Long-term grazing effects on soil greenhouse gases emission and soil microbial communities of Alberta grasslands

by

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Abstract

Grasslands cover a large area of the terrestrial surface and their soils store vast amounts of carbon (C) and nitrogen (N). Small changes in the processes driving C and N cycling can lead to either sequestration of these elements or their release as greenhouse gases (GHG) into the atmosphere. While livestock grazing is the primary use of grasslands worldwide, its effect on soil GHG fluxes and soil microbial communities remains unclear, especially in northern temperate grasslands. This thesis reports on the effect of long-term cattle grazing on soil GHG fluxes and soil microbial communities across the grasslands of Alberta, Canada (Central Parkland, CP; Dry Mixedgrass, MG; Foothills Fescue, FF subregions), over two growing seasons (2015 and 2016).

Using static chambers and gas chromatography, the CO_2, CH_4 and N_2O fluxes from soils were measured. Livestock grazing itself did not affect cumulative GHG fluxes in either year. However, the cumulative CO_2 emission was altered by a grazing and year interaction, being relatively lower from grazed than non-grazed areas in the dry year, while the opposite was true during the wet year. Among subregions the lowest cumulative CO_2 was observed within MG. The soil microbial communities (bacterial, archaeal and fungal) was quantitatively and qualitatively assessed using quantitative polymerase chain reaction and high-throughput sequencing. Livestock grazing did not affect the abundance of bacterial and archaeal communities, or richness and beta diversity in either year. However, fungal alpha diversity was lower in grazed areas during the dry year, but greater in the same areas during the wet year. Also, richness of bacterial and archaeal communities was greater within MG, while their abundance was greater within FF.

Overall this study showed that light to moderate long-term grazing had limited impact on soil GHG fluxes and soil microbial communities, compared to non-grazed areas of the

grasslands, while regional characteristics (climatic conditions) and soil properties had a greater impact.

"We know more about the movement of celestial bodies than about the soil underfoot"

Leonardo Da Vinci, circa 1500's

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List of Symbols and Abbreviations

ACIS, Alberta Climate Information Service
BD, bulk density
bp, base pair
C, carbon
C/N, carbon to nitrogen ratio
CH_4, methane
CO_2, carbon dioxide
CP, Central Parkland
DNA, deoxyribonucleic acid
FF, Foothills Fescue
G, grazing
GHG, greenhouse gases
GWC, gravimetric water content
GWP, global warming potential
ISA, indicator species analysis
ITS, internal transcribed spacer
km, kilometer
MAP, mean annual precipitation
MAT, mean annual temperature
MG, Dry Mixedgrass
N, nitrogen
N_2O, nitrous oxide
NG, non-grazed
NS, Natural Subregion
OTU, operational taxonomic unit
PCR, polymerase chain reaction
qPCR, quantitative polymerase chain reaction
SOC, soil organic carbon
SON, soil organic nitrogen
TN, total nitrogen

Chapter 1. Literature review

1.1. Overview

The three main greenhouse gases (GHG) that accumulate in the atmosphere, alter the Earth's radiative balance and cause "global warming", are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O; Oertel et al., 2016). Since the late 1700s, when the industrial era began, the concentration of these gases has increased on 40, 20 and 150% and reached the level of ~400, 2 and 0.3 ppm for CO_2, CH_4 and N_2O, respectively (IPCC, 2014). Thus, rising concentrations of GHG in the atmosphere and global climate change increase the need for data about the global carbon (C) and nitrogen (N) cycles (LeCain et al., 2002).

Grasslands cover up to 40% of the Earth's terrestrial surface and play an important role in global biogeochemical cycles of C and N (Wu et al., 2010). The upper meter of grassland soils can store 100 and 10 Mg (10^6 gram) per hectare of soil organic carbon (SOC) and soil organic nitrogen (SON), respectively, with the potential to sequester more under proper management (Jobbágy and Jackson, 2000; Piñeiro et al., 2010). This potential is an opportunity to remove GHG from the atmosphere, and thereby mitigate climate change (Soussana et al., 2010; Follett and Reed, 2010). On the other hand, due to ongoing biological processes grassland soils can produce GHG with the resulting magnitude of gas emission or consumption dependent, in part, on land use (Leahy et al., 2004; Liebig et al., 2010). Livestock grazing is the primary use of grasslands worldwide, however, the role that it plays in altering GHG exchange between soil and atmosphere remains unclear (Bremer et al., 1998; Liebig et al., 2010; Healy et al., 1996; Shi et al., 2017).

1.2. Grasslands of Alberta, Canada

Alberta's landscape is the most diverse of any region in North America (Downing and Pettapiece, 2006). The province was divided into 6 Natural Regions and subdivided into 21 Natural Subregions, which provided an ecological context for the planning resource management activities in the province (Downing and Pettapiece, 2006). More than 70% of Alberta's grazing lands are located within the Grassland and Parkland Natural Regions (Alberta Agriculture and Forestry, 2015). In this study, I focused on three subregions within these two Natural Regions. The Dry Mixedgrass and Foothills Fescue Natural Subregions both lay within the Grassland Natural Region. The Dry Mixedgrass subregion is located in the south-east corner of the province and is characterized by high temperatures and low summer precipitation that lead to large moisture deficits, and low-growing and drought-tolerant mixedgrass vegetation (Adams et al., 2013). The dominant soil type in this region is a Brown Chernozem, which forms with low organic matter input into the soil and high mean annual temperatures (Downing and Pettapiece, 2006). The Foothills Fescue is the wettest Natural Subregion and is characterized by cool summers and higher precipitation. The dominant vegetation is diverse and productive (Adams et al., 2003). Black Chernozem is the main soil type within the subregion, which forms with high organic matter input into the soil and moderate temperatures (Alberta Environment and Parks, 2015). The largest Natural Subregion within the Parkland Region is the Central Parkland, covering 88% of the Region. This area is a broad transitional zone located between the cool, moist boreal forests to the north and dry, warm grasslands to the south and thus is a matrix of aspen forest, shrubland and grassland. The dominant soil type is Black Chernozem (Downing and Pettapiece, 2006). More detailed description of the subregions, e.g. plant community composition, is provided in Chapter 2 of the thesis. The diversity and uniqueness of the

grasslands in Alberta allows to reveal response to long-term grazing under various conditions, which might be variable across the province.

1.3. Grasslands management in Alberta, Canada

Grazing by large herbivores is the natural conditions of the World's rangelands (LeCain et al., 2000). Bison were the dominant grazer in the Canadian prairies until 1880, at which time they were extirpated (Willms et al., 2011). In 1881, there were only 9000 cattle in the northwest of Canada, but this number increased markedly thereafter with advancing European settlement (Wang et al., 2014). From 1911 to 1941, the number of cattle doubled and reached 1.35 M, which led to overgrazing (Willms et al., 2011). Grasslands deterioration was further exacerbated by a 12-year drought lasting from 1917 to 1930 that in turn, increased soil erosion. All of these factors together, heavy and unregulated grazing pressure, coupled with an extended drought, affected soil quality and led to high losses of soil organic carbon (Wang et al., 2014). Since then, government land managers and livestock producers have both gained much knowledge about the agronomic and ecological impacts of grazing, which pushed them to the develop an improved grazing management practices, which included regulating of grazing duration, frequency and intensity (Wang et al., 2014).

1.4. Grazing effect on grasslands

Livestock grazing can affect grasslands in many ways by defoliation, nutrient redistribution (dung and / or urine deposition) and trampling (Liu et al., 2015). Duration, frequency and intensity of grazing are the factors that determine and regulate all above- and belowground processes in grasslands (Hodel et al., 2014, Bardgett and Cook, 1998). Over

stocking lead to plant groundcover reduction, increase wind and water soil erosion, which leads to losses of soil organic carbon and nutrients, breaks soil pore continuity, which reduces water infiltration and air permeability (Eyles et al., 2015; Greenwood and McKenzie, 2001; Liebig et al., 2014). Whereas, good grazing management practice improve the productivity of grasslands, soil health and nutrient cycling (Liebig et al., 2014; Follet and Reed, 2010). These effects are apparent in Alberta, high intensity grazing in early spring reduced vegetation cover and soil organic matter, and increased bare ground, while light intensity of grazing did not change these factors (Naeth et al., 1991). Similarly, 45-years of high intensity grazing in Alberta's fescue grassland decreased fertility and water-holding capacity, while light grazing provided better soil characteristics (Dormaar and Willms, 1998). Thus, grazing itself is a tool to control and maintain soils quality and ecosystem functionality, as well as goods and services provided by grasslands (Sanjari et al., 2008).

1.5. Greenhouse gases and grassland soils

Soils of grasslands are an important component of the global biogeochemical cycles as they have wide geographical distribution and can store more than 100 and 10 Mg (1Mg = 10^6 gram) ha^{-1} of C and N in their top meter (Conant et al., 2005; Jobbágy and Jackson, 2000; Piñeiro et al., 2010). Ongoing biological processes in soils lead to generation or consumption of the major greenhouse gases, namely CO_2, N_2O and CH_4 (Baldock et al., 2012). Carbon dioxide is emitted into the atmosphere through respiration of soil microorganisms, which use an organic material as a source of energy and nutrients, and roots (Conrad, 1996). Methanogens and methanotrophs are responsible for the production and consumption of methane, respectively (Conrad, 1996). Nitrous oxide is generated in soils due to nitrogen transformation by

microorganisms during the processes of nitrification (conversion of ammonium to nitrate) and denitrification (conversion of nitrate to N_2O and N_2; Wrage et al., 2001). However, the exchange rate of the gases between soil and atmosphere depends on management practice and consequently, soil properties (Conant, 2010; Smith et al., 2003; Oertel et al., 2016). Thus, management of the duration, frequency and intensity of livestock grazing, which can alter soil properties, regulates the potential of grassland soils to reduce or enhance greenhouse gases concentration and as a result the magnitude of climate change (Baldock et al., 2012). However, there is inconsistent information regarding livestock grazing effects on GHG emission which does not enable a clear determination of the role grasslands and grazing play in regulating global GHG flux (Leahy et al., 2004; Wang and Fang, 2009; Liebig et al., 2010). These contradictory results of GHG emissions from grasslands, besides history and management of grazing, might be also associated with variable features of study sites, including climatic and hydrological conditions (Wang and Fang, 2009; Chen et al., 2015; Oertel et al., 2016).

1.6. Soil microbial communities of grasslands

Studying the soil microbial community is a challenging task as soil is a complex and variable system, which supports an enormous diversity of soil microorganisms (Tiedje et al., 1999). Moreover, less than 1% of the community has been cultivated or characterized, which is commonly known as "the great plate count anomaly", and thus, soil still can be considered as a "black box" in terms of our understanding of the microbial community and the processes it regulates (Torsvik and Øvreås, 2002; Staley and Konopka, 1985). Next-generation sequencing (NGS) technologies revolutionized the area of microbial ecology, as they enable a comprehensive analysis of microbial communities and provide insight into their interaction with

the environment (Boughner and Singh, 2016; Shokralla et al., 2012). Prokaryotes (bacteria and archaea) and eukaryotes (fungi) are involved in a wide range of soil biochemical processes, such as nutrients transformation and cycling, maintain the sustainability of an ecosystem (Wagg et al., 2014; Delgado-Baquerizo et al., 2016; Bardgett and Putten, 2014). In particular, soil microorganisms play an important role in climate regulation through their key role in soil processes, which lead to either consumption or formation of GHG (Classen et al., 2015). Grassland soils maintain a vast and diverse community of microorganisms (Bardgett and Cook, 1998; Macdonald et al., 2015); however, they remain poorly characterized (Evans et al., 2017; Griffin, 2016). Additionally, the impact of domestic livestock grazing on the community composition and abundance of soil bacteria, archaea and fungi is not well studied and results of previous studies are inconsistent across various microbial groups and different grazing intensities (Brussaard et al., 1997; Aldezabal et al., 2015; Eldridge et al., 2017; Radl et al., 2007; Chroňáková et al., 2009; Jirout et al., 2011; Elhottová et al. 2012; Chroňáková et al., 2013; Chroňáková et al., 2015; Gou et al., 2015; Huhe et al., 2017; Zhou et al., 2010; Qu et al., 2016). However, knowing the effect of cattle on these microbial groups is critical to understanding effects on ecosystem function, maintaining ecosystem sustainability and overall, human well-being (Eldridge et al. 2017).

1.7. Thesis format and research objectives

The overall goal of the research presented in this thesis was to investigate the effects of long-term livestock grazing on soil GHG emissions and soil microbial communities in Alberta's grasslands. The research was divided into two distinct parts.

The first study (Chapter 2) investigated whether livestock grazing altered emissions of GHG from grassland soils in northern temperate grasslands of Alberta, and if so, what is the direction and magnitude of this alteration? Specific objectives were to (i) estimate and compare emission rates of CO_2, N_2O and CH_4 from rangeland soils exposed to long-term grazing and grazing exclusion across a broad climatic gradient, (ii) evaluate cumulative GHG emission in relation to long-term grazing history.

The second study (Chapter 3) investigated the effect of grazing on soil microbial communities, in particular, the bacterial, fungal and archaeal communities. Specific objectives for this study were to (i) to assess abundance of bacteria, archaea and fungi in soil samples collected from grazed and non-grazed subplots from the three natural subregions of Alberta in 2015 and 2016 (ii) to examine the diversity of the bacterial, archaeal and fungal communities in grazed grassland soils in comparison with non-grazed treatment.

Chapter 4 is a synthesis of the work conducted, and reviews management implications for ranchers and policy makers interested in mitigating atmospheric GHG, and discusses future research needs.

Chapter 2. Effect of long-term grazing on greenhouse gas fluxes in northern temperate grasslands

2.1. Introduction

Grasslands cover up to 40% of the Earth terrestrial surface and they are a significant component of the global C and N cycles (LeCain et al., 2002; Wu et al., 2010). The upper meter of grassland soils can store 100 and 10 Mg (1 Mg = 10^6 gram) per hectare of soil organic carbon (SOC) and soil organic nitrogen (SON), respectively (Jobbágy and Jackson, 2000; Piñeiro et al., 2010). Even small changes in the processes driving soil cycles of C and N might alter the magnitude of soil fluxes and lead to releasing of large amounts of GHG into the atmosphere (Johnston et al., 2004; Mohammed, 2015). Livestock grazing is the primary use of grasslands worldwide (Bremer et al., 1998; Fynn et al., 2010). Duration, frequency and intensity of livestock grazing might alter plant productivity, soil physical and chemical properties (Greenwood and McKenzie, 2001) which, in turn, regulate biogeochemical cycling of C and N within the grasslands, the balance between C and N input (e.g. photosynthesis) and the output (gaseous C and N release; Piñeiro et al., 2010; Roy and Misra, 2005). While grasslands can mitigate climate change through their potential for C and N sequestration (Wu et al., 2010; Conant et al., 2005; Follett and Reed, 2010; Piñeiro et al., 2010; Soussana et al., 2010), however, the role that livestock grazing plays in altering gaseous C and N losses from grassland ecosystems remains unclear (Bremer et al., 1998; Liebig et al., 2010; Healy et al., 1996; Shi et al., 2017). However, the understanding of livestock grazing effect on the C and N release is essential to assess the contribution of grazed lands into the global C and N cycles and budgeting (Cao et al., 2004).

Biological transformation of C and N compounds in soils lead to emission or consumption of GHG, which are causing climate change through their rising concentrations in the atmosphere and alteration to the Earth's radiative balance (Abbasi and Müller, 2011; Oertel et al., 2016; IPCC, 2014). The most common GHG in the agriculture sector are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Asgedom and Kebreab, 2011; Soussana et al., 2010). While CO_2 is the most abundant (400 vs. 0.3 and 2 ppm for CH_4 and N_2O, respectively) and longest lasting (300 vs. 12 and 120 years for CH_4 and N_2O, respectively) gas in the atmosphere, the global warming potential (GWP) over a 100-year period for CH_4 and N_2O is 25 and 298 times higher than that for CO_2 (Oertel et al., 2016; Abbasi and Müller, 2011). Soil respiration, which includes heterotrophic and autotrophic sources, is the primary pathway for CO_2 release to the atmosphere (Wang and Fang, 2009). The net-exchange of CH_4 and N_2O between soil and atmosphere is a result of gas consumption and production processes, namely methanogenesis and methanotrophy for CH_4, and denitrification and nitrification for N_2O (Conrad, 1996; Flechard et al., 2007).

There is no global consensus on the effect of livestock grazing on GHG fluxes within grassland soils, with some studies demonstrating increased fluxes (Frank et al., 2002; LeCain et al., 2000), others no effect (Risch and Frank, 2006), and yet others showing reduced emission (Samal et al., 2015; Bremer et al., 1998) in comparison with soils without grazing. Relatively few studies have examined GHG in northern temperate grasslands of the Great Plains, and those that have were typically done at few locations (Gao et al., 2017, Thomas et al., 2018). Thus, the widespread effect of grazing on GHG from soils of the region remain poorly understood. Given that grasslands occupy a vast area with a diversity of grassland types, and that GHG emissions or

consumption from the area may be large, a quantitative assessment of the GHG under livestock grazing is necessary.

The objectives of this study were to (i) compare emission rates of CO_2, N_2O and CH_4 from grassland soils with a long-term history of light to moderate cattle grazing to soils where grazing had been excluded, across a broad climatic gradient, (ii) evaluate cumulative GHG emission in relation to long-term grazing history.

2.2. Materials and methods

2.2.1. Study sites

The study was conducted across south-central Alberta, Canada within three different natural subregions, including the Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) (Fig. 2.1; Downing and Pettapiece, 2006). These subregions represent major grasslands of the northern temperate Great Plains with wide ranging climatic conditions, soil types, vegetation composition and topography (Table 2.1). Briefly, the main features of the MG subregion is high temperatures and low precipitation during the summer as well as dominance of low-growing drought tolerant vegetation (Adams et al., 2013). The dominant vegetation is a *Stipa-Bouteloua-Agropyron* community, which is resilient to low grazing stocking rates (Dormaar et al., 1997) The main soil type is a Brown Chernozem, which forms with low organic matter input into soil and high mean annual temperatures (Downing and Pettapiece, 2006). The FF is the wettest subregion, which characterized by cooler summers with higher precipitation level. The dominant vegetation is diverse and productive, including *Festuca campestris* and *Danthonia parryi* (Chuan et al., 2018; Adams et al., 2003). Black Chernozem is the main soil type, which forms with high organic matter input into the soil and moderate temperatures

(Alberta Environment and Parks, 2015). The CP is a broad transitional zone located between the cool, moist boreal forests to the north and dry, warm grasslands to the south. Vegetation represents a matrix of forests, shrublands and grasslands, including *Populus tremuloides*, *Symphoricarpos occidentalis*, *Festuca hallii*, *Hesperostipa curtiseta*, *Pascopyrum smithii*, *Koeleria macrantha* (Chuan et al., 2018). The dominant soil type is Black Chernozem (Downing and Pettapiece, 2006). Livestock grazing has been an important land use across these regions for the past 140 years since European settlement (Willms et al., 2011).

Fifteen locations were examined in this study, with five in each natural subregion (Fig. 2.1). Of the sites, 12 were on public land and part of the Alberta Rangeland Reference Area program, a long-term monitoring program of ongoing grazing impacts on grasslands vegetation (Willoughby et al., 2004). Two additional locations were at the University of Alberta Mattheis and Kinsella Research Ranches, and one other location was on private land. Each location included a fenced area that excluded cattle, and was compared to the surrounding grasslands that had been annually grazed by cattle, typically during the summer growing season (late May-October). Exclosures were rectangular or circular in shape and varied in size from 0.05 to 0.2 ha, varied in age from construction between 15 and 60 years (Willoughby et al., 2004). Studied subplots were established on areas with a uniform ecosite (aspect, slope, soil texture). This study design created a total of 30 subplots, half of which were non-grazed (NG), with each paired to a grazed (G) subplot on the same ecosite in a blocked design. There was no specific information available on the localized stocking rates associated with grazing within each grazed subplot as the large size of each pasture did not ensure uniform use throughout. However, cattle stocking rates on all pastures containing the exclosures were generally considered light to moderate, consistent with allowable grazing practices on public land in Alberta. Additionally, while

exclosures did not exclude wildlife (e.g., deer, elk, moose, antelope) from accessing them, the small size of exclosures was likely to limit use by these species (Knight et al., 2014).

2.2.2. Gas sampling and measurements

Two static chambers were installed randomly within each of the G and NG subplots at each location (Livingston and Hutchinson, 1995). To protect equipment from damage by cattle, wire cages (~1 m in diameter) were placed over each chamber after their installation in grazed areas (Gao et al., 2017; Baah-Acheamfour et al., 2016). However, chambers were removed from the ground during the winter of 2015-2016 to protect them from freeze-thaw damage, and reinstalled in 2016 at a new random location to avoid grazing exclusion effects created by the cages the previous year. Overall, 17 gas sampling events were performed at all sites during the course of two grazing seasons in 2015 (n=8 samplings) and 2016 (n=9 samplings), generally beginning in early to mid-May and ending in October (Appendix A). Because of the large area over which the study locations were distributed (260 km in latitude and 450 km in longitude), more frequent gas sampling was considered infeasible (Gao et al., 2017).

All chambers were permanently inserted into the ground to approximately 2-3 cm depth at least a day prior to the first sampling each year to avoid capture of gas emission splashes after soil disruption (Saurette et al., 2008). Each static chamber consisted of an acrylic base (17 cm wide x 15 cm high x 66 cm long) that lacked a bottom and was covered with a detachable lid. The lid was equipped with a sampling port with rubber septa 15 mm in diameter and fan (25 x 25 x 10 mm, ADDA, Orange, CA, USA). Lids were tightly clamped onto the chambers during gas sampling. Chambers and lids were covered with silver tape to reflect sunlight and thereby reduce warming of air inside chambers during the gas sampling (Rochette and Eriksen-Hamel, 2008).

During each gas sampling event, 20 ml of air were taken at 0, 10, 20 and 30 minutes after the closure of the chamber, using 20 ml syringes fitted to 26G ½ needles (Becton Diskinson, Franklin Lakes, NJ, USA). The samples were then force-filled into pre-evacuated 12 ml glass exetainers® (Labco Ltd., Lampeter, CGN, UK). As removing of vegetation from the inside of chamber might imitate defoliation by cattle on the non-grazed subplots, we did not clip vegetation in the static gas chambers (Collier et al., 2016). A previous study showed limited difference in gas fluxes between chambers where vegetation was clipped or when vegetation was folded to fit in the chamber; however, the authors suggested estimation of biomass volume, when possible (Collier et al., 2016). In the present study, assessment of biomass was not possible, because chambers were installed permanently into the ground and biomass mass varied through the growing seasons.

Gas concentrations were subsequently measured by gas chromatography (Varian-3800, Varian Instruments, Palo Alto, CA, USA) in the laboratory no later than one week after sampling to prevent leakage and contamination of gas samples (Rochette and Eriksen-Hamel, 2008). The analyzer was equipped with a thermal conductivity-, flame ionization- and electron capture detectors to measure concentrations of CO_2, CH_4 and N_2O, respectively. To calibrate the gas chromatograph, certified standards of CO_2, CH_4 and N_2O were used (Praxair, Edmonton, AB, CA).

2.2.3. Calculations

The emission rate of gases was calculated using the equation provided by Saurette et al. (2008):

$$F = \frac{\Delta C \times T \times h}{\Delta t},$$

where F is the emission rate of CO_2, CH_4 or N_2O (μmol m^{-2} s^{-1}), $\Delta C / \Delta t$ is the slope of the gas concentration change over the 30 min measurement period (μmol mol^{-1} s^{-1}), h is the height of the chamber (m), and T is the air temperature adjustment for the molecular volume of gas (mol m^{-3}). To prevent underestimation of gas fluxes, all data were fitted to both linear and quadratic models (Stolk et al., 2009; Silva et al., 2015). The model, and thus slope, was selected based on the highest adjusted R^2 (Baah-Acheamfour et al., 2016; Stolk et al., 2009; Silva et al., 2015). Afterwards, observed fluxes were converted to kg C ha^{-1} d^{-1}, g C ha^{-1} d^{-1} and g N ha^{-1} d^{-1} for CO_2, CH_4 and N_2O, respectively, using the corresponding molecular weight (g mol^{-1}) of each gas.

To assess cumulative emission or consumption of each gas during 2015 and 2016, the area under the curve was calculated by summing mean gas fluxes for each interval multiplied by the number of days within each interval (Gao et al., 2017).

2.2.4. Soil sampling and analysis

Soil samples were collected in May, July and October of 2015 and 2016. During each sampling period ten soil cores were randomly collected from throughout each subplot from the 0-5 cm mineral soil layer using a soil corer 5 cm in diameter. Composite samples for each subplot were transported to the laboratory, where they were stored at -20 °C prior to further analysis. Soil pH was measured in a 1:2 soil-water mixture (Accumet® Basic A150, Fisher Scientific, Hampton, NH, USA). To determine soil organic carbon (SOC) and total nitrogen (TN) contents, soil samples were dried (60 °C), sieved (2 mm), ground to a fine powder using a ball mill (Spex^{TM} SamplePrep 8000D, Metuchen, NJ, USA), treated with HCl to remove carbonates, and

then analyzed using a LECO TruSpec C/N analyzer (LECO Corporation, Saint Joseph, MI, USA).

To determine bulk density (BD), two soil cores per subplot were collected from the 0-5 cm mineral soil layer using a 6 cm diameter soil corer in early August of 2016. Soil samples were placed into paper bags separately for each replicate and transported to the laboratory, where they were oven-dried (60 °C) to a constant weight. Values of BD were calculated by dividing the soil sample dry mass by the volume of the core, after removing any rocks and debris larger than 2 mm (i.e. larger size fraction was removed; Blake and Hartge, 1986).

2.2.5. Climatic conditions, soil temperature and soil water content measurements

Air temperature and precipitation data were downloaded from the Alberta Climate Information Service (ACIS) website (http://agriculture.alberta.ca) for both study years. The closest weather station to each of the 15 locations was selected and data were averaged for each subregion (n=5) for comparison to long-term norms. Additionally, to directly examine the relationship between environmental factors and GHG, soil volumetric water content (m^3 m^{-3}) and temperature (°C) were continuously monitored at the 5 cm soil depth for each subplot using an automated data collection system (EM50, Decagon Devices Inc., Pullman, WA, USA) equipped with moisture and temperature sensors (5TE, Decagon Devices Inc., Pullman, WA, USA).

2.2.6. Statistical data analysis

All statistical analysis and graphical output were done using RStudio, version 1.0.136 (RStudio Inc., Boston, MA, USA). Differences were considered significant at p-values <0.10. Linear mixed-effect models were used to test the impact of historical grazing treatment (grazed

vs. non-grazed) on soil properties, emission or consumption rates of GHG, cumulative fluxes and GWP over the growing season.

As suggested by Gao et al. (2017), analyses of GHG emission rates need to be performed separately for each year to account for variation among the dates of gas sampling, fluctuations in weather and changes in chamber locations between years. Thus, data were analyzed separately for 2015 and 2016 to isolate effects of grazing treatments, as well as geographic (subregion) and temporal (sampling dates) variation, together with their interactions, on the GHG emission rates during each year. Treatment (grazed vs. non-grazed) and subregions were included as fixed effects, while sampling dates within a year were treated as a repeated measures fixed factor. Duplicate chambers within a subplot were averaged prior to analysis. Individual study sites nested within subregions and treatment subplots within sites were treated as random effects. To check the distribution of residuals and equality of variances for the models, diagnostic plots were used. CO_2, CH_4 and N_2O gas data were normalized with a normal quantile transformation applied (Moulin et al., 2014). While analyses were done on transformed data, original (non-transformed) data were presented in all tables and figures to maintain interpretability.

