Carbon Dynamics across Contrasting Temperate Agroecosystems:

Soil Organic Matter Pools, Mycorrhizae, Water Use-efficiency, Methane and Carbon Dioxide Fluxes

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Soil Science

Department of Renewable Resources
University of Alberta

ABSTRACT

Keunbae, Kim. MSc., University of Alberta, October 2021. Carbon Dynamics across Contrasting Temperate Agroecosystems: Soil Organic Matter Pools, Mycorrhizae, Water Use-efficiency, Methane and Carbon Dioxide Fluxes. Supervisor: Hernandez Ramirez, Guillermo.

Perennial grain crops have been proposed as a sustainable alternative to conventional annual grain crops, since they potentially hold multiple desirable features including increased soil C sequestration and biodiversity, mitigation of greenhouse gases (GHGs), and reduction of water loss and others. These attributes are accomplished by their no-tillage management, longer growing season, and pervasive root systems. Nonetheless, the ramifications of implementing novel perennial grain cropping systems to the atmosphere, lithosphere, and biosphere remains elusive. In this research, comprehensive comparisons were made to enhance current understanding of how annual versus perennial grain cropping differ in soil organic matter (SOM) pools, GHGs emissions, and mycorrhizae (i.e., AMF). Five contrasting cropping systems — fallow, annual-grain, biennial-grain, perennial-grain, and perennial-forage — were compared with and without N fertilizer addition. A randomized complete block design consisting of four replicates was initiated at sites located in Edmonton and Breton, Alberta, Canada. In addition to the replicate plots, two adjacent 4-ha fields, including annual vs. perennial grain crops, were set up for eddy covariance measurements at the Breton site. By doing so, it was expected that they address a limitation of the chamber measurements with respect to instantaneous and heterogeneous capture, as well as evapotranspiration (ET) comparison. As a result of SOC fractionation, perennial grain crops increased soil C concentration in both transitory and stable pools compared with annual grain compartments, although overall soil C sequestration occurred as a function of N fertilizer, cropping systems, and underlying soil texture. Moreover, substantial CH_4 and CO_2 uptake were observed in perennial grain cropping system compared with annual grain crops likely due to a shift to favorable GHGs sink conditions such as aeration-moisture balance, photosynthetic activity, and microbial decomposition. However, overall cumulative ET and associated ecosystem-water use efficiency were similar with between the two cropping systems. Lastly, arbuscular mycorrhizal fungi (AMF) community compositions between annual and perennial grain cropping systems exhibited no significant difference, likely due to the genetic similarity of two crops. In addition, the two contrasting study sites had different patterns of AMF alpha and beta diversity, which may be a result of differing inherent soil properties and management legacy effect across field sites. In sum, AMF communities appear to be impervious to annual vs perennial grain crops, rather their most pronounced difference are field site specific. In sum, my research has been conducted for an overall assessment of whether perennial grain crops play a multifunctional role in agroecosystems toward better sustainability.

PREFACE

This thesis is an original work accomplished by Keunbae Kim under the supervision of Dr. Guillermo Hernandez Ramirez. Overall, my roles on the thesis were conceptualization, investigation, formal analysis, visualization, writing-original draft and reviewing. However, each thesis component has been completed with contribution, commitment, and dedication of those involved in the Perennial Cereal Research Program – in particular, Ms. Erin Daly who co-led the overall program.

Chapters 1, 2, 3, and 4 in the thesis are aimed for publication.

Chapter 1 "Perennial Grain Cropping Enhances the Soil Methane Sink in Temperate Agroecosystem" has been already published in Geoderma journal.

Dr. Monika Gorzelak provided feedback on the writing in Chapter 2 "Soil Carbon Response to Perennial Grain Cropping and Nitrogen Fertilizer". This chapter has been submitted to Soil & Tillage Research journal.

Dr. Monika Gorzelak and Patrick Neuberger contributed to the experimental design, data analyses, and writing of Chapter 3 "Do arbuscular mycorrhizal fungi community composition differ with between annual and perennial grain crops?". This chapter has been submitted to Applied Soil Ecology journal.

Drs Thomas K. Flesch and Trevor W. Coates contributed to data collection, data processing, and provided feedback on writing in Chapter 4 "Carbon and Water Dynamics of a Perennial Grain Crop in Temperate Agroecosystems". This chapter is under the 2nd round of revisions of Agriculture and Forest Meteorology journal.

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to Dr. Guillermo Hernandez-Ramirez for his supports, inspiration, and patience. It was an invaluable experience to learn a myriad of lessons in responsibility, communication, and optimistic attitude as well as fruitful accomplishment of research. Without his supports, I would not have been able to complete my degree.

To dear members of my advisory committee and collaborators, Dr. Monika Gorzelak for the cutting-edge discipline of AMF molecular ecology and visiting stay at Lethbridge Research and Development Centre. Dr. Thomas K Flesh and Dr. Trevor Coates guided experimental design, data processing, and writing processing for micrometeorological research. It was indeed fascinating and enjoyable to learn these two research subjects. Multidisciplinary indoctrination also enables me to span a wide scope of research interests and curiosities.

To the multiple sources of funding that makes it possible to pursue my degree: Alberta Agriculture and Forest, Agriculture and Agri-Food Canada, Agricultural Greenhouse Gas Program, and University of Alberta for financial supports to implement robust experiments in diverse research fields.

To members of our lab for collaboration and consultation: Erin Daly, Sisi Lin, Carmen Cecilia Roman Perez, Ekene Iheshiulo, Alan Lee, Kris Guenette, Shakila Ekanayaka, and Chloe Ducholke. To numerous field assistants including Alessia Marchesan, Megan Pudde, Keifer Klimchuk, Delainie Sutherland, Dylan Fath, Jeffrey Robbins, Jefferson Sawyer, Beatriz Nunez Acosta, Elisa Yukie Yokoyama, Oliva Grace Masters, Alanna Victoria Zubler, Melissa Wheatley, Jichen Li, Sanat Kanekar, and Evans Batung. To field technicians across campus

Germar Lohstraeter, Leigh-Ann Powers, Dick Puurveen for their patience, guidance and advise. To my fellow graduate students for being supportive and sharing experiences during graduate program including Jinbiao Li, Zhenfeng An, Mengmeng Liu, Xuehui Sun and many others.

To all my dedicated former professors at Chonnam National University. Dr. Young-Mi Choi for encouragement, sincere advice, and insights to study abroad for dream. Without her support, I would not have been able to experience professional developing and learning opportunities. Dr. Kwang-Sik Yoon and Dr. Han-Young Kim for supportive feedback to keep me on the right track.

To my beloved family. My father, mother, and brother, for unwavering support through this process. I am so thankful that he was my father, and I was able to live so close to him. I will remember his warmth, cherish his memory in my heart, and put the best effort into supporting mother and brother. Finally, the special thanks to my other half, Hye-in Yang, for her belief and support as well as motivation for persistent study.

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INTRODUCTION

Climate change effects including escalating ambient temperatures, atmospheric carbon dioxide (CO_2) levels, and irregular rainfall bring challenging circumstances threaten human life, societal prosperity, and biodiversity (IPCC, 2014). Moreover, accelerating climate change has detrimental impacts on food security and the environment especially in agroecosystems, as it affects soil and water resources that play a crucial role in growth of crops, forage, and livestock (Bowling et al., 2020). In addition, the human population expansion concurrently urges to reshape conventional agriculture systems to increase crop productivity (Bowling et al., 2020; Glover and Reganold, 2020). Currently, annual monoculture crops occupy more than three quarters of cropland, they require intense labor every year, bare soil for part of the year, and high fertilizer applications (de Oliveira et al., 2020). Such management subsequently generates significant greenhouse gas (GHGs) emissions, degrades soil organic matter (SOM), and even contaminates the environment (Glover and Reganold, 2010). Furthermore, annual grain cropping systems are less resilient to increased environmental stress potentially caused by contemporary circumstance. Consequently, it is essential to introduce sustainable agroecosystems for better adaptation to climate change, environmental challenges, and a growing human population (Glover and Reganold, 2010).

Perennial grain crops have been proposed as sustainable alternative to conventional annual grain crops, since they have abundant belowground biomass, no-tillage management practice, and a longer growing season (de Oliveira et al., 2020). Perennial grain cropping systems can not only confer economically stable productivity, but they also achieve environmental sustainability (Snapp et al., 2019). For instance, the extensive root systems can readily capture soil nutrients and water (Duchene et al., 2020), while at the same time no-tillage management for multiple years possibly enables to sequester a substantial amount of additional carbon in the soil profiles beneath perennial grain crops (Haddaway et al., 2017). In addition, perennial grain cropping systems can reduce expenses associated with field operations such as seedling, fertilizer, and pesticide inputs (Glover and Reganold, 2010). However, several challenges and shortfalls can be identified when using perennial grain crops to replace their annual counterparts. Some perennial crops can act as disease refugia, and annual grain crops can have advantages in terms of management flexibility over perennial crops (Cox et al., 2005). The relative short-life of annual crops enables farmer to quickly alter crop sequences responding to market opportunities and prices as well as to crop disease outbreaks. Besides, harvest index of annual grain crops in terms of dry matter allocation to harvested grain is in general much greater than of perennial grain crops. In spite of these difficulties, strategically integrating perennial grain cropping systems in arable land still holds unique opportunities for multifunctional agriculture as well as a variety of ecosystem options (Ryan et al., 2018).

Nitrogen (N) fertilizer application is a common practice to increase crop yield in agriculture (Guenette et al., 2019). As opposed to their annual counterparts, N fertilizer can bring about beneficial interactive effects in perennial grain cropping systems, likely due to their greater belowground biomass production (Liu et al., 2017, Kim et al., 2021). In other words, extensive root systems in perennials can effectively assimilate a substantial amount of N nutrients from added fertilizer and from the native soil N, which feasibly induce and benefit from an oscillation in N availability and associated crop productivity (Sprunger et al., 2018; Junger et al., 2019). Therefore, such difference in N accessibility and belowground biomass could differently influence C and N dynamics via stoichiometric microbial decompositions of SOM and plant residues (Sprunger et al., 2019; Miller et al., 2019; Roman-Perez and Hernandez-Ramirez, 2020).

Yet, the repercussions of how N fertilizer affect soil organic matter (SOM), greenhouse gases (GHGs), and microbial community compositions across contrasting cropping systems remain understudied.

Concerns about climate change have raised interest in ways to improve soil organic carbon (SOC) storage as it is known that SOC storage capacity is susceptible to cropping systems, inherent soil quality, and climate (Hernandez-Ramirez et al., 2009; Carter and Gregorich, 2010). Many studies document that perennial grain crops can accrue greater SOC and promote longer C retention time in the soil profile than annual grain crop in part because of massive belowground biomass, no-tillage, and longer growing season (Crew and Rumsey, 2017; Sprunger et al., 2018; Ryan et al., 2018). Therefore, increased soil C sequestration by perennial grain cropping systems may help mitigate climate change as a long-term plan. Nonetheless, it has not been fully understood how soil C accruals change over time across contrasting cropping systems. To better understand SOM dynamics, there is a need to evaluate the fast-cycling pool of SOM comprising heterogeneous continuum pools (Hernandez-Ramirez et al., 2009). Through physical size fractionation, particulate organic matter (POM), an initial indicator responsive to cropping systems, is generally utilized for interpretation of labile and stable pools (Sprunger et al 2018; Cates et al., 2019). Through examination of C and N concentration and distribution in the POM fraction, we pursue deciphering the C dynamics and provide insights on how to maximize soil C sequestration in agroecosystems.

Methane (CH_4) and carbon dioxide (CO_2) are important GHGs, and anthropogenic activities in agroecosystems contributes to remarkably increase atmospheric GHGs. Net outcomes of GHGs exchanges likewise seem to largely depend on cropping system choices and field management practices as theses collectively altered soil C accumulation, turnover, soil erosion, and crop productivity (Zenone et al., 2013; Bowling et al., 2020; Kim et al., 2021). Moreover, ongoing climate change (e.g., change of precipitation distribution and temperature) may also feedback impact GHGs dynamics at agriculture landscapes since major factors influencing variability of GHGs are known to be soil moisture and soil temperature (Van den Pol-van Dasselaar et al., 1998). Consequently, we embarked in developing a constructive framework for unravelling and enhancing soil C sequestration by quantifying GHGs flux exchange across contrasting cropping systems as well as understanding the environmental controlling environmental factors of GHGs in broad variety of agroecosystems (Gelfand et al., 2016).

In addition to C dynamics, water cycling is also a key component in that ecosystems link crop productivity through physiological and metabolic process (Eichelmann et al., 2016: Zhao et al., 2021). With climate change, increased frequency of drought and dry spells is expected to exacerbate water availability in agroecosystems (Hatfield and Dold, 2019). However, innovative perennial grain cropping systems can ameliorate deficiency of water through pervasive root systems, which presumably draw water from deep soil layers and use stored water from past rainfall (de Oliveira et al., 2020). It is further postulated that perennial grain cropping systems are capable of stabilizing grain crop even regions and season with erratic, intermittent rainfall (de Oliveira et al., 2020). Therefore, it becomes important to assess the impact of crop productivity and water budget (i.e., water use efficiency; WUE) across contrasting cropping systems (Jiang et al., 2020).

Soil microorganisms, and in particular arbuscular mycorrhizal fungi (AMF), are critical components for sustainable agroecosystems, because they possess a variety of functional roles associated with crop growth and soil health (Bakhshandeh et al., 2017). Moreover, more than

80% of terrestrial plant species form mutualisms with AMF, which enable AMF to exchange soil nutrients and water for plant-derived photosynthate (Smith and Read et al., 2010; Gorzelak et al. 2015; Mahmoudi et al., 2019). As the network of AMF has greater absorptive surface than plant root hair, which further influence and the course of plant succession (Yang et al., 2011). Thus, AMF richness, diversity, and community composition play a pivotal role in soil quality and crop performance at the individual and ecosystem level (Alami et al., 2020). Yet, AMF assemblages are strongly influenced by anthropogenic activities including tillage, fertilizer addition, and host crop identity (Alguacil et al., 2019; Ezeokoli et al., 2020; Azeem et al., 2020). Novel perennial grain cropping systems, such as crop growth habit and no-tillage management, may alter belowground AMF diversity and community compositions. Therefore, it is warranted to examine how differently contrasting cropping systems shift AMF diversity and community compositions across contrasting cropping systems.

The general goal of this thesis is to elucidate how perennial grain crops can influence the multifunctional outcomes of agroecosystems compared with conventional annual counterparts. Properties of agroecosystems that are investigated include: soil organic matter sequestration, GHGs emissions, WUE, and AMF diversity and community composition. In addition to testing the effects of contrasting cropping systems on these properties, the impact of N fertilizer on the abovementioned factors has been considered. This thesis hypothesizes that perennial grain crops can be a sustainable alternative to conventional annual grain crop in terms of agroecosystem multifunctionality, encompassing GHGs emissions, soil C sequestration, WUE, and AMF community compositions.

Previous studies suggest that perennial grain crops can have a myriad of potential benefits that in many different ways underpin the sustainability of agroecosystems. However, it underscores that comprehensive assessments to date are insufficient to validate multifunctionality of perennial grain cropping systems compared with conventional annual grain crops. Also, uncertainty of C dynamics as a response to N fertilizer additions across contrasting cropping systems remains. Thus, the objectives of this study are (i) to quantify soil C and N pools, (ii) to assess seasonal and inter-annual variations of GHGs emissions as well as water fluxes, (iii) to investigate how the mycorrhizae community composition shift across contrasting cropping systems.

CHAPTER 1. Perennial Grain Cropping Enhances the Soil Methane Sink in Temperate Agroecosystems

Kim, K., Daly, E.J., Hernandez-Ramirez, G., 2021. Perennial grain cropping enhances the soil methane sink in temperate agroecosystems. Geoderma 388, 114931.
https://doi.org/10.1016/j.geoderma.2021.114931

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1.1. Abstract

The impact of contrasting land use types on the soil methane (CH_4) sink remains elusive. We examined for the first time how annual versus perennial grain cropping influences CH_4 fluxes. Experimental sites were located in Edmonton and Breton, Canada. Treatments included perennial-forage, perennial-grain, fall-grain, spring-grain and fallow. We measured surface CH_4 fluxes, as well as root density, temperature and moisture at multiple soil depths over two years. Overall, Breton was a 58% larger CH_4 sink than Edmonton. Moreover, clear differences across cropping systems were evident in Edmonton. Both perennial-grain and perennial-forage were stronger CH_4 sinks than both spring-grain and fallow ($P < 0.01$). Notably, perennial-grain was a 67% stronger CH_4 sink than spring-grain (444 vs. 265 g CH_4-C ha^{-1} over two growing seasons). These divergences were indiscernible in Breton, where all cropping systems sustained uniformly higher CH_4 sinks than in Edmonton. The sites responded differently to the same cropping systems likely due to their contrasting land use histories. A long-term cropping of perennial forages prior to experiment establishment in Breton was conducive to maintaining a higher CH_4 sink, masking treatment effects. Conversely, a background of recurrent annual cropping (as in Edmonton) resulted in an enhanced CH_4 sink when transitioning into perennial-grain. The Q_{10} responses of the daily CH_4 fluxes to temperature further pointed to the greater CH_4 sink by perennial-grain. Irrespective of site, perennial-grain had a Q_{10} at least 24% larger than spring-grain. Additionally, perennial-grain had deeper-denser roots and lower water-filled pore space (WFPS) by 19% than spring-grain, likely enhancing gas exchange through the perennial-grain soil profile. The highest sensitivity of CH_4 fluxes to temperature and WFPS emerged from measurements taken at 40 cm depth, suggesting peak CH_4 consumption at this subsurface layer.

Overall, results indicate that as a consequence of increased root growth, perennial-grain can favorably shift the aeration-moisture balance in the soil towards increased CH_4 uptake.

Keywords:

Methane sink, perennial grain, land use systems, Q_{10}, moisture

Highlights:

- A CH_4 sink ranking: perennial-grain and perennial-forage > spring-grain and fallow.
- Shifting from long-term annual to perennial grain cropping prompts methane uptake.
- Land-use legacy of perennial forage occludes divergence due to cropping systems.
- Surface CH_4 fluxes best predicted by Q_{10}-moisture measured at 40 cm soil depth.
- Moisture emerged as key in mediating CH_4 uptake across cropping systems.

1.2. Introduction

Methane (CH_4) is an important greenhouse gas 28 times more potent than carbon dioxide (CO_2) on a mass basis (IPCC, 2014). Soils can act as a sink or a source of CH_4, mainly as a function of soil moisture, with unsaturated upland soils typically removing CH_4 from the atmosphere (Smith et al., 2011; Brachmann et al., 2020; Chai et al., 2020b). Nevertheless, the global CH_4 sink by upland soils remains uncertain, with estimations widely ranging between 9 to 51 Tg CH_4 year^{-1} (Dutaur and Verchot, 2007; Wanyama et al., 2019; Brachmann et al., 2020). When soils are unsaturated, biological CH_4 consumption by methanotrophs can continuously occur throughout the soil profile (Smith et al., 2011; Brachmann et al., 2020). This is linked to adequate soil aeration being a prerequisite for CH_4 oxidation to CO_2 (Chen et al., 2011; Díaz et al., 2018). However, the net outcome of CH_4 uptake seems to largely depend on the land use system (Dalal et al., 2008; McDaniel et al., 2019). Lang et al. (2019) and Wanyama et al. (2019) further pointed to land use history and management changes over time, emphasizing the importance of land use changes on soil water, aeration, soil mineral N status and their interaction as crucial predeterminants of surface CH_4 fluxes.

Croplands cover 11% of the total terrestrial surface, with annual crop species being dominant (FAO, 2011). A novel alternative to annual croplands is perennial grain cropping (Ryan et al., 2018; Duchene et al., 2019). Shifting to emerging perennial cropping systems holds the potential to deliver grain production while proactively managing environmental challenges, such as mitigating greenhouse gases (Ryan et al., 2018; Daly and Hernandez-Ramirez, 2020). Examples of perennial grain cultivars under development are wheat, rice, sorghum and rye (Ryan et al., 2018). Relative to annuals, perennial croplands have inherently longer growing seasons, deeper and denser rooting, and reduced soil disturbance over their multiyear growth (Hebb et al., 2017; Kiani et al., 2017; Sprunger et al., 2019). However, given the contrasting nature of annuals vs. perennials, it is unclear how a shift to perennial grain cropping could influence soil CH_4 fluxes. The few available reports suggest that perennial croplands dedicated to biomass production are a potential CH_4 sink (Don et al., 2012; Gauder et al., 2012). In addition to an overall paucity of data examining the putative effects of perennial grain cropping on CH_4 uptake, there is also a lack of understanding of possible underlying mechanisms. An earlier report by Culman et al. (2013) suggests that perennial grain crops have the potential to decrease soil moisture at depth. Hence, it can be postulated that perennial grain cropping could enlarge the CH_4 sink by influencing soil aeration. Indeed, this driving role of plants on CH_4 fluxes is just starting to be documented across several biomes (Meier et al., 2016; Welch et al., 2019; Chai et al., 2020b). Nonetheless, to date, there is no evidence available for how perennial grain cropping might strengthen the CH_4 sink in soils. Moreover, a common practice in croplands is the application of nitrogen fertilizers (Jungers et al. 2017; Guenette et al. 2019). Previous studies have shown that N additions and increased availability of inorganic N can hinder CH_4 uptake, in particular in forests (Butterbach-bahl and Papen, 2002, Fender et al., 2012; Wu et al., 2020). In the case of perennial grain cropping systems, it remains to be tested whether N fertilization affects soil CH_4 fluxes. Our study addresses these various knowledge gaps with respect to perennial grain cropping and the effects of N fertilization.

The mediating effects of soil moisture and temperature on CH_4 fluxes across contrasting cropping systems and land use changes are still unknown (Luo et al., 2013; Kern et al., 2012; McDaniel et al., 2019; Ning et al., 2020). Elucidating and accounting for these complex underlying mechanisms in generalized models can enable predictions of soil CH_4 uptake (Borken et al., 2003; Dijkstra et al., 2013; Dondini et al., 2016). Both moisture and temperature simultaneously influence gas exchange, permeability and diffusion in soil profiles, thus regulating CH_4 oxidation to CO_2 (Philippot et al., 2008; Díaz et al., 2018; Welch et al., 2019). Methanotroph activity is also stimulated by soil temperature (Dalal et al., 2008**;** Li et al., 2019) and to some extent by moisture (Dijkstra et al., 2013; Chai et al., 2020b). As perennial grain cropping could differentially influence both soil moisture and temperature when compared to annual cropping, deciphering such mediating effects on the CH_4 sink can aid in developing and parameterizing empirical models for CH_4 uptake across a broad range of management systems.

This study was conducted: (i) to examine how perennial grain cropping influences CH_4 fluxes relative to contrasting cropping systems including annual grain cropping and perennial forage, (ii) to identify relationships between surface CH_4 fluxes, root mass, moisture and temperature at multiple soil depths and across contrasting cropping systems, and (iii) to establish combined models of temperature and moisture to predict CH_4 uptake via empirical equations. To address these objectives, we assessed the aforementioned cropping systems in two field sites that had different long-term land use histories: perennial forage versus annual cropping. We hypothesize that perennial grain cropping will enhance the soil CH_4 sink, with crucial ramifications for CH_4 budgets and negative feedbacks to climate change due to potential impacts from land use change in temperate agroecosystems.

1.3 Materials and Methods

1.3.1. Study site

The experimental sites were located in Edmonton (53°29'42"N, 113°32'1"W) and approximately 100 km to the southwest in Breton (53°05'16.4", 114°26'32"W). Both sites are located in Alberta, Canada. The Edmonton site is characterized by gently undulating topography, whereas the Breton site is a rolling landscape with moderate relief. The Breton site has approximately 25 years of land use history as perennial forage for hay [alfalfa (*Medicago sativa* L.) and bromegrass (*Bromus spp.*)], whereas the Edmonton site has been continuously annually cropped to barley (*Hordeum vulgare* L.) silage for at least three decades.

1.3.2. Experimental design

A randomized complete block design with four replications was established at each study site. The full description of the experimental treatments is available in Table 1.2. Five contrasting cropping systems were applied: perennial-forage, perennial-grain, fall-grain, spring-grain and fallow, creating a broad range of divergent biophysical conditions. Spring-grain represents a typical annual grain cropping system, with active growth periods in the Canadian prairies from mid-May to mid Sept. In addition to cropping system treatments, N fertilize effects were examined simultaneously. Therefore, each of the assessed cropping systems included experimental plots both with and without N fertilization, except for the fallow treatment. In total there were nine treatments and 36 experimental plots at each study site. The N fertilizer source was a granular blend of urea and polymer-coated urea (environmentally smart nitrogen, ESN™) in a ratio of 2:1. The N fertilizer application rate was 56 Kg N ha^{-1}, which was broadcast on all 16 N-receiving plots at each study site on the seeding date of spring-grain (Table 1.2). The plot dimensions were 4 × 8 m. The field activities in the study represent typical agricultural management practices for the area (Table 1.2). Provided that the land use histories of the Breton and Edmonton sites were contrasting as noted above, it is presumed that within the context of this study the perennial-forage and spring-grain treatments resembled the continuations of the previous land uses in Breton (perennial) and Edmonton (annual), respectively.

1.3.3. Soil properties and weather data

Soil total carbon (C) and total nitrogen (N) analysis was completed via dry combustion in an Elemental Analyzer (*Thermo Fisher Scientific, Delft, Netherlands*). Soil pH and EC was measured using a 1:2 soil:water slurry with a Fisher AR20 pH/EC Meter (*Fisher Scientific, Pittsburg, USA*). Bulk density was determined using the core method (Hernandez-Ramirez et al., 2011; Soil Survey Laboratory Staff, 1996) and soil texture was determined via the hydrometer method (Gee and Or, 2002). Soil classification and initial soil properties at the surface layer were contrasting between the two sites (Table 1.1). Prior to these analyses, soil samples have been collected in three composites per each plot, which were thereafter bulked for homogeneity.

A set of 5TM soil sensors (*Meter, Washington, USA*) connected to an automated EM50 data logger (*Meter, Washington, USA*) were deployed in each block replicate. The soil sensors were installed at 7.5 cm, 22.5 cm and 40 cm soil depths to continuously measure and record hourly soil temperature (°C) and volumetric soil moisture (% v/v). As a common metric of moisture and aeration in soils that incorporates bulk density data and soil volumetric water content (Lin and Hernandez-Ramirez, 2020), we calculated water-filled pore space (WFPS) as follows:

$$\mathrm{WFPS} = \frac{\mathrm{VWC}}{\mathrm{f}} \qquad [1]$$

where WFPS% is water-filled pore space (%), VWC is volumetric water content (cm^3 H_2O cm^{-3} soil) and f is the total porosity (cm^3 cm^{-3}). Soil porosities were calculated from bulk density data (Supplementary Table S1.1) collected at multiple soil depths in June 2018, assuming a particle density of 2.65 g cm^{-3}.

The air temperature, ambient pressure and precipitation were recorded by a permanent on-site weather station at each site within 1 km of the plots (ACIS, 2020). Air temperature and pressure measurements were taken 2 m above ground surface. Breton is typically wetter than Edmonton by 110 mm yr^{-1} and slightly colder (ACIS, 2020) (Table 1.1).

1.3.4. CH_4 flux measurements

Fluxes were measured using a non-flow-through, non-steady-state chamber method (static, closed) (Lin *et al.*, 2017; Chai *et al.*, 2020a). One PVC chamber base (15 cm wide × 65 cm length × 17 cm height) was installed using a chisel and mallet into soil to a depth of 5 cm, perpendicular to the crop rows in each experimental plot. Once installed, the chamber bases remained in the field for successive flux measurements and only removed for seeding and harvest activities. At least one flux measurement event was conducted every week during the growing season from May to Sept. in 2018 and from April to Sept. in 2019, for totals of 52 and 54 flux measurement dates in Edmonton and Breton, respectively. Measurement frequency was increased to twice-weekly during spring-thaw, following major rainfalls and fertilizer application. The chamber headspace was sealed using an opaque detachable lid outfitted with a rubber septa. Three 20 mL air samples were taken at 20, 40 and 60 min after chamber closure through a septum port using a needle and injected into 12 mL pre-evacuated vials. To represent time zero, three ambient gas samples were collected at the beginning, middle and end of each flux measurement campaign at 10 cm above the ground surface. Samples were obtained between 10 am and 2 pm on sampling days for consistency. When crop growth necessitated, biomass in the chamber headspace was partially clipped to maintain a leak-proof seal with the chamber lid.

The gas samples were analyzed within two weeks of field sampling for CH_4 concentration by a gas chromatograph (Thermo Fisher Scientific Trace 1310 Gas Chromatography) equipped with a flame ionization detector (FID) at 250 °C. The CH_4 fluxes were calculated by plotting a linear or exponential regression between measured CH_4 concentration verses time using a modified ideal gas law as follows:

$$F = \frac{SPV}{ART} \times 12.01\ g\ C\ mol^{-1} \qquad [2]$$

where flux (F) is gaseous rate of analyte (μg CH_4-C m^{-2} hr^{-1}); S is the slope of the linear regression or derivative at time zero of the quadratic regression; P is ambient pressure (P_a); V is headspace volume of each chamber (L); A is the surface area with the chamber (m^2); R is gas constant (P_a L K^{-1} mol^{-1}) and T is ambient air temperature (K). The multiplication by 12.01 g C mol^{-1} is a conversion to C mass of CH_4 flux. The sign convention followed in this study was positive fluxes correspond to CH_4 sink or uptake. Growing season cumulative fluxes were calculated by linear interpolation between consecutive flux measurement dates.

1.3.5. Root sampling and analysis

Roots were sampled in June 2019 from the soil profile by obtaining undisturbed soil cores from 0 to 60 cm depth (5.7 cm diameter, n= 4 per plot). Root separation from soil was done by wet sieving (>180 μm size) followed by oven drying at 60 °C for 48 hrs to quantify total dry root mass (Hernandez-Ramirez et al., 2014). Root C and N was analyzed via dry combustion in a Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Delft, Netherlands).

1.3.6. Data analysis

The temperature sensitivity of oxidation was examined by using non-linear regression models (nls) (Eq. [3]). An offset data transformation of +10 CH_4-C (g C ha^{-1} day^{-1}) was applied to all daily flux data to enable a subsequent log-transformation during derivation of Arrhenius equation parameters as follows:

$$F = -10 + e^{(lnA - \frac{Ea}{R} \times \frac{1}{273+T})} \qquad [3]$$

where F stands for flux (CH_4-C g ha^{-1} day^{-1}), A is a constant for each process velocity according to collision theory, E_a denotes the activation energy of the process (kJ mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} k^{-1}) and T is temperature (K).

Using average monthly soil moisture and temperature measured at 40 cm depth, Arrhenius-Q_{10} was combined with linear residual-WFPS as follows:

$$F = -10 + e^{\left(b + a \times \frac{1}{273.15+T}\right)} + \beta_0 + \beta_1 \times WFPS + \varepsilon \qquad [4]$$

where parameters a (a= $-E/R_t$) and b [b= Ln(A)] control the flux as derived by Arrhenius equation (Eq. [3]) using automated algorithms (Supplementary material: R Code for Q_{10} calculation) (Pinheiro and Bates, 2000), β_0 is the intercept and β_1 is the regression coefficient (slope) of the linear regression of unexplained flux residuals against WFPS (in percentage) and ε is the error term. For instance, the Edmonton flux data for May to Sept. 2018 and Apr. to Sept. 2019 was used to fit an Arrhenius-Q_{10} against soil temperature (Fig. 1.3A), then the residual fluxes were regressed against WFPS (Fig. 1.3B) and finally the two components were merged (Fig. 1.3C).

Treatment effects were analyzed using a linear mixed effect (LME) model. Normality and homoscedasticity of the model residuals were assessed using the Shapiro-Wilk and plot functions, respectively (NLME package of 3.2.1; R Core Team, 2015). Fixed factors for one-way analysis of variance (ANOVA) were (i) “cropping system” (perennial-forage, perennial-grain, fall-grain, spring-grain, fallow), (ii) “treatment combinations” [fallow, spring-grain (with and without N), fall-grain (with and without N), perennial-grain (with and without N), perennial-forage (with and without N)]. Two-way ANVOA was also used to determine “cropping systems” (perennial-forage, perennial-grain, fall-grain, spring-grain) and “nitrogen fertilizer” (with and without N) effects. Block was considered as a random factor in the LME model. Fluxes were aggregated and evaluated at various timescales: daily, average by month, growing-season cumulative and two growing-season cumulative (2018 and 2019). Tukey’s Honest Significant Difference (HSD) test was conducted for pairwise comparisons of treatment means.

1.4. Results

1.4.1. CH_4 fluxes as a function of cropping systems

Cropping systems affected CH_4 fluxes over time (Fig. 1.1, Table 1.3, Supplementary Fig. S1.1). Daily soil fluxes of CH_4 peaked on certain dates at each of the two study sites (Fig. 1.1). For instance, perennial-grain showed substantially increased CH_4 uptake at the Breton site in late May 2018. Notably, during the same period, soil moisture beneath the perennial-grain was much lower than all other cropping systems (Supplementary Fig. S1.2). Irrespective of cropping system, most daily fluxes indicated uptake of CH_4 taking place over the summer months (e.g., June to Aug), while only 14 % CH_4 release events were registered within the colder and wetter periods (i.e., April and the first half of May) (Supplementary Fig. S1.2, Supplementary Fig. S1.3).

Cumulative CH_4 fluxes over both growing seasons (2018 and 2019) revealed contrasting effects of cropping systems (Table 1.3). At the Edmonton site, both the perennial-grain and perennial-forage had significantly greater CH_4 uptake than the spring-grain and fallow ($P < 0.01$). Specifically, perennial-grain had a 67% stronger CH_4 two-growing season cumulative uptake than spring-grain (443.6 ± 70.3 vs. 265.1 ± 71.7 g CH_4-C ha^{-1}). Notably, the divergence in CH_4 uptake across cropping systems was increasingly discernable in the second year of the study (2019) ($P < 0.01$). Moreover, the two-growing season cumulative uptake of the fallow plots was 19% lower than spring-grain, whereas fall-grain showed intermediate CH_4 uptake between the spring-grain and both perennial systems. Contrary to these clear differences observed at the Edmonton site, the Breton site showed no major variations in the cumulative CH_4 uptake as a function of applied treatments ($P > 0.05$; Table 1.3). Additionally, we detected no consistent effects of N fertilizer addition on the CH_4 fluxes for either study site ($P > 0.05$; Supplementary Fig. S1.1, Supplementary Table S1.2). Overall, the two-growing season cumulative CH_4 sinks ranged from 216 to 444 g C ha^{-1} in Edmonton, while Breton experienced a greater sink between 506 and 601 g C ha^{-1} (Table 1.3).

1.4.2. Flux sensitivities to changes in temperature and soil moisture

Cropping systems influenced soil temperature and moisture at both sites (Ps < 0.01). While perennial-grain and perennial-forage showed the lowest two-growing season average temperature and moisture, the fallow treatment experienced the overall warmest, wettest conditions. Spring-grain was also significantly wetter than perennial-grain (Ps < 0.05; Supplementary Table S1.3, Supplementary Table S1.4, Supplementary Fig. S1.2, Supplementary Fig. S1.3).

On flux measurement dates, air temperature ranged widely from 1.8 to 32.6 °C in Edmonton and from 1.0 to 25.8 °C in Breton (Supplementary Table S1.3; Supplementary Table S1.4). Comparing the three soil profile depths (7.5, 22.5 and 40 cm), soil temperature and moisture at the 40 cm depth showed the steadiest and most stable conditions (Table 1.4).

The sensitivity of CH_4 fluxes measured at the soil surface to temperature was quantified using a Q_{10} Arrhenius relationship (Eq. [3]). When comparing daily air temperature and soil temperatures at 7.5 cm, 22.5 cm and 40 cm depths, the highest Q_{10} sensitivity of surface CH_4 fluxes occurred with soil temperature at 40 cm depth, irrespective of site. Additionally, goodness-of-fits and statistical significance were largely improved when using soil temperature at 40 cm depth as a flux predictor compared to the other soil depths (Table 1.4, Supplementary Fig. S1.4). For instance, when shifting from using air to 40 cm soil temperature, the perennial-grain in Breton showed increases in Q_{10} from 1.73 to an overall peak of 2.92 while R^2 also raised from 14 to 38%. Moreover, this Q_{10} for perennial-grain was significantly greater than other cropping systems ($P < 0.001$). Irrespective of site, perennial-grain exhibited a Q_{10} at least 24% larger than spring-grain.

Yearly cumulative fluxes responded to changes in soil moisture at depth (Fig. 1.2, Supplementary Fig. S1.5). This moisture effect was particularly pronounced at the Edmonton site. Notably, Edmonton soils overall were relatively drier than those in Breton, with average WFPS for all treatments in 2018 and 2019 being 24% and 24%, respectively, versus in Breton where average WFPS for 2018 and 2019 was 27% and 31%. When aggregating fluxes individually by growing season, the highest magnitude of cumulative CH_4 uptake was consistently found in the perennial-grain for both years (Fig. 1.2, means ± SE are available in Table 1.3). More importantly, the variation in cumulative CH_4 uptake across the range of assessed cropping systems was significantly related to average soil moisture (expressed as WFPS at the 40 cm depth) (R^2s of 90 and 64%; Fig. 1.2) for each growing season at the Edmonton site. While the two inverse regression equations had similar regression coefficients (slopes of -3.43 and -3.56 g CH_4-C ha^{-1} per % unit of WFPS), the difference between the two intercepts indicates the more substantial cumulative CH_4 uptake in the second study year as noted above – a net 81 g CH_4-C ha^{-1} higher in 2019 than 2018. In general, cumulative CH_4 uptake sharply increased with decreasing WFPS. Likewise, at a finer timescale, daily CH_4 fluxes also increased with decreasing WFPS at 40 cm soil depth in particular for perennial-grain and perennial-forage at the Edmonton site ($P<0.1$ and $P<0.05$; Supplementary Fig. S1.5).

1.4.3. Developing models by combining temperature and moisture to predict CH_4 fluxes

To capture the observed effects of temperature and moisture simultaneously in empirical equations, the nonlinear Arrhenius CH_4 flux-Q_{10} (Eq. [3]) was further supplemented with the prediction contribution of unexplained flux residuals linearly regressed against WFPS. With the aim of aggregating data heterogeneity while still representing both the overall flux seasonality and the influence across cropping systems, we used monthly averages of point measurements for model development (Supplementary Table S1.5). Hence, the developed models provide predictability at time resolution by month.

Enhancements in flux predictability from amalgamating Q_{10} sensitivity with moisture effects differed across study sites. Flux models emerging from the Edmonton dataset appeared to benefit from applying temperature and WFPS as simultaneous predictors (Table 1.5, Fig. 1.3). For instance, the contribution of including the WFPS-flux-residuals component into the model generated an improvement in goodness of fit (R^2) from 16 to 27% in Edmonton in 2019 (Table 1.5). It is noticeable that all β_1 regression coefficients were negative, indicating increased surface

CH_4 uptake with less wetter soils as noted above. The contribution to predictability by the WFPS-flux-residuals component was much lesser at the Breton site. As noted above, Breton showed more homogeneous, wetter conditions in terms of precipitation and WFPS, whereas the differences witnessed in Edmonton were wider and more consistent across cropping systems (Table 1.1; Supplementary Table S1.5). Overall, a parameterized model equation to predict CH_4 fluxes was postulated for the Edmonton site including both temperature and moisture simultaneously (R^2 of 27%; Fig. 1.3C), while for the Breton site, temperature alone was the predictor with a large goodness of fit (R^2 of 59%; Fig. 1.4). In general, increasing temperatures, which frequently coincided with decreasing moisture, drove larger CH_4 uptakes in all experimental plots and across all cropping systems (Fig. 1.3C).

1.4.4. Root mass across cropping systems

Root mass strongly differed across cropping systems as they are linked to distinct plant growth habits (Table 1.6). The overall largest root mass, C and N concentration are found with perennial-forage and the lowest with spring-grain. Notably, perennial-grain had double the root mass density than spring-grain at the 15-30 cm subsurface soil layer, which was consistent at both Breton and Edmonton ($Ps < 0.01$). Furthermore, perennial-grain had a root C concentration four times larger than spring-grain in the 30-60 cm depth increment in Edmonton ($P < 0.01$). At both the Edmonton and Breton sites, perennial-forage showed significantly greater root N density than the spring-grain from 0-60 cm ($Ps < 0.05$ and 0.01, respectively). Notably, perennial-grain root N density showed no significant differences from the perennial-forage at either site.

1.5. Discussion

1.5.1. Impacts of perennial grain cropping on the soil CH_4 sink

Several parallel mechanisms may explain the increased soil CH_4 sink in perennial grain cropping systems: (i) longer growing seasons, (ii) dense, deep rooting, and (iii) the absence of soil disturbance. While the active growth period observed in annual crops lasts from mid-May to mid-September, perennial grains experience growing seasons from April (following the onset of the spring thaw) throughout October (until the start of winter freezing conditions) over at least 2 full years and potentially more with continued breeding efforts (Table 1.2) (Cattani et al., 2019; Hayes et al., 2012). By using the full extent of the growing season to develop their canopy (Hebb et al., 2017; Ryan et al., 2018; Zhang et al.2018), perennials generate a more stable microclimate compared to their annual counterparts. Furthermore, although the fallow fields experienced the same boundary-layer weather conditions, perennial-grain and perennial-forage profoundly altered the microclimate in terms of surface soil temperature and moisture (Supplementary Table S1.3, Supplementary Table S1.4, Supplementary Fig. S1.2, Supplementary Fig. S1.3). A more stable microclimate beneath continual, dense perennial canopies can shelter and facilitate methanotroph activity (McDaniel et al., 2019). The exception to this assertion is the periods following harvest when grain and aboveground biomass are removed, which leads to desiccation of the exposed soil surface while reduced evapotranspiration simultaneously increases overall soil moisture (Table 1.2).

The extensive root system of perennial grains is able to uptake water from deeper layers within the soil profile, hence enhancing soil aeration when compared to annual grains. Indeed, we found much greater root growth by perennial grains than by annual grains in the subsurface soil layers at the two study sites (Table 1.6). In addition to water uptake, greater root mass improves soil pore volume (Hernandez-Ramirez et al., 2014), pore connectivity (Schwen et al., 2011), aggregation and structure (Hebb et al., 2017; Kiani et al., 2017; Díaz et al., 2018), collectively leading to enhanced gas exchange within soil profiles beneath perennial fields. Along with an increased C and N contribution from root mass by perennial grain cropping (Table 1.6), the associated increases in root exudation further fosters overall microbial activity and organic matter cycling (Philippot et al., 2009; Sprunger et al., 2019; Daly and Hernandez-Ramirez, 2020).

Surface CH_4 fluxes integrate consumption and production processes throughout the entire soil profile. In unsaturated soil profiles, the intensity of bacterial CH_4 oxidation reaches a peak at subsurface layers where soil conditions (temperature and moisture) are more consistent than in the topsoil (Meier et al., 2016; Li et al., 2019). In contrast, the soil surface experiences abrupt fluctuations in temperature and moisture. Thus, the transient nature of rewetting by precipitation and desiccation by evaporation creates an unstable environment for methanotrophs in the topsoil (Hernandez-Ramirez et al., 2014; Lin et al., 2017; Chai et al., 2020a). Furthermore, microbes at deeper soil layers experience gradually decreasing availability of dissolved organic C sources, which serve as electron donors and are instead required to oxidize atmospheric CH_4 (Li et al., 2019; McDaniel et al., 2019). Our results suggest that the 40 cm soil depth has the largest activity of CH_4 consumption as detected in the study (Table 1.4, Supplementary Fig. S1.4). Consistently at both study sites and across cropping systems, temperature and moisture measured at the 40 cm depth emerged as the best predictors for surface CH_4 fluxes (Table 1.4, Supplementary Fig. S1.4, Supplementary Fig. S1.5). Furthermore, our Q_{10} sensitivity results suggest that a “hot layer” (analogous to a spatial “hot spot”) of CH_4-monooxygenase enzyme activity manifested in the vicinity of 40 cm depth (Table 1.4). From these results, it remains uncertain whether the hottest active layer for CH_4 oxidation is located between 22.5 and 40 cm, or even deeper than 40 cm. Overall, our findings agree with Li et al. (2019) who found that the deepest soil layer in their incubation study (20-30 cm depth) had the highest Q_{10} for CH_4 oxidation regardless of land vegetation cover. In our study, an intense Q_{10}-CH_4 consumption and increased root growth at the subsurface soil layers beneath perennial-grain plots further elucidates the mechanisms by which perennial grain cropping systems can enlarge the CH_4 sink.

Perennial grain cropping systems reduce soil disturbance operations by their nature, whereas annual croplands can receive a varying intensity of tillage – one or even multiple times during a growing season. An absence of tillage increases CH_4 uptake by likely improving soil aggregation, C and N accruals and microbial diversity over time (Pawlowski et al., 2017). Cessation of mechanical cultivation further enhances soil structure and porosity (Powlson et al., 2011; Kiani et al., 2017; Díaz et al., 2018). Concomitantly, it is known that methanotroph activity is sensitive to soil disturbance (Meier et al., 2016; Chai et al., 2020b) and therefore perennial grains and perennial forages that inherently experience less disturbance result in beneficial gains in CH_4 sinks.

To our knowledge, this is the first study to quantify soil CH_4 uptake specifically addressing the influence of perennial grain cropping systems. Additionally, this study further unravels the underlying driving factors of CH_4 uptake, such as soil moisture and temperature, while examining the contrasting attributes and effects of perennial vs. annual grain cropping. Most existing literature has focused on CH_4 fluxes from bioenergy perennials (Don et al., 2012; Drewer et al., 2012; Gauder et al., 2012; Walter et al., 2015). However, bioenergy crops differ from perennial grain cropping in terms of N cycling. While bioenergy croplands experience higher N removals due to the harvest of the entire aboveground biomass for feedstock purposes, perennial grain cropping typically retains plant residues and associated N (Walter et al., 2015;

Kiani et al., 2017). Moreover, our study indicates the lack of an interaction effect of N fertilization and perennial cropping on the CH_4 sink. This is in contrast to numerous studies documenting the inhibitory effect of N fertilization on CH_4 uptake, largely due to ammonia competitively inhibiting CH_4 oxidation by methanotrophs (Jassal et al., 2011; Stiehl-Brain et al., 2011; Sun et al., 2016). However, our findings are in agreement with previous findings in similar N-rich environments, wherein no effect of N fertilization on CH_4 oxidation was ascertained (Hernandez-Ramirez et al., 2009; Smith et al., 2011). These contradictory findings indicate that no generalizations can yet be made with respect to the effect of N fertilizer on CH_4 flux, due to the interplay of several factors including microbial community composition and soil chemical properties and should be the subject of future studies (Bodelier et al., 2004).

The magnitudes of the fluxes captured in our study are in line with earlier literature, which reported flux magnitudes ranging from 8 to 360 g CH_4-C ha yr^{-1} (Hernandez-Ramirez et al., 2009; Smith et al., 2011; Don et al., 2012). Overall, results suggest that under certain conditions, perennial grain cropping systems can enhance the soil CH_4 sink relative to conventional annual cropping systems. As perennial cropping enables grain harvests (Powlson et al., 2011), they can signify a move towards a sustainable land use (Ryan et al., 2018; Duchene et al., 2019). Yet, additional studies can further contribute to the enhancement of agronomic management and productivity of perennial grain cropping.

1.5.2. The mediating role of soil moisture on surface CH_4 uptake

In addition to revealing the overarching effects of cropping systems on the soil CH_4 sink, results further document the mediating effects of soil moisture (expressed as WFPS). Across assessed cropping systems, WFPS, which represents the balance between water content and aeration, was a main driver in surface CH_4 fluxes (Fig. 1.2; Supplementary Fig. S1.5). WFPS encompasses soil aeration by including treatment specific bulk density values in its calculation (Supplementary Table S1.1).

The mediating effects of moisture at the Edmonton site were evident by witnessing the similar CH_4 sinks that occurred in perennial-grain and perennial-forage, as they both had relatively low WFPS at depth, although they are plant species with dissimilar phenologies. Likewise, the cumulative CH_4 sink of the fall-grain was intermediate between perennial-grain and spring-grain (Table 1.3); this can be understood as a reflection of the WFPS in fall-grain soils being also in-between in the gradient of these cropping systems ranking from fully perennial to annual growth habit. In fact, the spring-grain soil was much wetter than perennial-grain (with a typical magnitude difference of 19% WFPS), resulting in perennial grain cropping having an enhanced CH_4 sink compared to the annual grain crops. In the same way, increased moisture in annual cropping and fallow soils counteracted the potential stimulation of the CH_4 uptake due to increasing heat availability in the warmer summer months ($P < 0.01$; Supplementary Fig. S1.4, Supplementary Fig. S1.5). We hypothesize that the effect of moisture was more pronounced at the Edmonton site than the Breton site primarily due to differences in annual precipitation affecting WFPS at each site. This effect was particularly pronounced by precipitation patterns in 2019, wherein Breton experienced 36% more precipitation than Edmonton during the growing season (ACIS, 2020). Thus, the Edmonton site experienced more significant shifts in soil moisture relative to the Breton site, which experienced more constant soil moisture conditions regardless of cropping system. This is supported by previous research that has highlighted the comparatively increased importance of soil temperature, as opposed to moisture, on CH_4 oxidation in wetter soils (Christensen et al., 1993; Wu et al., 2010).

That soil moisture mediates CH_4 fluxes have been inferred in recent studies across contrasting biomes (Wanyama et al., 2019; Brachmann et al., 2020; Chai et al., 2020b). Based on regression trees, Brachmann et al. (2020) identified specific thresholds of CH_4 uptake vs. soil moisture changes and postulated that CH_4 uptake was strongest when soil moisture was below 0.21 water fraction by volume. Likewise, using quadratic equations, Chai et al. (2020b) found 10 –30% volumetric water content to be optimum moisture for CH_4 uptake, with reduced CH_4 uptake at higher soil moisture. Wanyama et al. (2019) used multiple regression to establish a highly significant ($p < 0.001$) relationship between WFPS and CH_4 uptake, whereby CH_4 uptake from all land use types studied decreased with increases in mean annual WFPS. Their analyses collectively revealed that the ideal conditions for CH_4 uptake reside in the intermediate moisture range, but the optima seem to be specific to different soils and ecosystems. Thus, our study encompassing unique agroecosystems contributes by further informing how CH_4 fluxes respond to moisture. Moisture increases beyond an optimum range diminish and even obstruct gas exchange and diffusion (Fender et al., 2011; Welch et al., 2019; Wu et al., 2020). In fact, moisture increases that approach soil saturation can gradually shift the net CH_4 flux at soil surface towards becoming a CH_4 source (Brachmann et al., 2020). On the other hand, excessive drying can cause methanotrophs to encounter physiological water stress and even desiccation (Van den Pol-Van Dasselaar et al., 1998). Both moisture extremes inhibit or even impede the CH_4 sink (Rong et al. 2015; Miller et al., 2019; Chai et al., 2020b).

1.5.3. Land use history considerations and CH_4 uptake

The two study sites had contrasting land use histories, enabling certain interpretations while comparing annual vs. perennial cropping. Not only did Breton have a 58% overall larger CH_4 sink than Edmonton, but also cropping systems did not differ in their CH_4 sinks in Breton as they strongly did in Edmonton (Table 1.3). These differential responses across sites to the same cropping systems can be attributable in part to their previous land uses. Breton had a long-term history of perennial forage cropping prior to the beginning of the experiment. As the perennial-forage treatment in our study was consistently one of the highest CH_4 sinks, we hypothesize that having a recent background of long-term perennial forage cropping is conducive to maintaining a high CH_4 sink, even two years following a conversion to annual cropping (e.g., annual-grain). We further interpret that the legacy effects of previous perennial forage cropping in Breton were sufficiently persistent to mask the potential effects of contrasting cropping systems on the CH_4 uptake (e.g., fallow = perennial-forage in Breton). On the other hand, the long-term land use history prior to initiating the experiment in Edmonton was annual cropping for several decades. Beginning with a likely low CH_4 uptake baseline, such a background of recurrent annual cropping (as in Edmonton) offered ample opportunities to enhance the soil CH_4 sink by transitioning to perennial-grain. Shifting annual croplands to perennial grain cropping manifested a consistently enlarged CH_4 sink over two consecutive growing seasons in our study. Further research in these novel cropping systems and associated land use conversions can focus on organic matter cycling, soil health responses, C and water-use efficiencies as well as nitrous oxide and CO_2 fluxes (Daly and Hernandez-Ramirez, 2020)

It is noticeable that the magnitude of cumulative CH_4 uptake in fallow treatment at the Breton were almost equivalent to that of perennial-grain in Edmonton, particularly during the second study year (Table 1.3, Supplementary Fig. S1.1). With the aim of deepening the current understanding of these responses, future assessment can focus on the genomics of methanotrophs by quantification of the functional gene involved in anaerobic CH_4 oxidization, *pmoA*, with changes in land use (Han and Gu, 2013). Potential responses of microbial community structure and mycorrhiza associations with perennial grain crop species remain also unknown.

