SHORT-TERM DYNAMICS OF SOIL CHEMICAL AND HEALTH PROPERTIES UNDER AN INTERMEDIATE WHEATGRASS (*Thinopyrum intermedium*) FORAGE-GRAIN SYSTEM

By

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ABSTRACT

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Overwintering of beef cattle on pasture during the late fall/winter is a common practice in western Canada due to the reduced cost compared to feeding in confinement. However, producers rely on single-purpose annual and perennial forage species. Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey) exhibits superior characteristics as a forage species for cattle grazing during the cold season compared to other forage species currently in use in western Canada. As such, it offers potential as a dual-purpose forage for both grain for human consumption and cattle feed within the same growing season. After introducing a novel plant species into a diverse agricultural cropping system, it is crucial to evaluate the impact of incorporating that species on the resilience and diversity of the soil ecosystem. Therefore, this 3-yr study examined the dynamics of soil chemical and health properties under three IWG-based perennial forage treatments: IWG with no fertilizer post-establishment (IWGP), IWG with synthetic fertilizer applied after the first grain harvest (IWGF), and IWG in a mixed stand with a legume (Alsike clover, *Trifolium hybridum* L.) (IWGL). A forage-only perennial crop consisting of a 50:25:25 mix of Coutenay tall fescue/Algonquin alfalfa/Oxley II cicer milkvetch was included as a control. The experiment was laid out in a randomized complete block design with a one-way treatment structure at each of four field sites in Manitoba and Saskatchewan, Canada. Available nitrate-nitrogen (NO_3^--N) supply rates were measured in situ using plant root simulator (PRS) probes while all other nutrient analyses were conducted in the laboratory.

Soil NO_3^--N concentrations in the 0-15 and 15-60 cm soil layers during the growing season were similar for the IWGL and IWGF treatments in 2020 and 2021. Soil NO_3^--N supply rates were also similar for IWGF and the IWGL following urea application after grain harvest in 2021.

Significant treatment effects were not detected for any of the soil health indicators (24-hour CO_2 respiration, permanganate-oxidizable carbon [POXC], and bioavailable [ACE protein]) N tested. However, partial least squares (PLS) analysis showed that baseline soil properties measured prior to treatment establishment in 2019 explained > 72% of the variation in the three soil health indicators and > 79% of the variation in number of spikelets/head, average number of florets/head and head count. Results from this study indicate the potential of a legume intercrop as an alternative source of N for an IWG perennial forage-grain system. The results also demonstrate the potential of PLS analysis to model short-term soil productivity and crop attributes in an IWG perennial forage-grain system.

Keywords: cold-tolerant forage species, Intermediate Wheatgrass, *Thinopyrum intermedium*, Kernza®, soil chemical properties, soil health, partial least squares analysis

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FOREWARD

This thesis was prepared in a manuscript format in accordance with the Department of Soil Science, University of Manitoba guidelines. The thesis consists of four chapters. Chapter 1 is the general introduction, providing the background and a literature review on Intermediate Wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) in western Canadian prairies and importance of soil quality evaluation. Chapters 2 and 3 are research chapters prepared as manuscripts for journal submission. Chapter 2 focuses on soil chemical property dynamics under an IWG perennial forage-grain system. Chapter 3 examines the dynamics of selected soil health attributes under an IWG perennial forage-grain system and explores partial least squares (PLS) modeling of IWG phenological development and soil health attributes using baseline soil properties. Chapter 4 is the overall synthesis of the findings reported in Chapters 2 and 3 and includes implications of the results and recommendations for future research.

Contributions of Authors

The contributions of all authors are as follows for Chapters 2 and 3

- Nikisha Muhandiram: Conducted experiments; collected, organized, and analyzed data; interpreted results; wrote the original manuscript draft.
- Francis Zvomuya: Conceptualized the experiments; provided primary administration and supervision of the project; acquired project funding; reviewed and edited manuscript drafts.
- Douglas J. Cattani: Conceptualized the experiments; administered and supervised the project; acquired funding; reviewed and edited manuscript drafts.
- Emma McGeough: Administered the project; acquired funding; reviewed and edited the manuscript draft.

- Timothy Crews: Conceptualized the experiments; reviewed and edited the manuscript draft.

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1. GENERAL INTRODUCTION

1.1 The beef cattle industry in the Canadian prairies

Canada is the tenth largest beef producer in the world (FAO, 2021) and ranks among the world's largest exporters of beef, exporting 47% of domestically produced cattle and beef, primarily to the United States (Canadian Cattlemen's Association, 2020). In Canada, there are 10.4 million beef cattle (Statistics Canada, 2022) produced in three phases: cow-calf, backgrounding and feedlot/finishing. Global demand for beef is estimated to increase by 1.2% annually until 2050 (Pogue et al., 2018). A part of this global demand will likely be fulfilled by increasing bovine meat production in Canada, despite the confined agricultural lands for beef herd management. Most importantly, more than 80% of the Canadian beef herd comes from the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba (Pogue et al., 2018). In Manitoba, there are 1.07 million cattle (Beef Cattle Profile - Province of Manitoba, 2022) with most beef operations classified as cow-calf, which rely heavily on pasture and conserved forages as the main dietary ingredients.

During late fall and winter seasons, the cow-calf herds, including replacement heifers, are mostly managed intensively, causing a spike in expenditure. Under these circumstances, extended grazing in late fall or winter plays a major role in improving the efficient management of nutrient recycling in the cow and calf production system (Jungnitsch et al., 2011; Stonehouse et al., 2003).

1.2 Late fall/winter grazing of beef cattle

1.2.1 Extending the grazing season

The high cost of production is a major constraint for the beef cattle industry in Canada. During late fall and winter, feed costs account for 60-65% of total on farm costs for cow-calf herds (McCartney et al., 2004). Extending the grazing season by maintaining cattle on pasture during the fall/winter period reduces the cost of production compared to feeding in confinement (Kelln et al., 2011) while mitigating the need for mechanical harvesting and storage of forages and significantly lowering labour requirements (McCartney et al., 2008; McGeough et al., 2017). When extending the beef cattle grazing period, it is important to consider nutrient requirements of the target animal and, where possible, forages should be supplied to meet nutrient demand for, inter alia, maintenance, growth, and pregnancy. Therefore, forage quality is an important consideration when considering the type of overwinter grazing strategy to be utilized. Strategies for extended grazing include stockpile grazing, bale grazing, swath grazing, standing corn grazing and chaff grazing. Stockpile grazing, the grazing of accumulated forage regrowth following a haying or grazing event, is one of the lowest cost strategies (McGeough et al., 2017). However, the nutritive value tends to be relatively low in late fall/early winter (Biligetu at al., 2014); thus, it is predominantly utilized for mature cows with lower nutrient requirements. Utilization of novel forages to increase nutritive value would offer potential benefits to other classes of cattle such as replacement heifers or backgrounders.

From 2011 to 2016, tame or seeded pasture lands and natural pasture lands showed a declining trend while croplands increased (Statistics Canada, 2011, 2016). The temporal decrease in land area under natural pasture, which resulted in low-productivity land areas for grazing of cattle (McGeough et al., 2017), reflects the growing demand for annual crops for both humans and animals.

1.2.2 Annual vs. perennial forage–grain crops

Some hybrid forage corn varieties require fewer crop heat units, ensuring optimal yields under cold climate conditions (Lardner et al., 2012). Annual cool-season crops, such as ryegrass (*Lolium multiflorum* Lam.), are suitable for extended grazing in the winter season. Normally, growing annual forage is not cost-effective as there is an annual seeding cost, but when compared to intensive (in-house) feeding of beef cattle during the cool season, it is better to grow cold-tolerant annual crops. However, maximum performance is achievable when annual forages are grown with a perennial forage as a supplemental forage for optimum utilization of the land area (McCartney et al., 2008).

Perennial grain crops have a greater potential to support or enhance ecosystem services than annual grain crops by ensuring the long-term, proper functioning of soil and water resources. Examples include the cultivation of perennial grain crops to retain nutrients in the soil by minimizing soil erosion on sloping lands, and the cultivation of perennial grain crops for a number of consecutive years to enhance soil health by improving soil carbon and nitrogen cycling (Culman et al., 2013; Ryan et al., 2018; Zhang et al., 2016).

Some annual cool season-tolerant forages can support extended grazing during the winter (Mullenix & Rouquette, 2018). However, perennial forages are preferred over annual forages because they have a less negative impact on the environment (Jungers et al., 2017) in addition to their lower input (e.g., seed, fertilizers, and agrochemicals) requirements compared to annual forage–grain systems (Pimentel et al. 2012; Ryan et al., 2018).

1.2.3 Single-purpose vs. dual-purpose forage–grain systems

Among perennial forage-grain crops, dual-purpose forages (within a single growing season) may offer benefits over and above those from single-purpose perennial forages. In addition to providing high-quality feed for cattle during the vegetative stage of the crop, dual-purpose crops can serve as cash grain crops if allowed to reach the reproductive stage (Harrison et al., 2011). Dual-purpose forage-grain crops can also reduce pest problems in the field as, after harvesting grains for human consumption, the remaining biomass is used for grazing purposes (Ryan et al., 2018).

The use of common annual wheat (*Triticum aestivum* L.*)* as a dual-purpose crop for forage and grain is a low-risk strategy for farmers as they can earn an income from both crop functions (Diaz et al., 1986; Redmon et al., 1995). However, Edwards et al. (2011) reported that cattle grazing and planting prior to the optimal date reduced grain yield by 14%. Therefore, to ensure the effective use of a perennial forage, improved management of cattle grazing as well as the grain crop is crucial.

Research on feed gaps in the grain and graze system indicates that feed deficit directly affects livestock productivity (Moore et al., 2009). According to the researchers, evaluating feed quality and the marginal value of feed is important for the assessment of feed deficit. The allocation of different forage types for different seasons is an effective way to overcome this issue to some extent. Moore et al. (2009) suggested incorporating a dual-purpose forage-grain crop for winter, a perennial forage-grain crop for late spring, and forage shrubs for the remaining period (summer and autumn seasons).

Dual purpose crops, which provide grain harvest in summer then regrowth accumulation thereafter, potentially offer biomass for grazing that is highly vegetative and high in crude protein and energy, at a time when single-use perennial forages, which have been stockpiled since an early season grazing or haying event, have significantly declined in nutritive value. The high concentration of these chemical constituents in vegetative plant growth offer high productivity potential, thus making dual-purpose forages potentially attractive options for cattle with higher nutrient requirements, such as replacement heifers or backgrounding cattle.

1.3 Intermediate wheatgrass

Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) belongs to the family Poaceae and the tribe Triticeae (Mahelka et al., 2011) and is genetically related to common wheat (Zhang et al., 2016). Kernza® is the registered trademark name of the grain of IWG. Intermediate wheatgrass is widely grown in the Great Plains of North America (Canada and the USA) and has the potential for use as a perennial forage–grain crop for fall/winter grazing of beef cattle (Cattani and Asselin, 2017). It has promising agricultural traits, such as relatively large grain size and yield for an undomesticated grass, and high biomass yield, while the high nutrient content and edibility are comparatively high compared to most of the other perennial forage–grain crops (Zhang et al., 2016). Its deep, extensive, laterally well-spread root system maximizes the contact between plant and soil, which ensures extensive capture of available nutrients and moisture in the soil (Wagoner, 1995; Culman et al., 2013). As a perennial crop, IWG can utilize more sunlight and sequester more carbon into deeper soil layers compared to annual grain crops (Culman et al., 2010). Therefore, IWG can contribute to a more functional agroecosystem by significantly reducing nitrogen (N) leaching and increasing soil carbon (C) (DeHaan et al., 2018).

One study conducted in several Midwestern states of the USA suggest that harvesting of IWG as a dual-purpose forage–grain crop improves grain and forage production, thus improving the profitability of the overall crop production system while enhancing nutrient cycling and availability (Pugliese et al., 2019). Another advantage of IWG is that it can withstand winter annual weeds without affecting grain productivity (Zimbric et al., 2020). Therefore, farmers can incorporate this forage-grain crop into their cropping systems under existing conditions.

Dick et al. (2018) observed no adverse effect on seed production during the first two years when IWG was grown in mixed stands with alfalfa (*Medicago sativa* L.), sweet clover (*Melilotus officin*alis (L.) Pall.), and white clover (*Trifolium repens* L.). However, fall grazing after grain harvest improved IWG seed production relative to the removal or chopping of forage residue. Nonetheless, alfalfa and sweet clover took over the stand in the third year of the study.

1.4 Alsike clover

Alsike clover (*Trifolium hybridum* L.) is a perennial crop native to northern Europe and has shown greater N fixation compared with red clover (Rice, 1980). It can withstand cool climate conditions and therefore is well-suited to northern US and Canadian climates; also, it can tolerate adverse environmental conditions, such as acidity, low soil fertility, and harsh winters while maintaining yield stability (Taylor and Townsend, 1985). Alsike clover grows well in silty clay loams, which provide adequate moisture to the growing crop (John, 2008).

Typically, in crop rotations, legumes are followed by a cash crop to reduce N fertilizer requirements. A 3-yr study examining N fixation and dry matter production showed greater N fixation potential for alsike clover compared with red clover (Rice, 1980).

1.5 Soil health

Soil health, which is the continued capacity of the soil to function as a vital living system and maintain ecological stability while ensuring optimum above-ground and below-ground ecological diversity, is an important concept in sustainable agriculture (Doran and Zeiss, 2000; Cardoso et al., 2013). It is therefore important to examine the dynamics of soil health attributes in perennial forage-grain cropping systems. Healthy soils not only support crop production under ideal climate conditions but may also improve crop resilience under conditions of climate change and extreme weather events (Congreves et al., 2015). Soil properties such as organic matter, soil microbial biomass, cation exchange capacity (CEC), bulk density and porosity are major determinants of soil health. Maintaining the balance among these properties is important for sustainable land management (Larson and Pierce, 1994).

1.5.1 Soil health indicators

Researchers have attempted to qualify or evaluate soil health under different soil environments and land management systems by using specific soil physical, chemical, and biological measurements (e.g., Williams et al., 2020). Changes in these soil attributes are thought to be good indicators of soil health dynamics following changes in management or environmental conditions. The selected set of indicators must be sensitive to often subtle changes in land management practices and soil functions (Andrews et al., 2004; Allen et al., 2011; Williams et al., 2020).

Aggregate stability, total porosity, air-filled porosity, moisture content, and bulk density are important physical indicators of soil health while chemical attributes can include organic carbon, total nitrogen, mineral nutrients, CEC, pH, and electrical conductivity. However, most of these soil properties change very slowly with management practice (Büchi et al., 2017). Therefore, long-term experiments are necessary to satisfactorily detect changes in soil chemical and physical properties in a cropping system.

Biological properties generally have a faster response to management practices when compared to physical and chemical properties (McDaniel et al., 2014). Autoclaved-citrate extractable (ACE) soil protein, soil organic matter, permanganate-oxidizable carbon (POXC), soil respiration, microbial biomass C and N, soil enzymes and macro and meso fauna are some of the biological assays that have proven useful for evaluating changes in soil health on time scales of under 10 years (Cardoso et al., 2013; Williams et al., 2020).

Microorganisms show rapid responses to changes in the surrounding environment. Researchers are still learning what functions specific taxa of microorganisms perform, but in aggregate, microorganisms are frequently considered to be very efficient and effective indicators of soil health changes (Nielsen, 2002). This ability to show a quick response is a result of their high surface-to-volume ratios and reproductive rates.

During animal grazing in forage lands, soil compaction due to overgrazing or excessive stocking rate is a huge problem causing reduced plant growth due to reduced aeration and changes in soil physical properties such as bulk density, air-filled porosity, soil aggregate size distribution, and aggregate stability. Soil compaction affects soil structure, especially when the soil is wet (Steinfeld et al., 2006; Drewry et al., 2008).

1.5.2 Value of perennial forages in improving soil health

Nelson et al. (2011) reported that the grains of organically-managed modern hard red spring wheat cultivars are high in Zn and Cu than conventionally grown hard red wheat. Microbial populations are normally higher in organically-managed soils than in conventional cropping systems (Stockdale and Watson, 2009). However, microbial diversity varies with land management practices since changes in soil properties directly affect the microbial community (Nelson et al., 2011). Soil microbes play an important role in the decomposition of organic matter, soil aggregation, N mineralization and immobilization, and dissolution of minerals (Davis and Abbott, 2006).

Ryan et al. (2018) reported that soil degraded by continuous tillage with annual crops can be regenerated by crop rotation with annual and perennial forages. Their review also highlighted studies on how perennials can act as buffers on the edges of the fields as well as on sloping lands as they help mitigate both soil and nutrient loss and preserve groundwater quality. Further, intercropping perennial forages with legumes may reduce the N fertilizer requirements of forage–grain crops as most legumes have the ability to fix N. Another advantage of incorporating perennial forage–grain crops into a cropping system is that they may enhance carbon sequestration in deeper soil layers by capturing carbon in late fall and winter when annual cropping systems are non-productive (Cattani and Asselin, 2017).

1.6 Soil carbon

The soil carbon pool is greater than other carbon pools in the terrestrial ecosystem (Harrison et al., 2011) and it is twice the atmospheric carbon content and even larger than the combination of carbon in plants and the atmosphere (Liang et al., 2017). Soil carbon storage is commonly underestimated due to shallow sampling of soil (20 cm or less). Such an approach is often used because of the expense and difficulty of sampling in deeper layers and the assumption that carbon in deeper soil horizons is not as sensitive to changes in management practices. Changes in the soil carbon pool involve microbial activities, which can either release carbon into the atmosphere via microbial catabolism or store carbon in the soil in hardly decomposable complex structures (Liang et al., 2017).

Soil carbon can be classified into two pools: active (or labile) carbon and non-labile carbon. A commonly used method for characterizing labile carbon is by oxidizing C with potassium permanganate (permanganate oxidizable carbon, POXC) whereas the non-labile fraction is operationally defined as the C that cannot be oxidized by potassium permanganate (Blair et al., 1995). The POXC fraction can be sensitive to changes in land management practices, including crop rotation, tillage, fertilizer management, and cover crops (DuPont et al., 2010).