To compare the effect of long-term grazing on cumulative gas emission or consumption, the same model was applied as described above; however, year was considered the repeated measure factor (instead of individual sampling dates) to capture the overall effect of long-term grazing treatment between 2015 and 2016 (Gao et al., 2017). To make the data comparable between years, we examined only those data from the growing season of each year that had overlapping sampling periods, thereby ensuring that the number of days used to calculate cumulative fluxes were the same for each year.

Soil pH, SOC and C/N ratio from the three sampling periods in each of the two years were averaged, as were the two replicate values for bulk density, prior to statistical analysis. Treatments and subregions were considered as fixed factors in the model, while individual study sites nested within subregions and treatment subplots within sites were treated as random effects. To test the effects of soil moisture, soil temperature and their separate interactions with grazing treatments and subregion on GHG emissions, the latter were fit to a linear fixed-effect model, where random effects were as described above.

2.3. Results

2.3.1. Climatic conditions and soil properties

Climatic conditions varied between years and subregions (Fig. 2.2A, B). Overall, 2015 was drier than 2016. In 2015, MAP was lower by 23, 17 and 32% compared to the 30-yr average for the CP, MG and FF, respectively. In 2016, MAP was 12 and 9% greater than the 30-yr average for the MG and FF, while being 3% lower in the CP (Fig. 2.2A). MAT was generally above that of the 30-yr average for all study locations and years by more than 1°C, although the difference between 2015 and 2016 never exceeded 0.5°C (Fig. 2.2B).

Soil pH and C/N ratio did not differ between grazing treatments and subregions (Table 2.2). Differences in soil properties between grazed and non-grazed subplots were limited to SOC and BD (Table 2.2). Overall, SOC values were greater ($P=0.002$) in grazed areas (5.95 ± 0.32 %) than non-grazed areas (4.96 ± 0.26 %). Similarly, bulk densities were greater ($P=0.013$) in grazed areas (1.19 ± 0.07 g cm^{-3}) than adjacent non-grazed grassland (1.05 ± 0.06 g cm^{-3}). SOC and BD values also varied among subregions (Table 2.2). Observed SOC were lower within MG compared to the both other subregions ($P<0.10$). Lower soil BD values ($P<0.10$) were detected within FF, higher within MG and intermediate within CP.

Mean daily soil temperature increased under long-term grazing (P=0.019) in 2015 and was 17.6 and 16.7 °C for the G and NG, respectively (Table 2.3). Soil temperature did not vary in relation to grazing on the year later. In 2015, soil temperatures were 16.9, 19.1 and 15.1°C for the CP, MG and FF, respectively, with higher values within MG, lower values within FF and intermediate values within CP. In 2016, soil temperature followed a similar pattern, at 16.9, 18.1 and 14.8°C for the CP, MG and FF, respectively, with higher values within CP and MG, lower values within FF (P<0.10). Mean soil water content was not affected (P>0.10) by long-term grazing (Table 2.3), but varied among subregions. Greater soil moisture (P<0.10) was found within the within CP and FF relative to the MG for both study years (Table 2.3).

2.3.2. GHG fluxes

Long-term grazing did not affect N_2O emission, but CO_2 and CH_4 exhibited main effects of grazing (P<0.10) in 2015 (Table 2.4). Mean emission rates of CO_2 were lower for G areas compared to adjacent NG grassland, 21.2 ± 1.3 and 24.4 ± 1.3 kg C ha^{-1} d^{-1}, respectively. Consumption rates of CH_4 were also reduced under grazing and was -10.7 ± 0.9 and -13.4 ± 0.9 g C ha^{-1} d^{-1} for G and NG, respectively. Consumption of CH_4 was additionally affected by the interaction treatments x subregions (Table 2.4). The lowest consumption rate of CH_4 was observed within the MG subregion regardless of grazing treatment (Fig. 2.3). In contrast, CH_4 uptake was intermediate in the FF, and was particularly high in the CP, but only for soils under long-term grazing.

Fluxes of CO_2 and CH_4 varied with a subregion x sampling date interaction (P<0.10) in both 2015 and 2016 (Table 2.4). However, the pattern of CO_2 emissions and CH_4 consumption remained highly variable among subregions and throughout the growing season (Fig. 2.4A, B,

2.5A, B; Appendix B and Appendix C). Among all three subregions in 2015, CO_2 emission rates were lower within MG (15.6 ± 1.7), higher within FF (27.2 ± 1.4) and intermediate within CP (24.7 ± 2.3 kg C ha^{-1} d^{-1}). Among all three subregions in 2016, lower CO_2 emission rates occurred within MG (25.1 ± 1.9), higher within CP (43.6 ± 2.9) and intermediate within FF (36.7 ± 2.5 kg C ha^{-1} d^{-1}).

The mean consumption rate of CH_4 in 2015 was lower within the MG (-7.5 ± 0.6) compared to the both, CP (-14.4 ± 1.5) and FF (-14.5 ± 1.1 g C ha^{-1} d^{-1}). Among all three subregions in 2016, the mean consumption rate of CH_4 was once again lower within MG (-7.8 ± 0.4), higher within CP (-13.2 ± 1.1) and intermediate within FF (-10.1 ± 0.7 g C ha^{-1} d^{-1}). No differences in N_2O emissions were detected between grazing treatments or subregions, though it varied throughout the 2016 growing season (P=0.02; Table 2.4). The lowest N_2O emission was observed at the end of May with higher emission of N_2O revealed at the beginning of July (data not shown).

2.3.3. Cumulative emission of GHG

Cumulative CO_2 emission were affected by the interaction between treatments and subregions, years and subregions (Table 2.5; Fig. 2.6A). While grazing led to lower emission of CO_2 in 2015 relative to non-grazed subplots, the opposite pattern was detected in 2016 (Fig. 2.5A). Also, grazed areas experience a much greater increase in CO_2 emission between years (+50%), while the increase in CO_2 emission from non-grazed subplots between years was much less (+23%). Among all three subregions, lowest cumulative CO_2 emission was observed within MG (2758 ± 249) compared to the both, CP (4149 ± 451) and FF (4117 ± 274 kg C ha^{-1}). Overall, cumulative emission of CO_2 varied between years with lower values observed in 2015and it was 2861 ± 221 and 4512 ± 275 kg C ha^{-1} for 2015 and 2016, respectively.

Cumulative CH_4 consumption was affected by subregions, year of sampling, and their interaction (Table 2.5). Lowest cumulative CH_4 consumption was observed within MG compared to FF in 2015 (Fig. 2.6B). Among all three subregions, lowest cumulative CH_4 consumption was observed within MG (1.04 ± 0.07) compared to the both, CP (1.91 ± 0.20) and FF (-1.62 ± 0.13 kg C ha^{-1}). Overall, in 2015 soils consumed more CH_4 compared to 2016 and cumulative CH_4 consumption was -1.6 ± 0.2 and -1.4 ± 0.1 kg C ha^{-1} in 2015 and 2016, respectively. While cumulative emission of N_2O was unaffected by grazing, however it varied only in relation to years, being lower during 2015 (0.03 ± 0.03 kg N ha^{-1}) compared to 2016 (0.11 ± 0.04 kg N ha^{-1}).

2.3.4. GHG and environmental conditions

Emission of CO_2 and consumption of CH_4 were associated with soil water content and its interaction with grazing treatments in both 2015 and 2016 (Table 2.6). Overall, emissions of CO_2 and CH_4 had a positive linear relationship with soil water content. At the same level of soil water content in 2015, emissions of CO_2 tended to be higher from soils without grazing (Fig. 2.7A). In 2016, soil moisture levels below 0.10 m^3 m^{-3} led to emissions of CO_2 that were greater from the NG, while being higher from G when soil moisture was high (Fig. 2.7B). The CP generally had higher CO_2 emissions than the MG, while FF did not have a significant regression fit (Fig. 2.7C). CH_4 consumption rates were greater for soils without grazing under the same soil water content in 2015 (Fig. 2.7D); however, the relationship between CH_4 and soil water content did not have a significant regression fit. In 2016, MG had lower consumption rates than the FF, while the CP did not have a significant regression fit (Fig. 2.7E). Overall in 2016, lower consumption CH_4

rates were associated with higher soil water content, except NG within MG (Fig. 2.7F). Soil water content did not affect N_2O fluxes (Table 2.6).

Emission of CO_2 and consumption of CH_4 were associated with soil temperature and its interaction with treatments and subregions (Table 2.6). In 2015, CO_2 and soil temperature as well as its interaction with grazing treatments did not have a significant regression fit (Fig. 2.8A). In 2016, NG subplots emitted less CO_2 than that from G (Fig. 2.8B) and emission of CO_2 within CP was higher than that within MG, while FF did not have a significant regression fit (Fig. 2.8C). CH4 consumption rate was lower within MG compared to the CP and FF (Fig. 2.8D), however a significant regression fit was not observed. Soil temperature did not affect N_2O fluxes (Table 2.7).

2.4. Discussion

Exchange of GHG between soil and atmosphere is a large component of the C and N balance within grassland ecosystems (Bremer et al., 1998). This study addressed a critical gap in our knowledge of how long-term grazing affects GHG fluxes from grassland soils by examining these processes across a broad climatic gradient of northern temperate grasslands in the Great Plains. Unlike many previous studies that have been done only at a single or select few locations (Gao et al., 2017; Thomas et al. 2018), this study examined GHG fluxes in response to long-term grazing at 15 different locations with widely varying vegetation, soils and climate. Importantly, in the present study it was reported that a history of long-term grazing altered cumulative CO_2 emission depending on climatic conditions. Given that grassland ecosystems occupy a large area of the terrestrial surface, these results improve our understanding of their role in supporting

global cycles of C and N and reducing GHG fluxes (C and N release), and in turn, provide an important component for C and N balance of grasslands under grazing.

2.4.1. Long-term grazing effect on GHG

The results of this study showed reduction of averaged CO_2 emission over growing season in 2015 under long-term grazing, while in 2016 averaged CO_2 emission was similar between grazed and non-grazed treatments. This finding is consistent with previous studies of soil CO_2 emissions within the more arid regions (MAP < 410 mm) of the Great Plains such as the shortgrass steppe (LeCain et al., 2002), mixed-grass prairie (LeCain et al., 2000). None of the latter studies detected differences in CO_2 emission between grazed and non-grazed soils, while CO_2 emissions from soils in more mesic areas of the tall-grass prairies (MAP 856 mm) were reduced under grazing (Bremer et al., 1998, Johnson and Matchett, 2000). Notably, the reversal of long-term grazing effects on cumulative CO_2 emissions from soils between the two years of the study suggests that environmental conditions are likely to dictate when and where grazing may alter GHG in northern temperate grasslands. In the current study, the lower precipitation conditions of 2015 led to lower cumulative CO_2 emission under long-term grazing, while the subsequent wetter (i.e. near normal rainfall) year led to more CO_2 release under long-term grazing.

Emission of CO_2 comes from two sources, namely autotrophic (roots) and heterotrophic (microorganisms) pathways (Rastogi et al., 2002; Wang et al., 2017), which can potentially be affected by grazing through defoliation, trampling, as well as the redistribution of nutrients via dung and urine. Removal of aboveground biomass, subsequent litter residue, and reductions of leaf area by defoliation can increase soil temperature and accelerate respiration of both sources

(Bremer et al., 1998). In the present study, we observed increased soil temperatures under long-term grazing subplots in 2015, this year was also associated with reduced precipitation across all subregions, which likely led to a lower input of decomposable organic material (living roots, exudates) into soil (Bremer et al., 1998), which in turn decreased CO_2 emission from soils under long-term grazing. Livestock excreta can affect soils properties (Eyles et al., 2015) and stimulate microbial activity (Bardgett and Cook, 1998), which in turn, can increase heterotrophic CO_2 emission from soils (Wang et al., 2017). In the present study the short-term impact of grazing on soil respiration was eliminated within chambers by the protection of cages, and thus the absence of fresh urine and dung within chambers. SOC is closely and positively correlated with CO_2 emission from soils (Liebig et al., 2010) and higher SOC contributes to higher soil respiration (Liebig et al., 2013). In the present study SOC was higher under long-term grazed soils, however as mentioned above soil moisture was likely the driving factor for the CO_2 reduction from grazed soils in 2015. Grazed areas also demonstrated greater inherent variation in GHG flux from year-to-year compared to non-grazed areas, thereby highlighting the need for multi-year studies to fully quantify and characterize observed patterns in GHG flux within these ecosystems. Finally, high litter mass on the non-grazed subplots studied here (Chuan et al., 2018) may have protected microbial communities from severe climatic alterations between years, which was confirmed by more stable CO_2 emission between years within non-grazed areas.

The results of this study showed a reduction in average CH_4 consumption over the growing season from soils under long-term grazing in 2015, while remaining similar non-grazed areas in 2016. These findings supported previous observations that rangeland soils serve as a small sink for atmospheric methane (Mosier at al., 1997; Liebig et al., 2010; Wei et al., 2012). The negative values obtained for CH_4 net-flux indicated that methane consumption prevailed

over the process of methanogenesis (Topp and Pattey, 1997; Schönbach et al., 2012). This result occurred despite grazed subplots having higher bulk density, which likely reduced diffusion of CH_4 and O_2 (substrates for methanotrophs activity) from the atmosphere into soil (Chen et al., 2011), thereby inhibiting CH_4 uptake to some degree. Soil methanotroph activity may also have been inhibited on grazed areas by increased evaporation from soil and associated water stress (Chen et al., 2011). The balance between the fore-mentioned processes determine the overall grazing effect on methanogenesis and methanotrophy, and in turn, ultimately regulate overall CH_4 fluxes.

Long-term grazing did not affect averaged N_2O emissions over both growing seasons from the grassland soils studied here. This might be attributed to the high variability of N_2O emission, which is commonly observed in many field experiments and explained by complex interaction between microbiological processes, namely nitrification and denitrification, climatic conditions and soil properties, including soil moisture and temperature, oxygen supply and others (Cowan et al., 2015; Liebig et al., 2010; Gao et al., 2017; Butterbach-Bahl et al., 2013; Luo et al., 2013). Furthermore, grazing increases the spatial and temporal variability of N_2O emission due to local soil compaction, and the re-distribution of urine and dung (Oenema et al., 1997; Gao et al., 2017). Finally, because of the inherent greater variability of N_2O flux, low sampling frequencies may be insufficient to detect differences in this GHG, and indeed the errors around the flux values observed in this study were high (Wei et al., 2012). Due to the large area encompassed by the study sites examined in this research, more frequent gas sampling was infeasible (Gao et al., 2017; Baah-Acheamfour et al., 2016).

2.4.2. Geographical and temporal variability in GHG flux

In the present study, differences between natural subregions in their cumulative CO_2 emission rates were observed. Spatial variability of CO_2 emission was attributed to soil organic matter content, soil water content and live root biomass, in the study of three contrasting temperate steppe types in northern China (Shi et al., 2017), as well as strong climatic gradients of temperature and precipitation, which are considered a main driving factor of soil organic matter decomposition, and therefore CO_2 formation (Follet et al., 2012). Lower CO_2 emissions within the MG can be attributed to the low SOC content in the region (in comparison with CP and FF), which otherwise positively influences soil respiration (Liebig et al., 2013). The lowest cumulative CH_4 uptake observed was within the MG as well. Soil water and organic matter content, together with soil texture, are the main drivers of spatial variability of CH_4 uptake (Shi et al., 2017). Ultimately, the balance of interactions among these factors determines the rate of CH_4 uptake. While a coarse (high sand content) soil texture of soils within the MG subregion might promote methanotrophy due to the availability of O_2 from the atmosphere (Bock, 2016), water limitations in the region due to low precipitation can reduce the magnitude of the CH_4 consumption and overall activity of methanotrophs (Wang et al., 2005; Luo et al., 2013).

Cumulative CO_2 emissions were affected by interannual variability of climatic conditions, with the lowest soil respiration observed in the dryer year. Soil water content has often been shown to be a controlling factor for GHG emission or consumption (Oertel et al., 2016; Rastogi et al., 2002; Wang and Fang, 2009). Both microbial and root respiration increase with greater water availability (Liu et al., 2009; Wang and Fang, 2009; Rastogi et al., 2002), and may explain differences in CO_2 cumulative emission between years; the lower cumulative emission in 2015 was likely related to lower precipitation levels. Cumulative CH_4 and N_2O emissions were different between years as well with lower CH_4 consumption and higher N_2O

emission in 2016. Both processes are known to be highly dependent on soil water content, as a regulator of soil microbial activity (Soussana et al., 2010; Liebig et al., 2010; Samal et al., 2015; Oertel et al., 2016; Wu et al., 2010). More specifically, higher consumption of CH_4 in 2015 was likely attributed to lower precipitation level, which increased diffusion of substrates for methanotrophs activity (CH_4 and O_2) into soils (Chen et al., 2011). While, as discussed above, wet, anaerobic conditions are likely to favor CH_4- and N_2O-producing bacteria (Oertel et al., 2016).

2.5. Summary

While average CO_2 and CH_4 fluxes over growing season in 2015 were reduced by long-term grazing, in 2016 both averaged over season fluxes was on the same level between grazed and non-grazed subplots. Cumulative emission of CO_2 varied widely between years and treatments, indicating that climatic conditions have importance in the processes regulating GHG dynamics. In dry year cumulative CO_2 emission was lower from grazed area and it was 50% higher in wet year, while for non-grazed area the differences between years was 23% with higher emission in wet year as well. This is an important information for range managers and livestock producers, as well as for C and N balance calculation for the grassland ecosystems globally and locally. It should be stressed that while the grazing intensity applied within the studied locations was light to moderate, higher intensities of grazing might have led to different effects.

The finding that cumulative CO_2 emission varied with climatic conditions among treatments and years is an important result as future climate scenarios predict increases in mean annual precipitation and temperature (Hufkens et al., 2016), however the effect of these changes on GHG fluxes remains uncertain. Any short-term moisture increases could increase the potential

for GHG emissions, while these fluxes could be reduced by the increased risk of more frequent and severe drought years.

Table 2.1. Main characteristics of the studied subregions according to Downing and Pettapiece, 2006.

Factor	Central Parkland		Dry Mixedgrass		Foothills Fescue	
Area, km^2	53706		46937		13623	
Average elevation, m above sea	750		800		1100	
Main soil type	Black Chernozem		Brown Chernozem		Black Chernozem	
Annual heat-moisture index	27.9		42.6		29.6	
MAT, °C	2.3		4.2		3.9	
MAP, mm	441.2		333.3		469.6	
Ecologically sustainable stocking rates[a], AUM ha^{-1}	0.04-3.08		0.1-0.88		0.63-3.25	
Vegetation cover[b], %	97.6 ± 2.5	91.0 ± 2.5	59.5 ± 2.6	59.2 ± 2.8	81.7 ± 4.4	83.4 ± 4.9
Grass mass[b], kg ha^{-1}	2403 ± 97	2664 ± 102	1789 ± 203	2020 ± 21	1772 ± 158	1954 ± 249
Herbage mass[b], kg ha^{-1}	2905 ± 147	3031 ± 115	1989 ± 171	2176 ± 48	2807 ± 301	2323 ± 210
Litter mass[b], kg ha^{-1}	1215 ± 290	2840 ± 645	1378 ± 128	2442 ± 247	1071 ± 279	3452 ± 760
Vegetation	Aspen (*Populus tremuloides*), plains rough fescue (*Festuca hallii*)		Blue grama grass (*Bouteloua gracilis*), western wheatgrass (*Pascopyrum smithii*), needle and thread grass (*Hesperostipa comata*)		Foothills rough fescue (*Festuca campestris*), Parry's oatgrass (*Danthonia parryi*), Idaho fescue (*Festuca idahoensis*)	

[a] Data were taken from Range plant communities and range health assessment guidelines (2013);
[b] Data were taken from Chuan et al., 2018

Table 2.2. Grassland soil properties (0-5 cm mineral layer) and ANOVA test output for long-term grazed (G) and non-grazed (NG) treatments for the three subregions of Alberta. The values represent mean ± standard error.

Subregion	Treatment	pH_{H2O}	SOC, %[1]	C/N	BD, g cm^{-3}
Central	G	6.13 ± 0.11	7.20 ± 0.42	11.43 ± 0.30	1.24 ± 0.08
Parkland	NG	6.13 ± 0.09	5.39 ± 0.32	11.34 ± 0.23	1.03 ± 0.04
Dry	G	6.54 ± 0.06	2.89 ± 0.15	11.73 ± 0.27	1.36 ± 0.11
Mixedgrass	NG	6.62 ± 0.07	2.48 ± 0.12	11.63 ± 0.47	1.23 ± 0.05
Foothills	G	6.36 ± 0.06	7.81 ± 0.44	11.31 ± 0.35	0.98 ± 0.10
Fescue	NG	6.43 ± 0.09	7.11 ± 0.37	11.57 ± 0.32	0.89 ± 0.10

ANOVA

Factor	Statistics[2]	pH_{H2O}	SOC, %	C/N	BD, g cm^{-3}
Treatment (GT)	P	0.474	**0.002**	0.875	**0.013**
	F	0.545	14.687	0.026	8.771
	df (n, d)	1, 12	1, 12	1, 12	1, 11
Subregion (S)	P	0.277	**<.0001**	0.763	**0.022**
	F	1.431	37.320	0.277	5.449
	df (n, d)	2, 12	2, 12	2, 12	2, 11
GT x S	P	0.868	0.102	0.559	0.642
	F	0.143	2.782	0.609	0.462
	df (n, d)	2, 12	2, 12	2, 12	2, 11

[1] SOC, soil organic carbon; C/N, ratio of carbon and nitrogen; BD, bulk density
[2]P, probability value; F, F-statistics; df (n, d), degrees of freedom for numerator and denominator, respectively

Table 2.3. Effect of long-term grazing treatment exposure, geographic (subregion) variability, and their interactions, on the measured soil temperature and water content of northern temperate Great Plains grasslands during each of 2015 and 2016.

Subregion	Treatment	Soil temperature, °C		Soil water content, m^3 m^{-3}	
		2015	2016	2015	2016
Central Parkland	G[1]	18.13 ± 0.25	17.17 ± 0.21	0.15 ± 0.003	0.17 + 0.004
	NG	16.12 ± 0.18	16.61 ± 0.17	0.14 ± 0.003	0.16 ± 0.003
Dry Mixedgrass	G	19.55 ± 0.23	18.12 ± 0.19	0.10 ± 0.003	0.12 ± 0.002
	NG	18.75 ± 0.22	18.11 ± 0.21	0.10 ± 0.002	0.10 ± 0.002
Foothills Fescue	G	15.18 ± 0.19	14.89 ± 0.16	0.16 ± 0.003	0.21 ± 0.003
	NG	15.03 ± 0.18	14.60 ± 0.16	0.13 ± 0.002	0.19 ± 0.003

ANOVA

Factor	Statistics[2]	2015	2016	2015	2016
Treatment (GT)	P	**0.019**	0.229	0.149	0.223
	F	7.317	1.615	2.376	1.669
	df (n, d)	1, 12	1, 11	1, 12	1, 11
Subregion (S)	P	**0.004**	**0.007**	**0.009**	**0.035**
	F	9.302	7.680	7.149	4.504
	df (n, d)	2, 12	2, 11	2, 12	2, 11
GT x S	P	0.186	0.950	0.117	0.955
	F	1.941	0.051	2.581	0.046
	df (n, d)	2, 12	2, 11	2, 12	2, 12

[1]G, grazed; NG, non-grazed

[2]P, probability value; F, F-statistics; df (n, d), degrees of freedom for numerator and denominator, respectively

Table 2.4. Effect of long-term grazing treatment exposure, geographic (subregion) and temporal (sampling date) variation, as well as their interactions, on the emission rates of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from soils of the northern temperate Great Plains grasslands during each of 2015 and 2016.

Factor	2015				2016			
	df_{num}*	df_{res}	F	P	df_{num}	df_{res}	F	P
CO_2 flux, kg C ha^{-1} d^{-1}								
Treatment (GT)	1	12	4.979	**0.011**	1	11	1.018	0.335
Subregion (S)	2	12	4.465	**0.027**	2	11	6.354	**0.014**
Sampling date (SD)	7	107	11.218	**<.0001**	8	135	22.092	**<.0001**
S x GT	2	12	0.708	0.463	2	10	0.009	0.991
GT x SD	7	108	0.949	0.324	8	138	0.827	0.580
S x SD	14	106	3.281	**<.0001**	16	135	3.426	**<.0001**
S x GT x SD	14	107	0.888	0.573	16	137	0.313	0.995
CH_4 flux, g C ha^{-1} d^{-1}								
Treatment (GT)	1	12	5.213	**0.041**	1	11	0.134	0.721
Subregion (S)	2	12	3.316	**0.071**	2	11	4.796	**0.031**
Sampling date (SD)	7	124	2.203	**0.019**	8	140	9.199	**<.0001**
S x GT	2	12	0.192	0.828	2	11	3.337	**0.072**
GT x SD	14	125	0.717	0.658	8	141	1.669	0.111
S x SD	7	124	2.198	**0.011**	16	140	6.438	**<.0001**
S x GT x SD	14	125	0.616	0.848	16	141	0.444	0.968
N_2O flux, g N ha^{-1} d^{-1}								
Treatment (GT)	1	11	0.451	0.514	1	9	0.307	0.592
Subregion (S)	2	11	0.681	0.526	2	11	0.067	0.935
Sampling date (SD)	7	87	1.464	0.191	8	93	2.371	**0.023**
S x GT	2	11	0.942	0.419	2	9	1.464	0.281
GT x SD	7	87	0.728	0.649	8	93	0.707	0.685
S x SD	14	86	0.643	0.822	16	92	1.250	0.247
S x GT x SD	14	86	0.679	0.789	16	92	0.910	0.560

Values in bold indicate significant differences at $p<0.10$

* Df_{num}, numerator degrees of freedom; df_{res}, residuals degrees of freedom, F, F-statistic, P, probability value.

Table 2.5. Effect of long-term grazing, spatial (subregion) and temporal (year) variabilities as well as their interactions, on the cumulative emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from rangeland soils in Alberta, Canada.

Factor	df_{num}	Df_{res}	F	P
CO_2, kg C ha^{-1}				
Treatment (GT)	1	12	0.007	0.934
Subregion (S)	2	12	3.363	**0.069**
Year (Y)	1	23	56.232	**<.0001**
S x GT	2	12	0.383	0.689
GT x Y	1	23	12.152	**0.002**
S x Y	2	23	1.963	0.164
S x GT x Y	2	23	0.005	0.995
CH_4, kg C ha^{-1}				
Treatment (GT)	1	12	0.069	0.797
Subregion (S)	2	12	3.951	**0.048**
Year (Y)	1	23	3.031	**0.095**
S x GT	2	12	2.751	0.105
GT x Y	1	23	2.200	0.151
S x Y	2	23	3.529	**0.046**
S x GT x Y	2	23	0.349	0.709
N_2O, kg C ha^{-1}				
Treatment (GT)	1	12	0.186	0.647
Subregion (S)	2	12	0.171	0.845
Year (Y)	1	23	3.467	**0.075**
S x GT	2	12	0.136	0.874
GT x Y	1	23	0.665	0.423
S x Y	2	23	0.332	0.721
S x GT x Y	2	23	0.785	0.468

Values in bold indicate significant differences at $p<0.10$

Table 2.6. Effect of soil moisture and its interaction with grazing treatment (G/ NG) and geographic subregions, on the emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from northern temperate Great Plains grassland soils during 2015 and 2016.

