application in soil has no significant effects on soil CH_4 fluxes (Mosier et al., 2005). Furthermore, many studies on N fertilizer application and its effect on emissions typically focus on N_2O rather than CH_4 fluxes. CH_4 fluxes are typically much lower than N_2O emissions and are more difficult to accurately measure compared to N_2O emissions. The studies of Preza-Fontes et al., (2020), Lynn (2019), and Fontes et al., (2017) generated data on greenhouse gas fluxes from different agricultural cropping systems. They established field experiments that included different cropping system treatments to evaluate cover crops and crop rotations. Another study by Gehl et al., (2020) also collected greenhouse gas samples from dryland grain sorghum receiving

different N rates, using closed static chambers that were installed in plots where greenhouse gases were sampled and quantified. A summary of the different field experiments is provided in Table 4.

The focus of all their research was solely on the N_2O emissions and therefore CH_4 data from these efforts have not been analyzed. There currently are no data in the literature on CH_4 fluxes in different crop production systems in the Central Great Southern Plains, therefore, we assessed the CH_4 data from these studies to provide this missing information. Our objectives were (a) to compile and analyze the data from these studies for the significant linear response of CH_4 concentrations in static chambers used for N_2O studies, (b) evaluate the frequency, and magnitude of CH_4 consumption and emission, and (c) assess treatment effects on the CH_4 fluxes. Our overall goal is to provide information to serve as a reference for the magnitude and extent of CH_4 flux at the soil surface in cropping systems of the region and provide information on the rate, quantity, and variability of methane flux from soil surfaces across various cropping systems in the Central Great Southern Plains. By capturing the flux rate, degree, and variability over time, these data can shed light on the sources and sinks of methane in these agricultural areas. This information can be used to develop strategies to reduce emissions and provide insight into the potential impacts of cropping system factors that influence the CH_4 dynamics at the soil surface.

METHODOLOGIES

General Description of Field Experiments Where Soil Methane Samples Were Taken

A total of 11,837 gas samples were collected from six independent experiments across five sites at in 362 sampling events between 2012 and 2019. Different agricultural field experiments were carried out in Ashland Bottoms, Colby, Tribune and Topeka, Kansas and Goodwell, Oklahoma (Figure 10).

A study in Ashland Bottoms, Kansas was conducted to determine the effects of fallow management, N fertilization and their interactions on N_2O emissions on all phases of a 3-year winter wheat-grain sorghum-soybean rotation located on a Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll) (39°11¢ N, 96°35¢ W). This experiment in Ashland Bottoms began in 2007, but the collection of greenhouse gas samples did not start until 2012 and lasted until 2019. The experimental treatment structure was a split-split plot design with 4 replications where the whole plot was a cropping rotation phase (winter wheat-grain sorghum- soybean). These whole plots were first split by cover crop treatment applied between wheat harvest and planting of the grain sorghum. These sub-plots were split again by fixed N rates of 0, 45, 90, 135, and 180 kg N ha^{-1} at planting of the grain sorghum. The CH_4 data presented as Ashland Bottoms 1 were collected in 2012 to 2014 from treatments which all received 90 kg N ha^{-1} at planting of grain sorghum with the following treatments imposed between wheat harvest and sorghum planting: 1) chemical fallow, 2) Daikon radish

cover planted in June and terminated in November, and 3) sorghum-sudan cover crop planted in June terminated in October (4 replicates of 3 treatments). The CH_4 data presented as Ashland Bottoms 2 were collected between 2014 and 2016 from plots receiving 0, 90, and 180 kg N ha^{-1} at planting of the grain sorghum that were subjected to the following treatments between wheat harvest and planting of grain sorghum: 1) chemical fallow, 2) Daikon radish cover planted in June and terminated in November, 3) sorghum-sudan cover crop planted in June terminated in October, and 4) double crop soybean grain production (4 replicates of 12 treatments). Ashland Bottoms 3 presented data collected in 2017 to 2019 from treatments receiving 0, 45, 90, 135, and 180 kg N ha^{-1} at planting of the grain sorghum that were subjected to the following treatments between wheat harvest and planting of grain sorghum: 1) chemical fallow, 2) sorghum-sudan cover crop planted in June terminated in October, and 3) double crop soybean grain production (4 replicates of 15 treatments). A detailed description of the experimental design is reported by Fontes et al., (2017). The nitrous oxide data collected between 2014 and 2016 (Ashland Bottoms 2) is presented by Preza-Fontes et al., (2020).

The study presented here as Ashland Bottoms 4 was located on a Belvue Silt loam (Coarse-silty, mixed, superactive, nonacid, mesic Typic Udifluvent) (39˚08’ N 96˚37’ W) and utilized a randomized complete block design with 4 replicates of 5 treatments. The treatments include: 1) a zero N check treatment, 2) soil test recommendation (2016=185 kg N ha^{-1}, 2017=235 kg N ha^{-1}), 3) split soil test recommendation (1/3 at planting, 2/3 at Vegetative growth stage (V) 7), 4) sensor

based recommendation (56 kg N ha^{-1} at planting and, 22 and 37 kg N ha^{-1} at V8 and V12 in 2016 and 2017, respectively), and 5) Arial Normalized difference vegetation index (NDVI) based recommendation (56 kg N ha^{-1} at planting and, 63 and 112 kg N ha^{-1} at V8 and V12 in 2016 and 2017, respectively). In 2017, an additional location was added near Topeka, Kansas on a Eudora silt loam (Coarse-silty, mixed, superactive, mesic Fluventic Hapludoll) (39˚04' N 95˚46' W) with the same treatment structure where treatment included 1) a zero N check treatment, soil test recommendation (258 kg N ha^{-1}), 2) split soil test recommendation (1/3 at planting, 2/3 at V7), 3) sensor based recommendation (56 kg N ha^{-1} at planting and 38 kg N ha^{-1} at V8, and 4) Arial NDVI based recommendation (56 kg N ha^{-1} at planting and 112 kg N ha^{-1} at V8). A detailed description of the experimental design as well as crop yield response and N_2O emissions can be found in Lynn (2019) for data from Ashland Bottoms 4 and Topeka.

The studies presented as Colby, Goodwell and Tribune were established to evaluate the impact of locally relevant nitrogen management strategies on N_2O emissions and grain yield. The treatment structure contained 4 replicates and generally included the following treatments 1) a zero N check treatment, 2) soil test recommendation to provide 20 kg N Mg^{-1} of expected yield, 3) soil test recommendation to provide 20 kg N Mg^{-1} of expected yield minus 50%, and 4) soil test recommendation to provide 20 kg N Mg^{-1} of expected yield plus 50%. These treatments were all surface applied as 32-0-0 liquid urea ammonium nitrate. In 2019 at Goodwell, the four standard treatments described above were applied as well as a treatment where the soil test recommended N rate was injected with a no-till single

disk injection unit. In 2018 at Goodwell the treatments only included 1) a zero N check treatment, 2) soil test recommendation to provide 20 kg N Mg^{-1} of expected yield, and 3) soil test recommendation to provide 20 kg N Mg^{-1} of expected yield injected with a no-till single disk injection unit. At Colby in 2018 an additional treatment was included where the soil test recommended N rate was treated with the urease inhibitor Agrotain ® (Koch Agronomic Services, Wichita, KS). The Goodwell experiment was located on a Gruver clay loam (Fine, mixed, superactive, mesic Aridic Paleustoll) (36”35’N, 101˚36’W). The Colby experiment was located on a Keith silt loam (Fine-silty, mixed, superactive, mesic Aridic Argiustoll) (39˚23’N, 101˚4’W). The Tribune experiment was located on a Richfield Clay Loam (Fine, smectitic, mesic Aridic Argiustoll) (38˚28’N, 101˚47’W). Precipitation and fractional water index data were also collected from Mesonet station close to Goodwell as supplemental information because it was only in Goodwell that a large emission event was observed.

