Effect of land use and land use management on methane oxidation

by

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Abstract

Methane (CH_4) is a potent greenhouse gas that has increased dramatically since the beginning of the industrial era. According to the IPCC 5th assessment report, global warming potential of CH_4 is 28 times higher than CO_2 for a 100 year time scale. Therefore, it is an utmost importance to investigate the factors that affect the CH_4 budget. Methane oxidation is a biological process that reduces atmospheric CH_4 and is affected by land use and land management. The objectives of this study were to: (i) investigate the biotic and abiotic factors that affect CH_4 oxidation in native tallgrass prairie with 'prescribe fire' and (ii) investigate the effect of three land-uses (native, restored prairie and cropland) on CH_4 oxidation across two locations with distinct precipitation regimes in Kansas. The first study was conducted at three selected watersheds (C1A, C3A, and C3B) at the Konza Prairie Biological Station (KPBS) in Manhattan, Kansas. The second study was conducted at the Agricultural Research Center in Hays, KS and the Konza Prairie Biological Station in Manhattan, KS.

Laboratory incubation experiments investigated the CH_4 oxidation rate at different soil water contents (Gravimetric Water Content-GWC 9%, 20%, 25%, 30%, and 35%). The CH_4 oxidation rate was maximum at a soil water content of 25%, and CH_4 oxidation decreased at soil water contents of 9% and 35%. The addition of ammonium (NH_4^+, 50µg N g^{-1} soil) inhibited CH_4 oxidation by 48%. Higher CH_4 oxidation occurred for a 3-year burned versus annually burned tallgrass prairie. Methane oxidation was correlated with total soil copper content.

In the second study, soil samples were collected at a depth of 0-5cm. The soil was measured for NH_4^+, total C, pH, total Cu, extractable Cu, total PLFA biomass and methanotrophic PLFA biomass. A laboratory incubation study investigated the effect of land use and management on CH_4 oxidation. Higher CH_4 oxidation was measured at Konza (0.033 nmol

hr $^{-1}$ g^{-1} soil) than Hays (0.021 nmol hr $^{-1}$ g^{-1} soil) sites. The highest CH_4 oxidation rate was measured at the Konza restored prairie (0.050 nmol hr $^{-1}$ g^{-1} soil) and the lowest at the Hays native prairie (0.023 nmol hr $^{-1}$ g^{-1} soil). Land use had a significant ($p<0.05$) impact on soil microbial biomass, methanotrophic biomass, soil pH, extractable Cu, and total Cu content. Methane oxidation was significantly correlated to soil pH at the Konza site. Location with soil pH greater than 7 inhibited CH_4 oxidation. Total Cu and CH_4 oxidation were significantly correlated at both sites as Cu is a key factor for methanotrophic enzymatic activity. Further, higher pH decreases available Cu in the soil. Together these results suggest that soil Cu content is a crucial driving factor for CH_4 oxidation.

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Dedication

This thesis is dedicated to my wife, Nilusha, who has been a constant source of support and encouragement throughout my graduate studies.

Chapter 1 - Introduction

Methane (CH_4) is a greenhouse gas that atmospheric concentration has increased since the industrial era due to anthropogenic interventions. According to the world meteorological organization, atmospheric CH_4 concentration was 1859± 2 ppb in 2017, representing a 257% increase since the pre-industrial era (WMO, 2018). Emissions of CH_4 represent 17% of the global greenhouse gas budget (WMO, 2018). The global warming potential of CH_4 is 28 times higher than CO_2 at a 100 year time scale (Stocker et al., 2013). The fossil fuel industry and agricultural activities add CH_4 to the atmosphere. Approximately 90% of the atmospheric CH_4 is destroyed by atmospheric radicals (Kirschke et al., 2013). Upland soils, including native grasslands and temperate forests, are a biological sink for CH_4 (Dutaur and Verchot, 2007). Therefore, it is important to investigate the factors affecting CH_4 oxidation by soils.

Wetlands, lakes, rivers, termites, wildfires, wild animals, and other geological sources are natural sources of CH_4. Wetland methanogens are the highest natural methane-producing microorganisms in natural ecosystems contributing177 -284 Tg CH_4 yr^{-1} globally (Reay et al., 2018). The agriculture sector, including livestock production, rice cultivation, landfills, and waste-water handling, contributed 56% of global anthropogenic CH_4 emission from 2000 to 2017 (Saunois et al., 2019). Methanogens are CH_4 producers, and methanotrophs are CH_4 consumers (Hanson and Hanson, 1996). Methanogens are specific microbes that produce CH_4 gas in highly reduced conditions. Methanotrophic bacteria are methane oxidizers (Hanson and Hanson, 1996).

Based on the physiology, morphology, phylogeny, and metabolic pathways, methanotrophs microorganisms are divided into type I, type II, and type III methanotrophs groups (McDonald et al., 2008). Methane monooxygenase enzymes are divided into particulate

methane monooxygenase (*pMMO*) and soluble methane monooxygenase (s*MMO*). Both *pMMO* and *sMMO* enzymes contain copper mediated molecule for methane monooxygenase enzymatic activity (Kim et al., 2004). Type I and Type II methanotrophs are classified on the basis of methane monooxygenase enzymatic activity. Type I predominantly express pMMO which has a di-copper active center while type II expresses sMMO with a di-iron active center (Fru, 2011). Intracytoplasmic membrane (ICM) is a unique phenotypic feature that is used to identify type I and type II methanotrophic bacteria. Primarily, CH_4 oxidation mechanism occurs within the ICMs, which is controlled by bioavailable copper content. Both pMMO and sMMO enzymes are contained in type III methanotrophic bacteria. Further, methanotrophs can be divided into aerobic and anaerobic methanotrophs. Those aerobic methanotrophs are mainly gram-negative bacteria and absorb both atmospheric CH_4 with oxygen and assimilate carbon into their biomass (Hanson and Hanson, 1996). Methane monooxygenase is the enzyme that is involved in methane oxidation (Semrau et al., 2010).

Since methanotrophs bacteria are important for consuming atmospheric methane, it is essential to investigate the factors that affect CH_4 oxidation and how grassland management practices enhance methane oxidation. Various biotic and abiotic factors that affect methane oxidation by soil include temperature, moisture, pH, microbial diversity, soil nutrients levels, and soil trace metals (Nesbit and Breitenbeck, 1992; Mancinelli, 1995; Albanna and Fernandes, 2009). Native grassland management practices may affect the methanotrophic abundance as well as their activity. The CH_4 oxidation capabilities of the soil depend on the quantity and the quality of methanotrophic bacteria. Therefore, molecular techniques assess the abundance, and the activity of methanotrophs will be discussed in this chapter.

Methanotrophic bacteria are a subset of the methylotrophic physiological group (Hanson and Hanson, 1996). Methanotrophic bacteria use CH_4 as the energy source and metabolize it to methanol by methane monooxygenase enzyme in the first step (Hakemianet al., 2007). Soluble methane monooxygenase (sMMO) and particulate methane monooxygenase (pMMO) are two major forms of enzymes in the methanotrophic bacteria. Further two distinct carbon assimilation pathways; ribulose monophosphate pathways (RuMP) and Serine can be used to identify the different methanotrophic bacteria communities. During the assimilation, aerobic methanotrophs consume atmospheric O_2. These dioxygen bonds split and generate H_2O and formaldehyde (CH_3O). As discussed earlier, all the methanotrophs formed membrane-bound MMO or particulate MMO and which is a copper-mediated enzyme. All methanotrophs are capable of expressing the pMMO gene in the presence of copper. Previous studies show that Type I methanotrophic bacteria require a higher level of copper, higher CH_4 and lower O_2. Therefore Type I methanotrophic bacteria are dominant where higher CH_4 and lower O_2 concentration availability in the soil. In contrast, Type II methanotrophs are dominant when CH_4 is low and O_2 is at higher levels (Hanson and Hanson, 1996). Methanotrophs that contain higher pMMO have the potential to oxidized CH_4 at higher rates. Carbon assimilation into serineuses formaldehyde. Interestingly, there are physiological similarities for both methanotrophic bacteria and autotrophic ammonia-oxidizing bacteria. Though they are phylogenetically diverse, ammonia-oxidizing bacteria share several similar properties with methane-oxidizing bacteria. Ammonia-oxidizing bacteria oxidized CH_4 and assimilate CO_2 with ammonia or methane as substrates (Hanson and Hanson, 1996). Under certain environmental conditions, ammonia-oxidizing bacteria can oxidize CH_4, and CH_4-oxidizing bacteria are capable of oxidizing ammonia in the atmosphere.

Soil Water, Temperature and pH

Soil water content is one of the factors that affect soil microbial activity (Macinelli 1995). Low soil water content stresses soil microbes and limits microbial functions by affecting microbial physiology. Soil water content is negatively correlated with microbial enzymatic functions (Brocketter et al., 2012). Higher soil water content reduces aerobic methanotrophic activity due to decreased O2 in the soil. Higher soil water content enhances the anaerobic methanotrophic activity, which is not the focus of our discussion. Based on CH_4 oxidation, methanotrophic bacteria can be divided into low-affinity and high-affinity methanotrophic bacteria. High-affinity methane oxidating (HAMO) microbes that oxidized lower concentrations of CH_4 are dominant in upland soils (Cai et al., 2016). Low-affinity methanotrophs are capable of oxidizing higher CH_4 concentrations where CH_4 is produced. As soil water changes CH_4 and O_2 concentrations, it is important to consider soil water level to evaluate the CH_4 oxidation in upland soils.

As the diffusion of gases is limited at higher soil water contents, CH_4 oxidation and production dynamics change. Higher soil water content supports more anaerobic conditions with higher CH_4 production as well as higher low-affinity CH_4 oxidation by anaerobic methanotrophic communities. Gulledge et al. (1998) showed that maximum CH_4 oxidation at 20%-40% of water-holding capacity in taiga and tundra upland and wetland soils. Hydromorphic soils have higher CH_4 oxidation capabilities than upland soils (Christiansen et al., 2012). Precipitation is a key factor for the CH_4 oxidation in upland soils and type II methanotrophs relative abundance is reduced with higher precipitation (Horz et al., 2005). Furthermore, Horz et al.(2005) found that there was a significant difference in type II methanotrophs abundance for soils with different water contents, but the mechanism influencing that abundance was unclear.

Most methanotrophs are mesophiles, but certain methanotrophic species can thrive under extreme conditions (Hanson and Hanson, 1996). Methane oxidation varies with temperature. Methane oxidation ranges from 0°C to 10°C or more than 35°C at certain extreme conditions. The optimum temperature for methane oxidation is 25°C (Hanson and Hanson, 1996). Special methanotrophs that thrive in freezing conditions have different optimum temperatures for CH_4 oxidation (He et al., 2012). Significant differences in methane oxidations and carbon assimilation with temperature have been reported. With an increase of temperature of 25°C, CH_4 oxidation was enhanced by 198%; however, carbon conversion efficiency decreased significantly (Roslev et al., 1997). Therefore CH_4 oxidation and abundance may change with the seasonal fluctuation of temperature. Soil pH levels can affect the methanotrophic community composition and activity. Most studies suggested that neutral pH values favored methane oxidation, but optimum pH can vary between pH 3-7 (Hanson and Hanson, 1996; Malyan et al., 2016).

Nitrogen inputs- NH^+_4

Nitrogen is a major nutrient for plant productivity and is available in the soil as inorganic N, nitrate (NO^-_3), and ammonium (NH^+_4). Many studies have investigated the effect of NH^+_4 on CH_4 oxidation and methanotrophic activity. The classic research by Nesbit et al. (1991) showed that the addition of NH_4^+ inhibited CH_4 oxidation in soil. Three levels of Nitrogen inputs (10, 20, and 40 kg N $ha^{-1}Yr^{-1}$) from three different N fertilizers (NH_4Cl, $(NH_4)_2SO_4$, KNO_3) inhibited CH_4 oxidation in Alpine meadow soils in Qinghai-Tibetan plateau (Fang et al., 2014). Soil NH^+_4 interacts with CH_4 monooxygenase inhibiting CH_4 oxidation (Hanson and Hanson 1996; Walkiewicz et al., 2018).

Copper

The Cu level in soil affects methanotrophic activity (Lindley et al., 2004). Since Cu is a component of CH_4 monooxygenase, bioavailable Cu plays a major role in the activity of CH_4 monooxygenase (Morton et al., 2000). As discussed earlier in the classic review by Hanson and Hanson (1996), Cu is a regulatory element in CH_4 oxidation. The addition of Cu to methanotrophic growth media enhanced methane oxidation (Prior et al., 1985). Particulate methane monooxygenase (pMMO) has a higher requirement for Cu; thus CH_4 oxidation activity could be affected by the bioavailability of soil Cu. The methanobactin peptide is the Cu binding compound in methane monooxygenase.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) is an important parameter when considering the soil metal speciation. Bioavailable Cu can be decreased with organic matter content. Soil organic matter limits free metal activity when referring to Cu; on the other hand, DOC can enhance Cu metal solubility (McBride et al., 1997). Sullivan et al. (2013) observed that DOC was positively correlated with CH_4 oxidation. Dissolved organic carbon might have a strong influence on the annual methane budget (Zhou et al., 2014).

Prescribe burning in native grasslands

Prescribe fire is a management technique used to manage native grasslands to enhance productivity. A meta-analysis by Dooley et al. (2011) suggests that there was no effect of fire on microbial biomass. Prescribe fires are different from natural wildfires and are a well-established

practice since early ages (Alcañiz et al., 2018). Further, prescribed fires are used to manage the natural ecosystem by enhancing vegetative productivity (Anderson et al., 2006).

Fire can change the soil properties and soil chemical components, which indirectly affects soil microbes (Dooley et al., 2017; Fultz et al., 2016). Direct heat transfer could reduce the soil microbial abundance. Further nutrient volatilization during an intense fire can reduce the long term nutrient availability. Soil chemistry, soil physical structure, and soil microbiota can be affected by grassland burning (Muñoz-Rojas et al., 2016). However, fire intensity determines the degree of physical, chemical, and biological changes. Grassland fires enhance the mineralization of nutrients, especially organic C and organic N (Reich et al., 2001; González-Pérez et al., 2004). Higher NH_4^+ may persist in the soil for almost one year (Hobbs 1983). As discussed earlier, NH_4^+ can inhibit CH_4 monooxygenase activity.

Methanotrophs

Previous research suggested that microbial community composition and activity may influence soil methane oxidation (Schimel et al., 2012; Semrau et al., 2010). There are several techniques to quantify methanotrophic bacterial abundance in the soil. Phospholipid fatty acid (PLFA) biomarkers and 16s rRNA biomarkers can be used to identify those methanotrophic communities (McDonald et al., 2008). The unique functional enzyme, methane monooxygenase with the pmoA gene, can be used as an ecological marker (Horz et al., 2005). However, the phylogenetic resolution of soil microbial PLFA is lower than other molecular techniques. Stable isotope (^{13}C) probing (SIP) techniques have identified the distinct pattern of methanotrophic communities in the soil. Comparing the PLFA database with 16S rRNA and pmoA gene sequences by multivariate analysis increases the phylogenetic resolution of PLFA (Bodelier et

al., 2009). Moreover, PLFA analysis with ^{13}C tracers provides in depth information on the soil microbiota. Application of ^{13}C -PLFA has investigated aerobic methanotrophic bacteria (Yao et al., 2015). As summarised in Table 1.1, Unique PLFA biomarkers like 16:0, 14:0, 16:1ω7c, 16:1ω5t, 16:1ω8c, 16:1ω7c, 16:1ω5c, 18:1ω7c, 16:1ω6c are associated with type I methanotrophs and 18:1ω8c, 18:2ω6c, 18:2ω12c, 18:2ω7c associated with type II methanotrophs. PLFA biomarkers i14:0, a15:0, 18:0 are assoicated with verrucomicrobial methanotrophs.

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Table 1.1 PLFA (Phospholipid Fatty Acid) biomarkers of methanotrophic bacteria.