1.5.4. Escalating climate change and feedbacks

As soils beneath perennial grain cropping consistently showed the highest temperature sensitivity – in terms of flux-Q_{10} response (Table 1.4, Supplementary Fig. S1.4), warming (gradually increasing heat availability) can lead to a beneficial feedback of an even greater CH_4 uptake (Luo et al., 2013, Shimizu et al., 2013, Zhang et al., 2018). Likewise, based on our models (Table 1.5, Fig. 1.3), slightly drier, longer growing seasons in comparable temperate regions can also be conducive to increases in the CH_4 sink. These predictions apply as long as the changing conditions still support plant growth (i.e., heat units and water supply) and hence, perennial grain cropping could beneficially buffer, mitigate and adapt to such ongoing effects. In addition to acclimation to warming and drying, high-enzymatic-affinity methanotrophs could be selected for in these soil profiles over time (McDaniel et al., 2019; Li et al., 2019).

Our empirical modelling for CH_4 fluxes effectively followed a similar approach as previously devised by Borken et al. (2003) for CO_2 fluxes, by combining Arrhenius-temperature-Q_{10} with moisture to account for flux residuals (Fig. 1.3). However, it needs be recognized that this approach does not account for hystereses of fluxes as a function of both thermal and moisture effects over daily and seasonal time courses (Hernandez-Ramirez et al., 2011, Kariyapperuma et al., 2018). Additionally, other biophysical factors such as soil texture, structure, oxygen profile and acidity (Wang et al., 2019, Reddy et al., 2019, Guo et al., 2020) need to be accounted for in process-based modelling. Overall, the field-derived Q_{10} data from our study encompasses a broad range of cropping systems and land use histories and thus contributes valuable input parameters for terrestrial modelling efforts elsewhere.

1.6. Conclusion

Our results substantiate that an enhancement in the CH_4 sink can be realized by converting long-term annual croplands into perennial grain cropping. However, the reversal does not apply; changing from a land use of perennial forage to annual cropping does not manifest a reduction in the CH_4 sink, at least within two years following transition. Furthermore, perennial grain cropping following long-term perennial forages sustains the same considerable CH_4 sink as in perennial forage systems. The mediating mechanisms by which perennial grain cropping enhances the CH_4 uptake are influenced by increased root growth that favorably shifts the aeration-moisture balance at the soil subsurface layers facilitating a CH_4 sink. Overall, the choice of cropping system and land use conversion has profound impacts on soil CH_4 uptake in temperate, upland landscapes.

1.7. References

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Tables

Table 1. 1. Soil classification and properties (0–15 cm depth increment) for Edmonton and Breton sites.

	Units	Edmonton	Breton
Classification		Black Chernozem	Orthic Gray Luvisol
TOC †	g C kg^{-1} soil	53.7±2.9	27.5±2.9
TN	g N kg^{-1} soil	4.67±0.29	2.21±0.18
Texture		Clay	Silty clay loam
Clay †	%	47.0	31.1
Silt	%	36.0	49.1
Sand	%	17.0	19.8
pH‡		7.2	5.7
EC‡	dS m^{-1}	1.5	0.83
Cumulative Precipitation	mm yr^{-1}	441.5§ [314.3]¶	551.4§ [388.8]¶
Air Temperature	ºC	3.15§ [12.36]¶	3.07§ [11.46]¶

† Soil total organic carbon (TOC) and total nitrogen (TN) were measured by dry combustion (Brachmann et al., 2020) for each block replicate; these are means of four replicates (±SE). Texture was measured by hydrometer (Hebb et al., 2017).

‡ 1:1 soil to solution. EC stands for electric conductivity.

§ These are long-term normals (Alberta Climate Information Service).

¶ Values inside the brackets correspond to the measurement period: April to September, 10-years average (2009–2019; Alberta Climate Information Service).

Table 1. 2. Description of cropping systems and field activities at both Edmonton and Breton.

Abbreviated ID	Description	Seeding Dates		Harvest Dates	
		Edmonton	Breton	Edmonton	Breton
Fallow	Continuous fallow using Roundup® and D ester herbicide since June 2017				
Spring-grain	Spring rye cropping system for grain production. *Secale cereale* L. cv. Gazelle. Tillage was used for crop establishment every year in the mid spring. Annual rye or summer rye are alternative designations in the literature.	22 May 2018; 23 May 2019	23 May 2018; 21 May 2019	19 Sep 2018; 25 Sep 2019	19 Sep 2018; 24 Sep 2019
Fall-grain	Fall rye cropping system for grain production. *Secale cereale* L. cv. Hazlett. Tillage was used for crop establishment every year in the late summer. Winter rye or biennial rye or are alternative designations in the literature.	29 Aug 2017; 3 Sep 2018	30 Aug 2017; 03 Sep 2018	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 24 Sep 2019
Perennial-grain	Perennial rye cropping system for grain production. *Secale cereale* L. × *S. montanum* Guss cv. ACE-1. Tillage was used for crop establishment only in the late summer 2017.	29 Aug 2017	30 Aug 2017	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 16 Sep 2019
Perennial-forage	Perennial forage cropping system for hay production. Alfalfa *Medicago sativa* L. and bromegrass *Bromus spp*. Aboveground biomass is cut and carry two times a year for hay with livestock feeding purposes. Tillage was used for stand establishment only in the mid summer 2017.	16 June 2017	8 June 2017	26 June 2018; 31 Aug 2018; 16 July 2019; 17 Sep 2019	26 June 2018; 30 Aug 2018; 15 July 2019; 16 Sep 2019

Table 1. 3. Cumulative CH_4 uptake across cropping systems at Edmonton and Breton sites averaged over fertilizer treatments in 2018 and 2019 as well as cumulative of the two growing seasons (2018 plus 2019) (n= 4 ± SE).

	2018	2019	2018 & 2019 Cumulative
Cropping system	g CH_4-C ha^{-1}		
		Edmonton	
Fallow	76.3±49.0	139.4±28.2 a†	215.7±74.2 a†
Spring grain	93.1±60.0	172.0±19.8 a	265.1±71.7 a
Fall grain	146.6±45.2	230.3±37.3 ab	376.9±80.1 ab
Perennial grain	176.3±38.8	267.3±37.2 b	443.6±70.3 b
Perennial forage	166.7±34.8	271.1±30.7 b	437.8±58.1 b
Overall mean ± SE	137.9±20.8 a¶	224.5±16.0 a	362.5±28.0 a
Crop ANOVA *P*	*0.13*	*< 0.01*	*< 0.01*
		Breton	
Fallow	221.4±50.4	284.8±61.8	506.2±112.1
Spring grain	251.7±36.9	326.3±26.0	578.0±55.3
Fall grain	258.7±27.5	342.3±13.4	601.0±29.9
Perennial grain	266.4±35.7	328.9±23.4	595.3±51.6
Perennial forage	279.9±26.6	276.9±27.0	556.8±46.6
Overall mean ± SE	259.4±14.6 b	314.9±12.2 b	574.3±23.1b
Crop ANOVA *P*	*0.74*	*0.09*	*0.54*
Site ANOVA *P*	*<0.001*	*<0.001*	*<0.001*

† Pairwise crop comparisons based on Tukey HSD test after ANOVA.

¶ Pairwise site comparisons based on Tukey HSD test after ANOVA

Lowercase letters denote significant differences between treatments based upon post hoc analysis after one way ANOVA. The same letters indicate no significant difference within column, different letters indicate significant differences within column at $p < 0.05$.

Table 1. 4. Temperature sensitivities (Q_{10}) of daily CH_4 uptake flux by cropping systems at the Edmonton and Breton sites as based on Arrhenius (Eq.[3]).

	Q_{10}				R^2			
Crop	Air	7.5 cm	22.5 cm	40 cm	Air	7.5 cm	22.5 cm	40 cm
				Edmonton				
Fallow	1.07	1.27	1.44	1.49	0.00	0.01	0.04	0.05
Spring grain	1.16	1.38	1.48	1.52	0.00	0.02	0.03	0.04
Fall grain	1.07	1.14	1.44	1.50	0.00	0.00	0.02	0.04
Perennial grain	1.22	1.52	1.82	1.89	0.00	0.02	0.07	0.09
Perennial forage	1.31	1.69	1.97	2.07	0.01	0.04	0.10	0.12
				Breton				
Fallow	1.31	1.43	1.72	1.83	0.07	0.08	0.22	0.28
Spring grain	1.64	1.95	2.17	2.29	0.26	0.12	0.14	0.19
Fall grain	1.64	1.81	2.19	2.34	0.11	0.15	0.26	0.31
Perennial grain	1.73	2.08	2.78	2.92	0.14	0.21	0.36	0.38
Perennial forage	1.95	2.53	2.61	2.67	0.19	0.30	0.35	0.35

Table 1. 5. Soil temperature and combined temperature Q_{10}-moisture model parameters for CH4 uptake flux (averages by month) as a function of temperature and water filled pore space (WFPS as % at 40 cm soil depth).

	Eq.[3]				*Eq.[4]*				
Year	E_a	A	Q_{10}	$_{adj}R^2$	a†	b‡	β_0	β_1	$_{adj}R^2$
					Edmonton				
2018	4108.8	59.97	1.62	*0.03*	-494.17	4.09**	1.61***	-0.0407***	*0.44*
2019	3356.9	45.34	1.53	*0.16*	-403.74*	3.81***	0.58	-0.0147*	*0.27*
2018 and 2019	2896.4	36.72	1.44	*0.06*	-348.35*	3.60***	0.96***	-0.0244***	*0.27*
					Breton				
2018	12732.8	2406.53	3.17	*0.54*	-1531.40***	7.78***	1.36	-0.0282	*0.60*
2019	8949.1	509.23	2.52	*0.77*	-1076.32***	6.23***	0.13	-0.0025	*0.77*
2018 and 2019	9242.7	566.70	2.56	*0.59*	-1111.46***	6.33***	0.20	-0.0040	*0.59*

, ** and * are significance at alpha critical levels < 0.05, < 0.01 and < 0.001, respectively.*

† a = -E/R_t where R_t is 8.314 J mol^{-1} k^{-1}.

‡ b = Ln(A). This transformation enables the statistical testing of the linearity of b (H_0: b=0).

Table 1. 6. Root mass, C and N densities across cropping systems by soil depth increments (i.e., 0–15, 15–30, 30–60 and 0–60 cm) for the two field experimental sites in June 2019*.

	Root-mass				Root-C				Root-N			
	(mg root mass cm^{-3} soil)				(mg root C cm^{-3} soil)				(µg root N cm^{-3} soil)			
Cropping system	0-15 cm	15-30 cm	30-60 cm	0-60 cm	0-15 cm	15-30 cm	30-60 cm	0-60 cm	0-15 cm	15-30 cm	30-60 cm	0-60 cm
							Edmonton					
Spring grain	4.42±1.64	0.40±0.06 a†	0.25±0.05 a	5.08±1.62 a	1.52±0.59 a†	0.12±0.01 a	0.06±0.01a	1.71±0.59 a	52.40±10.77	6.26±1.14 a†	3.55±0.63 a	62.23±10.73 a
Fall grain	5.42±0.98	0.85±0.16 b	0.83±0.22 bc	7.1±1.11 ab	2.09±0.35 ab	0.27±0.06 a	0.23±0.04 b	2.59±0.37 ab	84.28±15.06	12.10±3.30 a	11.55±2.38 bc	107.94±15.47 ab
Perennial grain	7.48±3.13	0.79±0.07 b	0.68±0.13 ab	8.96±3.05 ab	3.01±1.34 ab	0.24±0.01 a	0.23±0.04 b	3.48±1.31 ab	105.18±39.48	8.58±0.54 a	9.57±2.21 ab	123.34±38.81 ab
Perennial forage	36.88±22.92	5.83±2.31 c	2.40±0.76 c	45.12±25.86 b	16.1±10.8 b	2.16±1.10 b	0.88±0.30 c	19.13±12.14 b	461.00±295.79	113.98±78.34 b	33.32±10.21 c	608.31±382.45 b
P-value	>0.05	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.05	>0.05	<0.01	<0.01	<0.05
							Breton					
Spring grain	6.85±2.06 a	1.05±0.20 a	0.70±0.13 a	8.61±2.08 a	1.97±0.41 a	0.30±0.05 a	0.13±0.01 a	2.40±0.44 a	69.59±18.18 a	7.40±1.17 a	4.02±0.73 a	81.02±18.28 a
Fall grain	15.11±3.83 ab	1.70±0.16 b	1.04±0.10 a	17.86±3.83 ab	5.00±1.45 ab	0.54±0.10 ab	0.23±0.04 a	5.77±1.45 ab	155.34±48.71 ab	14.60±3.29 b	6.85±1.33 a	176.80±48.48 a
Perennial grain	14.35±2.55 ab	2.12±0.37 b	0.79±0.12 a	17.27±2.92 ab	5.32±1.10 ab	0.65±0.15 b	0.23±0.03 a	6.20±1.24 ab	171.46±18.27 ab	17.35±4.24 b	6.29±0.62 a	195.11±21.92 ab
Perennial forage	39.55±17.06 b	7.07±1.16 c	2.25±0.26 b	48.87±17.59 b	14.5±6.28 b	2.31±0.49 c	0.67±0.06 b	17.51±6.53 b	501.37±176.75 b	62.11±10.98 c	16.48±2.03 b	579.96±179.64 b
P-value	<0.05	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

* The given data represents mean ± standard error (n=4),

† Pairwise comparisons based on Tukey HSD test after ANOVA.

Figures

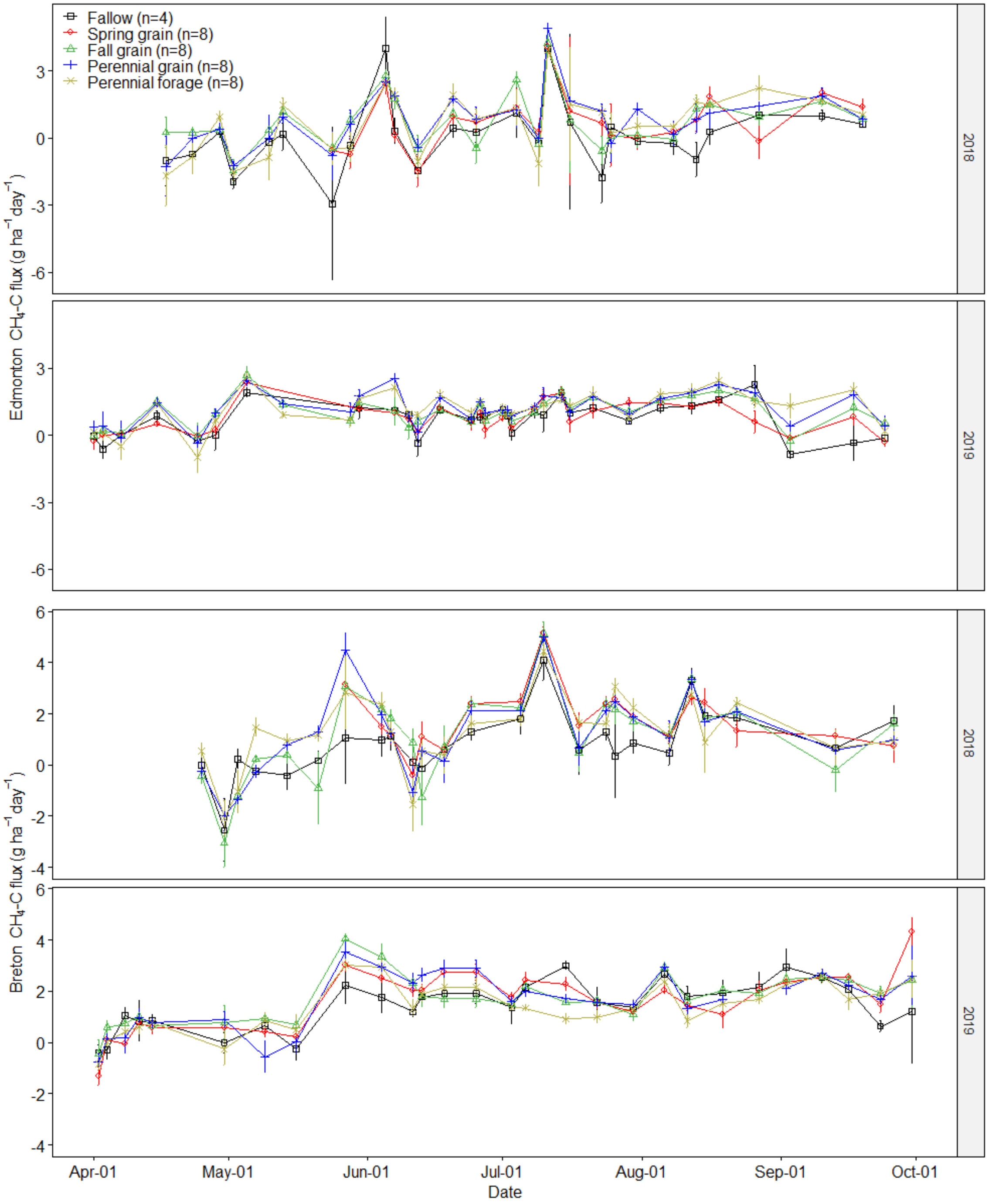

Fig. 1. 1. Daily CH_4 uptake fluxes across cropping systems at the Edmonton and Breton sites over two consecutive growing seasons. Positive fluxes indicate CH_4 uptake, error bars indicate standard error.

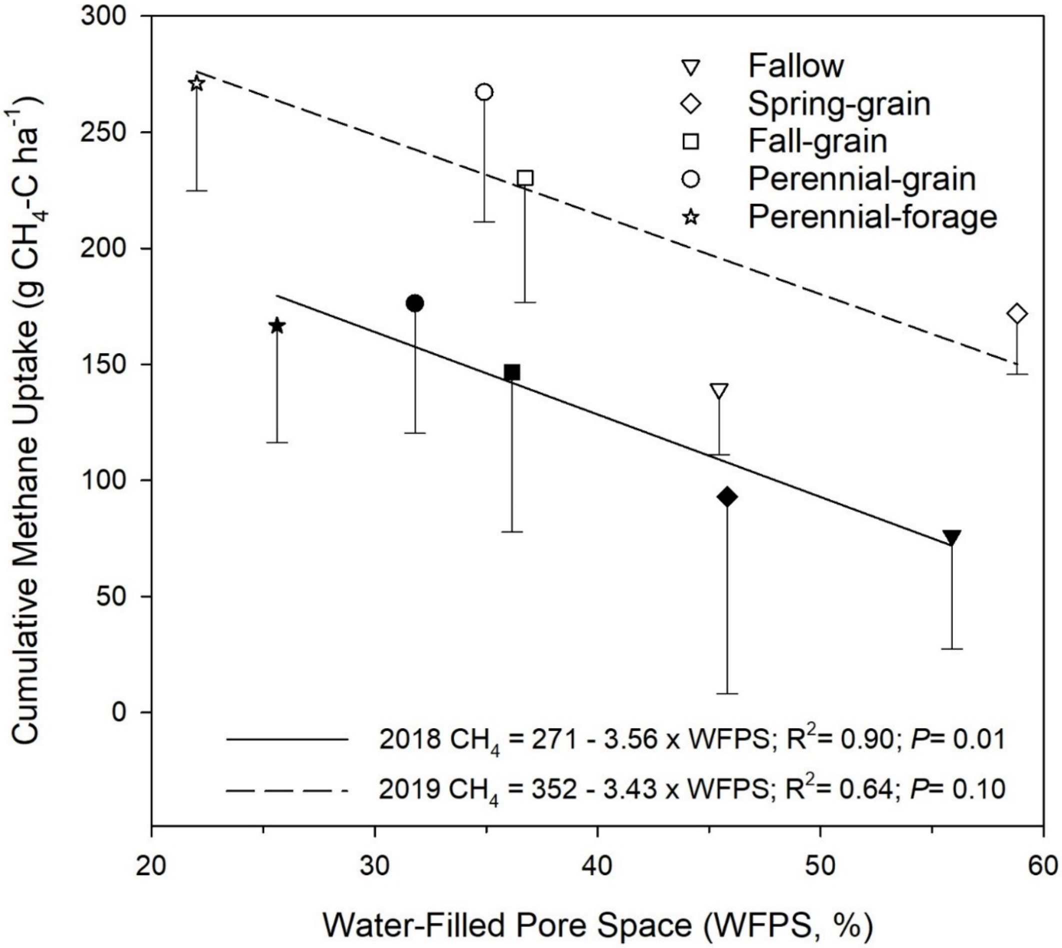

Fig. 1. 2. Annual cumulative CH_4 uptake at the soil surface as a function of soil moisture (water-filled pore space) across cropping systems in the Edmonton site in 2018 (closed symbols) and 2019 (open symbols). Circles are perennial-grain, stars are perennial-forage, squares are fall-grain, diamonds are spring-grain and triangles are fallow. For clarity, unidirectional error bars (as standard errors of the means) are shown for cumulative methane flux. Linear regression fittings are shown separately for 2018 and 2019. Soil moisture was derived by averaging hourly measurements at the 40 cm soil depth at the time of flux measurements (Supplementary Fig. S2).

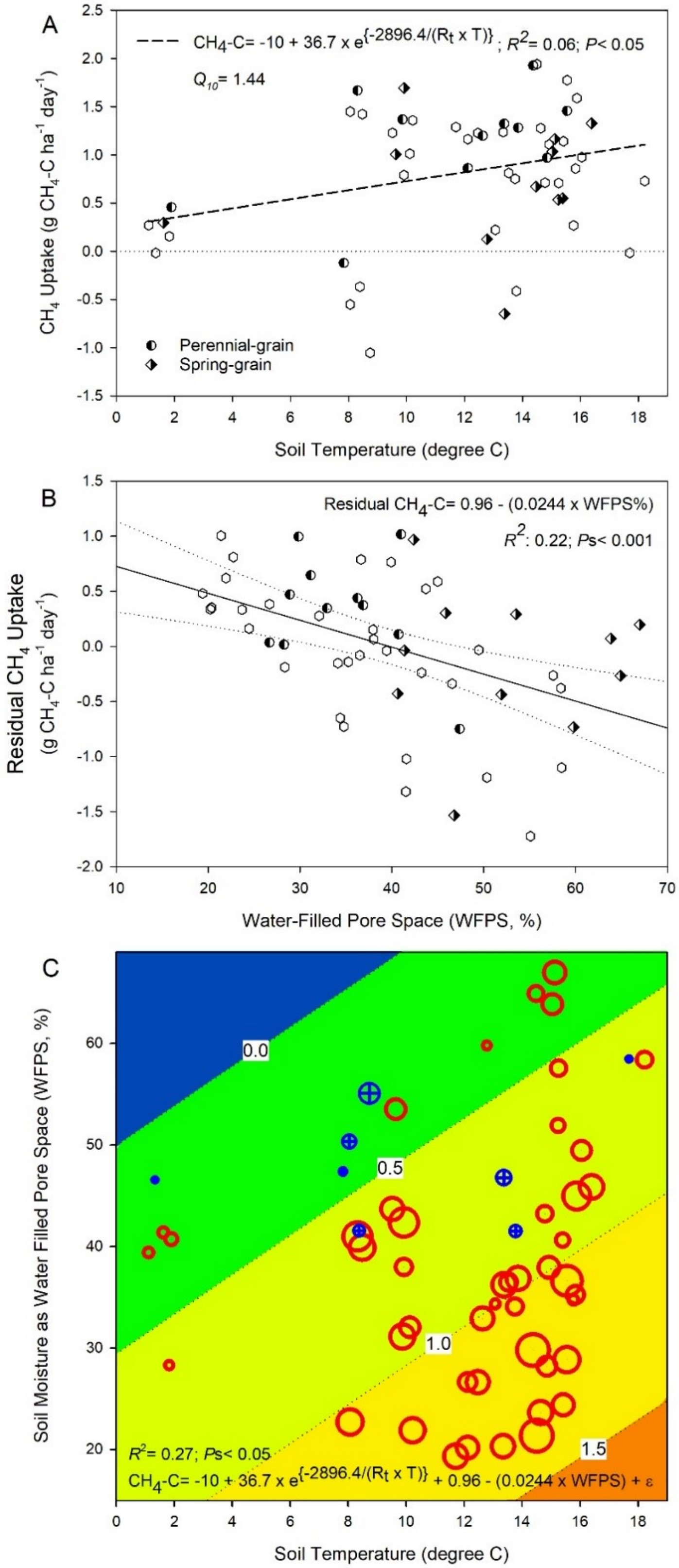

Fig. 1. 3. Daily CH$_4$ uptake flux at the soil surface as a function of (A) soil temperature, (B) soil moisture and (C) combined soil temperature and moisture at 40 cm soil depth across cropping systems at the Edmonton site. For comparison purposes in (A) and (B), data points from perennial-grain and spring-grain are highlighted with specific symbols as indicated in the legend. Shown flux data points are averages of the measurements within each month throughout the study (these averages by month are available in Supplementary Table S5). Soil temperatures were derived by averaging hourly measurements at the 40 cm soil depth at the time of flux measurements. (A) Arrhenius equation fitting (Eq. [3]) and Q_{10} for CH$_4$ uptake are provided. (B) Linear fitting of residual CH$_4$ uptake (after predicting CH$_4$ using Arrhenius equation in panel A) regressed against soil moisture. This CH$_4$ residual model informs the integration of temperature-Q_{10} and moisture into the combined model equation (as Eq. [4]) in panel C. (C) The combined Q_{10} and moisture model equation is provided and represented by the background color bands where the band boundaries are labelled as daily CH$_4$ fluxes (g CH$_4$-C ha^{-1} day^{-1}). Bubble radii are proportional to the CH$_4$ fluxes; empty bubbles are uptake and crossed bubbles are emission. Note that temperature for Arrhenius equation is in Kelvin. ε stands for model error.

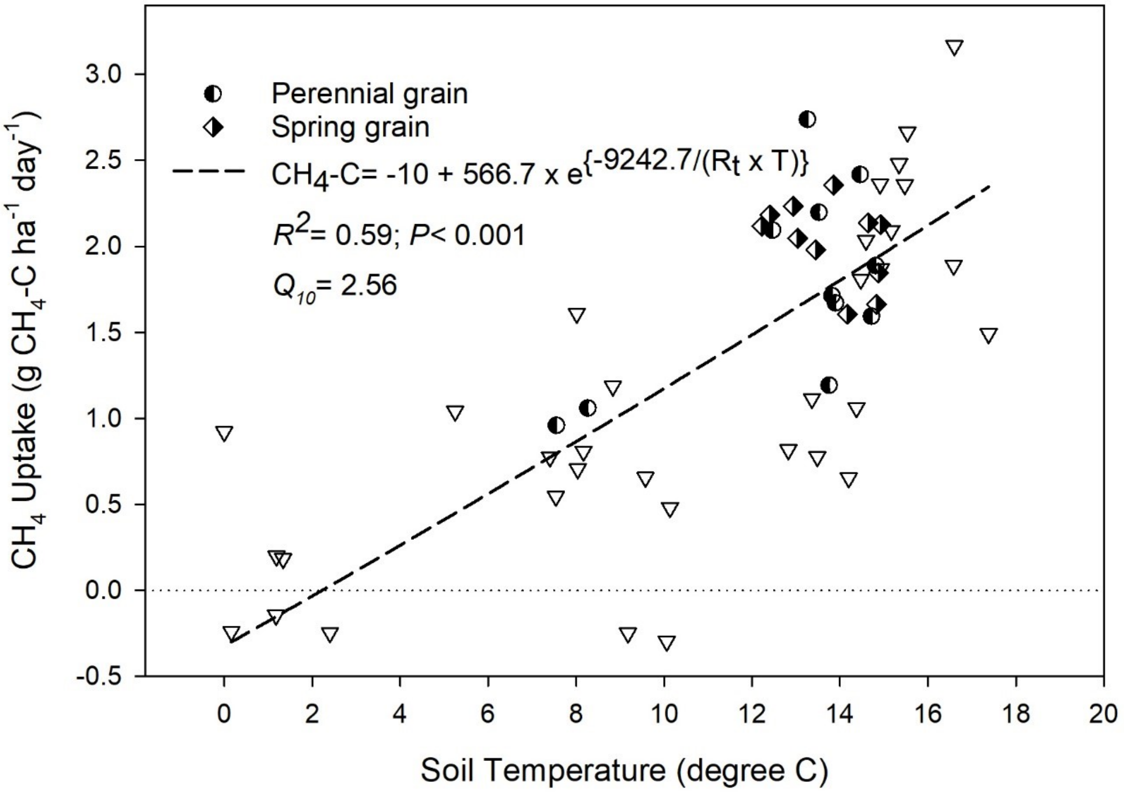

Fig. 1. 4. Daily CH_4 uptake flux at the soil surface as a function of soil temperature at 40 cm soil depth across cropping systems at the Breton site. For comparison purposes, data points from perennial-grain and spring-grain are highlighted with specific symbols as indicated in the legend. Shown flux data points are averages of the measurements within each month throughout the study (these averages by month are available in Supplementary Table S5). Soil temperatures were derived by averaging hourly measurements at the 40 cm soil depth at the time of flux measurements. Arrhenius equation fitting (as Eq. [3]) and Q_{10} for CH_4 uptake are provided.

CHAPTER 2. Soil Carbon Response to Perennial Grain Cropping and Nitrogen Fertilizer

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2.1. Abstract

Carbon sequestration in agricultural soils can play a pivotal role in the mitigation of accelerating climate change. Our research evaluated a continuum of agricultural cropping systems, including innovative perennial grain cropping, to assess which systems promote increases in and stabilization of soil organic matter (SOM). In comparison with conventional annual cropping systems, perennial grain cropping may increase C accrual resulting from the no-tillage management, longer growing seasons, and extensive root growth associated with these novel systems. Furthermore, the effects of N fertilizer addition on SOM dynamics under contrasting cropping systems was examined. We conducted physical SOM fractionation into particulate (POM) and mineral-associated organic matter (MAOM) in samples taken over two years from two experimental sites in Central Alberta, Canada. Five contrasting cropping systems [perennial-forage, perennial-grain, fall-grain, spring-grain, and fallow] both with and without N fertilizer were tested. Our findings demonstrate that perennial-grain cropping was consistently superior in sequestering SOM-C compared to annual-grain crops at the surface soil layer (0-15 cm depth, $Ps < 0.05$). Over the duration of this experiment, perennial-grain cropping considerably boosted C accumulation into the recalcitrant SOM pools as represented by increasing MAOM, particularly at the Edmonton site, which is characterized by a clay-rich, Black Chernozemic soil ($P < 0.05$). However, recurrent N fertilizer additions diminished C sequestration by perennial grain cropping in both POM and MAOM fractions ($Ps < 0.05$). Correlation analysis indicated that accrual and allocation of C within the soil profile was more closely related to aboveground plant biomass productivity as opposed to root growth, particularly generating more intermediate-labile POM. Our results shed light on how to achieve greater soil C sequestration as a function of cropping system options, N fertilizer addition, and underlying soil texture.

Keywords:

Particulate organic matter, mineral-associated organic matter, perennial grain cropping, soil C sequestration, N fertilizer, soil organic matter.

Highlights:

- Perennial grain crops increased C accrual into labile and stable soil organic matter pools.
- N fertilizer addition significantly attenuates C sequestration in perennial grain crops.
- Carbon in particulate organic matter in shallow layer was related to aboveground biomass C.

2.2. Introduction

Soil carbon sequestration is an invaluable strategy to mitigate escalating atmospheric CO_2 levels and associated global climate change (IPCC, 2014; Chendev et al., 2015; Hebb et al., 2017). Within agricultural systems, this is achievable by implementing farming practices that ultimately facilitate the stabilization of soil organic carbon (SOC), thus reducing anthropogenic greenhouse gases emissions into the atmosphere (Curtin et al., 2014; Kiani et al., 2017; Li et al., 2018). Sustainable agricultural management options that replenish SOC stocks include reduction of tillage, continuous cropping, and inclusion of perennial species in crop rotations (Bhattacharyya et al., 2013; Lin et al., 2020; Thilakarathna and Hernandez-Ramirez, 2021). Additional benefits of these improved management practices include, but are not limited to, improved soil structure, soil fertility, and soil water-holding capacity (Kiani et al., 2020; Poffenbarger et al., 2020; Guenette and Hernandez-Ramirez, 2018; Smith et al., 2020).

Emerging perennial grain crops such as rye, wheat and rice, is a promising approach that have the potential to promote multifunctionality and ecosystem services across agricultural landscapes (DeHaan and Ismail, 2017; Ryan et al., 2018). Compared to conventional annual cropping systems, perennial grain crops are characterized by distinct growth habits and field management practices (Ryan et al., 2018, Sprunger et al., 2019), which may be conducive to increased SOM accrual (Hebb et al., 2017; Kiani et al., 2017; Poffenbarger et al., 2020). More specifically, perennial grain cropping systems entail no-tillage management, longer growing seasons, and extensive roots that collectively may favor C accumulation in soil profile (Crews and Rumsey, 2017, Kiani et al., 2017; Kan et al., 2020, Mary et al., 2020). Previous studies that focused on an intermediate perennial plant as dual-use crop or unequal cultivar comparison (i.e., cereal vs. forage), have shown inconsistent effects of cropping system options on C accrual, likely because of interacting effects of numerous factors such as local climate, soil type, topography, agricultural management, legacy effects, and soil C saturation (Sprunger et al., 2018a; Sprunger et al., 2018b; Cates et al., 2019). However, our research has been conducted by involving novel perennial rye grain crops, the identical rye grain cultivar to annual grain crop counterparts. Additionally, there is a lack of information on how soil organic matter (SOM) accretion changes over time (i.e., inter-annual variation and seasonal dynamics) across contrasting cropping systems, which this study aims to quantify by assessing SOM dynamics during different crop growth stages over multiple growing seasons (Lin et al, 2020).

Nitrogen (N) fertilizer application in croplands is a common practice of critical economic importance to boost primary productivity (Guenette et al., 2019). However, adding N to soils can strongly influence SOM dynamics (Thilakarathna and Hernandez-Ramirez, 2021). Two parallel hypotheses can be postulated: (i) increased net primary productivity caused by N fertilizer application may directly contribute biomass-derived C into SOC stocks, or (ii) narrower C:N ratios in the soil as a result of N fertilizer addition may accelerate SOM mineralization rate (Liu et al., 2017; Daly and Hernandez-Ramirez, 2020). The latter hypothesis is also referred as priming of SOM, which is triggered by stoichiometric microbial decomposition, whereby N additions satisfy the nutritional prerequisites of soil microbes which are then able to decompose and mineralize recently-added plant residues or preexisting SOM (Roman-Perez and Hernandez-Ramirez, 2021). Overall, N input may have varying effects on SOM dynamics (Chen et al., 2014; King and Blesh, 2018). Moreover, in the case of perennial-grain crops, N fertilizer additions could interact with their abundant belowground biomass production (Liu et al., 2017; Kim et al., 2021). Hence, perennial cropping systems might actually rely less on exogenous nutrient additions compared with annual crops likely due to their pervasive root systems (King and Blesh, 2018). Such interactive effects between N fertilizer application and contrasting cropping systems suggest divergent responses of SOM dynamics to N fertilizer in different agroecosystems. Therefore, identifying best management of N fertilizer is pivotal to enhancing SOC storage, and hence, there is a need to investigate how N fertilization impacts SOM accrual in annual vs. perennial grain cropping systems (Silveira et al., 2013).

SOM comprises a highly heterogeneous continuum of decomposing organic compounds, which represents their ongoing decay as mediated by microbial activity ranging from recently-added plant residues to stable humus forms (Curtin et al., 2014; Kantola et al., 2017; Kiani et al., 2017). To begin understanding SOM dynamics, there is a need to assess the more rapidly cycled pools of SOM across a range of contrasting cropping systems (Hernandez-Ramirez et al., 2009). This can enable early detection of the direction of change and functionality of relevant young SOM fractions as altered by management practices (Samson et al., 2020b). One such fraction, particulate organic matter (POM; 53 μm< particle size < 2mm), is termed an intermediate decomposable pool (i.e., a transitory pool in the continuum decay of SOM) (Li et al., 2018). POM consists of recent crop residues, added manure and microbial residues, and can be considered a sensitive indicator of recent alterations in cropping practices and their effects on SOM dynamics (Cates et al., 2016; Smith et al., 2020; Lin et al., 2020; Kooch and Noghre, 2020). Hence, examining C and N concentrations and distributions in the POM fraction is an effective method to understand C sequestration mechanisms across contrasting cropping systems, as opposed to simply measuring total organic carbon (TOC) and total nitrogen (TN) (Franzluebbers and Stuedemann, 2008). In contrast, mineral-associated organic matter (MAOM) represents a relatively recalcitrant, stable SOM pool against the more labile, decomposable SOM. Stable MAOM pools enable to persist SOM protection for much longer than POM pools

(Lavallee et al., 2020). Such fractionation of SOM can further reveal insights about the sources and stability of SOC. Moreover, the C:N ratios of the separated SOM pools can in part indicate the extent of microbial decomposition (Poffenbarger et al., 2020).

Carbon input into soil primarily depends on plant biomass production both above and belowground (Hernandez-Ramirez et al., 2011; Mary et al., 2020). Our study addresses the allocation of SOM into the labile POM fraction versus stable MAOM pools across contrasting cropping systems, which differ in their above- and below-ground biomass production and tillage practices. Our study objectives were to: (i) quantify soil C and N pools at different depths across contrasting cropping systems including a perennial grain crop, (ii) characterize the impacts of N fertilizer on soil C transitory (POM) and stable (MAOM) pools, and (iii) investigate temporal variations in SOM over two consecutive growing seasons in two contrasting soils.

2.3. Materials and Methods

2.3.1. Study site

The experimental sites were located in Edmonton (53°29'42" N, 113°32'1" W) and Breton (53°05'16.4" N, 114°26'32" W), Alberta, Canada. Soil classification and initial soil properties at the surface layer were contrasting between the two sites (Table 2.1). Notably, soils at the Edmonton site were richer in organic matter with roughly double the carbon and nitrogen concentrations and also greater clay content by 16% compared with soils at the Breton site. Breton is typically wetter than Edmonton by 110 mm yr^{-1} and slightly colder (Table 2.1) (ACIS, 2020). The Edmonton site is characterized by gently undulating topography, whereas the Breton site is a rolling landscape with moderate relief.

2.3.2. Experimental Design

A randomized complete block design with four replications was established at each study site. Five contrasting cropping systems were applied: perennial-forage, perennial-grain, fall-grain, spring-grain and continuous fallow (Table 2.2), creating a broad range of divergent biophysical conditions. Spring-grain represents a typical annual grain cropping system, with active growth periods in the Canadian prairies from mid-May to mid Sept. Except for fallow, each of the assessed cropping systems included experimental plots both with and without N fertilization. In total there were nine treatments and 36 experimental plots at each study site. The N fertilizer source was a granular blend of urea and polymer-coated urea (environmentally smart nitrogen, ESN™) with a ratio of 2:1. The N fertilizer application rate was 56 Kg N ha^{-1}, which was broadcast on all 16 N-receiving plots at each study site on the seeding date of spring-grain (Table 2.2). The plot dimensions were 4 m × 8 m. Field activities represented typical agricultural management practices (Table 2.2).

2.3.3. Soil sample collection and preparation

Field sampling campaigns were conducted to examine inter-annual and season dynamics of SOM pools at both Edmonton and Breton sites. Soil samplings were conducted on 27 Sep 2018 shortly following harvest for both sites, 25 June 2019 and 26 June 2019 approximately coinciding with crop anthesis, and 1 Oct 2019 and 30 Sep 2019, shortly after harvest for Edmonton and Breton, respectively. A total of 5 soil cores were collected from each plot using a handheld auger from two depth increments (i.e., 0 - 7.5 cm and 7.5 - 15 cm). The two depths of field moist soil samples were homogenized, resulting in one composite sample per plot for each depth, then passed through a sieve of 8 mm mesh in the lab. Samples were then stored at 4°C prior to further processing.

2.3.4. Particulate organic matter (POM) fractionation

The POM size in our study was defined by a physical size fractionation between 0.053 mm and 2 mm in size diameter (Hernandez-Ramirez et al., 2009; Li et al., 2018). Physical fractionation was implemented in the lab using field moist soil samples to preserve and capture the original integrity of SOM. To account for soil moisture, water contents were first measured using 10 g soil sub-samples, which were oven dried at 60 ºC for 24 hours. Field moist soil equivalents were then corrected to an oven-dry of mass 25 g. These moisture-corrected soils were transferred into 125 mL plastic bottles with ten glass beads each of 5 mm in diameter, and distilled water was added until the bottle had a total water content of 90 mL. Prepared bottles were then placed on a horizontal reciprocal shaker at 180 rpm for 16 hours. The resultant soil suspension was passed through 2 mm and 0.053 mm sieves in size diameter. The pebbles and large plant residues retained on the 2 mm sieve were transferred into a beaker after removal of the glass beads. Likewise, the material retained on the 0.053 mm sieve was transferred into another beaker; i.e., this is the 0.052 to 2 mm POM size fraction. POM was oven-dried at 60 °C for at least 72 hours prior to being weighed. Finally, the material was ground using a ball-mill and stored for C and N analysis (Pansu and Gautheyrou, 2006).

2.3.5. Aboveground and root biomass measurements

Aboveground biomass was taken by hand harvesting with a 50 cm × 50 cm quadrat at two locations within each replicated plot prior to harvest over consecutive two growing seasons. Three subsamples were collected to represent each plot using the quadrat. Root sampling was completed near the time of crop anthesis in mid-July 2018 and 2019. Undisturbed soil cores (5.7 cm diameter, n=4 for each plot) were taken from 0 to 60 cm depth. Root separation of 0-15, 15-30 and 30-60 cm depth increments was conducted by wet sieving (>180 µm size) (Hernandez-

Ramirez et al., 2014). Both aboveground and root biomass were oven dried at 60 °C for 48 hours to measure dry biomass weight. Subsamples were then ground using a ball-mill and encapsulated in tin capsules.

2.3.6. Soil and biomass C and N analyses

Soil (i.e., whole soils and POM fraction) and biomass (i.e., aboveground and root biomass) C and N concentration were measured by dry combustion (Brachmann et al., 2020) using an Elemental Analyzer (EA; Carlo Erba NA 2100, Carlo Erba Strumentazione, Milan, Italy). The N concentration in aboveground biomass was determined using a POSS DS2500 (*Foss Analytics, Denmark*) near infrared spectroscope (NIR) with NIR calibration curve obtained by EA analysis. Stable MAOM pool was calculated by simply subtracting the POM pool from measured total SOM. Fractions of POM-C and -N as well as MAOM-C and -N were presented on the basis of concentrations as well as proportions of the total SOM-N and -C, respectively.

2.3.7. Statistical analyses

Analyses of variance (ANOVA) were conducted using linear mixed effects models (LME) in R (NLME package of 3.2.1; R Core Team, 2015). Covariance structure was corrected by including a blocking factor as a random effect. Two-way ANOVA was used to test the effects of cropping system type, N fertilizer addition, and their interaction [Cropping systems (spring-grain, fall-grain, perennial-grain, perennial-forage) x Fertilizer (with N, without N)]. One-way ANOVA was used for cropping system effect comparison (fallow, spring-grain, fall-grain, perennial-grain, perennial-forage). The normality and homogeneity of the model residuals were evaluated by the Shapiro-Wilk's test and plot function. Box-Cox transformations were implemented to correct non-normality or heteroscedasticity when needed. Pairwise comparisons were run after significant ANOVA with Tukey's Honest Significant Difference (HSD) using the agricolae package in R (Mendiburu and Felipe de, 2020).

Repeated measures analysis was used to assess consecutive soil samplings with a LME model. Heterogeneous variances and correlation of model residuals were accounted for using a set of predefined structure options as follows: compound symmetry, first order auto-regressive correlation structure (AR1), second order auto-regressive correlation structure (AR2), power variance structure, and exponential variance structure. After accounting for lack of independence and heteroscedasticity, the models were re-assessed by the Shapiro-Wilk's test and plot function. The best model was chosen for ANOVA based on its Akaike information criterion (AIC), parsimony and simplicity (Pinheiro and Bastes, 2000).

Spearman correlation was used to explore and interpret the association across all variables.

2.3. Results

2.3.1. Soil total organic carbon and total nitrogen

Soil TOC and TN were significantly affected by cropping system type at the Breton site from 0 – 15 cm over two growing seasons. Based on postharvest soil sample collections, the overall ranking followed: perennial-forage> perennial-grain> spring-grain> fall-grain> fallow (*Ps* <0.05, Table 2.3). Moreover, these differences across cropping systems were more evident in the topsoil 0-7.5 cm depth (hereafter referred to as 'topsoil') increment than in the subsurface soil layer at 7.5-15 cm depth (hereafter referred to as 'subsurface') (*Ps* < 0.05, Supplementary Table S2.1, Supplementary Table S2.3). Interestingly, TOC, TN and the C:N ratio in the topsoil showed a significant interactive effect between crop and N fertilizer post-harvest in the second growing season (*Ps*$<$*0.05*, Supplementary Table S2.3). Soils receiving no N fertilizer showed increases in SOM pools, specifically in the perennial-grain and perennial-forage (*P*<0.01, Fig. 2.4).

Contrary to Breton, there was no significant difference in soil TOC, TN, and C:N ratio between cropping systems in the Edmonton site when averaged over the 0-15 cm depth (*Ps* >0.05, Table 2.4). However, the perennial-grain tended to have numerically higher soil TOC, TN, and C:N ratio than spring-grain in Edmonton (Supplementary Table S2.4, Supplementary Table S2.5, Supplementary Table S2.6). Interestingly, the highest TOC and TN in soils under the perennial-grain cropping system were found in the subsurface soil after harvest in the second growing season (*P* < 0.05, Fig. 2.5, Supplementary Table S2.6).

Least squared means of soil TOC for Breton and Edmonton from 0-15 cm ranged from 25.3 to 32.7 g C kg^{-1} soil, and from 57.6 to 62.6 g C kg-1 soil, respectively. Overall soil TOC and TN concentrations in Edmonton were about twofold higher than those in Breton. Perennial-grain cropping showed steadily increasing soil TOC and TN from 0-15 cm over the duration of this experiment compared to spring-grain, although statistical significance was not observed (Table 2.3, Table 2.4).

2.3.2. Cropping Systems influence on POM and MAOM

Significant effects of cropping systems on POM-C and POM-N concentrations from 0-15 cm were found in Breton after the first harvest in Sept. 2018 (i.e., perennial forage > perennial grain > fall grain > spring grain > fallow) (Ps <0.05, Table 2.3). Furthermore, POM-C and POM-N gradually increased over time during the study in all treatments (Table 2.3). Specifically, these differences across cropping systems were evident in the topsoil instead of the subsurface soil layer (P<0.01, Fig. 2.1, Supplementary Table S2.1).

In the case of Edmonton, the interactive effect of crop and fertilizer was significant on both POM-C concentration and POM-C fraction, however only for the topsoil. Notably, perennial-grain cropping receiving no N fertilizer had 47% higher POM-C concentration and 40% higher POM-C fraction relative to perennial-grain amended with N fertilizer (*Ps* < 0.05, Fig. 2.2).

Even though significant differences in POM-C, -N concentration, and fraction in the topsoil vanished in the middle of the growing season (soil sample collection at anthesis on late June 2019), significance differences were evident for the stable MAOM pool. For example, MAOM-N concentrations in the subsurface soil in Breton were significantly decreased by N fertilizer addition (*P* < 0.05, Supplementary Table 2.2). Likewise, in Edmonton, MAOM-C and MAOM-N fraction under perennial-grain cropping were significantly greater than the other cropping systems (*Ps* < 0.05, Supplementary Table 2.5, Fig. 2.3).

After two growing seasons, overall significance of POM-C concentration and fraction across cropping system types disappeared at both sites when considering the entire soil depth increment from 0 to 15 cm (*Ps*>0.05, Table 2.3, Table 2.4). However, once these data were analyzed separated by the two soil layers (0-7.5 and 7.5-15 cm), POM-C concentration at the topsoil showed to be significantly affected by cropping system, in similar fashion as shortly after the harvest of the first growing season (i.e., spring-grain < perennial-grain) (*Ps* < 0.05, Supplementary Table S2.3). Furthermore, differential responses of POM and MAOM to the N fertilizer addition were also observed. There was a marginally significant effect of N fertilizer addition on POM-C within the perennial-forage (*P* = 0.051, Fig. 2.4). Significance of N fertilization on MAOM-C and MAOM-N concentrations was also found in perennial-grain and perennial-forage cropping systems at the Breton site (*Ps* < 0.05, Fig. 2.4, Supplementary Table S2.3). Although there was no significance across cropping systems and N fertilizer additions in Edmonton, it can be noted that POM-C and MAOM-C concentrations under perennial-grain cropping were numerically greater than that in spring-grain by 21 and 9 %, respectively ($P>0.05$, Supplementary Table S2.6).

Overall C:N ratio in POM was higher than that in MAOM (Table 2.3, Table 2.4).

2.3.3. Changes in stable SOM pool over time: spring-grain vs. perennial-grain

A significant interactive effect of crop × fertilizer × time was determined for topsoil SOM, each of these three separate factors (i.e., crop, fertilizer, and time) had no significant impacts ($P > 0.05$, Supplementary Table S2.7). Notably, MAOM-C in perennial-grain crops in subsurface soil layer were significantly greater than spring-grain, while at the same time, overall TOC in perennial-grain was also increased (*Ps*<0.05, Fig. 2.5, Supplementary Table S2.7). Additionally, TOC and MAOM-C in perennial-grain fields exhibited similar increasing pattern over time (*Ps*<0.05, Fig. 2.5, Supplementary Table S2.7).

2.3.4. Correlation analysis

As expected, N parameters showed a positive linear relationship with C variables (*Ps* < 0.05, Fig. 2.8). Interestingly, root-C:N ratio in Breton was positively correlated with the POM-C:N ratio, whereas the aboveground biomass C:N ratio at the Edmonton site was negatively correlated with the POM-C:N ratio ($\rho = 0.480$, $\rho = -0.587$, respectively, *Ps* < 0.01; Fig. 2.8, Supplementary Table S2.9, Supplementary Table S2.10). Furthermore, at the Edmonton site, the C concentration in aboveground biomass was more highly correlated with the POM-C concentration, as opposed to the organic C concentration in the whole soil ($P < 0.01$, $P < 0.05$, respectively; Fig.2.9; Supplementary Fig. S2.2.).

2.4. Discussion

2.4.1. Perennial-grain cropping as a means of SOM sequestration

Sequestration of SOC is the result of stabilization mechanisms in soils in conjunction with a positive imbalance between C inputs (e.g., plant photosynthesis) and C removals (e.g., biomass harvest, microbial respiration, root respiration, leaching, and erosion) over time (Kantola et al., 2017; King and Blesh, 2018; Lin et al., 2020). Our study suggests that perennial-grain cropping is a beneficial alternative to conventional annual-grain crops, based on their effective contributions to increased SOC sequestration in both the labile and stable pools (Fig. 2.1, Fig. 2.3, and Fig. 2.5). The initial C accrual effects caused by perennial-grain cropping were detected in the transitory SOM pool (i.e., POM-C) at the Breton site in the first year. Although we could not find significant differences between cropping systems in the POM-C concentration at the Edmonton site in the first year, the significant increases in MAOM-C concentration and fraction became apparent in the second year of crop growing phase (i.e., in late June 2019) (Fig. 2.3). Furthermore, the recalcitrant MAOM-C pool has shown significant increases in concentration on Edmonton site over the study period (Fig. 2.5). Thus, perennial-grain cropping has the direct potential to increase the amount of C in labile pools (POM), or indirectly enhance C allocation into the recalcitrant pool (MAOM). Considering that residence time of MAOM-C ranges from 10 up to greater than 100 years, it is clear that increased MAOM-C concentrations by perennial-grain cropping is a substantially beneficial for C sequestration purposes (Valdez et al., 2017). Moreover, the fact that SOM pools increased over time – in particular the MAOM pool in the subsurface soil layer in Edmonton (Fig. 2.5) – showed that the Black Chernozemic soil with initially high SOM was not saturated in SOM. Collectively, these results highlight the strategic contribution of perennial-grain cropping to further SOC sequestration.