Soil Methane Sampling and Quantification

At all locations, a close vented chamber with a designed based on the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Greenhouse Gas Reduction through Agricultural Carbon Enhancement network (GRACEnet) Project Protocols was used to collect gas samples that were taken at 0-, 15-, 30-, and 45-minute intervals after the chamber was covered. Chambers had a surface area of 0.28 m^{-2} and a volume of 35.7 L. Twenty (20) ml vials were prepared and sealed with thick butyl rubber stopper and aluminum caps. Thirty (30) ml of air

was extracted using a syringe to create a partial vacuum. A syringe was also used to extract 20 ml of air from the rubber septum of the chamber lid and inject it into the appropriate pre-vacuumed vials. Samples were immediately transported to the Noble Research Center at Oklahoma State University for greenhouse gas quantification using a gas chromatograph (Varian 450-GC, Serial no. GCD912B060, The Netherlands) containing thermal conductivity detector for CO_2, flame ionization detector for CH_4 and electron capture detector for N_2O.

All data generated from all experimental fields from 2012 until 2019 were compiled and the gas fluxes were calculated using the linear relationship between CH_4 concentration in the chamber headspace versus the 45-minute deployment time as outlined in the GRACEnet protocol (Parkin & Venterea, 2010). This change in volumetric concentration was translated to a mass flow (mg CH_4 m^{-2} hr^{-1}). A significant flux was identified as an individual measurement with an R^2 of the linear regression equal to or greater than 0.8. A positive flux value indicates that there was an emission of gas from the soil to the atmosphere and a negative flux value implies a removal of gas from the atmosphere into the soil. A critical t-test analysis as described by Venterea et al., (2015) was used to determine if the slope from the linear regression between time and headspace concentrations in the chambers was significantly different from zero and alpha value was set at 0.1.

Treatment effects Analysis

All the field experiments were designed to measure N_2O fluxes in response to treatments. However, because the majority of the CH_4 fluxes were insignificant, only the sampling events where more than 60% of the chambers showed significant fluxes were statistically analyzed to determine treatment effects. We used 60% as the threshold to identify sample events where the majority of the chambers had significant flux in order to concentrate our efforts on events with a higher likelihood of having significant treatment effects. It was assumed that in those events where less than 60% of the chambers showed a significant flux that inclusion of the zero values would force treatment means near zero and thus limit the likelihood of significant treatment effects. If 60% of the chambers produced significant flux during a sample event, this would mean that 40% of the chambers produced no significant flux which was treated as zero flux. These zero fluxes were included in calculating the treatment mean.

Prior to analysis for treatment effects the data were initially checked for normality using PROC Univariate in SAS statistical software (SAS 9.4, 2016). When data was found to be normally distributed PROC GLM was used to analyze the for analysis of variance (ANOVA) (Tribune, Ashland Bottoms 1), and PROC NPAR1WAY for Kruskall-Wallis test was used for none normal data (Goodwell).

RESULTS AND DISCUSSION

General Dynamics, Frequency and Magnitude of Methane Fluxes in Various Agricultural Cropping Systems

In the 7-year duration of different experiments conducted, approximately 11,837 individual measurements were collected and analyzed for significant CH_4 flux in 362 sampling events. All non-significant flux measurements were set at zero and fluxes that were significantly different from zero were compiled and evaluated. There were 2,453 measurements or only 21% of all measurements that manifested a significant flux of CH_4 into or out of the soil. These measurements were collected during 348 sampling events or 96% of the sampling events where at least one chamber provided a significant flux.

The number of chambers generating significant fluxes was less than half of the number chambers deployed at all locations except Tribune (Table 5). This shows that the occurrence of significant fluxes is not common. Particularly in the sorghum/wheat/cover crop rotation study presented as Ashland Bottoms 1, 2, and 3, which had an average of 26%, 16%, and 14% of chambers having significant fluxes, respectively. The percentage of chambers measuring a significant flux was also generally low at Colby, Topeka, and Goodwell with 20%, 20%, and 25% of the chambers showing significant fluxes, respectively. Ashland Bottoms 4 was intermediate with 35% of the chambers showing a significant flux. Tribune was the most active site but still only had 51% of the chambers showing a significant flux. A negative flux indicates that

methane is being consumed by the soil and positive flux means that methane is produced from the soil. Across all locations, 85% of the fluxes were negative. Our result was consistent with the observation of Bosco, et al., (2019), where daily CH_4 fluxes were negative in approximately 80% to 90% of their sampling days. De Bernardi et al., (2022) also had an average CH_4 flux that were negative throughout their study period. Kim et al., (2021) found that the majority of the daily fluxes were negative indicating CH_4 consumption into the soil.

In our current study the highest rate of CH_4 consumption was detected at the Tribune site where the average flux was -0.0065 mg m^{-2} hr^{-1}. The smallest average negative flux of -0.0008 mg $m^{-2}hr^{-1}$ was observed in Topeka. Although the majority of significant fluxes were negative at Goodwell (88%), the average flux value was positive. The positive value for the average flux observed at Goodwell was the result of a large CH_4 emission of 0.24 mg $m^{-2}hr^{-1}$ that occurred during a single sampling event on June 14, 2018. All the other observation events at Goodwell showed small fluxes that without this large event would have provided a negative averaged for this location.

Only 16 of the 348 sampling events resulted in more than 60% of the chambers measuring significant flux (Table 5). Tribune had nine (9) sampling events in which more than 60% of its chambers produced significant fluxes whereas Goodwell had 2, Ashland Bottoms 1 had 2, and Ashland Bottoms 4 had 3 events. There were no events at Topeka, Ashland Bottoms 2, and 3 that had 60% or more chambers with significant fluxes. Sample events in Tribune, Goodwell, Ashland Bottoms 1, and Ashland Bottoms 4 were selected to screen the data for treatment effects because it was assumed that

when less than 60% of the chambers generated zero flux the likelihood of observing a treatment effect was low because the means would approach zero.

Methane Dynamics Across Location and Time

Fluxes that were found to be significantly different than zero occurred at all locations and throughout the 7 years of monitoring. In general, the fluxes ranged from 0.005 to -0.015 mg CH_4-C m^{-2} hr^{-1} except for the previously mentioned event at Goodwell where 0.24 mg CH_4-C m^{-2} hr^{-1} was observed (Figure 11).