Cotrufo et al. (2019) proposed a framework for understanding soil C accumulation, persistence, and changes with the availability of N by dividing soil organic matter (SOM) into two fractions, namely, particulate organic matter (POM) and mineral-associated organic matter (MAOM). Particulate organic matter is primarily made of plant parts. Thus, POM is low in N compared to MAOM, which is composed mainly of high-N microbial products. Particulate organic matter is also more vulnerable to disturbance, and, therefore, has a shorter turnover time than MAOM. Mineral-associated organic matter is the largest and most persistent organic pool in the environment as it forms strong chemical bonds with minerals such as goethite and forms small soil aggregates. These small structures create physical protection by limiting access to microbes and enzymes. This fraction of organic matter can be degraded by oxidative degradation or desorption and if the bonds are weaker between organic matter and minerals, MAOM is even prone to biological degradation (Kaiser and Guggenberger, 2007; Kogel-Knabner et al., 2008; Cotrufo et al., 2019).

1.7 Nitrogen cycling

Nitrogen is an essential nutrient for plants and is subject to various inflows to and outflows from the soil (Russelle, 1992). In a cropping system, available forms of N are supplied by the soil to meet crop demand. Soil organic N becomes available to plants through mineralization and mineralized N becomes less available to plants through microbial immobilization (Mary et al., 1996).

Nitrogen inflows to the soil and losses from the soil depend on spatial and temporal variables (Russelle, 1992). Deposition of N from the atmosphere, symbiotic and non-symbiotic N fixation, and synthetic fertilizer N are identified as N inputs to the soil. Nitrogen losses occur via ammonia volatilization, denitrification, nitrate leaching, wind and water erosion, and fire and animal processes. During these inflows and outflows, N transfers among several pools, including plants, the soil, and even among large herbivores. This whole system is generally recognized as the cycling of N in the environment. Organic amendments can improve N cycling by altering the C:N ratio in the soil. This can lead to a balance between the demand and supply of N in the soil ecosystem and ultimately improves soil fertility and agronomic productivity (Drinkwater, 1998).

The use of a legume in lieu of inorganic fertilizer also reduces the carbon footprint (Hauggaard-Nielsen et al., 2016). During their 3-yr study, Hauggaard-Nielsen et al. (2016) observed that biological N fixation by a pure-stand red clover performed similarly to a high N fertilizer rate (326 kg ha^{-1} yr^{-1}) applied to a pure-stand ryegrass (*Lolium perenne* L.) as there was no significant difference in the yield of the successive winter wheat crops between these two systems. Intercropping legumes with perennial forage–grain crops is recommended to ensure long-term N supply to the cash crop in the absence of inorganic fertilizer N (Dick et al, 2018).

1.8 Nutrient supply rates

A valuable method for measuring the nutrient-supplying capacity of soils over time involves the deployment of ion exchange resins such as plant root simulator (PRS) probes in cropped soils. Plant root simulator probes consist of anion and cation exchange membranes. Anion probes have a positively charged membrane saturated with HCO_3^- and cation probes have a negatively charged membrane saturated with Na^+ (Western Ag, Saskatoon, SK). These resin membranes in a sense mimic plant root surfaces when buried in the soil. After burying in the soil, membrane counter ions desorb easily and are replaced by ions in the soil solution.

Nutrient supply rate measurement with PRS probes depends on soil factors. Sulewski et al. (2002) reported that soil moisture, soil temperature, and root environment influence PRS measurements. However, they mentioned that this sensitivity of PRS probes to soil environmental conditions improves their usefulness as the objective of using PRS probes is to simulate plant roots. They recommended recording soil moisture and temperature during the PRS probe burial period to get a better understanding of nutrient supply rates.

1.9 Research Objectives

Intermediate wheatgrass is a perennial plant species with adaptive dual-purpose capacity. There is little or no literature available in the western Canadian context to demonstrate the potential of IWG as a perennial forage-grain crop to refine the late fall/winter grazing of beef cattle. Therefore, to date, the impact on soil quality and soil health of incorporating IWG into diverse agricultural cropping systems has not been clearly defined. Therefore, the objectives and hypotheses of this study are as follows:

Objective 1: to evaluate the impact of IWG on changes in selected soil chemical properties and soil health indicators

> **Hypothesis 1:** Soil chemical properties vary with IWG-based perennial forage treatment.
>
> It is expected that intercropping IWG with a legume (IWGL) will increase soil available N and N supply rate relative to IWG without fertilizer added treatment (IWGP). Additionally,

IWG with fertilizer added (IWGF) is expected to increase soil available N and therefore N supply rate relative to IWG without fertilizer added (IWGP).

Hypothesis 2: Soil health properties differ among IWG-based perennial forage treatments.

It is conceivable that IWG intercropped with a legume (IWGL) will increase bioavailable N in the soil relative to IWG without fertilizer added (IWGP). Also, the IWGF and IWGL treatments may produce greater yields than the IWGP treatment, which receives less N, and this may support a greater accumulation of soil C and POXC, and support greater microbial activity, hence greater soil respiration.

Objective 2: to model crop attributes and soil health indicators using baseline soil variables.

Hypothesis 1: Soil health indicators measured at the end of the 3-yr study can be modeled using baseline soil properties measured before establishment of the forages.

Hypothesis 2: Phenological development indicators measured at the end of the 3-yr study can be modeled using baseline soil properties measured before establishment of the forages.

1.9.1 Thesis Layout

This thesis consists of four chapters. Chapter 1 is a review of literature, and provides a general overview of the beef cattle industry in the North American region and the Canadian prairies, late fall/winter grazing of beef cattle, IWG as a potential perennial forage for late fall/winter grazing, and the value of perennial forage systems in improving soil health. Chapter 2 focuses on the temporal changes in soil N supply rate (supply rates of soil nitrate-N and ammonium-N to plants, estimated using PRS probes) and selected soil chemical properties as affected by IWG forage-grain system. Chapter 3 addresses soil health indicators (24-hour CO_2 respiration, bioavailable

(ACE protein) N and POXC and explores the key soil properties for modeling soil and plant attributes under the dual-purpose, multi-year cropping system. Chapter 4 is the overall synthesis of the research project.

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2. SOIL CHEMICAL PROPERTY DYNAMICS UNDER A PERENNIAL INTERMEDIATE WHEATGRASS FORAGE-GRAIN SYSTEM

2.1 Abstract

Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) is a perennial, dual-purpose forage-grain crop which can potentially be used for late fall/winter grazing of beef cattle. This experiment assessed changes in soil chemical properties under threedifferent IWG-based perennial forage treatments: IWG with no fertilizer post-establishment (IWGP), IWG with synthetic fertilizer applied after the first grain harvest (IWGF), IWG in a mixed stand with a legume (Alsike clover, *Trifolium hybridum* L.) (IWGL), and a single-purpose perennial crop consisting of a 50:25:25 mix of Courtney tall fescue (*Schedonorus arundinaceus*)/Algonquin alfalfa (*Medicago sativa*)/Oxley II cicer milkvetch (*Astragalus cicer*) (control). The experiment was laid out in a randomized complete block design with a one-way treatment structure and four replicates (blocks) per treatment. The experiment was conducted at a large pasture plot site in Manitoba and at three small plot sites in Manitoba and Saskatchewan. Soil nitrogen (N) supply rates were measured in situ in the large pasture plots using plant root simulator (PRS) probes while other soil properties were measured in the laboratory. The PRS probes were switched every 14 d during the growing season and analyzed for ammonium- (NH_4^+) and nitrate- (NO_3^-) N. There was an overall decrease with time in NO_3^--N supply rate in the 2020 and 2021 growing seasons. This was observed for all four treatments in 2021 and, in this growing season, the NO_3^--N supply rate following urea application was significantly higher in the IWGF treatment (48.9 μg cm^{-2} 2 wk^{-1}) than the IWGP treatment (12.3 μg cm^{-2} 2 wk^{-1}) on the last sampling day (Day 100). However, there was no significant difference between IWGF and IWGL during the same period. In small plots, soil NO_3^--N concentration in the 0-15 layer for the IWGL treatment was significantly greater

in 2020 than in 2021. In both growing seasons, there were no significant differences between the IWGF and the IWGL treatment in the 0-15 cm layer. In the large pasture plot study at Glenlea, NO_3^--N concentration in the 15-60 cm layer was significantly greater in the IWGL treatment than the control and the IWGP treatment in the 2020 growing season. Our results indicate the adequacy of a legume intercrop as an alternative source of N for an IWG perennial forage-grain system.

Keywords: PRS probes, soil N supply rate, legume intercrop, *Thinopyrum intermedium*, intermediate wheatgrass, Kernza®, dual use crop.

2.2 Introduction

The western Canadian prairies contribute significantly to the beef cattle industry in Canada (Pogue et al., 2018). Maintaining this high contribution is a challenge as the industry faces an increasingly high cost of production, especially during the late fall and winter seasons when farmers have to spend appreciable amounts of money on cattle feed (confined feeding) (Stonehouse et al., 2003; Jungnitsch et al., 2011; Hibbard et al., 2021). Under western Canadian conditions, extending the grazing season in late fall and early winter is commonly practised by cow-calf producers to reduce costs associated with overwintering beef cattle (McCartney et al., 2008; McGeough et al., 2017). Stockpile grazing of perennial forage is a commonly practised strategy (Sheppard et al., 2015, WCCCS, 2017). However, the nutritive value of stockpile perennial grasses and legumes during the late fall/early winter period is often insufficient to meet the nutrient requirements of the cow-calf herd (Beck et al., 2006; Biligetu at al., 2014), particularly under extreme cold conditions when requirements are increased for thermoregulation. Therefore, the main challenge facing Canadian prairie farmers is the availability of a cold-tolerant forage species which can meet cattle nutrient demands during the cool season. For example, a 2-yr study to evaluate the grain yield production of perennial cereal rye (*Secale cereale* L. x *S. montanum*) in Manitoba yielded poor results as the plants were less cold-tolerant and affected by a fungal disease (ergot), thus impeding further improvement of the crop for perennial grain production (Cattani, 2019). Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey) presents an opportunity to overcome this challenge as it has favorable characteristics that allow it to withstand cold conditions with less disease (Park et al.,1994; Hibbard et al., 2021).

Intermediate wheatgrass is a perennial plant species with adaptive dual-purpose capacity. Currently, beef cattle producers in Canada mainly depend on single-purpose annual or perennial forages for feeding cattle during winter. As a dual-purpose forage, IWG offers additional benefits, including serving as a cash grain crop for human consumption while providing much-needed high-quality forage for cattle during late fall and early winter (Hunter et al., 2020); sequestering carbon (C) into deeper soil layers (van der Pol et al. 2022) and capturing most of the available nutrients and moisture in the soil via its extensive root system (Culman et al., 2010; Culman et al., 2013); and minimizing water accumulation in the field for the subsequent growing season by utilizing solar energy during early spring and late fall (Cattani and Asselin, 2017). Intermediate wheatgrass (or Kernza) is still developing through selection and breeding, mainly focusing on grain yield (Cattani and Asselin, 2017; Cattani and Asselin, 2018).

Intermediate wheatgrass has a higher grain yield and grain size than other perennial forage species (Zhang et al., 2016). As a perennial, it goes through multiple harvesting seasons, resulting in a higher regrowth and eventually higher grain and forage production, demonstrating its potential use as a feed for cattle during the cool season (Pugliese et al., 2019).

Soil chemical and physical properties typically change very slowly over time. Therefore, significant changes in such soil properties are not expected within a short period of time (Acuña and Villamil., 2014; Büchi et al. 2017). However, soil biological properties are more sensitive to management practice in the short term (Utobo and Tewari, 2015; Paz-Ferreiro and Fu, 2016). Nonetheless, soil chemical properties such as pH and electrical conductivity can provide insights into nutrient availability in the soil, nutrient leaching from the topsoil layers, soil microbial activity, phytotoxicity, and soil salinity, attributes which are ultimately useful as soil health indicators (Haynes, 2009; Rengel, 2011; Obade and Lal, 2016).

Measurement of soil nutrient concentrations is mainly carried out by collecting soil samples and testing in a laboratory. While laboratory measurement could provide the quantity of available nutrients in the soil, it fails to quantify the portion of these available nutrients that a plant would essentially uptake. Ion exchange resins can provide a more useful measure of nutrient availability under field conditions (Qian and Schoenau, 2002a). Plant root simulator (PRS) probes (Western Ag Innovations, Saskatoon, SK, Canada) are an example of ion exchange resins that are increasingly used to measure nutrient supply rates. Such measurements provide a better representation of temporal changes in nutrient availability compared with laboratory measurements of available nutrient concentration at a point in time.

Although the productivity of a legume/forage intercrop varies with the forage and legume crop, this intercropping has been shown to improve grain protein content of barley (Eskandari et al., 2009). Since legumes can fix atmospheric nitrogen (N), when IWG is grown in a mixed stand with a legume, it can benefit from the increased N availability in the soil relative to IWG grown as a pure stand without applying N fertilizer. However, research evidence indicates that there can be a time lag between N fixation by the legume intercrop and year of establishment (Crews et al., 2022). However, when selecting a legume to intercrop with IWG, the competition between the legume and the main cash crop should be considered. Recent field studies in Manitoba assessing the performance of IWG in a mixed stand with a legume revealed that alfalfa and sweet clover are too competitive with IWG whereas white clover is not competitive enough and alsike clover is intermediate, falling in between the competitiveness of the other legume species (D. Catani, personal communication, 2021).

Recent studies have demonstrated many ecosystem services associated with IWG relative to annual grain cropping systems, such as greater N uptake efficiency from enhanced absorption and retention of N due to the laterally well-spread root system, lower nitrate leaching due to deep root system (Culman et al., 2013; Sprunger et al., 2018; Jungers et al., 2019) and higher water use efficiency (De Oliveira et al., 2020). Nonetheless, further evaluation of soil chemical property dynamics is essential before releasing this forage-grain crop. Therefore, the objective of this study was to evaluate the impacts of IWG-based perennial forage treatments on soil chemical quality attributes. Specifically, we compared the effects of (1) IWG with no fertilizer post-establishment, (2) IWG with synthetic fertilizer applied after the first grain harvest, (3) IWG in a mixed stand with a legume (Alsike clover, *Trifolium hybridum* L.) and (4) a single-purpose perennial crop control consisting of a 50:25:25 mix of Courtney tall fescue/Algonquin alfalfa/Oxley II cicer milkvetch. Specific objectives were to (1) evaluate the impact of the above IWG-based perennial forage treatments on selected soil chemical properties and (2) characterize temporal changes in soil N supply rate as a function of different IWG-based perennial forage treatments.

2.3 Materials and Methods

2.3.1 Study sites

The study was conducted at three locations in Manitoba and one location in Saskatchewan. Pasture-size plots (1.1 to 1.2 ha) were set up at the Glenlea Research Station in Manitoba (49.6491° N 97.1189° W) and seeded in May of 2020. Small plots (49 – 73 m^2) were established at the Agriculture and Agri-Food Canada (AAFC), Brandon Research and Development Centre (BRDC), Manitoba (49.8694 °N, 99.9791 °W) and seeded in May 2019; the Ian N. Morrison Research Farm, Carman, Manitoba (49.5016° N, 98.0285° W) and seeded in July 2019; and the Livestock and Forage Centre of Excellence, Clavet, Saskatchewan (51.9349° N, 106.3791° W) and seeded in May 2019. The soil was a Gleyed Rego Black Chernozemic clay (Gleyed Humic Vertisol) at the Glenlea site, a Newdale clay loam Orthic Black Chernozem (Typic Haplocryoll) at the Brandon site, a Hibsin Orthic Black Chernozem (Udic Boroll) with a sandy loam texture at the Carman site, and a well-drained, loamy to fine sandy Dark Brown Chernozem at the Clavet site.

Plot sizes were 10 m × 5.5 m at Brandon, 6.6 m × 11 m at Carman, and 7 m × 7 m at Clavet. All sites were previously under annual crops: oats at Carman, field peas (*Pisum sativum* var. *arvense* (L.) Poir.) at Brandon, and oats and barley (*Hordeum vulgare*) at Glenlea, whereas the Clavet site was summer-fallowed in 2018 but was cropped to canola (*Brassica napus*) in 2017. The Glenlea site had a history of hog manure application and the Clavet site had previously received cattle manure applications.

2.3.2 Experimental setup

2.3.2.1 Experimental layout

The experimental design at each site was a randomized complete block with a one-way treatment structure. The treatments were (1) a control consisting of a single-purpose perennial crop (Courtney tall fescue/Algonquin alfalfa/Oxley II Cicer milkvetch [50:25:25]) (Control); (2) dual-purpose IWG in a pure stand (IWGP); (3) dual-purpose IWG in a pure stand plus synthetic fertilizer (50 kg N ha^{-1} post grain harvest) (IWGF); and (4) dual-purpose IWG in a mixed stand (50:50) with a legume (Alsike clover; *Trifolium hybridum*) (IWGL). The IWG seed source for these studies is Syn-2 seed of a ten-clone synthetic developed at the University of Manitoba from selections for long-term grain yield capability (Cattani 2017). The control treatment was a high-performing grass and legume mix assessed in a previous stockpile forage extended grazing study (Peng, 2017). The IWGL treatment received no fertilizer application post-establishment and therefore allowed evaluation of the effect of alsike clover on N availability for IWG

2.3.2.2 Treatment application

Treatments were established after soil sampling in 2019. No fertilizer was applied prior to treatment establishment. Nitrogen fertilizer was applied to control plots in the spring of 2020 based on the initial soil test results and to the IWGF treatment after the first harvest in August 2020 and 2021, dependent upon the year of establishment.

2.3.3 Plant root simulator probe installation and sampling

Anion and cation exchange resin (plant root simulator, PRS) probes were installed in three blocks at the Glenlea site after seeding in 2020 and at the start of the 2021 growing season. The probes were switched after 14 d, with this cycle repeated until the soil was frozen, giving a total of 8 consecutive 2-wk periods in each growing season. The PRS probes used in this study were 15 cm × 3 cm × 0.5 cm in dimensions (Western Ag, Saskatoon, SK), and the membranes were enclosed inside the plastic support. Anion probes have positively charged membranes, which attract and adsorb anions, such as nitrate, phosphate, and sulfate, in the soil. Cation probes have negatively charged membranes to attract cations such as ammonium, potassium, calcium, and magnesium. In this study, PRS probes were used to determine the supply rates of NO_3^--N and NH_4^+ -N in the soil.

