Assessment of Perennial Cereals in Central Alberta: Environmental Performance and Productivity

by

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Abstract

Despite their paramount importance, modern, high-input agroecosystems used for annual grain production are often characterized as degraded systems with substantial greenhouse gas (GHG) emissions, namely nitrous oxide (N_2O), a GHG 273x more potent than carbon dioxide (CO_2) on a mass basis. Perennial grain crops represent a novel hybrid between annual grain crops and perennial forage crops and are of great interest due to their purported ability to rectify several environmental challenges while continuing to deliver agricultural products. This body of work aimed to improve our understanding of soil nitrogen (N) cycling and N_2O production and release from agroecosystems and investigate the environmental performance and productivity of perennial grain cropping. Chapter 2 of this dissertation is a synthesis paper that establishes the conceptual foundations for the N_2O priming effect, defined as the short-term increase or decrease in the rate of soil organic N (SOM-N) mineralization in response to a stimulus, such as the addition of carbon (C) and/or N to the soil. Chapter 3 documents a laboratory incubation conducted to examine the effects of N fertilizer and artificial root exudate (ARE) on N_2O priming and changes in N_2O production from nitrification and denitrification sources. Additions of ARE or N fertilizer alone caused positive N_2O priming; while additions of ARE and urea concurrently resulted in an antagonistic interactive effect that diminished the N_2O production derived from SOM-N mineralization ($p < 0.05$). However, results indicate that the combination of elevated root exudation from a perennial cropping system with N fertilization has the potential to amplify N_2O emissions due to increases in both nitrification and denitrification sources. Chapters 4-7 document the results of a multi-year field study conducted at two sites in Central Alberta (Edmonton and Breton). Perennial cereal rye (*Secale cereale L.* × *S. montanum Guss* cv. ACE-1) was used as a model perennial grain crop for this research. At both sites, an experimental continuum of perenniality (perennial forage, perennial grain, fall grain, spring grain [or annual grain], and fallow) was established. Chapter 4 investigates the productivity of perennial grain cropping systems. Grain yield of the perennial rye in year one averaged 64% and 51% of the fall and spring rye yields at the Breton and Edmonton sites, respectively. Conversely, perennial rye at both sites yielded substantial aboveground biomass compared to other rye crops over both years. Overall, perennial rye cropping beyond two years faced issues of winter survival and weed competition. In Chapters 5, 6, and 7 the environmental performance of perennial grain cropping was assessed by: (1) evaluating how contrasting cropping systems might differentially alter soil physical and hydraulic properties, (2) measuring growing season N_2O emissions from contrasting cropping systems and (3) investigating how GHG emissions and soil organic C stocks respond when perennial crops are transitioned to annual crops after multiple growing seasons. While moderate improvements in soil physical and hydraulic properties manifested under the perennial grain crop at both sites when compared to the annual grain crop, they did not do so to the extent of the perennial forage crop. We attribute this to the inclusion of tap-rooted alfalfa in the perennial forage, and the overarching beneficial influence of root density on soil properties. Further, perennial grain crops reduced cumulative N_2O emissions at the Breton site by 60% and 94% in years two and three of the study, respectively ($Ps < 0.0001$). Correlation analyses for both sites revealed that the average root density was negatively correlated with soil available N ($p < 0.05$) and cumulative N_2O emissions, specifically at the Breton site ($p < 0.01$), suggesting that the enhanced root density of perennial crops reduced soil N availability, which translated into reduced cumulative N_2O emissions. However, when the perennial grain crop was transitioned to an annual crop via tillage, gains in soil C achieved at the Edmonton site during multi-year perennial grain cropping were lost to the atmosphere as CO_2. In sum, the environmental performance and agronomic productivity of perennial grain cropping suggest that while these novel systems have the potential to contribute to sustainable agroecosystems, yield reductions, winter mortality, and weed pressure suggest that perennial grain cropping is not yet a feasible option for cold temperate conditions.

Preface

This dissertation is an original work by Erin J. Daly. I was primarily responsible for project management, field work, laboratory analysis, data curation, manuscript writing, journal submission, and revisions for each of the chapters included herewith, which were supervised by Dr. Guillermo Hernandez Ramirez. Dr. Guillermo Hernandez Ramirez was responsible for funding acquisition, methodology, project administration, and manuscript editing. In addition, Mr. Keunbae Kim co-led the Perennial Cereal Research program alongside me and contributed greatly to field work and data curation.

Chapter 2 of this dissertation has been submitted for publication as “Soil organic nitrogen priming to nitrous oxide: a synthesis” to Soil Biology and Biochemistry. Dr. Kate A. Congreves, Dr. Tim Clough, Dr. Carolina Voigt, and Dr. Reiner Ruser aided with manuscript writing and editing. Dr. Eliza Harris contributed to data curation and visualization, as well as manuscript editing.

Chapter 3 of this dissertation has been published as “Sources and priming of soil N_2O and CO_2 production: Nitrogen and simulated exudate additions” in Soil Biology and Biochemistry. Dr. Guillermo Hernandez-Ramirez aided with data analysis and manuscript writing and editing.

Chapter 4 of this dissertation has been published as “Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges” in Agronomy Journal. Dick Puurveen, Chloe Ducholke, and Keunbae Kim helped with field work and data collection, Lori Oatway aided in laboratory analysis, and Dr. Guillermo Hernandez Ramirez aided with manuscript writing and editing.

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1.0 General introduction

The world population is expected to increase to 9.73 billion people by 2050, which will increase the pressure on already strained agroecosystems worldwide (FAO, 2017). Despite their paramount importance, modern, high-input agricultural lands used for annual grain production are often characterized as degraded systems that rely on inorganic nutrient inputs, tillage, and recurrent equipment traffic, resulting in increased erosion, compaction, and depleted soil quality (Abid and Lal, 2008; Crews and Rumsey, 2017). In addition, agricultural management exerts significant control over greenhouse gas (GHG) emissions, or fluxes, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) (Johnson et al., 2007). Notably, agriculture is the largest anthropogenic source of global N_2O emissions, a result of agricultural nitrogen (N) fertilizer use (Forster, 2021; Reay et al., 2012). A potent greenhouse gas, N_2O has a global warming potential 273 times that of CO_2 on mass basis over a 100-year time horizon and is the dominant ozone depleting substance emitted (Ravishankara et al. 2009; Forster et al. 2021).

Current systems of agricultural production are dominated by annual crops, which require large inputs of N fertilizer and can result in losses of total carbon (C) and N from the soil system as well as reduced biodiversity and ecosystem services (Glover and Reganold, 2010). More than 50% of the Earth's population currently rely on approximately 43.7 million km^2 of marginal land deemed to be at high risk of degradation resulting from annual grain production (Eswaran et al., 1999; Zhang et al., 2011). Therefore, the future of agriculture will require significant improvements to agroecosystem sustainability, while also meeting increasing demand for agricultural products from the same land base, a so-called "sustainable intensification" approach (Glover and Reganold, 2010; Tilman et al., 2011).

1.1 Nitrous oxide priming

A mechanistic understanding of the production and release of N_2O from soil is essential for the quantification and prediction of N_2O emissions, and for the development of effective adaptation and mitigation strategies (Congreves et al., 2018). The magnitude of N_2O emission may be amplified or diminished by the so-called priming effect (PE). Specifically, the N_2O priming effect (henceforth referred to as N_2O priming) is the short-term acceleration (positive N_2O priming) or deceleration (negative N_2O priming) in the rate of soil organic nitrogen (SOM-N) mineralization in response to stimuli such as additions of C and N substrates to soil, measured as changes in the proportion of N_2O evolved from SOM-N. Due to the complexity of N cycling processes in soil, N_2O priming integrates the complex and interactive effects of a multitude of soil N transformations such as SOM-N mineralization, immobilization, nitrifying and denitrifying processes, which are inherently linked with C cycling and C availability in soil (Daly and Hernandez-Ramirez, 2020).The complex nature of N cycling, N_2O producing processes, and the interlinkages with the soil C cycle has proved challenging for researchers aiming to investigate N_2O priming. As such, it is currently unclear how substantial the contribution of N_2O priming is to net N_2O emissions and researchers do not fully understand how interactions between external N additions, such as N fertilizer, and native soil N affect the various N_2O producing processes (Xu et al., 2023).

1.2 Perennial grain crops

Perennial grain crops, which are novel amalgam of annually harvested grain crops and perennial grasses that are seeded once and can be harvested for 2-3 subsequent seasons, are a possible solution to the challenges facing agricultural production (Cox et al., 2006; Ryan et al., 2018). Perennial grain crops are characterized by low soil disturbance and stand persistence over several years (Crews and Rumsey, 2017). When compared to annuals, perennial grain crops may require fewer inputs such as time and N fertilizer. Perennial grain crops have longer growing seasons than annual crops, potentially allowing for the more efficient utilization nutrients, water, and sunlight (Culman et al., 2013; De Oliveira et al, 2020; Kim et al., 2022a). On average, perennials have greater root depth and density than annual plants, which can reduce leaching of nutrients and erosion of topsoil (Cox et al., 2004; Glover et al., 2010; Kim et al., 2021). In addition, the extensive root systems of perennial grain crops, in conjunction with no-till management, have the potential to facilitate enhanced stabilization of soil organic matter (SOM), and thus act as a C sink (Kim et al., 2022b).

Perennial grain crops presently undergoing evaluation include wheat, rye, rice, and sorghum (Cox et al., 2006). Breeding efforts for perennial grains are categorized into direct domestication or wide hybridization (Cox et al., 2006). Wide hybridization involves the crossing of a wild perennial with a related annual grain crop to produce perennial progeny with grain yields higher than those from the wild perennial (Acharya et al., 2004; Reimann-Philipp, 1995). Conversely, direct domestication involves selection for desirable variants in wild perennial individuals and reiteratively breeding those individuals for multiple successive generations (Dehaan et al., 2020).

For the purposes of this research project, we selected perennial cereal rye [cv. ACE-1] as a model perennial grain crop, based on preliminary findings from Lethbridge, Alberta that reported superior winter hardiness of ACE-1 perennial cereal rye relative to several perennial wheat cultivars (Daly et al., 2022; Hayes et al., 2018). The perennial rye cultivar selected for this study was developed via hybridization of a wild perennial [perennial wild rye (*Secale montanum* L.)] crossed with a compatible annual grain [rye (*Secale cereale* L.)] (Acharya et al., 2004; Cox et al., 2006; Reimann-Philipp, 1995).

1.2 Perennial grain crops and soil nitrous oxide emissions

Human manipulation of the global N cycle is a direct result of N fertilizer application in agricultural systems and indirectly the result of increased N deposition in natural ecosystems (Smith, 2017). This perturbation has enhanced the production and release of gaseous N forms from soil, including as N_2O (Chen et al., 2014; Steffen at al., 2015). There are many complex soil N transformations leading to N_2O production and release, and multiple processes can operate concurrently in adjacent soil microsites due to soil heterogeneity (Hernandez-Ramirez et al., 2009; Liu et al., 2010). Microbially-mediated processes that produce N_2O include nitrification, denitrification, nitrifier-denitrification, codenitrification, and dissimilatory nitrate reduction to ammonium (DNRA). Each of these processes is controlled by the soil microbial community composition, air and water diffusion through the soil profile, soil temperature, pH, the availability of C and N substrates, and the interplay of each of these factors with one another (Sanchez-Martin et al., 2008; Liang et al., 2015; Putz et al., 2018).

Agricultural management decisions such as crop selection regulates the controls of N_2O production and release via processes such as soil water use (Kim et al., 2022a; Mårtensson et al., 2022) root architecture (Abalos et al., 2016; Daly et al., 2020; Smith et al., 2013), N cycling (Gelfand et al. 2016), growing season length (Behnke and Villamil, 2019; Thomas et al., 2017) and tillage practices (Lognoul et al. 2017). Consequently, perennial grain crops may reduce N_2O emissions (or fluxes) compared to annual grain crops due to their deeper, denser root systems, increased mineral N [NO_3^- and ammonium (NH_4^+)] uptake, and longer growing seasons (Abalos et al., 2016; Ferchaud et al., 2015 Gregorich et al., 2005; Rochette et al., 2018).

However, conflicting research has shown that N_2O emission patterns from soil may increase with the implementation of a perennial cropping system due to increased root tissue decay, temporarily increased soil bulk density and water-filled pore space, and proportional increases in root exudation with increased root density (Basche et al., 2014; Langarica-Fuentes et al., 2018; Thomas et al., 2017). Root exudates are mixtures of labile, low molecular weight C and N compounds that are released from plant roots into the soil environment that can alter nutrient mobilization, nutrient availability, and consequently the production of N_2O via N_2O priming (Pausch & Kuzyakov, 2018).

1.3 Perennial grain crops and soil physical quality

Soil physical quality is intrinsically linked to sustainability and land productivity and is an important basis for overall soil health (Li et al., 2011; Xu et al., 2017). Metrics of soil physical quality include those related to soil structure, porosity, and associated functions and processes such as water movement and gas diffusion including bulk density, total porosity, pore volume fractions and hydraulic conductivity (Hebb et al., 2017; Reynolds et al., 2009, Shahab et al., 2013). In agricultural soils, maintaining or improving soil physical quality has dual benefits of improving crop productivity and reducing environmental degradation (Reynolds et al., 2009).

The nature of perennial grain crops negates the use of tillage, which may improve soil physical quality by reducing aggregate disruption and soil compaction, and by promoting soil biological activity and increased SOM content (Crittenden et al., 2015; Pimentel et al., 2012). In addition, previous literature has demonstrated the increased belowground investment by perennials into extensive root systems (Duchene et al., 2020; Sprunger et al., 2018), which can promote macroaggregate formation and stabilization that aids in water and air movement through the soil profile (McGowan et al., 2019). Currently, it is not clear if soil physical quality benefits will manifest after 2-3 years, which is the expected lifespan of a perennial grain crop. Additionally, the longevity of any benefits such as increased SOC and improved soil structure remains uncertain after termination of the perennial grain, namely if the next phase of the crop rotation is an annual crop.

1.4 Productivity of a perennial grain crop

Currently, perennial grain crops do not exist in any considerable commercial sense. One concern over perennial crop feasibility lies with their ability to produce comparable yields to annual crops due to the potential trade-off between perenniality and yield. Seed yield and allocation to reproductive structures is typically viewed as being lower in perennial crops than their annual counterparts, and critics of perennial grain crops suggest that perennials are ecologically unfit for large-scale cultivation for grain due to the necessary resource allocation to maintaining perenniality (Ploschuk et al., 2004; Smaje, 2015). However, there is potential for high yields in perennial crops and previous research has shown that grain yield can be increased while protecting the perenniality of the cultivar (Moffat, 1996, Cox et al., 2006). Indeed, different theories suggest that the aforementioned trade-off does not limit seed yield, as perennial cereals are able to assimilate more nutrients and sunlight over the growing season than annuals, thus they acquire more total energy to allocate to biomass and seeds (Pimentel et al., 2012; DeHaan, et al., 2005).

In addition, there is evidence that perennial crops can utilize N more efficiently than annual counterparts, perhaps due to beneficial relationships with microorganisms in the soil (Dawson et al., 2008; Sprunger et al, 2018). However, a lack of published literature exists to date regarding the nitrogen use efficiency (NUE) of a perennial grain crop, and how the crop allocates N between vegetative and reproductive structures compared to an annual counterpart. Increased NUE in perennial grain crops has the potential to counterbalance high N fertilizer costs, namely when managed as a dual-purpose grain and forage crop, potentially making perennials a profitable option for producers (Bell et al., 2008).

1.5 Purpose of the study

Comprehensive, conceptual theories on the processes and controls governing N_2O emissions from agricultural soils exists in literature, however, gaps in our understanding of N_2O priming exist, and substantial uncertainty accompanies the prediction N_2O emissions at the field-scale. Soil and crop interactions are increasingly complex and little research has been done regarding how the inclusion of a perennial grain crop in a diversified rotation may affect N_2O emission patterns relative to continuous annual cropping (Gelfand et al., 2016). Therefore, a mechanistic understanding of the production and release of N_2O from soil, including the impact of N_2O priming, is essential for the quantification and prediction of N_2O emissions and for the development of effective adaptation and mitigation strategies, including perennial grain crops (Congreves et al., 2018).

In this context, Chapter 2 of this dissertation presents a synthesis paper that was developed to integrate the various mechanisms controlling N_2O priming to reconcile a unified understanding of the phenomenon. By compiling the findings of the existing literature investigating N_2O priming, this chapter attempts to discern the controls on N_2O priming from available experimental evidence and identify gaps in our current understanding to direct future research.

Further, in Chapter 3 of this dissertation, the effects of labile C via artificial root exudate and N fertilizer addition on the priming of SOM to CO_2 and N_2O were investigated in a controlled laboratory setting, with the aim of improving our understanding of how the enhanced root exudation of a perennial grain crop might alter the magnitude of N_2O and CO_2 emissions via priming, as well as the contribution from nitrification and denitrification to N_2O production. We hypothesized that due to the heterogeneous nature of soil, it is possible that the observed priming effect is the result of multiple mechanisms acting congruently in soils.

Chapter 4-7 document the findings from a multi-year field trial investigating the productivity and environmental performance of perennial grain cropping at two identical field sites in Alberta, Canada (Edmonton and Breton). Chapter 4 examines the potential trade-offs between biomass, grain, and NUE, which is needed to assess if perennial grain crops are a sustainable option to produce grain and straw for feed, fuel, and fiber. As such, this chapter was designed to assess the agronomic potential of a perennial grain cultivar, with the objectives of assessing yearly biomass and grain yields, protein productivity, NUE, survival, and competitiveness.

In Chapter 5, we aimed to address the knowledge gap of the ability for a perennial grain crop to make meaningful improvements in soil physical quality over their 3-year lifespan. This knowledge gap was addressed by measuring indicators related to soil structure, porosity, and associated functions and processes such as water availability and movement as well as air exchange including, but not limited to, bulk density, total porosity, pore volume fractions and hydraulic conductivity. We hypothesized that the perennial grain would impart moderate benefits to soil physical quality as an intermediate between a perennial forage and an annual grain crop.

Chapter 6 of this dissertation documents the effect of perennial grain crops on soil N_2O emissions and how controlling factors (i.e., soil moisture, soil mineral N, and root mass density) that alter N_2O emissions diverge under different cropping systems under field conditions with comparable management and N fertilizer application. We hypothesized that the longer growing season and increased root density of the perennial grains would reduce N_2O emissions relative to an annual grain.

Finally, in Chapter 7 of this dissertation, the two field trials in Alberta were maintained beyond the termination of the perennial grain crop to investigate the impacts of transitioning from a perennial grain phase into an annual grain phase within a multi-year crop rotation, thus capturing the variability caused by different crops, management practices, and weather on agricultural GHG emissions, and investigating the longevity of the impact of a perennial grain crop on soil properties and subsequent yields in an annual crop (Tenuta et al., 2019). We hypothesized that tillage of soil after 3 years of perennial grain cropping will result in increased SOC-sourced CO_2 emissions due to soil aggregate destruction and subsequent transfer of soil C from slow pools to active pools that may counteract any previously accrued SOC benefits from multi-year perennial cropping (Mondal and Chakraborty, 2022).

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2 Soil organic nitrogen priming to nitrous oxide: a synthesis

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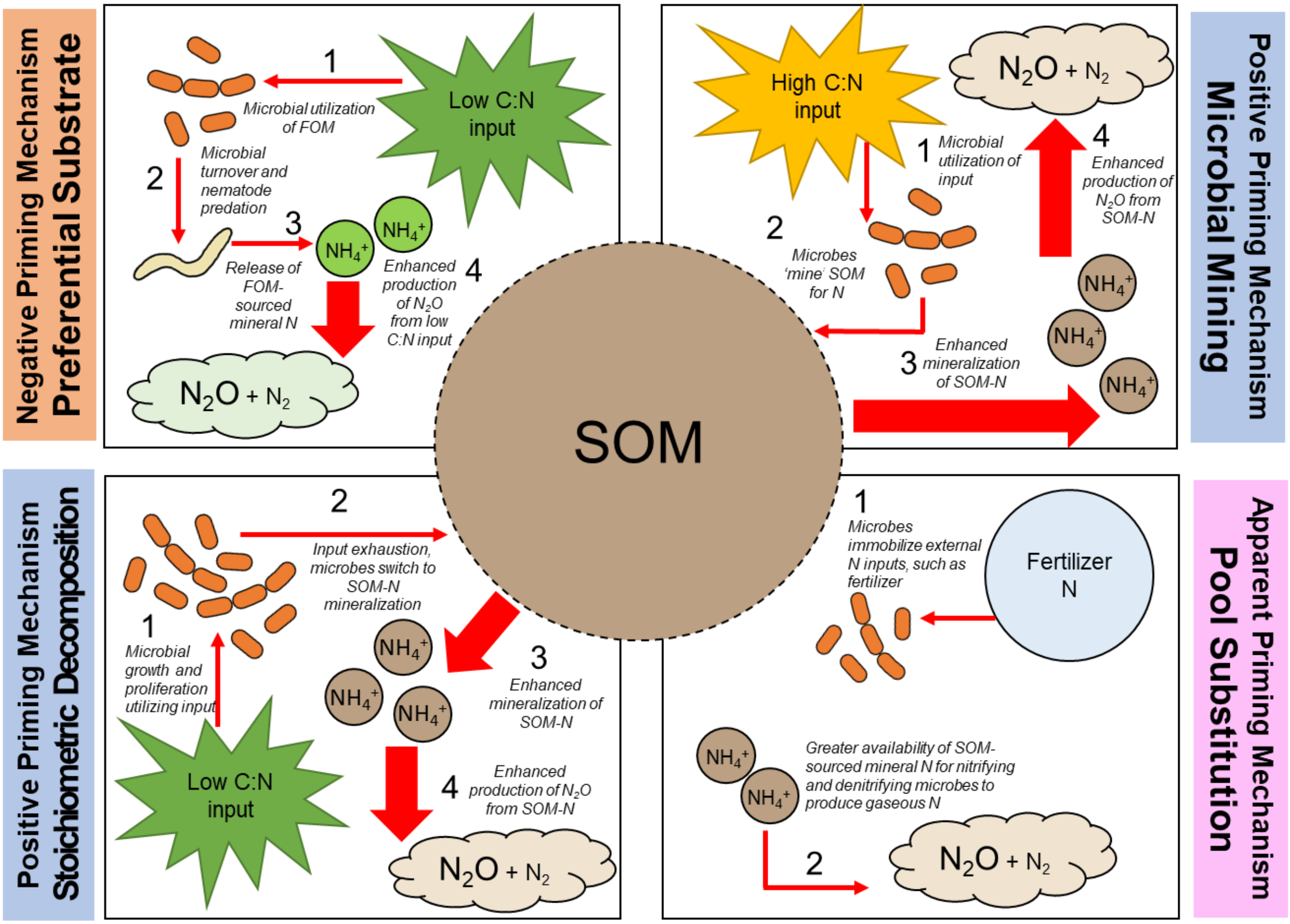
2.1 Graphical Abstract



2.2 Abstract

The priming effect (PE) is the short-term increase or decrease in the rate of soil organic matter mineralization in response to a stimulus, such as the addition of carbon (C) and/or nitrogen (N) to the soil. Literature has generally framed the PE in terms of CO_2 evolved from soil organic C mineralization, but fewer publications have focused on how the PE affects the soil N cycle and nitrous oxide (N_2O) production from soil organic N mineralization (SOM-N), despite the potency of N_2O as a greenhouse gas and ability to destroy stratospheric ozone. This review summarizes our current understanding of how the PE can alter the rates of SOM-N mineralization and subsequently amplify, diminish, or maintain N_2O production in and release from soils, henceforth referred to as N_2O priming. Additionally, the concept of process priming, the differential augmentation of N_2O-producing processes (e.g., priming of nitrification) is introduced. Diverse results across studies suggest that the mechanisms of N_2O priming cannot be fully explained by a single hypothesis. It is currently unclear how significant the contribution of N_2O priming to net N_2O emissions is, but a preliminary estimate suggests that N_2O emissions resulting from priming mechanisms can range from -39 – 76% following C and N amendments compared to a control. To disentangle the complexity of N_2O priming, an expansion of current research efforts is required. The promotion of open data sharing and publication of full datasets will facilitate the development and validation of models that can accurately simulate the complexity of soil N dynamics and account for the feedback effects of climate change on N_2O priming, which is a key research gap. This is particularly the case in under-studied areas such as permafrost-affected soils of arctic, subarctic, and alpine regions, and vulnerable tropical regions, where climate warming may amplify N_2O priming.

2.3 Introduction

A mechanistic understanding of the production and release of nitrous oxide (N_2O) from soil is essential for the quantification and prediction of N_2O emissions, and for the development of effective adaptation and mitigation strategies (Congreves et al., 2018). As a potent greenhouse gas with a global warming potential of 273 times that of CO_2 on mass basis over a 100-year time horizon, N_2O is also the dominant ozone depleting substance emitted from natural and anthropogenic sources at the earth's surface (Ravishankara et al. 2009; Forster et al. 2021). The majority of N_2O emissions are related to microbially-mediated soil processes, which contribute up to 70% of total global N_2O emissions (Butterbach-Bahl et al., 2013; Syakila and Kroeze, 2011).

Over 90% of soil nitrogen (N) is comprised of soil organic N (SOM-N), a component of soil organic matter (SOM), which is a heterogeneous mixture of organic substances of different forms and degradability (Chen et al., 2014; Enggrob et al., 2020). Depolymerization of high molecular weight SOM-N to peptides and amino acids by extracellular proteases allows for rapid microbial utilization of these compounds as energy and nutrient sources (Mooshammer et al., 2014). Oligopeptides can also be further broken down to amino acids and mineralized to inorganic ammonia (NH_3), which reacts with soil water to form ammonium (NH_4^+) (Mooshammer et al., 2014; Mullen, 2011). This NH_4^+ is also available for microbial immobilization and incorporation into biomass, microbial metabolic activities, or plant uptake. Microbial mineralization of SOM-N is key to soil N stabilization as a major product of this process, NH_4^+, is the primary substrate required to kick-start several processes that can result in the eventual loss of N from the soil system in gaseous forms, including N_2O (Butterbach-Bahl et al., 2013; Enggrob et al., 2020). These processes are both biotic (e.g., as a direct substrate for nitrification, and by providing the substrate for denitrification after NH_4^+ oxidation) and abiotic (e.g., chemodenitrification after NH_4^+ oxidation) (Butterbach-Bahl et al., 2013).

Soil N transformations leading to N_2O production and release are numerous and complex, as multiple processes may operate concurrently in adjacent soil microsites due to soil heterogeneity

(Hernandez-Ramirez et al., 2009; Liu et al., 2010). Processes that produce N_2O include nitrification, denitrification, nitrifier-denitrification, codenitrification, and dissimilatory nitrate reduction to ammonium (DNRA) (Butterbach-Bahl et al., 2013. Autotrophic nitrification sequentially oxidizes NH_4^+ to hydroxylamine (NH_2OH), nitric oxide (NO), nitrite (NO_2^-), and nitrate (NO_3^-) (Stein, 2019). During this process, ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) can generate N_2O as a result of abiotic and biotic transformations of their metabolic intermediates under aerobic conditions (Stein, 2019). Under hypoxic soil conditions AOB, but not AOA, may also generate N_2O via the process of nitrifier-denitrification, where NO_2^- is reduced via NO to N_2O (Stein, 2019). The AOB may also generate N_2O from NH_2OH under anaerobic conditions, but our current understanding suggests that nitrifiers lack the ability to reduce N_2O to N_2 (Stein, 2019). Denitrification and DNRA are processes that reduce NO_3^- through various intermediate steps to N_2 and NH_4^+, respectively. Denitrification produces N_2O when conditions are not favourable for N_2O reduction to N_2, while DNRA releases N_2O as a by-product (Giles et al., 2012; Holtan-Hartwig et al., 2002; Németh et al., 2014; Olaya-Abril et al., 2021).

Each of these N_2O-producing processes are mediated by the soil microbial community, air and water diffusion through the soil profile, and the complex interactions between carbon (C) and N cycling in soils. The effects of C availability on N_2O production include, but are not limited to: (i) increasing the energy supply for denitrifiers and thus the rate, duration, and end product (i.e., N_2O vs. N_2) of denitrification, (ii) increased aerobic respiration rates and the creation of anaerobic microsites favouring denitrification, and (iii) increasing rates of microbial growth triggered by increased C availability shifting the balance of SOM-N mineralization and immobilization and thus the availability of N substrates for N_2O production (Sanchez-Martin et al., 2008; Li et al., 2022; Liang et al., 2015; Putz et al., 2018).

The magnitude of N_2O emission may be further amplified or diminished by the so-called priming effect (PE). Specifically, the N_2O priming effect is the short-term acceleration (positive N_2O priming; henceforth referred to as N_2O priming) or deceleration (negative N_2O priming) in the rate of SOM-N mineralization in response to stimuli such as additions of C and N substrates to soil, measured as changes in the proportion of N_2O evolved from SOM-N, a definition adapted from Kuzyakov et al. (2000). Due to the complexity of N cycling processes in soil, N_2O priming integrates the complex and interactive effects of a multitude of soil N transformations such as SOM-N mineralization, immobilization, nitrifying and denitrifying processes, which are inherently linked with C cycling and C availability in soil (Daly and Hernandez-Ramirez, 2020). The complex nature of N cycling, N_2O producing processes, and the interlinkages with the soil C cycle has proved challenging for researchers aiming to investigate N_2O priming.

The phenomenon of N_2O priming was first described by Lohnis (1926), who found that mineralization of SOM-N increased following the application of green manure. However, the phenomenon was not a primary focus of research until the 1980s and 1990s, when researchers examined what was at the time coined the “added N interaction (ANI)”. The ANI was described as the greater uptake of SOM sourced N by plants in soils that received applications of fertilizer N, as opposed to those that received no fertilizer N (Azam et al., 2002; Jenkinson et al., 1985; Kuzyakov, 2010). Notably, these studies did not address the consequences of the PE increasing the substrate availability for subsequent N_2O production; instead most of the priming research over the past 20 years has focused on how the PE controls C and N cycling in terrestrial ecosystems, quantified as changes in CO_2 derived from soil organic C (SOM-C) (Bastida et al., 2019; Kuzyakov et al., 2000; Kuzyakov, 2010; Li et al., 2018; Zhu et al., 2014).

Recently, N_2O priming has been suggested as an important consideration with respect to the potential risks of native soil N pools contributing to N_2O emissions (Daly and Hernandez-Ramirez, 2020). However, it is currently unclear how substantial the contribution of N_2O priming is to net N_2O emissions and researchers do not fully understand how interactions between external N additions, such as N fertilizer, and native soil N affect the various N_2O producing processes (Xu et al., 2023). Due to the inherent complexity of N_2O production and release from soils, N_2O emission events are highly episodic in nature (Wagner-Riddle et al, 2020). As such, it is a significant challenge to disentangle the direct effects of N_2O emission triggering events on N_2O production and consumption processes from the N_2O priming effect, which is a consequence of ephemerally altered rates of SOM-N mineralization.

More complexity is added when considering that N_2O priming can also be defined as being real or apparent: Real N_2O priming is a direct result of the acceleration or retardation of SOM-N mineralization due to increased activity or concentration of microbial biomass, whereas apparent N_2O priming does not involve a change in SOM-N mineralization rates (Blagodatskaya and Kuzyakov, 2008; Daly et al., 2020; Kuzyakov et al., 2000). Apparent priming can occur due to: i) enhanced microbial activity leading to increased N_2O evolution sourced from biomass turnover but not from enhanced SOM-N mineralization, ii) isotopic exchange in which isotopically labeled ^{15}N substrates applied to soil displace native unlabeled N from a 'bound' pool or iii) pool substitution, the process by which added isotopically labeled ^{15}N is immobilized by microbes in place of native unlabeled N thus increasing the availability of native N for N_2O production (Jenkinson et al., 1985; Kuzyakov et al., 2000). Distinguishing between real and apparent N_2O priming remains a challenge, however, concurrent measurements of N_2O and CO_2 emissions can be conducted to understand SOM mineralization dynamics (Blagodatsky et al., 2010; Daly et al., 2020).

Finally, the multiple microbially-mediated processes by which N_2O is produced after SOM-N mineralization may also be altered by stimuli such as additions of C or N and can subsequently amplify N_2O priming (Arcand and Congreves, 2020; Daly and Hernandez-Ramirez, 2020). The differential augmentation or reduction of the microbial processes that produce N_2O is termed process priming (i.e., priming of nitrification, priming of denitrification) (Daly and Hernandez-Ramirez, 2020). As in Arcand and Congreves (2020) and Daly and Hernandez-Ramirez (2020), process priming has been shown to increase the proportion of N_2O emissions derived from denitrification when a C source is applied to soils. By providing substrate for heterotrophic denitrifiers, anoxic microsite formation is enhanced, which subsequently stimulates N_2O production via denitrification (Daly and Hernandez-Ramirez, 2020; Liang et al., 2015; Smith, 1997). However, addition of a C source and creation of anoxic microsites may also reduce the N_2O:N_2 ratio, due to enhanced stepwise denitrification to N_2 (Arcand and Congreves, 2020).

Current literature suggests that real N_2O priming can be altered by the soil microbial community (Henderson et al., 2010; Li et al., 2018), soil moisture (Roman-Perez and Hernandez-Ramirez, 2021c; Thilakarathna and Hernandez-Ramirez, 2021), soil pore architecture (Kim et al., 2022), root exudation (Daly and Hernandez-Ramirez, 2020; Kim et al., 2022; Langarica-Fuentes et al., 2018), inorganic and organic N fertilizer application (Leiber-Sauheitl et al., 2015; Lin and Hernandez-Ramirez, 2022; Schleusener et al., 2018), freeze-thaw cycles (Lin and Hernandez-Ramirez, 2022), C substrate type (Li et al., 2022), and management legacy (Lin and Hernandez-Ramirez, 2021; Thilakarathna and Hernandez-Ramirez, 2020; Thomas et al., 2017) (Table 2.1). To date, no publication has integrated the various factors affecting N_2O priming to reconcile a unified understanding of the phenomenon that is N_2O priming. The objectives of this synthesis are threefold: (1) compile the findings of contemporary literature investigating N_2O priming, specifically studies that directly quantify the change in N_2O-sourced from SOM-N, (2) attempt to discern the controls on N_2O priming from available experimental evidence, and (3) identify gaps in our current understanding to direct future research.

2.4 Measurements of N_2O priming and process priming

Calculation of N_2O priming requires quantifying the change in N_2O emissions sourced from SOM-N in response to stimuli relative to a control that was not subject to the stimuli. When an external N input is involved, a two-source mixing model is most commonly to distinguish between N_2O emissions derived from multiple N pools (i.e., SOM-N or external N), to calculate the magnitude and direction of N_2O priming. Most commonly, an isotopically-labelled N substrate is applied to soil, such as ^{15}N enriched N fertilizer or plant residues, and N_2O priming can be calculated as the difference in N_2O evolved from SOM-N in soils amended with an external input versus a control soil without as follows:

$$N_2O_{\text{SOM-N}} = N_2O_{\text{total}} \left(\delta^{15}N_2O_{\text{added}} - \delta^{15}N_2O_{\text{total}} \right) / \left(\delta^{15}N_2O_{\text{added}} - \delta^{15}N_2O_{\text{SOM-N}} \right) \qquad [2.1]$$

$$N_2O_{added} = N_2O_{total} - N_2O_{SOM\text{-}N} \quad [2.2]$$

$$N_2O\ priming = N_2O_{SOM\text{-}N\ treatment} - N_2O_{SOM\text{-}N\ control} \quad [2.3]$$

Where: $N_2O_{SOM\text{-}N}$ is the N_2O flux sourced from SOM-N, N_2O_{total} is the total N_2O flux, $\delta^{15}N_2O_{added}$, $\delta^{15}N_2O_{total}$ and $\delta^{15}N_2O_{soil}$ are the $\delta^{15}N$ values of the N_2O-N from the external input, the total N_2O, and SOM-N, respectively, and $N_2O_{SOM\text{-}N\ treatment}$ and $N_2O_{SOM\text{-}N\ control}$ represent SOM-N derived N_2O production from treated and control soils, respectively (Daly et al., 2020; Lu et al., 2018; Zang et al., 2016). In control soils (without receiving any external N or C input), N_2O_{total} and $N_2O_{SOM\text{-}N\ control}$ are assumed to be equivalent.

To isolate the effects of C addition alone on N_2O priming, one of the mineral N pools, e.g., the NO_3^- pool, can be isotopically labeled with a very small amount of highly enriched ^{15}N (Sehy et al., 2004). The amount of highly enriched N required for this purpose is very small compared to typical N fertilizer rates (<1%) and thus can be considered a negligible N input. Using the pool dilution approach (Davidson et al., 1991), gross SOM-N mineralization and nitrification rates can be calculated simultaneously. Therefore, assays of gross N transformation rates coupled with measurements of N_2O emissions could be used to estimate N_2O priming after C addition alone. However, this approach can lead to over- or underestimation of individual N_2O sources due to uneven application of isotopically enriched N resulting in two or more pools with different ^{15}N enrichment, such as a highly enriched NO_3^- pool near the applied label and a low enriched pool far from the applied label (Arah, 1992; Vandenheufel et al., 1988).

The use of isotopomers (defined as isomers having the same number of each isotopic atom but differing in their position) may be utilized to quantify the relative contribution of different N_2O producing processes such as nitrification and denitrification for quantification of N_2O process priming (Congreves et al., 2019; Daly and Hernandez- Ramirez, 2020; Harris et al., 2021). Briefly, this process uses mass spectrometry or laser spectroscopic techniques to determine the intramolecular distribution of ^{15}N in the

N_2O molecule. The partitioning of ^{15}N between the central [alpha (α)] and the terminal [beta (β)] N atoms of the linear N_2O molecule is expressed as site-preference (SP), with high SP values up to 35.6‰ (27.2‰ to 35.6‰) attributed to nitrification (hydroxylamine oxidation) and lower SP values of -6.9‰ (−6.9‰ to 1.4‰) indicative of nitrite or nitrate reduction (denitrification and nitrifier denitrification) (Congreves et al., 2019; Daly and Hernandez-Ramirez, 2020; Toyoda and Yoshida, 1999; Ostrom and Ostrom, 2012; Zou et al., 2014).

2.5 Theoretical mechanisms proposed for N_2O priming

2.5.1 Biotic mechanisms

Several contrasting hypotheses explain the mechanisms behind real N_2O priming observations (Fig. 2.1). One hypothesis, preferential substrate utilization, has been proposed as an explanation of negative N_2O priming (Blagodatskaya and Kuzyakov, 2008). The preferential substrate hypothesis states that microorganisms switch from using SOM-N and instead utilize more readily degradable N substrates when they become available, thus reducing SOM-N mineralization. For example, applications of mineral N fertilizer may provide an easily accessible source of N for soil microorganisms, leading to reduced mineralization of SOM-N to provide an N source for their metabolic needs. Additionally, soil microorganisms may also switch from SOM-N mineralization to decomposing the N-rich necromass of microbial populations that multiplied using fresh organic matter (FOM) substrates such as litter, crop residues, manure, or compost, after they are subject to predation (Bernard et al., 2021; Cui et al., 2020). This process can lead to mineralization of N derived from the FOM inputs, as nematode predation of bacteria releases NH_4^+ due to differences in C:N ratios, and subsequent utilization of this NH_4^+ by N_2O producing microorganisms (Zheng et al., 2022). As such, the preferential substrate hypothesis is controlled by soil N availability, and labile N inputs slow the mineralization of SOM-N by microorganisms, which switch to utilizing more readily available N sources instead. Consequently, N_2O emissions from SOM-N are reduced, resulting in negative N_2O priming (Daly and Hernandez-Ramirez, 2020; Lin and Hernandez-Ramirez, 2022; Qiao et al., 2016).

Real positive N_2O priming observations may be explained by the microbial mining hypothesis, which states that SOM-N mineralization is controlled by N limitation, and N-limited microorganisms may increase SOM-N mineralization to access N (Chen et al., 2014; Daly and Hernandez-Ramirez, 2020; Mason-Jones et al., 2018). As such, soils that have low baseline N availability may experience positive priming in response to additions with high C:N ratios, as microbes increase mineralization of SOM-N to access N to maintain microbial stoichiometry (Fontaine et al., 2003; Kumar et al., 2016). Consequently, this theory implies that soils supplied with external N inputs, such as mineral N fertilizer, should experience reduced or negative N_2O priming due to the retardation of SOM-N mineralization with increased labile N resources in soil (Liu et al., 2020a; Liu et al., 2020c; Roman-Perez and Hernandez-Ramirez et al., 2021c). Therefore, according to the N-mining hypothesis, SOM-N mineralization, and subsequent SOM-N sourced N_2O and thus N_2O priming, may be negatively correlated to native soil N mineral availability (Chen et al., 2013; Mason-Jones et al., 2018).

Conversely, Mason-Jones et al. (2018) concluded that N-mining is not a universal explanation for real N_2O priming. Instead, the authors proposed that energy-induced synthesis of exoenzymes, and thus N_2O priming, is regulated by microbial energy status as opposed to microbial N-limitation as defined by the microbial mining hypothesis. This hypothesis has been referred to in literature as stoichiometric decomposition (Chen et al., 2014; Daly and Hernandez-Ramirez, 2020; Roman-Perez and Hernandez-Ramirez, 2021c). Stoichiometric decomposition postulates that additions of labile C and/or N can stimulate microbial metabolism and biomass growth by fulfilling nutrient limitations until labile substrates are exhausted and microorganisms shift to using SOM. Consequently, increased SOM-N mineralization can result in enhanced SOM-N derived N_2O emissions, i.e., N_2O priming (Liu et al., 2020c; Mason-Jones et al., 2018; Roman-Perez and Hernandez-Ramirez, 2021c). Interestingly, microbial mining and stoichiometric decomposition promote opposite effects of N availability on N_2O priming. According to stoichiometric decomposition, nutrient availability promotes N_2O priming, whereas N_2O priming resulting from microbial mining is driven by resource scarcity (Chen et al., 2014).

Finally, N_2O priming may be the result of a process referred to as pool substitution, which is considered an apparent priming mechanism, as no acceleration of SOM mineralization occurs (Azam et al., 2002; Jenkinson et al., 1985; Kuzyakov, 2010). Apparent N_2O priming due to pool substitution occurs when microbes immobilize external N, such as N fertilizer, as opposed to utilizing mineral N sourced from SOM-N mineralization (Takeda et al., 2022). Thus, mineral N released from SOM is available for microbial conversion into N_2O, as microbial stoichiometric requirements are being met by external N. As such, it appears that more N_2O was sourced from SOM-N, despite no additional mineralization occurring (Kuzyakov et al., 2000; Takeda et al., 2022).

2.5.2 Abiotic mechanisms

Roman-Perez and Hernandez-Ramirez (2021c) suggested that N_2O priming may also be partially controlled via abiotic mechanisms, including pH. Soil pH alters the chemical form, concentration, and availability of substrates and subsequently influences soil microbial biomass and activity, which drives the mineralization of SOM (Kemmitt et al., 2006; Nicol et al., 2008). As such, pH exerts control over priming, and tends to be greater in neutral soils, relative to acidic soils (Fig 2, Supplementary Fig. S2) (Blagodatskaya and Anderson 1998; Blagodatskaya and Yuzyakov, 2008). Additionally, pH may alter the PE when additions of fertilizer urea undergo hydrolysis and temporarily increase soil pH and thus the solubility of SOM (Curtin et al., 1998; Magdoff and Weil, 2004). Increasing SOM solubility increases microbial access and thus urea application may contribute to N_2O priming (Roman-Perez and Hernandez-Ramirez 2020). However, N_2O priming has also been observed in soils upon the addition of N sources aside from urea (Häfner et al., 2021; Leiber-Sautheitl et al., 2015; Mehnaz et al., 2019; Schleusner et al., 2018) and therefore, while the impact of urea on SOM solubility may contribute to the phenomenon, it is not the sole driver.

Solubility of SOM may also increase due to the release of organic acids, such as oxalic acid, from roots or soil microorganisms (Clarholm et al., 2015; Jiang et al., 2021). Increased solubility of SOM with organic acid release can occur when organic compounds are abiotically liberated from organic-mineral associations by weathering, which can increase the concentration of small, soluble organic molecules in the soil solution, thus increasing their availability for microbial utilization (Jiang et al., 2021; Keiluweit et al., 2015). Again, Keiluweit et al. (2015) conclude that this indirect mechanism is not the sole driver, as N_2O priming has been observed in incubation experiments that lack living plants, but instead enhances microbial access to previously mineral-protected compounds, amplifying biotic priming mechanisms. Ultimately, due to the highly heterogeneous nature of soil, multiple priming mechanisms (i.e., real, apparent, biotic, abiotic) may be operating concurrently in the same soil, and priming mechanisms may shift over time.

2.6 Factors altering the magnitude and direction of N_2O priming

2.6.1 The effect of soil organic carbon on N_2O priming following N addition

The control of SOM content on priming has previously been proposed in the literature, in terms of SOM-C and measured as evolved CO_2 (Kuzyakov, 2002; Kuzyakov and Bol, 2006; Perveen et al., 2019). More recently, Roman-Perez and Hernandez-Ramirez (2021c) extended this notion to include SOM-C content as a control on N_2O priming, suggesting that in soils with high SOM-C content, N_2O priming may be proportional to SOM-C content when an N source is supplied, thus satisfying microbial stoichiometric prerequisites, and enhancing SOM-N mineralization as postulated by the stoichiometric decomposition hypothesis. The authors applied ^{15}N labeled urea to a mineral soil with high baseline fertility and measured N_2O priming by applying a two-source mixing model to determine the source of N_2O (urea versus SOM-N) (Roman-Perez and Hernandez-Ramirez, 2021c). Based on their findings, and those of other published studies, they suggested a positive relationship exists between the SOM-C concentration and the proportion of N_2O sourced from SOM-N versus total N_2O emissions when external N is applied (i.e., % N_2O priming). This is a natural extension of our understanding as soil C and N cycles are inextricably linked and highlights the need to develop an integrated view of these two important, coupled biogeochemical cycles (Daly and Hernandez-Ramirez, 2020; Li et al., 2022). However, the proposed relationship between SOM-C and N_2O priming in support of the stoichiometric decomposition hypothesis was based on only 5 available publications, all of which differed with respect to methodology, duration, management, biophysical conditions, and study type (incubation vs. field) (Buckthought et al., 2015; Guardia et al., 2018; Müller et al., 2014; Schleusner et al., 2018). Using ^{15}N labelled ammonium nitrate, Schleusner et al. (2018) also investigated the role of stoichiometric decomposition on N_2O priming. In mineral soils with a history of liquid manure application, and thus increased overall SOM-C content, they observed increased total N_2O emissions, as well as increased N_2O derived from SOM-N relative to soil that received no manure (i.e., greater N_2O priming in manure-applied soils). However, there was no influence of overall SOM-C content on % N_2O priming between manured and non-manured soils with the application of N fertilizer, meaning the pattern of N_2O priming remained the same despite the magnitude of N_2O priming differing. These findings support the stoichiometric decomposition hypothesis, as the C:N ratio remained relatively constant in the soils both with and without historical manure applications. Therefore, the stoichiometric imbalance was consistent between the two soils investigated.

Conversely, Ingold et al. (2018) observed increased N_2O priming when ^{15}N labelled manure was applied to mineral soils with lower SOM-C than those with a history of repeated manure application and thus increased baseline SOM-C. Indeed, preliminary correlation analyses conducted on data assembled from the current literature on N_2O priming suggests that SOM-C content is inversely related to % N_2O priming, with the greatest % N_2O priming occurring in soils with lower SOM-C ($R = -0.48$, $p < 0.001$) (Fig. 2). However, the analysis presented in Fig. 2 should be interpreted with caution due to the paucity of data points available and the relationships presented are to observe general patterns in the data. Ingold et al. (2018) did not propose a specific mechanism to explain these observations; however, similar findings were attributed to microbial N-mining in an experiment conducted by Thilakarathna and Hernandez-Ramirez (2020).

Thilakarathna and Hernandez-Ramirez (2020) conducted a laboratory incubation that examined N_2O priming after ^{15}N labeled urea applications to a mineral soil managed under multiple contrasting crop rotations for nearly 40 years. The rotations altered baseline SOM-C contents such that a range of treatments with SOM-C concentrations from comparatively low (16.01 g C kg^{-1}) to high (31.48 g C kg^{-1}) were included. In line with Roman-Perez and Hernandez-Ramirez (2021c), soils with high SOM-C (54.8 g C kg^{-1}) resulted in significantly greater net N_2O emissions than soils lower in SOM-C. However, while the increasing SOM-C content tended to increase the magnitude of net N_2O emissions, N_2O priming patterns were not consistent. This is because the soils highest in SOM-C experienced overall negative N_2O priming when urea was applied. That is, despite having large net N_2O emissions, when compared to the control (no N fertilizer application as urea), urea reduced the proportion of N_2O sourced from SOM-N mineralization, which is consistent with the N-mining hypothesis. This is in contrast with Roman-Perez and Hernandez-Ramirez (2021c) who found that 20% of total N_2O emissions were primed N_2O after urea application at a comparable water-filled pore space (WFPS) to Thilakarathna and Hernandez-Ramirez (2020) (40% vs. 44% WFPS, respectively), suggesting that the relationship between SOM-C and N_2O priming may not be straightforward, and more research is needed.

Further, if SOM-C content exhibited a consistent positive relationship with N_2O priming (i.e., consistent with the stoichiometric decomposition hypothesis) it might be expected that an organic soil, such as the drained peat soil used for an incubation study by Leiber-Sauheitl et al. (2015), would exhibit strong positive N_2O priming when subjected to applications of ^{15}N labelled urine and excrement, which was not the case. In fact, when exposed to excreta, N_2O experienced a source shift from peat to excreta, indicating a trend towards negative priming with a proposed mechanism of preferential substrate utilization. Notably, peat soil differs from mineral soil in its inherent decomposability; peat soil tends towards a greater abundance of recalcitrant C components (Aaltonen et al., 2022, Bader et. al., 2017). Additionally, the soil at this site was acidic (pH ~ 4). Correlation analysis indicates that pH exhibits a strong control over N_2O priming ($R = 0.47$, $p = 0.01$) (Fig. 2.2), which may override SOM-C effects, as acidic soils may alter biological activity and thus N_2O priming (Blagodatskaya and Anderson 1998; Blagodatskaya and Yuzyakov, 2008).

Alternate N_2O priming patterns and hypothesized mechanisms in these studies may be the result of discrepancies in OM recalcitrance, N fertilizer types and application rates, contrasting soil management histories, pH, and/or the presence or absence of growing plants during the experiment, all of which can alter the microbial community; a major control on the magnitude and direction of N_2O priming. Additionally, the findings of Schleusner et al. (2018) suggest that the C:N ratio of soil and applied substrates exerts a stronger control on N_2O priming than the SOM-C content alone, as stoichiometric constraints on the microbial community have been proposed as a mechanism for N_2O priming (Klemedtsson et al., 2005; Mooshammer et al., 2012; Schleusner et al., 2018; Qiao et al., 2016). Therefore, while evidence suggests that the magnitude and pattern of N_2O priming is altered by SOM-C content (Roman-Perez and Hernandez-Ramirez, 2021c; Thilakarathna and Hernandez-Ramirez, 2020; Schleusener et al., 2018), conflicting results suggest a complex relationship, and an initial analysis of available data is inconclusive (Fig. 2.2, Supplementary Fig. S2.2).

2.6.2 The effects of soil moisture on N_2O priming

Soil water is the medium in which microbially-mediated N transformations occur, and changes in soil moisture alter diffusion properties of gases and solutes. The relationship between net N_2O production and soil moisture has been thoroughly investigated, and a widely cited relationship developed by Davidson (1991) between N_2O production and soil moisture proposes that N_2O producing processes shift with soil moisture status, with nitrification-sourced N_2O dominating at WFPS < 60% and denitrification-sourced N_2O contributing the majority between 60-80% WFPS, above which the end product of denitrification shifts predominantly into N_2. Soil moisture therefore acts as a mediator for microbial activity, and coupled biotic and abiotic mechanisms may influence N_2O priming (Jiang et al., 2021; Thilakarathna and Hernandez-Ramirez, 2021; Lin and Hernandez-Ramirez, 2020). Indeed, an increase in WFPS from 53% to 65% in a Black Chernozem soil resulted in a transition from negative to positive N_2O priming in an incubation experiment by Thilakarathna and Hernandez-Ramirez (2021), and the magnitude of N_2O priming increased 12-fold in an experiment by Roman-Perez and Hernandez-Ramirez (2021c) when WFPS was increased from 28% to 64% in the same soil type.

Notably, the literature investigating soil moisture effects on N_2O priming reported N_2O priming responses – both positive and negative - in all treatments that also received N fertilizer application, regardless of soil moisture contents. This suggests that variations in soil moisture alone may not act as the sole stimulus for N_2O priming, but instead soil moisture mediates N_2O priming magnitude and direction, which can in part be ascribed to greater diffusion of SOM in the soil solution (Roman-Perez and Hernandez-Ramirez, 2021c). Changes in soil moisture can also influence the bioavailability of C and N substrates in soils, thus altering the supply and delivery of SOM substrates to microbes and subsequent N_2O priming responses.

In climates that experience a prolonged freezing period, previous research has stressed the importance of accounting for agricultural soil N_2O emissions during the spring thaw period as up to 70% of yearly N_2O emissions can occur during this short-lived event (Daly et al., 2021; Flesch et al., 2018; Flessa et al. 1995; Risk et al., 2014, Wagner-Riddle et al., 2017, Wagner-Riddle et al., 2008). Lin and Hernandez-Ramirez (2022) investigated the effects of increasing soil moisture resulting from a simulated spring thaw on N_2O priming. As such, their investigation integrated the effects of increased WFPS and the influx of priming-triggering labile substrates from root and microbial lysis that result from soil freezing (Congreves et al., 2018; He et al., 2023; Lin and Hernandez-Ramirez, 2022). As in He et al. (2023), N_2O priming during a freeze-thaw event may be triggered by the increased input of plant-derived organic compounds or dissolved organic C leachate during a thaw event.

Interestingly, mineral soils under the highest moisture treatment (75% WFPS during the simulated spring thaw) first experienced positive N_2O priming, and then shifted to negative N_2O priming after the soils had warmed to room temperature (23℃). This phenomenon was not observed in the medium (65%) or lowest (55%) soil moisture treatments, which maintained positive N_2O priming until tapering back to zero priming. These findings suggest that the mediating effect of soil moisture on N_2O priming is not a simple positive linear relationship, but instead becomes increasingly dynamic with increasing soil moisture. Lin and Hernandez-Ramirez (2022) attributed this shift from positive to negative N_2O priming to increased mineral N immobilization as more labile resources were depleted and microbes began utilizing substrate with larger C:N ratios, resulting in reduced N substrate for N_2O production (Gan et al., 2011). However, the combination of high soil moisture and the flush of labile substrate available after a freeze-thaw event can also alter the N_2O:N_2 product ratio of denitrification, as denitrifying microbes produce more N_2, in particular as soil warms and cold soil temperatures are no longer hindering the enzymic activity of N_2O reductase (Congreves et al. 2018, Daly et al., 2020).

Incubation studies are useful to isolate treatment effects without the numerous confounding effects that can impact a field study, but field data is invaluable to observe the dynamics of N_2O priming under real-world conditions. Studies examining N_2O priming in field settings are sparse, but a 2-yr field study by Häfner et al. (2021) investigated the importance of soil moisture on N_2O priming *in situ* due to distinctly different precipitation patterns between study years 1 and 2. In the second year, after application of different organic digestates, net N_2O emissions and N_2O priming were approximately half of the first year, due to the first year of the study experiencing 80% more rainfall in the first 30 days of the experiment. Notably, the % N_2O priming triggered by each treatment with respect to total N_2O emissions was relatively consistent between both study years. For example, the proportion of N_2O priming triggered by the addition of maize digestates in year 1 and year 2 was 51% of cumulative N_2O emissions, reinforcing the concept that while increases in soil moisture enhance microbial-substrate interaction and alter the magnitude of N_2O priming, soil moisture is not the sole driver behind the N_2O priming phenomenon. This finding is consistent with the incubation studies of Kim et al. (2022), Roman-Perez and Hernandez-Ramirez (2021c) and Lin and Hernandez-Ramirez (2022), as well as our analysis (Fig. 2.2, Supplementary Fig. S2.2).

2.6.3 Soil management and management legacy

Land management practices and their legacy effects on N_2O emissions are well documented in the literature. Organic amendments such as livestock manure (Lin et al., 2017; Lin et al., 2021; Lin et al., 2020; Zhou et al., 2017), biosolids (Roman-Perez and Hernandez-Ramirez, 2021a; Roman-Perez and Hernandez-Ramirez, 2021b), or digestates (Baral et al., 2017; Koster et al., 2015), crop residues (Pfab, 2011; Ruser et al., 2009), cropping system type (Abraha et al., 2018; Adler et al., 2018; Daly et al., 2022), and synthetic N fertilizer use (Shcherbak et al., 2014; van Kessel et al., 2013) can exhibit enduring control over N cycling and N_2O release from soil by altering N-cycling functional genes and soil N transformations (Chen and Peng, 2020). However, the effects of different land management practices with specific regard to how they transiently alter the rate of SOM-N mineralization and thus N_2O priming are less understood.

Application of organic amendments can alter the magnitude and direction of N_2O priming by introducing an organic C source, increasing the availability of mineral N substrate, altering the microbial community, and possibly increasing soil moisture (Buckthought et al., 2015; Häfner et al., 2021; Lazcano et al., 2021; Lin and Hernandez-Ramirez, 2021; Schleusner et al., 2018). For example, Häfner et al., (2021) applied organic digestates to a silt loam soil in a field experiment, and concluded that regardless of digestate source, soils receiving digestate experienced enhanced SOM derived N_2O (i.e., N_2O priming) immediately after application relative to those that did not receive digestates. In this study, all digestate types were highly enriched in NH_4^+, a consequence of the anaerobic digestion process (Häfner et al. 2021; Möller and Stinner, 2010), and therefore may have acted as a source of readily available N for soil microbes, alleviating stoichiometric constraints and triggering microbial growth and production of SOM-degrading exoenzymes as per the stoichiometric decomposition hypothesis (Kuzyakov, 2002; Kuzyakov et al., 2000; Liu et al., 2020b; Roman Perez and Hernandez-Ramirez, 2021c).

Similarly, Ingold et al. (2018) conducted an incubation experiment that tested the effects of ^{15}N-labelled goat manure application on sandy soils and found a 25-fold increase in cumulative N_2O

emissions after manure application, of which only 16% were sourced from the applied manure. This increase in N_2O emissions sourced from SOM-N coincided with an increase in microbial biomass N, demonstrating that significant N_2O priming can be triggered by the growth and proliferation of microbial biomass capable of enhancing N_2O emissions from native soil N under nutrient rich conditions (Chen et al., 2014; Ingold et al., 2018). Conversely, more readily available C and N compounds in organic amendments may be preferentially degraded by microorganisms and thus trigger negative N_2O priming as per the preferential substrate utilization hypothesis observed by Leiber-Sautheitl et al. (2015). Markedly, when an amendment of sheep urine with a low C:N ratio (0.85) was applied to a peat soil with a high C:N ratio (29.9) a source-shift from microbial utilization of SOM to the applied urine as an N source to produce N_2O lends further credibility to the role of amendment C:N ratios in determining the magnitude of N_2O priming (as discussed in Section 4.6.1).

Overall, organic substrates can vary widely in their properties and composition. Therefore, it is difficult to postulate a one size fits all hypothesis regarding how organic substrates may alter the direction and magnitude of N_2O priming. Importantly, no publication to date has investigated how different organic substrates may affect N_2O priming due to the alteration of the soil microbial community. Manure applications have been shown to promote fungal proliferation in soils (Lucas et al., 2014; Tang et al., 2020), altering the bacteria:fungi ratio, and subsequently shifting the production ratio of $N_2O:N_2$ towards N_2O, as fungi do not possess the *nosZ* gene that reduces N_2O to N_2 (Maeda et al., 2015). Fungi may be capable of metabolizing more recalcitrant organic matter than bacteria (Chen et al., 2022; Fierer et al., 2003), but it is not clear how this may alter the direction and magnitude of N_2O priming.

Agricultural management decisions such as crop selection or residue management may also alter N_2O priming. Crop residue management alters soil organic C and N dynamics, microbial community composition, and cumulative N_2O emissions (Chivenge et al., 2007; De Notaris et al., 2022; Pfab, 2011; Suleiman et al., 2018). However, little is known about how residue management may alter N_2O priming. Takeda et al. (2022) conducted a field study on two sugarcane systems with contrasting residue management: burning vs. green cane trash blanketing. They attributed enhanced immobilization of fertilizer N and subsequent increases in SOM derived N_2O in the green cane trash residue site to the increased labile C released from the residue on the surface. Interestingly, N_2O priming in this study was ascribed to pool substitution and thus apparent priming, as no concurrent increase in CO_2 emissions was detected (Kuzyakov et al., 2000; Takeda et al., 2022). Conversely, Xu et al. (2021) determined that increased SOM-N mineralization, and thus real N_2O priming, occurred when straw residue was incorporated, and N fertilizer was applied to a maize crop concurrently. They hypothesized that straw application and N fertilizer addition tended to increase cumulative and SOM derived N_2O emissions relative to those with just N fertilizer because of a greater stoichiometric limitation of available N when straw was incorporated. Therefore, microbes mined N from SOM, providing more SOM-N for N_2O production (Chen et al., 2014; Schleusner et al., 2018; Xu et al., 2021).

In terms of crop selection, root traits, including the quantity and composition of rhizodeposits, root architecture, and persistence, differ according to plant species and crop genotypes and can vary widely (Hallet et al., 2022). Due to their effects on soil physical, chemical, and biological functioning, contrasting cropping systems may have significant impacts on N_2O priming by altering the baseline biotic and abiotic conditions that may mediate N_2O priming responses when the system is exposed to a stimulus, such as N fertilizer application. A better understanding of how diverse crops might alter soil properties and N_2O priming responses could guide improved agricultural management practices (Paterson and Sim, 1999; Kuzyakov and Domanski, 2000).

Specifically, rhizodeposits are a diverse mixture of compounds including sugars, amino acids, organic acids, and other metabolites, the proportions of which differ between plant species (Hutsch et al., 2002; Tian et al., 2019; Wen et al., 2022). Daly and Hernandez-Ramirez (2020) investigated how enhanced rhizodeposition quantity under a simulated perennial cropping system might differentially alter N_2O priming relative to an annual cropping system using an artificial root exudate when N fertilizer is applied to the system. They found that enhanced rhizodeposition under a perennial system reduces N_2O

priming when N fertilizer is applied, relative to an annual system with proportionally reduced belowground rhizodeposit C inputs, in part due to more complete denitrification to N_2 (Daly and Hernandez-Ramirez, 2020; Langarica-Fuentes et al., 2018; Sprunger et al., 2019). To date, this is the only publication investigating how rhizodeposit quantity might alter N_2O priming in N fertilized cropping systems.

The impact of rhizodeposit composition on N_2O priming remains elusive, despite the importance of rhizodeposits for regulating microbial activity and soil nutrient cycling (Wen et al., 2022). Morley et al. (2014) found that the ratio of N_2O:N_2 is C substrate dependent; organic acid application resulted in reduced N_2O:N_2 ratios, thereby reducing net N_2O emissions when ^{15}N labeled KNO_3 was applied to microcosms, potentially by promoting microbial growth and triggering N limitation, a hypothesis supported by the findings of Giles et al. (2017), who determined that citric acid application resulted in the lowest N_2O:N_2 ratio by triggering N limitation, leading to more complete denitrification to N_2. Overall, evidence suggests that rhizodeposition may alter N_2O priming, and understanding which compounds minimize N_2O priming or trigger negative N_2O priming could aid in the selection of crop cultivars to promote reduced N_2O emissions (Giles et al., 2017). However, our current understanding is limited, therefore this is an area for continued research.

Finally, how these management decisions may impart a legacy effect onto N_2O priming is unknown. The magnitude of N_2O production and emission from soil has been shown to be affected not only by current soil conditions, but also the conditions previously experienced by the soil, a concept referred to as soil memory (Banerjee et al., 2016; Gabbarini et al., 2021; Lapsansky et al., 2016). Therefore, soil management practices that affect the production of microbial enzymes, which catalyze SOM mineralization in all proposed real biotic priming mechanisms, may result in different soil conditions than those that signaled the specific enzymatic production in the first place. This can lead to soil memory effects on N_2O priming (Banerjee et al., 2016; Chen et al., 2014; Zhu et al., 2021). For example, soils with a history of recurrent synthetic N additions may ‘train’ microbes to rapidly hydrolyse and utilize added urea (Rochette et al., 2013), which may result in contrasting N_2O priming patterns relative to comparable soils that have no experience of repeated N fertilizer additions (Thilakarathna and Hernandez-Ramirez, 2020). To date, no publication has investigated the long-term legacy effects of N fertilizer application on N_2O priming, despite abundant evidence that N fertilizer application has a persistent legacy effect on the composition of soil microbial communities as demonstrated in Katulanda et al., 2018, Liu et al., 2020b, and van der Bom et al., 2018.

2.7 Existing research gaps and the relevance of N_2O priming for predicting N-cycling processes

2.7.1 Building upon current research for improved understanding

Promising areas of future research include the potential control that the C:N ratios of the soil and of the added substrates exhibit on N_2O priming, as our review of the literature suggests that controls on N_2O priming are more complex than simply the SOM-C content. Investigation into the control C:N ratios exert over N_2O priming may provide greater insight into the mechanistic hypotheses of stoichiometric decomposition and N-mining, which currently suggest opposite effects of N and C availability on N_2O priming (Chen et al., 2014). Notably, there is currently a lack of N_2O priming research that reports the effects of C addition alone without N addition, as the most common method of measuring N_2O priming involves the application of an isotopically labelled ^{15}N substrate such as N fertilizer. To address this, studies may consider including assays of gross N transformation rates with measurements of N_2O emissions, which could provide insight into SOM-N mineralization.

Additionally, experiments that include measurements of both N_2 and N_2O could further elucidate the mediating effects of soil moisture on N_2O priming and allow for a deeper understanding of the underlying processes behind the phenomenon (Daly et al., 2020). The ratio of $N_2O:N_2$ can also shift with alterations in substrate composition and availability, enzymatic activity, and microbial community composition; all factors that may influence the magnitude and direction of N_2O priming (Congreves et al., 2018; Congreves et al., 2019; Kuzyakov, 2002; Ruser et al., 2006; Samad et al., 2016). Currently, measurements of N_2 emissions require low initial concentrations of N_2, which can be achieved in laboratory settings by replacing the experimental headspace with inert helium gas, but such conditions are difficult to achieve in the field (Molstad et al., 2007; Senbayram et al., 2012). Optionally, N_2 emissions can be monitored by utilizing very high ^{15}N abundancies in the NO_3^- pool, which are therefore restricted to N-fertilization methods (Russow et al., 1996).

Finally, despite the important role crops and crop management may play in altering N_2O priming dynamics, there is insufficient research of such effects. Studies of N_2O priming are often conducted under controlled experimental settings with (Thilakarathna and Hernandez-Ramirez, 2020), or without the presence of plants (Daly and Hernandez-Ramirez, 2020; Kim et al., 2022), and under different experimental treatments such as different substrate composition, rates of substrate addition, and whether substrate additions are pulsed or continuous. Each of these N_2O priming studies ascribed their findings to a different mechanism or did not specify a mechanism. Studies conducted under field conditions are less common, but also produce conflicting results (Table 2.1, Supplementary Table S2.1) (Lloyd et al., 2013; Takeda et al., 2022; Xu et al., 2021). Plants are capable of imparting substantial changes to SOM turnover rates in the rhizosphere, but studies focused on the influential role of plant selection and crop management on C and N cycling are currently under-represented in the N_2O priming literature (Bernard et al., 2022; Chen et al., 2014).

2.7.2 Incorporation of N_2O priming into process-based models

Developing and improving existing models capable of simulating N cycling to include priming could reduce uncertainty and improve N_2O emission estimates, allowing for extrapolation at the field scale for which mitigation measures can be designed and implemented (Leip et al., 2011). Existing models that are capable of simulating N_2O production processes include, but are not limited to, DNDC, DayCent, ecosys, and CoupModel (Berardi et al., 2020; Giltrap et al., 2020; He et al., 2016; Wang et al., 2021). The most frequently used of these are based on first-order kinetics, which consider only SOM pool size and environmental factors but lack the necessary complexity of soil microbial biomass function and physiology, and as such are incapable of accurately describing and predicting N_2O priming (Blagodatsky et al., 2010; Cheng et al., 2014; Perveen et al., 2014). Knowledge concerning the dominant priming drivers and potential feedback mechanisms is thus crucial for model development to accurately predict soil N dynamics and N_2O emissions (Chen et al., 2019; Perveen et al., 2014). However, incorporating N_2O priming into models is difficult due to the vast variability of N_2O priming responses under different conditions and the overall paucity of data, as N_2O priming is driven by multiple processes for which the regulation, drivers, and interactions are not sufficiently understood. This highlights the importance of open-source data sharing in which key soil variables and priming responses are reported, making site to site comparisons feasible and future model validation possible. To develop a mechanistic model for N_2O priming, equations describing the separate mechanisms are needed (Bernard et al., 2022), and our review offers a first step by compiling current research to elucidate relationships and drivers.

Incorporating N_2O priming into models will also inform how climate change will alter cumulative N_2O emissions and N_2O priming. Climate change is expected to alter climate patterns that govern precipitation and temperature (IPCC, 2021; Konapala et al., 2020; Orlowsky and Seneviratne, 2012). Moreover, extreme fluctuations in weather conditions may occur with increased frequency (Easterling et al. 2017; IPCC, 2021). In general, changes in soil moisture and temperature will alter microbial activity, subsequent mineralization rates, and microbially-mediated N_2O producing processes such as nitrification and denitrification. However, interactions between priming and environmental conditions such as soil N availability, plant growth, and precipitation changes are currently poorly constrained (Harris et al., 2022; Lin and Hernandez-Ramirez, 2022; Li et al., 2020).

2.7.3 Expansion of research efforts into under-studied northern latitudes

A growing body of literature shows that polar and alpine regions may contribute substantially to N_2O production and release in their current state (Abbott et al., 2015; Repo et al., 2009; Marushchak et al., 2011; Voigt et al., 2017a; Voigt et al., 2017b). While emissions of N_2O were formerly believed to be negligible in these regions due to low rates of SOM-N mineralization in cold soils (Nadelhoffer et al., 1991), this assumption was recently rebutted as SOM-N mineralization rates in high-latitude soils can be of the same magnitude as in temperate regions (Marushchak et al., 2021; Ramm et al., 2021). Our understanding of N_2O emissions – and potential N_2O priming – from the high-latitude and high elevation areas across the globe is sparse, due to the small number of measurements (Voigt et al., 2020). However, N cycling in these regions is likely to become become increasingly relevant in the face of climate change, the effects of which are amplified in polar and high elevation regions. Compared to the rest of the globe, these regions experience 4x greater warming (Rantanen et al., 2022), leading to wide-spread permafrost thaw (Biskaborn et al., 2019), nutrient release at the thaw front (Beermann et al., 2017; Keuper et al., 2012) and changes in local hydrology (Webb et al., 2022).