Enhanced SOC sequestration with perennial-grain cropping may result from: i) increased C input from higher above and belowground biomass production by perennials, ii) enhanced stability in the microclimate at the soil surface and diminished physical disturbance, and iii) reduction of erosion caused by year-round ground cover, or any combination of these various factors (Hernandez-Ramirez et al., 2009; Crews and Rumsey., 2017; King and Blesh, 2018). Both Cates et al., (2016) and Sprunger et al., (2018b) demonstrated that soils under perennial vegetation develop higher C storage than soils under annual crops as a result of greater activities of perennial roots and soil microbial community. Similarly, the root data collected in this study suggests significantly greater C contributions belowground by perennial-grain cropping relative to annual grain (Fig. 2.6). Moreover, this increased root biomass in perennial grain crops could also be linked to higher root exudation, which provides nutrients and energy to soil microbes that mediate the effective accretion of the transitory SOM pool (i.e., POM) by altering mineralization and likely optimizing also humification (Cates et al., 2019; Daly and Hernandez-Ramirez, 2020). Moreover, no-tillage management under perennial-grain crops can provide soil biota with favorable microclimatic conditions and enhance soil aggregate formation, improving soil structure (Cates et al., 2016; Kiani et al., 2017). Ameliorated soil structure facilitates microbial access to nutrients by maintaining aerobic soil conditions and at the same time physically protecting SOM pools (Sprunger et al., 2018b; Cates et al., 2019; Kiani et al., 2020). Furthermore, an increasing vertical stratification, a decrease in the diluting effect of mixing, and the reduced incorporation of surface residue-C inputs into the soil under no-tillage management might also slow down the mineralization rate (Hernandez-Ramirez et al., 2009; Gauder et al., 2016; Mary et al., 2020). Lastly, continuous ground cover and C input by year-round vegetation growth may generate an indirect protection of MAOM from mineralization. Newly-added nutrient inputs can cause priming effects even on stable SOM pools (Daly and Hernandez-Ramirez, 2020; Thilakarathna and Hernandez-Ramirez, 2021; Roman-Perez and Hernandez-Ramirez, 2021); However, year-round ground cover provided by perennial plants can reduce this priming effect on MAOM by maintaining the balance of r- and K- strategists within the microbial population dynamics (Gauder et al., 2016; Soares et al., 2020).

2.4.2. Response of SOM accretion under perennial grain cropping to N fertilizer addition

Notably, our study revealed that adding N fertilizer specifically in perennial-grain cropping systems has significant impacts on POM, MAOM, and TOC, for both the Edmonton and Breton study sites. Although responses occurred in different growing seasons and SOM pools, N fertilizer additions precluded increases in SOC concentration. Nitrogen fertilization had a marginal effect on the composition of aboveground plant biomass (Supplementary Table S2.8). It is likely that belowground biomass also experience the same. Narrower C:N ratios in plant biomass caused by N fertilization can facilitate decomposability in the initial transformation stages from recently-added plant residues into stable SOM. These inferences support the applicability of the stoichiometric decomposition hypothesis upon N addition as recently reported by Roman-Perez and Hernandez-Ramirez (2021). This hypothesis applies where exogenous N additions fulfill the nutrient prerequisites for faster microbial activity, which subsequently unleashes primed mineralization of SOM (Liu et al., 2017; Thilakarathna and Hernandez-Ramirez, 2021; Roman-Perez and Hernandez-Ramirez, 2021). Additionally, our results may also adhere to the optimal foraging theory, implying that perennial grain cropping invests in root expansion where the soil became rich in nutrients, and thus increased root mass under perennial-grain crops was capable of capturing relatively greater amounts of the added N fertilizer compared with annual crops (Sprunger et al., 2018b). Subsequently, the increased primary productivity influenced by N fertilizer additions could alter the magnitude of crop-derived residuals coupled with a rate of SOM decomposition. Moreover, previous research has found that N management alters microbial community structure and composition by shifting nutrient distribution (Somenahalley et al., 2020). As N fertilizer addition decreases the soil C:N ratio and increases crop-derived residues, microbial-mediated SOM decomposition rate may subsequently accelerate as noted above (Valdez et al., 2017; Borges et al. 2019). Collectively, perennial grain cropping might have dynamized the N cycling as triggered by N fertilizer addition when compared with annual grain crops.

2.4.3. Contrasting study sites and their SOM dynamics over time

Increases in SOM appear to depend on a range of site-specific factors such as soil texture, cropping history, and climate (Curtin et al., 2014; Gauder et al 2016; Eclesia et al., 2016; Lin et al., 2020; Thilakarathna and Hernandez-Ramirez, 2021). These factors influence C sequestration and stabilization through their influences on soil biota, physical preservation of SOM, and biochemical composition of SOM (Carter and Gregorich, 2010; Chen et al., 2014; Kiani et al., 2017; Lin et al., 2020). This research supports the idea that stabilization of SOM is mainly driven by the underlying texture and mineralogy of the soil matrix, and hence, soil texture may explain in part the differential responses of POM-C and MAOM-C to same set of assessed cropping systems across the two contrasting study sites. Relative to the Breton site, the higher percent clay at the Edmonton site provides greater surface area in the Black Chernozem soil, (Table 2.1) which may have caused the greater response observed in the stable mineral-associated SOM pool (i.e., MAOM) to the different cropping systems over the study period specifically in Edmonton

(Eclesia et al., 2016; Potffenbarger et al, 2020). Although the soil at the Edmonton site had an initial high baseline of SOM, a high percentage of clay likely enabled the perennial-grain cropping system to further contribute and augment SOC concentrations in this soil. Samson et al. (2020a) recently highlighted that the contributions of the MAOM-C pool to SOM formation in association with soil texture variations are independent of the SOM saturation status. Mirroring the MAOM outcome, the POM-C:N ratio was much narrower in Edmonton than in Breton, which can also support more decomposability of transitory SOM pools (i.e., POM) to be transformed into stable SOM pools such as MAOM. This notion is line with Li et al. (2018) who concluded that the POM losses were triggered by acceleration of biological decomposition and subsequent transformation into the recalcitrant SOM pool.

The POM pool is a sensitive indicator of SOM decomposability (Hernandez-Ramirez et al., 2009; Li et al., 2018; Sprunger et al., 2018b). Lability of the quantified POM pool was further supported by its relatively wider C:N ratio compared with MAOM. This can suggest that POM was more favorable to relatively faster decomposition rate than MAOM in terms of C mineralization; however, upon microbial access and remobilization, MAOM could become a source of mineralized N based on narrower C:N ratios. Furthermore, this comparatively wider C:N ratio within the transitory pool can potentially generate a priming effect by microbes mining preexisting SOM-N, with the consequent release of asymmetrically-larger mineralized C (Daly and Hernandez-Ramirez, 2020). Decoupled cyclings of C and N has been previously documented during both gains and losses of intermediate POM pools (Hernandez-Ramirez et al., 2009; Li et al., 2018).

The higher proportion of C within POM relative to MAOM may be derived from plant-C contributions. Furthermore, the response of the POM pool to contrasting cropping systems manifested earlier and faster over the time of the study than changes in whole SOC and TN. This further suggests that recently added, plant-derived C and N contributed to an increase in the transitory SOM pool (Hernandez-Ramirez et al., 2011; Lin et al., 2020). This assertion is supported by a significant correlation between aboveground biomass-C and POM-C, which was stronger than the correlation between aboveground plant biomass-C and SOM-C (Table 2.6; Supplementary Table S2.10).

The fact that there were no significant differences in SOM pools across cropping systems in the middle of the growing season (i.e., soil sample collection in late June 2018, in particular at the Edmonton site) could be the result of sampling concurrent with the peak of root expansion. This temporal effect may have transiently masked underlying differences in the POM pool, which then became evident in the fall (i.e., postharvest soil sample collection). Abundance of fine roots during plant anthesis can be captured within the POM size range; however, these fine roots would turnover and decompose quickly even before the end of the growing season (Hernandez-Ramirez et al., 2009; Soares et al., 2020). Conversely, harvest at the end of the growing season results in plant C inputs to the soil surface mostly in the form of residues from aboveground biomass, which stimulate and renew SOM decomposition and mineralization (Valdez et al., 2017; Pugliese et al., 2019; Lin et al., 2020). Therefore, the effects of cropping system on SOM dynamics became more evident in postharvest soil sampling than during anthesis.

Our finding of high MAOM-C accrual in the subsurface soil layer under perennial-grain cropping at the Edmonton site can provide further insights into the sources of SOM. As noted above, higher root mass under perennial-grain crops in the subsurface layer can translate into SOM accrual. This is in line with both King and Blesh (2018) and Sprunger et al. (2018b) who concurrently stated that perennial crops accumulate C much deeper in the soil profile and create soil aggregation capable of storing SOC when compared to their annual counterparts. Furthermore, a slower SOM decomposition at this subsurface layer could foster root-C conversion and retention in stable SOM pools (Valdez et al., 2017; Borges et al., 2019). This is in part due to both lower oxygen availability and higher clay content with increasing soil depth (Borges et al., 2019). In fact, Spearman correlations between aboveground plant-C and -N concentration vs. MAOM-C were not found. Overall, this can support that deeper SOM accretion – particularly into MAOM under perennial vegetation – is mostly derived from root contributions (Curtin et al., 2014; Pugliese et al., 2019; Liebmann et al., 2020), while shallower SOM allocation in the soil profile – particularly into POM – can primarily evolve from aboveground plant–C and –N sources (Hernandez-Ramirez et al., 2011; Valdez et al., 2017).

2.5. Conclusion

Our comprehensive assessment of SOM accrual and dynamics supports that perennial-grain cropping is more effective at contributing to and stabilizing SOM than annual cropping systems. Furthermore, perennial-grain cropping systems can substantially increase C sequestration in the stable MAOM pool in the absence of N fertilizer additions. Of the two studied soils, the clay-rich Black Chernozemic soil boosted the benefits of perennial-grain cropping on sequestering C in soil profile. Our results shed light on how to achieve greater soil C sequestration as a function of cropping system options, N fertilizer addition, and underlying soil texture. Future research can further document the temporal changes in C pools and allocations as well as to examine even deeper soil layers. This information can help to further understand the SOM turnover and how roots of perennial vegetation contribute to SOM accretion.

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Tables

Table 2. 1. Soil classification and properties (0–15 cm depth increment) on Edmonton and Breton sites.

	Units	Edmonton	Breton
Classification		Black Chernozem	Orthic Gray Luvisol
TC †	g C kg^{-1} soil	53.7±2.9	27.5±2.9
TN	g N kg^{-1} soil	4.67±0.29	2.21±0.18
Texture		Clay	Silty clay loam
Clay †	%	47.0	31.1
Silt	%	36.0	49.1
Sand	%	17.0	19.8
pH‡		7.2	5.7
EC‡	dS m^{-1}	1.5	0.83
Cumulative Precipitation	mm yr^{-1}	441.5§ [314.3]¶	551.4§ [388.8]¶
Air Temperature	ºC	3.15§ [12.36]¶	3.07§ [11.46]¶

† Soil organic carbon (SOC) and total nitrogen (TN) were measured by dry combustion (Brachmann et al., 2020) for each block replicate; these are means of four replicates (±SE). Texture was measured by hydrometer (Hebb et al., 2017).

‡ 1:1 soil to solution. EC stands for electric conductivity.

§ These are long-term normal (Alberta Climate Information Service).

¶ Values inside the parenthesis correspond to the measurement period: April to September (Alberta Climate Information Service).

Table 2. 2. Description of cropping systems and field activities at both Edmonton and Breton.

Abbreviated ID	Description	Seeding Dates		Harvest Dates	
		Edmonton	Breton	Edmonton	Breton
Fallow	Continuous fallow using Roundup® and D ester herbicide since June 2017				
Spring-grain	Spring rye cropping system for grain production. Secale cereale L. cv. Gazelle. Tillage was used for crop establishment every year in the mid spring. Annual rye or summer rye are alternative designations in the literature.	22 May 2018; 23 May 2019	23 May 2018; 21 May 2019	19 Sep 2018; 25 Sep 2019	19 Sep 2018; 24 Sep 2019
Fall-grain	Fall rye cropping system for grain production. Secale cereale L. cv. Hazlett. Tillage was used for crop establishment every year in the late summer. Winter rye or biennial rye or are alternative designations in the literature.	29 Aug 2017; 3 Sep 2018	30 Aug 2017; 03 Sep 2018	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 24 Sep 2019
Perennial-grain	Perennial rye cropping system for grain production. Secale cereale L. × S. montanum Guss cv. ACE-1. Tillage was used for crop establishment only in the late summer 2017.	29 Aug 2017	30 Aug 2017	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 16 Sep 2019
Perennial-forage	Perennial forage cropping system for hay production. Alfalfa Medicago sativa L. and bromegrass Bromus spp. Aboveground biomass is cut and carry two times a year for hay with livestock feeding purposes. Tillage was used for stand establishment only in the mid summer 2017.	16 June 2017	8 June 2017	26 June 2018; 31 Aug 2018; 16 July 2019; 17 Sep 2019	26 June 2018; 30 Aug 2018; 15 July 2019; 16 Sep 2019

Table 2. 3. Overall contrasting cropping systems effects on particulate organic matter (POM), mineral-associated organic matter (MAOM), and soil organic matter (SOM) at 0–15 cm soil depth increment on Breton site.

Treatment	POM-C Concentration (g C kg^{-1} soil)	POM-N Concentration (g N kg-1 soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C kg-1 soil)	MAOM-N Concentration (g N kg-1 soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C kg-1 soil)	TN (g N kg-1 soil)	Whole Soil C:N
September 2018													
Fallow	5.96a	0.42	13.94a	0.23	0.19	19.37ab	1.72a	11.23	0.77	0.81	25.32a	2.14a	11.77
Spring-grain	6.95ab	0.47	14.66ab	0.25	0.20	21.28ab	1.92ab	11.06	0.75	0.80	28.23ab	2.39abc	11.77
Fall-grain	7.12ab	0.47	14.83b	0.27	0.21	19.32a	1.78a	10.76	0.73	0.79	26.44a	2.26ab	11.65
Perennial-grain	7.51b	0.51	14.50ab	0.25	0.20	21.71ab	1.97ab	10.99	0.75	0.80	29.23ab	2.48bc	11.74
Perennial-forage	7.74b	0.51	14.97b	0.25	0.20	23.09b	2.08b	11.09	0.75	0.80	30.83b	2.59c	11.85
Overall mean	7.18	0.48	14.7	0.25	0.20	21.1	1.91	11.0	0.75	0.80	28.3	2.40	11.8
ANOVA-P	<0.01**	<0.05*	<0.05*	0.17	0.52	<0.01**	<0.01**	0.25	0.17	0.52	<0.01**	<0.001***	0.73
September 2019													
Fallow	7.83	0.50	15.74	0.27	0.18	20.85	2.17	9.60	0.73	0.82	28.68a	2.67a	10.75
Spring-grain	7.83	0.49	15.87	0.26	0.18	21.80	2.27	9.51	0.74	0.82	29.64ab	2.76ab	10.71
Fall-grain	7.58	0.47	16.04	0.26	0.18	21.14	2.20	9.57	0.74	0.82	28.72a	2.67a	10.74
Perennial-grain	8.44	0.54	15.74	0.28	0.19	21.94	2.27	9.55	0.72	0.81	30.38ab	2.81ab	10.78
Perennial-forage	8.70	0.55	15.99	0.26	0.18	23.96	2.43	9.71	0.74	0.82	32.66b	2.98b	10.89
Overall mean	8.11	0.51	15.9	0.26	0.18	22.1	2.28	9.65	0.73	0.81	30.2	2.79	10.8
ANOVA-P	0.11	0.15	0.26	0.71	0.54	0.09	0.06	0.77	0.71	0.54	<0.05*	<0.05*	0.67

*, **, and *** indicate statistical significance at *P* <0.05, *P* <0.01, *P* <0.001, respectively.

Table 2. 4. Overall contrasting cropping systems effects on particulate organic matter (POM), mineral-associated organic matter (MAOM), and soil organic matter (SOM) at 0–15 cm soil depth increment on Edmonton site.

Treatment	POM-C Concentration (g C kg-1 soil)	POM-N Concentration (g N kg-1 soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C kg-1 soil)	MAOM-N Concentration (g N kg-1 soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C kg-1 soil)	TN (g N kg-1 soil)	Whole Soil C:N
September 2018													
Fallow	15.51	1.30	11.98	0.27	0.25	42.10	3.85	10.99	0.73	0.75	57.61	5.08	11.24
Spring-grain	16.61	1.42	11.76	0.29	0.27	40.78	3.80	10.76	0.71	0.73	57.39	5.16	11.02
Fall-grain	17.93	1.54	11.69	0.30	0.28	42.78	3.97	10.83	0.70	0.72	60.71	5.41	11.05
Perennial-grain	16.80	1.44	11.72	0.28	0.27	42.07	3.84	10.96	0.72	0.73	58.88	5.20	11.15
Perennial-forage	18.06	1.54	11.79	0.30	0.29	41.21	3.82	10.81	0.70	0.71	59.27	5.28	11.07
Overall mean¶	16.9	1.44	11.8	0.29	0.27	41.5	3.82	10.9	0.71	0.72	58.4	5.27	11.1
ANOVA-P	0.33	0.31	0.38	0.68	0.74	0.91	0.94	0.35	0.68	0.75	0.56	0.54	0.20
September 2019⸸													
Fallow	17.56	1.48	11.85b	0.29	0.26	42.91	4.26	10.07	0.71	0.74	60.48	5.74	10.53
Spring-grain	17.08	1.49	11.51a	0.29	0.26	41.08	4.07	10.10	0.71	0.74	58.16	5.56	10.48
Perennial-grain	18.14	1.58	11.50a	0.29	0.26	44.51	4.40	10.10	0.71	0.74	62.62	5.98	10.48
Perennial-forage	17.98	1.55	11.63ab	0.29	0.27	43.09	4.22	10.23	0.71	0.73	61.07	5.77	10.61
Overall mean	17.65	1.52	11.6	0.29	0.27	42.8	4.23	10.14	0.71	0.73	60.5	5.75	10.5
ANOVA-P	0.72	0.68	<0.01	0.98	0.93	0.15	0.19	0.24	0.98	0.93	0.10	0.13	0.18

¶ Fall-grain data were excluded to compare yearly change within the site since data for September 2019 was excluded in overall mean.

⸸ Fall-grain data was not excluded because this treatment sampling was conducted after till management.

*, **, and *** indicate statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

List of Figure Captions

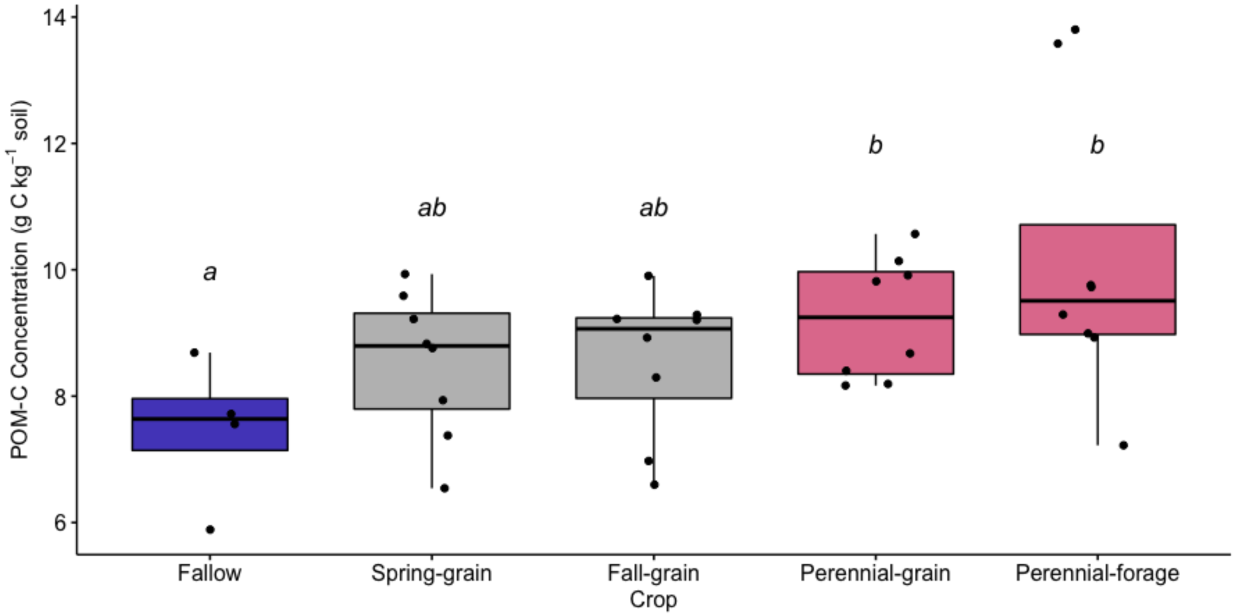


Fig. 2. 1. Particulate organic matter carbon (POM-C) concentration across cropping systems at the Breton site after the first growing season harvest at the 0–7.5 cm depth increment. Dots represent each POM-C concentration measurements, color and italic letters are shown for pairwise comparisons based on Tukey HSD test after ANOVA ($P<0.01$). An increasing gradient of POM accrual was observed ranking from fallow up to perennial–grain and –forage cropping.

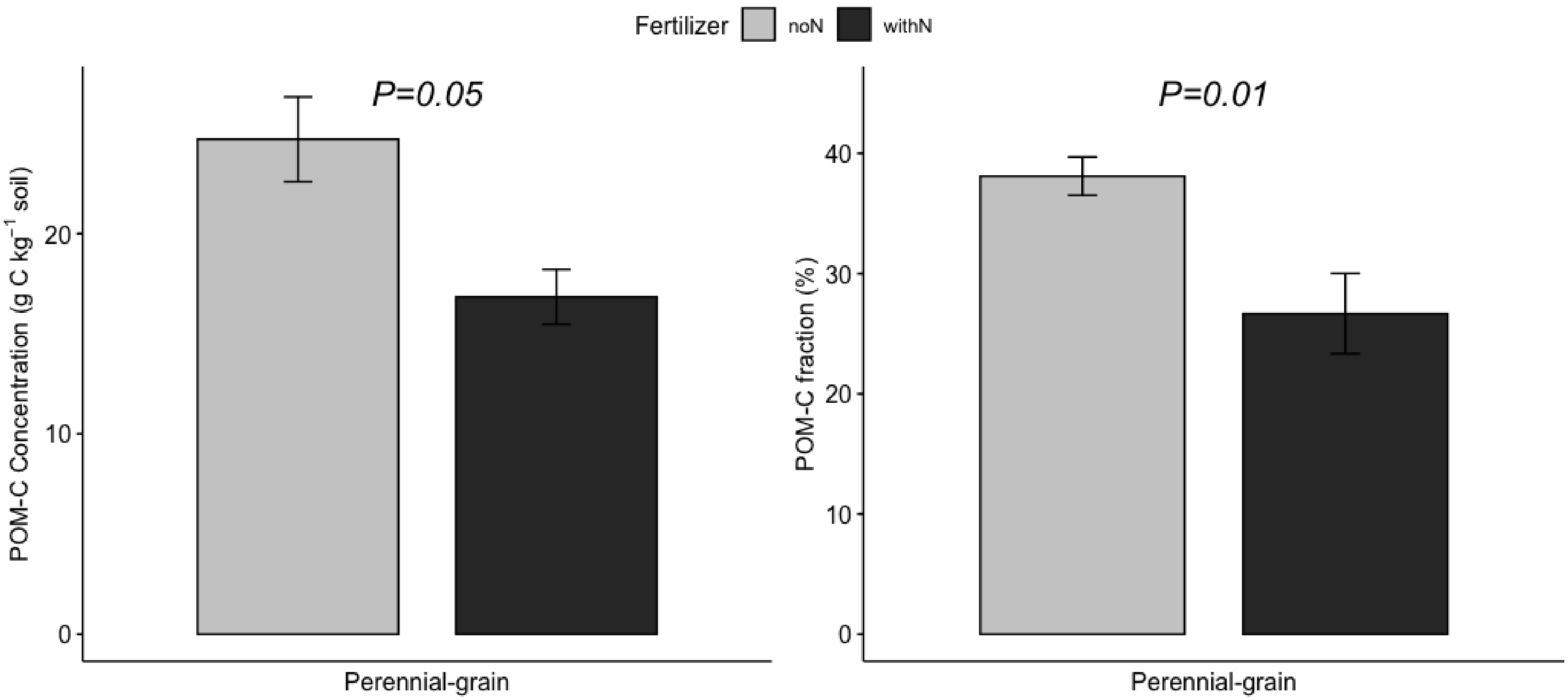
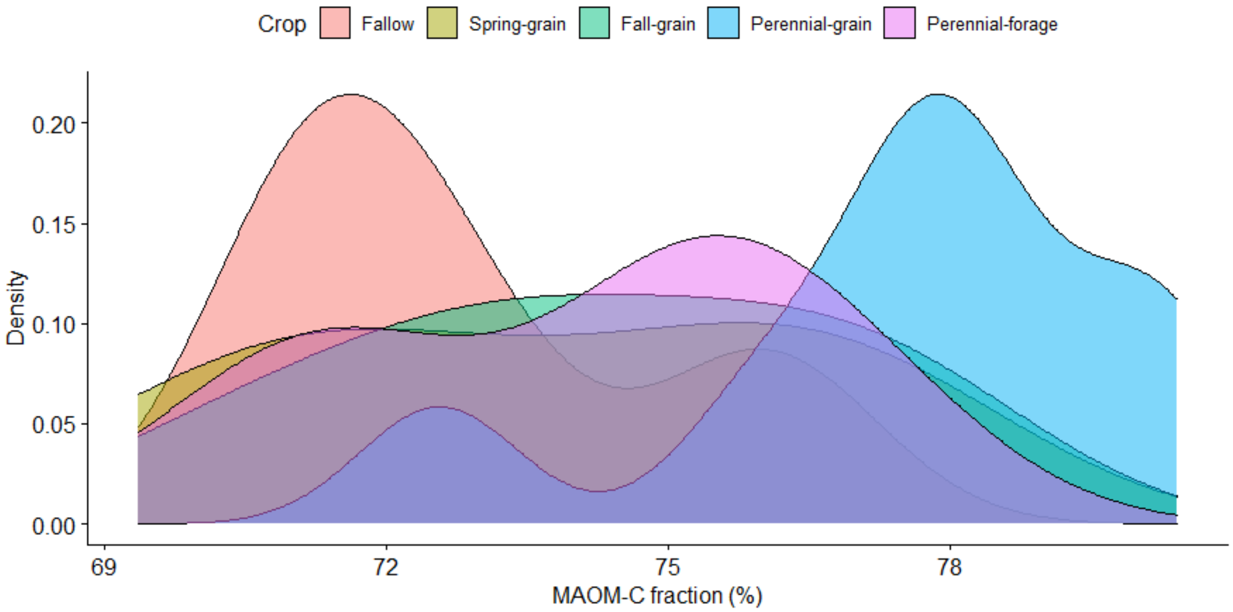


Fig. 2. 2. Particulate organic matter carbon (POM-C) concentration (left panel) and fraction (right) in perennial-grain crop according to N fertilizer effect on Edmonton site following the first-year harvest at 0–7.5 cm depth. ANOVA P-values are provided. Error bars show the standard errors of the mean.



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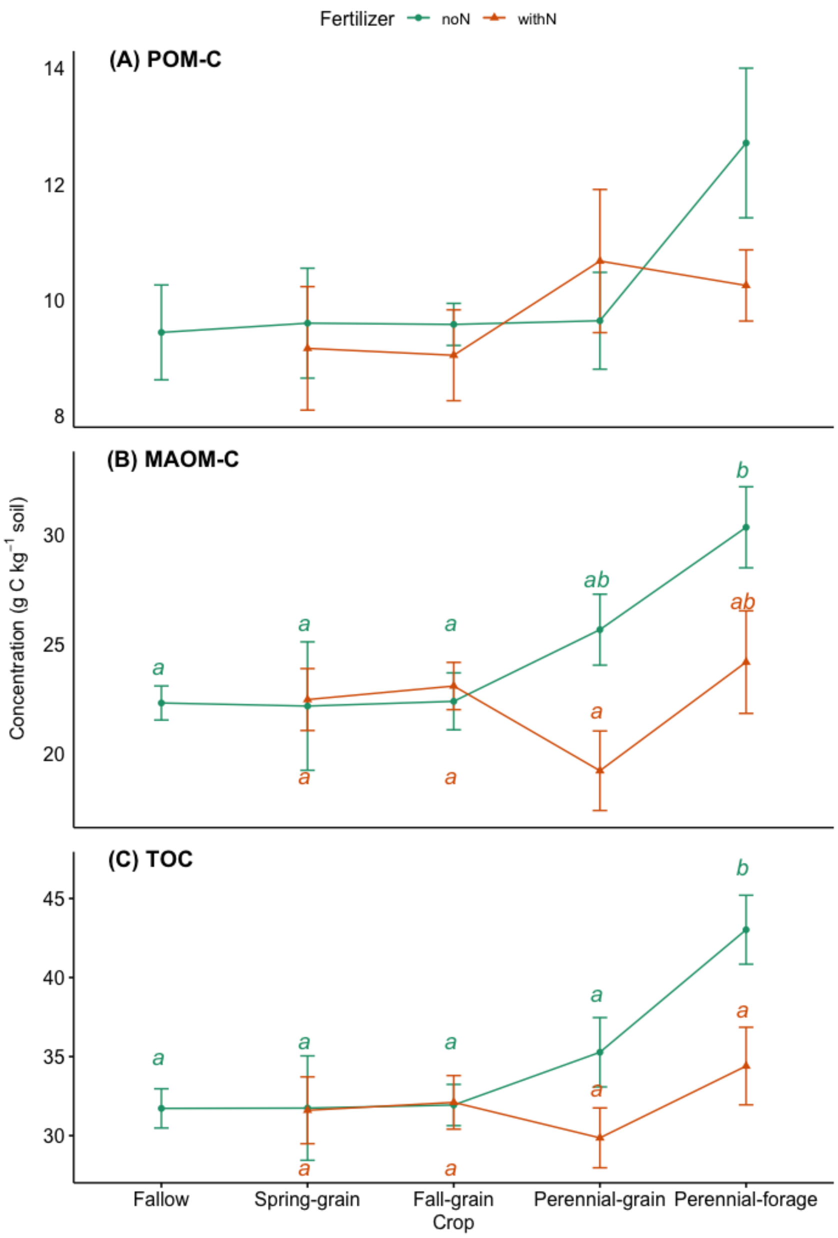

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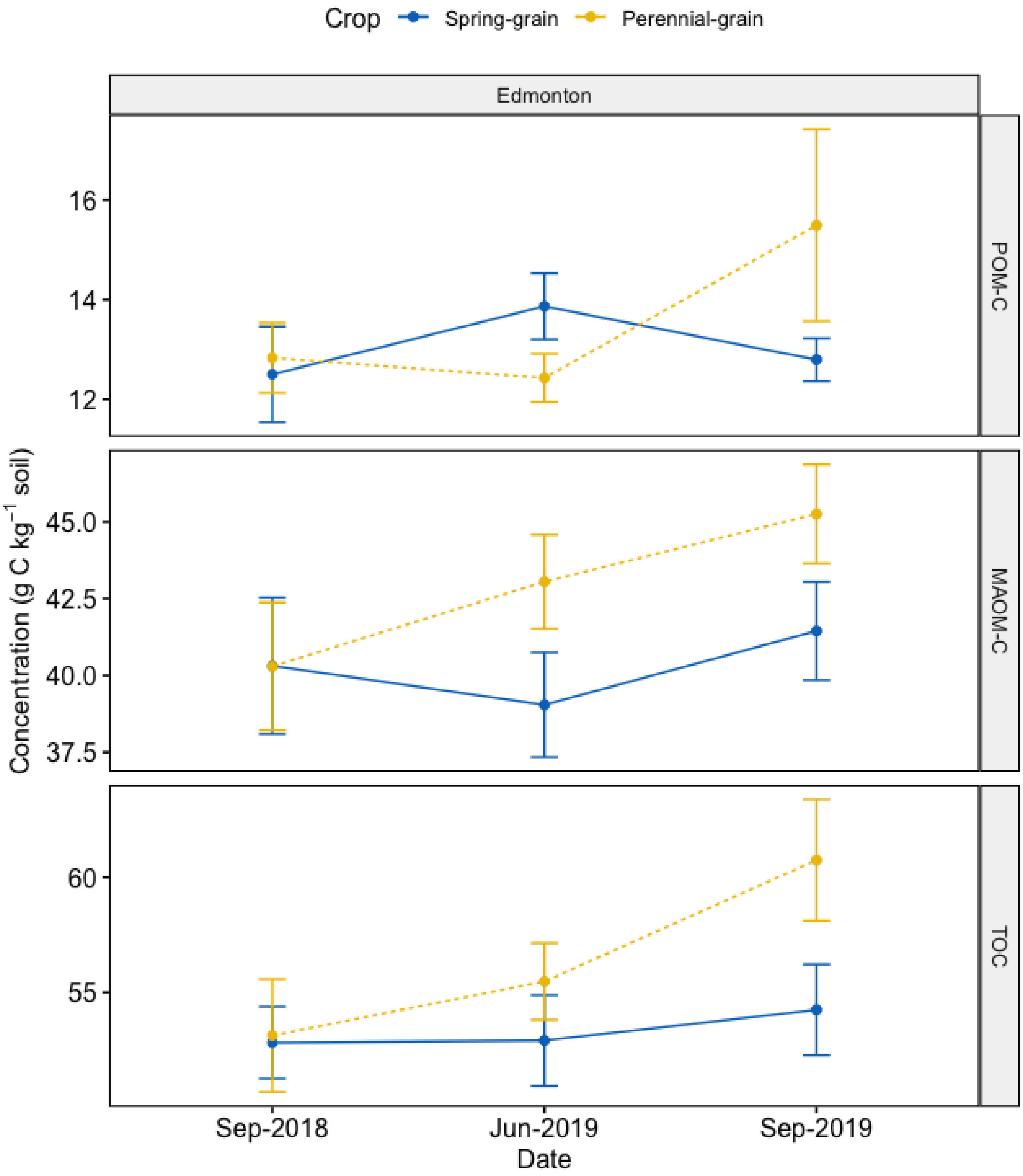

Fig. 2. 5. Particulate organic matter (POM), mineral associated organic matter (MAOM) and total organic matter carbon (TOC) concentrations for annual-grain and perennial-grain cropping at subsurface depth increment (7.5–15 cm) over time in the Edmonton site. At this subsurface soil layer, perennial-grain caused C accretion over time during the study, with more consistent effects within the MAOM pool. Error bars show standard errors of mean.

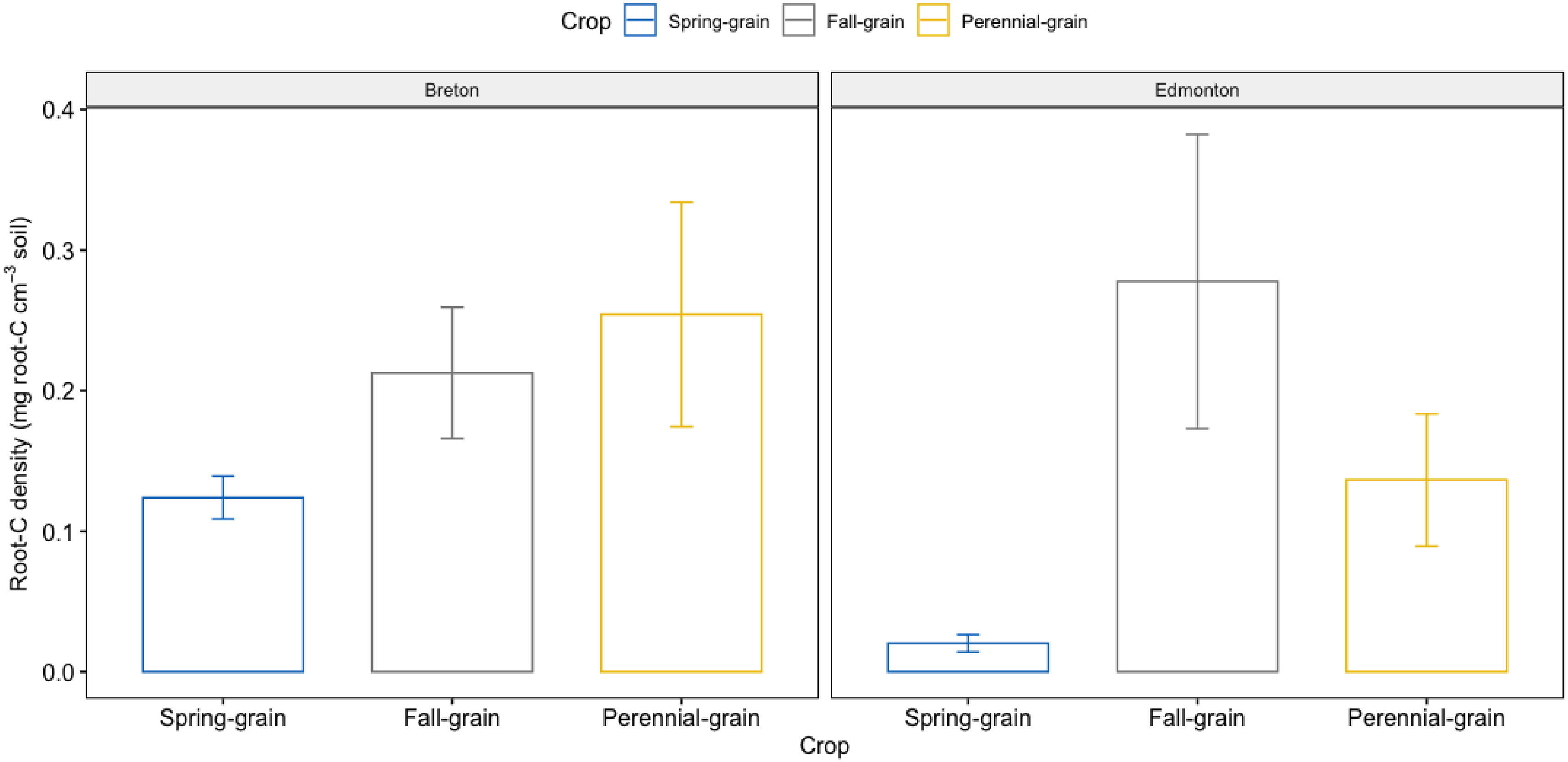

Fig. 2. 6. Root carbon (root-C) density for annual-, fall-, and perennial-grain crops at 0–15 cm soil depth increment on 11 July 2019 for Breton site, and on 12 July 2019 for Edmonton site. Error bar indicates standard error of mean.

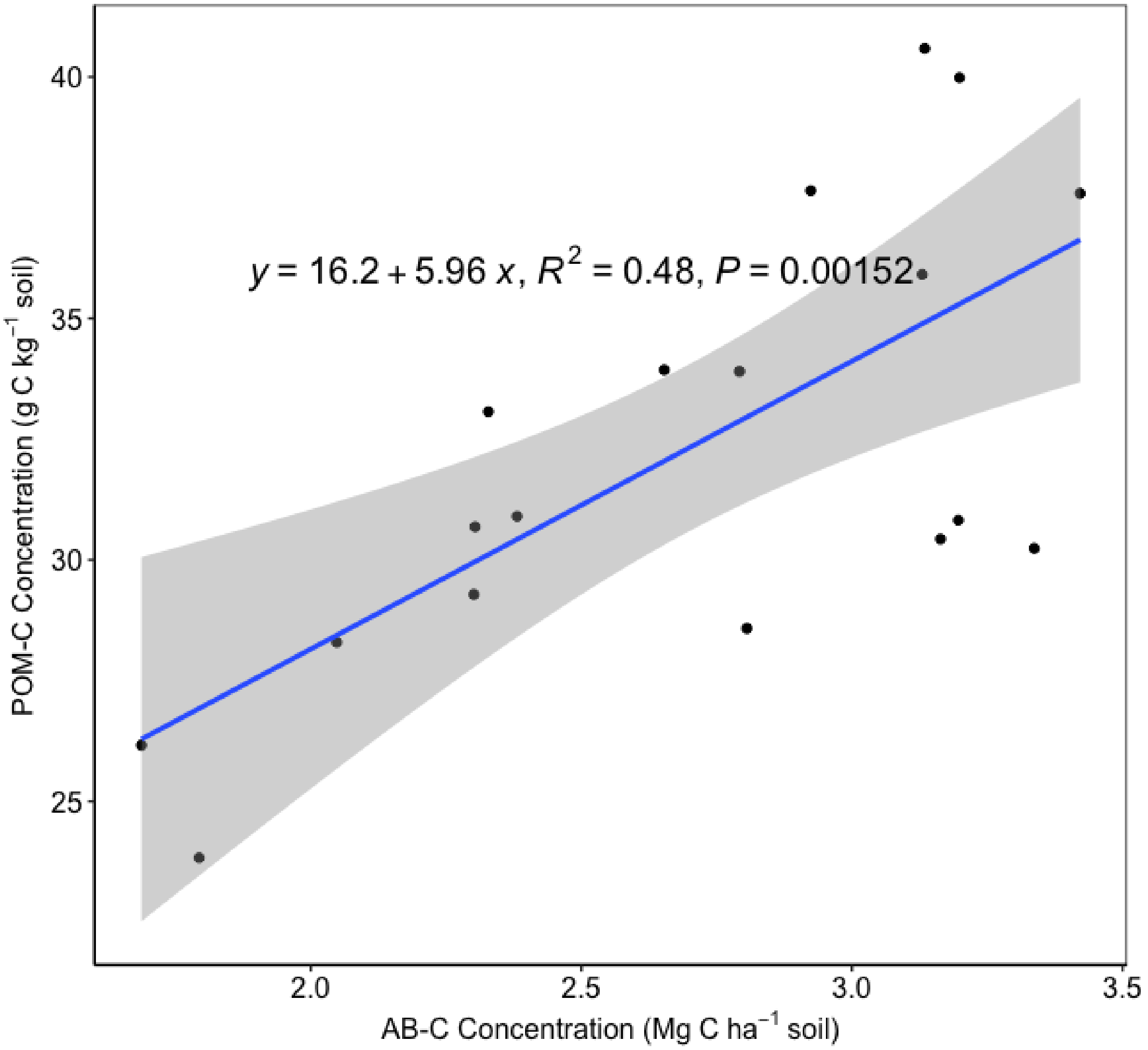

Fig. 2. 7. Aboveground plant biomass carbon (AB-C) as a function of particulate organic matter C (POM-C) concentration at the 0-15 cm soil depth at Edmonton site. Soil sample collection was in 1 Oct 2019. Points represent raw data, blue line shows the linear relationship as predicted. Grey area represents the 95% confidence interval predicted by linear correlation mode.

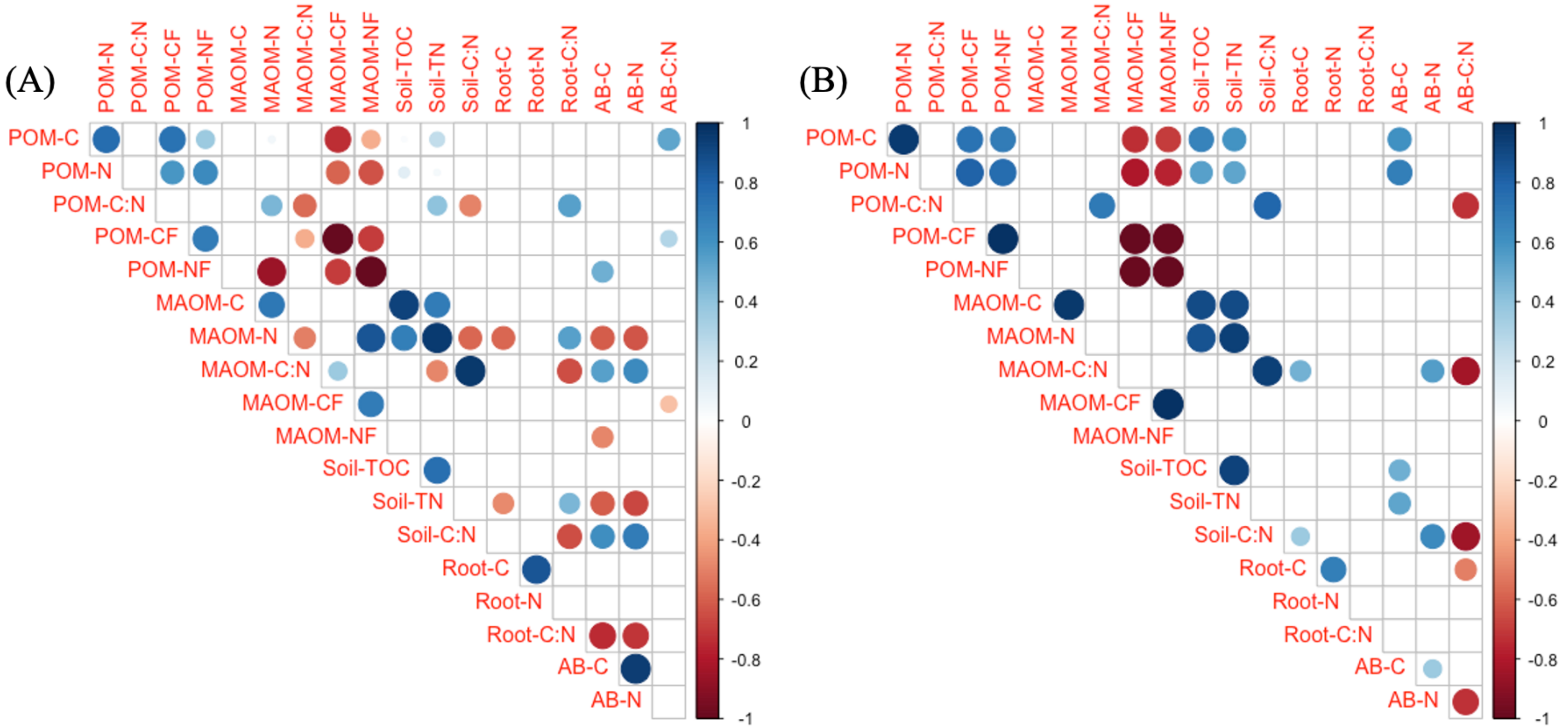

Fig. 2. 8. Spearman correlation analyses of the (A)Breton and (B) Edmonton soils at 0-15 cm depth increment. The size and color or circle denote P values and correlation coefficient signs. The circles were removed when P-value of the correlation is less than 0.05.

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; AB, aboveground biomass, TOC; total organic carbon concentration; TN; total nitrogen concentration; C, carbon concentration; N, nitrogen concentration; CF, carbon fraction; NF, nitrogen fraction.

CHAPTER 3. Arbuscular Mycorrhizal Fungi Community Linkages to Soil Nutrient Availability across Contrasting Agroecosystems.

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3.1 Abstract

Perennial grain crops have been proposed to establish sustainable agroecosystems, as their growth habit and no-till management likely invoke multifunctionality such as improvement of soil structure as well as increases in nutrient retention and carbon (C) sequestration. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts, which are ecologically important for over 80% terrestrial plants that form root associations with these fungi. Our study examined AMF diversity and community composition in annual and perennial grain crops; with and without nitrogen (N) fertilizer addition at two field sites within the Canadian prairies (Breton and Edmonton). Arbuscular mycorrhizal fungi diversity and community composition were significantly different between the two study sites. This was attributed to the contrasting cropping management histories of the sites; previous long-term land use in Edmonton was annual cropping, whereas Breton was perennial forage crop. AMF community composition was not different between annual versus perennial grain cropping in both the bulk soil and rhizosphere (roots). Overall, the addition of N fertilizer did not change AMF diversity and community compositions, but increased the abundance of *Archaeospora*. The most predominant genus in these temperate agroecosystems was *Paraglomus*, while *Archaeospora* and *Claroideoglomus* were observed in the rhizosphere but unfrequently and specifically at the Edmonton site. Interestingly, *Archaeospora* and *Paraglomus* were significantly correlated with changes in particulate organic matter C, while *Claroideoglomus* had a positive correlation with ammonium concentration. Results revealed associations between key soil properties and certain AMF genera, which showed to be site specific.

Keywords: Arbuscular mycorrhizal fungi, Perennial grain crop, Nitrogen fertilizer, *Paraglomus*, Legacy effect

Highlight:

- *Paraglomus* was the most ubiquitous AMF genus, and its abundance correlated with particulate organic C.
- Abundance of *Archaeospora* in roots increased as a response to N fertilizer addition.
- *Claroideoglomus* presence was associated with increasing soil ammonium concentration.
- Contrasting cropping systems (annual vs. perennial) have significant impacts on key soil attributes.
- AMF communities in soil and roots were similar between annual and perennial grain crops.

3.2. Introduction

Arbuscular mycorrhizal fungi (AMF), of the fungal division *Glomeromycota*, are an ancient lineage of obligate biotrophs formed in the roots of more than 80% of terrestrial plants (Smith and Read et al., 2010; Gorzelak et al., 2017, Gu et al., 2020, Jiang et al., 2020, Zhang et al., 2020). Arbuscular mycorrhizal fungi are capable of extending root bioactive zones by accessing surrounding soils using hyphae. This enables the AMF to access soil nutrients and water, which can in turn be provided to crops as they form large mycelia networks (Bakhshandeh et al., 2017, Pellegrino et al., 2020). These characteristics help host plants access nutrients such as phosphorus (P), nitrogen (N) and micronutrients (Jiang et al., 2020). Previous studies have shown that AMF in agricultural soils increase crop yield, improve tolerance to drought and pathogens, and enhance soil structure (Higo et al., 2015, Alguacil et al., 2019). However, AMF assemblages are strongly affected by anthropogenic activities including tillage, fertilizer, and host crop identity (Alguacil et al., 2019, Ezeokoli et al., 2020, Azeem et al., 2020). Understanding the impacts of land management decisions on the diversity of AMF communities is vital to obtaining positive outcomes in agroecosystems (Lopes and Fernandes, 2020, Xiao et al., 2020).

Sustainable agricultural systems attempt to balance soil quality with economic benefits (Wieme et al., 2020, Piazza et al., 2019). Perennial grain cropping systems have the potential to contribute to sustainable agriculture goals because of their unique growth habit and management (Ryan et al., 2018). Perennial grains promote carbon (C) sequestration and mitigate C loss by minimizing soil erosion and improving soil structure, nutrient retention, and water infiltration, while theoretically maintaining profitability (McGowan et al., 2019). Moreover, several studies have found that perennial crops have higher AMF richness and diversity than annual crops, since persistently growing root systems have greater opportunities to establish mycorrhizal fungal colonies (Benitez et al., 2016, Alguacil et al., 2019, Lopes and Feranades, 2020). Given a premise that microbial diversity has positive impacts on soil quality and crop performance, introducing perennial grain cropping systems feasibly approaches the first step for establishment of sustainable agroecosystems (Emery et al., 2017). This is because the premise is in line with enabling higher AMF diversity and community compositions in perennial grain cropping systems (Alami et al., 2020). For instance, perennial grain cropping systems do not require plowing for multiple years. Such management not only improves soil quality by promoting the formation of soil aggregates, but it also decreases disruption of AM fungal hyphal networks (Benitez et al., 2016, Piazza et al., 2019, Gu et al., 2020). However, under certain circumstances, deep tillage could enhance crop growth by potentially improving soil porosity and water availability within root-restricting soils layers (Schneider et al., 2017). This can enable the expansion of root systems, which in turn can facilitate the proliferation of AMF communities via nutrient exchange (Gu et al., 2020). A deeper understanding of how cropping systems impact soil quality and microbiomes can inform better management practices and the move towards more sustainable land systems.

Widespread N fertilizer used to improve crop yields by alleviating N deficiency stress has been a foundation in conventional agricultural management since the green revolution in the 1960s (Langholtz et al., 2021). However, over time, excessive N fertilizer application can hamper the growth of crops due to adverse impacts on soil quality (Hoang et al 2019, Zhang et al., 2020, Xiao et al., 2020). Furthermore, current research emphasizes the need to understand the AMF response to N fertilizer addition, as AMF are intrinsically linked to N cycling (Bakhshandeh et al., 2017, Somenahally et al., 2018). While AMF can transfer N to host plants under optimal conditions, they may also immobilize N into deeper soil layers or even compete with host plants in N deficient soils (Ingraffia et al., 2020). Nevertheless, not much is known about whether or how the structure and diversity of AMF communities shift between perennial and annual cropping systems when applying N fertilizer. Investigation of N fertilizer effects on AMF diversity and community composition across a range of agroecosystems will provide insight into how different AMF can influence on available N in contrasting cropping systems.

Total soil organic matter (SOM) typically does not respond rapidly to changes in management because of complex biogeochemical processes that can confer stability to SOM (Cotrufo et al., 2019). Therefore, particulate organic matter (POM), a labile SOM fraction, has been introduced as a detectable parameter to evaluate the effect of soil management practices on SOM dynamics (Ren et al., 2021). AMF provide host plants with nutrients in return for photosynthetic C as part of a mutualistic interaction (Wattenburger et al., 2020). Therefore, AMF growth can participate in and influences the cycling of C and N in the soil, potentially increasing SOM under perennial cropping. Moreover, an external stimulus such as N fertilizer addition may alter AMF community composition (Daly and Hernandez-Ramirez, 2020). In fact, investigating how AMF communities develop in response to labile C and N may provide insights into improving the resilience of cropping systems. Since POM is responsive to differences between cropping systems, and POM carbon (POM-C) is often considered an early indicator of soil C change, a relationship between the labile C pool, plant mediated supplies of photosynthetic energy and AMF community compositions may hypothetically exist (Cotrufo et al., 2019). In addition, soil inorganic N pools such as ammonium (NH_4^+) and nitrate (NO_3^-) can behave differently across contrasting cropping systems. Consequently, such alteration in N availability may moderate the mycorrhiza fungal community, since membership in communities of microorganisms is an outcome of physiological acclimation to environmental variables such as nutrient availability (Higo et al., 2015, Hahn et al., 2018, Lopes and Fernand, 2020). Furthermore, variations in soil attributes across cropping systems may determine the dominant AMF genera (Montes-Borrego et al., 2014; Jiang et al., 2020).

