IMPACTS OF SHORT-TERM COVER CROPPING ON SOIL MICROBIAL COMMUNITIES AND BIOGEOCHEMICAL FUNCTIONS IN PRAIRIE CANADA

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ABSTRACT

Cover crops have the potential to confer numerous benefits to agricultural soils. Many ecosystem services derived from cover crops are underpinned by activities of soil microorganisms, while the cover crop acts as a catalyst. While the biological impacts of cover crops are relatively well understood in temperate agroecosystems, research in semi-arid environments is limited. My research addressed this knowledge gap by focusing on the impacts of cover cropping on biological indicators of soil health in semi-arid agroecosystems. I analyzed phospholipid fatty acid (PLFA) abundance and composition, and extracellular enzyme activity (EEA) in soils at three locations in the Canadian prairies: Saskatoon, Saskatchewan; and Carman and Glenlea, Manitoba. The study had eleven treatments at each site, comprising four-year rotations with and without cover crops, two-year rotations without cover crops, and a perennial alfalfa check, arranged in a randomized complete block design with four replicates per site. Cover crops were first grown in Saskatoon and Carman in 2018, and in 2019 in Glenlea. Surface soils were sampled in fall 2020, spring 2021, and summer 2021. I hypothesized that cover cropping would support a more active, abundant soil microbial community and impose changes in microbial community composition, leading to improved soil health compared to non-cover cropped treatments. The perennial alfalfa had higher fungal PLFA abundance and lower stress indicators compared to rotation treatments. Sampling time affected total PLFA abundance ($p < 0.05$) at all locations, and EEA measurements at nearly all sampling times and locations. However, specific impacts of seasonality differed between sites. The inclusion of cover crops did not affect PLFA abundance, microbial community composition, nor EEA activity. These findings suggest that biological indicators of soil health in the short-term are more impacted by factors aside from cover cropping, such as soil properties or climatic differences, and do not support the use of cover crops to enhance biological soil health in the short-

term. These results may be partly due to the limited time cover crops had to establish sufficient biomass to induce effects on soil microbial communities. Longer-term studies may use these findings as a benchmark and should track changes attributable to cover cropping over a longer timeframe.

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LIST OF ABREVIATIONS

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
AP	Alkaline phosphatase
βG	β-glucosidase enzyme; degrades cellulose
EEA	Extracellular enzyme assays
F:B	Fungal to bacterial phospholipid fatty acid ratio
GC	Gas chromatography
G-	Gram-negative bacteria
G+	Gram-positive bacteria
MB	Microbial biomass
NAG	N-acetyl-β-D-glucosaminidase enzyme; degrades chitin
NMDS	Non-metric multidimensional scaling
PLFA	Phospholipid fatty acid [analysis]
SOC	Soil organic carbon
SOM	Soil organic matter

1.0 INTRODUCTION

Cover cropping is rapidly gaining traction as one of the management strategies that producers and researchers are examining to improve crop productivity while balancing environmental health. Cover crops are plants that are grown to cover the soil without the intention of being harvested for a profit. They can be grown alongside a cash crop or, more commonly, grown at alternate timing to cash crops, often referred to as shoulder-season cover crops. Because a large portion of cover crops cannot be sold, farmers are evaluating the long-term return-on-investments of implementing cover crops. In annual cropping systems, cover cropping extends the amount of time that living plants interact with the soil to confer ecosystem services. Living roots in the soil provide microbes with energy via the production of root exudates. In turn, soil microbes retain and cycle nutrients and carbon (C) and contribute to overall improved soil health and fertility (Tiemann et al., 2015; Finney et al., 2017; Krupek et al., 2022). Cover crops provide agronomic benefits including reduced soil erosion and water runoff, increased levels of soil organic matter (SOM) (Krupek et al., 2022), weed and disease suppression, fixation of atmospheric nitrogen in the case of legume cover crops, alleviation of compaction (Hartwig & Ammon, 2002), and overall improved soil health (Rankoth et al., 2019). Cover crops also provide environmental benefits including the potential to mitigate climate change by reducing fertilizer use, increasing nutrient retention (Kaye & Quemada, 2017; Bourgeois et al., 2022), and increasing soil organic carbon (SOC) sequestration (Abdalla et al., 2019).

Producers grow cover crops for a variety of reasons. In a recent survey of prairie producers, the most common rationales for growing cover crops, in order of popularity, were to build soil health, to keep living roots in the ground for longer, and to feed soil biology (Morrison, 2021). Thus, the ultimate goal of cover cropping for most producers is to improve the health of the soil

and they recognize the vital role that soil biota play. Indeed, soil microorganisms underpin most of these valuable agronomic and environmental ecosystem services that cover crops provide (Ritz et al., 2007; Ferris & Tuomisto, 2015). Cover crops affect soil microbial communities in two main ways: 1) extending the time with living roots in the soil as well as the amount of crop residue biomass input within a single growing season, which increases the quantity of energy and nutrients available for soil microbes (Finney et al., 2017); and 2) increasing plant diversity over time, which increases the quality and diversity of available energy (McDaniel et al., 2014).

In regions where the growing seasons are longer and more temperate than the semi-arid Canadian prairies, plenty of evidence supports the use of cover crops and their role in improving soil microbial diversity and function (Tiemann et al., 2015; McDaniel et al., 2014). However, the Canadian prairie growing season is considerably shorter than most regions evaluated in existing studies. Growing cover crops in the prairies is risky because the short growing season allows very limited time for cover crops to establish prior to freeze-up, and because water is an extremely limiting factor in plant growth in such semi-arid environments. It is thus unclear whether cover crops in the short Canadian prairie shoulder-season (i.e.: the time between spring thaw and planting, and between harvest and freeze-up) have enough time to accumulate sufficient biomass and produce enough root exudates to promote nutrient provisioning and cycling, SOC sequestration, and to perform activities that support improvements in soil-derived ecosystem services, as observed in regions that have longer cover crop seasons. While cover crops are used more broadly in central and eastern Canada, the shorter growing season in the Canadian prairies has limited the implementation of cover crops in the west (Morrison, 2021).

My research contributes to a larger research effort led by Dr. Yvonne Lawley at the University of Manitoba aimed to determine whether cover crops are a viable option for prairie

producers. Specifically, the research project examines whether yields differ between cover-cropped and conventionally cropped systems and identify other positive and negative aspects (i.e., economic, environmental, agronomic) of implementing cover crops into production systems in prairie Canada. Experimental sites were established across the prairies and a variety of agronomic (e.g., yields, soil nutrients) and environmental (e.g., greenhouse gas emissions, soil carbon) data were collected to evaluate the effect of including cover crops into annual rotations.

My thesis research aims to determine how cover crops affect soil biological properties. The main objective of the research is to determine whether growing cover crops confers soil health benefits in the Canadian prairies over the short term, with a focus on soil microbial abundance and community structure by way of phospholipid fatty acid analysis, and biogeochemical cycling using extracellular enzyme activities. Other members of the research team focused on measuring soil nutrients and carbon, crop yields, and other agronomic data. As such, these data are not presented here, but will be referenced where theses or publications are available. It is hypothesized that the use of cover crops will increase the abundance and activity of soil microbial communities.

2.0 LITERATURE REVIEW

2.1 Cover crops and their purpose

Cover crops are plants that are grown to cover the soil without the intention of being harvested for a profit. Long-term benefits of cover cropping include improved agroecosystem resiliency and yield stability (Liebig et al., 2015; Alahmad et al., 2018). Additionally, cover crops provide numerous ecosystem services when consistently implemented in a system (Daryanto et al., 2018). These include: reduced erosion potential; increased SOM and soil moisture content; weed control; increased microbial biomass (Daryanto et al., 2018); improved nutrient cycling; increased soil carbon sequestration; and higher resiliency to stress (Ritz et al., 2009). In many cases, cover cropping improves the yield of the following cash crop (Daryanto et al., 2018).

The living components of soil provide many of these ecosystem services via biological processes (Ritz et al., 2009). Therefore, understanding how soil biota and their processes respond to cover crop implementation can inform how cover crops can confer benefits and, ultimately, help producers make important management decisions regarding cover crops. Soils with more diverse and abundant soil organism communities are better equipped to exploit soil resources and optimize ecosystem services compared to less populated soils (Ferris & Tuomisto, 2015) and are likely to exhibit superior soil health. Through careful cover crop management, producers have the potential to manipulate the soil microbiome of their operation to improve soil health (Finney et al., 2017). Producers can choose different cover crop varieties depending on their management goals. For example, different cover crops are recommended to be grown for different purposes: tap-rooted or bulbous-rooting cover crops are used to reduce compaction, whereas crimson clover is used for nitrogen fixation, and field rye is used to combat weeds (Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), 2021).

For this research, the purpose and impacts of cover cropping will be studied with a focus on their relationship to soil microbial communities and biological indicators of soil health.

Cover cropping can improve the resiliency of agroecosystems, especially in situations of stress, such as during drought periods (Ritz et al., 2009; Alahmad et al., 2018; Bowles et al., 2020). This idea of increasing agroecosystem resiliency is linked with the idea of increasing crop diversity in a given system, wherein greater diversity tends to result in yield benefits over time (Bowles et al., 2020). In many cases, where precipitation levels are higher, implementing cover crops in rotation is a way for producers to diversify their crop inputs without being forced to extend their cash crop rotations and grow less profitable cash crops too often. Evidence collected from eastern Canada and the USA suggests that increasing crop rotational diversity with the inclusion of cover crops can lead to increased system resiliency and cash crop yields, particularly in corn (Bowles et al., 2020; Chahal et al., 2021). These findings are consistent with information commonly provided to growers in central and eastern Canada, wherein cover crop implementation is encouraged, in part, as a form of insurance to stabilize yields and protect against yield losses in years of weather extremes (OMAFRA, 2021). However, these claims have less scientific backing in semi-arid growing environments such as in the Canadian prairies and require further investigation, especially when it comes to the impact on soil microorganisms.

2.2 Challenges with cover cropping in a semi-arid growing environment

While the benefits of growing a cover crop are well researched and tend to show positive impacts on soil health, getting that cover crop well-established in order to confer the associated benefits represents a vital challenge in semi-arid environments. The Canadian prairies are situated in a semi-arid environment, where water availability is the main limiting factor in crop production.

Extended periods of drought years can be devastating for prairie growers. The time between harvest and winter freeze-up, and between spring thaw and seeding time in the prairies (referred to as the shoulder season) is extremely limited. The short growing season, extremely variable weather conditions, and insufficient precipitation levels combined lead to a high level of risk when it comes to successfully growing late-seeded shoulder season cover crops (Liebig et al., 2015).

Late-seeded cover crops have minimal time for establishment in short, semi-arid growing seasons, and, as such, cover crop biomass appears to be significantly less than in more temperate environments (Otchere et al., 2022). There are also some concerns that cover crops in semi-arid systems may use water and nutrients that would be more productively and profitably used by the intended cash crop (Reese et al., 2014). In some cases, there appears to be no differences in soil moisture and soil available nitrogen between cover cropped and non-cover cropped treatments in semi-arid cover crop studies (Liebig et al., 2015), whereas others have found that cover cropped treatments had lower soil moisture levels and lower soil available nitrogen compared to non-cover cropped treatments (Reese et al., 2014).