Methanotrophs	PLFA	References
Type I Methanotrophs	16:0, 14:0, 16:1ω7c, 16:1ω5t, 16:1ω8c, 16:1ω7c, 16:1ω5c, 18:1ω7c, 16:1ω6c	Nazaries et al. 2013; Yao et al. 2015; Sundh et al.,2018; Bodelier et al. 2009;
Type II Methanotrophs	18:1ω8c, 18:2ω6c, 18:2ω12c, 18:2ω7c	Nazaries et al. 2013; Yao et al. 2015; Sundh et al.,2018; Bodelier et al. 2009;
Verrucomicrobia	i14:0, a15:0, 18:0	Nazaries et al. 2013; Yao et al. 2015

Chapter 2 - Methane Oxidation in Native Prairie Soil

Abstract

Methane is a potent greenhouse gas that has increased significantly since the industrial era. Methanotrophic bacteria in native tallgrass prairie soils affect the atmospheric methane balance by consuming atmospheric methane. Previous research has demonstrated that prescribe burning increases methane oxidation. The objective of this study was to determine the effects of abiotic factors; soil water, NH_4^+, and total copper on methanotrophic activity in the native prairie soil. Soil samples were collected from Konza Prairie Biological Station (KPBS) in Kansas. Homogenized soil samples were processed and incubated at ambient temperature for 48 hrs at four soil water contents (Gravimetric Water Content-GWC 9%, 20%, 25%, 30%, and 35%). Methane concentration in headspace was adjusted to approximately 3.0 µl l^{-1} and incubated at room temperature (22°C- 25°C). Selected soil samples were amended with NH_4Cl at 50µg N g^{-1} soil. Soil water content significantly affected methane oxidation. Methane oxidation was optimum at soil 25% GWC at a rate of 0.054 nmol CH_4 h^{-1} g^{-1} soil. At lower soil water levels (9%GWC), methane oxidation decreased to 0.007 nmol CH_4 h^{-1} g^{-1} soil, due to the reduction of microbial activity. Methane oxidation was reduced at higher soil water contents (35% GWC, 0.008 nmol CH_4 h^{-1} g^{-1} soil) due to limited gas diffusion in the soil.

The addition of NH_4^+ inhibited methane oxidation by 48%.To investigate the effect of prescribed burning management on methane oxidation, C1A, C3A, C3B watersheds at KPBS were selected based on prescribed burning intervals. Higher methane oxidation rates were recorded for C3B watershed with a 3-yr burn cycle. Total soil copper and methane oxidation were correlated. Soil microbial PLFA biomass was not significantly different between

watersheds; however higher Type I methanotrophic bacteria was measured for the C3B watershed which had higher total Cu and higher CH_4 oxidation.

Introduction

Methane (CH_4) is a greenhouse gas that is 28 times more potent than CO_2 (Stocker et al., 2013). According to the 5th assessment report of IPPC (2014), the average global atmospheric concentration of CH_4 is over 1.8 ppm. The energy sector is considered as the highest CH_4 emitter alongside the agricultural sector (EPA, 2019). Natural biological cycles produce CH_4 by soil microbes under anaerobic conditions, specifically in rice cultivation and livestock (Dean et al., 2018). Well-aerated grasslands and forests soils' were considered as a major contributor to methane oxidation (Wang et al., 2014).

Methanotrophic bacteria can reduce atmospheric CH_4 by oxidizing CH_4 in both anaerobic and aerobic conditions in the soil (Dean et al., 2018; Aronson et al., 2013; Hanson et al., 1996). Soil moisture, temperature, substrate availability, enzyme activities are some of the biotic and abiotic factors that affect CH_4 oxidation (Kalyuzhnaya et al., 2019, Hanson et al., 1996). Aerobic methanotrophic bacteria assimilate CH_4 by oxidizing CH_4 to CO_2 under aerobic conditions. Particulate CH_4 monooxygenase *(pMMO*) and soluble CH_4 monooxygenase *(sMMO)* pathways are two pathways for CH_4 oxidation (Hanson et al., 1996; Hakemian et al., 2007).

Activities of these two enzymatic pathways are linked with copper (Cu) (Fru 2011; Semrau et al., 2010; Hakemian et al., 2007). The *pMMO* gene tends to express at higher Cu levels, increasing CH_4 oxidation activity. Perticulate methane monooxygenase enzymatic activity had altered when exceeding of Cu content by 1906.4 mg L^{-1} in the growth medium due to Cu toxicity (Fru, 2011).

Ammonium inhibits methanotrophic activity (Walkiewicz et al., 2018; Nesbit et al., 1991). Ammonium binds with methane monooxygenase, thus inhibiting CH_4 oxidation. Nitrogen fertilizer decreases methane oxidation in croplands (Hutsch et al., 1994).

Land-use change and management can impact on CH_4 oxidation by changing the composition and activity of methanotrophs in the soils (Tate, 2015). Natural fire and prescribe burnings are essential for the productivity of most grassland ecosystems around the globe (Alcañiz et al., 2018; Bento-Gonçalves et al., 2012). Prior to European settlement in North America, native inhabitants burned the grasslands (Kay, 2000). Human-induced fires or prescribe burnings were carried out by settlers for hunting, agriculture, and other reasons (Williams et al., 2003). Fires are used to control invasive vegetation and plant diseases, mitigate wildfire hazards, improve wildlife habitats, enhance plant productivity, facilitate the distribution of grazing lands, and restore native ecosystems (Stubbendieck et al., 2007). Prescribe fires in grasslands are well defined and well-studied management practices for the productivity of the ecosystem (Alcañiz et al., 2018).

Prescribe fires affect soil physical, chemical, and microbial properties (Alcañiz et al., 2018). An increase in soil temperature occurs only in the first few centimeters of the soil surface, sometimes reaching as high as 400°C but lasting only a few seconds during a fire. However, the effects differed with soil characteristics and vegetation cover. The impact of burning may alter the organic matter content of the soil surface and some soil properties (Alcañiz et al., 2018; Fultz et al. 2016). Long-term prescribed or natural fires alter total soil carbon inputs and high-frequency fire regimes lead to a reduction in total soil carbon (González-Pérez et al., 2004). Soil NH_4^+ content increases immediately after burning by 241% relative to pre-burn N concentrations (Fultz et al., 2016). The increase of mineralizable nitrogen content was observed immeadily after the burning but replenished in a shorter period of time (less than two months) in Konza prairie temperate grasslands (Garcia & Rice, 1994). However, the increase of nitrogen mineralization after a burning event was observed in mountain grasslands in the montane zone of Roky

mountains, which lasts for one year (Hobbs & Schimel, 1984). However, the available mineralized nitrogen content was reduced to original conditions with the development of vegetation during the post-fire period.

Since soil biological activities are linked with both soil chemical and soil physical characteristics, fire itself, directly and indirectly, affects soil biological properties. Burning affects the soil microbial community and activity at different time scales (Fultz et al. 2016; Knelman et al. 2015) Fire reduced total microbial biomass by an average of 33% and fungal abundance by an average of 47.6% in boreal and temperate forest ecosystems but not in grassland ecosystems (Dooley et al., 2017). Further soil microbial biomass and enzymatic activities were reduced along with total C, total N, available P and available S in annual and periodic burning of upland flatwood (Eivazi et al., 1995). The lack of research data on long-term response of prescribing fires to soil biological activities and its relationship with soil organic carbon in temperate grassland ecosystems had not comprehensively evaluated.

There are limited studies on the impact of burning on CH_4 oxidation. Tropical forest and temperate grassland burning increases CH_4 oxidation (Aronso et al., 2013; Rivera-Zayas, 2019). Three-year patch burning enhanced CH_4 oxidation by 30% compared with annual burning in a temperate grassland Konza Prairie (Rivera-Zayas, 2019). Net CH_4 oxidation may be enhanced due to the activity or abundance of methanotrophic bacteria in the soil.

The investigation of biotic and abiotic factors that affect methanotrophic abundance and activity would help to understand the methane oxidation process in ecosystems under prescribed burning management.

Prescribed burning frequency may influence CH_4 oxidation in native prairies by both biotic and abiotic factors. Therefore, it is important to investigate factors that influence the

activity and abundance of aerobic methanotrophic bacteria in the soil to better predict the impact of human-induced burning regimes on CH_4 oxidation in native prairie soil. The objectives of this study were to: (i) measure CH_4 oxidation rates at different soil gravimetric water contents; (ii) determine the effect of NH_4^+ on CH_4 oxidation; (iii) to determine the effect of annual and 3-y burning frequencies on CH_4 oxidation; (iv) determine the effect of total and extractable copper content of CH_4 oxidation, through laboratory incubation experimentation and (v) determine the abundance of methanotrophic community in the soil using PLFA biomarkers.

Material and Methods

Sampling sites and soil characteristics

The study was conducted at selected watersheds managed by Konza Prairie Long Term Ecological Research (LTER) program at Konza Prairie Biological Station (KPBS) in Manhattan Kansas (39°05’ N, 96°35’ W). The KPBS, a native tallgrass prairie area, consists of 3487 ha with rich biodiversity including big bluestem (*Andropogn gerardii*), Indiangrass *(Aorghastrum nutans*), and switchgrass (*Panicum virgatum*). Climate characteristics were similar to the continental climatic zone by warm, wet summers with dry and cold winters. Mean annual precipitation is 835 mm and mean monthly temperature fluctuates from 6.6 to 19.4 ^oC. The major soil series are Ivan, Benfield, Florence, Clime, Sogn, Tully, Irwin, and Reading (Konza Prairie Biological Station, 2009) and the dominant soil is the Benfield-Florance soil series (Fine, mixed, superactive, mesic Udertic Argiustolls). The topography of KPBS is complex, ranging from 320 to 444 m above sea level.

The research sites C1A (39°04'40.1"N 96°32'36.6"W), C3A (39°05'40.2"N 96°32'45.2"W), and C3B (39°05'25.8"N 96°32'41.3"W) are grazed watershed units with

perennial C_4 grasses as dominant vegetation. Annual prescribed burned (C1A) was burned every spring; the C3A site (2016 /2019) and C3B site (2014/2017) were burned every three years in spring on offset years. Site description, soil characteristics, and prescribe fire history are summarized in Table 2.1, Table 2.2 (Blair, 2018) and Table 2.3, respectively.

Soil processing and Moisture controlling

Soil core samples were taken from each site with standard soil sampling probes (5.5 cm diameter) at a depth of 0 to 10 cm. Collected soil samples were placed into collection bags (zip-lock bags) and stored in a cooler. The collected soil samples were homogenized, and large roots, rocks, and plant residues removed manually prior to sieving. The soil samples were sieved using 2 mm sieve and subsampled for chemical and biological analysis. Five subsoil samples with four replicates from each site were used for the laboratory incubation.

Soil methane oxidation-Lab incubation

Gravimetric water content (GWC, %) of the sieved soil samples were analyzed using standard laboratory procedures. For GWC 10 g of soil was dried at 105 °C for 24 hours and GWC was determined. The soil water content was adjusted by adding distilled water to 20%, 25%, 30%, and 35% GWC. Four replicates from each sample were taken for the analysis. After adjusting to the desired GWC of each soil sample, the samples were incubated at 25 °C with 95%-98% humidity for 5 days. CH_4 concentration in ambient air was approximately 3.0 $\mu l\ l^{-1}$ was adjusted in the headspace (925mL) at room temperature (22°C- 25°C). The jar lids were made to airtight by sealing the cap using Dow Corning high-vacuum grease. After adjusting the headspace CH_4 concentration of each soil sample in the mason jars, 10 mL of headspace gas was

collected to pre-evacuated glass vials (25ml) every 8 hours (0, 8, 16, 24, 32, 40 and 48) for 48 hours. Collected gas samples were analyzed for CH_4 concentration by gas chromatography (GC) using a Brucker model 456-GC with fully automated autosampler (Scion instruments in Livingston, Scotland). The GC was equipped with a flame ionization detector (FID), thermal conductivity detector (TCD) and electron capture detector (ECD). Temperature and detectivity of FID, TCD and ECD 450°C-1.4 pg C/sec, 450°C -300pg/ml, 450°C -7fg/s respectively and ECD equipped with 63Ni -15mCi radioactive source (555Mbq).

Soil Inorganic Nitrogen content

Soil inorganic N, NH_4^+ and NO_3^-, was extracted by adding 100 mL of 1M KCl to 25g of moist soil sample. The sample was shaken for 60 min on an orbital shaker at 300 rpm and filtered through Whatman No. 42 filter paper into a 20 mL scintillation vial. The extract was analyzed for NH_4-N and NO_3-N by colorimetric analysis (Gelderman et al., 1998) at KSU Soil Testing Laboratory.

Total and extractable Copper (Cu) content in the soil

Soil samples were dried at 40°C for 24 hours and ground with pestle and mortar prior to the digestion. For digestion, 1g of dry soil was digested with repeated addition of HNO_3 and H_2O_2 (Sposito et al., 1982). For extractable soil copper. 10 g of soil was analyzed by the DTPA extraction method (Sposito et al., 1982). Both soil digestion and DTPA extraction (Lindsay & Norvell, 1978) were carried out by Kansas State University soil chemistry lab using the Inductively coupled Plasma (ICP) spectrometer, model 720-ES ICP optical emission spectrometer, manufactured by Varian Australia (PVT) Ltd, Mulgrave, Victoria, Australia.

Phospholipid Fatty acid analysis (PLFA)

Soil microbial biomass and functional groups were determined by PLFA analysis. Standard soil probes were used to take soil samples from the topsoil (0-10 cm). Soil samples were collected into Ziplock bags and 40 g of fresh soil were sent to Ward Laboratories Inc., Kearny, NE for PLFA analysis. Sample results were reported as nanomole. Specific aerobic methanotrophic community's PLFA biomarkers (i16:0, 14:0, 16:1ω7c, 16:1ω5t, 16:1ω8c, 16:1ω7c, 16:1ω5c, 18:1ω7c, 16:1ω6c, 18:1ω8c, 18:2ω6c, 18:2ω12c, 18:2ω7c, i14:0, a15:0, 18:0) were analyzed for the methanotrophic abundance in the soil samples (Table 2.4). Abundance of Verrucomicrobial PLFA biomarkers was not analyzed separately as Verrucmicrobial abundance was distinctly lower than type I and type II methanotrophs.

Data analysis

Soil physical and chemical data of each watershed at KPBS were collected from Long-Term Ecological Research (LTER) dataset published on Konza LTER website (www. lter.konza.ksu.edu) and detailed information of protocols available in KPBS LTER website. Three watershed data were used in this study. KPBS fire history and physical, chemical properties of each watershed (C1A, C3A, C3B) were used for this study. Historical fire data were available since 1972 and soil physical/chemical content data were collected in 2010 and 2015. Soil pH, NH_4^+, total C were used from the metadata for this study. Analysis of Variance (ANOVA) and a regression model was used to analyze the data set with the 95% confidence interval. R statistical software (www.R-project.org) used for the data analysis.

Results

Methane oxidation was significant ($p<0.001$) with the incubation time (Fig. 2.2). After 48 hours, 50% of the methane was consumed at the rate of 0.054 nmol CH_4 h^{-1} g^{-1} soil. Furthermore, there was a significant effect of soil water content on CH_4 oxidation ($p<0.01$) (Fig. 2.3). Methane oxidation was maximum at 25% GWC with a rate of 0.054 nmol CH_4 h^{-1} g^{-1} soil. Lowest CH_4 oxidation rate 0.007 nmol CH_4 h^{-1} g^{-1} soil and 0.008 nmol CH_4 h^{-1} g^{-1} soil recorded at soil 9% GWC and 35% GWC, respectively.

The addition of NH_4^+ inhibited methane oxidation of the soil (Fig. 2.4). Methane oxidation was reduced from 0.045 nmol CH_4 h^{-1} g^{-1} soil to 0.023 nmol CH_4 h^{-1} g^{-1} soil with the addition of 50 µg N g^{-1} soil (NH_4Cl) to the soil. The addition of NH_4Cl inhibited CH_4 oxidation by 49%.