AMF remain difficult to characterize because of their obligate biotrophy and our inability to grow them in pure culture. However, their role in acquiring and delivering nutrients to plants is a compelling feature that ought to be studied and better understood in an effort to build sustainable agricultural systems. Here we compare the AMF communities associated with annual and perennial grain cropping systems at two different field sites. Our study also investigated the impact of N fertilizer addition as well as the influence of selected soil properties on AMF community composition. Our objectives were to investigate: (1) how the AMF community composition shifts across contrasting cropping systems, (2) whether N fertilizer addition has an impact on AMF community in annual vs. perennial cropping systems, and (3) if (and how) select soil attributes are correlated with taxa at the AMF genus level. Understanding soil-plant-mycorrhizae fungal interactions across contrasting agroecosystems will shed light on how to establish and manage sustainable agricultural systems for future generations.

3.3. Materials and Methods

3.3.1. Study sites

The experimental sites were located in Breton (53°05’16.4”, 114°26’32”W) and Edmonton (53°29’42”N, 113°32’1”W) within the Canadian Prairies. Breton and Edmonton have long-term mean annual precipitations of 526.8 and 441.5 mm yr^{-1} (596.0 mm and 399.6 mm yr^{-1} in 2019) and mean annual temperature of 3.51 and 3.15 °C (2.47 and 3.39 °C in 2019), respectively

(Alberta Climate Information Service). It is noted that the wetter 2019 growing season was attributed to large rainfall events in June and July 2019, which collectively represented 52 % of the annual precipitation. Soil classification and initial properties at the surface layer showed contrasting results between the two sites (Table 3.1). With respect to topography, Breton is described as a rolling and medium relief landform, whereas Edmonton is a uniformly flat (Alberta Soil Information Service). Prior to establishing the experiment, the long-term land use history at Breton was perennial forage for hay harvest (alfalfa *Medicago sativa* L. and bromegrass *Bromus spp.*), and annual cropping of barley (*Hordeum vulgare* L.) grown for silage in Edmonton.

3.3.2. Experimental Design

Research plots were established as randomized complete block designs with four replications, the dimensions of each plot measuring 4 m in width and 8 m in length at both sites (Breton and Edmonton). Five discrete agroecosystems [fallow, rye *Secale cereale* L. cv. Gazelle (spring grain), *S. cereale* L. cv. Hazlett (fall grain), *S. cereale* L. × *S. montanum* Guss cv. ACE-1 (perennial grain) and *Medicago sativa L.* alfalfa and *Bromus spp.* brome grass (perennial-forage)] were implemented (Table 3.2). With the exception of fallow, N fertilizer was assessed in the four vegetated cropping systems by including separate plots with and without N fertilization. The N fertilizer source was a 2:1 ratio of a granular blend of urea and polymer-coated urea (environmentally smart nitrogen, ESN^{TM}), the application rate was 56 kg N ha^{-1} applied at the time of spring grain seeding. The placement of fertilizer was surface broadcast on all 16 N-receiving plots. At each site, the total number of experimental plots were 36. The field activities followed conventional agricultural management practices (Kim et al., 2021; Table 3.2).

3.3.3. Soil and Roots Sampling

Samples of both roots (rhizosphere) and soil were conducted at anthesis of the spring grain crop. A total of three subsamples per compartment (i.e., soil and roots) from each plot were collected for high-throughput DNA sequencing using a pre-sterilized shovel at the soil surface layer (0-15 cm depth). Subsamples of soil and roots were then bulked. Additional root samples were collected separately for microscopic analysis in the same way as collected for DNA sequencing. The samples for high-throughput DNA sequencing were stored in a freezer at -20 ºC until processing while the microscopy samples were kept in a fridge at 4 ºC in 50% ethanol after washing with distilled water.

3.3.4. Staining and Microscope for AM fungal colonization

Root subsamples of approximately 1 g were submerged in room temperature 10% KOH solution overnight for bleaching, stained using a mixture of glycerol (50%), MilliQ water, acetic acid (0.25%), and trypan blue (0.001%, w/v), and destained in vinegar. Colonization was evaluated using a magnified intersection method (reference), which quantifies the proportion of the root length encompassing arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) (McGonigle et al., 1990). The percentage of colonization was acquired by dividing the number of identified mycorrhizal structures by the total number of observations and multiplying 100.

3.3.5. Molecular analysis

Total DNA was extracted from 0.25 g of samples using the PowerSoil DNA Isolation kit according to the manufacturers' guideline (MoBio Laboratories Inc., Carlsbad, California). DNA purity and concentration were measured by a Biodrop spectrophotometer (Biochrom, Cambridge, UK) and a Qubitv4 fluorimeter using a Qubit™ dsDNA BR Assay Kit (ThermoFisher Scientific, Massachusetts, USA), respectively. The amplificability of the AMF small subunit (SSU) region were confirmed with PCR by using primer pair NS31 (5’-TTGGAGGGCAAGTCTGGTGCC-3’) to AML2 (5’-GAACCCAAACACTTTGGTTTCC-3’) (Morgan and Egerton-Warburton, 2017). The PCR cycling conditions were: 94 ºC (3 min); 35 cycles of 94 ºC (45 s), 63 ºC (60 s), and 72 ºC (90 s); followed by final extension step 72 ºC (10 min) (Morgan and Egerton-Warburton, 2017). The fragment size and quality of amplification of the PCR product was verified by electrophoresis on 1% agarose gel. Sequencing was implemented using an Illumina MiSeq platform at a read length of 2 × 300 bp (Illumina Inc., San Diego, California, USA).

3.3.6. Bioinformatics and statistical analysis

The raw FASTQ data was processed with the Qiime2 pipeline (version 2019.10 https://qiime2.org/) (Bolyen et al., 2019). The first 21 bp and 22 bp in the forward and reverse reads were trimmed for removing primers, respectively. The forward and reverse reads were truncated at 295 and 283 bp, corresponding to average quality score (Phred Q score) of higher than 20, respectively. DADA2 algorithm was used to implement error correction, quality filtering, chimera removal and sequence variance of Illumina amplicon sequences (Callanhan et al., 2016). 25.6% and 21.1% of total reads remained after filtering for the Breton and Edmonton samples, respectively. Thereafter, amplicon sequence variant (ASVs) was clustered with ≥ 97% similarity in an open-reference picking process using classify-consensus-vsearch in Qiime2. Clustered ASVs (hereafter, referred as operational taxonomic units; OTUs) for taxonomic identification was retrieved from directly querying the MaarjAM database (Őpik et al., 2010). Unassigned OTUs were further aligned against a Silva 138 99% OTUs sequences reference database. Over the succeeding classification against two database, non-Glomeromycotina fungi for the 18S sequences were removed from subsequent analyses to constrain analysis to the target groups. OTU count tables for samples were depleted of singletons and OTUs that occured in less than three samples. The processed data were exported from Qiime2 to analyze and visualize within the R packages ‘phyloseq’ and ‘vegan’.

Alpha diversity of AMF communities was evaluated by Chao1 richness, Pielou’s evenness, Shannon’s diversity, and inverse Simpson’s diversity indices with a linear mixed model as the parametric test. The linear mixed model was used for analysis of variance (ANOVA) of AMF fungal colonization as well as ANOVA of alpha diversity. By doing so, a random effect (‘block’) was removed while statistically analyzing crop and fertilizer effect on colonization and alpha diversity using the R package. Normality and homoscedasticity of the model residuals were assessed using Shapiro-Wilk and function, respectively (NLME package of 3.2.1; R Core Team, 2015). Box-Cox transformation were applied to correct non-normality or heteroscedasticity when needed. Pairwise comparisons were conducted after significant ANOVA with Tukey’s Honest Significant Difference using agricolae package in R (Mendiburu and Felipe de, 2020).

Before statistical analysis of beta diversity (i.e., the ratio between regional and local species diversity), OTU absolute count data were transformed for even sampling depth based on ‘phyloseq’ tutorial (McMurdie and Holmes, 2013). The beta significance of AMF communities was assessed by permutational multivariate analysis of variance (PERMANOVA) and illustrated by non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrices (Anderson et al., 2018). The differential abundance analysis was performed by edgeR: a Bioconductor package (Robinson et al., 2010). Transformation-based canonical correspondence analysis (tb-CCA) and redundancy analysis (tb-RDA) were used to explain dissimilarity with environmental variables. Spearman correlation test was also performed to find relationships between dominant taxa and environmental variables.

3.4. Results

3.4.1. Mycorrhizal colonization assessment

Microscopic assessment showed that arbuscular mycorrhizas colonized the roots of the three different grain crops (i.e., spring, fall, and perennial rye). Breton showed a relatively higher hyphae, vesicle and arbuscular colonization rate compared with Edmonton site. Although cropping system and N fertilizer addition did not significantly influence the root colonization percentage at either site, spring grain had about 20% ($P > 0.05$) higher hyphal colonization than fall grain at Breton site. In addition, perennial grain exhibited 26% ($P > 0.05$) higher hyphae colonization compared with fall grain in Edmonton (Supplementary Table 3.1).

3.4.2. 18S sequences

A total of 1,371,001 and 693,204 reads (89.3 % and 52.2 %) were assigned to *Glomeromycota* against MaarjAM and Silva databases in Breton and Edmonton, respectively (Supplementary Fig. 3.5). 2.5% and 10.5% of total reads were not assigned to any phylum against these databases, while at the same time the targeting primers inadvertently amplified *Nematozoa* at the phylum level by up to 27.6 % of Edmonton samples. 431 unique AM fungal OTUs were clustered and found across samples in Breton and Edmonton sites. Altogether, 4 Orders (*Archaeosporales, Diversisiporales, Glomerales, Paraglomerales*), 8 families (*Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeracease, Diversisporaceae,, Gigasporaceae, Glomeracease, Paraglomeraceae*), 8 genera (*Acaulospora, Ambispora, Archaeospora, Clasroideoglomus, Diversispora,, Glomus, Paraglomus, Scutellospora*) were observed as well as 12 virtual taxa. The most abundant genera in Breton were *Paraglomus* (97.0 %), *Archaeospora* (1.14 %), *Clasroideoglomus* (0.6 %), *Glomus* (0.6 %), and *Diversispora* (0.4 %), while in Edmonton, the most abundant genera were *Paraglomus* (71.9%), *Archaeospora* (18.3 %), *Clasroideoglomus* (9.0 %) followed by *Ambispora* (0.1 %).

3.4.3. AMF alpha diversity

Breton had significantly higher Chao1 richness than Edmonton, whereas Pielou's evenness, Shannon's and Inverse Simpson's indices was lower ($P < 0.05$; Fig. 3.1). In Breton, fallow had at least 34 % higher Evenness, Shannon's and Inverse Simpson diversity than the crop treatments ($Ps < 0.05$; Table. 3.3). In contrast to the alpha diversity patterns at Breton site, perennial-forage in Edmonton has overall higher alpha diversity compared with other treatments (Table 3.3). Irrespective of site, there was no difference between spring-grain and perennial grain in alpha diversities (Table 3.3). Despite the lack of an overall N fertilizer effect on AMF alpha diversities, fall grain crop and perennial forage without N fertilizer addition showed slightly higher Inverse Simpson indices than those with N addition ($P > 0.05$; Supplementary Table 3.2; Supplementary Fig. 2). The comparison between soil and root samples had a contrasting pattern in respond to sites, and evenness indices showed significant difference at both sites (Table 3.4). Overall, the Peilou's evenness showed similar patterns as the alpha diversity indices, suggesting the alpha diversity was more strongly moderated by species evenness rather than by richness.

3.4.4. AMF community composition

Overall, AMF community composition differed significantly between the Breton and Edmonton sites (Fig. 2A). However, significant differences between cropping system type occurred only at the Breton site (Fig 2B; Supplementary Table 4). Nitrogen fertilizer application had no impact on AMF composition at either site. At the Breton site, fallow plots significantly differed in AMF composition from the perennial grain, and perennial forage, but not from spring grain and fall grain (Fig. 1B; Supplementary Table 5). Pairwise comparison also indicated no difference between spring and perennial grain crops (Supplementary Table 5). In comparison, we found a significant difference in root AMF community composition between spring and fall grain cropping treatments at Edmonton site ($P < 0.05$, Supplementary Table 5). It is noted that the AMF community composition in perennial grain roots was not assessed at Breton site. This is because the perennial grain plots were not sampled due to significant weed pressure from the seed bank and lack of effective weed control. It is intrinsically difficult to successfully control weeds in perennial grain crops as opposed to annual grain crops, where seasonal tillage management is possible. In addition, this excessive weed competition was also accompanied by abundant rainfall during the second year of the study, as mentioned earlier in the site description. Nevertheless, our research focused and evaluated AMF diversity and community composition in perennial-grain crops specifically in the soil compartment, based on the assumption that the potential effects of weed infestation on AMF-root systems would take time to translate into impacts on the soil compartment.

The differences in AMF community composition between the two study sites (in both bulk soil and roots) were significantly associated with changes in POM-C (Supplementary Fig. 4A and B). In addition to POM-C, ammonium (NH_4^+; NH_4-N), and nitrate (NO_3^-; NO_3-N) were also marginally or significantly correlated with AMF community composition when encompassing all the cropping treatments for both bulk soil and root compartments in the experimental two sites ($P < 0.1$, $P_S < 0.05$; Supplementary Fig. 4 C, D, and E). Moreover, the AMF community composition significantly differed between bulk soil vs. roots at both sites (Fig. 2 C; Supplementary Table 4). Using differential abundance analysis to compare between cropping systems, *Archaeospora*, *Glomus*, *Paraglomus*, and *Scutellospora* were significant different at the

Breton site (*Ps* < 0.05, Supplementary Fig 7 A). Also, *Glomus* and *Scutellospora* were significantly different at the Edmonton site (*Ps* < 0.05, Supplementary Fig. 7 B). Yet, considering that our main research focus was on contrasting annual vs. perennial grain croppings, we could only find a difference in *Archaeospora* at the Breton site within the soil compartment (*P* < 0.05, Supplementary Fig. 7). There were no significant differences in AMF across cropping systems within roots at the genus level at both sites (*Ps*> 0.05, Supplementary Fig. 6; Supplementary Table 6; Supplementary Table 7). Noticeably, *Archaeospora* abundance in the rhizosphere increased with N fertilizer application at Breton site (Supplementary Table 6).

Using differential abundance analysis to compare between bulk soil vs. roots at the genera level, *Archaeospora* and *Claroideoglomus* were significantly greater in the soil at Edmonton site (*Ps* < 0.05, Fig. 3; Supplementary Table 7). In contrast, AMF genera abundance in Breton did not differentiate between these two compartments (*P* > 0.05, Fig. 3; Supplementary Table 6). *Archaeospora* at Breton site had a negative correlation with POM-C, whereas *Paraglomus* had a positive correlation with POM-C (*Ps*< 0.05, ρ = -0.474, ρ = 0.387, respectively; Supplementary Fig. 7A). *Clariodeoglomus* at Edmonton site had a positive correlation with NH_4-N (*P* < 0.05, ρ = 0.431; Supplementary Fig. 7A). When differential abundance analysis was separately conducted within soil and roots compartments, *Archaeospora* was negatively correlated with POM-C only in root samples (*P* < 0.05; ρ = -0.732; Supplementary Fig.7).

3.5. Discussion

This study showed how AMF can shift as a function of cropping systems and soil properties such as POM-C and available N. The Breton and Edmonton sites harbored significantly different AMF communities, and AMF in roots and soil bulk compartments responded differently to experimental treatments applied at both sites. Although N fertilizer additions did not alter AMF diversity and community composition, we found *Archaeospora* increased with N fertilizer application. Moreover, there was certain correlations between N availability in soils and specific AMF genera; for instance, the presence of *Claroideoglomus* with increasing NH_4-N concentration in the topsoil.

3.5.1. AMF community composition changes across contrasting cropping systems

The AMF present in roots and bulk soil responded to experimental treatments differently across the two study sites, revealing no unified effect of cropping system across sites. In this study, we compared conventional annual grain crops to novel perennial grain crops, since the two cropping systems have contrasting field management and crop phenology. However, our findings indicate that these management and phenology differences across cropping systems are not the main drivers of differences in AMF diversity and community composition. It was expected that no-tillage management in perennial grain cropping would support AMF propagules in a manner similar to perennial plant species (Muller et al., 2017). Conversely, the AMF diversity and community in our study were unresponsive to the contrasting cropping systems (Table 3, Supplementary Table 5). When comparing multiple cropping systems (i.e., ranging from fallow to perennial forage), it can be postulated that host type was a determinant of alpha and beta diversity in the soil AMF community profiles (Gorzelak et al., 2017, Jiang et al., 2020). This is because the experimental treatments used for this study (i.e., spring grain, fall grain, and perennial grain) were cultivars of the same *S. cereale* species, despite having different growth habits and management. Although different cultivars of the same plant species can harbor different AMF communities in soil, it appears that *S. cereale* does not (Kavadia et al., 2020; Parvin et al., 2021). When investigating AMF community composition in roots, there was also no difference between annual and perennial grain crops. However, we observed differences between grain crops (i.e., spring vs. fall grain crops) at the Edmonton site. This finding suggests that there are indeed differences in AMF across the differing grain crop cultivars. Such differences in AMF community profiles between crop types may manifest in the bulk soil only after multiple seasons, as the differences captured in our study were observed two years after the experimental establishment.

The differential response of AMF to cropping systems across the two study sites could have been influenced by legacy effects of previous land management history. Historical effects on soil AMF community composition occurred in Breton, whilst this legacy effect was not discernible in Edmonton (Table 3; Supplementary Table 5). The long-term land use prior to establishing the experimental site at Breton was perennial forage. As the soil at the Breton site had an overall lower transitory C pool (i.e., POM fraction) compared with the Edmonton, we interpret that long-term perennial forage cropping systems had developed a great allocation of C to the stable organic matter pool, capable of sequestering C long-term (POM-C proportion were 27.6 ± 0.04 and 30.7 ± 0.07 % for Breton and Edmonton, respectively). This was supported by inferences from CCA and RDA, which showed how POM-C concentration can significantly account for the variance and difference in the community composition across study sites when focusing on both bulk soil and roots (Supplementary Fig. 4A and B). The Breton soil was conducive to maintaining overall lower POM-C fraction, even after two growing seasons of perennial cropping at the experimental site. Based on the premise that POM is readily utilized as a substrate by microbes compared with other more recalcitrant or protected SOM compounds (Cotrufo et al.,, 2019), the relatively lower POM fraction at Breton may have play an important role in regulating AMF community composition (Martinez-Gracia et al., 2018). In our research, we observed that *Archaeospora* and *Paraglomus* have significant correlations with POM-C only at Breton site. Moreover, our RDA analysis showed that POM-C was a marginally significant driver of introducing a divergence of AMF community composition at Breton within the root compartment. However, our RDA does not show any correlation with divergences of AMF community composition in the soil compartment. It is postulated that a sequential change of POM-C concentration was not captured appropriately to fully account for divergences of AMF community composition. The POM-C measurement was implemented only at the time of crop anthesis, which may attenuate or even mask cropping systems effects on POM-C compared with POM changes that occur at the end of the growing season or even in the long term (Supplementary Fig. 3A, Supplementary Fig. 4C). Nonetheless, our finding further implies that the AMF community composition reacts more sensitively to alternations derived from previous long-term land use changes under an initially lower labile SOM pool, which could have been inherited from the earlier management history. On the other hand, the Edmonton site had a history of long-term annual cropping for several decades before the beginning of this study. The Edmonton site had a comparatively higher labile SOM pool and overall C concentration compared to the Breton site, which may have concealed the potential impacts of contrasting cropping systems on AMF community composition. Further investigation is required to conclude to what degree AMF community composition is sensitive to alternations in POM-C abundance and when POM-C measurement timing can become representative of alterations in AMF community composition.

Shannon and inverse Simpson indices in the fallow treatment were comparatively higher or equivalent to in any of the grain cropping options (i.e., spring grain, fall grain, and perennial grain) at both study sites. Although the fallow treatment was managed to prevent any plant growth, native grasses sporadically occurred in spite of periodic herbicide applications, which may have increased AMF diversity. As an alternative explanation, earlier studies have shown that anthropogenic stressors such as continuous cropping systems profoundly decrease microbial diversity (Entry et al., 2002, Higo et al., 2019, Alami et al., 2020). Additionally, different plant species form AMF to a varying degree. While wheat crops form mycorrhizal associations, they do not appear to gain any benefits from doing so (Graham and Abbott, 2000). Similar to wheat, rye may also form symbioses with AMF without any net benefit. This may result in a decrease of alpha diversity as a result of cereal cropping, as they can successfully grow without the dependence of mycorrhizae (Phillips et al., 2019, Alguacil et al., 2019). Furthermore, the perennial forage treatment showed an AMF diversity pattern opposite to the grain cropping treatments, depending on the site. These differences between treatments may be due to interactions of environment × plant species or ecological behavior of AMF specific to either each experimental site or their land use history (Casazza et al., 2017; Hontoria et al., 2019). Our measured environmental factors explained about 11.7 % variation in AMF community composition, thus untested factors are clearly at play (Montes-Borrego et al., 2014). Collectively, previous management history, intrinsic soil properties, and ecological behavioral differences may have impacted AMF alpha and beta diversity as well as initial AMF inoculum available in the soil to colonize new plant roots (Ramos-Zapata et al., 2012; Njeru et al., 2015; Phillips et al., 2019; Gu et al., 2020).

The differential length of the growing seasons across crop types (annual vs. perennial grain) may be a more significant driver of beta diversity than agricultural practices. Interestingly, contrasting cropping systems influenced the AMF community structure. Moreover, AMF community composition shifted when comparing fallow to perennial forage (Fig. 2B). This can suggest a gradual shift in AMF community composition over time, which further implies that AMF community composition can be associated with plant succession during cropping development. To examine the hypothesis that vegetation type impacts AMF community, there is a need to further investigate how AMF community composition changes following vegetation establishment over the long term.

In addition, contrasting cropping systems produce significant differences at the AMF genus level (Supplementary Fig. 7). In other words, AMF taxa differences at the genus level were relatively inconsistent across contrasting cropping systems, which further implies that the AMF taxa present across cropping systems have specific functional roles (Higo et al., 2019).

3.5.2. AMF community composition response to N fertilizer

As AMF are typically involved in plant nutrient acquisition, studies have shown that their abundance and diversity respond either specifically to N fertilizer addition, or to other agricultural management practices (Benitez et al., 2016; Bakhshandeh et al., 2017, Xiao et al., 2020). Although N fertilizer may have a marginal impact on symbioses associated with AMF directly, we hypothesized that an overall increase in plant growth due to the application of N fertilizer would decrease plant dependence on AMF symbioses and their associated benefits, such as the acquisition of available N. In our study, N fertilizer addition solely decreased inverse Simpson diversity at Edmonton sites, but did not significant change between identical cropping systems (Supplementary Fig. 2). In general, our results revealed no alteration in overall AMF diversity and community composition as a function of N fertilizer application across any of the widely contrasting cropping systems (Supplementary Table 2; Supplementary Table 3; Supplementary Table 4). It is possible that N fertilizer addition (56 kg N ha^{-1}) was not enough to reshape AMF assemblage (Emery et al., 2017). Additionally, the lack of response in AMF diversity and community composition may depend on background nutrient availability in soils (Xiao et al., 2020). Phosphorous availability may have played a pivotal role in AMF community structure amongst other nutrients. This is based on the presumption that AMF can access and source up to 90 % of P and 20 % of N for host plants through the hyphal network (Egerton-Warburton et al., 2007; Williams et al., 2017; Bakhshandeh et al 2017). To be specific, phosphorous is immobile in soils relative to available nitrogen by forming insoluble P complexes with cations such as iron, aluminum, and calcium. This slows diffusion of P which becomes subsequently depleted near root surfaces; however, AMF hypha networking can extend and increase absorption and transportation of P into root surfaces, whereas N fertilizer may have less impacts on AMF community compositions because of greater mobility of N resources within the soil pores (Knerr et al., 2018). Consequently, this advantage in P acquisition could presumably impact and augment the interaction of AMF community composition as a function of N fertilization across the contrasting cropping systems.

Notably, the perennial grain fields that received N fertilizer showed overall higher Shannon and inverse Simpson diversity than the perennial grain plots without any N fertilizer addition although this comparison was not a statistical significance (Supplementary Fig. 1). Notably, root biomass of the perennial grain crop was significantly higher than that of the annual crop (Kim et al., 2021). Consequently, perennial plants with increased root density produce more root exudates than annual crops (Daly and Hernandez-Ramirez, 2020). This higher root exudation consists of diverse organic C and N compounds, which in the presence of N fertilizer, may synergistically bolster AMF diversity (Xiao et al., 2020).

Differential abundance analysis at genus level showed N fertilizer addition had a positive correlation with *Archaeospora* at the Breton site, which suggests that this genus was able to benefit and adapt to higher N fertilizer conditions.

3.5.3. Soil properties and AMF community

The labile C pool (i.e., POM-C) and inorganic N concentrations are used as an energy source for soil microorganisms and for plant nutrient acquisition, respectively. Collectively, these properties can act as meaningful indicators of soil fertility (Jemo et al., 2018; He et al., 2018). The contrasting cropping systems in our study had significant impacts on the aforementioned soil attributes (Supplementary Fig. 3). Likewise, these key soil properties have also significant impacts on AMF community composition, since they can moderate AMF community structure (Zhang et al., 2020; Alami et al., 2020). In turn, alteration of the AMF community can also impact ecological processes such as nutrient absorption and translocation by plants (Xiao et al., 2020). From a broader perspective, AMF community composition can also influence overall C and N dynamics in ecosystems (Wang et al., 2018). In our study, resource availability diverged across grain cropping systems; however, the measured soil properties accounted for only 2 % of the shifts in AMF community composition. Thus, even though labile pools of soil C and N changed with treatments in our study, they were not the main drivers altering the overall AMF assemblages.

Additionally, a better understanding of how cropping systems impact AMF can emerge from correlation analyses between AMF community taxa and selected soil properties (Alami et al., 2020). Certain AMF genera were significantly affected by specific soil properties as abovementioned. For instance, in Breton, POM-C was negatively correlated with *Archaeospora*, but positively correlated with *Paraglomus*, while NH_4-N was positively correlated with

Claroideoglomus specifically at the Edmonton site. Interestingly, these individual genera responded differently to these two key soil properties at the two contrasting study sites. NO_3-N did not have any significant correlations with any particular AMF taxa; this can be attributed to AMF preference for ammonium. This finding is in line with He et al. (2018) who reported that AMF have higher uptake and assimilation rate of NH_4-N than that of NO_3-N while facilitating enhanced plant N acquisition.

3.5.4. Dominance of Paraglomus in the Canadian Prairie Soils

Paraglomus was the most ubiquitous genus across treatments at both sites (Breton, 90.5%; Edmonton, 74.2%). Both sites may be favorable habitat for *Paraglomus* species, likely in association with predominant soils and climate (Ezeokoli et al. 2020). Several studies have demonstrated that *Paraglomus* is well adapted to agricultural soils. Long term agricultural practices can lead to gradual soil acidification, which actually becomes favorable for *Paraglomus* species (Bainard et al., 2014; Rezacova et al., 2019; Avio et al., 2020). Bainard et al., (2014) has shown that *Paraglomus* was highly dominant in AMF colonization of common crop roots within the Canadian Prairies, under a similar climatic condition as our study sites. Although *Paraglomous* in our study was widespread in both bulk soil and root compartments, the incidence of *Paraglomus* species was even more dominant in the bulk soil than with the roots.

3.5.5. AMF Community Composition in Bulk Soil and Roots

Shifts in AMF community in bulk soil and roots have been observed across diverse crop species and varying environmental conditions (Higo et al., 2015). In line with previous studies, which reported the discrepancy of AMF diversity and community composition at different microsites (bulk soil vs. rhizosphere), our findings also showed significant changes in AMF community structure between soils and roots irrespective of cropping systems (Azeem et al., 2020, Zhao et al., 2020). Possibly, bulk soil compartment included AMF hyphae and inactive AMF structures, while root samples represented AMF that were actively colonizing and more likely to be engaged in nutrient exchange with the crops. Some AMF genera produce more hyphae than others or colonize shorter distances from roots into bulk soil compartment with relatively more abundance in the roots compartment (Hempel et al., 2007). Therefore, AMF genera may be underrepresented in the bulk soil over roots. In addition, uneven nutrient availability across microsites within the soil might be a decisive factor driving the spatial distribution of AMF. Overall, growth of microbes such as AMF are sensitive to resource availability such as root exudates, plant litter input, and photosynthates (Xiao et al., 2020; Azeem et al., 2020).

AMF selectively colonized particular host crop roots. *Paraglomus* genus was frequently found even more in the roots at both study sites. The dominance of *Paraglomus* is likely due to their widespread distribution and adaptation in our climatic regions. Conversely, *Archaeospora* and *Claroideoglomus* genera had lower presence in the roots compared to the bulk soil at the Edmonton site. This observation indicates host selection preference for particular genera. Additionally, AMF genera in the two compartments (i.e., bulk soil vs. roots) responded differently to selected soil properties (Supplementary Fig 8B and C), indicating that particular genera are better adapted to certain environmental conditions (Zhang et al., 2020). This study provides the first evidence of particular AMF genera association with specific environmental factors in contrasting cropping systems.

3.6. Conclusion

Our study explored AMF community composition across contrasting cropping systems (e.g., annual vs. perennial grain crops). AMF community composition under perennial grain cropping did not differ from annual grain, likely due to the genetic similarity of the two crops. Furthermore, the two contrasting study sites had different patterns of AMF alpha and beta diversities. This may be a result of differing inherent soil properties and management legacy effects across field sites. Environmental variables such as labile C and N in the soils were correlated with AMF community composition and specific AMF genera. *Archaeospora* were relatively sensitive to environmental alternations, whilst the most prevalent AMF genus was *Paraglomus* across all assessed agroecosystems. Thus, AMF communities appear to be impervious to annual vs. perennial cropping approaches, rather their most pronounced differences are field site specific.

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Tables

Table 3. 1. Overview of the main soil characteristics (0–15 cm a depth) in Breton and Edmonton (Kim et al., 2021).

	Units	Breton	Edmonton
Classification		Orthic Gray Luvisol	Black Chernozem
TOC†	g C kg^{-1} soil	27.5±2.9	53.7±2.9
TN†	g N kg^{-1} soil	2.21±0.18	4.67±0.29
Texture		Silty clay loam	Clay
Clay	%	31.1	47.0
Silt	%	49.1	36.0
Sand	%	19.8	17.0
pH‡		5.7	7.2
EC‡	dS m^{-1}	0.83	1.5
Cumulative Precipitation	mm yr^{-1}	551.4§ [388.8]¶	441.5§ [314.3]¶
Air Temperature	ºC	3.07§ [11.46]¶	3.15§ [12.36]¶

†Soil total organic carbon (TOC) and total nitrogen (n=4±SE) were measured by dry combustion (Brachmann et al., 2020), texture measured by hydrometer (Hebb et al., 2017).

§Annual cumulative precipitation (Alberta Climate Information Service).

¶Growing season cumulative precipitation (April-September; Alberta Climate Information Service).

Table 3. 2. Description of cropping systems and field activities at both Edmonton and Breton (Kim et al., 2021).

Abbreviated ID	Description	Seeding Dates		Harvest Dates	
		Edmonton	Breton	Edmonton	Breton
Fallow	Continuous fallow using Roundup® and D ester herbicide since June 2017				
Spring-grain	Spring rye cropping system for grain production. *Secale cereale* L. cv. Gazelle. Tillage was used for crop establishment every year in the mid spring. Annual rye or summer rye are alternative designations in the literature.	22 May 2018; 23 May 2019	23 May 2018; 21 May 2019	19 Sep 2018; 25 Sep 2019	19 Sep 2018; 24 Sep 2019
Fall-grain	Fall rye cropping system for grain production. *Secale cereale* L. cv. Hazlett. Tillage was used for crop establishment every year in the late summer. Winter rye or biennial rye or are alternative designations in the literature.	29 Aug 2017; 3 Sep 2018	30 Aug 2017; 03 Sep 2018	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 24 Sep 2019
Perennial-grain	Perennial rye cropping system for grain production. *Secale cereale* L. × *S. montanum* Guss cv. ACE-1. Tillage was used for crop establishment only in the late summer 2017.	29 Aug 2017	30 Aug 2017	24 Aug 2018; 25 Sep 2019	23 Aug 2018; 16 Sep 2019
Perennial-forage	Perennial forage cropping system for hay production. Alfalfa *Medicago sativa* L. and bromegrass *Bromus spp*. Aboveground biomass is cut and carry two times a year for hay with livestock feeding purposes. Tillage was used for stand establishment only in the mid summer 2017.	16 June 2017	8 June 2017	26 June 2018; 31 Aug 2018; 16 July 2019; 17 Sep 2019	26 June 2018; 30 Aug 2018; 15 July 2019; 16 Sep 2019

Table 3. 3. Arbuscular mycorrhizal fungi alpha diversity. Average and standard errors [n=4 (fallow) and 8 (spring-grain, fall-grain, perennial-grain and perennial-forage) ± SE] of soil samples in Breton and Edmonton across treatments in metrics including Chao1 richness, Peilou's evenness, Shannon's diversity, and inverse Simpson's diversity. Italic number means P-value.

Treatment	Chao1	Evenness	Shannon	InvSimpson
	Breton			
Fallow	76.55±17.39	0.44±0.11b¶	1.91±0.54b	3.65±2.03b
Spring-grain	79.82±32.04	0.31±0.08ab	1.36±0.45ab	2.25±0.78ab
Fall-grain	80.11±14.77	0.32±0.07ab	1.42±0.38ab	2.12±0.59ab
Perennial-grain	74.58±13.53	0.29±0.07a	1.21±0.30a	1.82±0.42ab
Perennial-forage	66.39±24.56	0.24±0.06a	1.00±0.37a	1.57±0.30a
Overall mean ± SE	75.37±21.46	0.31±0.09	1.32±0.45	2.13±0.98
P-value	*0.49*	*<0.01*	*<0.01*	*<0.01*
	Edmonton			
Fallow	30.30±10.10	0.48±0.12	1.57±0.39	3.33±01.17ab
Spring-grain	31.39±20.81	0.47±0.26	1.32±0.62	2.76±1.03ab
Fall-grain	35.08±15.61	0.54±0.16	1.71±0.21	3.55±0.82ab
Perennial-grain	40.95±20.79	0.46±0.15	1.40±0.45	2.72±1.11a
Perennial-forage	40.40±9.30	0.51±0.07	1.87±0.29	4.44±1.55b
Overall mean ± SE	36.22±16.33	0.49±0.16	1.58±0.45	3.36±1.28
P-value	*0.65*	*0.87*	*0.06*	*<0.05*

¶Pairwise comparisons were performed with Tukey HSD test after ANOVA.
Lowercase letters signify significant difference between treatments based on Tukey HSD test after ANOVA

Table 3. 4. Arbuscular mycorrhizal fungi alpha diversity. Average and standard errors (n=16 at Breton, n=24 at Edmonton) of soil and root samples on Breton and Edmonton sites across treatments in metrics including Chao1 richness, Peilou's evenness, Shannon's diversity, and inverse Simpson's diversity. Italic number means P-value.

Treatment	Chao1	Evenness	Shannon	InvSimpson
	Breton			
Soil	79.96±24.10	0.32±0.08a¶	1.39±0.40	2.18±0.67
Roots†	70.46±17.46	0.38±0.08b	1.61±0.39	3.00±1.51
	0.17	*<0.05*	*0.13*	*0.06*
	Edmonton			
Soil	35.81±18.80	0.49±0.19b	1.48±0.47	3.01±1.03
Roots	39.61±14.12	0.36±0.17a	1.32±0.70	3.05±2.06
	0.42	*<0.05*	*0.36*	*0.42*

¶Pairwise comparisons were performed with Tukey HSD test after ANOVA.

Lowercase letters signify significant difference between treatments based on Tukey HSD test after ANOVA

†Roots dataset for Breton site only excludes perennial grain crop.

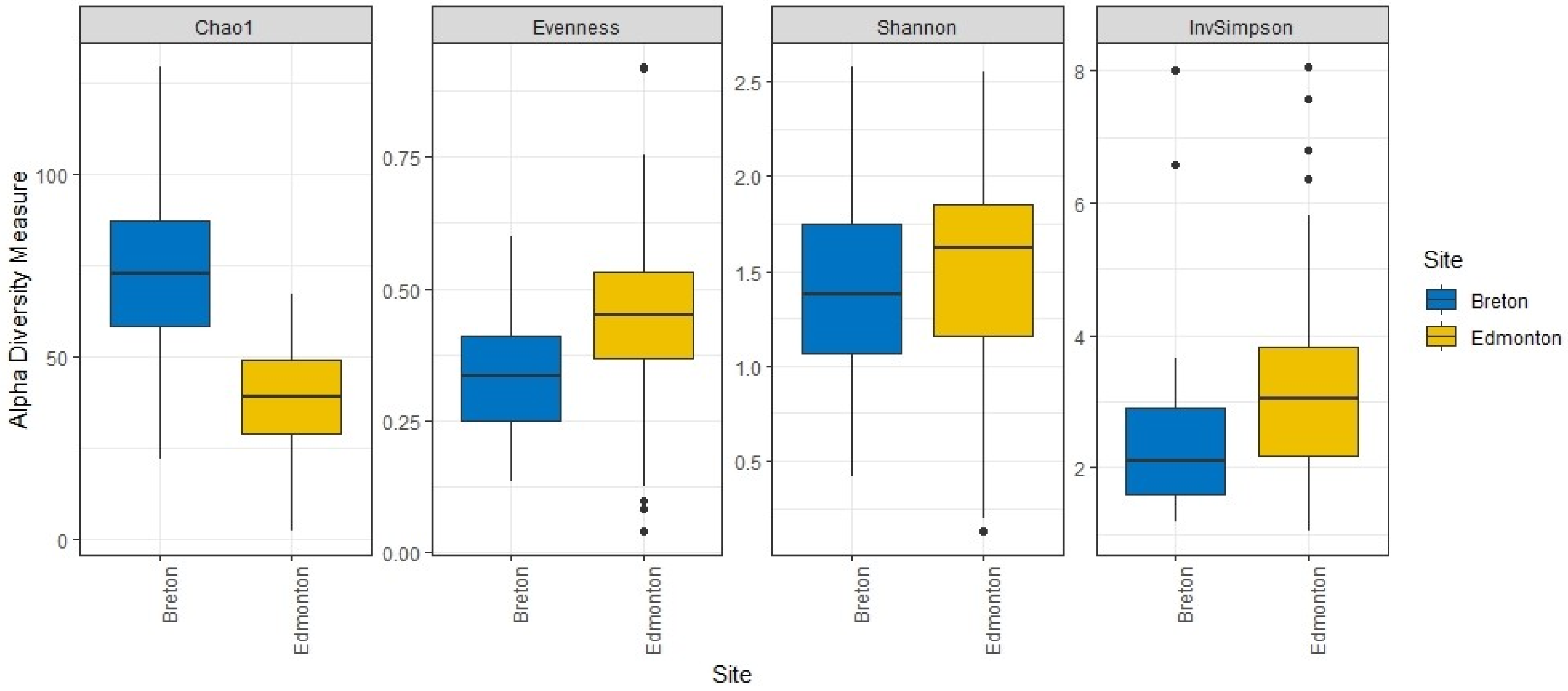

Fig. 3. 1. Arbuscular mycorrhizal fungi alpha diversity index including significance of Chao1 (*P<0.001*), Evenness (*P<0.01*), Shannon (*P=0.48*) and Inverse Simpson (*P<0.01*) between sites.

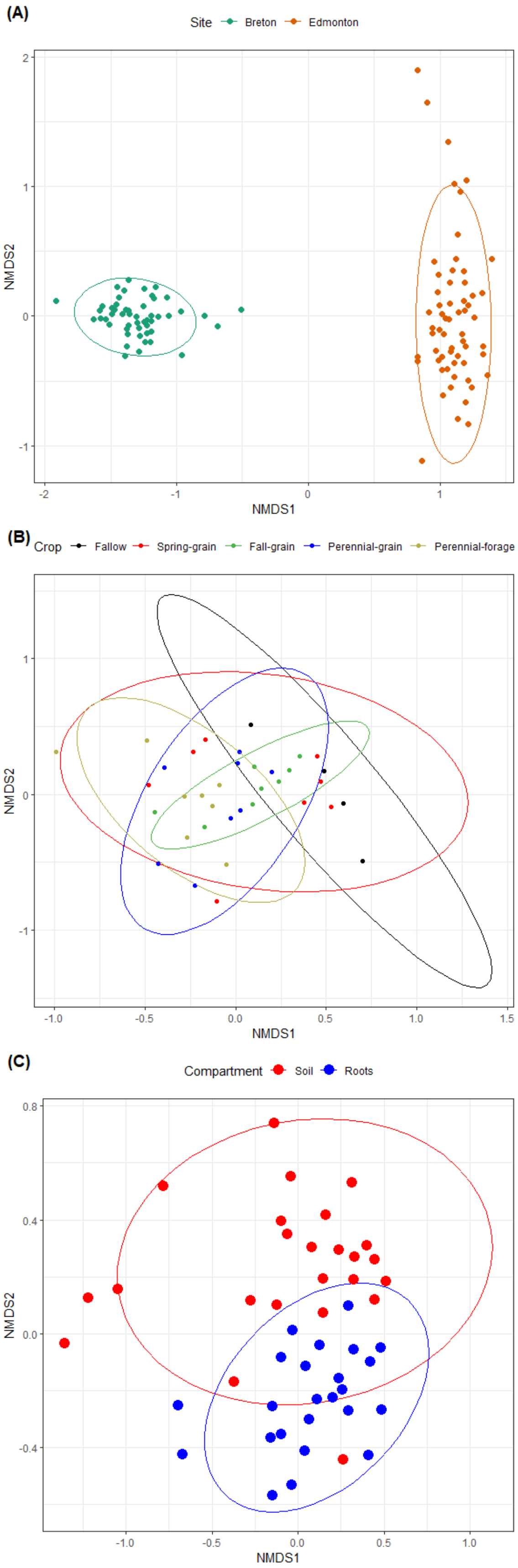

Fig. 3. 2. Non-metric multidimensional scaling (NMDS) plots of arbuscular mycorrhizal fungi communities based on Bray-Curtis distances. Circles are 95 % confidence ellipses of the comparison, community composition significantly differs between: (A) site; Breton and Edmonton (PERMANOVA, *F* = 60.075, *P* < 0.001), (B) cropping systems in Breton; fallow, spring-grain, fall-grain, perennial-grain, perennial-forage (PERMANOVA, *F* = 2.644, *P* = 0.004), (C) compartments in Edmonton; soil and roots (PERMANOVA, *F* = 8.962, *P* = 0.001).

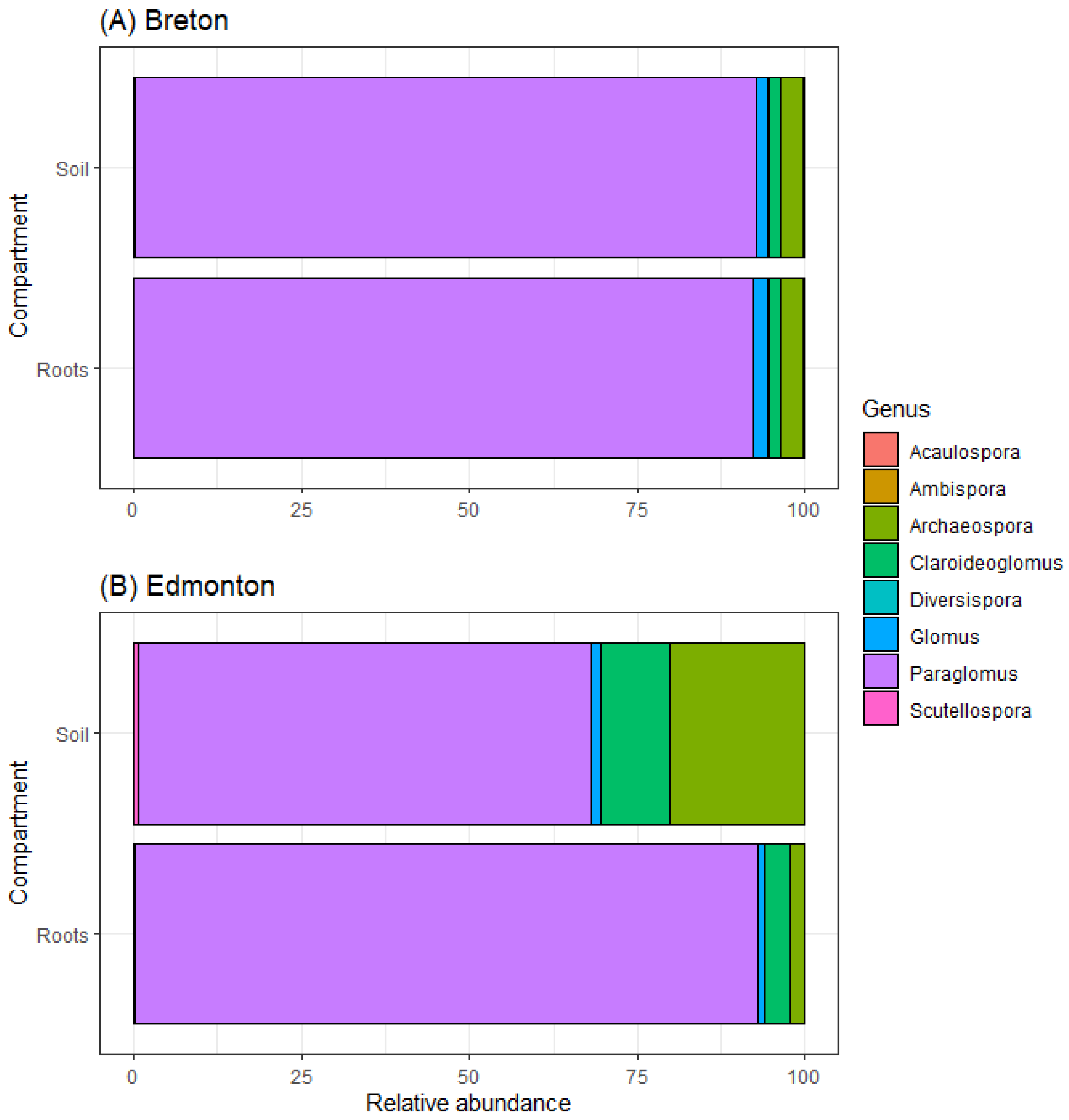

Fig. 3. 3. Proportional composition of arbuscular mycorrhizal fungi (AMF) root and soil communities. Panel indicates proportional sequence abundance at site: (A) Breton, (B) Edmonton, and legend shows phylogenetic level to identified AMF genus.

CHAPTER 4. Carbon and Water Dynamics of a Perennial Grain Crop in Temperate Agroecosystems

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4.1. Abstract

Interest in perennial grain crops has increased over the past decades as a sustainable alternative to conventional annual grain crops due to their carbon sequestration and water use efficiency (WUE) potentials. This study has quantified carbon dioxide (CO_2) and water (H_2O) fluxes over contrasting cropping systems, including a perennial grain crop (i.e., *Secale cereale* L. × *S. montanum* Guss cv. ACE-1 perennial rye) vs. an annual grain crop (i.e., *S. cereale* L. cv. Gazelle spring rye). The experiment was conducted over two continuous growing seasons using co-located eddy covariance systems in two adjacent 4-ha fields situated in Breton, Central Alberta, Canada. Substantially greater sequestration of atmospheric C was observed in the perennial grain systems compared to the annual grain cropping systems through both net ecosystems exchange (NEE) and net ecosystem carbon balance (NECB) (yearly NEE of -44.7 and 13.9 g C m^{-2} yr^{-1} vs. -445.4 and -696.9 g C $m^{-2}yr^{-1}$; yearly NECB of 357.3 and 386.3 g C m^{-2} yr^{-1} vs. 27.6 and -448.9 g C m^{-2} yr^{-1}). In addition to longer growing periods, which enhanced the cumulative C captured via photosynthesis, the significantly greater net CO_2 sink in perennial grain crops was attributed to relatively lower ecosystem respiration (R_e), which was influenced by soil temperature sensitivity (Q_{10}), reduced soil disturbance, soil moisture, and weather factors. Overall, ecosystem carbon use efficiency (CUE_e) of perennial grain crops was greater than annual grain crops (yearly CUE_e of 0.04 and -0.01 g C g C^{-1} vs. 0.35 and 0.46 g C g C^{-1}). Despite perennial grain crops registering higher evapotranspiration (ET) at the beginning of growing season, overall cumulative ET for the entire growing season and the associated ecosystems-water use efficiency (WUE_e) were alike across the two cropping systems. As such, perennial grain crops can sequester significantly more carbon into croplands, without strongly altering terrestrial water balance.

Keywords:

Perennial grain crop, eddy covariance, net ecosystem exchange, carbon use efficiency, water use efficiency

Highlights:

- Perennial grain crops enhance carbon sequestration compared to annual grain crops at the ecosystem level.
- Temperature and precipitation play an important role in regulating net ecosystem exchange.
- Perennial grain cropping systems do not strongly alter terrestrial water balance.

4.2. Introduction

The effects of global climate change are increasingly evident in rising ambient temperatures and sea levels (IPPC, 2007). Continually increasing atmospheric carbon dioxide (CO_2) levels are of major concern, as CO_2 is the foremost contributor to the acceleration of climate change processes in terrestrial ecosystems (Hernandez-Ramirez et al., 2011; Zanotelli et al., 2013; Amiro et al., 2017). From the perspective of agroecosystems, croplands can be a carbon sink or source of atmospheric $CO_{2,}$ based on the ecosystem C balance between soil organic C formation and decomposition mechanisms (Abraha et al., 2018; Sharma et al., 2019, Moore et al., 2020). However, a general trend of soil C loss has been observed in the majority of conventional row-cropping systems for decades (Amiro et al., 2017). Concurrently, sustainable development goals for 2030 require the mitigation of detrimental climate change repercussions by implementation of improved land management practices, while maintaining beneficial ecosystems services (United Nations, 2015).

Notably, cropping systems in the Canadian Prairies have continuously evolved to achieve sustainable agriculture, including the introduction of new crop species, cultivar development, and appropriate land management practices (Amiro et al., 2017). Perennial grain crops have been particularly spotlighted as a sustainable alternative to conventional annual grain crops, as they hold potentials for multiple desirable characteristics, including (i) mitigation of greenhouse gas emissions, (ii) high soil organic carbon accruals, (iii) a wide range of habitat adaptation, (iv) reduction in nutrient leakage, (v) increase in biodiversity, and (vi) low management input requirements, likely owing to their longer growing seasons, pervasive root systems, no-tillage management, and continuous ground cover (Eichelmann et al., 2016; Maleski et al., 2019; de Oliveira et al., 2020, Abraha et al., 2020; Kim et al., 2021). Subsequently, the merits of introducing perennial grain cropping systems may satisfy the demand for economically, ecologically, and socially sustainable cropping systems.

In addition to their favorable potential to diversify agroecosystems, novel perennial grain cropping systems likely alter terrestrial C dynamics by changing rates of soil C turnover as well as crop productivity (Zenone et al., 2013; Sharma et al., 2019). A comprehensive understanding of C dynamics plays a critical role in discerning management options that can regulate atmospheric CO_2 concentration and subsequent climate change. Nevertheless, the net ecosystem C exchange as a function of establishing of perennial grain cropping systems remains elusive (Wagle et al., 2019; de Oliveira et al., 2020). Thus, our multi-year study addressed and evaluated net ecosystem carbon balance (NECB), seasonal variations in the components of net ecosystem exchange (NEE), and their environmental controls in two contrasting cropping systems: annual vs. perennial grain cropping. In addition, carbon use efficiency (CUE) was also investigated to determine how efficiently perennial grain crops could sequester carbon from the atmosphere (Zanotelli et al., 2013; Chen et al., 2018).