The success of cover crops in semi-arid systems appears to be highly dependent on precipitation: when post-seeding rainfall was insufficient, cover crops did not establish sufficiently prior to a killing frost and therefore benefits were not conferred to the same degree (Reese et al., 2014). Contrastingly, a timely rainfall after cover crop seeding led to sufficient cover crop establishment and some of the benefits associated with cover cropping were conferred (Liebig et al., 2015). Reese et al. (2014) classified their experimental locations by water stress risk levels. At the high water-stress location, cover crops had a neutral to positive impact on the following cash crop yields, and in the moderate and low water-stress locations, grain yields were negatively impacted and not impacted, respectively (Reese et al., 2014). That is to say that in drought-prone areas, cover crops may help protect against yield losses. This supports the claim of

cover crops being useful as yield stabilizers, especially in years of drought (Alahmad et al., 2018; OMAFRA, 2021).

Both studies referred to were performed over a short-term period. As such, the medium and long-term impacts of cover cropping on soil biology in semi-arid environments requires more research. It should also be noted that Reese et al. (2014) focused their study on corn crops, which would likely yield different results compared to crops more commonly grown in the Canadian prairies, such as wheat or canola.

2.3 No-till and cover crops

No-till is an agricultural management practice wherein soil disturbance is minimal and tillage is not practiced (Agriculture and Agri-Food Canada (AAFC), 2017). On a similar note, reduced tillage management, also referred to as low-till or minimal tillage, encompasses some tillage but is less than conventional tillage (AAFC, 2017). Cover crops are often implemented in systems together with no-till practices. These practices fit well with each other because cover crops can be used to aerate the soil and help suppress weeds where tillage would traditionally be used for those purposes (OMAFRA, 2021).

Since the 1980's, reduced tillage has increased in popularity across the country, and particularly in the Canadian prairies (AAFC, 2017). In Saskatchewan, almost 75% of agricultural land is no-till managed, with an additional 18% of agricultural land being managed using reduced tillage practices (Statistics Canada, 2017). In Manitoba, approximately 20% of agricultural land is being no-tilled and almost 40% is managed using reduced tillage practices (Statistics Canada, 2017). To sum up, over 90% of Saskatchewan's agricultural land is managed using low or no-till

methods and in Manitoba, that number is almost 60%. The discrepancy between the two prairie provinces is likely due to differences in climates and crops grown.

Between the years 2011 and 2016, winter cover crop usage increased by almost 20% in Saskatchewan and there was an increase of just over 10% in Manitoba (Statistics Canada, 2017). This is to say that no-till management has been steadily gaining traction in the Canadian prairies which, by extension, creates more opportunities for cover cropping practices to be implemented. No-till systems are known to mitigate soil erosion, reduce required labour hours, improve soil moisture and nutrient retention, and increase biodiversity (AAFC, 2017). At the same time, some farmer-identified challenges with the system mean that no-till management practices are not adhered to 100% of the time on many farms. Some issues that may lead predominantly low or no-till farmers to till their fields include problems with pest and weed pressure, overly moist soils in the spring, tillage requirements for specialty crops, and excessive crop residue sitting on the soil surface (AAFC, 2017). In studying cover cropping in terms of how it relates to producers, it is important to consider the system, as a whole, which often includes low or no-till management.

2.4 Cover crop effects on biological indicators of soil health

While there are many definitions of the term "soil health" in the literature, the common denominator is the implication that soil health is associated with the living component of the soil. Soil health refers to:

> the ecological attributes of the soil which have implications beyond its quality or capacity to produce a particular crop. These attributes are chiefly those associated with the soil biota; its biodiversity, its food web structure, its activity and the range of functions it performs… the term soil health encompasses the living and dynamic nature of soil…
>
> -(Pankhurst et al., 1997, as cited by Bünemann et al., 2018).

Consolidating findings from measurements of numerous biological properties of soil can give indications as to changes in soil health over time. Biological indicators of soil health can include analyses of microbial diversity, microbial abundance, pathogen presence, insect residents, etc.. While growing in popularity, comprehensive biological indicators of soil health remain disproportionately underrepresented in most soil health testing protocols used today, especially compared to physical and chemical soil tests (Toor et al., 2021; Bünemann et al., 2018). Common soil testing tends to focus on soil health as it pertains directly to crop production, with total organic matter and soil pH being the most frequently used indicators of soil quality (Bünemann et al., 2018). However, soil organisms play a vital role in soil function and are highly responsive to changes in their host environment (Bünemann et al., 2018). As such, biological testing should be more widely included in soil health testing.

Limited research has been done on the effects of cover crops on soil biological properties generally, with most research focusing on the effects of cover crops in relation to cash crop yield (Van Eerd et al., 2023). This research gap is especially prevalent in semi-arid environments. However, research conducted in sub-tropical USA suggests that adding cover crops can reduce the gap in soil health parameters, according to a relative soil health index, between non-cover cropped soils and control soils (Krupek et al., 2022). The control soil was typically a nearby lesser-disturbed soil, such as that of perennial grassland, and that would be expected to have superior soil health measurements than the bare cropped soil (Krupek et al., 2022). Krupek et al. (2022) noted that the management histories of the fields and soil physical properties, such as soil texture, were important factors in the magnitude of response to cover cropping.

Phospholipid fatty acid (PLFA) profiling and enzyme activity assays are novel biological indicators that are not commonly included in soil health testing, but that could have immense potential in better informing soil health tests. For the purpose of this research, I focus on PLFA

profiling and extracellular enzyme activity (EEA) assays as novel indicators of biological soil health.

2.4.1 PLFA profiling

Phospholipid fatty acids (PLFAs) are a component of all cells. As such, the degree of PLFA presence in soils can indicate the amount of living organisms present (Palojarvi, 2006). PLFA membranes degrade rapidly post-mortem, wherein the fatty acid tails in the molecule detach from the phospholipid head. PLFA analyses targets intact PLFA molecules and are thus ultra-sensitive to changes in microbial populations over time (Palojarvi, 2006). There is no set benchmark number that producers should strive for in a PLFA test, but rather results are compared across time and/or locations (Kroeger & Gunderson, 2020). A field whose PLFA test results has twice the PLFA value as another field can be interpreted to have more soil microbes and likely more soil-microbially derived ecosystem services (Kroeger & Gunderson, 2020; Ferris & Tuomisto, 2015). It should also be noted that soil microbiota follow a natural seasonal cycle. Therefore, it is important to sample fields with this cyclical pattern in mind: growers and researchers should aim to sample fields around the same time each year (or each crop rotation) so that results can be compared across locations (Kroeger & Gunderson, 2020). Measurements of PLFA profiles will provide insight into fungal and bacterial populations and microbial stress ratios, and how they may vary between soils of various cover cropping practices. Moreover, PLFAs can be used to assess how different management practices might shift soil microbial community composition (Norris, Swallow et al., 2023).

Through a meta-analysis of 81 studies with 134 paired PLFA extractions, Muhammad et al. (2021) found that agricultural fields managed with cover crops enhanced the PLFA abundance by an average of 24% when compared to non-cover cropped treatments. PLFAs had the greatest

positive responsive to cover cropping on clay loam soils and when cover crop residue was incorporated into the topsoil (Muhammad et al., 2021)—a practice not likely to occur in Prairie Canada where no-till is commonplace (Statistics Canada, 2017). Overall, cover cropping was found to increase microbial community abundance and structure and therefore improve the biological health of the soils (Muhammad et al., 2021).

Contrastingly, Singh & Kumar (2021) found that PLFA profiles between cover cropped and non-cover cropped treatment groups within the rotations were not statistically different in a 26-year long-term study with a 4-year crop rotation and a 2-year crop rotation. Average PLFAs for the 4-year rotation were greater when compared to the average PLFA for the 2-year rotation (Singh & Kumar, 2021). This may imply that longer crop rotations (i.e., with greater temporal crop diversity) have the potential to support greater soil microbial populations. PLFAs from the fall 2018 sampling period were significantly greater in the cover cropped compared to non-cover cropped treatments (Singh & Kumar, 2021).

An earlier study by Zhang et al. (2014) looked at the impact of tillage and crop rotation on various soil quality indicators, including PLFAs. The independent treatments tested were continuous corn versus a corn- soybean rotation, and conventional tillage versus no-till. Although cover crops were not utilized in this study, findings can be extended to cover crop experiments due to the fact that cover crops are often used to increase plant diversity over time, in a similar way that crop rotations are used. Similarly to Singh & Kumar (2021), Zhang et al. (2014) found no benefits to the diverse rotation in terms of PLFAs. In fact, Zhang et al. (2014) found the continuous corn treatments to have greater fungal biomass, specifically arbuscular mycorrhizal fungi, compared to the corn-soybean rotation and there was no effect on bacterial communities between the treatments. This brings about questions surrounding potential competitive interactions between soil microbial species associated with each respective cash crop. It should

also be noted that this study was short-term and that it may take multiple years to realize positive impacts of diverse crop rotations and, by extension, cover cropping.

Additionally, this and many other studies focused their efforts on rotations which included corn and soybean, or did not state the cash crop type (Muhammad et al., 2021; Singh & Kumar, 2021; Zhang et al., 2014). Corn and soybean crops are commonly grown in eastern and central Canada, but are less common in the Canadian prairies. My research will seek to provide data that is more relevant to Prairie producers by using crops commonly grown in western Canada including canola and wheat.

2.4.2 Enzyme assay testing

Soil microbial extracellular enzymes play a vital role in nutrient cycling and plant debris decomposition and, by extension, microbial metabolism in soils. Enzyme assays aim to provide an estimate of the "total enzyme pool" in a given soil sample (Hargreaves & Hofmockel, 2015). Soil microorganisms are the main source of most soil enzymes and, as such, soil enzyme assays offer a good reflection of soil microbial activity (Dick, 2011). Enzymes from plant debris are rapidly degraded by soil microorganism and are beneficial for microbial population growth (Dick, 2011). Extracellular enzymes are considered a reliable indicator of soil quality due to their immediate response to different management practices and because they are relatively easy to measure (Rankoth et al., 2019). Soil enzyme activities are positively correlated to soil quality indicators, specifically soil biogeochemical processes, and are therefore good indicators of changing soil health (Rankoth *et al.*, 2019). While soil quality tends to refer to the more physical, unchangeable aspects of soil, such as texture or parent material, and soil health tends to refer more to biologcal, dynamic properties of soil, the terms are often used nearly interchangeably in modern soil science (Toor et al., 2021).