While some evidence of N_2O priming in high-latitude regions exists (Marushchak et al, 2021; Rousk et al., 2016; Voigt et al., 2017a), the majority of the N_2O priming literature focuses on agricultural regions in temperate climates, even though permafrost soils contain 50% of global SOM-N stocks, a portion of which may be liberated and available for N_2O production following thawing over the next century (Batjes, 1996; Voigt et al., 2020). Crucially, incorporation of N_2O priming into models may enhance our understanding of how N_2O priming may affect N cycling and net N_2O emissions in underrepresented ecosystems including, but not limited to the subarctic, arctic, and alpine regions. It is noted that tropical regions are also underrepresented in priming literature, as very few studies have been conducted to investigate priming across the diverse land use systems despite that fact that these ecosystems will also be sensitive to global climate change (Mganga and Kuzyakov, 2018; Nottingham et al., 2022)

Efforts must be made towards constraining the N dynamics in understudied regions, including the unique conditions of permafrost-affected ecosystems, which are currently limited in their assessment of permafrost thaw, increased nutrient availability, N_2O priming, and subsequent N_2O emissions in the face of climate change (Lacroix et al., 2022). To advance our understanding of N_2O priming, the potential consequences of ongoing climate change, and to better quantify the effects of N_2O priming in remote regions where field-scale data is lacking, future research must focus on the development of models capable of simulating the complexity of soil N dynamics and the collection of robust data required to validate these models (Denk et al., 2017; Voigt et al., 2020).

2.8 Future research directions

Overall, the phenomenon of N_2O priming is highly complex and mediated by a multitude of biotic and abiotic factors, C and N cycling processes, and their interactions. The literature summarized by this synthesis estimates that N_2O emissions resulting from priming mechanisms can range from -39 – 76% following C and N amendments compared to a control. Given the importance of peak emissions following perturbations such as N fertilization, manure application, and crop residue addition, we expect that N_2O priming plays a significant role in cumulative N_2O emission. However, when compared to soil SOM-C priming, N_2O priming research is still in its infancy, and we need to design future experiments to better understand and quantify N_2O production and emission. While there is a substantial body of literature documenting N mineralization dynamics, a limited number of those studies reported the changes in proportion of N_2O evolved from SOM-N and thus N_2O priming. This synthesis reveals several research priorities to better understand the phenomenon of N_2O priming including:

1. An expansion of current research efforts, including: (i) the effect of different soil C:N ratios and substrate C:N ratios on the direction and magnitude of N_2O priming, which can be used to substantiate hypothesized priming mechanisms, (ii) increasing our understanding of how different plant species (namely crops in agricultural settings) and management practices may exert control over N_2O priming by altering the baseline biotic and abiotic conditions that may mediate N_2O priming responses, and (iii) including measurements of N_2 as well as N_2O to better understand soil N cycling and quantify total N losses from enhanced SOM-N mineralization.
2. Promotion of open data sharing and publication of full datasets, with a particular focus on key variables relevant to priming (i.e., pH, WFPS, initial soil C and N, % N_2O priming, N_2O emissions in per kg of soil and per area).

3. Development and validation of models capable of simulating N_2O priming, with emphasis on models that can accurately simulate the complexity of N dynamics in understudied regions, including permafrost-affected areas of the arctic, subarctic, and alpine regions, and models capable of simulating the feedback effects of climate change on N_2O priming.

Using this knowledge, we can guide policy decisions and develop management practices that reduce N_2O emissions, a key forcing agent of anthropogenic climate change, which is expected to have disproportionately increased effects on sensitive, understudied regions including polar, alpine, and tropical areas. Notably, research interest into N_2O priming is increasing, with most publications on the subject published in the last 2 years. This synthesis provides guidance for future research to improve our understanding of N dynamics and N_2O priming so that effective mitigation strategies may be developed.

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Tables

Table 2.1. Compilation of currently available literature assessing soil organic nitrogen priming to nitrous oxide. For an expanded version of this table, refer to supplementary materials, Table S2.1.

Study	Study No.	Type	Proposed Priming Mechanism(s)	Study Description
Daly and Hernandez-Ramirez 2020	1	Incubation	Microbial mining and preferential substrate utilization	Applied variable rates of artificial root exudate and urea.
Hafner et al., 2021	2	Field	No specific mechanism identified	Applied organic digestates from various sources to soils.
Kim et al., 2022	3	Incubation	No specific mechanism identified	Investigated the effect of decomposing switchgrass roots in small and large pore soil.
Leiber-Sautheitl et al., 2015	4	Incubation	Preferential substrate utilization	Applied animal excreta to peat soils.
Lin and Hernandez-Ramirez, 2022	5	Incubation	Preferential substrate utilization	Simulated a soil freeze-thaw cycle at different moisture contents on soils with and without a history of manure application.
Mehnaz et al, 2019	6	Incubation	Microbial mining	Applied phosphorus and carbon substrates of varying recalcitrance to soil.
Roman-Perez and Hernandez-Ramirez, 2021	7	Incubation	Stoichiometric decomposition	Applied urea to soils at varying moisture contents.
Thilakarathna and Hernandez-Ramirez, 2020	8	Incubation	Microbial mining	Applied urea and nitrification inhibitors to soils with contrasting agricultural management histories.
Thilakarathna and Hernandez-Ramirez, 2021	9	Incubation	Stoichiometric decomposition	Applied urea with nitrification and urease inhibitors to a soil at various moisture contents.
Schleusner et al., 2018	10	Incubation	No specific mechanism identified	Applied N fertilizer to soils with or without a history of liquid manure application.
Liao et al., 2021	11	Field	No specific mechanism identified	Applied biochar and urea to soil.
Takeda et al., 2022	12	Field	Pool substitution	Applied N fertilizer and alternative sugarcane residue management to field sites.
Xu et al., 2021	13	Field	No specific mechanism identified	Applied N fertilizer to soils with and without wheat straw incorporation.
Li et al., 2022	14	Incubation	No specific mechanism identified	Nitrate and three carbon substrates were applied to three different soils.

Figures

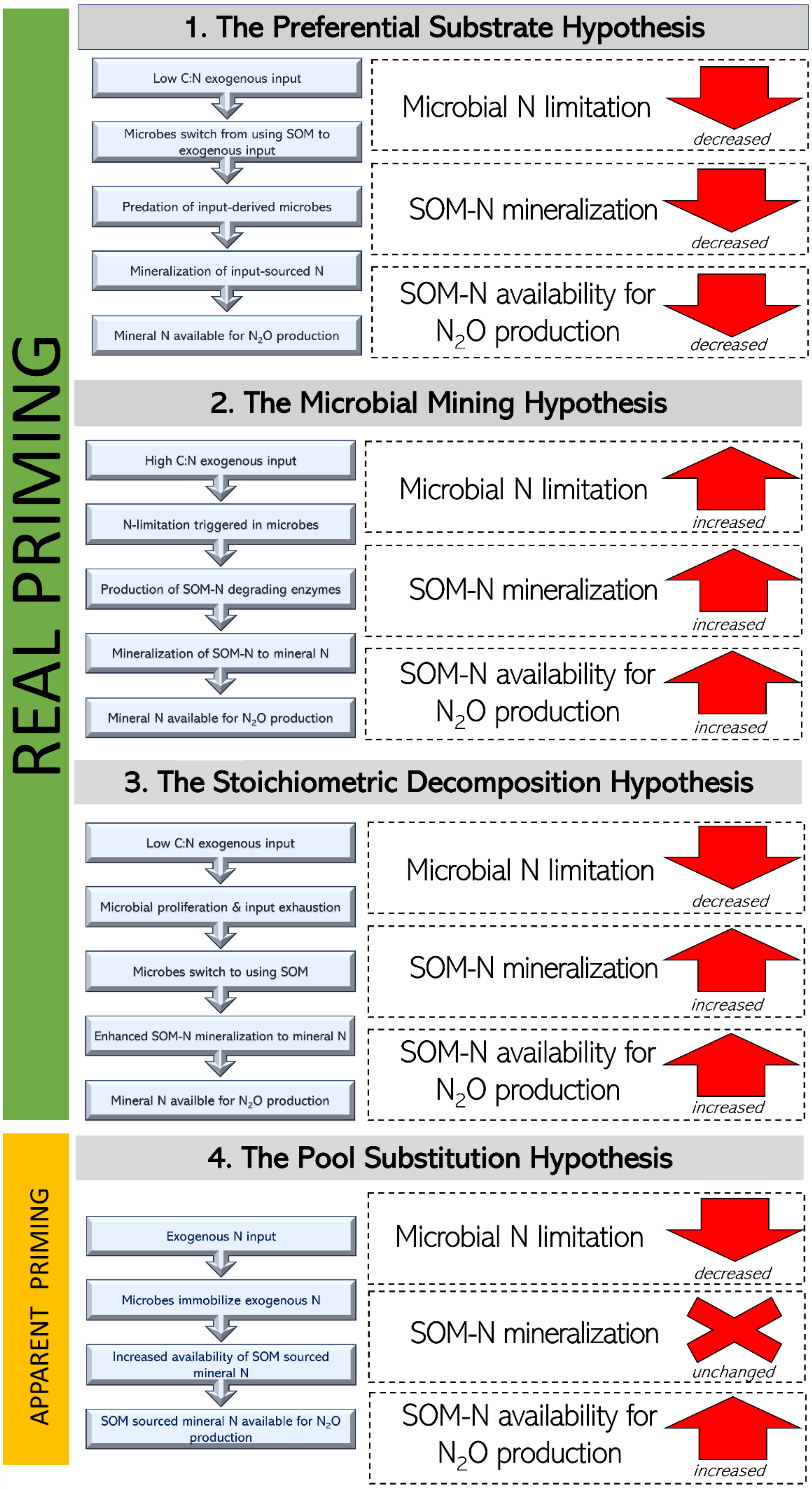

Figure 2.1. Proposed mechanisms of real N_2O priming and the consequences for soil N-cycling. **(1)** The preferential substrate hypothesis; **(2)** The Microbial mining hypothesis; **(3)** The stoichiometric decomposition hypothesis. Acronyms used include N, nitrogen; FOM, fresh organic matter; SOM, soil organic matter; N_2O, nitrous oxide.

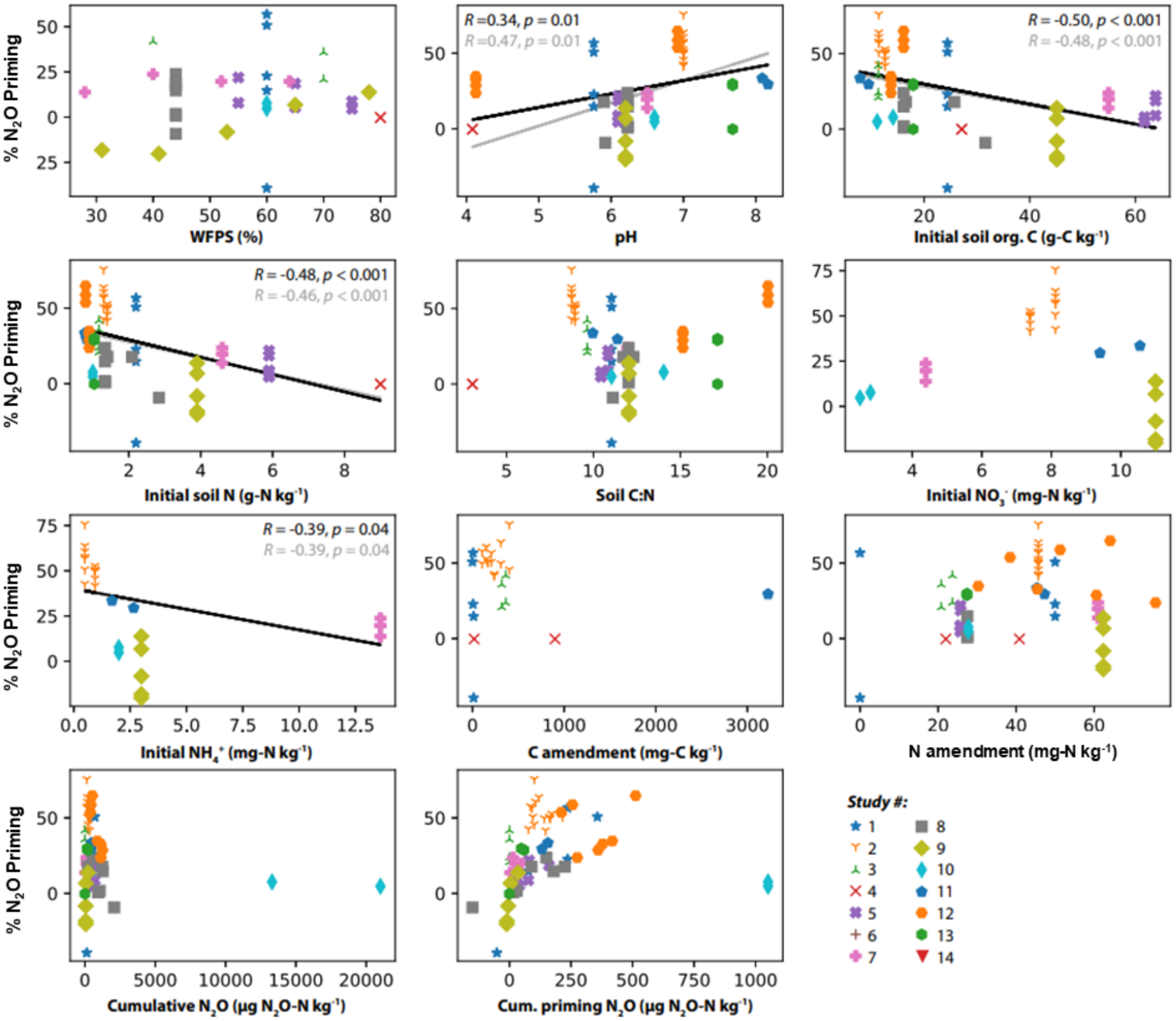

Figure 2.2. Correlation scatter plots for the % of cumulative N_2O derived from N_2O priming versus (from top left to bottom right): water-filled pore space (WFPS), pH, initial soil organic C content, initial soil N content, soil C:N, initial soil nitrate concentration (NO_3^-), initial soil ammonium concentration (NH_4^+), C content of C amendment, N content of N amendment, cumulative N_2O emissions, cumulative N_2O priming. Black trendlines include study 12, whereas grey trendlines do not (as study #12 was ascribed to apparent priming). For additional information on studies 1-14, see supplementary table S2.1.

3 Sources and priming of soil N_2O and CO_2 production: nitrogen and simulated exudate additions

Daly, E. J., & Hernandez-Ramirez, G. (2020). Sources and priming of soil N_2O and CO_2 production: Nitrogen and simulated exudate additions. Soil Biology and Biochemistry, 149, 107942.

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3.1 Abstract

Identifying the sources of nitrous oxide (N_2O) and carbon dioxide (CO_2) production from soil is central to enhancing the understanding and prediction of these emissions to the atmosphere. The magnitude of N_2O and CO_2 production derived from soil organic matter (SOM) can asymmetrically change due to stimuli from root exudation and nitrogen additions – a response termed the priming effect. We conducted an incubation to examine the effects of N and artificial root exudate (ARE) additions on the priming of SOM. We also evaluated the changes in N_2O production from nitrification and denitrification by measuring ^{15}N-N_2O site preference (SP). ARE consisted of a mixture of 99 atom% ^{13}C labelled compounds at three rates (0, 6.2, 12.5 mg C kg^{-1} soil day^{-1}) applied daily to microcosms with or without urea, a subset of which was also labelled with 5 atom% ^{15}N. Additions of ARE or urea alone caused positive priming effects; however, addition of ARE and urea concurrently resulted in an antagonistic interactive effect that diminished the N_2O production derived from SOM mineralization ($p < 0.05$). Moreover, CO_2 production from SOM decreased in urea-treated microcosms ($p < 0.01$) such that all soils receiving ARE and urea exhibited reduced positive priming relative to their unfertilized counterparts. Based on SP results, the contributions of denitrification and nitrification to total N_2O production were both amplified due to the combined inputs of ARE and urea compared to the untreated control (49.9 ± 10.1 and 28.3 ± 8.0 μg N_2O-N kg^{-1}, respectively). In soils receiving only ARE, N_2O derived from denitrification decreased relative to a control, thus reducing overall N_2O production (-9.5 ± 12.3 μg N_2O-N kg^{-1}); conversely, nitrification-derived N_2O was differentially augmented (+17.2 ± 9.0 μg N_2O-N kg^{-1}). Results indicate that a combination of elevated root exudation with N fertilization has the potential to asymmetrically amplify N_2O emissions due to increases in both nitrification and denitrification sources.

Keywords: Priming; Exudate; Carbon; Nitrogen; Nitrous oxide; Carbon dioxide

3.2 Introduction

Globally, agriculture is regarded as the largest threat to ecosystem function of any single human activity, in part due to the continually increasing emissions of greenhouse gases, which currently account for 10-12% of total global anthropogenic emissions (Smith et al., 2007; Kang et al., 2013; Chai et al., 2020). In Canada alone, agriculture contributes significantly to overall emissions, which increased from 57 Mt in 1990 to 72 Mt of CO_2 equivalent in 2017 (Environment Canada, 2019; Snyder et al., 2009). Specifically, agricultural soils cycle large amounts of carbon dioxide (CO_2), the single most abundant greenhouse gas with a residence time of 5-200 years (Hallett et al., 2002) and nitrous oxide (N_2O), which has a global warming potential 298 times greater than carbon dioxide on a mass basis, an atmospheric residence time of a century or more and the ability to decompose stratospheric ozone (Ravishankara et al., 2009; Stocker et al., 2013).

The influence of root exudation on N_2O production is just beginning to be understood. Notably, root exudation differs across plant phenotypes (Paterson and Sim, 1999; Kuzyakov and Domanski, 2000). For instance, it has been found that perennial plants produce more exudate than annual plants (Langarica-Fuentes et al., 2018; Sprunger et al., 2019). Ongoing crop innovation efforts are focused on developing perennial grain crops with the underlying premise being that perennial grain cropping can alleviate the numerous environmental challenges associated with annual cropping systems (Cox et al., 2010; Ryan et al., 2018). Perennial crops presently undergoing evaluation include wheat, rye, rice, and sorghum. Currently there is no consensus regarding the effects of perennial grain crops on N_2O and CO_2 emissions, given their greater exudation rates and increased carbon (C) input to the soil. Several studies have reported reductions in N_2O emissions from perennial vegetation relative to annual crops (Gelfand et al., 2016; Gregorich et al., 2005; Smith et al., 2013; Smith et al., 2011); however, conflicting research suggests that perennial agriculture may increase emissions (Meier, 2017; Thomas et al., 2017). In sum, it is currently unclear how N_2O emissions can be impacted by an increased exudation (e.g., from perennial grain crops). When considering the potential effects of increased root exudation on soil organic matter

(SOM) mineralization and subsequent fluxes of N_2O and CO_2 from soil, an important consideration is the hypothetical mediating role of root exudation on microbial activity, triggering a priming effect (PE) on SOM mineralization.

As in Kuzyakov et al. (2000), we define the priming effect (PE) as the short-term change in the mineralization of SOM in response to external stimuli such as additions of C and nitrogen (N) to soil. Previous studies have demonstrated that a rhizosphere PE on soil organic nitrogen (SOM-N) and soil organic carbon (SOM-C) and subsequent N_2O and CO_2 fluxes exists (Langarica-Fuentes et al., 2018; Zang et al., 2016), but none have yet to address PE within the context of increased root exudation that is typical of perennial cropping systems. In earlier laboratory experiments, PE has been investigated with a single incorporation of a very energy rich substrate (such as a simple sugar) into a soil. However, the effects of small, continuous inputs of an artificial exudate mixture can be considered more representative of field conditions. Therefore, under laboratory conditions, we aimed at isolating the individual and combined effects of adding labile C (as simulated root exudation) and N (as urea) without the confounding effects of live plant roots while also controlling soil moisture and temperature.

It is well known that aerobic soil respiration from roots and microbes is the primary path by which CO_2 is released to the atmosphere (Schlesinger et al., 2000; Smith et al., 2011). However, the specific N transformations related to N_2O production and release are more complex and less well understood (Hernandez-Ramirez et al., 2009; Liu et al., 2010). Autotrophic nitrification sequentially oxidizes ammonium (NH_4^+) into hydroxylamine (NH_2OH), nitrite (NO_2^-), and to nitrate (NO_3^-), whereas denitrification is a reduction converting NO_3- to NO_2-, nitric oxide (NO), N_2O or dinitrogen (N_2) (Morley and Baggs, 2010; Butterbach-Bahl et al., 2013; Grant et al., 2020). As a function of the soil heterogeneity, aeration and availabilities of C and N substrates, nitrification and denitrification can take place concurrently and interlinked through microbial mediation (Sanchez-Martin et al., 2008; Liang et al., 2015; Putz et al., 2018). In addition to the N_2O produced from NO_3- during denitrification, the intermediate NH_2OH and NO_2^- during nitrification can be utilized as substrates to produce N_2O (Liu et al., 2010;

Ostrom and Ostrom, 2012; Butterbach-Bahl et al., 2013). Also, NO_2^- can be utilized as initial substrate for N_2O production by both nitrifier denitrification and chemodenitrification.

Unravelling the multiple potential sources of N_2O production is necessary to design better strategies for mitigating emissions as well as for improving modelling predictions of terrestrial N_2O fluxes (Grant et al., 2020; Chai et al., 2020). Measuring the site preference (SP) of ^{15}N within the N_2O molecule is a way to gain insights into the N_2O sources (Toyoda and Yoshida, 1999; Ostrom and Ostrom, 2012; Congreves et al., 2019). The conversion from hydroxylamine (NH_2OH) into N_2O results in an SP different than both nitrifier denitrification and heterotrophic bacterial denitrification. This differentiation has been recently used by Congreves et al. (2019) to apportion total N_2O production of N-rich agricultural soils into two categories of microbial processes sourcing N_2O: nitrification (i.e., with NH_2OH as substrate) versus denitrification (encompassing both nitrifier denitrification and heterotrophic bacterial denitrification as their SPs overlap) (Sutka et al., 2003; Zou et al., 2014). Other processes such as fungal denitrification and chemo-denitrification may source N_2O in forest soils with acidic pH, low disturbance, and limited N availability, therefore these sources are typically negligible in most agricultural soils (Liu et al., 2010; Rohe et al., 2014; Wei et al., 2017; Wei et al., 2019; Kabir, 2005; Sanaullah et al., 2020).

In addition to using SP to determine the proportion of N_2O production made up by the two process categories – nitrification and denitrification, the augmentation or reduction of N_2O production from the nitrification and denitrification contributions can also be quantified by comparing soils receiving C or N addition with the corresponding control soils (used as reference baseline). For the purposes of this study, this differential augmentation or reduction of microbial processes to produce N_2O is termed N_2O-producing process priming (i.e., priming of nitrification, priming of denitrification).

Here we investigate the effects of labile C addition via artificial root exudate (ARE) and N via urea on CO_2 and N_2O production rates, SOM priming and the processes by which N_2O is produced in a controlled laboratory setting. The main objectives of this study where to (1) determine how daily ARE additions and subsequent priming effects may alter the magnitude of CO_2 and N_2O production from soil, (2) assess the effects of N addition and the potential interaction effects with ARE on soil N and C transformation processes, and (3) examine how ARE and urea additions alter the contributions of nitrification and denitrification to N_2O production in a simplified soil system receiving exudates (i.e., simulating root exudation rates typically experienced in a perennial cropping system).

3.3 Materials and Methods

3.3.1 Field soil collection

Soil for this experiment was collected from the upper 15 cm of the 39-year continuous barley (*Hordeum vulgare*) Hendrigan rotation at the Breton Plots of the University of Alberta (53°05’22” N, 114°26’ 27” W), 110 km southwest of Edmonton, Alberta. The fields are tilled annually for seed bed preparation. The soil is classified as Orthic to Dark Gray Luvisol, with a moderately fine texture of silty loam to loam (Table 3.1). Soil was collected and stored at 4.6° C prior to the beginning of the experiment. Field moist soil (58% WFPS) was sieved to < 8 mm and roots and plant residues were removed prior to packing the soil into containers.

3.3.2 Experimental design and incubation

The experiment was a fractional factorial design consisting of four replicates with 3 levels of 99 atom% ^{13}C-labelled artificial root exudate (ARE) applications (0, 6.2 and 12.5 mg C kg^{-1} soil day^{-1}) designed to mimic 0x, 0.5x and 1x the average exudation of a model perennial grass species (Langarica-Fuentes et al., 2018; Paterson and Sim, 1999) and 2 levels of either 5 atom% ^{15}N-labelled urea or unlabeled urea (0 or 50 mg N kg^{-1} soil). Please see Table 3.2 for detailed treatment description. The N addition rate (50 mg N kg^{-1} soil) was based on the urea fertilization rate typically applied to barley crops in the field plots from where the soil was collected (i.e., 90 kg urea-N ha^{-1} yr^{-1}), considering that a topsoil layer of 15 cm is treated by the urea application in the field. Each treatment container measured 10.3 cm in height and 11.5 cm in diameter and were individually packed by incremental layers with 1.041 kg of soil to a bulk density of 1.2 g cm^{-3}. ARE was a mixture of 60% ^{13}C-glucose and 40% ^{13}C-malonic acid, a simplified mixture consistent with earlier literature (Langarica-Fuentes et al. 2018; Paterson et al., 2007). Prior to the incubation, a preincubation of 3 days was completed where each soil microcosm was maintained at a constant water filled pore space (WFPS) and temperature to allow microbial activity to equilibrate prior to beginning measurements. One bulk mixture of ARE was prepared by diluting solid glucose and malonic acid with ultrapure water on day 1 of the experiment and stored at 4.6° C for the duration of the experiment and stirred using a magnetic stirrer prior to each use. This bulk ARE solution had a concentration of 1.986 g C L^{-1}. ARE was applied drop wise to four locations distributed in the four quadrants of the microcosm surface using syringes. To better simulate the exudation by plant roots, ARE was added every day for a total of 20 consecutive days. The 0.5x and 1x treatment microcosms received 3.15 and 6.30 mL of solution kg^{-1} soil day^{-1}, respectively. Urea in powder consistency was applied on incubation day 1 by mixing and incorporating to a soil depth of 5 cm. This disturbance was not applied to the unfertilized microcosms. All treatments were maintained at 60% WFPS throughout the duration of the experiment by weighing each individual microcosm (after the application of exudate) and adjusting the total weight with deionized water as follows:

$$WFPS = \frac{(GWC * BD) * 100}{f} \tag{3.1}$$

where WFPS is the water-filled pore space (%); GWC is the gravimetric water content (g g^{-1}); BD is the bulk density (g cm^{-3}) and f is the total porosity (cm^3 cm^{-3}).

The ambient room and cooler temperatures were continuously recorded by two temperature data loggers (*UX100-001, Onset, Bourne, USA*) and ambient pressure was measured on an hourly basis during CO_2 and N_2O measurements using a barometric pressure meter *(Testo 500, Testo, West Chester, USA).*

3.3.3 Nitrous oxide and carbon dioxide production and isotopic measurements

We utilized a non-steady state, closed measurement system that allowed for simultaneous measurements of CO_2 and N_2O production, the bulk ^{15}N-N_2O value and the alpha (α) and beta (β) values of the ^{15}N-N_2O molecule. A cavity ring down spectroscope (*G2508, Picarro Instruments, Santa Clara,* *USA*) and a thermoelectrically-cooled, mid-infrared quantum cascade laser spectroscope (*QC-TILDAS, Aerodyne Research Inc., Billerica, USA*) were connected to an automatic chamber with a headspace of 2.4 L where incubation microcosms were placed for measurements.

Mixing ratios of ^{14}N-^{14}N-^{16}O, ^{14}N-^{15}N-^{16}O (α) and ^{15}N-^{14}N-^{16}O (β) were quantified in continuous mode using the quantum cascade laser (wavenumber of 2188 cm^{-1}) equipped with 200-m path length analytical cell (2 L volume at 30 Torr vacuum), nafion (*Perma Pure, Lakewood, USA*) and coupled in recirculation with a non-steady-state chamber as further described below. Temperature (20 °C) and sample flow rate (1.85 standard L min^{-1}) were held constant in the instrumentation. The TDLWintel software provided system control as well as data acquisition and recorded at a 1 Hz resolution.

The α and β isotopic ratios were calibrated as in Mohn et al. (2014) with primary gas standards: A ($\delta^{15\alpha}N_2O$: 15.70‰, $\delta^{15\beta}N_2O$: -3.21‰) and B ($\delta^{15\alpha}N_2O$: 5.55‰, $\delta^{15\beta}N_2O$: -12.9‰) at a concentration of 0.8 ppm. For routine calibration every 120 minutes, secondary gas standards included synthetic N_2O ($\delta^{15\alpha}N_2O$: -1.8‰, $\delta^{15\beta}N_2O$: 0.2‰) at concentrations of 0.5, 1.2 and 1.9 ppm, breathing air ($\delta^{15\alpha}N_2O$: 15.4‰, $\delta^{15\beta}N_2O$: -2.7‰, 342 ppb), and ultra-high purity dinitrogen for background absorption spectra subtraction. Analytical precision of $\delta^{15\alpha}N_2O$ and $\delta^{15\beta}N_2O$ at 1.2 ppm was 0.4 ‰ (standard deviation of thirty continuous measurements using integration of five seconds).

Chambers were closed for a total of 3.5 minutes and allowed to flush and return to ambient concentrations for 1.5 minutes prior to beginning the next measurement. Measurements were obtained every 1-3 days throughout the 32-day incubation. The N_2O and CO_2 production rates were calculated using a modified ideal gas law as follows (Pennock et al. 2010; Yates et al. 2006):

$$PR = \frac{P*slope*W*3600*24*V}{R*T*mass} \quad [3.2]$$

where PR is the production rate of the gas (μg N_2O-N kg $soil^{-1}$ day^{-1}, or mg CO_2-C kg $soil^{-1}$ day^{-1}); P is pressure at chamber headspace (atm); slope is the regression coefficient derived from a linear regression fit to the measured concentration data for time between 30 and 180 seconds of the chamber enclosure periods (nL L^{-1} s^{-1}), W is the weight of N (28 g mol^{-1}) or C (12 g mol^{-1}) within a mole of N_2O or CO_2, V is the chamber headspace including recirculation tubing (L); R is the universal gas constant (atm nL K^{-1} μmol^{-1}), T is temperature at chamber headspace (K) and mass is the soil mass (kg). Cumulative CO_2 and N_2O productions for the duration of the incubation were calculated by multiplying the average gas production rate of two consecutive measurements by the time interval between measurements.

Bulk ^{13}C-CO_2 samples were taken via a sampling port at 30s and 180s during chamber closure and stored in N_2-flushed, pre-evacuated, 12 mL exetainer vials (*Labco, Lampeter, Wales*) prior to analysis by an isotope ratio mass spectrometer (*Finnigan Delta V Plus IRMS, Thermo Electron, Bremen, Germany*) connected to a gas bench (*Gasbench2, Thermo Fisher Scientific, Bremen, Germany*) at the Alberta Agriculture and Forestry Research Centre in Lethbridge, Alberta.

3.3.4 Soil analyses

All soil in the microcosms were homogenized and stored at 4.6 °C immediately after concluding the incubation on day 32, prior to being randomly subsampled for soil analyses. Total C, total N, soil $\delta^{15}N$ and $\delta^{13}C$ analysis was completed via dry combustion in an Elemental Analyzer (*Thermo Fisher Scientific, Delft, Netherlands*) interfaced with a continuous flow IV (*Thermo Fisher Scientific, Bremen, Germany*) to a Finnigan Delta V Plus Isotope Ratio Mass Spectrometer (*Thermo Electron, Bremen, Germany*).

The retention of the added urea-N in the soil at the end of the incubation experiment was determined by using measured data (soil $\delta^{15}N$ and total N concentration) and standard mass balance calculations for the following treatments: 0U, 0.5U and 1U.

Dissolved organic C was extracted by inverting field moist soil in 32 mL of solution with 5 mmol L^{-1} $CaCl_2$ for 1 minute, followed by centrifugation (6,000 g for 10 minutes) then syringe filtering the supernatant (0.45 µm nylon filter, *Fisher Scientific, Edmonton, Canada*). The supernatant was analyzed using a Combustion TOC Analyzer (*Shimadzu, Kyoto, Japan*) and corrected by method blank and a moisture factor calculation (Hernandez-Ramirez et al., 2009).

Available nitrogen (NH_4^+ and NO_3^-) was quantified by extracting 5 g of field moist soil with 50 mL of 2M KCl, shaken in a reciprocal shaker for 30 minutes, filtered using fine porosity 15 cm diameter filters (*Fisher Scientific, Pittsburg, USA*) and analyzed using colorimetry (EPA/600/4-79/020) (*Beermaster Plus, Thermo Fisher, Bremen, Germany*). Soil pH was measured using a 1:5 soil: water slurry with a pH meter. Bulk density was determined using the core method. Soil texture was determined via the hydrometer method.

3.3.5 Calculations and statistical analyses

The Keeling plot method was used to discern the isotopic composition of the CO_2 and N_2O as well as the intramolecular distribution of ^{15}N-N_2O from the soil. This method accounts for the ambient atmospheric concentrations of each component by plotting inverse concentration values against the isotopic composition to pinpoint the signature of the source process (Chen et al., 2016). The mathematical representation of the graphical method is as follows:

$$\delta^{13/15}X_{sample} = x_{background}\left(\delta^{13/15}X_{background} - \delta^{13/15}X_{soil}\right)\left(\frac{1}{x_{sample}}\right) + \delta^{13/15}X_{soil} \qquad [3.3]$$

where $\delta^{13/15}X_{sample}$, $\delta^{13/15}X_{background}$ and $\delta^{13/15}X_{soil}$ are the isotope ratios of measured, background (ambient) and soil ^{13}C-CO_2, ^{15}N-N_2O or the intramolecular distribution of ^{15}N-N_2O, respectively. The $x_{background}$ and x_{sample} represent the concentrations in the ambient air and in the total measured of the target component (Pataki et al., 2003).

A two-source mixing model was used to separate CO_2 and N_2O derived from SOM or from exogenous inputs of exudate carbon or urea as follows:

$$X_{soil} = X_{total}(\delta^{13/15}X_{added} - \delta^{13/15}X_{total})/(\delta^{13/15}X_{added} - \delta^{13/15}X_{soil}) \qquad [3.4]$$

$$X_{added} = X_{total} - X_{soil} \qquad [3.5]$$

where X_{soil} is the microbial utilization of SOM-C or SOM-N, X_{total} is the total CO_2 or N_2O from the treatment, $\delta^{13/15}X_{added,}$ $\delta^{13/15}X_{total}$ and $\delta^{13/15}X_{soil}$ are the $\delta^{13}C$ or $\delta^{15}N$ values of the CO_2-C or N_2O-N from the exogenous inputs (ARE or urea), total CO_2 or N_2O and from SOM-C or SOM-N, respectively (Lu et al., 2018).

Priming effects were calculated according to the following equation:

$$PE = [CO_2 \ or \ N_2O_{treatment}] - [CO_2 \ or \ N_2O_{control}] \qquad [3.6]$$

where [CO_2 or N_2O treatment] and [CO_2 and N_2O control] represent CO_2 or N_2O production in treatments and control soils, respectively (Zang et al., 2016).

Nitrous oxide measurements from the first 10 days of the experiment (when production rates ≥ 5 μg N_2O-N kg^{-1} soil day^{-1} consistently occurred) were used for calculations of site preference (SP) as high N_2O production significantly increase the precision of the measurements of α and β (Waechter et al., 2008). SP was calculated as follows:

$$SP = \delta\,^{15}N^{\alpha} - \delta\,^{15}N^{\beta} \qquad [3.7]$$

where $\delta^{15}N_{\alpha}$ and $\delta^{15}N_{\beta}$ are the ratios of $^{15}N/^{14}N$ in the alpha and beta positions relative to a universal standard in ‰ delta notation (Sutka et al., 2003). Subsequently, a two-process mixing model was used to allocate N_2O production into nitrification and denitrification with end members of 35.6‰ for nitrification and -6.9‰ for denitrification (Sutka et al., 2003, Zou et al., 2014). The isotopic effect of the conversion from N_2O to N_2 was accounted for using established relationships between available $\delta^{15}N_2O$ and SP (Congreves et al., 2019). The resultant changes in the magnitudes of SP were small, with a median of -1.1‰ and in only 6% of the measurements. This is in line with the WFPS used in this experiment (i.e., 60%), which typically exhibits relatively low conversion of N_2O to N_2 compared to much wetter soil conditions. This is further supported by Congreves et al. (2019) who recently examined SP responses to a wide range of moistures in agricultural soils of the Canadian Prairies. Moreover, 90% of these corrections corresponded to soils receiving exudate additions; nearly all of them receiving the 1x exudate addition rate (i.e., 12.5 mg C kg^{-1} dry soil day^{-1}).

All statistical analyses were performed using version 1.1.383 of R Studio software (R Core Team, 2017). Data normality was tested by the Shapiro-Wilk test, homogeneity of variance was tested by the Bartlett or Levene tests. Cumulative N_2O and CO_2 production, SOM-C priming, as well as urea-N retention in the soil were tested using two-way analysis of variance (ANOVA) tests with ARE and urea as fixed effects. SOM-N priming and urea-N retention were tested using one-way ANOVA with treatment as the fixed effect. Final available N was tested using a one-way Welch's ANOVA as the assumption of homogeneity was not met. Log transformation was applied to variables that were non-normal. All analyses were tested at alpha critical value of 0.05, and Tukey's Honest Significant Difference was used for post-hoc comparisons of means.

3.4 Results

3.4.1 N_2O production and priming of organic matter

Generally, N_2O production from all treatments peaked three days after urea application, followed by a steady decline in N_2O production on average from all treatments (Fig. 3.1). The maximum N_2O production on this day was measured from the 0U treatment (36.4 µg N kg^{-1} soil day^{-1}), which was approximately 5 times greater than from the 0nU treatment (7.4 µg N kg^{-1} soil day^{-1}), the treatment with the lowest N_2O production on this day (day 3). This general pattern remained constant throughout the duration of the experiment, with daily N_2O production from the 0U treatment remaining the highest and the 0nU the lowest. All treatments showed no discernable differences in daily N_2O production 30 days after urea application.

As expected, cumulative N_2O production from the treatments with applied urea were greater, on average, than those without (Table 3.3). In regards to the effects of urea, exudate and their interaction on cumulative N_2O production, the effect of urea on cumulative N_2O production was highly significant (p <

0.001), whereas exudate application and the interaction of urea and exudate on N_2O production were not significant ($p > 0.05$ and $p = 0.08$, respectively). Post-hoc analysis found statistically significant differences in cumulative N_2O production between the control soil (0nU) and the 0U treatment, the 0U treatment and the 1nU treatment, and no significant difference between the 1U treatment and 1nU treatment (Table 3.3).

Cumulative source-pool priming of soil organic matter (SOM) was calculated for all treatments with added N by partitioning the proportion of N_2O production derived from SOM-N or exogenous inputs of urea ^{15}N (Table 3). Both urea and exudate were found to significantly alter SOM-N priming ($p < 0.01$ and 0.05, respectively). Further, a significant interactive effect of exudate and N additions was discerned ($p < 0.05$), indicating that the application of urea alters SOM-N priming with respect to exudate additions and vice versa. Overall, only the 0U treatment experienced a significant ($p < 0.05$) positive priming effect on SOM-N relative to the control (0nU) soil (Table 3.3). Both the 0.5U and 1U treatments showed cumulative N_2O production with reduced contributions from SOM-N that were not significantly different from the control (0nU), indicating less native soil N mineralization contributed to N_2O formation in treatments with added exudate when N is applied. Overall, no significant difference in utilization of SOM-N was found between the 0nU, 0.5nU, 0.5U, 1nU and 1U treatments (Table 3.3).

3.4.2 Urea-nitrogen retention in soils

The greatest urea-N retention was found in the 0U soil (30.3 ± 7.3%), followed by the 0.5U (23.8 ± 7.3%) and the lowest retention was found in the 1U (20.9 ± 5.6%) treatment. This is a numerical trend of decreasing soil N retention (~increased N removals and losses) with increasing rates of exudate addition; however, an analysis of variance showed no statistical significance ($p > 0.05$) due to significant variability in the dataset.

3.4.3 Available N (NH_4^+ and NO_3^-) at completion of incubation

The final available nitrogen (NH_4^+ and NO_3^-) concentrations in each treatment were significantly ($P < 0.05$) affected by both urea and exudate application, but no statistically significant interactive effect was found. Treatments with urea application had significantly greater ($p < 0.01$) final available N than those without, as expected. Further, exudate application was found to significantly ($p < 0.05$) reduce available N. Notably, the greatest available N was found in the 0U treatment, while the lowest available N was found in the 1nU treatment (Fig. 3.2). When total available N was analyzed as its individual components, NH_4^+ and NO_3^-, NH_4^+ content was not significantly different between treatments ($p > 0.05$), however NO_3^- was significantly affected by both urea ($p < 0.001$) and exudate ($p < 0.05$). Again, addition of urea resulted in greater NO_3^- content in the soil, whereas exudate application reduced NO_3^-. The interaction of urea and exudate was not significant.

3.4.4 Priming of nitrification and denitrification

Nitrous oxide production was high enough (≥ 5 μg N_2O-N kg^{-1} soil day^{-1}) to enable the analytical detection and estimation of ^{15}N-N_2O site preference (SP) for the first 10 days of the incubation for the 1U and 1nU treatments and the first 7 days for the 0nU treatment. Examination of the 1U treatment showed that the dominant process contributing to N_2O production was denitrification for the entirety of the dataset (Fig 3.3). This is in contrast to the 1nU treatment, where denitrification was the dominant process for the first 4 days, followed by nitrification dominance for two sampling days before both processes were of similar magnitude on day 10 of the incubation (Fig 3.3). Finally, the 0nU treatment had no discernable pattern with respect to nitrification or denitrification trends. Still, denitrification was the overall dominant process (Fig 3.3). It should be noted that the variability and uncertainty in SP values increased as N_2O production decreased, as evidenced by the standard error bars on each data point increasing as the magnitude of daily N_2O production gradually reduced from the 1U to 0nU treatments.

The cumulative priming of nitrification and denitrification processes were calculated for the 1nU and 1U treatments for the 5 days at the onset of the experiment to capture the peak N_2O production (Fig.

3.4). Cumulative denitrification and nitrification were both positively primed in the 1U treatment (49.9 ± 10.1 and 28.3 ± 8.0 μg kg^{-1}, respectively), whereas for the 1nU treatment, denitrification was negatively primed (-9.5 ± 12.3 μg kg^{-1}), and nitrification was positively primed (17.2 ± 9.0 μg kg^{-1}).

3.4.5 CO_2 production and priming of organic matter

Carbon dioxide production for all treatments remained steady throughout the duration of the experiment following a minor peak in CO_2 production that was observed from treatments 0U, 0.5U and 1U on day 2 of the incubation shortly after the initial exudate application and urea addition, which rapidly returned to pre-treatment CO_2 production rate by day 3 (Fig. 3.5). Both exudate application and urea contributed to differences in the magnitude of CO_2 production ($p < 0.01$ and 0.05, respectively), with treatments receiving exudate at the 1x addition rate having a significantly greater production rate than treatments without (0), but not significantly different from the 0.5x addition rate, and treatments receiving urea having reduced production rates relative to no urea. No significant interaction effect was discerned (Table 3.4).

Analysis of SOM contribution to the whole CO_2 production revealed distinct patterns with respect to the effects of N and ARE additions on priming effects (Table 3.4). Similar to cumulative CO_2 production, both urea and exudate were found to have significant effects on SOM priming (*p*s < 0.01). Addition of C via exudate application resulted in significant positive SOM-C priming when compared to a control soil (0nU), however no significant difference was found between the 0.5 and 1 treatments (Table 3.4). Finally, the addition of urea reduced positive SOM-C priming, such that all treatments receiving urea had reduced SOM-C contribution to CO_2 production compared to their exudate-only treated counterparts (i.e., 0.5nU showed greater priming than 0.5U).

3.5 Discussion

3.5.1 Cumulative N_2O production and soil nitrogen priming

The response of N_2O production rate to added N has been widely reported in literature (Breitenbeck et al., 1980; Bouwman, 1996; Bouwman et al., 2002; Chai et al., 2020). As expected, we found that added N significantly enhanced cumulative N_2O production relative to a control ($p < 0.001$). The interactive effect ($p = 0.08$) of N and C additions reinforces the connection between N and C biogeochemical cycles and the concept that both resources are important drivers of the magnitude of N_2O production (Azam et al., 2002; Liang et al., 2015, Tiedje et al., 1983). A meta-analysis by Shan and Yan (2013) showed that N_2O emissions decrease by 12% when soils simultaneously receive N fertilization and surface crop residue; however, when applied separately both N fertilization and surface crop residue increased emissions. Collectively, our findings further substantiate an antagonistic effect of concurrent C and N additions to diminishing N_2O production.

Interestingly, the cumulative N_2O production from the fertilized treatments with exudate (0.5U and 1U) were found not to be significantly different from the control (0nU), suggesting that the application of a labile C source in the form of an artificial root exudate (ARE) mixture reduced cumulative N_2O production from the soil system in this experiment relative to the 0U treatment. This may be the result of increased N immobilization by microbes, reducing the N substrate availability for nitrification and denitrification processes (Fisk et al., 2015; Sanchez-Martin et al., 2008). As reported in Liang et al. (2015), a low N_2O production rate associated with C inputs comparable to those used in our study (~0-3 g C kg^{-1} soil) suggests that more N is being used to build soil microbial biomass as additional C can be distributed to growth, resulting in a greater N use efficiency by microbes and subsequent reduction of N_2O production.

Reduced cumulative N_2O production from the soil system with the application of exudate could also be the result of more complete denitrification when a C source is supplied and heterotrophic microbial transformation of N_2O to the environmentally inert N_2 occurs. Denitrification is a modular process whereby several soil properties, including labile C content, can alter the ratio of $N_2O/(N_2O+N_2)$ emitted from the soil (Richardson et al., 2009). The addition of a labile C source is known to stimulate biological oxygen demand, creating anoxic conditions for denitrification to occur, as well as maintaining the denitrification process as organic C provides electrons required for microbes to further reduce N_2O to N_2 (Liang et al., 2015, Meijide et al., 2007, Senbayram et al., 2012). Therefore, the reduced N_2O production rate may be concurrent with increased N_2 production when readily utilizable C is available for microbial N_2O reduction, which has been demonstrated in field studies (Morley and Baggs, 2010). This is further supported by the soil urea-N retention values measured at the end of the incubation, which showed a distinct pattern of decreasing N retention in soil with increasing additions of labile C as noted above. Therefore, it is more likely that complete denitrification to N_2 was occurring and converting N into this gaseous form in soils receiving exudate-C than an overall reduction in N_2O production rate. Moreover, in addition to N_2, other gaseous N species such as ammonia and nitric oxide losses could have further impacted the divergences in soil N retention across exudate treatments.

The lowest cumulative N_2O production was recorded from the 1nU treatment, which was significantly lower than the 0U treatment ($P < 0.05$) and similar in magnitude to the control soil (0nU), suggesting that additions of labile C alone, without the interactive effects of urea, may not increase N_2O production from soil. This finding is consistent with earlier literature that has found that organic C inputs are not the sole driver of N_2O production in soil systems and controlling factors such as soil NO_3^- concentrations, soil management and climatic conditions are important regulatory predictors as well (Basche et al., 2014, Thomas et al., 2017; Chai et al., 2020).

Notably, reduced cumulative N_2O production from treatments with the addition of labile C suggests that a perennial cropping system, which has a greater root system and increased exudate input into soil, has the potential to reduce N_2O production rate relative to an annual counterpart (Paterson and Sim, 1999; Kuzyakov and Domanski, 2000). This is contrary to research that has found increases in N_2O with labile C additions (Henderson et al., 2010; Thomas et al., 2017; Schleusner et al., 2018), or variable effects on N_2O production (Leiber-Sauheitl et al., 2015; Langarica-Fuentes et al., 2018) (Table 3.5; Supplementary Table S3.1). However, Congreves et al. (2017) found that dissolved organic C in soil was negatively correlated to peak N_2O production and suggested the reason for this was more complete stepwise denitrification to N_2. The conflicting results of these studies are the outcome of the multitude of factors contributing to N cycling and N_2O production from soils and thus no generalization can be applied to all scenarios; however, our findings reinforce that specific cropping system effects on N_2O production exist. It is noted that available literature on the subject is scarce, fragmented, and inconclusive (Table 3.5; Supplementary Table S3.1). Based on the lack of consistency in these few existing reports, more studies are needed to fill the knowledge gap of how priming effects of C and N additions on SOM-N impact the N_2O production.

In our study, the application of labelled ^{15}N-urea was utilized to calculate the priming effect on SOM-N and its contribution to the N_2O production observed. The microbial mining hypothesis states that SOM mineralization is controlled by N limitation, with SOM mineralization decreasing with increased labile N resources in soil (Chen et al., 2014, Mason-Jones et al., 2018). Therefore, soils supplied with N in the form of urea should experience a reduced or negative SOM-N priming effect due to the increased availability of labile N (Fontaine et al., 2004; Kumar et al., 2016). The results of this experiment revealed a significantly different phenomena, demonstrating that the microbial mining hypothesis in this form does not explain microbial activity in response to applied N in this soil. The greatest SOM-N utilization, thus the greatest positive priming, was observed in the 0U treatment, whereas the 0.5U and 1U treatments had reduced contributions from SOM-N, indicating less N mineralization in treatments with added exudate and reduced priming effects ($p < 0.05$). No difference in priming of the SOM-N pool was found between the 0.5U and 1U treatments ($p > 0.05$) due to high variability in the dataset. However, when examining the overall pattern, the increase in labile C in the 1U treatment relative to the 0.5U treatment shows a reduced utilization of SOM-N, i.e., reduced positive priming to minimal or nonexistent amounts.

Contrary to the N-mining hypothesis, increased N availability is capable of triggering C limitation in microbes. Carbon limitation can then result in increased production of extracellular enzymes to break down SOM to access SOM-C, which can result in the simultaneous release of native C and N due to the narrow C:N ratio of the native SOM (Drake et al., 2013; Liu et al., 2017; Schimel and Weintraub, 2003). Notably, extracellular enzyme production is an energy intensive process requiring ample amounts of N that would be available in the 0U treatment (Allison 2005; Lu et al., 2018). The observed pattern of reduced positive SOM-N priming when N and C are added together (0.5U and 1U treatments) relative to the nitrogen only (0U) treatment supports our hypothesis that C limitation in the 0U treatment lead to increased extracellular enzyme production, microbial mineralization and utilization of SOM-N and is a function of the C:N ratio of the added substrate and SOM (Chen et al., 2014; Murphy et al., 2015; Kuzyakov and Bol, 2006; Sparling et al., 1982). Therefore, the SOM-N priming effect observed was in part controlled by reduced labile C availability, which is a consistent feature of continuously cropped, Gray Luvisolic soils such as those used for this experiment (Table 3.1) (Izaurralde et al. 2001; Soon and Arshad, 1996). Thus, increased root exudation from an extensive perennial root system compared to an annual cropping system (Paterson and Sim, 1999; Rasche et al., 2017) has the potential to lessen microbial demand for C and subsequently reduce SOM-N mineralization under similar conditions to those in this experiment.

3.5.2 Separating the soil N_2O production from denitrification and nitrification

Denitrification was the dominant N_2O-producing process for all treatments (0nU, 1nU and 1U) during the first 4 days of the incubation, comprising 59%, 71% and 72% of the total N_2O production, respectively (Figs. 3.3a, 3.3b, 3.3c). Denitrification has been shown to be the dominant N_2O forming process when soil WFPS is high (70-80%) and can be dominant in soils with a high clay content, such as those used for our experiment, at 60% WFPS due to anoxic microsite formation in small pores (Balaine et al., 2016; Butterbach-Bahl et al., 2013, Pihlatie et al., 2004). Likewise, inversely related metrics to WFPS such as aeration and gas diffusivity have also been found to adequately explain rates of denitrification

(Balaine et al., 2016, Chamindu Deepagoda et al., 2020). Soil WFPS is capable of explaining as much as 95% of variation in N_2O fluxes and can be considered, along with temperature, to be the main driver of denitrification (Hernandez-Ramirez et al., 2009; Butterbach-Bahl et al., 2013). Therefore, similar patterns of denitrification activity throughout all treatments were not unexpected as WFPS and temperature were maintained for all treatments throughout the duration of the experiment.

Denitrification remained the dominant contributing process in the 0nU and 1U treatments from days 5 to 8 of the incubation, contributing 81% and 67% percent of the total N_2O production, but nitrification became the dominant contributing process in the 1nU treatment from days 5 to 8, comprising 63% of the total N_2O production. This shift in the dominant N_2O producing process in the 1nU treatment is due to a significant decrease in the denitrification contribution to total N_2O production, rather than a substantial increase in the contribution of nitrification, which is supported by the low cumulative N_2O production from the 1nU treatment. Increased soil microbial immobilization of available nitrogen (NH_4^+ and NO_3^-) with the addition of simple C substrates reduced the denitrification potential of the soil by reducing the availability of NO_3^- for denitrifying bacteria, evidence of which can be seen in total available nitrogen concentrations determined at the completion of the experiment (Fig. 3.2), which show reduced concentrations in the 1nU treatment relative to the 0nU and 1U treatments (Cheng et al., 2017). Interestingly, while the total available N concentrations (NH_4^+ and NO_3^-) are lowest in the 1nU treatment, the concentration of NH_4^+ in the 1nU treatment is not significantly different than other treatments, indicating that the reduced total available N concentration is more so due to the low concentrations of NO_3^-. The comparatively unreduced concentrations of NH_4^+ in the 1nU treatment may be the result of a process known as dissimilatory nitrate reduction to ammonium (DNRA), wherein NO_3^- is converted to NH_4^+ in systems with high carbon availability (Morley and Baggs, 2010, Putz et al., 2018). The process of DNRA may release small amounts of N_2O as a byproduct but overall leads to a conservation of reactive nitrogen in the soil system (Putz et al., 2018). Unfortunately, there is a limited understanding of ^{15}N SP arising from the DNRA process, and therefore we are unable to definitively make any conclusions with our current experiment and future research into this area is required (Wu et al., 2016).

Further, using SP data we are able to determine how the processes of denitrification and nitrification are primed by exogenous inputs relative to the control (0nU) soil (Fig. 3.4). The positive cumulative priming of the denitrification process in the 1U treatment is the result of additional substrates (both C and N) and formation of anoxic microsites stimulating denitrifying microbes in the soil system. Similarly, positive priming of nitrification in the 1U treatment is the result of the addition of urea providing substrate for nitrifying bacteria. In the 1nU treatment, a very different pattern is observed, which may be the result of a lack of substrate availability for denitrification, or our inability to measure N_2 production in this experiment (Fig. 3.4). Denitrification-derived N_2O was negatively primed relative to the 0nU treatment, suggesting that the application of root exudate alone hindered N_2O production via denitrification in the 1nU treatment, as neither treatment received urea. However, the ratio of $N_2O/(N_2O+N_2)$ has been shown to shift in favor of N_2 production when NO_3^- is limiting and when C availability is high (Weier et al., 1993). The fact that most soils requiring SP correction (to account for the N_2O to N_2 reduction effect, as aforementioned) were soils receiving the 1x exudate addition rate suggests that N_2O production in these soils was altered by complete denitrification to N_2. This also supports the reduced cumulative N_2O production observed in the 0.5U and 1U treatments as discussed above. Therefore, we propose that the application of root exudate did not hinder the denitrification process but instead stimulated complete denitrification and the reduction of N_2O to N_2, thus reducing N_2O production in favor of N_2 production. This postulated hypothesis requires further examination, particularly when considering the implications of a perennial cropping system and potential N_2O emission reductions. Perennial cropping systems have increased ability to remove NO_3^- from soil due to increased rooting mass (Cox et al., 2006), an extended growing season that increases nutrient uptake potential, greater labile carbon inputs to soil, and thus have the potential to alter the carbon to NO_3^- balance relative to an annual cropping system and shift microbial activity in favor of environmentally benign N_2 production.

3.5.3 Cumulative CO_2 production and soil carbon priming

The temporary peak in daily CO_2 production on incubation day 2 from treatments 0U, 0.5U and 1U was likely the result of hydrolysis from the addition of urea on day 1, rather than an increase in microbial respiration. Urea is 20% C, and the enzyme urease is responsible for catalyzing the chemical reaction by which CO_2 is simply released from the soil (Lee et al., 2011). The abiotic process of urea hydrolysis is rapid, with research showing complete hydrolyzation within days of the initial application, which explains why this phenomenon was observed only in the treatments with urea and quickly dissipated (Choi et al., 2007; Clay et al., 1990; Lee et al., 2011).

The highly significant effect ($p < 0.01$) of applied exudate on cumulative CO_2 production compared to the control (0nU) soil is the result of increased microbial respiration triggered by the addition of C in the form of daily artificial exudate (Blagodatskaya et al., 2007; de Graaf et al., 2010). The observed increases in cumulative CO_2 production were significant but muted, likely as a result of the small exudate additions chosen to deliberately mimic real root exudation rates and avoid priming artefacts (Berthrong et al., 2013; Blagodatskaya and Kuzyakov, 2008). The greatest cumulative CO_2 production was measured from the 1nU treatment. The 0.5nU, 1U and 0.5U treatments also showed increased CO_2 production relative to the control soil (0nU); however, the 0U treatment was shown to have no difference in cumulative CO_2 production relative to the control (Table 3.4). This is consistent with previous research findings in which soils amended with mineral N alone show similar CO_2 production compared with a control treatment (Sanchez-Martin et al., 2008; Chen et al., 2014). The results of this experiment validate that labile C is a key factor stimulating increased CO_2 production from SOM. Additionally, the effect of N addition on CO_2 production in this experiment was statistically significant ($P < 0.05$). In treatments with exogenous inputs of both C and N (0.5U and 1U), CO_2 production was reduced relative to their unfertilized counterpart (0.5nU and 1nU), indicative of a greater C use efficiency by microbes in the presence of available N (Liang et al., 2015; Manzoni et al., 2012; Spohn et al., 2016). This trend did not hold for the 0U and 0nU treatments, due to the comparatively high variability in the 0nU measurements.

These findings are relevant when considering the effects of cropping system choices (perennial vs. annual) on CO_2 production from soil. Proportional increases in root exudation from perennial systems with increased root mass have the potential to increase soil CO_2 production when considering the effects of labile C and N availability alone. However, perennial cropping systems also are characterized by reduced tillage, erosion and soil temperatures that may offset the increased microbial activity and lead to overall reduced CO_2 production from soil (Lal, 2003; Pimentel et al., 2012).

The source from which the CO_2 production originated (exogenous root exudate vs. SOM-C) was also significantly altered by both exudate and applied N (ps < 0.01). Addition of labile C in the form of ARE induced a positive priming effect relative to the control treatment (0nU), suggesting that microbial communities in the presence of ample C resources are able to utilize SOM-C more than those with limited C because they are not energy limited, but N limited (Fang et al., 2018; Liu et al., 2017). However, when urea was applied, positive priming was significantly reduced and even slightly negative in the 0U and 0.5U treatments relative to when ARE was applied alone. This observation is indicative of altered microbial community function with respect to organic C and mineral N availability. When soil N availability is low, but labile C is abundant, such as in the 1nU treatment, microbes must utilize SOM to obtain required N (microbial mining). However, in treatments with added N (0.5U and 1U), SOM-C positive priming is reduced, as microbes partially switch to utilizing readily available, labile sources of both C and N (i.e., preferential substrate utilization) (Ramirez et al., 2012; Zang et al., 2016). Thus, multiple priming mechanisms can occur in a single soil as priming is complex and varies with respect to C and N availabilities as well as spatially and temporally (Murphy et al., 2015). In the same soil, different exogenous inputs can favor microbial communities that utilize readily available sources of C and N or those capable of decomposing recalcitrant organic matter (Dijkstra et al., 2013; Fontaine et al., 2004; Zang et al., 2016).

3.6 Conclusion

Results showed the need to postulate multiple hypotheses operating in parallel to comprehend priming complexity. No single mechanism can explain the different patterns of priming of SOM in response to exogenous inputs of labile C and N due to the inherent complexities of SOM, microbial communities, and the combined biogeochemical cycles of C and N. Addition of urea fertilizer increased the production of N_2O and the positive SOM-N priming to produce N_2O, both of which were reduced when labile C was applied via daily artificial root exudate. Additionally, N and C additions altered the magnitude and proportion of the processes by which N_2O is produced, nitrification and denitrification. Alternatively, additions of labile C via root exudation increased CO_2 production and SOM-C priming to generate CO_2, but addition of urea reduced both. Generally, C inputs are increased in a perennial cropping system relative to an annual cropping system and our findings demonstrate that increasing exudation rate (i.e., 0, 6.2, 12.5 mg C kg^{-1} soil day^{-1}) is capable of altering C and N interactions in beneficial (reducing both N_2O production and SOM-N mining towards additional N_2O production) and adverse (increased both whole CO_2 production and SOM-C priming for asymmetrically increased CO_2 production) ways. In a broader sense, the outcomes of this study are not confined to understanding the dynamics of N_2O and CO_2 production from perennial cropping systems alone but may be applied generally to understand how root exudation and N additions in soil systems alter the magnitude and source of greenhouse gas emissions. However, soil systems in field conditions are inherently more complex than those in a controlled incubation study and more research is required to more comprehensively elucidate the effects of a shift from annual to perennial cropping systems.

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Tables

Table 3.1. Soil classification and descriptive properties of the soils (0-15 cm) used in the experiment.

Canadian classification	Gray Luvisol
FAO classification	Albic Luvisol[1]
USDA classification	Boralf[1]
C (%)	2.43 ± 0.03
N (%)	0.22 ± 0.004
C/N	11
Dissolved organic carbon (g C kg^{-1})	11.95 ± 0.32
Available nitrogen (NH_4^+ and NO_3^-) (mg N kg^{-1})	8.70 ± 0.37
Original soil δ^{13}C (‰)	−27.67 ± 0.1
Original soil δ^{15}N (‰)	7.30 ± 0.09
pH (1:5 H_2O)	5.76 ± 0.02
Bulk density (g cm^{-3})	1.24
Soil texture	
% clay	18.95 ± 0.1
% silt	40.22 ± 0.5
% Sand	40.83 ± 0.6

[1] Lavkulich, L. M., Arocena, J. M. 2011. Luvisolic soils of Canada: genesis, distribution, and classification. Canadian Journal of Soil Science, 91, 781-806.

Table 3.2. Complete list of experimental treatment combinations indicating the applied carbon and nitrogen substrates.

Treatment	Rate of artificial root exudate‡ mg C kg^{-1} dry soil day^{-1})	Rate of urea addition (mg N kg^{-1} dry soil)
0nU†	0	0
0U*	0	50
0.5nU	6.2	0
0.5U*	6.2	50
1nU	12.5	0
1U*	12.5	50
0U⸸	0	50
1U⸸	12.5	50

† the numbers 0, 0.5 and 1 in the treatment acronyms indicate the three exudate addition rates, U stands for urea addition, and nU stands for no urea addition.

* 5 atom% ^{15}N-labelled urea

⸸ unlabeled urea

‡ ARE were applied daily as a mixture of glucose:malonic acid at a C ratio of 60:40.

Table 3.3. Cumulative whole N_2O production and allocations of N_2O production derived from SOM-N and urea sources.

Treatment	Whole N_2O Production	N_2O production from SOM-N source	N_2O production from urea addition
		μg N_2O-N kg^{-1} soil	
0nU	179.1 ± 39.4 a	179.1 ± 39.4 a	0
0U	698.5 ± 233.4 b	536.46 ± 150.34 b	162.04 ± 47.07 a
0.5nU	415.6 ± 92.3 ab	415.6 ± 92.3 ab	0
0.5U	471.72 ± 116.1 ab	289.9 ± 71.2 ab	181.82 ± 49.16 a
1nU	128.3 ± 19.7 a	128.3 ± 19.7 a	0
1U	505.12 ± 79.3 ab	256.19 ± 104.56 ab	248.93 ± 50.24 a

Values are means of treatments ± SE (n=4 for 0nU, 0U, 0.5nU, 0.5U; n=8 for 1nU, 1U).
Letters indicate differences between treatments ($p < 0.05$) within each table column.

Table 3.4. Cumulative whole CO_2 production and allocations of CO_2 production derived from SOM-C and simulated exudate (ARE) sources.

Treatment	Whole CO_2 production	CO_2 production from SOM-C source ‡	CO_2 production from simulated exudate (ARE) addition
		mg CO_2-C kg^{-1} soil	
0nU	408.89 ± 23.54 Aa	408.89 ± 23.54 Aa	0
0U†	397.06 ± 6.81 Ba	397.06 ± 6.81 Ba	0
0.5nU	459.96 ± 13.74 Aab	454.71 ± 13.54 Aab	4.85 ± 1.43 a
0.5U†	413.79 ± 14.09 Bab	405.72 ± 12.66 Bab	8.07 ± 3.89 a
1nU	488.11 ± 9.27 Ab	471.14 ± 9.61 Ab	16.97 ± 1.34 b
1U†	446.08 ± 7.37 Bb	424.68 ± 10.76 Bb	21.40 ± 4.10 b

Values are means of treatments ± SE (n=4 for 0nU, 0U, 0.5nU, 0.5U; n=8 for 1nU, 1U).
Letters indicate differences between treatments ($p < 0.05$) within each table column.
‡ Uppercase and lowercase letters denote pairwise comparisons for the urea and exudate addition effects, respectively.
† To account for CO_2 derived from urea molecule (the urea addition rate was 50 mg N kg^{-1} dry soil), 21.4 mg C kg^{-1} soil were subtracted from the cumulative CO_2 production from the SOM-C source in treatments 0U, 0.5U and 1U.

Table 3.5. Compilation of experimental reports currently available in the literature assessing priming effects of C and N additions on N2O emissions. Equivalent results from our study are shown in Table 3. Note that this is a selected portion of the full table which is shown in Supplementary Table S3.1. This table shows applied treatments and measured results of five available studies, while Supplementary Table S3.1 provides detailed description of the five studies along with their results and a summary of their findings.

Study	Type	Length	C amendment	C rate	N amendment	N Rate	Cumulative N_2O Emissions	Absolute change in N_2O emissions compared with unamended control	Relative change in N_2O emissions compared with unamended control (%)
Thomas et al., 2017	Field	2 yrs	fall rye root exudate and decomposing root tissue	variable	NH_4NO_3	45 kg N Ha^{-1}	yr 1: 419 g N Ha^{-1}	15 g N Ha^{-1}	3
					composted beef cattle manure	100 kg N Ha^{-1}	yr 2: 120 g N Ha^{-1}	52 g N Ha^{-1}	43
			oilseed radish root exudate and decomposing root tissue		NH_4NO_3	45 kg N Ha^{-1}	yr 1: 312 g N Ha^{-1}	-92 g N Ha^{-1}	-29
					compost	100 kg N Ha^{-1}	yr 2: 173 g N Ha^{-1}	105 g N Ha^{-1}	61
Schleusner et al., 2018	Incubation	1 week	historical applications of liquid manure (LM +)	140-170 kg manure-N Ha^{-1} yr^{-1}	NH_4NO_3	100 kg N Ha^{-1}	141 ± 18 mmol N g^{-1}	76 mmol N g^{-1}	54
			no liquid manure application (LM -)	n.a.			124 ± 12 mmol N g^{-1}	71 mmol N g^{-1}	57
			historical applications of liquid manure (LM +)	140-170 kg manure-N Ha^{-1} yr^{-1}	n.a.	0	65 ± 27 mmol N g^{-1}	n.a.	
			no liquid manure application (LM -)	n.a.			53 ± 29 mmol N g^{-1}		
Leiber-Sauheitl et al., 2015	Incubation	21 days	Sheep feces and/or urine	urine: 6.79 g C m^{2} faeces: 335.01 g C m^{2}	Sheep faeces and/or urine	urine: 7.92 g N m^{2} faeces: 15.20 g N m^{2}	0.2 - 3.3 g N m^{-2} (21 days)$^{-1}$	insignificant	insignificant
Henderson et al., 2010	Incubation	72 hrs	glucose	1000 mg C kg^{-1} (one time application)	KNO_3	500 mg N kg^{-1}	94 mg N_2O-N kg^{-1}	94 mg N_2O-N kg^{-1}	n.a.
			soybean residue				39 mg N_2O-N kg^{-1}	39 mg N_2O-N kg^{-1}	
			red clover residue				36 mg N_2O-N kg^{-1}	36 mg N_2O-N kg^{-1}	
			barley residue				60 mg N_2O-N kg^{-1}	60 mg N_2O-N kg^{-1}	
Langarica-Fuentes et al., 2018	Incubation	7 days ARE addition, 4 hrs N_2O measurement	ARE mix (glucose, sucrose, fructose, ribose, arabinose, glycine, valine, glutamine, serine, alanine, malic acid, citric acid, malonic acid, oxalic acid, fumaric acid in equimolar C concentrations)	0.375 mg C day^{-1}	KNO_3	100 mg N kg^{-1}	negligible	n.a.	
				0.75 mg C day^{-1}					
				1.5 mg C day^{-1}					
				3 mg C day^{-1}					
				0.375 mg C day^{-1}					
				0.75 mg C day^{-1}					
				1.5 mg C day^{-1}					
				3 mg C day^{-1}					
				0.375 mg C day^{-1}			6.5 ng N_2O-N g hr^{-1}	6.5 ng N_2O-N g hr^{-1}	n.a.
				0.75 mg C day^{-1}				n.d.	
				1.5 mg C day^{-1}					
				3 mg C day^{-1}			27.6 ng N_2O-N g hr^{-1}	27.6 ng N_2O-N g hr^{-1}	n.d.