Additionally, the eco-hydrological cycle is one of the major drivers involved in plant productivity through physiological and metabolic processes (Eichelmann et al., 2016; Zhao et al., 2021). Previous studies have shown that the frequency and extent of widespread drought events will increasingly affect North America as a result of escalating climate change. This underscores the need for a critical understanding of the evaporative water (H_2O) fluxes and overall water balance at terrestrial surfaces as a function of land use conversions (Eichelmann et al., 2016; Hatfield and Dold, 2019). Two parallel hypotheses focusing on water cycling in novel perennial grain cropping systems can be postulated. Perennial grain crops may lead to reduced runoff and higher soil water content due to the absence of tillage and relatively higher plant litter remaining on the soil surface (Abraha et al., 2015). Conversely, the longer growing season of perennial grains has the potential to increase water usage, which subsequently reduces moisture in the soil profile. To diminish water stress and satisfy water demands, the pervasive roots system of perennial grain crops could enable the extraction of groundwater from deep soil layers (Virgilio et al., 2019). The findings from this study will provide a basis for future research to examine how distinct perennial grain crops could have differential impacts on hydrological and edaphic processes compared to conventional annual grain crop counterparts (Anapalli et al., 2019; de Oliveira et al., 2020).

To our knowledge, this is the first empirical assessment on how perennial grain crops affect net ecosystem C and water exchanges compared to an adjacent, conventional annual counterpart. This research addressed the following questions by utilizing continuous eddy covariance (EC) measurements in co-located, paired contrasting cropping systems: i) what are the seasonal and inter-annual variations of NEE and evapotranspiration (ET) dynamics in these two contrasting cropping systems?, ii) how do weather factors influence the NEE components?, and iii) what are the outcomes of net ecosystem carbon balance (NECB), CUE, and WUE in these two contrasting cropping systems?

4.3. Materials and Methods

4.3.1 Site description

The experimental site (53°08’52” N, 114°42’23” W) was located at the University of Alberta Breton Research Station, Alberta, Canada. The study region has a humid continental climate based on the Köppen classification system, with an annual cumulative precipitation of

526.8 mm and an annual mean air temperature of 3.51 ºC (2010-2019; 10 yrs; Table 4.1). The soils at the site are classified as Orthic Gray Luvisols with silty clay loam texture. The landscape features are lightly sloping with moderate relief. Initial soil physiochemical properties of the surface layer (0-15 cm) are presented in Table 4.1.

Perennial-grain crops (*Secale cereale L.* × *S. montanum Guss cv. ACE-1 rye*) and annual-grain crops (*Secale cereale L. cv. Gazelle rye*) were planted at two adjacent 4-ha fields (Supplementary Fig. S4.1). Conventional management practices and fertilization rates for the region were followed for the duration of the experiment. Typical growing seasons for annual crops in the Canadian prairies are from May to October. Seeding for the annual grain crop was conducted every year (i.e., 2018 and 2019) in late May (Table 4.2). In contrast, perennial grain crops were sown in early September in 2017 (the growing season prior to the first seeding of the annual crop). Note that once seeded in September 2017, the perennial grain field did not require additional seeding or tillage operations (Table 4.2). Nitrogen (N) fertilizer additions for both crops were applied concurrent with the seeding of the annual grain crop each year at a rate of 56 kg N ha^{-1}. The N fertilizer source was a granular blend of urea and polymer-coated urea (environmentally smart nitrogen, ESN™) in a 2:1 ratio.

4.3.2. Eddy covariance and weather station measurements

Open-path eddy covariance (EC) systems were established near the center of each 4-ha filed in May 2018 and have been continuously recording for the 2 years of this study to calculate CO_2 flux [i.e., the sum of gross primary production (GPP) and respiration ecosystem (R_e)] and total evaporative water flux (i.e., the sum of plant transpiration and evaporation from soil and canopy surfaces). EC instrumentation was comprised of a LI-7500 open-path infrared gas analyzer (IRGA, LI-COR bioscience, Lincoln, NE, USA) for measuring CO_2 and water concentration and a 3-D CSAT3 sonic anemometer (Campbell Scientific Inc.; CSI, Logan, UT, USA) for recording lateral, longitudinal, and vertical wind speed and sonic temperature. These units were oriented to align with the dominant wind direction (i.e., facing to the west). Wind components and densities for CO_2 and H_2O were collected at a frequency of 10Hz to compute high-quality eddy flux. The height of the EC measurement was adapted according to the growing crop height (h) (approximately >1.8 m + h). Ancillary measurements of soil temperature (°C) and soil volumetric water contents (VWC; % v/v) were conducted at three depths (7.5, 22.5, and 40 cm) below the soil surface using a set of 5TM soil sensors (*Meter, Washington, USA)* connected to automated EM50 data loggers *(Meter, Washington, USA).* Soil VWC data was converted into water filled pore space (WFPS) using soil bulk density data to account for soil porosity.

A permanent weather station was deployed within 500 m from the research site over a grassed area. This instrumentation provided continuous measurements of air temperature, solar radiation, precipitation, relative humid, and vapor pressure deficit (VPD) (https://acis.alberta.ca/weather-data-viewer.jsp; Supplementary Fig. S4.1).

4.3.3.. Data processing

Data processing of eddy-covariance measurements was implemented in three sequential steps; correction, filtering, and gap-filling to obtain reliable 30-min CO_2 and water fluxes. Primary data processing was computed by using Eddypro 7.0.6 software (LI-COR Bioscience, Lincoln, NE, USA). This processing approach was performed for standard flux corrections including 'W-boost' bug corrections, tilt corrections, frequency response corrections, spike removal, and Webb-Pearman-Leuning (WPL) corrections (Webb et al., 1980). Rigorous data filtering processes were implemented by specific standards, which consist of quality control parameters, field origin rates of footprint calculation, low friction velocity (u* threshold < 0.1 m/s), and implausible CO_2 and water fluxes. Less than 70 % of flux measurements derived from within the filed were rejected by a backward Lagrangian footprint model (Flesch, 1996). Occasionally, data was missed due to power failure or maintenance. Initial 30-min flux measured data points numbered 18371 for the annual grain crop and 19064 for the perennial grain crop derived from the EC systems; of which, about 52.9 % and 52.9 % of the collected data in the annual and perennial grain crops were filtered and passed the quality check criteria, respectively. Gap-filling and partitioning procedures were conducted using REddyproc package in R from the Max Planck Institute for Biogeochemistry, Germany (Reichstein et al., 2005; Wutzler et al., 2018). This function enabled the estimation of half-hourly NEE, GPP, and R_e. The marginal distribution sampling (MDS) gap-filling method was implemented, as well as a combination of Look-up tables and mean diurnal variation (Wutzler et al., 2018; Fig. 4.5, Supplementary Fig. S4.2, Supplementary Fig. S4.3, Supplementary Fig. S4.4).

Negative values of NEE are indicative of a CO_2 sink, whereas positive values of NEE indicate CO_2 release from the fields in this study. Partitioning of NEE into GPP and R_e was completed by using an Arrhenius-based model for nighttime fluxes and using a common rectangular hyperbolic light-response curve for daytime fluxes (Keenan et al., 2019). Gross primary production was simply calculated by subtracting R_e from NEE.

4.3.4. Flux terminology

Based on the eddy covariance data, the ecosystem CO_2 autotrophic fixation per unit of time and area is defined as gross primary productivity (GPP; negative sign), while the ecosystem respiration per unit of time and area is called ecosystem respiration (R_e; positive sign). As noted above, net ecosystems exchange (NEE) represents the amalgamation between GPP and R_e fluxes.

$$NEE = GPP + R_e \tag{1}$$

Net ecosystem production (NEP) refers to the net flux of CO_2 from ecosystem where NEP equals to negative NEE.

$$NEP = -NEE \tag{2}$$

Carbon use efficiency (CUE) represents how efficiently cropping systems store C from the atmosphere (Chen et al., 2018). Ecosystem carbon use efficiency (CUE_e) is commonly defined as NEP over GPP (Chen et al., 2018; Mendes et al., 2020). Harvest carbon use efficiency (CUE_h) represents an agronomic perspective of CUE where only harvested biomass (C removals as harvest) is considered, it is defined as dry matter or C amount in biomass over GPP.

$$CUE_e = \frac{NEP}{GPP} \tag{3}$$

$$CUE_h = \frac{DM_{harvested}}{GPP} \ or \ \frac{C_{harvested}}{GPP} \tag{4}$$

The cumulative NEE is added to C content in harvested biomass to obtain net ecosystems carbon balance (NECB); the values are then integrated over the total measurement period (Hernandez-Ramirez et al., 2011; Virgilio et al., 2019).

$$NECB = NEE_{cumulated} + C_{harvested} \tag{5}$$

Water use efficiency (WUE) describes how efficiently the crop can assimilate carbon from the atmosphere over a given period relative to the water usage in the same period via evapotranspiration to the atmosphere, encompassing the ground and canopy levels (Eichelmann et al., 2016; Jiang et al., 2020). Ecosystem water use efficiency (WUE_e) was defined as the GPP over ET, and the harvest water use efficiency (WUE_h) was defined as dry matter or carbon in harvested biomass over ET or. In agronomy, WUE_h is commonly used interchangeably with crop water productivity (CWP), which is a measurement of crop yield per unit of water consumed (Peddiniti et al., 2020).

$$WUE_e = \frac{GPP}{ET} \tag{6}$$

$$WUE_h = \frac{DM_{harvested}}{ET} \ or \ \frac{C_{harvested}}{ET} \tag{7}$$

To determine difference in energy partitioning of turbulent fluxes, Bowen ration (β) was calculated as sensible heat (H) over latent heat (LE) fluxes (Hernandez-Ramirez et al., 2010).

In order to conduct comparisons of cumulative seasonal fluxes of CO_2 and water across the assessed crops and growing seasons, we considered the following duration of growing seasons: i) 2018 May 28 – 2018 October 31, and ii) 2019 May 15 – 2019 October 31.

4.3.5. Soil chamber measurements

To examine and compare fluxes of CO_2 based on EC systems with soil surface chamber measurements, seven chambers were arranged as a transect within each 4-ha field during the 2019 spring-thaw season. The non-flow-through, non-steady-state chamber method was used. Dimensions of the chamber were 15 cm width by 65 cm length and 17 cm height (Kim et al., 2021). The chamber base was inserted to a depth of 5 cm in the soil. During measurements, the chamber headspace was sealed using an opaque detachable lid outfitted with a rubber septum. Three 20 mL gas samples were taken at 20, 40 and 60 min after chamber closure through the septum port using a syringe and injected into 12 mL pre-evacuated vials. Sample collection was conducted between 10 am and 2 pm for consistency.

The gas samples were analyzed by a gas chromatograph (Trace 1310 Gas Chromatograph, *Thermo Fisher Scientific, Delft, Netherland*) equipped with thermal conductivity detector (TCD). The CO_2 fluxes were calculated by plotting a linear or exponential regression between measured CO_2 concentration versus time using a modified idea gas law as follows:

$$F = \frac{SPV}{ART} \times 12.01\ g\ C\ mol^{-1} \tag{8}$$

Where flux (F) is gaseous rate of analyte (μg CO_2-C m^{-2} hr^{-1}); S is the slope of the linear regression or derivative at time zero of the quadratic regression; P is ambient pressure (Pa); V is headspace volume of each chambers (L); A is the surface area with the chamber (m^2); R is gas constant (Pa L $K^{-1}mol^{-1}$), and T is ambient air temperature (K). The multiplication by 12.01 g C mol^{-1} is a conversion to C mass of CO_2 flux.

4.3.6. Additional plots for replicated measurements of crop productivities and soil attributes

Crop growth and soil properties were examined in experimental plots (4 m × 8 m) established in a randomized complete block design (RCBD) with four replicates. These replicated plots were located adjacent to the 4-ha fields where eddy covariance systems were set up (Supplementary Fig. S4.1). A detailed description of agronomic activities was previously provided in Kim et al. (2021). Aboveground dry biomass data was destructively sampled one week prior to the dates of grain harvest by using 0.5 m × 0.5 m quadrats. Grain and biomass samples were oven dried at 65°C for 7 days to reach constant weight. Thereafter, dried biomass fractions were further pulverized by using a ball mill. Ground sub-samples were weighted into tin capsules and analyzed for C content via dry combustion.

4.3.6.1. Statistical analyses

Treatment effects for crop biomass were analyzed using a linear mixed effects (LME) model to account for block effects as confounding, random factors. Normality and homoscedasticity of the model residuals were evaluated by Shapiro-Wilk and plot functions, respectively (NLME package of 3.2.1; R core Team, 2015). If these assumptions were violated, the Box-Cox transformation was applied to the dataset. Tukey's Honest Significant Difference (HSD) test was used for post hoc comparison of means.

4.4. Results

4.4.1. Weather conditions

Cumulative annual precipitation during research years was drier in 2018 (486.5 mm) and wetter in 2019 (596.0 mm) than the 10-year (2010-2019) mean (526.8 mm) (Table 4.1). Correspondently, cumulative precipitation over each growing season (i.e., planting to harvest; May-October) was 341.0 mm and 501.0 mm in 2018 and 2019, respectively, whereas cumulative 10-year means of the same period were approximately 401.3 mm. In comparison, total and growing season precipitation in 2019 were 22% and 46% wetter than in 2018, respectively (Table 4.2). This can be attributed to substantial rainfall events in June and July 2019, which were collectively 52 % of the annual precipitation (Fig. 4.1).

Regarding air temperature, the 2019 growing season had a mean temperature 2.7 °C lower than the 2018 growing season (Table 4.2). As a result, the 2019 growing season was colder and wetter than the 2018 growing season.

The overall wetter soil status in 2019 may have contributed to the large failure in perennial crop establishment, which resulted in comparatively higher weed emergence and growth within the study location. The plant community composition at harvest in 2019 was perennial-grain rye (54.2 ± 9.8 %), miscellaneous grass species (20.5 ± 4.8 %), clover (12.5 ± 2.1 %), timothy (11.0±5.7 %), and rye grass (1.6±1.5 %) (Table 4.2). The weed growth was challenging to control, and this reduced grain yields through direct competition. Consequently, the perennial grain crop was harvested primarily as forage biomass at an earlier grain development stage in 2019. Excess soil moisture in 2019 also resulted in inconsistent vegetation cover in both the annual and perennial crop fields.

Soil moisture was sensitive to the magnitude and timing of precipitation (Fig. 4.1). Precipitation caused increases in WFPS at 7.5 cm depth, despite an overall gradual decline in WFPS was observed over the growing season likely due to continual ET by the crops (Fig. 4.1). During the non-growing seasons, plants did not actively uptake water and the soil profile was recharged to its maximum soil water content (Fig. 4.1). Noticeably, perennial grain crops at 7.5 cm depth had higher WFPS than annual grain crops during the second growing season.

Variability in air and soil temperature, solar radiation, vapor pressure deficit (VPD), relative humid (rH) are presented in Fig. 4.2 and Fig. 4.3. In general, seasonal patterns of soil temperature and VPD matched for the two cropping system types. Relative humidity was less than 50 % on average and VPD was consistently similar for both crops (Fig. 4.3).

4.4.2. Crop growth and phenology

The perennial grain crop developed faster in the early growing season, relative to the annual crop, maintaining a higher crop height until mid-July. The maximum height of the perennial grain crop ranged from 135 to 150 cm. Towards to the end of the growing season, the height difference between the perennial and annual grain crops narrowed. In fact, the height of the annual grain crop surpassed the perennial grain crop by August in both growing seasons (Fig. 4.4).

Dry matter (DM) grain yields of annual grain crops were 3.52 ± 0.59 and 2.86 ± 0.18 Mg DM ha^{-1} in 2018 and 2019, which was significantly higher than the grain yields for the perennial; 2.81 ± 0.18 Mg DM ha^{-1} for 2018 and 1.45 ± 0.12 Mg DM ha^{-1} for 2019, respectively ($Ps < 0.05$; Table 4.2). Relatively higher grain yield and strong wind conditions led to the lodging of the annual grain crop prior to harvest in 2018 (Supplementary Table 4.1).

As abovementioned, the productivity of the perennial grain crop in 2019 was compromised by weather and weed pressure therefore the harvest of these plots was implemented in two ways: perennials as a grain crop, and as forage. Aboveground biomass (i.e., grain + straw) for the annual crop was comparatively reduced relative to the perennial crop for both consecutive growing seasons, when considering the perennial crop solely as forage in 2019, although statistical significance was not found ($Ps > 0.05$; Table 4.2). In contrast, a substantial reduction of grain, straw, and total aboveground biomass in perennial crop was observed compared to annual grain crop when perennials were considered as a grain crop in 2019 ($Ps < 0.05$, Table 4.2). Although the perennial grain crop overall had numerically higher C content in the aboveground biomass than the annual grain crop, this comparison was not statistically significant for both 2018 and 2019 ($Ps > 0.05$; Table 4.2).

4.4.3. CO_2 fluxes

4.4.3.1. Ecosystems respiration (R_e)

Estimated daily respiration fluxes from the perennial grain crop were 5.61 ± 0.32 and 3.20 ± 0.16 g C m^{-2} day^{-1} for the 2018 and 2019 growing seasons, respectively. Over the same period, average daily respiration fluxes in annual grain crops were 5.80 ± 0.34 and 8.30 ± 0.43 g C m^{-2} day^{-1}. Over the perennial grain crop field, the respiration fluxes peaked on 17 July 2018 with 15.7 g C m^{-2} day^{-1} and on 12 July 2019 with 10.8 g C m^{-2} day^{-1}, whereas respiration fluxes from the annual grain crop field peaked on 29 July 2018 at 17.3 g C m^{-2} day^{-1} and on 31 July 2019 at 23.1 g C m^{-2} day^{-1} (Fig. 4.8). At these peak times, daily respiration fluxes of the perennial grain crop were comparatively lower than the annual grain crop; which was particularly pronounced in the second year. In addition, the annual grain crop field reached its peak R_e fluxes later than the perennial grain crop. Although the magnitude of fluxes between annual and perennial grain crops differed, the overall pattern of R_e fluxes agreed over both growing seasons (Fig. 4.8). The two crops showed small R_e peaks at harvest, thereafter daily respiration fluxes of CO_2 were minimal until the onset of winter. Cumulative growing-season R_e in the perennial grain crop field amounted to 875.7 and 541.0 g C m^{-2} in 2018 and 2019, respectively. In comparison, the annual grain crop had 904.1 and 1402.0 C m^{-2} in 2018 and 2019, respectively (Table 4.2 & Fig. 4.8)

4.4.3.2. Gross primary production (GPP)

Estimated daily mean GPP fluxes from the perennial grain crop were 8.27 ± 0.53 and 9.06 ± 0.35 g C m^{-2} day^{-1} for the two successive growing seasons, while at the same time the fluxes in annual grain crop averaged 6.70 ± 0.52 and 8.52 ± 0.53 g C $m^{-2}day^{-1}$. Peak daily GPP fluxes for the perennial grain crop were 20.9 and 20.2 g C m^{-2} day^{-1} on 2018 June 12 and on 2019 July 12, respectively. Annual grain crops exhibited peaks of 22.0 and 25.7 g C $m^{-2}day^{-1}$ on 2018 July 26 and 2019 July 25, respectively. In general, the daily peak GPP fluxes occurred at similar times to peak daily respiration fluxes.

The perennial grain crop had higher CO_2 uptake than annual grain crop during the initial months of the two growing seasons (i.e., May and June). Cumulative GPP fluxes from the perennial grain crop from May to June were significantly higher than those from the annual grain crops (i.e., in 2018, the perennial grain crop was 461.3, while the annual grain crop was only 187.5 g C m^{-2}; in 2019, the perennial grain crop was 594.1, while the annual grain crop was 249.2 g C m^{-2}). Conversely, the annual grain crop registered slightly higher or similar daily GPP fluxes than perennial grain crop during the remaining of the growing season (Fig. 4.8). As expected, substantial declines in GPP fluxes were observed following harvest operations. Overall, cumulative growing season GPP fluxes from the perennial grain crop were 1289.8 g C m^{-2} in 2018 and 1591.6 g C m^{-2} in 2019, which were greater than from the annual grain crop, with 1044.4 g C m^{-2} in 2018 and 1439.6 g C m^{-2} in 2019 (Table 4.2, Fig. 4.7, Fig. 4.8).

4.4.3.3. Net ecosystem exchange (NEE)

Symmetrical diurnal NEE cycles were observed from May to October, with peak fluxes observed around 1:00 PM (Fig. 4.5, Supplementary Fig. S4.2B, Supplementary Fig. S4.3B). The perennial grain crop had remarkable CO_2 uptake via higher photosynthetic activity during the daytime in June as well as in May. In addition, the CO_2 uptake for the perennial grain crop was relatively more discernible than the uptake for the annual grain crop in September and October, as the perennial crop registered regrowth after harvest. Focusing on the partitioning of NEE, the observed negative NEEs from the perennial grain crop were generated by comparatively higher

GPP fluxes than R_e fluxes. In contrast, the annual grain crop had very reduced photosynthetic capacity at the period of crop emergence and initial canopy development (i.e., May), which resulted in measured NEE fluxes that paralleled estimated respiration fluxes. Moreover, the annual grain crop showed stronger respiration compared to the perennial grain crop during the nighttime within the growing season for both growing seasons (Fig 4.5, Fig. 4.8). In general, nighttime CO_2 fluxes were negligible after August, likely due to crop senescence and freezing temperatures.

Daily NEE fluxes from the perennial grain crop averaged -2.86 ± 0.28 g C m^{-2} day^{-1} in 2018 and -4.12 ± 0.31 g C m^{-2} day^{-1} in 2019, while daily NEE fluxes from the annual grain crops were approximately C neutral (-0.28 ± 0.24 g C $m^{-2}day^{-1}$ in 2018, and -0.07 ± 0.23 g C $m^{-2}day^{-1}$ in 2019). The maximum CO_2 uptake NEE fluxes of the annual grain crop were -8.02 g C m^{-2} day^{-1} on 12 July 2018 and -7.30 g C m^{-2} day^{-1} on 16 July 2018. However, in the case of the perennial grain crop, the maximum NEE fluxes were -13.2 g C m^{-2} day^{-1} on 22 June 2018 and -12.1 g C $m^{-2}day^{-1}$ on 10 June 2019. Recall that the NEE fluxes from the annual grain crop were balanced out by R_e and GPP, so the NEE of the annual crop became very close to zero fluxes when encompassing both growing seasons as a whole. Furthermore, positive NEE fluxes were observed from the annual grain crop after crop senescence. Cumulative NEE fluxes from the perennial grain crop rapidly decreased when approaching harvest, after which time the perennial grain crop began accumulating carbon slowly for two growing seasons (Fig. 4.7). Overall, across the two assessed cropping systems, cumulative NEE of the annual grain crops showed neutrality (-44.7 g C m^{-2} in 2018, 13.3 g C m^{-2} in 2019), while the perennial grain crop field continuously exhibited strong net CO_2 uptakes (-445.4 g C m^{-2} in 2018, -697.0 g C m^{-2} in 2019) (Fig. 4.7,

Table 4.2). Thus, the perennial grain crop had a higher GPP and at the same time lower respiration fluxes when compared to the annual grain crop (Fig. 4.8).

4.4.4. Net ecosystem carbon balance (NECB) and carbon use efficiency (CUE)

Estimates of annual C extraction via NECB were 357.3 and 386.3 g C m^{-2} for the annual grain crop, and 27.6 and -448.9 g C m^{-2} for the perennial grain crop in 2018 and 2019, respectively. Net ecosystem carbon balance of perennial grain crop during the second year was substantially greater in terms of carbon sequestration at the ecosystem level.

Differences in harvest carbon use efficiency (CUE_h), as calculated based on dry matter and C content, were undiscernible across two crops. Yet, the perennial grain crop consistently exhibited considerably higher ecosystem carbon use efficiency (CUE_e) compared to the annual grain crop (i.e., the perennial grain crop was 0.35 and 0.46 g C g C^{-1}; annual grain crops were only 0.04 and -0.01 g C g C^{-1} in 2018 and 2019, respectively, Table 4.2). Comparably, weekly CUE_e in the perennial grain crop was continuously higher than the annual grain crop over the two growing seasons, except shortly following harvest events (Fig. 4.9).

4.4.5. Response of GPP and R_e to major climatic variables

Carbon dioxide (CO_2) fluxes (i.e., GPP and R_e) were strongly influenced by seasonal climate elements. Cumulative GPP for both crops had a strong linear relationship with cumulative precipitation (adjusted R^2s= 0.97 for both perennial grain and annual grain crops; Fig. 4.10A). Additionally, weekly R_e increased with increasing air temperature as described by the Arrhenius equation (i.e., perennial grain crop: R^2= 0.37, $P < 0.05$; annual grain crop: R^2= 0.61, $P < 0.05$; Fig. 4.10B). Soil temperature sensitivity (Q_{10} coefficient) under the perennial grain crop was 2.72, while the Q_{10} of the soil under the annual grain crop was much higher at

3.93. Optimum GPP occurred with a VPD of 5 hPa for annual grain crops, while for the perennial grain crop the optimum VPD was higher, at 5.6 hPa (Fig. 4.10C).

4.4.6. Water fluxes

The daily mean ET fluxes during both growing seasons were comparable across both crops, being 2.30 ± 0.12 and 2.51 ± 0.11 mm H_2O day^{-1} for the annual grain crop, and 2.65 ± 0.12 and 2.78 ± 0.10 mm H_2O day^{-1} for the perennial grain crop in 2018 and 2019, respectively. However, average daily ET fluxes during May and June from the perennial grain crop were slightly higher than for the annual grain crop (i.e., 2.55 ± 0.16 mm and 2.24 ± 0.13 mm for annual grain crop, and 3.95 ± 0.25 mm and 3.26 ± 0.15 mm for perennial grain crop in 2018 and 2019, respectively). Late in the growing season, the magnitudes of ET were similar between perennial and annual grain crops. ET for both crops increased at the beginning of growing season, and it thereafter declined until reaching grain fill or crop senescence. Annual grain crops peaked at ET at 5.88 mm day^{-1} on 29 July 2018, and at 8.07 mm day^{-1} on 25 July 2019, whilst the perennial grain crop peaked at 6.31 mm day^{-1} on 2018 June 20, and at 7.12 mm day^{-1} on 2019 July 25. Cumulative ET for the perennial grain crop was 413.58 mm in 2018 and 469.92 mm in 2019, whereas cumulative ET for annual grain crops was 358.8 mm in 2018 and 423.5 mm in 2019, respectively (Fig. 4.6, Fig. 4.7, Table 4.2). Overall, the perennial grain cropping system had slightly more evaporative water loss during the growing season compared to the annual grain cropping system. Cumulative ET of annual and perennial grain crops was 105.0 % and 121.0 % of the growing-season precipitation received in 2018, respectively. In contrast, annual and perennial grain crops evaporated about 84.6% and 93.8 % of the growing-season precipitation in 2019.

Although the perennial crop had relatively lower WUE_h based on grain yield and C content of the grain when considering it as a grain crop, higher WUE_h based on aboveground biomass and C content in the aboveground biomass was observed when the perennial crop was regarded as a forage crop. Ecosystem water use efficiency between the two cropping systems did not greatly differ across years (Table 4.2). Weekly WUE_e illustrated that the perennial grain crop had higher efficiency compared to the annual grain crop, particularly at the beginning of the growing seasons (Fig. 4.9).

4.4.7. Turbulent energy fluxes

The perennial grain crop registered more intense LE during the beginning of growing season than the annual grain crop. As the canopy developed, the LE fluxes from the annual grain cropping system gradually increased from May to August, which was opposite to the H fluxes for each growing season. As a result, the annual grain crop had a higher Bowen ratio, in particular in May and June, as opposed to the perennial grain crop. Collectively, higher Bowen ratios were observed later in the growing seasons for both crops (Fig. 4.12). The Bowen ratio of the cumulative energy fluxes for the perennial grain crop was less than that for the annual grain crop (i.e., perennial grain crop was 0.12 and 0.10; annual grain crop, 0.20 and 0.24 for 2018 and 2019, respectively; Table 4.2).

4.5. Discussion

4.5.1 Impacts of perennial grain cropping on the soil CO_2 sink

Perennial grain crops have been recently underscored as a means to mitigate greenhouse gas emissions. This notion further provokes the investigation into how different eco-physiological mechanisms and controls on greenhouse gas emissions operate in the perennial grain system compared to a conventional annual grain crop. Within a broader context, enhanced understanding of the aforementioned mechanisms and controls can aid in the development of sustainable agricultural polices to mitigate global climate change.

Over two consecutive growing seasons, the perennial grain cropping system behaved as a C sink, although it is noted that weed control represented a considerable challenge in the perennial grain crop in the second year. On the contrary, the NEE for the annual grain crop indicated a neutral C outcome. Overall, the ecosystem C assimilation (i.e., negative NEE) under the perennial grain crop was substantially greater than that under the annual grain crop. The discrepancy between two crops was particularly extreme in the early and late periods of both growing seasons. This is because perennial grain crop had a longer period covering the ground compared to the annual grain crops, in particular with earlier green ups in the spring and extend growing season in fall. The longer growing season and overall greater canopy height translated into extended and intense photosynthetic activity by the perennial grain crop. This greater cumulative GPP by the perennial grain crop supported a net C gain in the form of vegetative biomass (i.e., the perennial grain crop produced 461.3 and 594.1 g biomass-C m^{-2}, while the annual grain crop produced 187.5 and 249.2 g biomass-C m^{-2} for 2018 and 2109, respectively). This phenomenon is frequently observed in boreal perennial ecosystems, where greater C gains are a response to greater GPP, given that other conditions are favorable (Taylor et al., 2013).

In contrast, the perennial grain crop exhibited relatively lower R_e fluxes due to lower soil temperature in the spring. Likewise, our soil chamber measurements, which followed the spring thaw in 2019 indicated a coherent pattern in soil CO_2 fluxes, within which the annual grain crop had generally heightened CO_2 release than the perennial grain crop (Fig. 4.11).

Our partitioning of CO_2 into GPP and R_e showed that the annual grain crop had greater cumulative respiration fluxes, supporting that tillage management enhances soil organic matter decomposition by disrupting aggregates, mixing and exposing C in the soil (Ruan and Robertson, 2020; Abraha et al., 2018; Menefee et al., 2020). Additionally, the early senescence of roots and recently added residues in annual grain crop were probably readily decomposable, which differs in the conditions of the continuously growing root system of perennial grain crop. These attributes in annual grain crop may have contributed to increase soil respiration by biota after harvest towards the end of growing season and also during the subsequent early spring (Abraha et al., 2018; Kim et al., 2021). Notwithstanding, perennial grain crops have greater root biomass, which is capable of generating autotrophic respiration (Tang et al., 2020; Kim et al., 2021). This contribution from root respiration seems to have negligible impacts on CO_2 effluxes. Future research can quantify and elucidate the autotrophic and heterotrophic contribution to respiration in perennial cropping systems.

The substantial CO_2 uptake observed from the perennial grain crop in the second year of the study was coupled with both increased cumulative GPP and reduced cumulative R_e. The high precipitation in 2019 significantly increased soil moisture, in particular in the perennial grain crop field. This excessively wet soil over extended periods may have precluded or at least reduced soil respiration. Greater R_e was observed in the annual grain cropping system in 2019 than in 2018, suggesting that decomposing plant residues and senesced roots from the previous growing season likely boosted CO_2 emissions in 2019 (Zenone et al., 2013). In addition, it should be considered in parallel that unintendedly high volunteer growth may have also contributed to enhanced GPP in the perennial grain crop field in the second year.

NECB was evaluated to derive net soil C balance after accounting for harvest removals. The annual grain crop had considerable net C loss over the duration of this study, whilst perennial grain cropping showed significant C gains, particularly in the second year. Notably, greater NEE in the perennial grain crop enabled the sequestration of significant amounts of C, despite C being removed via harvest. In line with this assertion, perennial grain cropping systems have shown abundant soil and root C compared to annual grain crops (Kim et al., 2021). Thus, the greater CO_2 uptake by perennial grain crops is well distributed between crop biomass and soil profile C. Overall NECB substantiates the significant potential for C storage with perennial grain cropping systems. Our assessment of annual C budget, however, does not account for root C exudation and non-CO_2-C emissions (VOC, CO, CH_4) components, as well as the possibility for ecosystem C saturation.

The effect of perennial grain cropping on harvest carbon use efficiency (CUE_h) was negligible, which implies that the C obtained via GPP was not allocated to crop biomass and grain in the same proportions in perennial and annual cropping. Genetic and phenotypic divergences between perennial and annual grain crops may have dictated the differential C allocation and distribution mechanisms from photosynthesis into biomass vs. grain compartments across perennial and annual crops. Conversely, ecosystem carbon use efficiency (CUE_e), as opposite to CUE_h, had consistent differences between the two cropping systems. The perennial grain crop maintained higher CUE_e than the annual grain crop, indicating increased soil C sequestration by perennials at the ecosystem level. Our findings provide a baseline to estimate environmental benefits in terms of C sequestration under perennial grain cropping in the Canadian Prairies and comparable eco-physiological regions.

4.5.2. Fluxes vs. climatic and vegetation factors

Deciphering the dynamic responses of fluxes to climatic and vegetative factors may provide insights of how ecosystem processes can vary as a function of escalating climate change (Sharma et al., 2019; Jiang et al., 2020). In our study, variations in air temperature were consistent with soil temperature, VPD, GPP and ET fluxes over time. Simultaneously, other fluctuations were attributed to different plant phenological stages. Crop senescence and cloudiness, for instance, reduced photosynthetic activity, which impacted overall NEE fluxes.

Notably, differences in precipitation and temperature explained variations in NEE fluxes across years (Sharma et al., 2019; Ruan and Robertson, 2020; Menefee et al., 2020). Substantial rainfall in 2019 apparently triggered disproportional biomass productivity across both annual and perennial crops, and this effect may have also impacted the cumulative GPP and R_e. Our regression analysis revealed a robust linearity between cumulative precipitation and cumulative GPP, and this finding is in agreement with the assertion that interannual variability of water availability often impacts the interannual variability in C sequestration in temperate regions (Zenon et al., 2013). Moreover, soil temperature under the annual grain crop was slightly higher than under perennial grain crops, and the temperature sensitivity (Q_{10}) for ecosystem respiration of the annual grain crop was likewise higher than of the perennial grain crop. As a result, the higher soil temperature and Q_{10} contributed to neutral NEE fluxes by releasing substantial CO_2 as soil respiration in the annual grain cropping system.

Optimum VPD for crop growth can vary for the same crop due to different environmental conditions, stage of crop development, genotypes, and degree of water stress (Wagle et al., 2019). Optimal VPD for GPP of the perennial grain crop was higher compared to the annual grain crops, suggesting that the perennial grain crop might be more resilient to drought events.

Although WUE was not significantly different between two crops, perennial grain crops may have the potential to act as a larger CO_2 sink, even under relatively drier conditions.

4.5.3. Impacts of perennial grain cropping on the soil water fluxes

The perennial grain field had slightly higher evaporative losses than annual grain crops, which can be attributed to sparse vegetative growth and water use by the perennial during the late fall, dormant season and early spring (Abraha et al., 2015). However, overall water losses were not significantly different between two cropping systems. Our result is contrary to the expectation that perennial vegetation increases water usage by extending the growing season and the extensive root system. This is in line with earlier studies indicating that a transition from row crops to perennial bioenergy crops does not strongly alter terrestrial water balance in a humid temperate climate (Abraha et al., 2015).

Moderate weather variations over the two growing seasons in the study drove the annual water balance. Compared with the 10-year precipitation average, precipitation was higher in 2019 and relatively lower in 2018. The measured ET was proportional to annual precipitation, which suggests that water exchange was dependent on water input and the overall water balance. Surplus ET over annual precipitation would have been derived from other existing sources, such as groundwater, subsurface lateral flow or soil water storage. In contrast, ET that was lower than annual precipitation implies losses through water run-off and deep percolation (de Oliveira et al., 2020).

Harvest water use efficiency (WUE_h) for grain yield was lower for the perennial grain crop when considering the perennial crop as a grain crop (instead of a forage crop). This result confirms that the perennial grain crop not only produced lower grain yields but overall ET was also similar between two crops. WUE_h for aboveground biomass and overall WUE_e showed no distinctive differences. This suggests that the potential drought resilience of perennial grain crops may not be as robust as we had initially expected.

4.5.4. Turbulent energy fluxes

Surface energy exchange and balance between the vegetation surface and the atmosphere can be affected by agricultural practices through changes in leaf area, biomass, surface roughness, and albedo (Zenone et al., 2013; Sharma et al., 2019). Contrasting crop canopy growths and phenologies may contribute differentially to the energy partitioning at the terrestrial surface (Moore et al., 2020). For example, the Bowen ratio near the end of two growing seasons clearly showed the gradual senescence of the crops and decreasing canopy conductance (Eichelmann et al., 2016). Perennial grain crops tend to have a lower albedo than annual grain crops, which typically indicates that higher energy availability can be used in evaporative and photosynthetic process throughout the growing season (de Oliveira et al., 2020). In our study, the perennial grain crop had higher LE and lower H at beginning of growing season, as well as in the late fall, likely a result of the extended ground coverage. Higher LE in the perennial grain crop means greater atmospheric humidity associated with soil moisture and also increased canopy conductance. In addition, the lower cumulative Bowen ratio for perennial grain crop further highlights the partitioning of available energy from net radiation as mediated by the cooling effect of the dense perennial canopy.

4.6. Conclusion

High frequency CO_2 and water fluxes over two contrasting cropping systems during two successive years provided valuable insights of how a perennial grain crop sequesters C without impacting water use efficiency relative to annual grain crops in the Canadian Prairies. Our research suggested that a lower soil temperature sensitivity (Q_{10}) for R_e and greater soil moisture under perennial grain cropping enhanced CO_2 uptake. Concomitantly, the perennial grain crop had a higher NECB and CUE_e compared with the annual grain crops. Furthermore, the perennial grain crop showed more capacity to assimilate CO_2 through photosynthesis under relatively drier conditions, as shown by a higher optimum VPD. Although the seasonal distribution of ET fluxes was slightly shifted between the two cropping systems, the overall cumulative ET and WUE was similar between the two crops. Collectively, our results shed light on the capacity of perennial grain crops to become a sizeable terrestrial sink of atmospheric CO_2 at the ecosystem level, leading to C sequestration in soil profile and plant biomass.

4.7. References

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Table 4. 1. Soil classification and properties from 0–15 cm depth for the Breton site.

	Units	Breton
Classification		Orthic Gray Luvisol
TC †	g C kg^{-1} soil	27.5±2.9
TN	g N kg^{-1} soil	2.21±0.18
Texture		Silty clay loam
Clay †	%	31.1
Silt	%	49.1
Sand	%	19.8
pH‡		5.7
EC‡	dS m^{-1}	0.83
Cumulative Precipitation	mm yr^{-1}	526.8§ [401.3] ¶
Air Temperature	ºC	3.51§ [10.32] ¶

† Soil organic carbon (OC) and total nitrogen (TN) were measured by dry combustion (Brachmann et al., 2020) for each block replicate; these are means of four replicates (±SE). Texture was measured by hydrometer (Hebb et al., 2017).

‡ 1:1 soil to solution. EC stands for electric conductivity.

§ These are long-term normals (Alberta Climate Information Service).

¶ Values inside the parenthesis correspond to the measurement period: May to October (Alberta Climate Information Service).

Table 4. 2. Seeding and harvest dates, crop biomass, net ecosystem CO$_2$ exchange (NEE), net ecosystem carbon balance (NECB), carbon use efficiency (CUE), evapotranspiration (ET), and water use efficiency (WUE) for annual and perennial grain crops.

		2018			2019			
		Annual grain	*Perennial grain*	P-value	*Annual grain*	*Perennial grain*†	*Perennial grain*‡	P-value
Seeding	Date	2018 May 23	2017 Sep 1		2019 May 22	---		
Harvest	Date	2018 Oct 5	2018 Oct 3		2019 Oct 11	2019 Aug 25		
Grain	(Mg DM ha^{-1})	3.52±0.59	2.81±0.18	< 0.05*§	2.86±0.18	1.45±0.12		< 0.001***
Straw	(Mg DM ha^{-1})	5.96±0.18	8.37±4.76	< 0.05*	5.87±0.08	4.44±0.37		< 0.05*
Aboveground biomass; (Grain + Straw)	(Mg DM ha^{-1})	9.48±0.24	11.18±0.53	> 0.05	8.73±0.26	5.87±0.50	11.60±1.61 ($P > 0.05$)	< 0.01**
Grain-C	(Mg C ha^{-1})	1.47±0.02	1.20±0.08	> 0.05	1.19±0.78	0.62±0.05		< 0.001***
Straw-C	(Mg C ha^{-1})	2.55±0.08	3.52±0.20	< 0.05*	2.54±0.03	1.86±0.15		< 0.05*
Aboveground biomass-C; (Grain-C + Straw-C)	(Mg C ha^{-1})	4.02±0.10	4.73±0.22	> 0.05	3.73±0.10	2.48±0.21	5.15±0.70 ($P > 0.05$)	< 0.01**
Soil-C¶	(Mg C ha^{-1})	46.58±2.79	50.15±2.28	> 0.05	48.91±1.93	51.95±1.98		> 0.05
Cumulative NEE	(g C m^{-2})	-44.7	-445.4		13.3	-696.9		
Cumulative GPP	(g C m^{-2})	1044.4	1289.8		1439.6	1531.6		
Cumulative Reco	(g C m^{-2})	904.1	875.7		1402.0	541.0		
NECB	(g C m^{-2})	357.3	27.6		386.3	-448.9	-181.9	
CUE$_h$ -DM (Grain)	(g C g DM^{-1})	0.34	0.22		0.20	0.09		
CUE$_h$-DM (Aboveground biomass)	(g C g DM^{-1})	0.91	0.87		0.61	0.38	0.76	
CUE$_h$-C (Grain)	(g C g C^{-1})	0.14	0.09		0.08	0.04		
CUE$_h$-C (Aboveground biomass)	(g C g C^{-1})	0.38	0.37		0.26	0.16	0.34	
CUE$_e$	(g C g C^{-1})	0.04	0.35		-0.01	0.46	0.46	
Cumulative ET	mm	358.89	413.58		423.58	469.92		
Cumulative Bowen ratio		0.20	0.12		0.24	0.10		
Precipitation	mm	486.5 (341.6)			596.0 (501)			
Temperature	ºC	3.08 (11.28)			2.47 (8.56)			
WUE$_h$-DM (Grain)	(g DM kg H$_2$O^{-1})	0.98	0.68		0.68	0.31		
WUE$_h$-DM (Aboveground biomass)	(g DM kg H$_2$O^{-1})	2.64	2.70		2.06	1.25	2.47	
WUE$_h$-C (Grain)	(g C kg H$_2$O^{-1})	0.41	0.29		0.28	0.13		
WUE$_h$-C (Aboveground biomass)	(g C kg H$_2$O^{-1})	1.12	1.14		0.88	0.53	1.10	
WUE$_e$	(g C kg H$_2$O^{-1})	2.91	3.12		3.40	3.26		

† Shown results were calculated when considering the perennial crop as perennial grain crop.

‡ Shown results were calculated when considering the perennial crop as perennial forage crop.

§ asterisks denote the statistical significance based on ANOVA results (* < 0.05, ** < 0.01, ***<0.001).

¶ This is based on a soil depth of 0-15 cm after accounting for bulk density.

¤ Crop and weeds distribution based on dry matter in the 2019 perennial cropping system as follows: perennial rye 52.83 ± 5.80, rye grass 1.94 ± 1.16, timothy 9.55 ± 3.25, clover 18.20 ± 5.45, and various grass species 17.47 ± 3.04.

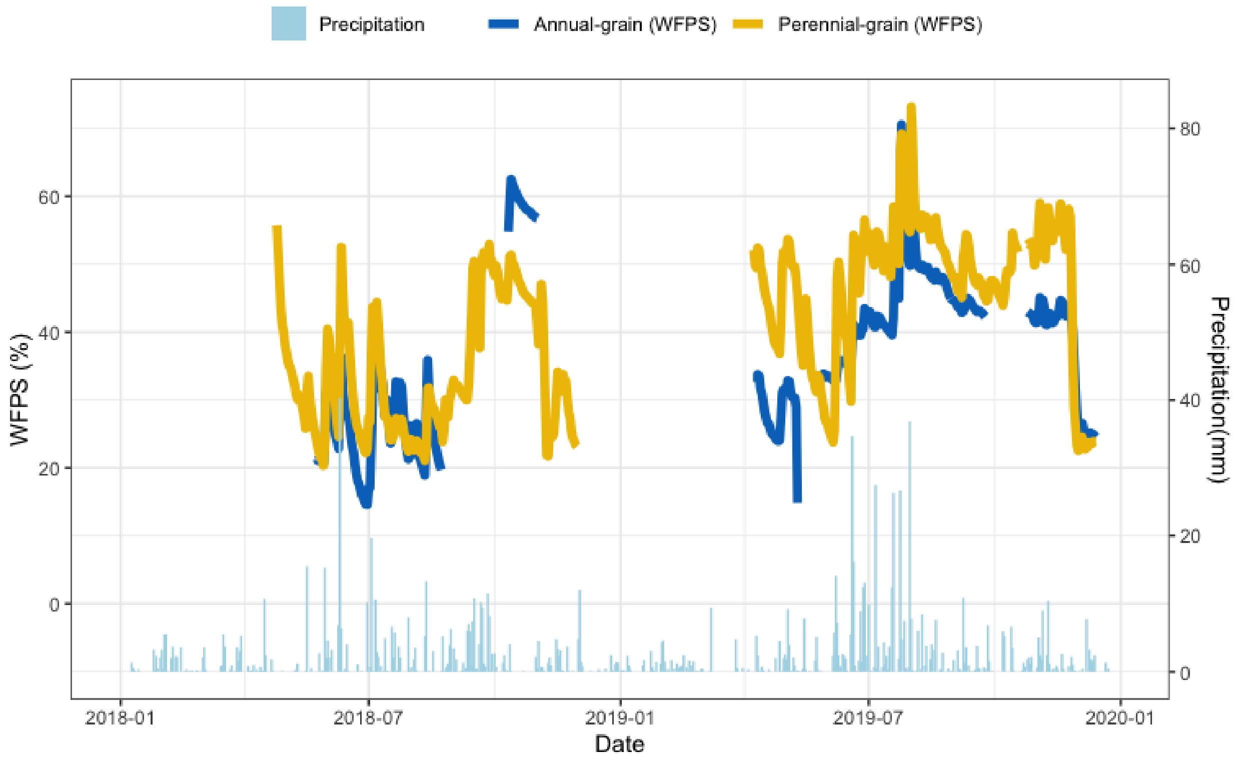

Fig. 4. 1. Daily average of water filled pore space (WFPS) at the 7.5 cm depth and precipitation for the contrasting cropping systems (i.e., annual grain cropping vs. perennial grain cropping) in 2018 and 2019.

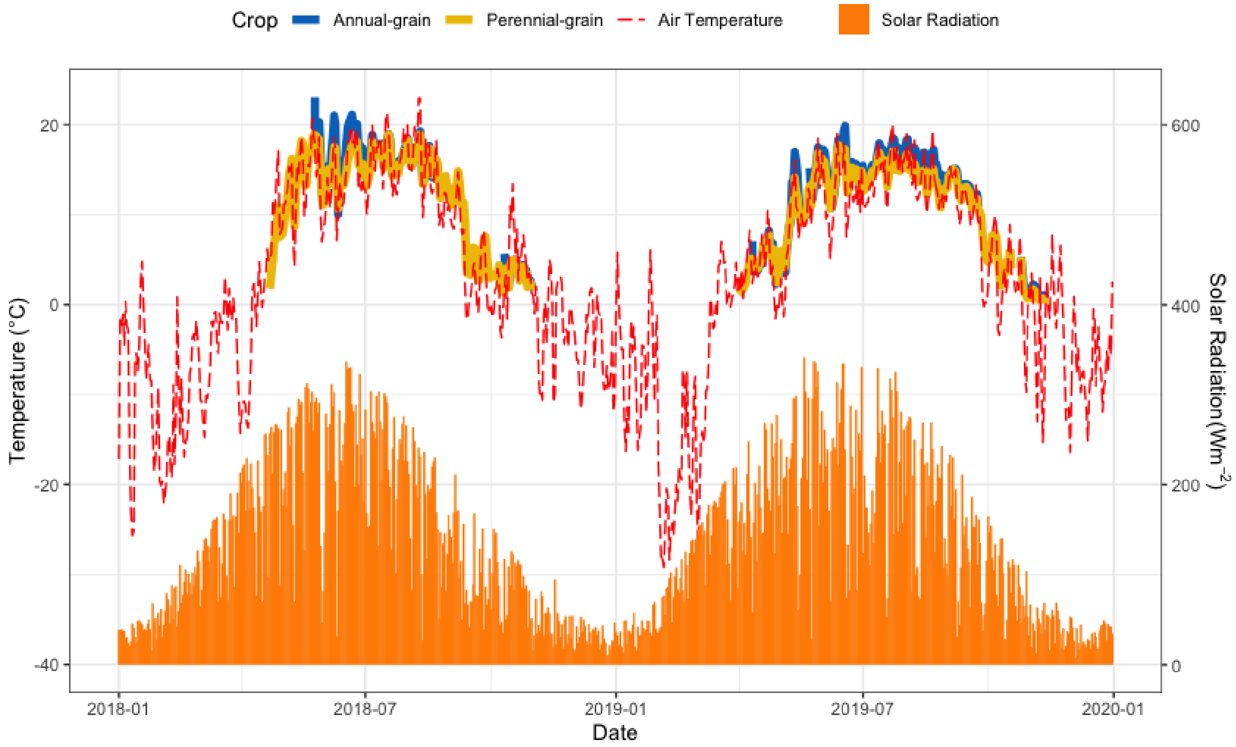

Fig. 4. 2. Daily average of soil temperature at 7.5 cm depth, air temperature, and solar radiation in the contrasting cropping systems (annual grain crops vs. perennial grain crops) in 2018 and 2019.

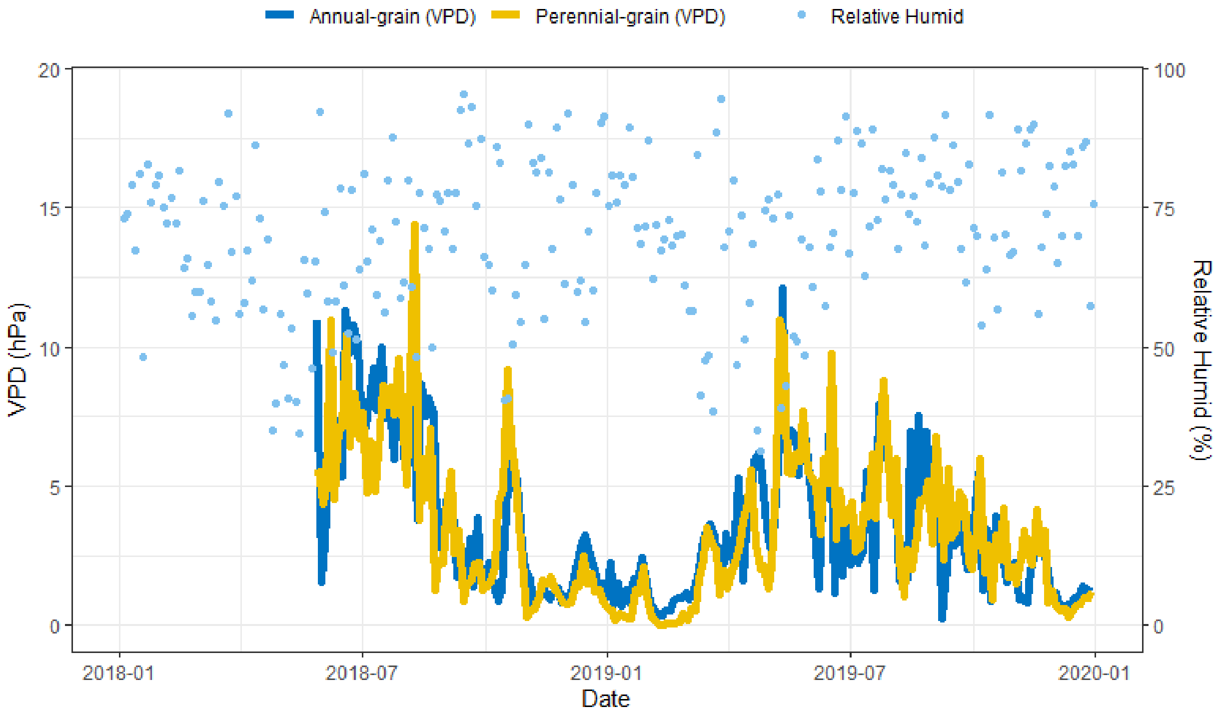

Fig. 4. 3. Daily average of vapor pressure deficit (VPD) and relative humidity (rH) in annual and perennial grain cropping systems in 2018 and 2019.