The emission on June 14, 2018 exceeded the normal flux by two orders of magnitude in comparison to positive fluxes that occurred during any other sampling event. For example, four days later, a sampling event yielded a positive flux of 0.0016 mg CH_4-C m^{-2} hr^{-1}, while all other fluxes from Goodwell were negative. The closest Mesonet station indicated that there was 20 mm of rain on June 12, 2018, in Goodwell. This sizeable rain event resulted in the relatively high degree of saturation of the soil surface as indicated by the average 2-inch fractional water index of (0.9) and (0.7) a day before and during the sampling date, respectively. The monitored location in Goodwell was under fallow rotations which could allow moisture to accumulate in the soil profile and following a sizeable rain event, may have created a low oxidation-reduction potential necessary for CH_4 production in the soil or in anaerobic microsites inside soil aggregates. Low redox potential in soil microsites can lead to the production of CH_4. The generation of CH_4 following a rain event is highly possible. In fact, Burgin et al., (2011) reported reactions in upland soils frequently varies over much smaller

spatial scales and fluctuates on short temporal scales of only hours to days. Soil aggregates may have anoxic microsites within aggregates which may limit the diffusion of gases hence delaying CH_4 emissions into the surface possibly suggesting that the CH_4 emission on June 14, 2018 could have been generated by microbial activity days earlier. The presence of large amounts of water in soil pore spaces is key to methane generation. Soil macroaggregates of 2-6 mm have been shown to produced methane with 40% water-filled pore space (WFPS) while soil microaggregates both produced and consumed CH_4 at higher water contents (Sey et al., 2008). In addition, Brewer et al., (2018) found that methanogenesis does not require high soil moisture because it was detected across a wide range of WFPS (10% - 95%) in their study, demonstrating that prevalence of methanogenesis in dry areas is not an exception and occurs throughout a range of soil moisture levels. It should be noted that the Goodwell site received some N enrichment. In croplands that are subjected to N enrichment, methanotrophic bacteria have the potential to oxidize less CH_4, which can result in more CH_4 being emitted into the atmosphere (Yue et al., 2019). Data available at the Goodwell location does not allow for a mechanistic understanding of why this flux occurred but does demonstrate that infrequent, relatively large, emission events can offset the more consistent consumption of methane in cropland soils.

Although observed at extremely low rates, CH_4 fluxes at the Ashland Bottoms, Topeka, Tribune, and Colby locations were mostly negative indicating influx or consumption of the gas into the soil, in all locations (Figure 11). The CH_4 flux during the sample events from each location ranged from -0.014 to 0.001 mg CH_4-C m^{-2} hr^{-1} in

Ashland Bottoms 1, from -0.0054 to 0.0006 mg CH_4-C m^{-2} hr^{-1} Ashland Bottoms 2, from -0.0032 to 0.0022 mg CH_4-C m^{-2} hr^{-1} in Ashland Bottoms 3, from -0.0097 to 0.0046 mg CH_4-C m^{-2} hr^{-1} in Ashland Bottoms 4, from -0.0041 to 0.001 mg CH_4-C m^{-2} hr^{-1} in Colby, from -0.012 to 0.24 mg CH_4-C m^{-2} hr^{-1} in Goodwell, from -0.005 to 0.001 mg CH_4-C m^{-2} hr^{-1} in Topeka, and from -0.014 to 0.000 mg CH_4-C m^{-2} hr^{-1} in Tribune. Except for the high emission value from Goodwell, the temporal fluxes of CH_4 ranged from -0.0142 to 0.0046 mg CH_4-C m^{-2} hr^{-1}. While some studies have established seasonal trends in CH_4 fluxes (Chang et al., 2021; and Yue et al., 2016) emphasizing low flux in the winter and higher flux in the summer, the data in this study were unable to confirm that trend, because of the uneven distribution of sample events throughout the year because most of the sample events occurred during the summer growing season to capture the N_2O emissions prevalent during the growing season after fertilization.

The influx of CH_4 into the soil denotes the potential for soil to be a sink for CH_4. Studies by Oliveira et al., (2021); Sainju et al., (2021); Musafiri et al., (2020); Wang et al., (2015); Tian et al., (2013); and Ussiri et al., (2009), generally found croplands of different cropping systems, soil fertility management, and tillage practices to be net sinks for CH_4 since their average CH_4 fluxes were also negative. Chan and Parkin (2001) emphasized that agricultural soil acts as a small sink. Methanotrophs that may be present in the soil can absorb CH_4 that has diffused from the atmosphere, resulting in CH_4 consumption and a negative flux.

Many factors can affect the occurrence of negative fluxes of CH_4 which includes diffusion of gases into the soil and methanotrophic activity (von Fischer et al., 2009).

The amount of methane that goes into the soil is largely controlled by diffusion of atmospheric methane into the soil which, is strongly influenced by other soil factors such as soil moisture (Wang et al., 2014).

Effects of Nitrogen Fertilization on the Dynamics of Methane

As mentioned, the majority of observations did not result in a significant flux (79%), which makes experimental treatment comparisons difficult. However, efforts were made to conduct ANOVA and Kruskall-Wallis test on data from those sampling events where more than 60% of the chambers showed significant fluxes. Analysis showed no significant treatment effects in the 16 sample events analyzed for treatment effects. Despite a lack of significant differences, data from Tribune (Table 3) and Goodwell (Table 4) are presented because they provided unique observations. Tribune was unique because it presented significant fluxes most frequently (Table 5). Goodwell was also unique because it generated a large emission on June 14, 2018.

The field experiment in Tribune tested the impact of different rates of N fertilizers applied. The experiment in Tribune had nine sample events where there was greater than 60% of chambers showing flux significantly different than zero. The mode of fertilizer application was tested on the experimental field in Goodwell in 2018, and the results only produced 2 sample events with more than 60% of chambers showing significant flux values. The field experiment in Ashland Bottoms 1 also produced 2 sample events with more than 60% of chambers showing significant flux values and treatments effects were not significantly different (Table 5).

The lack of significant impact of N rate (Tribune) and application method (Goodwell) is consistent with studies of Kim et al., (2021); Jin et al., (2019); Álvaro-Fuentes et al., (2016); Wang et al., (2014); Alluvione, et al., (2009); and Amos (2005) who found that soil CH_4 flux is not affected by N fertilizer application.

In contrast, Hutsch (2001) emphasized that a short-term effect of ammonium (NH_4^+) or urea fertilization is an important factor in inhibiting CH_4 oxidation because the methanotrophic enzyme system is blocked, and the long-term effect of repeated NH_4^+ fertilization can change the composition of the soil microbial community. Most methanotrophs can co-oxidize NH_4^+ and CH_4 because they have comparable structures and sizes (Sun et al., 2016). When the ratio of NH_4^+ to CH_4 is high enough, methanotrophs prefer to oxidize NH_4^+ rather than CH_4, which reduces their consumption of CH_4 (Yang et al., as cited by Sun et al., 2016). Ammonium is a more energetically advantageous substrate. Additionally, these researchers stated that compared to the process of breaking down CH_4 molecules, which required more energy, methanotrophs can use the N in NH_4^+ more effectively and with less energy. This suggests that the ammonium forms of N are those predominately responsible for disrupting methanotrophic activity which may explain why our results did not show a significant impact on CH_4 fluxes. At Tribune, urea ammonium nitrate (32-0-0) was applied as the N source. The urea in this fertilizer form generally hydrolyzes to NH_4^+ rapidly followed by nitrification to NO_3^-. Kirschke et al., (2019) showed that this transformation to NO_3^- can be completed in 30 days after urea applications. This may explain the lack of significant differences resulting from N applications. At Tribune in

2019 the N fertilizer treatments were applied on June 10 and at Goodwell in 2018 the urea ammonium nitrate fertilizer was applied on June 2. Only the first observation dates presented in Tables 2 and 3 were collected within a month after these treatment applications. Therefore, although NH_4^+ may possibly influence CH_4 dynamic, the resolution of this field-based data may have been insufficient to measure significant effects.