Factor	2015				2016			
	df_{num}	df_{den}	F	P	df_{num}	df_{den}	F	P
CO_2 flux, kg C ha^{-1} d^{-1}								
Moisture (M)	1	23	2.591	0.121	1	21	0.039	0.843
M x GT	1	12	10.053	**0.008**	1	11	6.832	**0.024**
M x S	2	14	0.635	0.544	2	13	4.142	**0.039**
M x GT x S	2	12	0.309	0.739	2	10	0.672	0.532
CH_4 flux, g C ha^{-1} d^{-1}								
Moisture (M)	1	20	0.377	0.546	1	18	0.327	0.574
M x GT	1	12	5.156	**0.043**	1	11	1.039	0.329
M x S	2	14	2.685	0.101	2	13	4.279	**0.038**
M x GT x S	2	12	0.338	0.719	2	11	3.535	**0.067**
N_2O flux, g N ha^{-1} d^{-1}								
Moisture (M)	1	17	1.116	0.305	1	16	0.034	0.857
M x GT	1	12	1.005	0.335	1	11	0.650	0.438
M x S	2	12	0.267	0.769	2	12	0.337	0.718
M x GT x S	2	13	0.429	0.660	2	11	0.416	0.670

GT, treatment; S, subregion;

Values in bold indicate significant differences at the $p<0.10$. Df_{num}, numerator degrees of freedom; df_{den}, denominator degrees of freedom, F, F-statistic, P, probability value.

Table 2.7. Effect of soil temperature and its interactions with grazing treatment (G/ NG) geographic subregion, on the emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) of northern temperate Great Plains grassland soils during 2015 and 2016.

Factor	2015				2016			
	df_{num}	df_{den}	F	P	df_{num}	df_{den}	F	P
CO_2 flux, kg C ha^{-1} d^{-1}								
Temperature (T)	1	23	3.453	**0.076**	1	21	0.416	0.526
T x GT	1	12	6.715	**0.023**	1	10	4.549	**0.058**
T x S	2	13	1.677	0.225	2	11	5.471	**0.022**
T x GT x S	2	12	0.122	0.886	2	11	0.384	0.691
CH_4 flux, g C ha^{-1} d^{-1}								
Temperature (T)	1	22	2.546	0.124	1	19	2.089	0.165
T x GT	1	13	2.696	0.125	1	10	0.881	0.369
T x S	2	13	2.499	0.121	2	11	4.786	**0.032**
T x GT x S	2	12	0.596	0.566	2	11	2.409	0.136
N_2O flux, g N ha^{-1} d^{-1}								
Temperature (T)	1	16	0.307	0.587	1	19	0.002	0.965
T x GT	1	13	2.134	0.168	1	10	0.538	0.479
T x S	2	12	0.047	0.954	2	11	0.545	0.589
T x GT x S	2	13	1.085	0.367	2	12	0.378	0.694

Values in bold indicate significant differences at the $p<0.10$. Df_{num}, numerator degrees of freedom; df_{den}, denominator degrees of freedom, F, F-statistic, P, probability value.

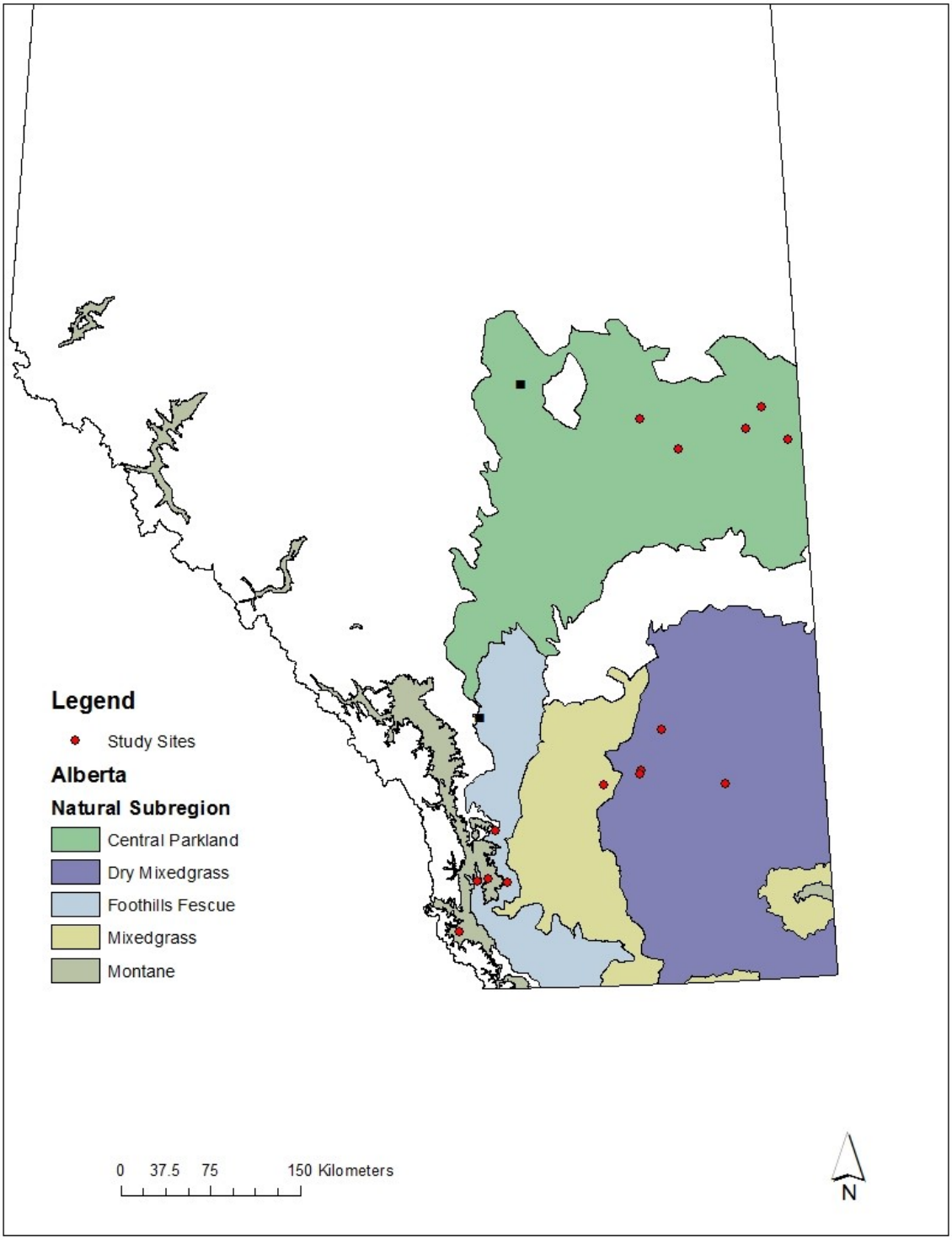

Fig 2.1. Location of the study sites in the Central Parkland, Dry Mixedgrass and Foothills Fescue subregions of Alberta, Canada. Map credit to M. Kohler.

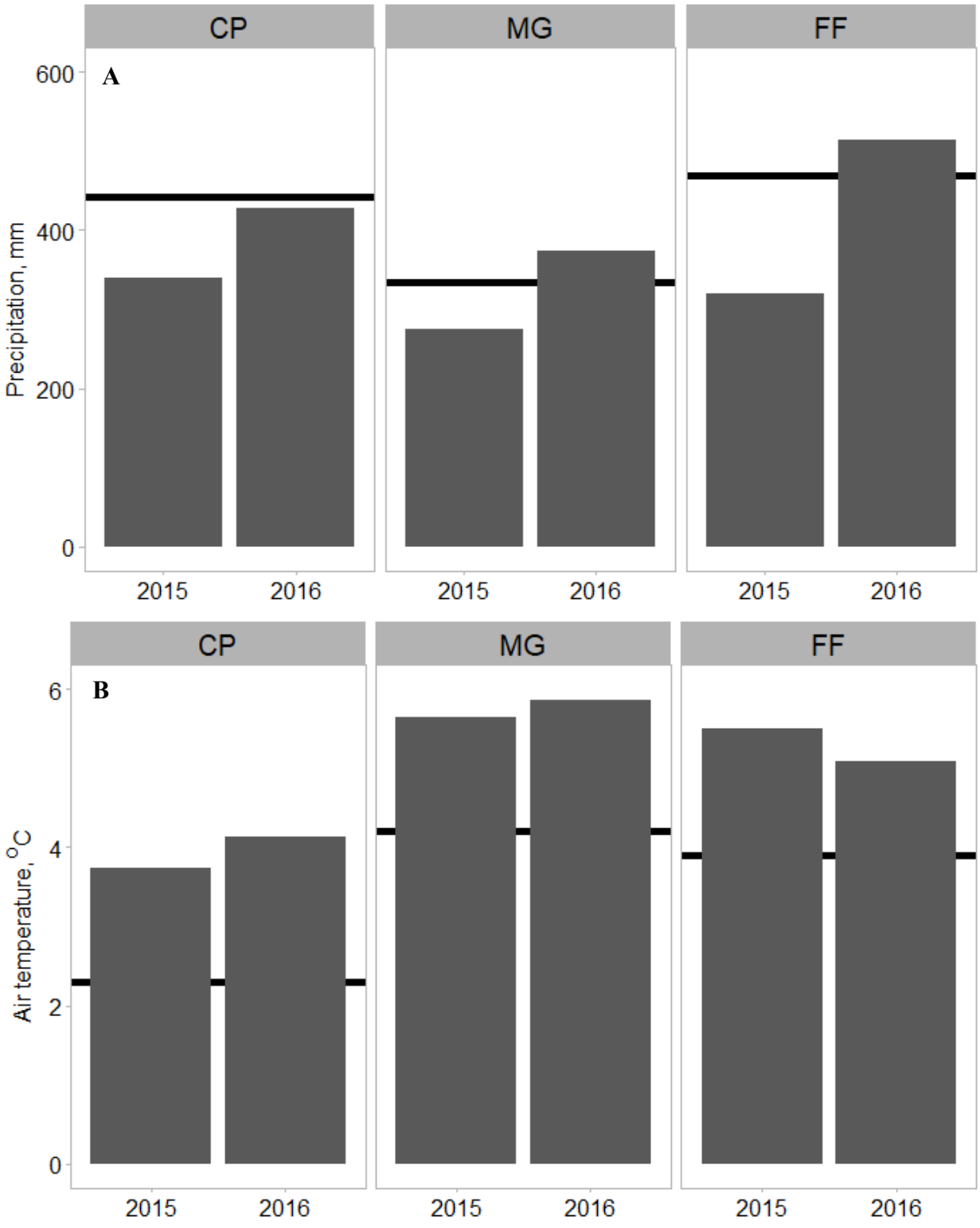

Fig. 2.2. Mean annual precipitation (MAP, A) and mean annual temperature (MAT, B) for the Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions during 2015 and 2016. Horizontal lines within each subregion represent the MAP and MAT from Downing and Pettapiece, 2006. Data were downloaded from http://agriculture.alberta.ca.

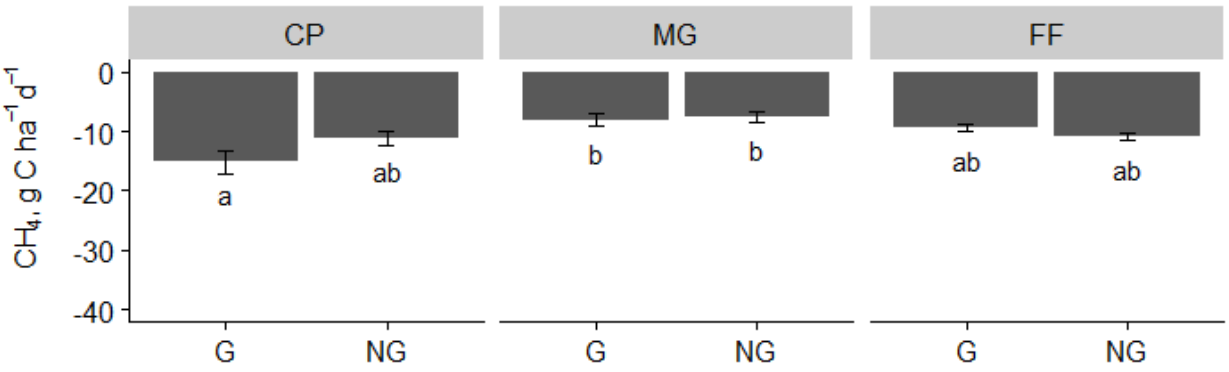

Fig. 2.3. Consumption rates of CH_4 between grazed (G) and non-grazed (NG) treatments and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2016. Different lowercase letters indicate 2-way interaction between treatments and subregions.

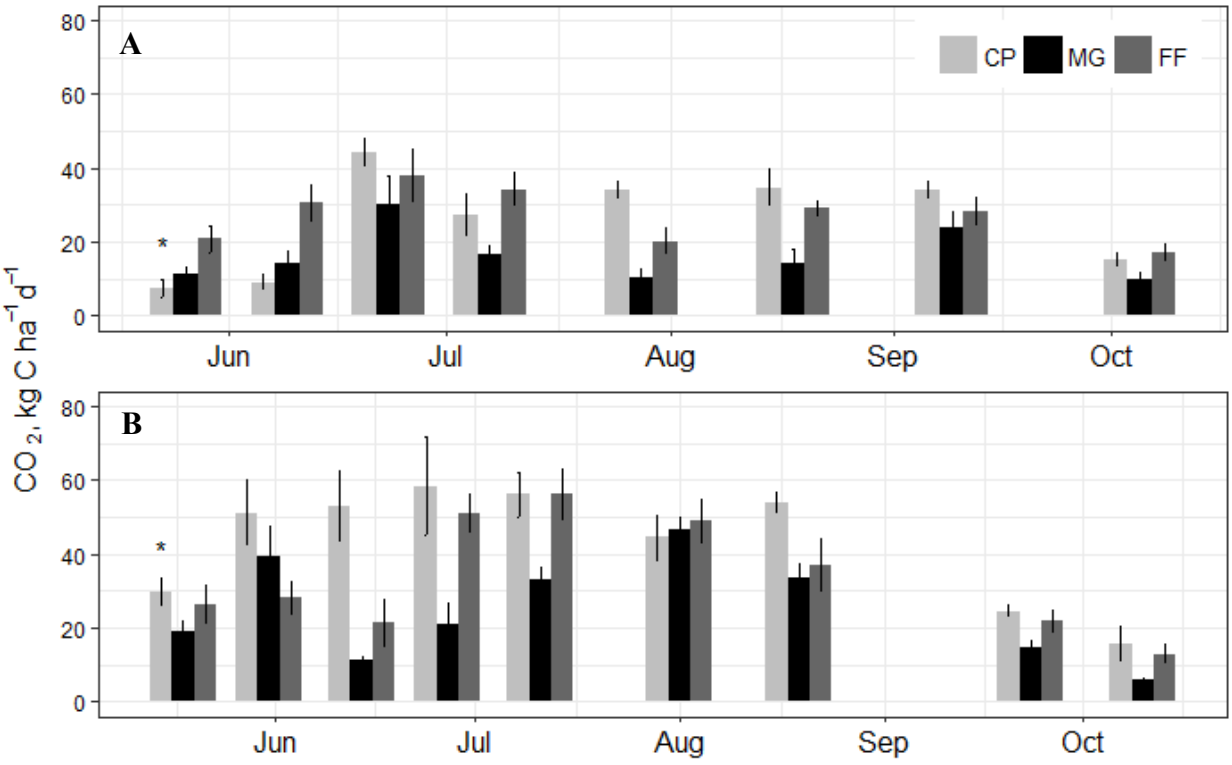

Fig. 2.4. CO_2 emission rates (kg C ha^{-1} d^{-1} ± standard error) during 2015 (A) and 2016 (B) from northern temperate grasslands of the Great Plains.
*Due to high variation of CO_2 emission rates across subregions and sampling dates in both study years, post-hoc tests are provided in Appendix 3.

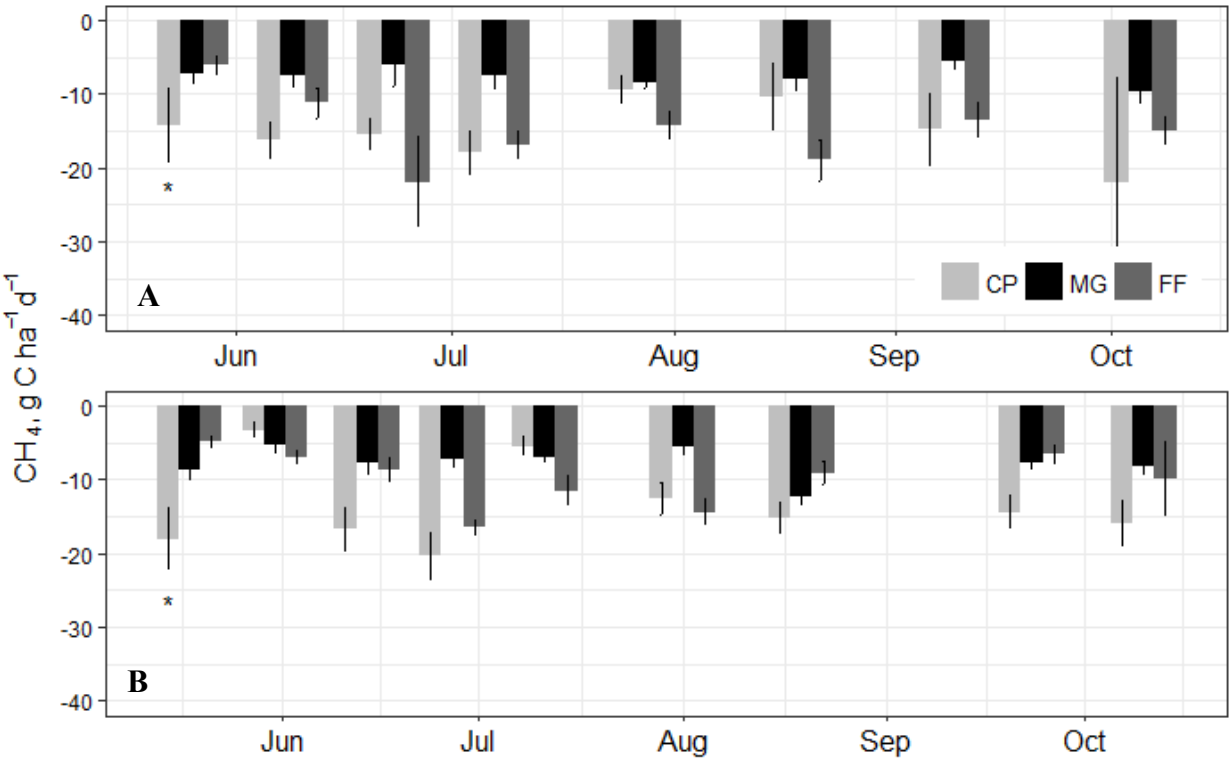

Fig. 2.5. Consumption rates of CH_4 (g C ha^{-1} d^{-1} ± standard error) in 2015 (A) and 2016 (B) from northern temperate grasslands of the Great Plains.
*Due to high variation of the CH_4 consumption rate across subregions and sampling dates in 2016 the posthoc test is provided in the Appendix 4.

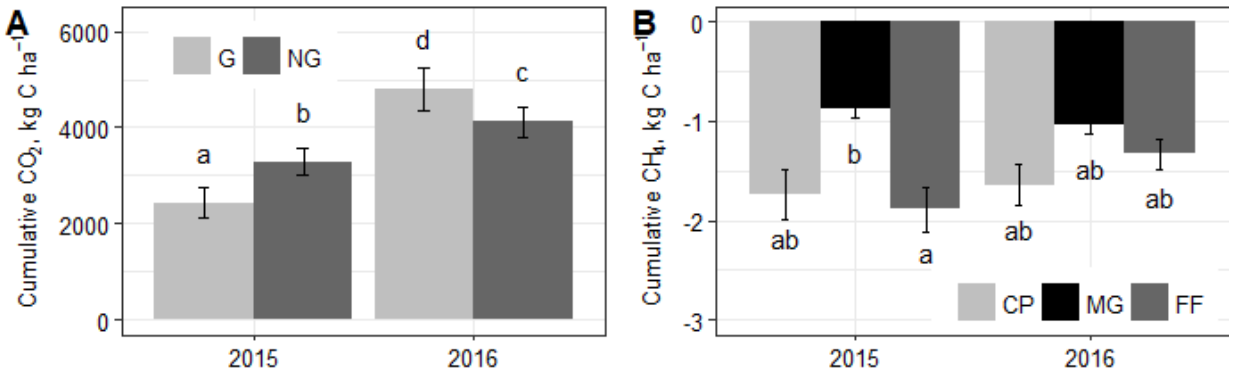

Fig. 2.6. Cumulative CO_2 emission (A), cumulative CH_4 consumption (B) in each of 2015 and 2016 from northern temperate grasslands of the Great Plains. Different lowercase letters indicate (A) 2-way interaction between grazed (G) and non-grazed (NG) treatments and years; (B) 2-way interaction between and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions and years.

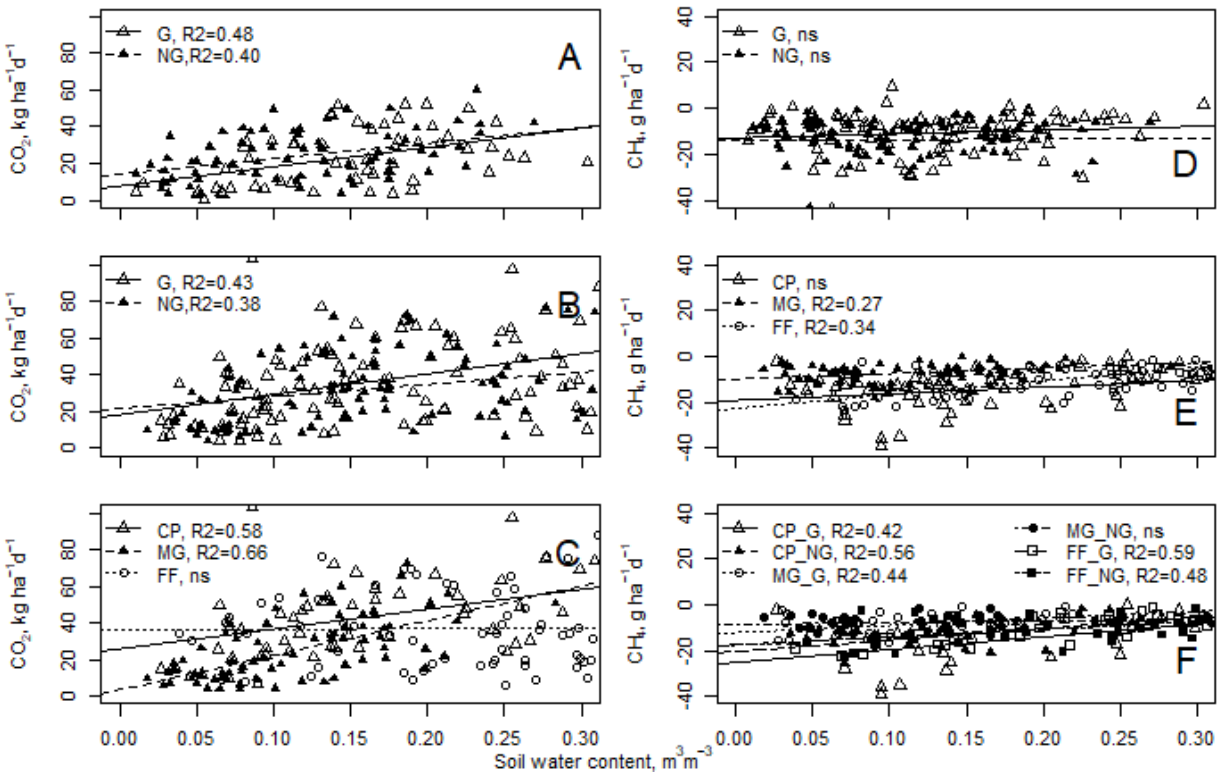

Fig. 2.7. Relationship between soil water content (m^3 m^{-3}) and carbon dioxide (CO_2) emission (kg C ha^{-1} d^{-1}) in 2015 (A) and 2016 (B, C) and CH_4 consumption (g C ha^{-1} d^{-1}) in 2015 (D) and 2016 (E, F).

(A, B) represent 2-way interaction between soil water content and grazed (G) and non-grazed (NG) treatments for CO_2 emission in 2015 and 2016, respectively; (C) represent 2-way interaction between soil water content and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions for CO_2 emission in 2016; (D) represent 2-way interaction between soil water content and grazed (G) and non-grazed (NG) treatments for CH_4 consumption in 2015; (E) represent 2-way interaction between soil water content and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions for CH_4 consumption in 2016; (F) represent 3-way interaction between soil water content, grazed (G) and non-grazed (NG) treatments and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions for CH_4 consumption in 2016.

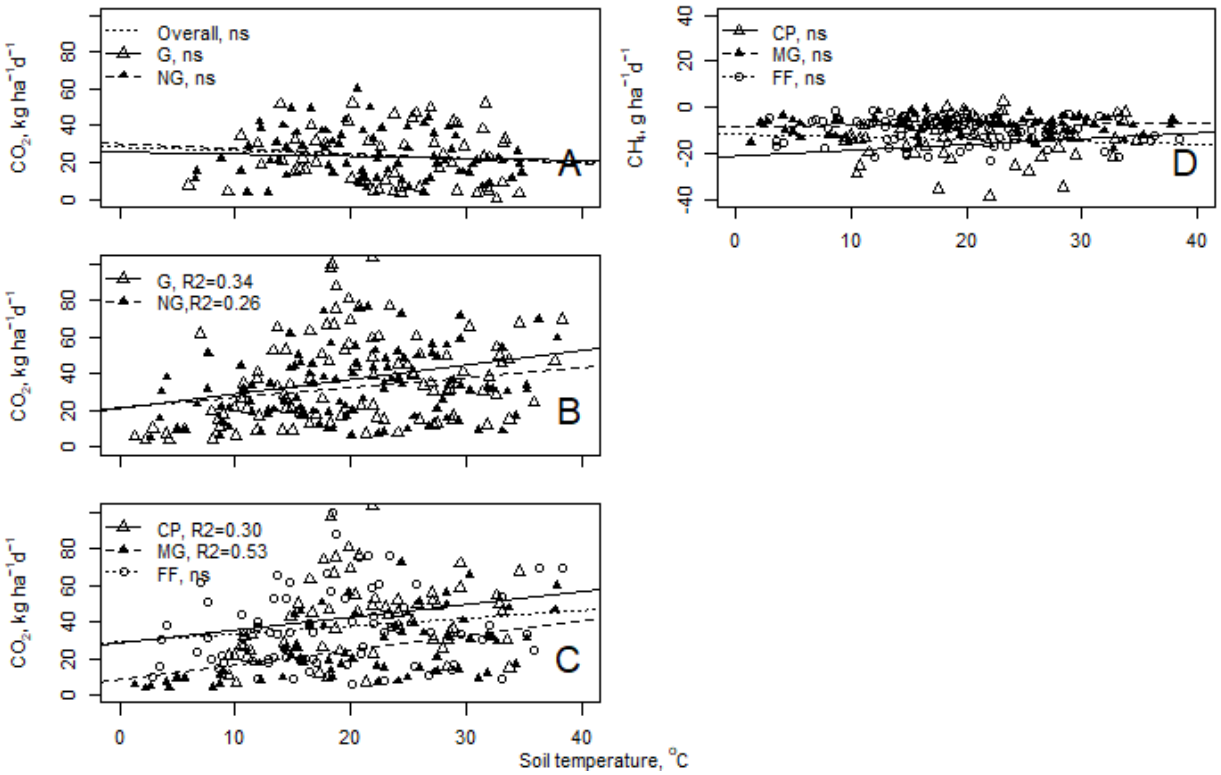

Fig. 2.8. Relationship between soil temperature (°C) and carbon dioxide (CO_2) emission (kg C ha^{-1} d^{-1}) in 2015 (A) and 2016 (B, C), as well as CH_4 consumption in 2016 (D).