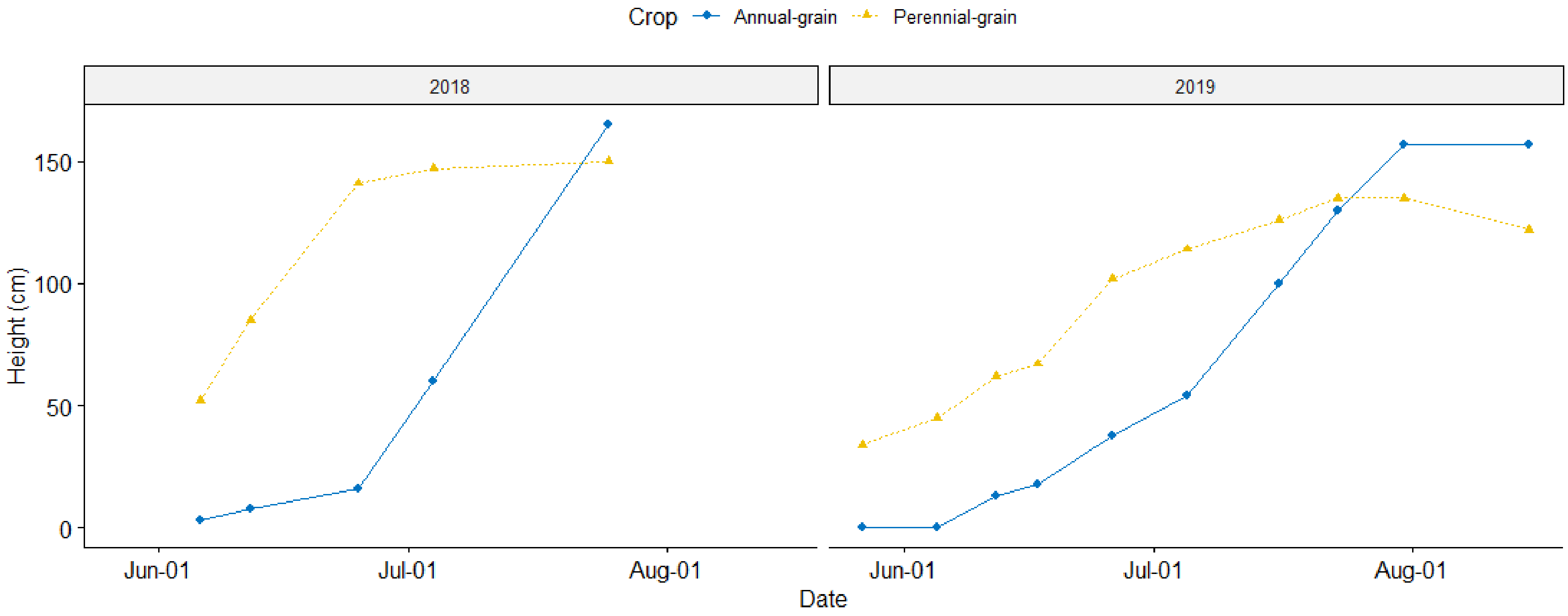

Fig. 4. 4. Crop height average during two growing seasons in the two 4-ha plots measured every two weeks. Crop height was determined from soil surface to the top of the apical bud. Means correspond to ten random measurements per field.

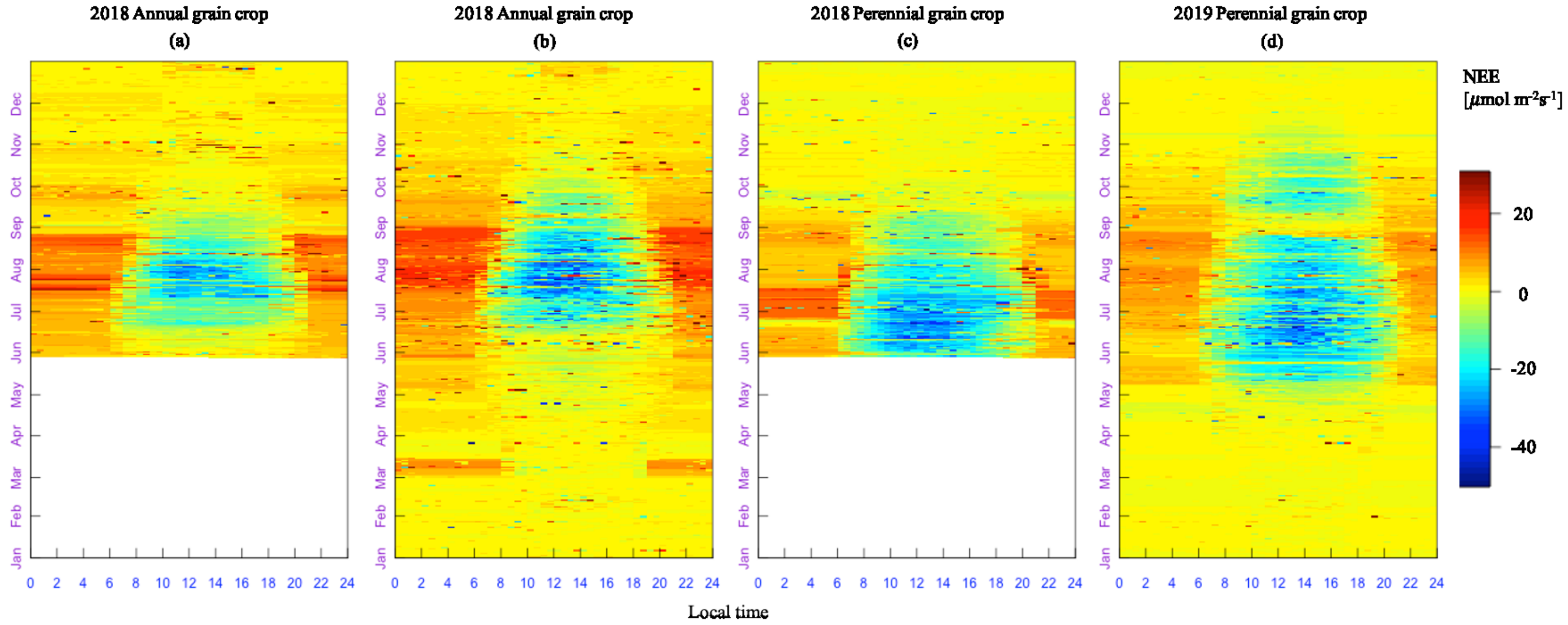

Fig. 4. 5. Diel net ecosystems exchange (NEE) fingerprint for annual vs. perennial grain crops for two growing seasons.

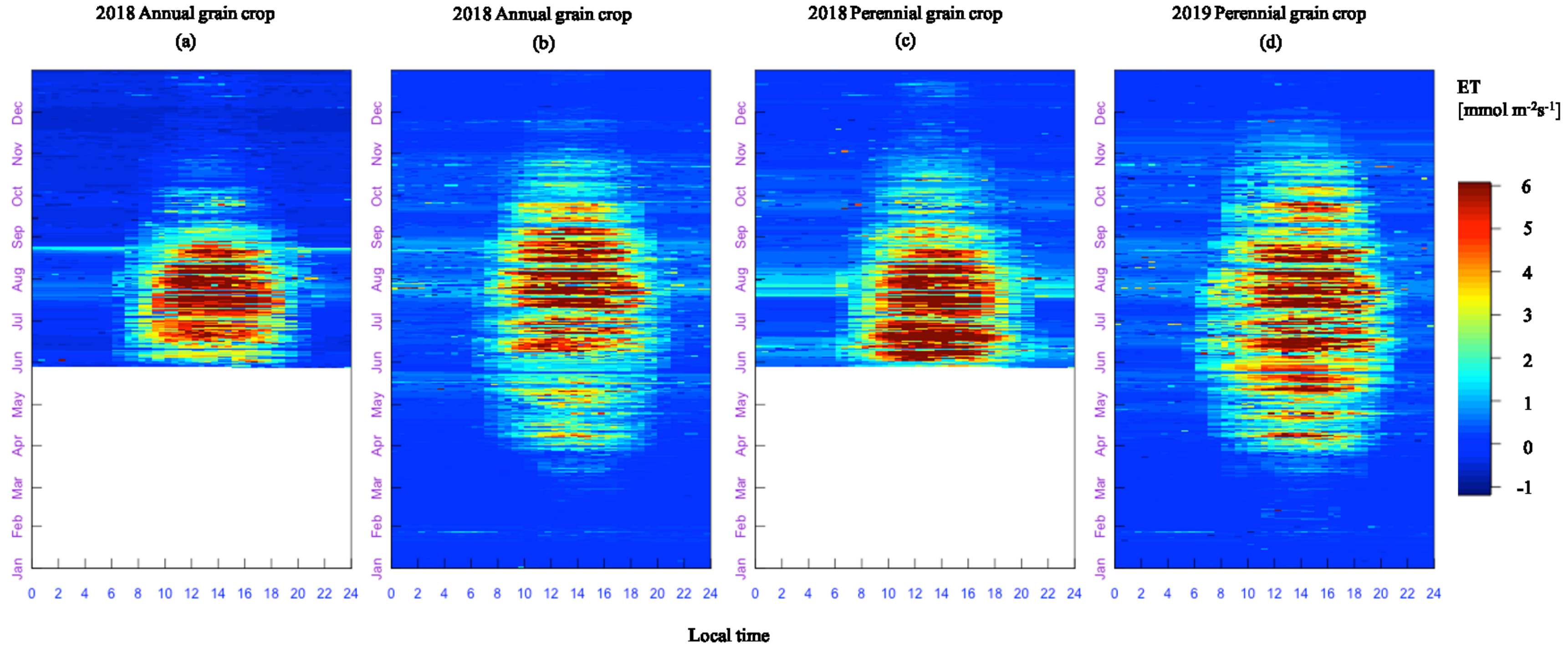

Fig. 4. 6. Diel evapotranspiration (ET) fingerprint for annual vs. perennial grain crops for two growing seasons.

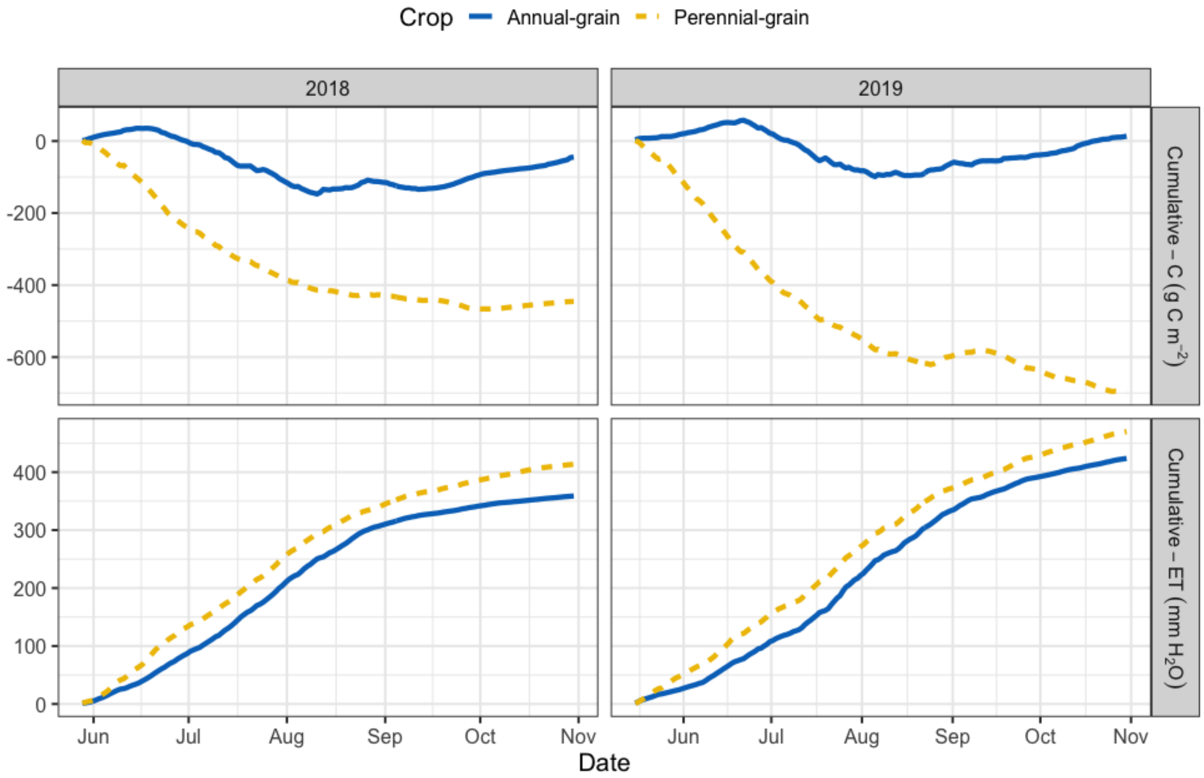

Fig. 4. 7. Cumulative carbon (C) and evapotranspiration (ET) for the contrasting cropping systems (i.e., annual vs. perennial) during consecutive two growing seasons.

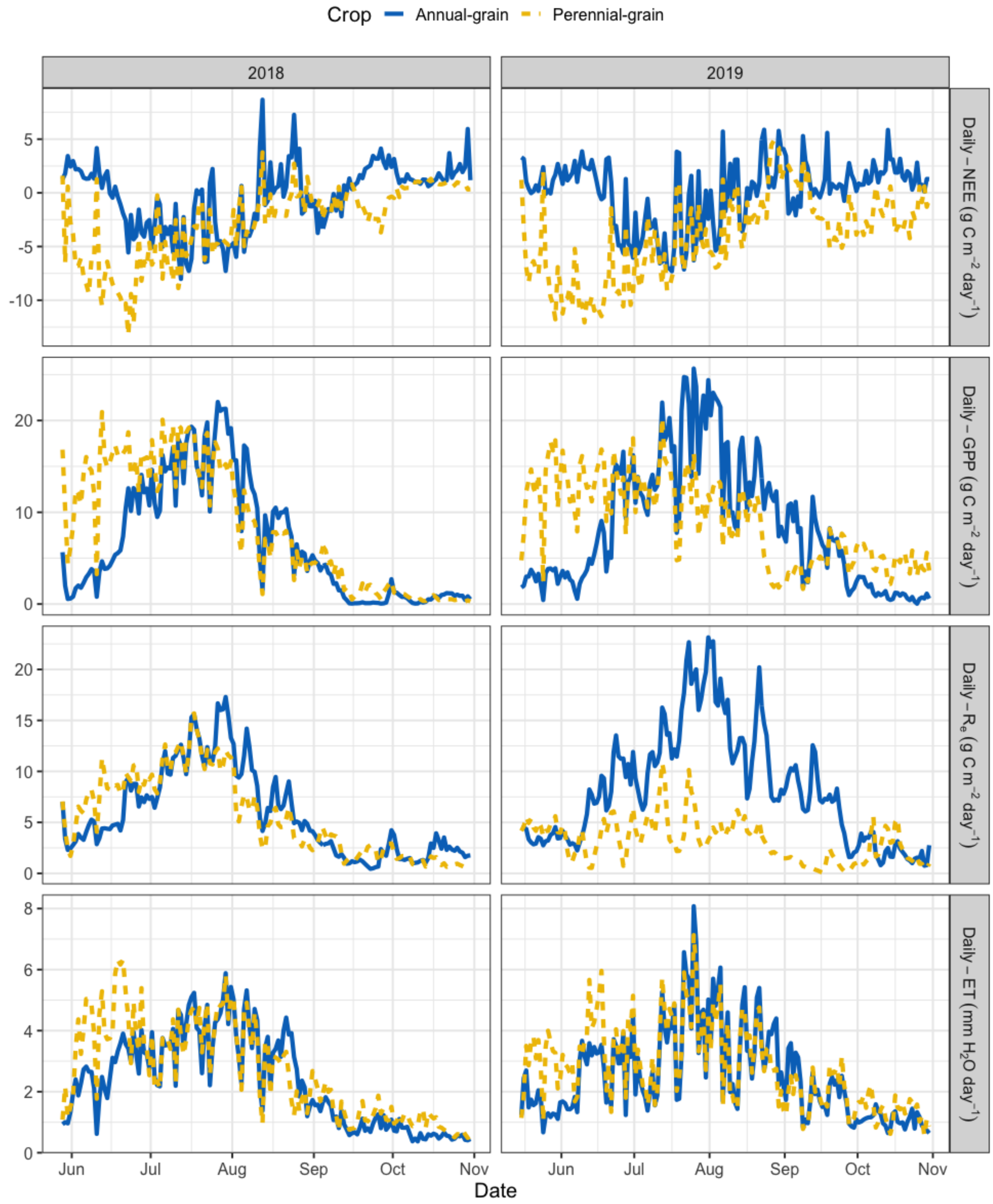

Fig. 4. 8. Daily net ecosystem exchange (NEE), gross primary production (GPP), ecosystem respiration (R$_e$), evapotranspiration (ET) over contrasting cropping systems (i.e., annuals vs. perennials) during two consecutive growing seasons (2018 May 28 –2018 Oct 31, 2019 May 15 –2019 Oct 31).

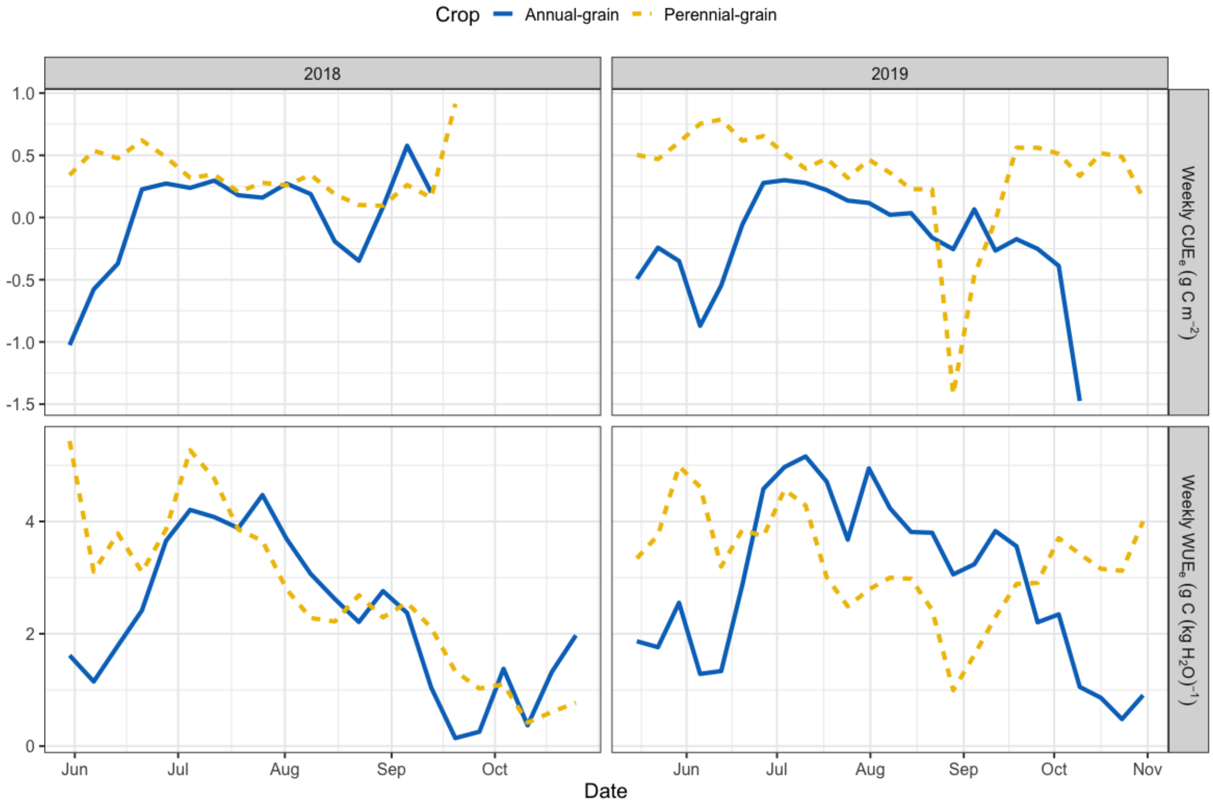

Fig. 4. 9. Weekly ecosystem carbon use efficiency (CUE_e) and ecosystem water use efficiency (WUE_e) for two growing seasons for the contrasting cropping systems (i.e., annual vs. perennial grain crops).

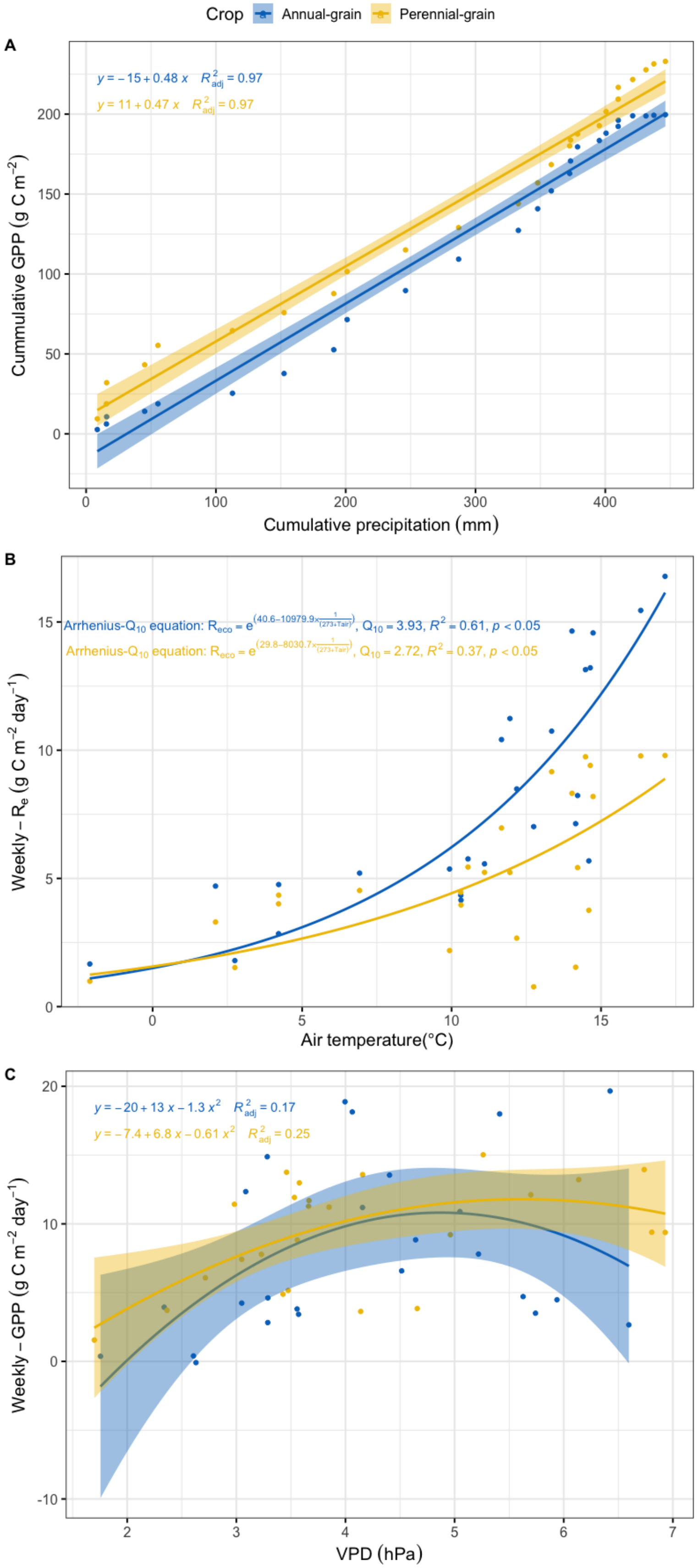

Fig. 4. 10. Regression analyses of A. linear relationship between weekly cumulative gross primary production (GPP) and cumulative precipitation, B. Arrhenius equation based exponential relationship between weekly ecosystem respiration (R_e) and air temperature, and C. Quadratic relationship between weekly GPP and vapor pressure deficit (VPD) for annual and perennial grain cropping systems during the 2019 growing season (15 May to 31 Oct.).

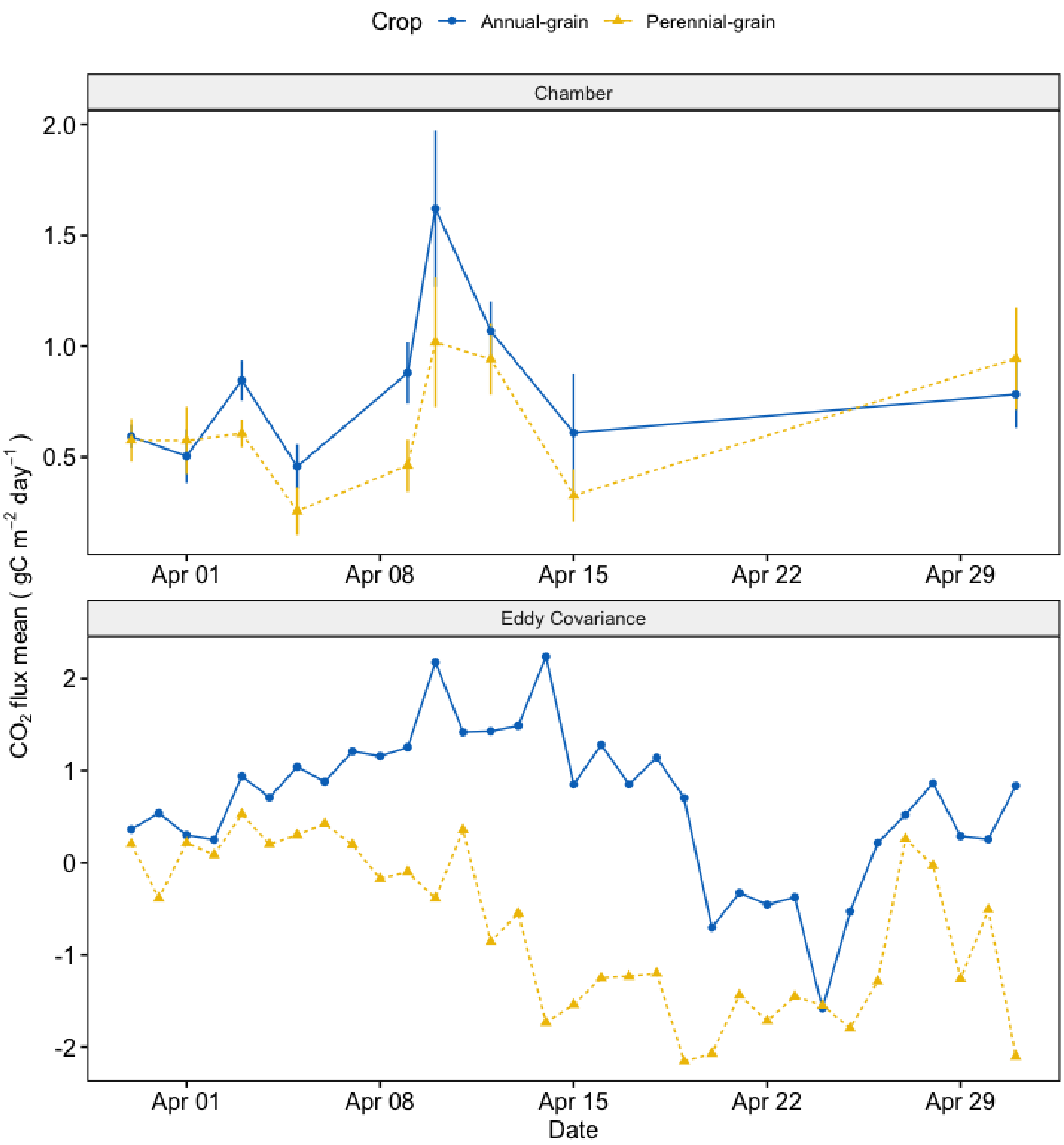

Fig. 4. 11. Daily CO_2 fluxes from the contrasting cropping systems (i.e., annual vs. perennial crops) from chamber and eddy covariance systems. Chamber-based flux measurements (top panel) correspond to seven chambers located within each 4-ha field. Error bars indicate standard error (n=7). These data only show the measurement period during spring thaw (i.e., from 2019 March 31 to 2019 May 1).

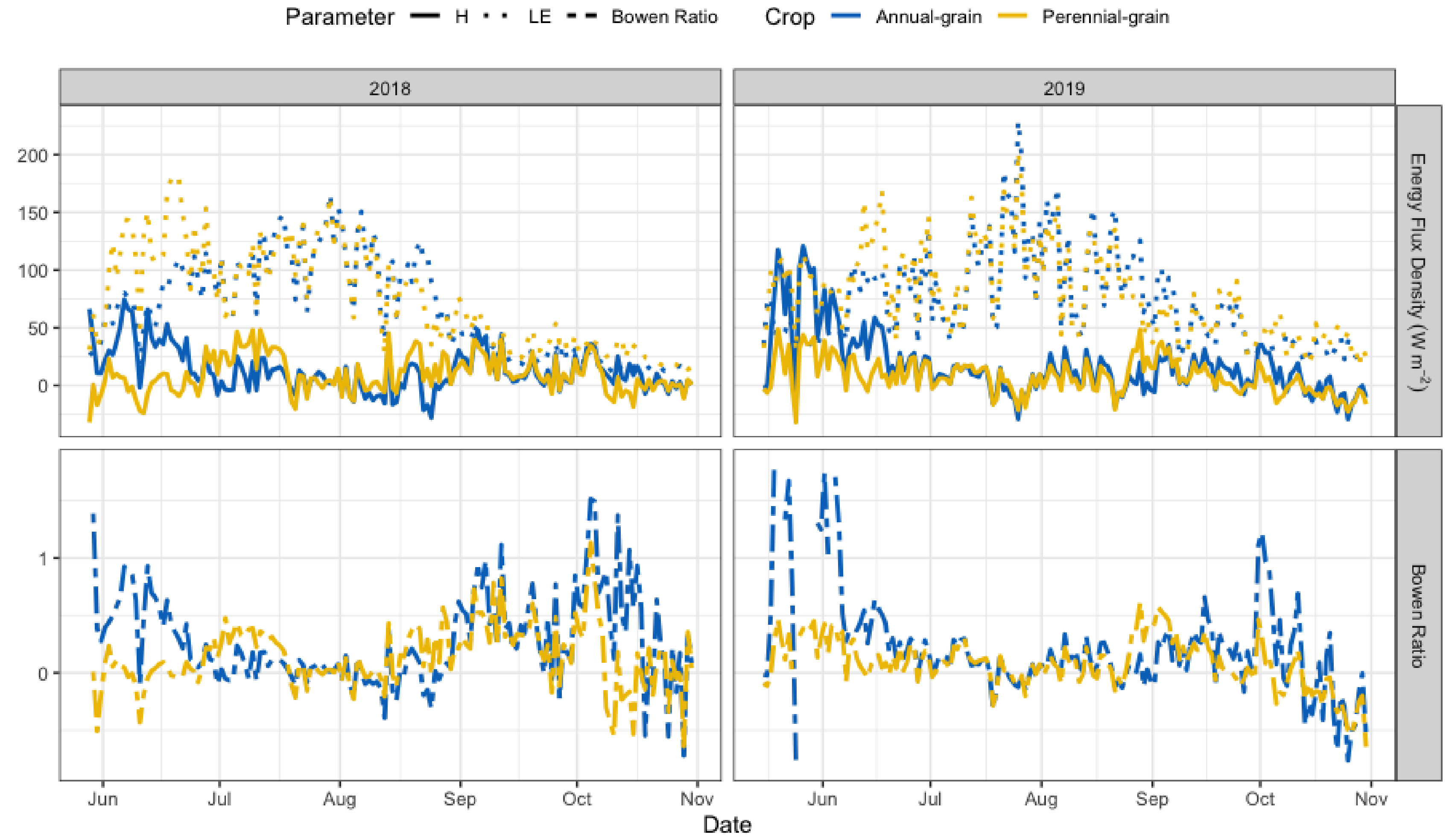

Fig. 4. 12. Daily mean energy flux density encompassing turbulent fluxes (*H*: heat flux; *LE*: latent flux) and Bowen ratio during two growing seasons.

CONCLUSION

Summary of Findings

In this research, comprehensive assessments about novel perennial grain cropping systems have been addressed through investigation of soil C sequestration, greenhouse gases (GHGs) emissions, mycorrhizae, and water use efficiency. Perennial grain cropping systems have substantial benefits over conventional annual grain cropping systems in pursuit of multifunctional agriculture. Our findings indicate that perennial grain cropping contributed to increase soil organic carbon (SOC) sequestration in both labile and stable pools. This appears to be site-specific factors such as soil texture, cropping history, and climate. This study provides insight of how to maximize soil C sequestration by selected cropping and N fertilizer management options. Secondly, we demonstrated that perennial grain cropping systems become a sizeable terrestrial sink of CH_4 and CO_2. Perennials also registered much greater net ecosystem exchange balance and ecosystem carbon use efficiency compared with annual grain cropping systems. As a result, substantially different C sequestration was observed between perennial and annual grain crops. Our research revealed that the mediating mechanisms by perennial grain cropping systems favorably shift the aeration-moisture balance, photosynthetic activity, and microbial temperature sensitivity. Surprisingly, perennial grain cropping systems did not show significant differences in arbuscular mycorrhizal fungi (AMF) diversity, community compositions and water use efficiency compared with annual grain crops. Although N fertilizer additions showed to significantly decrease soil C sequestration in perennial grain crops under certain condition, overall effects of N fertilizer were inconsistent across cropping systems and sites.

In conclusion, perennial grain cropping systems hold a prominent opportunity to beneficially convert agricultural systems by pursuing multifunctionality. Therefore, we anticipate that perennial grain cropping systems will gradually be implemented as a sustainable agriculture system in near future.

Synthesis and Future Research Prospects

Perennial grain cropping systems face feasibility limitations in terms of practical agronomy. This is because of the inherent difficulty to successfully practice mechanical weed controls which were closely associated with declines in crop grain productivity. Consequently, our experiment was also terminated at the end of second year. This notions underline the vulnerable stability of perennial grain cropping systems as well as several unanswered questions of how to improve their deployability and practicality. It is proposed that additional multi-year studies may enhance the further understanding of perennial grain cropping systems.

Focusing on a broader perspective for future research scopes, it will be important to uplift the general understanding on C dynamics by undertaking deeper and finer spatiotemporal measurements of soil properties, gaseous fluxes, and mycorrhizae attributes. Furthermore, additional investigation of soil properties related to C pools can account for unsolved C flow in the overall C cycling. For instance, aspects of root exudation and non-carbon emissions can be further examined in future studies. On the same topic, measuring additional soil attributes as potential explanatory factors of AMF might be able to account for the divergence of AMF community compositions across soils and cropping systems. In our study, AMF molecular analysis elucidated only species identity and how much they were distributed. In the view of the assumption that each identity has different functional role in soil, identifying the functional role of particular species through culture-based determination and could provide better insights for designing and managing sustainable agroecosystems. Both methanotroph genomics and partitioning of CO_2 respiration sources (i.e., autotrophic vs. heterotrophic) may also improve the current understanding on GHGs emissions and controlling mechanisms.

Even though our field research offered unique opportunities to study C dynamics across contrasting cropping systems, the results might only fit to comparable eco-physiological regions. In the case of our result, the differential effects of cropping systems were exhibited for each corresponding to site. Consequently, this indicates the requirement for multiple-site experiments to be able to generalize the impacts of perennial grain cropping systems. Alternatively, model development can be considered for future studies with the aim of exploring knowledge gaps of space, economy, and time.

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APPENDIX

SUPPLEMENTARY MATERIAL

CHAPTER 1. Perennial Grain Cropping Enhances the Soil Methane Sink in Temperate Agroecosystems

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Table S1. 1. Means and standard errors of soil bulk density (n = 5 ± SE) at 5–10 cm and 25–30 cm depths. Samples collected in June 2018 with the same method implemented by Hernandez-Ramirez et al., 2014.

	Edmonton		Breton	
	5–10 cm	25–30 cm	5–10 cm	25–30 cm
Fallow	1.04±0.11	0.97±0.17	1.09±0.09	1.10±0.04
Spring-grain†	1.07±0.14	1.12±0.14	1.16±0.14	1.12±0.10
Perennial-grain	1.00±0.13	0.91±0.14	1.05±0.13	1.15±0.15
Perennial-forage	0.94±0.09	0.97±0.17	1.09±1.66	1.00±0.18

† For porosity calculations, we assumed that bulk density of spring-grain was representative of the bulk density of fall-grain (spring-grain was considered the most similar cropping system within the experiment; cropping system description can be found in Table 2).

Table S1. 2. Cumulative CH_4 uptake by cropping systems (n= 4 ± SE) at Edmonton and Breton. To evaluate the experimental factors (i.e., crop and fertilizer), the fallow plots were not included enabling a balanced model.

	F-value†	P
	Edmonton 2018	
Fertilizer	1.94	0.20
Crop	1.92	0.19
Fertilizer × Crop	2.01	0.14
	Edmonton 2019	
Fertilizer	2.42	0.13
Crop	4.97	**<0.01**
Fertilizer × Crop	0.99	0.41
	Breton 2018	
Fertilizer	0.69	0.38
Crop	0.18	0.89
Fertilizer × Crop	0.03	0.98
	Breton 2019	
Fertilizer	0.39	0.53
Crop	3.18	**0.04**
Fertilizer × Crop	0.95	0.43

† The denominator degrees of freedom (df) was 21 across all two-way ANOVA models. The numerator df for crop was 3 and for fertilizer was 1.

Table S1. 3. Means and ranges for soil temperature and water-filled pore space by soil depths × cropping systems in Edmonton.

	Temperature (ºC)				WFPS (v/v %)			
Crop	Min	Max	Median	Average	Min	Max	Median	Average
Air								
	1.8	32.6	17.9	17.6				
7.5 cm								
Fallow	0.4	26.7	15.6	14.5	15.0	63.9	49.5	46.9
Spring grain	0.4	22.3	15.3	14.3	31.2	59.7	48.0	47.6
Fall grain	0.3	24.7	16.3	14.6	11.2	56.0	33.9	32.7
Perennial Grain	0.1	21.6	15.0	13.5	11.7	57.5	31.0	31.0
Perennial forage	0.2	19.4	13.9	12.4	22.9	58.6	30.4	34.1
22.5 cm								
Fallow	-0.2	21.5	15.4	13.8	32.3	60.6	49.1	50.5
Spring grain	0.0	19.6	14.9	13.1	35.9	62.5	44.5	46.2
Fall grain	-0.2	18.7	14.9	13.1	26.0	68.8	47.1	49.4
Perennial Grain	-0.3	17.2	14.0	12.3	29.1	57.3	39.1	40.4
Perennial forage	-0.3	17.0	13.1	11.8	29.3	53.5	35.0	37.4
40 cm								
Fallow	-0.4	20.4	15.1	13.4	29.2	59.2	49.7	49.4
Spring grain	-0.5	18.7	14.6	12.7	35.0	68.2	54.2	53.9
Fall grain	-1.0	17.1	14.3	12.3	30.3	48.0	36.0	36.5
Perennial Grain	-0.6	16.1	13.1	11.8	25.0	54.7	30.9	33.7
Perennial forage	-0.7	16.0	12.3	11.4	20.0	40.1	23.2	24.8

Table S1. 4. Means and ranges for soil temperature and water-filled pore space (WFPS) by soil depths × cropping systems in Breton.

	Temperature (ºC)				WFPS (v/v %)			
Crop	Min	Max	Median	Average	Min	Max	Median	Average
				Air				
	1.0	25.8	18.1	16.6				
				7.5 cm				
Fallow	1.8	19.8	14.7	13.1	45.5	58.4	53.5	53.3
Spring grain	1.5	18.1	15.0	12.9	19.7	56.7	35.9	37.7
Fall grain	1.9	19.8	15.8	13.6	25.4	55.0	48.6	42.9
Perennial Grain	1.8	17.7	14.1	12.5	21.4	54.8	45.9	40.9
Perennial forage	0.8	18.6	14.0	12.5	21.7	59.3	47.1	43.1
				22.5 cm				
Fallow	2.3	19.5	14.9	13.4	51.6	72.8	56.4	58.0
Spring grain	2.1	17.1	14.3	12.6	24.2	62.7	43.3	41.9
Fall grain	2.3	17.5	14.1	12.6	24.6	60.8	46.9	43.8
Perennial Grain	2.6	16.3	14.0	12.2	37.3	62.0	56.0	51.2
Perennial forage	0.4	17.4	14.0	12.4	29.1	49.3	42.4	39.8
				40 cm				
Fallow	2.1	19.2	14.8	13.4	53.5	91.8	58.6	64.6
Spring grain	2.0	16.8	14.6	12.8	46.2	64.4	50.2	52.7
Fall grain	2.3	16.8	14.6	12.8	37.6	71.7	53.9	54.4
Perennial Grain	2.4	16.3	13.7	12.2	29.1	66.3	50.6	46.6
Perennial forage	0.2	16.4	14.0	12.0	15.9	32.4	22.2	22.4

Table S1. 5. Temperature, water filled pore space and methane flux for the cropping systems over two years. These are averages by month. Positive fluxes are uptake. Temperature and water-filled pore space (WFPS) are measurements at the 40 cm soil depth.

	Edmonton			Breton		
Crop	Temperature (ºC)	WFPS (%)	CH_4 uptake flux (g CH_4–C ha^{-1} day^{-1})	Temperature (ºC)	WFPS (%)	CH4 uptake flux (g CH_4–C ha^{-1} day^{-1})
May 2018						
Fallow	8.75	55.1	-1.05	10.06	51.2	-0.30
Spring grain	13.38	46.8	-0.65	16.60	53.5	3.17
Fall grain	8.05	50.3	-0.55	9.18	55.5	-0.25
Perennial grain	7.84	47.4	-0.12	10.13	43.4	0.48
Perennial forage	8.39	41.6	-0.37	7.54	47.3	0.55
June 2018						
Fallow	15.25	57.6	0.71	14.20	56.4	0.65
Spring grain	15.23	51.9	0.54	14.37	55.4	1.06
Fall grain	13.53	36.5	0.81	13.36	57.6	1.12
Perennial grain	13.37	36.3	1.32	12.83	41.5	0.82
Perennial forage	12.47	26.7	1.23	13.48	NA	0.78
July 2018						
Fallow	18.22	58.4	0.73	17.38	57.6	1.49
Spring grain	16.39	45.9	1.33	15.53	51.9	2.66
Fall grain	15.83	35.3	0.86	15.47	53.6	2.36
Perennial grain	15.53	28.9	1.46	14.91	33.8	2.36
Perennial forage	15.41	24.4	1.14	15.34	40.1	2.48
August 2018						
Fallow	17.70	58.5	-0.02	16.58	56.0	1.89
Spring grain	15.39	40.6	0.55	14.93	47.3	1.87
Fall grain	15.76	34.8	0.27	15.17	46.4	2.09
Perennial grain	14.85	28.2	0.97	14.59	31.7	2.04
Perennial forage	14.63	23.7	1.28	14.48	36.0	1.81
September 2018						
Fallow	9.92	38.0	0.79	8.83	54.8	1.19
Spring grain	9.92	42.4	1.69	NA	46.4	0.92
Fall grain	10.12	32.1	1.01	8.03	53.3	0.71
Perennial grain	9.87	31.2	1.37	7.40	42.6	0.78
Perennial forage	10.22	21.9	1.36	8.17	42.9	0.81
April 2019						
Fallow	1.36	46.6	-0.02	1.18	52.1	0.20
Spring grain	1.62	41.4	0.30	2.40	53.9	-0.25
Fall grain	1.11	39.4	0.27	1.34	41.2	0.18
Perennial grain	1.89	40.7	0.46	1.18	48.5	-0.14
Perennial forage	1.82	28.3	0.16	0.16	37.1	-0.24
May 2019						
Fallow	9.52	43.7	1.23	9.58	54.1	0.66
Spring grain	9.63	53.5	1.01	8.27	47.1	1.06
Fall grain	8.48	39.9	1.42	8.01	50.7	1.61
Perennial grain	8.31	41.0	1.67	7.55	55.5	0.96
Perennial forage	8.06	22.7	1.45	5.25	37.3	1.04
June 2019						
Fallow	14.78	43.2	0.71	13.82	64.4	1.71
Spring grain	14.47	64.9	0.67	14.46	48.6	2.42
Fall grain	13.75	34.1	0.75	13.52	41.2	2.20
Perennial grain	12.63	32.9	1.20	13.26	53.7	2.74
Perennial forage	11.70	19.4	1.29	12.47	28.6	2.09
July 2019						
Fallow	16.04	49.5	0.98	14.81	71.1	1.89
Spring grain	15.12	67.0	1.16	14.87	55.8	1.85
Fall grain	14.92	38.0	1.11	14.72	61.0	1.59
Perennial grain	13.85	36.9	1.28	13.90	60.4	1.67
Perennial forage	13.34	20.4	1.24	13.75	31.3	1.19
August 2019						
Fallow	15.88	45.0	1.59	14.64	86.4	2.14
Spring grain	15.03	63.8	1.04	14.83	61.5	1.66
Fall grain	15.54	36.6	1.77	14.92	67.7	2.13
Perennial grain	14.37	29.8	1.93	13.45	66.0	1.98
Perennial forage	14.50	21.4	1.94	14.17	34.8	1.61
September 2019						
Fallow	13.79	41.5	-0.41	13.03	68.7	2.05
Spring grain	12.78	59.8	0.13	12.93	52.8	2.23
Fall grain	13.07	34.4	0.22	13.85	58.4	2.36
Perennial grain	12.12	26.7	0.86	12.41	57.3	2.18
Perennial forage	12.11	20.2	1.16	12.23	28.5	2.12

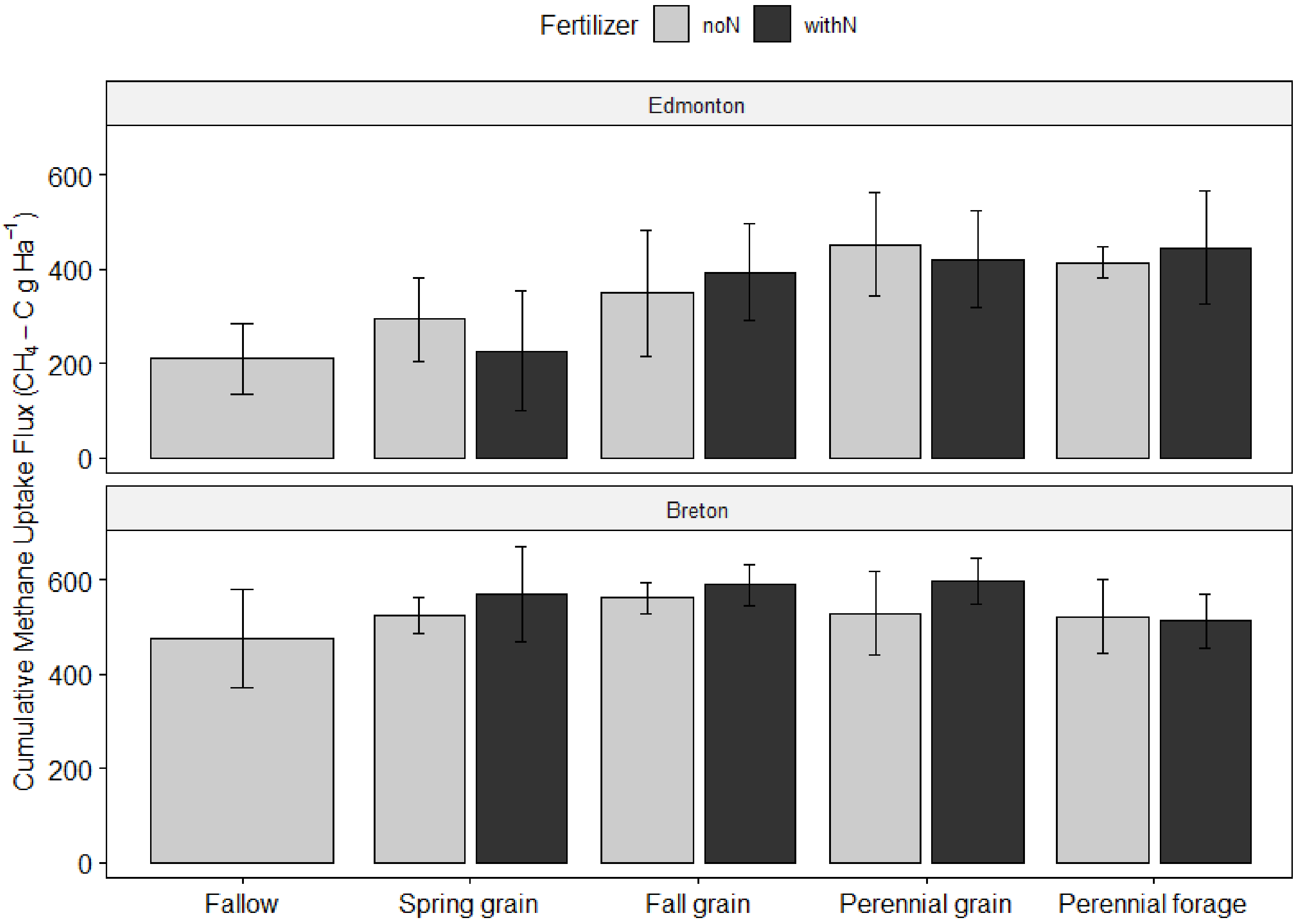

Fig. S1. 1. Cumulative CH_4 flux during the entire study showing cropping × nitrogen treatment combinations in Breton and Edmonton (integrating measurement from 26 April 2018 to 18 Oct 2018 and from 26 March 2019 to 30 Oct 2019). Means correspond to four field plots. Error bars are standard errors of the means.

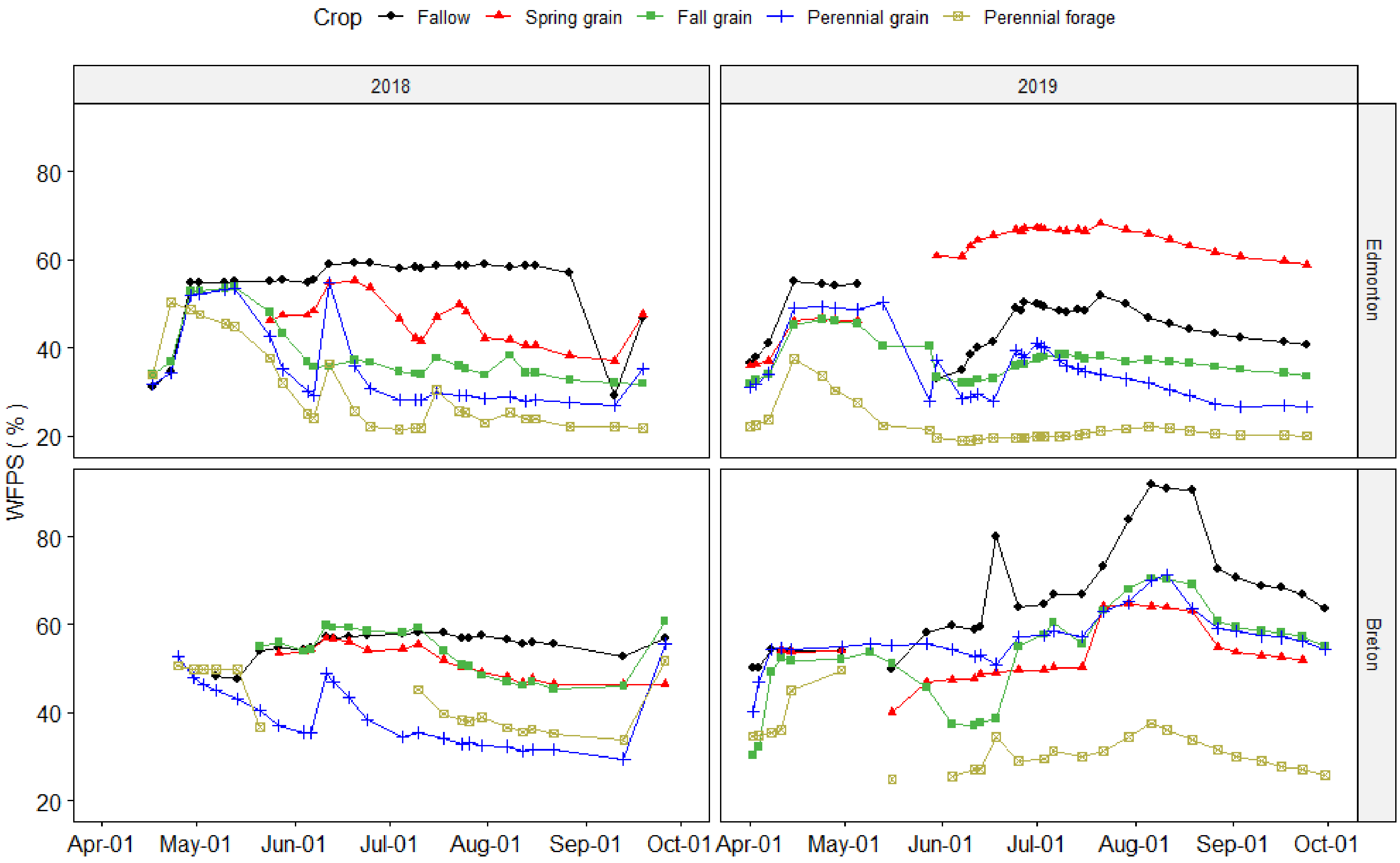

Fig. S1. 2. Soil water filled pore space (WFPS; 40 cm depth) at the time of flux measurements over two consecutive growing seasons in Edmonton and Breton sites.