Probe installation locations were isolated by inserting PVC cylinders (10 cm diam. and 45 cm ht.) into the soil after removing plant roots from the insertion area to exclude plant roots, hence root uptake of nutrients. Four cation and four anion probes were installed at each of two locations in each plot. The four probe pairs at each plot location constituted one PRS sample. On each retrieval day, 24 PRS probe samples from the three blocks were removed and replaced with new probes. The new probes were carefully inserted into the same slots as the previous, ensuring complete soil to membrane contact. A garden knife was used to make slots in the soil for burying the probes and a "back cut" was applied to maximize the contact between the ion exchange membrane and the soil. The total number of PRS probe samples deployed and retrieved over the two growing seasons was 384 (24 probe samples × 8 burial periods × 2 years).

Retrieved probes were shipped to Western Ag laboratories for colorimetric determination of ammonium and nitrate ions using an automated flow injection analysis system (Technicon Autoanalyzer II) after immersing probes in 0.5 M HCl solution for one hour to remove adsorbed ions. This provided data on the total quantity of ammonium and nitrate ions adsorbed per unit area of membrane over the 2-wk installation period.

2.3.4 Soil sampling

Soil samples for nutrient analysis were collected from the 0-15 and 15-60 cm soil layers in September each year. Three subsamples were collected from each depth interval in each plot at AAFC-Brandon, Carman, and Clavet. The three subsamples from each depth interval were composited and placed in a labeled poly bag. At the Glenlea pasture site, 3 samples were collected from each plot and were placed in three separate poly bags. All samples were stored at 4°C until processing and analysis.

2.3.5 Soil Chemical Properties

2.3.5.1 Available nitrogen

Soil NO_3- and NH^+-N were extracted from soil samples using 2 M KCl (Maynard et al., 2006). Briefly, 5 g of moist soil were weighed into an Erlenmeyer flask (125 mL), followed by the addition of 50 mL of 2 M KCl. After 30 min of shaking, the solution was filtered through a Whatman #42 filter paper, and the filtrate was analyzed for NO_3^--N concentration by the cadmium reduction procedure (Cortas and Wakid, 1990) while NH_4^+-N concentration was measured by the phenate method (Maynard and Karla, 1993) using a Technicon autoanalyzer III (Bran + Luebbe, Germany).

2.3.5.2 Available phosphorus

Available phosphorus (Olsen P) concentration was determined by the sodium bicarbonate method (Olsen et al., 1954). One gram of soil was weighed into a 50-mL Erlenmeyer flask and 20 mL of 0.5 M $NaHCO_3$ was added. The pH was maintained at 8.5. After 30 min of shaking and filtration through a Whatman #42 filter paper, P in the filtrate was analyzed by the colorimetric molybdate blue method (Murphy and Riley,1962) using a flow-injection analysis (FIA) spectrophotometer (FIAlyzer-1000 series, FIAlab Instruments, USA).

2.3.5.3 Calcium, magnesium, and potassium

Concentrations of Ca, Mg, and K were measured following extraction of 3 g soil with 30 mL Mehlich III solution (Mehlich, 1984). After shaking for 5 min on a reciprocating shaker and filtering through a Whatman No. 42 filter paper, the filtrate was stored in plastic vials at 4°C. Deionized water and a $CsCl$-$LaCl_3$ solution were added to the filtrate followed by analysis for Ca and Mg by atomic absorption spectroscopy and for K by flame emission spectroscopy (Carter and Gregorich, 2007).

2.3.5.4 Sulfate

Soil SO_4-S concentration was measured colorimetrically (400 nm) following reduction to sulfide using hydriodic acid and bismuth reagent (Carter and Gregorich, 2007).

2.3.5.5 Electrical conductivity and pH

Soil pH was measured in a 1:2 soil:H_2O (mass/volume) suspension using a pH meter (Model 290A, Orion, Boston, MA), after which the suspension was analyzed for electrical conductivity (EC) using a conductivity meter (Model 125A, Orion, Boston, MA).

2.3.5.6 Cation exchange capacity

Cation exchange capacity (CEC) was measured in ammonium acetate extracts at pH 7 (Gillman et al, 1983). In this method, 2 g of soil were shaken with 20 mL of ammonium acetate solution for 2 h, followed by centrifugation. After removing the supernatant, the soil was washed with 95% ethanol to remove any remaining base cations in the soil sample. As the final step, the soil sample was washed with 10% NaCl solution to remove NH_4^+ ions trapped in the soil. Cation exchange capacity was calculated based on the NH_4^+ ion concentration of the soil solution.

2.3.6 Statistical Analysis

Analysis of variance of N supply rate data was performed using the generalized linear mixed model procedure (PROC GLIMMIX) for repeated measures in SAS OnDemand for Academics using the gamma distribution (SAS Institute, 2014) with forage system and year as fixed effects, sampling day as the repeated measures factor, and block as a random effect. Various covariance structures were compared, and the compound symmetry (CS) structure was selected as the best fit for the repeated measures analysis. When treatment effects were significant, means were compared using the Tukey multiple comparison procedure at $\alpha = 0.05$.

Analysis of covariance of soil chemical properties data was performed using PROC GLIMMIX of SAS. Lognormally-distributed data were analyzed by specifying DIST = LOG in the MODEL statement of PROC GLIMMIX.

Data for soil properties were analyzed separately for the large pasture plot study (Glenlea) on the one hand and the three small plot sites on the other as the plot configuration differed between the two groups. As urea fertilizer was only applied in 2021 post-grain harvesting at the Glenlea site, nutrient data were analyzed separately for the two growing seasons. For small plot sites, forage system and year were modeled as fixed factors while site and block nested within site [block(site)] were modelled as random factors. For the Glenlea pasture plot site, forage system was modeled as a fixed factor and block was modelled as a random factor.

2.4 Results and Discussion

2.4.1 Weather

Monthly and seasonal precipitation and temperature data for the four study sites are presented in Fig. 2.1 and Table 2.1, respectively.

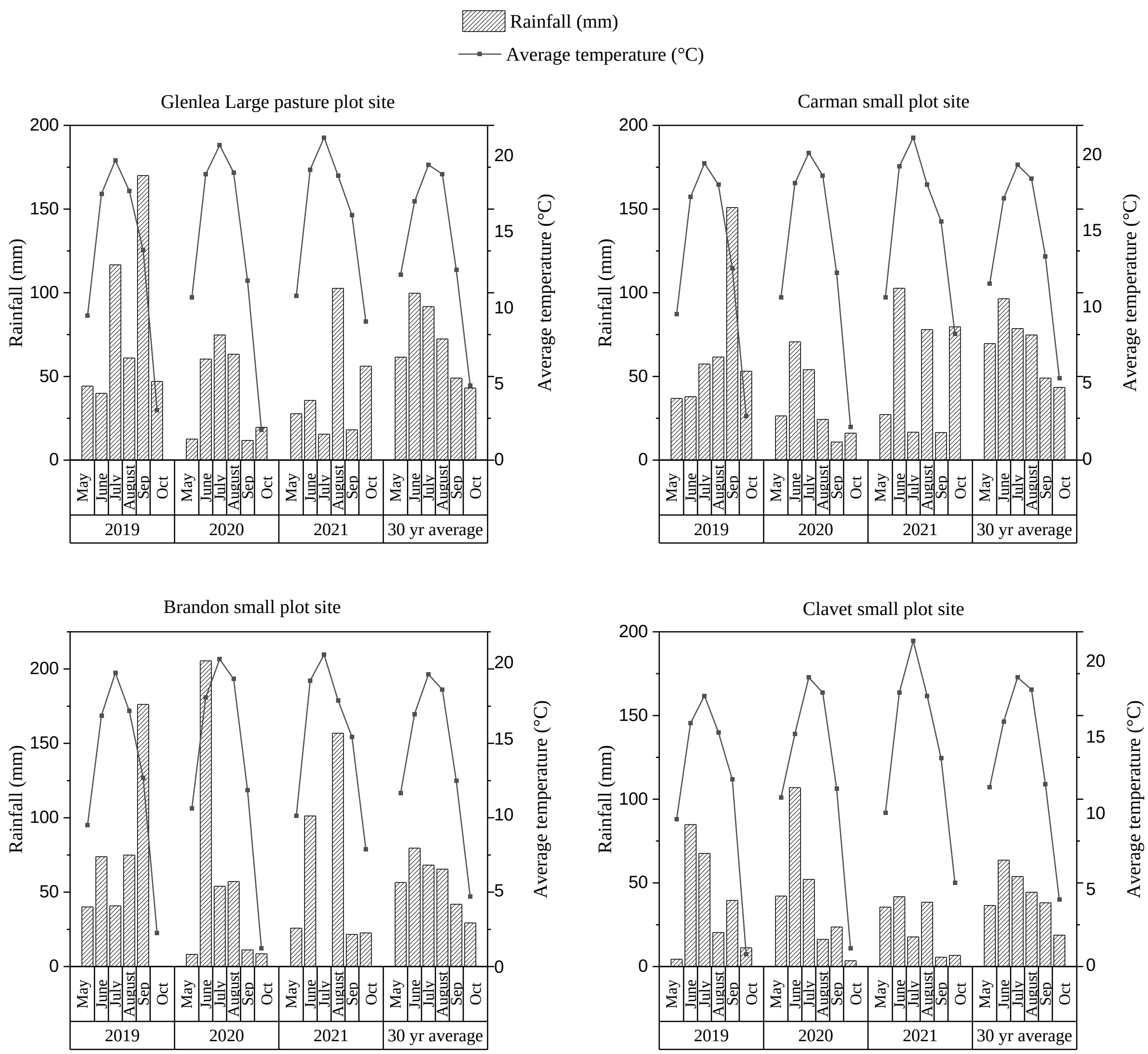

Figure 2.1 Monthly precipitation and air temperature at the study sites.

Long term (1981-2010) total annual precipitation and average annual temperature are, respectively, 543 mm and 2.8 °C for the Glenlea site, 545 mm and 3.5 °C for the Carman site, 462 mm and 2.7 °C for the Brandon site, and 340 mm and 3.3 °C for the Clavet site (Environment and Climate Change Canada, 2022) (Table 2.1). Mean annual precipitation values at all study sites in 2020 and 2021 were lower than the 30-yr averages for the sites.

Table 2.1 Total seasonal (May-October) and annual precipitation and average air temperature at the study sites.

Site	Period	Total precipitation (mm)				Average temperature (°C)			
		2019	2020	2021	30-yr average	2019	2020	2021	30-yr average
Glenlea	May-Oct	479	243	256	417	13.6	13.8	15.8	14.1
	Annual	546	327	361	543	1.8	3.7	4.7	2.8
Carman	May-Oct	398	202	321	412	13.3	13.7	15.6	14.3
	Annual	473	267	380	545	1.9	3.7	4.7	3.5
Brandon	May-Oct	406	345	328	341	12.8	13.3	14.9	13.7
	Annual	470	401	382	462	1.4	3.1	3.7	2.7
Clavet	May-Oct	228	244	146	255	12.0	12.7	14.4	13.6
	Annual	266	297	181	340	1.2	2.0	3.1	3.3

Monthly and annual weather data for the four study sites were recorded within 0.4 to 35 km of the sites.

2.4.2 Baseline soil measurements

Table 2.2 Selected baseline soil properties based on soil tests conducted prior to establishment of the treatments in 2019 and 2020.

Soil Property [a]	Glenlea	Carman	Brandon	Clavet
NO_3^--N (0-15 cm, mg kg^{-1})	25±9.86 [b]	14±5.13	25±6.00	43±5.07
NO_3^--N (15-60 cm, mg kg^{-1})	31±14.2	39±14.4	74±20.8	107±27.5
Olsen P (0-15 cm, mg kg^{-1})	48±11.3	19±8.82	46±6.01	9±2.85
K (0-15 cm, mg kg^{-1})	551±107	279±45.5	424±53.0	527±49.0
Ca (0-15 cm, mg kg^{-1})	4042±196	2527±248	3455±497	2015±232
Mg (0-15 cm, mg kg^{-1})	1537±116	644±151	457±48.5	447±65.2
SO_4^{2-}-S (0-15 cm, mg kg^{-1})	12±7.50	6±2.09	13±2.43	45±19.1
SO_4^{2-}-S (15-60 cm, mg kg^{-1})	49±38.4	26±32.1	28±6.34	103±62.7
Na (0-15 cm), mg kg^{-1})	63±17.1	24±5.35	10±1.41	38±9.60
SOC (0-15 cm), g kg^{-1})	60±9	26±2.9	46±5.8	27±4
CCE (0-15 cm), g kg^{-1})	0.55±0.19	0.47±0.13	0.96±0.44	0.14±0.14
CEC (0-15 cm), $cmol_c$ kg^{-1})	35±1.58	19±1.88	22±2.66	15±1.58
pH (0-15 cm)	7.2±0.16	6.5±0.46	7.4±0.14	6.2±0.38
pH (15-60 cm)	7.6±0.43	7.6±0.54	8.0±0.18	7.1±0.39
EC (0-15 cm, mS cm^{-1})	0.53±0.08	0.20±0.05	0.30±0.03	0.61±0.61
EC (15-60 cm, mS cm^{-1})	0.68±0.41	0.31±0.08	0.25±0.04	1.39±1.39
24-hr CO_2 respiration (0-15 cm, mg kg^{-1})	269±46.3	153±36.3	176±18.3	157±49.2
POXC (0-15 cm, mg kg^{-1})	1009±86.4	680±36.1	737±73.6	616±68.4
ACE protein N (0-15 cm, mg g^{-1})	7.6±1.44	6.7±0.98	6.9±0.84	6.6±0.73
Bulk density (0-20 cm, g cm^{-2})	1.25±0.02	1.36±0.03	1.29±0.05	-
Bulk density (20-60 cm, g cm^{-2})	1.38±0.03	1.59±0.03	1.42±0.04	-

[a] SOC, soil organic carbon; CCE, $CaCO_3$ equivalent; CEC, cation exchange capacity; EC, electrical conductivity; POXC, permanganate-oxidizable carbon; ACE protein N, autoclaved citrate-extractable protein N

[b] Mean ± standard deviation (n = 16)

2.4.3 Nitrogen supply rate

Soil NO_3^--N supply rate was determined in two phases corresponding to the period before urea application to the IWGF plots on August 18, 2021 (following grain harvest) and the post-urea application period (i.e., after August 18, 2021). Prior to urea application to the IWGF plots, the IWGP and IWGF treatments were the same (Table 2.3).

The year × sampling day interaction was significant for NO_3^--N supply rate prior to urea application (Table 2.3). However, the treatment (forage system) effect was not significant. Averaged across treatments, supply rate of soil NO_3^--N ranged from 30.3 to 37.8 µg cm^{-2} 2 wk^{-1} and over the 8 sampling days it ranged from 22.0 to 63.3 µg cm^{-2} 2 wk^{-1}.

Table 2.3 Effect of forage treatment, sampling day and year on NO_3^--N supply rate in 2020 and 2021

Effect	NO_3^--N supply rate (μg cm^{-2} 2 wk^{-1})	
	Pre-urea application[c]	Post-urea application[d]
Treatment[a]		
Control	36.1a[b]	26.4
IWGP	30.3a	19.7
IWGF	-	28.2
IWGL	37.8a	29
Sampling day		
Day 1	63.3	48.1
Day 15	51.2	30.9
Day 29	24	13.6
Day 43	24.6	8.7
Day 57	27.9	12.3
Day 70	50.3	61.9
Day 84	34.5	58.9
Day 100	22	23
Year		
2020	47.3	-
2021	25.3	-

	P value	
Treatment	0.06	0.03
Sampling day	<0.0001	<0.0001
Year	0.06	-
Year × Sampling day	**<0.0001**	-
Year × Treatment	0.54	-
Sampling day × Treatment	0.19	**0.004**
Year × Sampling day × Treatment	0.47	-

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

[c] Control, IWGP, and IWGL treatments that spanned the 2020 and 2021 growing seasons

[d] Control, IWGP, IWGF and IWGL treatments that spanned the 2021 growing season only.

There was an overall temporal decrease in NO_3^--N supply rate in the 2020 and 2021 growing seasons (Fig. 2.2). This is consistent with previous studies, which have also shown that nutrient supply rates from PRS probes are strongly correlated with nutrient uptake by plants (Dessureault-Rompré et al., 2022; Qian and Schoenau, 2005; Nyiraneza et al., 2009; Sharifi et al., 2009). Absorption of NO_3^--N from the same location will reduce the bioavailable NO_3^--N concentration in subsequent burials (Qian and Schoenau, 2000, Qian and Schoenau, 2002a). This is due to N mining by PRS probes over the growing season. Similar to plant roots, PRS probe measurements are sensitive to surrounding environmental conditions, especially soil moisture and soil temperature (Sulewski et al., 2002; Scherer-Lorenzen et al., 2003). Moreover, there is a balance between N immobilization and mineralization in soil and soil moisture near field capacity and warm temperatures increase the rates of these process. Therefore, supply rates of NO_3^--N also depend on the quantity of N mineralized from soil organic N pools (Havlin et al., 2005).