Soil enzymes commonly assessed include β-glucosidase (βG), N-acetyl-β-D-glucosaminidase (NAG), and phosphatase as they relate to C, nitrogen (N) and C, and phosphorus (P) and C cycling, respectively (Stott et al., 2013; McDaniel & Grandy, 2016; Norris et al., 2020). βG is an exocellulase enzyme that responds, albeit slowly, to changes such as cropping systems, and is therefore beneficial to include due to its low seasonal or environmental variability (Dick, 2011; Ekenler & Tabatabai, 2002). βG is used an indicator of simple soil organic matter quality since it is a cellulose degrader (Dick, 2011). βG is also important in nitrogen mineralization in soils (Ekenler & Tabatabai, 2002). NAG is an enzyme involved with chitinase systems. NAG is a major component of polysaccharides that make up bacterial cell walls. The degradation of these cellulose and chitin cell walls releases simple, carbon-rich sugars. As such, NAG is used as an indicator of carbon cycling and, by proxy, nitrogen cycling (Dick, 2011). Phosphatase is a ubiquitous, Cu-containing enzyme that is responsible for dephosphorylation and is therefore used as an indicator of phosphorous cycling in soils (Dick, 2011). Enzyme ratio measurements will allow for correlations to be made surrounding soil organic matter quality as well as nutrient supply and demand (Dick, 1997). Findings from enzyme ratios can be used to assess nutrient and carbon cycling and thus are useful indicators of soil health.

Using fluorometric extracellular enzyme activity assays, McDaniel et al. (2016) evaluated the effects of various cover crop treatments in a 12-year rotation study. Treatment groups included between 1 and 5 crops grown in 3-year rotations. NAG was more abundant in springtime in more diverse cropping systems, indicating that those treatment groups may have a better nitrogen mineralization ability during peak nitrogen requirement time (early season) and increased nitrogen-retaining capacity compared to less diverse rotations (McDaniel et al., 2016). Furthermore, small changes in crop diversity to agricultural systems with less complex rotations

improved microbial community size and function; inclusion of cover crops supported the largest increases in soil function and microbial community size (McDaniel et al., 2016).

Rankoth et al. (2019) conducted a study comparing the extracellular enzyme activity in long-term cover cropped versus non-cover cropped farmer fields in corn-soybean rotations in Missouri, USA. Various extracellular soil enzymes were compared between treatments. It was concluded that using cover crops improves soil microbial enzymatic activities and soil organic matter content (Rankoth et al., 2019). Colorimetric extracellular enzyme activity assays revealed that, by year 4 of continuous cover cropping, βG activity in cover cropped fields was greater at the 0-10 cm depth (Rankoth et al., 2019). By year 6, βG was also significantly greater at the 1020 cm and 20-30 cm depths as well (Rankoth et al., 2019). However, some soil enzymes tested did not respond linearly to cover crop inclusion over time as βG did. As such, it is implied that cover crops have mixed effects on extracellular soil enzymes, with high spatial and temporal variability in farmer-managed fields (Rankoth et al., 2019). This is especially true when comparing the results of Rankoth et al. (2019) to results found in small-plot research experiments where there seems to be less variability (McDaniel et al., 2016).

A previous study by VeVerka et al. (2019), in the same location as the Rankoth et al. (2019) study in Missouri, found that βG levels between the cover cropped and non-cover cropped plots were not statistically different and found there to be a narrower range of βG activity amongst all treatments. In 2014, βG measurements ranged from 35 -105 μg pnp g^{-1} dry soil h^{-1}, whereas the range increased to 35 -145 μg pnp g^{-1} dry soil h^{-1} by 2018 (VeVerka et al., 2019; Rankoth et al., 2019). This implies that the benefits of cover cropping, in terms of soil microbial enzymatic activity, may take multiple years to be conferred.

However, it should be noted that there are potentially confounding variables in both the Rankoth et al. (2019) and VeVerka et al. (2019) studies, wherein cover cropped treatments all

received no-till management and non-cover cropped treatments all received vertical-till management. In both cases, statistical analysis failed to evaluate potential interactions between tillage type and cover crop treatment, and also failed to include appropriate controls in the experimental design to account for the potential interactions. This raises the question of whether cover cropping or tillage, or both, were the causes of differing enzyme activity between the treatments. As mentioned earlier, Zhang et al. (2014) tested monoculture versus rotation treatments, and no-till versus conventionally tilled treatments, and found there to be significant differences in PLFA and enzyme activity only between the tillage treatments. My research will avoid this issue of potential confounding variables by implementing appropriate controls and using the same tillage method across treatments as much as possible. Although it should be noted here that in my research, one experimental location includes potato plots which will require soil disturbance due to hilling requirements.

2.5 Role of soil microbiota in nutrient cycling

Soil contains billions of unique microorganisms. Many of these organisms play an important role in cycling nutrients from the soil and the atmosphere into forms that are accessible to the crop. Nutrient demands of soil microbiota are thus inherently linked to nutrient cycling in the soil (Paul, 2015).

Soil bacteria tend to be incredibly flexible in terms of their substrate utilization for energy. For example, some bacterial species in the genus *Pseudomonas* are capable of utilizing and degrading over 100 different carbon substrates (Paul, 2015). While some soil bacteria are generalists, others thrive in specific niches. For example, rhizobium bacteria are specifically associated with legume cover crops, like those in the clover and pea families. Rhizobium fixes atmospheric nitrogen and increases nitrogen availability for the intended cash crop (Tribouillois

et al., 2016). Perhaps one of the most important activities of soil bacteria is their role in the decomposition of cellulose. Plant matter is comprised of ~60% cellulose, representing the primary source of soil organic matter, and thus energy, in soils (Paul, 2015). However, the energy contained in cellulose is not plant-available due to its semi-crystalline structure. Through a series of soil bacterial and fungal depolymerization processes, cellulose (and hemicellulose) is biodegraded into its glucose sub-units (Paul, 2015). Through this process, soil carbon becomes highly available in the form of glucose, and important soil nutrients including nitrogen, phosphorus and sulfur are immobilized, which improves soil nutrient retention (Paul, 2015).

Soil fungi are effective in nutrient cycling due to their ability to break down plant litter and other soil organic matter through the secretion of various oxidases and glycosidases, as well as their fibrous, hyphal growth pattern (Paul, 2015). Hyphae are microscopic, fibrous branches that reach out into the rhizosphere (Tribouillois et al., 2016). The hyphal network increases the underground surface area and improves the ability of the plant to uptake resources (Tribouillois et al., 2016). Mycorrhizal fungi, which are abundant in soils, engage in symbiotic relationships with plant roots, wherein the plant delivers sugars to the fungus and the fungus delivers soil minerals and water to the plant. Mycorrhizal fungi develop extensive hyphal networks that act as an annex of the roots. Many saprophytic fungi excrete cell wall degrading enzymes that decompose dead plant and animal materials (Venturini & Delledonne, 2014). This decay is involved in nutrient cycling through the decomposition of soil organic matter into simpler chemical compounds that are more easily taken up by other soil microbes and plant roots (Venturini & Delledonne, 2014; Paul, 2015).

When considering the role of soil microbiota in nutrient cycling for research purposes, microbial function and abundance are often measured. Some of the most common biological measurements used in soil science research analyze the extracellular enzymatic activity, community shifts, and

abundance of soil fungi and bacteria (Paul, 2015). In my research, these characteristics will be studied using PLFAs and extracellular enzyme assays.

3.0 MATERIALS AND METHODS

3.1 Site description, treatments, & plot management

A field study was initiated in 2018 at Saskatoon, Saskatchewan (52°09'11.0"N 106°36'44.2"W), Carman, Manitoba (49°30'05.9"N 98°01'42.7"W), and Glenlea, Manitoba (49°38'57.4"N 97°07'08.0"W). Each site tested treatments that included a four-year crop long rotation including cover crops, a four-year crop long rotation without cover crops, a two-year wheat-canola short rotation check, and a perennial alfalfa check. Cash crop and cover crop species were chosen to represent broader taxonomic groups (e.g., brassicas, oilseeds, grains, tubers). Crops grown differed between sites based on common growing practices and scientific interest. Specifically, soybean was grown in Carman and Glenlea but not in Saskatoon because this crop is more commonly grown in Manitoba than in Saskatchewan. Potato was included at the Saskatoon site to examine the experimental effects on a horticultural crop. The field experiment was organized as a fully phased randomized complete block design that consisted of four replicates for a total of 44 plots per site (Tables 3.1, 3.2, 3.3). Plots measured 6 m x 6 m, 6 m x 8 m, and 8 m x 8 m at the Saskatoon, Carman, and Glenlea sites, respectively.

This cover crop experiment was initiated in 2018 at the Saskatoon and Carman sites and in 2019 at the Glenlea site. Soil samples were collected in fall 2020, spring 2021 and summer 2021 at each site. In relation to the years of research for this project, cash crops at all sites were seeded in May 2020 and 2021. Alfalfa check plots were established at all three sites prior to the start of the study. Alfalfa plots were planted in Saskatoon in 2018. The planting dates for alfalfa at the Carman and Glenlea sites are unavailable. Alfalfa plots were managed through mowing at all three research sites. Agronomic data, such as crop yield and cover crop biomass, from the Glenlea and

Carman sites were unavailable at the time of writing. See **Appendix A** for additional agronomic information.

The Saskatoon and Carman sites first included cover crops in rotation in 2018, and Glenlea started including cover crops in 2019 (Tables 3.1, 3.2, 3.3). The sites were all no-till managed, except for the potato plots in Saskatoon which required some disturbance for management. In Saskatoon, the soil is an Orthic Dark Brown Chernozem with a sandy loam texture and low organic matter content (Table 3.4). The Glenlea site has Rego Black Chernozem soils with a clay texture and relatively high baseline SOM content (Table 3.4) (University of Manitoba, 2016). The soils at the Carman site have a sandy loam texture and are classified as Rego Black Chernozems (Table 3.4).

Table 3.1: Cash crop and cover crop rotation at the Saskatoon, Saskatchewan field site. Crops in brackets represent cover crops that were planted following the cash crop in a given season.

Trt Description	Trt ID	2018	2019	2020*	2021*
4-yr rotation with cover crops	1	Wheat (red clover)	Canola (berseem clover/oat mix)	Potato (fall rye)	Pea (tillage radish)
	2	Canola (berseem clover/oat mix)	Potato (fall rye)	Pea (tillage radish)	Wheat (red clover)
	3	Potato (fall rye)	Pea (tillage radish)	Wheat (red clover)	Canola (berseem clover/oat mix)
	4	Pea (tillage radish)	Wheat (red clover)	Canola (berseem clover/oat mix)	Potato (fall rye)
4-yr rotation with no cover crops	5	Wheat	Canola	Potato	Pea
	6	Canola	Potato	Pea	Wheat
	7	Potato	Pea	Wheat	Canola
	8	Pea	Wheat	Canola	Potato
2-yr rotation with no cover crops	9	Wheat	Canola	Wheat	Canola
	10	Canola	Wheat	Canola	Wheat
Perennial	11	Alfalfa	Alfalfa	Alfalfa	Alfalfa

***Soil sampling occurred in all treatments (Trt) in Fall 2020, Spring 2021, and Summer 2021**

Table 3.2: Cash crop and cover crop rotation at the Carman, Manitoba field site. Crops in brackets represent cover crops that were planted following the cash crop in a given season.