Higher CH_4 oxidation was observed in the C3B soil (0.083 nmol CH_4 hr^{-1} g^{-1} soil) and lowest CH_4 oxidation in the C3A soil (0.031 nmol CH_4 h^{-1} g^{-1} soil) (Fig 2.5). Total Cu content was higher in the C3B soil(Fig 2.6). There was no significant correlation with total Cu content and methane oxidation in the soil (Fig 2.7).

Total microbial biomass of the C1A, C3B, and C3B sites were 742, 664, and 746 nmol PLFA g^{-1}soil, respectively (Fig. 2.8). A similar trend was observed with the distribution of methanotrophic PLFA biomass, where C1A, C3B, and C3B methanotrophic biomass was 309, 275, and 312 nmol PLFA g^{-1} soil, respectively. There were no significant differences in total biomass and the methanotrophic biomass. Type I, type II and verrucomicrobiota were investigated (Fig. 2.9) with available PLFA biomarkers. Type I methanotrophic biomarkers were higher in C3B than the other sites. Type I methanotrophic abundance of C1A, C3B, and C3B were 140, 146 and 180 nmol PLFA g^{-1} soil. Type II methanotrophic abundance was higher at

C1A site (124 nmol PLFA g^{-1} soil). Verrucomicrobial content was lower than type I and type II methanotrophic bacteria.

Discussion

The effect of the prescribed burning on CH_4 oxidation was addressed in this study. The changes in soil water content and the addition of NH_4^+ impacted CH_4 oxidation. High CH_4 concentration can enhance methane oxidation rates (Roslev et al., 1997). The CH_4 oxidation rates of this study were lower than the results reported by others (Dunfield et al., 1995; Albanna et al., 2009) as the initial CH_4 concentration of this study was lower (~3 µl l^{-1}). However, CH_4 oxidation rates were similar to that reported reported by Christiansen et al. (2017).

The optimum soil water content (20-30%) for CH_4 oxidation was similar to that reported by Dasselaar et al. (1998). As methanotrophic bacterial depend on available CH_4 and available O_2, soil water content can reduce gas diffusion into the soil At lower water content, microbial activity is reduced due to desiccation (Semrau et al., 2010). The optimum water content for the CH_4 oxidation can vary with soil type; this study suggests that 20-30% of water content as the optimum soil water content in silty-clay- loam soil on Konza Prairie.

The addition of NH_4^+ inhibited CH_4 oxidation rate by 49%. Previous studies reported the addition of N inhibited the CH_4 oxidation (Nesbit et al., 1991; Fang et al., 2014). Direct or indirect effects of other soil properties might have influenced on CH_4 oxidation in field scale with reference to soil NH_4^+ content. Soil with higher CEC value can enhance the availability of or NH_4^+, thus influence the CH_4 oxidation. Further higher soil organic C content and clay can increase the soil CEC, which might increase the NH_4^+ content in the soil. Therefore, it is vital to investigate the soil texture with soil CEC, pH, and organic content to comprehensively understand the CH_4 oxidation process in the soil for future studies.

As reported by Rivera-Zayas (2019), net CH_4 oxidation was higher with a 3-yr burning (C3B, C3A) than an annual burn(C1A). In the laboratory, the soils from these watersheds also had higher CH_4 oxidation for the C3B site where prescribe fire was performed in 2014 and 2017. Oxidation of CH_4 was lower in soil from the C3A, which was burned in 2013 and 2016 before soil sampling in 2018.

Total PLFA biomass and methanotrophic PLFA biomass were not statistically different between sites. There was no significant difference in Type I and Type II methanotrophic biomass in C1A, C3A, and C3B sites. However, Type I methanotrophic bacterial abundance was higher in C3B than C1A and C3A. Higher Cu content in the soil might have influenced the higher type I methanotrophic biomass in the C3B site over C1A and C3A sites. Therefore greater CH_4 oxidation might have occurred due to higher type I methanotrophic biomass in C3B site.

Higher total Cu was present in C3B, which had higher CH_4 oxidation; Site C3A had lower levels of Cu which had lower CH_4 oxidation. Site C3B had higher soil organic C (50 g C kg^{-1}) than C1A (46 g C kg^{-1}) and C3A (42 g C kg^{-1}) (Rivera-Zayas, 2019). As available soil Cu content is directly linked with the soil organic C (McBride et al., 1997), higher organic C might influence available Cu. Further, DOC might influence on Cu availability. However, the correlation of DTPA extractable Cu and CH_4 oxidation was not significant, although a trend was apparent. As DTPA extractable Cu represents the plant available Cu content in the soil, it might not clearly represent the bioavailable Cu content for methanotrophic enzymatic activities. The effect of burning on CH_4 oxidation and methanotrophic activity is very complex and comprehensive research is needed to investigate this topic further.

Conclusion

It is important to understand the biotic and abiotic factors that affect CH_4 oxidation to mitigate the CH_4 emission in the globe. This experiment indicates that CH_4 oxidation and changes due to soil water content were significant in upland native prairie soils. Short term and long-term interval of prescribe burning soil burning influenced CH_4 oxidation capacity.

Methanotrophic biomasses were similar between watershed; however, type I methanotrophic bacteria were higher at the C3B site. Methane oxidation was higher and was correlated with total copper levels. This study suggests that total soil copper might be a key factor in CH_4 oxidation. Further studies are needed to comprehensively understand the effect of fire on CH_4 oxidation in native prairie soils.

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Table 2.1. Land management and location description of C1A, C3A and C3B sites at Konza Prairie Biological Station (KPBS)

C1A	Description:	Seasonally grazed (~01 May to ~ Sep. 01) by cattle and scheduled prescribed burned annually in the spring.			
	Bounding Coordinates:	Northern:	39.0835	Southern:	39.073
		Western:	-96.5508	Eastern:	-96.5385
C3A	Description:	Seasonally grazed (~01 May to ~ Sep. 01) by cattle and scheduled prescribed burned every 3 years in the spring.			
	Bounding Coordinates:	Northern:	39.098	Southern:	39.0906
		Western:	-96.5534	Eastern:	-96.5385
C3B	Description:	Seasonally grazed (~01 May to ~ Sep. 01) by cattle and scheduled prescribed burned every 3 years in the spring.			
	Bounding Coordinates:	Northern:	39.0961	Souther:	39.0874
		Western:	-96.5534	Eastern:	-96.5385

Table 2.2 Average mean values of pH, total soil C and NH_4^+ of C1A, C3A and C3B watersheds at Konza Prairie Biological Station.

Watershed	Year	Average pH	Average Total C-µg C g^{-1}	Average NH_4^+ - µg N g^{-1}
C1A	2010	6.23	4.25	7.85
	2015	6.50	4.47	6.89
C3A	2010	6.35	4.11	9.88
	2015	6.46	4.37	5.24
C3B	2010	6.43	3.90	10.7
	2015	6.61	3.99	5.70

(Blair, 2018)

Table 2.3 Prescribe burning history of C1A, C3A, C3B and HQ restorative plots at Konza Prairie Biological Station.

KONZA PRAIRIE -PRESCRIBE BURNING HISTORY			
C1A	**C3A**	**C3B**	**HQ Restorative**
1980	1980	1980	2003
1983	1983	1983	2007
1988	1989	1984	2009
1990*	1991	1988	2010
1991	1992	1989	2012
1992	1993	1990	**2013****
1993	1996	1991	
1994	2000	1992	
1995	2003	1993	
1996	2004	1994	
1997	2006	1995	
1998	2007	1996	
1999	2008	1997	
2000	2009	1998	
2001	**2010***	1999	
2002	**2013**	2000	
2003	**2016**	2001	
2004	**2019****	2002	
2005		2003	
2006		2004	
2007		2005	
2008		2006	
2009		2007	
2010		2008	
2011		2009	
2012		**2011***	
2013		**2014**	
2014		**2017****	
2015			
2016			
2017			
2018			
2019**			

* Current prescribe burning time interval started year, C1A annual burning started 1990, C3A -two-year time interval started 2011, C3B- two- year time interval started 2011

**Latest prescribed burn performed year

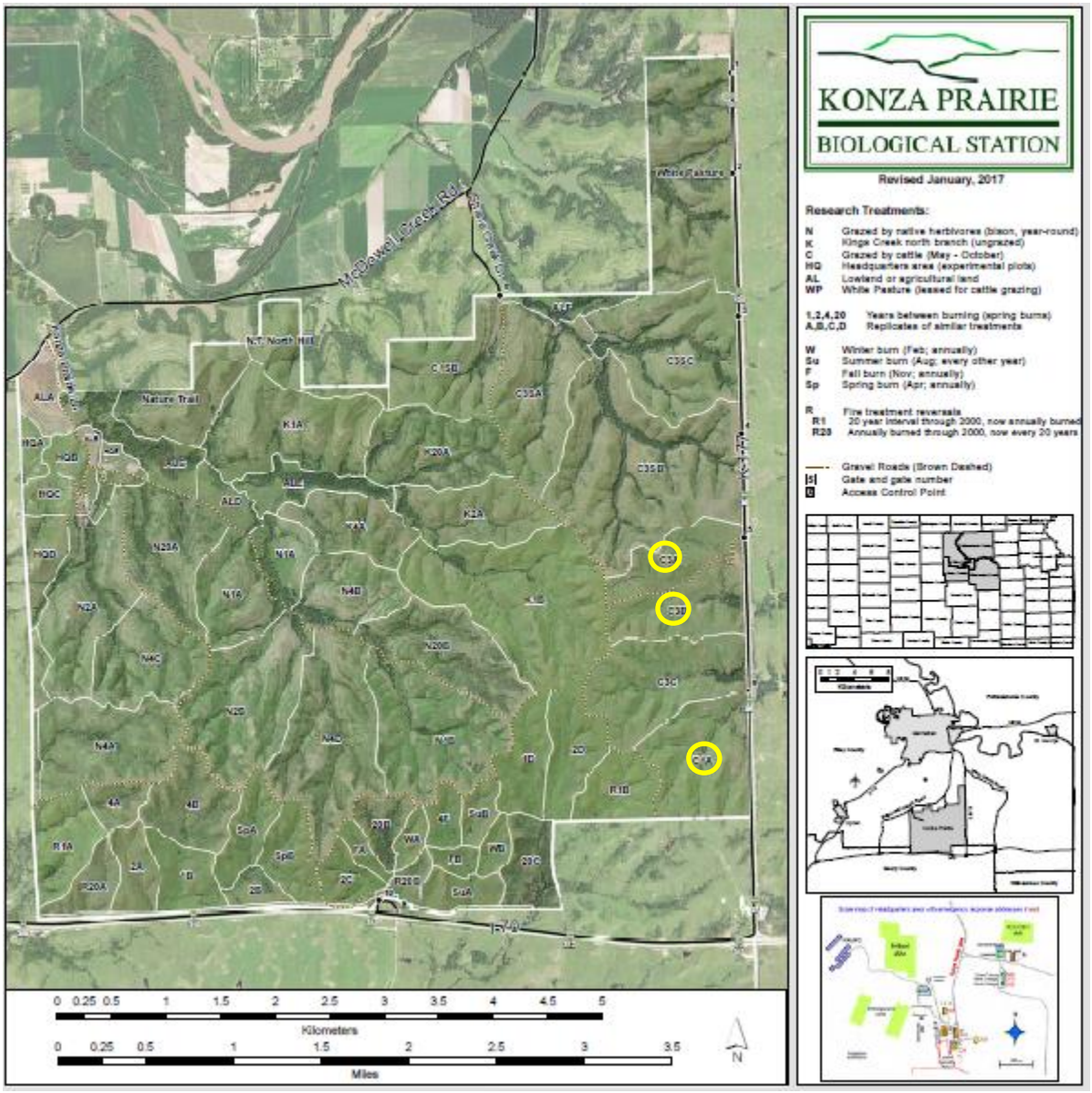

Figure 2.1 Map of Konza Prairie Biological Station, Kansas USA, and studied watersheds. C1A, C3A, and C3B. Figure 2.1 reproduced by using the original Konza prairie map. (kpbs.konza.k-state.edu/images/newkonzamap2017.pdf)

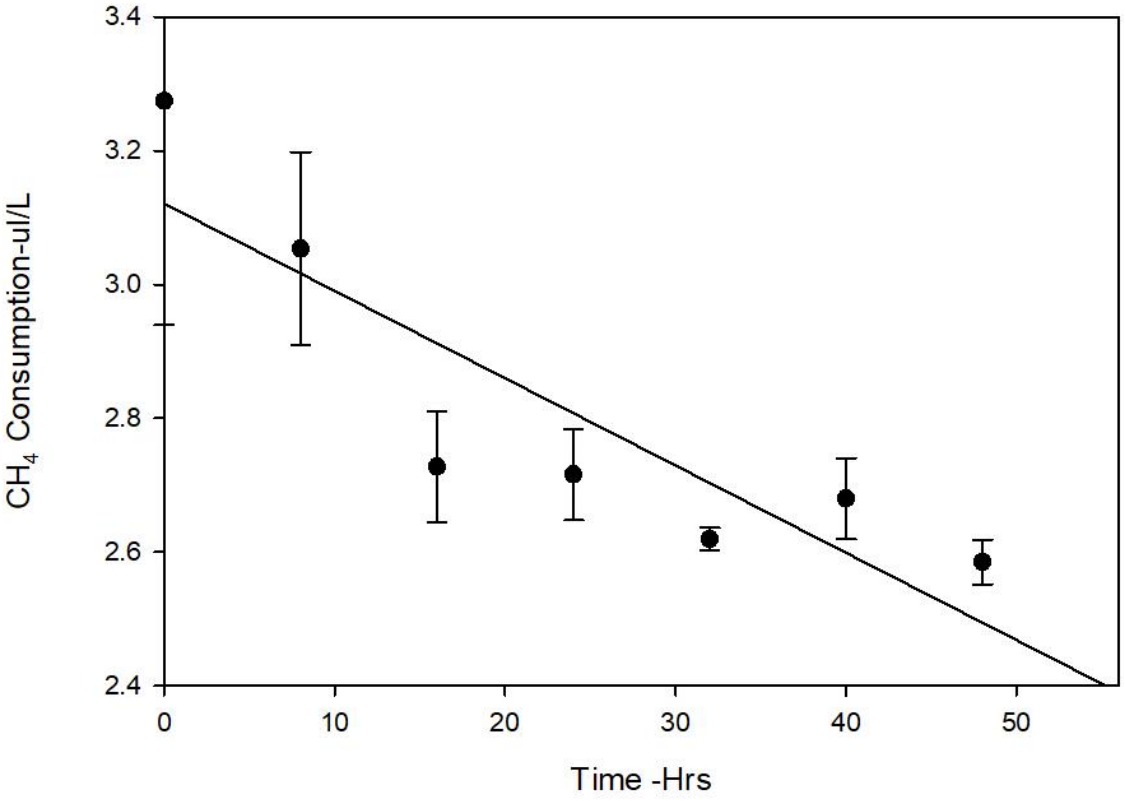

Figure 2.2 Change of methane concentration with time. Homogenized soil samples from Konza Prairie Biological Station site C3B incubated for 48 hours after adjusting the initial CH_4 concentration to 3 µl l^{-1}.