Aside from the select studies that show effects of N fertilization on CH_4 fluxes, other agriculture land management practices have little to no impact on these fluxes. For instance, tillage (Krauss et al., 2017), tillage and cropping system (Bayer et al., 2012), tillage and cover crop species (Behnke & Villamil, 2019), crop rotation and grazing (Abagandura et al., 2019) have been shown to not affect CH_4 fluxes. The data presented as Ashland Bottoms 1, 2 and 3 representing fluxes from a wheat-sorghum-soybean rotation with and without cover crops support these findings as no significant differences were observed in these experiments.

CONCLUSIONS

This study utilized a large dataset comprised of 11,837 individual measurements taken in 362 sampling events. At least one significant flux was measured during 96% of these sampling events with the remainder having no evidence of CH_4 transfer between the atmosphere and the soil. However, only 16 of the sampling events resulted in 60% or more of the chambers observing a significant flux. Therefore, the analysis of treatment effects was limited to these events because in the remaining events the treatment means were so near zero the likelihood of a significant treatment effect is low. Among those sample events subjected to analysis of variance, no significant differences were found. This suggests that N rate, N fertilizer placement, and cover crop utilization do not impact CH_4 fluxes from cropping systems in the Central Great Southern Plains region.

Across all locations only 26% of chambers showed a significant flux due to the small rate of CH_4 transfer between the soil and atmosphere. Even when a large average flux of 0.24 mg m^{-2} hr^{-1} was measured at Goodwell only 63 % of the chambers resulted in a significant flux based on the linear regression analysis. This combined with the fact that across all sampling events at Goodwell only 25% of them resulted in a significant flux measurement. This is similar to results from the remaining experiments except for Tribune where 51% of the chambers measured a significant flux.

When averaged across all locations and events the average CH_4 flux was -0.0016 mg m^{-2} hr^{-1}. This average includes 79% of observations where a significant flux was not observed and therefore set to zero. If this flux rate is used to calculate an average annual consumption of methane it equates to -0.187 kg CH_4-C ha^{-1} $year^{-1}$ or -0.0064 Mg CO_2 eq

ha^{-1} yr^{-1} (using 34 as CO_2 equivalent). This demonstrates that the magnitude of the sink that soil may serve in influencing atmospheric CH_4 is generally small on a hectare basis, however, if this consumption rate is multiplied by the 8.8 million hectares of cropland in Kansas, we find that annual consumption may approach 55,950 Mg CO_2 eq yr^{-1}.

This data set serves as a valuable preliminary assessment of the magnitude of CH_4 transfer between cropland soils and the atmosphere. However, the experimental designs were not developed for this purpose, therefore, there are weaknesses in the data that limit its value in determining treatment effects and season influences on CH_4 dynamics. Due to the small fluxes future research should utilize chambers with much smaller volume/surface area ratio, which would increase the sensitivity to gas emissions to the headspace of the chamber. Monitoring the oxidation state of the soil profile would also improve the interpretation of CH_4.

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TABLES AND FIGURES

Table 4. Summary of the different agriculture field experiments established across Kansas and Oklahoma.

Data identification	Collection Years	Soil Series	Crop planted	Treatments	Experimental Design	No. of Experimental Units	References
Ashland Bottoms 1	2012-2014	Wymore silty clay loam	Sorghum	2012-2013 • Sorghum-Sudan (June-October) • Chemical Fallow • Tillage radish (June-November) on subplots receiving 90 kg N ha^{-1} in grain sorghum phase	Split plot randomized complete block design	12	Fontes et al., (2017)
Ashland Bottoms 2	2014-2016		Wheat	2014-2016 • Double crop soybeans (June-October) • Sorghum-Sudan (June-October) • Chemical Fallow • Tillage radish (June-November) in subplots receiving 0, 90, and 180 Kg N ha^{-1} during grain sorghum phase	Split plot randomized complete block design	48	Preza-Fontes et al., (2020)
Ashland Bottoms 3	2014-2016		Cover Crops/ Fallow	2012-2013 • Sorghum-Sudan (June-October) • Chemical Fallow • Tillage radish (June-November) on subplots receiving 90 kg N ha^{-1} in grain sorghum phase 2014-2016 • Double crop soybeans (June-October) • Sorghum-Sudan (June-October) • Chemical Fallow • Tillage radish (June-November) in subplots receiving 0, 90, and 180 Kg N ha^{-1} during grain sorghum phase	Split plot randomized complete block design	24 48	Fontes et al., (2017) Preza-Fontes et al., (2020)

				2017-2018 • Double crop soybeans (June-October) • Sorghum-Sudan (June-October) • Chemical Fallow • Tillage radish (June-November) in subplots receiving 0, 50, 90, 135, and 180 Kg N ha^{-1} during grain sorghum phase		60	
Ashland Bottoms 4	2016-2017	Belvue silt loam	Corn	• Zero N check treatment • Soil test recommendation (2016=185 kg N ha^{-1}, 2017=235 kg N ha-1) • Split soil test recommendation (1/3 at planting, 2/3 at V7) • Sensor based recommendation (56 kg N ha^{-1} at planting and 22 and 37 kg N ha^{-1} at V8-V12 in 2016 and 2017, respectively) • Arial NDVI based recommendation (56 kg N ha^{-1} at planting and 63 and 112 kg N ha^{-1} at V8-V12 in 2016 and 2017, respectively)	Randomized complete block design	20	Lynn, 2019
Colby	2018-2019	Keith silt loam	Grain Sorghum	• Zero N check treatment • Soil test recommendation, • Soil test recommendations plus 50%, • Soil test recommendations minus 50%	Randomized complete block design	16	Gehl et al., (2020).
Goodwell	2018-2019	Gruver clay loam	Grain Sorghum	2018 • Zero N check treatment • Soil test recommendation surface applied • Soil test recommendations injected	Randomized complete block design	12	

				2019 • Zero N check treatment • Soil test recommendation surface applied • Soil test recommendations minus 50% • Soil test recommendations plus 50% • Soil test recommendations injected.		15	
Topeka	2017	Eudora silt loam	Corn	• Zero N check treatment • Soil test recommendation to supply 1.12 (258 kg N ha^{-1}) • Split soil test recommendation (1/3 at planting, 2/3 at V7) • Sensor based recommendation (56 kg N ha^{-1} at planting and 38 kg N ha^{-1} at V8) • Arial NDVI based recommendation (56 kg N ha^{-1} at planting and 112 kg N ha^{-1} at V8).	Randomized complete block design	20	Lynn, 2019
Tribune	2019	Richfield clay loam	Grain Sorghum	• Zero N check treatment • Soil test recommendation • Soil test recommendation plus 50% • Soil test recommendations minus 50%	Randomized complete block design	12	

Table 5. Summary of information in each experimental field on the number of chambers installed, number of significant fluxes, and average CH_4 fluxes.