Trt Description	Trt ID	2018	2019	2020	2021
4-yr rotation with cover crops	1	Wheat (red clover)	Canola (barley/pea)	Oat (fall rye)	Soybean (tillage radish)
	2	Canola (barley/pea)	Oat (fall rye)	Soybean (tillage radish)	Wheat (red clover)
	3	Oat (fall rye)	Soybean (tillage radish)	Wheat (red clover)	Canola (barley/pea)
	4	Soybean (tillage radish)	Wheat (red clover)	Canola (barley/pea)	Oat (fall rye)
4-yr rotation with no cover crops	5	Wheat	Canola	Oat	Soybean
	6	Canola	Oat	Soybean	Wheat
	7	Oat	Soybean	Wheat	Canola
	8	Soybean	Wheat	Canola	Oat
2-yr rotation with no cover crops	9	Wheat	Canola	Wheat	Canola
	10	Canola	Wheat	Canola	Wheat
Perennial	11	Alfalfa	Alfalfa	Alfalfa	Alfalfa
***Soil sampling occurred in all treatments (Trt) in Fall 2020, Spring 2021, and Summer 2021**					

Table 3.3: Cash crop and cover crop rotation at the Glenlea, Manitoba field site. Crops in brackets represent cover crops that were planted following the cash crop in a given season. Note that cover crops were first planted at this site in 2019, whereas in Saskatoon and Carman, cover crops were first planted in 2018.

Trt Description	Trt ID	2019	2020*	2021*	2022
4-yr rotation with cover crops	1	Wheat (red clover)	Canola (barley/pea)	Oat (fall rye)	Soybean (tillage radish)
	2	Canola (barley/pea)	Oat (fall rye)	Soybean (tillage radish)	Wheat (red clover)
	3	Oat (fall rye)	Soybean (tillage radish)	Wheat (red clover)	Canola (barley/pea)
	4	Soybean (tillage radish)	Wheat (red clover)	Canola^{+} (barley/pea)	Oat (fall rye)
4-yr rotation with no cover crops	5	Wheat	Canola	Oat	Soybean
	6	Canola	Oat	Soybean	Wheat
	7	Oat	Soybean	Wheat	Canola
	8	Soybean	Wheat	Canola^{+}	Oat
2-yr rotation with no cover crops	9	Wheat	Canola	Wheat	Canola
	10	Canola	Wheat	Canola^{+}	Wheat
Perennial	11	Alfalfa	Alfalfa	Alfalfa	Alfalfa

***Soil sampling occurred in all treatments (Trt) in Fall 2020, Spring 2021, and Summer 2021**

$^{+}$ There was a canola crop failure at the Glenlea site in 2021 due to herbicide error

Table 3.4: Soil properties of samples taken from Saskatoon, Carman, and Glenlea research sites.

Property	Saskatoon	Carman	Glenlea
Soil texture	Sandy loam	Sandy loam	Clay
Soil type	Orthic Dark Brown Chernozem	Rego Black Chernozem	Rego Black Chernozem
Soil pH	7.13	4.91	7.05
Soil organic matter	3.7%	4.3%	6.3%

Average daily temperatures followed similar trends at Saskatoon, Carman, and Glenlea over the course of the sampling period: May 2020 – July 2021 (Figs. 3.1, 3.2, 3.3). The lowest temperatures at all three research sites were recorded in February 2021. The 2021 growing season was abnormally hot and dry across growing locations: Between the years 1981 – 2010, the average daily temperature in June was 16.1°C, 17.2°C, and 17.0°C at the Saskatoon, Carman and Glenlea sites respectively (Government of Canada, 2023a; Government of Canada, 2023b; Government of Canada, 2023c). In comparison, the averag daily temperatures in June 2021 at the Saskatoon, Carman, and Glenlea sites were 18.1°C, 19.3°C, and 19.8°C respectively (Government of Canada, 2023a; Government of Canada, 2023b; Government of Canada, 2023c). Carman experienced significantly higher rainfall in June 2021 compared to the Saskatoon and Glenlea sites respectfully (Figs. 3.1, 3.2, 3.3).

The problem of limited precipitation in 2021 was compounded by the fact that the previous 2020 season was also dry, which led to lower soil water levels than normal. Similar to Saskatoon and Carman, Glenlea experienced a relatively dry spring in 2021. However, the higher clay content in the Glenlea soils likely retained more moisture compared to the Saskatoon site.

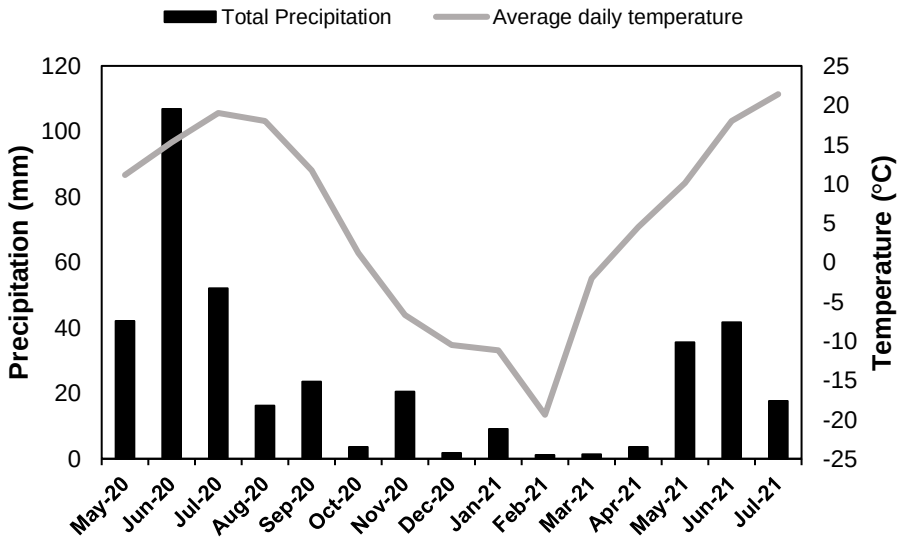

Fig 3.1: Total precipitation and average monthly temperature from the time period May 2020 until July 2021 at the Saskatoon, SK site (Government of Canada, 2023c).

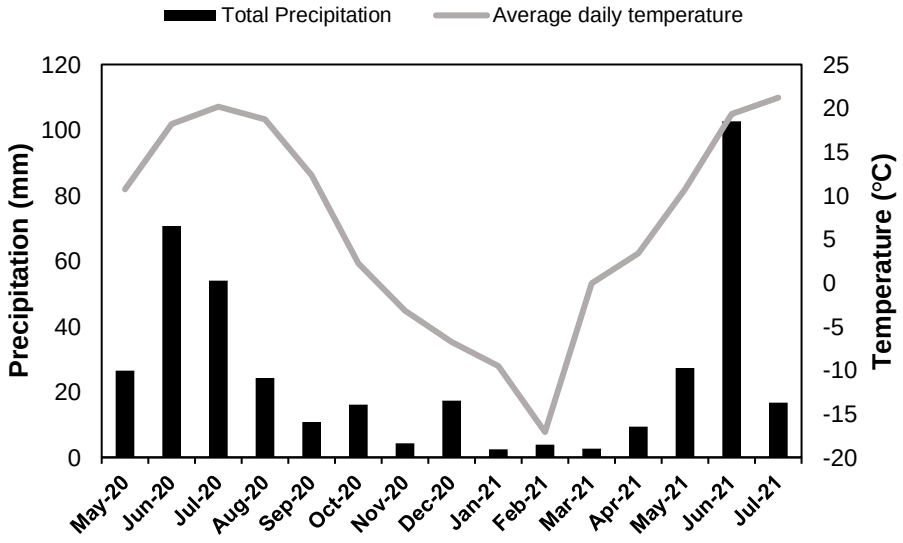

Fig 3.2: Total precipitation and average monthly temperature from the time period May 2020 until July 2021 at the Carman, MB site (Government of Canada, 2023a).

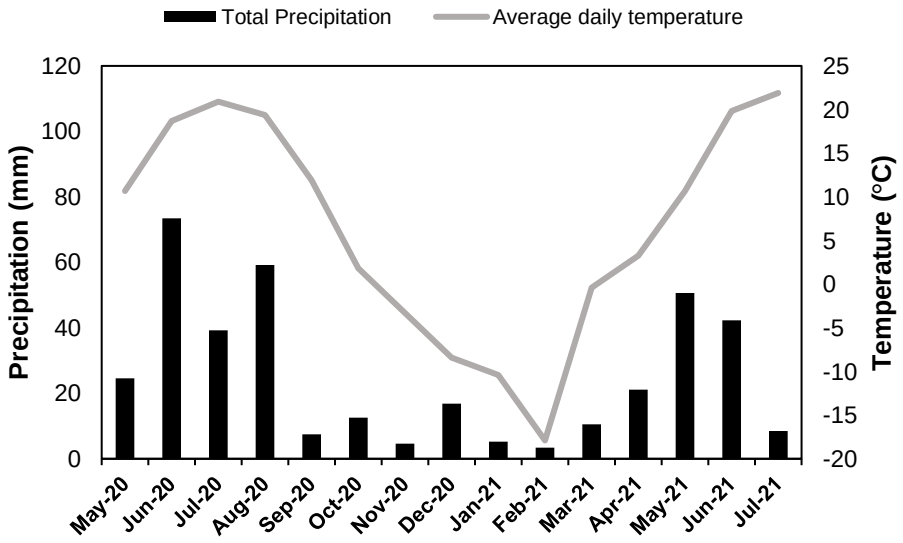

Fig 3.3: Total precipitation and average monthly temperature from the time period May 2020 until July 2021 at the Glenlea, MB site. Winnipeg was the closest government weather station to the Glenlea site (approximately 30 km), so data collected from Winnipeg was used for Glenlea (Government of Canada, 2023b).

3.2 Soil sampling & analysis

Surface soils (0-15 cm) were collected using 2.5 cm diameter JMC Backsaver soil probes (JMC Soil Samplers, Newton, IA). To capture seasonal variability, samples were collected in fall 2020, following cash crop harvest and cover crop establishment, in spring 2021 prior to seeding, and in summer 2021 coinciding with cash crop flowering/anthesis. Soil cores were collected using the judgement sampling technique in each plot at each of the sites, wherein a non-random selection of sample locations is chosen based on what is considered to be most typical of the plot.Potato plots were sampled within and between hills separately. Depending on the year, four or five soil cores were composited into a single sample per plot. In the field, soils were placed in labelled polypropylene bags and stored in coolers with ice packs. Equipment was sterilized between plots using ethanol and samplers wore clean latex gloves throughout the sampling process. Soils from the Saskatoon site were refrigerated at 4°C overnight and sieved using sterilized 2 mm sieves the next day. After sieving, field-moist soils were stored frozen at -20°C to be preserved for further

processing. Soil samples from Carman and Glenlea sites were frozen at -20°C post-sampling and shipped to Saskatoon in coolers with ice packs. Upon delivery, these soils were sieved using sterilized 2 mm sieves and then stored at -20°C.

3.3 Microbial community structure & abundance – PLFA

Phospholipid fatty acids (PLFAs) were extracted to determine microbial abundance and community structure between treatments and sampling sites. After initial processing, which included sieving using 2mm sterilized sieves and storing at -20°C, soil subsamples of ~20 g from each plot were transferred into labelled glass vials, covered with parafilm, and capped with a plastic lid to be used for PLFA analysis. Vials were stored at -20°C, freeze-dried, and then ground to a fine powdery texture using a mortar and pestle. The mortar and pestle were cleaned with soap and water, ethanol, and then flame-sterilized between samples to mitigate risk of microbial contamination between samples. Grinding the soil allows for better retrieval of fungal lipids because their cell walls are more robust compared to bacteria, for example; without grinding, chemical solvents are not likely to break fungal cell walls down as efficiently as other groups.