(A) represent overall relationship between soil temperature and CO_2 emission as well as 2-way interaction between soil temperature and grazed (G) and non-grazed (NG) treatments for CO_2 emission in 2015; (B) represent 2-way interaction between soil temperature and grazed (G) and non-grazed (NG) treatments for CO_2 emission in 2016; (C) represent 2-way interaction between soil temperature and Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF) subregions for CO_2 emission in 2016; (D) represent 2-way interaction between soil temperature and grazed (G) and non-grazed (NG) treatments for CH_4 consumption in 2016.

Chapter 3. Long-term grazing effect on soil microbial communities of Alberta grasslands

3.1. Introduction

Soil microorganisms are key drivers of nutrient transformation and cycling, are sensitive indicators of soil quality and play a critical role in regulating the functionality of an ecosystem (Doran and Zeiss, 2000; Delgado-Baquerizo et al., 2016). While high microbial diversity promotes a variety of ecosystem functions, simplification of community composition negatively alters ecosystems processes and sustainability (Wagg et al., 2014; Delgado-Baquerizo et al., 2016; Bardgett and van der Putten, 2014). However, microbial communities within grasslands remain poorly characterized (Evans et al., 2017) and the impact of livestock grazing on community composition of soil bacteria, archaea and fungi is not well known (Brussaard et al., 1997; Aldezabal et al., 2015; Eldridge et al., 2017).

Up to 90% of soil processes are mediated by soil microorganisms (Nannipieri et al., 2003). Prokaryotes (bacteria and archaea) are highly diverse phylogenetically and functionally (Killham and Prosser, 2015), they are catalyzing reactions involved in greenhouse gases production or consumption (Chroňáková et al., 2015). Archaeal contribution to the total activity of prokaryotes in soils is ~1%, which makes them an important component of ongoing biological processes (Nicol et al., 2003). Similar to bacteria and archaea, fungal community in soils are extremely species rich (Aislabie and Deslippe, 2013; Taylor and Sinsabaugh, 2015), and have profound impact on biogeochemical cycles due to the wide range of substrates they utilize for growth (Taylor and Sinsabaugh, 2015).

Microbial communities are highly sensitive to soil disturbances and environmental conditions (Kotzé et al., 2017). Human activities, such as cultivation of lands and deforestation,

are known to alter the composition and richness of microbial communities, whereas less is known about the influence of livestock grazing on soil microorganisms. Moreover, published data provided contradictory findings (Hodel et al., 2014; Brussaard et al., 1997; Aldezabal et al., 2015; Eldridge et al., 2017; Radl et al., 2007; Chroňáková et al., 2009; Jirout et al., 2011; Elhottová et al. 2012; Chroňáková et al., 2013; Chroňáková et al., 2015; Gou et al., 2015; Huhe et al., 2017; Zhou et al., 2010; Qu et al., 2016). For example, livestock grazing can lead to no changes of soil microbial abundance (assessed as microbial biomass; Hodel et al., 2014; Medina-Roldán et al., 2012; Tom et al., 2006), increase (Aldezabal *et al.*, 2015; Bardgett *et al.*, 1997), or decrease (Ingram et al., 2008). While severe grazing has been shown to increase the biomass of aerobic bacteria, archaea and fungi, in turn resulting in the development of a qualitatively new community profile compared to areas without grazing (Elhottová et al. 2012), others have found that the type of grassland has a much greater impact on soil microbial communities than grazing itself (Ford et al., 2013).

Livestock grazing is an important factor regulating all above- and belowground processes in grasslands (Hodel et al., 2014, Bardgett and Cook, 1998). Defoliation, trampling, and the re-distribution of dung and urine by large herbivores can alter vegetation, soil thermal, hydrological, physical and chemical properties, which in turn, can affect soil microbial communities (Bremer et al., 1998; Bardgett and Cook, 1998; Aldezabal et al., 2015; Hodel et al., 2014). Consequently, grazing has the potential to positively, negatively or have no effect on soil microorganisms depending on the balance between these different processes (Bardgett and Wardle, 2003). Also, soil microbial communities vary markedly across environmental gradients (soil type and properties, topography) and through time (season of soil sampling) (Tracy and Frank, 1998; Hodel et al., 2014). Grasslands of the Northern Great Plains are highly diverse in

their climatic conditions, topography, soil types and vegetation composition, but all are commonly used for livestock grazing. Thus, studies examining the effects of livestock grazing on microbial communities under different environmental and climatic conditions through time are needed to fully understand the range of potential responses by soil microbial communties to ongoing land use.

In the present study, the response of soil microbial communities to long-term livestock (cattle) grazing was examined across a broad agro-climatic gradient of northern temperate grasslands of Alberta over two growing seasons. The specific objectives of the study were: (i) to quantify the abundance of bacteria, archaea and fungi in these grasslands, and (ii) compare the compositional structure of bacterial, archaeal and fungal communities in soils associated with long term grazing with that present in adjacent non-grazed areas.

3.2. Materials and methods

3.2.1. Study locations

The study locations were situated within three subregions of Alberta, Canada, including the Central Parkland (CP), Dry Mixedgrass (MG) and Foothills Fescue (FF; Downing and Pettapiece, 2006; Table 2.1; Table 2.2). Briefly, the main features of the MG subregion is high temperatures and low precipitation during the summer as well as dominance of low-growing drought tolerant vegetation (Adams et al., 2013). The dominant vegetation is a *Stipa-Bouteloua-Agropyron* community, which is resilient to low grazing stocking rates (Dormaar et al., 1997) The main soil type is a Brown Chernozem, which forms with low organic matter input into soil and high mean annual temperatures (Downing and Pettapiece, 2006). The FF is the wettest subregion, which characterized by cooler summers with higher precipitation level. The dominant

vegetation is diverse and productive, including *Festuca campestris* and *Danthonia parryi* (Chuan et al., 2018; Adams et al., 2003). Black Chernozem is the main soil type, which forms with high organic matter input into the soil and moderate temperatures (Alberta Environment and Parks, 2015). The CP is a broad transitional zone located between the cool, moist boreal forests to the north and dry, warm grasslands to the south. Vegetation represents a matrix of forests, shrublands and grasslands, including *Populus tremuloides*, *Symphoricarpos occidentalis*, *Festuca hallii*, *Hesperostipa curtiseta*, *Pascopyrum smithii*, *Koeleria macrantha* (Chuan et al., 2018). The dominant soil type is Black Chernozem (Downing and Pettapiece, 2006). A total of 15 locations were examined in this study, with five locations in each of the three subregions. Each location was comprised of an exclosure wherein livestock (i.e. cattle) grazing was excluded for at least 15 and up to 60 years, each of which in turn, were compared to the surrounding grazed area to determine the long-term grazing effect on soil microbial communities. Cattle grazing has occurred in these regions for approximately 140 years (Wang et al., 2014), and recent grazing practices typically include light to moderate stocking rates between May and October annually (see Table 2.1). This created a total of 30 subplots, half of which were non-grazed (NG), with each paired to a grazed (G) subplot. For the present study, there was no specific information available on the localized stocking rates associated with grazing within each grazed subplot as the large size of grazing paddocks did not necessarily lead to uniform use across each pasture.

3.2.2. Soil sampling and DNA extraction

Soil samples were collected in the late spring, middle of summer and fall over two years (May 26-29 and 18-23, July 28-31 and 12-15, October 5-9 and 1-9, during 2015 and 2016, respectively). Ten soil cores were randomly collected per grazed and non-grazed subplot of each

of the 15 sites from the 0-5 cm mineral soil layer using a soil corer Ø 5 cm, placed into plastic bags, and thoroughly mixed. Overall 180 composite samples were collected. The composite samples were transported on dry ice to the laboratory, where they were stored at -20°C prior to DNA extraction.

Total DNA was extracted from 250 mg of each composite sample using Power Soil DNA Isolation Kits (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol. The kit was chosen as it eliminates humic substances as well as other PCR inhibitors, and provides DNA that is ready to use in downstream analysis, such as PCR, qPCR and NGS (mobio.com). Quality and quantity of the DNA were assessed with a NanoDrop2000C spectrophotometer (ThermoScientific, Wilmington, DE, USA) before further analysis. The extracted DNA was stored at -80°C prior to molecular analysis.

3.2.3. Quantitative polymerase chain reaction (qPCR)

To assess the abundance of soil bacteria, archaea and fungi, a quantitative polymerase chain reaction (qPCR) assay was applied to the DNA obtained from the previous step as the template and 10x diluted in nuclease-free water (dilution was revealed beforehand). The sets of primers used for the qPCR assays are listed in Table 3.1. The volume of the reaction was 10 µl, which included 5 µl of PowerUP^{TM} SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA), 0.8 (bacteria), 0.4 (archaea) and 0.6 (fungi) µl of 10µM primers, 1 µl of template DNA, and an adjusted volume of H_2O (to 10 µl). For the no-template control, 1 µl of nuclease-free water was added instead of template DNA. All the samples were analyzed in duplicate on the reaction plates (Applied Biosystems, Foster City, CA, USA). The thermocycler (StepOne, Applied Biosystems, Foster City, CA, USA) program was: 50°C for 2 min, 95°C for 2 min

followed by either 40 (bacteria and fungi) or 35 (archaea) cycles of 95°C for 15 sec, 55°C (bacteria) / 66°C (archaea) / 56°C (fungi) for 15 sec and 72°C for 1 min. To confirm the absence of non-specific amplification and that amplified products had the appropriate size (180, 570 and 380 bp for bacteria, archaea and fungi, respectively), melting curve and gel electrophoresis analyses were performed, respectively. The qPCR efficiencies were 98-104% (R^2 = 0.997-0.999) for bacteria, 90-92% (R^2 = 0.997-0.998) for archaea and 90-98% (R^2 = 0.992-0.996) for fungi.

The 10^8 to 10 copy numbers standards were made using serially diluted custom-made gBlocks® (Integrated DNA Technology, Caroalville, USA), the design of which was based on partial sequences of *Clostridium thermocellum*, *Methanoscarcina mazei* and *Aspergiillus niger* strain P-19 for bacterial, archaeal 16S rRNA and fungal 18S rRNA, respectively. Copy numbers of bacterial and archaeal 16S rRNA and fungal 18S rRNA genes were expressed as the copy number per gram of dry soil weight (oven-dried at 105°C for 48 hrs).

3.2.4. Next-generation sequencing (NGS)

To explore alpha and beta diversity of soil bacterial, archaeal and fungal communities, 16S / ITS amplicon sequencing was applied. The sets of primers used are listed in Table 3.1. For bacteria, the amplicon libraries were prepared according to the Illumina protocol (Part #15044223 Rev. B). A modified Illumina protocol was utilized for archaea and fungi as described below.

Selection of archaeal and fungal primer sets were based on the following criteria: amplicon length, coverage and selective amplification (Toju et al., 2012). For the 1st stage PCR annealing temperature, the number of cycles and PCR mix were optimized. For all samples, the volume of PCR reaction was 25 µl and contained 10 µl of Platinum Hot Start PCR 2x Master

Mix (Invitrogen, Lithuania, Vilnus), and 0.5 µl of each 10µM forward and reverse primers, 1 µl of the diluted DNA (5 ng μl^{-1} in 10mM Tris pH 8.5) and 13 µl of nuclease-free water (www.earthmicrobiome.org). The thermocycler (ProFlex PCR system, Applied Biosystems, Foster City, CA, USA) program was as follows: (i) *archaea*, 94°C for 2 min with subsequent 40 cycles of 94°C for 30 sec; the annealing temperature was lowered from 67°C by 1°C every cycle until it reached 55°C, which was used for the remaining cycles, and for 30 sec, 72° for 30 sec; and 72°C for 5 min (Fontana et al., 2016; Takahashi et al., 2014); (ii) *fungi*, 94°C for 2 min followed by 40 cycles of 94°C for 30 sec, 50°C for 30 sec, 72° for 30 sec, and a final extension of 72°C for 5 min.

Subsequently, all PCR products were purified using AMPure XP beads (Beckman Coulter, Pasadena, CA, USA) following the Illumina protocol, however, 45 µl of beads to 25 µl reaction were added as suggested by the AMPure XP beads manufacturer protocol (# 001298v001). For the 2nd stage PCR, the volume of PCR reaction was 50 µl and contained 25 µl of Platinum Hot Start PCR 2x Master Mix (Invitrogen, Lithuania, Vilnus), 5 µl of each Nextera XT Index Primer 1 and 2 (Illumina, San Diego, CA, USA), 5 µl of the DNA obtained after 1st stage PCR / clean up step and 10 µl of nuclease-free water. The thermocycler (ProFlex PCR system, Applied Biosystems, Foster City, CA, USA) program was as follows: 94°C for 2 min with subsequent 8 cycles of 94°C for 30 sec, 55°C for 30 sec, 72° for 30 sec and 72°C for 5 min. A second PCR clean-up as well as further library preparation was performed according to the Illumina protocol without any modifications. As an internal control, 10% PhiX was used. Paired-end sequencing (2x300) was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at the University of Alberta, AFNS department (Edmonton, AB, Canada).

3.2.5. Bioinformatics analysis

Obtained FASTQ files were processed with LotuS pipeline (Hildebrand et al., 2014). Briefly, paired-end reads were merged and quality filtered with default parameters. Further, sequences were clustered at a 97% similarity using UPARSE (Edgar, 2013). UCHIME was used alongside the *gold.fna* database for the detection and elimination of chimeric sequences (Edgar et al., 2011). Representative sequences of each OTU were taxonomically classified by similarity search with Lambda (Hauswedell et al., 2014) against SILVA v.128 and UNITE databases for bacteria / archaea and fungi, respectively. The representative sequences, biom file, and OTU tree were merged into a phyloseq object for further statistical analysis (McMurdie and Holmes, 2013).

3.2.6. Statistical analysis

All statistical analysis and graphical output were done using Rstudio, version 1.0.136 (RStudio Inc., Boston, USA). Differences were considered statistically significant with $p<0.10$. As 2015 was drier than 2016, data from the two years were analyzed separately (see Chapter 2).

The effects of treatments (long-term grazing, non-grazed), geographic location (subregions) and time of sampling (months), on the gene copy abundance of bacteria, archaea and fungi were tested with a linear mixed effects model (lme4, Bates et al., 2017). Grazing treatments and subregions were fixed effects, while month of sampling was treated as a repeated measure. Individual study sites nested within subregions and treatment subplots within sites were treated as random effects. To check the distribution of residuals and equality of variances of the model, diagnostic plots were used. A logarithmic transformation was applied to normalize qPCR data. While analyses were done on transformed data, untransformed data were presented in all tables and figures to maintain interpretability. To summarize the effects of factors compact letter

display (cld) method along with least-squares means (lsmeans / emmeans) was used (Lenth, 2017).

To assess the coverage of libraries rarefaction curves were built by applying the R function provided on the http://genoweb.toulouse.inra.fr website. To assess richness and the alpha diversity metrics (Shannon and Simpson diversity indexes), the phyloseq package in R was used (McMurdie and Holmes, 2013). To test long-term grazing effects, as well as geographic (spatial) and seasonal (temporal) variabilities on richness and alpha diversity metrics, a linear mixed effect model with the same model structure described above was used. To assess beta diversity, PerMANOVA analysis was performed based on phylogenetic (weighted and unweighted UniFrac) and non-phylogenetic (Bray-Curtis) dissimilarities, and ordination plots (non-metric multidimensional scaling, NMDS) were created using the vegan package (Oksanen et al., 2013).

The specific OTUs that characterize long-term grazing or non-grazed subplots were identified using an indicator species analysis (ISA) with the IndVal package in R (Dufrene and Legendre, 1997). The predictive value of A is the probability that a subplot was either grazed or non-grazed based on the OTUs found there, while the predictive value B is the probability of finding a particular OTUs in the long-term grazing / non-grazed subplots (Dufrene and Legendre, 1997). While the ISA revealed the OTUs specific for each treatment, a differential abundance analysis tested differences in relative abundance of OTUs that occurred in both treatments (DESeq2); the Benjamini & Hochberg approach was used for multiple mean comparison adjustment (Love et al., 2014).

3.3. Results

3.3.1. Abundance of bacteria, archaea and fungi

Long-term grazing itself did not affect the abundance of bacterial, archaeal or fungal gene copies in either of the study year (Table 3.2). However, gene copy values varied across subregions and months (Table 3.2). In 2015, a 3-way interaction treatments x subregions x months affected the abundance of soil bacterial gene copies (Table 3.2). The latter was significantly higher within the FF subregion under grazing in July, while the lowest abundance was observed within the MG in May for both treatments (Fig. 3.1A). Overall, lowest gene copies abundance was observed within MG (2.74×10^9), highest within FF (5.56×10^9) and intermediate within CP (4.13×10^9 gene copies g^{-1} soil d.w.). Bacterial gene copies abundance also varied between months, and was higher in July (4.84×10^9) and October (4.37×10^9) than May (3.31×10^9 gene copies g^{-1} soil d.w.). In 2016, the abundance of bacterial gene copies was affected by a subregion x month interaction (Table 3.2). The lowest bacterial gene copies abundance was observed within the MG, while the highest were in the FF during May (Fig. 3.1B). Overall, the gene copies abundance of bacteria in the different subregions was 5.11×10^9, 3.27×10^9 and 6.59×10^9 gene copies g^{-1} soil d.w. for the CP, MG and FF, respectively.

In 2015, soil abundance of archaeal gene copies was affected by a treatments x subregions x months interaction (Table 3.2). Closer examination of the latter revealed lowest abundance of archaeal gene copies for the non-grazed treatment in May within the CP, while the highest for grazed treatment in July within the FF and the non-grazed treatment in July within the MG (Fig. 3.2). Overall, archaeal abundance differed over time with lowest values in May (5.36×10^8), higher values in July (7.29×10^8) and intermediate values in October (6.43×10^8 gene copies g^{-1} soil d.w.). Gene copies abundance of archaea also varied between subregions and was lower within the

CP (4.20 x 10^8), while being significantly higher within the MG (7.04 x 10^8) and FF (7.84 x 10^8 gene copies g^{-1} soil d.w.). In 2016, the abundance of archaeal gene copies varied between subregions and months (Table 3.2). Among subregions the lowest values were detected within the CP (3.08 x 10^8), highest values within the FF (1.19 x 10^9) and intermediate values within MG (6.21 x 10^8 and gene copies g^{-1} soil d.w.). Between months the values were higher in May (8.17 x 10^8), lower in July (5.61 x 10^8) and intermediate in October (6.90 x 10^8 gene copies g^{-1} soil d.w.).

In 2015, an interaction subregions x months had an effect on fungal abundance as well as subregions and months (Table 3.2). The lowest abundance of fungi was observed in May for all subregions, while being highest in October within the FF (Fig. 3.3A). Among subregions fungal gene copies varied and was 7.14 x 10^7, 7.71 x 10^7 and 1.42 x 10^8 gene copies g^{-1} soil d.w. for the CP, MG and FF, respectively. Between months the lowest values were observed in May (1.20 x 10^7), while highest in July (9.62 x 10^7) and October (1.18 x 10^8 gene copies g^{-1} soil d.w.). In 2016, a 3-way interaction treatments x subregions x months affected the gene copies abundance of soil fungi as well as months (Table 3.2). Within the MG fungal abundance was lower during July in the grazed treatment, while being higher in October for the same (grazed MG) treatment (Fig. 3.3B). Overall, fungal abundance was higher in October (9.39 x 10^7), lower in July (5.01 x 10^7) and intermediate in May (1.02 x 10^8 gene copies g^{-1} soil d.w.).

3.3.2. Soil water content and bacterial, archaeal and fungal abundance

Bacterial gene copies abundance was affected by GWC x subregions x months, GWC x subregions and GWC x months interactions in 2015 (Table 3.3). Closer examination showed positive linear relationships between GWC and bacterial gene abundance within each of the MG and FF subregion, as well as in May, July and October with higher abundance in October at the

same level of GWC (Fig. 3.4A, B). In July within the CP subregion, relationships between GWC and bacterial gene abundance were negative, while within the MG in July and October they were positive and linear (Fig. 3.4C). In 2016, bacterial gene copies abundance generally had positive and linear relationships with GWC (Table 3.3; Fig. 3.4D), with additional interactions between GWC x months and GWC x subregions x months (Table 3.3). Bacterial gene copies abundance were positively associated with GWC in May and July, particularly within the MG in May and July, and the FF in July (Fig. 3.4E, F).

Archaeal gene copies abundance was affected by the GWC x subregions x months, GWC x subregions and GWC x months interactions in 2015 (Table 3.3). Closer examination however, showed a non-significant regression fit for all relationships (Fig. 3.5A, B, C). In 2016, GWC, GWC x subregions, GWC x months and GWC x subregions x months affected archaeal gene copies abundance (Table 3.3). Overall, the relationships detected were typically linear and positive ($p=0.07$, $R^2=0.20$). Archaeal gene copies abundance was positively associated with GWC within the FF (Fig. 5D), and stronger in May (Fig. 3.5E). Similar positive relationships were evident within the CP in May, and within the MG in May and July, as well as within the FF in October (Fig. 3.5F).

Fungal gene copies abundance were affected by GWC x subregions, GWC x months and GWC x subregions x months in 2015 (Table 3.3). The relationships detected were positive and linear within the CP and MG (Fig. 3.6A), but non-linear overall in May, July and October (Fig. 3.6B), while being linear and positive within the MG during July and October (Fig. 3.6C). In 2016, fungal gene copies abundance was positively and linearly associated with GWC in May (Fig. 3.6D).

3.3.3. Bacterial, archaeal and fungal taxonomic characteristics

A total 2,394,992 high quality bacterial sequences were obtained with an average of 13,764 sequences per sample. These sequences were distributed on average between 1512 different OTUs at 97% identity. The rarefaction curve showed that all study samples tended to approach a saturation plateau (Appendix D). Approximately 99% of the sequences were assigned to 42 phyla across all samples. The predominant phyla were Actinobacteria (6-42%), Proteobacteria (14-32%) and Acidobacteria (10-20%) (Appendix E).

A total of 7,286,502 high quality archaeal sequences were obtained with an average of 42,862 sequences per sample. These sequences were distributed on average between 105 different OTUs at 97% identity. Rarefaction curves showed that all study samples tended to approach a saturation plateau (Appendix D). Approximately 99% of the sequences were assigned to 8 phyla across all samples. The predominant phyla were Thaumarchaeota (81-100%) and Euryarchaeota (<19%; Appendix F).

A total of 8,521,071 high quality fungal sequences were obtained with an average of 48,415 sequences per sample. These sequences were distributed on average between 502 different OTUs at 97% identity. The rarefaction curve showed that all study samples tended to approach a saturation plateau (Appendix D). Almost 100% (99.99%) of the sequences were assigned to 9 phyla across all samples. The predominant phyla were Ascomycota (8-94%), Basidiomycota (3-89%) and Zygomicota (0.1-21%; Appendix G).

3.3.4. Alpha diversity metrics of bacterial, archaeal and fungal communities

Long-term grazing itself did not affect bacterial richness or Shannon's diversity index in either of the study years; however, the Simpson index was lower in 2016 under non-grazed

subplots compared to grazed, with values of 0.990 ± 0.001 and 0.991 ± 0.001 for the NG and G, respectively (Table 3.4). In 2015, richness varied between months and was lower in May (1210 ± 41), higher in July (1292 ± 39) and intermediate in October (1237 ± 36). Also, a 2-way interaction between treatment and months affected bacterial richness (Table 3.4). The latter was higher within the MG for NG treatment, while being lower within the CP for NG treatment as well as within the MG for G treatment (Fig. 3.7A). Shannon's index also varied between months and was lower in May (5.89 ± 0.06), higher in July (6.03 ± 0.06) and intermediate in October (5.97 ± 0.08). Shannon index was affected by a treatment x month interaction (Table 3.4). Shannon's index was higher in July for the NG, while being lower in May for both treatments and in October for the NG (Fig. 3.7C). Simpson diversity index varied among subregions and between months in 2015 (Table 3.4). Lower Simpson values were observed within the CP (0.987 ± 0.002) and FF (0.989 ± 0.001) compared to the MG (0.993 ± 0.001). Between months, the Simpson diversity index varied and was 0.989 ± 0.001, 0.991 ± 0.001 and 0.989 ± 0.003 for May, July and October, respectively. In 2016, richness of the bacterial community was affected by subregions and months, as well as the interaction of treatment x months (Table 3.4). Among subregions significantly lower values was observed within the CP (1139 ± 38), significantly higher within MG (1325 ± 26) and intermediate within FF (1198 ± 36). Between months, lower values of richness were observed in July (1162 ± 34), the highest values in October (1277 ± 41) and intermediate in May (1235 ± 36). Bacterial richness was higher in October for the G treatment, while being lower in July for the G (Fig. 3.7B). Shannon diversity index was lower within the CP (5.86 ± 0.07), higher within MG (6.18 ± 0.02) and intermediate within FF (5.89 ± 0.04). The Simpson index was lower within the CP (0.989 ± 0.001) and FF (0.990 ± 0.001), while remaining higher within the MG (0.993 ± 0.001).

Archaeal richness or diversity indexes were not affected by long-term grazing itself (Table 3.5). In 2015, archaeal richness varied among subregions and was lower within the CP (79 ± 2) and FF (79 ± 3) compared to the MG (95 ± 2). Richness of archaea was also affected by a 3-way interaction treatments x subregions x months; richness was higher within the MG for NG treatment in May and July, while being lower within the CP for NG in May, and also the G in July (Fig. 3.8A). Shannon's index for archaeal diversity varied between months, and was lower in May (1.78 ± 0.06), higher in October (1.90 ± 0.04) and intermediate in July (1.87 ± 0.03). Also, Shannon's index varied between subregions x months (Table 3.5); while this metric did not change within the CP and MG during the growing season, it was lowest within the FF during May and higher within the FF during July and October (data not shown). The Simpson index varied among subregions and between months (Table 3.5). Lower Simpson indices were observed within the MG (0.739 ± 0.005) and FF (0.734 ± 0.019) compared to the CP (0.786 ± 0.011). Between months the lowest values were observed in May (0.730 ± 0.018), while in July (0.762 ± 0.009) and October (0.769 ± 0.010) they were higher. In 2016, archaeal richness, Shannon's and Simpson indexes varied among subregions (Table 3.5). Higher richness was observed within the MG (94 ± 2), while it was lower within the CP (79 ± 2) and FF (74 ± 2). Shannon's diversity was lower within the FF (1.66 ± 0.04), higher within the CP (1.95 ± 0.05) and intermediate within the MG (1.79 ± 0.02). Simpson diversity index was higher within the CP (0.785 ± 0.010), lower within the FF (0.721 ± 0.012) and intermediate within the MG (0.732 ± 0.007). Finally, Simpson diversity was affected by the interaction of subregions x months, whereby it was higher in May for NG and lowest in July for NG (Fig. 3.8B).