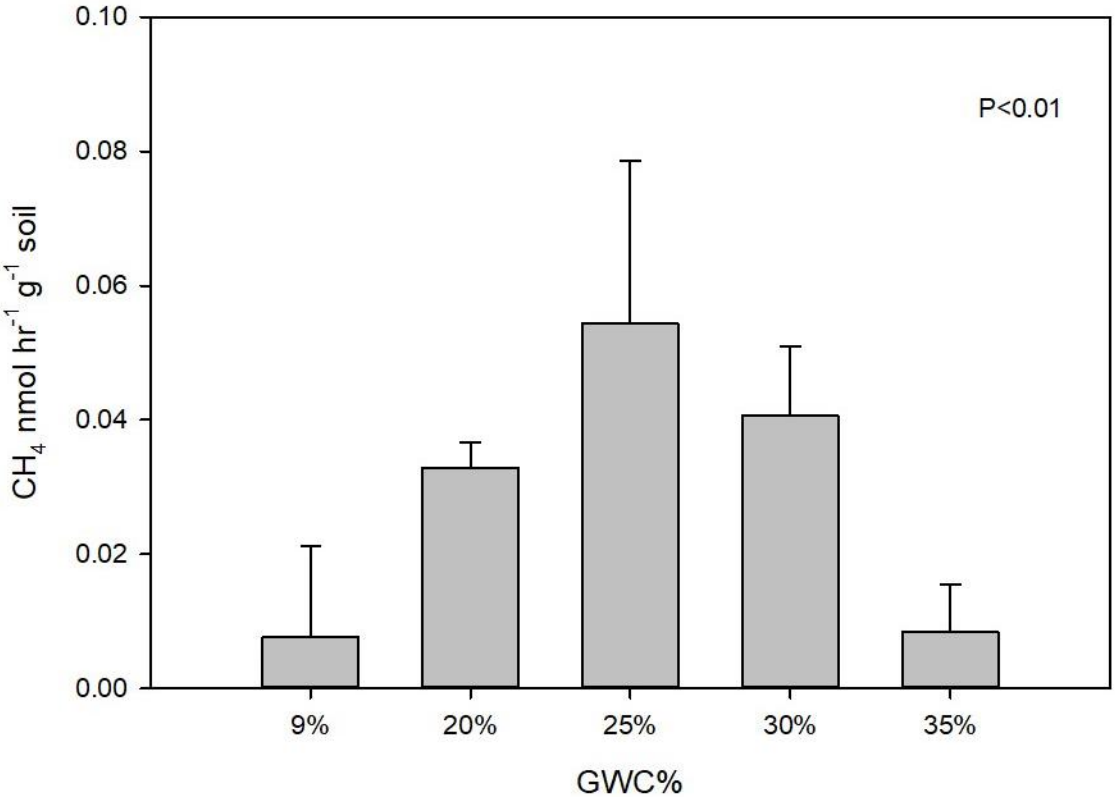

Figure 2.3 Methane oxidation rates for different gravimetric soil water contents (GWC%) of soil collected from Konza Prairie Biological Station C3B site.

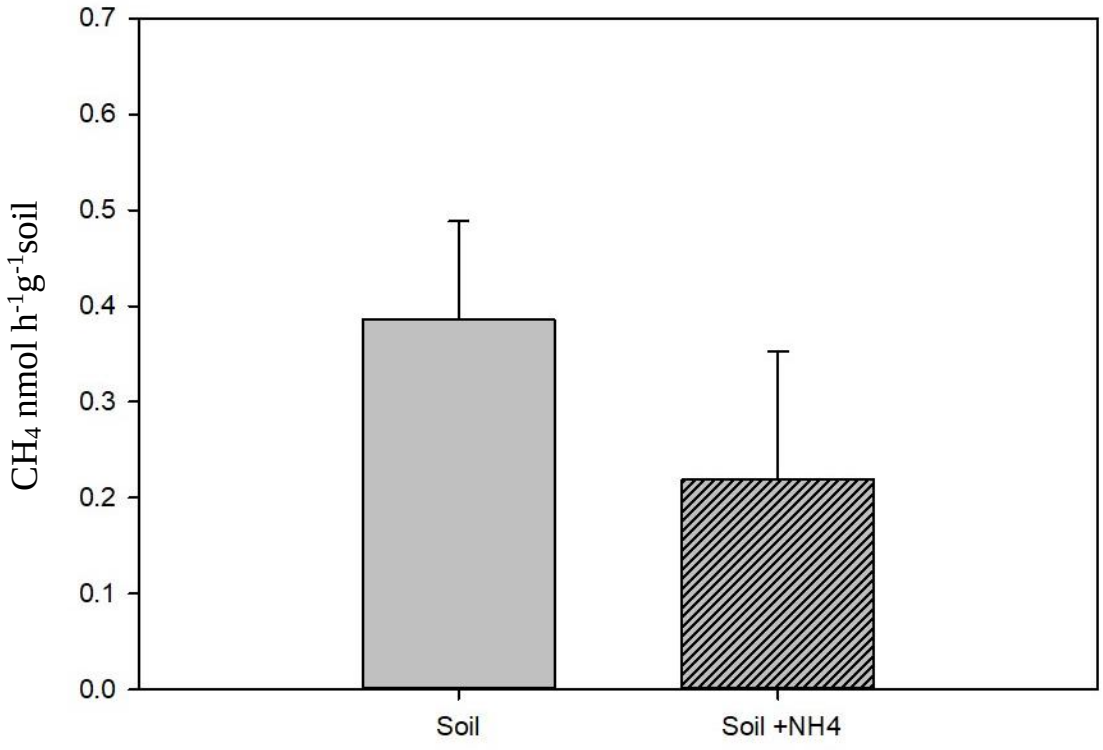

Figure 2.4 Effect of NH_4^+ addition on methane oxidation. Homogenized soil samples from Konza Prairie Biological Station (C3B) incubated with the addition of 50 µg N g^{-1} as NH_4Cl.

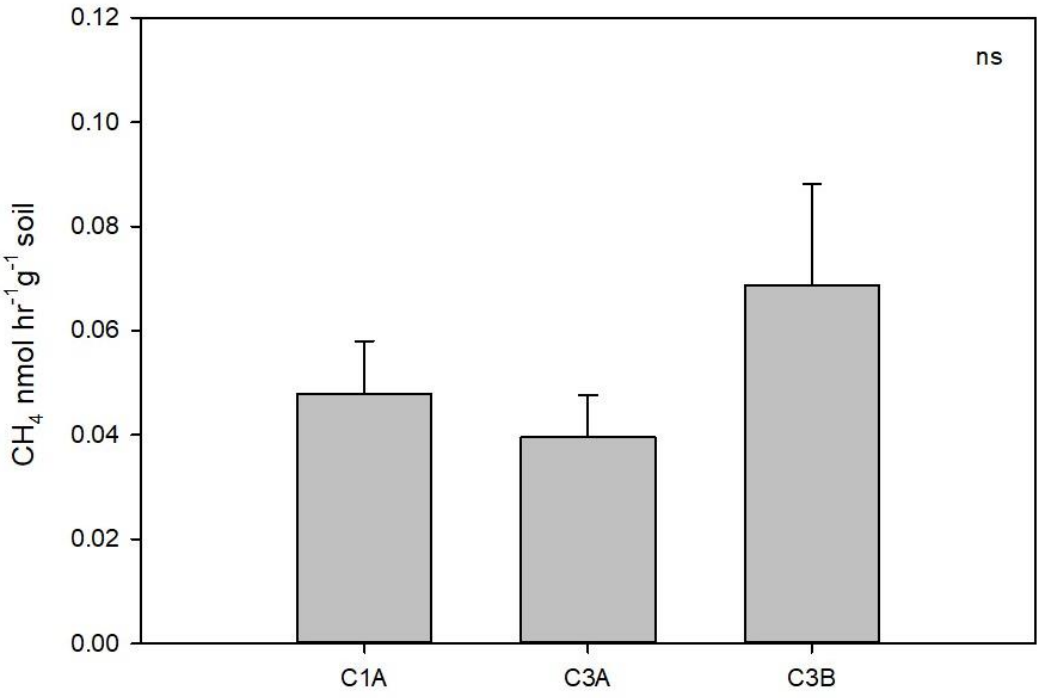

Figure 2.5 Methane oxidation rate of C1A, C3A, and C3B sites at Konza Prairie Biological Station

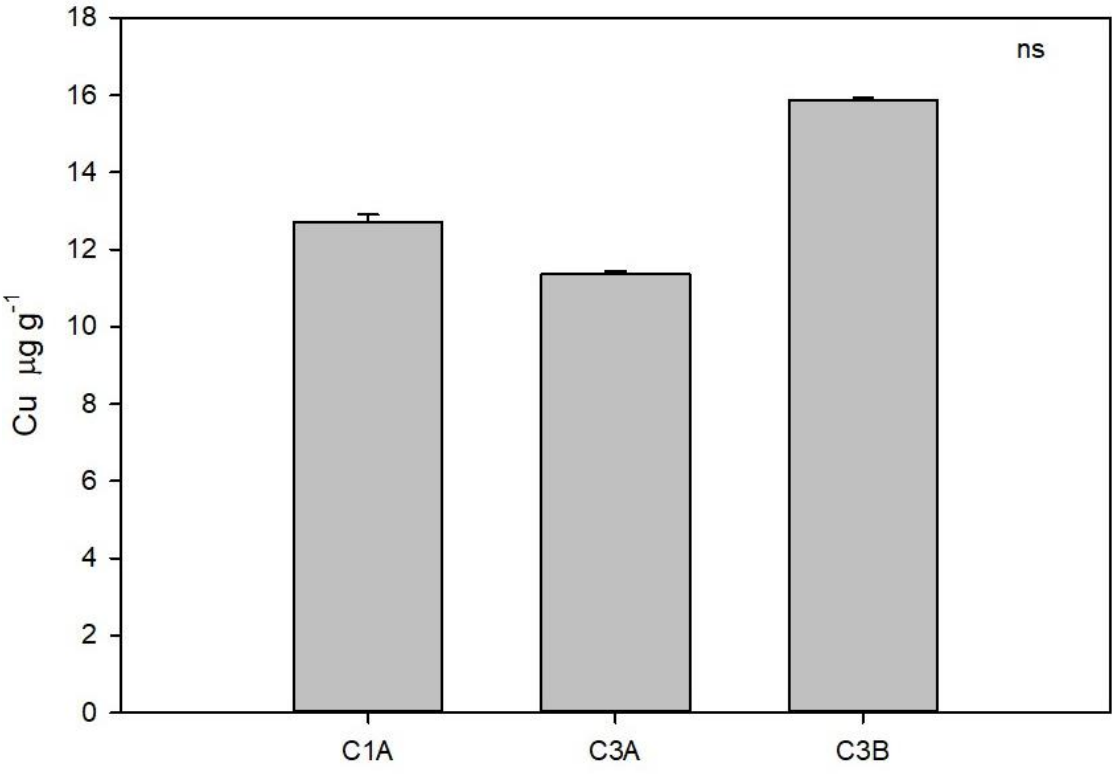

Figure 2.6 Total Copper concentration (µg g^{-1}) of C1A, C3A, and C3B sites at Konza Prairie Biological Station.

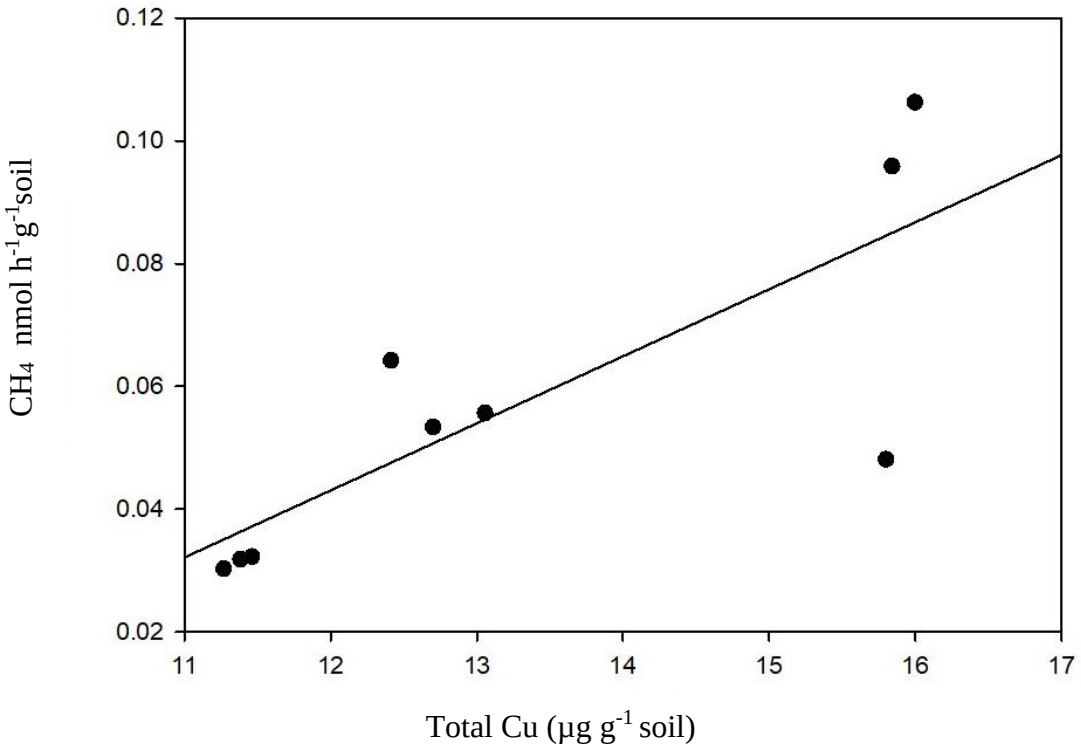

Figure 2.7 Correlation of CH_4 oxidation rates at C1A, C3A, and C3B sites with total Cu (P< 0.05, R2=0.67)

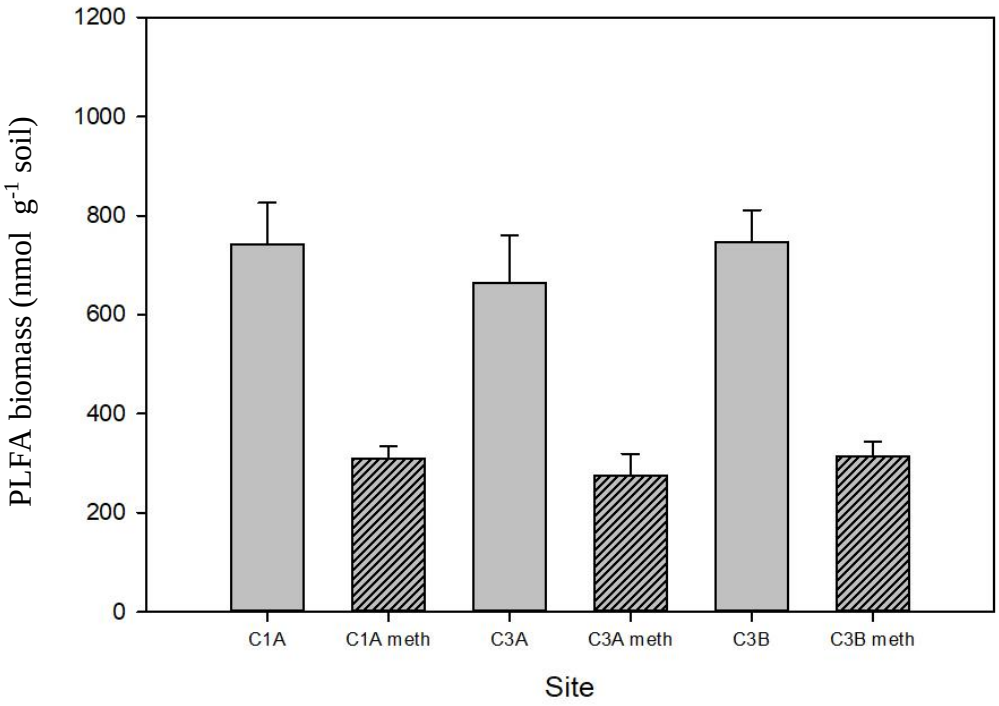

Figure 2.8 Comparison of Total PLFA biomass and methanotrophic biomass (meth) of C1A, C3A and C3B sites at Konza Prairie Biological Station. C1A total biomass (C1A), C3A total biomass (C3A) and C3B total biomass (C3B) and C1A methanotrophic biomass (C1A meth), C3A methanotrophic biomass (C3A meth), C3B methanotrophic biomass (C3B meth).