PLFAs were extracted using methods outlined by Helgason, Walley, and Germida (2010). In brief, 4 g freeze-dried and ground soils were measured into test tubes and shaken with 19 mL modified Bligh and Dyer extractant (Bligh & Dyer, 1959; White et al., 1979) Lipids were extracted using 500 mg silicon fractionation columns (Bond Elut, Agilent Technologies, Santa Clara, CA) to be left with only intact phospholipids. Following methylation of the isolated phospholipids [which were thusly reduced to fatty acid methyl esters (FAMEs)], methyl nanodecanoate (19:0) was included in each sample to allow for peak calibration. The samples were read on a Bruker 436 gas chromatography flame ionization detector (GC FID) (Bruker Corporation, Billerica, MA). GC

output was reprocessed to account for environmental changes over the course of sample reading. PLFAs were identified based on comparing FAME peaks relative to a library of known standard peak biomarkers.

General bacterial biomarkers consisted of 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:1 ω7c, 16:0 10-methyl, 17:0 iso, 17:0 anteiso, 17:0 cyclo ω7c, 17:0 10-methyl, 18:1 ω7c, 18:0 10-methyl, and 19:0 cyclo ω7c. Gram positive bacteria biomarkers were 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, and 17:0 anteiso. Gram negative bacteria biomarkers were 16:1 ω9c, 16:1 ω7c, 18:1 ω7c, 18:1 ω9c, 17:0 cyclo ω7c, 19:0 cyclo ω7c. Actinobacteria biomarkers were 16:0 10-methyl and 18:0 10-methyl. Arbuscular mycorrhizal fungi presence was determined by the presence of 16:1 ω5c. Only one biomarker was used as an estimate for fungal abundance: 18:2 ω6c. Stress indices of G- bacteria (Stress 1 and Stress 2) were determined using ratios of 17:0 cyclo ω7c/16:1 ω7c and 19:0 cyclo ω7c/18:1 ω7c respectively. Fungi:bacteria ratio was calculated by dividing the total 18:2 ω6c by the sum of bacterial biomarkers.

3.4 Soil biological properties – Extracellular enzyme assays

Extracellular enzyme activity (EEA) was measured fluorometrically following 4-methylumbelliferone (4-MUB) protocols outlined by Dechka & Arcand (2016), which were primarily adapted from Bell et al. (2013) and Hargreaves & Hofmockel (2015). β glucosidase, (βG), N-acetyl-β-D-glucosaminidase (NAG), and alkaline phosphatase (AP) enzyme activities were all measured fluorometrically (Table 3.5).

Table 3.5: Enzymes assayed for each research location, along with their respective substrates, pH, and rationale (Dick, 2011; Arcand et al., 2016).

Enzyme	Substrate	Buffer pH	Rationale
β-glucosidase (βG)	4methylumbelliferyl- α-D-glucopyranoside	5.5* (Eivazi & Tabatabai, 1988)	Indicates labile soil organic matter quality (Dick, 2011)
N-acetyl- β-D glucosaminidase (NAG)	4-methylumbelliferyl N-acetylglucosaminide	5.5 (Parham & Deng, 2000)	Involved in chitin degradation and proxy indicator of N cycling (Dick, 2011; Stott, 2019)
Alkaline Phosphatase (AP)	4-methylumbelliferyl phosphate	11 (Dick, 2011)	Important for P cycling (Dick, 1997)

***Note the optimal buffer pH of βG is 6.0. A pH of 5.5 was used based on preliminary laboratory trials which yielded superior results at lower pH.**

Soil subsamples (approximately 50 g) used for assays were air-dried at 22°C for 48 h and stored in labelled 50 dram plastic vials. Assays were conducted at optimal enzyme pH: pH 11.0 for AP, and pH 5.5 for βG and NAG. Note that the optimal pH for βG and NAG are 6.0 and 5.5 respectively. However, both enzymes tested at both pH levels yielded consistently superior results at pH 5.5 for both enzymes. Therefore, it was determined that assaying βG and NAG at the same pH was beneficial in terms of results and resource management for this study. Briefly, 1.0 g of air-dried soil was blended for 30 s with 120 mL pH-adjusted modified universal buffer and transferred to a beaker on a stir plate with a stir bar. 1800 μL soil slurry will be pipetted into 5 mL plastic centrifuge tubes. 450 μL of substrate solution were added to the tubes (Sigma-Aldrich Corp., St. Louis, MO). Substrates were made to concentrations of 2000 μM for βG and NAG, and 4000 μM for AP. Reagents used for substrates were 4-methylumbelliferyl-β-D-glucopyranoside, 4-methylumbelliferyl N-acetyl-glucosaminide, or 4-methylumbelliferyl phosphate salt for βG, NAG, and AP assays respectively. Tubes were wrapped in foil to protect against light degradation and were incubated for three h, shaking laterally at a speed of 142 rpm and 22°C. After incubation,

tubes were centrifuged at 2000 rpm for five minutes. Supernatant subsamples of 250 μL were pipetted into black 96-well microplates. 4-MUB fluorescence was measured on the Thermo Scientific Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, Waltham, MA) at 360 nm excitation and 465 nm emission wavelengths. There were six technical replicates per sample, with an additional six technical replicates every tenth sample. Technical replicates were averaged to obtain the final data point used for statistical analysis. A consistent check soil sample was also included on each microplate to ensure accuracy across the experiment as a whole.

3.5 Statistical analysis

Statistical analysis for this research used multiple approaches due to the unbalanced experimental design with respect to presence of cover crop in the treatment (Tables. 3.1, 3.2, 3.3) and the change of crop species during the temporal sampling arc for this study (Fig. 3.4). It is important to note that the cash crop grown in a given plot changed between spring 2021 and summer 2021 sampling points. For the purposes of this study, I considered the most recent crop to be the most recently harvested cash crop (Fig. 3.4). Each research site was analyzed independently because different crops were grown at each site. Trends were compared between locations.

Fig 3.4: Visual explanation of soil sample timing in relation to the growing cycle. Fall 2020 samples were taken post-harvest, spring 2021 samples were taken pre-plant, and summer 2021 samples were taken mid-season.

I analyzed the effect of treatment (i.e., treatments 1 through 11) and sampling time (i.e., fall 2020, spring 2021, and summer 2021) on PLFA biomarkers and enzyme activity using two-way ANOVA where treatment and sampling time were the independent variables. This analysis enabled me to look at each of the 11 treatments and identify patterns within cropping systems or crops grown. Due to the unbalanced nature of the experimental design with respect to the number of treatments in the rotation types (i.e., short rotation *vs*. long rotation), cross-comparison were not necessarily analogous, so further investigation was required. One-way ANOVA was performed on summer data separately since a different crop was grown in summer than in the fall and spring at each plot (Fig. 3.4). Analyzing summer 2021 independently from fall 2020 and spring 2021 allowed me to examine the effect of the living crops on soil PLFA and enzyme activities within the growing season, versus evaluating the effect of the previous crop as the independent variable. Due to sample collection timing, there was no living cash crop during fall 2020 sampling (post-harvest) or spring 2021 sampling (pre-plant) (Fig 3.4), but this was intentional to capture when cover crops were established (fall) and in the early stages of cover and cash crop residue decomposition before seeding (spring).

All statistical analyses were performed using R Version 4.2.1, "Funny-Looking Kid". Analysis of variance (ANOVA) with repeated measures was performed using linear mixed effects (lme) models to test the effects of sampling time and treatment in PLFA abundance and extracellular enzyme activity. Sampling time and treatment (crop species grown, rotation type) were assigned as fixed effects and block/plot were the random effects. Effects were considered significant if $p < 0.05$. If so, post-hoc testing was executed using estimated marginal means (EMM), and pairwise contrasts were used to test differences between treatments at individual timepoints using Tukey honest significant difference (HSD). Shapiro-Wilk and Levene's tests

were used to evaluate normality of residuals and homogeneity of variance, respectively. Where distributions failed to meet the assumptions of the ANOVA, data were log transformed. Correlations were performed on non-transformed data using Spearman's rank correlation tests and were considered meaningfully correlated if the r values were $> \pm 0.5$ and were significant if $p < 0.05$. NMDS plots were created using Primer-E statistical software, but were excluded due to unacceptable R^2 values (see **Appendix B**).

4.0 RESULTS

4.1 Soil microbial PLFA abundance and composition

In general, total PLFA abundance was highest at Glenlea, followed by Carman and Saskatoon, with average total PLFA abundances of 29.43, 27.97, and 23.53 nmol g^{-1} respectively.

There were limited significant interactions between crop rotation treatment and sampling time across the range of biomarker groups and ratios: significant interactions were found only for the stress 2 ratio at the Saskatoon and Carman research sites, and the G+:G- bacteria ratio at the Carman site (Table 4.1). However, the main factor of crop rotation significantly affected PLFA abundance in Saskatoon for the fungal biomarker group and the F:B and stress 1 ratios (Table 4.1). In Carman and Glenlea, crop rotation affected fungal and AMF abundance and the F:B, G+:G- bacteria, and stress 2 ratios (Table 4.1). Crop rotation did not affect abundance for total PLFA, general bacteria, G+ bacteria, G- bacteria, actinobacteria and AMF at any of the sites (Table 4.1). Sampling time always impacted PLFA abundance regardless of biomarker group. This finding was consistent across locations, with a single exception at Glenlea, where sampling time did not affect fungal PLFA abundance (Table 4.1).

I also had similar findings in the compositional data (i.e., mol %), wherein sampling time affected PLFA composition across all biomarker groups and ratios at all three research locations (see **Appendix C**), while treatment effects were limited to fungal biomarkers only. Treatment had a significant impact on fungal PLFA composition at all locations, and AMF was affected by all factors (treatment, sampling time, and the interaction between treatment and sampling time) at all locations as well (see Table A13). Like compositional PLFA abundance, AMF relative abundance tended to be greatest in the perennial treatments across locations compared to the rotation treatments (**Appendix C**).

Table 4.1: Two-way ANOVA F-test results for the effect of treatment & sampling time on phospholipid fatty acid (PLFA) abundance at all sites in fall 2020, spring 2021, and summer 2021 sampling times; values are F-statistics and values in brackets are p values.