While long-term grazing did not alter richness of the fungal community, it affected alpha diversity indices in both years (Table 3.6). In 2015, the Shannon's and Simpson indices were lower

in soils under long-term grazing than those without grazing, with values of 3.69 ± 0.11 and 3.96 ± 0.09 (Shannon's) and 0.89 ± 0.02 and 0.92 ± 0.01 (Simpson) for the G and NG, respectively. In 2016, both the Shannon's and Simpson indices were higher in soils under long-term grazing than in areas without grazing, with values of 3.69 ± 0.13 and 3.39 ± 0.15 (Shannon's), and 0.89 ± 0.02 and 0.83 ± 0.03 (Simpson) for the G and NG, respectively. Richness of the fungal community also varied across subregion in 2015 and months in 2016 (Table 3.6). Among subregions fungal community richness was higher within the MG (507 ± 15), lower within the CP (415 ± 17) and intermediate within the FF (473 ± 19) in 2015. Between months lower richness was observed in May (402 ± 15) and July (403 ± 17) compared to October (457 ± 21) in 2016. Shannon's index varied among subregions in 2015, and was lower within the CP (3.53 ± 0.17), higher in the MG (4.10 ± 0.07), and intermediate in the FF (3.84 ± 0.11).

3.3.5. Beta diversity metrics of bacterial, archaeal and fungal communities

Long-term grazing did not affect beta diversity metrics (Bray-Curtis, unweighted UniFrac and weighted UniFrac) of the bacterial and archaeal communities in either study year (Table 3.7, Appendix H, I). However, dissimilarities of the bacterial community were affected by subregions. This was confirmed by NMDS ordination based on Bray-Curtis dissimilarities, which showed strong clustering of bacterial and archaeal communities into the three groups, which were associated with the CP, MG and FF subregions (Fig. 3.5). While bacterial abundance was associated with increased SOC, TN, GWC, MAP within the FF subregion, in general, richness and Shannon's diversity of the bacterial community were elevated in the MG for both study years (Fig. 3.5, Appendix J). While archaeal abundance increased in the FF subregion, richness as well as Shannon's and Simpson diversity indexes of the archaeal community were all elevated within the

MG (Fig. 3.5, Appendix J). Long-term grazing affected beta diversity metrics of the fungal community (Table 3.7, Appendix H, I). Additionally, dissimilarities of the fungal community were affected by subregions. This was confirmed by NMDS ordination based on Bray-Curtis dissimilarities, which showed strong clustering of the fungal community within each of the CP, MG and FF regions (Fig. 3.5).

A summary of findings obtained from the Tables 3.2, 3.4-3.7 about long-term grazing effects on soil microbial communities is provided in Table 3.8, which includes the abundance (qPCR), richness (observed OTUs), and alpha and beta diversity metrics, of bacterial, archaeal and fungal communities. Overall, long-term grazing itself had limited effects on soil bacterial and archaeal communities, while fungal communities were more sensitive to long-term grazing.

3.3.6. Indicator species- and differential abundance analyses

In 2015, indicator species analysis for the bacterial community revealed 38 OTUs, 16 of which were associated with soils under long-term grazing, while another 22 were associated with soils without grazing impact (Table 3.9). In 2016, ISA revealed 17 OTUs associated with soils under long-term grazing, while another 19 OTUs were associated with soils without grazing. In 2015, two OTUs were strongly associated with long-term grazing as indicated by a positive predictive value equal to 1 (Table 3.9). Within non-grazed subplots, seven strongly associated OTUs were found. For the archaeal community, ISA analysis did not reveal any indicator OTUs for the long-term grazed treatment or non-grazed soils. In 2015, indicator species analysis for the fungal community revealed 37 OTUs, 11 of which were associated with long-term grazed soils, while another 26 were associated with non-grazed subplots (Table 3.10). In 2016, ISA on the fungal community revealed 24 OTUs, eight of which were associated with soils under long-term

grazing, while other 16 with soils without grazing. For soils under long-term grazing, 3 OTUs overlapped between 2015 and 2016, while for soils without grazing 6 OTUs overlapped (Table 3.10). Another 2 OTUs were strongly associated with long-term grazing, while 8 OTUs were strongly associated with soils without grazing.

Differential analysis for bacteria and archaea did not reveal any differences between long-term grazed and non-grazed treatments for both study years. For fungi, in 2015 Mortierellaceae, Microascaceae, Cordycipitaceae, Chaetomiaceae dominated soils with grazing (Fig. 3.10A), while in 2016 Mortierellaceae, Microascaceae and Entolomataceae prevailed in soils with long-term grazing (Fig. 3.10B).

3.4. Discussion

Examining the response of soil microbial communities to long-term grazing under various environmental and climatic conditions can improve our understanding of microbial ecology and the role of microorganisms in regulating grassland ecosystem function. The uniqueness of the present study is that it assessed the effects of long-term grazing on the abundance, richness, alpha and beta diversity metrics of soil bacterial, archaeal and fungal communities in different northern temperate grasslands. Moreover, the study was conducted across a broad geographic area (260 km in latitude and 450 km in longitude) over two growing seasons. With a few exceptions, we observed that microbial communities were generally resistant to long-term grazing, but changed markedly across climatic regions and seasonally through time.

3.4.1. Long-term grazing effect on soil microbial communities

We observed no main effects of long-term grazing on bacterial, archaeal and fungal abundance, despite known effects of grazing at these sites on vegetation (RRA, 2016; RRA, 2017), litter decomposition rates (Chuan et al. 2018) and soil properties, namely BD and SOC (see Chapter 2, Hewins et al. 2018). These findings are consistent with previous studies of soil microbial abundance assessed as microbial biomass carbon under long-term grazing in Australia (50 years; Tom et al., 2006), Switzerland (100 years; Hodel et al., 2014) and England (>16 years; Medina-Roldán et al., 2012), while other investigations have found that light grazing intensities increased microbial biomass (Bardgett et al., 1997). Soil organic carbon is closely and positively correlated with soil microbial biomass (McGonigle and Turner, 2017), and though we found higher SOC under long-term grazing this did not increase microbial abundance at our study locations. It should be noted that the current study locations were exposed to only light to moderate grazing intensities, and higher intensities of grazing can induce more change to soil properties and therefore lead to greater changes in microbial abundance (Tom et al., 2006).

Grazing affected the composition of microbial communities to different extents; fungal communities responded directly to grazing, while bacterial and archaeal communities responded via interactions with other factors. Despite grazing induced changes in vegetation composition within our study region (RRA, 2016; RRA, 2017), concomitant effects on the microbial community may not be expected due to a low correlation between the alpha diversity of plants and soil microorganisms (Prober et al., 2015) as well as shift absence of major plants (Hodel et al., 2014). There were numerous OTUs that characterized bacterial and fungal communities under long-term grazing, but no archaea indicator OTUs were found for this same treatment. While other studies have found that the archaeal community can be affected by livestock grazing

(Chroňáková et al., 2015), in the present study the main effect of grazing did not affect the community.

In 2015, the Proteobacteria phylum was revealed as an indicator for long-term grazing treatment. This is a metabolically diverse group divided on α-, β-, γ-, δ- subphyla. While α-, β- and δ-Proteobacteria prevail in soils with recalcitrant carbon (cellulose, lignin), β- and γ-Proteobacteria are dominant under low-molecular-weight carbon (root exudates) input into soils (Aislabie and Deslippe, 2013). In 2015, Firmicutes, Actinobacteria, Bacteroides, Chloroflexi, Microgenomates and Chlamydiae were associated with non-grazed soils. While little is known about the ecology of the latter three phyla, the other three have been characterized (Aislabie and Deslippe, 2013). Members of the Firmicutes degrade various carbon sources, members of Bacteroidetes degrade complex organic molecules (starch, proteins, cellulose, and chitin) as well as plant material, and members of Actinobacteria dominate in soils with inputs of labile carbon (Aislabie and Deslippe, 2013). Thus, the main feature of the indicator phyla is that they participate in the decomposition of various substrates and may potentially impact carbon and nitrogen cycling.

Low richness and diversity of archaeal community has previously been observed (Chroňáková et al., 2015; Auguet et al., 2010). While there were no indicator archaeal OTUs between treatments, Thaumarchaeota was the dominant phylum in soils, which was consistent with a previous study (Xie et al., 2017). Members of the Thaumarchaeota play an important role in carbon and nitrogen cycles (Pester et al., 2011). The second most abundant phylum of archaea in our study was Euryarchaeota, which includes methanogens, suggesting that methane flux from these soils may not change. Overall, the dominant phyla of bacterial and archaeal communities did not change due to long-term grazing, indicating that the dominant phyla of soil bacteria and

archaea are stable under long-term grazing (Zhang et al., 2016), at least when grazing is light to moderate intensity.

Fungal microbial communities under long-term grazing were characterized by the Basidiomycota phylum, while soils without grazing included Ascomycota, Zygomycota and Chytridiomycota. Fungal communities play an important role in the carbon cycle as they are involved in the decomposition of various organic matters, such as plants, cellulose, keratin and collagen (Aislabie and Deslippe, 2013). Within the fungal community of soils under grazing were found microorganisms that are associated with animal origins, such as dung, meat and living tissue (e.g. Mortierellaceae, Microascaceae; hbm-mnb.ca).

3.4.2. Regional effect on soil microbial communities

Communities of all three soil microbial groups differed markedly among the three distinct study subregions. Spatial studies of soil microbial components conducted on scales ranging from the micro (from μm^2 to mm^2)-, through plot- (from m^2 to hundreds m^2) and regional- (from km^2 to hundreds km^2), to global (>100000 km^2; Constancias et al., 2015) level revealed significant spatial structuring of soil microbial communities (Constancias et al., 2015). Abiotic factors, namely edaphic (e.g. soil pH, texture) and climatic (precipitation, temperature) variables determine the distribution of soil microorganisms at a regional scale (Chen et al., 2016). The regions in this study were selected to represent broad differences in climate (and therefore vegetation and soils) and this likely altered the beta diversity clustering of bacterial, archaeal and fungal communities across the subregions. Similarly, beta plant diversity has been used to predict soil microbial beta diversity (Prober et al., 2015); the pronounced differences in

vegetation across the studied subregions therefore likely affected the soil microbial community in each region.

The abundance of each of the three microbial groups generally increased with soil moisture. A number of other studies have similarly reported positive relationships between soil moisture and the abundance of soil microorganisms, as well as species richness (Wang et al., 2015; Hawkins et al., 2003). However, other environmental factors such as soil properties might explain microbial abundance and community composition (Bachar et al., 2010). We observed higher abundance of bacteria and archaea within the FF subregion, which is one of the wettest subregions in the province. Interestingly, the richness of bacterial and archaeal communities tended to be higher in the MG, the driest, warmest and generally least productive subregion (Downing and Pettapiece, 2006). One possibility for this difference is soil texture, which within the MG subregion was mostly coarse (high sand content, Ø 0.5-1 mm), while the CP and FF had more medium textured soils (high silt content, Ø 0.002-0.5; Bock, 2016; www.fao.org). Compared to fine textured soils that have more water-filled pores, coarse textured soils tend to have isolated water films at the same soil water content (Chau et al., 2011), which creates isolated microhabitats that prevent competition between microorganisms and can increase species richness (Chau et al., 2011).

3.5. Summary

The amount of research on soil microbial ecology has increased sharply in recent years because of the importance that microbial communities play in regulating ecosystem function. However, within the Canadian Prairies this information is sparse. Given the spatial extent of grazing and the increasing demand for understanding the processes regulating carbon storage and

GHG emissions, these results suggest that microbial communities are largely resistant to moderate levels of grazing, such as those found in the study sites. However, it is essential that we understand the effects of higher levels of grazing in order to ensure the conservation of microbial resources (Aislabie and Deslippe, 2013) and provide capacity for these communities and the ecosystems in which they reside to adapt to future environmental changes.

Table 3.1. Description of the primers used for the quantitative polymerase chain reaction (qPCR) and next-generation sequencing (NGS).

Target	Primer sequence (5'-3')	Primer name	Reference
	qPCR		
Bacteria	*ACT CCT ACG GGA GGC AGC AG* *ATT ACC GCG GCT GCT GG*	Eub338 Eub518	Fierer et al., 2005
Archaea	*CGG GGY GCA SCA GGC GCG AA* *GTG CTC CCC CGC CAA TTC CT*	A364a A934b	Kemnitz et al., 2007
Fungi	*TTA GCA TGG AAT AAT RRA ATA GGA* *TCT GGA CCT GGT GAG TTT CC*	nu-SSU-0817 nu-SSU-1196	Borneman and Hartin, 2000
	NGS*		
Bacteria	*CCT ACG GGN GGC WGC AG* *GAC TAC HVG GGT ATC TAA TCC*	341F 805R	Klindworth et al., 2013
Archaea	*ACG GGG YGC AGC AGG CGC GA* *GGA CTA CVS GGG TAT CTA AT*	344F 806R	Takahashi et al., 2014
Fungi	*CTT GGT CAT TTA GAG GAA GTA A* *GCT GCG GTT CTT CAT CGA TGC*	ITS1 ITS2	Orgiazzi et al., 2012

* Illumina adapters TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G were added to the forward and reverse primers, respectively

Table 3.2. Results of a mixed-model testing the effects of long-term grazing, geographical location (subregion) and temporal (monthly) variability, and their interactions, on abundance (gene copies per gram of soil, dry weight) of bacteria, archaea and fungi in rangeland soils of Alberta, Canada.

Factor	df_{num}	2015			2016		
		df_{res}	F	P	df_{res}	F	P
Bacterial abundance, gene copies g^{-1} soil, d.w.							
Treatment (GT)	1	12	0.116	0.739	11	0.998	0.338
Subregion (S)	2	12	10.169	**0.003**	12	2.869	**0.097**
Month (M)	2	47	13.707	**<.0001**	38	1.488	0.238
S x GT	2	12	0.845	0.454	11	0.389	0.686
GT x M	2	47	0.238	0.789	39	0.086	0.917
S x M	4	47	1.389	0.252	38	2.149	**0.093**
S x GT x M	4	47	2.533	**0.053**	39	1.791	0.151
Archaeal abundance, gene copies g^{-1} soil, d.w.							
Treatment (GT)	1	12	0.040	0.844	12	1.075	0.319
Subregion (S)	2	12	3.514	**0.063**	12	8.028	**0.006**
Month (M)	2	48	5.879	**0.005**	43	2.695	**0.079**
S x GT	2	12	0.491	0.624	12	0.226	0.801
GT x M	2	48	0.835	0.440	44	1.719	0.191
S x M	4	48	1.025	0.404	43	0.606	0.661
S x GT x M	4	48	2.743	**0.039**	44	0.794	0.536
Fungal abundance, gene copies g^{-1} soil, d.w.							
Treatment (GT)	1	12	2.652	0.129	14	0.111	0.745
Subregion (S)	2	12	3.002	**0.088**	13	1.445	0.349
Month (M)	2	39	109.68	**<.0001**	35	4.713	**0.015**
S x GT	2	12	0.239	0.791	13	0.081	0.922
GT x M	2	39	1.147	0.328	34	0.589	0.561
S x M	4	39	2.728	**0.042**	34	1.747	0.162
S x GT x M	4	39	1.053	0.393	34	3.185	**0.025**

Values in bold indicate significant differences at $p<0.10$

Table 3.3. Results of a mixed-model testing the effects of soil water content (GWP), long-term grazing, geographic location (subregions), temporal (months) variabilities, and their interactions, on the bacterial, archaeal and fungal abundance within rangeland soils of Alberta, Canada in 2015 and 2016.

Factor	df_{num}	2015			2016			
		df_{den}	F	P	df_{num}	df_{den}	F	P
Bacterial abundance, gene copies g^{-1} soil, d.w.								
Water content (GWC)	1	62	1.697	0.197	1	52	4.530	**0.038**
GWC x Treatment (GT)	1	17	0.410	0.531	1	11	0273	0.612
GWC x Subregion (S)	2	25	8.182	**0.002**	2	21	0.333	0.721
GWC x Month (M)	2	52	10.976	**0.001**	2	40	3.764	**0.032**
GWC x GT x S	2	16	0.558	0.583	2	11	0.349	0.713
GWC x S x M	4	54	3.094	**0.023**	4	40	2.566	**0.053**
GWC x GT x M	2	51	0.076	0.927	2	38	0.113	0.893
GWC x GT x T x M	4	52	0.782	0.542	4	38	1.805	0.148
Archaeal abundance, gene copies g^{-1} soil, d.w.								
Water content (GWC)	1	55	2.562	0.115	1	61	4.474	**0.038**
GWC x Treatment (GT)	1	20	0.471	0.500	1	11	1.569	0.235
GWC x Subregion (S)	2	31	5.607	**0.008**	2	22	7.131	**0.004**
GWC x Month (M)	2	50	5.394	**0.008**	2	46	2.833	**0.069**
GWC x GT x S	2	18	0.008	0.992	2	12	0.301	0.745
GWC x S x M	4	53	3.762	**0.009**	4	47	2.479	**0.056**
GWC x GT x M	2	53	0.790	0.458	2	42	0.329	0.721
GWC x GT x T x M	4	53	0.787	0.539	4	43	0.433	0.784
Fungal abundance, gene copies g^{-1} soil, d.w.								
Water content (GWC)	1	45	1.622	0.209	1	49	0.620	0.435
GWC x Treatment (GT)	1	20	0.689	0.416	1	13	0.142	0.712
GWC x Subregion (S)	2	20	7.499	**0.004**	2	25	0.254	0.778
GWC x Month (M)	2	48	59.044	**<.001**	2	39	3.819	**0.030**
GWC x GT x S	2	17	1.037	0.376	2	14	0.417	0.666
GWC x S x M	4	46	2.418	**0.062**	4	40	0.910	0.467
GWC x GT x M	2	45	0.501	0.609	2	37	1.039	0.364
GWC x GT x T x M	4	45	0.774	0.548	4	NA	NA	NA

Values in bold indicate significant differences at $p<0.10$

Table 3.4. Results of a mixed-model testing the effects of long-term grazing, geographic location (subregion) and temporal (month) variability, and their interactions, on alpha diversity characteristics of the bacterial community in rangeland soils of Alberta, Canada.

Factor	df_{num}	2015			2016		
		df_{den}	F	P	df_{den}	F	P
Bacterial richness							
Treatment (GT)	1	11	2.099	0.176	12	0.001	0.973
Subregion (S)	2	12	2.712	0.106	12	5.416	**0.021**
Month (M)	2	35	3.000	**0.063**	40	3.966	**0.027**
GT x S	2	10	5.062	**0.029**	12	0.397	0.681
GT x M	4	34	1.379	0.266	40	3.365	**0.044**
S x M	2	34	1.053	0.394	39	0.806	0.528
GT x S x M	4	34	0.615	0.655	39	0.316	0.866
Shannon diversity index (bacteria)							
Treatment (GT)	1	12	1.075	0.321	12	0.035	0.855
Subregion (S)	2	12	2.672	0.109	12	4.628	**0.032**
Month (M)	2	32	8.003	**0.001**	38	2.020	0.146
GT x S	2	12	1.800	0.209	12	1.256	0.319
GT x M	4	32	2.802	**0.075**	38	1.906	0.162
S x M	2	32	1.601	0.198	38	0.713	0.588
GT x S x M	4	32	0.234	0.917	38	0.253	0.906
Simpson diversity index (bacteria)							
Treatment (GT)	1	12	2.021	0.181	12	4.112	**0.065**
Subregion (S)	2	12	6.646	**0.011**	12	10.221	**0.002**
Month (M)	2	33	5.512	**0.008**	39	2.425	0.102
GT x S	2	12	1.341	0.298	12	2.538	0.120
GT x M	4	33	2.135	0.134	39	1.064	0.355
S x M	2	32	1.072	0.386	38	1.362	0.265
GT x S x M	4	32	0.191	0.941	38	0.847	0.504

Values in bold indicate significant differences at $p<0.10$

Table 3.5. Results of a mixed-model testing the effects of long-term grazing, geographic location (subregion) and temporal (month) variability, and their interactions, on alpha diversity characteristics of the archaeal community in rangeland soils of Alberta, Canada.

Factor	df_{num}	2015			2016		
		df_{den}	*F*	*P*	df_{den}	*F*	*P*
Archaeal richness							
Treatment (GT)	1	12	0.962	0.345	12	0.927	0.355
Subregion (S)	2	12	9.688	**0.003**	12	18.852	**0.001**
Month (M)	2	39	2.153	0.129	45	0.829	0.443
GT x S	2	12	1.410	0.281	12	0.240	0.790
GT x M	4	39	1.342	0.273	45	0.014	0.986
S x M	2	39	1.317	0.281	45	1.342	0.269
GT x S x M	4	39	3.483	**0.016**	45	0.567	0.688
Shannon diversity index (archaea)							
Treatment (GT)	1	13	0.212	0.653	12	0.001	0.981
Subregion (S)	2	12	1.573	0.246	12	5.780	**0.017**
Month (M)	2	37	4.839	**0.014**	44	0.782	0.464
GT x S	2	12	0.155	0.858	12	0.048	0.953
GT x M	4	38	1.573	0.221	45	1.632	0.207
S x M	2	37	3.305	**0.021**	44	0.074	0.989
GT x S x M	4	37	0.349	0.843	45	0.885	0.481
Simpson diversity index (archaea)							
Treatment (GT)	1	13	0.107	0.749	12	0.124	0.731
Subregion (S)	2	12	2.959	**0.089**	12	5.453	**0.021**
Month (M)	2	38	2.988	**0.062**	44	0.409	0.667
GT x S	2	12	0.007	0.993	12	0.074	0.929
GT x M	4	38	0.686	0.509	45	2.757	**0.074**
S x M	2	37	1.918	0.128	44	0.025	0.999
GT x S x M	4	38	0.188	0.943	45	0.844	0.505

Values in bold indicate significant differences at $p<0.10$

Table 3.6. Results of a mixed-model testing the effects of long-term grazing, geographic location (subregion) and temporal (month) variability, and their interactions, on alpha diversity characteristics of the fungal community in rangeland soils of Alberta, Canada.

Factor	df_{num}	2015			2016		
		df_{den}	*F*	*P*	df_{den}	*F*	*P*
Fungal richness							
Treatment (GT)	1	11	1.206	0.295	12	0.008	0.929
Subregion (S)	2	12	4.955	**0.027**	12	0.449	0.648
Month (M)	2	45	1.297	0.283	42	3.489	**0.039**
GT x S	2	11	1.097	0.367	12	1.485	0.265
GT x M	2	45	1.446	0.246	43	0.903	0.413
S x M	4	45	1.774	0.151	42	1.995	0.113
GT x S x M	4	45	0.598	0.666	43	0.990	0.423
Shannon diversity index (fungi)							
Treatment (GT)	1	11	3.489	**0.087**	12	3.858	**0.073**
Subregion (S)	2	12	3.310	**0.072**	12	0.611	0.559
Month (M)	2	45	0.501	0.609	43	1.024	0.368
GT x S	2	11	0.092	0.912	12	2.734	0.106
GT x M	2	45	0.709	0.498	44	1.387	0.261
S x M	4	45	1.496	0.219	43	0.832	0.513
GT x S x M	4	45	0.348	0.844	43	1.477	0.226
Simpson diversity index (fungi)							
Treatment (GT)	1	11	3.638	**0.082**	12	4.789	**0.049**
Subregion (S)	2	12	1.694	0.226	12	0.794	0.474
Month (M)	2	45	0.132	0.877	43	0.912	0.409
GT x S	2	11	0.103	0.903	12	2.219	0.152
GT x M	2	45	0.239	0.788	44	1.598	0.214
S x M	4	45	1.073	0.381	43	1.445	0.236
GT x S x M	4	45	0.395	0.811	43	1.403	0.249

Values in bold indicate significant differences at $p<0.10$

Table 3.7. Results of permutational analysis of variance (Bray-Curtis distance) testing the effect of treatment (grazed / non-grazed), geographic location (subregion) and temporal (month) variability, and their interactions, on each of the bacterial, archaeal and fungal communities in rangeland soils of Alberta, Canada.

Factor	2015						2016					
	df	SS	MSS	F	R^2	p	df	SS	MSS	F	R^2	p
Bacteria												
Treatment (GT)	1	0.145	0.145	1.015	0.013	0.382	1	0.149	0.149	1.029	0.013	0.348
Subregion (S)	2	1.736	0.868	6.062	0.160	**0.001**	2	1.726	0.863	5.934	0.145	**0.001**
Month (M)	2	0.252	0.126	0.881	0.023	0.646	2	0.247	0.124	0.849	0.021	0.759
GT x S	2	0.289	0.145	1.012	0.027	0.385	2	0.262	0.131	0.899	0.022	0.644
GT x M	2	0.188	0.094	0.657	0.017	0.985	2	0.231	0.115	0.793	0.019	0.849
S x M	4	0.393	0.098	0.686	0.036	0.994	4	0.439	0.109	0.755	0.037	0.983
GT x S x M	4	7.444	0.099	0.697	0.037	0.987	4	0.427	0.107	0.734	0.036	0.991
Residuals	52	10.847	0.143		0.686		58	8.436	0.145		0.708	
Archaea												
Treatment (GT)	1	0.133	0.133	1.690	0.019	0.142	1	0.058	0.058	0.814	0.007	0.535
Subregion (S)	2	1.565	0.782	9.922	0.226	**0.001**	2	2.415	1.207	16.908	0.313	**0.001**
Month (M)	2	0.170	0.085	1.080	0.025	0.383	2	0.064	0.032	0.447	0.008	0.935
GT x S	2	0.099	0.049	0.629	0.014	0.775	2	0.142	0.071	0.995	0.018	0.432
GT x M	2	0.046	0.023	0.289	0.006	0.981	2	0.071	0.036	0.499	0.009	0.891
S x M	4	0.186	0.047	0.591	0.027	0.900	4	0.149	0.037	0.524	0.019	0.958
GT x S x M	4	0.073	0.018	0.230	0.010	1.000	4	0.105	0.026	0.369	0.014	0.994
Residuals	59	4.652	0.079		0.672		66	4.713	0.071		0.610	
Fungi												
Treatment (GT)	1	0.515	0.514	1.565	0.016	**0.034**	1	0.394	0.394	1.124	0.013	0.231
Subregion (S)	2	4.376	2.188	6.657	0.140	**0.001**	2	4.017	2.008	5.730	0.129	**0.001**
Month (M)	2	0.722	0.361	1.099	0.023	0.230	2	0.571	0.286	0.815	0.018	0.921
GT x S	2	0.714	0.357	1.086	0.023	0.262	2	0.780	0.390	1.113	0.025	0.183
GT x M	2	0.421	0.210	0.640	0.013	1.000	2	0.545	0.272	0.778	0.017	0.963
S x M	4	1.225	0.306	0.932	0.039	0.688	4	1.202	0.300	0.857	0.039	0.920
GT x S x M	4	0.919	0.229	0.699	0.029	1.000	4	1.176	0.294	0.839	0.038	0.957
Residuals	68	22.351	0.329		0.715		64	22.432	0.351		0.721	

Table 3.8. Summary findings of long-term grazing effect on the abundance (qPCR), richness (observed OTUs), alpha diversity (Shannon and Simpson diversity indexes) and beta diversity (Bray-Curtis, unweighted UniFrac, weighted UniFrac) measures of soil bacterial (B), archaeal (A) and fungal (F) communities.