Figures

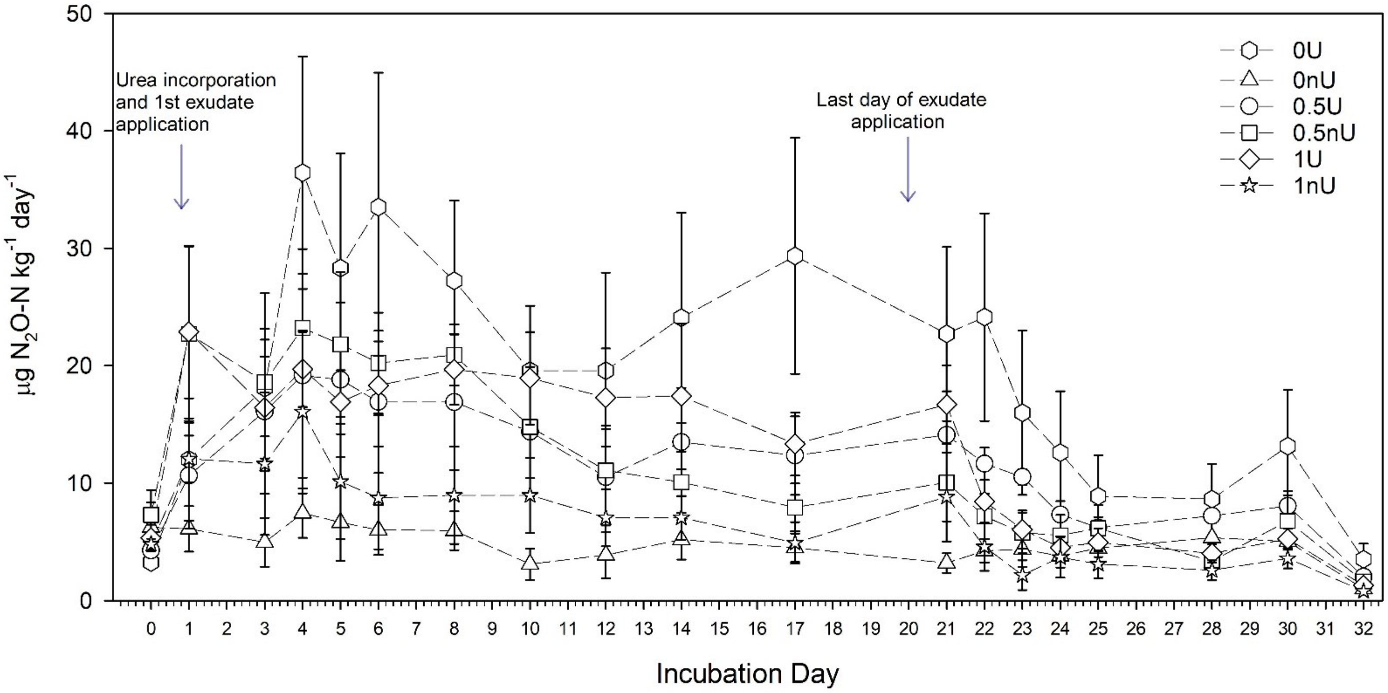

Figure 3.1. Daily N_2O production (μg N_2O-N kg^{-1} soil day^{-1}) during the incubation period for all treatments. Error bars represent ± 1SE for each treatment. Arrows indicate when the urea and artificial root exudate addition took place during the incubation.

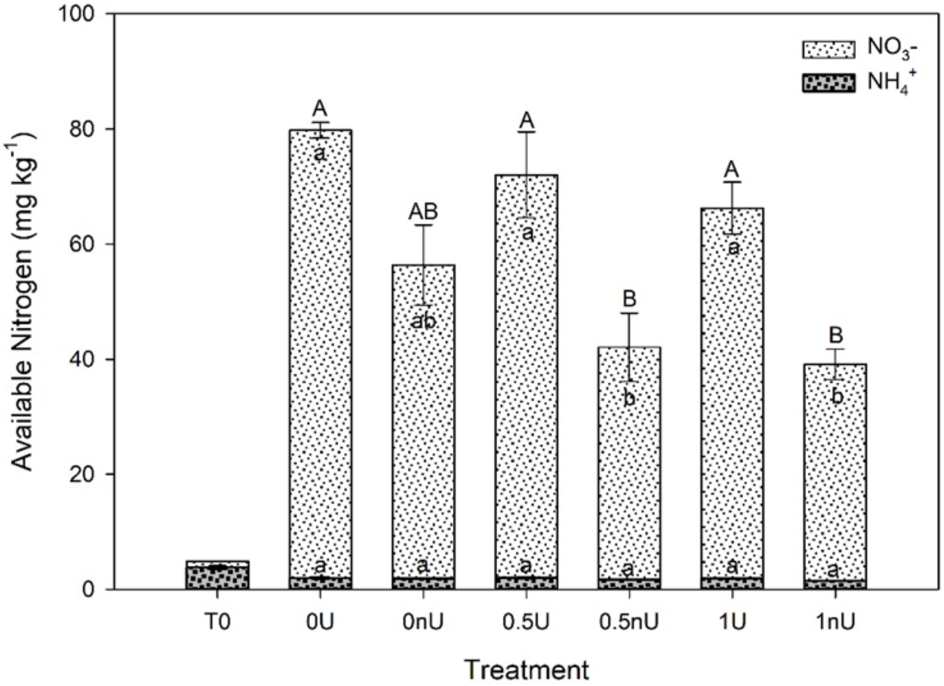

Figure 3.2. Final N concentrations of nitrate, NO_3^- (top) and ammonium, NH_4^+ (bottom). Lowercase letters indicate statistical differences between treatments for NO_3^- and NH_4^+. Uppercase letters indicate statistical differences between the total available nitrogen ($NO_3^- + NH_4^+$) for each treatment. Statistical significance based on $p < 0.05$ using a one-way ANOVA. Error bars represent ± 1SE for each nitrogen form for each treatment. Note the SE for initial NO_3^- is too small to see at this y-scale.

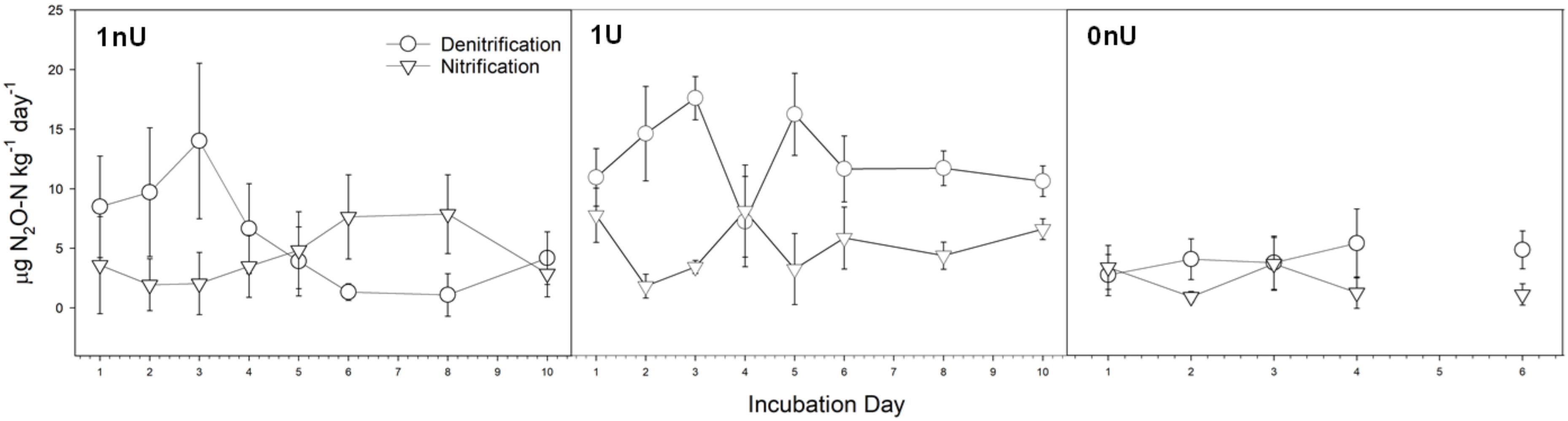

Figure 3.3. Daily N_2O production partitioned into nitrification and denitrification (μg N_2O-N kg^{-1} day^{-1}) for the 1nU, 1U and 0nU treatments. Error bars represent ± 1SE for each average.

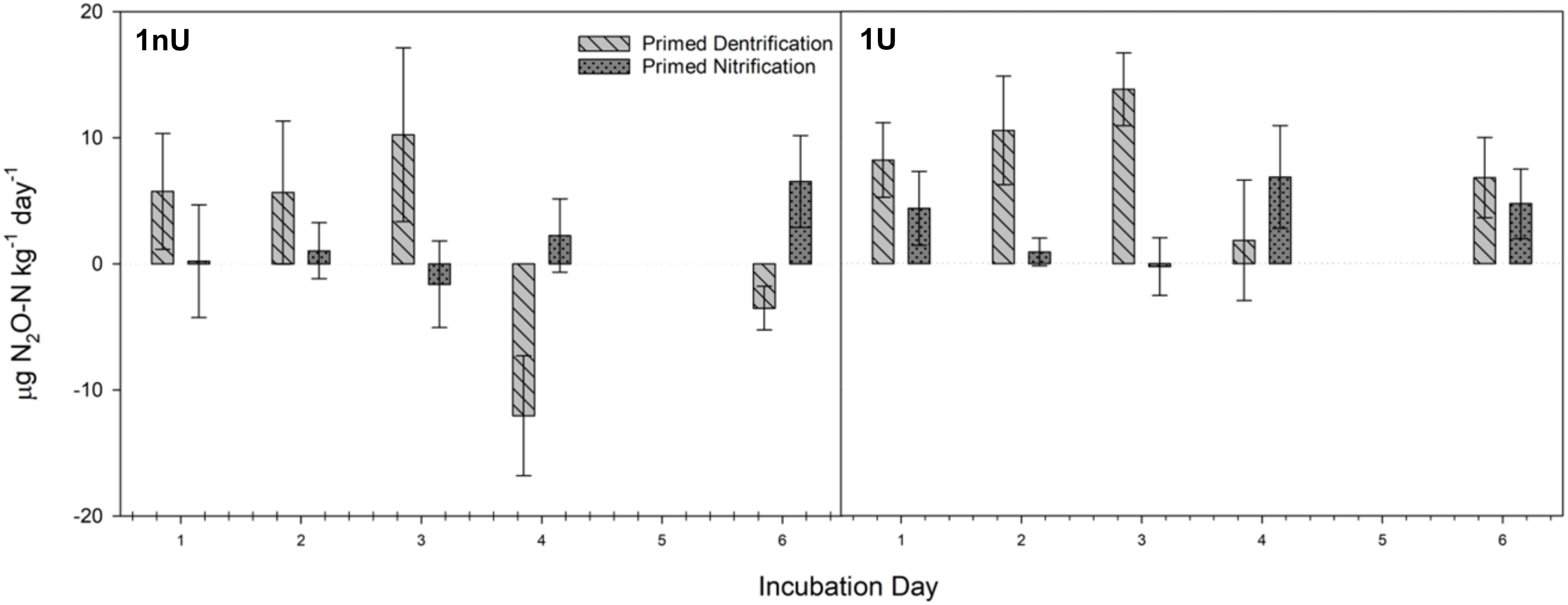

Figure 3.4. Daily priming of nitrification and denitrification (µg N_2O-N kg^{-1} day^{-1}) for the 1nU and 1U treatments. Error bars represent ± 1SE for each process for each treatment.

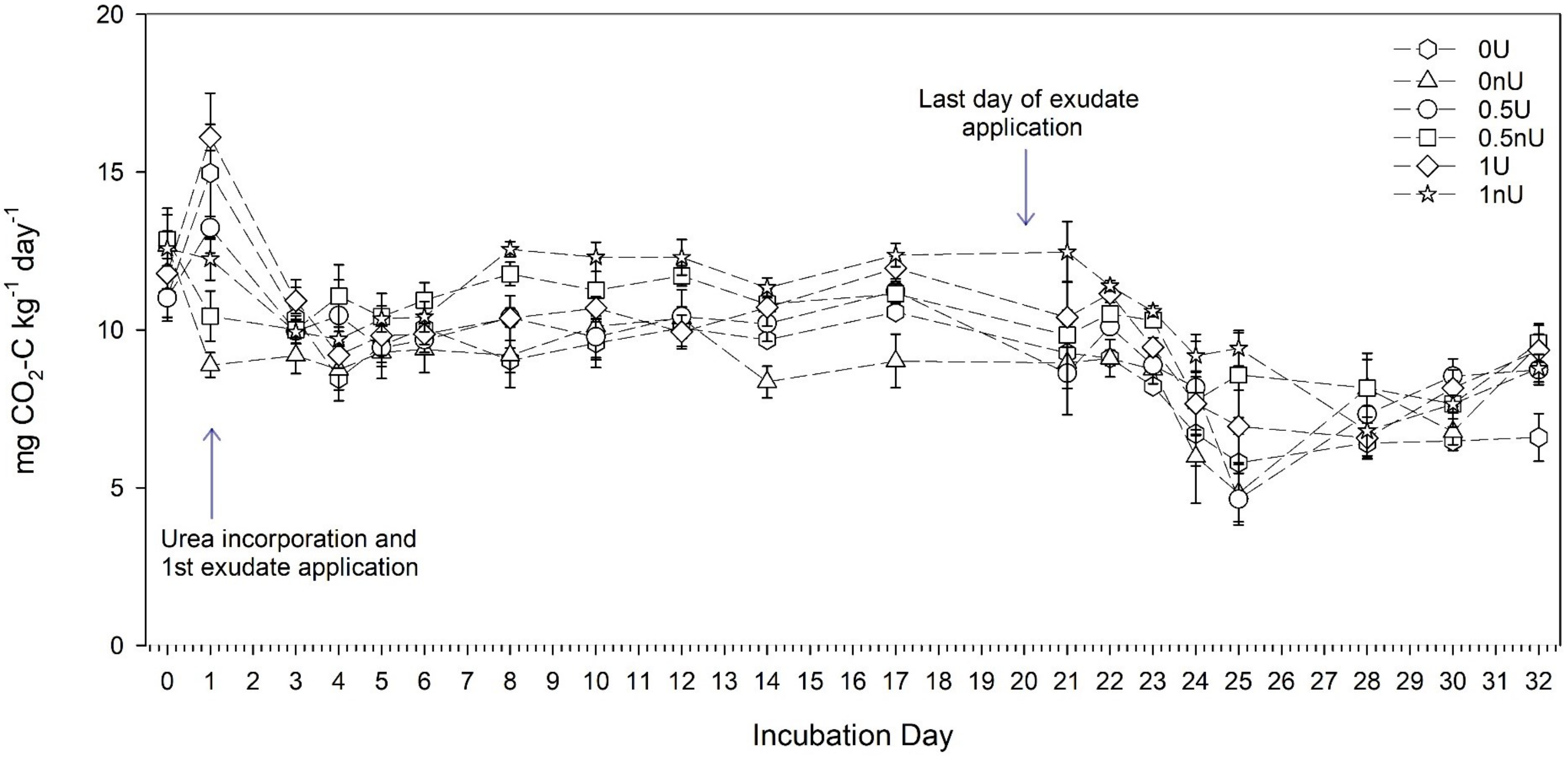

Figure 3.5. Daily CO_2 production (mg CO_2-C kg^{-1} day^{-1}) during the incubation period for all treatments. Error bars represent ± 1SE for each treatment. Arrows indicate important events during the duration of the incubation

4 Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges

Daly, E. J., Hernandez-Ramirez, G., Puurveen, D., Ducholke, C., Kim, K., & Oatway, L. (2022). Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges. Agronomy Journal, 114(1), 471-489.

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4.1 Core Ideas

- Perennial rye grain yield was up to 47% less than that of fall rye in year one.
- Grain yield of perennial rye was substantially reduced in year two.
- Grain protein productivity of perennial rye can match that of spring rye.
- Abundant tillering of perennial rye may be an opportunity to improve grain yield.
- Winter mortality and weed pressure can undermine multi-year perennial rye cropping.

4.2 Abstract

Perennial crops may present an opportunity to produce grain in a more environmentally and economically friendly manner. We examined principal agronomic traits of perennial cereal rye (*Secale cereale L.* × *S. montanum Guss* cv. ACE-1) at two field sites in Alberta, Canada over two consecutive growing seasons. Treatments included perennial rye, fall rye (*Secale cereale L.* cv. Hazlett), spring rye (*Secale cereale L.* cv. Gazelle) and perennial forage [meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa*)] with and without nitrogen fertilizer addition. Grain yield of the perennial rye in year one averaged 64% and 51% of the fall and spring rye yields at the Breton and Edmonton sites, respectively. Grain yield of the perennial rye in year two at the Edmonton site averaged 42% of the fall and spring rye. Perennial rye at the Breton site in year two was subject to competition with weeds, resulting in minimal grain productivity. Perennial rye at the Edmonton site yielded significantly more aboveground biomass (without grain) than the other rye crops over both years. Likewise, perennial rye at the Breton site produced 1.5x more aboveground biomass than the perennial forage in year one. The experiment was terminated after virtually nonexistent regrowth at both sites in the spring after two growing seasons. Overall, perennial rye may be an option as a dual-purpose forage-grain crop, however, perennial rye cropping beyond two years faces issues of winter survival and weed competition; hence, multi-year perennial rye cropping is not yet a feasible option for cold temperate conditions.

4.3 Introduction

Novel perennial grain crops are of great interest due to their purported ability to rectify several environmental challenges originating from modern agricultural production while continuing to deliver food products (Glover et al., 2010b; Ryan et al., 2018). Annual monocrops are often associated with adverse environmental effects such as the loss of soil physical quality, reduced biodiversity, emissions of greenhouse gases and substantial erosion (Jaikumar et al., 2012; Ryan et al., 2018; Zhang et al., 2011). Current efforts to breed perennial grain crops can be divided into two approaches, direct domestication and wide hybridization (Cox et al., 2006). Wide hybridization is effectively a shortcut of direct domestication, wherein a wild perennial is crossed with a compatible annual grain and their progeny are selected for perenniality (Acharya et al., 2004; Reimann-Philipp 1995). Higher grain yields from perennial grains developed via hybridization relative to those developed via direct domestication make them more comparable to annual grain crops (Jaikumar et al. 2012, Newell and Hayes 2018, Ryan et al., 2018). Irrespective of these breeding approaches, current perennial grain crops undergoing development include rye, wheat, rice, sorghum, and intermediate wheatgrass (IWG) (Ryan et al., 2018).

Currently, perennial grain crops do not exist in any considerable commercial sense, as their profitability is a fundamental consideration for producers and is often a driver of management decisions (Hayes et al., 2012). Perennial crops must produce comparable grain yields or offset yield losses by increased aboveground biomass (i.e., vegetative growth that does not include grain, henceforth referred to as just 'biomass') for forage and/or by reducing fertilizer input costs. Seed yield and allocation to reproductive structures is typically viewed as being lesser in perennial crops than their annual counterparts, due in part to competing resource sinks within perennial plants and the fact that annual crops have been selected for yield gains for much longer (Bell et al., 2008; Jaikumar et al., 2012; Ploschuk et al., 2005). However, there is potential for considerable yields in perennial crops and studies have shown that grain yield can be increased while preserving the perenniality of the new cultivars (Cox et al., 2006; Moffat, 1996). Additionally, protein contents of grain and biomass, which may differ in a perennial grain relative to an annual counterpart, are necessary considerations relevant to overall quality for human and animal consumption (Newman et al., 2009; Nuttall et al., 2017). Finally, increased fertilizer nitrogen use efficiency (NUE) in perennial crops has the potential to counterbalance high fertilizer costs. An economic assessment by Bell et al. (2008) found that, if used as a dual-purpose grain and forage crop with reduced fertilizer inputs relative to an annual grain crop, perennial wheat could be a profitable option for Australian producers in areas of poor or intermediate soil quality.

Previous research efforts suggest that perennial crops can utilize nitrogen (N) more efficiently than annual counterparts potentially due to beneficial relationships with microorganisms in the soil, increased root mass and length, longer growing seasons, internal recycling of N resources or a combination of the above (Dawson et al., 2008; Glover et al., 2010a; Lewandowski and Schmidt, 2006). However, a lack of published literature exists to date regarding the NUE of a perennial grain cultivar, as well as how the crop allocates N between vegetative and reproductive structures compared to an annual counterpart based on efficiency metrics such as the N harvest index (NHI), physiological efficiency (PE) and uptake efficiency (UE). Sprunger et al. (2018) found that regardless of N fertilizer application rate, IWG had greater whole plant NUE than annual wheat due to the perennial's greater root mass and enhanced uptake of soil N. However, it is noteworthy that IWG is not a true perennial counterpart of an annual grain, but instead a domesticated forage.

Finally, further research into how perennial grain crops allocate resources to different plant yield components as well as crop harvest index (HI), a common measure of yield physiology, relative to an annual counterpart can inform future breeding goals as well as the feasibility of incorporating perennial grain crops into long, diversified rotations (Wiebe et al., 2016). Other important considerations for researchers and producers alike include winter survival, spring regrowth and lodging susceptibility of perennial grain crops. These seasonality aspects and agronomic considerations are crucial to the successful implementation of perennial cropping in temperate regions worldwide where these novel production systems could be impacted by harsh winter conditions and early snowfall events (Fowler, 2012; Fowler et al., 1989).

To understand the agronomic potential of a perennial grain cultivar, it must be studied over multiple consecutive years, as production may change with stand age (Jaikumar et al., 2012). Therefore, multi-year field trials were designed and implemented at two sites in central Alberta, Canada (Edmonton and Breton, 2 years each) to gather essential agronomic information on ACE-1 perennial rye, a model perennial grain cultivar. Perennial rye was selected based on preliminary findings from Lethbridge, Alberta summarized by Hayes et al. (2018), who reported on the superior performance of ACE-1, relative to several perennial wheat cultivars. This study is the first of its kind to compare a perennial grain with analogous spring (annual) and fall (biennial) grain, utilizing spring rye (cv. Gazelle) and fall rye (cv. Hazlett), respectively. As well, a perennial forage crop [meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa*)] typical of the area was included in the experimental design to compare the potential of perennial rye as a dual-purpose forage-grain crop. The objectives of this study were to assess yearly biomass and grain yields and compare the 2nd year perennial rye yield components to those of annual and biennial counterparts (spring rye and fall rye, respectively). Additionally, we assessed the protein productivity, HI, NHI, NUE, PE and UE and the survival, competitiveness and lodging susceptibility of perennial rye compared to spring and fall rye with contrasting growth habits. Overall, the objective of this study was to determine the possibility for perennial rye cropping in central Alberta, where long, cold winters and short growing seasons have limited perennial crop production to highly cold-hardy species (Fowler, 2012).

4.4 Materials and Methods

4.4.1 Sites and experimental design

Field sites were established in Edmonton, Alberta, Canada (53° 29' 43.33", 113° 31' 59.24") and Breton, Alberta, Canada (53° 5' 16.72", 114° 26' 29.35"). Soils at the Edmonton site are a clay texture and are classified as Orthic Black Chernozems. Soils at the Breton site are a loam texture and are classified as Orthic Grey Luvisols, according to the Canadian System of Soil Classification (Table 4.1). Mean annual air temperature at the Edmonton and Breton sites is 4.2 and 3.4°C respectively, with average yearly precipitation of 446 and 479 mm (Environment Canada, 2020). Hourly temperature and precipitation data was obtained for both sites from permanent weather stations within 1 km of the experimental plots at both sites.

Both sites were arranged in an identical randomized complete block design consisting of four replicates and eight treatments per block replicate. Treatment structure consisted of two factors, crop (4) and N fertilizer application (2). Crop type consisted of three contrasting grain crop growth habits (perennial rye grain cv. ACE-1, fall rye grain cv. Hazlett, spring rye grain cv. Gazelle) and a perennial forage crop [meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa*)] (Table 4.2). Within each block replicate, two plots of each cropping treatment were seeded, with one receiving no N fertilizer and one receiving 56 kg N ha^{-1} yr^{-1} in the form of a urea and polymer-coated urea (i.e., environmentally smart N; ESN) blend (2:1 ratio) (henceforth referred to as 'unfertilized' and 'fertilized', respectively). This rate was chosen using preliminary soil test results obtained in May 2018 and the Alberta Farm Fertilizer Information and Recommendation Manager (Government of Alberta, 2021). Each experimental plot measured 8 m in length and 4 m in width, for plots totalling 32 m^{2}.

4.4.2 Plot management

Plot management activities, which varied between crops and sites, are summarized in Supplementary Table S4.1. The perennial and fall rye were seeded at 90 kg ha^{-1} with a 23 cm row spacing to a depth of 2.5 cm. The spring rye treatment was seeded at 60 kg ha^{-1} with a 23 cm row spacing to a depth of 2.5 cm. For all rye treatments, 15 kg of phosphorus per ha in the form of phosphate was placed with the seed. Perennial forage treatments were broadcast seeded at 55 kg ha^{-1} and incorporated. The N fertilizer was broadcasted at the aforementioned rate on the selected plots for each corresponding N treatment concurrent with the spring rye seeding every year. Broadleaf weeds were controlled using a combination of Stellar™ XL herbicide (*Corteva Agriscience, Calgary, Canada*) applied at 0.9 L ha^{-1} using a backpack sprayer and hand weeding.

4.4.3 Field Measurements

Grain and biomass yields were measured by hand harvesting 1-meter lengths of two adjacent rows at two locations within each replicated plot, at least 1-meter from the plot edges. The harvested material was then bagged, threshed, weighed and oven dried until a constant weight was reached for determination of grain and biomass dry matter (DM). Specific to the Breton site in year 2, dried plant material from the perennial rye plots was sorted after drying, prior to the final weigh, to differentiate perennial rye biomass from weed growth.

Plant yield components including tiller count and kernels per spike were assessed in year two by counting each component within three 1-meter lengths in each grain plot. The purpose of the counts was to characterize the yield components of perennial rye, relative to an annual counterpart. This was done on 30 May 2019, 5 July 2019, 23 July 2019, and 29 August 2019 at the Breton site and 31 May 2019, 11 July 2019, 17 July 2019, 30 July 2019, 13 August 2019, and 28 August 2019 at the Edmonton site.

Lodging estimates were completed as per the method described by Caldicott and Nuttall (1979). A square meter quadrat was delineated and marked with flags. A wooden stake was driven into the soil, perpendicular to the soil surface. Proportions of leaning (5° to 45° from vertical), lodged (45° to 85° from vertical) and lodged flat (85° to 90° from vertical) crop were recorded and the lodging index was determined as follows:

$$Lodging\ index\ = 1/3\ (\%\ area\ leaning)\ +\ 2/3\ (\%\ area\ lodged)\ +\ (\%\ area\ lodged\ flat) \qquad [5.1]$$

Crop stage was assigned based on the Crop Identification and BBCH (Biologische Bundesanstalt Bundessortenamt und Chemische Industrie) Staging Manual by Lancashire et al. (1991) (Agriculture and Agrifood Canada, 2011). Because of our focus on perennial cropping, phenology staging was done biweekly during the growing season 2019 in all rye plots by observing leaf counts, tillering, flag leaves, and spikelets.

Normalized difference vegetation index (NDVI) readings were taken early in the growing season (June-July) after fertilizer application to detect differences between the fertilized and unfertilized counterparts for each crop. Measurements of NDVI were taken using a Trimble Greenseeker Handheld Crop Sensor (*Vantage Canada, Calgary, Canada*) with a plumb bob hung 30 cm from the sensor to ensure measurements were taken consistently from the same height above the growing crop. Three readings were taken from each plot at random to account for spatial differences.

4.4.4 Laboratory analyses

Grain and biomass protein were determined using a FOSS DS2500 (*Foss Analytics, Denmark*) near infrared spectroscope (NIR). Samples were scanned from 400 – 2500 nm as whole grain samples or ground biomass samples using a large product cup with a removable top. To create an NIR calibration curve for each grain and biomass, samples harvested in the first year of cropping from each experimental plot were ground and encapsulated in tin capsules (*Elemental Microanalysis, Devon, UK*) and total N was determined using dry combustion in a Flash 2000 Organic Elemental Analyzer (*ThermoScientific, Walthum, USA)*. Model calibration statistics including R^2, standard error (SE) and standard error of prediction (SEP) for grain, biomass and perennial forage biomass are as follows: $R^2 = 0.94$, SE = 0.07, SEP = 0.11; $R^2 = 0.94$, SE = 0.05, SEP = 0.06 and $R^2 = 0.91$, SE = 0.17, SEP = 0.24, respectively. Conversion from N concentration into protein concentration was done by multiplying N content by the widely used Jones' Factor of 6.25 (Jones, 1931).

4.4.5 Calculations and statistical analyses

Using the dry matter (DM) weight and total N content determination from NIR spectroscopy, harvest index (HI), grain N partitioning (NHI), fertilizer-N use efficiency (NUE) in grain production, uptake efficiency (UE), physiological efficiency (PE) and protein productivity were estimated by comparing each rye treatment with fertilization, to their respective controls (no added N) as follows (Hernandez-Ramirez et al., 2011; Thilakarathna et al., 2020):

$$Aboveground\ HI = \frac{Grain\ Yield}{Grain\ Yield + Aboveground\ Biomass + Residue} \quad [4.2]$$

$$NUE\ of\ grain = \frac{Grain\ DM_{@N\ rate} - Grain\ DM_{control}}{N\ fertilizer\ rate} \quad [4.3]$$

$$UE = \frac{Aboveground\ N\ accumulation_{@N\ rate} - Aboveground\ N\ accumulation_{control}}{N\ fertilizer\ rate} \quad [4.4]$$

$$PE = \frac{Grain\ DM_{@N\ rate} - Grain\ DM_{control}}{Aboveground\ N\ accumulation_{@N\ rate} - Aboveground\ N\ accumulation_{control}} \quad [4.5]$$

$$Aboveground\ NHI = \frac{Grain\ N}{Aboveground\ Biomass\ N + Residue\ (stubble)\ N + Grain\ N} \quad [4.6]$$

$$Protein\ productivity = protein\ content \times biomass\ DM\ yield\ or\ grain\ DM\ yield \quad [4.7]$$

All statistical analyses were performed using version 1.1.383 of R Studio software (R Core Team, 2020). Data normality was tested by the Shapiro-Wilk test, homogeneity of variance was tested by the Bartlett test and plot functions. Non-normality and heteroscedasticity were corrected, when necessary, using a Box-Cox transformation. Significant differences between fertilized and unfertilized NDVI readings were determined using Welch's Two-Sample T Tests. Biomass yield, grain yield, HI, NHI, tiller count, kernel count, thousand kernel weight (TKW), grain protein, biomass protein, grain protein productivity and biomass protein productivity were analyzed using two-way analysis of variance (ANOVA) tests with crop and fertilizer as fixed effects. The NUE, PE and UE were tested using one-way ANOVA with crop as the fixed effect. In some cases, when Box-Cox transformations did not rectify heteroscedasticity, Welch's ANOVA was used in place of ANOVA. All analyses were tested at alpha critical level of 0.05, and Tukey's Honest Significant Difference from the agricolae (v. 1.3-3) package was used for post-hoc comparisons of means (de Mendiburu, 2019). To assess if grain and biomass yields of the perennial rye were significantly different in year two compared to year one, a repeated measures analysis was completed with fertilizer and year as fixed effects, plot ID as the random effect and a first order autoregressive correlation structure to account for temporal autocorrelation.

4.5 Results

4.5.1 Weather conditions

At the Edmonton site, temperatures were generally similar to the 30-year average, except for a colder September 2018 and February 2019 (Fig. 4.1A). In September 2017 and 2018, precipitation was greater than the 30-year average by 39% and 51%, respectively. Conversely, May 2018 and 2019 experienced less rainfall than usual. Conversely, June 2019 was increasingly wet, with a 54% increase in precipitation this month (Fig. 4.1B).

Monthly air temperature at the Breton site deviated from the 30-year monthly average (1980-2010) in May 2018, which was slightly warmer and September 2018, February 2018, and February 2019, which were all colder than average (Fig. 4.2A). Average monthly precipitation greatly differed from the 30-year average for the 2018-2019 (year two) growing season, as conditions were substantially wetter in the months of June and July (Fig. 4.2B).

4.5.2 Yield and yield components

4.5.2.1 Year one

At the Edmonton site, crop was the only factor affecting grain yield in year one ($p < 0.001$), whereas fertilizer and the interaction of fertilizer and crop were insignificant (Table 4.3). Perennial rye had lower grain yield than both fall and spring rye, yielding 46% and 56% of fall and spring rye, respectively. In terms of biomass, the perennial rye crop had greater biomass ($p < 0.001$) than the other rye crops but did not differ from the perennial forage. Harvest index was affected by crop alone ($p < 0.001$). The HI ranked from lowest to highest was as follows: perennial rye < spring rye < fall rye (Table 4.3). Likewise, for TKW at the Edmonton site, the only significant factor was crop ($p < 0.001$). On average, TKW was lowest in perennial rye (31.8 ± 0.5 g) and greater in fall (33.0 ± 0.4 g) and spring rye (35.9 ± 0.5 g) (Table 4.4).

Crop type was the only significant factor determining grain yield at the Breton site in year one, similar to the Edmonton site (Table 4.3). Perennial rye yields were the lowest; both spring and fall rye had greater grain yields ($p < 0.001$). There was no effect of fertilizer on grain yield for any treatment, nor an interaction between crop and fertilizer. On average, perennial rye yield was 52% and 64% of fall and spring rye yield, respectively. Similarly, crop type was the only factor determining biomass at the Breton site in year one ($p < 0.001$). Neither fertilizer, nor the interaction of crop and fertilizer was significant. Notably, the greatest biomass productivity was from the perennial rye crop and the lowest from the perennial forage crop. The perennial rye crop produced over 1.5x more biomass than the perennial forage plots. Analysis of HI indicated an effect of crop ($p < 0.05$), but not fertilizer nor the interaction of fertilizer and crop. No difference in HI between perennial rye and fall rye was found, but perennial rye HI was significantly lower than that of spring rye (Table 4.3).

Breton TKW was affected by the interaction between crop and fertilizer ($p < 0.05$), due to the increase in TKW in fall rye when no N was applied. Crop type was also significant ($p < 0.001$), but fertilizer was not. Consequently, fall rye without N addition had the highest TKW (39.1 ± 0.6 g), whereas perennial rye TKW (31.8 ± 0.5 g, on average) did not differ from any of the other treatments (Table 4.4).

4.5.2.2 Year two

Grain yield at the Edmonton site was affected by crop ($p < 0.01$) (Table 4.3). Perennial rye produced, on average, 38% and 46% of the grain yields of fall and spring rye, which were not different from one another. Conversely, perennial rye at the Edmonton site in year two produced more biomass than all the other crops ($p < 0.001$). Specifically, the perennial rye produced 68% more biomass than the perennial forage crop, on average. Neither fertilization, nor the interaction of crop and fertilizer were significant. Differences in HI were found for all crops ($p < 0.001$), with perennial rye having the lowest HI values and fall rye the highest (Table 4.3).

Notably, perennial rye had more total tillers per plant than the other crops, but less kernels per spike ($p < 0.001$ and $p < 0.001$), which had a lower TKW than both fall and spring rye crops ($p < 0.001$). Each yield component had no effect of fertilizer, nor was an interactive effect detected. Estimated productive tillers as a percent of total tillers was substantially reduced in the perennial rye crop treatments relative to the spring and fall rye. Notably, the proportion of productive tillers in the perennial rye crop was on average less than half of that of the spring rye crop (Table 4.4).

Grain yield quantification was challenging at the Breton site in year two for the perennial rye treatments, due to strong competition from weeds. Only certain areas of the plot with perennial rye dominance were sampled when possible, thus grain yield measurements from Breton in year two are not an accurate mean estimate and are included solely to demonstrate that grain production is possible for consecutive seasons (Table 4.3). For fall rye and spring rye, both crop and fertilization affected grain yield ($p < 0.05$ and $p < 0.05$), but their interaction did not. Spring rye had greater grain yield than fall rye, and unfertilized plots yielded less grain than their fertilized counterparts did. Biomass yield was affected by crop alone ($p <0.001$). No differences were discerned between contrasting rye growth habits (i.e., perennial, fall and spring), however, all three rye crops produced more biomass than the perennial forage.

Perennial rye had a greater number of total tillers, relative to the other two rye crops, similar to the Edmonton site ($p < 0.001$). However, perennial rye had a lower number of kernels per spike ($p < 0.001$). Notably, none of the aforementioned yield components were affected by fertilization nor the interaction between fertilization and crop. Representative TKW data was not possible for perennial rye at the Breton site in year two, due to lack of sample collection in all the field replicates. No difference in TKW was found between the fall rye or spring rye crops. Again, estimated productive tillers were much lower for the perennial rye crop relative to the fall and spring rye crops, with the average proportion of productive tillers relative to the total being only 43% of the spring rye crop (Table 4.4).

4.5.2.3 Changes in perennial rye grain and biomass yields from year one to year two

Year was found to be a significant factor when assessing the capability of a 2-year-old perennial rye crop to maintain grain and biomass yields (Supplementary Table S4.2). Year was a factor for grain yield at the Edmonton site from year one to year two. Grain yield showed a decline in the 2nd season of perennial rye growth ($p < 0.001$). There was no effect of fertilizer, nor was there an interactive effect of year and fertilizer. Biomass yield was affected by year at both Edmonton and Breton sites ($p <0.001$ and 0.01, respectively). The Edmonton site showed increases in biomass from year one to year two, whereas the Breton site showed a decline from year one to year two.

4.5.3 Grain and biomass protein and protein productivity

At the Edmonton site, both grain protein and biomass protein concentrations were solely dependent on crop in both year one ($p < 0.001$ and $p <0.001$) and year two ($p < 0.001$ and $p <0.001$) (Table 4.5). Grain protein of the perennial rye was higher than the other rye growth habits (fall and spring rye) for both years. Predictably, biomass protein was highest for the perennial forage plots for both years. In year one, perennial rye had the second greatest biomass protein, which was greater than spring rye. In year two, no discernable differences were detected across the three rye crops in terms of biomass protein (Table 4.5).

Grain protein productivity was lower in the perennial rye plots for both year one and year two at the Edmonton site, based on crop type alone, and no differences were detected between the fall and spring rye crops ($p < 0.001$ and 0.01). Similar to the Breton site, biomass protein productivity for perennial rye in both years was greater ($p < 0.001$ and 0.01) than the other rye crops, but lower than the perennial forage plots. In year two this trend held for fall and spring rye, however, no difference between perennial rye and perennial forage was established in terms of biomass protein productivity. No effect of fertilizer, nor the interaction of crop and fertilizer, was found for the Edmonton site for any of the protein concentration or protein productivity calculations in either year (Table 4.5).

At the Breton site, both grain protein and biomass protein concentrations were solely dependent on crop in year one ($p < 0.001$ and $p < 0.001$) and year two ($p < 0.05$ and 0.001, respectively). Grain protein content, from highest to lowest in year one: perennial rye > fall rye > spring rye. Fall rye was higher in protein than spring rye. As expected, biomass protein for both year one and year two was highest for the perennial forage plots. Notably, in year one, perennial rye biomass had the second greatest biomass protein after the perennial forage crop; but switched to having the lowest biomass protein in year two (Table 4.5).

With respect to grain protein productivity at the Breton site, a crop effect ($p < 0.001$) was detected at the Breton site in year one. Perennial rye showed no difference in protein productivity from spring rye, but both perennial and spring rye were lower than fall rye. In year two, there was a clear reduction in perennial rye protein productivity as a result of severely reduced grain yield. No differences between the spring and fall rye were discerned. Conversely, when considering biomass protein productivity, perennial rye had greater protein productivity than the other rye crops but did not differ from the perennial forage ($p <0.001$). In year two, biomass protein productivity was affected by crop ($p < 0.001$) as well as fertilizer ($p < 0.01$). Perennial rye plots had lower biomass protein productivity than all other crops (when encompassing grain and forage), and fertilized treatments had greater biomass protein productivity than those without fertilizer addition (Table 4.5).

4.5.4 Nitrogen use efficiency metrics: NUE, UE, PE, NHI

Neither site showed differences in NUE between grain crops in year one. Correspondingly, no numerical trends in NUE were discernible due to high variability in the dataset for both Edmonton and Breton. Notably, only the Edmonton site in year two showed significance ($p < 0.05$), wherein perennial rye had greater NUE than fall rye but was not different from spring rye (Supplementary Table S4.3).

Similarly, no differences in UE were discernible between rye crops at both sites in year one, and all treatments showed a low or even negative uptake efficiency. For both years at the Edmonton site, perennial rye showed no difference from either the fall or spring treatments, despite the UE being numerically higher (Supplementary Table S4.3).

Consistently, no differences in PE in year one at either site could be discerned. However, despite statistical insignificance, perennial rye at the Breton site showed an apparent reduction in PE relative to spring rye in year one. Perennial rye at the Edmonton site in year two had lower PE than fall rye ($p < 0.01$), but was not different than spring rye, despite being markedly diminished (Supplementary Table S4.3).

Perennial rye consistently had the lowest numerical NHI across years and sites, but this reduction was not statistically significant at the Breton site in year one. Conversely, at the Edmonton site in year one, NHI was lower for the perennial rye than the fall and spring rye ($p < 0.001$). This trend held consistent at the Edmonton site in year two, where the perennial rye had lower NHI than the other two rye crops ($p < 0.001$) (Supplementary Table S4.4). Overall, differences in NHI were dependent on crop type alone, meaning fertilizer application did not affect NHI for any crop type in any year.

4.5.5 Canopy greenness – NDVI

Few differences were discerned between fertilized and unfertilized counterparts of the same crop over both growing seasons (Figs. 4.3, 5.4). At the Edmonton site, fertilized fall rye had significantly higher NDVI than in the corresponding unfertilized fields on two of the sampling dates over both year one and year two of the study ($p <0.05$ and $p <0.05$). However, in year two the difference between fertilized and unfertilized fall rye became insignificant later in the season. In year two at the Edmonton site, fertilized spring rye exhibited higher NDVI than unfertilized spring rye ($p < 0.05$). At the Breton site, only perennial forage in year two showed higher NDVI due to the fertilizer addition and on only one sampling date.

Phenological differences between the spring rye crop and the perennial and fall rye crops can be seen in the NDVI measurements for year one (Fig. 4.3). A trend of declining NDVI values for the perennial and fall rye crops are indicative of declining leaf area index (LAI), whereas the spring rye crop shows a trend of increasing LAI over the measurement period for each site.

4.5.6 Staging

Detailed staging was completed for both sites in year two (Fig. 4.5). No differences were discerned between fertilized and unfertilized plots of the same crop, thus only crop is shown as a factor. At both sites, the perennial rye matured faster than the spring rye, maintaining a significant lead in maturity over most of the growing season, until all rye crops reached similar maturity in late August 2019.

4.5.7 Susceptibility to Lodging

Lodging measurements were done when visual evidence of lodging was apparent at either site. This corresponded to two dates at the Breton site, 19 September 2018 and 22 July 2019. Substantial lodging in the perennial rye and spring rye plots was observed in 2018 after an early autumn snowfall prior to harvest of the aforementioned plots. The lodging indices for the perennial rye + N and the spring rye + N were 68.5 ± 2.7 and 97.9 ± 0.8%, respectively. Conversely, when crop standability measurements were done on 22 July 2019, lodging was only recorded in the perennial rye + N plots with no evidence of lodging in the other rye crops. The average lodging index of the perennial rye + N was 65.2 ± 6.1%.

4.5.8 Competitiveness of perennial rye crops

Weed pressure from the soil seed bank in the perennial rye plots at the Breton site in year two resulted in significantly reduced plant density and yield of the perennial rye. Specifically, only 40 ± 5% of perennial rye plots were composed of perennial rye, on average. At the Breton site, the remaining ~60% of the plant matter in perennial rye plots was comprised of Timothy (*Phleum pretense*) (10 ± 3%), white clover (*Trifolium repens*) (12 ± 3%), ryegrass (*Lolium perenne*) (2 ± 10%) and various unidentified perennial grasses (36 ± 4%). Notably, the prevalence of perennial grasses hindered attempts to control them with herbicides due to the risk of damaging the perennial rye crop itself.

4.5.9 Winter Survival

Winter survival of the perennial rye crop was 50% and 48% in the fertilized and unfertilized plots in year one at the Edmonton site, and 58% and 52% for the fertilized and unfertilized plots in year one at the Breton site, respectively. After the second winter, survival of the perennial rye crop at the Edmonton site in year two was highly diminished, with only 31% and 24% of the plants remaining in the fertilized and unfertilized plots. Winter survival at the Breton site in year two showed an increase from year one, with 61% of the original plant count for both fertilized and unfertilized plots. However, at the time of this plant count early in the growing season, we were unable to differentiate between the various grass species detailed in section 3.6 and therefore there is a high probability that the winter survival of the perennial rye in year two at the Breton site was much lower. In year three, the experiment was concluded because perennial rye crop exhibited negligible survival at the Breton site and no survival at the Edmonton site.

4.6 Discussion

4.6.1 Yield potential of a perennial rye crop

The perennial rye crop at both the Edmonton and Breton sites showed decreased grain yield relative to fall and spring rye, which is consistent with earlier reports (Table 4.3) (Cattani, 2019; DeHaan and Van Tassel, 2014; Hayes et al., 2018). Yield reduction in the perennial rye crop in this instance can be attributed to reduced kernels per spike and TKW for both sites in the first and second years of cropping (Table 4.4). Reduced grain yield in perennials is the result of natural selection, as energy is allocated to structures such as roots and stems that increase competitiveness and longevity over seeds, thus seed size in perennial plants is generally smaller than in annuals (Cox et al., 2018; DeHaan et al., 2005; Wagoner and Schaeffer, 1990). Notably, Huang et al. (2018) reported no declines in yield in a perennial rice cultivar, PR23, when grown at several sites in China and Laos, indicating that the tradeoff between perenniality and yield is not definite, and thus continued research can improve perennial grain prospects

(Cox et al., 2006). Preliminary research on the model perennial chosen for this study, ACE-1 perennial rye, also suggested issues with floret fertility and chromosome pairing during meiosis may reduce yield in tetraploid varieties of perennial rye, such as ACE-1 (Acharya et al., 2004; Hayes et al., 2018). However, diploid varieties of perennial rye, such as Reimann-Phillip, have shown improved spike fertility, suggesting that informed breeding efforts can reduce sterility (Hayes et al., 2018). Overall, annual crops have the advantage of intensive, long term breeding efforts whereas perennial grains are a relatively new breeding endeavor, with much of the current research being led by The Land Institute in Kansas, USA since the early 1990s (Cox et al., 2002; Jackson and Jackson, 1999).

Although this study did not directly measure productive versus unproductive tillers in the field, we conducted post hoc estimations of the proportion of productive tillers (Table 4.4). These back-calculations indicated that the proportion of productive tillers in perennial rye were substantially reduced relative to the spring and fall rye crops. This illustrates why the increased total tiller count of the perennial rye crop was not conducive to increased rye yield in this crop. The majority of perennial rye tillers did not bear grain, which is a common attribute in perennial rye crops (Cattani, 2019; Wagoner and Schaeffer, 1990). These findings present an opportunity for the development of breeding goals and should inform future research into perennial rye improvement, whereby perennial rye crop yield can be optimized by selecting offspring that confer increased yields by trading off with reduced unproductive tillering.

A major concern with perennial grain crops is a decline in grain yield with increasing stand age (Jungers et al., 2018; Murphy et al., 2010; Pimentel et al., 2012; Ploschuk et al., 2005). This is consistent with our results, as the grain yield of the perennial rye crop declined in year two relative to year one at the Edmonton site (Supplementary Table S4.2). This is in contrast to a study by Jaikumar et al. (2012) that found no reductions in grain yield between 1-year old and 2-year-old perennial rye and wheat. We hypothesize that this may be the result of different perennial rye cultivars used (Rival rye vs. ACE-1 rye), or the result of environmental conditions that favored vegetative growth over grain production in our experiment, as evidenced by grain yield reductions in both spring and fall rye crops in year two as well.

Notably, both sites experienced overall decreases in grain yield across all grain treatments, potentially a result of the abnormally cold and wet growing season at both sites in year two (Figs. 4.1, 5.2) (ACIS, 2020). Colder temperatures affect seed filling, ultimately reducing grain yield and increased precipitation can diminish yields, specifically in rye crops (Mantri et al., 2012; Peltonen-Sainio et al., 2011). Thus, year two reductions in grain yield may have been the result of the specific environmental conditions of this growing season, instead of perennial rye genetic shortfalls.

An earlier economic analysis by Bell et al. (2008) ascertained that a perennial grain crop could be profitable if it produced 40% of the grain yield of an analogous annual crop and the harvested biomass was used as forage. According to Bell et al. (2008), both the Breton site and the Edmonton site in year one may be profitable despite averaging only 55% grain yield of the other grain crops. In year two, the Edmonton site barely achieved this threshold, averaging 42% of the other crops' grain yield. While grain yield in year two was minimal at the Breton site, we hypothesize that had the Breton perennial rye plots not experienced such substantial reductions in yield as a result of poor competition with weed growth, reductions in grain yield could still have materialized, due to colder than average fall and winter conditions that may have resulted in winter damage and consequently reduced grain yield (Peltonen-Sainio et al., 2011). Notably, the economic analysis by Bell et al. (2008) accounted for reduced fertilizer and seeding costs of a perennial grain as well as the reduced market price, but neglected the potential benefits of reduced erosion, increases in soil organic matter, and other ecosystem services which are postulated with the adoption of perennial grains (Ryan et al., 2018). Further, the analysis by Bell et al. (2008) estimated a set price for perennial grain, which in reality would be subject to change depending on markets and grain quality. Thus, a more detailed economic analysis is still required to fully capture these several externalities and uncertainties to conclude if a perennial grain crop is profitable in the long term.

Aboveground biomass yields of perennial rye at the Edmonton and Breton sites in year one were the greatest of all treatments including the perennial forage, regardless of fertilization (Table 4.3). This is partially the result of greater tillering in perennial rye relative to the other rye crops (Table 4.4). Several studies have emphasized that prolific biomass production is a consistent trait of many perennial grasses (Acharya et al., 2004; Fedenko et al., 2013; Shinners et al., 2010). Notably, this can present the breeding opportunity for reallocation of this assimilated carbon away from unproductive tillers to grain via breeding as mentioned above (Cox et al., 2006; Jaikumar et al., 2012). Interestingly, in year two at the Edmonton site, biomass production of perennial rye increased relative to year one, whereas this trend was not evident at the Breton site. Based on our observations, this was less the result of reduced biomass from individual plants of perennial rye and instead the result of weed growth competition in the perennial rye plots at the Breton site. Indeed, by the end of year two, less than half of each perennial rye plot was occupied by perennial rye.

The relationship of grain to total aboveground biomass of a crop, referred to as HI, is a measure of efficiency for plants producing grain. HI of the perennial rye was universally reduced in this study relative to the spring and fall rye, due to less proportion of assimilated carbon being allocated to grain over biomass (Table 4.3). Reduced HI of perennial rye relative to annual counterparts is consistent with literature, as the evolutionary advantage of a wild perennial is highly dependent on the survival of vegetative structures and thus more photosynthate is allocated to nonsexual growth (Cox et al., 2006; Culman et al., 2013; DeHaan et al., 2005; Jaikumar et al., 2012). However, this metric places the importance of grain production over total plant productivity (DeHaan and Van Tassel, 2014). Total primary productivity includes vegetative biomass, which is an important characteristic of perennial grains that may serve as dual-purpose forage and grain crops (Ryan et al., 2018; Snapp et al., 2019).

Interestingly, no effect of fertilization was found for grain yield, biomass yield or HI for any treatment in year one, or at the Edmonton site in year two (Table 4.3). The Edmonton site is characterized by Black Chernozemic soil, which is highly fertile. Highly fertile soils may not show a yield response to fertilizer addition (Tausz et al., 2017; Thilakarathna et al., 2020). Specifically, Campbell et al. (2005) studied the effects of fertilization on grain and biomass yields in a Chernozemic soil and found that when compared to an unfertilized control, fertilization negligibly altered yields in the first years of the experiment and obvious yield increases took several growing seasons to materialize. As well, while the Breton site is underlain by a generally less fertile Gray Luvisolic soil, its land use history was that of a mixed perennial grass stand grown for forage harvest for at least 60 years prior to this experiment. The soil was tilled for the first time in June 2017 prior to experiment establishment. Thus, there was ample N from mineralizing roots and grass residues and increased soil organic matter decomposition from the tillage disturbance, a legacy effect that can last up to three years after conversion of a perennial grass stand (Mukumbuta and Hatano, 2020; Thilakarathna and Hernandez-Ramirez, 2021). Thilakarathna and Hernandez-Ramirez (2021) documented how growing perennial forage in Breton raises soil organic matter and N concentrations, which is then available for subsequent crop uptake upon simulated tillage and cropping. This is supported by the lack of differences found between fertilized and unfertilized NDVI readings in the crop canopies for the majority of sampling dates, indicating that crops in the unfertilized plots did not experience reductions in N availability or uptake that may have translated into lower NDVI readings, relative to their fertilized counterparts. Indeed, only 12% of average NDVI readings showed differences between fertilized and unfertilized crops. Overall, several more growing seasons and successive grain harvests may be required to detect consistent differences in fertilized vs. unfertilized plots for both the Edmonton and Breton sites.

4.6.2 Substantial protein productivity of a perennial rye crop

Total grain protein is one component that determines the profitability of a grain crop (Asseng et al., 2002). In accordance with the present study, ample literature has documented increased protein in perennial grain crops relative to annual grain crops (Pimentel et al., 2012; Marti et al., 2016; Ryan et al., 2018). For both the Edmonton and Breton sites, the perennial rye had increased grain protein relative to the fall and spring rye, whose protein contents were within the expected range (Table 4.5). Protein content of annual rye grain in Alberta is generally 12% but can reach as high as 14.5% depending on cultivar (Alberta Agriculture and Forestry, 2016; Arendt and Zannini, 2013) Protein productivity is the product of grain protein concentration and grain yield; thus, it encompasses both metrics into a single density parameter and represents the overall ability of a crop to produce grain protein (Asseng et al., 2002). As a result of greater grain protein, the grain protein productivity of the perennial rye at the Breton site in year one was comparable to that of spring rye. Interestingly, increased protein concentration in the perennial rye was able to overcome lower yields and deliver the same grain protein productivity in the perennial crop as an annual crop. Conversely, in both years at the Edmonton site, the protein productivity was lower than the fall and spring rye despite having greater grain protein concentration, due to insufficient grain yields. Thus, the grain protein productivity of a perennial rye crop may be comparable to an annual rye crop in specific circumstances, but more research is needed as accurate yield measurements were precluded at the Breton site in year two.

Similarly, the perennial rye biomass protein was greater in year one at both sites compared to the biomass protein of the other rye treatments, but was lower than the perennial forage biomass, likely due to the presence of alfalfa in the perennial forage mix, a legume with a higher crude protein content than most grasses (Table 4.5) (Deng et al., 2020). Notably, increased biomass yield in the perennial rye resulted in comparable protein productivity between the perennial rye and perennial forage at the Breton site in year one and the Edmonton site in year two. Forages are the main source of food for ruminants and those with a legume are highly valued as animal feed because they are an inexpensive source of protein (Radovic et al., 2009; Wilkins and Humphreys, 2003). Thus, comparable protein productivity from perennial rye biomass highlights its practicality as a dual-purpose forage-grain crop.

4.6.3 Nitrogen use efficiency of a perennial rye crop

Common measures of N use efficiency include NUE, PE, UE and NHI and optimizing these metrics is a significant challenge for world agriculture, particularly in grain crops (Jamil, 2020). Both NUE (a measure of how grain yield increases with fertilizer application) and UE (a measure of whole-aboveground plant N increase with fertilizer application) were small for all rye crops across both sites and years (Supplementary Table S4.3) . Our NUE values were lower when compared to previous research on grain crops in Black Chernozemic soils comparable to the Edmonton site (Thilakarathna et al., 2020) and

Gray Luvisolic soils similar to the Breton site (Malhi et al., 2011). We hypothesize that this may be attributed to a combination of two things: primarily, the fertilizer application method in our study. To reduce damage to the perennial rye plots, fertilizer was broadcast onto the plots and left unincorporated; a method that can suffer significant losses due to NH_3 volatilization (Alberta Agriculture and Forestry, 2016; Romero et al., 2017). Secondly, baseline soil fertility conditions at both Edmonton and Breton sites were relatively high. Thus, a combination of these two factors may have resulted in low NUE and UE and masked the effects of fertilizer application.

Further, perennial rye showed no improvement in NUE or UE relative to annual. We postulate that this null result may be because we did not measure root contributions to overall plant NUE in our study, as a previous study by Sprunger et al. (2018) found increases in the whole plant NUE (when accounting for the roots) of the perennial IWG compared to annual wheat. Notably, Sprunger et al. (2018) calculated NUE for aboveground and belowground components separately as well as for the whole plant and found that the aboveground NUE was not different between the perennial and annual, which is congruent with the findings of the present study. The increase in whole plant NUE reported by Sprunger et al. (2018) was the result of increased root biomass and root N content in the perennial crop. This finding was further confirmed in related work by Kim et al. (2021), who found twofold the root mass in perennial rye in the 15-30 cm subsurface soil layer and greater root N density than a spring rye crop. Additionally, the abovementioned lodging of the perennial rye crop observed at the Breton site in year two is likely indicative of an over application of fertilizer, which would mask any NUE effects.

The NHI is the ability of a plant to partition N into grain over other vegetative sinks, which is an important metric for the economy of grain quality and allocation efficiency (Dobermann, 2007; Jamil, 2020). Lower NHI for the perennial rye is predictable, based on the overall perennial life strategy for longevity, which prioritizes allocation to vegetative structures over grain (DeHaan and Van Tassel, 2014; DeHaan et al., 2007; Snapp et al., 2019). Notably, Cox et al. (2002) postulated that the aforementioned tradeoff between vegetative structures and grain in perennial rye crops would only be required in the first year. Results for the Edmonton site contradict this hypothesis, as the NHI of the perennial rye was reduced relative to fall and spring rye in year one and again in year two (Supplementary Table S4.4). While no differences in NHI were determined for the Breton site in year one between any of the rye crops, the perennial rye had reduced NHI on average. This indicates transfer of N to grain in the perennial rye crop was reduced relative to fall and spring rye crops, despite perennial rye having the highest grain protein content (Lopez-Bellido and Lopez-Bellido, 2001). Similarly, reduced PE in the perennial rye at the Edmonton site in year two supports the diminished ability for perennial rye to translate increased whole plant N content into increased grain yield.

4.6.4 Challenges with growth, survival and competitiveness of perennial rye

Perennial rye initially matured faster than its fall and spring counterparts but slowed considerably as the season progressed, eliminating the ability for an earlier grain harvest, a prospect that would have reduced the risk of an early season snowfall damaging yields, inducing lodging and often even impeding harvest in Western Canada. However, the rapid vegetative growth may allow for a forage harvest early in the season and not impede the perennial rye crops ability to regrow and produce a considerable grain harvest (Ates et al., 2017; Pugliese et al., 2019). Testing this hypothesis should be included in a future study, as a second biomass harvest for forage may improve the profitability of the perennial rye, but a mismatch in the timing of biomass harvest could unintentionally deplete root carbohydrate reserves and compromise successful re-growth of perennial rye (Ferraro and Oesterheld, 2002).

Unfortunately, the current climate of central Alberta does not lend itself to the over winter success of existing grain crops, let alone novel perennial grains (Cattani et al., 2019; Salmon et al., 2015). In addition to reduced yields, high winter mortality also precludes the ability to study the long-term benefits on soil health that are purported in the literature with perennial grain cropping (Ryan et al., 2018; Crews and Cattani, 2018). Furthermore, the competitiveness of the perennial rye against weed pressure at the Breton site was insufficient to support a considerable grain yield past one year, despite worries that perennial grains could become invasive (Schlautmann et al., 2018).

4.7 Conclusion

The perennial grain, ACE-1 perennial rye, may be a viable option relative to spring and fall rye in cold temperate environments if harvested as a dual-purpose forage and grain crop; however, grain yields may be reduced after the first year, reducing its feasibility as a cash crop. Biomass yields and unproductive tillering of the perennial rye were considerably elevated, indicating the significant potential for perennial rye to fix atmospheric carbon, lending to the strategic possibility for breeding efforts to physiologically redistribute resource allocation from vegetative structures to grain. The perennial rye crop produced ample grain protein productivity as a result of increased grain protein concentration, but only when grain yields were sufficient to sustain a considerable harvest in the first year of growth. Generally, N use efficiency parameters did not differ between growth habits (perennial vs. fall vs. spring) for aboveground biomass, indicating that gains in NUE in perennial rye crops likely come from increased belowground allocation or at lower fertilization rates. Challenges associated with increasing weed pressure and winter mortality of perennial rye crops further prevented more than two production cycles, as well as their monitoring beyond two years at either study site. Overall, perennial rye requires further development prior to consideration as a suitable option as a grain crop in agroecosystems that experience conditions comparable to those in central Alberta, Canada.

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Tables

Table 4.1. Baseline soil properties at the Edmonton and Breton field sites from 0-30 cm depth.

Soil Properties	Edmonton	Breton
Canadian classification	Black Chernozem	Gray Luvisol
TC (g C kg^{-1}) (0-30 cm)	41.6 ± 7.5	19.2 ± 3.9
TN (g N kg^{-1}) (0-30 cm)	3.6 ± 0.5	1.7 ± 0.3
Available nitrogen (NH_4^+ + NO_3^-) (mg N kg^{-1}) (0-15 cm)ͳ	55.5 ± 2.5	48.3 ± 4.5
pH (1:5 H_2O) (0-30 cm)	7.3 ± 0.09	6.1 ± 0.08
Bulk density (g cm^{-3}) (5-30 cm)	1.0 ± 0.06	1.1 ± 0.06
Soil texture (0-30 cm)	clay	loam
% clay	48.3	24.8
% silt	35.7	41.8
% sand	16.0	33.3

ͳAvailable nitrogen samples obtained from the Edmonton and Breton sites on 1 May 2018.

Table 4.2. Detailed description of crops at Edmonton and Breton field sites (adapted from Kim et al., 2021).

Crop	**Description**
Perennial rye	Perennial rye crop for grain production. *Secale cereale* L. × *S. montanum* Guss cv. ACE-1.
Spring rye	Spring rye crop for grain production. *Secale cereale* L. cv. Gazelle. Annual rye or summer rye are alternative designations in the literature.
Fall rye	Fall rye crop for grain production. *Secale cereale* L. cv. Hazlett. Winter rye or biennial rye are alternative designations in the literature.
Perennial forage	Perennial forage crop for hay production. Alfalfa *Medicago sativa* L. and bromegrass *Bromus spp*. Aboveground biomass is cut and removed two times a year for hay for livestock feeding purposes.

Table 4.3. Dry matter (DM) of aboveground biomass yield (without grain), grain yield and harvest index (HI) for perennial rye, fall rye, spring rye crops and perennial forage at the Edmonton and Breton sites for 2018 and 2019.

Crop + Fertilization	Grain Yield (kg DM Ha^{-1})		Biomass Yield (aboveground biomass without grain) (kg DM Ha^{-1})		HI (kg grain DM kg^{-1} grain and biomass DM)	
	Year One					
	Breton	Edmonton	Breton	Edmonton	Breton	Edmonton
Perennial rye + N	2810 ± 190 Aa	2170 ± 130 Aa	8370 ± 470 Aa	2980 ± 190 Aa	0.19 ± 0.05 Aa	0.43 ± 0.02 Aa
Perennial rye + 0 N	3190 ± 220 Aa	2450 ± 160 Aa	9880 ± 190 Aa	3550 ± 190 Aa	0.17 ± 0.06 Aa	0.41 ± 0.008 Aa
Fall rye + N	5640 ± 280 Ba	4890 ± 240 Ba	6450 ± 310 ABa	2050 ± 110 Ca	0.31 ± 0.1 ABa	0.71 ± 0.007 Ba
Fall rye + 0 N	5840 ± 180 Ba	5120 ± 320 Ba	6570 ± 420 ABa	2100 ± 150 Ca	0.30 ± 0.1 ABa	0.71 ± 0.005 Ba
Spring rye + N	3520 ± 60 Ca	4030 ± 190 Ba	5960 ± 190 Ba	2480 ± 100 BCa	0.36 ± 0.004 Ba	0.62 ± 0.005 Ca
Spring rye + 0 N	3610 ± 180 Ca	4140 ± 270 Ba	6180 ± 300 Ba	2320 ± 140 BCa	0.36 ± 0.006 Ba	0.64 ± 0.008 Ca
Perennial forage+ N	n.a.		3470 ± 280 Ca	2500 ± 190 ABa	n.a.	
Perennial forage + 0 N			3750 ± 320 Ca	3020 ± 380 ABa		
	Year Two					
Perennial rye + N	860₸	860 ± 40 Aa	4050 ± 720 Aa	6500 ± 370 Aa	₸	0.12 ± 0.01 Aa
Perennial rye + 0 N	500₸	1040 ± 170 Aa	4000 ± 630 Aa	9000 ± 1250 Aa	₸	0.11 ± 0.02 Aa
Fall rye + N	2220 ± 270 Aa	2430 ± 400 Ba	4940 ± 380 Aa	3320 ± 430 Ba	0.31 ± 0.03 Aa	0.39 ± 0.02 Ba
Fall rye + 0 N	1820 ± 170 Ab	2600 ± 150 Ba	4350 ± 410 Aa	3650 ± 140 Ba	0.30 ± 0.02 Aa	0.42 ± 0.01 Ba
Spring rye + N	2870 ± 180 Ba	2070 ± 240 Ba	5870 ± 90 Aa	4400 ± 360 Ca	0.33 ± 0.01 Aa	0.33 ± 0.01 Ca
Spring rye + 0 N	2130 ± 73 Bb	2060 ± 210 Ba	4770 ± 250 Aa	4990 ± 380 Ca	0.31 ± 0.01 Aa	0.29 ± 0.01 Ca
Perennial forage+ N	n.a.		2620 ± 220 Ba	2340 ± 190 Da	n.a.	
Perennial forage + 0 N			2640 ± 260 Ba	2600± 159.0 Da		

₸ Breton – year two perennial rye values are not representative due to sampling bias but are included to demonstrate grain production possibility in the absence of significant weed pressure. Only one replication of grain yield for each fertilizer treatment was possible, thus no standard errors are presented, and perennial rye was not included in statistical analyses.

Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column ($\alpha = 0.05$).

Table 4.4. Yield components for perennial rye, fall rye and spring rye crops for year two at the Edmonton and Breton sites.

Crop + Fertilization	Tiller Count (# per plant)	Kernel Count (# per spike)	Thousand Kernel Weight (g)	Estimated Productive Tillers[δ] (% of total)
			Breton	
Perennial rye + N	8.6 ± 0.5 Aa	28.4 ± 1.9 Aa	31.0[ǂ]	25.3
Perennial rye + 0 N	7.8 ± 0.6 Aa	28.4 ± 1.9 Aa	30.9[ǂ]	17.4
Fall rye + N	5.0 ± 0.1 Ba	33.0 ± 1.1 Ba	30.6 ± 1.9 Aa	50.3
Fall rye + 0 N	6.1 ± 0.9 Ba	33.2 ± 1.0 Ba	34.1 ± 0.6 Aa	35.8
Spring rye + N	3.8 ± 0.1 Ca	36.0 ± 1.1 Ba	30.6 ± 0.7 Aa	61.4
Spring rye + 0 N	3.8 ± 0.1 Ca	35.2 ± 0.9 Ba	30.4 ± 1.3 Aa	47.4
			Edmonton	
Perennial rye + N	17.4 ± 1.2 Aa	28.3 ± 2.8 Aa	27.4 ± 0.3 Aa	12.5
Perennial rye + 0 N	17.3 ± 1.7 Aa	25.8 ± 1.3 Aa	27.7 ± 0.6 Aa	15.2
Fall rye + N	6.4 ± 0.1 Ba	34.0 ± 1.4 Ba	28.5 ± 0.4 Ba	28.4
Fall rye + 0 N	6.4 ± 0.2 Ba	34.7 ± 1.3 Ba	29.6 ± 1.0 Ba	26.4
Spring rye + N	5.6 ± 0.3 Ca	37.0 ± 1.1 Ba	31.3 ± 0.2 Ca	33.9
Spring rye + 0 N	6.1 ± 0.2 Ca	38.0 ± 1.0 Ba	31.9 ± 0.1 Ca	30.5

[ǂ] Breton - year two had minimal grain productivity. Perennial rye values are not included in the statistical analysis as only one replication for each fertilizer treatment was possible, thus no standard errors are presented.

[δ] Estimated productive tillers per plant was calculated as grain productivity (g m^{-2}) / [kernel weight (g) x kernel count (# per spike) x plant count (plants m^{-2})]

Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column ($\alpha = 0.05$).

Table 4.5. Grain and aboveground biomass protein for perennial rye, fall rye, spring rye crops and perennial forage.

Crop + Fertilization	Grain Protein (%)		Biomass Protein (%)		Grain Protein Productivity (kg protein Ha^{-1})		Biomass Protein Productivity (kg protein Ha^{-1})	
	Year One							
	Breton	Edmonton	Breton	Edmonton	Breton	Edmonton	Breton	Edmonton
Perennial rye + N	16.7 ± 0.2 Aa	18.4 ± 0.1 Aa	5.1 ± 0.4 Aa	7.5 ± 0.2 Aa	470 ± 30 Aa	400 ± 20 Aa	430 ± 50 Aa	220 ± 20 Aa
Perennial rye + 0 N	16.7 ± 0.3 Aa	18.4 ± 0.2 Aa	5.0 ± 0.4 Aa	7.5 ± 0.3 Aa	530 ± 30 Aa	450 ± 30 Aa	490 ± 40 Aa	260 ± 20 Aa
Fall rye + N	12.6 ± 0.3 Ba	13.9 ± 0.2 Ba	4.3 ± 0.4 Ca	6.3 ± 0.3 ABa	710 ± 40 Ba	680 ± 30 Ba	270 ± 20 Ba	130 ± 10 Ba
Fall rye + 0 N	12.5 ± 0.2 Ba	13.9 ± 0.2 Ba	4.1 ± 0.2 Ca	6.4 ± 0.3 ABa	730 ± 20 Ba	710 ± 50 Ba	270 ± 14 Ba	130 ± 10 Ba
Spring rye + N	14.5 ± 0.3 Ca	15.1 ± 0.3 Ca	4.6 ± 0.2 ACa	5.5 ± 0.2 Ba	510 ± 10 Aa	610 ± 30 Ba	280 ± 10 Ba	140 ± 10 Ba
Spring rye + 0 N	14.6 ± 0.5 Ca	15.2 ± 0.6 Ca	4.7 ± 0.2 ACa	5.5 ± 0.2 Ba	530 ± 30 Aa	630 ± 60 Ba	290 ± 30 Ba	130 ± 10 Ba
Perennial forage+ N	n.a.		11.7 ± 0.9 Ba	14.6 ± 1.4 Ca	n.a.		400 ± 40 Aa	370 ± 40 Ca
Perennial forage + 0 N			10.2 ± 0.6 Ba	15.5 ± 0.6 Ca			490 ± 40 Aa	470 ± 50 Ca
	Year Two							
Perennial rye + N	16.0₸	14.1 ± 0.2 Aa	5.2 ± 1.3 Aa	5.4 ± 0.2 Aa	140₸	130 ± 10 Aa	230 ± 40 Aa	350 ± 20 Aa
Perennial rye + 0 N	17.2₸	13.7 ± 0.6 Aa	3.9 ± 0.04 Aa	4.9 ± 0.3 Aa	90.0₸	150 ± 30 Aa	160 ± 30 Ab	440 ± 60 Aa
Fall rye + N	13.8 ± 0.5 Aa	13.2 ± 0.7 Ba	6.5 ± 0.4 Aa	5.5 ± 0.3 Aa	300 ± 30 Aa	280 ± 40 Ba	320 ± 10 Ba	180 ± 30 Ba
Fall rye + 0 N	13.7 ± 0.4 Aa	13.1± 0.5 Ba	6.6 ± 0.4 Aa	5.7 ± 0.6 Aa	250 ± 20 Aa	330 ± 20 Ba	280 ± 10 Bb	220 ± 10 Ba
Spring rye + N	13.4 ± 0.1 Ba	12.1 ± 0.3 Ba	5.3 ± 0.3 Aa	4.1 ± 0.3 Aa	370 ± 20 Aa	250 ± 20 Ba	380 ± 10 Ba	230 ± 20 BCa
Spring rye + 0 N	12.8 ± 0.1 Ba	12.3 ± 0.4 Ba	6.0 ± 0.6 Aa	5.5 ± 0.3 Aa	270 ± 10 Aa	250 ± 20 Ba	250 ± 30 Bb	280 ± 30 BCa
Perennial forage+ N	n.a.		12.2 ± 0.9 Ba	13.1 ± 0.8 Ba	n.a.		320 ± 20 Ba	310 ± 20 ACa
Perennial forage + 0 N			11.8 ± 1.4 Ba	12.8 ± 0.3 Ba			320 ± 30 Bb	330 ± 30 ACa

₸ Breton - year two had minimal grain productivity. Perennial rye values are not included in the statistical analysis as only one replication for each fertilizer treatment was possible, thus no standard errors are presented.

Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column (α = 0.05).

Figures

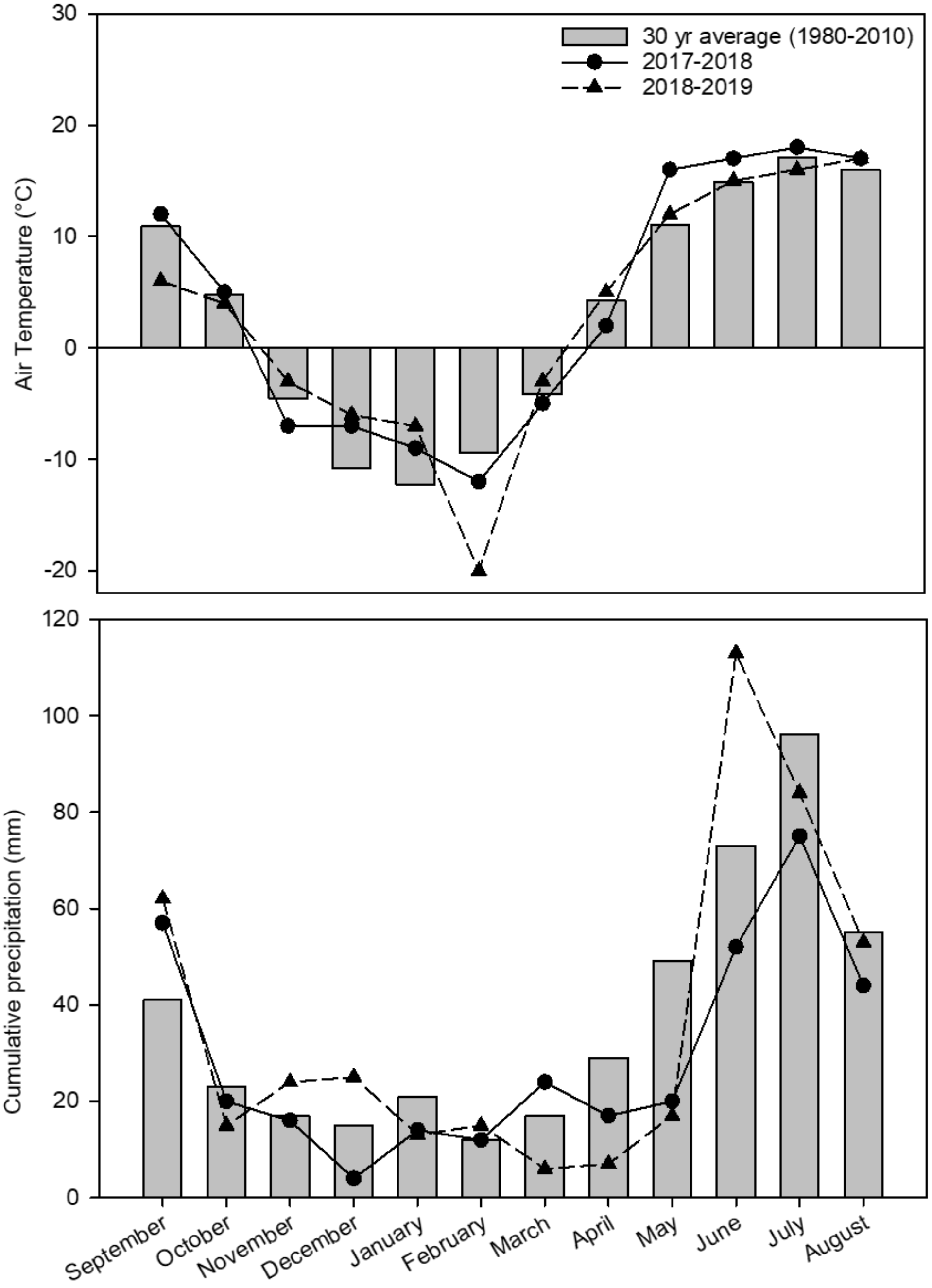

Figure 4.1. Temperature (A) and precipitation (B) obtained from Alberta Information Service (ACIS, 2020) for the Edmonton site for year one (2017-2018) and year two (2018-2019) of the filed experiment.

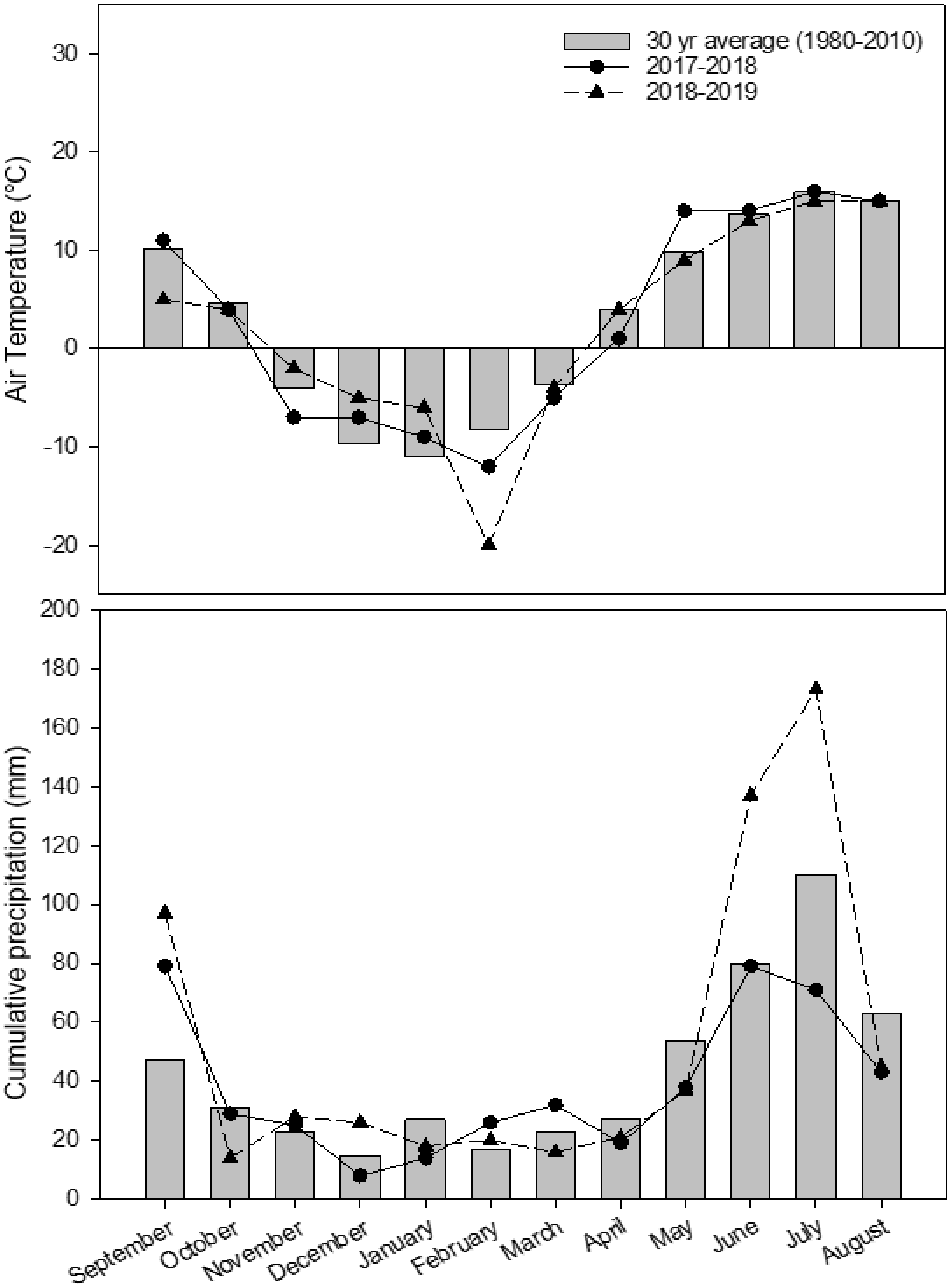

Figure 4.2. Temperature (A) and precipitation (B) obtained from Alberta Information Service (ACIS, 2020) for the Breton site for year one (2017-2018) and year two (2018-2019) of the field experiment.

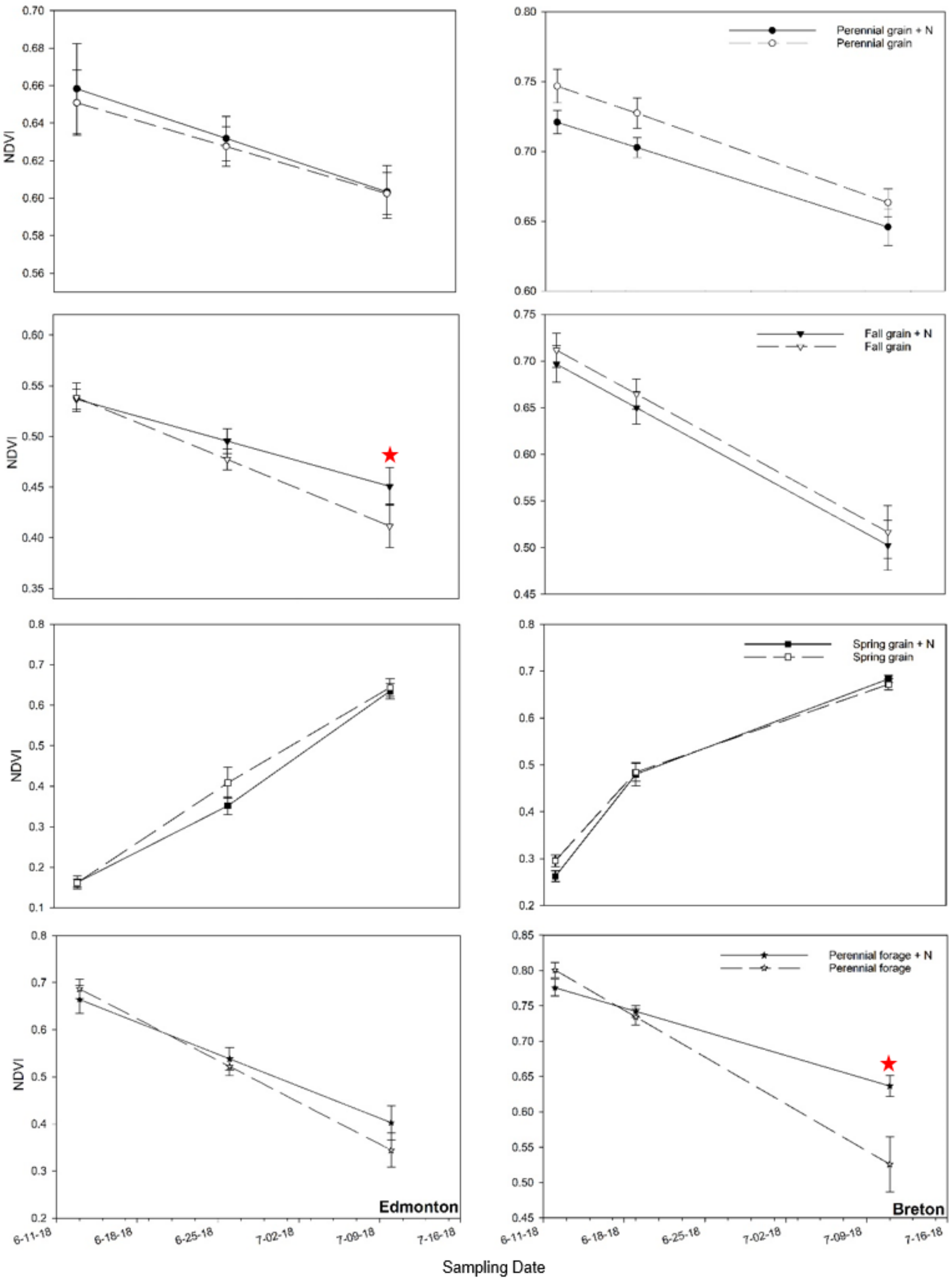

Figure 4.3. Normalized difference vegetation index (NDVI) measurements of perennial rye (circles), fall rye (triangles), spring rye (squares) and perennial forage (stars) at the Edmonton (left) and Breton (right) sites for both fertilized (filled symbols) and unfertilized (unfilled symbols) treatments in 2018. Red stars indicate significant differences between the fertilized and unfertilized counterparts of each crop type based on a Welch's Two Sample T-Test (α = 0.05). Note the different y-axis scales across panels.

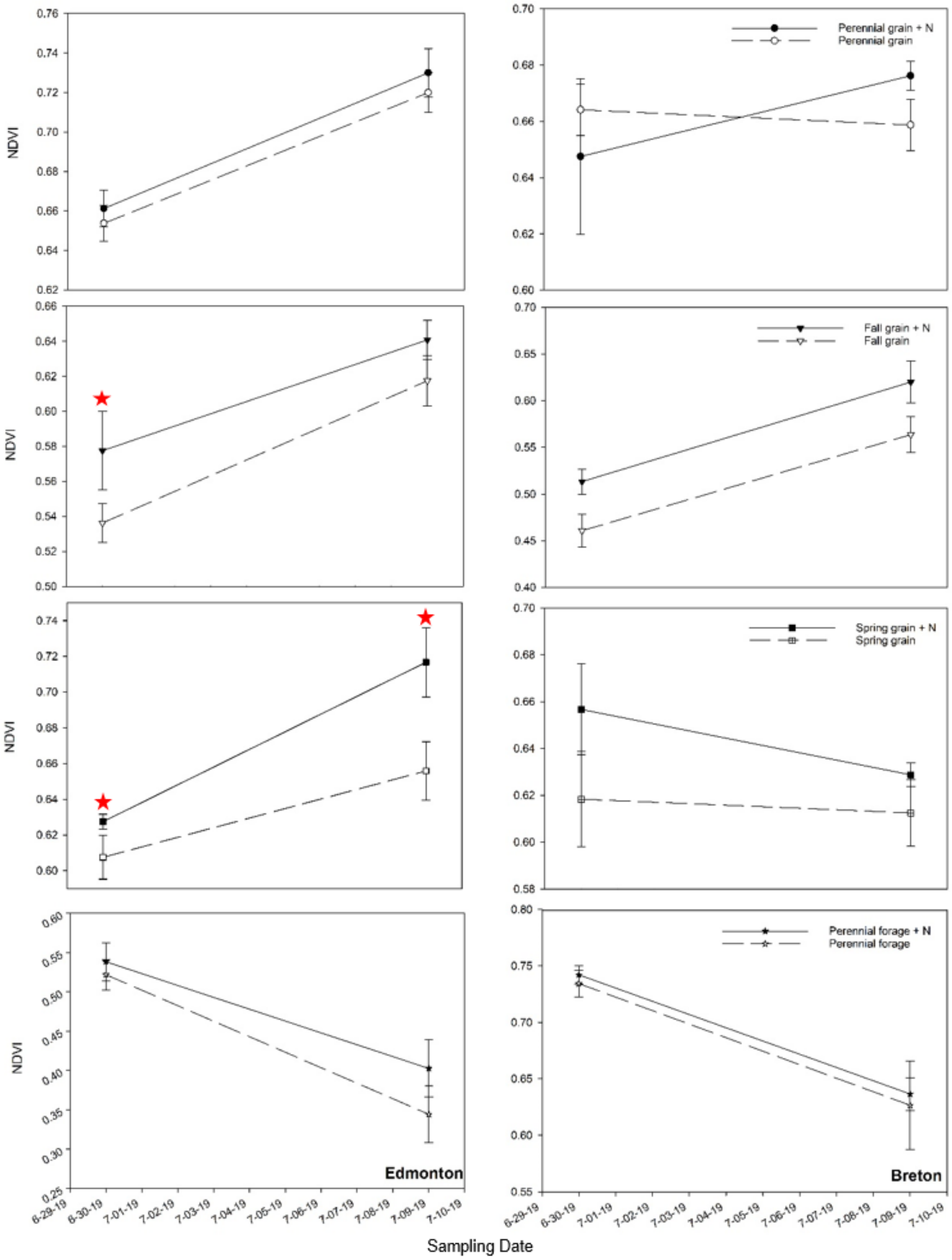

Figure 4.4. Normalized difference vegetation index (NDVI) measurements of perennial rye (circles), fall rye (triangles), spring rye (squares) and perennial forage (stars) at the Edmonton (left) and Breton (right) sites for both fertilized (filled symbols) and unfertilized (unfilled symbols) treatments in 2019. Red stars indicate significant differences between the fertilized and unfertilized counterparts of each crop type based on a Welch's Two Sample T-Test (α = 0.05).

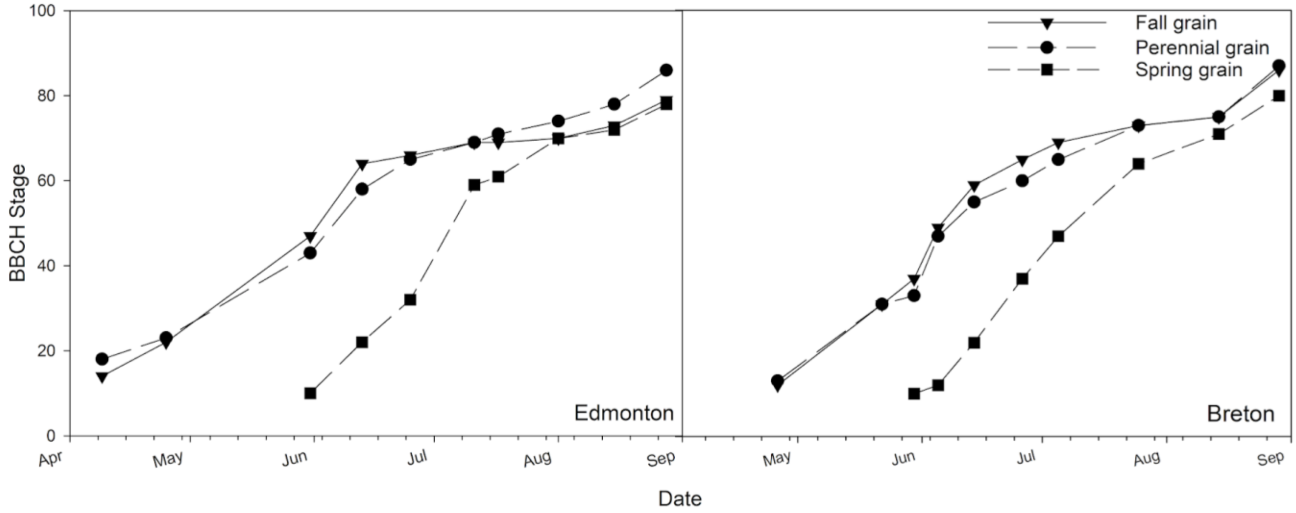

Figure 4.5. Perennial rye, fall rye and spring rye staging based on the BBCH staging manual for year two (2019) for the Edmonton (left) and Breton (right) sites.

5 The response of soil physical quality parameters to a perennial grain crop

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5.1 Highlights

- The perennial rye crop (*Secale cereale* L. x *S. montanum* L.) increased soil C and N compared to an annual rye crop (*S. cereale L*.)
- Alfalfa-brome forage improved soil macroporosity and effective porosity.
- Root mass density was positively correlated with total porosity and macroporosity.

5.2 Abstract

Soil physical quality is paramount for root growth, water, and air movement, and for its subsequent effects on chemical and biological processes in the soil. Management practices and their legacies can impact soil physical quality, and perennial grain cropping has been proposed as a solution to maintain or improve soil physical quality in agroecosystems due to their provision of year-round ground cover and increased root growth. An alfalfa-brome perennial forage crop, a perennial rye crop (*Secale cereale* L. x *S. montanum* L.), and an annual rye crop (*S. cereale* L.) were evaluated at two sites in Central Alberta with contrasting management histories (Edmonton and Breton) over 3 years to determine the effects on soil physical and hydraulic properties. Compared to the annual crop, the perennial forage crop reduced the bulk density of the uppermost soil depth sampled (5-10 cm depth increment) ($p < 0.05$) at the Edmonton site and increased soil macroporosity ($p < 0.05$) and pore connectivity ($p < 0.05$) in the deeper subsurface soil layer (25-30 cm depth increment) at both sites. While moderate improvements in soil physical and hydraulic properties manifested under the perennial rye crop when compared to the annual rye crop, they did not do so to the extent of the perennial forage crop. We attribute this to the inclusion of tap-rooted alfalfa in the perennial forage, and the overarching beneficial influence of root mass density on soil properties. Root mass density from highest to lowest consistently ranked as perennial forage > perennial rye > annual rye for both sites. Root mass density was negatively correlated with bulk density at both Breton ($r = -0.77$, $p < 0.05$) and Edmonton ($r = -0.69$, $p < 0.05$) sites. Furthermore, at Breton, root mass density positively correlated with macroporosity ($r = 0.88$, $p < 0.01$). Notably, the perennial rye crop enhanced soil carbon mass density relative to the annual rye crop in the clayey topsoil of the Edmonton site ($p < 0.05$), but treatment effects were muted at the Breton site due to the influence of previous land use. Despite moderate improvements in soil physical quality, our results suggest that 3 years of perennial rye monocropping falls short of the major improvements seen under a perennial alfalfa-brome forage crop over the same timeframe.

Keywords: perennial grain, perennial forage, soil quality, soil hydraulic properties, HYPROP®

5.3 Introduction

Landscape conversion from native grasslands to agricultural use has contributed to increased erosion, compaction, and disturbance of the soil physical structure (Abid and Lal, 2008; Crews and Rumsey, 2017). Additionally, conversion from perennial systems to annual croplands results in reductions of belowground plant biomass and decreases in soil carbon and aggregate stability, even in no till agricultural systems (DuPont et al., 2014; Milne and Haynes, 2004). The roots of perennial plants are denser, longer-lived, and extend deeper in the soil profile than their annual counterparts and are often linked with improved soil physical properties (DuPont et al., 2014). Land management practices such as tillage and frequent equipment traffic in annual agricultural systems can also adversely modify pore structure and subsequent air and water movement in soils, compared to perennial counterparts (Hebb et al., 2017; Kiani et al., 2017). In sum, the detrimental impacts on soil quality from perennial grassland conversion to annual agriculture can be considered ecosystem disservices and are a consequence of unsustainable soil management practices.

However, soil quality is intrinsically linked to sustainability and land productivity. Abundant literature has stressed the importance of improving the soil quality of agricultural land, with the aim of enhancing soil carbon sequestration, disease suppression, and water filtration (Lal, 2016; Palm et al., 2014; Powlson et al., 2011). Previous research has shown moderate improvements in soil quality under agricultural production with the implementation of beneficial management practices such as reduced tillage (Krauss et al., 2020; Six et al., 2000), diverse crop rotations (Karlen et al., 2006; Kiani et al., 2017), and cover cropping (Adetunji et al., 2020; Mbuthia et al., 2015). Moreover, restoration of agricultural land to perennial grassland has shown significant improvements in soil quality metrics such as bulk density, aggregate stability, soil carbon and erodibility (Cui et al., 2019; Milne and Haynes, 2004; O'Brien and Jastrow, 2013; Rosenzweig et al., 2016). However, the combined effects of global population growth and changing diets is increasing the demands placed on agriculture to supply food for human consumption, animal feed, and fuel (Alexander et al., 2015). Therefore, widespread restoration of agricultural land to perennial grassland systems is an unrealistic solution. The recent development of perennial grain breeding programs has introduced the possibility for the inclusion of a perennial grain crop phase into traditional annual crop rotations to support the transition to multifunctional agroecological systems that may impart the benefits of a perennial grassland onto an annual crop-dominated agricultural system.

Perennial grain crops are novel amalgam of annually harvested grain crops and perennial grasses, which are seeded once and harvested for multiple subsequent seasons, thus minimizing tillage and equipment traffic. Additionally, increased root density in perennial grain systems has been consistently reported (Duchene et al., 2020; Kim et al., 2021; Sprunger, 2018). Therefore, in addition to their ability to be used as dual-purpose forage and grain crops (Daly et al., 2022; Zimbric et al., 2021), they may present an opportunity to restore ecosystem services provided by perennial grasslands while maintaining profitability, a key driver in management decisions (Ryan et al., 2018). Because perennial grain crops are in the early stages of development, perennial grain yields tend to be lower than annual grain yields; therefore, soil quality improvements will be a key adoption incentive to offset reduced revenue (Bell et al., 2008; Daly et al., 2022). Previous research into perennial grain systems has shown improvements in soil properties relative to annual croplands (Culman et al., 2013; Duchene et al., 2020; Su et al., 2009). However, it is unclear if soil quality will improve under a perennial grain crop that is seeded as a monocrop and only survives 2-3 years in the harsh winter conditions of Central Alberta, as opposed to the diverse plant assemblages of perennial grasslands adapted to northern prairie ecosystems (Cattani, 2019; Daly et al., 2022). For this study, we selected perennial rye [cv. ACE-1] as the model perennial grain crop, based on preliminary findings from Lethbridge, Alberta, which reported superior winter hardiness of perennial rye relative to several perennial wheat cultivars (Hayes et al., 2018). The perennial rye cultivar selected for this study was developed via wide hybridization, a process wherein a wild perennial [perennial wild rye (*Secale montanum* L.)] is crossed with a compatible annual grain [rye (*Secale cereale* L.)] (Acharya et al., 2004; Daly et al., 2022; Reimann-Philipp, 1995).

Previous land management practices and their legacy effects may play a pivotal role in the ability of perennial grain crops to improve or maintain soil quality. The soil legacy concept describes the persistent consequences of previous management on soil biotic and abiotic properties (Jing et al., 2022). Upon establishment of a perennial grain crop, soils that were previously managed as a perennial system may benefit from legacy effects that promote biopore reuse, food web stability, and continued protection of stored soil organic carbon (Or et al., 2021). Indeed, the beneficial legacy effects of a perennial system may persist several years into the establishment of a conventionally tilled annual cropping system, beyond the expected lifespan of a perennial grain crop (Or et al., 2021; Keller et al., 2021). Conversely, annual systems can impart detrimental legacy effects such as compaction and reduced microbial community diversity and function that may not recover after several growing seasons of improved management practices (Keller et al., 2021; Kim et al., 2021; Longepierre et al., 2021). Therefore, examining how contrasting land use histories respond after conversion to a perennial grain cropping system can inform how soil management history can influence soil quality recovery rates (Keller et al., 2021).

Soil quality metrics encompassing soil physical and hydraulic properties have proven sensitive to management effects and can act as useful metrics to measure soil quality changes in contrasting land management regimes (Hebb et al., 2017; Kiani et al., 2017; Guenette et al., 2019). Soil physical quality has been referred to as the foundation of overall soil quality, due to its subsequent effects on soil chemical and biological processes (Li et al., 2011; Xu et al., 2017). As such, insightful indicators of soil physical quality are those related to soil structure, porosity, and associated functions and processes such as water movement and air exchange including bulk density, total porosity, pore volume fractions, and hydraulic conductivity (Hebb et al., 2017; Jiang et al., 2018; Kalu et al., 2021; Reynolds et al., 2009, Shahab et al., 2013). Additionally, the S-index as proposed by Dexter (2004) is a measure of soil physical quality derived from the slope of the moisture retention curve at the inflection point. The usefulness of this metric has been reported in literature to illustrate contrasting soil quality between different land use choices and cropping systems (Czyz and Dexter, 2009; Guenette et al., 2019; Hebb et al., 2017; Kiani et al., 2017).

Consequently, there is a knowledge gap regarding the ability for a perennial rye crop to make meaningful improvements in soil physical quality over their 3-year lifespan. We hypothesize that as a hybrid of an annual grain and a perennial grass, the perennial rye will act as an intermediate between a perennial forage and an annual rye, imparting moderate improvements to soil physical quality relative to an annual rye system, but not to the extent of a perennial forage crop. Additionally, we hypothesize that different land use histories will affect the response of soil physical and hydraulic properties to short-term perennial rye cropping; namely that soil previously managed as a perennial system may maintain a high level of soil physical quality when converted to a perennial grain system due to legacy effects. Accordingly, specific objectives of this study were to: i). determine the effects of perennial rye, annual rye, and perennial forage on soil physical and hydraulic properties in a soil with a history of perennial forage cropping versus a soil with a history of recurrent tillage and annual grain cropping, and ii). relate potential differences in physical and hydraulic properties to differences in root mass density, soil carbon and nitrogen storage, and management legacy between cropping systems upon completion of one perennial rye life cycle. This is the first study of its kind to assess the effects of perennial rye on soil physical and hydraulic properties with contrasting land management histories and can be used as a baseline to inform beneficial management practices that restore ecosystem services and improve agricultural sustainability.

5.4 Materials and methods

5.4.1 Study sites

Field sites were established within Alberta, Canada in Edmonton (53° 29' 43.33", 113° 31' 59.24") and Breton (53° 5' 16.72", 114° 26' 29.35") in August 2017. Soil at the Edmonton site is classified as Black Chernozem and has a long-term management history of continuous annual barley for approximately 20 years prior to experiment establishment. Tillage was conducted yearly at this site to prepare the seedbed for spring barley seeding. Soil at the Breton site is classified as Grey Luvisol and was managed for hay forage production for at least 60 years prior to the experiment. Tillage was not utilized at this site until experiment establishment. Detailed baseline soil properties for each site are presented in Table 5.1.

5.4.2 Experimental Design and Management

Experimental sites were arranged in identical randomized complete block designs consisting of four block replicates and established in June 2017 by rotary tilling the entire experimental area for plot preparation. After this initial tillage, only the annual rye treatments were subjected to yearly rotary tillage immediately prior to seeding. Each experimental plot measured 32 m^2 (8 m length × 4 m width). For this study, the three treatments of focus were two analogous grain cultivars: perennial [ACE-1 rye (*Secale cereale L.* × *S. montanum Guss]* and annual [Gazelle rye (*S. cereale L.*)], as well as a perennial forage [(meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa* var. 4010 BR)], henceforth referred to as perennial AB.

The perennial and annual rye treatments were seeded with a 22.9 cm row spacing to a depth of 2.5 cm. Perennial AB treatments were broadcast seeded at a rate of 55 kg ha^{-1} for meadow brome and 4 kg ha^{-1} for alfalfa, then incorporated. Each crop received yearly applications of a urea-ESN blend (2:1 ratio) at 56 kg N ha^{-1}. For all rye treatments, 15 kg of phosphorus per ha in the form of phosphate was placed with the seed. The perennial AB plots were harvested twice per season for forage and the rye treatments were harvest once per season for grain using a small forage harvester. Due to poor regrowth of the perennial rye at the Edmonton site in 2020, the experiment was concluded at this site after soil sample collection in May 2020. Detailed management activities for each site are summarized in Supplementary Table S5.1.

5.4.3 Soil and root sample collection

Undisturbed soil cores for soil physical and hydraulic property analysis were collected from the perennial AB, perennial rye, and annual rye treatments from three depth increments: 5-10 cm, 15-20 cm, and 25-30 cm in May (Edmonton) and July (Breton) of 2020 by hammering a cylindrical stainless-steel core (80 mm i.d.) into the soil using a hammer holder designed to fit the steel cores and a rubber mallet. These depth increments were chosen to provide an overview of soil physical and hydraulic properties with increasing depth; however, it is noted that they do not provide a continuous profile from 0-30 cm. Specifically, the first 5 cm was not sampled to avoid litter, undecomposed organic matter, and surface crusts that may mask treatment effects (Hebb et al., 2017). Cores were taken from the soil near the center of the plots to avoid edge effects. In the rye treatments, cores were sampled immediately adjacent to a crop row, whereas core locations were randomly selected for the perennial AB treatment. Cores were excavated with a shovel, leveled off using a soil knife and sealed with a plastic cap at each end to prevent sample loss and drying. Two replicate cores were taken for each depth increment in each plot and averaged to prevent pseudo-replication, for a total of 72 cores per site. Cores were stored at 4°C for up to 3 months until analysis.

Soils for total carbon (TC) and total nitrogen (TN) analysis were sampled using a truck-mounted auger from 0-30 cm prior to experiment establishment in August 2017, and again in May and July 2020 (Edmonton and Breton sites, respectively). Roots were sampled at both sites in June 2018 and 2019 by obtaining undisturbed soil cores from the 0 to 30 cm depth increment (5.7 cm diameter, n = 4 per plot). Root separation from soil was done by wet sieving (>180 μm mesh size) followed by oven drying at 60 °C for a minimum of 48 hrs to quantify total dry root mass (Kim et al., 2021; Hernandez-Ramirez et al., 2014).

5.4.4 Soil analyses and calculations

Soil physical and hydraulic properties were obtained using a HYPROP ® instrument system (*Meter Environment, Munich, Germany*) using the simple evaporation method (Schindler and Müller, 2017) in combination with WP4 potentiometer® dewpoint method, for the very dry range (*Decagon Devices, Pullman, USA)*. The HYPROP ® instrument system has been effectively used to characterize soil water characteristics for various soil classifications (Bezerra-Coelho et al., 2018; Guenette et al., 2019;

Hebb et al., 2017; Kiani et al., 2017) and has the advantage of providing a large amount of comparatively accurate and continuous data in a short time frame.

In the lab, soil cores were saturated, and 2 holes (3.75 and 1.25 cm in depth) were augured into each core to insert ceramic-tipped tensiometers attached to a pressure transducer base, which measures matric potential from 0 to -100 kPa. Bases were interfaced with a computer, which recorded tension measurements in 10-minute intervals. Upon completion of the HYPROP ® measurements, subsamples were run through a WP4 dewpoint tensiometer according to the Operator's Manual V2 (Decagon Devices, 2010).

Data was analyzed using the HYPROP-FIT ® software, which uses measured data values and manually input supplemental WP4® data points to fit the constrained van Genuchten model (van Genuchten, 1980) for moisture retention as follows:

$$\theta = \theta_r + \frac{(\theta_{s-}\theta_r)}{[1+(\alpha h)^n]^m} \tag{5.1}$$

Where: θ is the water content (cm^3 cm^{-3}), θ_r is the residual water content (cm^3 cm^{-3}), θ_s is the saturated water content (cm^3 cm^{-3}), α is the inverse of the air entry potential (kPa^{-1}), h is the matric potential (kPa), and n and m are shape parameters. It is noted that saturated water content (θ_s) was interpreted as effective porosity (EP), as this represents the volume of pores that water can occupy; in other words, this indicates the soil porosity excluding occluded pores.

Pore volume fractions were calculated using the relationship between points on the water retention curve (kPa) and pore diameters (μm) as follows: macro (0 to -5 kPa, >60 μm), meso (-5 to -33 kPa, 60-9 μm), micro (-33 to -50 kPa, 9-6 μm) and residual (< -50 kPa, < 6 μm) as in Hernandez-Ramirez et al. (2014) and Guenette et al. (2019). The effective porosity (EP) and field capacity (FC) of the soil were estimated from each water retention curve at tensions of 0 and −33 kPa, respectively. Unsaturated hydraulic conductivity (UHC) classes were analyzed similarly, with large, medium, and small hydraulic conductivity classes corresponding to −1 to −10 kPa, −10 to −20 kPa, and −20 to −33 kPa tensions, respectively (Guenette et al., 2019).

The S-index, which is a measure of soil physical quality and is the slope of the moisture retention curve at its inflection point, was calculated from the fitted van Genuchten θ(h) function (Eq. (1)) via Dexter (2004) as follows:

$$S = -n\,(\theta_s - \theta_r)\left(\frac{2n-1}{n-1}\right)^{\left(\frac{1}{n}-2\right)} \qquad [5.2]$$

Soil dry bulk density (BD) was calculated from the stainless-steel cores with a known volume (250 cm^3) using soil weights determined after oven drying the sample at 100°C for at least 48 hr. Soil total porosity (TP) was estimated using BD values, assuming a soil particle density of 2.65 g cm^{-3}. The soil TC and TN concentrations were analyzed via dry combustion in an Elemental Analyzer (*Thermo Fisher Scientific, Delft, Netherlands*), then converted to mass densities using BD. Soils were tested for the presence of carbonate-C by subtracting total organic C from TC. Edmonton soils contained <10% TC as carbonate-C, and Breton soils contained <5% TC as carbonate-C. Soil pH was measured using a 1:5 soil:water slurry with a pH meter. Soil texture was determined via the hydrometer method.

5.4.5 Statistical analysis

All statistical analyses were performed with R Studio software version 4.0.3 (R Core Team, 2021) with an alpha critical value of 0.05. Assumptions of normality and homoscedasticity were checked by the Shapiro-Wilk and Levene tests, respectively, and data transformations including logarithmic, square root or Box-Cox were utilized when needed to meet assumptions. Each depth was analyzed separately. One-way analysis of variance (ANOVA) was performed on linear models developed for all measured variables, except when transformation did not correct heteroscedasticity and Welch's ANOVA was performed to account for unequal variance (Welch, 1951). Post hoc investigation was completed after significant ($p < 0.05$) ANOVA using Tukey's Honest Significant Difference test for comparison of treatment means using the Agricolae package (de Mendiburu, 2020). Correlation analyses were conducted using the rcorr function in the Hmisc package in R, which computes a matrix of Pearson's r for all possible pairs in a matrix (Harrell, 2021).

5.5 Results

5.5.1 Bulk density and total porosity

The BD from 5-10 cm at the Edmonton site was reduced in the perennial AB treatment compared to the perennial and annual rye treatments, which did not differ from one another ($p < 0.05$). Statistically significant differences were not detected between treatments in the other depths; however, annual rye BD was consistently elevated relative to the perennial rye and AB treatments for the 15-20 and 25-30 cm depth increments.

Related to BD, TP in the 5-10 depth increment also differed between treatments. Perennial AB TP was greater than the TP of the perennial rye ($p < 0.05$) (Table 5.2). Neither the perennial AB nor the perennial rye differed from the annual rye, which had an intermediate TP. Mirroring the BD findings, perennial rye and perennial AB generally had numerical increases in TP relative to the annual rye for the 15-20 and 25-30 cm soil depth increments, respectively (Table 5.2).

At the Breton site in the 5-10 cm and 25-30 soil depth increments, BD from highest to lowest ranked as follows: annual rye > perennial rye > perennial AB, however the differences were not significant. Similarly, the trend in the 15-20 cm depth was annual rye > perennial rye = perennial AB. The TP for all depths trended from highest to lowest as follows: perennial AB > perennial rye > annual rye, but the treatments did not statistically differ from one another (Table 5.3).

5.5.2 Effective porosity and field capacity

Differences in EP materialized at the Edmonton site in the 25-30 cm depth increment. The EP represents connected soil porosity that contributes to fluid flow and is determined from the water retention curve when tension corresponds to 0 kPa (i.e., soil cores at water saturation). The perennial AB treatment had greater EP than the annual rye but did not differ from the perennial rye ($p < 0.05$) (Table

5.2). On average, perennial treatments increased EP in the 25-30 cm soil depth increment. Conversely, differences in FC were evident only at the 5-10 cm soil depth increment. The annual rye treatment had the highest FC, which was higher than the perennial AB but did not differ from the perennial rye ($p < 0.001$).

Similarly, significant differences in EP were only evident in the 25-30 cm soil depth increment at the Breton site. Perennial AB had higher EP than the annual rye but did not differ from the perennial rye treatment ($p < 0.01$). No differences in FC between any treatments were discerned for any soil layer at the Breton site (Table 5.3).

5.5.3 Pore volume fractions

Edmonton showed changes in pore size distribution between treatments for all depths (Fig. 5.1). Macroporosity trends at the Edmonton site were consistent for the 5-10 and 15-20 cm depth increments from highest to lowest: perennial AB > perennial rye > annual rye. Only the 25-30 cm depth showed statistically significant differences between treatments; perennial AB had increased macroporosity compared to the perennial and annual rye treatments, which did not differ from one another ($p < 0.05$) (Table 5.2).

No trends or significant differences were found between treatments for mesoporosity or microporosity at any depth, except that the perennial AB treatment was generally highest. Residual porosity was greater in the annual and perennial rye treatments than the perennial AB treatment in the 15-20 cm depth increment ($p < 0.05$) (Table 5.2).

Changes in pore size distribution also manifested at the Breton site (Fig. 5.1). Macroporosity from 5-10 cm was highest in the perennial AB and lowest in the annual rye treatment, but no treatment differed significantly from one another. Within the 15-20 cm depth increment, perennial AB had the greatest macroporosity, which was significantly greater than the annual rye ($p < 0.05$). Perennial rye did not differ from either treatment. From 25-30 cm, macroporosity was greater in the perennial AB treatment than both the perennial and annual rye treatments ($p < 0.001$) (Table 5.3).

Differences in mesoporosity were evident at the 5-10 cm depth. Perennial rye had significantly greater mesoporosity than annual rye ($p < 0.05$). Perennial AB did not differ from either the perennial rye or the annual rye. Differences in mesoporosity between treatments did not materialize for the other depth increments (15-20 and 25-30 cm). No differences were detected for microporosity or residual porosity in any depth increment (Table 5.3).

5.5.4 Unsaturated hydraulic conductivity

Differences in large UHC were detected in the 25-30 cm depth increment at the Edmonton site. Perennial AB had greater large hydraulic conductivity than annual rye ($p < 0.05$). Perennial rye acted as an intermediate and did not differ from either the perennial AB or the annual rye. No differences were evident in the medium or small UHC (Table 5.2).

Similar to the Edmonton site, differences in large UHC materialized in the 25-30 cm depth increment at the Breton site; however, the pattern differed. The perennial rye treatment had greater large UHC than the perennial AB ($p < 0.05$). And the annual rye did not differ from the perennial rye or perennial AB (Table 5.3).

5.5.5 S-index

At the Edmonton site, significant differences in the S-index materialized at the 25-30 cm soil depth increment, where the perennial AB treatment > perennial rye = annual rye. Notably, despite significance not being found at the other soil depths, the S-index for perennial AB was consistently greater than that of the annual rye treatment (Fig. 5.2, Table 5.2). No trends or significant differences materialized at the Breton site; however, all S-index values at the Breton site were much higher than those at the Edmonton site overall.

5.5.6 Root density

Root mass density at the Edmonton site in 2018 and 2019 was consistently higher in the perennial treatments relative to the annual rye. Root density trends from highest to lowest were consistently:

perennial AB > perennial rye > annual rye (Table 5.4). Even when the sum of root density for the 2018 and 2019 samplings for annual rye was compared to the 2-year-old perennial treatments in 2019 [i.e., 2018 (YR 1) + 2019 (YR 2) vs. 2019 (YR 2)], the cumulative annual rye root density was less than the perennial AB for all soil depth increments, and less than the perennial rye for the 15-30 cm depth increment ($p < 0.001$) (Fig. 5.3).

A similar trend was observable at the Breton site. For each depth increment (0-15 and 15-30), perennial AB had increased root mass density relative to the annual rye treatment. Perennial rye acted as an intermediate, with greater root density than the annual rye but lower than the perennial AB (Table 5.4). Again, when the sum of annual rye root density for 2018 and 2019 was compared against the root growth of the 2-year-old perennial treatments, annual rye had consistently reduced root mass density compared to the perennial AB for all depths. However, the root density of the 2-year-old perennial rye did not differ from the cumulative annual rye root density at this site (Fig. 5.3).

5.5.7 Soil carbon and nitrogen

Mass densities of TC and TN were significantly greater in the perennial rye relative to the annual rye treatment at the Edmonton site in the 0-15 cm depth increment ($p < 0.01$ and $p < 0.01$, respectively) (Table 5.6). The 15-30 cm depth increment lacked statistical significance, but on average, the perennial treatments increased TC and TN relative to the annual rye. The TC and TN trended as follows: perennial rye > perennial AB > annual rye. At the Breton site, no differences in TC or TN were found for either soil depth increment and no discernible trends were noted.

5.5.8 Correlation analyses

Significant correlations of independently measured variables are detailed below. Correlations between variables derived from the fitted Van Genuchten model are not presented, as they are not independent from one another. At the Edmonton site in the 5-10 cm soil depth increment, root mass density from 0-15 cm in 2018 was significantly correlated with BD ($r = -0.69$, $p < 0.05$) and consequently

TP ($r = 0.68$, $p < 0.05$). In the 15-20 cm soil depth increment, root density from 0-15 cm in 2019 was negatively correlated with residual porosity and positively with the S-index ($r = -0.90$, $p < 0.001$ and $r = 0.68$, $p < 0.05$).

At the Breton site, root density from 0-15 cm in 2019 was strongly positively correlated with macroporosity in the 5-10 cm soil depth increment ($r = 0.88$, $p < 0.01$). In the 15-20 cm soil depth increment, root density from 15-30 cm in 2019 was negatively correlated with soil BD ($r = -0.77$, $p < 0.05$), and positively with macroporosity ($r = 0.65$, $p < 0.05$). Interestingly, TC was also correlated with BD, TP and macroporosity at this soil depth ($r = -0.62$, $p < 0.05$; $r = 0.53$, $p <0.05$ and $r = 0.59$, $p <0.05$, respectively). Linear regressions of TC with BD, TP and macroporosity highlight these relationships (Figs. 5.4A-C). In the 25-30 cm soil depth increment, root density from 15-30 cm in 2019 correlated with macroporosity ($r = 0.76$, $p <0.05$) and EP ($r = 0.60$, $p < 0.05$). Again, linear regressions emphasize the significant relationships between root density and macroporosity as well as root density and EP (Fig. 5.5A, Fig. 5.5B).

5.6 Discussion

5.6.1 Cropping system effects on soil physical and hydraulic properties

Differences in soil physical properties were evident three years after treatment establishment at the Edmonton and Breton sites. At the Edmonton site, the perennial AB treatment improved soil quality compared to the annual rye, as evidenced by increased TP and reduced BD, namely in the 5-10 cm depth increment (Table 5.2). Increases in TP can be partially attributed to enhanced macroporosity (i.e., pore diameter > 60 μm) under the perennial AB, presumably due to the greater root density, which is supported by the strong positive correlation found between root density and TP, and negative correlation between root density and soil BD at this depth. Perennial root systems have been shown to increase macroporosity compared to soils under annual vegetation, which is key for promoting water movement and aeration, as well as facilitating future root growth (Marshall et al., 2016; Udawatta et al., 2006; Uteau et al., 2013).

Indeed, McCallum et al. (2004) reported an increase in pores > 4 mm after 4 years of perennial pasture and attributed this increase to both the density and structure of the perennial roots.

Interestingly, despite elevated root density in the perennial rye treatment relative to the annual rye in the 5-10 cm depth increment, reductions in BD were not measured in the perennial rye treatment (Table 5.2). Although perennial rye had greater root density than the annual rye treatment, the perennial AB had 4x the root density of the perennial rye treatment from 0-15 cm. Notably, conversion to no till has been shown to increase BD and negatively impact the structure of clayey soils in the short term (< 6 years after initiation of no till) (Holthusen et al., 2018; Grant and Lafond, 1993; Li et al., 2020). Thus, in the clay soil of the Edmonton site, the increased root mass and activity of the perennial rye relative to the annual rye may be preventing the short-term effect of no till increasing BD, but not to the extent of the exceptionally densely-rooted perennial AB treatment, which developed significantly lowered BD relative to the annual rye (Table 5.4).

The effects on BD due to the cessation of tillage in the perennial treatments at the Edmonton site may be counteracted by increased aggregation. A global meta-analysis by Mondal and Chakraborty (2022) found that after the implementation of no till, macroaggregates increased by up to 31% <10 years after conversion, even when BD increased. Increasing macroaggregates can serve as a protective mechanism for soil organic C accrual, as evidenced by the increased C storage in the 0-15 cm soil depth increment in the perennial rye at the Edmonton site relative to the annual rye (Table 5.6). In turn, soil C increases under no till have been shown to improve soil's ability to resist compaction and reduce maximum BD; the underlying mechanism being the low density, high specific surface area, and significant water absorbency of soil organic matter (Blanco-Canqui et al., 2009; Soane, 1990).

Significantly increased soil C under the perennial rye at the Edmonton site may be a consequence of the increased aboveground biomass of the perennial rye relative to the perennial AB. Namely, in 2019, the perennial rye produced 213% more aboveground biomass than the perennial AB, on average (Daly et al., 2022). Moreover, Kim et al. (2022) reported that high clay soils, such as those at the Edmonton site, had greater C accumulation due to increased mineral-associated organic matter under a perennial rye crop compared to an annual rye crop. Conversely, soils at the Breton site are much lower in clay and were subjected to land use change from a perennial hay stand only three years prior. Declines in soil C stocks related to land use change may take decades to reach a new equilibrium, therefore the legacy effects of the previous management may be masking treatment effects in terms of soil C stocks at the Breton site (Oberholzer et al., 2014; Poeplau et al., 2011). In fact, soil C loss was found to be highest 30-50 years after conversion from pasture to agricultural cropping in a meta-analysis by Guo and Gifford (2002).

Of note, soil C accruals in no till systems can misleadingly be the result of a redistribution of C to the uppermost soil layers and comparisons to tilled systems should be based on samples taken beyond the deepest tillage depth (Gal et al., 2007). In our experiment, rotary tillage was conducted to approximately 8 cm in the annual rye treatment, and therefore soil samples for C analysis were obtained to a depth of 60 cm. However, the duration required to ensure the differences in soil C accrual between different management practices are significantly detectable can range from 8-100 years due to the high spatial variability of soil, and therefore, while we can identify significant preliminary trends, continued monitoring will be required to strengthen these findings (Necpalova et al., 2014).

At the Breton site, differences manifested in the 5-10 cm and 15-20 cm depth increments in terms of reallocation of pore size fractions in both perennial treatments relative to the annual, without significant differences in overall TP or BD (Table 5.3). Daynes et al. (2013) reported that TP can remain unchanged despite shifts and rearrangements in pore size distributions due to the regulatory activities of plant root growth. Increased mesoporosity in the 5-10 cm depth increment in the perennial treatments relative to the annual treatment at the Breton site is consistent with Hebb et al. (2017), who found that perennial vegetation such as native grasslands and introduced pastures had increased mesoporosity relative to annual croplands. Notably, mesopores play a key role in the regulation of plant available water, and thus increases in mesoporosity may improve plant available water in soils under perennial rye crops

(Brady and Weil, 2002; Hebb et al., 2017). Conversely, Hebb et al. (2017) found no differences in macroporosity, whereas macroporosity was elevated in both perennial treatments in the 15-20 cm depth at the Breton site. This difference may be attributed to the effect of cattle grazing in Hebb et al. (2017), a factor that was absent in our study. Further, macroporosity was positively correlated with TC in this soil depth increment (Fig. 5.4B), despite only observing trends in increased TC under perennial cropping, but no statistical significance. Therefore, the processes occurring at the Edmonton site related to increased aggregation and subsequent organic C accrual may be less pronounced at the Breton site in part due to the comparatively lower clay content, as clay provides increased surface area and active sites for mineral-organic matter associations (Kim et al., 2022; Poffenbarger et al., 2020).

Differences in macroporosity were also evident in the deepest subsurface soil depth increment in our study (i.e., the 25-30 cm depth increment) at both sites. We hypothesize that this finding is due to the inclusion of alfalfa in the perennial AB mixture. Alfalfa grows deep taproots that can reorient and shift soil pores and increase stable macropore formation in subsurface soil layers more than the fibrous root systems of the rye treatments (i.e., perennial and annual) (Bodner et al., 2014; Cuef et al., 2021; McCallum et al., 2004). In fact, Han et al. (2015) observed differences in macroporosity after only one year of cropping with a tap-rooted species, which the authors attributed to the tap roots' ability to penetrate deeper soil layers. This would be paramount for the Edmonton site, as the soil has 48% clay content (Table 5.1). Increases in EP for the perennial AB and rye treatments were also discernable at this same subsurface soil layer, which is consistent with a review by Strudley et al. (2008). They found that while results under no tillage management can be mixed, under perennial vegetation, no till soils tended to have increased macropore connectivity compared to conventional tillage, in part due to the greater vertical pore connectivity that results from enhanced root growth under perennials (Strudley et al., 2008).

At the Breton site, macroporosity and EP in the 25-30 cm depth increment were strongly positively correlated with root mass density from 15-30 cm in 2019 (Fig. 5.5A, Fig. 5.5B). Potentially, the increased root density in the perennial treatments and inclusion of tap-rooted alfalfa in the perennial AB treatment are causing a parallel effect in Edmonton and Breton. By overriding differences in inherent soil properties and management histories, identical patterns in the findings of EP and macroporosity at both experimental sites emphasize the significant driving role of root growth in perennial systems. Notably, our study did not include alfalfa in the perennial rye treatment, which was seeded as a monoculture, unlike the perennial AB, which was a polyculture of meadow brome and alfalfa. Therefore, soil quality improvements more comparable to those seen under the perennial AB treatment may be an option if a perennial grain polyculture, such as a polyculture of intermediate wheatgrass, alfalfa, and perennial sunflower, is established (Ryan et al., 2018).

Differences in UHC between the three treatments were primarily in the 25-30 cm soil depth increment and paralleled trends in EP and macroporosity at this same layer for the Edmonton site. Water movement is facilitated by large continuous pores, such as those formed by dense perennial root systems that are predominantly vertical in orientation and display high pore connectivity (Holthusen et al., 2018; Iversen et al., 2003). Notably, the opposite was found at the Breton site; UHC was lowest under the perennial AB. This may be due to the timing of field sampling, as hydraulic conductivity in perennial systems can transiently fluctuate based on root decay and regrowth, which typically increases as the growing season progresses. As perennial root systems gradually decay over time, stable pores can form, increasing UHC in perennial systems with deeper, denser root systems (Fuentes et al., 2004; Mitchell et al., 1995). Therefore, differences in the length of the growing season and the time of field sampling can impact UHC findings producing inconsistent results across sites.

Overall, cropping system effects are muted at the Breton site, as evidenced by the shape of the soil moisture retention curves (Figure 5.1). Notably, the soil moisture retention curves at the Breton site show less differentiation between treatments compared to the Edmonton site. Development of soil structure and aggregation are dynamic properties that depend upon soil parent material as well as climate and management factors, therefore it is expected that these two sites, with initial differences in soil organic matter, texture, and contrasting management histories, show different overall responses to the same perennial cropping systems (Strudley et al., 2008). Namely, over 60 years of perennial cropping at the Breton site prior to the establishment of this experiment is likely a major contributing factor to this finding. It is well established that previous land management can impart legacy effects on soil for several years (Or et al., 2021; Keller et al., 2021), therefore, it is possible that the Breton site requires more time than the duration of this experiment for the annual grain treatment to substantially alter soil hydraulic properties. The steeper soil moisture retention curves at the Breton site are indicative of more varied pore size distribution, which indicates that good soil structure was previously developed under long-term perennial hay and was maintained despite three years of tillage and vegetation-free periods under the annual cropping treatment (Cueff et al., 2021; Daynes et al., 2013; Hebb et al., 2017).

As evidenced by the flatter soil moisture retention curves, the clay soil of the Edmonton site likely started from an overall more degraded state due to years of intensive annual cropping previously practiced at this study site, which may be the reason more significant beneficial effects emerged from establishing the perennial AB and perennial rye treatments. Additionally, the increased clay content of the Edmonton site may have promoted aggregation and pore size rearrangement more readily than the Breton site, as increased clay content in soil has been shown to contribute to soil structure recovery by enhanced reactive surfaces promoting organomineral complexation and formation of macroaggregates (Bach et al., 2010; Baer et al., 2010; Tisdall and Oades, 1982).

Comparatively elevated S-index results at the Edmonton site in the 25-30 cm soil depth increment under the perennial AB treatment can in part be explained by increases macroporosity in the corresponding soil layer (Table 5.1, Fig. 5.2). Higher values of S-index are indicative of improved soil structure and are sensitive to management practices, namely soil compaction, which tends to be higher in deeper soil layers due to lower soil organic matter (Dexter, 2004; Holthusen et. al., 2018). In general, an S-index value of 0.035 indicates the boundary between “good” and “poor” soil quality. However, all values at the Edmonton site are below this boundary, which is typical for agricultural soil that has been frequently tilled over decades and continuously cropped to shallow rooted annual plants (Dexter, 2004;

Hebb et al., 2017). This contrasts to the Breton site, which was a perennial hay stand for at least 60 years prior. Therefore, improvements in the S-index at the Edmonton site and not the Breton site are likely due to a more degraded baseline soil quality upon treatment establishment, and thus the Edmonton soil became more responsive to the range of assessed land management options in our study due to the overarching influence of land use history.

5.6.2 Bridging the soil health gap

The difference between the modelled S-index and the boundary of good soil physical quality proposed by Dexter (2004) (S = 0.035) characterizes the soil physical quality deficit in our study (Fig. 5.2). Because soil physical quality is an important basis for overall soil health (Li et al., 2011; Xu et al., 2017), the S-index deficit at the Edmonton site can be considered a soil health gap, a term proposed by Maharjan et al. (2020) to describe the difference between undisturbed native soil and the soil health of an adjacent agroecosystem. By using the S-index value of 0.035 as a benchmark for soil health, we can assess the success of land management practices, such as the cultivation of perennials, in agroecosystems without the need for undisturbed benchmark data. The S-index provides a robust metric to describe multiple soil classifications and management practices (Dexter, 2004; Guenette et al., 2018; Hebb et al., 2018; Kiani et al., 2017; Rezaee et al., 2018; Xu et al., 2017), therefore we propose it is a useful reference to assess the soil health gap. At the Edmonton site, the implementation of the perennial AB treatment improved the S-index by 21% relative to the annual rye treatment in the 25-30 cm soil depth increment. While the perennial AB was unable to fully ameliorate the soil to the proposed benchmark of S = 0.035 in the limited timeframe of this experiment, the main goal of characterizing the soil health gap is to determine if a given management practice is moving towards a heathier soil (Morgan and Cappellazzi, 2021), which was evident in the case of the perennial AB treatment at the Edmonton site.

5.6.3 Implications for agricultural production

If reduced soil physical quality is linked to declining crop performance and profitability, improving, or maintaining soil physical quality may improve and stabilize yield and profitability due to increased soil water availability (Basche and DeLonge, 2019), improved structure from increased aggregation (Milne and Haynes, 2004), and enhanced biological activity (Culman et al., 2010). However, the longevity of increased soil C, TP, EP, and macroporosity and reduced BD remains unclear, namely if the next phase of the crop rotation is an annual crop. McCallum et al. (2004) posit that effects will be better preserved if beneficial practices, such as no till, are maintained in the proceeding annual crop, as evidenced by the maintenance of perennial phase-induced increases in macroporosity in the 2 years following an alfalfa crop in soils cropped with wheat and canola. Conversely, Wahlstrom et al. (2021) found that only 3.1% of previously established tap-root macropores formed by fodder radish were reused by the roots of a subsequent crop of annual barley. Thus, further research is required to assess the overall effects of the inclusion of perennial crops in long-term diversified crop rotations to determine whether the improvements in soil physical quality derived from perennials persist into the next phases of a rotation.

5.7 Conclusion

Divergences in soil physical and hydraulic properties across agricultural management systems were evident upon completion of the three-year experiment. Perennial AB improved the bulk density and porosity in the 5-10 cm soil depth at the Edmonton site, and increased soil macroporosity and pore connectivity in the deeper subsurface soil layer (25-30 cm depth increment). These findings are attributed to the influence of abundant root growth by perennials, the importance of land use history, the more degraded starting point of the soil after years of tillage and annual cropping, and the high clay content at this site. Perennial rye cropping counteracted the short-term consolidation effects of no till on soil BD but was not capable of significant improvements within the timeframe that these crops are expected to grow in the climate of Western Canada. Additionally, it is still unclear if the short-term implementation of a perennial phase in a cropping system can maintain soil physical and hydraulic properties after the conversion from a perennial hay stand due to the influence of legacy effects at the Breton site. Finally, future research is required to determine if a perennial cropping phase manifests in meaningful improvements in productivity of a subsequent annual crop.

5.8 References

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Tables

Table 5.1. Select baseline soil properties from the Edmonton and Breton sites.

Soil Properties	**Edmonton**	**Breton**
Canadian classification	Black Chernozem	Gray Luvisol
USDA classification[¥]	Udic Boroll	Boralf
FAO classification[¥]	Chernozem	Albic Luvisol
Total carbon (TC) (g C kg^{-1}) (0-30 cm)	41.6 ± 7.5	19.2 ± 3.9
Total nitrogen (TN) (g N kg^{-1}) (0-30 cm)	3.6 ± 0.5	1.7 ± 0.3
pH (1:5 H_2O)	7.3 ± 0.09	6.1 ± 0.08
Bulk density (g cm^{-3}) (5-30 cm)	1.0 ± 0.06	1.2 ± 0.06
Soil texture	silty clay	loam
% clay	48.3	24.8
% silt	35.7	41.8
% sand	16.0	33.3

[¥] Canadian Agricultural Services Coordinating Committee. Soil Classification Working Group, National Research Council Canada, Canada. Agriculture, & Agri-Food Canada. Research Branch. (1998). The Canadian system of soil classification (No. 1646). NRC Research Press.

Table 5.2. Mean values of soil physical and hydraulic properties for the annual rye, perennial rye, and perennial AB treatments at the Edmonton site.

Cropping System	BD (g cm^{-3})	TP (%)	EP (%)	FC (%)	S-index (unitless)	Macro (cm^3 cm^{-3})	Meso (cm^3 cm^{-3})	Micro (cm^3 cm^{-3})	Residual (cm^3 cm^{-3})	Large (cm d^{-1})	Medium (cm d^{-1})	Small (cm d^{-1})
						5-10 cm						
Annual rye	1.02 ± 0.02 a	61.57 ± 0.53 ab	56.52 ± 1.45	35.25 ± 0.92 a	0.026 ± 0.002	0.076 ± 0.007	0.092 ± 0.007	0.032 ± 0.003	0.358 ± 0.009	0.149 ± 0.028	0.007 ± 0.001	0.002 ± 0.0002
Perennial rye	1.05 ± 0.02 a	61.00 ± 0.81 b	56.66 ± 1.05	35.04 ± 0.99 a	0.027 ± 0.001	0.100 ± 0.014	0.096 ± 0.008	0.031 ± 0.003	0.347 ± 0.026	0.172 ± 0.019	0.007 ± 0.001	0.002 ± 0.0020
Perennial AB	0.93 ± 0.03 b	64.93 ± 1.19 a	54.70 ± 1.11	28.19 ± 1.64 b	0.029 ± 0.002	0.106 ± 0.008	0.109 ± 0.009	0.032 ± 0.001	0.293 ± 0.018	0.229 ± 0.028	0.007 ± 0.007	0.001 ± 0.0002
P-value	*0.016 **	*0.015 **	*0.45*	*0.00077 ****	*0.46*	*0.087*	*0.26*	*0.91*	*0.51*	*0.097*	*0.95*	*0.58*
						15-20 cm						
Annual rye	1.09 ± 0.03	58.93 ± 1.23	53.63 ± 1.54	33.10 ± 0.91	0.025 ± 0.001	0.090 ± 0.009	0.083 ± 0.003	0.026 ± 0.001	0.337 ± 0.009 a	0.223 ± 0.060	0.008 ± 0.001	0.002 ± 0.0002
Perennial rye	1.02 ± 0.03	61.50 ± 1.08	54.04 ± 1.10	32.54 ± 1.11	0.025 ± 0.001	0.109 ± 0.019	0.082 ± 0.005	0.030 ± 0.002	0.324 ± 0.010 a	0.184 ± 0.040	0.007 ± 0.001	0.002 ± 0.0003
Perennial AB	1.03 ± 0.03	61.25 ± 1.19	54.44 ± 1.90	30.96 ± 0.57	0.028 ± 0.001	0.118 ± 0.008	0.099 ± 0.007	0.033 ± 0.022	0.289 ± 0.035 b	0.175 ± 0.010	0.006 ± 0.001	0.002 ± 0.0001
P-value	*0.27*	*0.27*	*0.58*	*0.21*	*0.064*	*0.44*	*0.063*	*0.89*	*0.0016***	*0.9*	*0.19*	*0.45*
						25-30 cm						
Annual rye	1.12 ± 0.06	56.43 ± 2.20	51.68 ± 1.60 b	30.76 ± 0.94	0.024 ± 0.001 b	0.103 ± 0.013 b	0.086 ± 0.004	0.028 ± 0.003	0.280 ± 0.025	0.153 ± 0.020 b	0.008 ± 0.001	0.002 ± 0.0002
Perennial rye	1.04 ± 0.04	60.88 ± 1.62	53.70 ± 1.26 ab	32.48 ± 1.01	0.025 ± 0.001 b	0.096 ± 0.010 b	0.084 ± 0.005	0.027 ± 0.002	0.318 ± 0.022	0.250 ± 0.011 ab	0.007 ± 0.001	0.002 ± 0.0002
Perennial AB	1.01 ± 0.03	61.64 ± 1.03	58.33 ± 1.64 a	30.40 ± 0.68	0.029 ± 0.002 a	0.136 ± 0.008 a	0.095 ± 0.005	0.033 ± 0.002	0.316 ± 0.028	0.272 ± 0.04 a	0.007 ± 0.001	0.002 ± 0.0001
P-value	*0.28*	*0.27*	*0.018 **	*0.59*	*0.017 **	*0.046**	*0.24*	*0.17*	*0.15*	*0.028**	*0.49*	*0.23*

Note: BD, dry bulk density; TP, total porosity; EP, effective porosity; FC, field capacity water content at −33 kPa; S-index, soil physical quality metric; Macro, pore volume diameters > 60 µm; Meso, pore volume diameters between 9 and 60 µm; Micro, pore volume diameters between 6 and 9 µm; Residual, pore volume diameters < 6 µm; Large, unsaturated hydraulic conductivity between −1 and −10 kPa; Medium, unsaturated hydraulic conductivity between −10 and −20 kPa; Small, unsaturated hydraulic conductivity between −20 and −33 kPa. Values displayed are mean values per treatment per depth increment (5-10, 15-20 and 25-30 cm) ± SE (n=8). Lowercase letters indicate significant differences between treatment means for each column within each depth at α = 0.05. Saturated water content, field capacity, S-index, macro, meso, micro, and residual pore volume fractions (PVF), and large, medium, and small unsaturated hydraulic conductivity were derived from raw data fit to the van Genuchten model. Bulk density and total porosity are measured directly from dry weights.

Table 5.3. Mean values of soil physical and hydraulic properties for the annual rye, perennial rye, and perennial AB treatments at the Breton site.

Cropping System	BD (g cm^{-3})	TP (%)	EP (%)	FC (%)	S-index (unitless)	Macro (cm^3 cm^{-3})	Meso (cm^3 cm^{-3})	Micro (cm^3 cm^{-3})	Residual (cm^3 cm^{-3})	Large (cm d^{-1})	Medium (cm d^{-1})	Small (cm d^{-1})
5-10 cm												
Annual rye	1.24 ± 0.03	53.31 ± 1.02	51.25 ± 1.15	38.91 ± 1.07	0.036 ± 0.001	0.051 ± 0.003	0.052 ± 0.003 b	0.027 ± 0.002	0.392 ± 0.016	0.093 ± 0.021	0.031 ± 0.006	0.015 ± 0.003
Perennial rye	1.19 ± 0.02	55.00 ± 0.79	53.42 ± 1.13	37.44 ± 1.74	0.033 ± 0.002	0.057 ± 0.005	0.066 ± 0.005 a	0.030 ± 0.002	0.375 ± 0.015	0.143 ± 0.040	0.047 ± 0.009	0.014 ± 0.003
Perennial AB	1.19 ± 0.04	55.06 ± 1.55	53.61 ± 0.43	37.86 ± 1.91	0.037 ± 0.002	0.067 ± 0.013	0.062 ± 0.004 ab	0.033 ± 0.002	0.377 ± 0.015	0.126 ± 0.042	0.049 ± 0.010	0.016 ± 0.005
P-value	*0.48*	*0.49*	*0.18*	*0.81*	*0.29*	*0.58*	*0.042 **	*0.18*	*0.66*	*0.69*	*0.4*	*0.96*
15-20 cm												
Annual rye	1.42 ± 0.04	46.38 ± 1.44	46.71 ± 1.46	35.03 ± 0.49	0.032 ± 0.001	0.019 ± 0.002 b	0.051 ± 0.003	0.030 ± 0.003	0.359 ± 0.01	0.129 ± 0.006	0.047 ± 0.009	0.017 ± 0.003
Perennial rye	1.34 ± 0.02	47.19 ± 1.79	46.60 ± 1.30	35.64 ± 1.25	0.034 ± 0.002	0.037 ± 0.006 ab	0.049 ± 0.003	0.027 ± 0.002	0.349 ± 0.01	0.090 ± 0.010	0.036 ± 0.006	0.014 ± 0.002
Perennial AB	1.34 ± 0.05	49.29 ± 1.57	46.17 ± 0.78	34.31 ± 1.31	0.033 ± 0.002	0.054 ± 0.009 a	0.045 ± 0.001	0.024 ± 0.002	0.348 ± 0.02	0.098 ± 0.011	0.032 ± 0.007	0.011 ± 0.002
P-value	*0.24*	*0.8*	*0.95*	*0.69*	*0.71*	*0.0096 ***	*0.38*	*0.24*	*0.78*	*0.19*	*0.37*	*0.96*
25-30 cm												
Annual rye	1.45 ± 0.04	44.39 ± 1.46	42.08 ± 0.86 b	33.33 ± 0.78	0.031 ± 0.002	0.025 ± 0.006 b	0.045 ± 0.003	0.029 ± 0.004	0.323 ± 0.032	0.120 ± 0.021 ab	0.039 ± 0.008	0.014 ± 0.003
Perennial rye	1.43 ± 0.04	46.00 ± 1.39	43.51 ± 0.70 ab	33.14 ± 1.10	0.028 ± 0.002	0.030 ± 0.004 b	0.044 ± 0.002	0.026 ± 0.002	0.336 ± 0.011	0.153 ± 0.055 a	0.041 ± 0.010	0.027 ± 0.008
Perennial AB	1.34 ± 0.07	49.56 ± 2.08	45.57 ± 0.62 a	33.86 ± 1.74	0.029 ± 0.002	0.072 ± 0.008 a	0.044 ± 0.003	0.025 ± 0.002	0.323 ± 0.023	0.048 ± 0.007 b	0.025 ± 0.007	0.008 ± 0.002
P-value	*0.19*	*0.17*	*0.0089 ***	*0.92*	*0.52*	*0.00014****	*0.9*	*0.75*	*0.34*	*0.027 **	*0.19*	*0.095*

Note: BD, dry bulk density; TP, total porosity; EP, effective porosity; FC, field capacity water content at −33 kPa; S-index, soil physical quality metric; Macro, pore volume diameters > 60 µm; Meso, pore volume diameters between 9 and 60 µm; Micro, pore volume diameters between 6 and 9 µm; Residual, pore volume diameters < 6 µm; Large, unsaturated hydraulic conductivity between −1 and −10 kPa; Medium, unsaturated hydraulic conductivity between −10 and −20 kPa; Small, unsaturated hydraulic conductivity between −20 and −33 kPa. Values displayed are mean values per treatment per depth increment (5-10, 15-20 and 25-30 cm) ± SE (n=8). Lowercase letters indicate significant differences between treatment means for each column within each depth at α = 0.05. Saturated water content, field capacity, S-index, macro, meso, micro, and residual pore volume fractions (PVF), and large, medium, and small unsaturated hydraulic conductivity were derived from raw data fit to the van Genuchten model. Bulk density and total porosity are measured directly from dry weights.