Although the main effect of year on NO_3^--N supply rate was not significant, the supply rates on Days 15, 29, 43 and 57 were significantly greater in 2020 (80.2, 40.2, 67.3 and 60.1μg cm^{-2} 2 wk^{-1}, respectively) than in 2021 (32.8, 14.4, 9 and 13, μg cm^{-2} 2 wk^{-1}, respectively) (Fig. 2.2a). Only on Day 84 was the supply rate significantly higher in 2021 (52.8 μg cm^{-2} 2 wk^{-1}) than in 2020 (22.5 μg cm^{-2} 2 wk^{-1}). A possible explanation for this is the rainfall received during the 2-wk burial period in each year. In 2020, sampling Day 84 was on September 30 whereas in 2021 it was on September 2 and the total rainfall received during sampling Days 70 to 84 (September 16-30) was only 0.2 mm in 2020, compared with 55.8 mm in 2021 (August 19-September 2). Nitrate supply rate in soil originates from the mineralizable pool of soil N (Havlin et al., 2005) and soil moisture near field capacity enhances N mineralization due to increased microbial activity in the soil (Li et al., 2019). This is consistent with PRS probes measurements showing a positive correlation with rainfall during the sampling (burial) period. In our study, the Glenlea site was seeded in 2020. Nitrogen fertilizer was not applied in 2020 as N was not removed from the soil with the harvest during this establishment year when the forage was not harvested. A study by Fernandez et al. (2020) evaluating changes in IWG yield with N fertilizer application and planting density showed that IWG grain yield declined after the year of establishment and N fertilizer application alleviated the problem. Even though the grain was not harvested in 2020, the plants had passed all the initial growth stages (germination, vegetative, and elongation) within that growing season, which usually requires a large amount of N. Nitrate N concentration in the surface soil layer (0-15 cm) for IWGP, IWGL and the control was significantly lower in 2021 (9.1 mg kg^{-1}) than in 2020 (19.4 mg kg^{-1}), which could explain the lower supply rates in 2021 than in 2020.

In 2020, there was a significant decrease in NO_3^--N supply rate on Day 29 (40.2 μg cm^{-2} 2 wk^{-1}) relative to the previous two sampling dates (79.7 and 80.2 μg cm^{-2} 2 wk^{-1} on Day 1 and Day 15, respectively). However, the supply rate increased significantly on Day 43 (67.3 μg cm^{-2} 2 wk^{-1}) relative to Day 29. After Day 43 in 2020, the decreasing trend of the supply rate started again. The drop in supply rate on Day 29 (August 6) was likely related to the rainfall received after the previous sampling. The PRS probes were initially installed on June 25 and the first sampling (retrieval) of probes was on July 9. Total rainfall during June 25 through July 9 was 32.9 mm compared with 55.1 mm during July 9 – 23 (Day 15). However, July 23 – August 6 (Day 29) was very dry, with only 1 mm rainfall recorded. The dry soil conditions likely negatively impacted the NO_3^--N supply rate measurements. This is consistent with the significant increase in NO_3^--N supply rate on Day 43 (August 20), which was preceded by a total of 47.7 mm of rainfall during the 2-week burial period. Previous studies have also shown that dry soil conditions are quite challenging for measuring nutrient supply rate using PRS probes as they limit ion movement in the soil (Sulewski et al., 2002; Dijkstra et al., 2016). Additionally, under dry conditions, reduced microbial N mineralization can lower supply rates. In 2021, there was a gradual decrease in the supply rate until Day 43 (9 μg cm^{-2} 2 wk^{-1}). The supply rate on Day 57 (13 μg cm^{-2} 2 wk^{-1}) was also not significantly different from that on Day 43. The PRS probes at Glenlea were installed at the end of May and therefore Days 1 through 57 spanned early June through early August. Total rainfall amounts in June (35.7 mm) and July (15.5 mm) were very low compared with 2020 (60.4 mm and 74.8 mm, respectively). Therefore, another possible reason for the declining trend in supply rate other than the low available N content in the soil might be the drier soil conditions. More importantly, under dry soil conditions, it is difficult to ensure proper contact between the soil and the PRS probe. Although plant roots can compete with PRS probes for NO_3^- uptake, this was negligible since the probe installation locations were shielded from plant roots with PVC root-exclusion cylinders (RECs). Still, there is a possibility of microbial immobilization of N (Dijkstra et al., 2016). A complication with the use RECs when measuring NO_3^--N supply is that NO_3^--N can be lost by leaching or denitrification and this might be enhanced by higher soil moisture content within the RECs because plants are not utilizing the moisture (E. Bremer, personal communication, 2022). Although some studies have shown minimal differences in soil moisture and temperature between RECs and bulk soil (Huang et al., 1997), contrasting results have been observed under different circumstances, especially with plants growing under drought conditions, which may deplete soil moisture to a much greater degree outside than inside of RECs (E. Bremer, personal communication, 2022).

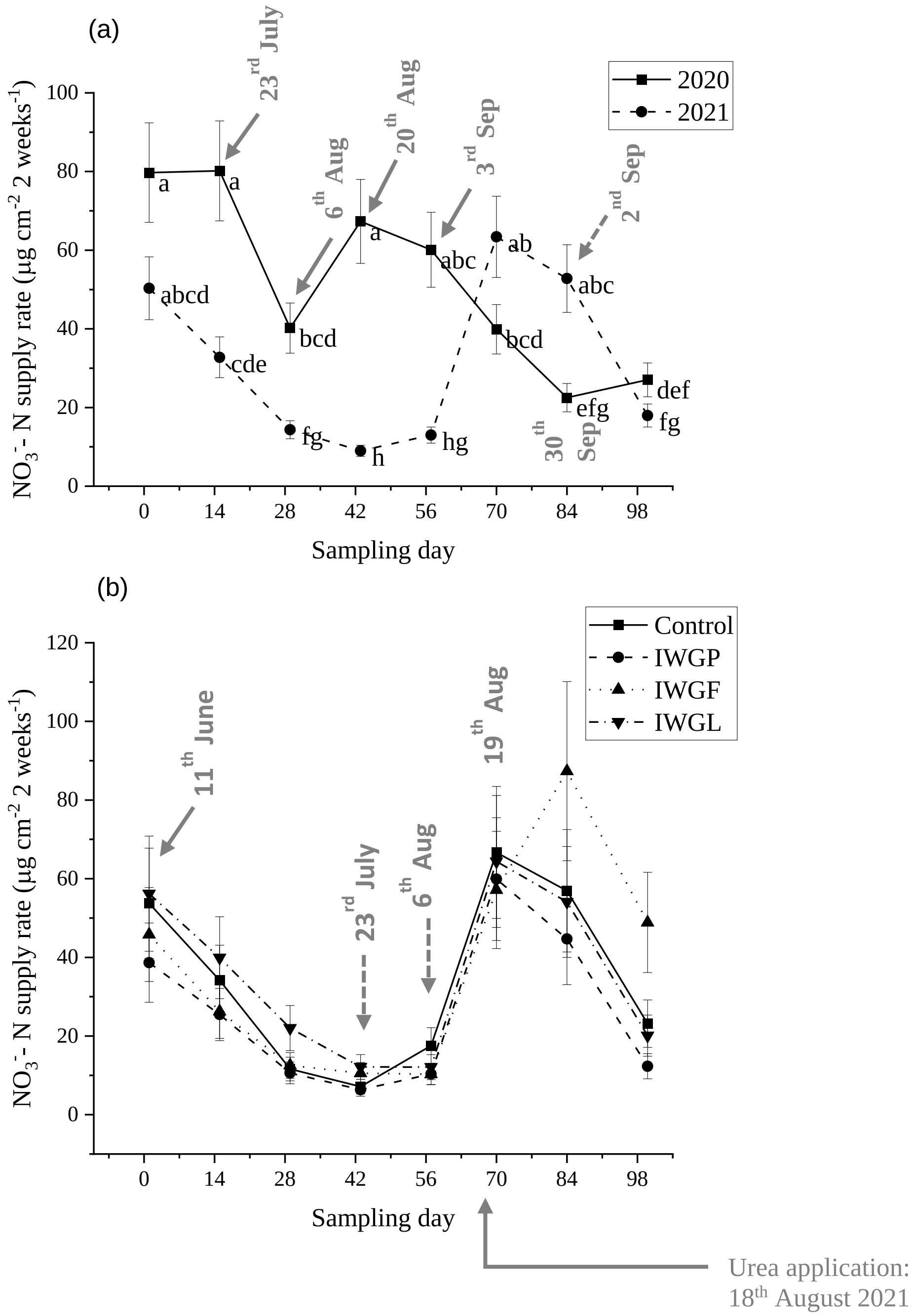

Figure 2.2 Temporal changes in NO_3^--N supply rate, (a) averaged across Control, IWGP and IWGL treatments, during the growing season in 2020 and 2021; and (b) for individual forage treatments during the growing season in 2021. Vertical bars represent standard errors of the mean. Bars with the same letter are not significantly different at α = 0.05 according to the Tukey multiple comparison procedure. Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

However, longer burial times may be useful to detect differences in NO_3^--N supply rates under dry conditions such as those in 2021 (Drohan et al., 2005). The supply rate increased to 63.4 μg cm^{-2} 2 wk^{-1} on Day 70 and was significantly higher than on Day 57. Total precipitation was high in August after Day 57 (August 6, 96.8 mm), ensuring higher supply rates. However, after Day 70, the supply rate showed a decreasing trend. In both years, the supply rates on Day 100 (27 and 18 μg cm^{-2} 2 wk^{-1}, respectively, for 2020 and 2021) were significantly lower than those on Day 1 (79.7 and 50.3 μg cm^{-2} 2 wk^{-1}). As explained before, this is due to the removal of NO_3^- ions by probes during the sampling period. Another possible explanation could be immobilization of soil N towards the end of the growing season.

The sampling day × treatment interaction was significant for NO_3^--N supply rate in the 2021 growing season (post-urea application), which included all four treatments (control, IWGP, IWGF and IWGL) that spanned the 2021 growing season only (Table 2.3). Averaged across sampling dates, NO_3^--N supply rate ranged from 19.7 µg cm^{-2} 2 wk^{-1} for IWGP to 29 µg cm^{-2} 2 wk^{-1} for IWGL. Overall, the supply rate was lowest on Day 43 (8.7 µg cm^{-2} 2 wk^{-1}) and highest on Day 70 (61.9 µg cm^{-2} 2 wk^{-1}).

Nitrate-nitrogen supply rates for all forage treatments (post-urea application to IWGF plots) were significantly greater on Day 1 than on Days 43 and 57 (Fig. 2.2b). Also, except for the IWGL treatment, the supply rates for the other three treatments were significantly greater on Day 1 than on Day 29. However, the IWGL treatment started to show significant depletion in supply rate after the third sampling day (Day 29). John (2008) reported that, while alsike clover is adaptable to different types of soils, soil moisture is a critical factor for its performance as it has a low tolerance for prolonged drought conditions. In our study, June and July in 2021 were drier than the other months during which supply rates were measured.

There was a spike in the supply rate following urea fertilizer application post-grain harvest in August 2021 (Day 70) (Fig. 2.2b) and this increase was significant for all forage treatments relative to the previous sampling day. Sampling Day 57 (August 6) was at the end of the 2-wk burial period during which only 6.6 mm of rainfall were recorded. By comparison, the 2-wk period preceding the next sampling day (Day 70 on August 19) received 41 mm of rainfall. The higher precipitation could be the reason for the elevated supply rates for all treatments on Day 70 despite the fact that fertilizer was only applied to IWGF plots. After Day 70, while the supply rates for the control, IWGP, and IWGL treatments started to decrease, the supply rate of the IWGF treatment further increased until Day 84, indicating the effect of urea application. Additionally, the similar NO_3^--N supply rates on Day 100 (48.9 μg cm^{-2} 2 wk^{-1}) and Day 1 (45.8 μg cm^{-2} 2 wk^{-1}) were only detected in the IWGF treatment but not in the other two IWG treatments. Once again, this result might also be explained by the application of urea before Day 70.

On Day 100, the supply rate for the IWGF treatment was significantly greater than that of the IWGP treatment. More importantly, on all three sampling days following application of urea (Days 70, 84, and 100), there were no significant differences in supply rate between IWGF and IWGL. This performance of the IWGL treatment indicates that the alsike clover in the IWGL forage system in 2020 and 2021 provided sufficient N for the standing perennial forage crop and performed as well as the treatment that received inorganic fertilizer (IWGF). This result is consistent with observations by Khanal et al. (2021), who tested a 4-yr rotation that included legumes and annual forages, and found that 2-year cropping of red clover or white clover following wheat and canola in successive first and second years was adequate to supply the N requirement of the wheat crop and part of the N requirement of the canola crop.

Analysis of variance excluding the IWGF treatment (which was not present in 2020) showed significant year × sampling day and year × forage system (treatment) interactions for cumulative NO_3-N supply rate (Table 2.4).

Table 2.4 Effect of Forage treatment, sampling day and year on cumulative NO_3^--N supply rate in 2020 and 2021

Effect	Cumulative NO_3^--N supply rate (μg cm^{-2})	
	Pre-urea application[c]	Post-urea application[d]
Treatment[a]		
Control	178	128ab[b]
IWGP	160	98.9b
IWGF	--	116ab
IWGL	188	144a
Sampling day		
Day 1	63.1	47.8f
Day 15	115	78.7e
Day 29	140	92.5d
Day 43	169	102cd
Day 57	199	114c
Day 70	260	178b
Day 84	304	239a
Day 100	325	263a
Year		
2020	246	-
2021	124	-
P value		
Treatment	0.051	0.048
Sampling day	<.0001	**<.0001**
Year	0.02	-
Year × Sampling day	**<.0001**	-
Year × Treatment	**0.047**	-
Sampling day × Treatment	0.99	0.74
Year*Sampling day × Treatment	0.99	-

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b]Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

[c]Control, IWGP, and IWGL treatments that spanned the 2020 and 2021 growing seasons

[d] Control, IWGP, IWGF and IWGL treatments that spanned the 2021 growing season only.

Cumulative NO_3^--N supply rates were significantly greater in 2020 than in 2021 at all sampling times, but differences between the two years increased with time until Day 43, after which there was no further increase (Fig. 2.3). As explained previously, depletion of soil available N could be the reason for the lower cumulative supply rates in 2021. During the last three sampling days in both years, differences in cumulative supply rates between successive sampling days were not significant (Fig. 2.3). Sequential burials in laboratory studies generally show the most rapid adsorption initially, followed by slower rates (Qian and Schoenau, 2000; Qian and Schoenau, 2002a). The pattern depends on the quantity of NO_3^--N initially present in the soil solution, rate of mineralization/immobilization, and volume of soil. This is consistent with the curves obtained in the current study for cumulative supply rates data and it was likely related to the reduction in soil available N towards the end of the growing season due to NO_3^--N mining by the probes sampling from the exact same location. Additionally, cumulative supply rates were also affected by rainfall, as evident after Day 57 (August 6).

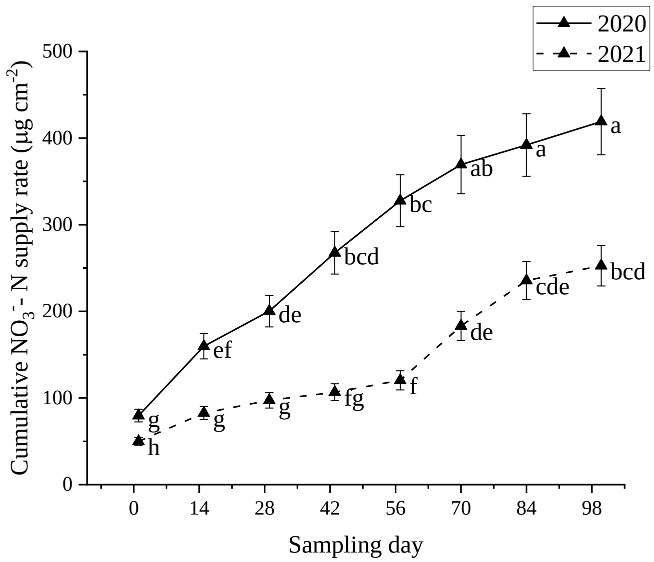

Figure 2.3 Cumulative NO_3^--N supply rates during the growing season in 2020 and 2021, calculated by summing the supply rates of successive 2-week burial periods, averaged across treatments. Vertical bars represent standard errors of the mean. Means with the same letter are not significantly different at α = 0.05 according to the Tukey multiple comparison procedure.

There were no significant differences in cumulative NO_3^--N supply rates among treatments in the 2020 growing season. However, the cumulative supply rates for all three treatments were significantly lower in the 2021 growing season than in 2020 (Fig. 2.4. Moreover, the cumulative supply rate for the IWGL treatment was significantly greater than that of the IWGP treatment in 2021. This is consistent with the expected lag in N inputs from the legume as the N in the legume is initially in an organic form that must be mineralized before becoming bioavailable.

Since fertilizer was not applied prior to the start of the growing season in 2021, supply rates were expected to decline relative to 2020 as soil available N was progressively depleted. Particularly, legume N fixation presumably feeds from the mineralizable N pool resulting in lower quantities in soil for plant uptake. The greater supply rates for IWGL than for IWGP in 2021 may have been due to N fixation by alsike clover in the former treatment.

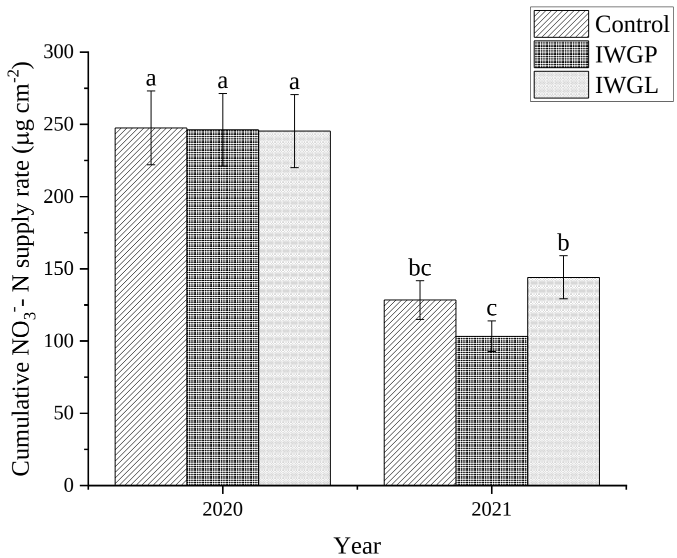

Figure 2.4 Cumulative NO_3-N supply rate for different forage treatments during the growing seasons in 2020 and 2021, calculated by summing the supply rates of successive 2-week burial periods. Vertical bars represent standard errors of the mean. Bars with the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey multiple comparison procedure. Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

Analysis of variance based on all four forage systems in 2021 indicated a significantly higher cumulative NO_3^--N supply rate for the IWGL treatment than the IWGP treatment (Table 2.4). This was likely due to greater N fixation by the legume intercrop during the 2021 growing season. This result is consistent with significantly greater grain protein content for the IWGL treatment than the IWGP treatment in the same year (Le Heiget, unpublished data, 2022). NO_3^--N supply rates for IWGP and IWGF were not significantly different, likely because the IWGF plots only received fertilizer N on Day 70, prior to which it was the same treatment as IWGP.