Location/ Experimental Field	Number of chambers installed	Average No. of Chambers with Significant Fluxes	No. of sampling events where greater than 60% of the chambers had significant fluxes	Number of Significant Fluxes		Average CH_4 flux (mg CH_4-C m^{-2} hr^{-1})
				Negative	Positive	
Ashland Bottoms 1	24	6	2	226	31	-0.0020
Ashland Bottoms 2	48	8	0	579	135	-0.0012
Ashland Bottoms 3	60	8	0	240	76	-0.0005
Ashland Bottoms 4	40	14	3	333	41	-0.0023
Colby	20	4	0	133	28	-0.001
Goodwell	24	6	2	296	39	0.0024
Topeka	24	5	0	69	16	-0.0008
Tribune	16	8	9	206	4	-0.0065

Table 6. Treatment means of methane fluxes (mg CH_4-C m^{-2} hr^{-1}) observed in 2019 on different sampling event when greater than 60% of the chambers installed in Tribune generated significant flux. P-value presented in this table were from the ANOVA. (N rates presented here are the zero N check and N rates resulting from soil test recommendations minus 50%, soil test recommendations, and soil test recommendations plus 50%)

	Sampling events (dates)								
Treatments	June 13	July 17	Aug 7	Aug 21	Aug 28	Sept 4	Sept 18	Sept 25	Oct 16
Kg N ha^{-1}	mg CH_4-C m^{-2} hr^{-1}								
0	-0.0157	-0.0047	-0.0136	-0.0125	-0.0092	-0.0142	-0.0163	-0.0120	-0.0073
72	-0.0113	-0.0040	-0.0105	-0.0089	-0.0131	-0.0120	-0.0101	-0.0105	-0.0108
144	-0.0064	-0.0122	-0.0156	-0.0081	-0.0174	-0.0160	-0.0105	-0.0071	-0.0111
217	-0.0012	-0.0051	-0.0059	-0.0115	-0.0049	-0.0146	-0.0141	-0.0096	-0.0089
p-values	0.5493	0.6634	0.1699	0.8085	0.1969	0.7600	0.3817	0.7774	0.8749

Table 7. Treatment means of methane fluxes (mg CH_4-C m^{-2} hr^{-1}) observed in 2018 on different sampling event when greater than 60% of the chambers installed in Goodwell generated significant flux (Fertilized treatments received 56 kg N ha^{-1}). P-value presented in this table were from the non-parametric Kruskall-Wallis test.

Treatments	Sampling events (dates)	
	June 14	July 10
	------ mg CH_4-C m^{-2} hr^{-1}-----	
No fertilizer	0.2149	-0.00355
Surface applied	0.1447	-0.00471
Injected	0.3633	-0.00320
p-value	0.1911	0.9260

Table 8. Treatment means of methane fluxes (mg CH_4-C m^{-2} hr^{-1}) observed on different sampling events when greater than 60% of the chambers installed in Ashland Bottoms 1 generated significant flux (Fertilized treatments received 90 kg N ha^{-1}).

Treatments	Sampling events (dates)	
	Dec. 13, 2012	Feb 6, 2013
	------ mg CH_4-C m^{-2} hr^{-1}-----	
Chemical fallow	-0.0075	-0.0157
Daikon radish cover crop	-0.0065	-0.0122
Sorghum Sudan Cover crop	-0.0095	-0.0155
p-value	0.7718	0.8731

Table 9. Treatment means of methane fluxes (mg CH_4-C m^{-2} hr^{-1}) observed in 2017 on different sampling events when greater than 60% of the chambers installed in Ashland Bottoms 4 generated significant flux (Fertilized treatments received 56 kg N ha^{-1}).

Treatments	Sampling events (dates)		
	May 15	June 19	June 26
	------ mg CH_4-C m^{-2} hr^{-1}-----		
Zero N check	0.0092	-0.0199	-0.0057
Soil test recommendation	0.0001	-0.0086	-0.0094
Split soil test recommendation	0.0052	-0.0062	-0.0076
Sensor based recommendation	0.0027	-0.0062	-0.0111
Arial NDVI based recommendation	0.0046	-0.0126	-0.0082
p-value	0.4096	0.1711	0.2631

Figure 10. Map of the State of Kansas and Oklahoma showing the five locations where the different field experiments were conducted.

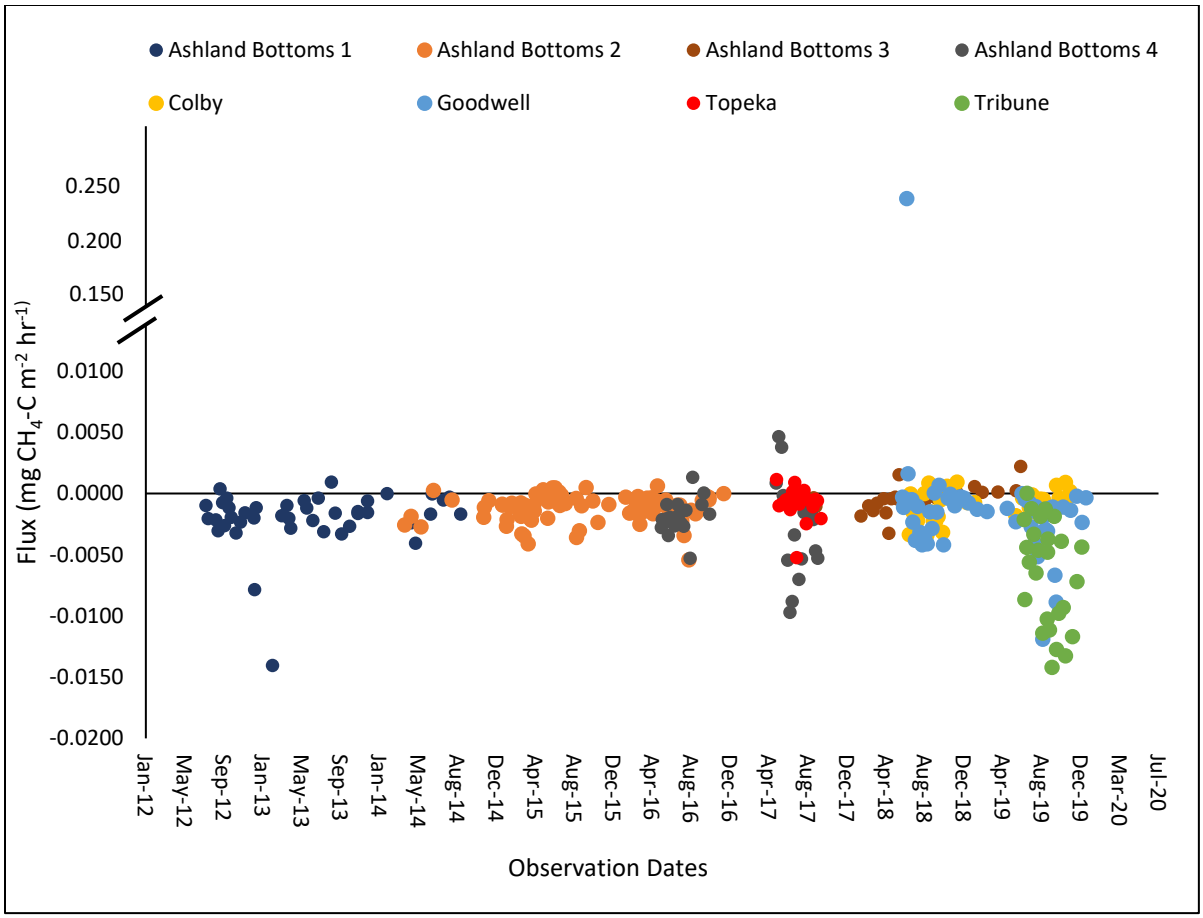

Figure 11. Fluxes of CH_4-C averaged across sample events in all field experiments at different locations from July 2012 to December 2019.