Measure	2015			2016			Source
	B	A	F	B	A	F	
Abundance (qPCR)	-	-	-	-	-	-	Table 2
Richness	-	-	-	-	-	-	
Shannon	-	-	+	-	-	+	Table 4, 5, 6
Simpson	-	-	+	+	-	+	
Bray-Curtis	-	-	+	-	-	-	
Unweighted UniFrac	-	-	+	-	-	+	Table 7, Appendix 5, 6
Weighted UniFrac	-	-	+	-	-	-	

"+" indicates that significant effect of long-term grazing on bacteria, archaea or fungi was found

Table 3.9. Indicator OTUs analysis of soil bacterial communities associated with grazed and non-grazed treatments in 2015 and 2016.

	OTU	Phylum	A	B	S	P	OTU	Phylum	A	B	S	P
	2015						**2016**					
Grazed	3507	Actinobacteria	0.764	0.487	0.61	0.010	1076	Acidobacteria	0.813	0.342	0.527	0.027
	1808	Actinobacteria	0.794	0.297	0.486	0.035	3418	Actinobacteria	0.771	0.447	0.587	0.008
	1229	Bacteroidetes	0.865	0.378	0.572	0.006	3463	Actinobacteria	0.760	0.289	0.469	0.036
	2560	Bacteroidetes	0.832	0.405	0.581	0.007	3809	Actinobacteria	**1.000**	0.158	0.397	0.027
	2407	Bacteroidetes	0.831	0.351	0.54	0.020	4008	Bacteroidetes	0.889	0.237	0.459	0.028
	2443	Bacteroidetes	0.906	0.243	0.469	0.039	3033	Bacteroidetes	0.882	0.237	0.457	0.011
	2610	BRC1	0.768	0.324	0.499	0.048	9453	Bacteroidetes	0.933	0.184	0.415	0.023
	2436	Chloroflexi	0.885	0.243	0.464	0.022	6405	Bacteroidetes	0.933	0.184	0.415	0.031
	2116	Chloroflexi	0.794	0.216	0.414	0.049	1665	Chlorobi	0.786	0.316	0.498	0.020
	2667	Gemmatimonadetes	0.908	0.216	0.443	0.035	1312	Firmicutes	0.838	0.342	0.535	0.011
	1095	Proteobacteria	0.763	0.459	0.592	0.016	1740	Planctomycetes	0.79	0.342	0.52	0.014
	1133	Proteobacteria	0.784	0.432	0.582	0.018	3056	Proteobacteria	0.695	0.421	0.541	0.028
	2835	Proteobacteria	0.751	0.405	0.552	0.037	1034	Proteobacteria	0.792	0.368	0.54	0.032
	2674	Proteobacteria	0.926	0.216	0.447	0.018	636	Proteobacteria	0.714	0.368	0.513	0.043
	2953	Proteobacteria	**1.000**	0.162	0.403	0.027	2393	Proteobacteria	0.95	0.237	0.474	0.006
	5285	Proteobacteria	**1.000**	0.135	0.368	0.050	3346	Verrucomicrobia	0.804	0.289	0.482	0.046
							2075	Verrucomicrobia	0.750	0.237	0.421	0.040
Non-grazed	4111	Acidobacteria	0.955	0.182	0.417	0.024	1276	Actinobacteria	0.829	0.342	0.533	0.011
	2321	Acidobacteria	0.931	0.182	0.411	0.038	2828	Bacteroidetes	0.889	0.263	0.484	0.012
	2229	Actinobacteria	0.849	0.273	0.481	0.034	6551	Bacteroidetes	0.900	0.184	0.407	0.045
	7586	Actinobacteria	**1.000**	0.121	0.348	0.039	2391	Bacteroidetes	**1.000**	0.158	0.397	0.025
	2331	Bacteroidetes	**1.000**	0.212	0.461	0.002	3336	BRC1	0.709	0.368	0.511	0.043
	12635	Bacteroidetes	**1.000**	0.121	0.348	0.045	3302	Gracilibacteria	0.765	0.421	0.567	0.019
	2530	Chlamydiae	0.768	0.515	0.629	0.009	6744	Microgenomates	0.92	0.211	0.440	0.018
	6322	Chlamydiae	**1.000**	0.152	0.389	0.014	1444	Planctomycetes	0.808	0.474	0.619	0.005
	11029	Chloroflexi	**1.000**	0.152	0.389	0.029	2230	Planctomycetes	0.765	0.500	0.618	0.004
	3216	Elusimicrobia	0.864	0.242	0.458	0.024	2001	Planctomycetes	0.867	0.237	0.453	0.026
	4001	FCPU426	0.858	0.394	0.581	0.003	1881	Planctomycetes	0.764	0.421	0.567	0.029
	9857	Firmicutes	**1.000**	0.121	0.348	0.050	1218	Planctomycetes	0.809	0.368	0.546	0.025
	5879	Firmicutes	0.959	0.242	0.482	0.009	2429	Proteobacteria	0.786	0.342	0.518	0.015
	8089	Microgenomates	**1.000**	0.152	0.389	0.029	1296	Proteobacteria	0.78	0.342	0.517	0.022

	5318	Parcubacteria	0.925	0.152	0.374	0.043	3192	Proteobacteria	0.771	0.342	0.514	0.013
	6953	Peregrinibacteria	0.842	0.242	0.452	0.046	2538	Proteobacteria	0.913	0.237	0.465	0.017
	7371	Planctomycetes	0.771	0.242	0.432	0.040	1862	Proteobacteria	0.714	0.342	0.494	0.032
	6308	Proteobacteria	0.883	0.242	0.463	0.029	460	Proteobacteria	0.985	0.184	0.426	0.038
	16798	Proteobacteria	0.953	0.182	0.416	0.023	1872	Proteobacteria	0.917	0.184	0.411	0.047
	4348	Proteobacteria	0.940	0.152	0.377	0.036						
	1763	Verrucomicrobia	0.909	0.303	0.525	0.005						
	2268	Verrucomicrobia	0.857	0.303	0.51	0.003						

Text in bold indicates strong association of species with grazed or non-grazed treatment

Table 3.10. Indicator OTUs analysis for soil fungal communities associated with grazed and non-grazed treatments in 2015 and 2016.

GT	OTU	Phylum	A	B	S	P	OTU	Phylum	A	B	S	P
			2015						**2016**			
Grazed	*626*	*Ascomycota*	*0.946*	*0.250*	*0.486*	*0.013*	486	Ascomycota	0.924	0.600	0.744	0.006
	776	*Ascomycota*	*0.722*	*0.386*	*0.528*	*0.035*	*350*	*Ascomycota*	*0.922*	*0.400*	*0.607*	*0.049*
	965	Ascomycota	0.856	0.227	0.441	0.032	*776*	*Ascomycota*	*0.879*	*0.300*	*0.513*	*0.024*
	1140	Ascomycota	0.995	0.205	0.451	0.008	519	Ascomycota	0.949	0.250	0.487	0.032
	350	*Ascomycota**	*0.850*	*0.546*	*0.681*	*0.006*	*626*	*Ascomycota*	*0.986*	*0.225*	*0.471*	*0.002*
	152	Basidiomycota	0.867	0.409	0.595	0.035	1559	Ascomycota	0.897	0.150	0.367	0.037
	228	Basidiomycota	0.939	0.341	0.566	0.024	2443	Ascomycota	0.967	0.125	0.348	0.037
	1291	Basidiomycota	**1.000**	0.159	0.399	0.015	208	Basidiomycota	0.954	0.200	0.437	0.041
	1961	Basidiomycota	0.829	0.318	0.514	0.014						
	2920	Basidiomycota	0.978	0.136	0.365	0.045						
	4869	Basidiomycota	**1.000**	0.136	0.369	0.027						
Non-grazed	629	Ascomycota	0.886	0.619	0.741	0.001	*441*	*Ascomycota*	*0.974*	*0.524*	*0.714*	*0.001*
	572	Ascomycota	0.835	0.595	0.705	0.010	*239*	*Ascomycota*	*0.991*	*0.381*	*0.615*	*0.001*
	441	*Ascomycota*	*0.904*	*0.548*	*0.704*	*0.001*	581	Ascomycota	0.829	0.476	0.628	0.013
	611	Ascomycota	0.808	0.595	0.694	0.008	*1711*	*Ascomycota*	*0.917*	*0.262*	*0.490*	*0.008*
	837	Ascomycota	0.863	0.452	0.625	0.013	647	Ascomycota	0.826	0.262	0.465	0.020
	239	*Ascomycota*	*0.876*	*0.429*	*0.613*	*0.027*	*689*	*Ascomycota*	*0.971*	*0.167*	*0.402*	*0.041*
	543	*Ascomycota*	*0.86*	*0.429*	*0.607*	*0.004*	*543*	*Ascomycota*	*0.978*	*0.309*	*0.550*	*0.006*
	4121	Ascomycota	**1.000**	0.167	0.408	0.008	2529	Basidiomycota	**1.000**	0.262	0.512	0.001
	1593	Ascomycota	0.988	0.119	0.343	0.04	762	Basidiomycota	0.699	0.309	0.465	0.032
	500	Ascomycota	0.821	0.357	0.541	0.007	1078	Basidiomycota	**1.000**	0.167	0.408	0.012
	1604	Ascomycota	0.979	0.19	0.432	0.006	135	Basidiomycota	0.887	0.405	0.599	0.017
	1087	Ascomycota	0.739	0.381	0.531	0.016	43	Basidiomycota	0.921	0.524	0.694	0.049
	992	Ascomycota	0.941	0.190	0.423	0.039	504	Basidiomycota	0.811	0.476	0.621	0.016
	1711	*Ascomycota*	*0.842*	*0.286*	*0.490*	*0.020*	*316*	*Basidiomycota*	*0.997*	*0.167*	*0.408*	*0.048*
	1147	Ascomycota	0.86	0.262	0.475	0.014	682	Chytridiomycota	**1.000**	0.167	0.408	0.010
	1819	Ascomycota	0.981	0.214	0.458	0.004	836	Zygomycota	**1.000**	0.143	0.378	0.027
	689	*Ascomycota*	*0.969*	*0.214*	*0.456*	*0.005*						
	221	Ascomycota	**1.000**	0.190	0.436	0.002						
	2037	Basidiomycota	0.876	0.190	0.409	0.021						
	832	Basidiomycota	0.792	0.357	0.532	0.046						

	316	*Basidiomycota*	*0.972*	*0.167*	*0.402*	*0.012*	
	731	Basidiomycota	0.873	0.286	0.500	0.018	
	167	Basidiomycota	0.819	0.405	0.576	0.048	
	891	Glomeromycota	0.777	0.381	0.544	0.011	
	2276	Zygomycota	**1.000**	0.119	0.345	0.021	
	3268	Zygomycota	**1.000**	0.095	0.309	0.037	

*Italic text indicates overlap between years within grazed or non-grazed treatments; text in bold indicates a strong association of species with grazed or non-grazed treatment

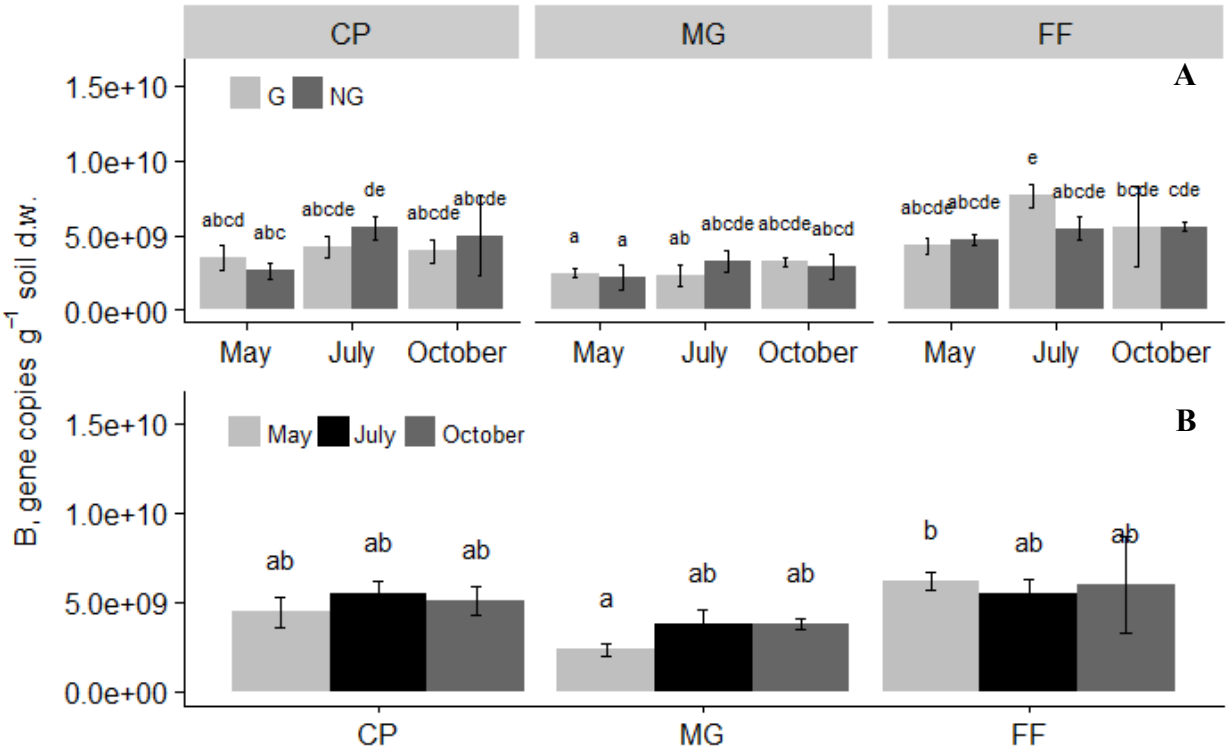

Fig. 3.1. Abundance of soil bacteria (B, gene copies per gram of soil, dry weight) in grassland soils of Alberta, Canada. (A) different lowercase letters indicate significant differences for the 3-way interaction between grazed (G) and non-grazed (NG) treatments, Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions, and month in 2015; (B) different lowercase letters indicate significant differences for the 2-way interaction between months and Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2016. Vertical bars represent ± standard error.

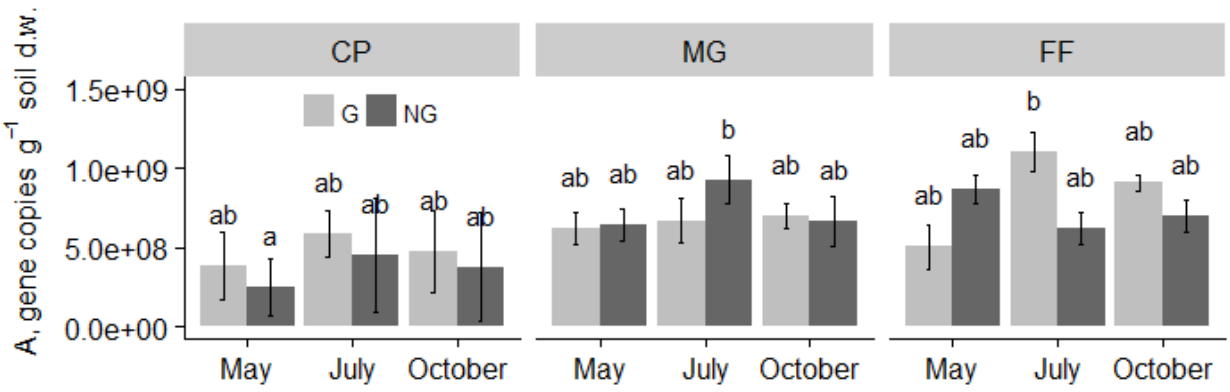

Fig. 3.2. Abundance of soil archaea (gene copies per gram of soil, dry weight) in grassland soils of Alberta, Canada in 2015. Vertical bars represent ± standard error.

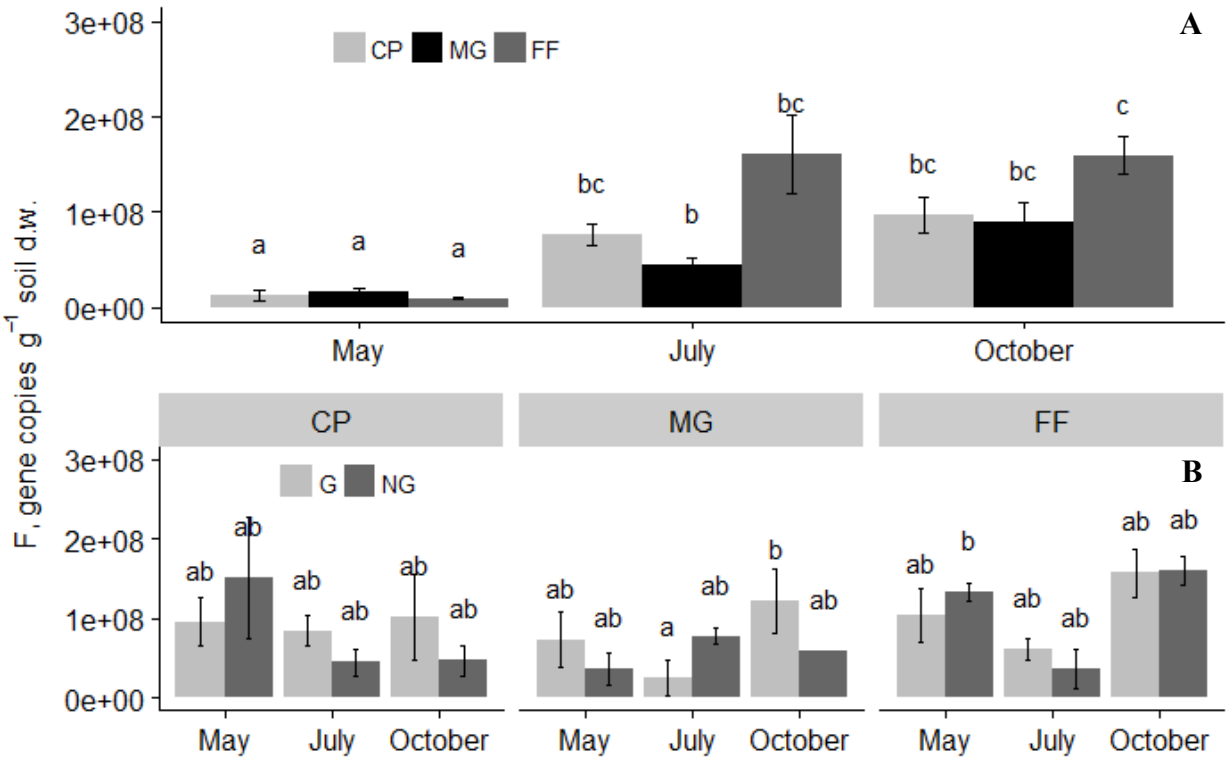

Fig. 3.3. Abundance of soil fungi (gene copies per gram of soil, dry weight) in grassland soils of Alberta, Canada. (*A)* different lowercase letters indicate significant differences for the 2-way interaction between months and Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2015; (*B)* different lowercase letters indicate significant differences for the 3-way interaction between grazed (G) and non-grazed (NG) treatments, Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions and months in 2016. Vertical bars represent ± standard error.

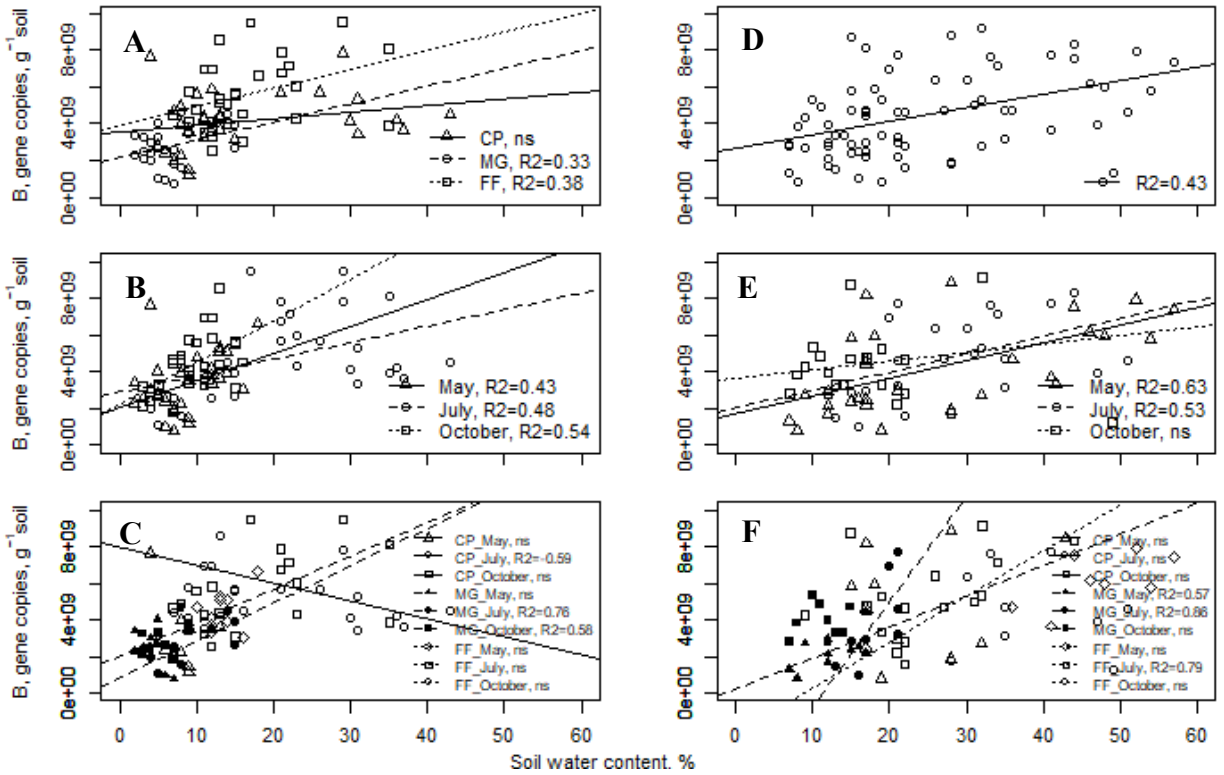

Fig. 3.4. Relationship between gravimetric soil water content (GWC) and the abundance of soil bacteria (B) in 2015 (A, B, C) and 2016 (D, E, F). (A) represents the 2-way interaction between GWC and Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2015; (B) represents the 2-way interaction between GWC and months in 2015; (C) represents the 3-way interaction between GWC, subregions and months in 2015; (D) represents the overall interaction between GWC and bacterial abundance in 2016; (E) represents the 2-way interaction between GWC and months in 2016; (F) represents the 3-way interaction between GWC, subregions and months in 2016. Only significant lines are presented in the graphs C and F.

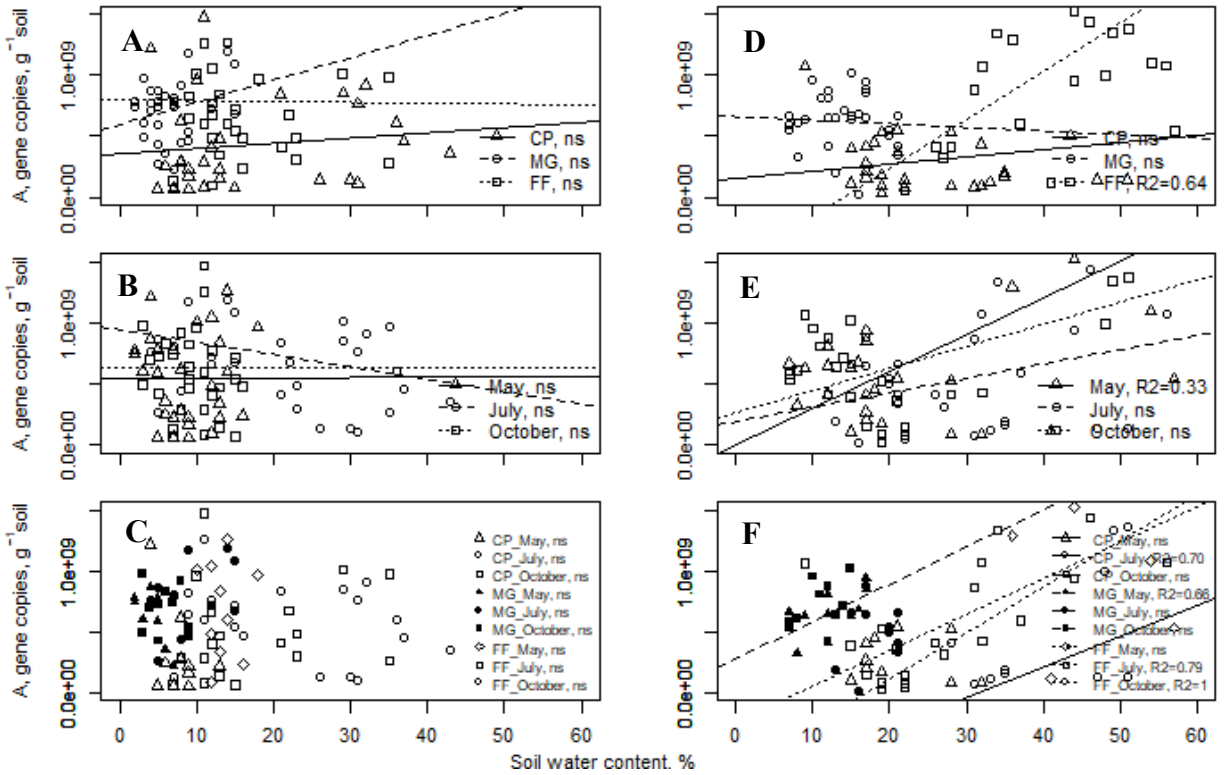

Fig. 3.5. Relationship between gravimetric soil water content (GWC) and abundance of soil archaea (A) in 2015 (A, B, C) and 2016 (D, E, F). (A) represents the 2-way interaction between GWC and Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2015; (B) represents the 2-way interaction between GWC and months in 2015; (C) represents the 3-way interaction between GWC, subregions and months in 2015; (D) represents the 2-way interaction between GWC and subregions in 2016; (E) represents the 2-way interaction between GWC and months in 2016; (F) represents the 3-way interaction between GWC, subregions and months in 2016. Only significant lines are presented in the graphs C and F.