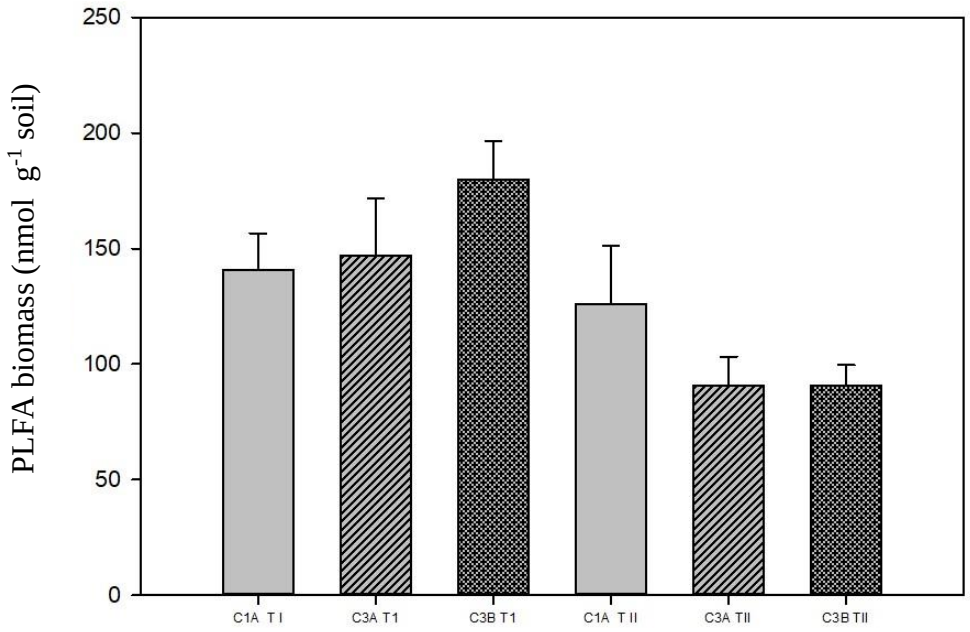

Figure 2.9 Comparison of Type I, Type II methanotrophic PLFA biomass of C1A, C3A and C3B sites at Konza Prairie Biological Station.

Chapter 3 - Land use effect on Methane Oxidation

Abstract

Methane (CH_4) is one of the critical anthropogenic greenhouse gases that has increased significantly with the beginning of the industrial era. Methane oxidation by methanotrophic bacteria mitigates CH_4 emissions. The effect of land use (native prairie, cropland and restored grassland) on CH_4 oxidation is not well known. The objective of this study was to investigate the CH_4 oxidation of soil from different land uses (native prairie, agriculture, and restored prairie) at two precipitation regimes in Kansas (Konza-850 mm y^{-1}, Hays-579 mm y^{-1}). Soil samples were collected from Konza Prairie Biological Station and at Hays Agricultural Experiment Station. The soils were classified as Reading silt loam at Konza and Harney silt loam at Hays. Homogenized soil samples were incubated at 25 °C at a gravimetric water content of 25% for 32 hrs. Methane (~2.5 µl L^{-1}) was added at the beginning of the incubation, and headspace gas samples analyzed over 32 hrs. Total copper, extractable copper, pH, soil extractable NH_4^+, and methanotrophic phospholipid Fatty Acids (PLFA) were measured for each soil sample. Methane oxidation was higher for the Konza location (0.033 nmol h^{-1} g^{-1} soil) compared to the Hays location (0.021 nmol h^{-1} g^{-1} soil). Methane oxidation was higher for restored grassland (0.050 nmol h^{-1} g^{-1} soil) and cropland (0.025 nmol h^{-1} g^{-1} soil) soils compared to native prairie (0.023 nmol h^{-1} g^{-1} soil) at the Konza location. For the native prairie, restored grassland, and cropland sites at Hays, methane oxidation was 0.021nmol h^{-1} g^{-1}soil, 0.020nmol h^{-1} g^{-1} soil and 0.022 nmol h^{-1} g^{-1} soil, respectively. Total soil microbial PLFA biomass, methanotrophic PLFA biomass, total Cu, extractable Cu content had significant differences with land use. Methane oxidation was significantly negatively correlated (P=0.07) to soil pH at the Konza site, where with soil pH greater than 7 inhibited CH_4 oxidation. Total Cu and CH_4 oxidation were significantly positively

correlated (P=0.007) at both sites as Cu is a key factor for methanotrophic enzymatic activity. A significant relationship of soil extractable Cu and CH_4 oxidation was not observed. Further, pH decreases available Cu content in the soil. These results suggest that soil Cu content is key driving factor for CH_4 oxidation.

Introduction

Land use management is one factor that affects global anthropogenic greenhouse gases (CO_2, CH_4, N_2O, etc.) (Stocker et al., 2013). Soils are both a source and sink of greenhouse gases (IPCC, 2019). As discussed by Ignell et al. (2009), land change related studies are needed to address global environmental changes. Transformation of native ecosystems to agricultural ecosystems alter environmental sustainability. Agriculture, forestry and other land-use practices were responsible for 23% of total anthropogenic greenhouse gas emission between 2007-2012 (IPCC, 2019).

From the years 2003 to 2012, global CH_4 emissions were estimated to be 558 Tg CH_4 $yr^{-1,}$ and about 60% of global emissions were anthropogenic (Saunois et al., 2016). Approximately 9-47 Tg CH_4 yr^{-1} is consumed by soil microbes, which is approximately 4-7% of the total CH_4 budget (Sabine et al., 2013). Direct human interventions have changed the atmospheric CH_4 balance by extensive industrial and agricultural development. (Sabine et al., 2013). Global CH_4 emission for agriculture and waste category for 2000-2012 was 56% of global anthropogenic CH_4 emissions (Saunois et al., 2016).

The impacts on soil physical, chemical, and biological properties from the conversion of native lands have been studied extensively. Soil water content, soil bulk density, porosity, soil C, N, and pH are altered when native grasslands were converted to cultivated lands (Macdonald et al., 2009)

Methane oxidation is a biological process that mitigates the atmospheric CH_4 concentration (Hanson et al., 1996; Saunois et al., 2016). Soil water content, temperature, pH, organic matter content, texture, soil redox, $NH4^+$, and copper are soil physio-chemical factors that affect methane oxidation by aerobic methanotrophic bacteria (Macinelli 1995; Fru 2011; Malyan et al., 2016). The impact of land use and management systems on soil CH_4 oxidation is not well understood. Therefore, it is vital to investigate the impact of land use on aerobic methanotrophic communities and factors that affect the methanotrophic abundance and activity comprehensively.

Conversion of native forest lands and grassland to croplands have led to a decline in the CH_4 oxidation by soil microbes. CH_4 oxidation was higher in forest lands (4.5 kg CH_4 ha^{-1} yr^{-1}) and upland grassland/shrublands (3.7 kg CH_4 ha^{-1} yr^{-1}) systems than in agricultural lands (1.5 kg CH_4 ha^{-1} yr^{-1}) (Singh et al., 2016; Aronson et al., 2013). Land use has affected the aerobic methanotrophic community's composition by altering the diversity of soil microbial communities and long-term N fertilizer usage had altered the methanotrophic composition in paddy soil (Singh et al., 2016). The addition of N fertilizer inhibits CH_4 oxidation soil (Labs, 2010). No-till improves CH_4 oxidation compared to tilled soils (Prajapati et al., 2014).

Methanotrophic communities behave differently with different land uses. Type I methanotrophs communities were dominant in pastures compared with Type II methanotrophs (Tate et al., 2012). Clusters of methanotrophic bacteria change with temperature regimes (Tate 2012; Mohanty 2007).

Soil type influences aerobic methanotrophic bacteria. Coarse textured soils have higher CH_4 oxidation than fine-textured soils due to better aeration (Mohanty et al., 2007). Soil organic matter also influences CH_4 oxidation as it influences soil water content and aeration. The

increase of soil organic matter significantly affected on CH_4 oxidation by enhancing the oxygen diffusion and reducing the water stress under different temperatures. (Christophersen et al., 2000).

Soil water and temperature impact CH_4 oxidation. Distinct aerobic methanotrophic community differences and activities were evaluated in three different precipitation zones: Konza prairie in Kansas, shortgrass steppe in Colorado, and Sevilleta in New Mexico (Judd et al., 2016). Methane oxidation kinetics suggested that higher K_m and V_{max} at Konza than Colorado and New Mexico sites. Further lower methanotrophic biomass e occurred at Konza (Judd et al., 2016).

With the rapid global environmental changes, land restoration is important. The prime goal of restoration is to restore, initiate and expedite the conversion of disturbed ecosystems to native ecosystems (Vaughn et al., 2010). As the native lands were used extensively for crop production, there is a need for ecological restoration. Tallgrass prairie restoration may enhance the biodiversity and re-established the original abiotic and biotic factors to compete with future environmental variabilities. Methane oxidation rates took more than 100 years to reach pre-cultivation level of woodlands in Denmark and Scotland (Priemé et al., 1997). The restoration of degraded lands can improve CH_4 oxidation by methanotrophic bacteria (Singh et al., 2016).

As net CH_4 oxidation may change due to the activity or the abundance of aerobic methanotrophic bacteria, it is important to investigate how land use affects CH_4 oxidation. The objectives of this study were to (i) investigate the CH_4 oxidation rates at different land use (native, restored and croplands) at two different precipitation regimes and (ii) investigate the effect of NH_4, total Cu, extractable Cu, soil C, PLFA, and pH on CH_4 oxidation.

Material and Methods

Study site

Two sites Konza and Hays, were selected with three land-use practices; native, restored grassland and cropland. Land use management practices and soil characteristics are summarized in Table 3.1.

Konza site is located at Konza Prairie Biological Station at Manhattan, Kansas. Three land use practices: Konza native (KNZ-N), Restored grassland (KNZ-R), and Crop (KNZ-C) were used for the experiment. The average annual rainfall and average temperature is 850 mm and 12.7°C respectively (Allen et al., 2018).

The plant community of the KNZ-N was dominated by perennial C_4 grasses, e.g. big bluestem, Indian grass and C3 herbaceous forb species (Heisler-White et al., 2009). The soil type of the study site was Tully-fine, mixed, superactive, mesic, pachic, argiustoll. The KNZ-N was annually burned with no-grazing. Prescribed burning management started in 1980 and the site was last burned in 2019 spring prior to soil sampling. The KNZ-R plots were cultivated prior to restoration and locally collected seeds source were introduced to restore the site in 1998. The soil series of the KNZ-R is a Reading silt loam fine silt, mixed, superactive, mesic Pachic Agriudoll. The KNZ-R site started in 2003 and burned on two-year interval and prescribed burning interval had changed time to time due various research needs. KNZ-R was last burned in 2019. The KNZ-C soil was a Reading silt loam fine, mixed, superactive mesic pachic vertic Agriudoll.

The Hays site was located at the Agricultural Research Extension facility at Ellis county (38°50’N, 99°19’W) Kansas. The average annual rainfall was 590 mm in Hays and an average monthly temperature of 12.1 °C (Allen et al., 2018). Hays native (HYS-N) site was mixed-grass prairie consisting of upland and lowland and used for cattle grazing. Little bluestem

(*Schizachyrium scoparium*), Sideoats Grama (*Bouteloua curtipendula*) were dominant C4 plants and forbs; Cuman ragweed (*Ambrosia psilostachya),* Purple prairie clover (*Dalea purpurea*) C3 plant was also present (Heisler-White et al., 2009). HYS-N soil is a Harney silt loam fine smectitic, mesic, pachic, Argiustoll. Hays restored (HYS-R) prairie was agricultural land and restored as a pasture with no recording of prescribed burning or natural wildfires.

The Hays restorative (HYS-R) site was a cultivated fruit orchard and it was killed by late freeze event during 1940's. Since then, the site was allowed grow back to native grass and HYS-R was used as pasture operations since 1999.

The Hays cropland (HYS-C) site was Harney silt loam as well. HYS-C site has historically been farmed as wheat-sorghum -fallow rotation with minimum tillage and sorghum was replaced by forage sorghum for feed. Nitrogen and P fertilizer were yearly applied 78 kg N ha^{-1} and 11.2-16.8 kg P ha^{-1} respectively. Soil samples were taken in May 2019.

Soil sampling and soil process

Soil samples were taken from each site with standard soil sampling probes (5.5 cm diameter) at a depth of 10 cm as described in sampling soils for nutrient management brochure (USDA-NRCS, 2005). Four random soil samples were collected and placed into collection bags (zip-lock bags) manufactured by SC Johnson & Sons company in Racine, Wisconsin, and stored in a cooler on ice. The collected soil samples were homogenized, and large roots, rocks, and plant residues were removed manually prior to sieve. The soil samples were sieved through a 2 mm sieve and subsampled for chemical and biological analysis. Four subsoil samples from each site were used for the lab incubation. The gravimetric water content of each soil sample was adjusted to 20-25% GWC.

Soil methane oxidation-Lab incubation

Gravimetric water content (GWC %) of the sieved soil samples were analyzed following standard lab procedures. Ten grams of soil samples were dried at 105°C for 24 hours to determine GWC for each sample. Soil (10g) was added into 250 mL Erlenmeyer flasks for lab incubation and GWC was adjusted to 25% prior to the CH_4 addition. Four replicates from each plot were taken for the analysis. After adjusting to the desired GWC the soils were pre-incubated at 22 ^oC for 5-7 days. The Erlenmeyer flasks were placed in mason jars (940mL) and made airtight by sealing the cap. Silicone gel was applied to all lids to minimize air leaks. Headspace CH_4 concentration was adjusted (~ 2.8 µl L^{-1}) by injecting CH_4. 10 mL of headspace gas was collected to pre-evacuated glass vials (25ml) at every 8 h (0, 8,16, 24, 32) for 32 hours to analyze for CH_4 concentration were analyzed by gas chromatography (GC) using a Brucker model 456-GC with fully automated autosampler (Scion instruments in Livingston, Scotland). GC was equipped with flame ionization detector (FID), thermal conductivity detector (TCD) and electron capture detector (ECD). Temperature and detectivity of FID, TCD and ECD 450°C-1.4 pg C/sec, 450°C -300pg/ml, 450°C -7fg/s respectively and ECD equipped with ^{63}Ni -15mCi radioactive source (555Mbq).

Ideal gas law equation used for the calculation. All calculations considered the ambient condition. Air pressure and temperature were taken as 0.96 atm and 298.5 K. Headspace volume was 0.925 L (including mason jar and Erlenmeyer flask).

Soil Inorganic Nitrogen content

Soil inorganic N, NH_4^+ and NO_3^-, was extracted by adding 100 mL of 1M KCl to 25g of moist soil, shaken for 60 min on an orbital shaker at 300 rpm and filtered through Whatman No.

42 filter paper into a 20 mL scintillation vial. The extract was analyzed for NH_4-N and NO_3-N by colorimetric analysis at KSU Soil Testing Lab Provide reference.

Total and extractable Copper (Cu) content in the soil

For total and extractable Cu, samples were dried at 40°C for 24 hours and ground with pestle and mortar prior to the digestion.Both soil digestion and DTPA extraction procedure were conducted by Kansas State University soil chemistry lab. For the digestion, 1g of dry soil was digested with repeated additions of HNO_3 acid and H_2O_2 (Sposito et al., 1982). For extractable soil Cu, 10 g of soil was analyzed by the DTPA extraction method (Lindsay & Norvell, 1978). Soil digestion and DTPA extractions were analyzed by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP optical emission spectrometer, manufactured by Varian Australia (PVT) Ltd, Mulgrave, Victoria, Australia.