Location	Factor	Df	Total PLFA	Gen. bac.[a]	Fungi	F:B[b]	G+[c]	G-[d]	Actino[e]	AMF[f]	G+:G-[g]	Stress 1	Stress 2
Saskatoon	Trt	10	1.3128 (0.2643)	1.2829 (0.2798)	3.6625 (0.0023)**[h]	3.4653 (0.0033)**	1.1573 (0.3529)	1.4320 (0.2099)	1.3538 (0.2443)	1.2834 (0.2795)	2.749 (0.0140)*	2.301 (0.0352)*	3.901 (0.0015)**
	Sample time	2	9.7214 (0.0002)***	9.2309 (0.0003)***	10.1871 (0.0001)***	14.7390 (<0.0001)***	5.9859 (0.0041)**	16.5301 (<0.0001)***	6.3125 (0.0031)**	10.6422 (0.0001)***	192.245 (<0.0001)***	99.721 (<0.0001)***	110.730 (<0.0001)***
	Trt × Sample time	20	1.1796 (0.2996)	1.2054 (0.2786)	1.1666 (0.3105)	1.5330 (0.0998)	1.1509 (0.3241)	1.2009 (0.2822)	1.2442 (0.2491)	1.1548 (0.3207)	0.968 (0.5101)	0.887 (0.6033)	3.423 (0.0001)***
Carman	Trt	10	0.8667 (0.5719)	0.7952 (0.6338)	5.7022 (0.0001)***	7.4221 (<0.0001)***	0.6106 (0.7935)	1.6053 (0.1485)	0.7284 (0.6925)	2.9065 (0.0101)*	3.111 (0.0067)**	1.351 (0.2459)	3.579 (0.0027)**
	Sample time	2	21.5053 (<0.0001)***	16.1132 (<0.0001)***	20.4401 (<0.0001)***	9.3634 (0.0003)***	19.0802 (<0.0001)***	22.4200 (<0.0001)***	8.5529 (0.0005)***	41.2844 (<0.0001)***	113.113 (<0.0001)***	116.472 (<0.0001)***	54.326 (<0.0001)***
	Trt × Sample time	20	0.6149 (0.8877)	0.5797 (0.9132)	1.3130 (0.2028)	1.0469 (0.4242)	0.4741 (0.9677)	0.7950 (0.7101)	0.6365 (0.8701)	0.9208 (0.5639)	2.165 (0.0101)*	1.165 (0.3121)	2.489 (0.0029)**
Glenlea	Trt	10	1.6453 (0.1369)	1.4561 (0.2001)	4.76720 (0.0003)***	4.9029 (0.0002)***	1.3653 (0.2390)	1.7696 (0.1062)	1.1207 (0.3767)	2.3495 (0.0318)*	3.913 (0.0014)**	1.313 (0.2645)	3.104 (0.0068)**
	Sample time	2	13.7800 (<0.0001)***	18.7848 (<0.0001)***	1.86206 (0.1639)	7.2912 (0.0014)**	21.1510 (<0.0001)***	6.0448 (0.0040)**	33.4250 (<0.0001)***	5.0794 (0.0091)**	54.877 (<0.0001)***	116.945 (<0.0001)***	58.322 (<0.0001)***
	Trt × Sample time	20	0.6292 (0.8752)	0.8270 (0.6728)	0.57091 (0.9181)	1.1419 (0.3341)	0.7687 (0.7388)	0.5333 (0.9405)	1.0222 (0.4513)	0.7879 (0.7174)	1.194 (0.2899)	1.502 (0.1132)	1.371 (0.1722)

[a] Gen. bac. = General Bacteria
[b] F:B = fungal to bacterial PLFA ratio
[c] G+ = Gram positive bacteria
[d] G- = Gram negative bacteria
[e] Actino = Actinobacteria
[f] AMF = Arbuscular mycorrhizal fungi
[g] G+:G- = Gram positive bacteria to Gram negative bacteria PLFA ratio
[h] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table 4.2: One-way ANOVA F-test results for the impact of treatment on phospholipid fatty acid (PLFA) abundance at the Saskatoon, Carman, and Glenlea research sites at the summer 2021 sampling time; values are F-statistics and values in brackets are p values.

Location	Factor	Df	Total PLFA	Gen. bac.[a]	Fungi	F:B[b]	G+[c]	G-[d]	Actino[e]	AMF[f]	G+:G-[g]	Stress 1	Stress 2
Saskatoon	Trt	10	1.37773 (0.2333)	1.36087 (0.241)	2.06468 (0.0576)	2.2506 (0.0391)*[h]	1.19706 (0.3282)	1.62825 (0.1417)	1.2734 (0.2848)	1.28908 (0.2765)	3.841 (0.0016)**	2.258 (0.0385)*	7.501 (<.0001)***
Carman	Trt	10	0.6082 (0.7955)	0.6065 (0.7969)	1.75524 (0.1094)	5.2418 (1e-04)***	0.6229 (0.7833)	0.5962 (0.8052)	0.6369 (0.7716)	0.8577 (0.5796)	1.849 (0.0902)	0.717 (0.7022)	2.7196 (0.0148)*
Glenlea	Trt	10	1.0988 (0.3915)	1.1756 (0.3414)	2.94689 (0.0093)**	6.7495 (<0.0001)***	1.1134 (0.3816)	1.2001 (0.3264)	1.0982 (0.3919)	1.7797 (0.104)	4.259 (8e-04)***	2.295 (0.0357)*	2.667 (0.0165)*

[a] Gen. bac. = General Bacteria
[b] F:B = fungal to bacterial PLFA ratio
[c] G+ = Gram positive bacteria
[d] G- = Gram negative bacteria
[e] Actino = Actinobacteria
[f] AMF = Arbuscular mycorrhizal fungi
[g] G+:G- = Gram positive bacteria to Gram negative bacteria PLFA ratio
[h] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

In Saskatoon, total PLFA was higher in spring 2021 than it was in fall 2020 and summer 2021 (Fig. 4.1). Total PLFA at the Carman site was highest in the fall and not statistically different between spring 2021 and summer 2021 (Fig. 4.1). In Glenlea, the total PLFA abundance was higher in summer 2021 compared to fall 2020 and spring 2021 sampling times (Fig. 4.1). This illustrates that seasonality was important for total PLFA abundance at every research location; however, the impact that seasonality had on PLFA abundance was different at each of the three sites.

Like total PLFA, fungal abundance in Saskatoon was highest in the spring and lowest in the summer (Fig. 4.1). Fungal PLFA abundance in Carman declined across the growing season arc—like total PLFA—where fall 2020 had the highest fungal PLFA abundance, spring 2021 was significantly lower, and summer 2021 was significantly lower still (Fig. 4.1). Like Saskatoon, fungal abundance follows a similar trend as total PLFA abundance at the Carman site. Seasonality appears to be important for fungal abundance; however, similar to total PLFA, the effect of seasonality on fungal abundance differed between locations (Fig. 4.1).

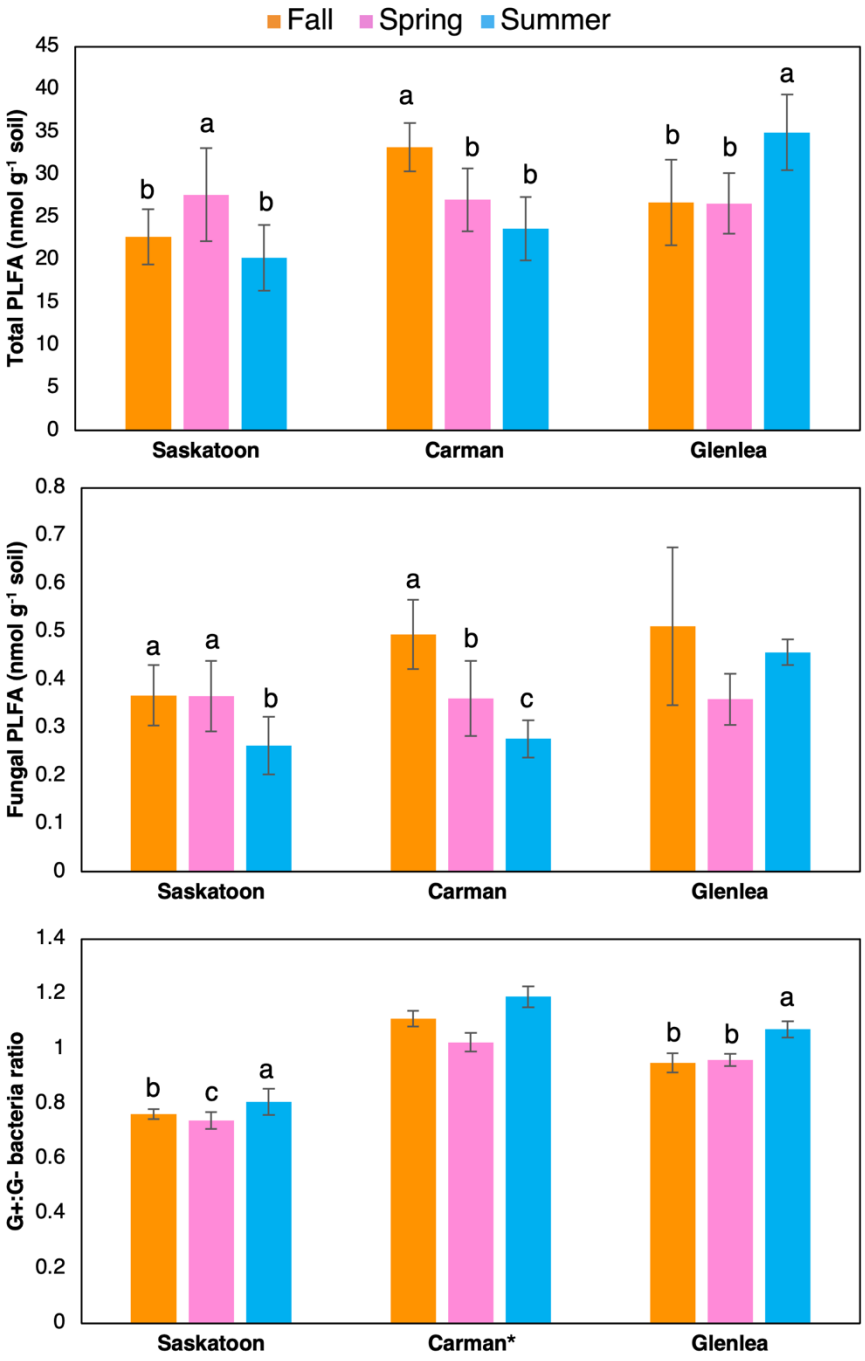

Fig 4.1: Total phospholipid fatty acid (PLFA) abundance, fungal PLFA abundance, and G+:G- bacteria ratio at all research sites at fall 2020, spring 2021, and summer 2021. Different letters above bars indicate significant differences between means according to Tukey's HSD, $p<0.05$. *There was a significant interaction between the factors of treatment and sampling time for the G+:G- ratio in Carman, therefore significance letters were not assigned.

At all research locations, the perennial alfalfa treatment had the greatest fungal abundance. This is true both when treatment effects were grouped across time (Fig. 4.2) and when treatment effects were analyzed from the summer 2021 sampling time on its own when living cash crops and alfalfa were present (Fig. 4.3). When summer was analyzed independently, there was stronger treatment differentiation and higher fungal abundance at the Glenlea site compared to the Saskatoon and Carman sites (Fig. 4.3). However, there was no difference in fungal abundance in any of the cover cropped treatments compared to any of the long or short rotation treatments without cover crops. Additionally, average total PLFA abundance was greater at Glenlea in summer 2021 (34.92 nmol g^{-1} soil) compared to the values for Saskatoon (20.25 nmol g^{-1} soil) and Carman (23.65 nmol g^{-1}) (Fig. 4.1). Perhaps the higher clay content in the Glenlea soils retained more soil water and supported greater total PLFA abundance, and particularly fungal abundance.