APPENDICES

Appendix Table 1. Simple linear regression of average methane fluxes, and weather and soil parameters near a dairy wastewater lagoon measured from June 2020 to June 2021.

Simple Linear Regression	R^2	P value
Average flux = $-3.108 \times 10^{-3} + 4.433 \times 10^{-5}$ Relative humidity	0.033	0.229
Average flux = $3.031 \times 10^{-4} - 1.128 \times 10^{-5}$ Air temperature	0.001	0.825
Average flux = $-2.76 \times 10^{-4} + 2.52 \times 10^{-4}$ Precipitation	0.093	0.041*
Average flux = $-3.814 \times 10^{-2} + 3.886 \times 10^{-3}$ Air pressure	0.004	0.650
Average flux = $1.169 \times 10^{-3} - 2.876 \times 10^{-6}$ Solar radiation	0.028	0.269
Average flux = $2.757 \times 10^{-4} + 1.915 \times 10^{-6}$ Average wind direction	0.002	0.765
Average flux = $2.68 \times 10^{-3} – 7.30 \times 10^{-4}$ Average Wind speed	0.090	0.044*
Average flux = $5.628 \times 10^{-4} - 2.604 \times 10^{-5}$ Soil Temperature	0.005	0.632
Average flux = $-1.931 \times 10^{-3} + 2.816 \times 10^{-3}$ Fractional water index	0.045	0.161

Appendix Table 2. Soil methane and carbon dioxide fluxes measured at different distance near a dairy wastewater lagoon from June 2020 to June 2021.

Days	Cumulative Days	Sampling Date	Methane				Carbon Dioxide			
			45 m	9 m	Berm	Average	Berm	9 m	45 m	Average
1	1	6/18/2020	-0.0118	-0.0124	-0.0114	-0.0119	42.9737	35.8328	29.4320	36.0795
2	5	6/22/2020	0.0036	0.0006	-0.0016	0.0009	186.2725	163.9545	106.4644	152.2304
3	7	6/24/2020	0.0014	-0.0014	-0.0007	-0.0002	181.7703	111.8443	104.3610	132.6586
4	8	6/25/2020	0.0072	-0.0011	-0.0078	-0.0006	152.0245	89.7564	108.8599	116.8803
5	9	6/26/2020	0.0023	-0.0015	-0.0105	-0.0032	105.0939	69.4032	102.8364	92.4445
6	10	6/27/2020	0.0041	0.0010	-0.0075	-0.0008	85.5941	54.2383	58.0714	65.9679
7	12	6/29/2020	0.0041	0.0010	-0.0075	-0.0008	78.3836	49.3036	71.7270	66.4714
8	13	6/30/2020	0.0042	-0.0006	-0.0092	-0.0019	86.6256	33.5081	30.7490	50.2942
9	14	7/1/2020	-0.0157	-0.0073	-0.0131	-0.0121	82.4100	44.8225	30.8002	52.6775
10	15	7/2/2020	-0.0151	-0.0073	0.0108	-0.0039	157.2914	130.9593	101.6245	129.9584
11	26	7/13/2020	0.0020	-0.0009	-0.0096	-0.0029	199.7123	140.0951	146.7019	162.1698
12	27	7/14/2020	0.0012	-0.0009	-0.0041	-0.0013	168.6590	102.3904	115.8680	128.9725
13	29	7/16/2020	-0.0009	0.0005	-0.0013	-0.0006	118.1809	65.5007	90.1808	91.2875
14	33	7/20/2020	-0.0002	0.0000	-0.0095	-0.0032	77.2762	63.3012	63.3863	67.9879
15	34	7/21/2020	-0.0007	-0.0001	-0.0016	-0.0008	86.2909	67.0797	86.3688	79.9132
16	46	8/3/2020	0.0083	0.0039	0.0023	0.0048	70.3461	77.1993	62.2466	69.9307
17	47	8/4/2020	0.0036	0.0072	0.0004	0.0037	128.4398	107.0560	152.4987	129.3315
18	49	8/6/2020	0.0006	0.0071	-0.0045	0.0011	144.7729	110.8467	116.9191	124.1796
19	54	8/11/2020	-0.0021	-0.0060	0.0001	-0.0027	188.7891	114.8477	107.9648	137.2005
20	84	9/10/2020	-0.0009	0.0002	0.0002	-0.0002	202.9360	67.9274	116.3379	129.0671
21	112	10/8/2020	-0.0002	0.0054	-0.0007	0.0015	139.2009	11.0229	49.7193	66.6477
22	118	10/16/2020	-0.0019	-0.0076	-0.0021	-0.0039	47.1725	12.8593	13.4213	24.4844
23	124	10/22/2020	0.0006	-0.0055	-0.0098	-0.0049	35.5394	24.8122	7.5403	22.6306

24	133	10/31/2020	0.0000	0.0005	-0.0012	-0.0002	31.1451	21.3302	18.0102	23.4952
25	140	11/7/2020	-0.0008	0.0041	-0.0036	-0.0001	41.3078	16.3976	21.9071	26.5375
26	147	11/14/2020	-0.0025	0.0070	-0.0098	-0.0018	28.9936	7.0992	3.3848	13.1592
27	154	11/21/2020	-0.0020	0.0006	-0.0007	-0.0007	20.5843	8.6964	7.7998	12.3602
28	161	11/28/2020	-0.0098	0.0012	-0.0015	-0.0034	21.7639	10.9235	6.8140	13.1671
29	168	12/5/2020	0.0009	0.0001	0.0003	0.0004	17.3250	11.5873	4.2168	11.0430
30	175	12/12/2020	0.0020	-0.0007	-0.0004	0.0003	18.3863	10.0377	4.9723	11.1321
31	182	12/19/2020	-0.0013	-0.0012	0.0016	-0.0003	18.9770	11.1667	7.8201	12.6546
32	189	12/26/2020	-0.0021	0.0001	-0.0021	-0.0014	14.6155	9.7808	7.7693	10.7219
33	210	1/16/2021	-0.0006	-0.0006	-0.0001	-0.0004	17.1888	23.6628	7.9708	16.2741
34	217	1/23/2021	0.0002	-0.0014	-0.0025	-0.0012	44.5841	42.9167	26.2541	37.9183
35	221	2/27/2021	0.0007	-0.0009	-0.0031	-0.0011	64.3884	57.0285	29.0276	50.1482
36	242	3/20/2021	-0.0003	0.0021	0.0006	0.0008	90.0658	56.2940	61.4635	69.2744
37	249	3/27/2021	0.0009	0.0029	-0.0006	0.0011	90.2210	46.1548	45.0073	60.4610
38	256	4/3/2021	0.0018	-0.0001	-0.0003	0.0005	115.7578	68.3992	39.5302	74.5624
39	263	4/10/2021	-0.0005	0.0023	-0.0055	-0.0013	122.6331	44.4368	56.0021	74.3574
40	284	5/1/2021	-0.0017	0.0025	-0.0063	-0.0018	78.3624	61.8829	31.8098	57.3517
41	297	5/14/2021	-0.0013	0.0040	-0.0065	-0.0013	108.4739	118.0818	104.4842	110.3466
42	310	5/27/2021	0.0300	0.0184	0.0069	0.0184	136.9740	101.0533	113.4672	117.1648
43	325	6/11/2021	0.0015	0.0139	-0.0211	-0.0019	147.7029	75.4590	96.2353	106.4658
44	332	6/18/2021	-0.0029	0.0040	-0.0040	-0.0010	123.1746	36.4810	56.3446	72.0001
45	339	6/25/2021	-0.0080	0.0079	-0.0083	-0.0028	100.5581	88.2431	68.9300	85.9104

Appendix Table 3. Weather and soil parameters during observations dates from June 2020 to June 2021 as measured and reported by the Stillwater Mesonet station.