Phospholipid Fatty acid analysis (PLFA)

Phospholipid Fatty Acid (PLFA) analysis used to identify the abundance of soil microbial biomass and analyzed the various group functional groups of soil microbiota by using the known PLFA biomarkers. Soil was sampled from the topsoil (0-5 cm). All the fresh soil samples were collected to plastic zip lock freezer bags and unique sample identification was attached. Samples were frozen at -4oC. A Styrofoam cooler with regular ice packs was used for the storage of soil samples. Samples were shipped to Ward Laboratories Inc, Kearney, NE for PLFA analysis. Sample results were analyzed as nanomole (nmol). Specific aerobic methanotrophic community's PLFA biomarkers (i16:0, 14:0, 16:1ω7c, 16:1ω5t, 16:1ω8c, 16:1ω7c, 16:1ω5c, 18:1ω7c, 16:1ω6c, 18:1ω8c, 18:2ω6c, 18:2ω12c, 18:2ω7c, i14:0, a15:0, 18:0) were analyzed for

the methanotrophic abundance in the soil samples. Total abundance of soil microbial biomass and methanotrophic bacteria biomass were and analyzed for each land-use (Table 3.3)

Statistical Analysis

All the data were presented as the mean values and standard error (Table 3.2). Analysis of variance (ANOVA) was used to evaluate differences in microbial and chemical properties and significant differences of each variable were analyzed by Tuckey pairwise comparison with a p=0.05. Minitab 19 (2019) statistical software used to conduct statistical analysis (www.minitab.com). All the figures (vertical bar plots and simple scatter plots) were prepared using Sigma plot 12.5 software (Systat Software, San Jose, CA). Further NRCS Pedon description data (MAPS project) and Konza Prairie Biological Station 's Long Term Ecological Research (KPBS LTER) online data (Konza Prairie LTER) were used to evaluate the soil biological, chemical and physical characteristics of each site.

Results

Methane Oxidation

Methane oxidation was not statistically significant between soils (Fig. 3.1). The highest CH_4 oxidation rate was 0.033 nmol g^{-1} soil h^{-1} from Konza soil compared with Hays of 0.021 nmol g^{-1} soil h^{-1}. Land use affected CH_4 oxidation. The highest CH_4 oxidation was 0.050 nmol g^{-1} soil h^{-1} for the KNZ-R. CH_4 oxidation rate was 0.026 nmol g^{-1} soil h^{-1} for KNZ-C and 0.023 nmol g^{-1} soil h^{-1} for KNZ-N. The lowest rate of CH_4 oxidation was 0.020 nmol g^{-1} soil h^{-1} for the HYS-R. Methane oxidation rate 0.022 nmol g^{-1} soil h^{-1} for HYS-C and 0.021 nmol g^{-1} soil h^{-1} for HYS-N.

Total Copper and extractable Cu

Total Copper (TCu) was significantly affected by land use ($p<0.05$) at Konza (Fig. 3.2). A significantly higher concentration of 17.2 µg g^{-1} TCu was measured at KNZ-R. T Cu of KNZ - C and KNZ-R were 15.3 µg g^{-1} and 11.7 µg g^{-1} respectively. The highest TCu of 11.9 µg g^{-1} was at HYS-C at Hays. T Cu of HYS-N and HYS-R were 11.2 µg g^{-1} and 11.3 µg g^{-1}, respectively. Overall higher TCu was at the Konza (14.8 µg g^{-1}) compared to Hays (11.4 µg g^{-1}).

Extractable Copper (ExCu) followed the same trend as TCu for all sites (Fig. 3.3). Land use affected ExCu ($p<0.05$) significantly. Significantly higher ExCu of 2.95 µg g^{-1} was at KNZ-R and in contrast to HYS-R with the lowest ExCu content of 1.17 µg g^{-1}. Overall significantly higher levels of ExCu were measured at Konza than Hays.

PLFA biomass, ratios and Methanotrophic specific activity

There was a significant difference in total PLFA biomass and methanotrophic biomass ($p<0.05$) (Fig. 3.4 & Fig. 3.5). For both KNZ-N and HYS-N sites, total PLFA was 804 nmol g^{-1}soil and 763 nmol g^{-1}soil, which were significantly higher than 173 nmol g^{-1}soil for HYS-C. Both KNZ-R and HYS-R had similar trends for total PLFA. Land use significantly affected total PLFA and both sites showed a similar trend for total PLFA. Methanotrophs biomass was significantly affected by land use ($p<0.05$). Methanotrophic biomass was significantly greater in the native prairie sites, 299 nmol g^{-1}soil PLFA and 322 nmol g^{-1}soil PLFA at HYS-N and KNZ-N, respectively. Methanotrophic biomass was lower at the agricultural sites; 81 nmol g^{-1}soil and 160 nmol g^{-1}soil for HYS-C and KNZ-C, respectively. Both HYS-R and KNZ-R had 265 nmol g^{-1}soil and 273 nmol g^{-1}soil of methanotrophic PLFA, respectively. Both Hays and Konza had similar trends in methanotrophic biomass PLFA with land use. Methanotrophic PLFA biomass to

total biomass ratios were not significant (Fig.3.6). However, lower biomass ratios were observed in native sites than rethe restored prairie and crop sites.

Methanotrophic specific activity was calculated for the sites (Fig.3.7.). The HYS-C site had the highest methanotrophic specific activity for all sites. The lowest methanotrophic specific activity was for the native site.

Total Carbon (TC) and pH

Total Carbon was generally higher at Konza than Hays (Fig 3.8). Native prairie was higher and the cropland with restored grassland were intermediate. Significant differences for pH was observed (Fig.3.9). Average pH of KNZ-N and KNZ-C was pH- 7.3 and KNZ-R was pH 6.9. Lower pH (<6.9) were observed at Hays sites. HYS-N had lowest pH 5.9 and HYS-R and HYS-C demonstrated pH 6.6 and pH 6.8, respectively.

Soil NH_4^+ content

Land use affected soil NH_4^+ (Fig 3.10). The HYS-C had significantly higher levels of NH4+ ($p<0.05$) of 1.18 µg N g^{-1} while the other soils were below 0.5 µg N g^{-1}.

Correlations of and Total Cu and pH

Methane oxidation for both sites was significantly correlated with TCu content (<0.05) (Fig 3.11). Methane oxidation was clustered around 9 -15 ppm of TCu and a slight positive increase of CH_4 oxidation. However, there were significant p=0.007 the R-sq was 0.32 and which suggested that regression models did not explain much variation.

There was a significant correlation with soil pH and CH_4 oxidation in Konza ($P<0.1$) (Fig. 3.12). This negative relationship suggests that higher pH values inhibited CH_4 oxidation. Values of pH ranging from 6.8 to 7.0 were associated with higher CH_4 oxidation than for pH >7.5 suggesting inhibition of CH_4 oxidation in Konza soils. There was no significant relationship of pH, and CH_4 oxidation was observed in the Hays site.

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Discussion

Comparison of CH_4 oxidation rates with soil chemical, physiological and biological characteristics offers a comprehensive understanding of aerobic methane oxidation in soil. Methane oxidation rates of both sites (Hays and Konza) were distinct.

The methane oxidation rates measured in this study were similar to others (Powlson et al., 1997; Amaral et al., 1998) in various ecosystems, including grassland and agricultural ecosystems. Grassland and reclaimed sites had oxidized 0.024 to 0.004 nmol $CH_4\ g^{-1}\ h^{-1}$ (Jacinthe and Lal, 2006). The high CH_4 oxidation rates for the KNZ-R site in contrast to most studies where higher oxidation usually occurred in native soils (Chan et al., 2001).

There was a significant correlation of CH_4 oxidation for the Konza site with pH ($p<0.05$). High pH (pH >7) inhibited CH_4 oxidation and pH 6-7 had high CH_4 oxidation rates. Previous studies demonstrated methanotrophic species had optimum CH_4 oxidation rates with different pH levels. *Methulosinus trichosporium*, *Methylobacter luteus* and M*ethylosinus trichosporium* had optimum CH_4 oxidation between 6-8 pH. *Methylobacter luteus* and *Methylosinus trichosporium* oxidation was inhibited at pH >7 (Amaral et al.,1998), The Hays sites had lower pH <7.0, and narrow with no relationship of pH with CH_4 oxidation.

The KNZ-R prairie had higher CH_4 oxidation than all other sites. KNZ-R site was 26 years of restored prairie and was not burned since 2013. Total Cu and extractable Cu were

significantly different for the KNZ-R site which had higher CH_4 oxidation. Copper is a co-factor of the methane monooxygenase enzyme. Soil Cu levels were correlated with CH_4 oxidation rates.

Dissolved organic carbon content might be a key driving factor for higher CH_4 oxidation at KNZ-R (Zhou et al., 2014). DOC can complex Cu^{2+} by N-bearing moieties. This can be especially significant at low solution Cu concentration. The S-curve isotherm can usually describe the adsorption of Cu in soil. At low solution Cu concentrations, complexation of Cu with DOC tends to keep Cu in solution. Increased Cu concentration in soil solution promotes the adsorption of Cu, once the Cu concentration exceeds to complexation capacity of the DOC. (Essington, 2005).

Soil methanotrophic PLFA biomass was affected by land use. There were no distinct differences in biomass abundance when comparing the two sites (Hays and Konza). Relatively higher total microbial biomass and methanotrophic biomass were observed at native plots compared to restorative plots, however, the CH_4 oxidation rates were lower in native soils than restorative plots. The methanotrophic enzymatic activity might play a key role at these conditions when it comes to aerobic CH_4 oxidation. The expression of methane monooxygenase is dependent upon soil Cu (Chi Fru, 2007). However, other chemical and physiological factors also contribute to methanotrophic community distribution in the soil. The abundance and diversity of methanotrophic bacteria were strongly related to iron oxyhydroxide (Chi Fru, 2007). Ion hydroxides considered as the most available iron mineral and which is capable of retaining the complexation of Cu in the soil. Further, the di-iron atoms in the soluble methane monooxygenase enzymatic activity might influence by the available iron content in the soil as it

associates with a di-iron molecule (Banerjee et al., 2019). Additional studies are needed to investigate the factors that effect on CH_4 oxidation in soil.

Conclusion

A comprehensive understanding of biotic and abiotic factors that affect CH_4 oxidation is important for the future greenhouse mitigation decision-making process. The above finding shows that CH_4 oxidation was affected by land use. Methanotrophic biomass was significantly different from land-use. Total Cu was significantly correlated with CH_4 oxidation. Extensive investigation on soil chemical, methanotrophic enzymatic activities and high-resolution methanotrophic community composition measurements might need to comprehend the methane oxidation process in future researches.

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Table 3.1 Site description, land use management practices and soil characteristics (0-5 cm)

site	Land use	Lat: & Long:	Soil series	Soil type	Silt	Clay	Sand	C
					%			
Konza Native (KNZ-N)	Annual Burn, No grazing, Last burn 2019 spring	39° 6' 20.02" north 96° 36' 36.14" west	Tully	Fine, mixed, superactive, mesic Pachic Argiustoll	61.0	28.3	10.7	3.53
Konza restorative (KNZ-R)	Typically burn in 4 yrs, last burn 2013, No grazing	39° 6' 11.77" north 96° 36' 15.34" west	Reading	Fine, mixed, superactive, mesic Pachic Vertic Argiudoll	61.0	33.0	6.0	2.70
Konza Crop (KNZ-C)	Agricultural land	39° 42' 14.04" orth 96° 36' 25.81" west	Reading	Fine-silty, mixed, superactive, mesic Pachic Vertic Argiudoll	53.3	39.1	7.6	1.63
Hays Native (HYS-N)	Native pasture, Grazing, no burn records	38° 50' 7.70" north 99° 18' 12.20" west	Harney	Fine, smectitic, mesic Pachic Argiustoll	56.9	28.8	14.4	1.77
Hays Restorative (HYS-R)	Restored Pature, Grazing, No burn records	38° 50' 39.48" north 99° 18' 58.58" west	Harney	Fine, smectitic, mesic Typic Argiustoll	10.0	30.0	10.0	2.90
Hays Crop (HYS-C)	Agriculture land	38° 50' 34.10" orth 99° 18' 52.90" west	Harney	Fine, smectitic, mesic Pachic Argiustoll	44.0	36.0	20.0	1.07

Table 3.2 Methane oxidation (nmol h^{-1} g^{-1} soil), NH_4 (µg g^{-1}), Extractable Cu (µg g^{-1}) Total Cu (µg g^{-1}), pH and Total C (g kg^{-1}).

Site	CH_4 nmo g^{-1} h^{-1}	NH_4 µg g^{-1}	Extract Cu-µg g^{-1}	Total Cu-µg g^{-1}	Total C	PLFA-nmol	pH
HYS-N	0.0243	0.413	1.32	11.2	18	299	6.0
HYS-R	0.0225	0.340	1.17	11.2	29	265	6.6
HYS-C	0.0248	1.18	1.61	11.9	11	81.1	6.9
KNZ-N	0.0258	0.448	1.66	11.7	35	322	7.3
KNZ-R	0.0571	2.94	2.94	17.2	27	274	7.0
KNZ-C	0.0292	2.13	2.13	15.3	16	160	7.3

Table 3.3 PLFA biomarkers of methanotrophic bacteria

Methanotrophs	PLFA	References
Type I Methanotrophs	16:0, 14:0, 16:1ω7c, 16:1ω5t, 16:1ω8c, 16:1ω7c, 16:1ω5c, 18:1ω7c, 16:1ω6c	Nazaries et al. 2013; Yao et al. 2015; Sundh et al.,2018; Bodelier et al. 2009;
Type II Methanotrophs	18:1ω8c, 18:2ω6c, 18:2ω12c, 18:2ω7c	Nazaries et al. 2013; Yao et al. 2015; Sundh et al.,2018; Bodelier et al. 2009;
Verrucomicrobia	i14:0, a15:0, 18:0	Nazaries et al. 2013; Yao et al. 2015

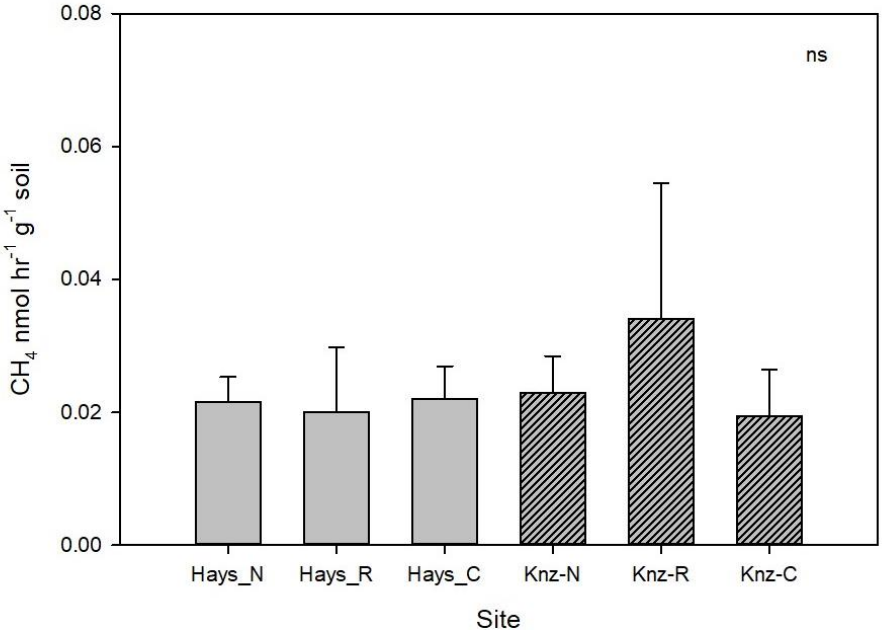

Figure 3.1 Methane Oxidation of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). Methane oxidation values were in nanomole per hour per gram of soil (nmol h^{-1} g^{-1} soil)

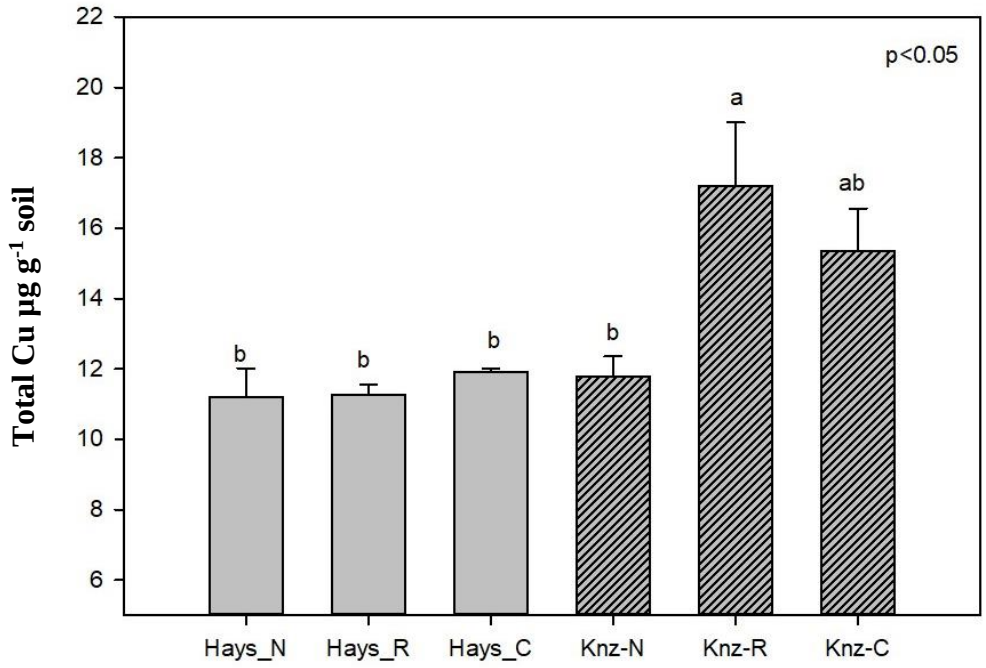

Figure 3.2 Total Copper content (µg g^{-1}) of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C).