The cumulative NO_3^--N supply rate was significantly different between consecutive sampling days except Days 29 vs. 43 and 43 vs. 57 when the increments were not significant. Days 29 through 57 spanned early July through early August. Total rainfall amount from Day 29 (July 9) to 43 (July 23) was 15.5 mm while from Day 43 to 57 (August 06) it was 6.6 mm. This entire period was dry compared to the total rainfall in August after Day 57 (96.8 mm), as explained earlier.

More than 61.2% of the NH_4^+ -N supply rate data were below the method detection limit (MDL) and were therefore not subjected to ANOVA or other parametric statistical tests. Low NH_4^+ -N supply rates are typical in moist soils whereas detectable, elevated NH_4^+ -N supply rates are commonly recorded in anaerobic soils (wetland and waterlogged soils), acidic soils (pH around 4), or just after the application of NH_4^+-N rich fertilizer (Western Ag Innovations, n.d.). Data collected from cropland since 1996 shows NO_3^--N supply rates averaging 60 μg cm^{-2} and NH_4^+ -N supply rates of about 1 μg cm^{-2} for 1- to 3-wk burial periods (Western Ag Innovations, n.d.).

2.4.4 Soil properties

2.4.4.1 Small plot sites

The year × treatment interaction was significant for soil NO_3^--N concentration in the 0-15 cm layer. Soil NO_3^--N concentration ranged from 2 to 6 mg kg^{-1} in the 0-15 cm layer and 6 to 12 mg kg^{-1} in the 15-60 cm layer. The NO_3^--N concentration across the 15-60 cm depth was greater than that in the 0-15 cm layer, suggesting possible leaching of NO_3^- ions into deeper soil layers. Several studies have shown that nitrate mobility in the soil varies with factors such as soil physical and chemical properties, precipitation, and management practices such as fertilizer application (Thomas, 1970; Sollins, 1988). Nitrate has greater mobility in coarse-textured soils where it can leach into deeper soil layers after the application of fertilizer (e.g., Vinten, 1994). However, as a perennial grass with a deep, well-spread root system, IWG has the ability to capture the leached nitrate in subsoil layers (Wagoner, 1995; Culman et al., 2013).

Nitrate N concentration in the 0-15 cm layer under the IWGL treatment was significantly greater in 2020 (5.85 mg kg^{-1}) than in 2021 (2.15 mg kg^{-1}) (Fig. 2.5). The 0-15 cm soil layer in the 2021 growing season showed a greater NO_3^--N concentration for the control treatment (4.89 mg kg^{-1}) than the IWGL treatment (2.15 mg kg^{-1}). Importantly, in both growing seasons, there were no significant differences between the IWGF and the IWGL treatments.

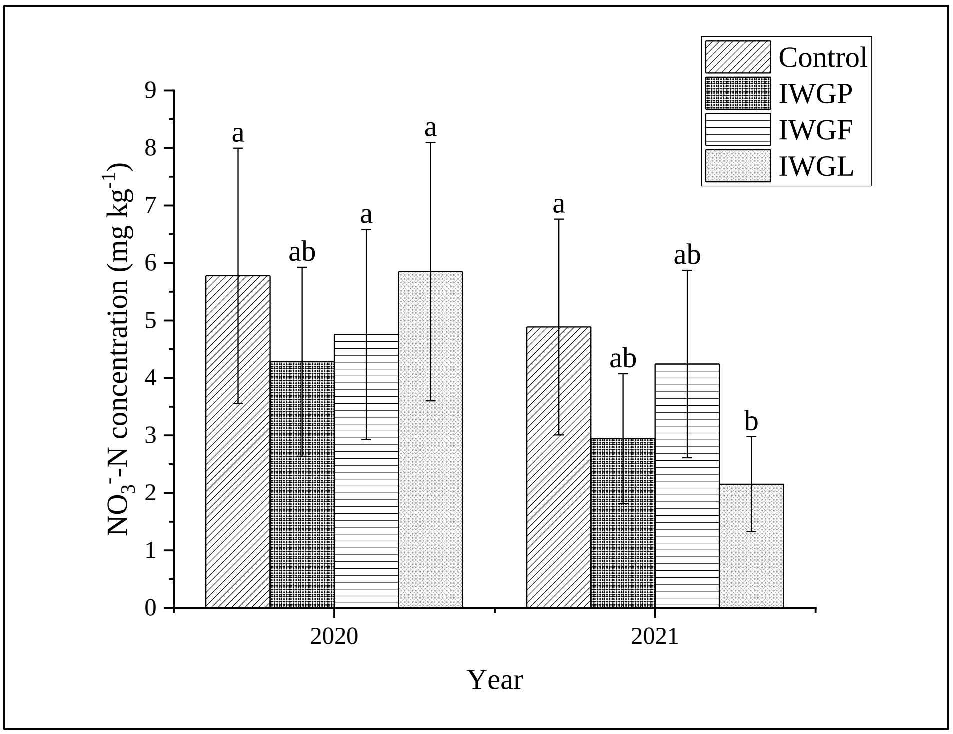

Figure 2.5 Soil NO_3^--N concentration in the 0-15 cm for different forage systems during the 2020 and 2021 growing seasons. Vertical bars represent standard errors of the mean. Bars with the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey multiple comparison procedure. Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

The IWGL treatment showed greater dry matter N concentration in 2020 than in 2021 (Le Heiget, unpublished data, 2022). Therefore, uptake of soil available N was likely greater for IWGL in 2020 than in 2021. Simultaneously, soil available N concentration was also greater in IWGL in 2020 than in 2021. This is in contrast to the expectation for greater uptake (mining) of N resulting in lower available N in the soil. These results can be possibly explained by the greater baseline soil NO_3^--N concentration (inherent fertility) in the soils at the small plot sites prior to establishment of the forage treatments (Table 2.2). Also, the higher utilization of N by the crop in the second year, consistent with the findings by Fernandez et al. (2020), may have reduced available N concentration in the soil at the end of the growing season in 2021.

There was no significant treatment effect on any of the other soil properties (Table 2.5). However, the soil depth effect was significant for pH and EC. Soil pH and EC were significantly higher in the 15-60 cm layer (7.64, 0.89 mS cm^{-1}) than in the 0-15 cm layer (6.85, 0.26 mS cm^{-1}).

Smith and Doran (1997) reported that even though the optimum pH and EC values for crop production are specific to the intended crop, the range of soil pH from 6.5 to 7 and the EC values from 0 to1.5 mS cm^{-1} are generally acceptable for crop growth and for sustaining soil chemical and biological processes.

Table 2.5 Effect of forage treatment and year on soil properties.

Effect	NO_3^--N (0-15 cm)	NO_3^--N (15-60 cm)	Olsen P	K	Mg	Ca	CEC
			mg kg^{-1}				$cmol_c\ kg^{-1}$
Treatment[a]							
Control	5.28	7.41a [b]	26.1a	382a	522a	2497a	18.8a
IWGP	3.53	6.08a	24.3a	372a	526a	2503a	19.0a
IWGF	4.46	7.31a	24.7a	376a	560a	2663a	20.1a
IWGL	3.53	6.89a	24.8a	388a	540a	2504a	18.7a
Year							
2020	5.07	6.63a	27.6a	392a	525b	2526a	18.1b
2021	3.35	7.22a	22.4b	367b	548a	2558a	20.2a
				P value			
Treatment	0.03	0.38	0.83	0.66	0.09	0.35	0.09
Year	0.0004	0.31	**0.0004**	**0.01**	**0.05**	0.67	**<0.0001**
Treatment × Year	**0.02**	0.01	0.87	0.81	0.57	0.15	0.08

Table 2.5 (continued)

Effect	SO_4^--S		EC		pH	
	0-15 cm	15-60 cm	0-15 cm	15-60 cm	0-15 cm	15-60 cm
	mg kg^{-1}		mS cm^{-1}			
Treatment[a]						
Control	16.4a[b]	55.7a	0.26a	0.93a	6.82a	7.6a
IWGP	17.2a	54.2a	0.25a	0.86a	6.81a	7.63a
IWGF	18.0a	52.6a	0.27a	0.81a	6.90a	7.67a
IWGL	17.9a	55.2a	0.26a	0.98a	6.88a	7.65a
Year						
2020	24.7a	69.8a	0.26a	0.95a	6.98a	7.79a
2021	10.0b	42.2b	0.26a	0.84a	6.71b	7.48b
	P value					
Treatment	0.96	0.97	0.59	0.32	0.73	0.78
Year	**<0.0001**	**<0.0001**	0.99	0.10	**0.0002**	**<0.0001**
Treatment × Year	0.77	0.82	0.12	0.73	0.22	0.25

[a]Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure

2.4.4.2 Glenlea large pasture site

In 2020, nutrient concentration data were analyzed for three treatments (control, IWGP, and IWGL since there was no IWGF prior to urea application in August 2021) and in 2021 for four treatments (control, IWGP, IWGF, and IWGL). In the first growing season, the NO_3^--N concentration in the 15-60 cm layer was significantly greater for the IWGL treatment than the control and the IWGP treatment (Table 2.6). Electrical conductivity in the 15-60 cm layer was significantly greater for the control than the IWGP and IWGL treatments (Table 2.6).

Greater NO_3^--N concentration in the sub-soil of the IWGL plots could not be easily explained but may indicate greater leaching under the legume intercrop relative to the other treatments. Equally inexplicable was the higher EC in the control treatment than in the IWGP treatments. In 2021, soil pH in the 0-15 cm layer was the only soil property which showed a significant treatment effect and was significantly higher for IWGP than IWGF (Table 2.7).

There was no significant treatment effect on any of the other soil properties (Tables 2.6 and 2.7). However, in this large pasture experiment, the soil depth effect was only significant for soil pH. Soil pH was significantly higher in both the 2020 and 2021 growing seasons in the 15-60 cm layer (7.68, 7.55, respectively, for 2020 and 2021) than in the 0-15 cm layer (7.15, 7.13).

Table 2.6 Effect of forage treatment on soil properties in 2020.

Effect	NO_3^--N (0-15 cm)	NO_3^--N (15-60 cm)	Olsen P	K	Mg	Ca	CEC
	mg kg^{-1}						cmol$_c$ kg^{-1}
Treatment[a]							
Control	15.4a[b]	8.69b	96.8a	632a	1536a	4124a	35.4a
IWGP	23.1a	12.0b	76.8a	653a	1584a	4244a	36.5a
IWGL	20.8a	16.5a	65.0a	639a	1446a	4254a	34.9a
			P value				
Treatment	0.28	**0.004**	0.17	0.91	0.31	0.9	0.63

Table 2.6 (continued)

Effect	SO_4^--S		EC		pH	
	0-15 cm	15-60 cm	0-15 cm	15-60 cm	0-15 cm	15-60 cm
	mg kg^{-1}		mS cm^{-1}			
Treatment[a]						
Control	18.7a[b]	26.7a	0.47a	0.58a	7.08a	7.66a
IWGP	18.7a	27.7a	0.54a	0.47b	7.05a	7.69a
IWGL	23.3a	48.8a	0.58a	0.53ab	7.31a	7.69a
	P value					
Treatment	0.58	0.05	0.17	**0.03**	0.08	0.91

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different (α = 0.05) according to the Tukey multiple comparison procedure.

Table 2.7 Effect of forage treatment on soil properties in 2021.

Effect	NO_3^--N (0-15 cm)	NO_3^--N (15-60 cm)	Olsen P	K	Mg	Ca	CEC
	mg kg^{-1}						$cmol_c$ kg^{-1}
Treatment[a]							
Control	10.4a[b]	14.4a	67.4a	532a	1522a	4377a	36.9a
IWGP	9.8a	14.4a	58.2a	699a	1514a	4176a	35.6a
IWGF	14.2a	30.1a	67.6a	660a	1564a	4128a	36.3a
IWGL	15.0a	24.7a	73.2a	728a	1516a	4098a	35.2a
			P value				
Treatment	0.22	0.27	0.68	0.62	0.88	0.77	0.73

Table 2.7 (continued)

Effect	SO_4^--S		EC		pH	
	0-15 cm	15-60 cm	0-15 cm	15-60 cm	0-15 cm	15-60 cm
	mg kg^{-1}		mS cm^{-1}			
Treatment[a]						
Control	17.6a[b]	50.4a	0.66a	0.67a	7.09ab	7.64a
IWGP	15.9a	51.1a	0.66a	0.70a	7.28a	7.56a
IWGL	13.4a	38.4a	0.67a	0.76a	6.97b	7.41a
	P value					
Treatment	0.7	0.67	0.97	0.5	**0.02**	0.34

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

During a short-term study, most of the soil chemical properties are not changed significantly as they are less sensitive to management practices. In a study examining near-surface soil quality improvement in a semi-arid region under perennial grass, legumes, and mixtures of grass and legumes over a 5-yr period, treatment effects were confined to the surface 0-10 cm soil layer, with the differences in soil treatments more significant after the fourth year of the study (Liebig et al., 2018). Ernst and Siri-Prieto (2009) concluded that their 12-yr study evaluating soil quality indicators under no-till cropping and rotation of crops with pastures needed to be continued over a greater duration in order to detect significant changes in soil properties. These studies show that, within the 3-yr duration of our study, it would be difficult to detect significant treatment effects.

2.5 Conclusion

Measurements from PRS probes (ion exchange membranes) showed a temporal decline in the supply rate of NO_3^--N in the soil for both 2020 and 2021 growing seasons. In 2021, this overall temporal declining trend in the supply rate was observed for all treatments. Except for NO_3^--N, pH and EC, treatment effects on soil properties in 2020 and 2021 provide evidence for the slow-changing nature of the soil chemical properties tested. Changes in these soil properties should be further examined in a longer-term study. The similar soil available nutrients between the widely used control treatment and the three IWG-based treatments provide evidence that IWG can be introduced to forage systems in the Canadian prairies without adversely affecting soil productivity. The similar soil NO_3^--N supply rates and soil available N concentrations in the IWGF and the IWGL treatments indicate that there is potential for a legume intercrop as an alternative source of N for an IWG perennial forage-grain system. Furthermore, the higher cumulative soil NO_3^--N supply rate for the IWGL treatment relative to the IWGP treatment suggests that low-N input legume-based IWG forage-grain systems can provide an alternative to N-fertilized conventional system.

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2.7 Transition to Chapter 3

In this first study, changes in soil chemical properties under IWG forage-grain systems were evaluated. However, significant treatment differences were not detected for most of the soil available nutrients tested during this 3-yr study. It is known that soil chemical properties take longer durations to change significantly as they are not sensitive enough to management practices. As a next step, we examined the dynamics of soil health indicators as these are known to be more sensitive to management practices and environmental variables.

3. MODELING INTERMEDIATE WHEATGRASS PHENOLOGICAL DEVELOPMENT AND SOIL HEALTH ATTRIBUTES USING BASELINE SOIL PROPERTIES

3.1 Abstract

Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey) is a perennial plant species with adaptive dual-purpose capacity which can potentially be used for late fall/winter grazing of beef cattle. However, the impact of IWG on soil health dynamics and resiliency remains largely undefined. This three-year study examined soil health attributes under different IWG-based perennial forage treatments: IWG with no fertilizer post-establishment (IWGP), IWG with synthetic fertilizer applied after the first grain harvest (IWGF), and IWG in a mixed stand with a legume (Alsike clover, *Trifolium hybridum* L.) (IWGL). A single-purpose perennial forage treatment consisting of a 50:25:25 mix of Tall fescue (*Schedonorus arundinaceus*)/Algonquin alfalfa (Medicago sativa L. 'Algonquin')/Oxley II cicer milkvetch (*Astragalus cicer* L.) was included for comparison (Control). The experimental design was a randomized complete block with a one-way treatment structure at each of four sites in Manitoba and Saskatchewan, Canada. Treatment effects were not significant for soil health indicators 3 yr after the start of the experiment. However, partial least squares (PLS) analysis indicated that the soil health indicators 24-h CO_2 respiration, permanganate-oxidizable C (POXC), and bioavailable (autoclaved citrate-extractable (ACE) protein) nitrogen (N) levels and plant phenological development attributes (total biomass yield, seed yield, dry matter crude protein content, head count, average number of spikelets/head and average number of florets/head) 3 yr post-treatment could be adequately modeled using baseline (pre-treatment) soil properties measured 3 yr prior. The highest model percentage of variation explained (PV) (79% to 82%) was observed for the average number of spikelets/head, average number of florets/head and head count. Model PVs for

all three health indicators tested were greater than 72%. Olsen P, Mg, Ca, CEC and organic carbon (OC) content were the most important baseline soil properties for predicting soil health indicators while baseline CEC was the most common predictor in models for phenological development indicators except seed yield. These findings indicate the adequacy of PLS analysis to quantitatively model short-term soil health indicator dynamics and some phenological development attributes based on baseline soil properties.

Keywords: forage system, soil health, permanganate-oxidizable carbon, bioavailable nitrogen, soil respiration, partial least squares analysis

3.2 Introduction

Soil health depends on the proper balance among soil physical, chemical, and biological properties and controls the functioning of soil as a vital living ecosystem amidst agricultural intervention (Doran and Zeiss, 2000, Kibblewhite et al., 2008). Therefore, when introducing a novel plant species into a diverse agricultural cropping system, a major consideration is to optimize crop yield and quality while maintaining a sustainable soil-plant ecosystem. Intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey), a perennial, dual-purpose forage with favorable characteristics that allow it to withstand cold conditions (Hibbard et al., 2021; Park et al.,1994), can potentially be used for late fall/winter grazing of beef cattle in the Canadian prairies. While research has demonstrated the beneficial effects of IWG on many ecosystem services (Culman et al., 2013; Sprunger et al., 2018; Jungers et al., 2019), its impacts on key soil health attributes are currently poorly understood.