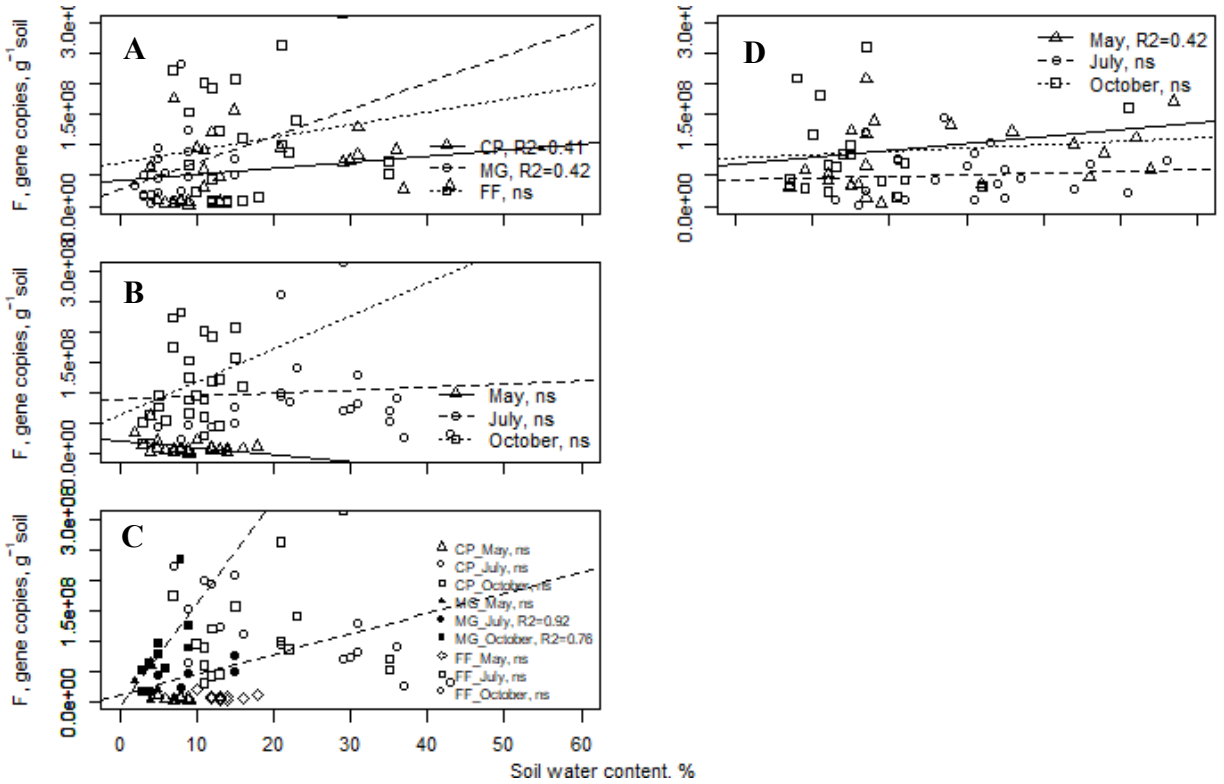

Fig. 3.6. Relationship between gravimetric soil water content (GWC) and abundance of soil fungi (F) in 2015 (A, B, C) and 2016 (D, E, F). (A) represents the 2-way interaction between GWC and Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions in 2015; (B) represents the 2-way interaction between GWC and months in 2015; (C) represents the 3-way interaction between GWC, subregions and months in 2015; (D) represents the 2-way interaction between GWC and months in 2016. Only significant lines are presented in the graph C.

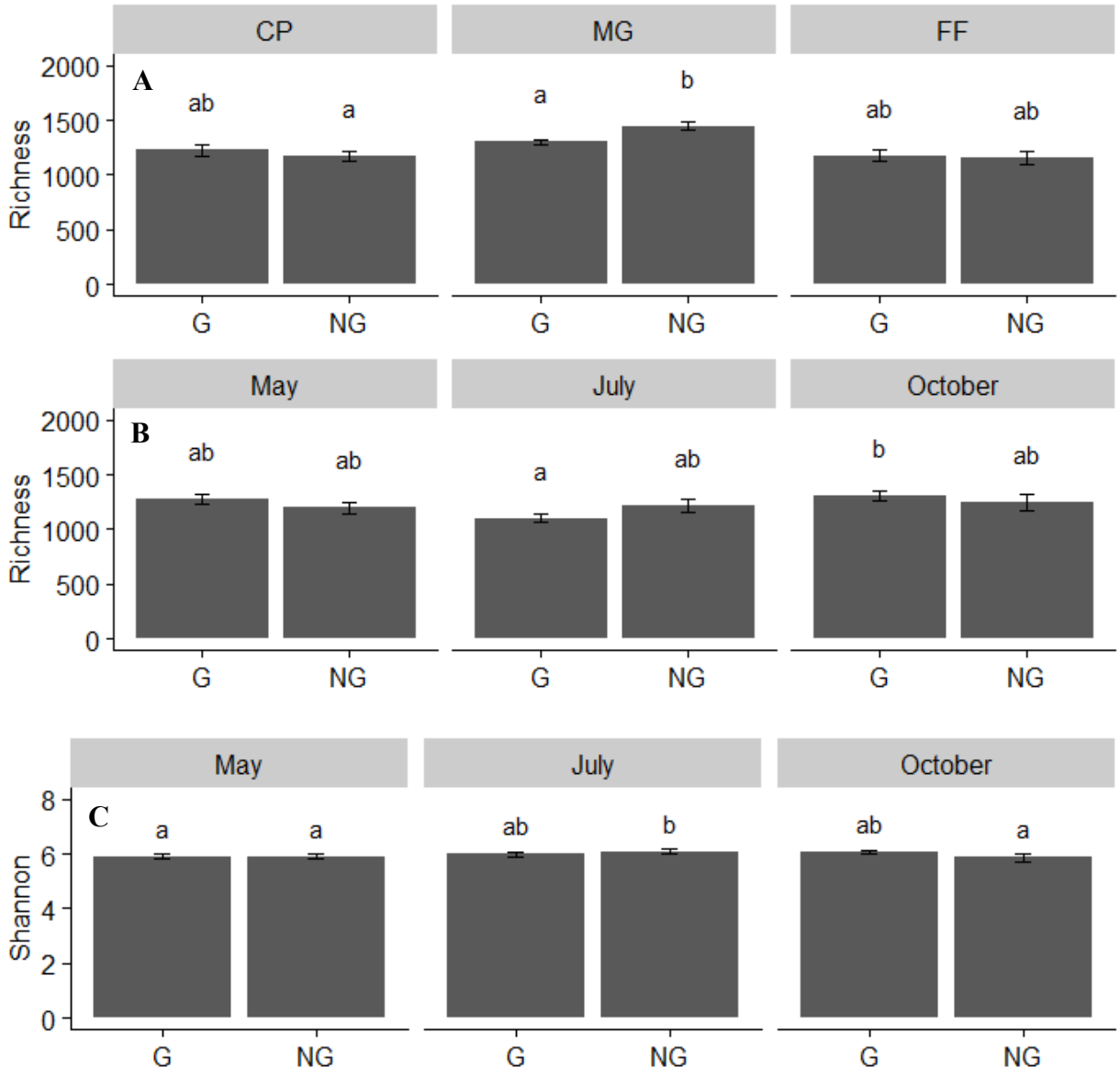

Fig. 3.7. Bacterial richness and Shannon diversity index in 2015 (A, C) and 2016 (B). (A, B) represent the 2-way interaction between Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions and grazed (G) and non-grazed (NG) treatments in 2015 and 2016, respectively; (C) represents the 2-way interaction between months and grazed (G) and non-grazed (NG) treatments in 2015.

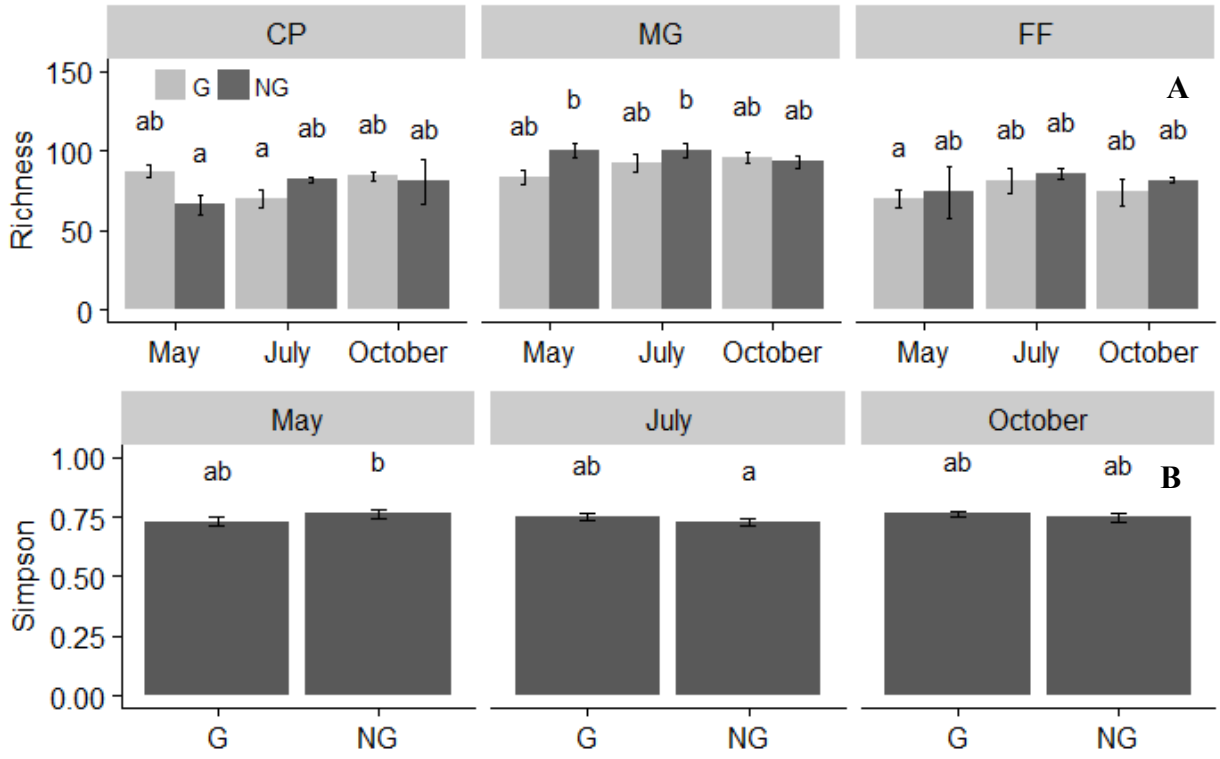

Fig. 3.8. Archaeal richness and Simpson index in 2015 (A) and 2016 (B). (A) represents the 3-way interaction between months, Central Parkland (CP), Mixedgrass (MG) and Foothills Fescue (FF) subregions as well as grazed (G) and non-grazed (NG) treatments in 2015; (B) represents the 2-way interaction between months and grazed (G) and non-grazed (NG) treatments in 2016.

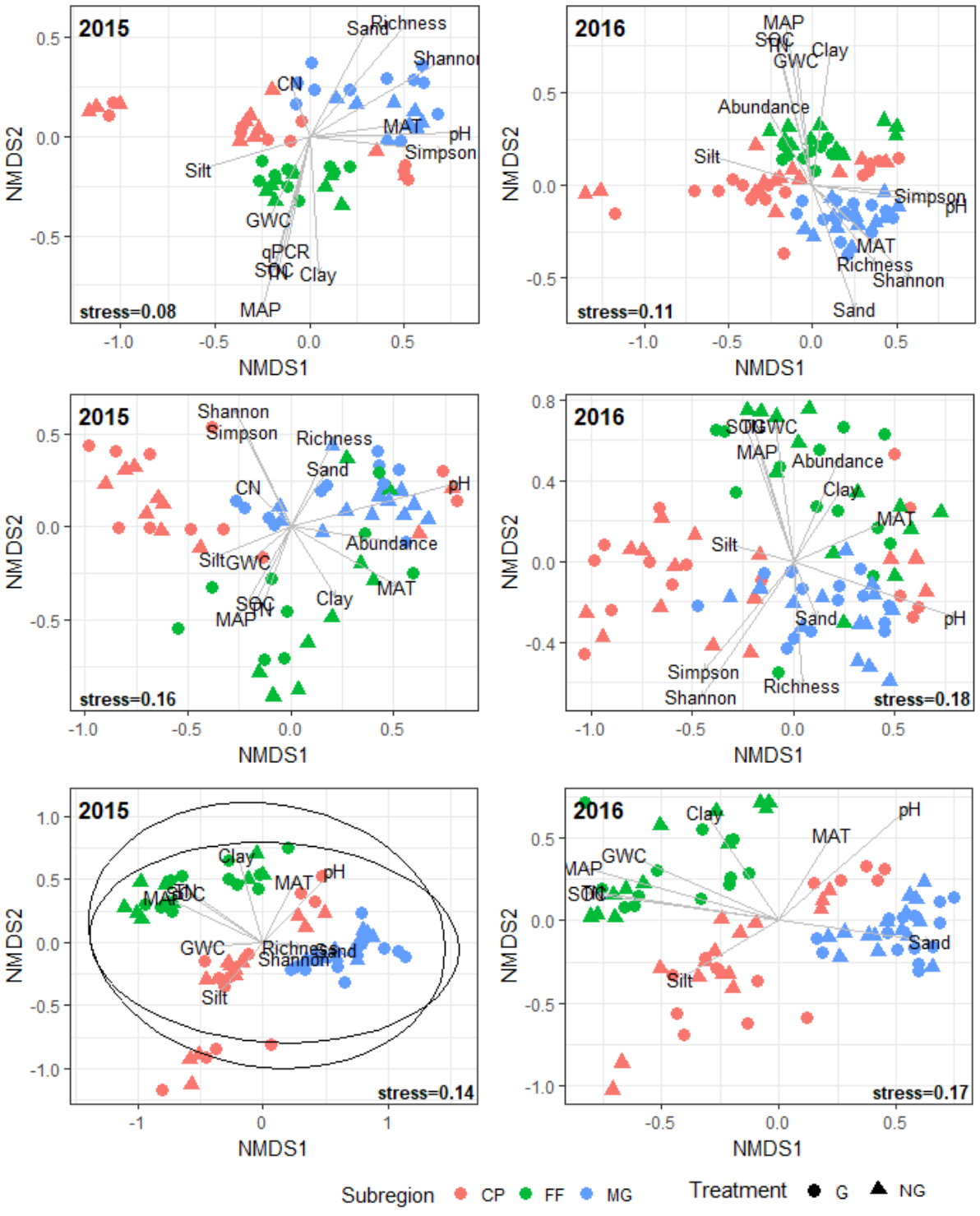

Fig. 3.9. Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities between bacterial (upper), archaeal (middle) and fungal (lower) communities of rangeland soils. Only significant variables are shown. Direction of arrows indicates increasing values of the variables, while length of arrows indicates stronger relationship.

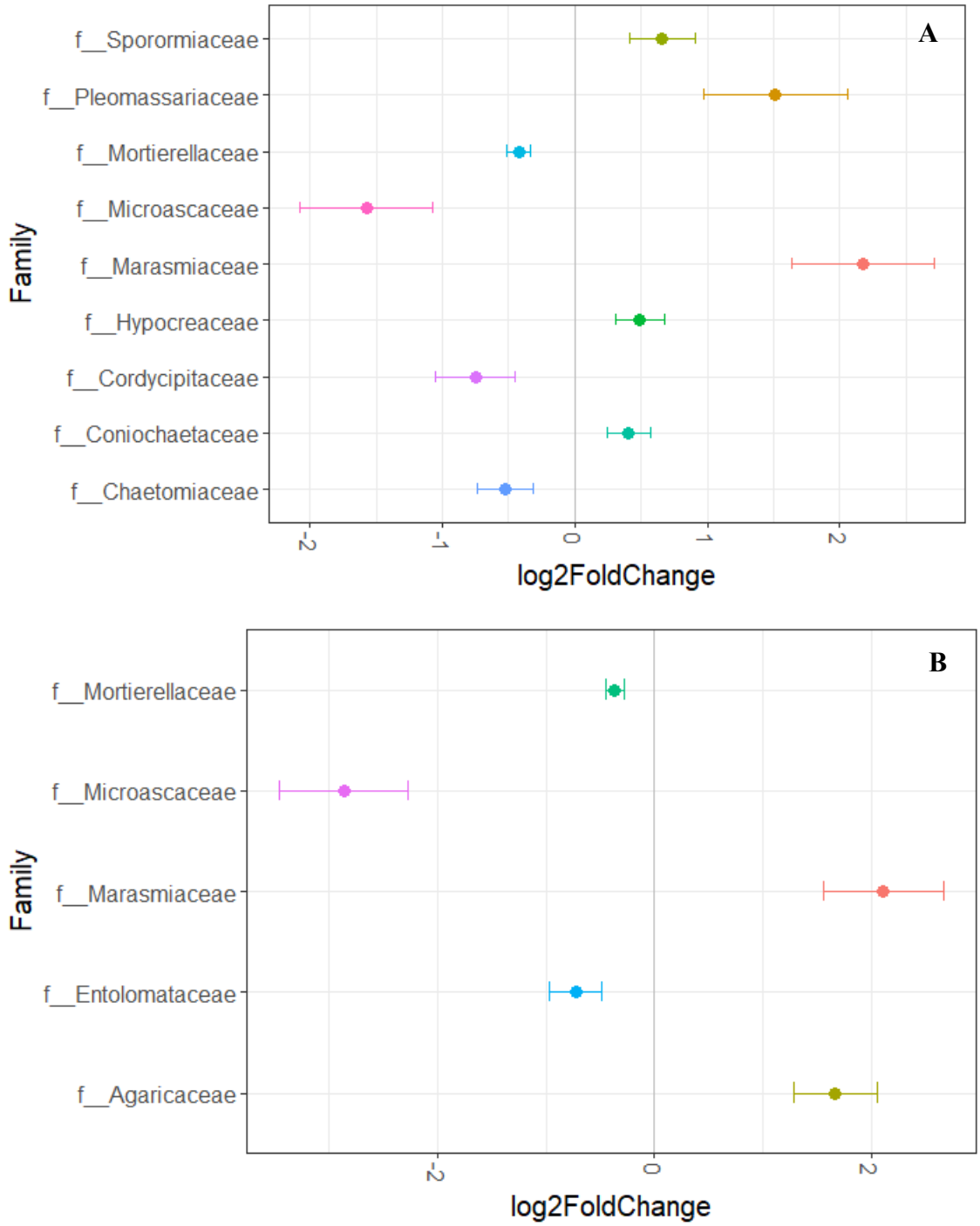

Fig. 3.10. Fungal community composition differences between the NG and G treatments in 2015 (A) and 2016 (B). Differences were calculated using the DESeq2 package in R as log2(NG/G). Negative values indicate higher representation of the individual family under long-term grazing.

Chapter 4. General discussion and conclusion

Grasslands cover up to 40% of the Earth's terrestrial surface and provide a wide range of benefits for the environment, economy and society (Wu et al., 2010; Follet and Reed, 2010). The maintenance of healthy and productive rangelands is a critical task for the well-being and survival of millions of people (Evans et al., 2017). However, development of appropriate grassland management to promote their conservation is impossible without comprehensive studies of the ecosystem and an understanding of their underlying processes.

Sequestration of carbon and nitrogen by grassland soils have the potential to play an important role in regulating climate change, as grasslands cover a large amount of the terrestrial surface and can be a significant sink for GHG (Piñeiro et al., 2010; Soussana et al., 2010). However, the specific role livestock grazing plays in altering GHG exchange between soil and atmosphere is still unclear, especially in Alberta, where only a few studies have been conducted at a limited number of locations (Gao el., 2017; Tomas et al., 2018). While soil microbial communities play a critical role in ecosystem functions, including producing or consuming of GHG (Doran and Zeiss, 2000; Classen et al., 2015; Delgado-Baquerizo et al., 2016), the study of long-term grazing effects on soil microorganisms is largely neglected, especially in grasslands (Eldridge et al., 2017).

The overall purpose of this thesis was to investigate the effect of long-term livestock grazing on GHG and soil microbial communities across Alberta grasslands. In Chapter 2, I investigated the long-term grazing effect on GHG exchange between soil and atmosphere. In Chapter 3, I assessed the impact of long-term grazing on soil microbial communities. Below, I highlight the findings from both chapters and provide suggestions for future research.

In Chapter 2, I provided a quantitative assessment of soil GHG emission / consumption from rangelands widely distributed across Alberta. The study's uniqueness is that it was the first to do so across a broad range of edaphic and climatic conditions. GHG were quantified in areas with and without long-term grazing over two growing seasons. I then used these data to calculate the global warming potential of soils. The cumulative values of CO_2 fluxes during both years were not affected by livestock grazing itself, but fluxes varied further with the grazing x year interaction, which indicated strong inter-annual variation in grazing-induced changes. Hence, grazing effects on GHG were strongly mediated by variation in growing conditions such as climate. This study was conducted during two years that differed highly in climatic conditions; higher emissions of CO_2 were evident from long-term grazing in the wet year, while remaining lower compared to the non-grazed area in the dry year. The broad geographic range of the study further allowed subregion-specific properties (i.e., content of soil organic carbon and soil water content) to be assessed, both of which had a high importance in regulating GHG fluxes. Overall, the study provides important information on the carbon and nitrogen balance calculations for these grasslands. Moreover, the large spatial coverage and multiple years evaluated allowed quantification of GHGs across the various climatic conditions, which provide important information for the development / modification of rangeland management practice within each individual subregion.

In Chapter 3, I investigated long-term grazing effects on soil microbial communities by examining the abundance and community composition of bacteria, archaea and fungi using molecular biology techniques. The uniqueness of the study is that it is one of the first to examine belowground biodiversity in northern temperate grasslands across multiple locations. Soil microbial communities in grazed areas had few differences compared to non-grazed areas. While

abundance of microbial communities was not affected by long-term grazing itself as well as alpha and beta diversity of bacterial and archaeal communities, however alpha and beta diversity of fungal communities were affected by main grazing effect, while the abundance of these communities was not affected. Also, I identified numerous individual OTUs within the fungal community that were associated with either grazed or non-grazed areas. Hence, the difference within the fungal community may have been associated with animal impacts, such as the provision of extra substrate for them (tissues, urine etc.). Strong clustering of the bacterial, archaeal and fungal communities was found, which in turn, were associated with the three subregions studied. This likely indicates that climatic conditions and environmental factors other than long-term grazing have greater impact on soil microbial communities. Moreover, the richness and abundance of communities were also associated with regional (spatial) variability. These findings were attributed to the unique soil characteristics of the different subregions. Thus, this investigation helps to understand the biotic component of rangeland soils as well as the effect of long-term grazing within them.

Combined, the two data chapters in this thesis show that long-term grazing itself had limited impacts on overall GHG and soil microbial communities. In contrast, subregion specific characteristics (soils as well as climatic conditions) had greater impact on the observed GHG and soil microorganisms. The results from this study are important as they demonstrate that these soils and associated processes related to GHG fluxes appear to be relatively resistant to the main effect of light to moderate levels of grazing, and provide a broad assessment of microbial diversity across Alberta grassland ecosystems.

Further direction

While I have provided new information about GHG and microbial diversity across Alberta's grassland ecosystems, there are few questions that remain uncovered. First, I identified numerous OTUs that responded to grazing, and the link between ecosystem processes and the identity of these microorganisms remains unclear. Further testing of functional features (e.g. functional genes) of specific microbes and the microbial communities and their effects on ecosystem processes (e.g. GHG flux) can be achieved by applying bioinformatic tools (e.g. PICRUSt) based on the data that were obtained in the present study, or further molecular analysis (e.g. shotgun metagenomics) or microarrays (e.g. GeoChip). Characterizing the linkages between key biogeochemical processes and functional community composition will provide further understanding of how these processes can be managed in grasslands under long-term grazing. Also, a further analysis can be done, e.g. connection diversity of plants and microbial community's composition as well as GHG and microbial taxa. Secondly, in this study I examined two distinctly divergent systems, grazed or non-grazed, while in actuality grazing management employs a broad spectrum of grazing systems that are known to have differential effects on plant communities (Vermeire et al., 2008). Furthermore, the grazed sites in this study were provincial lands that were light to moderately grazed, at stocking rates lower than those potentially practiced on private land. Further work could explore the impacts of a larger range of both grazing systems and grazing intensities to provide further information on how grazing management can be modified to offset greenhouse gases.