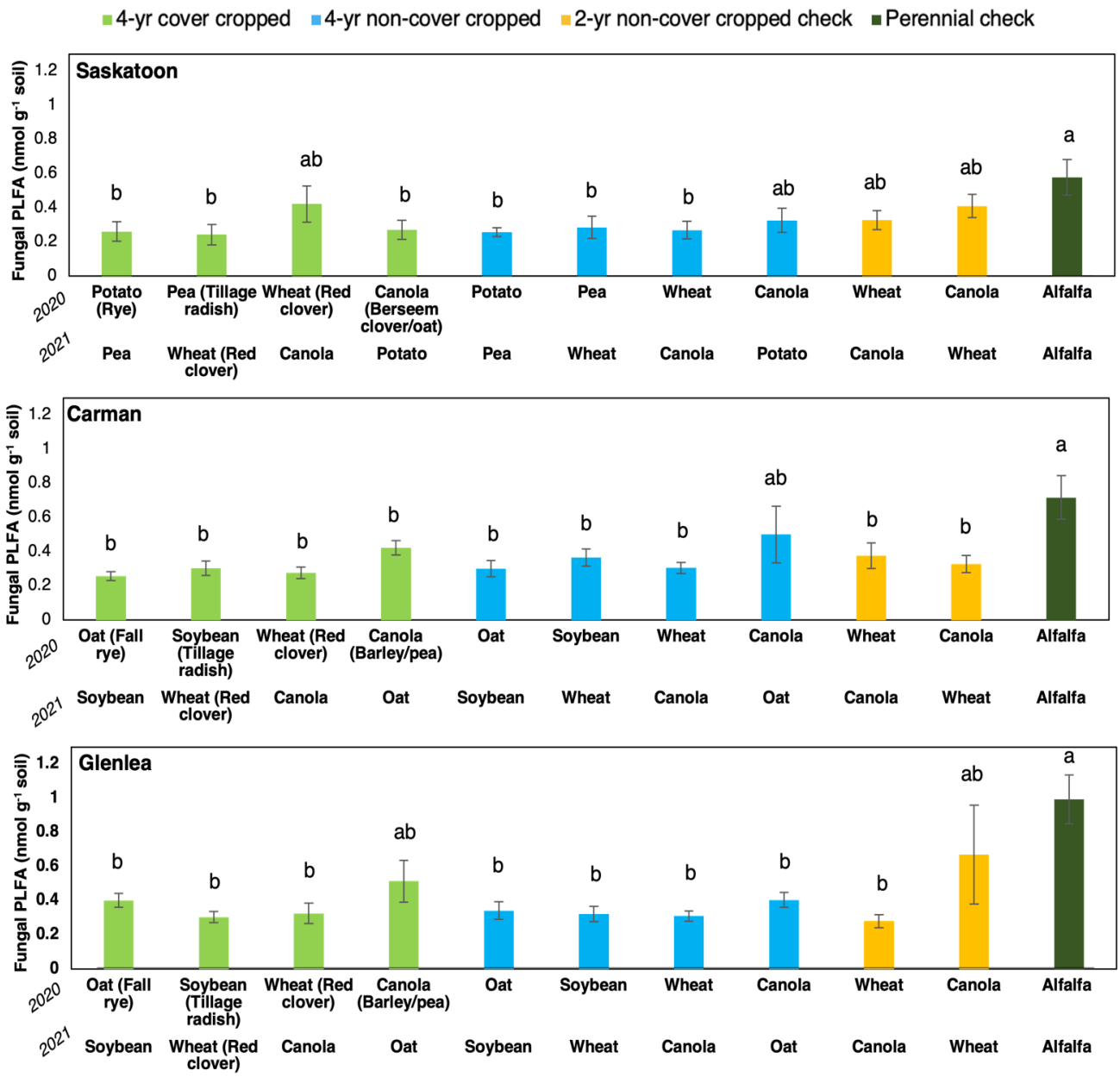

Fig 4.2: Fungal phospholipid fatty acid (PLFA) abundance by treatment at the Saskatoon, Carman, and Glenlea research sites averaged across sampling times. Crops listed were grown during the 2020 and 2021 growing seasons; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

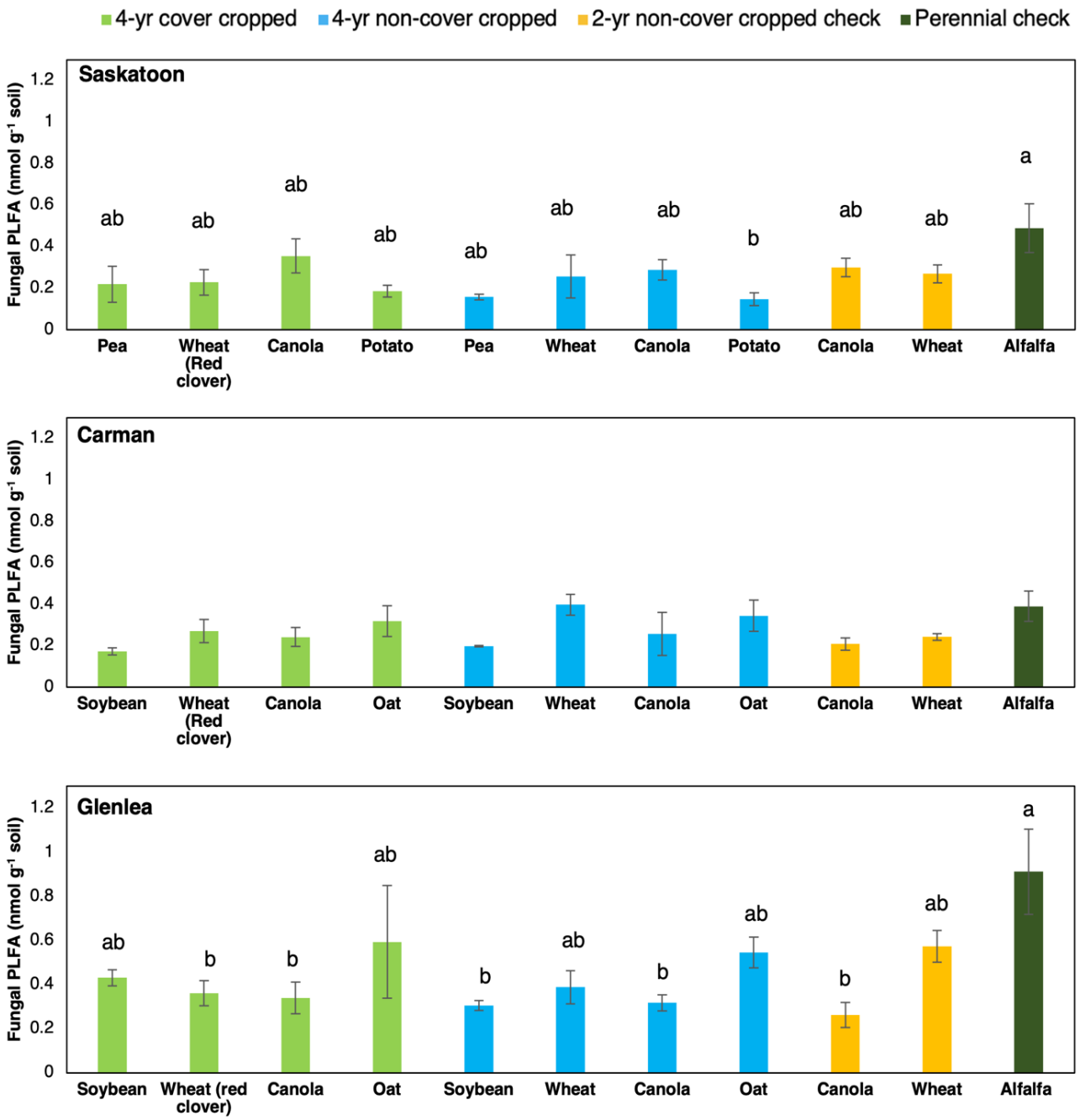

Fig 4.3: Fungal phospholipid fatty acid (PLFA) abundance at the Saskatoon, Carman, and Glenlea research sites in summer 2021. Crops listed were grown during the 2021 growing season; crops in brackets are the associated cover crops in select rotations growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

The effect of treatment on stress 2 indicators at Saskatoon and Carman was dependent on sampling time (Table 4.1). In Saskatoon at the fall 2020 and spring 2021 sampling times, there were no significant differences in stress 2 ratios between treatments (Fig. 4.4). During summer 2021 in Saskatoon, however, where short rotation canola was planted following short rotation wheat, stress 2 was lowest, followed by the perennial alfalfa and the four-year cover cropped canola plots (previously cover cropped wheat in 2020) (Fig. 4.4). The 2021 potato plots that were previously canola in 2020 had the highest stress 2 levels in Saskatoon in summer 2021 in both the cover cropped and non-cover cropped four-year rotations (Fig. 4.4). This is likely due to the high level of soil disturbance required to cultivate potatoes which can stress soil microorganisms.

At the Carman site, the perennial alfalfa treatment was significantly less stressed compared to the other treatments at fall 2020 and summer 2021 sampling times (Fig. 4.5). In spring 2021, cover cropped four-year rotation soybean plots, as well as perennial alfalfa, were least stressed (Fig. 4.5).

In Glenlea, there were no significant interactions between treatment and sampling time for stress 2 indicators, but the two factors were significant on their own (Table 4.1). Glenlea plots that had wheat in 2020 and canola in 2021 had the highest levels of stress regardless of cropping system, and the perennial alfalfa had the lowest stress 2 levels (Fig. 4.7). While the effect of treatment on stress 2 ratios in Glenlea during summer 2021 was significant (Table 4.2), the pairwise means comparisons did not pull out any one treatment over another (**Appendix D**). Perennial treatments tended to have the lowest stress 2 indices across sampling times and locations.

In terms of seasonal differences at all sites, stress 2 was highest in summer 2021 compared to the other sampling times (Figs. 4.4, 4.5, 4.6). It is likely that stress was highest in summer due to extreme drought conditions that occurred during summer 2021 in both Saskatchewan and

Manitoba, thereby impacting all research sites for this experiment. By mid-summer, when the summer 2021 samples were collected, soil water content was likely the lowest and microbial communities were stressed.