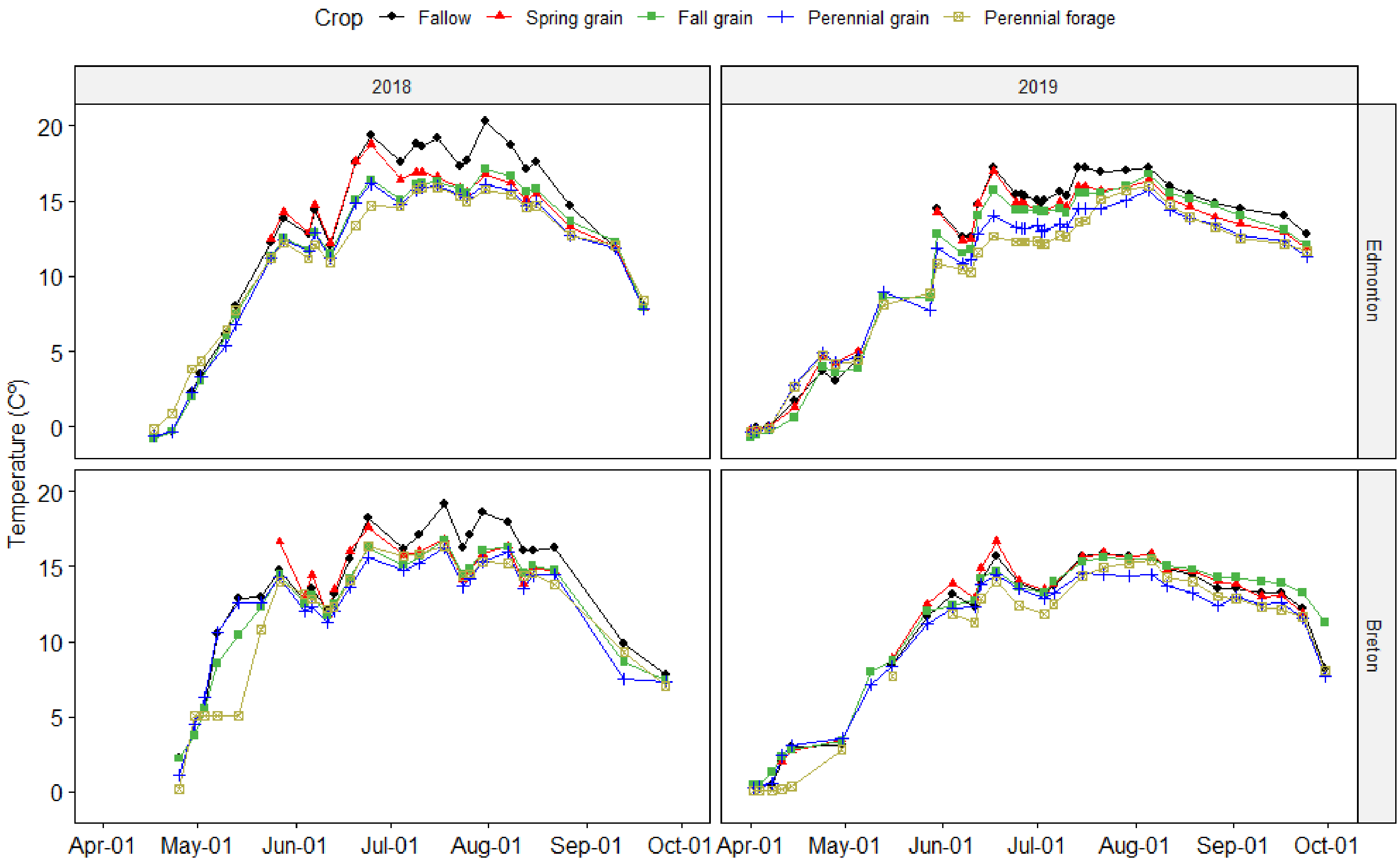

Fig. S1. 3. Soil temperature (40 cm depth) at the time of flux measurements over two consecutive growing seasons at the Edmonton and Breton sites.

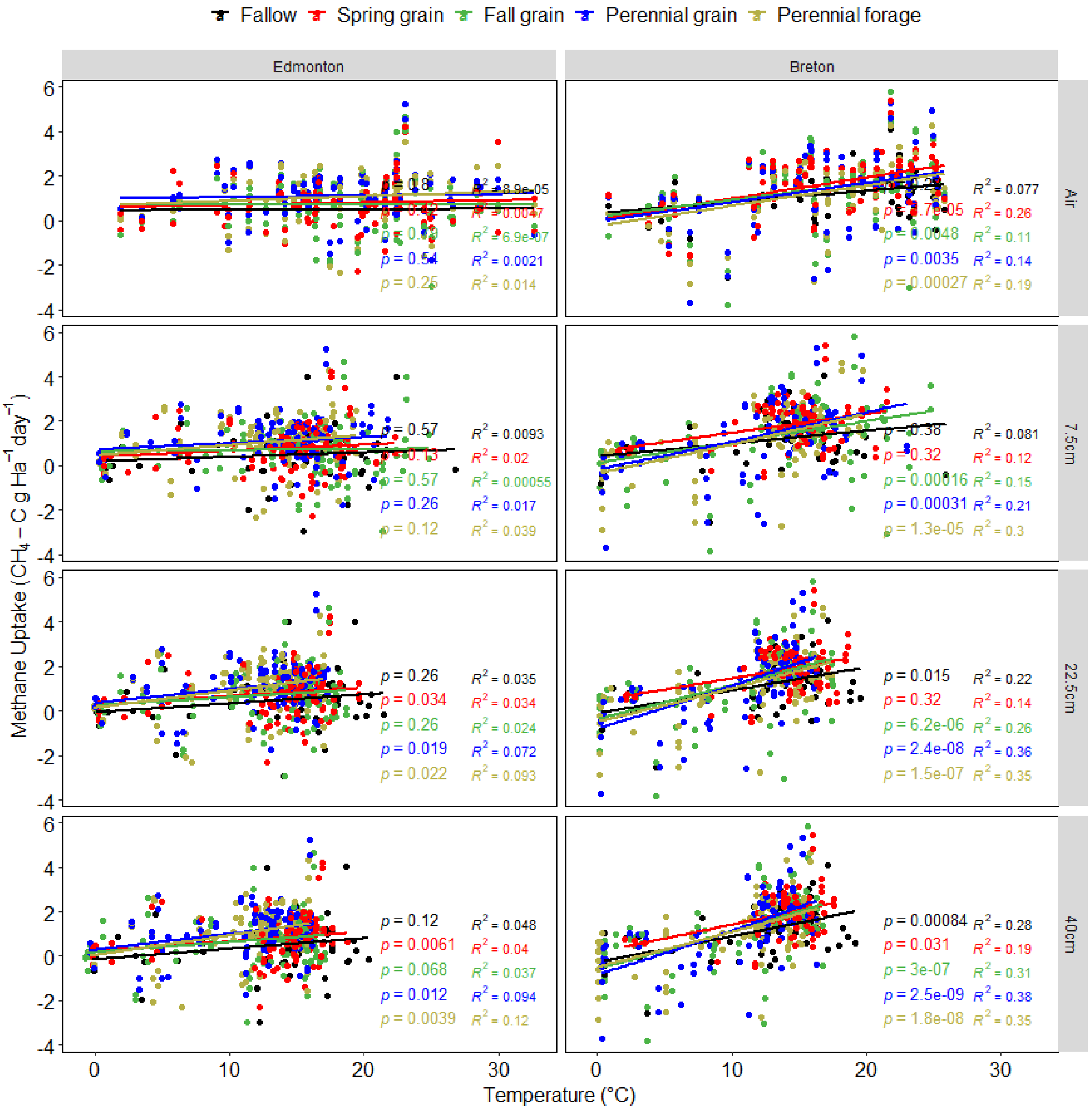

Fig. S1. 4. Arrhenius non-linear relationships of surface methane fluxes across cropping systems with air and soil temperatures at various depths (7.5, 22.5 and 40 cm) encompassing two consecutive growing season data in Edmonton and Breton. Predicted CH_4 fluxes (bold line) are shown. These are measured daily fluxes.

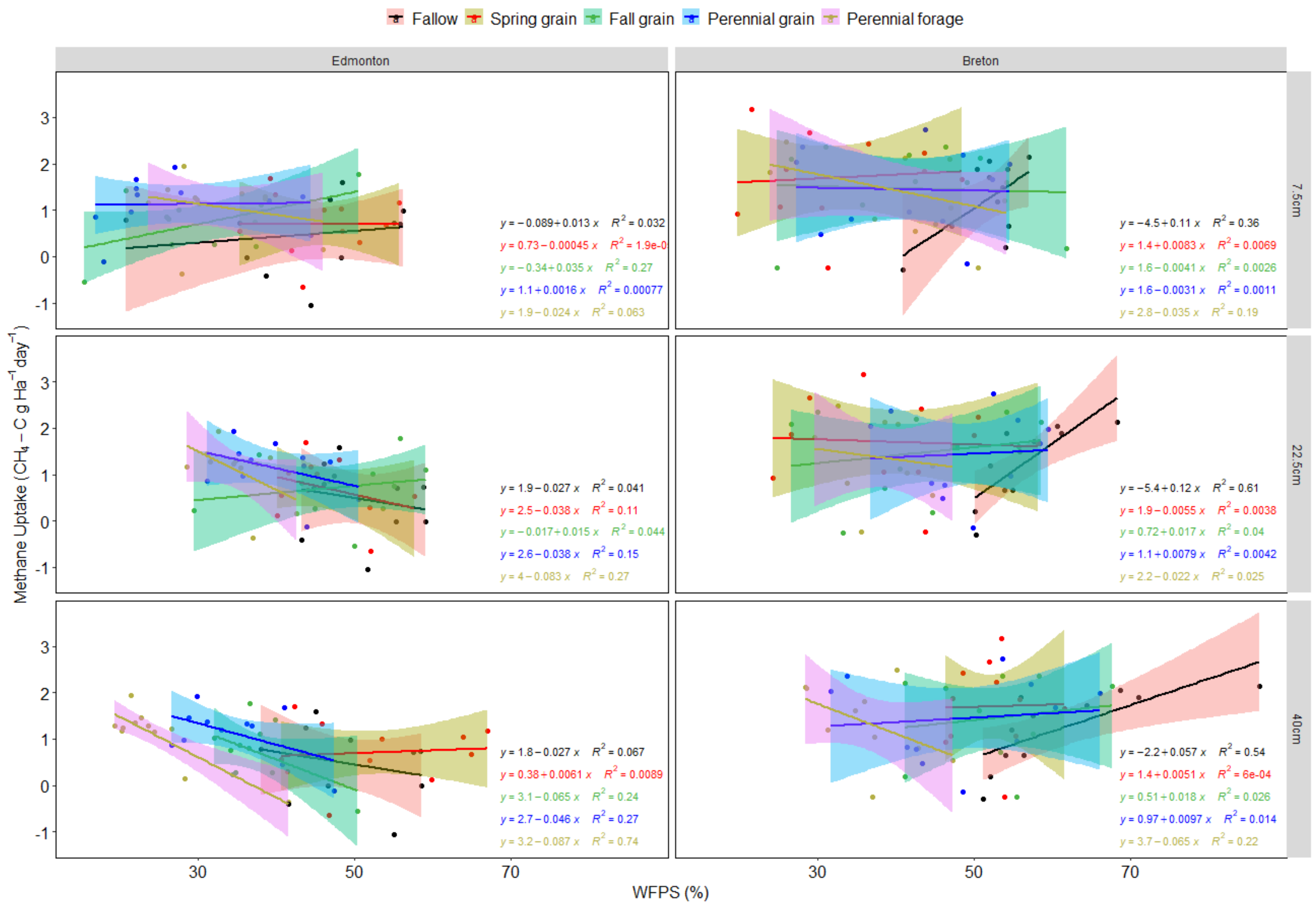

Fig. S1. 5. Relationships between surface CH_4 uptake rate and soil water filled pore space across cropping systems at three soil depths (7.5, 22.5 and 40 cm depth) encompassing the two-year growing season data in Edmonton and Breton. Predicted CH_4 fluxes (bold line) and 95% confidence intervals are shown. These are averages by months during the entire study.

Supplementary material: R Code for Q_{10} calculation

##

For the Arrhenius Equation [3] in the method section:
Using k=flux, T= Temperature, non-leaner model (nls) enables lnA and -(Ea/R) to have the best fit values. The lnA and -(Ea/R) were replaced with b and a, respectively.

```
k=e^(b+a*(1/T),
```

Transformations:

- All fluxes are expressed with methane uptake being positive.
- Add 10 units to all methane fluxes to have all fluxes as positive.
- The absolute temperature is expressed in Kelvin.

```
k=-10+e^(b+a*(1/(273.15+T))),
```

```
Model=nls(Flux~-10+exp(b+a*(1/(273.15+Temperature))),start = list(a=2,b=0.02),data=data)
```

The model gives the best fit value of a and b, which enables to identify temperature (T1) when the flux is 1. The next step names T1+10 as T2
The flux of T2 can be calculated by the model equation, and the flux ratio of T1 and T2 become a Q_{10}.

```
T2=a/(log(11)-b)-273.15
```

```
Q10=(-10+exp(b+a*1/(273+(a/(log(11)-b)-273.15+10))))/1
```

##

The model residuals were explained by the soil water contents as water filled pore space (WFPS in percentage).

model Flux= data$PredicF
measure Flux = data$Flux

```
data$sub=data$Flux-data$PredicF
```

the linear model was created by soil water content with residual flux

```
ModelA=lm(sub~WFPS%,data)
```

##

SUPPLEMENTARY MATERIAL

CHAPTER 2. Soil Carbon Response to Perennial Grain Cropping and Nitrogen Fertilizer

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Table S2. 1. Breton soil organic matter fractionation in September 2018.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
0-7.5cm													
Spring-grain	8.52a	0.55ab	15.94	0.27	0.21	23.92ab	2.08ab	11.27	0.73	0.78	32.93ab	2.72ab	12.11
Fall-grain	8.55ab	0.54a	16.28	0.29	0.22	20.98a	1.93a	10.88	0.71	0.78	29.64a	2.49b	11.91
Perennial-grain	9.23ab	0.61ab	15.87	0.28	0.22	23.54ab	2.11ab	11.10	0.72	0.77	32.93ab	2.74ab	12.00
Perennial-forage	10.16b	0.64b	16.09	0.27	0.21	26.11b	2.28b	11.40	0.73	0.78	36.27b	2.95b	12.29
No-N	9.22	0.59	15.86	0.28	0.22	23.41	2.10	11.08	0.72	0.78	32.82	2.74	12.01
With-N	9.01	0.57	15.99	0.27	0.22	23.97	2.10	11.24	0.73	0.78	33.35	2.73	12.15
Crop	***<0.05****	***<0.05****	*0.44*	*0.68*	*0.89*	***<0.05****	*0.05*	*0.17*	*0.72*	*0.94*	***<0.01*****	***<0.01*****	*0.23*
Fertilizer	*0.61*	*0.40*	*0.47*	*0.63*	*0.65*	*0.61*	*0.98*	*0.34*	*0.64*	*0.69*	*0.62*	*0.93*	*0.30*
Crop: Fertilizer	*0.74*	*0.63*	*0.78*	*0.87*	*0.65*	*0.89*	*0.90*	*0.36*	*0.90*	*0.79*	*0.68*	*0.71*	*0.14*
7.5-15 cm													
Spring-grain	5.38	0.38	14.23	0.22	0.18	19.10	1.76	10.87	0.78	0.82	24.49	2.14	11.45
Fall-grain	5.68	0.40	14.28	0.26	0.20	17.71	1.64	10.77	0.74	0.80	23.39	2.03	11.47
Perennial-grain	5.79	0.41	14.10	0.22	0.18	20.02	1.84	10.92	0.78	0.82	25.81	2.25	11.49
Perennial-forage	5.31	0.36	14.66	0.21	0.16	20.16	1.87	10.80	0.79	0.84	25.47	2.23	11.43
No-N	5.60	0.39	14.32	0.23	0.18	19.20	1.78	10.82	0.77	0.82	24.80	2.17	11.44
With-N	5.48	0.38	14.33	0.22	0.18	19.30	1.78	10.86	0.78	0.82	24.78	2.16	11.48
Crop	*0.79*	*0.62*	*0.39*	*0.32*	*0.20*	*0.49*	*0.38*	*0.93*	*0.32*	*0.20*	*0.60*	*0.50*	*0.99*
Fertilizer	*0.76*	*0.73*	*0.97*	*0.60*	*0.63*	*0.94*	*0.99*	*0.81*	*0.60*	*0.63*	*0.99*	*0.95*	*0.78*
Crop: Fertilizer	*0.31*	*0.34*	*0.74*	*0.40*	*0.39*	*0.32*	*0.36*	*0.60*	*0.40*	*0.39*	*0.23*	*0.30*	*0.33*

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. NS means non-significance between variables.

Table S2. 2. Breton soil organic matter fractionation in June 2019.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
						0-7.5cm							
Spring-grain	9.10	0.61	14.90	0.29	0.22	22.32	2.18	10.25	0.71	0.78	31.42	2.79	11.28
Fall-grain	9.08	0.61	14.92	0.29	0.22	22.45	2.14	10.47	0.71	0.78	31.53	2.75	11.47
Perennial-grain	9.41	0.64	14.79	0.29	0.23	22.93	2.17	10.55	0.71	0.77	32.33	2.80	11.51
Perennial-forage	10.43	0.69	15.00	0.29	0.23	25.12	2.37	10.61	0.71	0.77	35.55	3.06	11.61
No-N	9.70	0.65	14.86	0.29	0.22	23.70	2.27	10.44	0.71	0.78	33.39	2.92	11.44
With-N	9.31	0.62	14.95	0.29	0.22	22.71	2.16	10.50	0.71	0.78	32.02	2.78	11.50
Crop	*0.10*	*0.19*	*0.65*	*0.99*	*0.94*	*0.14*	*0.20*	*0.09*	*0.99*	*0.94*	***<0.05****	*0.08*	***<0.05****
Fertilizer	*0.36*	*0.37*	*0.45*	*0.99*	*1.00*	*0.29*	*0.21*	*0.54*	*0.99*	*1.00*	*0.21*	*0.15*	*0.42*
Crop: Fertilizer	*0.91*	*0.93*	*0.69*	*0.71*	*0.80*	*0.54*	*0.44*	*0.57*	*0.71*	*0.80*	*0.63*	*0.49*	*0.74*
						7.5-15 cm							
Spring-grain	7.32	0.48	15.19	0.27	0.20	20.03	1.98	10.12	0.73	0.80	27.47	2.47	11.14
Fall-grain	6.65	0.44	15.18	0.26	0.19	19.26	1.87	10.26	0.74	0.81	25.96	2.31	11.23
Perennial-grain	6.46	0.43	15.13	0.26	0.19	18.37	1.80	10.17	0.74	0.81	24.98	2.24	11.14
Perennial-forage	6.85	0.45	15.22	0.26	0.19	20.33	2.00	10.13	0.74	0.81	27.42	2.47	11.10
No-N	6.90	0.45	15.20	0.26	0.19	20.19	1.99b	10.13	0.74	0.81	27.22	2.45	11.10
With-N	6.73	0.44	15.16	0.27	0.20	18.81	1.84a	10.21	0.73	0.80	25.70	2.29	11.21
Crop	*0.50*	*0.59*	*0.98*	*0.78*	*0.86*	*0.34*	*0.21*	*0.82*	*0.78*	*0.86*	*0.28*	*0.18*	*0.82*
Fertilizer	*0.68*	*0.72*	*0.80*	*0.54*	*0.48*	*0.10*	***<0.05****	*0.49*	*0.54*	*0.48*	*0.15*	*0.08*	*0.28*
Crop: Fertilizer	*0.71*	*0.76*	*0.76*	*0.82*	*0.74*	*1.00*	*0.98*	*0.45*	*0.82*	*0.77*	*0.97*	*1.00*	*0.32*

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at P <0.05, P <0.01, P <0.001, respectively. NS means non-significance between variables.

Table S2. 3. Breton soil organic matter fractionation in September 2019.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
						0-7.5cm							
Spring-grain	9.38	0.59	15.88	0.30	0.20	22.29a	2.34a	9.48a	0.70	0.80	31.67a	2.93a	10.78a
Fall-grain	9.31	0.59	15.91	0.29	0.20	22.71a	2.38a	9.53a	0.71	0.80	32.02a	2.97a	10.79a
Perennial-grain	10.15	0.66	15.58	0.32	0.22	22.41a	2.33a	9.53ab	0.68	0.78	32.56a	2.99a	10.87a
Perennial-forage	11.48	0.73	15.78	0.30	0.21	27.23b	2.70b	10.05b	0.70	0.79	38.71b	3.43b	11.27b
No-N	10.38	0.66	15.77	0.29	0.21	25.11b	2.54b	9.84b	0.71	0.79	35.49b	3.19b	11.06b
With-N	9.78	0.62	15.80	0.31	0.21	22.21a	2.34a	9.45a	0.69	0.79	31.99a	2.96a	10.79a
Crop	**_<0.05*_**	_0.0796_	_0.534_	_0.7727_	_0.5787_	**_<0.01**_**	**_<0.01**_**	**_<0.05*_**	_0.7727_	_0.5787_	**_<0.001***_**	**_<0.001***_**	**_<0.01**_**
Fertilizer	_0.3073_	_0.3907_	_0.8917_	_0.4469_	_0.7283_	**_<0.01**_**	**_<0.05*_**	**_<0.01**_**	_0.4469_	_0.7283_	**_<0.01**_**	**_<0.01**_**	**_<0.01**_**
Crop: Fertilizer	_0.2_	_0.2174_	_0.2591_	_0.0944_	_0.1444_	**_<0.05*_**	**_<0.05*_**	**_<0.05*_**	_0.0944_	_0.1444_	**_<0.05*_**	**_<0.05*_**	**_<0.05*_**
						7.5-15 cm							
Spring-grain	6.29	0.40	15.86	0.23	0.15	21.31	2.20	9.62	0.77	0.85	27.60	2.59	10.60
Fall-grain	5.85	0.36	16.17	0.23	0.15	19.58	2.02	9.64	0.77	0.85	25.42	2.38	10.65
Perennial-grain	6.73	0.42	15.90	0.24	0.16	21.48	2.21	9.66	0.76	0.84	28.20	2.63	10.68
Perennial-forage	5.93	0.37	16.20	0.23	0.15	20.68	2.17	9.48	0.77	0.85	26.60	2.54	10.49
No-N	6.22	0.39	16.07	0.23	0.15	21.25	2.19	9.62	0.77	0.85	27.47	2.58	10.62
With-N	6.18	0.39	15.99	0.23	0.15	20.27	2.10	9.58	0.77	0.85	26.45	2.49	10.59
Crop	_0.10_	_0.08_	_0.31_	_0.81_	_0.52_	_0.52_	_0.34_	_0.80_	_0.81_	_0.52_	_0.25_	_0.19_	_0.66_
Fertilizer	_0.89_	_0.95_	_0.60_	_0.73_	_0.74_	_0.33_	_0.30_	_0.77_	_0.73_	_0.74_	_0.32_	_0.31_	_0.80_
Crop: Fertilizer	_0.88_	_0.89_	_0.64_	_0.72_	_0.88_	_0.31_	_0.48_	_0.14_	_0.72_	_0.88_	_0.25_	_0.44_	_0.13_

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at P <0.05, P <0.01, P <0.001, respectively. NS means non-significance between variables.

Table S2. 4. Edmonton soil organic matter fractionation in September 2018.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
						0-7.5cm							
Spring-grain	20.71	1.80	11.55	0.34	0.32	41.24	3.91	10.54	0.66	0.68	61.38	5.71	10.87
Fall-grain	20.32	1.75	11.62	0.31	0.30	45.51	4.30	10.59	0.69	0.70	64.33	6.05	10.91
Perennial-grain	20.77	1.80	11.57	0.32	0.31	43.85	4.04	10.78	0.68	0.69	63.50	5.85	11.05
Perennial-forage	21.53	1.86	11.68	0.34	0.32	42.39	4.01	10.55	0.66	0.68	63.34	5.87	10.89
No-N	21.58	1.88	11.54	0.34	0.33	41.49	3.88	10.65	0.66	0.67	62.12	5.76	10.95
With-N	20.09	1.72	11.67	0.31	0.29	45.01	4.25	10.58	0.69	0.71	64.15	5.97	10.91
Crop	*0.85*	*0.86*	*0.79*	*0.69*	*0.82*	*0.68*	*0.73*	*0.49*	*0.69*	*0.82*	*0.83*	*0.80*	*0.57*
Fertilizer	*0.15*	*0.10*	*0.17*	*0.07*	*0.07*	*0.19*	*0.16*	*0.52*	*0.07*	*0.07*	*0.40*	*0.40*	*0.70*
Crop: Fertilizer	***<0.01*****	***<0.01*****	*0.38*	***<0.05****	*0.06*	*0.64*	*0.63*	*0.14*	***<0.05****	*0.06*	*0.75*	*0.64*	*0.34*
Spring-grain	12.50	1.05	12.03	0.240	0.23	40.32	3.69	10.96	0.76	0.77	52.82	4.73	11.17
Fall-grain	15.54	1.33	11.81	0.280	0.27	40.04	3.64	11.03	0.72	0.73	55.58	4.97	11.19
Perennial-grain	12.83	1.08	11.94	0.242	0.23	40.30	3.64	11.07	0.76	0.77	53.13	4.72	11.26
Perennial-forage	14.59	1.23	11.99	0.269	0.26	40.04	3.63	11.06	0.73	0.74	54.62	4.87	11.26
No-N	14.27	1.21	11.90	0.266	0.25	39.43	3.57	11.08	0.73	0.75	53.70	4.78	11.24
With-N	13.46	1.13	11.99	0.250	0.24	40.91	3.73	10.98	0.75	0.76	54.37	4.87	11.19
Crop	*0.29*	*0.30*	*0.73*	*0.57*	*0.57*	*1.00*	*1.00*	*0.82*	*0.57*	*0.60*	*0.58*	*0.63*	*0.62*
Fertilizer	*0.53*	*0.50*	*0.56*	*0.51*	*0.47*	*0.46*	*0.41*	*0.28*	*0.51*	*0.49*	*0.68*	*0.60*	*0.37*
Crop: Fertilizer	*0.81*	*0.81*	*1.00*	*0.59*	*0.63*	*0.13*	*0.20*	*0.93*	*0.59*	*0.67*	*0.05*	*0.10*	*0.82*

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at P <0.05, P <0.01, P <0.001, respectively. NS means non-significance between variables.

Table S2. 5. Edmonton soil organic matter fractionation in June 2019.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
0-7.5cm													
Spring-grain	22.56	1.99	11.35	0.35	0.34	41.15	3.88	10.61ab	0.65	0.66	63.72	5.87	10.86ab
Fall-grain	22.66	1.99	11.38	0.35	0.34	41.14	3.92	10.49a	0.65	0.66	63.80	5.91	10.79a
Perennial-grain	23.11	2.03	11.42	0.35	0.34	42.59	3.99	10.66ab	0.65	0.66	65.70	6.02	10.91ab
Perennial-forage	23.62	2.06	11.51	0.36	0.34	41.73	3.88	10.76b	0.64	0.66	65.35	5.93	11.01b
No-N	22.63	2.00	11.37	0.35	0.34	41.42	3.90	10.61	0.65	0.66	64.06	5.90	10.86
With-N	23.35	2.04	11.46	0.36	0.34	41.88	3.93	10.65	0.64	0.66	65.23	5.97	10.92
Crop	*0.89*	*0.96*	*0.54*	*0.97*	*0.96*	*0.76*	*0.79*	*0.06*	*0.97*	*0.96*	*0.63*	*0.84*	**<0.05***
Fertilizer	*0.51*	*0.66*	*0.30*	*0.59*	*0.70*	*0.68*	*0.75*	*0.61*	*0.59*	*0.70*	*0.40*	*0.56*	*0.16*
Crop: Fertilizer	*0.30*	*0.33*	*0.74*	*0.04*	*0.06*	*0.05*	*0.06*	*0.18*	**<0.05***	*0.06*	*0.91*	*0.89*	*0.26*
7.5-15 cm													
Spring-grain	13.87	1.18	11.74	0.26b	0.25	39.05	3.64	10.73	0.74a	0.75a	52.91	4.82	10.98
Fall-grain	14.10	1.21	11.70	0.26b	0.24	40.88	3.78	10.80	0.74a	0.76a	54.98	4.98	11.03
Perennial-grain	12.43	1.05	11.81	0.22a	0.21	43.05	3.92	10.96	0.78b	0.79b	55.48	4.98	11.14
Perennial-forage	13.79	1.16	11.96	0.26b	0.24	39.70	3.63	10.95	0.74a	0.76a	53.49	4.78	11.21
No-N	13.50	1.15	11.77	0.25	0.24	40.50	3.73	10.86	0.75	0.76	54.01	4.88	11.08
With-N	13.59	1.15	11.83	0.25	0.24	40.83	3.76	10.86	0.75	0.76	54.43	4.91	11.10
Crop	*0.19*	*0.20*	*0.10*	**<0.01****	**<0.05***	*0.16*	*0.26*	*0.10*	**<0.01****	**<0.05***	*0.65*	*0.69*	*0.06*
Fertilizer	*0.88*	*0.96*	*0.45*	*0.98*	*0.88*	*0.80*	*0.81*	*0.99*	*0.99*	*0.88*	*0.80*	*0.84*	*0.81*
Crop: Fertilizer	*0.91*	*0.92*	*0.66*	*0.81*	*0.74*	*0.90*	*0.92*	*0.78*	*0.81*	*0.74*	*0.95*	*0.97*	*0.94*

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at P <0.05, P <0.01, P <0.001, respectively. NS means non-significance between variables.

Table S2. 6. Edmonton soil organic matter fractionation in September 2019.

Treatment	POM-C Concentration (g C/kg soil)	POM-N Concentration (g N/kg soil)	POM C:N	POM-C fraction	POM-N fraction	MAOM-C Concentration (g C/kg soil)	MAOM-N Concentration (g N/kg soil)	MAOM C:N	MAOMC fraction	MAOMN fraction	TOC (g C/kg soil)	TN (g N/kg soil)	Whole Soil C:N
0-7.5cm													
Spring-grain	21.38	1.87	11.42	0.34	0.31	40.70	4.13	9.87	0.66	0.69	62.08	6.00	10.35
Perennial-grain	20.51	1.79	11.47	0.32	0.29	43.81	4.37	10.03	0.68	0.71	64.40	6.17	10.45
Perennial-forage	21.45	1.87	11.51	0.33	0.30	43.37	4.29	10.11	0.67	0.70	64.82	6.16	10.54
No-N	20.56	1.80	11.44	0.32	0.29	43.38	4.31	10.06	0.68	0.71	63.99	6.12	10.47
With-N	21.66	1.89	11.49	0.34	0.31	41.87	4.21	9.95	0.66	0.69	63.53	6.10	10.43
Crop	*0.75*	*0.73*	*0.76*	*0.53*	*0.60*	*0.42*	*0.61*	*0.09*	*0.53*	*0.60*	*0.47*	*0.72*	*0.14*
Fertilizer	*0.28*	*0.30*	*0.65*	*0.26*	*0.35*	*0.46*	*0.60*	*0.20*	*0.26*	*0.35*	*0.81*	*0.92*	*0.63*
Crop: Fertilizer	*0.11*	*0.05*	*0.46*	*0.41*	*0.28*	*0.46*	*0.43*	*0.90*	*0.41*	*0.28*	*0.11*	*0.12*	*0.97*
7.5-15 cm													
Spring-grain	12.79	1.10	11.60	0.24	0.22	41.45	4.02	10.33	0.76	0.78	54.25	5.12	10.60
Perennial-grain	15.49	1.34	11.53	0.25	0.23	45.26	4.44	10.20	0.75	0.77	60.75	5.79	10.50
Perennial-forage	14.51	1.23	11.75	0.25	0.23	42.81	4.14	10.35	0.75	0.77	57.32	5.38	10.67
No-N	13.85	1.20	11.60	0.24	0.22	42.92	4.21	10.21	0.76	0.78	56.77	5.40	10.52
With-N	14.67	1.26	11.65	0.25	0.23	43.43	4.19	10.37	0.75	0.77	58.11	5.45	10.66
Crop	*0.27*	*0.27*	*0.10*	*0.12*	*0.09*	*0.12*	*0.09*	*0.29*	*0.64*	*0.69*	*0.09*	*0.07*	*0.16*
Fertilizer	*0.54*	*0.58*	*0.55*	*0.72*	*0.93*	*0.72*	*0.93*	*0.07*	*0.57*	*047*	*0.56*	*0.82*	*0.05*
Crop: Fertilizer	*0.93*	*0.94*	*0.81*	*0.43*	*0.33*	*0.43*	*0.33*	*0.35*	*0.94*	*0.95*	*0.60*	*0.51*	*0.49*

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at P <0.05, P <0.01, P <0.001, respectively. NS means non-significance between variables.

Fall-grain data missing on September 2019.

Table S2. 7. ANOVA P-value in repeat measures analysis with crops (spring-grain and perennial-grain) x fertilizer x date effects on Particulate organic matter (POM-C), mineral-associate organic matter (MAOM-C), and total organic carbon (TOC) concentration at the Breton and Edmonton sites.

	Breton					
	0-7.5 cm			7.5-15cm		
	POM-C	MAOM-C	TOC	POM-C	MAOM-C	TOC
Crop	0.13	0.87	0.48	0.98	0.88	0.9015
Fertilizer	0.60	0.30	0.32	0.67	0.48	0.47
Time	0.92	0.48	0.88	<0.01**	<0.05*	<0.05*
Crop : Fertilizer	0.30	0.30	0.62	0.24	0.97	0.74
Crop : Time	0.25	0.90	0.98	0.15	0.20	0.09
Fertilizer : Time	0.72	0.16	0.28	0.75	0.38	0.36
Crop : Fertilizer : Time	0.18	<0.05*	0.05	0.41	<0.05*	<0.05*

	Edmonton					
	0-7.5 cm			7.5-15cm		
	POM-C	MAOM-C	TOC	POM-C	MAOM-C	TOC
Crop	0.99	0.14	0.19	0.86	<0.05*	<0.05*
Fertilizer	0.21	0.10	0.38	0.44	0.32	0.15
Time	0.03	0.89	0.69	0.90	<0.05*	0.05
Crop : Fertilizer	0.09	0.57	0.67	0.45	0.26	<0.05*
Crop : Time	0.77	0.92	0.97	0.18	0.16	0.27
Fertilizer : Time	<0.001***	0.09	0.77	0.87	0.89	0.88
Crop : Fertilizer : Time	0.08	0.16	0.88	0.95	0.16	0.08

Table S2. 8. ANOVA P-value with crops (spring-grain, fall-grain perennial-grain, perennial-forage) x fertilizer effects on above-biomass C:N (AB-C:N) ratio at the Breton and Edmonton sites.

	numDf	DenDf	F-value	P-value
		Breton		
Crop	1	41	41.00	<0.001***
Fertilizer	3	41	4.05	0.05
Crop : Fertilizer	3	41	0.83	0.48
		Edmonton		
Crop	1	50	11.64	<0.001***
Fertilizer	30	50	1.24	0.27
Crop : Fertilizer	30	50	0.11	0.9480

Table S2. 9. Spearman correlation coefficient for association to soil properties in Breton.

	POM-C	POM-N	POM-C:N	POM-CF	POM-NF	MAOM-C	MAOM-N	MAOM-C:N	MAOM-CF	MAOM-NF	Soil-TOC	Soil-TN	Soil-C:N	Root-C	Root-N	Root-C:N	AB-C	AB-N
POM-N	0.768***																	
POM-C:N	0.361 NS	-0.250 NS																
POM-CF	0.736*	0.589***	0.225 NS															
POM-NF	0.364*	0.632*	-0.400 NS	0.700***														
MAOM-C	-0.243 NS	-0.146 NS	-0.071 NS	-0.796 NS	-0.668 NS													
MAOM-N	0.054*	-0.196 NS	0.457**	-0.439 NS	-0.854*	0.711***												
MAOM-C:N	-0.443 NS	-0.057 NS	-0.568**	-0.368*	0.321 NS	0.196 NS	-0.504*											
MAOM-CF	-0.736***	-0.589***	-0.225 NS	-1.000***	-0.700***	0.796 NS	0.439 NS	0.368*										
MAOM-NF	-0.364*	-0.632***	0.400 NS	-0.700***	-1.000***	0.668 NS	0.854*	-0.321 NS	0.700***									
Soil-TOC	0.029***	0.129**	-0.061 NS	-0.607 NS	-0.521 NS	0.925***	0.686***	0.136 NS	0.607 NS	0.521 NS								
Soil-TN	0.246***	0.043**	0.400*	-0.321 NS	-0.711 NS	0.700***	0.954***	-0.486*	0.321 NS	0.711 NS	0.754***							
Soil-C:N	-0.264 NS	0.104 NS	-0.500*	-0.196 NS	0.461 NS	0.086 NS	-0.575*	0.968***	0.196 NS	-0.461 NS	0.096 NS	-0.521 NS						
Root-C	0.064 NS	0.218 NS	-0.164 NS	0.250 NS	0.564 NS	-0.339 NS	-0.579*	0.389 NS	-0.250 NS	-0.564 NS	-0.225 NS	-0.471*	0.504 NS					
Root-N	-0.054 NS	-0.061 NS	0.111 NS	0.146 NS	0.214 NS	-0.286 NS	-0.264 NS	0.079 NS	-0.146 NS	-0.214 NS	-0.229 NS	-0.218 NS	0.150 NS	0.857***				
Root-C:N	0.121 NS	-0.229 NS	0.543***	0.114 NS	-0.454 NS	-0.007 NS	0.543**	-0.646***	-0.114 NS	0.454 NS	0.054 NS	0.450*	-0.643***	-0.271 NS	0.068 NS			
AB-C	-0.050 NS	0.171 NS	-0.354 NS	0.021 NS	0.486*	-0.143 NS	-0.604*	0.550**	-0.021 NS	-0.486*	-0.182 NS	-0.607*	0.614***	0.311 NS	-0.114 NS	-0.746***		
AB-N	-0.182 NS	0.075 NS	-0.386 NS	-0.054 NS	0.464 NS	-0.161 NS	-0.625*	0.632***	0.054 NS	-0.464 NS	-0.189 NS	-0.661*	0.696***	0.325 NS	-0.054 NS	-0.718***	0.946***	
AB-C:N	0.529*	0.246 NS	0.314 NS	0.296*	-0.050 NS	0.079 NS	0.207 NS	-0.346 NS	-0.296 NS	0.050 NS	0.054 NS	0.289 NS	-0.350 NS	-0.196 NS	-0.232 NS	0.075 NS	-0.054 NS	-0.329 NS

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at *P* <0.05, *P* <0.01, *P* <0.001, respectively. NS means non-significance between variables.

Table S2. 10. Spearman correlation coefficient for association to soil properties in Edmonton.

	POM-C	POM-N	POM-C:N	POM-CF	POM-NF	MAOM-C	MAOM-N	MAOM-C:N	MAOM-CF	MAOM-NF	Soil-TOC	Soil-TN	Soil-C:N	Root-C	Root-N	Root-C:N	AB-C	AB-N
POM-N	0.955***																	
POM-C:N	-0.228	-0.406																
POM-CF	0.732***	0.806***	-0.307															
POM-NF	0.692***	0.765***	-0.311	0.990***														
MAOM-C	0.302	0.187	0.141	-0.375	-0.410													
MAOM-N	0.317	0.218	-0.013	-0.364	-0.408	0.967*												
MAOM-C:N	-0.205	-0.331	0.705***	-0.108	-0.071	-0.071	-0.263											
MAOM-CF	-0.732***	-0.806***	0.307	-1.000***	-0.990***	0.375	0.364	0.108										
MAOM-NF	-0.692***	-0.765***	0.311	-0.990***	-1.000***	0.410	0.408	0.071	0.990***									
Soil-TOC	0.666**	0.544**	0.073	0.026	-0.020	0.895***	0.864***	-0.065	-0.026	0.020								
Soil-TN	0.591**	0.525**	-0.152	-0.042	-0.092	0.891***	0.938***	-0.370	0.042	0.092	0.928***							
Soil-C:N	-0.088	-0.226	0.796***	0.051	0.088	-0.125	-0.329	0.938***	-0.051	-0.088	-0.055	-0.381						
Root-C	-0.263	-0.381	0.191	-0.280	-0.251	-0.084	-0.135	0.474**	0.280	0.251	-0.094	-0.257	0.360***					
Root-N	-0.401	-0.366	-0.352	-0.389	-0.375	-0.069	0.001	-0.094	0.389	0.375	-0.197	-0.129	-0.278	0.688***				
Root-C:N	-0.133	-0.187	0.519	0.007	-0.020	-0.082	-0.150	0.432	-0.007	0.020	-0.088	-0.203	0.480	-0.137	-0.484			
AB-C	0.602**	0.680***	-0.311	0.395	0.381	0.309	0.321	-0.346	-0.395	-0.381	0.488*	0.521**	-0.271	-0.323	-0.137	-0.445		
AB-N	0.373	0.218	0.523	0.121	0.143	0.311	0.156	0.558**	-0.121	-0.143	0.486	0.216	0.637**	0.352	-0.207	0.011	0.360*	
AB-C:N	0.125	0.300	-0.730***	0.156	0.110	-0.015	0.158	-0.831***	-0.156	-0.110	-0.057	0.247	-0.849***	-0.509*	0.112	-0.366	0.300	-0.723**

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC; Total organic matter; C, Carbon; N, Nitrogen CF, Carbon fraction; NF, Nitrogen fraction.

*, **, and *** shows significance correlation at *P* <0.05, *P* <0.01, *P* <0.001, respectively. NS means non-significance between variables.

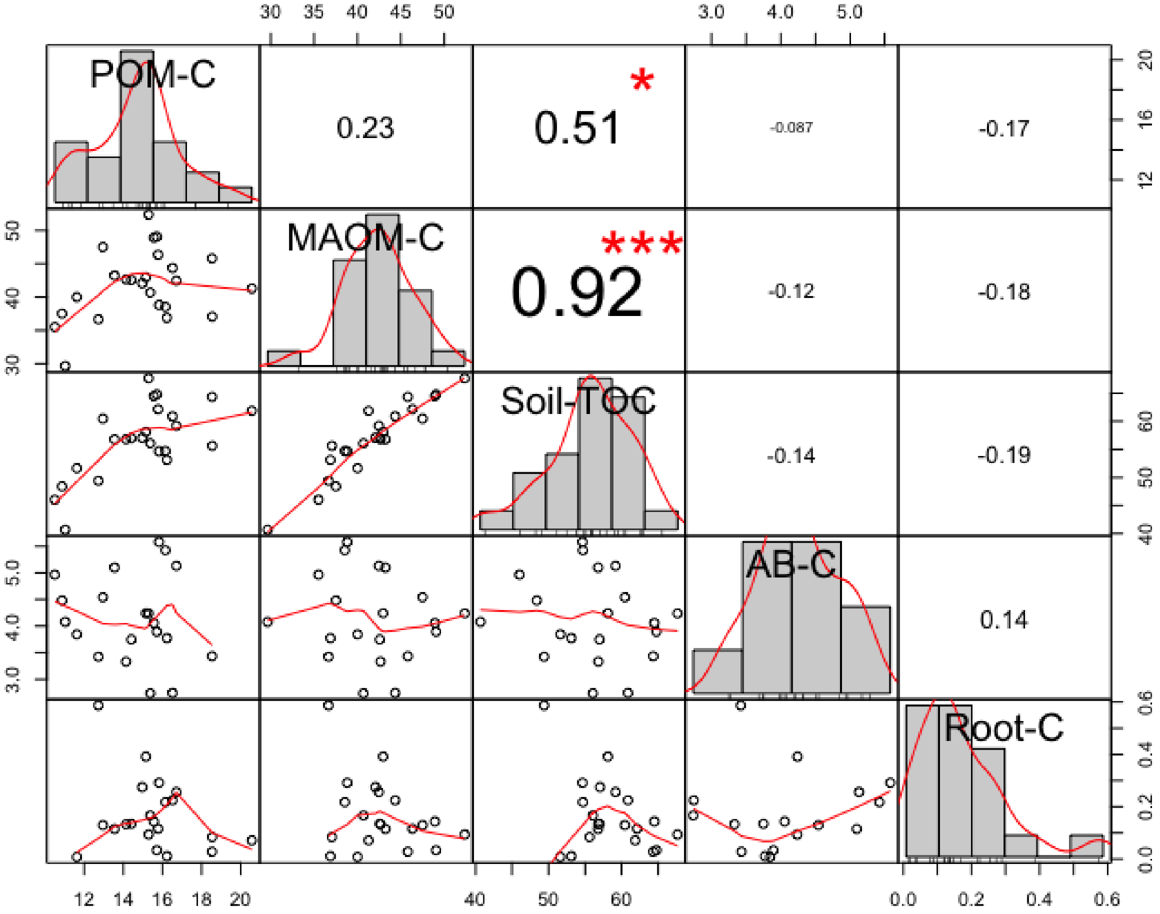

Fig. S2. 1. Spearman correlation analyses of the Breton soils at 0-15 cm depth increment. Distribution of variables shows bar graph, correlation coefficient is noted on right panels of diagonal, and paired correlation represents on left panels of diagonal. *, **, and *** indicate significance of less than 0.05,0.01, and 0.001, respectively.

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC, total organic matter; AB, above biomass; C, carbon; N, nitrogen.

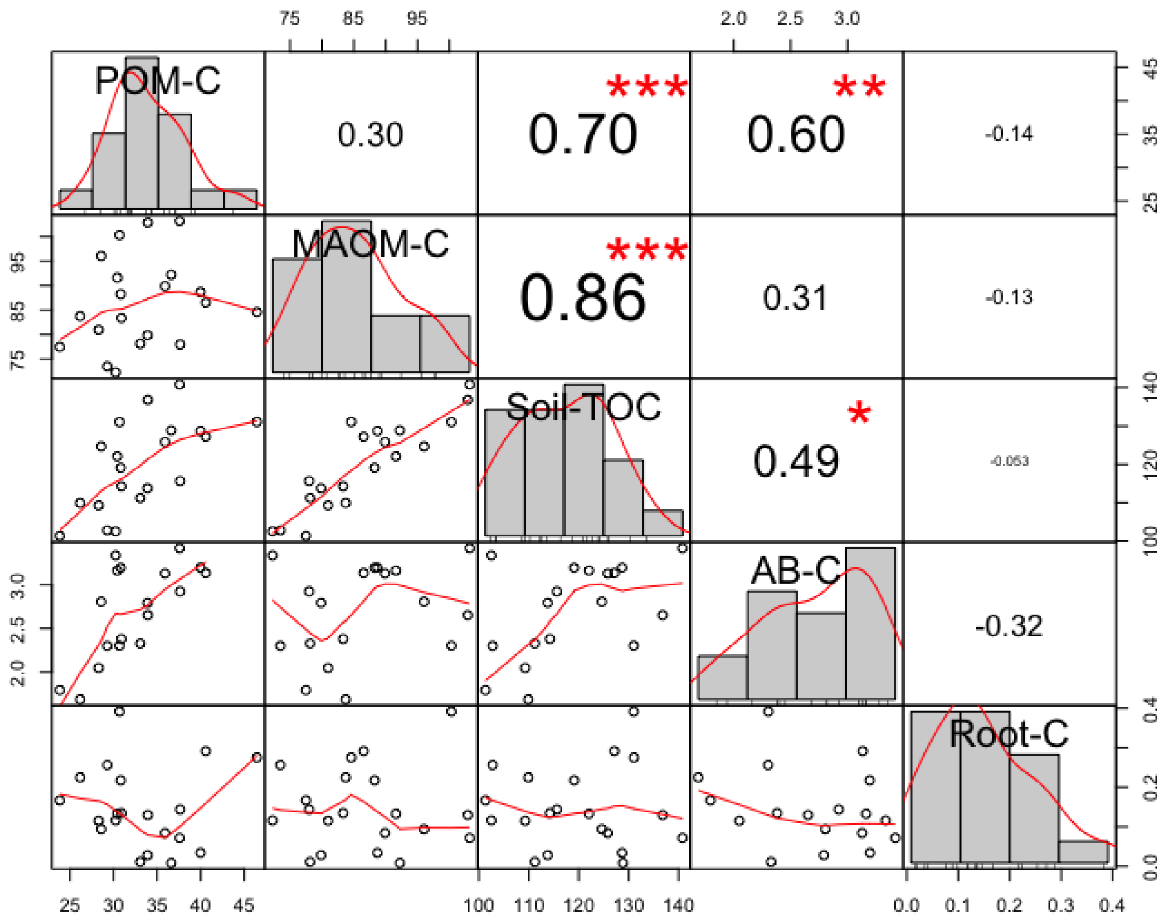

Fig. S2. 2. Spearman correlation analyses of the Edmonton soils at 0-15 cm depth increment. Distribution of variables shows bar graph, correlation coefficient is noted on right panels of diagonal, and paired correlation represents on left panels of diagonal. *, **, and *** indicate significance of less than 0.05,0.01, and 0.001, respectively.

Note: POM stands for particulate organic matter; MAOM, mineral-associate organic matter; TOC, total organic matter; AB, above biomass; C, carbon; N, nitrogen.

SUPPLEMENTARY MATERIAL

CHAPTER 3. Arbuscular Mycorrhizal Fungi Community Linkages to Soil Nutrient Availability across Contrasting Agroecosystems.

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Table S3. 1. Mean and standard errors (n=4) for arbuscular colonization (AC), vesicle colonization (VC), and hyphal colonization (HC). Italic number means *P*-value.

Treatment		AC	VC	HC
		Breton		
Crop	Spring	0.37±0.26	6.25±1.53	38.00±4.00
	Fall	0.37±0.26	6.87±1.34	31.62±1.46
		1	*0.74*	*0.19*
Fertilizer	noN	0.62±0.32	7.35±1.30	34.62±2.78
	withN	0.12±0.12	5.75±1.50	35.00±3.64
		0.15	*0.40*	*0.89*
		Edmonton		
Crop	Spring	-	0.62±0.49	6.37±1.67
	Fall	-	0.12±0.12	5.25±0.55
	Perennial		0.12±0.12	6.62±0.84
		-	*0.39*	*0.57*
Fertilizer	noN	-	0.25±0.13	5.83±0.85
	withN	-	0.33±0.33	6.33±0.97
		-	*0.80*	*0.66*

Table S3. 2. Alpha diversity in soil samples across the crop × fertilizer effects with two-way ANOVA.

		Breton		Edmonton	
Parameter	Treatment	F-value	P-value	F-value	P-value
Chao1	Crop	1.01	0.40	0.54	0.64
	Fertilizer	0.06	0.79	0.11	0.73
	Crop: Fertilizer	0.19	0.89	0.45	0.71
Evenness	Crop	2.29	0.10	0.37	0.77
	Fertilizer	2.02	0.16	1.24	0.27
	Crop: Fertilizer	0.12	0.94	0.43	0.72
Shannon	Crop	2.27	0.10	3.38	<0.05
	Fertilizer	1.68	0.20	1.02	0.32
	Crop: Fertilizer	0.09	0.96	1.69	0.19
InvSimpson	Crop	2.74	0.06	4.47	<0.05
	Fertilizer	3.54	0.07	4.91	<0.05
	Crop: Fertilizer	0.45	0.71	2.21	0.11

Number of degree freedom (NumDF) is [Crop = 3, Fertilizer = 1, Crop: Fertilizer = 3].
Denominator of Degree of freedom (denDF) is 21 and 20, in Breton and Edmonton, respectively.
Fallow was excluded to meet the statistical assumptions under Two-way ANOVA.

Table S3. 3. Alpha diversity in roots samples across the crop × fertilizer effects with two-way ANOVA

		Breton		Edmonton	
Parameter	Treatment	F-value	P-value	F-value	P-value
Chao1	Crop	0.02	0.88	1.09	0.35
	Fertilizer	0.09	0.76	0.04	0.82
	Crop: Fertilizer	3.12	0.11	3.45	0.05
Evenness	Crop	0.74	0.44	2.07	0.15
	Fertilizer	1.48	0.25	0.05	0.82
	Crop: Fertilizer	0.10	0.75	1.74	0.20
Shannon	Crop	0.70	0.42	2.41	0.11
	Fertilizer	1.73	0.22	0.04	0.84
	Crop: Fertilizer	0.35	0.56	1.49	0.25
InvSimpson	Crop	0.62	0.45	2.48	0.11
	Fertilizer	3.33	0.10	0.14	0.70
	Crop: Fertilizer	0.10	0.75	1.75	0.20

Number of degree freedom (NumDF) is [Crop = 1, Fertilizer = 1, Crop: Fertilizer = 1] and [Crop = 2, Fertilizer = 1, Crop: Fertilizer = 2] in Breton and Edmonton, respectively.
Denominator of Degree of freedom (denDF) is 8 and 15, in Breton and Edmonton, respectively
Fallow was excluded to meet the statistical assumptions under Two-way ANOVA.