Day	Cumulative Days	Sampling Date	Relative Humidity (%)	Air Temperature (ºC)	Precipitation (mm)	Air Pressure (mb)	Solar Radiation (W/m^2)	Wind direction (degree)	Wind speed (m/s)	Soil Temperature at 10 cm (ºC)	Fractional Water Index at 5 cm
1	1	6/18/2020	54.94	27.71	0.00	981.05	543.61	181.06	4.29	26.70	0.21
2	5	6/22/2020	74.22	23.73	12.45	979.77	591.22	325.44	2.60	22.52	0.98
3	7	6/24/2020	55.89	25.29	0.00	982.90	594.50	241.89	1.46	22.54	0.94
4	8	6/25/2020	69.44	27.29	0.00	983.33	608.25	184.78	3.07	25.32	0.86
5	9	6/26/2020	76.89	26.99	0.00	983.55	581.33	196.44	2.77	25.82	0.77
6	10	6/27/2020	75.11	25.16	0.00	980.45	341.45	174.78	3.80	26.37	0.69
7	12	6/29/2020	73.69	28.16	0.00	974.28	581.71	180.56	4.33	26.89	0.57
8	13	6/30/2020	73.32	28.91	0.00	974.67	585.22	176.16	3.98	27.18	0.52
9	14	7/1/2020	65.92	30.21	0.00	975.70	587.70	195.00	3.27	27.78	0.47
10	15	7/2/2020	73.20	30.17	0.00	981.75	581.75	147.44	2.45	29.00	0.46
11	26	7/13/2020	86.48	22.59	3.81	980.12	85.32	113.80	2.67	24.56	0.98
12	27	7/14/2020	81.92	27.48	0.25	977.75	262.40	124.64	3.57	26.16	0.97
13	29	7/16/2020	88.12	22.75	1.02	985.10	185.96	70.16	3.20	26.11	0.87
14	33	7/20/2020	65.68	29.18	0.00	982.72	561.16	185.16	2.41	27.96	0.60
15	34	7/21/2020	76.48	25.60	0.00	984.63	285.40	326.36	2.46	27.81	0.52
16	46	8/3/2020	69.52	23.78	0.00	987.33	575.24	36.76	2.68	23.88	0.90
17	47	8/4/2020	63.04	22.12	0.00	987.63	496.72	91.88	2.26	23.84	0.83
18	49	8/6/2020	90.48	22.38	0.00	983.66	133.76	155.24	2.22	23.42	0.98
19	54	8/11/2020	95.00	21.84	12.72	977.09	138.68	135.36	2.32	25.26	0.97
20	84	9/10/2020	92.00	11.91	13.46	982.30	161.96	337.12	3.35	16.97	0.94
21	112	10/8/2020	61.76	22.63	0.00	985.80	391.64	153.80	2.58	19.02	0.17
22	118	10/16/2020	43.28	13.43	0.00	997.26	399.60	245.92	1.56	16.09	0.15
23	124	10/22/2020	71.04	24.04	0.00	980.41	347.04	182.72	4.82	18.78	0.16

24	133	10/31/2020	62.76	11.67	0.00	987.73	348.80	177.80	3.76	7.64	0.97
25	140	11/7/2020	72.68	16.36	0.00	986.28	298.72	170.88	3.28	13.44	0.95
26	147	11/14/2020	82.64	16.12	0.25	974.60	245.12	194.12	4.07	12.51	0.80
27	154	11/21/2020	93.00	10.50	0.00	994.31	33.88	30.04	2.93	14.87	0.70
28	161	11/28/2020	81.68	2.69	0.00	992.39	221.76	205.48	0.39	4.20	0.96
29	168	12/5/2020	85.08	1.97	0.00	991.20	235.60	276.60	0.83	3.29	0.96
30	175	12/12/2020	74.00	3.06	0.00	985.69	97.50	306.12	2.97	5.32	0.96
31	182	12/19/2020	81.36	1.88	0.00	990.02	246.14	336.48	2.57	3.59	0.97
32	189	12/26/2020	57.00	5.03	0.00	983.32	269.94	155.16	0.56	2.72	0.96
33	210	1/16/2021	68.64	-0.87	0.00	985.81	146.88	283.52	1.14	2.30	0.65
34	217	1/23/2021	59.12	5.28	0.00	986.31	164.16	133.92	4.01	4.83	0.64
35	221	2/27/2021	100.00	8.11	0.00	980.81	113.00	140.76	3.66	6.28	0.74
36	242	3/20/2021	67.32	8.04	0.00	995.59	450.92	151.48	3.39	5.79	0.66
37	249	3/27/2021	61.00	16.74	0.00	979.78	459.92	290.12	1.98	11.26	0.66
38	256	4/3/2021	40.08	15.63	0.00	991.41	518.00	185.20	3.92	12.67	0.66
39	263	4/10/2021	66.60	10.20	0.00	980.50	473.72	322.32	4.70	11.08	0.53
40	284	5/1/2021	73.16	18.35	0.00	985.06	331.48	172.56	2.38	16.62	0.75
41	297	5/14/2021	59.36	18.22	0.00	991.53	490.08	119.64	2.74	15.91	0.69
42	310	5/27/2021	84.00	25.36	9.91	981.86	256.20	218.40	1.99	21.88	0.76
43	325	6/11/2021	73.76	28.57	11.18	976.08	615.76	171.08	3.69	26.07	0.60
44	332	6/18/2021	59.60	30.12	0.00	981.23	621.64	206.60	3.11	26.33	0.68
45	339	6/25/2021	67.56	30.30	0.51	978.58	612.56	190.36	4.59	27.01	0.55

Appendix Table 4. Cumulative flux of methane measured at different distance near a dairy wastewater lagoon from June 2020 to June 2021.