Table 5.4. Mean values of root mass density for the annual rye, perennial rye, and perennial AB treatments for the Edmonton and Breton sites for 2018 and 2019.

Cropping System	**Root Density (mg cm^{-3})** Edmonton	 Breton
	2018	
	0-15 cm	
Annual rye	0.40 ± 0.16 b	2.04 ± 0.47 b
Perennial rye	0.77 ± 0.15 b	2.88 ± 0.75 b
Perennial AB	3.35 ± 0.28 a	7.20 ± 0.90 a
P-value	*6e-06* ***	*0.002* **
	15-30 cm	
Annual rye	0.03 ± 0.01 c	0.22 ± 0.04
Perennial rye	0.16 ± 0.03 b	0.96 ± 0.51
Perennial AB	1.19 ± 0.45 a	1.34 ± 0.29
P-value	*3e-05* ***	*0.1*
	2019	
	0-15 cm	
Annual rye	1.97 ± 0.73	3.04 ± 0.92 b
Perennial rye	3.32 ± 1.39	6.37 ± 1.14 ab
Perennial AB	16.38 ± 10.18	17.57 ± 7.58 a
P-value	*0.05*	*0.02**
	15-30 cm	
Annual rye	0.18 ± 0.03 b	0.47 ± 0.09 b
Perennial rye	0.35 ± 0.03 b	0.95 ± 0.17 b
Perennial AB	2.59 ± 1.03 a	3.14 ± 1.03 a
P-value	*0.0001****	*0.0002* ***

Lowercase letters indicate significant differences between treatment means for each column within each depth at α = 0.05.

Table 5.5. Total carbon and nitrogen mass for the 0-15 and 15-30 cm soil depth increments for the Edmonton and Breton sites for the annual rye, perennial rye, and perennial AB treatments.

Cropping System	**Total Carbon (Mg ha^{-1})**	**Total Nitrogen (Mg ha^{-1})**	**Total Carbon (Mg ha^{-1})**	**Total Nitrogen (Mg ha^{-1})**
	Edmonton		Breton	
	0-15 cm			
Annual rye	83.09 ± 3.97 b	7.63 ± 0.35 b	55.35 ± 2.17	5.11 ± 0.19
Perennial rye	96.96 ± 2.42 a	8.97 ± 0.22 a	57.30 ± 2.43	5.05 ± 0.17
Perennial AB	89.72 ± 1.80 ab	8.16 ± 0.24 ab	56.64 ± 3.14	5.04 ± 0.18
P-value	*0.01**	*0.01**	*0.8*	*0.9*
	15-30 cm			
Annual rye	58.13 ± 9.19	5.07 ± 0.74	19.91 ± 1.70	2.11 ± 0.17
Perennial rye	62.62 ± 5.67	5.65 ± 0.50	18.53 ± 1.32	1.90 ± 0.12
Perennial AB	60.71 ± 7.62	5.25 ± 0.69	25.97 ± 3.19	2.51 ± 0.33
P-value	*0.7*	*0.7*	*0.07*	*0.2*

Lowercase letters indicate significant differences between treatment means for each column within each depth at α = 0.05.

Figures

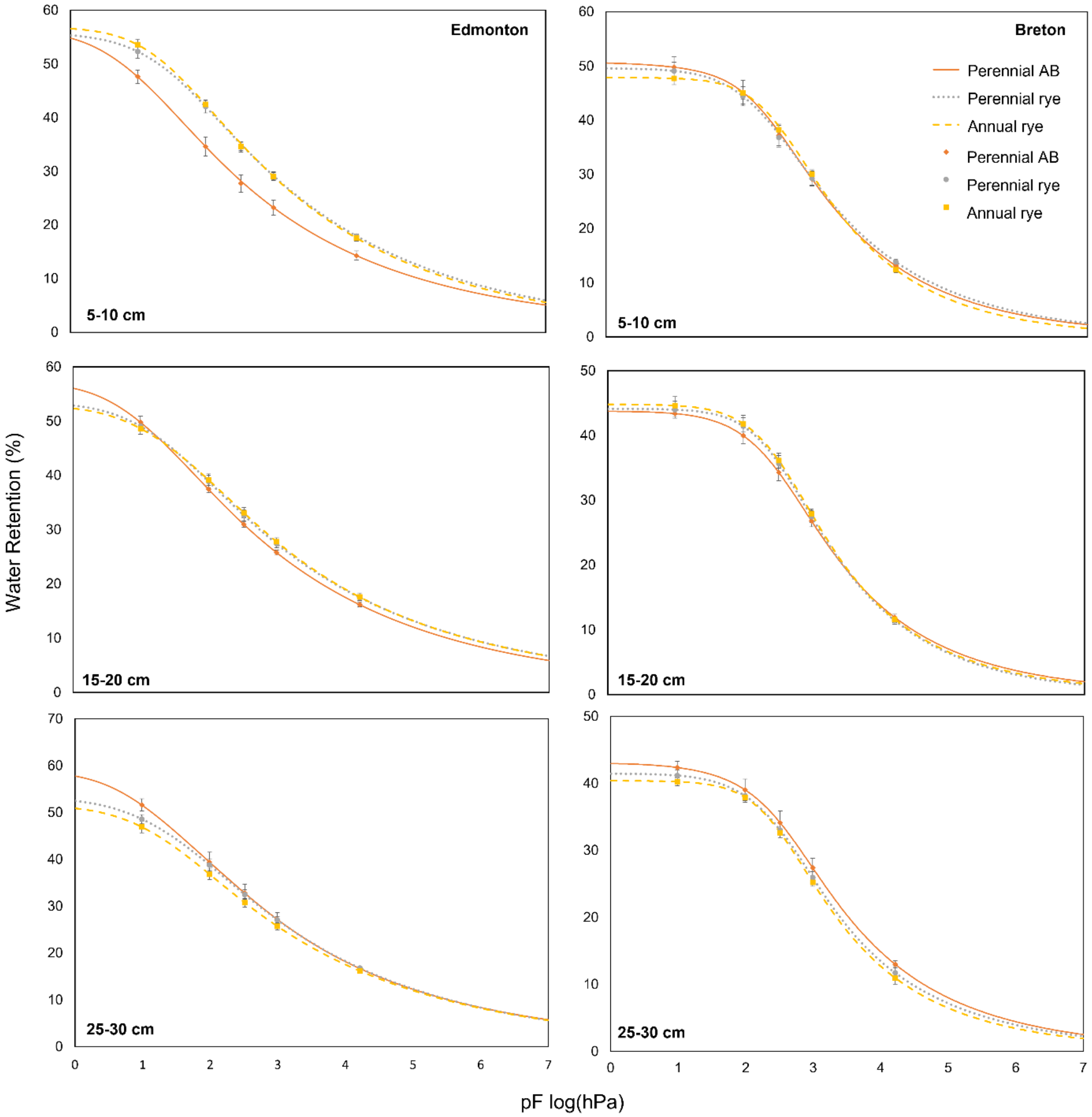

Figure 5.1. Water retention curves for the Edmonton (left) and Breton (right) sites for the 5-10, 15-20 and 25-30 cm depth increments. Curves are developed using the van Genuchten model on measured data for the perennial AB, perennial rye and annual rye treatments. Select points on the curve correspond to pF values of 1, 2, 2.5, 3 and 4.2, error bars are ± SE (n=8).

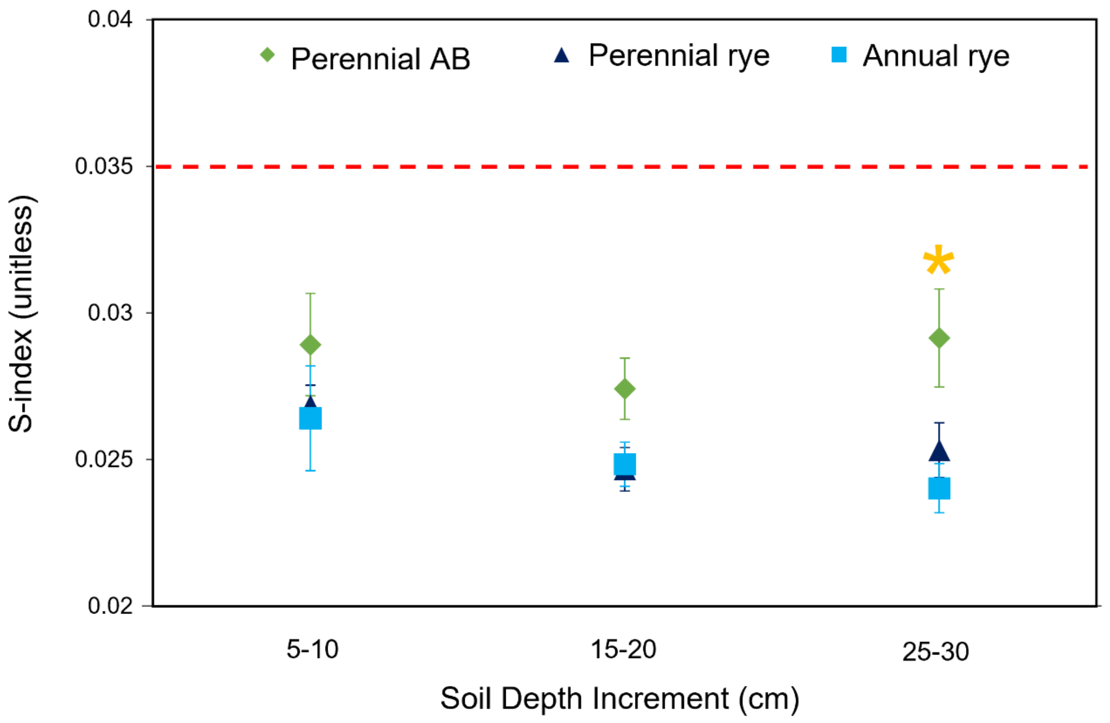

Figure 5.2. Soil physical quality as described by the S-index for all depths for the perennial AB, perennial rye and annual rye treatments for the Edmonton site. The dashed line indicates the threshold between good ($S > 0.035$) and poor ($S < 0.035$) soil physical quality. The star indicates significantly improved S-index in the perennial forage treatment relative to the perennial rye and annual rye in the 25-30 cm soil depth increment at $\alpha = 0.05$.

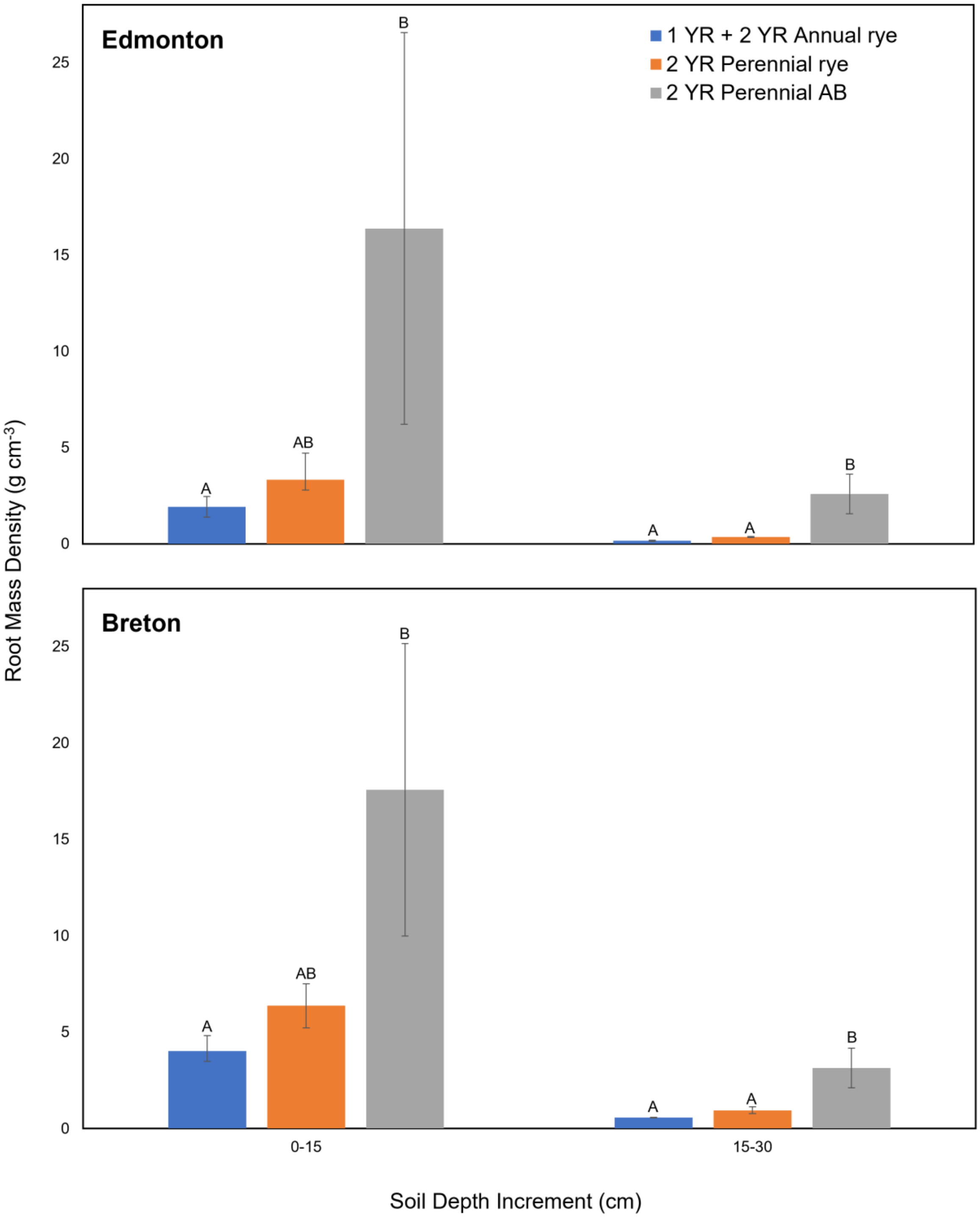

Figure 5.3. Cumulative root density from 2018 & 2019 for annual rye (1 YR + 2 YR), compared to 2019 root density for 2 YR old perennial treatments, rye and forage for the Edmonton site (top panel) and the Breton site (bottom panel). Uppercase letters denote significant differences between treatments within each depth and site at $\alpha = 0.05$. Error bars are ± SE (n=4).

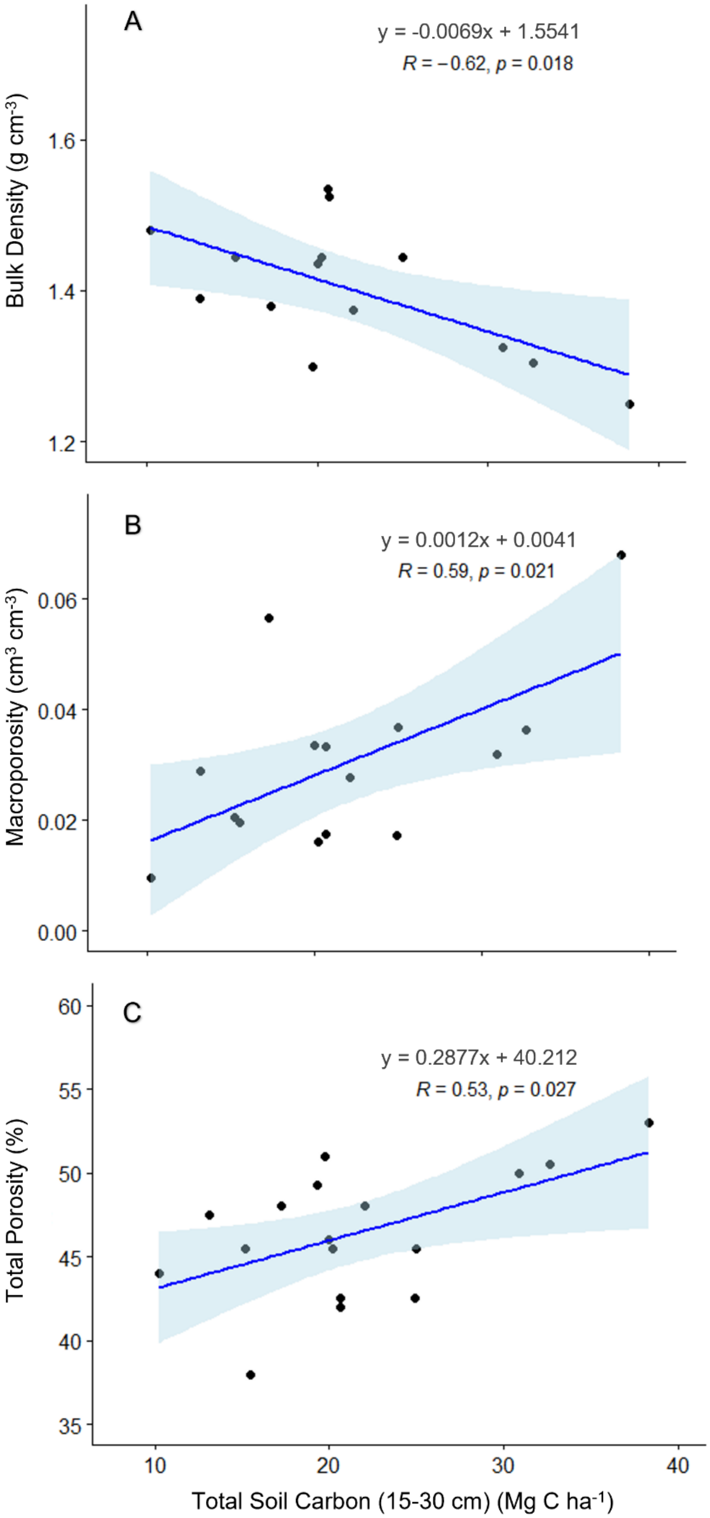

Figure 5.4. Linear regressions of (A) bulk density, (B) macroporosity and (C) total porosity across experimental treatments from the 15-20 cm depth increment as a function of total soil carbon in the 15-30 depth increment at the Breton site.

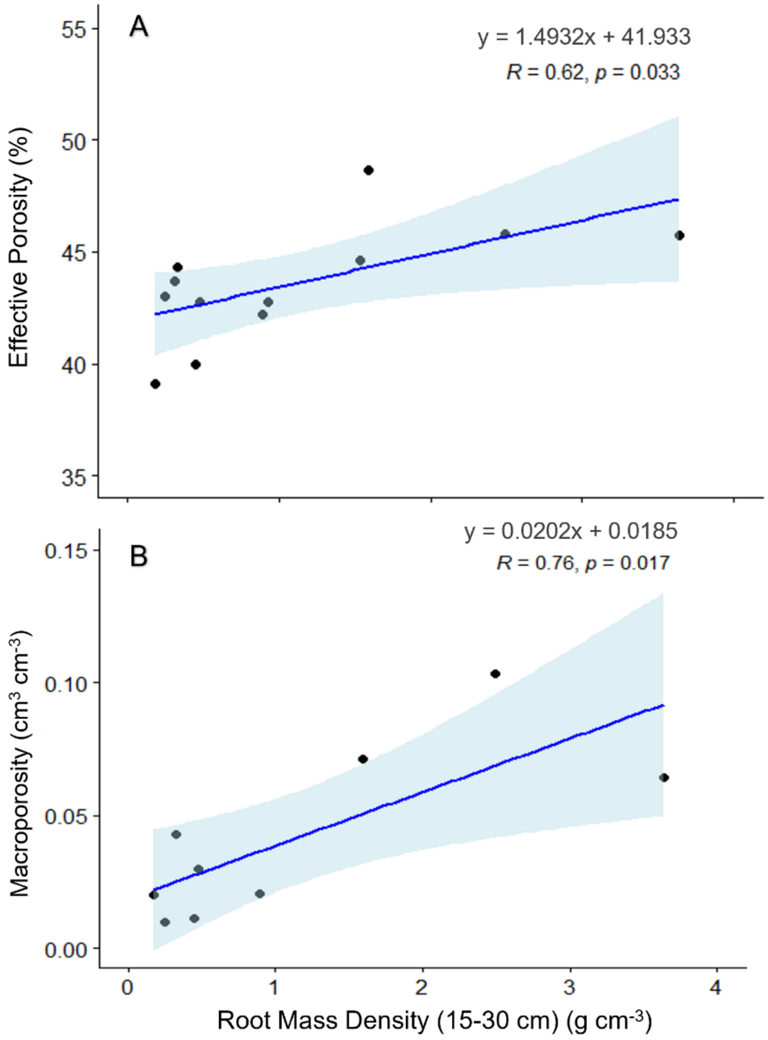

Figure 5.5. Linear regressions of (A) effective porosity (EP) and (B) macroporosity from the 25-30 cm depth increment as a function of root mass density across experimental treatments in the 15-30 cm depth increment at the Breton site.

6 Perennial grain crops reduce N_2O emissions under specific site conditions

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6.1 Abstract

Perennial grain crops represent a novel hybrid between annually harvested grain crops and perennial forage crops, which are seeded once and grow for multiple subsequent seasons. Previous research has shown comparatively reduced nitrous oxide (N_2O) emissions from perennial forage crops relative to annual grain crops, however, the effect of perennial grain cropping on N_2O emissions is unclear. We quantified field N_2O emissions along an experimental continuum of perenniality (perennial forage, perennial grain, fall grain, spring grain and fallow) established at two sites within Alberta, Canada with contrasting soils: Luvisolic at the Breton site and Chernozemic at the Edmonton site. We used static chambers and a micrometeorological technique based on an open-path Fourier-transform infrared gas sensor (OP-FTIR). Perennial grain crops reduced cumulative N_2O emissions at the Breton site by 60% and 94% in years two and three of the study, respectively ($Ps < 0.0001$). Conversely, no reduction in N_2O emissions by the perennial grain crop relative to the annual crop was evident at the Edmonton site. Correlation analyses for both sites revealed that the average root density from 0-60 cm was negatively correlated with soil available nitrogen (N) (0-15 cm depth) in years one ($Ps < 0.01$) and two ($Ps < 0.05$). Moreover, in year two, root density was negatively correlated with cumulative N_2O emissions, specifically at the Breton site ($P < 0.01$). Results suggest that the enhanced root density of perennial crops reduced soil N availability at the Breton site, which translated into reduced cumulative N_2O emissions in year two. Further, OP-FTIR measurements at the Breton site were in general agreement with static chamber measurements, which collectively informed that the bulk reduction in cumulative N_2O emissions occurred during spring thaw. Overall, the ability for perennial cereal grain crops to reduce N_2O emissions relative to annual crops appears to be site-specific.

Keywords: perennial, annual, crop, nitrous oxide, static chambers, Open-Path Fourier-transform infrared spectroscopy

6.2 Introduction

Nitrous oxide (N_2O) is a biogenic greenhouse gas that contributes to the radiative forcing of the atmosphere, with a global warming efficiency that is 273 times greater than that of carbon dioxide on a mass basis and an atmospheric residence time of a century or more (IPCC, 2021; Liebig et al., 2005). Notably, arable land occupies only about 37% percent of earth's land surface, but the agricultural sector contributes 84% of global anthropogenic N_2O emissions, which are largely influenced by management practices and the use of nitrogen (N) fertilizer (Liu et al., 2017; Smith et al., 2008). Due to rising global population, agricultural land area requirements and N_2O emissions are likely to continue increasing for the foreseeable future (van Groenigen et al., 2010; Reay et al., 2012).

Cropping systems regulate the biotic and abiotic factors driving N_2O emissions via processes including, but not limited to, soil water use and root characteristics (Abalos et al., 2016; Smith et al., 2013), N uptake (Gelfand et al. 2016), growing season length (Behnke and Villamil, 2019; Thomas et al., 2017) and tillage practices (Lognoul et al. 2017; Wang and Delal, 2015). These factors alter soil carbon (C) and N dynamics, structure, moisture, temperature, and microbial community composition, which in turn affect the dominant processes of N_2O formation in soil, nitrification and denitrification (Smith, 2017; Tenuta et al., 2019). Annual cropping systems currently dominate agricultural production, occupying 60 to 80% of global cropland (Pimentel et al., 2012). However, innovative crop breeding efforts have resulted in the development of novel perennial grain crops such as wheat, rice, rice, and sorghum, with the underlying premise being that perennial grain cropping can alleviate environmental challenges associated with annual cropping systems such as erosion and loss of soil C and N (Cox et al., 2007; Ryan et al., 2018).

Notably, perennial grain crops may differentially affect N_2O emissions compared to annual grain crops. Earlier studies suggest that increases in below-ground biomass via deeper, denser root systems, increased mineral N [nitrate (NO_3^-) and ammonium (NH_4^+)] uptake and longer growing seasons associated with perennial systems may reduce N_2O emissions relative to annual systems (Abalos et al., 2016; Ferchaud et al., 2015 Gregorich et al., 2005; Rochette et al., 2018). Conversely, conflicting research has shown that N_2O emissions from soil may increase with the implementation of a perennial cropping system. Increased availability of labile C and N substrates from proportionally augmented root exudation and tissue decay as well as the potential for temporarily increased soil bulk density and water-filled pore space after establishing perennial systems may provide ideal conditions for N_2O production (Basche et al., 2014; Daly and Hernandez-Ramirez, 2020; van Kessel et al., 2013; Thomas et al., 2017).

Interestingly, the abovementioned research has largely focused on perennial forages or bioenergy perennial crops, which both differ from perennial grain crops (Abalos et al., 2016; Ferchaud et al., 2020). Perennial forage systems are subject to different management practices (i.e., seeding rates and harvest timing) and reduced N fertilizer inputs (Abraha et al., 2018; Weißhuhn et al., 2017), whereas bioenergy crops harvest the entire aboveground biomass, which differs from perennial grain cropping in that the latter can retain plant residues and associated N (Kim et al., 2021, Walter et al., 2015, Kiani et al., 2017). Preliminary research indicates that current perennial grain cultivars may only survive 2-3 growing seasons in cold temperate agroecosystems, such as those in western Canada (Cattani, 2019). It is unclear if this is enough time for differences in N_2O emissions to materialize under contrasting crops (Kim et al., 2021). Indeed, Cusser et al. (2020) found that the effects of no-till management on soil water and N_2O emissions are only consistent after a longer term (> 10 yrs). Therefore, changes in soil N_2O emissions under perennial grain crops, which can be thought of as an intermediate system that integrates characteristics of both annual grain crops and perennial forages, must be investigated in detail over multiple years.

Previous research has stressed the importance of accounting for N_2O emissions during the spring thaw period that occurs after prolonged soil freezing during the winter (Flesch et al., 2018; Risk et al., 2014; Wagner-Riddle et al., 2017; Wagner-Riddle et al., 2008). It has been reported that up to 70% of yearly N_2O emissions can occur during this ephemeral season (Flesch et al., 2018; Lin et al., 2017; Kim et al., 2021; Thilakarathna et al., 2020). Freeze-thaw induced N_2O emissions originate primarily from denitrification and are affected by substrate availability, denitrifying enzyme activity and the physical release of trapped N_2O (Machado et al., 2021). During spring thaw, partial snow and ice cover can act as a physical barrier that reduces the diffusivity of N_2O from soil, but emission from snow-covered soil can occur when the underlying soil begins to warm to temperatures that support biological activity in thin films of liquid water (Congreves et al., 2018; Risk et al., 2014). In regions that experience months of frozen soil conditions, perennial forage crops, which utilize spring moisture and nutrients immediately upon spring thaw, have the potential to significantly reduce seasonal N_2O emissions relative to their annual counterparts (Flesch et al., 2018; Nemeth et al., 2014; Wagner-Riddle et al., 2017). To our knowledge, no research to date has measured spring thaw N_2O emissions from a perennial grain crop, which shares characteristics of both perennial forage and annual grain crops.

Measurement of N_2O emissions from soils is typically done using static flux chambers (Hernandez-Ramirez et al., 2009; Lin et al., 2017; Thilakarathna et al., 2020), although micrometeorological techniques can also be used (e.g., Wagner-Riddle et al., 2017; Flesch et al., 2018). The two approaches offer different strengths and weaknesses. Chambers have the advantage of simplicity and economy, whereas micrometeorological techniques have advantages in spring thaw conditions when access to static chambers is hindered by snow cover or surface water runoff. Further, micrometeorological techniques are suitable for making continuous measurements, an advantage in the transient and dynamic environment of spring thaw. Thus, continuous, field-footprint sampling methods that are deployable prior to full snowmelt, such as micrometeorological measurements, can be combined with static chamber measurements to obtain a more complete picture of annual emission patterns (Flesch et al., 2018; Grant et al., 1999).

Consequently, there is a knowledge gap regarding N_2O emission magnitudes and timing from perennial grain crops that needs to be addressed if they are to be adopted as a means of N_2O emission reduction. For this study, we selected perennial rye [cv. ACE-1] as a model perennial grain crop, based on preliminary findings from Lethbridge, Alberta, which reported a superior performance of perennial rye relative to several perennial wheat cultivars (Hayes et al., 2018). Accordingly, our research objectives are to: (1) quantify and examine patterns of N_2O emissions from a model perennial grain crop, using chamber and micrometeorological techniques, and compare them to N_2O emissions from spring grain, fall grain and perennial forage crops in two temperate field sites with contrasting soil types (Luvisol vs. Chernozem), (2) identify how major controlling factors and processes that alter N_2O emission (i.e., soil moisture, soil mineral N [nitrate {NO_3^-} and ammonium {NH_4^+} concentrations] and root mass density) differ with respect to spring, fall, perennial grain and perennial forage crops under field conditions with comparable management and N fertilizer applications and (3) evaluate yield-based emission factors (EFy) across the aforementioned continuum of perenniality, to assess the potential tradeoff between productivity and emission reduction.

6.3 Materials and Methods

6.3.1 Site characteristics and experimental design

Two field sites were established in Edmonton, Alberta, Canada (53° 29' 43.33", 113° 31' 59.24") and Breton, Alberta, Canada (53° 5' 16.72", 114° 26' 29.35"). Soil at the Edmonton site is classified as Orthic Black Chernozem and soil at the Breton site is classified as Orthic Grey Luvisol, according to the Canadian System of Soil Classification. Baseline soil properties and climatic normals for both sites are summarized in Table 6.1.

Both experimental sites were arranged in identical randomized complete block designs consisting of four block replicates and nine treatments per block. Treatment combinations consisted of two experimental factors: crop type and N fertilizer. Crop type consisted of three analogous grain cultivars: perennial [ACE-1 rye (*Secale cereale* L. × *S. montanum* Guss], fall [Hazlett rye (*S. cereale* L.)] and spring [Gazelle rye (*S. cereale* L.)], as well as perennial forage [(meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa*)], and fallow. Within each block, two plots of each vegetated treatment were seeded, with one receiving no N fertilizer and one receiving 56 kg N Ha^{-1} in the form of a urea and polymer-coated urea blend (2:1). This addition of N fertilizer did not show any treatment effect in our study, possibly due to the high background fertility of both soils and method of fertilizer application, as discussed in Daly et al. (2021). Therefore, this factor is not mentioned further.

Each experimental plot measured 32 m^2 (8 m length x 4 m width). The perennial, fall and spring grain treatments were seeded with a 22.9 cm row spacing to a depth of 2.5 cm. Perennial forage treatments were broadcast seeded at a rate of 55 kg ha^{-1} for meadow brome and 4 kg ha^{-1} for alfalfa, then incorporated. N fertilizer was broadcast at the aforementioned rate on the plots of select N treatments concurrent with spring rye seeding every year. Adjacent field-scale (4 ha) plots of perennial grain and spring grain were also established at the Breton site. The timing of major field activities is summarized in Table 6.2.

Measurements at both sites were conducted from 1 September 2017 to 31 August 2018 (year one) and from 1 September 2018 to 31 August 2019 (year two). Measurements for the Breton site were also conducted from 1 September 2019 to 31 August 2020 (year three).

6.3.2 Static chamber emission data collection

Custom acrylic chambers measuring 15.6 cm x 64.10 cm x 10 cm were installed on 5 September 2017 at both sites and removed only for seeding, tillage, or harvest. Otherwise, the chambers were left in the soil for the duration of the experiment. Each plot was equipped with a single chamber base, installed perpendicular to crop rows to a depth of 5 cm. Emissions were measured intermittently at least 1x weekly throughout the duration of the growing season, with sampling intensity increasing to 2x weekly during the spring thaw period, after fertilization and after major precipitation events. For consistency, gas samples were collected throughout the duration of the experiment between the hours of 10:00 AM and 2:00 PM.

Gas samples were collected from each chamber on a 20-, 40-, and 60-minute time step. In addition, ambient samples were taken 10 cm above the soil surface three times throughout the duration of the sampling interval, at the beginning, middle and end, to be used as time zero gas concentrations. Samples were injected into evacuated 12 mL Exetainer® vials and stored at 4°C until analysis via a gas chromatograph (*Varian 3800, Varian Inc., Walnut Creek, CA*) equipped with an electron capture detector (Lin and Hernandez-Ramirez, 2020). The flux detection limit was 0.4 g N_2O-N ha^{-1} day^{-1}.

The N_2O emissions were determined by plotting a linear or a quadratic relationship between measured N_2O concentrations against time, then applying the modified ideal gas law as follows:

$$\mathrm{N_2O\ Emission} = \frac{\mathrm{S\times P\times V}}{\mathrm{R\times T\times A}} \qquad [6.1]$$

where N_2O emission is the N_2O emission rate (mmol min^{-1} m^{-2}), S is the slope of the line from a simple linear regression or the first-order derivative at time zero for a quadratic regression curve (Yates et al., 2006; Pennock et al., 2010) (mL L^{-1} min^{-1}), P is the pressure of the gas (Pa), V is the volume of the gas chamber (L), A is the surface area of the gas chamber (m^2), R is the gas constant (Pa mL K^{-1} mmol^{-1}) and T is the temperature of the gas (K). The average of each replicate ± SE (n=8) was then calculated for each treatment measurement date.

Cumulative emissions were calculated between consecutive sampling dates by using linear interpolation. Annual cumulative emissions were calculated by considering one growing season as a full year between 1 September and 31 August of the following calendar year. Due to freezing soil temperatures, minimal soil biological activity and hindered gas transport processes, emissions were assumed to be negligible during winter (i.e., approximately November to March) (Lin et al., 2017; Thilakarathna et al., 2020).

6.3.3 OP-FTIR measurements

Emissions of N_2O during the spring thaw were measured at the Breton site using a micrometeorological technique. An open-path Fourier transform infrared sensor (OP-FTIR) was used to measure the vertical gradient of N_2O concentration in the atmosphere above the field site, with the N_2O emission rate calculated from the gradient. A detailed description of the measurement system is available in Flesch et al. (2016). Briefly, the OP-FTIR sensor measures the line-average N_2O concentration between the FTIR spectrometer (*Matrix-M IR cube, Bruker Optik, Ettlingen, Germany*) and a retro-reflector: an infrared beam is sent from the spectrometer to the reflector, which is then returned along the same path to the spectrometer for analysis. The vertical N_2O gradient is determined by sequentially aiming the OP-FTIR to two reflectors (using a pan-tilt aiming motor) vertically separated by approximately 2 m, which are mounted on a ladder approximately 100 m from the spectrometer. The aiming sequence was repeated automatically, with a dwell time of 2 minutes on each reflector. During operation, the measurement sequence was repeated automatically and continuously. The concentration measurements were averaged into 30-minute intervals and an emission rate calculated for each 30 minutes.

The OP-FTIR was deployed just prior to the beginning of spring thaw and emission measurements lasted approximately four weeks (until the snow had melted and soil moisture decreased). Measurements were made concurrently from the 4 ha perennial and spring grain fields, which were located adjacent to one another at the Breton site. The OP-FTIR was placed between the fields and sequentially aimed at reflector pairs located in each field. The 1-way path lengths for the perennial grain field in years one, two, and three were 96 m, 110 m, and 108 m, respectively. For the spring grain field, path lengths for years one, two, and three were 115 m, 122 m, and 124 m, respectively. Three-dimensional sonic anemometers were installed in each of the 4 ha fields at Breton and these provided the wind and turbulence information needed for the emission calculations.

6.3.4 Soil, plant biomass, and root sample collection and processing

Soil samples were collected from the treatment plots from May to October in year two at both sites and year three at the Breton site to identify temporal changes in soil mineral N concentrations between experimental treatments. Samples from 0-15 cm were collected from each plot by using a push probe (2.5 cm i.d.) to obtain three random cores, which were then homogenized in sterile plastic sampling bags for one composite sample per plot. Soil samplings occurred prior to seeding and N fertilization, one week after fertilization, several times throughout the growing season and immediately post-harvest. Samples were stored at 4°C until they were air-dried and ground for analysis. Soil mineral N was quantified by extracting 5 g of air-dried soil with 50 mL of 2M KCl, shaken in a reciprocal shaker for 30 minutes, filtered using fine porosity 15 cm diameter filters (*Fisher Scientific, Pittsburg, USA*) and analyzed via colorimetry using a SmartChem discrete wet chemistry analyzer (*Unity Scientific, Milford, USA*). Composite soil samples were taken in the manner described above after harvest each year for the determination of total soil organic carbon (TOC) and total nitrogen (TN) to discern potential differences between treatments. Samples were air-dried, ground, and analyzed using dry combustion using a Thermoscientific Flash 2000 Organic Elemental Analyzer (*Thermo Fisher Scientific Waltham, USA*). Additionally, soil pH was measured using a 1:5 soil:water slurry with a pH meter and soil texture was determined via the hydrometer method. Soil bulk density was determined using the core method.

Roots were sampled in late June 2018 and June 2019 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 and subsequently calculate root mass density (Hernandez-Ramirez et al., 2014).

Aboveground biomass yields for the grain treatments were measured by hand harvesting 1-meter lengths of two adjacent rows at two locations within each replicated plot, at least 1-meter from the plot edges. The harvested material was then bagged, threshed, weighed and oven dried until a constant weight was reached for determination of grain and forage dry matter (DM). The perennial forage plots were harvested by cutting a 1-meter length using a self-propelled flail type small plot forage harvester (*Swift Machine and Welding, Swift Current, Saskatchewan*)

6.3.5 Weather and soil moisture data collection

Hourly temperature and precipitation data was obtained for both sites from permanent weather stations within 1 km of the experimental plots. Soil temperature and volumetric moisture content were continuously measured hourly from Decagon 5TM sensors installed at each site at 7.5, 22.5, and 40 cm depth. Data from the sensors was logged on EM50 data loggers and collected monthly. In addition, soil moisture measurements were obtained from 5 cm depth from each plot using a Stevens Hydraprobe II (*Campbell Scientific, Edmonton, Alberta*) concurrent with static chamber measurements on sampling days.

6.3.6 *Calculations and statistical analyses*

Yield based emission factors for each treatment were calculated using the following equation:

$$EF_y = \frac{\text{Cumulative } N_2O \text{ emission}}{\text{Grain Yield}} \qquad [6.2]$$

Where EF_y is the intensity emission factor, cumulative N_2O emission is the yearly cumulative emission from each treatment (g N ha^{-1} yr^{-1}) and grain yield is the yield from each treatment (kg ha^{-1} yr^{-1}).

All statistical analyses were performed with R Studio software version 4.0.3 (R Core Team, 2020) with an alpha critical value of 0.05. Assumptions of normality and homoscedasticity were checked by the Shapiro-Wilk and Barlett tests and data transformations including logarithmic, square root or Box-Cox were utilized when needed to meet assumptions. One-way analysis of variance (ANOVA) was performed on linear mixed models developed using the NLME package (Pinheiro et al., 2020) for cumulative N_2O emissions, average mineral N concentration and EF_y, with crop as the fixed factor and block replicate as the random effect. Post hoc investigation was completed after significant ($P < 0.05$) ANOVA using Tukey's Honest Significant Difference test for comparison of treatment means using the Agricolae package (de Mendiburu, 2020). Pearson's correlation was used to explore relationships between N_2O emissions and measured variables.

6.4 Results

6.4.1 Weather conditions

Long-term normal (1981-2010) air temperature at the Breton and Edmonton sites is 3.4 and 4.2°C respectively, with an average yearly precipitation of 602 and 456 mm (Table 6.1) (Government of Canada, 2020). The first year at the Breton site was slightly warmer and significantly drier than the normal, with an average temperature of 3.6°C and only 465.2 mm of total precipitation. Conversely, years two and three at the Breton site were cooler (average of 2.4°C and 3.0°C, respectively) and substantially wetter than normal, with 630 mm and 724 mm of precipitation. Similarly, the first year at the Edmonton site was drier than normal, with 361 mm of precipitation, but similar to normal in temperature (4.1°C). Year two at the Edmonton site experienced similarly cool conditions as year two at the Breton site, with an average temperature of 3.2°C and 433 mm of precipitation (Government of Alberta, 2020).

6.4.2 Static chamber measurements of seasonal nitrous oxide emissions

6.4.2.1 Breton

Nitrous oxide emission pulses during the first year were detected following: (i) initial tillage for treatment establishment in the fall, (ii) spring thaw, (iii) N fertilizer application, and (iv) the first cut of the perennial forage crop (Figs. 6.1A-D). Specifics of each emission pulse are as follows:

> (i) Emissions measured on 6 September 2017 were elevated for all treatments. The highest emissions on this day were 13.28 ± 3.25 g N_2O-N ha^{-1} day^{-1} from the perennial grain crop. The subsequent emission measurement on 15 September 2017 was also high for all treatments, with spring grain emissions reaching 8.23 ± 0.23 g N_2O-N ha^{-1} day^{-1}.
>
> (ii) Chamber measurements captured amplified N_2O-N emissions corresponding to the spring thaw on 28 April 2018 from the perennial grain (15.29 ± 4.79 g N_2O-N ha^{-1} day^{-1}), fall grain (12.49 ± 5.92 g N_2O-N ha^{-1} day^{-1}) and perennial forage (6.39 ± 0.57 g N_2O-N ha^{-1} day^{-1}). These emissions corresponded with a spike in soil moisture associated with snowmelt during the thaw (Figs. 6.1B, 6.1C).
>
> (iii) Fertilizer application on 22 May 2018 followed by a significant precipitation event and subsequent spike in soil moisture (Figs. 6.1B, 6.1C) resulted in an emission pulse from all treatments on 5 June 2018, with the highest emissions originating from the spring grain (8.71 ± 4.77 g N_2O-N ha^{-1} day^{-1}) and the lowest from the fallow plot (2.89 ± 1.81 g N_2O-N ha^{-1} day^{-1}).

(iv) Ten days after the first cut of the perennial forage plots, an emission pulse was observed from that treatment on 6 July 2018, which peaked at 5.43 ± 2.12 g N_2O-N ha^{-1} day^{-1} (Fig. 6.1C).

Cumulative N_2O emissions for growing season one did not differ between treatments. Additionally, while a numerical trend of elevated emissions with N fertilizer addition was observed (average emissions for all treatments with and without N fertilizer addition were 396.02 ± 47.05 and 353.93 ± 62.60 g N_2O-N ha^{-1} day^{-1}, respectively), no effect of N fertilizer could be discerned. As such, the with and without N fertilizer treatments were pooled for each crop (Table 6.3). Interestingly, while there was no discernable difference in cumulative N_2O emissions across crops or N fertilizer addition, there was a difference in the proportion of emissions attributed to each measurement period (fall, spring thaw, summer) across treatments in year one (Table 6.3). Although most annual cumulative emissions occurred during the fall period for all crops, spring grain and fallow had greater proportions of cumulative emissions during the fall period compared to the other three treatments. Spring grain and fallow also had comparatively lower emissions during spring thaw and summer.

Daily emission pulses for year two corresponded with (i) spring thaw, (ii) the first cut of the perennial forage crop and (iii) a significant precipitation event (Figs. 6.2A-E). Specifics of each emission pulse are as follows:

(i) Emissions measured from 30 March 2019 to 15 April 2019 constitute the spring thaw emission pulse. The highest emissions during this period were observed from the spring grain (369.59 ± 191.24 g N_2O-N ha^{-1} day^{-1}). Notably, emissions from the perennial forage crop were substantially reduced on this day, with average emissions of 2.50 ± 1.10 g N_2O-N ha^{-1} day^{-1}.

(ii) On 16 July 2019, an emission pulse from the perennial forage (47.52 ± 29.10 g N_2O-N ha^{-1} day^{-1}) was captured, one day after the first cut of the perennial forage (Fig. 6.2D).

(iii) An emission pulse from the fallow from 31 Jul 2019 to 13 August 2019 coincided with a major precipitation event only hours earlier on the same day (36.8 mm of rainfall), which led to a sharp increase in soil moisture (Figs. 6.2A, 6.2B, 6.2D).

Cumulative N_2O emissions during the second year differed across crops ($P < 0.0001$), but not with respect to fertilizer nor their interaction. The fallow treatment had the highest annual cumulative emissions and significantly differed from the perennial forage and perennial grain, but not from the spring or fall grain (Table 6.3). Emissions from highest to lowest ranked as follows: fallow = spring grain = fall grain ≥ perennial grain = perennial forage ($P < 0.0001$). Notably, perennial grain did not differ from perennial forage; both crops' emissions were reduced relative to the other two grain crops and the fallow treatment. The allocation of annual cumulative emissions by seasonal intervals in year two differed from those in year one, largely due to the universal reduction of fall contributions to annual emissions in year two compared to year one. The perennial forage had the lowest contribution from spring thaw and the spring grain the highest – a trend that was reversed during the summer period. For both the spring thaw and summer periods, the proportion of perennial grain emissions acted as an intermediate between the two ends of the perenniality spectrum (i.e., annual spring grain to perennial forage).

The third and final year at the Breton site had daily emission pulses that corresponded with (i) spring thaw, (ii) the first cut of the perennial forage crop and (iii) multiple significant precipitation events (Figs. 6.3A-E). Specifics of each emission pulse are as follows:

(i) The spring thaw period occurred from 18 April 2020 to 24 April 2020. Both spring grain and the fallow showed elevated emissions during this period, with peak emissions originating from the spring grain on the 21 April 2020 (44.73 ± 25.09 g N_2O-N ha^{-1} day^{-1}) (Fig. 6.3D).

(ii) As with years one and two, an emission pulse from the perennial forage was evident post-harvest on 16 July 2020, which peaked in the perennial forage on 5 August 2020 (14.10 ± 7.09 g N_2O-N ha^{-1} day^{-1}) (Fig. 6.3D).

(iii). Precipitation events throughout the year increased soil moisture, which corresponded with emission pulses specifically from the spring grain and fallow treatments (Figs. 6.3A, 6.3D).

Cumulative N_2O emissions for the third year were affected by crop alone ($P < 0.0001$). The perennial grain and forage crops showed reduced emissions relative to the spring grain and fallow but did not significantly differ from one another. Emissions ranked from highest to lowest as follows: spring grain = fallow > perennial forage = perennial grain ($P < 0.0001$). Fall grain did not germinate in the spring of 2021; therefore, emissions were not included in the statistical analysis (Table 6.3). The proportion of emissions from each measurement period varied between crops. The two perennial treatments (grain and forage) had comparatively higher emissions during the fall period than the spring grain and fallow. Interestingly, the perennial grain treatment had the greatest proportion of emissions during the spring thaw period, whereas all other crops had diminished contributions during the 2020 spring thaw. However, cumulative emissions were so low from the perennial grain that despite having a greater proportion of total emissions occur during spring thaw, actual N_2O emission from the perennial grain was much reduced relative to the other treatments (Table 6.3).

6.4.2.2 Edmonton

Daily N_2O emission pulses from the Edmonton site in year one corresponded to the spring thaw, which occurred from 18 April 2018 to 3 May 2018. Peak emissions were recorded from the fall rye crop on the 18 April 2018 (352.78 ± 133.75 g N_2O-N ha^{-1} day^{-1}) (Figs. 6.4A-C). Annual cumulative emissions were affected by crop alone ($P < 0.05$), but no effect of N fertilizer application, nor their interaction, could be discerned. The fall grain treatment had significantly greater emissions than the perennial forage, whereas the perennial grain, spring grain, and fallow treatments did not differ (Table 6.4). In terms of the proportion of cumulative emissions contributed by each measurement period, the spring thaw period provided most annual cumulative emissions for all crops except the perennial forage treatment, which produced a greater proportion of annual emissions during the summer measurement period (Table 6.4).

The second and final year at the Edmonton site showed daily emission pulses that corresponded with (i) spring thaw, (ii) N fertilizer application followed by a precipitation event and (iii) the first cut of the perennial forage crop (Figs. 6.5A-D). Specifics of each emission pulse are as follows:

(i) Spring thaw occurred from 26 March 2019 to 16 April 2019, during which time the highest daily emission (170.97 ± 14.11 g N_2O-N ha^{-1} day^{-1}) was recorded from the fallow treatment on 29 March 2019.

(ii) Fertilizer application on 23 May 2019 followed by 33.1 mm of rainfall between 6 June 2019 and 9 June 2019 led to increased soil moisture (Figs. 6.5A, 6.5B, 6.5D) and heightened emissions from the spring grain, perennial grain, and perennial forage crops for approximately three weeks. The highest emissions during the post-fertilizer period occurred from the spring grain, which peaked at 29.74 ± 6.17 g N_2O-N ha^{-1} day^{-1} on 13 June 2019.

(iii) After the first harvest, an emission pulse from the perennial forage plots was detected, which peaked on 17 July 2019 at 29.52 ± 9.66 g N_2O-N ha^{-1} day^{-1} (Fig. 6.5D).

Despite detecting an effect of crop type on N_2O emissions (ANOVA $P < 0.05$), post hoc comparisons resulted in a loss of significant differences between crops. Cumulative emissions from highest to lowest were as follows: spring grain > perennial grain > fallow > fall grain > perennial forage. The majority of emissions for each treatment were contributed during the summer period, except for the fallow, for which the majority of emissions occurred over the spring thaw interval.

6.4.3 OP-FTIR measurements of spring thaw nitrous oxide emissions

6.4.3.1 Year One

The OP-FTIR measurements for the first year at the Breton site were conducted from 3 April 2018 to 4 May 2018. A strong emission pulse was observed from both the perennial grain and spring grain crops. The emission pulses coincided with high volumetric soil moisture characteristic of spring thaw (Figs. 6.1B, 6.1E). The emission pattern and magnitude were very similar between the two contrasting crops, with both reaching peak emissions within one day of each other. The perennial grain reached peak emissions on the 24 April 2018 at 903.8 ± 45.2 g N_2O-N ha^{-1} day^{-1} and the spring grain on 25 April 2018 at 960.9 ± 37.9 g N_2O-N ha^{-1} day^{-1}. Compared to years two and three, year one had the highest spring-thaw emissions from both crops (Table 6.5).

6.4.3.2 Year Two

Year two measurements began on 27 March 2019 and were finished with the end of the spring thaw emission pulse on 14 April 2019 (Fig. 6.2E). Emissions from the spring grain peaked on 31 March 2019 at 285.2 ± 71.4 g N_2O-N ha^{-1} day^{-1}, whereas the perennial grain emissions were considerably lower, peaking on 7 April 2019 at 69.1 ± 34.3 g N_2O-N ha^{-1} day^{-1}. Spring grain emissions paralleled peak soil moisture (Figs. 6.2B, 6.2E). Cumulative emissions for the measurement period were markedly diminished in the perennial grain treatment relative to the spring grain treatment (Table 6.5).

6.4.3.3 Year Three

The third year of OP-FTIR measurements was conducted from 28 March 2020 to 3 May 2020. The perennial grain crop showed consistently higher emissions during the first two weeks of measurement relative to the spring grain, but neither treatment showed a substantial emission pulse until later in the measurement period. On the 21 and 22 of April 2020, the perennial grain and spring grain treatments peaked, respectively, at 163.6 ± 38.2 and 278.0 ± 52.9 g N_2O-N ha^{-1} day^{-1}, which aligned with peak soil moisture (Figs. 6.3B, 6.3E). Due to the higher emissions from the perennial grain treatment earlier in the measurement period, cumulative perennial grain emissions surpassed those of the spring grain treatment by 18% (Table 6.5).

6.4.4 Soil mineral N concentrations

6.4.4.1 Breton

More frequent soil samplings were conducted in years two and three than in year one; however, differences in soil mineral N concentrations between crops were still detected in year one ($P < 0.001$). Average mineral N concentrations in year one ranked from highest to lowest as follows: spring grain = fallow > perennial grain > fall grain > perennial forage. In the second year of the study (Fig. 6.2C), differences between crops remained evident ($P < 0.001$). Spring grain and fallow crops had significantly greater inorganic N than the fall grain, perennial grain, and perennial forage treatments, which were not different from one another. From highest mineral N to lowest in year two: spring grain = fallow > fall grain > perennial grain > perennial forage. In the third year (Fig. 6.3C), the perennial grain and fallow crops had reduced mineral N relative to the spring grain and the perennial forage treatment was intermediate and did not differ from any other treatment ($P < 0.05$).

6.4.4.2 Edmonton

Similar to the Breton site, fewer soil samplings in year one did not preclude the discovery of crop effect on mineral N concentrations ($P < 0.001$). Highest to lowest mineral N concentrations in year one ranked as follows: fallow > fall grain = spring grain > perennial grain = perennial forage. In year two (Fig. 6.5C), differences between crops also existed ($P < 0.001$). From highest to lowest mineral N in year two: spring grain = fallow > fall grain = perennial grain = perennial forage.

6.4.5 Correlation and regression analyses

Correlation analyses between average root density from 0-60 cm and average mineral N from 0-15 cm for years one and two at the Breton and Edmonton sites showed a significant negative correlation at the Breton site in year one ($\rho = -0.68$, $P < 0.01$), year two ($\rho = -0.55$, $P < 0.05$), the Edmonton site in year one ($\rho = -0.69$, $P < 0.01$) and year two ($\rho = -0.52$, $P < 0.05$). Interestingly, at the Breton site in year two, average root density was also negatively correlated with cumulative N_2O emissions ($\rho = -0.61$, $P < 0.01$, Fig. 6.6B). A linear regression of average root density versus cumulative N_2O emissions reinforced the significant negative relationship between the two variables ($P < 0.01$, $R^2 = 0.39$.) (Fig. 6.7). As expected, soil N and soil C were consistently positively correlated (Ps <0.05).

6.4.6 Yield-based emission factors

Yield-based emission factors were calculated based on grain alone, forage alone (i.e., aboveground biomass without grain) and total aboveground biomass (i.e., forage and grain) for the Edmonton and Breton sites for years one and two. Year three at the Breton site was harvested entirely as forage (without separating grain) due to adverse weather severely affecting seeding and harvest timing, thus values would not be representative.

All EFy results (grain, forage, and total biomass) were affected by crop type in the first year at the Breton site ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively) (Table 6.6). The EFy grain of the perennial grain crop was significantly greater than that of fall grain, but neither differed from the spring grain. The EFy forage was highest for the perennial forage treatment and none of the three grain-bearing treatments significantly differed from each other. When total aboveground biomass was considered, the EFy for the perennial grain treatment did not significantly differ from the other grain–bearing treatments but was significantly reduced compared to the perennial forage. Year two at the Breton site showed no significant differences in EFy.

Crop type affected the EFy at the Edmonton site in year one for grain, forage, and total aboveground biomass ($P < 0.01$, $P < 0.001$ and $P < 0.05$) and for year two ($P < 0.001$, $P < 0.05$ and $P < 0.05$) (Table 6.7). For both years, based on grain alone (EFy grain), the perennial grain had a significantly higher EFy than the other crops. However, when forage was included and total aboveground biomass was considered, the perennial grain crop in year two had a reduced EFy relative to the perennial forage but did not differ in magnitude from the fall grain and spring crops.

6.5 Discussion

6.5.1 Cumulative N_2O emission reduction in Gray Luvisolic soils under multi-year perennial grain

Emission reduction by perennial grain cropping began to manifest at the Breton site in years two and three of the study, but no significant differences between treatments in cumulative N_2O emissions were discerned at the Edmonton site in the second year of flux measurements (Tables 6.3 and 6.4). Significant differences in year one at the Edmonton site were due to high emissions from the fall grain crop, a trend which was not evident in the second year. The higher potential for perennial grain cropping to reduce N_2O emissions at the Breton site than at the Edmonton site is likely due to differences in soil characteristics, climate, and land use history.

Soils at the Breton and Edmonton sites are classified as Gray Luvisols and Black Chernozems, respectively. Compared to Gray Luvisols, Black Chernozems have higher C and N contents, the cycles of which are strongly interlinked. Previous research has found that increased soil organic C (SOC) abundance and dynamics can promote anaerobiosis as a result of simultaneous microbial consumption of C substrate and O_2, thus triggering denitrification and increased N_2O emissions (Daly and Hernandez-Ramirez, 2020; Guenet et al., 2021; Mei et al., 2018). In fact, Abraha et al. (2018) found labile C to be a strong controlling factor for N_2O emissions, regardless of N fertilizer application rates or land use history. Additionally, Edmonton soils have twofold the amount of clay relative to the Breton site, which promotes denitrification at relatively lower soil moisture than soils lower in clay, due to the formation of anoxic microsites in small pores (Table 6.1) (Balaine et al., 2016; Butterbach-Bahl et al., 2013, Pihlatie et al., 2004). Thus, we hypothesize that these overarching soil characteristics at the Edmonton site overrode any crop specific effects on cumulative N_2O emissions.

Conversely, crop effects at the Breton site, such as the significant reduction in mineral N concentration with perennial cropping compared to annual, were able to translate into reduced N_2O emissions in this soil, which was lower in C, N, and clay (Figs 6.2C, 6.3C). While a reduction in mineral N concentrations under the perennial treatments was also evident at the Edmonton site, the overall availability of mineral N was markedly increased compared to the Breton site, suggesting that even with the increased N acquisition efficiency of perennial roots (Weih et al., 2011), there was still ample mineral N available for the formation of N_2O in the Chernozem soil at the Edmonton site. Notably, cumulative N_2O emissions and average mineral N concentrations at the Breton site in year two were negatively correlated with average root density from 0-60 cm (Fig. 6.6B), suggesting that the enhanced root density of perennial crops (both grain and forage) was an effective factor in reducing emissions, potentially because of enhanced water and N uptake efficiencies (Abalos et al., 2016; Kim et al. 2020; Sprunger et al., 2018). This is in contrast to literature that has suggested increases in root density may increase N_2O emissions due to increased C availability for denitrifying microbes (Meier, 2017; Thomas et al., 2017). However, solely focusing on increased C input does not account for the ability for dense perennial root systems to offset this effect with increased water and mineral N uptake. Essentially, perennial vegetation has the ecological advantage to access and utilize these resources over greater spatial-temporal extents thereby competing and preventing excess in their availabilities for microbial N_2O-producing processes.

Additionally, the Breton site is much wetter than the Edmonton site in terms of average yearly precipitation (Table 6.1). It is well established that soil moisture is a major, overriding control on N_2O emissions (Abraha et al., 2018; Butterbach Bahl et al., 2013; Firestone and Davidson, 1989; Roman-Perez and Hernandez-Ramirez, 2021), thus the potential for divergent crops to alter soil moisture dynamics was more pronounced at the wetter Breton site. Interestingly, correlation analyses at the Breton site indicated that average volumetric water content at 5 cm was positively correlated with average root density from 0-60 cm (Fig. 6.6B). This may seem counterintuitive, as one would expect increased root density to reduce soil water content. However, our findings coincide with those of Abalos et al (2016), who found that in comparison to an adjacent annual crop, the perennial forage was wetter in the top 5 cm because the dense, deeply penetrating roots sourced water from deeper in the profile than shallow-rooted annuals (Glover et al., 2007). Concurrently, this dense root system can improve soil structure by preventing and counteracting anaerobic microsite formation, as well as subsequent N_2O production (Hebb et al., 2017; Kim et al., 2021; van Kessel et al., 2013).

Land use history may have also played an important role with respect to the patterns in N_2O emissions observed at the Breton site. As opposed to the long-term annual cropping history of the Edmonton site, Breton was maintained as an unfertilized, hayed pasture for up to 60 years prior to treatment establishment. Previous research has shown that microbial communities harbored under long-term perennial vegetation are larger, more diverse, and capable of increased consumption of N_2O via denitrification resulting from an increased *nos-Z* bearing community, the gene for the terminal enzyme of denitrification, N_2O reductase, which reduces N_2O to N_2 (Domeignoz-Horta et al., 2015; Horn et al., 2006; Thompson et al., 2016). Additionally, historically unfertilized soils may select nitrifiers with low tolerance to high NH_4^+ inputs, resulting in low nitrification derived N_2O even when NH_4^+ is provided via fertilization (Liang and Robertson, 2021). Thus, N_2O emission reduction under restored perennial vegetation may have been supported by the reestablishment of the previous microbial community due to the powerful carry-over influence of historical land use on community gene diversity and composition (Herzberger et al., 2014; Jangid et al., 2011).

Further, land use history of long-term hay forage partially explains the lack of a response in N_2O emissions in the first year at Breton. Prior to treatment establishment, the perennial hay field in Breton was rotary tilled in June 2017. Ample N from mineralizing roots and grass residues, as well as increased soil organic matter decomposition from the tillage disturbance, masked any crop specific effect on N_2O emissions (Moore et al., 2020; Mukumbuta et al., 2020; Thilakarathna and Hernandez-Ramirez, 2021). This legacy effect, combined with the time required for perennial crops to build up belowground reserves and subsequently alter the soil environment (Smith et al., 2013), led to a delayed response in N_2O emission reduction that did not manifest until the second year of the experiment. However, despite being managed more similarly to an annual crop in terms of seeding rate, seed placement, harvest timing, N fertilization rate and diversity (i.e., grown as a monoculture), the perennial grain treatment mimicked the previously well-documented ability of perennial forage and bioenergy crops to reduce cumulative N_2O emissions after one year of growth (Drewer et al., 2012; Gelfand et al., 2016; Rochette et al., 2018).

6.5.2 OP-FTIR measurements reinforce differences in spring thaw emissions between annual and perennial grain crops

Static chamber measurements for year one at the Breton site captured only a portion of the emission pulse measured by the OP-FTIR micrometeorological set up, thus the magnitude of spring thaw emissions measured by static chamber was likely underestimated for both the perennial grain and the spring grain treatments (Figs. 6.1C, 6.1D). This is a common criticism of chamber methodology and reinforces the value of emission measurement systems deployable earlier in the season, such as OP-FTIR (Ball et al., 2000; Butterbach Bahl et al., 2002; Butterbach Bahl et al., 2013). Despite the missing chamber measurements at the onset of thaw, the pattern measured by the OP-FTIR instrumentation was consistent with the intermittent emissions that were captured by the static chambers. This supports our hypothesis that the legacy effect of tilling a long-term perennial hay stand overrode divergent emission patterns in the contrasting crops in the first year of measurements (Moore et al., 2020; Mukumbuta et al., 2020; Thilakarathna and Hernandez-Ramirez, 2021).

Similarly, close agreement between OP-FTIR and chamber measurements was observed in year two. Relative to the annual crops, reductions in N_2O emissions from the perennial grain crop during the spring thaw period was a major contributor to the overall reduction in cumulative N_2O emissions from the perennial grain treatment in year two. We hypothesize that this comparative reduction in emissions by the perennial grain relative to the spring grain is in large part due to differences in the length of the growing season. Perennials begin utilizing water and mineral N immediately after snowmelt, several weeks prior to seeding of an annual crop, thus preventing the formation of N_2O via denitrification in perennial fields (Abalos et al., 2016; Ferchaud et al., 2015 Gregorich et al., 2005; Rochette et al., 2018). Notably, while the perennial grain reduced N_2O emissions during the spring thaw period, it did not do so to the extent of the perennial forage. The perennial forage had lowest average mineral N content in year two and substantially reduced spring N_2O emissions in line with previous research (Dusenbury et al., 2008; Tenuta et al., 2019). Emission reduction by the perennial grain was not as substantial, likely due to lower plant diversity and density when compared to perennial forages; both characteristics that contribute to long-term N immobilization (Redin et al., 2014; Regehr et al., 2015).

Spring thaw emissions from year three at the Breton site measured by OP-FTIR and static chamber methods differed in their magnitude but maintained the pattern of higher emissions from the spring grain crop during peak thaw. However, the OP-FTIR measurements revealed slightly elevated N_2O emissions from the perennial grain crop in late March – a period when static chamber measurements had not commenced. These emissions in late March coincided with a minor, short-lived increase in soil moisture and temperature while the ground was still snow-covered, reinforcing the value of early spring emission measurements via the OP-FTIR when chamber locations are inaccessible. A proposed mechanism to explain these perennial grain crop emissions was postulated by Thomas et al. (2017) who stated that non-legume over-winter crops with substantial root density can increase the connectivity between denitrification microbes and their substrates (C and NO_3^-) within shallow rhizospheres that experience freeze-thaw cycles.

Notably, should climate change result in earlier, less predictable spring thaw events with multiple freeze-thaw cycles, underestimation of N_2O emissions from croplands could result without the adoption of field methods that can capture these early emissions, such as micrometeorological techniques like our OP-FTIR based system. Specifically, modelling has projected Alberta weather to become less predictable, subject to more fluctuations and overall warmer by 3 to 5°C relative to climate normals from 1961-1990 (Barrow and Yu, 2005; Forbes et al., 2011; Wheaton, 2001). Thus, the importance of continuous, large-footprint measurements during the crucial time of peak N_2O emissions following soil thawing will only continue to increase if accurate emission data is to be included in modelling efforts evaluating the paramount feedback to future climate change scenarios.

6.5.3 Perennial grain cropping as a dual-purpose crop to reduce EFy

Maximizing economic viability and environmental conservation by way of reducing N_2O emissions is an important consideration with respect to perennial grains, as previous research has indicated reduced grain yields from these novel perennial cultivars compared to their annual counterparts (Daly et al., 2022; Jungers et al., 2018; Murphy et al., 2010; Pimentel et al., 2012; Ploschuk et al., 2005). Economic productivity and environmental sustainability are inherently linked (van Groenigen et al., 2010), thus the tradeoff between the two must be investigated to assess if perennial grains have a net positive impact. We calculated three EFy metrics for each treatment studied, to account for both grain and forage components and the findings were consistent; perennial grains may be environmentally and economically viable if grain and forage yields are collectively considered, but not when perennial grain is considered solely a grain crop. Indeed, the EFy grain of the perennial grain was significantly increased compared to the fall and spring grain treatments in all years, except for the second year at the Breton site, when excessive moisture consistently reduced all grain yields. However, when the perennial forage was included, the EFy total was comparable among all grain-bearing treatments, a result of the prolific biomass production in the perennial grain crop (Daly et al., 2022). Granted, including a forage component into the calculation of EFy assumes both grain and forage have marketable value, which would require a detailed economic assessment and should be considered as an area for future research.

6.6 Conclusion

Our results suggest that the ability for perennial grain crops to reduce soil N_2O emissions relative to an annual counterpart is site-specific, depending on a multitude of factors including soil type, climate, and land use history. One proposed mechanism for this emission reduction is the greater root density of perennial grain crops reducing soil mineral N contents, as shown in our study. Further, micrometeorological measurements of sporadic, dynamic N_2O emissions during spring thaw indicate that there are benefits of using a micrometeorological measurement technique as a tool to capture emissions during this crucial time. Overall, perennial grain cropping may have the capability to balance environmental and economic considerations if harvested as a dual-purpose grain and forage crop.

6.7 References

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Tables

Table 6.1 Select baseline soil properties from the Edmonton and Breton sites.

Soil Properties	**Breton**	**Edmonton**
Canadian classification	Gray Luvisol	Black Chernozem
Total carbon (TC) (g C kg^{-1}) (0-30 cm)	19.2 ± 3.9	41.6 ± 7.5
Total nitrogen (TN) (g N kg^{-1}) (0-30 cm)	1.7 ± 0.3	3.6 ± 0.5
Available nitrogen (NH_4^+ & NO_3^-) (mg N kg^{-1}) (0 – 15 cm)	55.5 ± 2.5	48.3 ± 4.5
pH (1:5 H_2O)	6.1 ± 0.08	7.3 ± 0.09
Bulk density (g cm^{-3}) (5-30 cm)	1.1 ± 0.06	1.0 ± 0.06
Soil texture	loam	silty clay
% clay	24.8	48.3
% silt	41.8	35.7
% sand	33.3	16.0
Climate normals		
Average yearly temp (°C) §	3.4	4.2
Cumulative yearly precipitation (mm yr^{-1}) §	456	602

§ These are long-term 30-yr normals 1981-2010) (Government of Canada, 2020).

Table 6.2. Timing of major field activities at the Edmonton and Breton sites.