Table S3. 4. PERMANOVA across combination cropping systems (crop × N fertilizer) and rhizosphere compartments (soil vs roots).

		Breton			Edmonton	
Treatment	F.model	R^2	Pr(>F)	F.model	R^2	Pr(>F)
			Soil			
Crop	1.961	0.174	0.027*	1.540	0.137	0.089
Fertilizer	0.997	0.029	0.392	0.776	0.023	0.585
Crop : Fertilizer	0.530	0.049	0.946	1.425	0.127	0.126
			Roots			
Crop	0.235	0.017	0.946	2.287	0.182	0.035*
Fertilizer	0.952	0.072	0.397	0.436	0.017	0.883
Crop : Fertilizer	0.894	0.068	0.413	0.496	0.041	0.890
			Soil vs. roots			
Compartment	4.257	0.128	0.005**	8.962	0.163	0.001***

Significance codes: *P*<0.05, *; *P*<0.01, **; *P*<0.001, ***.
To avoid type 1 errors caused by unbalance design, the fallow plots were precluded.
The data follows the assumption of homogenous dispersion among the groups

Table S3. 5. Pairwise community composition comparison between crops in soil samples based on PERMANOVA. P value adjusted by false discovery rate (FDR).

	Fallow	Spring-grain	Fall-grain	Perennial-grain
		*Breton-Soil (F=2.644, P<0.004**)*		
Spring-grain	0.25	-	-	-
Fall-grain	0.11	0.99	-	-
Perennial-grain	0.03	0.15	0.12	-
Perennial-forage	0.03	0.03	0.04	0.15
		Breton-Roots (F=0.236, P>0.956)		
Fall-grain		0.96		
		Edmonton-Soil (F=1.506, P<0.081)		
Spring-grain	0.27	-	-	-
Fall-grain	0.29	0.27	-	-
Perennial-grain	0.27	0.95	0.27	-
Perennial-forage	0.37	0.29	0.27	0.29
		Edmonton-Roots (F=2.350, P<0.036)*		
Fall-grain		0.036		
Perennial-grain		0.303	0.243	

Significance codes: $P<0.05$, *; $P<0.01$, **; $P<0.001$, ***.

Table S3. 6. Differential abundance in genus rank impacted by treatments (crop × fertilizer × compartment) in Breton.

Genus	logFC	logCPM	LR	P-value	FDR
Fertilizer effects in soil compartment					
Acaulospora	2.642836	9.580914	2.166499	0.141047	0.919298
Ambispora	-0.55699	11.17158	0.546749	0.459649	0.919298
Archaeospora	0.182	15.0181	0.104635	0.746336	0.943827
Claroideoglomus	0.071661	13.89618	0.048413	0.825849	0.943827
Diversispora	-1.53564	11.92309	0.685517	0.407694	0.919298
Glomus	-0.20803	14.06627	0.164056	0.68545	0.943827
Paraglomus	-0.11856	19.87957	0.593174	0.441194	0.919298
Scutellospora	-0.04287	10.7842	0.001414	0.970005	0.970005
Cropping effects in root compartment (spring-grain vs fall-grain)					
Acaulospora	0.682645	6.335879	1.181428	0.277065	0.627046
Ambispora	0.567558	11.55047	0.490939	0.483509	0.627046
Archaeospora	0.41255	14.93505	0.786322	0.375215	0.627046
Claroideoglomus	0.136911	13.89087	0.136253	0.712035	0.712035
Diversispora	-0.68932	10.37202	0.460876	0.497215	0.627046
Glomus	0.863056	14.20747	3.981907	0.045991	0.367932
Paraglomus	-0.19049	20.55112	0.614587	0.433066	0.627046
Scutellospora	-0.43416	9.250149	0.359714	0.548665	0.627046
Fertilizer effects in roots compartment					
Acaulospora	1.262728	6.335795	0.711058	0.399093	0.456106
Ambispora	1.601079	11.55023	1.496333	0.221237	0.456106
Archaeospora	1.939533	14.93503	8.142036	0.004325	<0.05*
Claroideoglomus	-0.64372	13.89092	0.933912	0.333848	0.456106
Diversispora	-2.46323	10.37145	1.277358	0.258391	0.456106
Glomus	-0.7862	14.20747	0.879209	0.348418	0.456106
Paraglomus	0.726677	20.55111	2.232146	0.135166	0.456106
Scutellospora	1.600482	9.251566	0.496681	0.480962	0.480962
Compartment (soil vs. roots) difference					
Acaulospora	-1.02122	7.690594	2.900783	0.088537	0.708292
Ambispora	-0.3002	11.59216	0.714501	0.397954	0.944815
Archaeospora	0.02467	14.82294	0.021331	0.883882	0.944815
Claroideoglomus	0.055868	13.90856	0.109388	0.740843	0.944815
Diversispora	0.03485	11.28372	0.004791	0.944815	0.944815
Glomus	0.02727	14.06679	0.008401	0.926972	0.944815
Paraglomus	0.086244	20.30451	0.354503	0.551575	0.944815
Scutellospora	-0.78476	10.41876	1.801069	0.179583	0.718333

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at *P* <0.05, *P* <0.01, *P* <0.001, respectively.

Table S3. 7. Differential abundance at genus level analyzed by treatments (crop × fertilizer × compartment) at Edmonton sites.

Genus	logFC	logCPM	LR	P-value	FDR
Fertilizer effects in soil compartment					
Acaulospora	-1.77E-16	7.575127	0	1	1
Ambispora	1.765907	9.322814	0.493007	0.482589	0.721545
Archaeospora	-0.66958	17.58572	1.308163	0.252727	0.721545
Claroideoglomus	1.518271	16.87893	5.928463	0.014898	0.119186
Diversispora	-1.77E-16	7.575127	0	1	1
Glomus	-0.585	14.24991	0.373396	0.541159	0.721545
Paraglomus	0.692506	22.50767	0.955109	0.328422	0.721545
Scutellospora	-1.46681	12.75278	0.734741	0.391351	0.721545
Cropping effects in root compartment (spring-grain vs fall-grain)					
Acaulospora	8.21E-17	7.194283	0	1	1
Ambispora	1.970229	9.556909	1.022976	0.311814	0.825811
Archaeospora	0.174634	14.35988	0.109443	0.740779	0.987706
Claroideoglomus	0.315126	14.83909	0.696158	0.404078	0.825811
Diversispora	8.21E-17	7.194283	0	1	1
Glomus	0.6814	13.58865	0.670419	0.412905	0.825811
Paraglomus	-0.29125	20.21928	1.353077	0.244741	0.825811
Scutellospora	0.412786	10.60659	0.200567	0.654264	0.987706
Cropping effects in root compartment (spring-grain vs perennial-grain)					
Acaulospora	-7.72E-17	7.202059	0	1	1
Ambispora	1.155856	8.315646	0.275063	0.599956	1
Archaeospora	0.220081	14.21418	0.091191	0.762668	1
Claroideoglomus	0.682944	15.23821	2.135159	0.143956	1
Diversispora	-7.72E-17	7.202059	0	1	1
Glomus	-0.0826	11.94321	0.004739	0.945114	1
Paraglomus	-0.0401	19.84856	0.311358	0.576848	1
Scutellospora	-0.99721	10.93968	0.574508	0.448473	1
Cropping effects in root compartment (fall-grain vs perennial-grain)					
Acaulospora	-6.19E-17	7.084325	0	1	1
Ambispora	0.605332	9.755413	0.46463	0.495468	0.734964
Archaeospora	0.215669	14.19482	0.355131	0.551223	0.734964
Claroideoglomus	-0.23697	15.07349	0.382598	0.536216	0.734964
Diversispora	-6.19E-17	7.084325	0	1	1
Glomus	0.917365	13.32161	2.625323	0.105171	0.28518
Paraglomus	-0.33287	20.40849	2.598832	0.106942	0.28518
Scutellospora	1.352874	9.776214	3.220377	0.072727	0.28518
Fertilizer effects in roots compartment					
Acaulospora	-2.88E-17	7.189787	0	1	1
Ambispora	-0.60002	9.670269	0.046734	0.828847	1
Archaeospora	0.378928	14.39363	0.178546	0.672625	1
Claroideoglomus	-0.0272	15.2656	0.001841	0.965779	1
Diversispora	-2.88E-17	7.189787	0	1	1
Glomus	0.317163	13.35807	0.057463	0.810551	1
Paraglomus	0.006089	19.8383	0.002905	0.957016	1
Scutellospora	0.115984	10.86432	0.002759	0.958108	1
Compartment (soil vs. roots) difference					
Acaulospora	-9.57E-17	7.202749	0	1	1
Ambispora	0.006309	9.713074	0.000285	0.986535	1
Archaeospora	-1.68878	17.1707	43.03483	5.38E-11	<0.001***
Claroideoglomus	-0.54974	15.56886	8.645709	0.003278	<0.05*
Diversispora	-9.57E-17	7.202749	0	1	1
Glomus	-0.52825	13.4875	1.652242	0.198654	0.397308
Paraglomus	0.298097	20.66507	3.852521	0.049671	0.132457
Scutellospora	-0.12422	11.99503	0.062296	0.802904	1

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

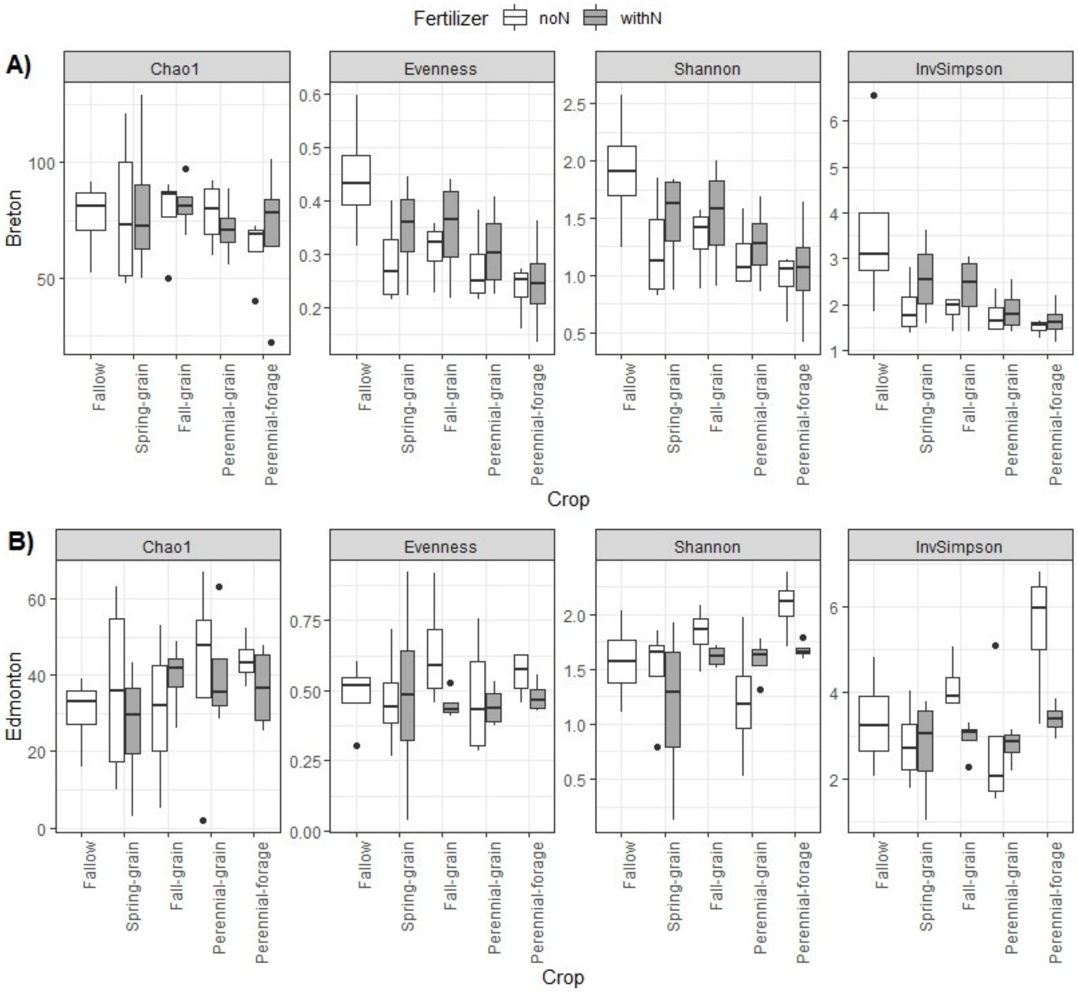

Fig. S3. 1. Arbuscular mycorrhizal fungi (AMF) alpha diversity including Chao1 richness, Peilou's Evenness, Shannon diversity and inverse Simpson diversity across cropping systems at (A) Breton and (B) Edmonton sites.

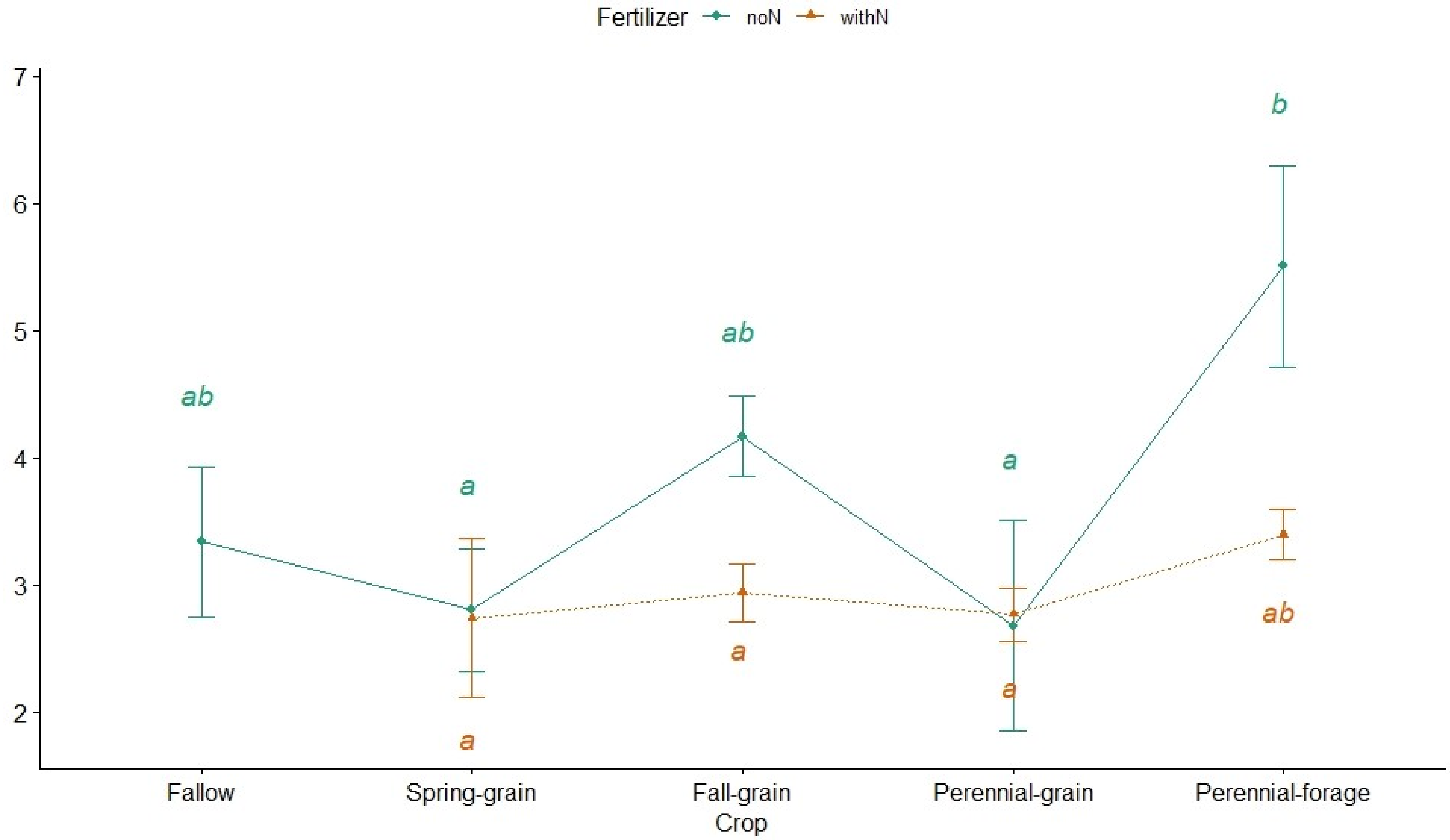

Fig. S3. 2. Arbuscular mycorrhizal fungi (AMF) inverse Simpson diversity in soil samples at Edmonton site ($P < 0.05$).

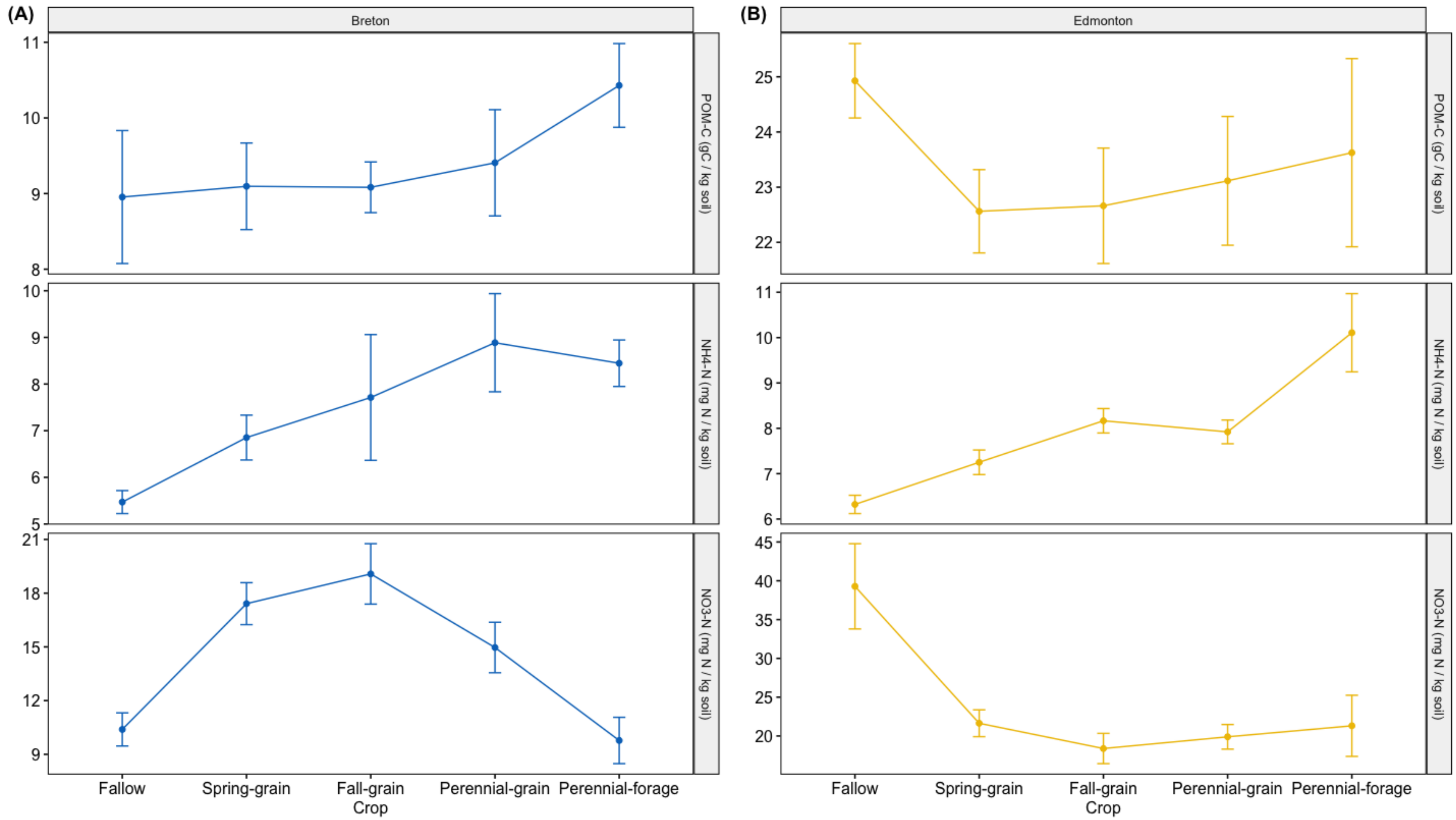

Fig. S3. 3. Soil properties (A) particulate organic matter carbon (POM-C), (B) ammonium concentration (NH_4^+; NH4-N), and (C) nitrate concentration (NO_3^-; NO3-N) across grain-cropping systems at both sites. Bar indicates standard error. Analysis of variance (ANOVA) *P* value of POM-C, NH4-N and NO3-N were less than 0.10, 0.01, and 0.001at the Breton site, and were 0.70, and less than 0.001, 0.01. In other word, all variables have significant difference corresponding to cropping systems expect to POM-C at the Edmonton site.

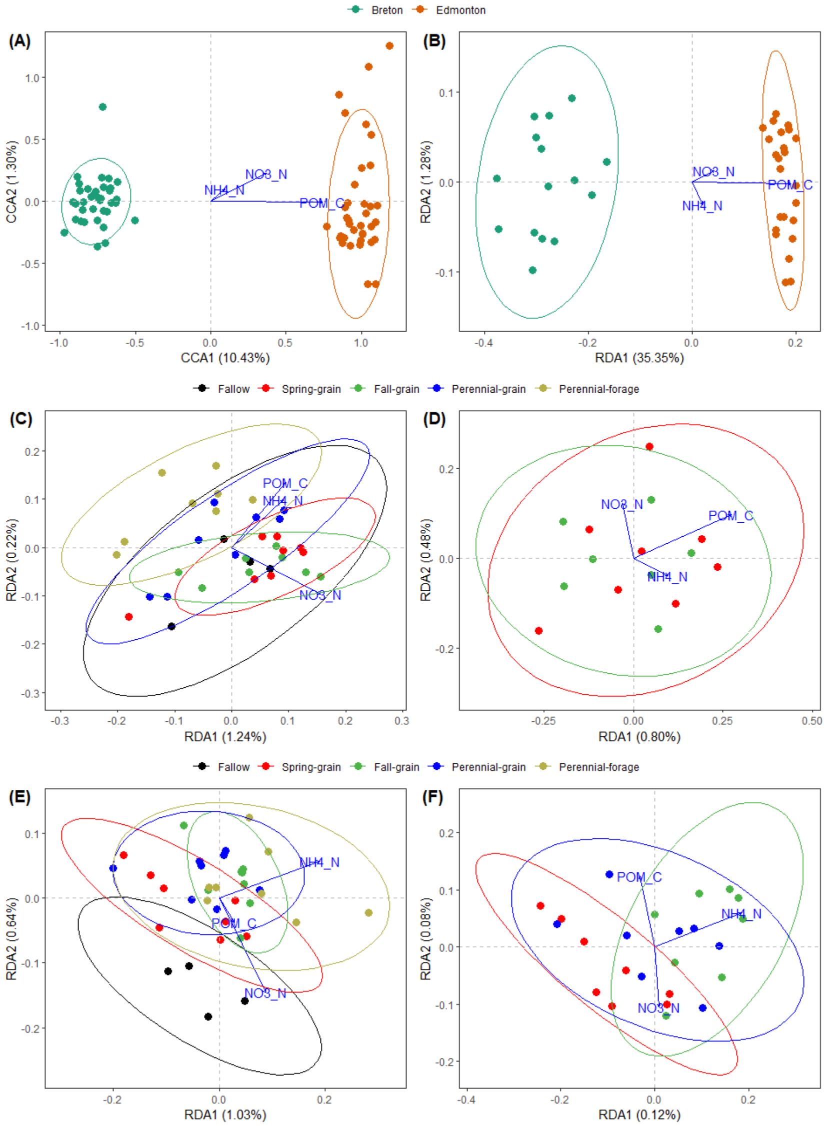

Fig. S3. 4. CCA of AMF taxonomic community composition among soil (A) and root (B) sample in two sites of contrasting historical land use agricultural field, RDA of AMF taxonomic community composition among Breton [soil (C), root (D)] and Edmonton [soil (E), root (F)] in current contrasting cropping systems (fallow, spring-grain, fall-grain perennial grain, and perennial-forage). Arrows in CCA and RDA graph represent significant fitted vectors.

Graph	Fitted vector	F-value	P-value
(A)	POM-C	8.4853	<0.001***
(B)	POM-C	23.196	<0.001***
(C)	NO_3-N	1.6781	<0.05*
(D)	POM-C	1.3966	<0.1
(E)	NH_4-N	1.7130	<0.05*

*, ** and *** are significance at alpha critical levels <0.05, < 0.01 and <0.001, respectively.

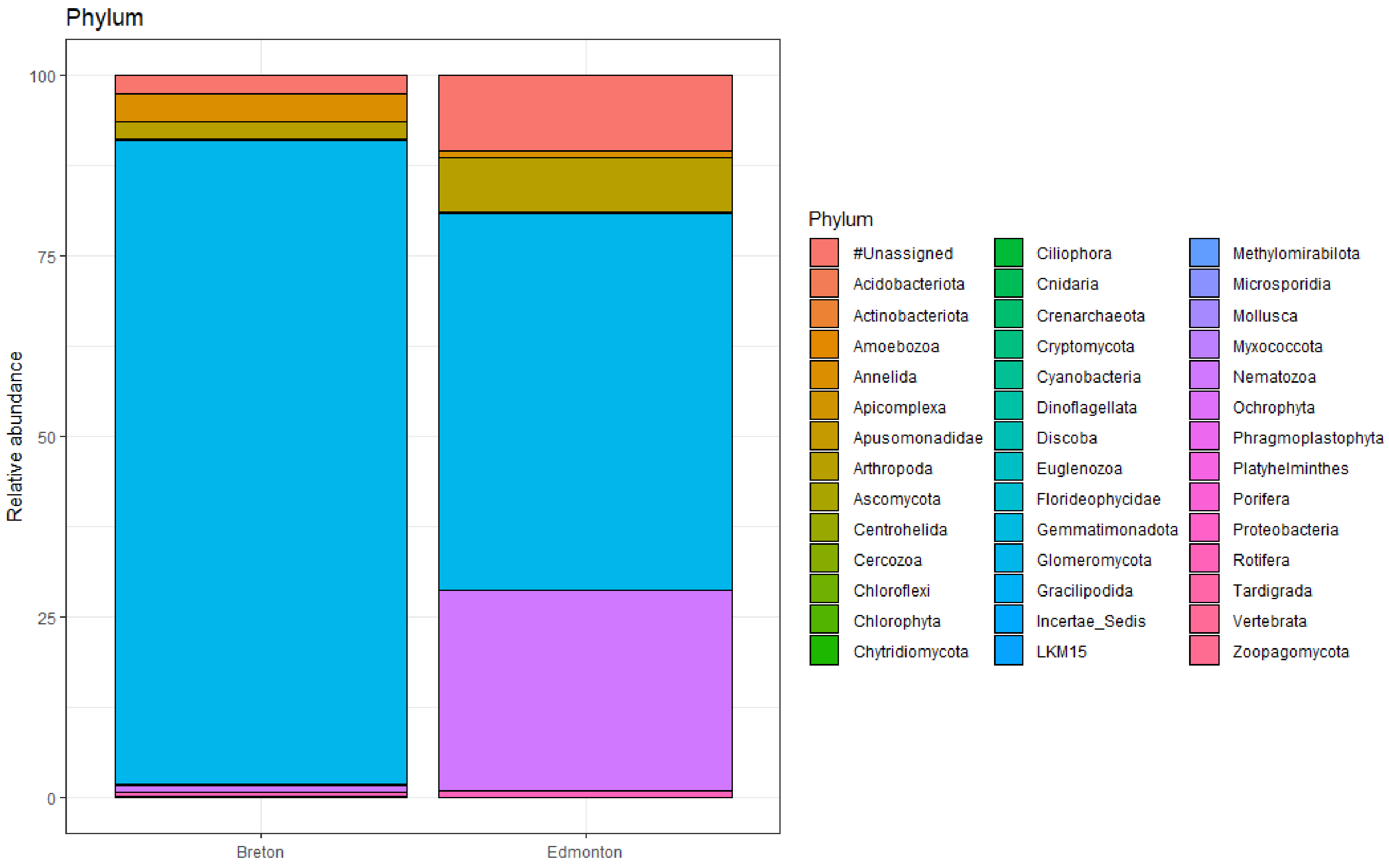

Fig. S3. 5.Proportional compositions of AMF communities in phylum level at the Breton and Edmonton sites.

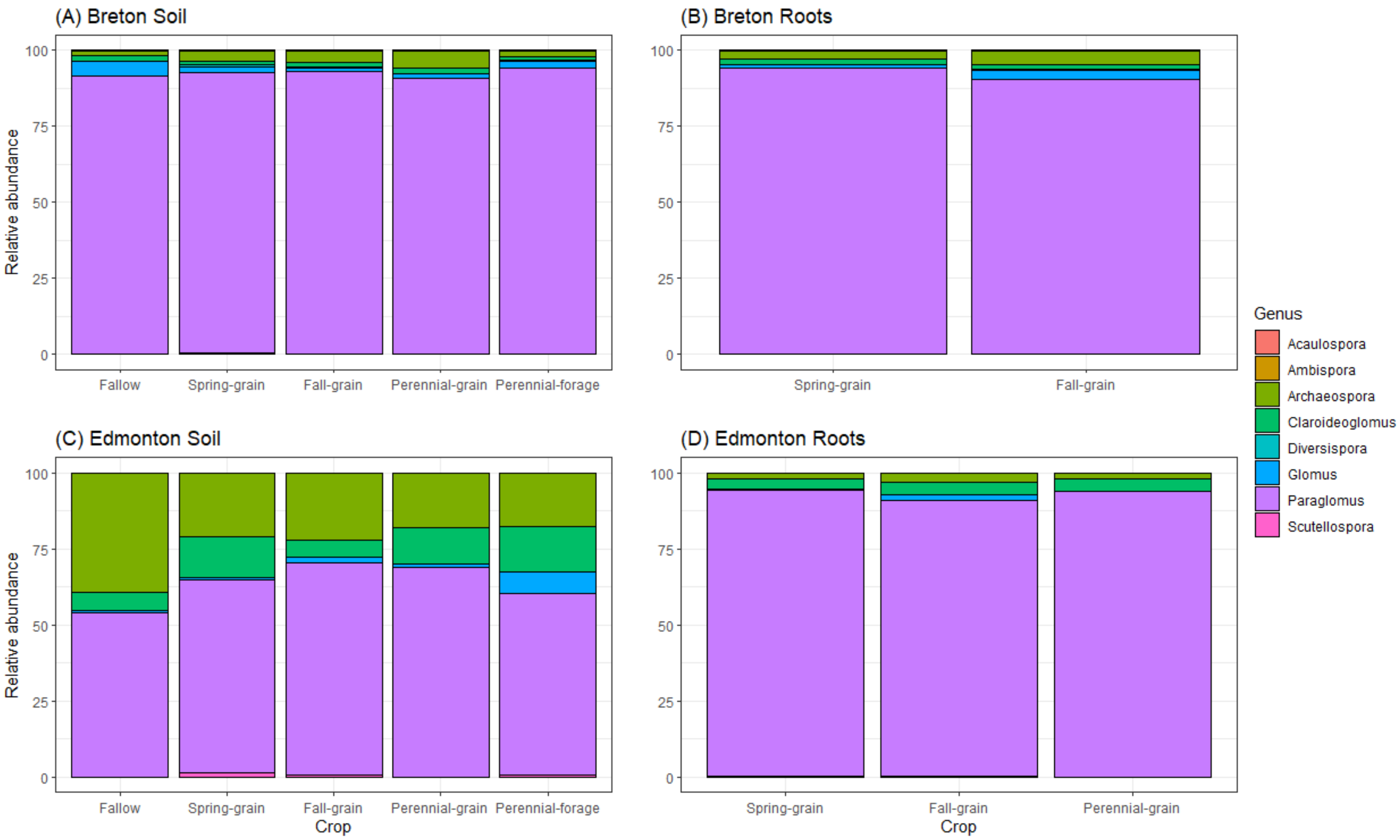

Fig. S3. 6. Proportional composition of AMF root and soil communities. Panel indicates proportional sequence abundance at site. Legend shows phylogenetic level to identified AMF genus.

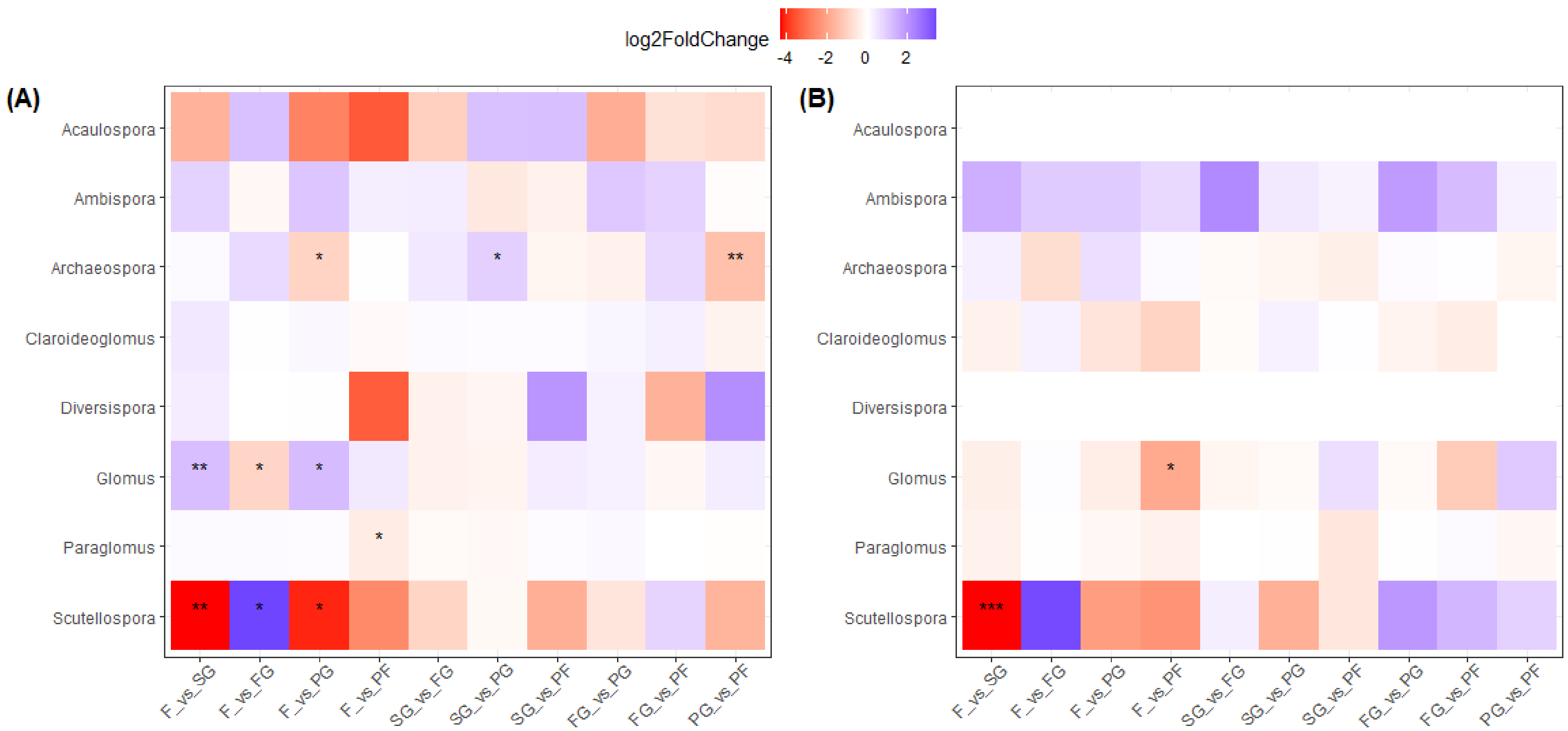

Fig. S3. 7. Heatmap of differential AMF genus across different cropping systems (F, Fallow; SG, spring-grain; FG, fall-grain; PG, perennial-grain; PF, perennial-forage). Left panel (A) represents Breton AMF soil samples, while right panel (B) shows Edmonton AMF soil sample. The log2 fold change between treatments is indicated by a color gradient. *, ** and *** are false discovery rate (FDR) at alpha critical levels <0.05, < 0.01 and <0.001, respectively.

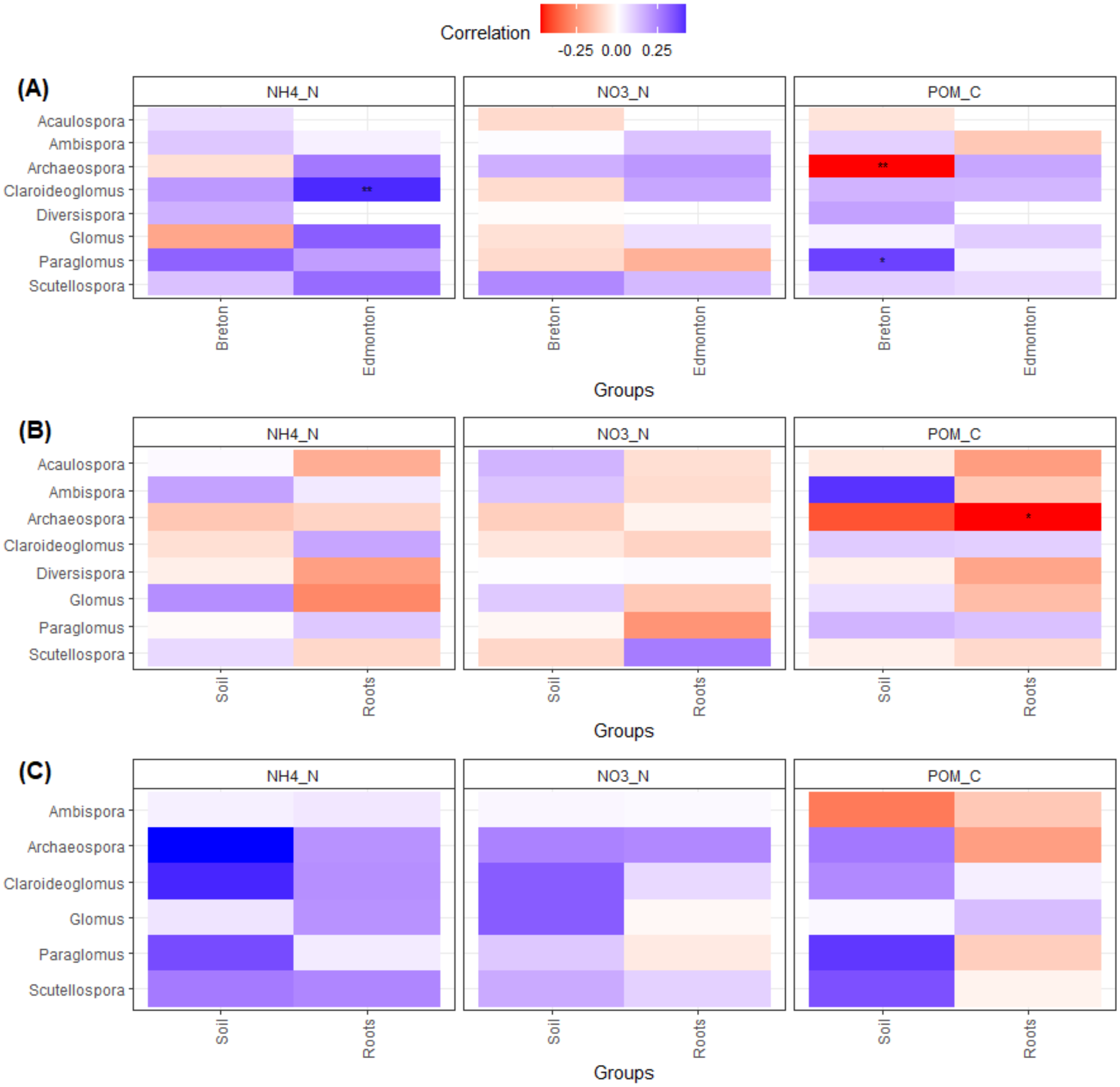

Fig. S3. 8. The heatmap of the correlation between AMF genus rank and physiochemical characteristics of (A) two site and (B) compartments in Breton and (C) compartments in Edmonton. Spearman's correlation analysis was used for creating the heatmap. Positive correlation is shown in red, whilst negative correlation is shown in blue. The asterisks mean significant correlations (*, $p<0.05$; **, $p<0.01$).

SUPPLEMENTARY MATERIAL

CHAPTER 4. Carbon and Water Dynamics of a Perennial Grain Crop in Temperate Agroecosystems

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Table S4. 1. Crop canopy lodging indices averaged from randomly collected measurements at pre-harvest (n=10).

	2018		2019	
Methodology	Rating1†	Rating2¶	Rating1	Rating2
Perennial grain crop	6.65±0.29 a	68.37±2.65 a	3.20±0.44	52.00±4.89
Annual grain crop	8.90±0.06 b	97.88±0.82 b	Non-lodging (NI)	NI
P-value	$P < 0.001$	$P < 0.001$		

† Scales from 0 to 9 (1= erect, 9= flat) on all replicates, lodging rating was evaluated when soil moisture content is less than 30 %.

¶ Berry et al., 2003. Methods for rapidly measuring the lodging resistance of cultivars.

Fig. S4. 1. University of Alberta Breton Research Plot configuration, red dots indicate the field locations of the eddy covariance systems within each 4-ha field.

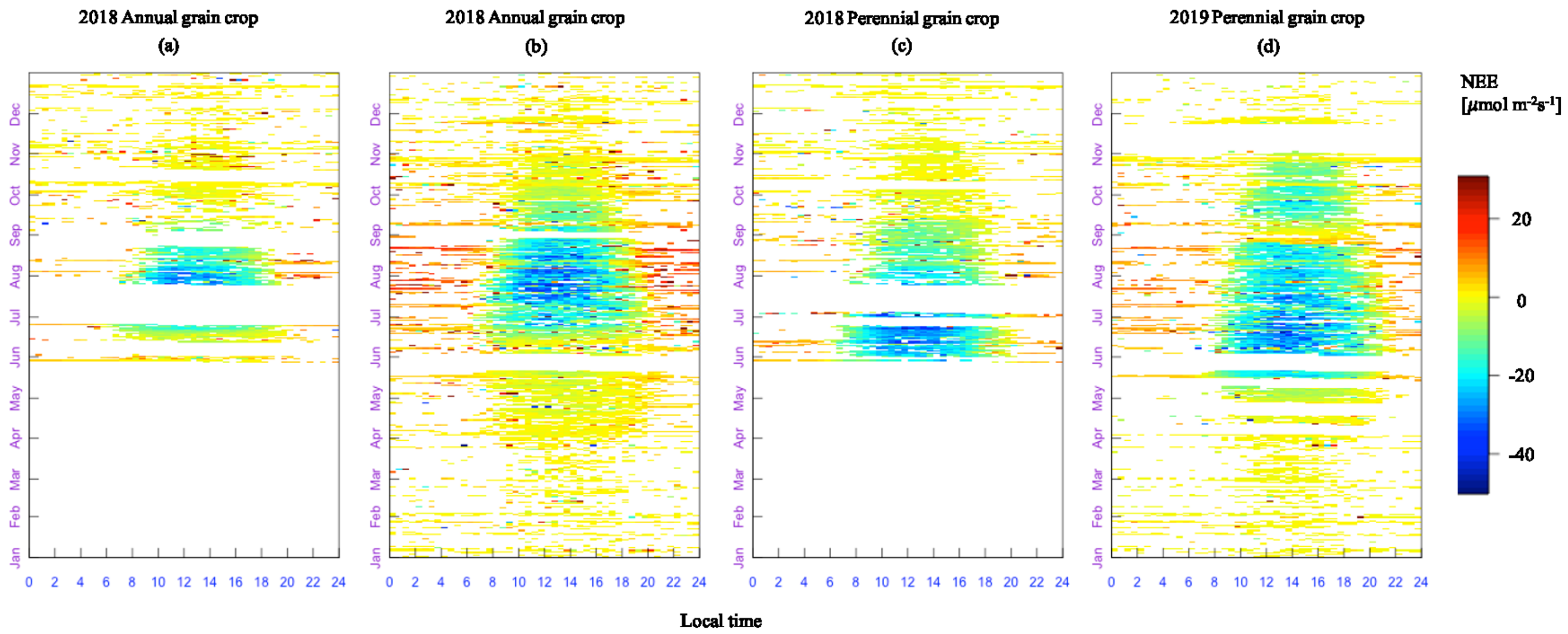

Fig. S4. 2. Diel net ecosystems exchange (NEE) fingerprint for annual vs. perennial grain cropping systems for two growing seasons before gap-filling.

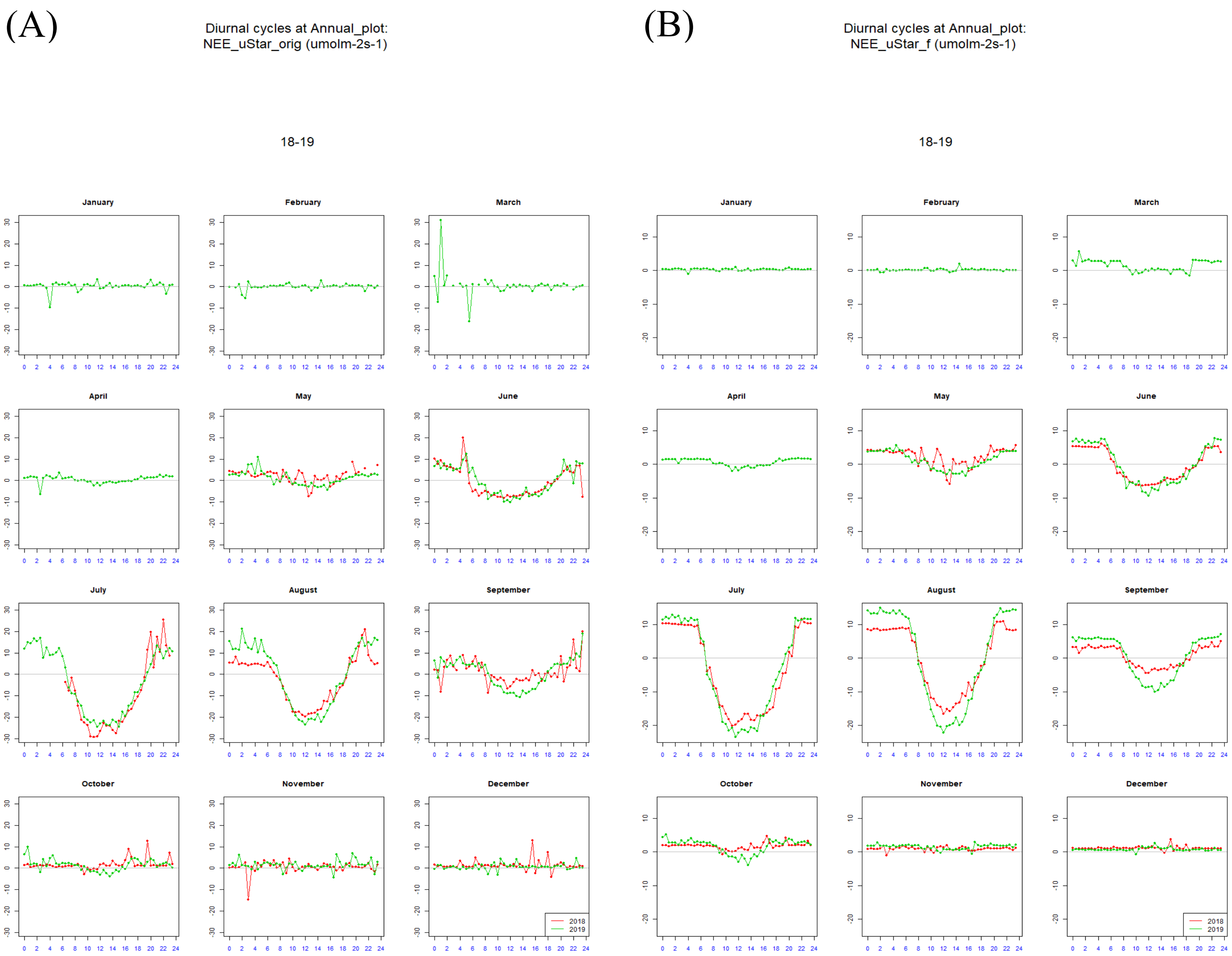

Fig. S4. 3. Monthly net ecosystems exchange (NEE) diel cycles in annual plot. Left panels (A) indicates original dataset while right panels (B) are final dataset after filtering and gap filling based on u-star scenario. Red lines are for 2018, while green lines are for 2019.

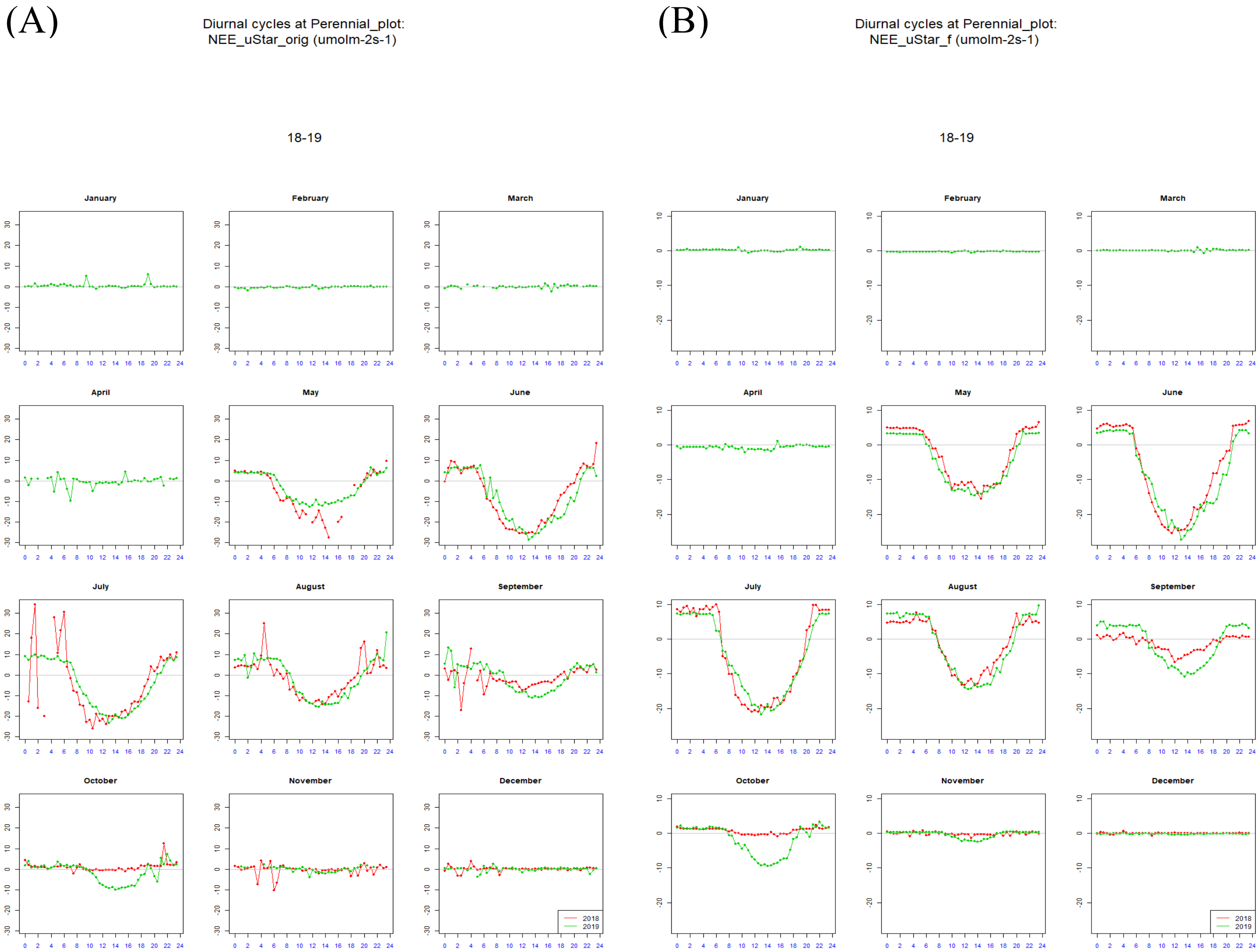

Fig. S4. 4. Monthly net ecosystems exchange (NEE) diel cycles in perennial plot. Left panels (A) indicate original dataset while right panels (B) are final dataset after filtering and gap filling based on u-star scenario. Red lines are for 2018, while green lines are for 2019