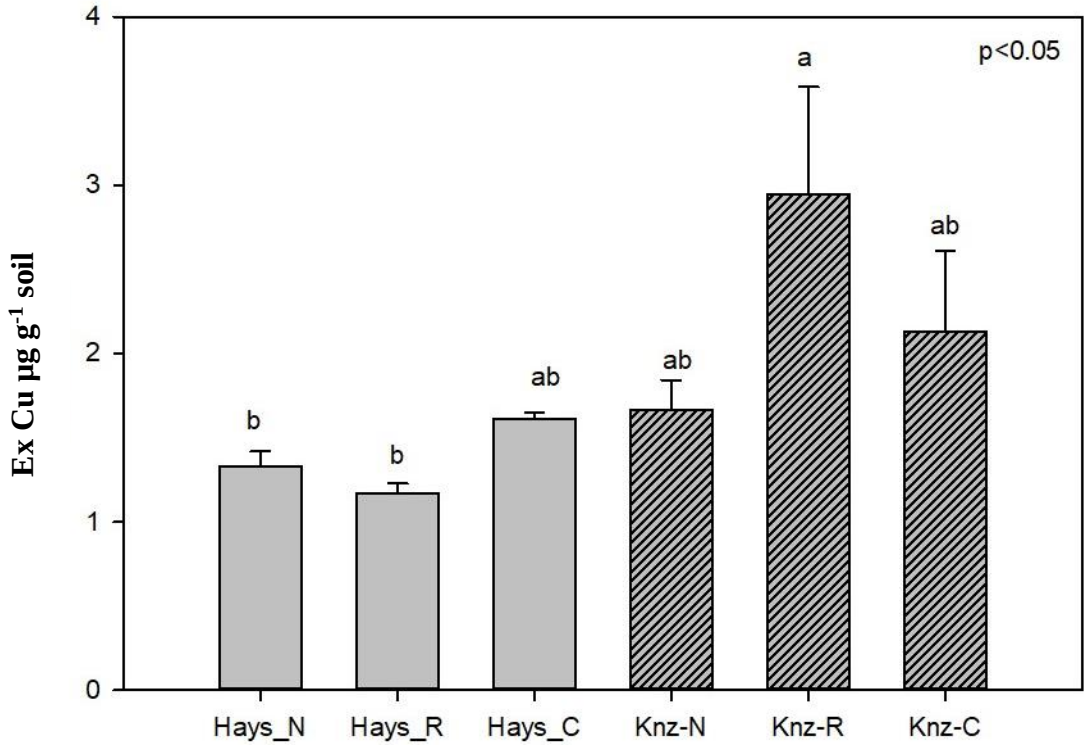

Figure 3.3 Extractable Copper content (µg g^{-1}) of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C).

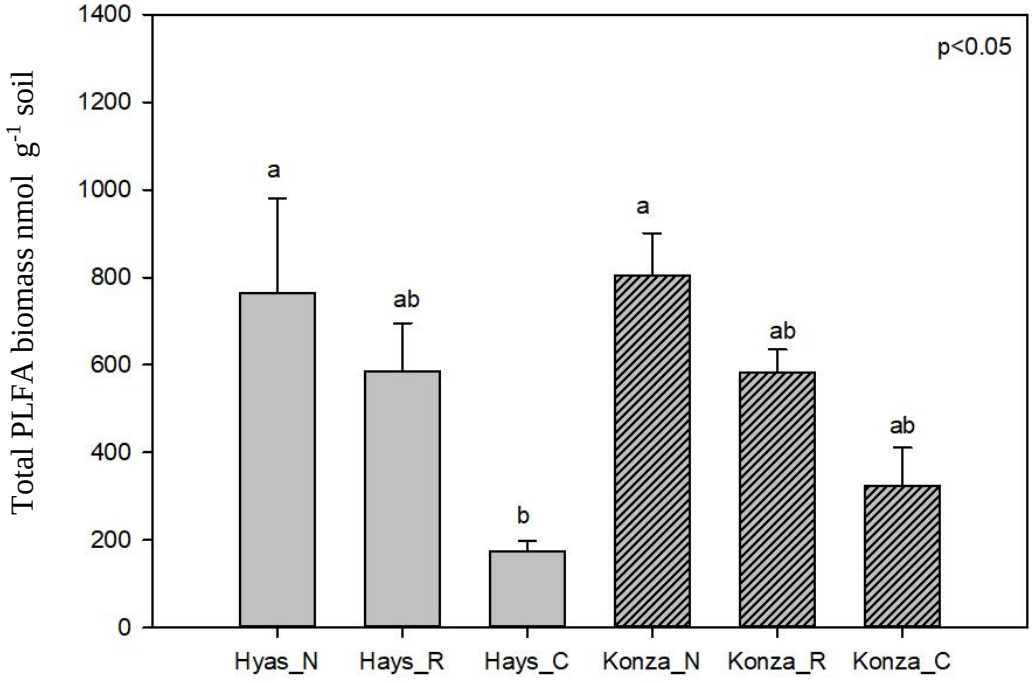

Figure 3.4 Total PLFA biomass of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). All values are in nmol-nano moles.

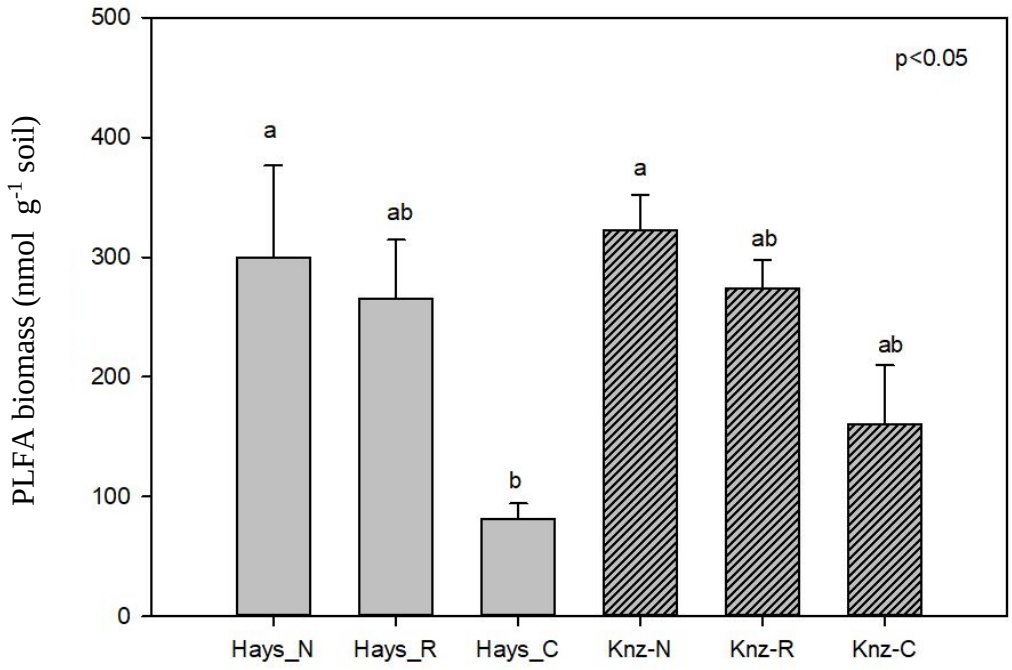

Figure 3.5. Methanotrophic PLFA biomass of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). All values are in nmol-nano moles.

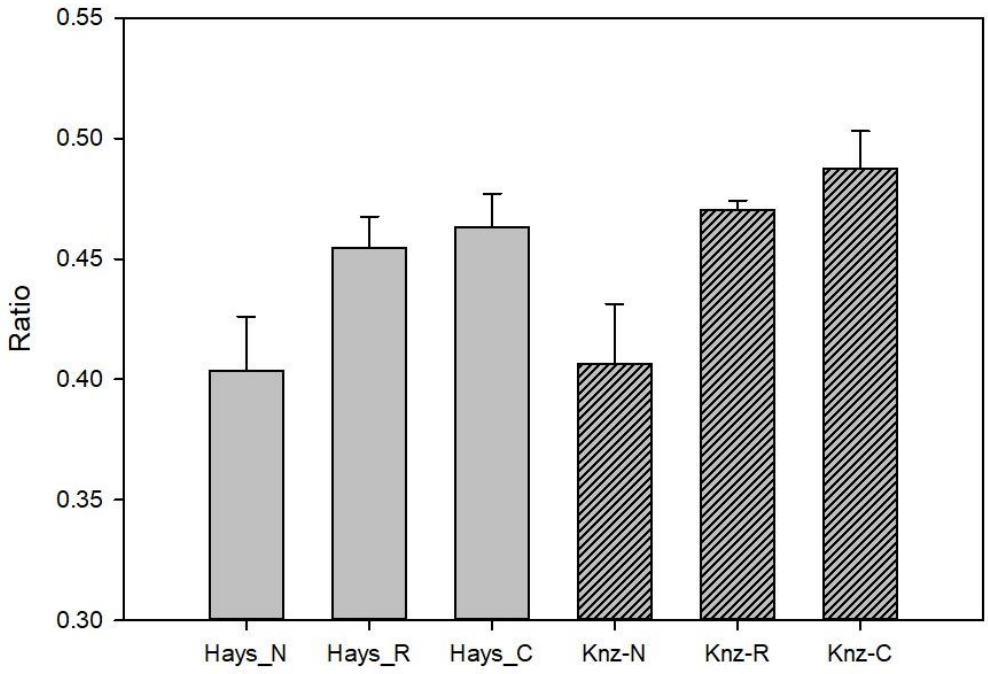

Figure 3.6 Methanotrophic PLFA biomass to total PLFA biomass ratios of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C).

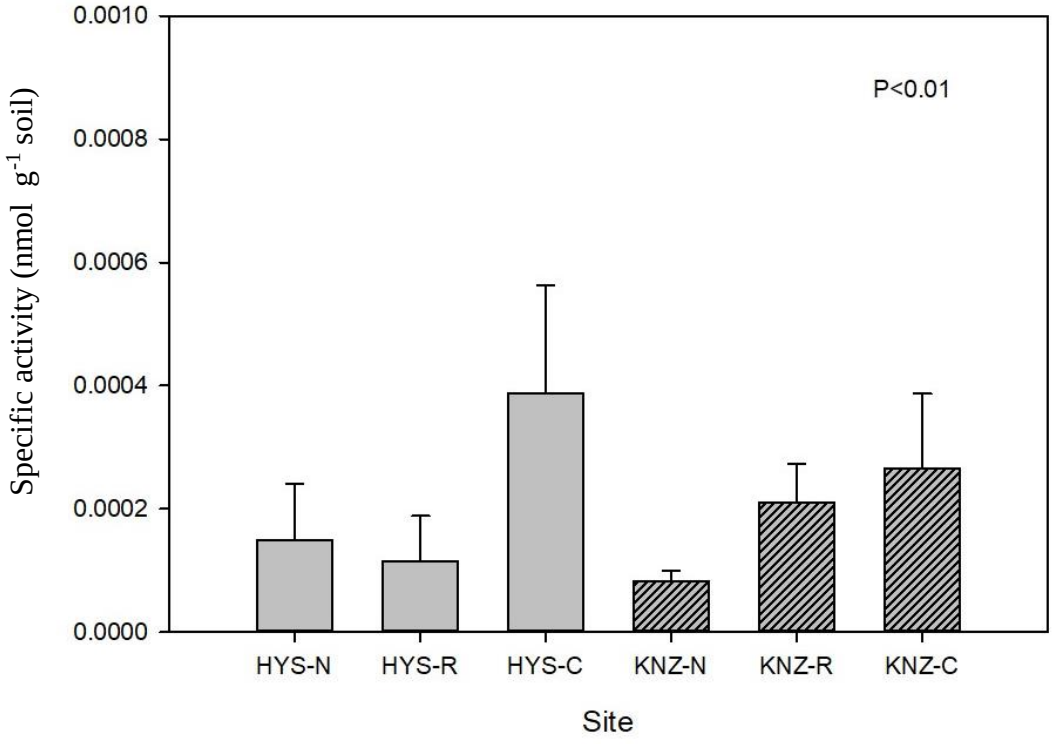

Figure 3.7 Specific activity of all the six sites at Hays and Konza. Methane oxidation Methanotrophic biomass to methane oxidation ratios are in Y-axis and experimental sites are on the X-axis.