Activity	2017-2018	2018-2019	2019-2020
		Edmonton	
Initial tillage[₸]	7-Jun-17	n.a.	n.a.
Perennial forage seeding	16-Jun-17		
Perennial grain seeding	30-Aug-17		
Fall grain seeding[⌈]	30-Aug-17	6-Sep-18	
Spring grain seeding[⌈]	22-May-18	23-May-19	
N fertilizer application	22-May-18	23-May-19	
1st harvest perennial forage	26-Jun-18	16-Jul-19	
2nd harvest perennial forage	31-Aug-18	17-Sep-19	
Fall grain harvest	31-Aug-18	2-Oct-19	
Spring grain harvest	31-Aug-18	2-Oct-19	
		Breton	
Initial tillage[₸]	6-Jun-17	n.a.	n.a.
Perennial forage seeding	8-Jun-17		
Perennial grain seeding	30-Aug-17		
Fall grain seeding[⌈]	30-Aug-17	6-Sep-18	4-Oct-19
Spring grain seeding[⌈]	21-May-18	21-May-19	29-Jun-20
N fertilizer application	21-May-18	21-May-19	7-May-20, 29-Jun-20[δ]
1st harvest perennial forage	26-Jun-18	15-Jul-19	16-Jul-20
2nd harvest perennial forage	30-Aug-18	16-Sep-19	17-Sep-20
Perennial grain harvest	23-Aug-18	25-Aug-19	17-Sep-20
Fall grain harvest	23-Aug-18	24-Sep-19	n.a[⸸]
Spring grain harvest	3-Oct-18	24-Sep-19	17-Sep-20

[₸] Entirety of experimental area tilled prior to treatment establishment.
[⌈] Plots for spring and fall grain treatments tilled concurrent with seeding each season.
[δ] Perennial grain and forage plots fertilized on 7 May 2020, spring grain plots fertilized on 29 June 2020.
[⸸] Fall grain did not germinate this year, thus no harvest was completed.

Table 6.3. Annual cumulative N_2O emissions (AnCumN_2O), the proportion of AnCumN_2O during fall, spring thaw, and summer periods as measured by static chambers for each treatment type at the Breton site.

Treatment	Proportion of AnCumN_2O (%)			AnCumN_2O (kg N_2O-N ha^{-1})[ǂ]
	Fall	Spring	Summer	
		Year One		
	1 Sept. 2017 - 25 Oct. 2017 (55 d)	28 April 2018 - 8 May 2018 (11 d)	9 May 2018 - 31 Aug. 2018 (105 d)	1 Sept. 2017 - 31 Aug. 2018
Perennial Forage	49.9	16.9	33.2	0.28 ± 0.058 a
Perennial Grain	50.3	21.3	28.4	0.35 ± 0.088 a
Fall Grain	49.3	15.3	35.4	0.33 ± 0.052 a
Spring Grain	69.8	5.4	24.8	0.30 ± 0.030 a
Fallow	70.9	5.5	23.6	0.29 ± 0.001 a
		Year Two		
	1 Sept. 2018 - 1 Nov. 2018 (62 d)	30 March 2019 - 15 April 2019 (17 d)	16 April 2019 - 31 Aug. 2019 (138 d)	1 Sept. 2018 - 31 Aug. 2019
Perennial Forage	4.2	7.5	88.3	0.87 ± 0.42 a
Perennial Grain	6.4	30.7	63.0	1.05 ± 0.26 ab
Fall Grain	6.9	43.4	49.7	2.06 ± 0.25 bc
Spring Grain	0.5	62.2	37.3	2.65 ± 0.52 c
Fallow	2.9	60.4	36.7	3.26 ± 1.02 c
		Year Three		
	1 Sept. 2019 - 18 Oct. 2019 (48 d)	18 April 2020 -24 April 2020 (7 d)	25 April 2020 - 31 Aug. 2020 (129 d)	1 Sept. 2020- 31 Aug. 2020
Perennial Forage	24.8	-1.1	76.3	0.49 ± 0.22 b
Perennial Grain	34.3	19.1	46.6	0.23 ± 0.04 b
Spring Grain	3.5	6.7	89.8	4.09 ± 1.42 a
Fallow	11.7	4.0	84.3	3.44 ± 0.99 a

[ǂ] Data represents mean ± standard error (n=8).

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 6.4. Annual cumulative N_2O emissions (AnCumN$_2$O), the proportion of AnCumN$_2$O during fall, spring thaw, and summer periods as measured by static chambers for each treatment type at the Edmonton site.

	Proportion of AnCumN$_2$O (%)			**AnCumN$_2$O (kg N_2O-N ha^{-1})ǂ**
Treatment	Fall	Spring Thaw	Summer	
		Year One		
	1 Sept. 2017 - 20 Oct. 2017 (50 d)	18 April 2018 - 3 May 2018 (16 d)	4 May 2018 - 31 Aug. 2018 (120 d)	1 Sept. 2017 - 31 Aug. 2018
Perennial Forage	20.8	38.2	40.9	1.45 ± 0.11 a
Perennial Grain	16.7	58.7	24.6	2.33 ± 0.40 ab
Fall Grain	16.2	64.1	19.7	3.32 ± 0.76 b
Spring Grain	20.2	60.0	19.8	1.85 ± 0.28 ab
Fallow	20.5	60.6	18.9	1.84 ± 0.25 ab
		Year Two		
	1 Sept 2018 - 31 Oct. 2018 (61 d)	26 March 2019 - 16 April 2019 (22 d)	17 April 2019 - 31 Aug. 2019 (137 d)	1 Sept. 2018 - 31 Aug. 2019
Perennial Forage	8.9	16.2	74.9	1.12 ± 0.15 a
Perennial Grain	5.2	32.7	62.1	1.75 ± 0.35 a
Fall Grain	9.0	39.9	51.1	1.23 ± 0.17 a
Spring Grain	4.1	43.1	52.8	1.77 ± 0.32 a
Fallow	0.0	63.3	36.7	1.69 ± 0.34 a

ǂData represents mean ± standard error (n=8).

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 6.5. Cumulative N_2O emissions measured during spring thaw at the Breton site from the perennial and spring grain treatments via the OP-FTIR micrometeorological measurements.

Treatment	Spring thaw cumulative N_2O (kg N_2O-N ha^{-1}) [ⱡ]		
	Year One	Year Two	Year Three
	3 April 2018 - 4 May 2018 (31 d)	27 March 2019 - 14 April 2019 (14 d)	28 March 2020 - 3 May 2020 (36 d)
Perennial grain	9.5 ± 0.3	0.3 ± 0.4	2.2 ± 0.5
Spring grain	9.0 ± 0.5	2.6 ± 0.6	1.8 ± 0.6

[ⱡ] Data represents mean ± standard error, which was calculated by summing daily standard errors, then scaled by the number of measurement days.

Table 6.6. Yield-based Emission Factors (EFy) in g N_2O-N kg^{-1} DM for each treatment for grain, forage (aboveground biomass without grain) and total aboveground biomass (grain and forage) for the Breton site.

	EFy grain[ⱡ]	**EFy forage**[ⱡ]	**EFy total**[ⱡ]
Treatment	Year One		
Perennial Forage	n.a.	0.08 ± 0.01 a	0.08 ± 0.10 a
Perennial Grain	0.12 ± 0.01 a	0.03 ± 0.01 b	0.02 ± 0.01 b
Fall grain	0.06 ± 0.01 b	0.03 ± 0.01 b	0.02 ± 0.001 b
Spring Grain	0.09 ± 0.01 ab	0.05 ± 0.001 ab	0.03 ± 0.001 b
	Year two		
Perennial Forage	n.a.	0.35 ± 0.13 a	0.35 ± 0.13 a
Perennial Grain	1.17 ± 0.49 a	0.50 ± 0.18 a	0.39 ± 0.10 a
Fall grain	1.06 ± 0.13 a	0.44 ± 0.04 a	0.42 ± 0.04 a
Spring Grain	1.09 ± 0.16 a	0.51 ± 0.08 a	0.49 ± 0.08 a

[ⱡ] Data represents mean ± standard error (n = 8 for all treatments & years except perennial grain in year two, for which n = 2).

Lowercase letters denote significant differences between crops 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 6.7. Yield-based Emission Factors (EFy) in g N_2O-N kg^{-1} DM for each treatment for grain, forage (aboveground biomass without grain) and total aboveground biomass (grain and forage) for the Edmonton site.

Treatment	EFy grain⸸	EFy forage⸸	EFy total⸸
	Year One		
Perennial Forage	n.a.	0.55 ± 0.07 a	0.55 ± 0.07 a
Perennial Grain	1.03 ± 0.12 a	0.76 ± 0.11 a	0.43 ± 0.06 ab
Fall grain	0.67 ± 0.1 ab	1.63 ± 0.25 b	0.47 ± 0.07 ab
Spring Grain	0.46 ± 0.06 b	0.77 ± 0.09 a	0.29 ± 0.03 b
	Year two		
Perennial Forage	n.a.	0.46 ± 0.07 a	0.46 ± 0.07 a
Perennial Grain	2.00 ± 0.12 a	0.24 ± 0.11 b	0.24 ± 0.06 b
Fall grain	0.57 ± 0.06 b	0.37 ± 0.03 ab	0.35 ± 0.03 ab
Spring Grain	0.84 ± 0.07 b	0.38 ± 0.04 ab	0.36 ± 0.04 ab

⸸Data represents mean ± standard error (n = 8).

Lowercase letters denote significant differences between crops, 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 $p < 0.05$.

Figures

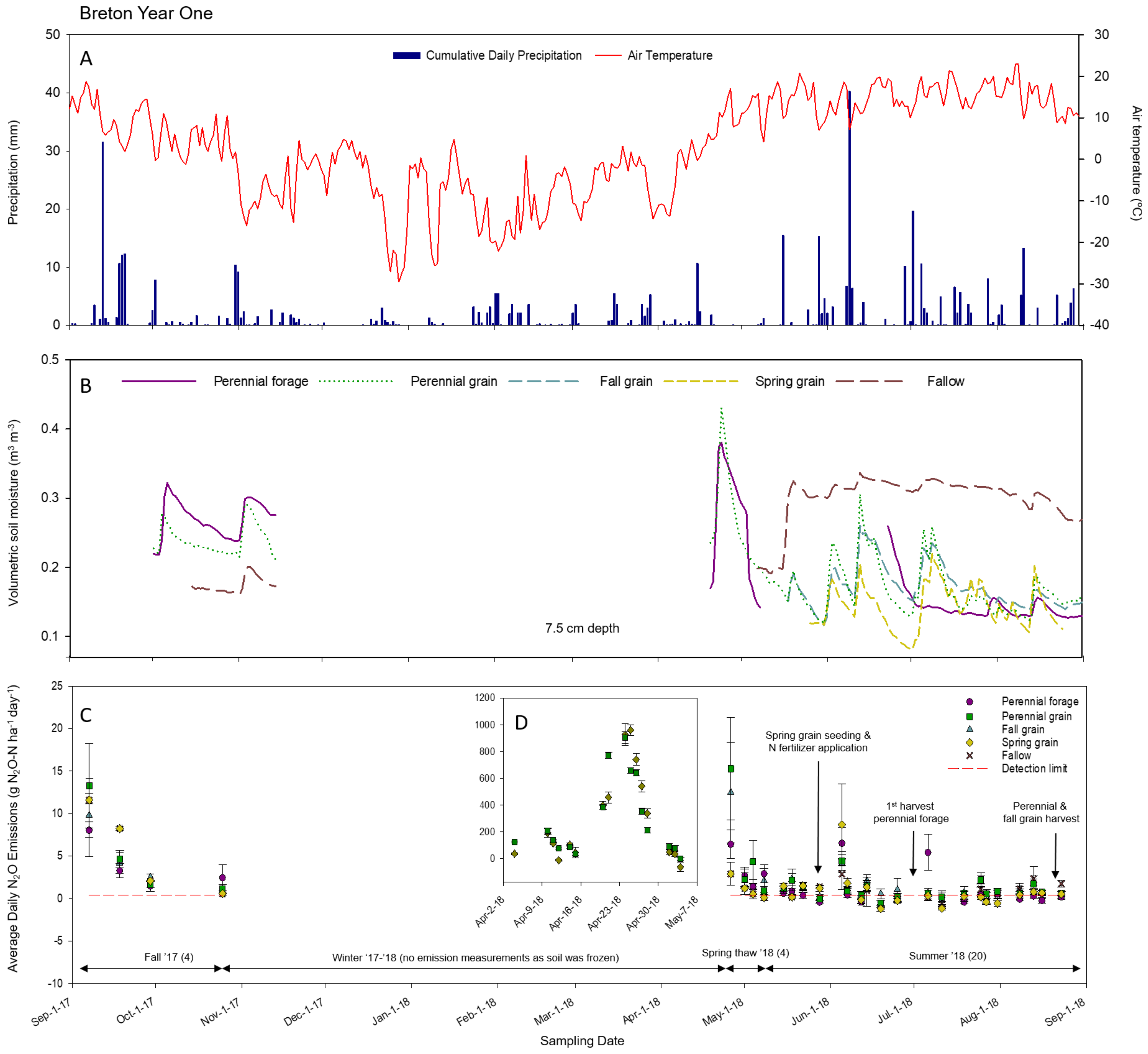

Figure 6.1. Field observations from the Breton site in year one. From top panel (A) to bottom panel (D): (A) precipitation and temperature, (B) volumetric soil moisture for all treatments at 7.5 cm depth, (C) daily chamber-measured N_2O-N emissions from the replicated plots where points represent the average of each replicate ± SE (n=8) and (D) daily OP-FTIR-measured N_2O-N emissions from the adjacent 4 ha fields. Note that the x-axis is the same for panels A to C.

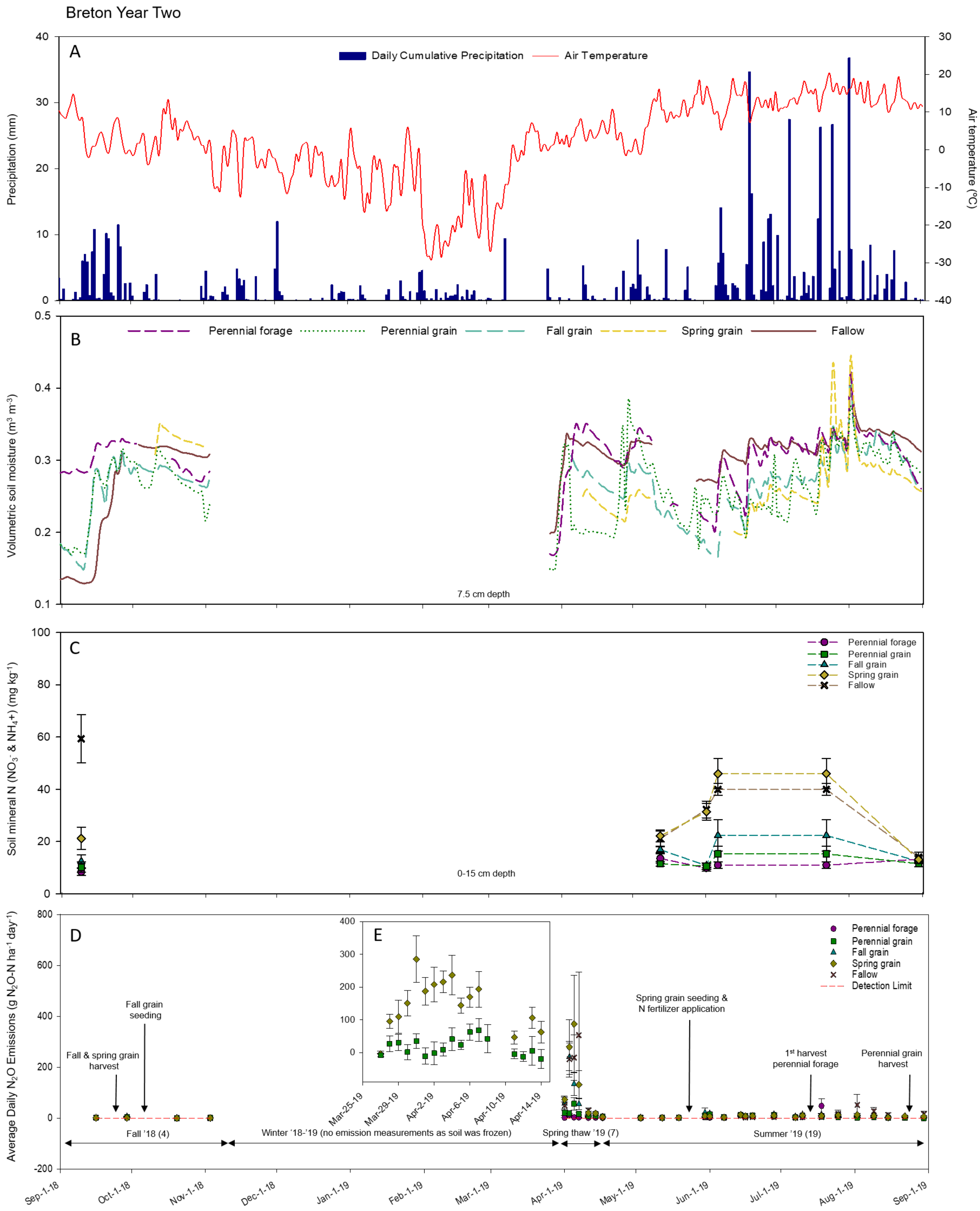

Figure 6.2 Field observations from the Breton site in year two. From top panel (A) to bottom panel (E): (A) precipitation and temperature, (B) volumetric soil moisture for all treatments at 7.5 cm depth, (C) soil mineral N concentrations from the replicated plots (0-15 cm) where points represent average of each replicate ± SE (n=8), (D) daily chamber-measured N_2O-N emissions from the replicated plots where points represent the average of each replicate ± SE (n=8) and (E) daily OP-FTIR-measured N_2O-N emissions from the adjacent 4 ha fields. Note that the x-axis is the same for panels A to C.

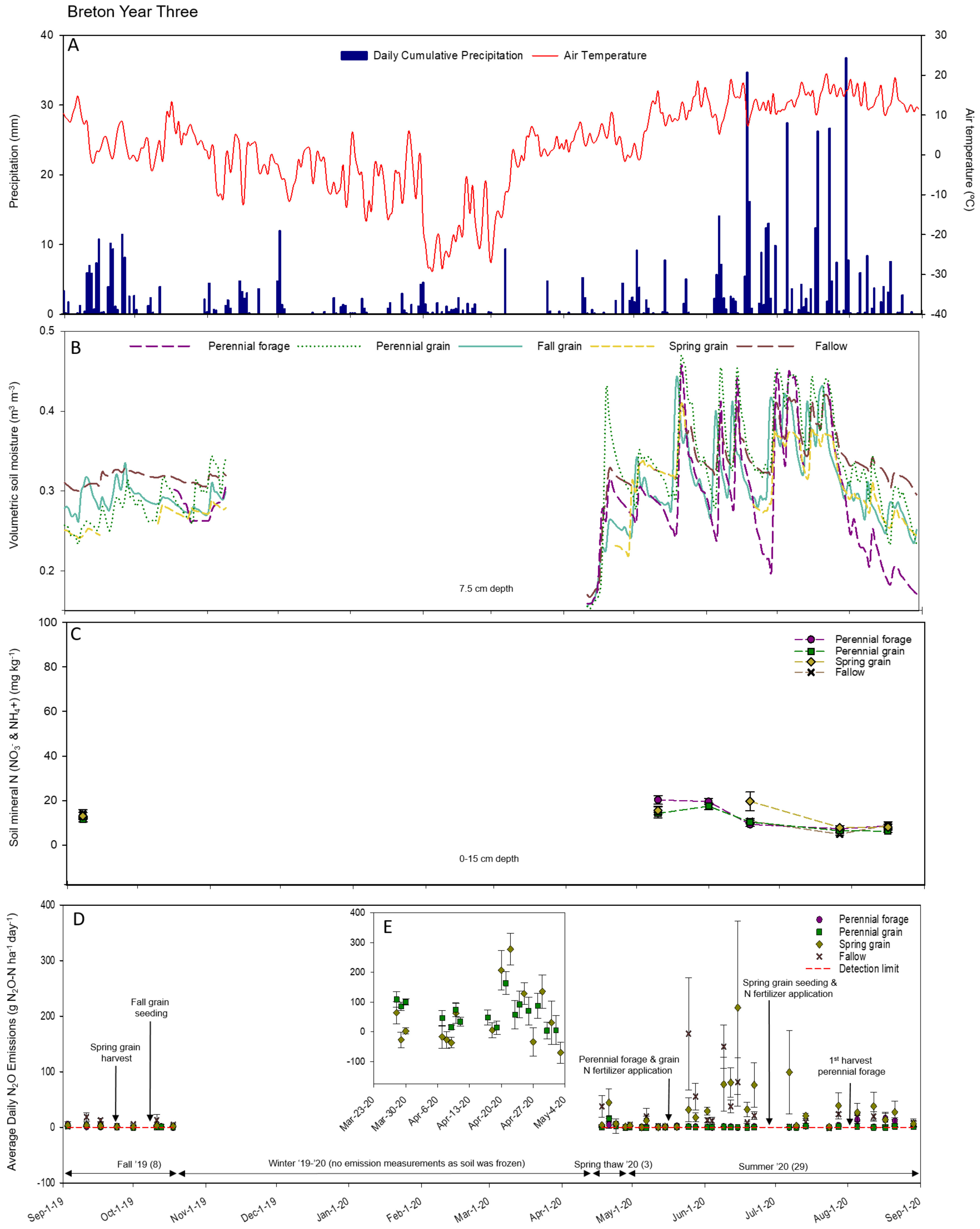

Figure 6.3. Field observations from the Breton site in year three. From top panel (A) to bottom panel (E): (A) precipitation and temperature, (B) volumetric soil moisture for all treatments at 7.5 cm depth, (C) soil mineral N concentrations from the replicated plots (0-15 cm) where points represent average of each replicate ± SE (n=8), (D) daily chamber-measured N_2O-N emissions from the replicated plots where points represent the average of each replicate ± SE (n=8) and (E) daily OP-FTIR-measured N_2O-N emissions from the adjacent 4 ha fields. Note that the x-axis is the same for panels A to C.

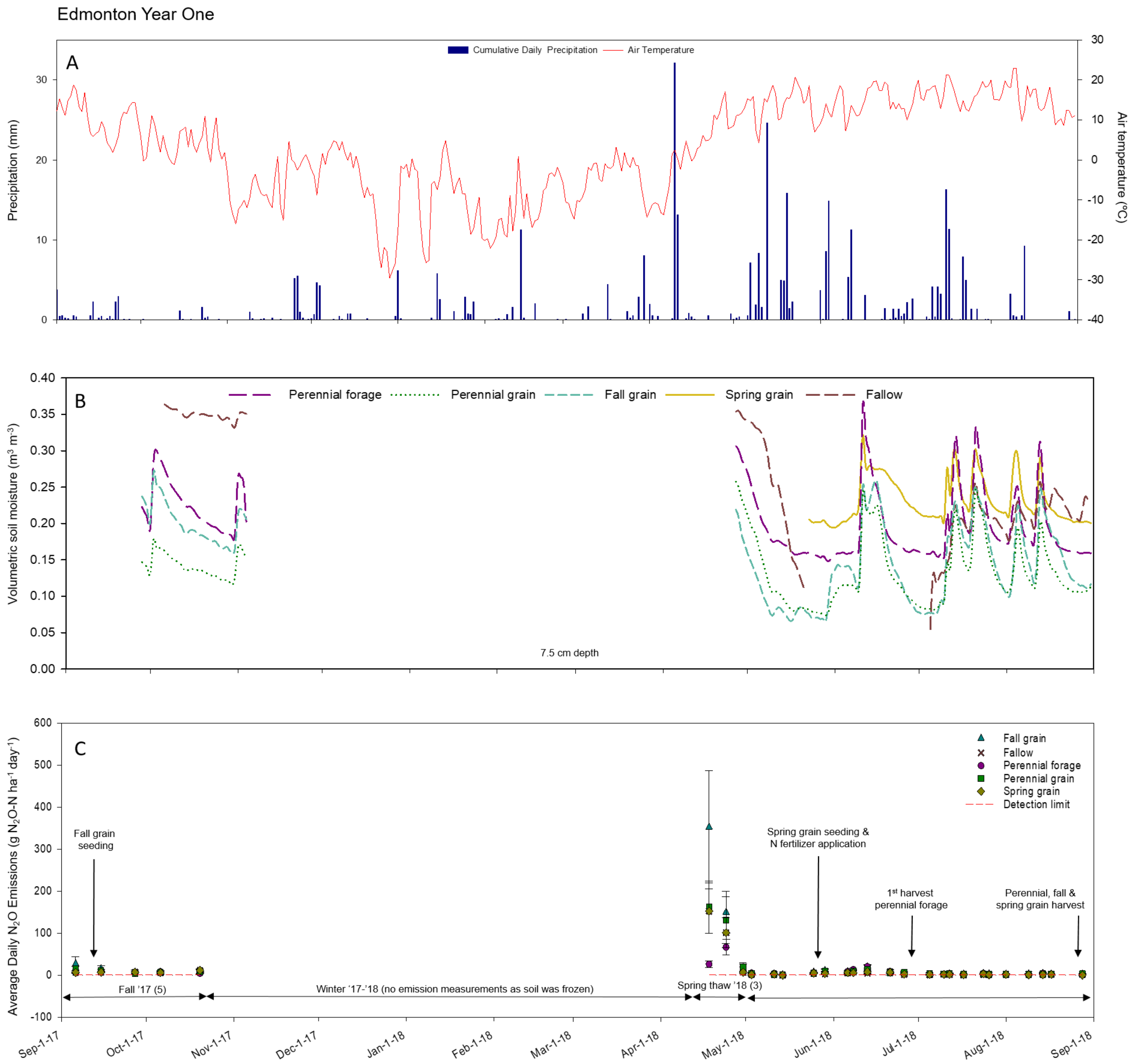

Figure 6.4. Field observations from the Edmonton site in year one. From top panel (A) to bottom panel (C): (A) precipitation and temperature, (B) volumetric soil moisture for all treatments at 7.5 cm depth and (C) daily N_2O-N emissions from the replicated plots where points represent average of each replicate ± SE (n=8). Note that the x-axis is the same for all panels.

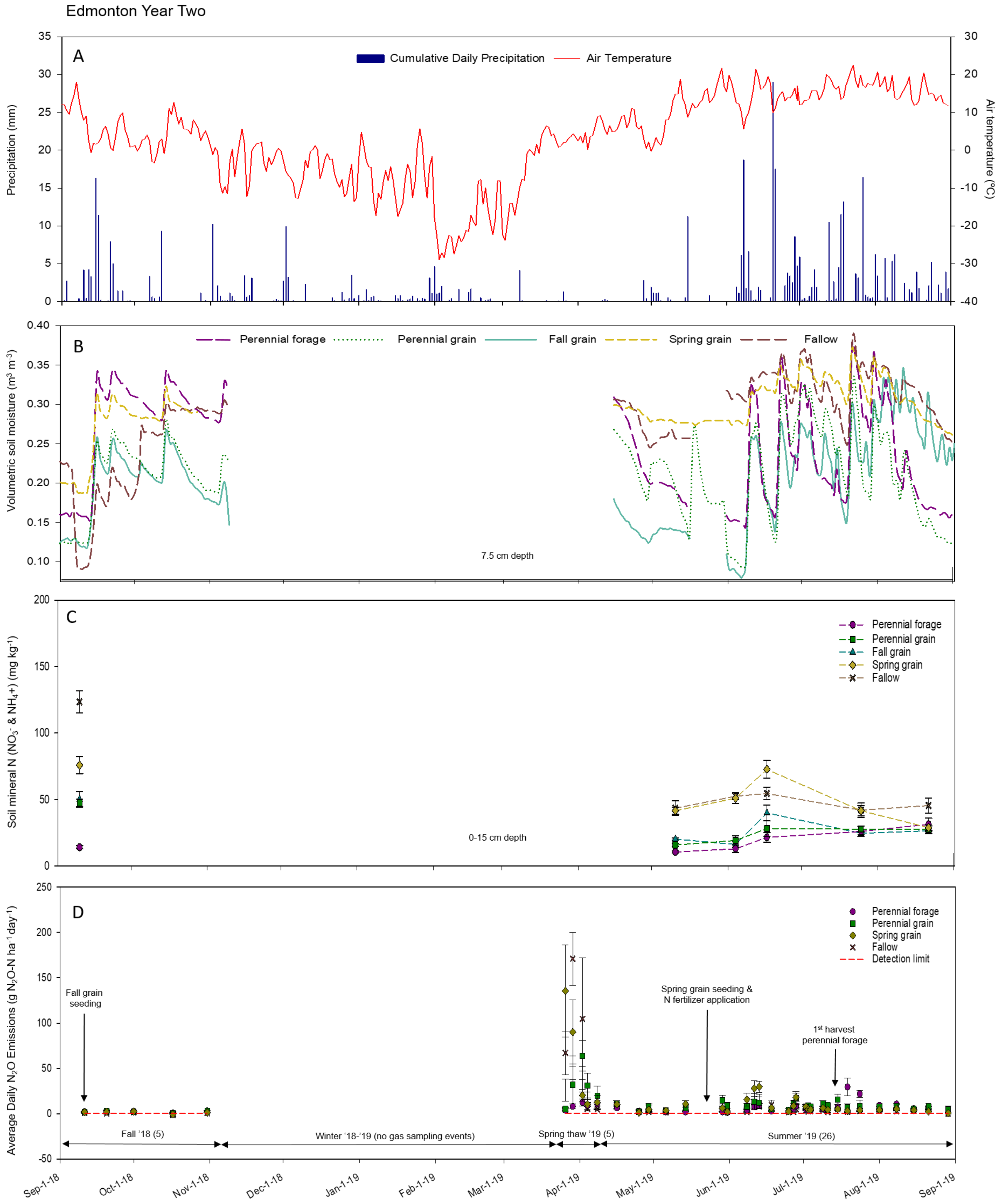

Figure 6.5. Field observations from the Edmonton site in year two. From top panel (A) to bottom panel (D): (A) precipitation and temperature, (B) volumetric soil moisture for all treatments at 7.5 cm depth, (C) soil mineral N concentrations from the replicated plots (0-15 cm) where points represent average of each replicate ± SE (n=8) and (D) daily N_2O-N emissions from the replicated plots, points represent average of each replicate ± SE (n=8). Note that the x-axis is the same for all panel

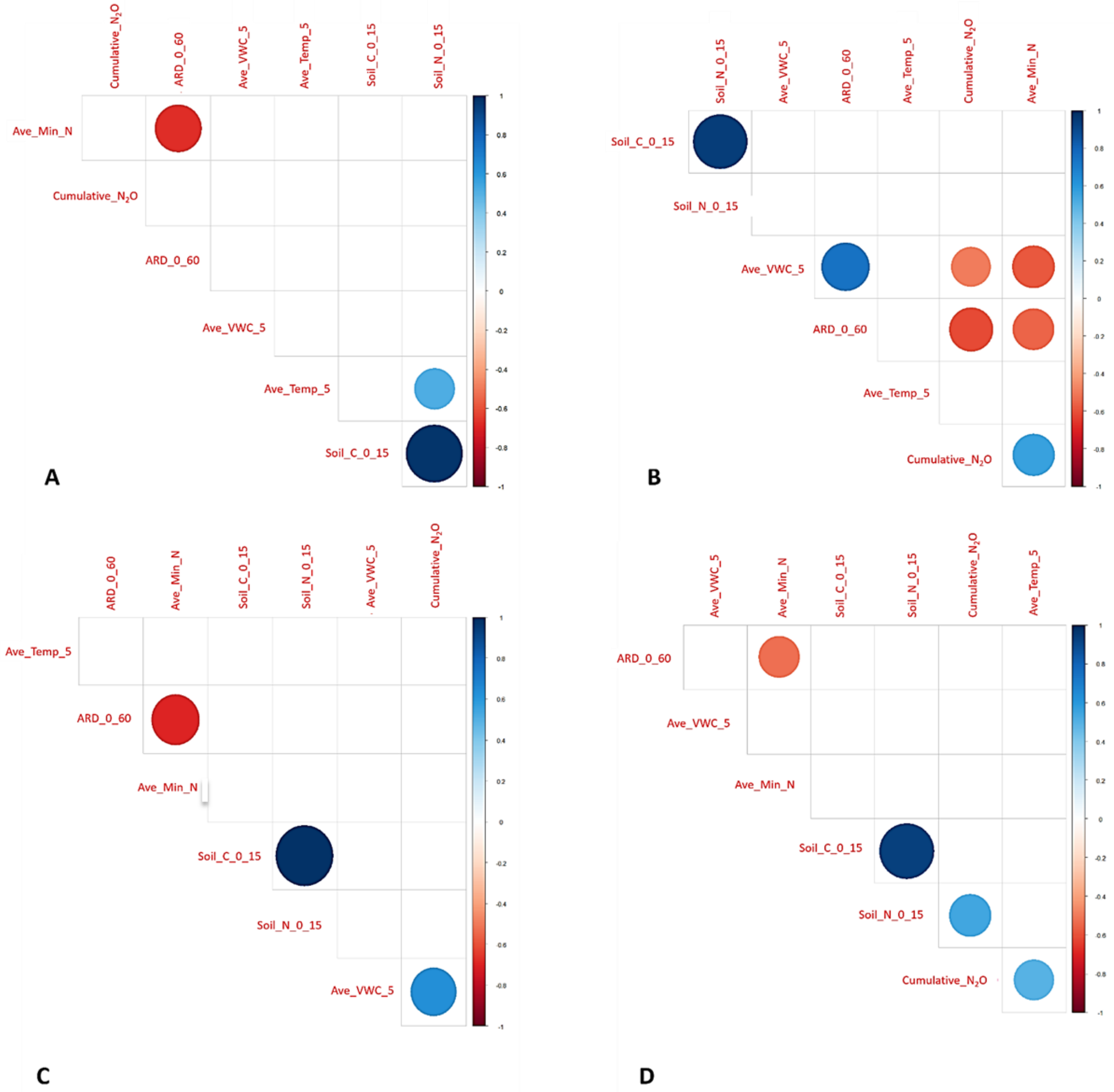

Figure 6.6. Pearson correlation analyses from top left to bottom right: (A) Breton year one, (B) Breton year two, (C) Edmonton year one, and (D) Edmonton year two. Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circle are proportional to the correlation coefficients. Circles only included when the P-value of the correlation was less than 0.05. Note: Cumulative_N$_2$O is the annual cumulative N$_2$O emission, Ave_Min_N is average mineral N (NH_4^+ & NO_3^-) from 0-15 cm, ARD_0_60 is average root density from 0-60 cm, Ave_VWC_5 is average volumetric water content at 5 cm, Ave_Temp_5 is average soil temperature at 5 cm, Soil C_0_15 is total soil carbon from 0-15 cm, Soil_N_0_15 is total soil nitrogen from 0-15 cm.

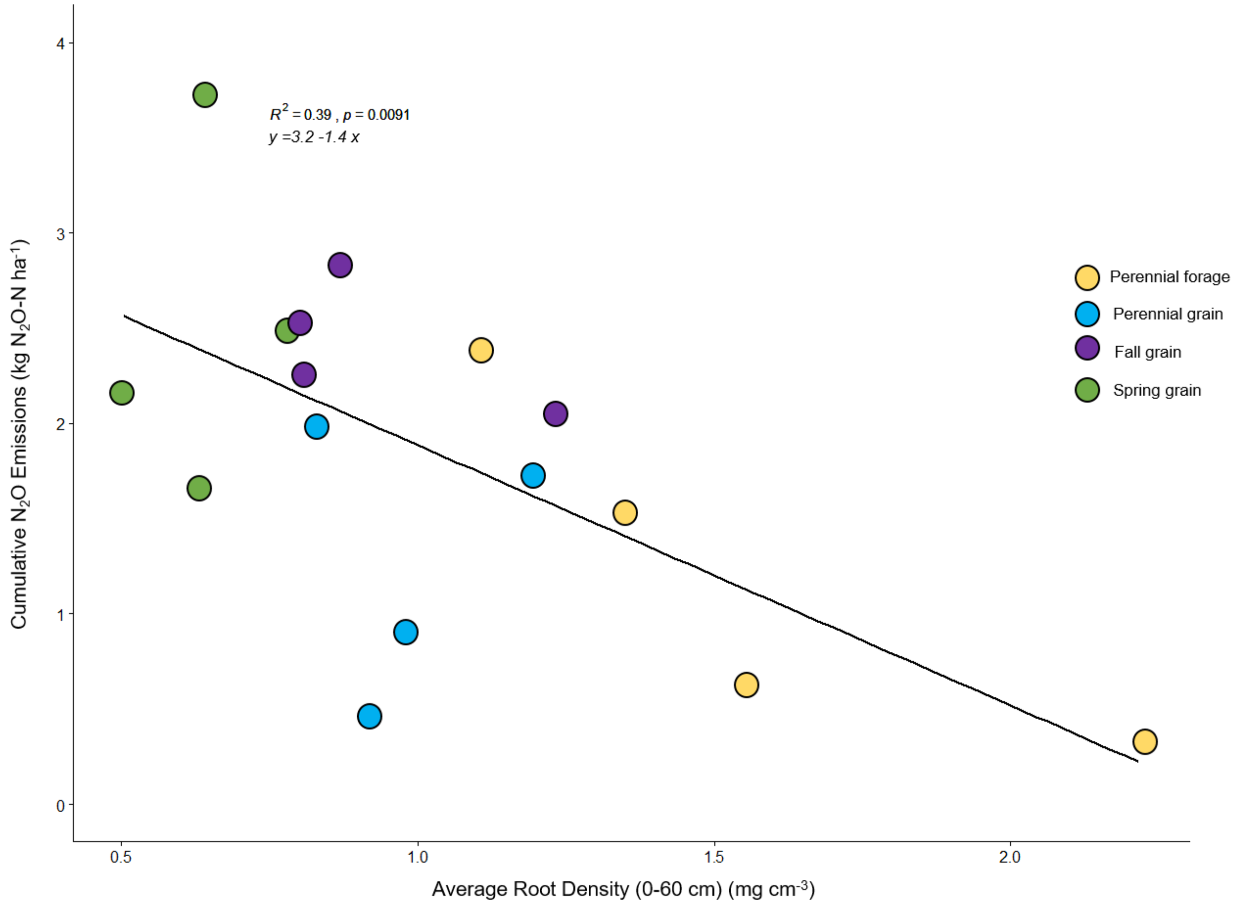

Figure 6.7. Cumulative N_2O emissions (kg N_2O-N ha^{-1}) for the Breton site in year two as a function of average root density, calculated from dry root mass from 0-60 cm soil depth.

7 Soil greenhouse gas dynamics following termination of multi-year perennial grain cropping

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7.1 Abstract

Previous research has shown that perennial grain crops, which combine attributes of annually harvested grain crops and perennial forages, can act as enhanced carbon (C) sinks, increase soil methane (CH_4) uptake, and substantially reduce nitrous oxide (N_2O) release compared to annual grain cropping. However, how greenhouse gas (GHG) fluxes and soil organic C (SOC) stocks respond when a perennial grain is transitioned to annual cropping in a diversified crop rotation is yet to be determined. We used static chambers at two experimental sites in central Alberta (Breton and Edmonton) with contrasting soil properties to quantify field carbon dioxide (CO_2), N_2O, and CH_4 fluxes during the transition from a perennial grain to annual cropping for barley silage via tillage ("the reversal"). We also investigated the effects of converting from perennial forage and fallow to annual cropping, as well as the continuation of annual cropping, for comparison purposes. To evaluate changes in SOC, soil samples from 0-30 cm were collected immediately prior to the reversal, and again after two consecutive growing seasons of annual cropping. At the Edmonton site, tillage of the perennial forage increased soil N_2O ($p < 0.05$) and CO_2 ($p < 0.001$) in year one following the reversal, and the perennial grain had elevated N_2O fluxes relative to the annual grain, which were attributed to a residue "sponge effect" and exposure of previously protected organic matter within soil aggregates disrupted by tillage. By year two, significant differences in GHG fluxes were not detected, but CO_2 fluxes from the legacy perennial grain and forage still trended higher. Importantly, gains in SOC achieved during multi-year perennial grain cropping in the 0-15 cm soil depth increment were lost after tillage and two growing seasons of annual cropping post-reversal. Legacy treatment effects did not manifest at the Breton site, possibly due to significant drought conditions hindering GHG production, and the long-term history of perenniality prior to experiment establishment. Overall, our multi-year experiment suggests that upon reversal to annual cropping, soils previously managed under perennial crops – both forage and grain, result in large C emissions to the atmosphere.

Keywords: Perennial grain, tillage, nitrous oxide, carbon dioxide, soil organic carboncarbon

7.2 Introduction

Agricultural management exerts significant control over greenhouse gas (GHG) fluxes, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) (Johnson et al., 2007). Management decisions regarding tillage and nitrogen (N) fertilizer use can alter soil organic matter (SOM) oxidation and the release of carbon (C) as CO_2, and stimulate nitrification and denitrification processes to produce N_2O, respectively (Daly et al., 2022a; Ruan and Robertson et al., 2013). Additionally, practices that induce soil compaction can alter soil water status and gas diffusion through the profile, forming anaerobic microsites that promote denitrification and methanogenesis and subsequent release of N_2O and CH_4 (Daly et al., 2022a; Kim et al., 2021). Simultaneously, agriculture can act as a sink for CO_2 and CH_4. When CO_2 is converted to organic C via photosynthesis, it can then be sequestered in soil as soil organic C (SOC), and the activity of methanotrophic microorganisms that oxidize CH_4 as a source of energy can be encouraged by promoting reduced disturbance cropping systems (Kim et al, 2021; Liebeg et al., 2005). Finally, N_2O fluxes can be mitigated through multiple different management practices, the majority of which focus on improving N fertilizer use efficiency in agroecosystems (Kanter et al., 2015).

Agricultural management practices that alter the soil environment to mitigate one GHG may increase the flux of another GHG, altering the overall GHG flux balance. To evaluate this, the concept of net global warming potential (GWP) can be used (Forster et al., 2021; Huang et al., 2013). The GWP, expressed at CO_2 equivalents (CO_2eq), provides a means for comparing the relative effects of one source or sink of GHGs by converting different GHGs to the same scale. To achieve this, the GWP is calculated as the global mean radiative forcing of 1 kg pulse fluxes of a GHG relative to 1 kg of the reference gas, CO_2. The GWPs of CO_2, CH_4 and N_2O are 1, 27, and 273 on a 100-year time horizon, respectively (Forster et al., 2021). By placing fluxes of CO_2, CH_4, and N_2O in common terms, it is possible to evaluate the net effects of a management practice (Robertson and Grace, 2004).

Identifying management practices that can minimize the contribution or maximize the sink capacity of CO_2, CH_4, and N_2O in agroecosystems is one step towards improved agricultural sustainability while maintaining or increasing land productivity. One such practice, crop rotation, can impact the pattern and magnitude of GHG fluxes in the long-term. Crop rotations can vary from single-species monocultures to diverse rotations of various crops planted without repetition for many years (Tenuta et al., 2019). Management decisions in crop rotations such as crop selection, N fertilizer application, residue management, and tillage can influence C and N dynamics, water balance, and soil aggregation (Lemaire et al., 2015; Li et al., 2018). As such, diverse rotations add complexity to the soil environment, altering soil microbial communities that can in turn affect the production and consumption of CO_2, N_2O, and CH_4 (Banerjee et al., 2016; Hu et al., 2015; Tenuta et al., 2019).

Annual crop rotations currently dominate agricultural production, occupying 60 to 80% of global cropland, but research suggests that incorporating perennials into crop rotations increases agroecosystem productivity, resiliency, and may reduce GHG fluxes (Agomoh et al., 2020; Bowles et al., 2020; Congreves et al., 2017; Tenuta et al., 2019). However, most available research focuses on perennial forages and legumes, and less research to date has focused on incorporating perennial grain crops into rotations. Innovative crop breeding efforts have resulted in the development of perennial grain crops, which are novel combinations of annually harvested grain crops and perennial grasses, thus they share some characteristics with perennial forages, but differ in several ways (Daly et al., 2022b; Daly et al., 2022c; Kim et al., 2021). Particularly, perennial forage systems are subject to dissimilar management practices including different seeding rates, harvest timing, and N fertilizer rates (Abraha et al., 2018; Weißhuhn et al., 2017).

Previous research from a multi-year experiment at two sites in central Alberta suggests that N_2O fluxes can be significantly reduced during a perennial grain phase (Daly et al., 2022a), CH_4 uptake can be improved (Kim et al., 2021), and SOC gains can be incurred (Daly et al., 2022b). However, little is known about the impacts on GHG fluxes when a perennial grain phase is reversed (henceforth referred to as “the reversal”), and the system is returned to an annual crop phase. Land conversion from perennials to annuals can disrupt soil C and N cycling and lead to significant CO_2, N_2O, and CH_4 fluxes (Abraha et al., 2018; Gelfand et al., 2015; Jabro et al., 2008). In particular, tillage of soil previously under no-till management can alter soil temperature and moisture, disrupt soil aggregates, and increase substrate availability for microbially-mediated SOM oxidation, nitrification, and denitrification (Ruan and Robertson, 2013; Moraru and Rusu, 2012). Understanding how the reversal from a perennial grain phase to an annual phase may affect GHG fluxes and SOC stocks is therefore a key component for weighing the net benefits of incorporating a perennial grain crop into a diversified rotation.

In addition, conflicting results on subsequent crop yields from the inclusion of perennial forages have been reported in literature, with increased, neutral, or reduced yields in subsequent annual crops (Cutforth et al., 2010; Entz et al., 2002; Franco et al., 2018; Hoyt, 1990). However, there is little information specific to the effects of a perennial grain on annual crop yields post-reversal. Annual crop yields may benefit from enhanced porosity and water infiltration rates, reduced bulk density, greater soil food web complexity and stability, and increased N cycling after multiple years of perennial grain (Culman et al., 2010; Ryan et al., 2018). Conversely, increased water use during a perennial phase may lead to soil water depletion and reduced yields in subsequent crops (Cutforth et al., 2010).

To fully understand the impacts of crop rotations, multi-year experiments must be conducted to capture the variability caused by different crops, management practices, and weather (Tenuta et al., 2019). Therefore, we designed a multi-year experiment at two sites in central Alberta to understand how the reversal of a perennial grain crop back to an annual crop in a diversified rotation affects GHG fluxes and subsequent annual crop yields, using ACE-1 perennial cereal rye as a model perennial grain. We selected perennial rye [cv. ACE-1] as the model perennial grain crop based on preliminary findings from Lethbridge, Alberta, which reported superior winter hardiness of perennial rye relative to several perennial wheat cultivars (Daly et al., 2022b; Hayes et al., 2018). For broader comparison purposes, our experiment also evaluated the transition from a perennial forage crop to an annual crop, the transition between two different annual crops, and an annual crop phase following a fallow period.

We hypothesize that legacy treatments managed without tillage (perennial grain and perennial forage) will show increased SOC-sourced CO_2 fluxes after tillage, due to soil aggregate destruction and subsequent transfer of soil C from slow pools to active pools that may counteract any previously accrued benefits from multi-year perennial cropping (Mondal and Chakraborty, 2022). Further, our previous research using an eddy covariance system has shown that in comparison to an annual crop, a perennial grain can have comparable water use efficiency and thus maintain the terrestrial water balance (Kim et al., 2022a). Therefore, we hypothesize that subsequent annual crop yields will not be hindered by water stress and instead benefit from prior multi-year perennial grain cropping due to enhanced C and N cycling.

The objectives of this research are as follows: (1) quantify and examine patterns of CO_2, N_2O, and CH_4 fluxes during the transition between various crop rotation phases at two sites in central Alberta with contrasting soil types (Luvisol vs. Chernozem), (2) examine how controlling factors including soil moisture, soil mineral N concentrations, soil type, and land use history alter individual GHG fluxes in each system, (3) calculate the GWP in terms of CO_2eq to evaluate the net effects of each system, and (4) evaluate subsequent annual crop yields, protein, and protein productivity following a perennial grain, perennial forage, annual grain, and fallow phase.

7.3 Materials and Methods

7.3.1 Site characteristics and experimental design

Two field sites were established in Edmonton, Alberta, Canada (53° 29' 43.33", 113° 31' 59.24") and Breton, Alberta, Canada (53° 5' 16.72", 114° 26' 29.35") in the summer of 2017. Soil at the Edmonton site is classified as Orthic Black Chernozem and soil at the Breton site is classified as Orthic Grey Luvisol, according to the Canadian System of Soil Classification. Baseline soil properties and climate normals for both sites are summarized in Table 7.1.

For the legacy treatments, both experimental sites were arranged in identical randomized complete block designs consisting of four block replicates and seven treatments per block. Treatment combinations consisted of two experimental factors: crop type and N fertilizer. Crop type consisted perennial grain [ACE-1 rye (*Secale cereale* L. × *S. montanum* Guss], annual grain [Gazelle rye (*S. cereale* L.)], perennial forage [meadow brome (*Bromus commutatus*) and alfalfa (*Medicago sativa*)], and fallow. Within each block, two plots of each vegetated treatment were established, with one receiving no N fertilizer and one receiving 56 kg N ha^{-1} in the form of a urea and polymer-coated urea blend (2:1). These plots were maintained with the same treatments for two (Edmonton) and three (Breton) growing seasons. Details on the historical management of these plots can be found in Supplementary Table S7.1 and in Daly et al., (2022a).

After two and three growing seasons at the Edmonton and Breton sites, respectively, the treatments were terminated via rotary tillage using a 2-m wide Sovema RP180 rotary tiller attached to a John Deere 5203 tractor. Two passes were necessary, as the perennial forage plots did not break up sufficiently in the first pass. A low speed second pass allowed for deeper penetration, with an overall tillage depth of approximately 10 cm. Following, all plots were seeded to barley [*Hordeum vulgare*]. All barley plots were fertilized with N at a rate of 56 kg N ha^{-1} using the same urea and polymer-coated urea blend (2:1) (Supplementary Figure S1). Measurements at the Edmonton site were conducted from September 2019 to August 2020 (year one) and from September 2020 to July 2021 (year two). Measurements at the Breton site were conducted from September 2020 to July 2021 (year one) and from August 2021 to August 2022 (year two). Note that flux measurements were concluded in year two after crop seeding at both sites.

7.3.2 Static chamber flux data collection

The static chamber methodology used for this experiment is the same one used by Daly et al. (2022a). Custom acrylic chambers measuring 15.6 cm x 64.10 cm x 10 cm were installed at the beginning of the experiment and removed only for seeding, tillage, or harvest. Otherwise, the chambers were left in the soil for the duration of the experiment. Each plot was equipped with a single chamber base, installed perpendicular to crop rows to a depth of 5 cm. Emissions were measured intermittently at least 1x weekly throughout the duration of the growing season, with sampling intensity increasing to 2x weekly during the spring thaw period, after seeding, tillage and fertilization, and after major precipitation events. For consistency, gas samples were collected throughout the duration of the experiment between the hours of 10:00 AM and 2:00 PM. Due to significant and prolonged soil freezing in Central Alberta, minimal soil biological activity, and hindered gas transport processes, emissions are assumed to be negligible during the winter (approximately November to March) and no gas sampling was conducted (Daly et al., 2022a; Lin et al., 2017, Thilakarathna et al., 2020).

Gas samples were collected from each chamber on a 20-, 40- and 60-minute time step. In addition, ambient samples were taken 10 cm above the soil surface three times throughout the duration of the sampling interval, at the beginning, middle and end, to be used as time zero gas concentrations. Samples were injected into evacuated 12 mL Exetainer® vials and stored at 4°C until analysis via a gas chromatograph (*Varian 3800, Varian Inc., Walnut Creek, CA*) equipped with an electron capture detector (Lin and Hernandez-Ramirez, 2020).

Emissions of CO_2, CH_4, and N_2O were determined by plotting a linear or a quadratic relationship between measured gas concentrations against time, then applying the modified ideal gas law as follows:

$$\text{GHG Flux} = \frac{\text{S}\times\text{P}\times\text{V}}{\text{R}\times\text{T}\times\text{A}} \qquad [7.1]$$

where GHG Flux is the gas flux rate (mmol min^{-1} m^{-2}), S is the slope of the line from a simple linear regression or the first-order derivative at time zero for a quadratic regression curve (Yates et al., 2006; Pennock et al., 2010) (mL L^{-1} min^{-1}), P is the pressure of the gas (Pa), V is the volume of the gas chamber (L), A is the surface area of the gas chamber (m^2), R is the gas constant (Pa mL K^{-1} mmol^{-1}) and T is the temperature of the gas (K). The average of each replicate ± SE was then calculated for each treatment measurement date.

To preclude the confounding effects that growing plants have on opaque chamber measurements of CO_2 emissions (i.e., due to shifts between photosynthesis and dark respiration within the plant canopy), we accounted for CO_2 emissions only during times when plants were not actively growing – therefore, CO_2 flux quantification only includes measurements taken over following study intervals: i) spring thaw prior to the reversal, ii) the reversal period prior to barley emergence, and iii) the subsequent spring thaw post-reversal prior to barley seeding. This approach enables the assumption that the CO_2 emissions measured via chambers represent CO_2 evolved from microbial decomposition of existing plant residues and SOM. With the purpose of enabling results to be comparable, excluding canopy-associated CO_2 was necessary to isolate and evaluate soil as a source or sink of atmospheric CO_2, and to simplify the interpretation of CO_2 flux contributions in relation to CH_4 and N_2O emissions over the same study intervals (Daly et al., 2020; Kuzyakov, 2006; La Scala et al., 2008).

Cumulative emissions were calculated between consecutive sampling dates using linear interpolation. Annual cumulative emissions for year one were calculated by considering one growing season as the time immediately after harvest of the previous crop to the harvest of the barley silage. In year two, flux measurements were concluded immediately prior to seeding.

7.3.3 Soil and plant sampling and laboratory analyses

Soil samples were collected from both sites over two growing seasons to identify temporal changes in soil mineral N concentrations between treatments. Samples from 0-15 cm were collected from each plot by using a push probe (2.5 cm i.d.) to obtain three random cores, which were then homogenized in sterile plastic sampling bags for one composite sample per plot. Samples were stored at 4°C until they were air-dried and ground for analysis. Soil mineral N was quantified by extracting 5 g of air-dried soil with 50 mL of 2M KCl, shaken in a reciprocal shaker for 30 minutes, filtered using fine porosity 15 cm diameter filters (*Fisher Scientific, Pittsburg, USA*) and analyzed via colorimetry using a SmartChem discrete wet chemistry analyzer (*Unity Scientific, Milford, USA*).

Composite soil samples were taken as above immediately prior to the reversal and after two seasons of barley silage cropping following biomass harvest for SOC and total nitrogen (TN) to discern potential differences between legacy treatments from 0-15 cm and 15-30 cm. Samples from the 15-30 cm depth at the Edmonton site were not taken due to logistical challenges, therefore this data is missing. Samples were air-dried, ground, and analyzed using dry combustion using a Thermoscientific Flash 2000 Organic Elemental Analyzer (*Thermo Fisher Scientific Waltham, USA*). The presence of carbonates was tested for by acidifying samples with of 50 µL 1M HCl. Soil pH was measured using a 1:5 soil:water slurry with a pH meter and soil texture was determined via the hydrometer method. Soil bulk density was determined using the core method.

Barely silage yields were measured by hand harvesting 1-meter lengths of two adjacent rows at two locations within each replicated plot, at least 1-meter from the plot edges. The harvested material was oven dried at 60°C until a constant weight was reached. Silage protein was determined using a FOSS DS2500 (*Foss Analytics, Denmark*) near infrared spectroscope (NIR).

Crop residue was collected after harvest of the legacy crops at both sites in legacy years one and two (2018 and 2019) by collecting all remaining plant material on the surface within a 0.5 m^2 quadrat that was randomly placed within each plot. The material was dried at 60°C until a constant weight was reached, ground, and analyzed using dry combustion using a Thermoscientific Flash 2000 Organic Elemental Analyzer (*Thermo Fisher Scientific Waltham, USA*).

7.3.4 Weather and soil moisture data collection

Hourly temperature and precipitation data was obtained for both sites from permanent weather stations within 1 km of the experimental plots. Soil temperature and volumetric moisture content were measured hourly using Decagon 5TM sensors installed at each site at 7.5, 22.5 and 40 cm depths. Data from the sensors was logged on EM50 data loggers and downloaded monthly.

7.3.5 *Calculations and statistical analyses*

Emissions were converted to CO_2 equivalents (CO_2eq) using the GWP coefficients for CO_2, CH_4, and N_2O, which are the cumulative radiative forcings between the present and 100 years in the future, caused by a unit mass of gas emitted now. The CO_2eq calculations were completed by accounting for N_2O and CH_4 only during the dates that CO_2 was measured using the following equation:

$$CO_2eq = CO_2 + (273\ x\ N_2O) + (27x\ CH_4) \qquad [7.2]$$

All statistical analyses were performed with R Studio software version 4.0.3 (R Core Team, 2020) with an alpha critical value of 0.05. Assumptions of normality and homoscedasticity were checked by the Shapiro-Wilk and Bartlett tests, and Box-Cox data transformations were applied to meet assumptions of normality and homoscedasticity. Two-way analysis of variance (ANOVA) was performed on linear mixed models developed using the NLME package (Pinheiro et al., 2020) on emissions data, soil mineral N, crop residue, barley yield, barley protein, and protein productivity with legacy crop and N fertilizer application as the fixed factors and block replicate as the random effect. Post hoc investigation was completed after significant ($P < 0.05$) ANOVA using Tukey's Honest Significant Difference test for comparison of treatment means using the Agricolae package (de Mendiburu, 2020). Soil C and N were analyzed using three-way repeated measures ANOVA, with legacy crop, N fertilizer, and year as fixed effects, plot ID as the random effect, and a first order autoregressive correlation structure to account for temporal autocorrelation. Post-hoc comparisons were completed using paired sample t-tests.

7.4 Results

7.4.1 *Weather conditions*

Long-term normal (1981-2010) air temperature at the Breton and Edmonton sites is 3.4 and 4.2°C respectively, with an average yearly precipitation of 602 and 456 mm (Table 7.1) (Government of Canada, 2020). Air temperature, precipitation, and volumetric soil moisture at 7.5 cm depth for the Breton and Edmonton sites are shown in Figs. 7.1 (A-B) and 7.2 (A-B). Mean daily air temperature for years one and two at the Breton site was 3.6 and 3.4°C. Cumulative precipitation was lower than normal, with 427.9 and

502 mm yr^{-1} of precipitation in years one and two. At the Edmonton site, mean daily air temperature for years one and two was 4.0 and 4.7°C. Cumulative precipitation was 508 mm in year one, and much lower than normal in year two with 270 mm (Government of Alberta, 2023).

7.4.2 Static chamber measurements of N_2O, CH_4, and CO_2 emissions

7.4.2.1 Breton

Pulses of N_2O in year one were detected following: (i) harvest of the legacy treatments (ii) spring thaw, and (iii) tillage of the legacy treatments, barley seeding, and N fertilizer application ("the reversal") (Fig. 3A). After harvest, emissions measured from 9 September 2020 to 5 October 2020 were elevated for the annual grain and fallow, but not for the perennial grain or perennial forage. The spring thaw flux pulse was short lived; elevated emissions were detected on 18 March 2021 from all treatments but returned to minimal emissions by the next field sampling. Conversely, after the reversal on 27 May 2021, a significant flux pulse was detected from all treatments for approximately one week. Elevated post-tillage N_2O emissions from the perennial forage continued for an additional week (Fig. 7.3A)

Cumulative N_2O emissions for year one did not differ, however, the legacy perennial grain treatments trended higher than the other treatments (0.35 ± 0.05 vs. 0.22 ± 0.04 kg N_2O-N ha^{-1}). No effect of legacy N fertilizer application was discerned (Table 7.2). The proportion of cumulative N_2O emissions attributed to the 14-day reversal period was substantial for every treatment.

On 28 July 2021, the barley crop was harvested, marking the start of year two. The single N_2O flux pulse detected in year two corresponded with the spring thaw in early April 2022 (Fig. 7.4A). Cumulative N_2O emissions did not differ from one another based on the legacy treatment, and no trends were noted. Compared to the fall period (37 days), the spring period (37 days) contributed the majority of cumulative N_2O emissions in year two (Table 7.2).

In general, soil at the Breton site in year one acted as a CH_4 sink, and few discernable seasonal trends were observed. In the fall period, the perennials (grain and forage) tended to act as greater CH_4

sinks than the others, but this trend was not observed in any other period (Fig. 7.3B). Cumulative CH_4 uptake, indicated by negative flux values, did not differ between legacy treatments, but trended higher for the perennial crops relative to the annual and fallow, on average (-0.26 ± 0.03 vs. -0.20 ± 0.03 kg CH_4-C ha^{-1}). No effect of legacy N fertilizer was discerned (Table 7.2). Similar to year one, soils at the Breton site in year two acted as a CH_4 sink, with over 93% of CH_4 uptake occurring during the fall period (Fig. 7.4B). Cumulative CH_4 uptake in year two was not affected by the legacy treatments (Table 7.2).

In year one, emissions of CO_2 were accounted for during the early spring, prior to any perennial grain and forage growth, and during the reversal period, prior to barley seedling emergence; as abovementioned, this was done to preclude the confounding effects of plant respiration on chamber measurements and subsequent data interpretation. Post-reversal, CO_2 emissions were elevated, peaking on 3 June 2021 for all treatments (average: 30 ± 3 kg CO_2-C ha^{-1} d^{-1}) (Fig. 7.C). Cumulative CO_2 emissions for year one did not significantly differ between legacy crop or N fertilizer application, however, cumulative CO_2 emissions trended as follows: perennial grain > perennial forage > annual grain > fallow, and previously N fertilized treatments on average had higher emissions than unfertilized treatments (420 ± 50 vs. 290 ± 30 kg CO_2-C ha^{-1}) (Table 7.2). In terms of the proportion of CO_2 flux attributed to each measurement period, the reversal period contributed > 80% of cumulative CO_2, on average.

CO_2 emissions for year two were conducted from spring thaw to barley seeding on 15 May 2022. As soils warmed with increasing air temperatures (Fig. 7.4C), emissions from all treatments increased, and the legacy perennial forage trended highest (Fig. 7.4C). Consequently, cumulative CO_2 emissions in year two were greater for the perennial forage than the annual grain ($p < 0.05$). Cumulative CO_2 emissions from the fallow and perennial grain were intermediate, not differing from the perennial forage or annual grain (Table 7.2). By year two, the effects of legacy N fertilizer applications were not detected.

7.4.2.2 Edmonton

Pulses of N_2O in year one were detected following: (i) spring thaw, (ii) the reversal, and (iii) a significant precipitation event (Fig. 7.5A). The spring thaw flux pulse was minimal, detected only on 4

April 2020 from the fallow treatment. Post-reversal on 12 June 2020, all treatments exhibited heightened emissions for approximately 10 days. Finally, a significant precipitation event at the end of June (> 40 mm over two days) resulted in a flux peak from the legacy perennial forage (Fig. 7.5A).

Cumulative N_2O emissions in year one from the legacy perennial forage were greater than those from the annual grain ($p < 0.05$) but did not differ from the perennial grain or fallow. No effect of legacy N fertilizer application was discerned (Table 7.3). The proportion of cumulative N_2O emissions attributed to the 10-day reversal period was substantial for every treatment.

Year two at the Edmonton site was marked by the barley harvest on 27 August 2020. A single N_2O flux pulse in year two was observed for all treatments, corresponding with the spring thaw in March 2021 (Fig. 6A). By the second year, differences in cumulative N_2O emissions between the legacy treatments were no longer evident and most emissions occurred during the spring thaw (Table 7.3).

Soils at the Edmonton site in year one acted as a weak CH_4 sink, and no trends were observed (Fig. 5B). Cumulative CH_4 uptake did not differ between legacy crop or N fertilizer (Table 7.3). Similarly, in year two, CH_4 uptake was minimal and did not differ between treatments. However, during the fall in year two, the legacy perennial grain briefly shifted from being a CH_4 sink to a CH_4 source (Fig. 7.6B, Table 7.3).

Similar to the Breton site, CO_2 emissions at the Edmonton site in year one were accounted for during the early spring, prior to plant growth in the perennial treatments, and during the reversal period, prior to barley seedling emergence. After the reversal, CO_2 emissions were elevated, peaking on 16 June 2020 for all treatments, but most notably for the fallow (28 ± 7 kg CO_2-C ha^{-1} d^{-1}) (Fig. 7.5C). Cumulative CO_2 emissions in year one were affected by the legacy crop ($p < 0.001$) and N fertilizer ($p < 0.05$) (Table 7.3). Emissions from the perennial forage treatment exceeded those from all other treatments, and legacy N fertilizer increased CO_2 emissions relative to the treatment without N fertilizer. The reversal period contributed the majority of CO_2 emissions from all treatments. Cumulative CO_2 emissions for year two were conducted from spring thaw to barley seeding on 4 May 2021 (Fig. 7.6C). By year two, the effects of the legacy treatments on CO_2 emissions were not detected (Table 7.3).

7.4.3 CO_2eq

The CO_2eq was calculated using N_2O and CH_4 emissions that corresponded to the dates that CO_2 emissions were measured (Fig. 7.7). At the Breton site, no differences in CO_2eq were observed for legacy crop or N fertilizer application for either year, however, both legacy perennial treatments trended higher than the fallow and annual grain in year one (Fig. 7.7A). At the Edmonton site, the legacy crop had a significant effect on CO_2eq in year one ($p < 0.001$). On average, the perennial forage crop increased CO_2eq relative to all other crops by approximately 40% (1.41 vs. 2.37 Mg CO_2eq ha^{-1}). This was due to CO_2 emissions after the reversal from the perennial forage, which contributed ~85% of the total CO_2eq (Fig. 7.7B). By the following spring, significant differences were not detectable, however, both perennial treatments still trended higher than the annual grain or fallow. No effect of N fertilizer on CO_2eq was evident for either year (Table 7.4).

7.4.4 Soil mineral N concentrations

7.4.4.1 Breton

Post-reversal, soil mineral N concentrations increased for all treatments, but returned to lower concentrations by the next sampling. In year two, a peak occurred after seeding and N fertilizer application (Supplementary Figs. S7.2A, S7.2B). The weighted average of mineral N concentrations over both years was not affected by legacy crop or N fertilizer (Table 7.5) but trended from high to low as follows: perennial forage > perennial grain > fallow > annual grain for both years.

7.4.4.2 Edmonton

In year one, soil mineral N increased for all treatments post-reversal (Supplementary Fig. S7.3A). Average mineral N was affected by crop ($p < 0.0001$) and N fertilizer ($p < 0.05$) (Table 7.5). Due to a higher concentration in the fall, the fallow treatment had greater average mineral N than the annual grain and perennial forage treatments, and treatments with a history of N application had greater mineral N than those without. In year two, mineral N increased noticeably after seeding and N fertilizer application, but the effect of the legacy crop on soil mineral N became marginal ($p = 0.05$) and legacy N fertilizer treatments no longer differed (Table 7.5, Supplementary Fig. S7.3B).

7.4.5 Soil organic carbon and total nitrogen

After two years of continuous annual cropping, differences in SOC and TN between legacy treatments were not detected for either site, nor for either depth increment at the Breton site (0-15 cm and 15-30 cm) (Table 7.6). Prior to the reversal, SOC and TN were significantly greater under the perennial grain relative to the annual grain at the Edmonton site in the 0–15 cm depth increment ($p < 0.01$ and $p < 0.01$, respectively) (data not shown; see Daly et al., 2022b). Repeated measures analysis showed that after two years of annual cropping, SOC and TN in the legacy perennial grain treatment was significantly reduced compared to SOC and TN immediately after multi-year perennial grain (Fig. 7.8) ($p < 0.01$). At the Breton site, no discernible trends were noted between sampling times.

7.4.6 Barley yields, protein, and protein productivity

7.4.6.1 Breton

Barley silage yields and protein content were affected by the legacy crops in year one, but not N fertilizer. Prior fallow and annual grain crops increased barley silage yield relative to the perennial treatments ($p < 0.05$). Conversely, barley silage protein was highest post-perennial forage, intermediate following perennial grain, and lowest after annual grain ($p < 0.05$). As such, only marginal differences ($p = 0.05$) materialized for protein productivity, which was highest after legacy fallow and lowest after perennial grain (Table 7.7). By year two, legacy crop effects had diminished and there were no differences in barley silage yield, protein content, or protein productivity (Table 7.7).

7.4.6.2 Edmonton

In year one, barley yields after perennial forage and fallow were reduced compared to the annual grain ($p < 0.01$). Barley yields after perennial grain were intermediate. Silage protein was higher after fallow than all other crops ($p < 0.01$). In terms of protein productivity, the increased protein content did not make up for reduced yields, and protein productivity followed similar trends as yields, with annual grain ≥ fallow = perennial grain > perennial forage ($p < 0.05$). No effect of legacy N fertilizer, nor the interaction between crop and fertilizer was found (Table 7.7).

By the second year, barley yields did not differ. Silage protein contents were higher after perennial grain than the fallow and perennial forage ($p < 0.05$), but this did not translate into differences in barley silage protein productivity (Table 7.7).

7.4.7 Legacy crop residue

Crop reside remaining on the soil surface post-harvest was consistently greater under the perennial forage crop than the perennial grain or annual grain for both sites (*p*s < 0.001) (Supplementary Table S7.2). In year two of the legacy cropping systems, residue from the perennial forage crops exceeded the average residue for the other crops by 70 and 64% at the Breton and Edmonton sites, respectively. The total C (TC) and TN contents of the crop residue were generally greater than the other crops as well, however, the inclusion of alfalfa in the perennial forage treatment tended to reduce the C:N ratio, except for year two at the Breton site, where no differences in the C:N ratio between legacy crop residue was observed (Supplementary Table S7.2).

7.5 Discussion

7.5.1 Residue incorporation triggered residue- and SOM-sourced CO_2 release

The reversal of the legacy perennial forage via tillage resulted in significantly increased cumulative emissions of CO_2 from the Edmonton site in year one, but the effects of the legacy treatments had diminished by the following year. Only trends were observed at the Breton site in year one, but CO_2 fluxes showed legacy treatment effects in year two. Overall, we observed common patterns in the CO_2 flux data, and variations are likely due to differences in inherent soil characteristics and weather patterns between the two sites.

At both the Edmonton and Breton sites, the soil that was managed under perennial forage released more CO_2 after tillage reversal than the annual grain or fallow (Tables 7.2, 7.3). Tillage improves residue-soil contact, increases the aeration of surface soil, and disrupts soil aggregates, all of which leads to organic matter decomposition and CO_2 release (Ruan and Robertson, 2013). Previous literature has found that the magnitude of residue-derived CO_2 flux is positively correlated with the rate of residue addition in tilled systems, and post-harvest residue was much greater under the perennial forage (Supplementary Table S7.2) (Mirzaei et al., 2022; Ruan and Robertson, 2013; Wang et al., 2019). In addition to the increased aboveground residue (Supplementary Table S7.2), the legacy perennial forage had significantly increased root mass density compared to the other treatments (Kim et al., 2021; Daly et al., 2022b). Therefore, greater residue inputs from the perennial forage led to increased mineralization, enhanced microbial biomass C, and augmented dissolved organic C, which subsequently resulted in increased residue-sourced CO_2 fluxes post reversal (Li et al., 2017; Mirzaei et al., 2022; Yang et al., 2012).