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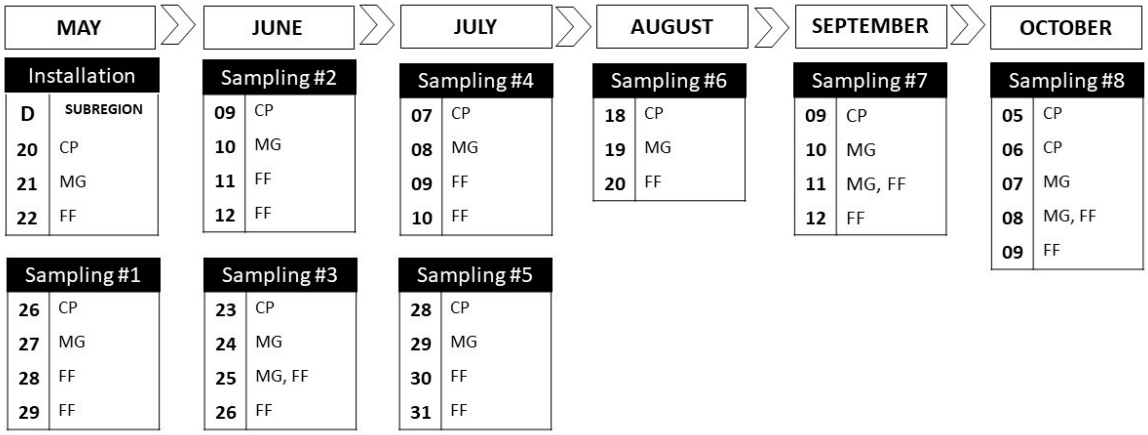
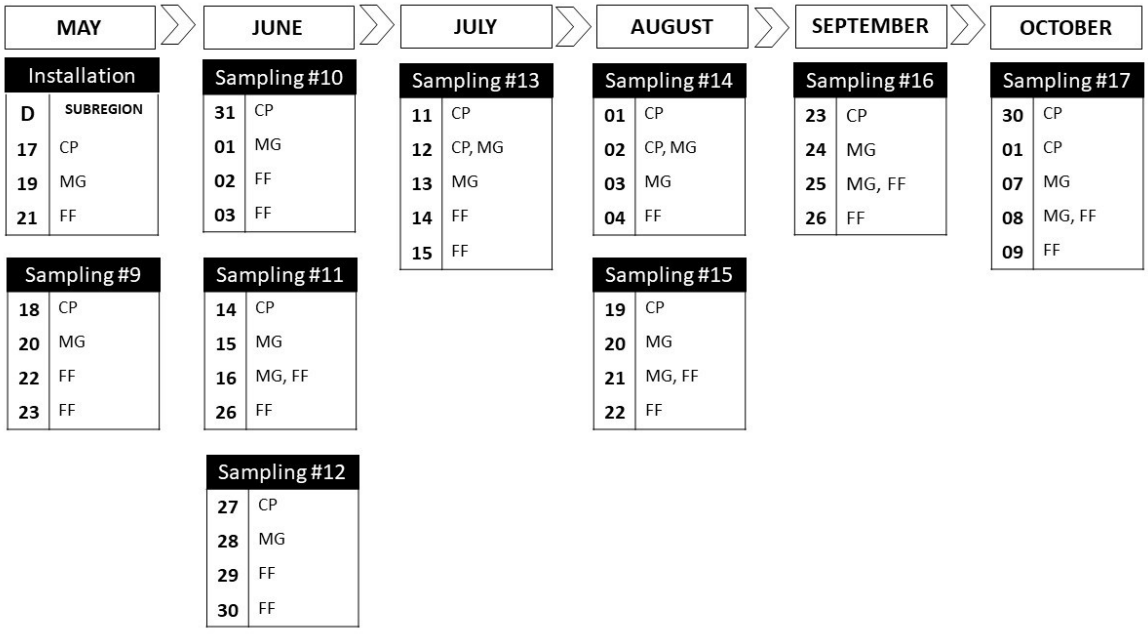
Appendices

2015



2016



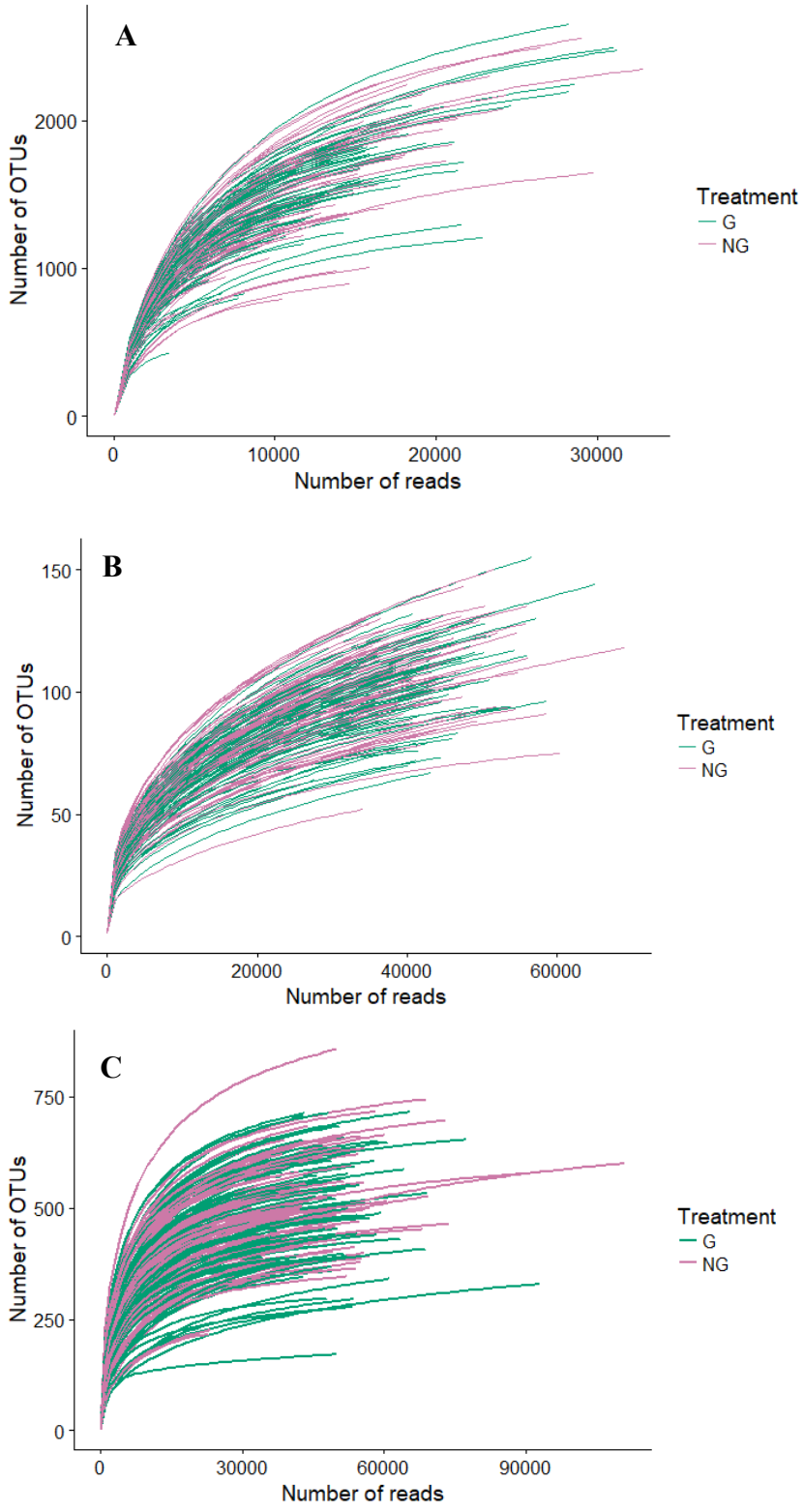
Appendix A. Gas sampling schedule conducted during each of 2015 and 2016; D, day of month; CP, Central Parkland; MG, Dry Mixedgrass and FF, Foothills Fescue subregions

Appendix B. Post-hoc test results for CO_2 emission rates for comparison between all subregions and all sampling dates in 2016

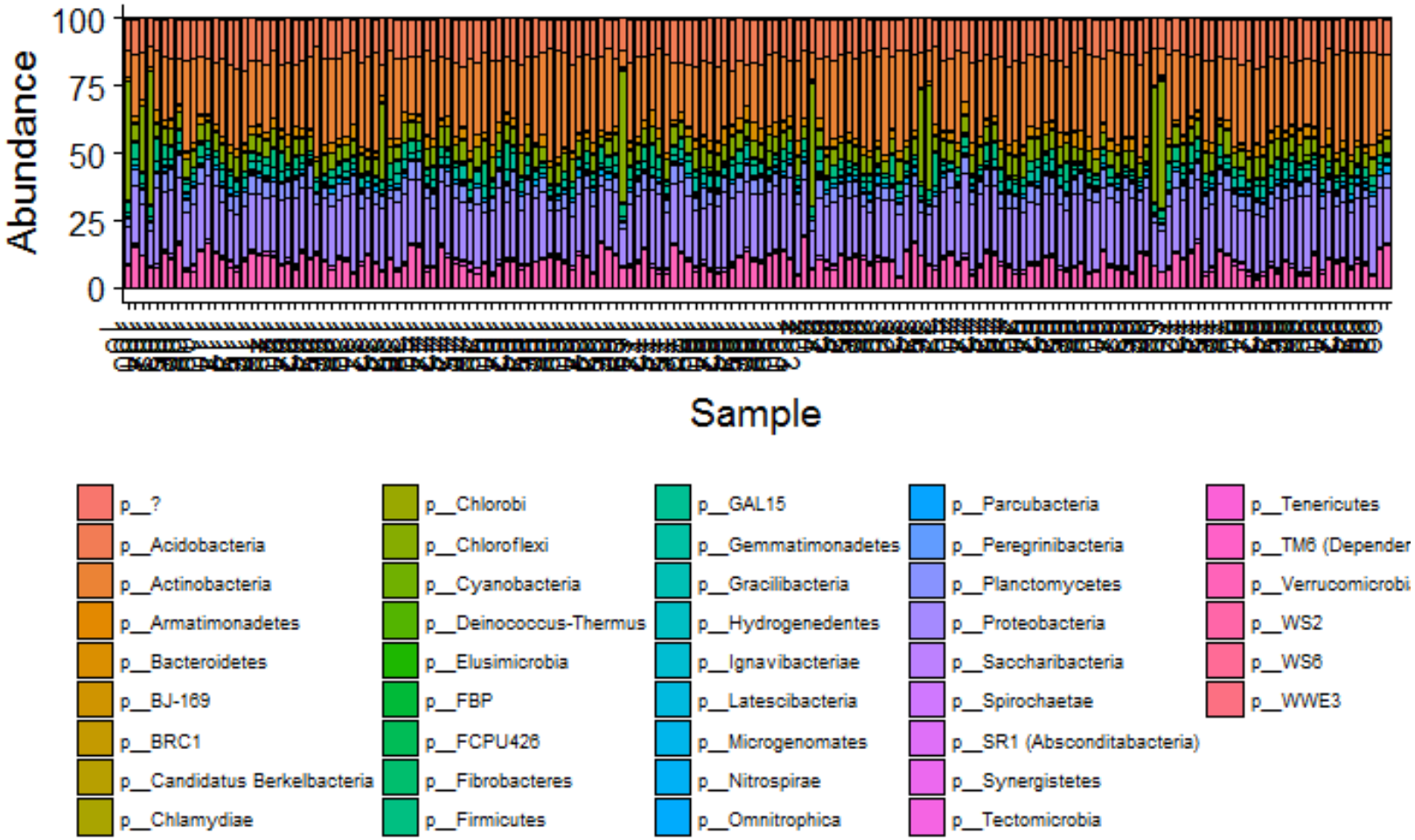
Subregion	2015		2016	
	Sampling #	Group	Sampling #	Group
CP	1	A	9	234567890ABCDE
	2	AB	10	67890ABCDE
	3	E	11	67890ABCDE
	4	ABCDE	12	67890ABCDE
	5	DE	13	CDE
	6	CDE	14	56789ABCDE
	7	CDE	15	67890ABCDE
	8	ABCDE	16	234567890AB
			17	1234
MG	1	ABC	9	2345689
	2	ABC	10	467890ABCDE
	3	ABCDE	11	12
	4	ABCDE	12	234568
	5	AB	13	34567890ABCDE
	6	ABCDE	14	70ABCDE
	7	ABCDE	15	467890ABCDE
	8	ABCD	16	235
			17	1
FF	1	ABCDE	9	234567890ABCDE
	2	BCDE	10	234567890ACD
	3	E	11	23456
	4	E	12	9ABDE
	5	ABCDE	13	BE
	6	BCDE	14	890ABCDE
	7	ABCDE	15	34567890ABCDE
	8	ABCDE	16	23456780C
			17	1234567

Appendix C. Post-hoc test results for CH_4 consumption rates for comparison between all subregions and all sampling dates in 2016

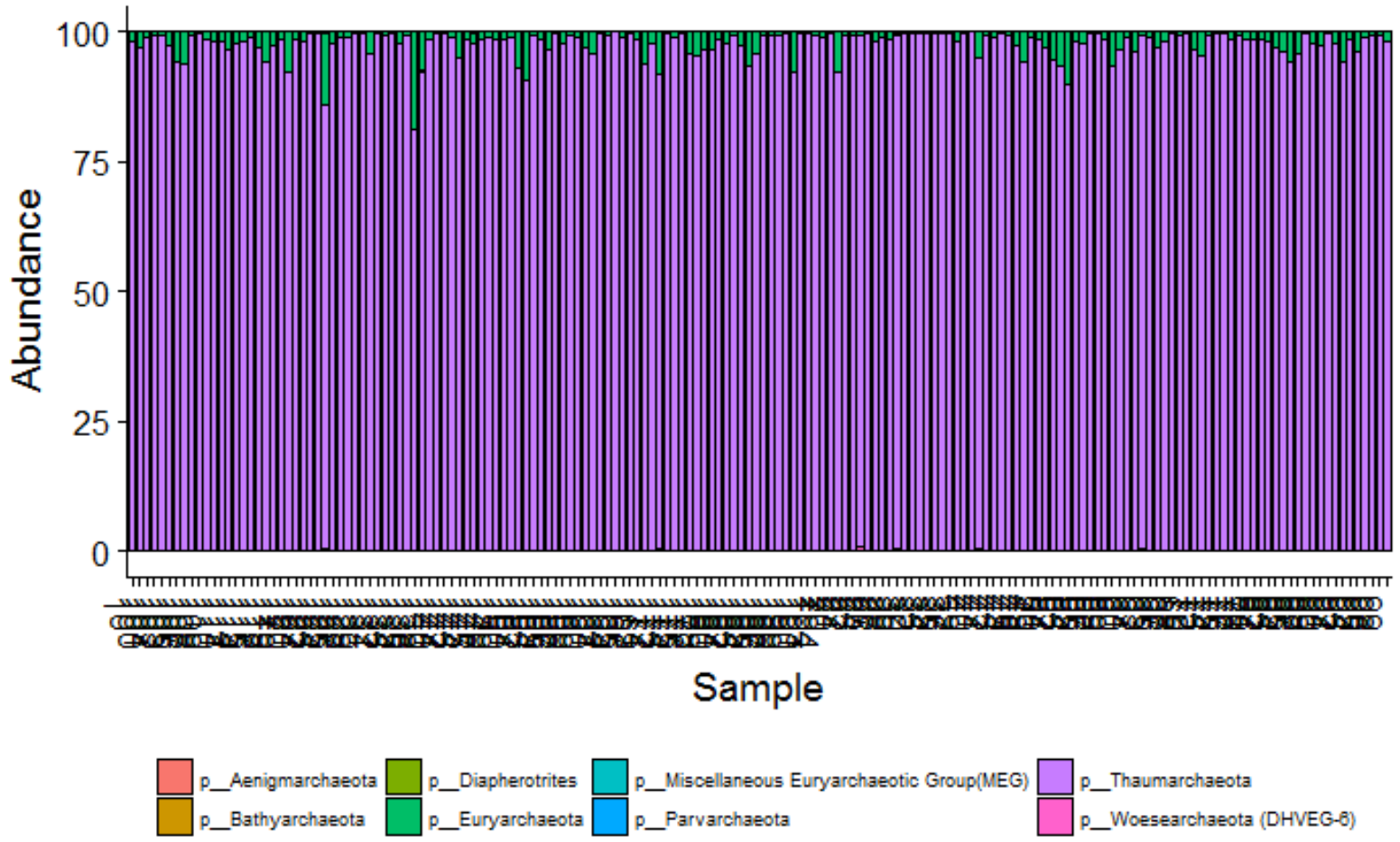
Subregion	2015		2016	
	Sampling #	Group	Sampling #	Group
CP	1	ABC	9	12345
	2	ABC	10	8
	3	ABC	11	12345
	4	ABC	12	12
	5	ABC	13	678
	6	ABC	14	1234567
	7	ABC	15	12345
	8	ABC	16	12345
			17	12345
MG	1	ABC	9	12345678
	2	ABC	10	45678
	3	ABC	11	12345678
	4	ABC	12	12345678
	5	ABC	13	345678
	6	ABC	14	45678
	7	ABC	15	12345678
	8	ABC	16	12345678
			17	12345678
FF	1	A	9	78
	2	AB	10	578
	3	C	11	245678
	4	BC	12	13
	5	BC	13	12345678
	6	BC	14	12346
	7	ABC	15	245678
	8	ABC	16	245678
			17	12345678



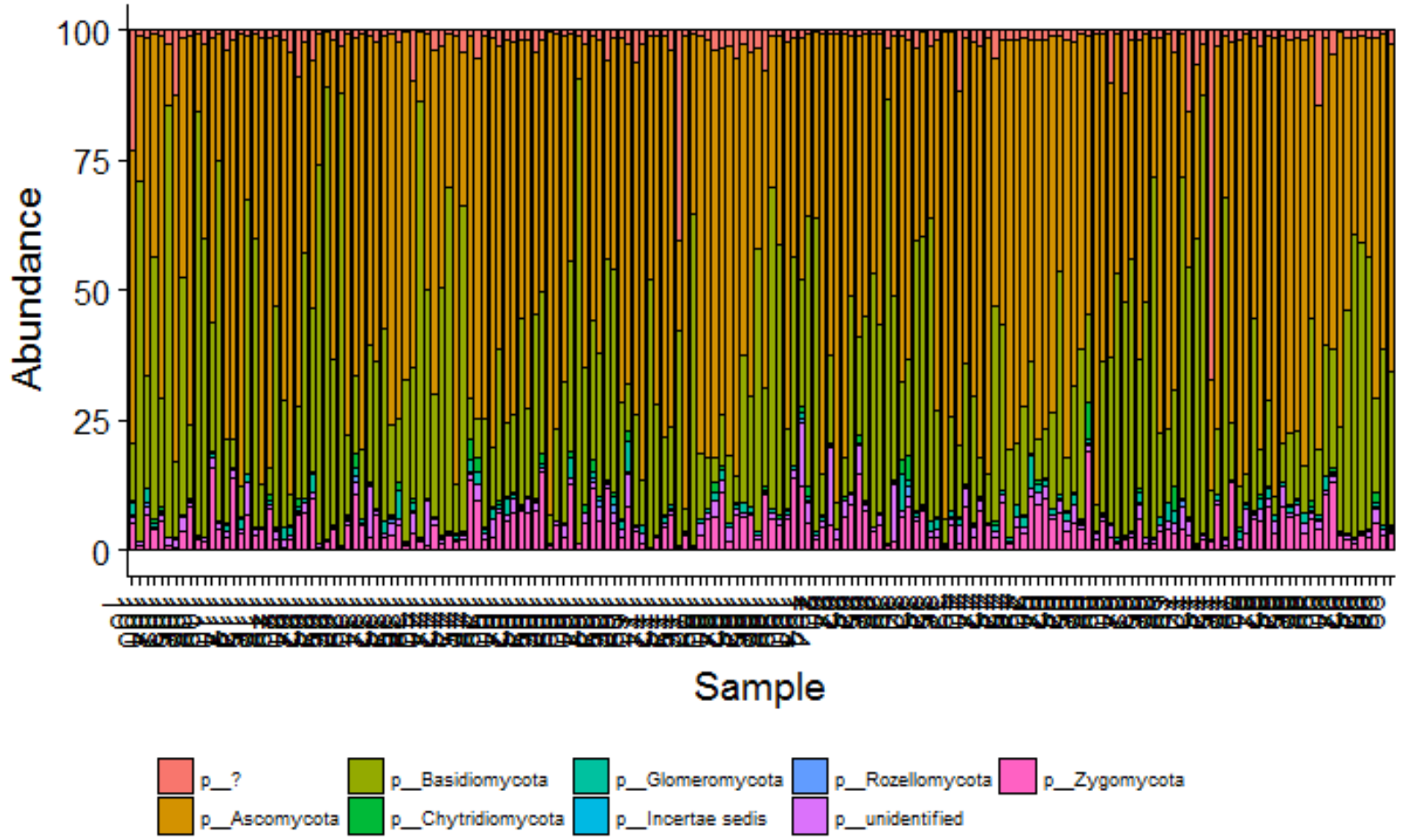
Appendix D. Rarefaction curves for (A) bacteria, (B) archaea and (C) fungi representing the number of OTUs versus the number of reads across all study samples. Each line within a graph represents an individual sample.



Appendix E. Relative abundance of the bacterial community at phylum level for each individual sample.



Appendix F. Relative abundance of the archaeal community at phylum level for each individual sample.



Appendix G. Relative abundance of the fungal community at phylum level for each individual sample.

Appendix H. Results of permutational analysis of variance (weighted UniFrac distance) testing effect of long-term grazing, geographic location (subregion) and temporal (month) variability, and their interactions, on bacterial, archaeal and fungal communities in rangeland soils of Alberta, Canada.

Factor	2015						2016					
	df	SS	MSS	F	R^2	p	df	SS	MSS	F	R^2	p
Bacteria												
Treatment (GT)	1	0.013	0.013	0.919	0.012	0.441	1	0.012	0.012	0.889	0.010	0.489
Subregion (S)	2	0.209	0.105	7.503	0.199	**0.001**	2	0.199	0.099	7.361	0.173	**0.001**
Month (M)	2	0.024	0.012	0.859	0.023	0.573	2	0.029	0.015	1.076	0.025	0.352
GT x S	2	0.020	0.010	0.713	0.019	0.705	2	0.024	0.012	0.868	0.020	0.591
GT x M	2	0.012	0.006	0.415	0.011	0.983	2	0.022	0.011	0.804	0.019	0.672
S x M	4	0.025	0.006	0.449	0.024	0.999	4	0.043	0.011	0.793	0.037	0.763
GT x S x M	4	0.026	0.007	0.474	0.025	0.990	4	0.035	0.008	0.646	0.030	0.949
Residuals	52	0.726	0.014		0.688		58	0.788	0.014		0.683	
Archaea												
Treatment (GT)	1	0.018	0.018	0.857	0.009	0.461	1	0.027	0.027	0.703	0.006	0.565
Subregion (S)	2	0.509	0.254	11.965	0.264	**0.001**	2	1.403	0.701	18.318	0.338	**0.001**
Month (M)	2	0.030	0.015	0.715	0.015	0.646	2	0.011	0.006	0.149	0.003	0.998
GT x S	2	0.012	0.006	0.294	0.006	0.964	2	0.057	0.029	0.747	0.014	0.636
GT x M	2	0.032	0.016	0.759	0.016	0.619	2	0.029	0.014	0.374	0.007	0.923
S x M	4	0.053	0.013	0.618	0.027	0.849	4	0.057	0.014	0.371	0.014	0.981
GT x S x M	4	0.016	0.004	0.189	0.008	0.999	4	0.043	0.011	0.284	0.010	0.997
Residuals	59	1.254	0.021		0.652		66	2.527	0.038		0.608	
Fungi												
Treatment (GT)	1	0.061	0.061	1.744	0.017	**0.068**	1	0.058	0.058	1.364	0.015	0.136
Subregion (S)	2	0.668	0.334	9.519	0.187	**0.001**	2	0.549	0.275	6.450	0.144	**0.001**
Month (M)	2	0.100	0.050	1.431	0.028	**0.071**	2	0.072	0.036	0.849	0.019	0.675
GT x S	2	0.059	0.029	0.855	0.017	0.662	2	0.096	0.048	1.129	0.025	0.232
GT x M	2	0.049	0.024	0.691	0.014	0.918	2	0.073	0.036	0.853	0.019	0.662
S x M	4	0.128	0.032	0.915	0.036	0.627	4	0.133	0.033	0.782	0.035	0.897
GT x S x M	4	0.113	0.028	0.806	0.032	0.850	4	0.113	0.028	0.662	0.029	0.987
Residuals	68	2.384	0.035		0.669		64	2.728	0.043		0.713	

Appendix I. Results of permutational analysis of variance (unweighted UniFrac distance) testing effects of treatment (grazed / non-grazed), geographic location (subregion) and temporal (month) variability, and their interactions, on bacterial, archaeal and fungal communities in rangeland soils of Alberta, Canada.

Factor	2015						2016					
	df	SS	MSS	F	R^2	p	df	SS	MSS	F	R^2	p
Bacteria												
Treatment (GT)	1	0.178	0.178	1.059	0.015	0.291	1	0.174	0.174	1.041	0.013	0.336
Subregion (S)	2	0.941	0.471	2.792	0.078	**0.001**	2	0.947	0.474	2.829	0.073	**0.001**
Month (M)	2	0.318	0.159	0.942	0.026	0.691	2	0.301	0.151	0.900	0.023	0.882
GT x S	2	0.337	0.169	1.000	0.028	0.460	2	0.342	0.171	1.022	0.026	0.336
GT x M	2	0.280	0.140	0.831	0.023	0.980	2	0.303	0.151	0.905	0.023	0.839
S x M	4	0.561	0.140	0.832	0.047	0.999	4	0.594	0.148	0.886	0.046	0.973
GT x S x M	4	0.613	0.153	0.909	0.051	0.894	4	0.619	0.155	0.924	0.048	0.884
Residuals	52	8.7652	0.169		0.731		58	9.709	0.167		0.747	
Archaea												
Treatment (GT)	1	0.176	0.176	1.215	0.015	0.188	1	0.141	0.141	0.934	0.011	0.538
Subregion (S)	2	1.588	0.794	5.478	0.133	**0.001**	2	1.540	0.770	5.089	0.114	**0.001**
Month (M)	2	0.269	0.134	0.927	0.022	0.589	2	0.207	0.104	0.684	0.015	0.972
GT x S	2	0.239	0.119	0.824	0.020	0.795	2	0.299	0.149	0.987	0.022	0.481
GT x M	2	0.222	0.111	0.766	0.019	0.892	2	0.234	0.117	0.773	0.017	0.900
S x M	4	0.489	0.122	0.844	0.041	0.852	4	0.527	0.132	0.871	0.039	0.841
GT x S x M	4	0.405	0.101	0.699	0.034	0.997	4	0.521	0.130	0.861	0.038	0.853
Residuals	59	8.553	0.145		0.716		66	9.988	0.151		0.742	
Fungi												
Treatment (GT)	1	0.339	0.339	1.419	0.015	**0.022**	1	0.308	0.308	1.277	0.014	**0.052**
Subregion (S)	2	2.731	1.365	5.699	0.122	**0.001**	2	2.512	1.256	5.201	0.117	**0.001**
Month (M)	2	0.496	0.248	1.035	0.022	0.337	2	0.483	0.241	0.999	0.023	0.431
GT x S	2	0.547	0.273	1.141	0.024	0.111	2	0.607	0.303	1.256	0.028	**0.033**
GT x M	2	0.359	0.179	0.749	0.016	0.999	2	0.386	0.193	0.799	0.018	0.993
S x M	4	0.839	0.209	0.876	0.038	0.967	4	0.892	0.223	0.923	0.042	0.798
GT x S x M	4	0.707	0.177	0.738	0.032	1.000	4	0.789	0.197	0.817	0.037	0.998
Residuals	68	16.291	0.239		0.730		64	15.456	0.242		0.721	

Appendix J. Regression values of non-metric multidimensional scaling (NMDS) analysis for environmental factors and microbial communities characteristics. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen; GWC, gravimetric water content.

Factor	2015		2016	
	r^2	p	r^2	p
Bacteria				
MAT	0.253	**0.003**	0.252	**0.001**
MAP	0.817	**0.001**	0.819	**0.001**
pH	0.639	**0.001**	0.747	**0.001**
SOC	0.477	**0.001**	0.676	**0.001**
TN	0.491	**0.001**	0.621	**0.001**
C/N	0.086	**0.092**	0.008	0.834
GWC	0.218	**0.006**	0.469	**0.001**
Abundance	0.346	**0.001**	0.264	**0.001**
Richness	0.622	**0.001**	0.317	**0.001**
Shannon	0.722	**0.001**	0.583	**0.001**
Simpson	0.492	**0.001**	0.499	**0.001**
Sand	0.407	**0.001**	0.507	**0.001**
Clay	0.471	**0.001**	0.559	**0.001**
Silt	0.366	**0.001**	0.413	**0.001**
Archaea				
MAT	0.370	**0.001**	0.309	**0.001**
MAP	0.307	**0.001**	0.335	**0.001**
pH	0.733	**0.001**	0.712	**0.001**
SOC	0.190	**0.003**	0.508	**0.001**
TN	0.212	**0.002**	0.498	**0.001**
C/N	0.090	**0.062**	0.037	0.320
GWC	0.075	**0.097**	0.459	**0.001**
Abundance	0.254	**0.001**	0.308	**0.001**
Richness	0.288	**0.001**	0.374	**0.001**
Shannon	0.477	**0.001**	0.653	**0.001**
Simpson	0.322	**0.001**	0.483	**0.001**
Sand	0.134	**0.016**	0.086	**0.084**
Clay	0.183	**0.001**	0.187	**0.003**
Silt	0.167	**0.007**	0.119	**0.020**
Fungi				
MAT	0.326	**0.001**	0.469	**0.001**
MAP	0.734	**0.001**	0.832	**0.001**
pH	0.675	**0.001**	0.795	**0.001**
SOC	0.484	**0.001**	0.779	**0.001**
TN	0.506	**0.001**	0.744	**0.001**
C/N	0.054	0.233	0.016	0.694
GWC	0.225	**0.001**	0.624	**0.001**
Abundance	0.077	0.121	0.045	0.354
Richness	0.083	**0.094**	0.062	0.243
Shannon	0.120	**0.034**	0.048	0.315
Simpson	0.051	0.242	0.061	0.224
Sand	0.365	**0.001**	0.425	**0.001**
Clay	0.532	**0.001**	0.555	**0.001**
Silt	0.330	**0.001**	0.253	**0.001**