Soil biological properties as health indicators are important to evaluate microbially-available soil carbon (C), which is an energy source for the microbial population, the biological activity of the soil, and readily available nitrogen (N) for microbial and plant uptake (Delgado and Gómez, 2016; Chu et al.,2019). Soil organic carbon (SOC) normally takes long timeframes to change in significant or detectable amounts, whereas changes in the labile SOC pool can be detectable over much shorter timeframes (Zou et al., 2005). Microbial and fungal communities use labile C as their energy source, releasing CO_2 into the atmosphere. Therefore, changes in soil labile carbon over time reflect changes in microbial biomass C (de Graaff et al., 2010; Schmidt et al, 2011).

Intermediate wheatgrass has a rapidly developing root system and a large root biomass which ensure a high potential to sequester C into deeper soil layers and to increase the turnover time of labile C pools by providing a large habitat for microbial communities (Bajgain et al., 2020, Duchene et al, 2020). Autoclaved citrate-extractable (ACE) protein is a useful component of potentially available organic N (bioavailable N) in soil and therefore an important indicator of soil health (Hurisso et al., 2018). However, attempts to evaluate the correlation between potentially mineralizable N and ACE protein have largely been hampered by a lack of precise estimations for potentially mineralizable N in soil (Vigil et al., 2002) and the possibility of humic substances influencing the measurements (Geisseler et al., 2019).

Some soil health indicators are more sensitive to management than chemical properties. A 20-yr study examining changes in soil properties and yield of corn under zero till and conventional tillage in New York showed that organic matter (OM), POXC, respiration, and protein content under zero till were more beneficial for growth and development of the crop (Nunes et al., 2018).

Phenological development attributes are important as indicators of plant productivity. However, the occurrence and timing of these physiological development phases may vary with environmental and crop factors (Wang and Engel., 1998; Ruml and Vulić., 2005). Duchene et al. (2021) developed a phenology model for IWG and identified changes in agronomic productivity of the crop depending on the timing and duration of different phenological development stages. Further, they suggested the implementation of phenology models developed by integrating different plant and soil factors to maintain favorable cropping system management.

Various statistical techniques have been employed to model soil and crop productivity indices using baseline soil properties. For example, Redulla et al. (2002) and Iqbal et al. (2005) utilized stepwise multiple linear regression and correlation analysis to determine the relationship between crop yield or quality and soil properties and landscape. Many other studies have employed ordinary least squares (OLS) regression, principal component analysis (PCA), canonical correlation analysis, and partial least squares (PLS) analysis to examine the relationships between crop growth, yield, nutrient concentration, and ecosystem management practices vs. baseline soil properties (Ping et al., 2004; McDonald, 2006; Anthony et al., 2012; Agomoh et al., 2018). Partial least squares analysis has recently been employed to explore the relationship between spring wheat biomass yield and baseline soil properties (Zvomuya et al., 2008) and the yield of irrigated barley vs. baseline soil properties under semiarid conditions (Agomoh et al., 2018). Therefore, PLS has the potential to adequately model soil health and productivity using baseline soil variables.

The objectives of this study were to (1) evaluate the impact of different IWG-based perennial forage treatments and a single-purpose perennial forage system on selected soil health properties and (2) employ PLS analysis to model short-term (within 3 yr) changes in soil health indicators and phenological development attributes using baseline soil properties.

3.3 Materials and Methods

3.3.1 Study sites

A detailed description of the study sites including plot sizes and soil types has been included in Chapter 2 of this thesis. Plots were established at the Glenlea Research Station (49.6491° N 97.1189° W) (1.1–1.2 ha) (seeded May 2020), Agriculture and Agri-Food Canada, Brandon Research and Development Centre (AAFC, BRDC) (49.8694 °N, 99.9791 °W) (10 m × 5.5 m) (May 2019), the Ian N. Morrison Research Farm, Carman (49.5016° N, 98.0285° W) (6.6 m × 11 m) (July 2019), and the Livestock and Forage Centre of Excellence, Clavet, Saskatchewan (51.9349° N, 106.3791° W) (7 m × 7 m) (May 2019). The soil is a Gleyed Rego Black Chernozemic clay (Gleyed Humic Vertisol) at the Glenlea site, a Newdale clay loam Orthic Black Chernozem (Typic Haplocryoll) at the Brandon site, a Hibsin Orthic Black Chernozem (Udic Boroll) with a sandy loam texture at the Carman site, and a well-drained, loamy to fine sandy Dark Brown Chernozem at the Clavet site. All sites were previously under annual crops, except the Clavet site, which was summer-fallowed in 2018.

3.3.2 Experimental setup

The experimental layout has previously been described in Chapter 2. Briefly, the experimental design was a randomized complete block with a one-way treatment structure. The treatments were (1) a single-purpose perennial grass and legume mix (Control); (2) dual-purpose IWG in a pure stand (IWGP); (3) dual-purpose IWG in a pure stand plus synthetic fertilizer (50 kg N ha^{-1} post grain harvest) (IWGF); and (4) dual-purpose IWG in a mixed stand (50:50) with a legume (Alsike clover; *Trifolium hybridum*) (IWGL).

Treatments were established after baseline soil sampling in 2019. Urea fertilizer was applied to control plots in the spring of 2020 based on soil test results and to the IWGF treatment after grain harvest in August 2020 and/or August 2021, depending on year of establishment, to enhance fall regrowth.

3.3.3 Soil sampling

Soil samples for determination of soil health attributes were collected from the 0-15 cm soil layer in 2019 and 2021. Three subsamples were collected from each plot at the Brandon, Carman, and Clavet sites. The subsamples were composited and placed in a labeled poly bag. At the Glenlea pasture site, three samples were collected from each plot and placed in three separate poly bags. All samples were stored at 4℃ until processing and analysis.

Soil samples for measurement of other baseline soil properties were collected using the procedure described above from the 0-15 and 15-60 cm soil layers prior to treatment establishment in 2019. Three subsamples from each depth interval were composited separately for each layer, placed in a labeled poly bag, and stored at 4℃ until processing and analysis.

3.3.4 Soil health indicators

3.3.4.1 Permanganate-oxidizable organic carbon

Permanganate-oxidizable carbon (POXC) provides an indirect measure of microbial biomass. Soil core samples were analyzed at the beginning (or seeding stage) and at the end of the experiment (fall 2021) for determination of POXC using a modification (Blair et al., 1995) of the method developed by Weil et al. (2003). Deionized water (18 mL) and 2 mL of 0.02 M $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) were added to 2.5 g of soil in a sterilized 50 mL centrifuge tube, followed by centrifugation for 2 min at 120 rpm. After settling for 10 min in the dark at room temperature, 0.5

mL of the supernatant was mixed with 49.5 mL of deionized water. Absorbance was measured within 24 h of extraction at a wavelength of 550 nm using a Bausch and Lomb 2500 spectrophotometer.

3.3.4.2 Soil respiration

Forty–gram subsamples of dried, sieved soil were wetted to 50% of water-filled pore space in 50 mL beakers. The wetted samples were transferred to 30 mL jars and the lids of the jars were placed along with the Solvita gel paddle. The samples were placed in an incubator set at 25 °C for 24 h. Accumulated CO_2 in the gel pad was measured using a Solvita digital reader (Haney et al, 2008).

3.3.4.3 Potentially-available organic nitrogen

Autoclaved citrate-extractable (ACE) protein was determined following extraction of 1 g air-dry soil with 8 mL of 0.02 M sodium citrate (pH 7.0), followed by autoclaving for 30 min at 121℃ and 103.4 kPa pressure (Hurisso et al, 2018). After cooling, the samples were centrifuged for 15 min at 16,652 rpm. The supernatants were decanted and stored at 4°C until further processing and analysis using a Packard SpectraCount colorimetric microplate reader (Packard Instrument Co.) at 590 nm. Protein concentration was determined from a standard curve according to the procedure described by Hurisso et al. (2018).

3.3.5 Statistical analysis

3.3.5.1 Analysis of variance

Analysis of variance (ANOVA) of soil health (24-h CO_2 respiration, POXC, and ACE protein N) data from 2021 was performed separately for the large plot experiment and the three small plot sites using PROC GLIMMIX in SAS OnDemand for Academics (SAS Institute Inc., 2014) with treatment (forage system) as the fixed factor and site and block nested within site (block(site)) as random factors. The baseline soil health indicator measurements taken prior to treatment establishment in 2019 were modeled as covariates in the models for the data collected in 2021. All data were normally distributed based on the Shapiro-Wilk's W statistic ($W > 0.9$) tested using the UNIVARIATE procedure of SAS.

3.3.5.2 Partial least squares analysis

Partial least squares (PLS) regression analysis was performed using PROC PLS in SAS OnDemand for Academics to identify baseline soil properties (predictor variables) that best explained the variability in the three soil health indicators (24-h CO_2 respiration, POXC, bioavailable N) and in selected phenological development attributes (heading, flowering, total biomass yield, seed yield, protein content, dry matter crude protein content, head count, average number of spikelets per head, and average number of florets per head) measured in the final year of the study (2021). Soil chemical properties measured in 2019 (Table 3.4) were included as explanatory variables in the model. All data were normally distributed, according to the Shapiro Wilk's W test from PROC UNIVARIATE in SAS.

The contribution of each explanatory variable to the PLS model was assessed using the variable importance in the projection (VIP). Predictor variables with VIP > 0.8 were deemed to have a significant contribution to the variation in the response variables (Wold, 1995). The number of extracted factors were determined using the split cross-validation method (CV = SPLIT) based on the minimum predictive residual sum of squares (PRESS) (Zvomuya et al., 2006). The CVTEST option in PROC PLS was used to compare the model with fewer factors with a model with the optimum or recommended number of factors. When there was no significant difference between the two models, the model with fewer factors (latent variables) was selected as the final model (Zvomuya et al., 2008). Separate models were developed for each response variable.

3.4 Results and Discussion

3.4.1 Weather

Monthly and seasonal precipitation and temperature data for the four study sites are presented in Chapter 2 (Fig. 2.1 and Table 2.1). Mean annual precipitation values at all study sites in 2020 and 2021 were lower than the 30-yr (1981-2010) averages for the sites.

3.4.2 Baseline soil properties

Table 3.1 Selected baseline soil properties based on soil tests conducted prior to treatment establishment in 2019.

Soil Property[a]	Glenlea	Carman	Brandon	Clavet
NO_3^--N (0-15 cm, mg kg^{-1})	25±9.86[b]	14±5.13	25±6.00	43±5.07
NO_3^--N (15-60 cm, mg kg^{-1})	31±14.2	39±14.4	74±20.8	107±27.5
Olsen P (0-15 cm, mg kg^{-1})	48±11.3	19±8.82	46±6.01	9±2.85
K (0-15 cm, mg kg^{-1})	551±107	279±45.5	424±53.0	527±49.0
Ca (0-15 cm, mg kg^{-1})	4042±196	2527±248	3455±497	2015±232
Mg (0-15 cm, mg kg^{-1})	1537±116	644±151	457±48.5	447±65.2
SO_4^{2-}-S (0-15 cm, mg kg^{-1})	12±7.50	6±2.09	13±2.43	45±19.1
SO_4^{2-}-S (15-60 cm, mg kg^{-1})	49±38.4	26±32.1	28±6.34	103±62.7
Na (0-15 cm, mg kg^{-1})	63±17.1	24±5.35	10±1.41	38±9.60
SOC (0-15 cm, g kg^{-1})	60±9	26±2.9	46±5.8	27±4
CCE (0-15 cm, g kg^{-1}	0.55±0.19	0.47±0.13	0.96±0.44	0.14±0.14
CEC (0-15 cm, cmol$_c$ kg^{-1})	35±1.58	19±1.88	22±2.66	15±1.58
pH (0-15 cm)	7.2±0.16	6.5±0.46	7.4±0.14	6.2±0.38
pH (15-60 cm)	7.6±0.43	7.6±0.54	8.0±0.18	7.1±0.39
EC (0-15 cm, mS cm^{-1})	0.53±0.08	0.20±0.05	0.30±0.03	0.61±0.61
EC (15-60 cm, mS cm^{-1})	0.68±0.41	0.31±0.08	0.25±0.04	1.39±1.39

24-hr CO_2 respiration (0-15 cm, mg kg^{-1})	269±46.3	153±36.3	176±18.3	157±49.2
POXC (0-15 cm, mg kg^{-1})	1009±86.4	680±36.1	737±73.6	616±68.4
ACE protein N (0-15 cm, mg g^{-1})	7.6±1.44	6.7±0.98	6.9±0.84	6.6±0.73

[a] SOC, organic carbon; CCE, $CaCO_3$ equivalent; CEC, cation exchange capacity; EC, electrical conductivity; POXC, permanganate-oxidizable carbon; ACE protein N, autoclaved citrate extractable protein N

[b] Mean ± standard deviation (n = 16)

Baseline soil NO_3^--N and Olsen P concentrations across the four sites ranged from medium to very high (Manitoba Soil Fertility Guide, 2007)). Initial K concentration was very high at all sites. This is typical of western Canadian prairie soils, as K is abundant in surface layers of medium and fine textured mineral soils (Havlin et al., 2005). Sulphate S concentration at all sites ranged from high to very high. Soil pH mostly ranged within neutral limits and was lowest at the Clavet site (6.2 in the 0- to 15-cm layer). Electrical conductivity at the four sites ranged from 0.2 to 1.39 mS cm^{-1}, indicating non-saline conditions at the start of the study. Soil organic C ranged from 26 to 60 g kg^{-1} and was therefore above the level (20 g kg^{-1}) considered critical for agricultural soils (Loveland and Webb, 2003).

3.4.3 Soil health indicators

There was a general increase in 24-h CO_2 respiration and POXC in 2021 relative to baseline measurements taken in 2019 (Table 3.1), whereas changes in ACE protein N were minimal (Tables 3.2 and 3.3). However, there were no significant differences in these soil health indicators among treatments.

Table 3.2 Forage system effects on soil health indicators at the Glenlea site in 2021.

Forage system[a]	24-hr CO_2 respiration	POXC	ACE protein N
	mg C kg soil^{-1} day^{-1}	mg kg^{-1}	mg g^{-1}
Control	363a[b]	1035a	6.72a
IWGP	367a	1047a	7.02a
IWGF	341a	1076a	7.43a
IWGL	341a	1104a	6.56a
		P value	
Treatment	0.46	0.65	0.78

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Table 3.3 Forage system effects on soil health indicators at Carman, Brandon, and Clavet in 2021.

Forage system[a]	24-hr CO_2 respiration	POXC	ACE protein N
	mg C kg soil^{-1} day^{-1}	mg kg^{-1}	mg g^{-1}
Control	197a[b]	742a	6.83a
IWGP	225a	765a	6.87a
IWGF	209a	739a	6.56a
IWGL	208a	753a	6.92a
		P value	
Treatment	0.15	0.74	0.72

[a] Control, single-purpose perennial crop; IWGP, dual-purpose IWG in a pure stand; IWGF, dual-purpose IWG with fertilizer post establishment; IWGL, dual-purpose IWG in a mixed stand with a legume.

[b] Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

The lack of significant treatment effects on the three soil health attributes is not surprising, considering the short duration of the study. Previous studies have demonstrated sensitivity to management of biological properties such as soil active C, potentially-mineralizable N, and organic matter under established experiments that had varying management histories and durations (e.g., Idowu et al., 2009). Culman et al. (2012) examined soil samples from 12 long-term studies under different tillage and cropping systems and reported that total soil organic C, particulate organic C, and microbial biomass organic C had a strong link with soil POXC and it was the most sensitive fraction to management practices and the growing environment. DuPont et al. (2010) observed changes in soil POXC and ACE protein in response to land use change from no-till perennial grassland to annual cropland. Unlike these previous studies, our study was only 3-yr in duration, which evidently was not long enough for the development of measurable treatment impacts.

3.4.4 Modelling soil health indicators and phenological development attributes

Table 3.4 Variable importance in the projection (VIP) and regression coefficients (*b*) for baseline soil variables measured in 2019 and used in models for predicting soil health indicators measured in 2021.

Variable[a]	24-hr CO_2 respiration		POXC		ACE protein N	
	VIP	b	VIP	b	VIP	b
NO_3^--N (0-15 cm, mg kg^{-1})	-	-	-	-	-	-
NO_3^--N (15-60 cm, mg kg^{-1})	-	-	-	-		
OlsenP (0-15 cm, mg kg^{-1})	0.86	0.12	1.15	0.2	1.26	0.30
Mg (0-15 cm, mg kg^{-1})	0.93	0.13	1.14	0.15	1.0	0.12
Ca (0-15 cm, mg kg^{-1})	0.99	0.14	0.86	0.02	0.85	-0.14
Na (0-15 cm, mg kg^{-1})	-	-	-	-	1.27	-0.47
K (0-15 cm, mg kg^{-1})	-	-	-	-	-	-
SO_4^{2-}-S (0-15 cm, mg kg^{-1})	-	-	0.91	-0.24	0.91	-0.06
SO_4^{2-}-S (15-60 cm, mg kg^{-1})	-	-	-	-	0.84	0.03
pH (0-15 cm)	-	-	0.96	0.12	1.10	0.16
pH (15-60 cm)	-	-	-	-	-	-

EC (0-15 cm, mS cm^{-1})	-	-	-	-	-	-
EC (15-60 cm, mS cm^{-1})	-	-	-	-	0.86	-0.04
CEC ($cmol_c$ kg^{-1})	1.06	0.15	1.08	0.09	0.81	-0.01
SOC (0-15 cm, g kg^{-1})	1.06	0.15	1.09	0.13	0.99	0.22
CCE (0-15 cm, g kg^{-1})	-	-	-	-	0.95	-0.08
24-hr CO_2 respiration (0-15 cm, mg kg^{-1})	1.04	0.14	0.87	0.01	-	-
POXC (0-15 cm, mg kg^{-1})	1.06	0.15	1.03	0.1	-	-
ACE protein N (0-15 cm, mg g^{-1})	-	-	-	-	0.86	0.24
Latent variables[b]	1		2		4	
Predictor variables[c]	7		9		12	
Overall model PV (%)[d]	76		88		72	
R^2 [e]	0.79		0.81		0.83	

[a] SOC, soil organic carbon; CCE, $CaCO_3$ equivalent; CEC, cation exchange capacity; EC, electrical conductivity; POXC, permanganate-oxidizable carbon; ACE protein N, autoclaved citrate extractable protein N

[b] Number of partial least square factors determined by split cross validation

[c] Number of explanatory variables in the final model as determined by split cross validation

[d] Percentage of variation explained by the latent variables in the cross-validation step

[e] Predictive strength assessed by linear regression of measured values of response variables versus predicted values obtained in the cross-validation step using predictors with VIP > 0.8.