In addition to driving changes in the magnitude of residue-sourced CO_2, residue management and tillage can also affect existing SOM stocks via the priming effect, whereby a positive priming effect increases the amount of SOM-sourced CO_2 when increased substrate availability accelerates microbial activity and therefore SOM decomposition (Mitchell et al., 2016; Plante and McGill, 2002). Prior to the reversal, two years of perennial grain cropping at the Edmonton site enhanced SOC and TN in the 0-15 cm depth increment relative to the annual grain, which is common in no-till plots as SOM accumulates in the surface layers (Daly et al., 2022b; Kim et al., 2022b; Malhi et al., 2011). We attributed this to the prolific aboveground biomass production of the perennial grain, coupled with the high clay content of the Edmonton soil supporting enhanced macro-aggregation that may have served as a protective mechanism for SOM accrual under the perennial grain that was disrupted when the soil was tilled (Daly et al., 2022b; Kim et al., 2022b; Mondal and Chakraborty, 2022). Notably, particulate organic matter (POM) is a SOM fraction that is sensitive to changes in soil management that has been shown to increase under perennial grain cropping relative to annual grain (Kantola et al., 2017; Kim et al., 2022b). The POM fraction can be protected within soil aggregates, decomposing on a decadal scale when undisturbed (Kantola et al., 2017; Liao et al., 2006). However, destruction of macroaggregates can transfer protected POM from slow pools to active pools, which is then available for microbial conversion to CO_2 (Conant et al., 2007; Grandy and Robertson, 2007; La Scala et al., 2008). In this study, we did not fractionate SOM to directly quantify the POM fraction; therefore, future experiments may be needed to examine these putative effects of tillage on the dynamics of SOM pools under perennial grain cropping and to evaluate our hypothesis.

However, after two years of annual barley at the Edmonton site, differences in SOC from 0-30 cm became undetectable, suggesting that a portion of SOC that was previously accrued under perennial cropping was lost to the atmosphere as CO_2 (Fig. 7.8). This finding is consistent with literature that observed losses from soil C stocks after tillage of a previously no-till system (Conant et al., 2007; Shahidi et al., 2014). However, our CO_2 results do not corroborate the loss of topsoil SOC experienced from the legacy perennial grain at the Edmonton site (Table 7.3). We attribute this to a methodological limitation. Opaque chambers do not facilitate continuous quantification of CO_2, but instead enable valid CO_2 quantification only in periods without active plant growth, whereas our SOC measurements represent SOC converted to CO_2 from May 2020 to August 2021, inclusive (Fig. 7.8). While chambers can capture the initial flush of tillage-induced CO_2, they may not capture the trend of enhanced CO_2 flux throughout the entire growing season, which can be supported using measurements of SOC (Malhi et al., 2011; Shahidi et al., 2014). This highlights the importance of multiple streams of data as used in this study to quantify CO_2 efflux and thus prevent underestimation of SOC losses.

Conversely, the lack of legacy treatment effects on cumulative CO_2 flux at the Breton site in year one is unlikely the result of a truncated CO_2 data set, as no differences in SOC were detected at this site prior to or post reversal. Instead, muted reversal effects on CO_2 emissions may be the result of a significant drought in Breton in 2021, as precipitation was 30% less than a normal year. Soil moisture is a major factor influencing CO_2 emissions; therefore, dryer than normal conditions may have hindered microbial activity, mineralization of residues, and subsequent CO_2 flux immediately after the reversal

(Jabro et al., 2008; Wang et al., 2019). Indeed, this may be the reason treatment differences were absent at the Edmonton site in year two as this site also experienced significantly reduced precipitation in 2021.

7.5.2 Multiple mechanisms promote N_2O production

Patterns of N_2O flux from the Edmonton site mirror patterns in CO_2 emissions, as the legacy perennial forage treatment had increased emissions over the annual grain. Emissions of N_2O from the perennial grain and fallow were also elevated relative to the annual grain, largely due to the pulse of N_2O emissions immediately following the reversal (Figs. 7.3, 7.5). Tillage can enhance SOM mineralization from previously untilled soil, as in the perennial grain and fallow, increasing mineral N supplies, which serve as substrates for nitrifying and denitrifying organisms to produce N_2O (Pinto et al., 2004; Ruan and Robertson, 2013).

Somewhat counter-intuitively, enhanced N_2O emissions do not correspond with enhanced mineral N availability in the perennial forage, as in the legacy perennial grain and fallow. Indeed, mineral N concentration in the perennial forage was significantly reduced relative to all other treatments, potentially a result of intense N immobilization from the very large quantity of accumulated aboveground plant residue that remained after the termination of this crop (Table 7.5, Table 7.8) (Guardia et al., 2016; Shapiro et al., 2001). When residues cannot meet microbial N requirements induced by residue C, active microbes will assimilate indigenous soil N into their biomass. Previous literature has reported that the coarse tap roots of alfalfa, a component in the legacy perennial forage, can induce temporary net immobilization of N and delayed N mineralization compared to other N-fixing species (Bolger et al., 2003; Louarn et al., 2014). In support of this hypothesis, Kim et al. (2021) reported root C:N ratios of the same perennial forage crop to be 29-34, which is above the threshold for stimulating net mineralization, which occurs around 20 (Bolger et al., 2003; Kumar and Goh, 1999).

Mineral N immobilization can reduce the substrate available for nitrification and/or denitrification and hence soil N_2O emissions (Chen et al., 2013; Frimpong and Baggs, 2010), however, the fertile soil at the Edmonton site still maintained considerable mineral N available for microbial transformation into N_2O. In fact, despite lowering mineral N concentrations, the abundant plant residue may have contributed to a "sponge effect" that promoted denitrification and N_2O release (Kim et al., 2022c; Kravchenko et al., 2017). Greater absorbency of plant residue can pull water from nearby pores, creating anoxic microsite conditions that are high in available organic C and hence favorable to N_2O production (Kim et al., 2020; Kim et al., 2022c).

Again, due to the drought conditions in year one at the Breton site, no differences in N_2O emissions were observed after the reversal of the legacy treatments, nor did treatment differences persist at the Edmonton site during the following spring, which also experienced very dry conditions. Production of N_2O is strongly controlled by precipitation and its effect on soil water-filled pore space, which exhibits control over soil redox potential and consequently over soil mineral N transformations that produce N_2O (Rochette et al., 2018; Rochette et al., 2008).

7.5.3 Disturbance effects on CH_4 uptake are site-specific

Kim et al. (2021) reported that perennial grain and perennial forage were stronger CH_4 sinks than the annual grain and fallow at the Edmonton site over two growing seasons. However, post reversal, differences in CH_4 uptake between the treatments did not persist. Methanotrophic bacteria are abundant in well-drained upland soils, thus these soils generally act as a CH_4 sink. However, methanotrophs are highly sensitive to environmental factors and stability tends to favor methanotroph activity (McDaniel et al., 2019; Tate, 2015). As such, when tillage was applied to convert the legacy treatments to barley silage and the perennial treatments were no longer facilitating a stable microclimate beneath their continual, dense perennial canopies and surface residue, differences in CH_4 uptake did not persist (Kim et al., 2021; McDaniel et al., 2019). Additionally, the dense root systems of both the perennial grain and perennial forage crops that previously maintained an aeration-moisture balance in subsurface soil layers that promoted CH_4 uptake were replaced by annual grain with a sparser, shallower root system (Daly et al., 2022a; Kim et al., 2021).

At the Edmonton site, the legacy perennial grain, perennial forage, and fallow treatments each took turns being net sources of CH_4 (Table 7.3). Again, this may be due to tillage of previously no-till treatments increasing microbial access to previously protected SOM, or in the case of the legacy perennials, the result of increased substrate from surface residue and decaying roots increasing microbial activity, creating anaerobic microsites, and supporting methanogen activity (Table 7.8) (Ussiri et al., 2009; Zhang et al., 2015). This effect was most pronounced in the perennial grain and least in the perennial forage, which may be due to the enhanced macroporosity and hydraulic conductivity found in the 25-30 cm depth increment under the legacy perennial forage (Daly et al., 2022b). Peak CH_4 consumption has previously been reported up to 40 cm depth in soil, therefore, the enhanced gas diffusivity in the subsurface of the perennial forage may have lessened the creation of anaerobic microsites, supported methanotroph activity, and minimized positive CH_4 emissions after the reversal (Daly et al., 2022b; Kim et al., 2021; Prajapati and Jacinthe, 2014).

Legacy treatment effects were not evident at the Breton site. In general, Breton maintained a higher CH_4 sink compared to the Edmonton site after the reversal to annual cropping. We ascribe this to the strong influence of previous land use prior to experiment establishment in 2017. For at least 60 years, the soils at the Breton site were managed as a perennial hay stand, whereas Edmonton was managed as an annual crop system for approximately 20 years. Long-term land management can impart legacy effects on soil for several years, therefore, it is possible that the Breton site requires more time than the duration of this experiment (~5 years) for treatments to substantially alter CH_4 patterns, which have been shown to maintain CH_4 uptake for multiple years after the conversion to annual cropping from perennial systems (Gelfand et al., 2011; Kim et al., 2021; Ruan and Robertson, 2013).

7.5.4 Reversal of perennial grain crops does not always enhance CO_2eq

The CO_2eq was calculated based on the emissions of CO_2, N_2O, and CH_4 to understand how the termination of a perennial phase in a cropping rotation might affect net GHG emissions. The GHG benefits of including a perennial phase, either grain or forage, into diversified crop rotations may be offset if CO_2eq emissions upon termination of the perennial crop are substantial, especially if the perennial phase was terminated via tillage and/or included an N-fixing crop (Lemke et al., 2018; Shahidi et al., 2020; Tenuta et al., 2019). Greatly enhanced CO_2eq emissions after tillage of a perennial phase have been reported in literature, with CO_2 emissions contributing the majority, consistent with our results (Ruan and Robertson, 2013). Our findings suggest, however, that the impacts of converting a perennial phase to annual cropping via tillage can be short-lived and, in some cases, may not be significantly greater than the CO_2eq from a strictly annual crop rotation. Notably, the transition from perennial grain to a barley crop did not result in augmented CO_2eq emissions relative to continued annual cropping at the either site, however, the CO_2eq results at the Edmonton site must be interpreted with caution, as results of soil sampling inform significant losses of SOC as CO_2 occurred at this site from the legacy perennial grain (Table 7.4, Fig. 7.8).

7.5.5 Yield impacts of a perennial grain phase

Previous literature has reported conflicting results on subsequent crop yields from the inclusion of perennial forages, with increased, neutral, or reduced yields in subsequent annual crops (Cutforth et al., 2010; Entz et al., 2002; Franco et al., 2018; Hoyt, 1990). Little data, however, is available on the benefits or limitations on subsequent crops from a perennial grain, which act as intermediates between perennial forages and annual grains. Barley yields in the first year after the reversal were consistently higher in the legacy annual grain plots at both the Edmonton and Breton sites than the legacy perennial forage. This is contrary to our original hypothesis, which downplayed the effect soil water depletion under the perennial grain. Soil water dynamics may have played a more important role during this study due to the significant drought conditions experienced at the Breton site in year one (Fig. 7.1C,). Indeed, Jefferson et al. (2013) determined that barley yield and protein tended to be greater after a perennial phase, however, this effect was not realized in dry years (Franco et al., 2018; Jefferson et al., 2013). Additionally, we hypothesize that the reduction in yields after perennial forage was primarily driven by the significant amount of crop residue on the soil surface, as residue can mechanically impede seedling establishment and thus reduce yields (Seehusen et al., 2016).

Elevated protein in the barley silage in the first year after perennial forage at the Breton site may be the result of slow mineralization of the alfalfa residues and eventual release of previously immobilized N (Bolger et al., 2003; Louarn et al., 2014). This is not reflected in our soil mineral N data, perhaps because of the rapid uptake by the barley crop (Supplementary Fig. S7.2). Additionally, it is possible that the barley was taking up organic N in the form of amino acids from the decaying perennial forage material and associated decomposing organisms (Jamtgard et al., 2008; Paungfoo-Lonhienne et al., 2012). The ability of barley to uptake organic N has been shown in literature, but its significance as a major N source is currently unclear (Farzadfar et al., 2021).

Protein productivity is the product of silage protein concentration and silage yield; thus, it encompasses both metrics into a single parameter and represents the overall ability of a crop to produce (Asseng et al., 2002; Daly et al., 2022c). In 3 of 4 barley growing seasons, protein productivity was not altered by the legacy treatments, and the significant reduction in protein productivity from the legacy perennial forage at the Edmonton site in year one was the result of residue-suppressed yields discussed above. As such, when considered in terms of protein productivity, the effects of the legacy treatments were generally neutral on subsequent barley silage crops.

7.6 Conclusion

Our multi-year experiment demonstrates that within a long, diversified rotation, the reversal of a perennial grain crop back to an annual cropping phase can alter the dynamics of CO_2, CH_4, and N_2O fluxes, but the effects are site specific. At the clay-rich Edmonton site, results collectively suggest that highly fertile soils managed under perennials – both forage and grain, are large emitters of C to the atmosphere after tillage and return to annual cropping. Conversely, legacy treatment effects did not manifest after the reversal at the Breton site, perhaps due to significant drought conditions hindering microbial activity and biogenic GHG production as well as the long-term history of perennial management prior to experiment establishment, which likely overrode the effects of land management changes implemented in this study. At both sites, yield penalties from the legacy perennial treatments were incurred in the annual crop in the first growing season post-reversal, but this did not persist into year two, and protein productivity was only reduced in 1 of 4 site-years post-perennial reversal.

7.7 References

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Tables

Table 7.1. Select baseline soil properties from the Breton and Edmonton sites

Soil Properties	**Breton**	**Edmonton**
Canadian classification	Gray Luvisol	Black Chernozem
Soil organic carbon (SOC) (g C kg^{-1}) (0-30 cm)	19.2 ± 3.9	41.6 ± 7.5
Total nitrogen (TN) (g N kg^{-1}) (0-30 cm)	1.7 ± 0.3	3.6 ± 0.5
pH (1:5 H_2O)	6.1 ± 0.08	7.3 ± 0.09
Bulk density (g cm^{-3}) (5-30 cm)	1.1 ± 0.06	1.0 ± 0.06
Soil texture	loam	silty clay
% clay	24.8	48.3
% silt	41.8	35.7
% sand	33.3	16.0
Climate normals		
Average yearly temp (°C) §	3.4	4.2
Cumulative yearly precipitation (mm yr^{-1}) §	456	602

§ These are long-term 30-yr normals 1981-2010) (Government of Canada, 2020).

Table 7.2. Cumulative N_2O, CH_4, and CO_2 emissions and the proportion of cumulative N_2O, CH_4, and CO_2 emissions during fall, spring, reversal, and summer periods as measured by static chambers for each legacy cropping system for years one and two at the Breton site.

Legacy Treatment	Year One					Year Two		
	Proportion of Cumulative N_2O (%)				Cumulative N_2O (kg N_2O-N ha^{-1})	Proportion of Cumulative N_2O (%)		Cumulative N_2O (kg N_2O-N ha^{-1})
	Fall 17 Sept. 2020 - 2 Nov. 2020 (46 d)	Spring 18 March 2021 - 27 May 2021 (70 d)	Reversal 28 May 2021 - 11 June 2021 (14 d)	Summer 12 June 2021 - 28 July 2021 (97 d)	17 Sept. 2020 - 28 July 2021	Fall 28 July 2021 - 25 Oct. 2021 (37 d)	Spring 4 April 2022 - 11 May 2022 (37 d)	18 Sept. 2021 - 11 May 2022
Fallow	17	28	32	23	0.21 ± 0.05	13	87	0.48 ± 0.05
Annual grain	31	16	33	19	0.19 ± 0.04	15	85	0.71 ± 0.21
Annual grain + N	14	33	36	17	0.28 ± 0.08	14	86	0.47 ± 0.08
Perennial grain	18	22	45	15	0.38 ± 0.05	19	81	0.52 ± 0.14
Perennial grain + N	10	30	39	22	0.31 ± 0.10	23	77	0.34 ± 0.04
Perennial forage	13	15	35	38	0.28 ± 0.04	18	82	0.78 ± 0.16
Perennial forage + N	23	22	25	31	0.17 ± 0.02	17	83	0.52 ± 0.14
ANOVA p values								
Crop					0.11			0.35
Fertilizer					0.35			0.09
Crop*Fertilizer					0.16			0.97
	Proportion of Cumulative CH_4 (%)				Cumulative CH_4[╢] (kg CH_4-C ha^{-1})	Proportion of Cumulative CH_4 (%)		Cumulative CH_4[╢] (kg CH_4-C ha^{-1})
	Fall 17 Sept. 2020 - 2 Nov. 2020 (46 d)	Spring 18 March 2021 - 27 May 2021 (70 d)	Reversal 28 May 2021 - 11 June 2021 (14 d)	Summer 12 June 2021 - 28 July 2021 (97 d)	17 Sept. 2020 - 28 July 2021	Fall 28 July 2021 - 25 Oct. 2021 (37 d)	Spring 4 April 2022 - 11 May 2022 (37 d)	18 Sept. 2021 - 11 May 2022
Fallow	4	24	47	26	-1.51 ± 0.29	1	99	-0.62 ± 0.17
Annual grain	5	18	33	44	-1.02 ± 0.20	6	94	-0.30 ± 0.07
Annual grain + N	7	13	35	45	-1.31 ± 0.18	5	95	-0.58 ± 0.18
Perennial grain	12	19	28	40	-1.18 ± 0.03	4	96	-0.64 ± 0.12
Perennial grain + N	13	16	24	47	-1.80 ± 0.35	9	91	-0.49 ± 0.18
Perennial forage	10	15	42	33	-2.09 ± 0.47	14	86	-0.42 ± 0.18
Perennial forage + N	9	11	51	29	-2.04 ± 0.35	8	92	-0.56 ± 0.22
ANOVA p values								
Crop					0.42			0.40
Fertilizer					0.30			0.88
Crop*Fertilizer					0.27			0.86
	Proportion of Cumulative CO_2 (%)				Cumulative CO_2 (Mg CO_2-C ha^{-1})[1]	Proportion of Cumulative CO_2 (%)		Cumulative CO_2 (Mg CO_2-C ha^{-1})[1]
	Fall	Spring 18 March 2021 - 31 March 2021 (13 d)	Reversal 28 May 2021 - 11 June 2021 (14 d)	Summer	18 March 2021 – 31 March 2021 and 28 May 2021 – 11 June 2021 (27 d)	Fall	Spring 4 April 2022 - 11 May 2022 (37 d)	4 April 2022 - 11 May 2022 (37 d)
Fallow	n.a.	15	85	n.a.	0.26 ± 0.06	n.a.	100	0.13 ± 0.03 ABa
Annual grain		18	82		0.23 ± 0.02		100	0.10 ± 0.01Aa
Annual grain + N		9	91		0.43 ± 0.10		100	0.11 ± 0.01 Aa
Perennial grain		14	86		0.29 ± 0.08		100	0.11 ± 0.01 ABa
Perennial grain + N		19	81		0.54 ± 0.11		100	0.12 ± 0.01 ABa
Perennial forage		20	80		0.40 ± 0.09		100	0.20 ± 0.04 Ba
Perennial forage + N		23	77		0.37 ± 0.11		100	0.14 ± 0.01 Ba
ANOVA p values								
Crop					0.57			0.04*
Fertilizer					0.10			0.88
Crop*Fertilizer					0.31			0.38

Data represents mean ± standard error (n=4).

Uppercase letters denote significant differences between legacy crop based upon post hoc analysis after two-way ANOVA. Lowercase letters denote significant differences between legacy fertilizer application upon post hoc analysis after two-way ANOVA. The same letters indicate no significant difference within column; different letters indicate significant differences within column at $p < 0.05$.

[╢] Negative values represent net soil CH_4 uptake.

[1] CO_2 emissions were only accounted for during times without active plant growth.

Table 7.3. Cumulative N_2O, CH_4, and CO_2 emissions and the proportion of cumulative N_2O, CH_4, and CO_2 emissions during fall, spring, reversal, and summer periods as measured by static chambers for each legacy cropping system for years one and two at the Edmonton site.

Legacy Treatment	Year One					Year Two		
	Proportion of Cumulative N_2O (%)				Cumulative N_2O (kg N_2O-N ha^{-1})	Proportion of Cumulative N_2O (%)		Cumulative N_2O (kg N_2O-N ha^{-1})
	Fall 2 Oct 2019 - 30 Oct. 2019 (28 d)	Spring 19 April 2019 - 12 June 2020 (57 d)	Reversal 13 June 2020 - 23 June 2020 (10 d)	Summer 24 June 2020 - 27 Aug. 2020 (64 d)	2 Oct. 2019 - 27 Aug. 2020	Fall 28 Aug. 2020 - 28 Oct. 2020 (61 d)	Spring 13 March 2021 - 5 May 2021 (53 d)	28 Aug. 2020 - 5 May 2021
Fallow	0	24	50	26	1.51 ± 0.29 ABa	1	99	0.62 ± 0.17
Annual grain	3	18	34	45	1.02 ± 0.2 Aa	6	94	0.30 ± 0.07
Annual grain + N	7	13	35	46	1.31 ± 0.18 Aa	5	95	0.58 ± 0.18
Perennial grain	12	19	28	41	1.18 ± 0.03 ABa	4	96	0.64 ± 0.12
Perennial grain + N	13	16	24	47	1.80 ± 0.35 ABa	9	91	0.49 ± 0.18
Perennial forage	10	15	42	33	2.09 ± 0.47 Ba	14	86	0.42 ± 0.18
Perennial forage + N	9	11	51	29	2.04 ± 0.35 Ba	8	92	0.56 ± 0.22
ANOVA p values								
Crop					0.03*			0.73
Fertilizer					0.25			0.43
Crop*Fertilizer					0.09			0.42
	Proportion of Cumulative CH_4 (%)				Cumulative CH_4 ╢ (kg CH_4-C ha^{-1})	Proportion of Cumulative CH_4 (%)		Cumulative CH_4 ╢ (kg CH_4-C ha^{-1})
	Fall 2 Oct 2019 - 30 Oct. 2019 (28 d)	Spring 19 April 2019 - 12 June 2020 (57 d)	Reversal 13 June 2020 - 23 June 2020 (10 d)	Summer 24 June 2020 - 27 Aug. 2020 (64 d)	2 Oct. 2019 - 27 Aug. 2020	Fall 28 Aug. 2020 - 28 Oct. 2020 (61 d)	Spring 13 March 2021 - 5 May 2021 (53 d)	28 Aug. 2020 - 5 May 2021
Fallow	12	63	6	19	-0.15 ± 0.03	134	-34	-0.01 ± 0.01
Annual grain	23	38	6	33	-0.14 ± 0.02	81	19	-0.01 ± 0.02
Annual grain + N	41	23	6	30	-0.15 ± 0.06	-9	109	-0.01 ± 0.03
Perennial grain	40	23	5	32	-0.10 ± 0.02	-372	472	-0.02 ± 0.01
Perennial grain + N	33	15	6	46	-0.15 ± 0.03	-118	218	-0.04 ± 0.05
Perennial forage	2	99	-3	2	-0.11 ± 0.05	112	-12	-0.01 ± 0.03
Perennial forage + N	36	49	-5	20	-0.15 ± 0.03	67	33	-0.02 ± 0.03
ANOVA p values								
Crop					0.88			0.93
Fertilizer					0.28			0.64
Crop*Fertilizer					0.87			0.87
	Proportion of Cumulative CO_2 (%)				Cumulative CO_2 (Mg CO_2-C ha^{-1})[1]	Proportion of Cumulative CO_2 (%)		Cumulative CO_2 (Mg CO_2-C ha^{-1})[1]
	Fall	Spring 19 April 2020 - 29 April 2020 (10 d)	Reversal 13 June 2020 - 23 June 2020 (10 d)	Summer	19 April 2020 – 29 April 2020 and 13 June 2020 – 23 June 2020 (27 d)	Fall	Spring 13 March 2021 - 5 May 2021 (53 d)	13 March 2021 - 5 May 2021
Fallow	n.a.	22	78	n.a.	0.23 ± 0.04 Aa	n.a.	100	0.14 ± 0.03
Annual grain		32	67		0.26 ± 0.02 Aa		100	0.19 ± 0.02
Annual grain + N		34	66		0.29 ± 0.03 Ab		100	0.15 ± 0.02
Perennial grain		42	58		0.24 ± 0.02 Aa		100	0.22 ± 0.04
Perennial grain + N		36	64		0.33 ± 0.01 Ab		100	0.21 ± 0.09
Perennial forage		41	59		0.42 ± 0.06 Ba		100	0.17 ± 0.03
Perennial forage + N		26	74		0.53 ± 0.06 Bb		100	0.23 ± 0.06
ANOVA p values								
Crop					0.0002***			0.68
Fertilizer					0.03*			0.55
Crop*Fertilizer					0.63			0.45

Data represents mean ± standard error (n=4).

Uppercase letters denote significant differences between legacy cropping system based upon post hoc analysis after two-way ANOVA. Lowercase letters denote significant differences between legacy fertilizer regime upon post hoc analysis after two-way ANOVA. The same letters indicate no significant difference within column; different letters indicate significant differences within column at $p < 0.05$.

╢Negative values represent net soil CH_4 uptake.

[1] CO_2 emissions were only accounted for during times without active plant growth.

Table 7.4. Cumulative CO$_2$eq emissions encompassed contributions of N$_2$O, CH$_4$ and CO$_2$ emissions over specific intervals within years one and two at the Breton and Edmonton sites. The study intervals when emissions data were included into these CO$_2$eq estimates can be identified under blue bars Figs. 7.3, 7.4, 7.5, and 7.6. This approach to data assemblage focuses on soil emissions by precluding the inclusion of CO$_2$ associated with canopy sources and sinks inside our opaque chambers over the periods of active plant growth.

Legacy Treatment	**Breton**	
	CO$_2$eq (Mg CO$_2$eq ha^{-1})	
	Year One	**Year Two**
Fallow	1.02 ± 0.21	0.67 ± 0.11
Annual grain	0.89 ± 0.10	0.65 ± 0.12
Annual grain + N	1.66 ± 0.40	0.58 ± 0.07
Perennial grain	1.18 ± 0.29	0.60 ± 0.12
Perennial grain + N	2.06 ± 0.52	0.55 ± 0.05
Perennial forage	1.50 ± 0.34	1.00 ± 0.10
Perennial forage + N	1.38 ± 0.41	0.72 ± 0.08
ANOVA p values		
Crop	0.51	0.10
Fertilizer	0.82	0.49
Crop x Fertilizer	0.07	0.43
	Edmonton	
	CO$_2$eq (Mg CO$_2$eq ha^{-1})	
	Year One	**Year Two**
Fallow	1.22 ± 0.23 a	0.79 ± 0.09
Annual grain	1.09 ± 0.13 a	0.81 ± 0.10
Annual grain + N	1.24 ± 0.16 a	0.78 ± 0.16
Perennial grain	1.05 ± 0.06 a	1.10 ± 0.18
Perennial grain + N	1.38 ± 0.05 a	0.92 ± 0.38
Perennial forage	1.85 ± 0.25 b	0.82 ± 0.17
Perennial forage + N	2.30 ± 0.28 b	1.08 ± 0.29
ANOVA p values		
Crop	< 0.001***	0.93
Fertilizer	0.23	0.98
Crop x Fertilizer	0.23	0.43

Data represents mean ± standard error (n=4).

Lowercase letters denote significant differences between legacy crops based upon post hoc analysis after two-way ANOVA. The same letters indicate no significant difference within column; different letters indicate significant differences within column at $p < 0.05$.

Table 7.5. Soil mineral N ($NH_4^+ + NO_3^-$) concentrations from 0-15 cm depth increment for years one and two at the Breton and Edmonton sites. Averages assembled from data available over each of the study years.

Legacy Treatment	Breton	
	Mineral N (mg kg^{-1})	
	Year One	**Year Two**
Fallow	4.51 ± 0.83	2.41 ± 0.4
Annual grain	4.45 ± 0.52	2.26 ± 0.11
Annual grain + N	3.85 ± 0.24	2.44 ± 0.25
Perennial grain	4.59 ± 0.29	2.65 ± 0.21
Perennial grain + N	4.74 ± 0.84	2.53 ± 0.2
Perennial forage	5.62 ± 0.43	2.64 ± 0.07
Perennial forage + N	5.39 ± 0.62	3.82 ± 0.64
ANOVA p values		
Crop	0.12	0.12
Fertilizer	0.11	0.25
Crop x Fertilizer	0.62	0.59
	Edmonton	
	Mineral N (mg kg^{-1})	
	Year One	**Year Two**
Fallow	49.66 ± 8.71 Aa	8.30 ± 0.86
Annual grain	26.38 ± 2.38 Ba	6.60 ± 0.10
Annual grain + N	29.16 ± 1.76 Bb	10.02 ± 1.20
Perennial grain	25.08 ± 2.67 ABa	10.12 ± 1.41
Perennial grain + N	40.15 ± 8.94 ABb	8.82 ± 0.59
Perennial forage	19.44 ± 1.04 Ca	10.81 ± 1.80
Perennial forage + N	22.62 ± 2.45 Cb	11.31 ± 1.18
ANOVA p values		
Crop	< 0.001***	0.05
Fertilizer	0.02*	0.14
Crop x Fertilizer	0.62	0.08

Data represents mean ± standard error (n=4).

Uppercase letters denote significant differences between legacy crops based upon post hoc analysis after two-way ANOVA. Lowercase letters denote significant differences between legacy fertilizer regime upon post hoc analysis after two-way ANOVA. The same letters indicate no significant difference within column; different letters indicate significant differences within column at $p < 0.05$.

Table 7.6. Soil organic carbon (SOC) and total nitrogen (TN) for the 0-15 and 15-30 cm depth increments for the Breton and Edmonton sites for the fallow, annual grain, perennial grain, and perennial forage treatments with and without nitrogen fertilization after two sequential barley crops.

Legacy Treatment	Breton					
	0-15 cm			15-30 cm		
	SOC (mg C kg^{-1})	TN (mg N kg^{-1})	C:N	SOC (mg C kg^{-1})	TN (mg N kg^{-1})	C:N
Fallow	26.31 ± 1.16	2.50 ± 0.01	10.55 ± 0.14	20.86 ± 3.16	1.97 ± 0.03	10.48 ± 0.30
Annual grain	29.84 ± 1.78	2.80 ± 0.01	10.68 ± 0.47	23.38 ± 1.01	2.22 ± 0.01	10.52 ± 0.24
Annual grain + N	25.91 ± 1.49	2.51 ± 0.01	10.34 ± 0.05	22.25 ± 1.90	2.10 ± 0.02	10.62 ± 0.14
Perennial grain	28.11 ± 0.95	2.63 ± 0.01	10.69 ± 0.11	22.15 ± 2.95	2.08 ± 0.02	10.50 ± 0.34
Perennial grain + N	27.39 ± 1.25	2.54 ± 0.01	10.77 ± 0.17	19.86 ± 1.89	1.90 ± 0.02	10.37 ± 0.21
Perennial forage	28.28 ± 1.10	2.62 ± 0.01	10.79 ± 0.12	21.22 ± 2.62	2.06 ± 0.03	10.29 ± 0.38
Perennial forage + N	29.22 ± 1.40	2.82 ± 0.01	10.35 ± 0.10	29.47 ± 1.67	2.54 ± 0.01	11.63 ± 0.98
ANOVA p values						
Crop	0.52	0.33	0.74	0.43	0.42	0.67
Fertilizer	0.26	0.50	0.19	0.50	0.87	0.25
Crop x Fertilizer	0.21	0.09	0.43	0.15	0.32	0.24
	Edmonton					
	0-15 cm			15-30 cm		
	SOC (mg C kg^{-1})	TN (mg N kg^{-1})	C:N	SOC (mg C kg^{-1})	TN (mg N kg^{-1})	C:N
Fallow	51.73 ± 2.49	4.65 ± 0.03	11.15 ± 0.32		n.d.	
Annual grain	52.89 ± 3.57	4.92 ± 0.03	10.75 ± 0.05			
Annual grain + N	54.75 ± 2.15	5.08 ± 0.02	10.78 ± 0.11			
Perennial grain	57.01 ± 0.76	5.24 ± 0.01	10.89 ± 0.30			
Perennial grain + N	57.03 ± 1.44	5.36 ± 0.01	10.64 ± 0.13			
Perennial forage	54.31 ± 3.90	5.05 ± 0.04	10.80 ± 0.20			
Perennial forage + N	57.13 ± 2.24	5.25 ± 0.01	10.87 ± 0.17			
ANOVA p values						
Crop	0.32	0.22	0.45			
Fertilizer	0.59	0.69	0.78			
Crop x Fertilizer	0.93	0.93	0.70			

Data represents mean ± standard error (n=4).

Table 7.7. Barley silage yields, protein content, and protein productivity for years one and two at the Breton and Edmonton sites.

	Breton					
	Year One			**Year Two**		
Legacy Treatment	Barley yield (kg DM ha^{-1})	Silage protein (%)	Barley silage protein productivity (kg protein ha^{-1})	Barley yield (kg DM ha^{-1})	Silage protein (%)	Barley silage protein productivity (kg protein ha^{-1})
Fallow	2692 ± 186 a	9.6 ± 0.4 ab	259 ± 16	6162 ± 703	7.4 ± 1.3	443 ± 80
Annual grain	2900 ± 637 a	8.1 ± 0.3 a	237 ± 59	5463 ± 698	6.6 ± 0.5	368 ± 68
Annual grain + N	2580 ± 63 a	8.7 ± 0.5 a	225 ± 17	6191 ± 156	6.6 ± 1.1	417 ± 139
Perennial grain	1780 ± 182 b	9.2 ± 0.3 ab	163 ± 13	6180 ± 798	8.0 ± 0.3	493 ± 58
Perennial grain + N	1677 ± 116 b	10.2 ± 1.1 ab	168 ± 9	6547 ± 690	9.5 ± 2.3	605 ± 117
Perennial forage	1819 ± 182 b	11.3 ± 0.6 b	205 ± 19	6754 ± 774	6.9 ± 0.7	475 ± 80
Perennial forage + N	2174 ± 117 b	10.4 ± 0.7 b	220 ± 19	5322 ± 748	7.4 ± 0.8	386 ± 48
ANOVA p values						
Crop	0.02*	0.02*	0.05	0.83	0.34	0.27
Fertilizer	0.84	0.63	0.66	0.65	0.49	0.97
Crop x Fertilizer	0.77	0.35	0.99	0.40	0.81	0.63
	Edmonton					
	Year One			**Year Two**		
	Barley yield (kg DM ha^{-1})	Silage protein (%)	Barley silage protein productivity (kg protein ha^{-1})	Barley yield (kg DM ha^{-1})	Silage protein (%)	Barley silage protein productivity (kg protein ha^{-1})
Fallow	9370 ± 407 a	10.3 ± 0.4 a	963 ± 33 ab	3924 ± 478	13.9 ± 0.2 a	545 ± 61
Annual grain	11428 ± 804 b	8.4 ± 0.1 b	960 ± 72 a	3116 ± 227	14.0 ± 0.3 ab	435 ± 30
Annual grain + N	11961 ± 1068 b	8.5 ± 0.2 b	1014 ± 86 a	2803 ± 371	14.2 ± 0.8 ab	403 ± 70
Perennial grain	10577 ± 786 ab	8.0 ± 0.5 b	837 ± 71 ab	3002 ± 406	15.3 ± 0.4 b	460 ± 62
Perennial grain + N	10582 ± 411 ab	8.4 ± 0.2 b	883 ± 24 ab	2757 ± 311	14.9 ± 0.2 b	410 ± 43
Perennial forage	8893 ± 335 a	8.8 ± 0.4 b	782 ± 39 b	4054 ± 273	14.1 ± 0.1 a	573 ± 43
Perennial forage + N	9417 ± 546 a	8.1 ± 0.5 b	763 ± 58 b	3280 ± 697	13.9 ± 0.2 a	461 ± 104
ANOVA p values						
Crop	0.006**	0.001**	0.01*	0.10	0.02*	0.25
Fertilizer	0.55	0.97	0.55	0.21	0.74	0.22
Crop x Fertilizer	0.91	0.47	0.84	0.79	0.67	0.80

Data represents mean ± standard error (n=4)

DM stands for dry matter.

Lowercase letters denote significant differences between legacy crops 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 $p < 0.05$.

Figures

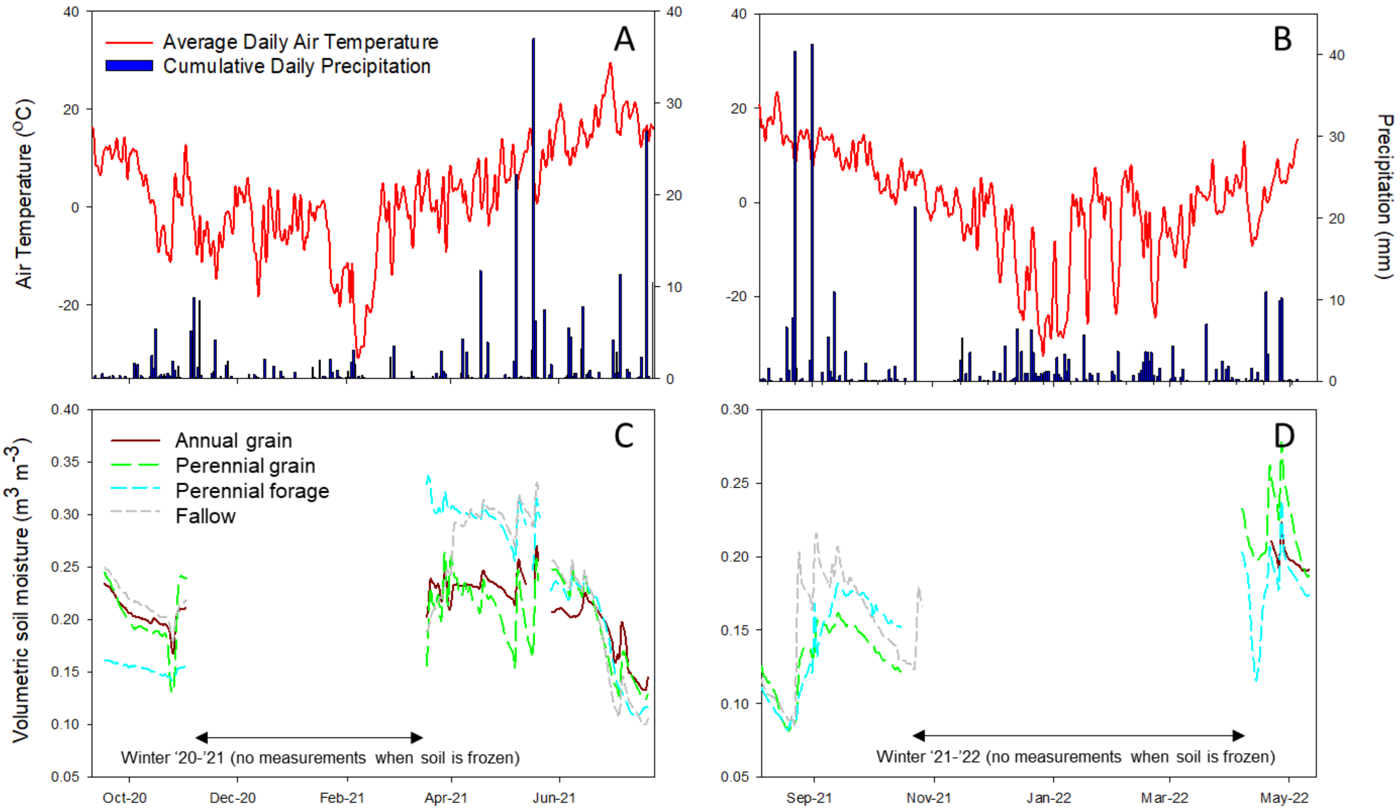

Figure 7.1. Daily precipitation and air temperature for (A) year one (2020-2021) and (B) year two (2021-2022), and average volumetric soil moisture at 7.5 cm depth for (C) year one and (B) year two at the Breton site. Note that the y-axes of panels C and D have different scales.

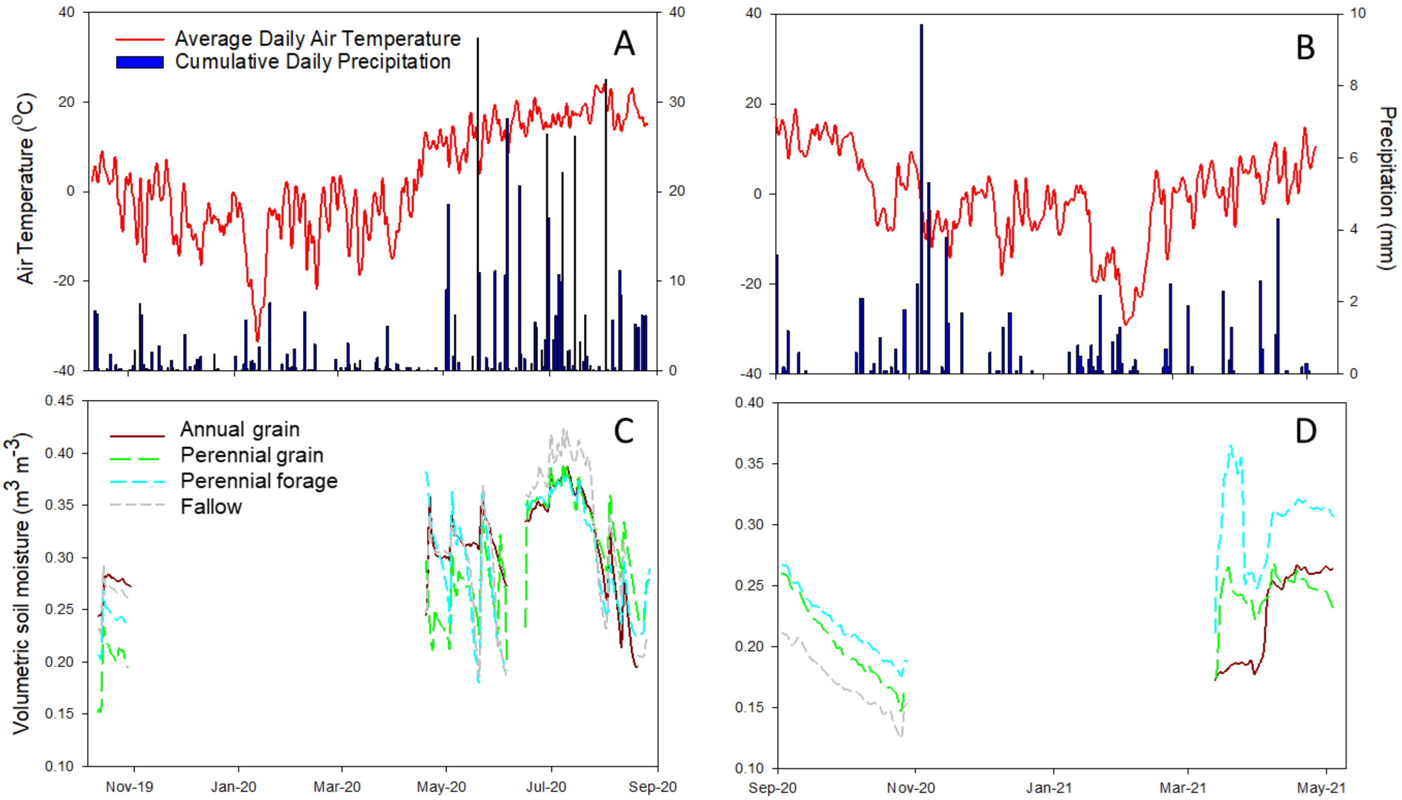

Figure 7.2. Daily precipitation and air temperature for (A) year one (2019-2020) and (B) year two (2020-2021), and average volumetric soil moisture at 7.5 cm depth for (C) year one and (B) year two at the Edmonton site. Note that the y-axes of panels C and D have different scales.

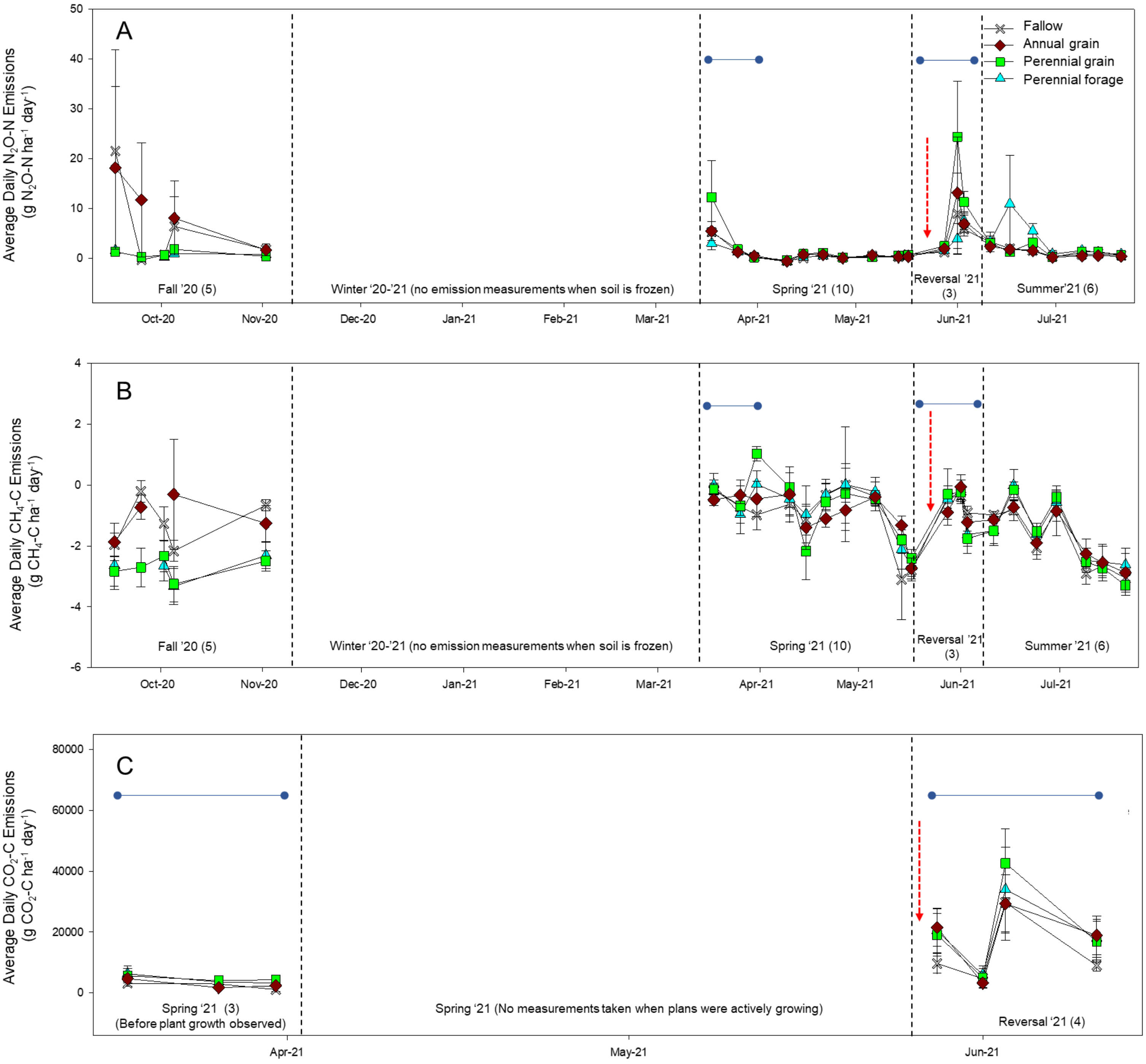

Figure 7.3. Daily (A) N_2O-N (B) CH_4-C and (C) CO_2-C emissions for year one (2020-2021) at the Breton site. Shown daily means ± standard errors (n=8) were calculated for each of the four legacy cropping systems. The dashed arrow indicates the date of the reversal of legacy treatments. The blue bars in panels A, B, and C correspond to the study intervals when emissions were accounted for in our CO_2eq estimates as shown in Figure 7.7.

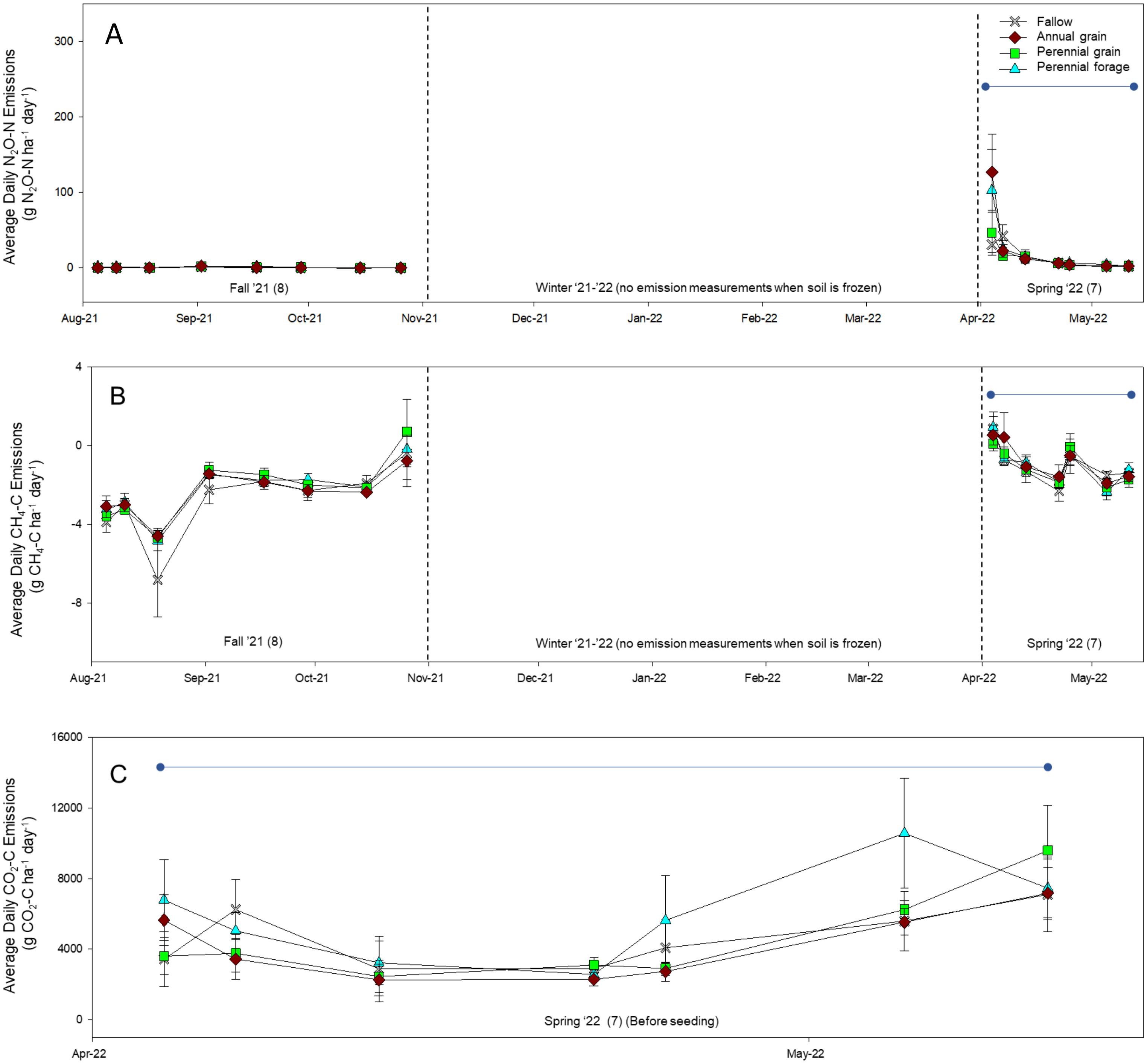

Figure 7.4. Daily (A) N_2O-N (B) CH_4-C and (C) CO_2-C emissions for year two (2021-2022) at the Breton site, where points represent the average of each crop replicate ± SE (n=8). The blue bars in panels A, B, and C correspond to the study intervals when emissions were accounted for in our CO_2eq estimates as shown in Figure 7.7. Flux measurements ended immediately prior to barley seeding and N fertilizer application in year two.

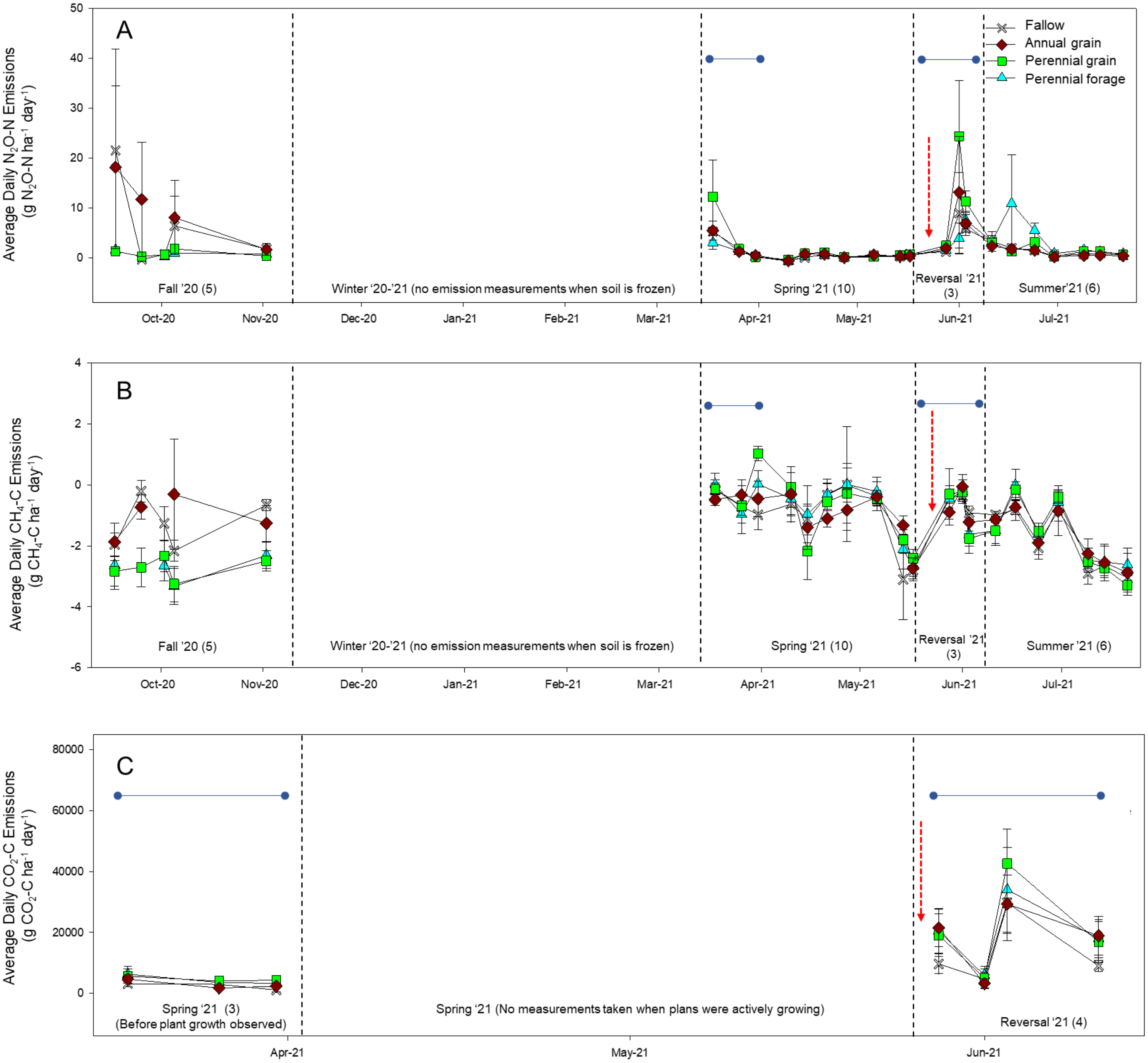

Figure 7.5. Daily (A) N_2O-N (B) CH_4-C and (C) CO_2-C emissions for year one (2019-2020) at the Edmonton site. Shown daily means ± standard errors (n=8) were calculated for each of the four legacy cropping systems. The dashed arrow indicates the date of the reversal of legacy treatments. The blue bars in panels A, B, and C correspond to the study intervals when emissions were accounted for in our CO_2eq estimates as shown in Figure 7.7.

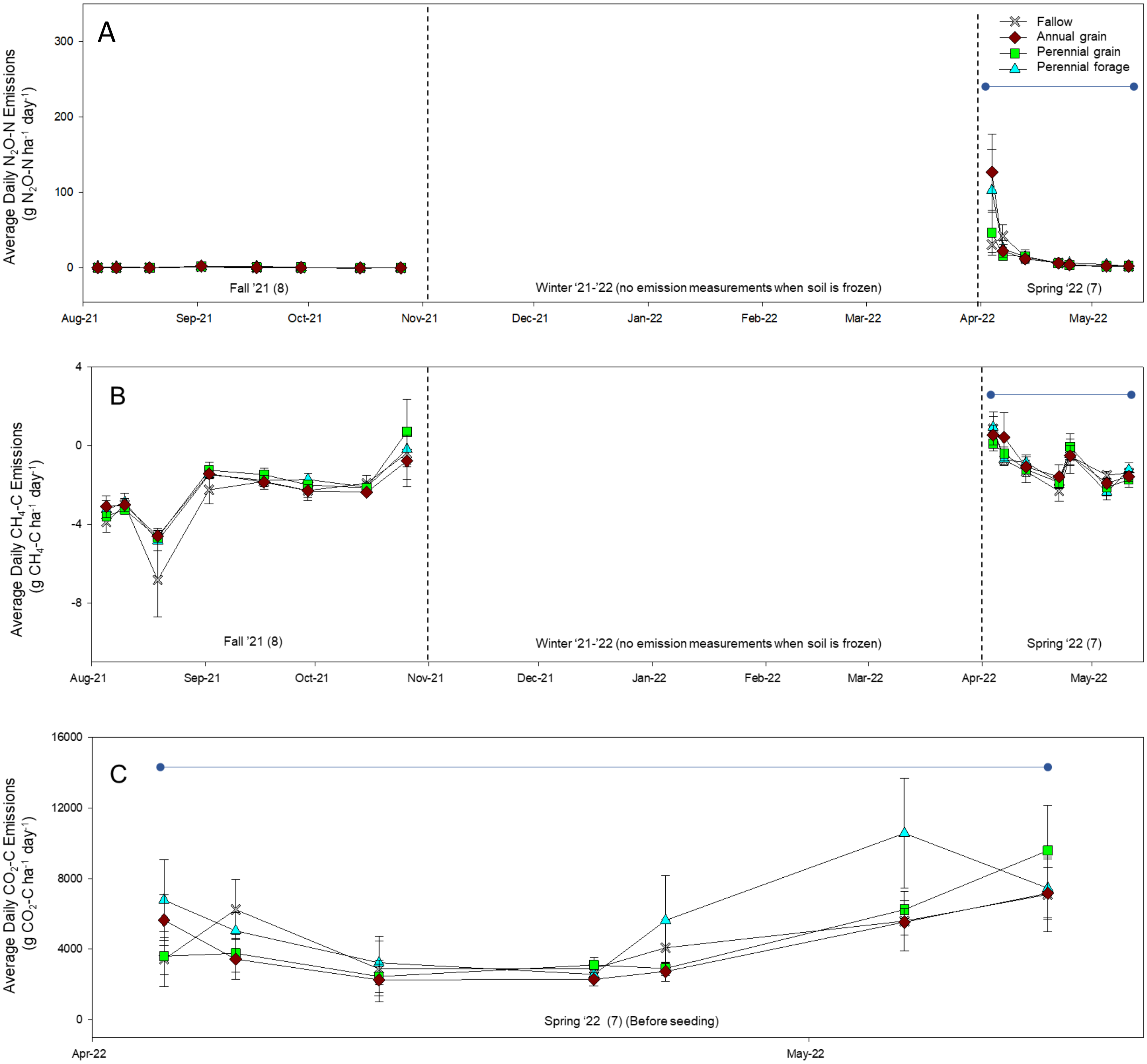

Figure 7.6. Daily (A) N_2O-N (B) CH_4-C and (C) CO_2-C emissions for year two (2020-2021) at the Edmonton site, where points represent the average of each crop replicate ± SE (n=8). The blue bars in panels A, B, and C correspond to the study intervals when emissions were accounted for in our CO_2eq estimates as shown in Figure 7.7. Flux measurements ended immediately prior to barley seeding and N fertilizer application in year two.

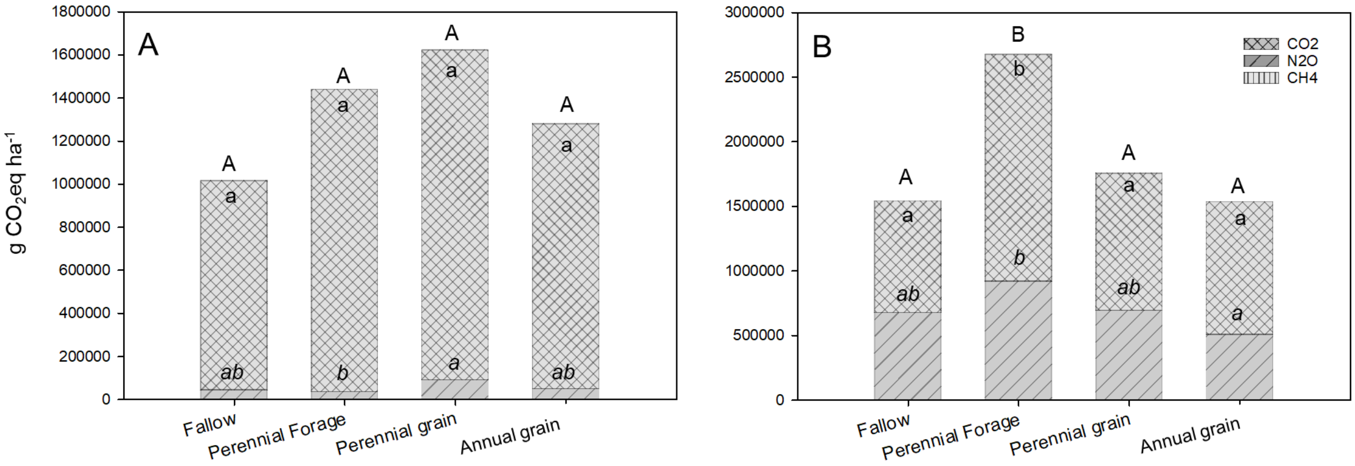

Figure 7.7. Aggregated CO_2eq of carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) emissions for the legacy fallow, perennial forage, perennial grain, and annual grain crops, averaged over legacy N fertilizer (n=8) calculated using a subset of the CH_4 and N_2O data from year one (A) for the Breton site and (B) for the Edmonton site. CH_4 contributions to CO_2eq were negligible and are not visible on the plot. The study intervals when emissions data were included into these CO_2eq estimates can be identified under blue bars in Figs. 7.3, 7.4, 7.5, 7.6. Uppercase letters denote significant differences in total CO_2eq between crops, lowercase letters denote significant differences in the CO_2 contribution to CO_2eq between crops, and lowercase italic letters denote significant differences in N_2O contribution to CO_2eq between crops at $p < 0.05$.

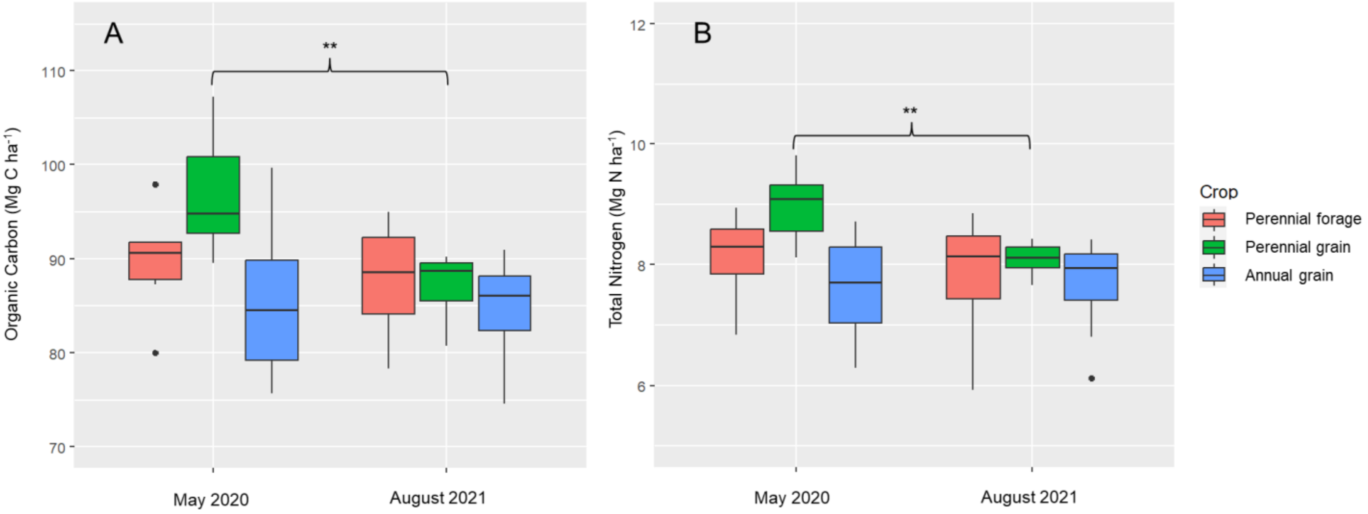

Figure 7.8. Boxplots of (A) soil organic carbon (SOC) and (B) total nitrogen (TN) contents from the 0-15 cm soil depth increment following 2.5 years of the legacy treatments (i.e., just prior to implementing the reversal via tillage in May 2020) and 15 months later (i.e., Aug. 2021) after two growing seasons of annual barley at the Edmonton site. Significant reductions in SOC and TN were found in the perennial grain treatment between May 2020 and August 2021 ($p < 0.01$).

8 Conclusions

The studies included in this dissertation improved our understanding of nitrogen (N) cycling in agroecosystems, namely with respect to nitrous oxide (N_2O) priming, and collectively assessed the environmental performance and agronomic productivity of perennial grain cropping. Generally, perennial grain cropping systems do present opportunities to improve agricultural sustainability, however, feasibility challenges remain, namely with respect to their longevity and agronomic performance, and future work will be required to improve their practicality and profitability.

To address outstanding questions regarding the underlying mechanisms behind the phenomenon of N_2O priming, we conducted a synthesis of literature in Chapter 2 and identified several key research needs to better understand and quantify N_2O production and emission from soils. Our synthesis determined that ~25% of N_2O emissions following C and N amendments may result from priming-related mechanisms, which suggests that N_2O priming plays a significant role in cumulative N_2O emissions, but the controls on N_2O priming have yet to be fully elucidated. Using this knowledge, we can guide future research that investigates agricultural management practices - including perennial grain cropping systems - that reduce N_2O emissions, a key forcing agent of anthropogenic climate change.

In Chapter 3, we investigated how the enhanced root mass of perennial grain crops may proportionally increase root exudation and affect soil organic matter (SOM) priming, and our findings demonstrated that increased root exudation altered soil carbon (C) and N interactions in beneficial (by reducing both cumulative N_2O production and soil organic N [SOM-N] priming to N_2O) and adverse (by increasing cumulative CO_2 production and soil organic C [SOM-C] priming to carbon dioxide [CO_2]) ways. Overall, the findings from this laboratory incubation are not confined to understanding the dynamics of N_2O and CO_2 production from perennial cropping systems alone, but also provide insight into how management of agroecosystems with respect to crop selection and N fertilization may alter the magnitude and source of greenhouse gas (GHG) emissions in general.

In addition to improving winter hardiness, in Chapter 4 we identified that the model perennial grain cultivar used for this study, ACE-1 perennial rye, may be a viable option when compared to existing annual rye cultivars if harvested as a dual-purpose forage and grain crop; however, reduced grain yields after the first year, minimized its feasibility as a cash crop. Notably, we identified that the prolific unproductive tillering of the perennial rye demonstrated the significant potential for perennial rye to fix atmospheric C, and thus suggested the possibility for breeding efforts to physiologically redistribute resource allocation from vegetative structures to grain production.

In Chapter 5, we investigated how soil physical and hydraulic properties are affected by contrasting agricultural management systems and determined that perennial grain crops increased topsoil SOM-C sequestration, which was attributed to their significantly increased aboveground biomass, and counteracted the short-term consolidation effects of no-till on soil bulk density, which provides insight into how to maximize SOM-C sequestration in no-till agroecosystems while minimizing compaction and associated consequences. However, we also concluded that perennial grain crops were not capable of significant improvements to overall soil physical and hydraulic properties relative to an annual counterpart within the timeframe that the perennial grain crop survived at our field sites in Central Alberta.

Our results in Chapter 6 suggest that the ability for perennial grain crops to reduce soil N_2O emissions relative to an annual counterpart is site-specific, depending on a multitude of factors, but can in part be attributed to the perennials' enhanced root mass reducing soil mineral N contents, thus providing guidance on how management decisions, such as crop selection, can be utilized to reduce N_2O emissions from agroecosystems. Importantly, the yield-based emission factor (EF) of the perennial grain crop was elevated relative to the other crops when calculated when considering grain yields alone. Therefore, perennial grain cropping requires further development to balance considerations of economic viability and N_2O emission reduction.

In our final chapter, we demonstrated that within a long, diversified rotation, the reversal of a perennial grain crop back to an annual cropping phase can alter the dynamics of GHG emissions, and that soils managed under perennial crops (forage and grain), were large emitters of C to the atmosphere as upon the transition to annual cropping via tillage, suggesting that alternative methods of transition between perennial and annual phases should be investigated to mitigate losses of SOC accrued under perennial grain cropping. Overall, this body of research presented a unique opportunity to assess the environmental performance and agronomic productivity of a perennial grain cropping system in Western Canada.

Based on our findings, while perennial grain crops provide opportunities to improve agricultural sustainability, feasibility challenges remain with respect to their agronomic performance and longevity, therefore future work will be required to optimize perennial grain management and improve their agronomic performance. In our field study, grain production beyond two growing seasons was severely hindered by weed pressure and winter mortality. In addition, several of our results were site specific. For example, in Chapter 5, we determined that the ability for perennial grain crops to substantially alter soil physical and hydraulic properties was enhanced at the clay-rich Edmonton site, whereas little convergence in soil properties between cropping systems was observed at the Breton site. Conversely, in Chapter 6, significant reductions in N_2O emissions were observed at the Breton site under the perennial grain crop, but no difference in N_2O emissions was observed at the highly fertile Edmonton site. These findings collectively inform the need for more long-term experiments at sites encompassing a wide range of eco-physiological regions and site histories, such that generalizations can be drawn regarding the impacts of perennial grain cropping systems to tailor specific management recommendations that maximize their benefits. Future research may also consider reduced N fertilizer rates for perennial grain crops, considering their increased ability to capture soil N resources, and the potential for perennial grain intercropping, to further emulate the synergistic species diversity of native perennial systems. Alternatively, the incorporation of perennial grain crops into process-based model development can be considered for future studies with the aim of reducing uncertainty and improving N_2O emission estimates, allowing for extrapolation at greater spatial and temporal scales, and improving our understanding of the mechanisms that control N cycling in soil to mitigate N_2O emissions from agroecosystems as a whole.