Observation Dates	45 m	9m	Berm
6/18/2020	0.0000	-0.2366	-0.2641
6/22/2020	0.3264	-0.2366	-0.2641
6/24/2020	0.4555	-0.2366	-0.3071
6/25/2020	0.4555	-0.2366	-0.4518
6/26/2020	0.4555	-0.2366	-0.4518
6/27/2020	0.4555	-0.2366	-0.6602
6/29/2020	0.4555	-0.2366	-1.0771
6/30/2020	0.4555	-0.1810	-1.1565
7/1/2020	0.4555	-0.1810	-1.1565
7/2/2020	0.4555	-0.1810	-1.1565
7/13/2020	0.8227	-0.1810	-2.6661
7/14/2020	0.8345	-0.1810	-2.7343
7/16/2020	0.8345	-0.1810	-2.7818
7/20/2020	0.8345	-0.0267	-3.3129
7/21/2020	0.8242	-0.0089	-3.3315
8/3/2020	2.6757	-0.0089	-3.3315
8/4/2020	2.7269	0.1587	-3.3315
8/6/2020	2.7269	0.4392	-3.3315
8/11/2020	2.7269	0.5022	-3.3315
9/10/2020	1.6809	0.5022	-3.3315
10/8/2020	1.6809	3.8878	-3.3315
10/16/2020	1.5326	3.6089	-3.5036
10/22/2020	1.7507	3.9071	-4.4872
10/31/2020	2.0778	3.9071	-4.7342
11/7/2020	1.7870	4.0473	-4.7342
11/14/2020	1.7870	5.0547	-4.7342
11/21/2020	1.6105	5.0547	-4.7342
11/28/2020	1.6105	5.0547	-4.7342
12/5/2020	1.7663	5.0547	-4.7342
12/12/2020	1.7663	5.0547	-4.8796
12/19/2020	2.4725	5.0547	-4.8796
12/26/2020	2.2440	4.9145	-4.8796
1/16/2021	2.2440	4.5718	-4.8796
1/23/2021	2.2440	5.0755	-5.1964
2/27/2021	2.2440	5.0755	-6.8840
3/20/2021	2.2440	5.0755	-6.8840
3/27/2021	2.2440	5.5169	-6.8840
4/3/2021	2.2440	5.5169	-6.8840

4/10/2021	2.2440	6.0102	-7.7304
5/1/2021	2.2440	6.0102	-7.7304
5/14/2021	1.9643	7.2735	-8.9937
5/27/2021	11.3379	13.0114	-7.3929
6/11/2021	11.3379	15.9601	-11.4877
6/18/2021	11.3379	15.9601	-11.4877
6/25/2021	10.1851	16.7182	-12.6353

Appendix table 5. Average concentration of greenhouse gases in the headspace during incubation across time.

GHG	Land use	Time (Hours)												
		1	24	48	72	96	120	144	168	169	193	241	289	337
CH_4	Cultivated	21.52^{a}	18.19^{a}	17.25^{a}	17.79^{a}	17.93^{a}	16.47^{a}	15.76^{a}	16.12^{ab}	16.56^{a}	15.79^{a}	16.00^{a}	14.25^{a}	16.13^{a}
	No-till	21.23^{a}	18.53^{a}	17.29^{a}	17.69^{a}	16.34^{a}	15.44^{a}	13.39^{b}	13.48^{c}	16.42^{a}	15.16^{a}	14.38^{a}	11.52^{b}	12.28^{b}
	Forest	21.52^{a}	17.99^{a}	16.28^{a}	17.40^{a}	17.78^{a}	17.07^{a}	15.44^{a}	16.61^{b}	16.37^{a}	15.54^{a}	13.05^{a}	7.97^{c}	6.83^{c}
	Grassland	21.75^{a}	18.29^{a}	17.34^{a}	17.40^{a}	17.24^{a}	16.18^{a}	15.22^{a}	15.74^{a}	16.65^{a}	15.25^{a}	16.24^{a}	13.50^{a}	16.13^{a}
	Sand	21.34^{a}	18.53^{a}	16.97^{a}	16.82^{a}	17.88^{a}	16.18^{a}	15.67^{a}	16.08^{ab}	16.19^{a}	15.50^{a}	16.88^{a}	14.40^{a}	17.37^{a}
CO_2	Cultivated	2120.75^{c}	5465.25^{d}	8441.98^{d}	10682.94^{d}	12615.40^{d}	13510.34^{d}	15038.26^{d}	15961.12^{d}	4023.60^{d}	6204.58^{d}	8410.57^{d}	10262.70^{d}	11995.19^{d}
	No till	2953.56^{b}	16954.90^{c}	24541.00^{c}	32282.47^{c}	35179.45^{c}	39851.06^{c}	40893.24^{c}	44592.13^{c}	3801.79^{c}	7121.68^{c}	11887.18^{c}	15877.84^{c}	21814.64^{c}
	Forest	3545.07^{a}	54407.02^{a}	79455.66^{a}	102664.85^{a}	114589.40^{a}	128640.30^{a}	135958.97^{a}	146536.17^{a}	8215.52^{a}	17974.50^{a}	32823.98^{a}	47300.16^{a}	61750.01^{a}
	Grassland	3467.17^{a}	40214.76^{b}	60051.64^{b}	76168.69^{b}	87334.77^{b}	96016.91^{b}	101950.71^{b}	108701.15^{b}	3668.28^{b}	11097.66^{b}	23485.56^{b}	36477.00^{b}	49651.51^{b}
	Sand	1828.90^{d}	1914.58^{e}	2224.59^{e}	2317.61^{e}	2338.67^{e}	2241.64^{e}	2270.14^{e}	2265.78^{e}	1552.46^{e}	1709.06^{e}	2464.08^{e}	2054.56^{e}	1627.99^{e}
N_2O	Cultivated	1.26^{a}	2.63^{b}	4.21^{b}	5.00^{b}	5.29^{c}	5.23^{c}	5.41^{c}	5.26^{c}	1.35^{cd}	1.37^{c}	1.69^{c}	1.71^{b}	1.49^{c}
	No till	1.29^{a}	20.70^{a}	35.79^{a}	38.60^{a}	36.78^{b}	38.48^{b}	37.05^{b}	37.75^{b}	1.87^{bd}	1.96^{bc}	2.37^{c}	2.34^{b}	2.71^{c}
	Forest	1.26^{a}	4.01^{b}	32.60^{a}	54.50^{a}	60.82^{a}	67.84^{a}	71.43^{a}	77.05^{a}	2.69^{a}	4.97^{a}	6.34^{a}	6.77^{a}	7.71^{b}
	Grassland	1.26^{a}	18.57^{a}	34.39^{a}	41.93^{a}	43.01^{ab}	44.65^{b}	44.59^{b}	45.73^{b}	2.02^{b}	2.63^{b}	4.46^{b}	6.60^{a}	10.34^{a}
	Sand	1.26^{a}	1.29^{b}	1.37^{b}	1.26^{b}	1.32^{c}	1.43^{c}	1.32^{c}	1.37^{c}	1.32^{c}	1.29^{c}	1.69^{c}	1.51^{b}	1.31^{c}

Note: Values with the same letters denote no significant difference.

Appendix table 6. Summary of p values derived from ANOVA of soil from different land use across incubation time.

	p value		
Time (hours)	CH_4	CO_2	N_2O
1	0.8153	<0.001	0.8312
24	0.0819	<0.001	0.0126
48	0.1428	<0.001	0.0133
72	0.6778	<0.001	0.0012
96	0.1784	<0.001	0.0005
120	0.0942	<0.001	0.0005
144	0.0003	<0.001	0.0003
168	0.0001	<0.001	0.0004
169	0.8853	<0.001	0.0025
193	0.5513	<0.001	<0.001
241	0.0862	<0.001	<0.001
289	<0.001	<0.001	<0.001
337	<0.001	<0.001	<0.001

VITA

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