A one latent variable PLS model consisting of 7 (CEC, OM, concentrations of Ca, Mg, Olsen P, POXC, and CO_2 respiration) of the 23 explanatory variables measured in 2019 accounted for 76% of the total variability in 24-hr CO_2 respiration (Table 3.4). All 7 explanatory variables were positively correlated with 24-hr CO_2. By contrast, a two-latent variable model containing all of the above explanatory variables plus pH (0-15 cm layer) and SO_4^{2-}-S (0-15 cm layer) explained 88% of the total variation in POXC. All variables except SO_4^{2-}-S in the 0-15 cm soil layer were positively correlated with POXC. A four-latent variable model containing 12 explanatory variables explained 73% of the total variation in ACE protein N. ACE protein N was positively correlated with 6 and negatively correlated with 6 of the explanatory variables.

As expected, soil health indicators at the end of the study in 2021 were positively correlated with baseline soil health indicators measured pre-treatment in 2019. Olsen P, Mg, Ca, CEC and OC were the most important baseline soil properties for predicting soil health indicators and Calcium concentration and CEC showed a negative correlation with bioavailable N. By contrast, initial CEC was positively correlated with POXC and soil respiration. Similar to our results, Chen et al. (2014) reported a positive correlation between soil organic C (organic matter) and soil respiration. They attributed this correlation to the microbial use of readily available soil organic C as an energy source. Similarly, Culman et al. (2012) reported a positive correlation between microbially-available organic C and POXC.

Table 3.5 Variable importance in the projection (VIP) and regression coefficients (b) for baseline soil variables measured in 2019 and used in models for predicting phenological development attributes measured in 2021 (Le Heiget, unpublished data, 2022).

Variable[a]	Total biomass		Head count		Seed yield		Crude protein		Spikelets/head		Florets/head	
	VIP	b	VIP	b	VIP	b	VIP	b	VIP	b	VIP	b
NO_3^--N (0-15 cm, mg kg^{-1})	-	-	1.16	0.26	0.92	-0.18	0.83	0.07	-	-	-	-
NO_3^--N (15-60 cm, mg kg^{-1})	1.17	-0.26	0.97	0.18	0.99	-0.23	-	-	0.90	-0.21	-	-
OlsenP (0-15 cm, mg kg^{-1})	-	-	1.13	-0.13	-	-	1.15	-0.1	-	-	-	-
Mg (0-15 cm, mg kg^{-1})	0.9	-0.03	1.13	-0.09	-	-	1.14	-0.1	-	-	0.94	-0.13
Ca (0-15 cm, mg kg^{-1})	1.04	0.16	-	-	1.06	0.19	-	-	1.23	0.21	1.17	0.24
Na (0-15 cm, mg kg^{-1})	-	-	-	-	1.01	0.18	1.08	0.09	1.25	0.25	1.16	0.27
K (0-15 cm, mg kg^{-1})	-	-	-	-	-	-	-	-	0.81	-0.06	0.86	0.03
SO_4^{2-}-S (0-15 cm, mg kg^{-1})	0.93	-0.2	-	-	-	-	0.80	0.07	-	-	-	-
SO_4^{2-}-S (15-60 cm, mg kg^{-1})	-	-	-	-	-	-	-	-	-	-	-	-
pH (0-15 cm)	-	-	1.07	-0.12	-	-	-	-	-	-	-	-
pH (15-60 cm)	-	-	0.90	-0.14	-	-	-	-	-	-	-	-
EC (0-15 cm, mS cm^{-1})	-	-	-	-	-	-	-	-	0.94	0.03	1.01	0.09
EC (15-60 cm, mS cm^{-1})	-	-	0.86	0.17	-	-	-	-	0.96	0.14	0.95	0.17
CEC (0-15 cm, $cmol_c$ kg^{-1})	0.99	0.07	0.98	-0.02	-	-	1.09	-0.09	1	0.09	1.01	0.07
SOC (0-15 cm, g kg^{-1})	-	-	0.95	-0.005	-	-	-	-	0.83	-0.04	0.89	0.01
CCE (0-15 cm, g kg^{-1})	-	-	0.91	-0.14	0.80	-0.15	0.82	-0.07	-	-	-	-
24-hr CO_2 respiration (0-15 cm, mg kg^{-1})	-	-	-	-	-	-	-	-	0.96	0.10	0.93	0.09
POXC (0-15 cm, mg kg^{-1})	0.94	0.09	0.88	-0.04	-	-	-	-	0.99	0.09	1.03	0.12

ACE protein N (0-15 cm, mg g^{-1})	-	-	-	-	-	-	-	-	-	-	-	-
Latent variables[b]	2		2		1		1		2		2	
Predictor variables[c]	6		11		5		7		10		10	
Overall model PV (%)[d]	39		79		35		24		81		82	
R^{2} [e]	0.86		0.68		0.44		0.68		0.86		0.86	

[a] SOC, soil organic carbon; CCE, $CaCO_3$ equivalent; CEC, cation exchange capacity; EC, electrical conductivity; POXC, permanganate-oxidizable carbon; ACE protein N, autoclaved citrate extractable protein N

[b] Number of partial least square factors determined by split cross validation

[c] Number of explanatory variables determined by split cross validation

[d] Percentage of variation explained by the latent variables in the cross-validation step

[e] Predictive strength assessed by linear regression of measured values of response variables versus predicted values obtained in the cross-validation step using predictors with VIP > 0.8.

A two-latent variable PLS model consisting of 6 explanatory variables [NO_3^--N (15-60 cm soil layer), SO_4^{2-}-S (0-15 cm soil layer), CEC concentrations of Ca, Mg, and POXC measured in 2019] explained 39% of the total variability in total biomass yield. Similarly, a two-latent variable model containing all the above explanatory variables except Ca and SO_4^{2-} -S (0-15 cm layer) concentrations, plus NO_3^--N (0-15 cm layer), Olsen P, pH (0-15, 15-60 cm layer), EC (15-60 cm layer), CCE and OC explained 79% of the total variation in head count m^{-2}. By contrast, a one-latent variable model containing concentrations of NO_3^--N (0-15 and 15-60 cm soil layers), Ca, Na and CCE accounted for 35% of the total variability in seed yield. In parallel, a one-latent variable model developed for crude protein contained 7 predictor variables (NO_3^--N and SO_4^{2-}-S in 0-15 cm layer, concentrations of Olsen P, Mg, Na, CEC, and CCE), which accounted for 24% of the total variability in crude protein concentration. Both the average number of spikelets per head and the average number of florets per head were explained by 2-latent variable PLS models containing 10 predictor/explanatory variables each. The model for the average number of spikelets per head consisted with concentrations of NO_3^--N in sub soil layer, Ca, K and Na, EC in both soil layers, CEC, OC, POXC, and 24-hr CO_2 respiration, explaining 81% of the total variability in the response variable. The model for the average number of florets per head contained all the above explanatory variables, except NO_3^--N concentration in the 15-60 cm soil layer, plus Mg concentration, and explained 82% of the total variability in the response variable.

Total biomass, seed yields and number of spikelets/head were negatively correlated with NO_3^--N concentration, perhaps reflecting the fact that reduced plant growth from factors such as drought results in lower N uptake, hence higher residual soil NO_3^-. This negative correlation is consistent with the findings of Agomoh et al. (2018), who reported a negative correlation between soil NO_3^--N and barley yield. McDonald (2016) also recorded a similar negative correlation between soil

NO_3^--N concentration and yield components of wheat and barley at maturity. For unclear reasons, our results also showed a negative correlation between SO_4^{2-}-S concentration and total biomass yield.

Soil quality indicators, including total organic and inorganic C, total N, light fraction C and N, mineralizable C and N, and available N and P have been identified as significant predictor variables for modeling spring wheat biomass yield under a semi-arid environment, with all soil properties except inorganic C showing a positive correlation with the biomass yield (Zvomuya et al., 2008). Although most of these soil properties were not included in our study, the positive correlation between biomass yield vs. POXC seems to be consistent with the relationship between biomass yield and total organic C, light fraction, and mineralizable C in the aforementioned study.

Malhi et al. (2009) reported an increase in dry matter crude protein content with application of N fertilizer alone and a decrease with the combined application of N and S fertilizers. Consistent with their observation from N fertilizer application, topsoil (0-15 cm layer) NO_3^--N concentration in our study was positively correlated with dry matter crude protein content. However, topsoil SO_4^{2-}-S (0-15 cm layer) concentration also showed a positive correlation with crude protein content in the model. This may be explained by the fact that fertilizer N was not applied prior to baseline soil sample collection in 2019 (except for control plots at Carman) and, therefore, the influence of inherent soil properties on response variables might be different compared to that from external sources (fertilizer application).

Seed head count and crude protein content were negatively correlated with baseline concentrations of Olsen P and Mg, CCE, and CEC. Similarly, POXC, soil pH and OC were also negatively correlated with seed head count. The reasons for these negative correlations are not clear. All the explanatory variables in the models for spikelets/head and average number of florets/head were positively correlated with these response variables except K, NO_3^--N concentration in the 15-60 cm layer, and OC for average number of spikelets/head and only Mg for average number of florets/head.

Baseline CEC was the common explanatory variable among models for 5 out of 6 response variables whereas baseline ACE protein was not correlated with any of the crop variables.

3.5 Conclusion

This study evaluated the short-term evolution of selected soil health indicators under three different IWG-based perennial forage-grain systems and one single-purpose perennial forage system and explored some of the key soil baseline properties controlling soil health and soil functions under these cropping systems. Significant treatment differences were not observed for any of the soil health indicators tested, which is not surprising, considering the short duration of the study. Nonetheless, the similar 24-hr CO_2 respiration, POXC and bioavailable N concentrations for the fertilized forage treatment (IWGF) and the treatment incorporating a legume (IWGL) indicate that there is a potential for a legume intercrop as an alternative source of N for an IWG perennial forage-grain system. Additionally, the similar soil health indicator levels for IWG-based forage treatments vs. the single purpose grain-legume treatment (Control) is a favorable observation when introducing a newly developed dual purpose perennial forage grain species. Based on overall model PV, PLS analysis was effective in quantitatively modelling 24-hr CO_2 respiration, POXC and bioavailable N based on baseline soil properties, but was less effective in modelling crop phenological development indicators. Overall, baseline CEC (correlated with 8 out of 9 response variables tested) and concentrations of Ca, Mg, and OC were the most common predictors (correlated with 7 out of 9 response variables tested). These results indicate that soil health and productivity can be effectively modeled using initial soil properties for fields under the IWG forage-grain systems tested in this study.

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4. OVERALL SYNTHESIS

4.1 Relevant Findings and Implications of the Research

To maintain competitiveness in the beef cattle industry, cost reduction strategies such as reducing labour and feed costs while maintaining high quality feed are critical. Extending the grazing season by using stockpile regrowth for grazing of cattle (cows, replacement heifers or backgrounders) on pasture in late fall/winter is considered a potential solution, which can be attained via use of perennial forages which can retain relatively high nutrient quality into this period. Normally, single-purpose annual and perennial forage species are used by Canadian farmers for feeding cattle over the cool season. However, Intermediate Wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey) shows superior characteristics over currently used forages as it is cold-tolerant, does not show leaf loss following frost as observed for commonly used legumes such as alflalfa, and its regrowth following grain harvest has been reported to be high in crude protein and total digestible nutrients, which are essential for both growing cattle and mature cattle under cold conditions (LeHeiget at al., 2022). However, perennial plant species are still being improved through selection and breeding to improve their agronomic productivity, phenological attributes, phytomorphology and survival under western Canadian conditions (Cattani and Asselin, 2018). Additionally, the beef industry is facing ever growing concerns regarding its environmental sustainability. Therefore, evaluation of the impact of forage selection on not only animal and agronomic performance but also soil performance and health is critical.

After incorporating IWG into the diverse agricultural cropping system, it is crucial to evaluate the changes in soil quality indicators in each growing season under varying regional climatic and soil conditions. When evaluating the soil quality, the IWG cropping system should improve soil quality or at least maintain it at the initial levels**.** If soil health significantly suffers even though IWG gives qualitatively or/and quantitatively higher yields compared to other forage–grain crops, then its benefits will be compromised. Therefore, this 3-yr (2019 to 2021) study was conducted to evaluate the changes in soil quality indicators under forage systems incorporating IWG. The main objectives were to evaluate the short-term impacts of IWG-based perennial forage treatments and a single purpose forage control on soil chemical properties (Chapter 2) and soil health indicators (Chapter 3). The study also examined temporal changes in N supply rates for different perennial forage treatments (Chapter 2) and explored models for soil health and phenological development indicators using baseline soil properties (Chapter 3).

The soil chemical properties evaluated in Chapter 2 demonstrated the low sensitivity of soil chemical properties to management practices in a short-term study, with available N, pH and electrical conductivity (EC) being the only soil chemical properties which showed significant differences within the 3-yr period. However, the similar soil chemical property levels for the control vs. IWG-based forage treatments indicates that the IWG has potential to perform similarly to previously tested forage-legume systems (Dahmer, 2017) with respect to its impacts on soil properties and soil health. Nitrate-N concentration in the 0-15 and 0-60 cm layers at the small plot sites under the IWG plus legume treatment (IWGL) was significantly greater in 2020 than in 2021. Greater available N concentrations in 2020 are consistent with the medium to very high baseline soil N concentrations across the three sites (Manitoba Soil Fertility Guide, 2007). Meanwhile, soil NO_3^--N concentrations in the IWGL treatment were significantly higher than for IWG in pure stand (IWGP). In particular, the performance of the IWGL treatment was similar to IWG with urea added treatment (IWGF) in both years (2020, 2021) for both layers. At the Glenlea pasture site, NO_3^--N concentration in 2020 in the 15-60 cm layer was significantly greater under IWGL treatment than IWGP and control treatments. These findings suggest the ability of a legume to provide N to an

IWG perennial forage-grain in lieu of fertilizer N. Additionally, these results from the alsike clover intercrop demonstrate the importance of intercrop species selection for the successful performance of a cropping system.

Nitrate-N supply rates measured during the 2020 and 2021 growing seasons showed a declining trend with time. The cumulative supply rate showed a rapid supply initially, followed by a more gradual increase and, finally, a plateau later in the growing season. Supply rates showed the strong influence of rainfall events, with large increases in upward trends typically following significant precipitation. Another crucial finding was the similar NO_3^--N supply rates between IWGF and IWGL treatments after the application of urea to the IWGF treatment. This finding indicates that a legume intercrop is an effective substitute for N fertilizer in an IWG perennial forage-grain system. Moreover, this implies improvement of soil fertility with a legume intercrop in a short period of time. Therefore, our results may assist researchers to improve the sustainability of an IWG perennial forage-grain system through forage-grain/legume (cereal-legume) intercropping.

Chapter 3 examined the effect of different IWG-based perennial forage treatments and a single-purpose forage control on 24-h CO_2 respiration, permanganate-oxidizable carbon (POXC), and bioavailable (autoclaved citrate-extractable (ACE) protein) nitrogen (N) tested in 2021. Significant treatment differences were not detected for any of the soil health indicators tested, reflecting the short duration of the study, and suggesting the need for a longer duration study to examine soil health dynamics under these forage systems.

Chapter 3 also explored the key soil baseline properties (chemical and health properties) controlling the evolution of soil health indicators and plant phenological development attributes in the final year of the study. Among soil health indicators tested the highest model percentage of variation explained (PV) was observed for POXC (87%) followed by 24-hr CO_2 respiration (74%)

and bioavailable (ACE protein) N (65%) while for phenological development indicators the average number of spikelets/head showed the highest PV (84%) followed by the average number of florets/head (81%) and head count/m^2 (77%). When predicting soil health indicators, Olsen P, Mg, Ca, CEC, and organic matter were the common predictor variables among all 3 models whereas for phenological development indicators POXC was the key predictor variable (except for seed yield). Results from this study indicate the effectiveness of using baseline soil properties to predict the selected soil health indicators but call for further evaluation for the phenological development indicators tested. The PLS models developed for soil health indicators can be implemented by industry to predict short-term changes in soil health and plant productivity using baseline soil properties without the need for annual measurements. The models developed for phenological development parameters will provide insights into how initial soil properties can influence phenological development in IWG-based perennial forage systems.

4.2 Recommendations

Our study provides information on the short-term dynamics of soil chemical properties and soil health attributes (Chapters 2 and 3) under the IWG perennial forage-grain system while presenting an effective approach for modelling soil health indicators and phenological development attributes using baseline soil properties (Chapter 3). Findings from the study indicate that even though soil chemical properties tested were slowly changing with time, soil NO_3^--N supply rate measured using PRS probes was sensitive to management practice (fertilizer application) and the surrounding environment (rainfall), with significant treatment effects detected within the 3-yr study.

Previous studies have indicated a strong correlation between plant uptake and nutrient supply rate (Qian and Schoenau, 2005; Nyiraneza et al., 2009; Sharifi et al., 2009). Therefore, future studies on IWG-based perennial forage systems should measure N uptake by IWG to check such a correlation. We also recommend that future studies should examine C:N ratio to evaluate N immobilization during the growing season and the effect of N immobilization on the declining trend of NO_3^--N supply rates in the course of the growing season.