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Appendix

Supplementary Materials
2 Soil organic nitrogen priming to nitrous oxide: a synthesis

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Supplementary Table S2.1. Compilation of currently available literature assessing soil organic nitrogen priming to nitrous oxide.

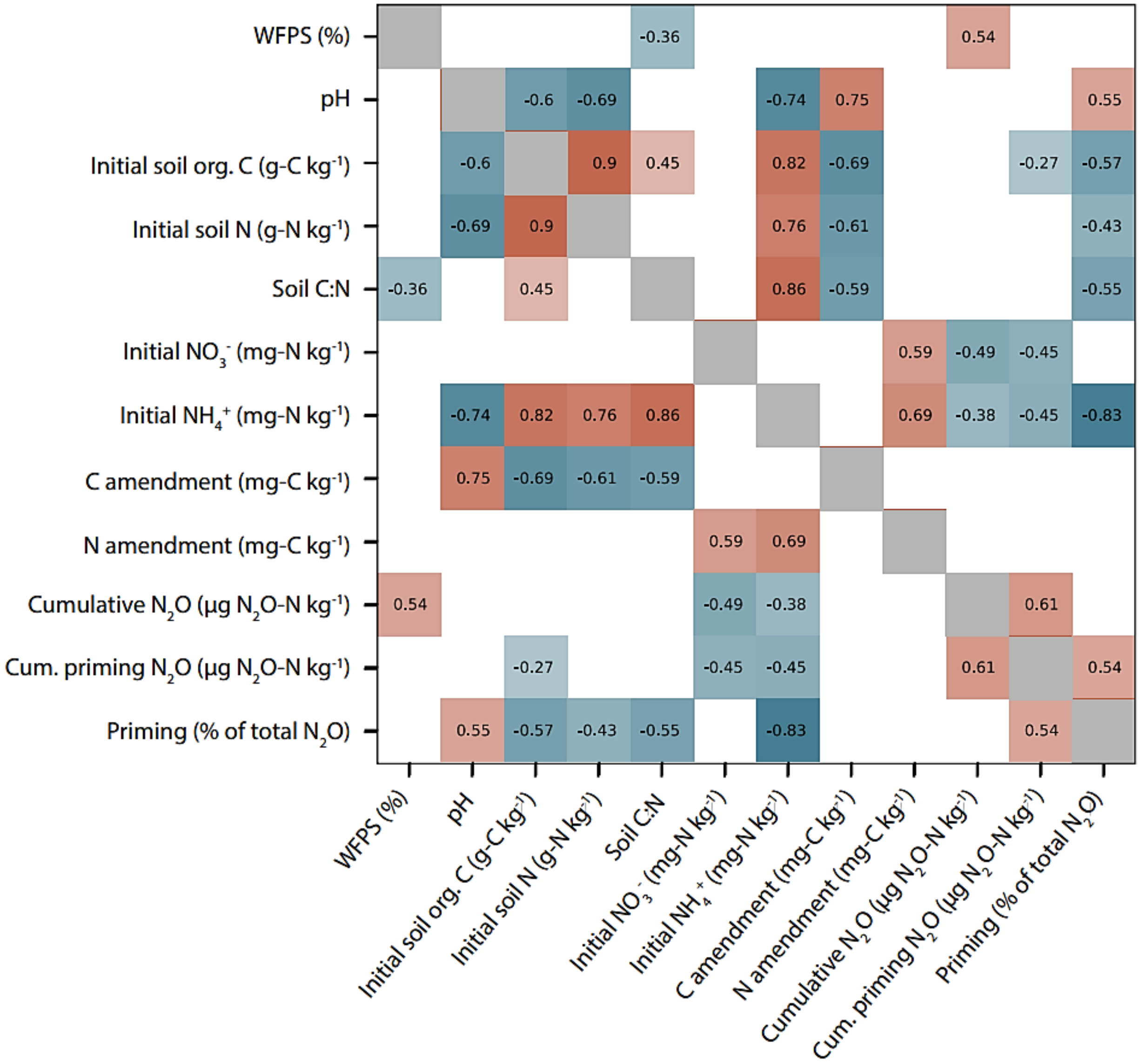
Study	Study No.	Type	Length	Soil Texture	Soil pH	Bulk Density (g cm^{-3})	Initial Soil Organic C Concentration (g C kg^{-1})	Initial Soil N Concentration (g N kg^{-1})	Soil C:N	Initial Available N (NO_3^- & NH_4^+)	Soil WFPS (%)	C Amendment	Characteristics of C Amendment	C Amendment Application Rate	N amendment	Characteristics of N Amendment	N Amendment Application Rate	Cumulative N_2O Emissions	Cumulative N_2O-N Priming	% of Priming Effect of Cumulative N_2O Emissions[‖]	Priming Index[†]	Study Description & Main Findings
Daly and Hernandez-Ramirez 2020	1	Incubation	32 days	Silt loam	5.76	1.24	24.3	2.2	11	NH_4^+ + NO_3^-: 8.70 mg N kg^{-1}	60% WFPS maintained for the entire experiment	Artificial root exudate (ARE) mix	60% glucose, 40% malonic acid blend 99 atom% ^{13}C labelled	0 (0x rate) 6.2 (0.5x rate) 12.5 (1x rate) mg C kg^{-1} soil day^{-1}	Urea	5 atom% ^{15}N labelled	0 mg N kg^{-1} soil (nU) 50 mg N kg^{-1} soil (U)	*All measurements are in µg N_2O-N kg^{-1} soil 0nU: 179.1 0U: 698.5 0.5nU: 415.6 0.5U: 471.7 1nU: 128.3 1U: 505.1	*All measurements are in µg N_2O-N kg^{-1} soil 0U: 357.4 0.5nU: 236.5 0.5U: 110.8 1nU: -50.8 1U: 77.1	0U: 51% 0.5nU: 57% 0.5U: 23% 1nU: -39% 1U: 15%	0U: 3.0 0.5nU: 2.3 0.5U: 1.6 1nU: 0.7 - 1U: 1.4	Daily applications for 20 consecutive days of artificial root exudate (ARE) to microcosms packed with soil from an agricultural Gray Luvisol topsoil (015 cm) that had been in continuous annual barley production for ~40 years. Urea, ARE, and their interaction were found to significantly alter N_2O priming ($P <0.01$, $P < 0.05$, and $P < 0.05$, respectively). Only the 0U treatment (no ARE, with urea) experienced a significant positive priming effect relative to the control, 0nU (no ARE, no urea). Less N_2O from native soil N mineralization may have occurred when ARE was applied. (antagonistic effect). The authors noted an antagonistic effect of C additions on total N_2O emissions. Increased C addition as ARE reduced SOM to N_2O priming. Potentially because of increased N immobilization by microbes or by more complete denitrification to N_2. Higher rates of denitrification in the 1U treatment suggest that N_2O conversion to N_2 occurred. Hypothesized that increased N availability (as in the 0U treatment) triggered C-limitation in microbes, triggering enzyme production that degraded SOM for a C-source and resulted in release of N because of the low C:N ration of SOM. This N was then available for microbial conversion to N_2O. SOM to CO_2 priming was enhanced by ARE addition, reduced or even negative due to urea addition. Suggesting that N limitation triggers SOM utilization to mine for N (microbial mining), but N provision in the form of urea switched microbes to preferential substrate utilization (when both C and N were added concurrently as in the 1U treatment).
Hafner et al., 2021	2	Field	2 yrs	Silt loam	7	1.24	YR 1: 12.5 YR 2: 11.3	YR 1: 1.4 YR 2: 1.3	YR 1: 8.9 YR 1: 9.0	YR 1: NH_4^+: 3.52 kg N ha^{-1} NO_3^-: 27.5 kg N ha^{-} YR 1: NH_4^+: 1.84 kg N ha^{-1} NO_3^-: 30.2 kg N ha^{-1}	Variable. First experimental year experienced ~2x the precipitation in the first 30 days of the experiment compared to year 2. In YR1, ~155 mm fell compared to 86.2 mm precipitation in YR 2.	Digestates: Maize, grass, sugar beet, sugar beet leaves, organic waste, food waste, cattle slurry	Combined with N amendment	n.d.	Digestates: Maize, grass, sugar beet, sugar beet leaves, organic waste, food waste, cattle slurry	Various atom% depending on source; all labelled with 5.36 to 12.2 atom% ^{15}N Variable C:N ratios, ranging from 2.3 (food waste) to 8.8 (cattle slurry). See Table 1 in paper for more details.	0 kg N ha^{-1} 170 kg N ha^{-1}	*All measurements are in g N_2O-N ha^{-1} YR 1: Control: 312 Maize:1166 Grass: 1293 Sugar beet: 1201 Sugar beet leaves: 1580 Organic waste: 1244 Food waste: 1060 Cattle slurry: 822 YR2: Control: 133 Maize: 690 Grass: 676 Sugar beet: 643 Sugar beet leaves: 602 Organic waste: 697 Food waste:545 Cattle slurry: 496	*All measurements are in g N_2O-N ha^{-1} YR 1: Maize: 604 Grass: 547 Sugar beet: 638 Sugar beet leaves: 804 Organic waste: 617 Food waste: 528 Cattle slurry: 376 YR2: Maize: 354 Grass: 289 Sugar beet: 394 Sugar beet leaves: 343 Organic waste: 446 Food waste: 318 Cattle slurry: 376	YR 1: Maize: 51% Grass: 42% Sugar beet: 53% Sugar beet leaves: 51% Organic waste: 50% Food waste: 50% Cattle slurry: 46% YR2: Maize: 51% Grass: 43% Sugar beet: 61% Sugar beet leaves: 57% Organic waste: 64% Food waste: 58% Cattle slurry:76%	YR 1: Maize: 2.9 Grass: 2.8 Sugar beet: 3.0 Sugar beet leaves: 3.6 Organic waste: 3.0 Food waste: 2.7 Cattle slurry: 1.1 YR2: Maize: 3.7 Grass: 3.2 Sugar beet: 4.0 Sugar beet leaves: 3.6 Organic waste: 4.4 Food waste: 3.4 Cattle slurry:2.9	Different digestates were applied to a field study on a Gray Luvisolic soil and GHG emissions were measured with static chambers. Maize, grass, sugar beet and sugar beet leaves were labelled with ^{15}N in situ while growing, hence referred to as N_t. Organic waste, food waste and cattle slurry were obtained from a biogas plant and thus only the NH_4^+ portion was labelled. In YR 1, all digestates resulted in increased total N_2O relative to the control. Sugar beet leaves had the greatest N_2O emissions compared to cattle slurry and food waste. Sugar beet leaves caused significantly greater positive N_2O priming than the cattle slurry treatment in YR 1, no significant differences in N_2O priming were found in YR 2. Ultimately, the results of the study suggest that the different digestate types influenced cumulative N_2O, flux rates and digestate derived N_2O only marginally. Hence, N_2O emissions were more strongly affected by environmental conditions. Found that increased precipitation in YR 1 increased the magnitude of primed N_2O, but the proportion of primed N_2O compared to total N_2O was similar for the N-labelled digestates for YR 1 and YR 2. In other words, increased soil moisture increased total N_2O and primed N_2O, but did not increase the proportion of primed N_2O. A major finding of this study was the large share of N_2O from the soil pool, showing that digestate application triggers "enhanced soil-derived N_2O", aka N_2O priming.
Kim et al., 2022	3	Incubation	39 days	Loam	n.d.	1.16	11.2	1.17	9.6	n.d.	Two different WFPS were maintained to test the effect on N_2O priming: 70% WFPS 40% WFPS	Decomposing switchgrass roots	Total C: 445 mg C kg^{-1} dry matter	n.d.	Decomposing switchgrass roots	Total N: 29 mg N kg^{-1} dry matter	n.d.	*All measurements are in mg N_2O-N kg^{-1} Values are estimates from Figure 4 in publication. Control: 0.05 LP40: 0.35 LP70: 1.4 Control: 0.18 SP40: 0.41 SP70: 0.55	*All measurements are in mg N_2O-N kg^{-1} Values are estimates from Figure 4 in publication. LP40: 0.15 LP70: 0.3 SP40: 0.1 SP70: 0.2	LP40: 42% LP70: 21% SP40: 24% SP70: 36%	LP40: 2.0 LP70: 4.5 SP40: 1.3 SP70: 1.5	Soil from a monoculture switchgrass plot was collected from 5-10 cm and packed into two pore size fractions (small pore and large pore). Switchgrass was grown in each rhizobox and labelled with ^{15}N and ^{13}C for 8 weeks. Plants were terminated after 8 weeks and rhizoboxes were separated into 40% and 70% WFPS treatments. Control refers to unplanted soil boxes incubated under each WFPS. The magnitude of N_2O priming was determined by separating fluxes from labelled ^{15}N switchgrass roots that were decomposing, and SOM. Higher microbial biomass C (MBC) in the small pore (SP) soil relative to the large pore (LP) soil. Overall, N_2O emissions were greater from the large pore soil than from the small pore soil (~21x greater), and greater from 70% WFPS than the 40% WFPS (~3.5x greater). Authors attribute higher N_2O emissions from the large pore soil to the "sponge effect" creating anoxic microsites in the large pore soil. In support of this, most N_2O emissions originated from the roots in the large pore soil. Suggest that the large pore soil is dominated more by fungal biomass. Saprotrophs prefer well aerated soil and require less N, the excess of which might have been available for denitrification. Roots decomposing in situ in the large-pore soil were not only a major source of N_2O; they also stimulated N_2O production from decomposition of intrinsic SOM via positive N_2O priming. N_2O priming was significantly greater in large pore soils rather than small pore soils. Suggest this is driven by higher enzyme activity near the decomposing roots, which simultaneously stimulated decomposition of SOM.
Leiber-Sautheitl et al., 2015	4	Incubation	21 days	Peat	Acidic	n.d.	27	9	3	n.d.	80% WFPS maintained for the entire experiment	^{15}N and ^{13}C labelled sheep urine and faeces	urine: 0.30 % C faeces: 39% C	urine: 6.79 g C m^{-2} faeces: 335.01 g C m^{-2}	^{15}N and ^{13}C labelled sheep urine and faeces	urine: 0.35% N faeces: 1.77% N	N rate of urine: 7.92 g N m^{-2} N rate of faeces: 15.20 g N m^{-2}	n.d.	n.d.	n.d.	Urine: 1.1 Faeces: 0.46 Urine + Faeces: 0.52	Undisturbed columns of peat were maintained at 80% WFPS and combinations of urine and faeces treatments (aka 'excreta') were applied. Cumulative N_2O emissions did not differ significantly between treatments. Found a source shift from peat-derived N_2O to excreta-derived N_2O when treatments were applied. Found no or negative N_2O priming of peat soil N by sheep excreta. Suggest preferential substrate utilization as the priming mechanism occurring, as microbes switch from decomposing peat to utilizing excreta.
Lin and Hernandez-Ramirez, 2022	5	Incubation	86 days	Clay	6.1	1.1	With history of manure application (SW): 63.7 Without history of manure application (CT): 61.6 [1]	SW: 5.9 CT: 5.9	10	n.d.	Three WFPS regimes in a simulated "spring thaw": Low (45-70-55% WFPS) Medium (55-80-65% WFPS) High (65-90-75% WFPS)	n.a.	n.a.	n.a.	Urea	Labelled with 5 atom% ^{15}N	N rate: 85 kg N ha^{-1}	*All measurements are in µg N_2O-N kg^{-1} Values are estimates from Figure 2 in publication. CT Low: 250 CT Medium: 350 CT High: 550 SW Low: 350 SW Medium: 600 SW High:700	*All measurements are in µg N_2O-N kg^{-1} Values are estimates from Figure 2 in publication. CT Low: 26.4 CT Medium: 39.9 CT High: 33.3 SW Low: 79.9 SW Medium: 160.5 SW High: 76.6	CT Low: 8.1% CT Medium: 5.6% CT High: 4.8% SW Low: 22.1% SW Medium: 18.8% SW High: 9.0%	CT Low: 1.2 CT Medium: 1.3 CT High: 1.1 SW Low: 1.4 SW Medium: 1.5 SW High: 1.1	Black Chernozem soils with (SW) and without (CT) a history of manure application were collected from 0-15 cm. Wheat was grown in the greenhouse in each pot, soil moisture was maintained at 57% WFPS. Pots were fertilized using a fertilizer blend that contained N during this time. Pots then underwent the "fall phase" where ^{15}N labelled N fertilizer was applied (85 kg N ha^{-1}) to simulate fall fertilizer application (day 0). Water was applied to achieve low, medium, and high WFPS treatments (45, 55 and 65%, respectively). Pots were kept at 2 degrees Celsius for 27 days. Control pots were unfertilized counterparts for all WFPS and manure treatments. To begin the freezing phase, pots were moved to -18 degrees Celsius for 27 days. DI water was added to simulate snow and ice inputs and bring the pots to the 6next stage of the low, medium, and high WFPS (55, 780 and 90% respectively). All pots were moved to 8room temperature on day 56 and WFPS was mai9ntained at 55, 65 and 75% for the low, med and high WFPS treatments. Fall applied N fertilizer as urea induced a positive priming effect on SOM during the subsequent spring thaw. Hypothesized that adding N satisfies microbial requisites for decomposition of existing SOM. Previously manured soil showed increased N_2O priming, suggesting that increasing SOM increased N_2O priming. Increasing WFPS significantly increased soil-derived N2O emissions (Low: 197, Med: 292, vs. High: 473). A history of manure application (SW) showed increased soil-derived N_2O emissions by 39% above those of the CT soil (374 vs. 268). Under the highest moisture, positive priming became negative before reaching zero, suggesting preferential substrate utilization. Microbes were required to immobilize soil N when decomposing more recalcitrant material later in the experiment, resulting in reduced N_2O priming.
Mehnaz et al, 2019	6	Incubation	20 days	Sand	6.1	n.d.	56	4	14	n.d.	14% (Gravimetric Moisture Content)	Glucose (GL) Oxalic acid (OX) Phenol (PH)	20 atom% ^{13}C	50 mg C kg^{-1}	Ammonium sulfate & potassium nitrate	$(NH_4)_2SO_4$ labelled with 33 atom% ^{15}N	15 mg N kg^{-1} (1 mg $(NH_4)_2SO_4$ + 14 mg KNO_3)	*All measurements are in µg N_2O-N kg^{-1} Values are estimates from Figure 5 in publication. No C no P: 24 No C with P: 13 GL no P: 4 GL with P: 15 OX no P: 12 OX acid with P: 21 PH P: 24 PH P: 35	n.a.	n.a.	n.a.	A laboratory incubation on a grassland soil to examine the effect of different C substrates (glucose, oxalic acid, phenol) and phosphorus (P) addition on priming and cumulative N_2O emissions. Found no effect of biochemical recalcitrance of C substrates on SOM priming (measured as CO_2 evolved). This may be due to different mechanisms for each substrate. Glucose can induce a priming effect by providing energy, whereas oxalic acid could desorb organic C compounds. Addition of P enhanced the priming effect. Increased availability of P and C may have induced N limitation, leading to microbial degradation of SOM for N acquisition. Oxalic acid and phenol increased gross N mineralization, but glucose did not. Glucose is energy-rich, microbes may not have mineralized N as their demand for N would be high. C substrate addition was indirectly related to N_2O emission. Daily CO_2 emissions were positively correlated with gross N mineralization rates. Phenol showed the highest N_2O emissions, potentially because of the high levels of N mineralization with phenol addition. Glucose on the other hand reduced N mineralization and showed insignificant increases in N_2O relative to the control. Alleviation of P limitation increased N_2O emission, suggesting denitrifiers are P limited in soil. P addition stimulated SOM priming, possible by inducing microbial demand for N, and or stimulating the growth of SOM degrading populations. Suggest that applying P and C can reduce C sequestration long term, may also increase N_2O emissions.
Roman-Perez and Hernandez-Ramirez, 2021	7	Incubation	35 days	Silty clay loam	6.5	1.03	54.8	4.6	11	NH_4^+: 13.61 mg N kg^{-1} NO_3^-: 4.39 mg N kg^{-1}	Multiple different WFPS were maintained to test the effect on N2O priming: 28% WFPS 40% WFPS 52% WFPS 64% WFPS	n.a.	n.a.	n.a.	Urea	5 atom% ^{15}N labelled	96 kg N ha^{-1} (or 61 mg N kg^{-1} soil)	*All measurements are in µg N_2O-N kg^{-1} 28% WFPS no N: 14.19 28% WFPS N: 27.17 40% WFPS no N: 20.23 40% WFPS N: 115.96 52% WFPS no N: 44.68 52% WFPS N: 246.85 64% WFPS no N: 93.42 64% WFPS N: 113.48	*All measurements are in µg N_2O-N kg^{-1} 28% WFPS N: 3.82 40% WFPS N: 15.10 52% WFPS N: 23.39 64% WFPS N: 48.97	28% WFPS N: 14% 40% WFPS N: 24% 52% WFPS N: 20% 64% WFPS N: 20%	28% WFPS N: 1.3 40% WFPS N: 1.7 52% WFPS N: 1.5 64% WFPS N: 1.5	Black chernozemic soils from 0-15 cm were collected to assess the effects of urea and WFPS on N_2O priming. Urea consistently triggered a strong positive priming of N_2O for all soil moisture levels, however, higher moisture levels intensified the priming effect. In general, priming was highest in the first 6 days of the experiment then neutralized. The magnitude of primed N_2O increased 12-fold from 3.82 to 48.47 ug N_2O-N kg^{-1} soil from 28% WFPS to 64% WFPS. Interestingly, the N_2O emissions from urea neutralized faster than the N_2O emissions from SOM, which lasted longer. Ascribes increased diffusion of dissolved organic matter with higher WFPS to increased priming. Hypothesize that urea addition fulfilled the stoichiometric requirements of soil microbes to promote growth and synthesis of exoenzymes to break down SOM. The majority of N_2O released was sourced from SOM in this SOM rich soil. Suggest increasing SOM to N_2O is in part a function of baseline soil SOM (i.e., higher SOM, higher N_2O priming).

Study	Study No.	Type	Length	Soil Texture	Soil pH	Bulk Density (g cm^{-3})	Initial Soil Organic C Concentration (g C kg^{-1})	Initial Soil N Concentration (g N kg^{-1})	Soil C:N	Initial Available N (NO_3^- & NH_4^+)	Soil WFPS (%)	C Amendment	Characteristics of C Amendment	C Amendment Application Rate	N amendment	Characteristics of N Amendment	N Amendment Application Rate	Cumulative N_2O Emissions	Cumulative N_2O-N Priming	% of Priming Effect of Cumulative N_2O Emissions╢	Priming Index˥	Study Description & Main Findings
Thilakarathna and Hernandez-Ramirez, 2020	8	Incubation	70 days	Loam	Continuous barley (CB): 6.23 Continuous fescue (CF): 5.90 Faba bean (FB): 6.23 Hay (H): 5.92	1.06	CB: 16.01 CF: 16.43 FB: 25.61 H: 31.48	CB: 1.34 CF: 1.41 FB: 2.09 H: 2.84	CB: 11.98 CF: 11.68 FB: 12.26 H: 11.07	n.d.	44% WFPS maintained for the entire experiment	Barley root exudates, root tissue and litter	Barley was grown in each experimental pot during the experiment	n.d.	Urea Urea + Nitrapyrin (nitra) Urea + DMPSA	3 different N treatments (same N rate, different inhibitors) and an unfertilized control. Urea was labelled with 5 atom% ^{15}N.	45 kg N ha^{-1}	*All measurements are in µg N_2O-N kg^{-1} Two treatments (management and fertilizer/inhibitor analyzed separately). Values are estimates from Figure 2 in publication. CB: 548 CF: 226 FB:1239 H: 2084 Control: 828 U: 1263 U+ nitra: 1056 U+ DMPSA: 949	*All measurements are in µg N_2O-N kg^{-1} Two treatments (management and fertilizer/inhibitor analyzed separately). Values are estimates from Figure 2 in publication. CB: 150 CF: 90 FB:225 H: -150 U: 180 U+ nitra: 30 U+ DMPSA: 15	CB: 24% CF: 18% FB:18% H: -9% U: 15% U+ nitra: 2% U+ DMPSA: 1%	U: 1.2 U+ nitra: 1.0 U+ DMPSA: 1.1	Soil samples from 0-15 cm were collected from 4 different rotations [continuous barley (CF), continuous fescue (CF), faba bean (FB), and hay (H)] at one site, representing a diverse assemblance of management practices on the same soil. Cumulative N_2O emissions were affected by management legacy. The cumulative N_2O emissions were found in the hay soil, as this soil was tilled for the first time upon treatment preparation after 3 years of no-till. Consequently, the hay soil also had the largest proportion of SOM derived N_2O of all management legacies. Conversely, cumulative N_2O priming across management legacies revealed a noticeable pattern. The percentage of cumulative N_2O derived from the priming of SOM tended to be the highest under CF and the lowest in hay soil (despite hay soil having the largest N_2O emissions (see above). The N_2O priming due to N addition was lowest in the hay soil. CB tended to have the greatest relative priming (expressed as percentage of the total N_2O emissions) among the four management legacies, potentially due to a history of urea additions. The hay soil had the lowest relative N_2O priming. Inhibitors tended to reduce primed emissions. Both DMPSA and nitrapyrin decreased the priming effect of urea addition on SOM mineralization by one order of magnitude on average (across all management legacies). Inherent priming effects of N_2O emissions are influenced by management legacies that differ in their stimulation of microbial turnover of SOM. High N losses and low N recovery capacity (soil retention and plant uptake) were evident in soils managed under long-term, continuous annual cropping. These annually cropped soils exhibited a positive priming effect that was attributable, in part, to acclimation to recurrent N fertilizer applications.
Thilakarathna and Hernandez-Ramirez, 2021	9	Incubation	30 days	Silty clay	6.2	1.07	45	3.9	12	NH_4^+: 3 mg N kg^{-1} NO_3^-: 11 mg N kg^{-1}	Multiple different WFPS were maintained to test the effect on N_2O priming: 31% WFPS 41% WFPS 53% WFPS 65% WFPS 78% WFPS	n.a.	n.a.	n.a.	Urea ^{15}N Urea ^{15}N Urea + nitrification inhibitor (urea+nitra) ^{15}N Urea + 2 urease inhibitors (urea+UI)	5 N treatments total (4 with N addition, one no N control). ^{15}N labelled treatments were labelled at 5 atom%.	100 kg N ha^{-1} (or 62.35 mg N kg^{-1})	*All measurements are in µg N_2O-N kg^{-1} Two treatments (WFPS and fertilizer/inhibitor analyzed separately). Values are estimates from Figure 5 in publication. 31% WFPS: 60 41% WFPS: 65 53% WFPS: 60 65% WFPS: 75 78% WFPS: 220 Control: 60 Urea: 110 Urea+nitra: 105 Urea+UI: 104	*All measurements are in µg N_2O-N kg^{-1} Two treatments (WFPS and fertilizer/inhibitor analyzed separately). Values are estimates from Figure 6 in publication. 31% WFPS: -10 41% WFPS: -11 53% WFPS: -5 65% WFPS: 5 78% WFPS: 35	Values are estimates from Figure 6 in publication. 31% WFPS: -18% 41% WFPS: -20% 53% WFPS: -8% 65% WFPS: 7% 78% WFPS:14%	31% WFPS: 0.8 41% WFPS: 0.8 53% WFPS: 0.9 65% WFPS: 1.2 78% WFPS:1.5	Black Chernozem soil was collected from a site previously cropped to wheat and/or barley and repacked into mesocosms for 30 days of flux measurements. Site preference measurements suggest that denitrification was the dominant source of N_2O during peak emission, and denitrification contributed more to N_2O emissions as WFPS increased. Examined priming of processes, i.e., how is nitrification or denitrification primed? In soils without labelled urea, both processes were positively primed, however the priming of denitrification was more substantial. Examining ^{15}N depletion, higher soil moistures resulted in greater ^{15}N depletion, supporting that denitrification was dominant as WFPS increased. Priming of SOM was strongly influenced by moisture. The priming of N_2O was unaffected by 10inhibitor treatments and contributed on average, 69% of total N_2O emissions. This study informs how primed N_2O production from SOM switches in rate and direction between negative priming and positive priming as a function of differences in soil moisture. At low soil moisture, negative N_2O priming was observed. At high soil moisture, positive N_2O priming was observed. Ranges from -20% (31 and 41% WFPS) to 14% (78% WFPS). Results suggest that pulses of N_2O emissions following N fertilization in croplands are triggered by soil moisture surpassing a threshold of 60% WFPS and causing positive priming of SOM, faster nitrification, and much enlarged denitrification.
Schleusner et al., 2018	10	Incubation	7 days	Loamy sand	6.6	1.2	Without manure [LM-]: 14 With manure [LM+]: 11	1	12.8	LM+ and LM-: NH4+: < 2.0 LM+: NO_3^-: <2.5 LM-: NO_3^- 2.8 mg N kg^{-1}	60% WFPS maintained for the entire experiment	n.a.	n.a.	n.a.	Ammonium nitrate	17 atom% ^{15}N	100 kg N ha^{-1}	*All measurements are in mmol N g^{-1} Values are estimates from Figure 2 in publication. LM-: 950 LM+: 1500	*All measurements are in mmol N g^{-1} Values are estimates from Figure 2 in publication. LM-: 75 LM+: 75	n.d.	LM-: 2.5 LM+: 2.2	Soil was collected from 0-10 cm from a potato-rye-maize rotation that received N fertilizer either as synthetic mineral N (LM-) or as liquid manure (LM+). Cumulative N_2O emission from SOM more than doubled after N fertilizer application to both soils (LM- and LM+). Cumulative N_2O emissions after mineral N fertilizer application were slightly higher in the soils with a history of manure application, likely because of their higher C and N content. Interestingly, N_2O derived from fertilizer was the largest fraction in this experiment, as opposed to N_2O from SOM. Found that manure history (and thus C and N content of soil) did not alter the magnitude of priming. Primed N_2O was similar in both LM- and LM+ soils.
Liao et al., 2021	11	Field	4 months	Sandy loam	Control: 8.09 Biochar: 8.17	Control: 1.47 Biochar:1.41	Control: 7.75 Biochar: 9.36	Control: 0.78 Biochar: 0.83	Control: 9.92 Biochar: 11.34	Control: NH_4^+: 1.70 mg N kg^{-1} NO_3^-: 10.55 mg N kg^{-1} Biochar: NH_4^+: 2.66 mg N kg^{-1} NO_3^-: 9.40 mg N kg^{-1}	Variable due to field conditions.	Biochar application	Pyrolyzed maize straw	12 t ha^{-1}	Urea	10.24 atom% ^{15}N	200 kg N ha^{-1}	*All measurements are in kg N_2O-N ha^{-1} Control no N: 0.58 Control N: 2.06 Biochar no N: 0.58 Biochar N: 1.89	*All measurements are in kg N_2O-N ha^{-1} Control: 0.69 Biochar: 0.56	Control: 33.7% Biochar: 29.7%	Control: 2.2 Biochar: 2.0	N_2O measurements were conducted using static chambers on an aquic Fluvent in China. Treatments included soils with or without biochar application. ^{15}N labelled urea was applied in a split application totalling 200 kg N ha^{-1} to all plots except for the ‘baseline’ plot, which received neither urea nor biochar. Found that urea application universally induced a priming effect on SOM. Biochar reduced N_2O priming in magnitude and proportion of total N_2O emissions. Biochar may reduce decomposition of SOM, which in turn reduces soil-derived NH_4^+-N concentrations. It has also been suggested that SOM, especially small aliphatic dissolved organic molecules, can enter into the biochar micropores or be adsorbed by its external surfaces, thus reducing microbial access to SOM.
Takeda et al., 2022	12	Field	4 months	Burdekin site: silt loam Mackay site: Sandy clay loam	Burdekin site: 6.92 Mackay site: 4.13	Burdekin site: 1.3 Mackay site: 1.1	Burdekin site: 16.0 Mackay site: 13.5	Burdekin site: 0.8 Mackay site: 0.9	Burdekin site: 20 Mackay site: 15	Burdekin site: 37 kg N ha^{-1} Mackay site: 31.8 kg N ha^{-1}	Variable due to field conditions.	Different sugarcane residues	Burdekin: burnt sugarcane residue Mackay: green cane trash blanketing	n.d.	Urea	5 atom% ^{15}N	Burdekin: 0 kg N ha^{-1} 150 kg N ha^{-1} 200 kg N ha^{-1} 250 kg N ha^{-1} Mackay: 0 kg N ha^{-1} 100 kg N ha^{-1} 150 kg N ha^{-1} 200 kg N ha^{-1} 250 kg N ha^{-1}	*All measurements are in kg N_2O-N ha^{-1} Burdekin 0N: 0.29 150N:1.52 200N: 1.68 250N: 3.07 Mackay 0N: 1.50 100N: 3.91 150N: 3.72 200N: 4.14 250N: 3.75	*All measurements are in kg N_2O-N ha^{-1} Burdekin 150N: 0.82 200N: 1.00 250N: 2.00 Mackay 100N: 1.38 150N: 1.25 200N: 1.19 250N: 0.91	Burdekin 150N: 54% 200N: 59% 250N: 65% Mackay 100N: 35% 150N: 33% 200N: 29% 250N: 24%	Burdekin 150N: 3.8 200N: 4.4 250N: 7.9 Mackay 100N: 1.9 150N: 1.8 200N: 1.8 250N: 1.6	A field experiment where sugarcane was grown under irrigated conditions at two sites with different residue management: burning (Burdekin site) or green cane trash blanketing (Mackay site). Burdekin experienced significant differences in N_2O emission because of different fertilizer rates, Mackay did not. Cumulative N_2O emissions from the Burdekin control treatment (0N) were ~1/5 of those at the Mackay site. N fertilizer loss increased exponentially with fertilizer rates. 60% of plant N uptake and N_2O emissions were derived from mineralised soil N and added fertilizer N increased the contribution of SOM to N_2O emissions. At the Burdekin site, SOM-derived N_2O emissions also increased with increasing N rates. In this study, cumulative CO_2 emissions did not respond to N fertilizer, suggesting that ‘real priming’ did not occur. Instead, added N may have undergone immobilization at both sites thus freeing up more native soil N for conversion to N_2O (apparent priming). At the Mackay site with residue, the labile C availability may have enhanced immobilization leading to greater pool substitution and even higher soil N sourced N_2O. This is supported by the increased fertilizer N remaining in the soil after experiment conclusion.
Xu et al., 2021	13	Field	4 months	Clay loam	7.68	1.52	17.8	1.04	17	n.d.	Variable due to field conditions.	Wheat straw incorporation	C/N = 98:1	0.86 kg straw m^{-2}	Urea	10.21 atom% ^{15}N	125 kg N ha^{-1}	*All measurements are in kg N_2O-N ha^{-1} Values are estimates from Figure 3 in publication. N0S0: 0.03 N0S1: 0.03 N1S0: 0.74 N1S1: 0.94	*All measurements are in kg N_2O-N ha^{-1} Values are estimates from Figure 3 in publication. N0S1: 0 N1S0: 0.22 N1S1: 0.27	N0S1: no priming N1S0: 30% N1S1: 29%	N0S1: no priming N1S0: 6.1 N1S1: 8.1	A field experiment conducted for one growing season to determine the effects of wheat straw incorporation and urea application on N_2O emissions and NH_3 volatilization. Conducted flux measurements using the closed chamber method. Note that soils were irrigated with N fertilizer application in accordance with local practices. Incorporation of straw reduced NO_3^- contents in soil after N fertilizer application relative to the fertilizer only treatment. Additionally, residual N remaining in the soil was greater with straw and N fertilizer than with N fertilizer alone. In the first 2-3 days after N fertilizer application, most N_2O was derived from fertilizer, however this decreased to <5% 13-17 days after fertilization. Non-fertilizer derived emissions after N fertilizer application were significantly greater than those without N fertilizer (positive priming). CO_2 emissions were also simulated by fertilizer application.
Li et al., 2022	14	Incubation	3 days	Ashley Dean (AD): Loam Lincoln University (LU): Loam Lincoln Demonstration (LD): Loam	Ashley Dean (AD): 6.2 Lincoln University (LU): 6.0 Lincoln Demonstration (LD): 5.8	1.1	Ashley Dean (AD): 32.3 Lincoln University (LU): 46.6 Lincoln Demonstration (LD): 45.5	Ashley Dean (AD): 3.3 Lincoln University (LU): 4.5 Lincoln Demonstration (LD): 4.8	Ashley Dean (AD): 9.8 Lincoln University (LU):10.4 Lincoln Demonstration (LD): 9.5	n.d.	Soils maintained at 3 matric potentials (in kPa): −3 − 5 − 7	acetate (ac) glucose (gl) butyrate (bu) water (control)	6 atom% ^{13}C	0.9 mL of the C substrate solution daily for three days at 80 µg C g$^{−1}$	KNO_3	40 atom% ^{15}N	300 µg N g$^{−1}$	*All measurements are in mg N_2O-N m^{-2} Values are estimates from Figure 3 in publication. ADac: 4 ADgl: 4 ADbu: 9 LUac: 11 LUgl: 11 LUbu: 30 LDac: 18 LDgl: 17 LDbu:30	n.d.	n.d.	n.d.	Soil samples were collected from 0 to 15 cm at three routinely cattle-grazed grassland sites all dominated by perennial ryegrass and white clover. Three C substrates were applied to soils at three different matric potentials. No significant effect of soil moisture (as different matric potentials) was detected. The increased SOM-derived N_2O emissions with glucose addition demonstrate that non ^{15}N-labelled N sources contributed to N_2O production. Non ^{15}N labelled sources include antecedent inorganic-N, turnover of antecedent microbial biomass-N, or soil organic N. Glucose is likely to favour the formation of anoxic microsites and thus promote N_2O emissions. A higher biological oxygen demand would explain the higher total emission of N_2O under glucose.

Abbreviations used include: N_2O, nitrous oxide; C, carbon; N, nitrogen; P, phosphorus; SOM, soil organic matter; WFPS, water-filled pore space; YR, year; ARE, artificial root exudate; DMPSA, 3,4-dimethylpyrazole succinic acid

╢Calculated as the proportion of emissions contributed by the N_2O priming effect divided by the total N_2O emissions.

˥Calculated as the ratio of SOM-N derived N_2O emission from the treatment to the SOM-N derived N2O emission from the control as in Leiber-Sauheitl et al. (2015).



	WFPS (%)	pH	Initial soil org. C (g-C kg^{-1})	Initial soil N (g-N kg^{-1})	Soil C:N	Initial NO_3^- (mg-N kg^{-1})	Initial NH_4^+ (mg-N kg^{-1})	C amendment (mg-C kg^{-1})	N amendment (mg-C kg^{-1})	Cumulative N_2O (µg N_2O-N kg^{-1})	Cum. priming N_2O (µg N_2O-N kg^{-1})	Priming (% of total N_2O)
WFPS (%)					-0.36					0.54		
pH			-0.6	-0.69			-0.74	0.75				0.55
Initial soil org. C (g-C kg^{-1})		-0.6		0.9	0.45		0.82	-0.69			-0.27	-0.57
Initial soil N (g-N kg^{-1})		-0.69	0.9				0.76	-0.61				-0.43
Soil C:N	-0.36		0.45				0.86	-0.59				-0.55
Initial NO_3^- (mg-N kg^{-1})									0.59	-0.49	-0.45	
Initial NH_4^+ (mg-N kg^{-1})		-0.74	0.82	0.76	0.86				0.69	-0.38	-0.45	-0.83
C amendment (mg-C kg^{-1})		0.75	-0.69	-0.61	-0.59							
N amendment (mg-C kg^{-1})						0.59	0.69					
Cumulative N_2O (µg N_2O-N kg^{-1})	0.54					-0.49	-0.38				0.61	
Cum. priming N_2O (µg N_2O-N kg^{-1})			-0.27			-0.45	-0.45			0.61		0.54
Priming (% of total N_2O)		0.55	-0.57	-0.43	-0.55		-0.83				0.54	

Supplementary Figure S2.1. Spearman correlation coefficients between all numeric variables. Positive correlations are displayed in blue and negative correlations in red. Color intensity is proportional to the correlation coefficients, which correspond to the numbers in each box. Only correlation coefficients with $p < 0.05$ are included in the figure. Abbreviations include: WFPS, water filled pore space; org., organic; Cum., cumulative.

Supplementary Materials

3 Sources and priming of soil N_2O and CO_2 production: nitrogen and simulated exudate additions

Daly, E. J., & Hernandez-Ramirez, G. (2020). Sources and priming of soil N_2O and CO_2 production: Nitrogen and simulated exudate additions. Soil Biology and Biochemistry, 149, 107942.

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Supplementary Table S3.1. Compilation of experimental reports currently available in the literature assessing priming effects of C and N additions on N_2O emissions. Equivalent results from our study are shown in Table 3.3.

Study	Type	Length	Soil Texture	Initial Soil Organic C Concentration	Initial Soil N Concentration	Soil C:N	Initial Available N (NO_3^- & NH_4^+)	Initial DOC	Soil WFPS (%)	C Amendment	Characteristics of C Amendement	C Amendment Application Rate	N amendment	Characteristics of N Amendment	N Amendment Application Rate	Cumulative N_2O Emissions	Absolute change in N_2O Emissions (compared with unamended control)	Relative change in N_2O Emissions (compared with unamended control) (%)	Study Description
Thomas et al., 2017	Field	2 yrs	clay loam	n.d.	n.d.	n.d.	n.d.	n.d.	variable	fall rye root exudate and decomposing root tissue	n.a.	variable	NH_4NO_3	n.a.	45 kg N Ha^{-1}	yr 1: 419 g N Ha^{-1}	15 g N Ha^{-1}	3	A 2-yr field study was conducted to quantify how cover crop type and fertilizer source affected N_2O emissions, soil water extractable organic carbon and NO_3^- dynamics. Year one and two cumulative N_2O emission values for each respective crop are averaged over nitrogen amendment type (compost or NH_4NO_3) as no significant difference was found between the two nitrogen amendment types. Concluded that fall rye increased non-growing season N_2O emissions, hypothesizing that cover crops concentrate denitrification substrates in the rhizosphere to enhance N_2O emissions.
													composted beef cattle manure	yr1: 171 g C kg^{-1}, 15 g N kg^{-1}, 30.2 mg NH_4-N kg^{-1}, 1140 mg NO_3-N kg^{-1} yr 2: 173 g C kg^{-1}, 16 g N kg^{-1}, 20.1 mg NH_4-N kg^{-1}, 607 mg NO_3-N kg^{-1}	100 kg N Ha^{-1}	yr 2: 120 g N Ha^{-1}	52 g N Ha^{-1}	43	
										oilseed radish root exudate and decomposing root tissue			NH_4NO_3	n.a.	45 kg N Ha^{-1}	yr 1: 312 g N Ha^{-1}	-92 g N Ha^{-1}	-29	
													composted beef cattle manure	yr1: 171 g C kg^{-1}, 15 g N kg^{-1}, 30.2 mg NH_4-N kg^{-1}, 1140 mg NO_3-N kg^{-1} yr 2: 173 g C kg^{-1}, 16 g N kg^{-1}, 20.1 mg NH_4-N kg^{-1}, 607 mg NO_3-N kg^{-1}	100 kg N Ha^{-1}	yr 2: 173 g N Ha^{-1}	105 g N Ha^{-1}	61	
Schleusner et al., 2018	Incubation	1 week	loamy sand	1.4 ± 0.1 % (total), 2.9 ± 0.0 % (SOM: LOI)	0.1 ± 0.0 %	12.7:1 ± 0.4	negligible	n.d.	60	historical applications of liquid manure (LM +)	Can assume addition of liquid manure added carbon to the soil, thus is considered a historical carbon amendment. Was not applied up to ten months prior to soil sampling for this experiment.	140-170 kg N Ha^{-1} yr^{-1}	NH_4NO_3	17% ^{15}N labelled	100 kg N Ha^{-1}	141 ± 18 mmol N g^{-1}	76 mmol N g^{-1}	54	An incubation experiment designed to test the contribution of native soil N to N_2O emissions after mineral N fertilizer application on the same soil with different SOM due to historical applications of liquid manure. The application of mineral fertilizer more than doubled N_2O production from native N sources compared to unfertilized controls, regardless of historical liquid manure application. Overall, N_2O sourced from fertilizer contributed the majority of N_2O emissions.
				1.1 ± 0.1 (total), 2.5 ± 0.0 % (SOM: LOI)	0.1 ± 0.0 %	12.9:1 ± 0.1				no liquid manure application (LM -)	n.a.	n.a.				124 ± 12 mmol N g^{-1}	71 mmol N g^{-1}	57	
				1.4 ± 0.1 % (total), 2.9 ± 0.0 % (SOM: LOI)	0.1 ± 0.0 %	12.7:1 ± 0.4				historical applications of liquid manure (LM +)	Can assume addition of liquid manure added carbon to the soil, thus is considered a historical carbon amendment. Was not applied up to ten months prior to soil sampling for this experiment.	140-170 kg N Ha^{-1} yr^{-1}	n.a.	n.a.	n.a.	65 ± 27 mmol N g^{-1}	n.a.		
				1.1 ± 0.1 (total), 2.5 ± 0.0 % (SOM: LOI)	0.1 ± 0.0 %	12.9:1 ± 0.1				no liquid manure application (LM -)	n.a.	n.a.				53 ± 29 mmol N g^{-1}			
Leiber-Sauheitl et al., 2015	Incubation	21 days	peat	27.0 ± 1.6 % (total)	0.90 ± 0.04 %	30:1	n.d.	n.d.	80	Sheep feces and/or urine	urine: 0.30 ± 0.15 % C faeces: 39.0 ± 0.13 % C	urine: 6.79 g C m^2 faeces: 335.01 g C m^2	Sheep faeces and/or urine	urine: 0.35 ± 0.03% N faeces: 1.77 ± 0.03 % N	urine: 7.92 g N m^2 faeces: 15.20 g N m^2	0.2 - 3.3 g N m^{-2} (21 days)$^{-1}$	insignificant	insignificant	An incubation experiment designed using sheep excreta amendments applied on the surface of undisturbed histic gleysol with degraded peat columns from a nutrient-poor grassland. Found that sheep excreta did not alter emissions of N_2O from anaerobic peat soil. However, the N_2O–N source shifted from peat to excreta, indicative of negative priming, but priming was not significant.
Henderson et al., 2010	Incubation	72 hrs	coarse loamy till	25.5 g kg^{-1}	1.70 g kg^{-1}	15:1	443 mg NO_3-N kg^{-1} negligible NH_4-N	816 mg C kg^{-1}	70	glucose	n.a.	1000 mg C kg-1	KNO_3	n.a.	500 mg N kg^{-1}	94 mg N_2O-N kg^{-1}	94 mg N_2O-N kg^{-1}	n.a.	An incubation experiment designed to elucidate the effects of glucose, red clover, soybean and barley plant residues on denitrifier abundance, denitrification gene mRNA levels, N_2O emissions and denitrification rates. The required quantity of carbon amendment was added as a one-time application. Cumulative N_2O emissions and denitrification rates increased over the incubation in both glucose and plant residue treatments.
								127 mg C kg^{-1}		soybean residue	Additional 18.8 g N kg^{-1}					39 mg N_2O-N kg^{-1}	39 mg N_2O-N kg^{-1}		
								127 mg C kg^{-1}		red clover residue	Additional 36.8 g N kg^{-1}					36 mg N_2O-N kg^{-1}	36 mg N_2O-N kg^{-1}		
								127 mg C kg^{-1}		barley residue	Additional 14.8 g N kg^{-1}					60 mg N_2O-N kg^{-1}	60 mg N_2O-N kg^{-1}		
Langarica-Fuentes et al., 2018	Incubation	7 days ARE addition, 4 hrs N_2O measurement	sandy loam	3.68% (total C)	0.12%	31:1	105.4 ± 3.3 mg N kg^{-1}	14.38 ± 2.65 mg kg^{-1}	50	ARE mix (glucose, sucrose, fructose, ribose, arabinose, glycine, valine, glutamine, serine, alanine, malic acid, citric acid, malonic acid, oxalic acid, fumaric acid in equimolar C concentrations)	Amino acids provide additional N, however amino-N accounted for less than 25% the total N added as KNO_3.	0.375 mg C day^{-1}	KNO_3	n.a.	100 mg N kg^{-1}	negligible	n.a.		A 7-day incubation experiment designed to understand the interaction between exudate addition and soil moisture on denitrifier community dynamics and denitrification rates. Significant N_2O production rates were observed only at 90% WFPS and increased with increasing C input.
												0.75 mg C day^{-1}				negligible			
												1.5 mg C day^{-1}				negligible			
												3 mg C day^{-1}				negligible			
									70			0.375 mg C day^{-1}				negligible			
												0.75 mg C day^{-1}				negligible			
												1.5 mg C day^{-1}				negligible			
												3 mg C day^{-1}				negligible			
									90			0.375 mg C day^{-1}				6.5 ng N_2O-N g hr^{-1}	6.5 ng N_2O-N g hr^{-1}	n.a.	
												0.75 mg C day^{-1}				n.d.	n.d.		
												1.5 mg C day^{-1}				n.d.			
												3 mg C day^{-1}				27.6 ng N_2O-N g hr^{-1}	27.6 ng N_2O-N g hr^{-1}	n.d.	

Supplementary Materials

4 Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges

Daly, E. J., Hernandez-Ramirez, G., Puurveen, D., Ducholke, C., im, K., & Oatway, L. (2022). Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges. Agronomy Journal, 114(1), 471-489.

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Supplementary Table S4.1. Key dates for management activities at Edmonton and Breton field sites.

Activity	**Growing season**	
	Year One (2017-2018)	Year Two (2018-2019)
	Edmonton	
Perennial forage seeding	16-Jun-17	n.a.
Perennial grain seeding	30-Aug-17	n.a.
Fall grain seeding	30-Aug-17	6-Sep-18
Spring grain seeding	22-May-18	23-May-19
Nitrogen fertilizer application*	22-May-18	23-May-19
Herbicide applicationǂ	5-Jun-18	27-Jun-19
1st harvest perennial forage	26-Jun-18	16-Jul-19
2nd harvest perennial forage	31-Aug-18	17-Sep-19
Perennial grain harvest	31-Aug-18	2-Oct-19
Fall grain harvest	31-Aug-18	2-Oct-19
Spring grain harvest	31-Aug-18	2-Oct-19
	Breton	
Perennial forage seeding	8-Jun-17	n.a.
Perennial grain seeding	30-Aug-17	n.a.
Fall grain seeding	30-Aug-17	6-Sep-18
Spring grain seeding	21-May-18	21-May-19
Nitrogen fertilizer application*	5-Jun-18	24-Jun-19
Herbicide applicationǂ	21-May-18	21-May-19
1st harvest perennial forage	26-Jun-18	15-Jul-19
2nd harvest perennial forage	30-Aug-18	16-Sep-19
Perennial grain harvest	23-Aug-18	25-Aug-19
Fall grain harvest	23-Aug-18	24-Sep-19
Spring grain harvest	3-Oct-18	24-Sep-19

*Nitrogen fertilizer is a 2:1 blend of urea and ESN.

ǂ Stellar^{TM} XL herbicide at a rate of 0.9 L ha^{-1}.

Supplementary Table S4.2. Repeated measures ANOVA results for annual grain yield and annual biomass yield at the Edmonton and Breton sites

Treatment or Statistic	Edmonton		Breton	
	F-value	**p-value**	**F-value**	**p-value**
	Annual Grain Yield			
Treatment	3.7211	0.1020		
Year	86.1505	0.001	n.a.[₸]	
Treatment x Year	0.1581	0.7046		
	Annual Biomass Yield			
Treatment	5.7190	0.0539	0.3312	0.5859
Year	121.6180	<.0001	14.157	0.0094
Treatment x Year	0.7260	0.4269	0.1516	0.7104

[₸] Repeated measures analysis not applicable due to lack of replicates for the perennial grain at Breton – year two. Breton - year two had minimal grain productivity.

Treatment factor includes perennial grain + N, perennial grain, fall grain + N, fall grain, spring grain + N and spring grain. Year factor includes 2018 and 2019 seasons.

Supplementary Table S4.3. Nitrogen use efficiency (NUE), uptake efficiency (UE) and physiological efficiency (PE) of each grain crop with applied N fertilizer for years one and two.

Crop	**NUE** **(kg grain DM kg^{-1} N fertilizer)**		**UE** **(kg N uptake kg^{-1} N fertilizer)**		**PE** **(kg grain DM kg^{-1} N uptake)**	
	Year One					
	Breton	Edmonton	Breton	Edmonton	Breton	Edmonton
Perennial grain + N	-4.2 ± 3.5 a	-3.2 ± 2.9 a	-1.3 ± 0.7 a	-0.2 ± 0.09 a	13.2 ± 9.1 a	35.6 ± 12.5 a
Fall grain + N	0.7 ± 4.7 a	-2.6 ± 2.3 a	0.8 ± 1.1 a	-0.05 ± 0.09 a	18.3 ± 19.0 a	29.3 ± 5.5 a
Spring grain + N	-1.0 ±2.6 a	-1.3 ± 3.2 a	-0.2 ± 0.2 a	-0.03 ± 0.09 a	41.5 ± 10.5 a	29.0 ± 12.9 a
	Year Two					
Perennial grain + N	n.d. ₸	3.1 ± 1.6 a	n.d. ₸	0.1 ± 0.1 a	n.d. ₸	8.4 ± 3.7 a
Fall grain + N	4.4 ± 3.4 a	-11.4 ± 2.9 b	0.2 ± 0.1 a	-0.4 ± 0.1 a	37.3 ± 12.7 a	30.3 ± 4.4 b
Spring grain + N	8.2 ± 1.8 a	-0.6 ± 3.2 a	0.3 ± 0.08 a	-0.08 ± 0.2 a	26.5 ± 2.1 a	18.4 ± 2.5 ab

₸ Breton- year two had minimal grain productivity and a lack or replication thus these values were not considered representative and excluded.

Lowercase letters denote significant differences between crops based upon post hoc analysis after ANOVA. The same letters indicate no significant differences within column; different letters indicate significant differences within column (α = 0.05).

Supplementary Table S4.4. Nitrogen harvest index (NHI) of each grain crop for years one and two.

Crop	NHI (kg grain N kg^{-1} N grain-and-biomass)	
	Year One	
	Breton	Edmonton
Perennial grain + N	0.38 ± 0.09 Aa	0.59 ± 0.02 Aa
Perennial grain	0.36 ± 0.1 Aa	0.58 ± 0.03 Aa
Fall grain + N	0.51 ± 0.1 Aa	0.79 ± 0.01 Ba
Fall grain	0.50 ± 0.1 Aa	0.80 ± 0.01 Ba
Spring grain + N	0.60 ± 0.009 Aa	0.74 ± 0.02 Ca
Spring grain	0.60 ± 0.009 Aa	0.76 ± 0.007 Ca
	Year Two	
Perennial grain + N	n.d.₸	0.25 ± 0.02 Aa
Perennial grain	n.d.₸	0.20 ± 0.03 Aa
Fall grain + N	0.43 ± 0.02 Aa	0.45 ± 0.02 Ba
Fall grain	0.41 ± 0.03 Aa	0.52 ± 0.03 Ba
Spring grain + N	0.48 ± 0.02 Aa	0.52 ± 0.08 Ba
Spring grain	0.47 ± 0.03 Aa	0.46 ± 0.02 Ba

₸ Breton - year two had minimal grain productivity and a lack or replication thus these values were not considered representative and excluded.

Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column (α = 0.05)

Supplementary Materials

5 The response of soil physical quality parameters to a perennial grain crop

Daly, E. J., Kim, K., Hernandez-Ramirez, G., & Klimchuk, K. (2023). The response of soil physical quality parameters to a perennial grain crop. Agriculture, Ecosystems & Environment, 343, 108265.

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Table S5.1. Timing of major field activities at the Edmonton and Breton sites.

Activity	2017-2018	2018-2019	2019-2020
		Edmonton	
Initial tillage[₸]	7-Jun-17	n.a	n.a.[¥]
Perennial AB seeding	16-Jun-17		
Perennial rye seeding	30-Aug-17		
Annual rye seeding[ʿ]	22-May-18	23-May-19	
N fertilizer application	22-May-18	23-May-19	
P fertilizer application	30-Aug-17, 22-May-18[¡]	23-May-19	
1st harvest perennial AB	26-Jun-18	16-Jul-19	
2nd harvest perennial AB	31-Aug-18	17-Sep-19	
Annual rye harvest	31-Aug-18	2-Oct-19	
Root sampling	3-Jul-18	19-Jul-19	
Hyprop sampling	n.a.		30-May-20
		Breton	
Initial tillage[₸]	6-Jun-17	n.a.	n.a.
Perennial AB seeding	8-Jun-17		
Perennial rye seeding	30-Aug-17		
Annual rye seeding[ʿ]	21-May-18	21-May-19	29-Jun-20
N fertilizer application	21-May-18	21-May-19	7-May-20, 29-Jun-20[δ]
P fertilizer application	30-Aug-17, 21-May-18[¡]	21-May-19	29-Jun-20
1st harvest perennial AB	26-Jun-18	15-Jul-19	16-Jul-20
2nd harvest perennial AB	30-Aug-18	16-Sep-19	17-Sep-20
Perennial rye harvest	23-Aug-18	25-Aug-19	17-Sep-20
Annual rye harvest	3-Oct-18	24-Sep-19	17-Sep-20
Root sampling	4-Jul-18	18-Jul-19	n.a.
Hyprop sampling	n.a.		13-Jul-20

[₸] Entirety of experimental area tilled prior to treatment establishment.

[¡] P fertilizer was placed with seed, therefore the perennial rye received P fertilizer on 30-Aug-17, the annual rye received P fertilizer on 22-May-18.

[ʿ] Plots for annual rye treatments tilled concurrent with seeding each season.

[¥] No harvest was completed at the Edmonton site in fall 2020 due to poor crop performance.

[δ] Perennial rye and forage plots received N fertilizer on 7-May-20, annual rye plots received N fertilizer on 29-Jun-2020

Supplementary Materials

7 Soil greenhouse gas dynamics following termination of multi-year perennial grain cropping

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Supplementary Table S7.1. Management activities at the Edmonton and Breton sites from 2017-2022.

Activity	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022
			Edmonton		
Initial tillage[╤]	7-Jun-17	n.a.	n.a.	n.a.	n.a.
Perennial forage seeding	16-Jun-17				
Perennial grain seeding	30-Aug-17				
Spring grain seeding[ᒥ]	22-May-18	23-May-19			
1st harvest perennial forage	26-Jun-18	16-Jul-19			
2nd harvest perennial forage	31-Aug-18	17-Sep-19			
Spring grain harvest	31-Aug-18	2-Oct-19			
Legacy treatment tillage (“the reversal”)	n.a.	n.a.	10-Jun-20		
Tillage, barley seeding & N fertilizer application			10-Jun-20	26-May-21	
Barley harvest			13-Aug-20	27-July-21	
			Breton		
Initial tillage[╤]	6-Jun-17	n.a.	n.a.	n.a.	n.a.
Perennial forage seeding	8-Jun-17				
Perennial grain seeding	30-Aug-17				
Spring grain seeding[ᒥ]	21-May-18	21-May-19	29-Jun-20		
1st harvest perennial forage	26-Jun-18	15-Jul-19	16-Jul-20		
2nd harvest perennial forage	30-Aug-18	16-Sep-19	17-Sep-20		
Perennial grain harvest	23-Aug-18	25-Aug-19	17-Sep-20		
Spring grain harvest	3-Oct-18	24-Sep-19	17-Sep-20		
Legacy treatment tillage (“the reversal”)	n.a.	n.a.	n.a.	26-May-21	
Tillage, barley seeding & N fertilizer application				26-May-21	12-May-22
Barley harvest				28-July-21	8-Aug-22

[╤] Entirety of experimental area tilled prior to treatment establishment in 2017.

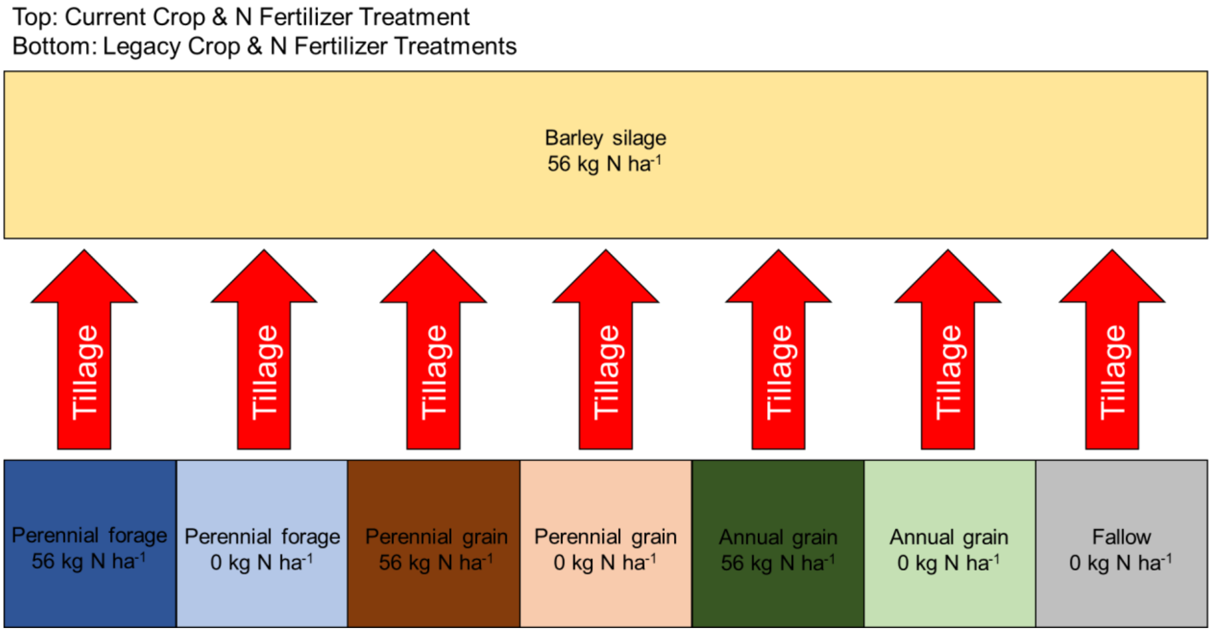
[ᒥ] Tillage was applied to the legacy annual grain treatments and N fertilizer was broadcast onto all legacy treatments concurrent with annual grain seeding each season.

Supplementary Table S7.2. Post-harvest crop residue dry matter (DM), total carbon (TC), total nitrogen (TN), and C:N ratio during legacy years one and two for the annual grain, perennial grain, and perennial forage crops with and without nitrogen fertilization at the Breton and Edmonton sites.

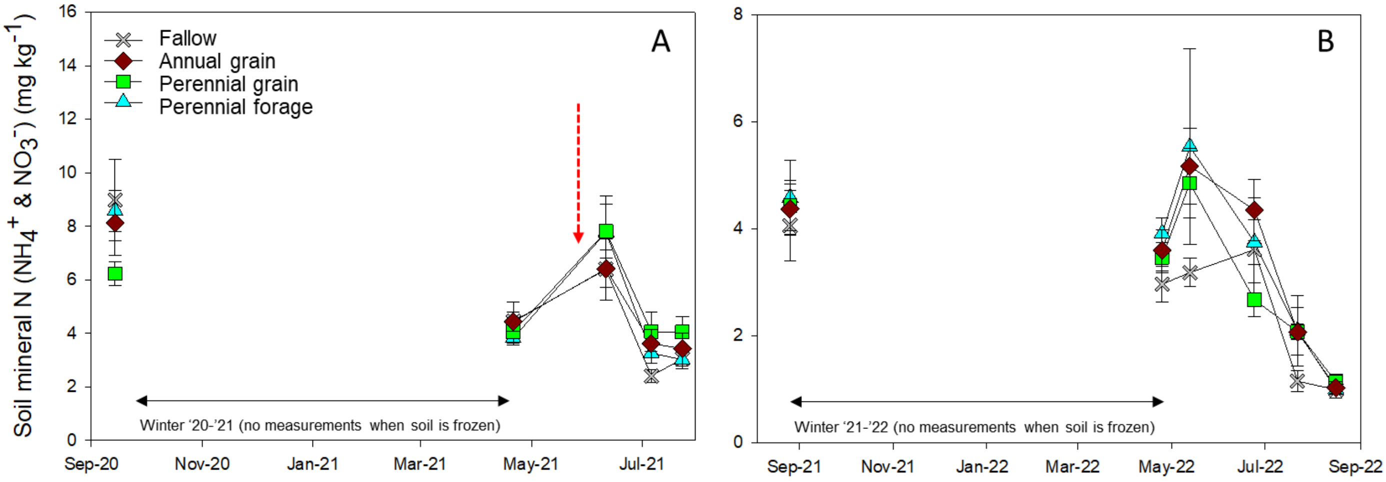
Legacy Treatment	**Breton**							
	Legacy Year One				Legacy Year Two			
	Crop Residue (kg DM ha^{-1})	TC (kg C ha^{-1})	TN (kg N ha^{-1})	C:N	Crop Residue (kg DM ha^{-1})	TC (kg C ha^{-1})	TN (kg N ha^{-1})	C:N
Annual grain	690 ± 45 a	339 ± 38 a	7 ± 1 a	49 ± 5 a	430 ± 23 a	301 ± 20 a	4 ± 1 a	79 ± 10
Annual grain + N	888 ± 139 a	464 ± 64 a	13 ± 4 a	42 ± 7 a	366 ± 53 a	367 ± 113 a	7 ± 3 a	59 ± 15
Perennial grain	400 ± 75 b	177 ± 35 b	6 ± 1 a	29 ± 6 b	363 ± 24 a	253 ± 17 a	5 ± 1 a	52 ± 5
Perennial grain + N	535 ± 162 b	239 ± 73 b	8 ± 2 a	30 ± 2 b	316 ± 81 a	218 ± 59 a	5 ± 1 a	47 ± 9
Perennial forage	1698 ± 165 c	917 ± 74 c	38 ± 1 b	24 ± 2 b	1371 ± 199 b	614 ± 69 b	15 ± 4 b	45 ± 8
Perennial forage + N	1453 ± 169 c	840 ± 81 c	34 ± 4 b	25 ± 1 b	1353 ± 125 b	665 ± 78 b	14 ± 2 b	48 ± 1
ANOVA p values								
Crop	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.14
Fert	0.62	0.28	0.24	0.85	0.93	0.76	0.80	0.45
Crop*Fert	0.5	0.46	0.20	0.44	0.91	0.59	0.43	0.38
	Edmonton							
	Legacy Year One				Legacy Year Two			
	Crop Residue (kg DM ha^{-1})	TC (kg C ha^{-1})	TN (kg N ha^{-1})	C:N	Crop Residue (kg DM ha^{-1})	TC (kg C ha^{-1})	TN (kg N ha^{-1})	C:N
Annual grain	771 ± 14 a	372 ± 43 a	15 ± 2 ab	25 ± 1 a	627 ± 67 a	433 ± 45 a	9 ± 2 a	51 ± 7 a
Annual grain + N	955 ± 89 a	566 ± 137 a	21 ± 6 ab	27 ± 1 a	673 ± 84 a	554 ± 34 a	9 ± 1 a	64 ± 7 a
Perennial grain	563 ± 144 b	240 ± 62 b	10 ± 2 a	24 ± 1 b	820 ± 187 a	568 ± 131 ab	16 ± 2 a	36 ± 7 b
Perennial grain + N	432 ± 161 b	184 ± 70 b	9 ± 3 a	21 ± 1 b	871 ± 183 a	600 ± 127 ab	14 ± 3 a	44 ± 4 b
Perennial forage	1468 ± 104 a	716 ± 237 a	34 ± 10 b	20 ± 1 c	2132 ± 308 b	714 ± 44 b	41 ± 4 b	18 ± 1 c
Perennial forage + N	765 ± 74 a	548 ± 73 a	31 ± 5 b	18 ± 1 c	1999 ± 369 b	803 ± 87 b	45 ± 7 b	19 ± 2 c
ANOVA p values								
Crop	< 0.001***	< 0.01**	< 0.01**	< 0.001***	< 0.001***	0.03*	< 0.001***	< 0.001***
Fert	0.08	0.97	0.81	0.19	0.66	0.28	0.83	0.11
Crop*Fert	0.21	0.54	0.75	0.05	0.68	0.79	0.74	0.64

Data represents mean ± standard error (n=4)

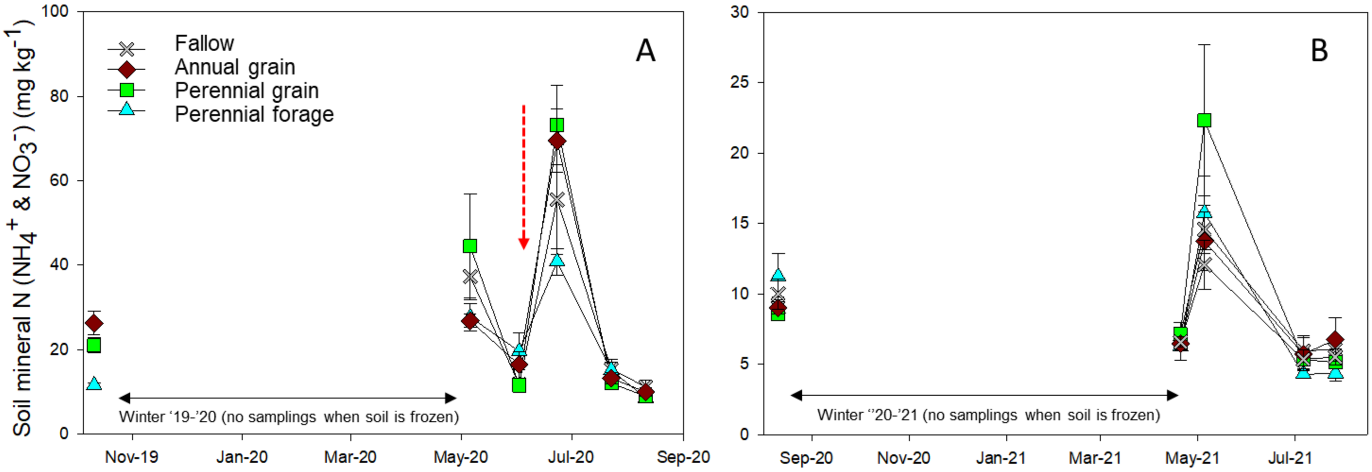
DM stands for dry matter.



Supplementary Figure S7.1. A schematic of the legacy treatments (bottom) that were converted via tillage (i.e., "the reversal") into a uniform treatment of barley silage (top).



Supplementary Figure S7.2. Soil mineral N (ammonium [NH_4^+] plus nitrate [NO_3^-]) concentrations for (A) year one (2020-2021) and (B) year two (2021-2022) at the Breton site from the 0-15 cm soil depth increment. The arrow indicates the date of tillage of the legacy treatments.



Supplementary Figure S7.3. Soil mineral N (ammonium [NH_4^+] and nitrate [NO_3^-]) concentrations for (A) year one (2019-2020) and (B) year two (2020-2021) at the Edmonton site from the 0-15 cm soil depth increment. The arrow indicates the date of tillage of the legacy treatments.