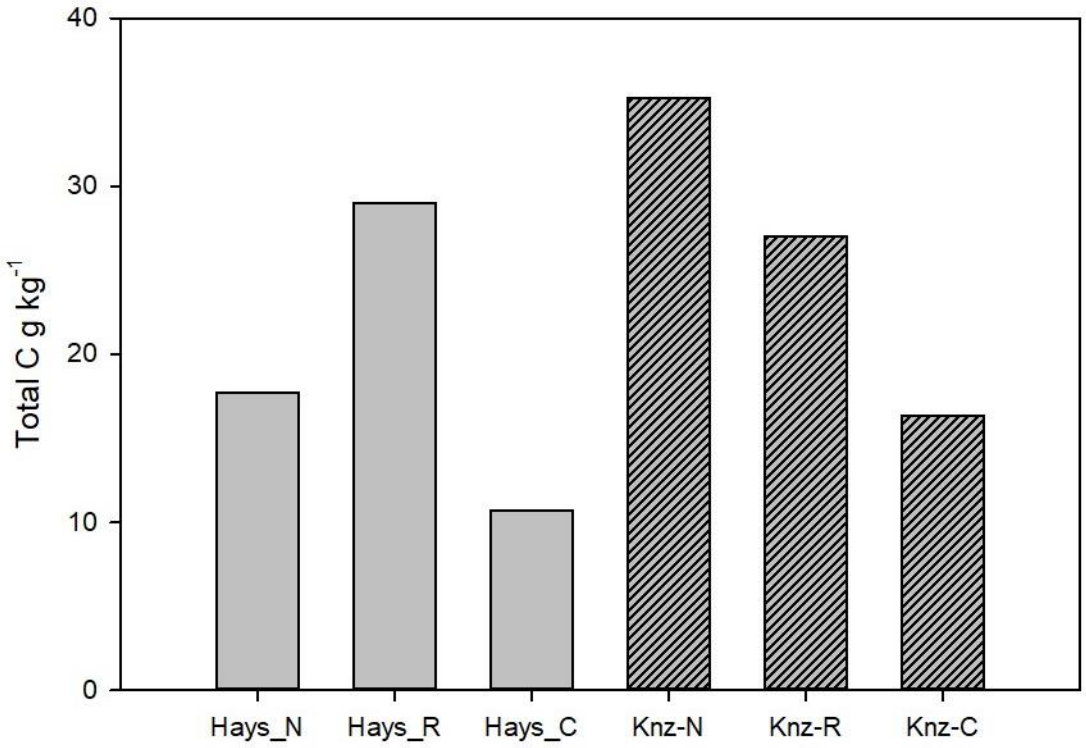

Figure 3.8 Total Carbon (TC g kg $^{-1}$) of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C)

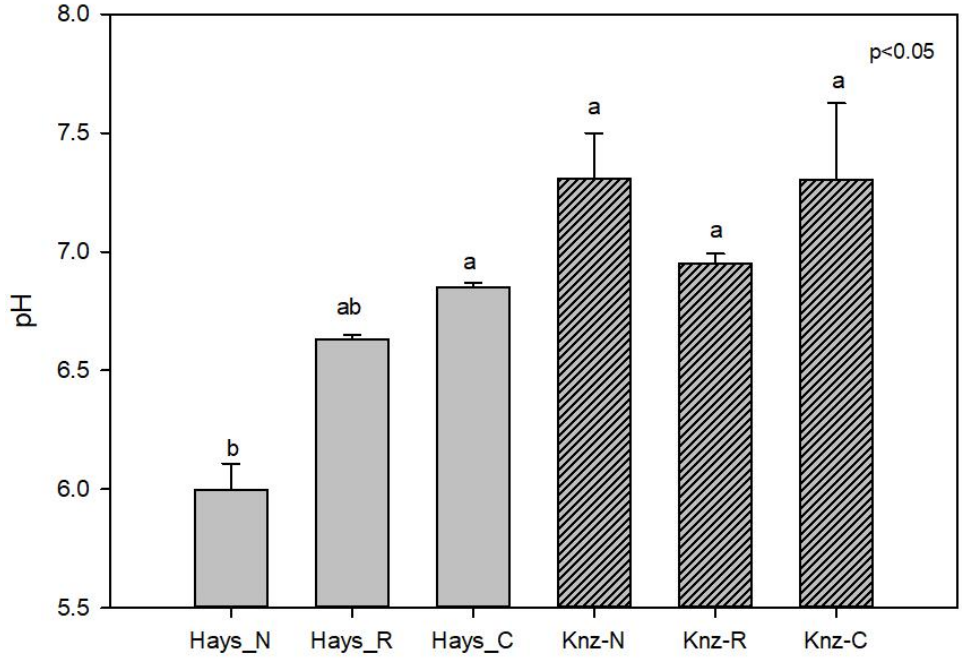

Figure 3.9: Soil pH of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C)

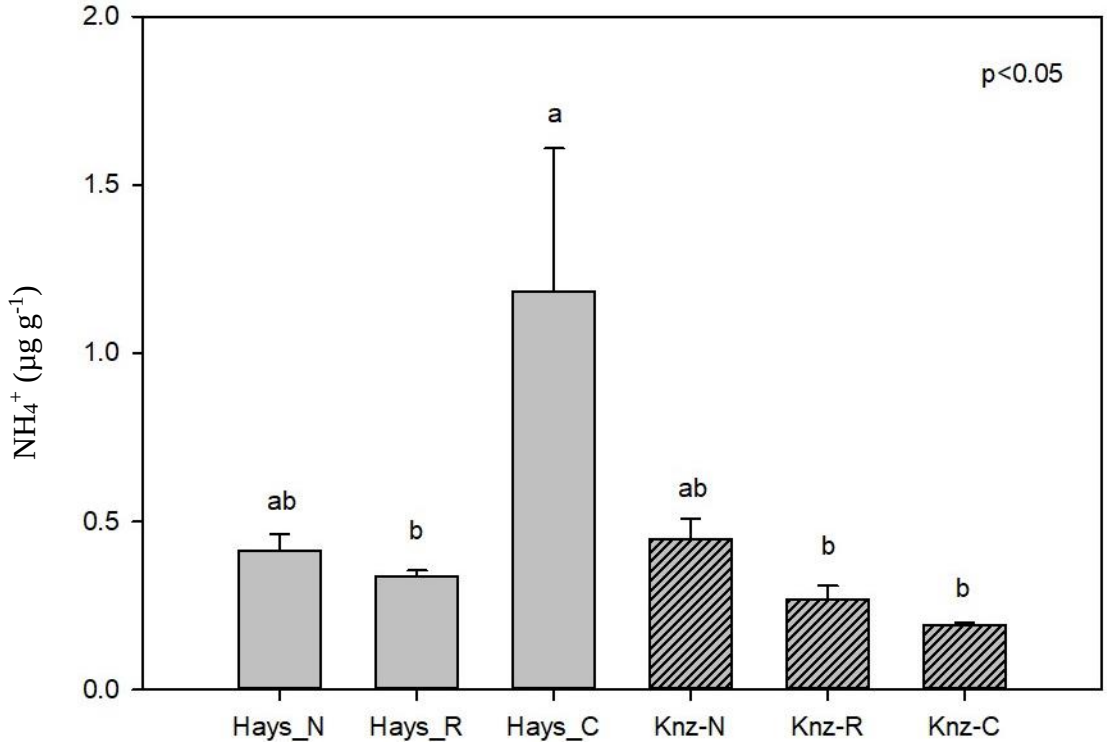

Figure 3.10 NH4 Concentration (µg g-1) of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). $NH4^+$ concentration- microgram per gram of soil (µg g^{-1} soil).

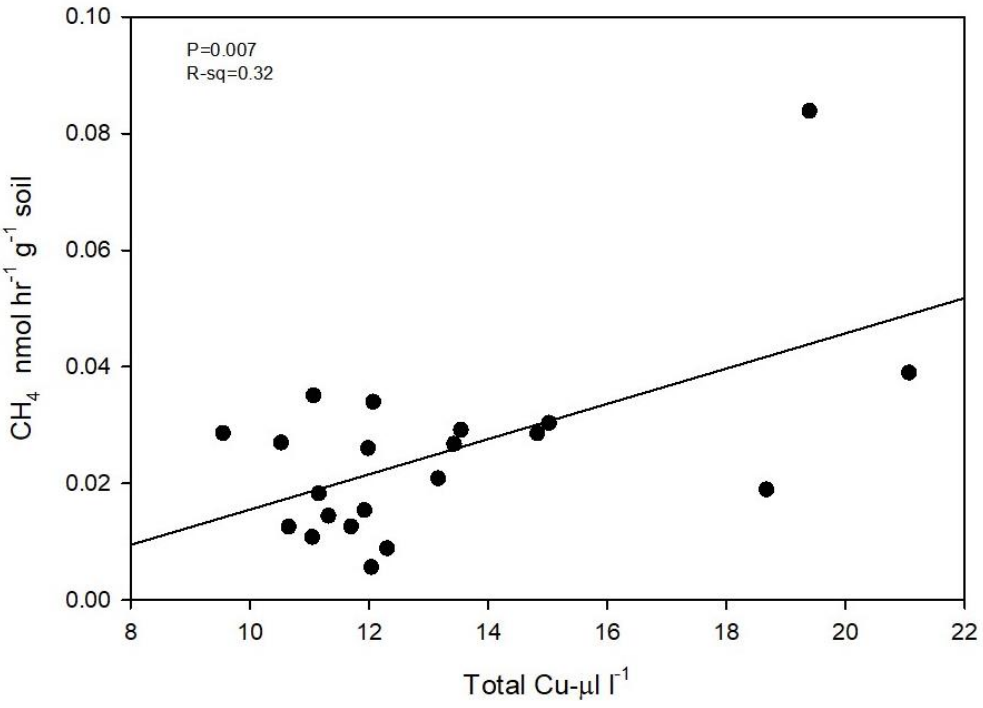

Figure 3.11 Significant relationship of CH_4 oxidation and Total Cu content of Hays native (Hays-N) Hays Restorative (Hays-R), Hays crop (Hays-C), Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). P=0.007 and R^2=0.32.

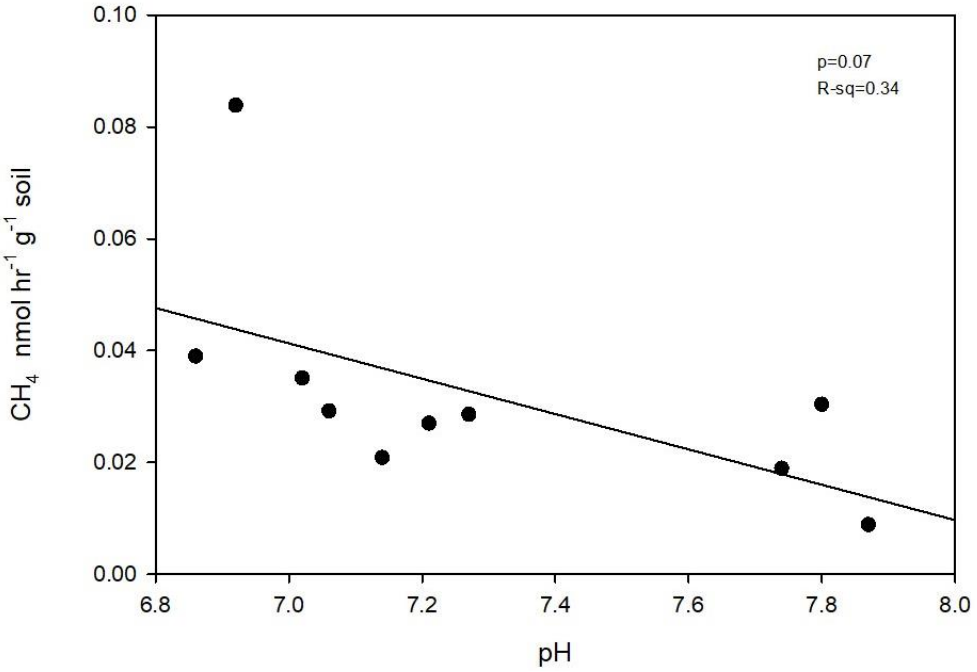

Figure 3.12 Significant difference of methane oxidation and soil pH at Konza native Knz-N), Konza Restorative (Knz-R) and Konza Crop (Knz-C). P=0.07 and R^2=0.34.

Chapter 4 - Summary

The increase in greenhouse gases in the atmosphere leads to global climate change. Methane (CH_4) is one of the important greenhouse gasses. Land use and land use management influence the CH_4 concentration in the global methane budget. Specifically, both agriculture and waste handling are representing 56% of total anthropogenic emissions. Land use and management are important factors in developing a methane budget. Land use and management affect CH_4 oxidation, which is a biological process that reduces atmospheric CH_4. Factors that govern CH_4 consuming microbial abundance and activity in the soil are uncertain.

Our results show that higher CH_4 oxidation rates in native prairie soil are sensitive to soil water content and NH_4^+ content in the soil. Methane oxidation rates were optimal 20-30% of gravimetric water content in the soil. The addition of NH_4^+ inhibited CH_4 oxidation. Burning frequency of native tallgrass prairie affects CH_4 oxidation. At Konsa Prairie Biological Station, higher CH_4 oxidation occurred at C3B site, which had a 3-yr burn cycle and was last burned in 2017. Site C3A also had a 3-yr burn cycle but was last burned in 2019 and had a lower CH4 oxidation rate. Annual prescribed burning had lower CH_4 oxidation rates compared with the C3B site. A higher total Cu was found at the C3B. Copper is key component of methane monooxygenase. However, the mechanisms for the higher Cu content in the C3B site is unknown. Type I methanotrophic biomass was highest for the C3B site. In the future, investigating biologically available Cu content might better determine the mechanism of soil Cu on CH_4 oxidation.

The second study demonstrated the effect of land use on methane oxidation. Effect of native, restored prairie, and cropland on CH_4 oxidation was evaluated in Hays and Konza sites in Kansas based on two precipitation regimes. Higher CH_4 oxidation was recorded at the restored

prairie at Konza. There were significant differences in NH_4, pH, total Cu, extractable Cu, and total C at both sites. A significant relationship between CH_4 oxidation and pH occurred at the Konza site but there was no relationship at the Hays site. There was a significant relationship with CH_4 oxidation and total Cu content at both Hays and Konza sites. There is a relationship between soil pH and Cu so it is unclear if the pH effect is direct or indirect through pH affecting the availability of Cu. There was no relationship between methanotrophic PLFA biomass and CH_4 oxidation. Together these results show that soil Cu content play a key role in methanotrophic activity as other important biotic and abiotic factors in the soil.

As discussed, CH_4 is a potent greenhouse gas and action is needed to reduce atmospheric CH_4. With the increase of the global population and extensive industrial developments in the world, CH_4 mitigation strategies should be implemented to control the methane emission. Soil CH_4 oxidation is a natural biological process, which could contribute to mitigating atmospheric CH_4 by implementing better land use and land management practices.

Soil management practices are important to maintain and enhance the CH_4 oxidation. For tallgrass prairie management, a 3-yr burn cycle enhanced CH_4 oxidation relative to the traditional annual burned prairie. Soil with higher organic matter content while minimizing the tillage practices would able to maintain a better CH_4 oxidation activity while enhancing the biodiversity of soil microbes, including methanotrophic bacteria.

Investigation of methanotrophic biodiversity, abundance with extensive analysis of soil physical and chemical characteristics will enhance the understanding of methanotrophic community's stability and CH_4 oxidation potentiality in each land use and land management systems.

Appendix A - Effect of nitrogen with higher methane concentration

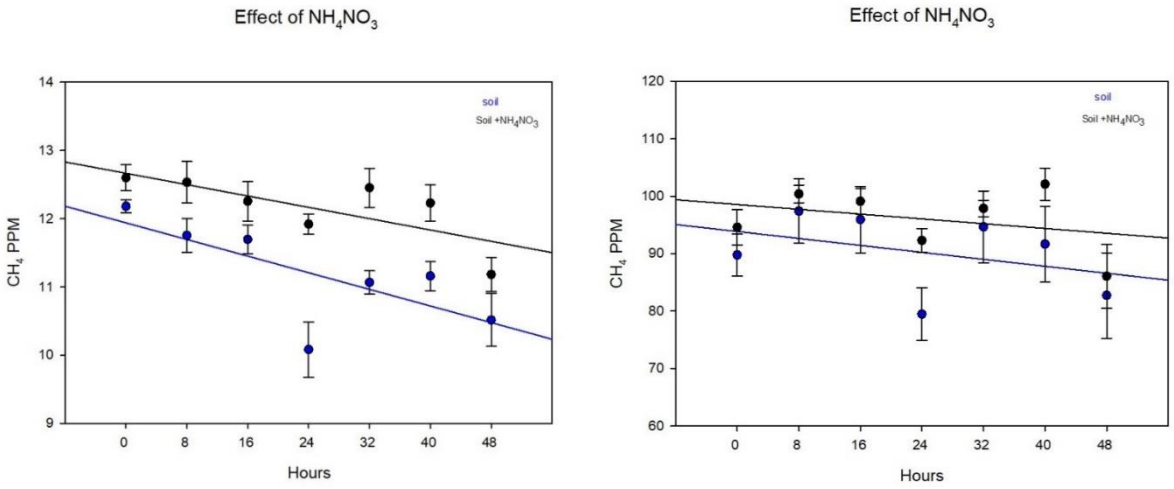

Figure A.1(a) & (b): Effect of NH_4NO_3 on native prairie soil samples (C3B) with higher CH_4 concentration levels (approximately 12-13 ppm and 100ppm) at 48 hours of incubation.

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