Since there are no plant roots to absorb moisture inside root exclusion cylinders (REC), moisture content is typically higher inside RECs compared to outside, which may enhance N leaching and denitrification compared to outside the REC (E. Bremer, personal communication, 2022). Therefore, measuring soil moisture and soil temperature inside and outside the RECs during each installation/retrieval day would provide more comprehensive insights into changes in supply rates during the growing season. This is particularly important during dry growing seasons such as 2020 and 2021: May to October total precipitation amounts during the 2020 and 2021 growing seasons were 242.5 and 255.7 mm, respectively, compared to the 30-yr (1981 to 2010) average of 417.4 mm. (Environment and Climate Change Canada, 2022). Cattle grazing at the Glenlea site occurred from October to November 2021. However, the soil samples were collected before cattle grazing started and therefore the effect of cattle grazing in the field was not captured by the soil sample analysis in 2021. To take this effect into consideration, samples should have been collected in fall 2022 and evaluated for the same soil chemical and health properties tested in the last 2 years. However, significant winter snowfall and catastrophic flooding in the spring of 2022 meant that all pastures were lost; thus, treatment effects could no longer be definitively delineated.

As a perennial, IWG has a long rooting depth. Therefore, soil sampling into deeper layers (e.g., 100 cm) would provide greater insight into NO_3^--N distribution in the soil profile.

This soil quality evaluation was conducted for only 2 yr at the Glenlea large pasture plot site and 3 yr at the small plot sites. If the study was continued for a few more years, it could have been possible to detect forage system effects on the selected soil health parameters. Other than extending the study duration, other sensitive indicators such as microbial biomass and species diversity, particulate organic matter (POM), changes of which could be observed within short study periods (Cotrufo et al., 2019) should be included in future studies. This is the first study to explore the use of PLS analysis to model soil health and plant development (Chapter 3) using baseline soil properties. Future studies should consider including other soil variables such as bulk density, porosity, and microbial measurements.

4.3 References

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APPENDICES

Appendix A: Supplementary figures

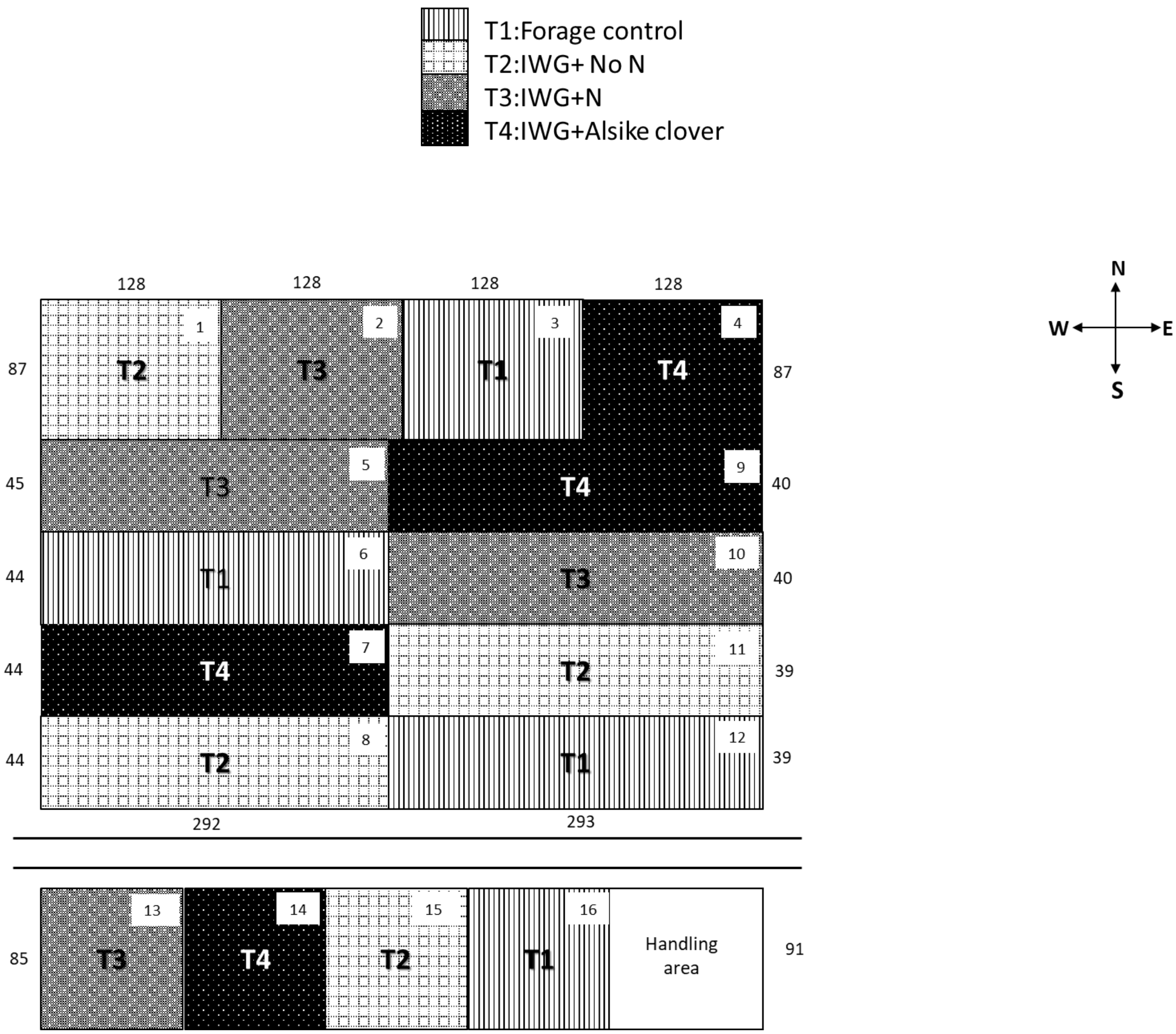

Figure S.1 Plot layout at Glenlea Research Station, University of Manitoba.

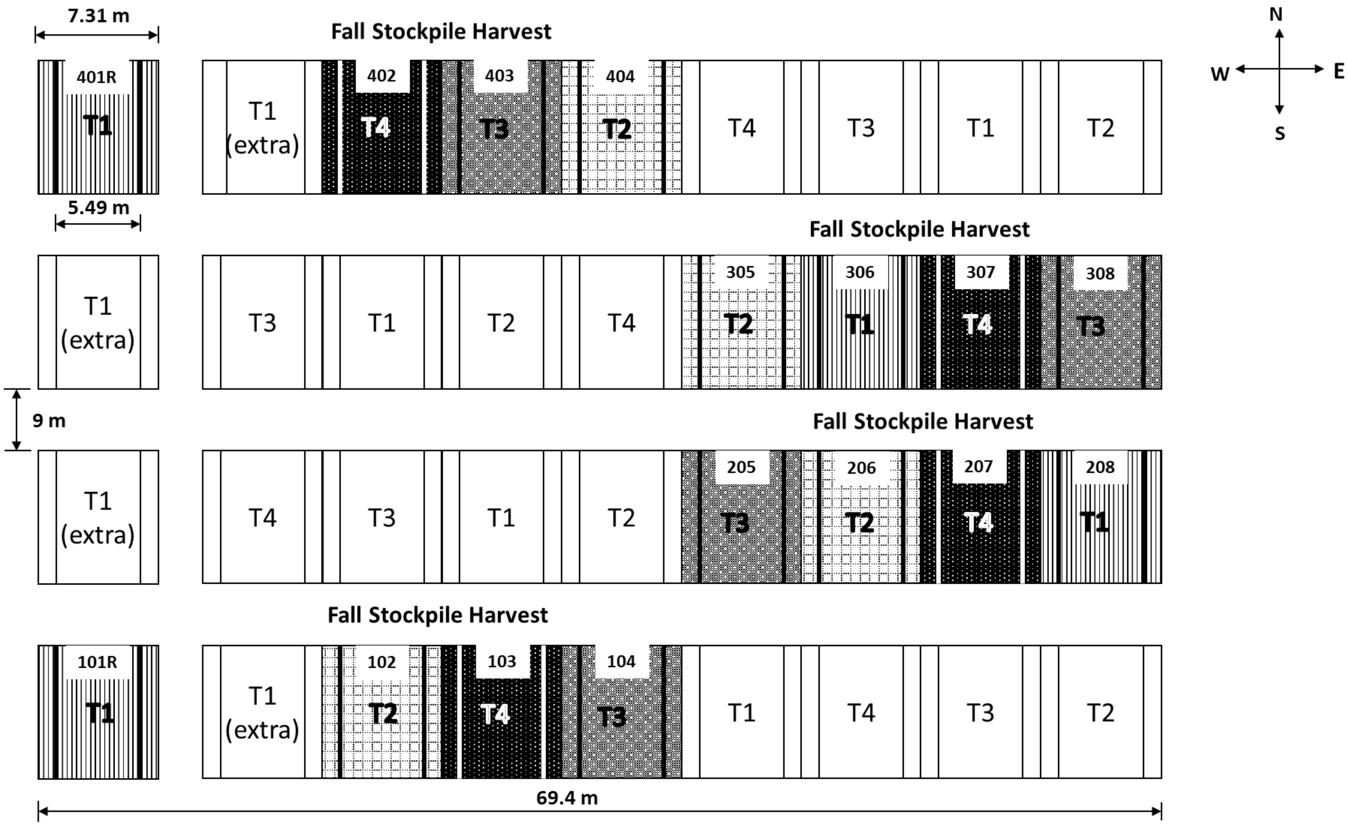

Figure S.2 Plot layout at Agriculture and Agrifood Canada, Brandon Research and Development Station, Manitoba.

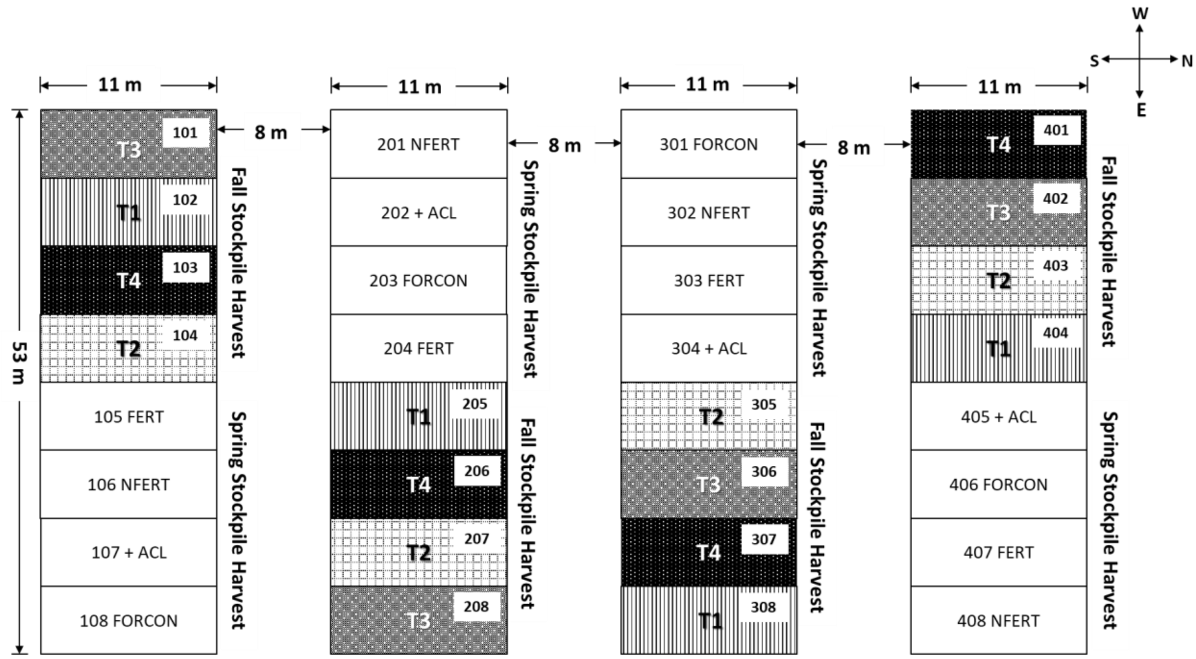

Figure S.3 Plot layout at Ian N. Morrison Research Farm, Carman, Manitoba.

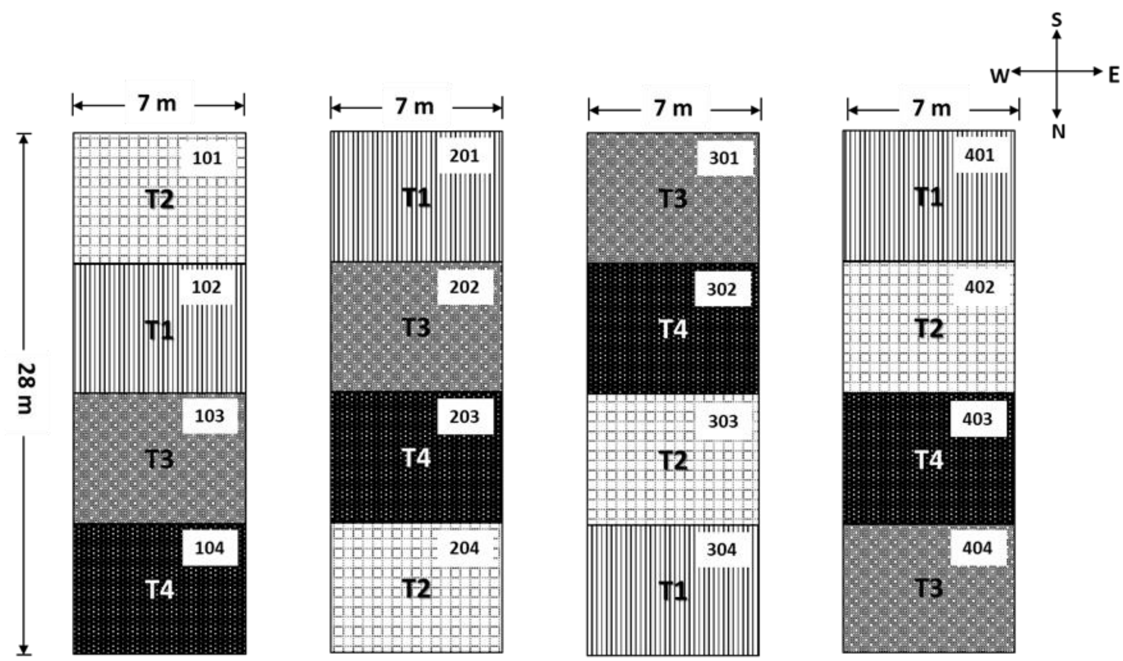

Figure S.4 Plot layout at Livestock and Forage Centre of Excellence, Clavet, Saskatchewan

Appendix B: Supplementary tables

Table S.1 Monthly, seasonal (May-October) and annual precipitation and air temperature at the study sites.

Site	Period	Total precipitation (mm)				Average temperature (°C)			
		2019	2020	2021	30 yr average	2019	2020	2021	30 yr average
Glenlea	May	44.2	12.6	27.7	61.5	9.5	10.7	10.8	12.2
	June	39.9	60.4	35.7	99.7	17.5	18.8	19.1	17.0
	July	116.7	74.8	15.5	91.7	19.7	20.7	21.2	19.4
	August	61.0	63.3	102.6	72.4	17.7	18.9	18.7	18.8
	Sep	170.0	11.8	18.1	49.0	13.8	11.8	16.1	12.5
	Oct	47.0	19.6	56.1	43.1	3.3	2.0	9.1	4.9
	May-Oct	478.8	242.5	255.7	417.4	13.6	13.8	15.8	14.1
	Annual	545.6	327.1	361.1	542.7	1.8	3.7	4.7	2.8
Carman	May	36.9	26.4	27.2	69.6	9.6	10.7	10.7	11.6
	June	37.9	70.7	102.7	96.4	17.3	18.2	19.3	17.2
	July	57.4	54.0	16.7	78.6	19.5	20.2	21.2	19.4
	August	61.6	24.3	78.0	74.8	18.1	18.7	18.1	18.5
	Sep	150.9	10.8	16.5	49.0	12.6	12.3	15.7	13.4
	Oct	53.1	16.1	79.6	43.4	2.9	2.2	8.3	5.4
	May-Oct	397.8	202.3	320.7	411.8	13.3	13.7	15.6	14.3
	Annual	473.0	266.8	379.9	545.0	1.9	3.7	4.7	3.5
Brandon	May	40.1	8.2	25.8	56.5	9.3	10.4	9.9	11.4
	June	73.8	205.5	101.2	79.6	16.5	17.7	18.8	16.6
	July	40.8	54.0	0.2	68.2	19.3	20.2	20.5	19.2
	August	74.9	57.1	156.8	65.5	16.8	18.9	17.5	18.2
	Sep	176.2	11.2	21.6	41.9	12.4	11.6	15.1	12.2
	Oct		8.6	22.6	29.3	2.2	1.2	7.7	4.6
	May-Oct	405.8	344.6	328.2	341.0	12.8	13.3	14.9	13.7
	Annual	469.6	401.4	382.2	461.7	1.4	3.1	3.7	2.7
Clavet	May	4.4	42.1	35.5	36.5	9.7	11.1	10.1	11.8
	June	84.8	106.9	41.7	63.6	16.0	15.3	18.0	16.1
	July	67.6	52.1	17.7	53.8	17.8	19.0	21.4	19.0
	August	20.3	16.2	38.4	44.4	15.4	18.0	17.8	18.2
	Sep	39.5	23.6	5.6	38.1	12.3	11.7	13.7	12.0
	Oct	11.2	3.5	6.7	18.8	0.8	1.2	5.5	4.4
	May-Oct	227.8	244.4	145.6	255.2	12.0	12.7	14.4	13.6
	Annual	266.4	297.4	180.7	340.4	1.2	2.0	3.1	3.3

Monthly and annual weather data for Glenlea, Carman, Brandon and Clavet study sites were recorded at KLeefeld (MAFRI), MB, 21 km from the study site, Carman U of M, MB, 0.4 km from the study site, Brandon RCS, MB, 4 km from the study site and Saskatoon RCS, SK, 35 km from the study site, respectively.

30 yr average (1981-2010) weather data were recorded at Glenlea, MB, 0.2 km from the study site, Carman, MB, 11.6 km from the study site, Brandon CDA, MB, 0.4 km from the study site and Saskatoon SRC, SK, 28 km from the study site, respectively.