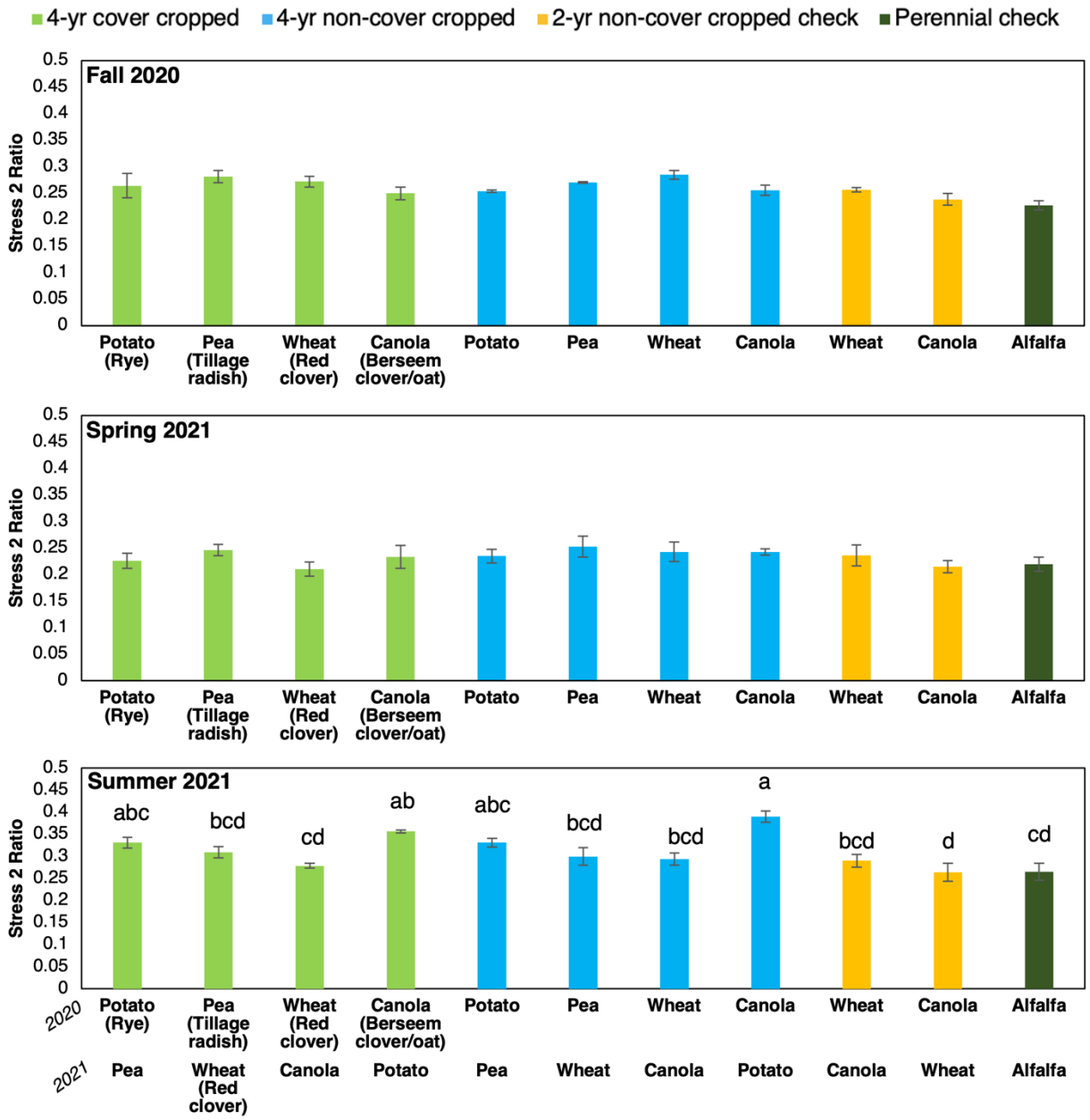

Fig 4.4: Stress 2 ratio at the Saskatoon site across sampling times. Crops listed were grown during the 2020 growing season, and both 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

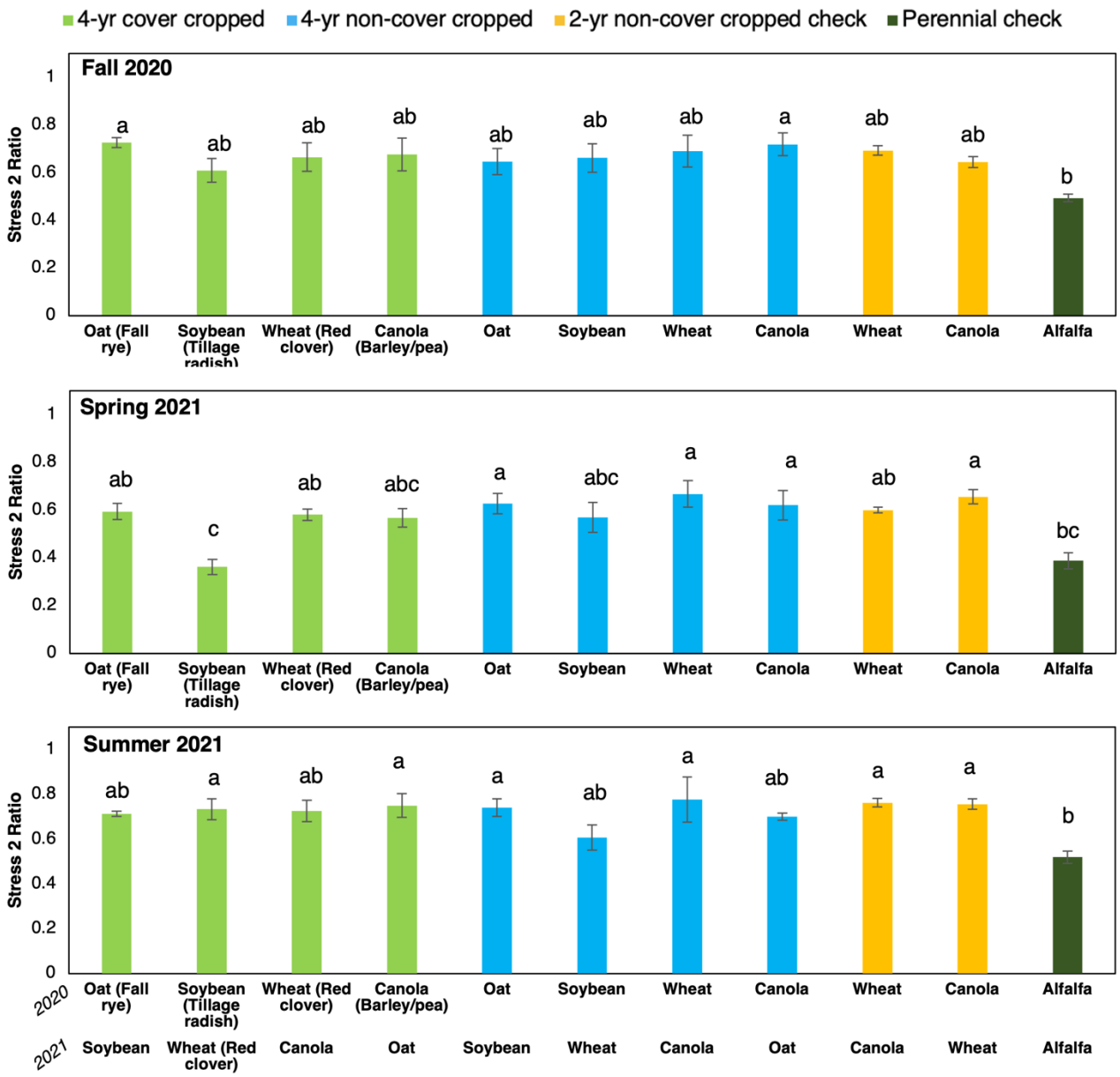

Fig 4.5: Stress 2 ratio at the Carman site across sampling times. Crops listed were grown during the 2020 growing season, and both 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

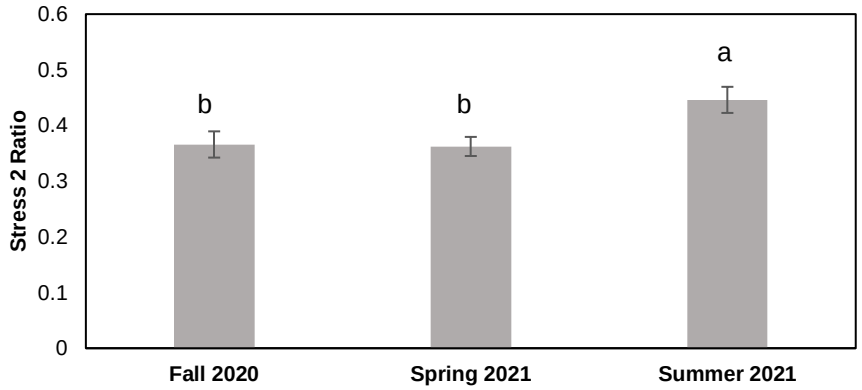

Fig 4.6: Stress 2 ratio at the Glenlea site across all sampling times. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

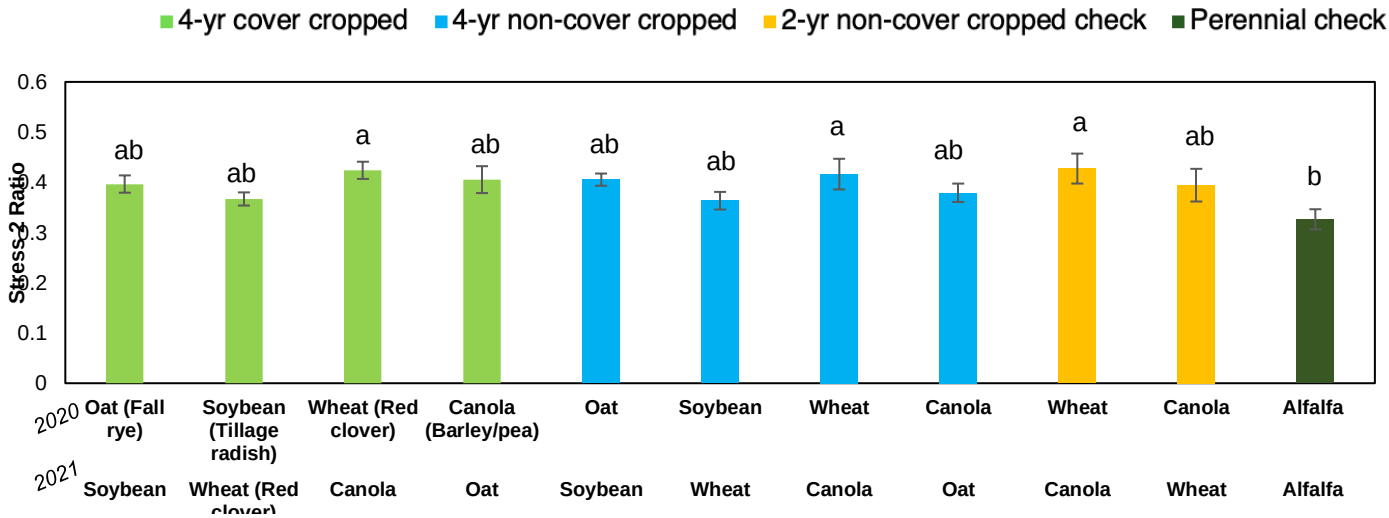

Fig 4.7: Stress 2 ratio at the Glenlea site averaged across all sampling times. Crops listed were grown during the 2020 and 2021 growing seasons; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

The Gram positive to Gram negative bacteria ratio (G+:G-) was significantly affected by treatment and sampling time at all three research locations (Table 4.1). In Carman, the interaction between the two factors was also significant, but not at the other two sites (Table 4.1). In Saskatoon and Glenlea, the G+:G- bacteria ratio was highest at summer 2021 sampling time (Fig. 4.1). This appears to be the case in Carman as well (Fig. 4.9). Although not statistically significant, there was a consistent trend where the perennial alfalfa treatment had a low G+:G- bacteria ratio across locations compared to the other treatments (Fig. 4.8, 4.9).

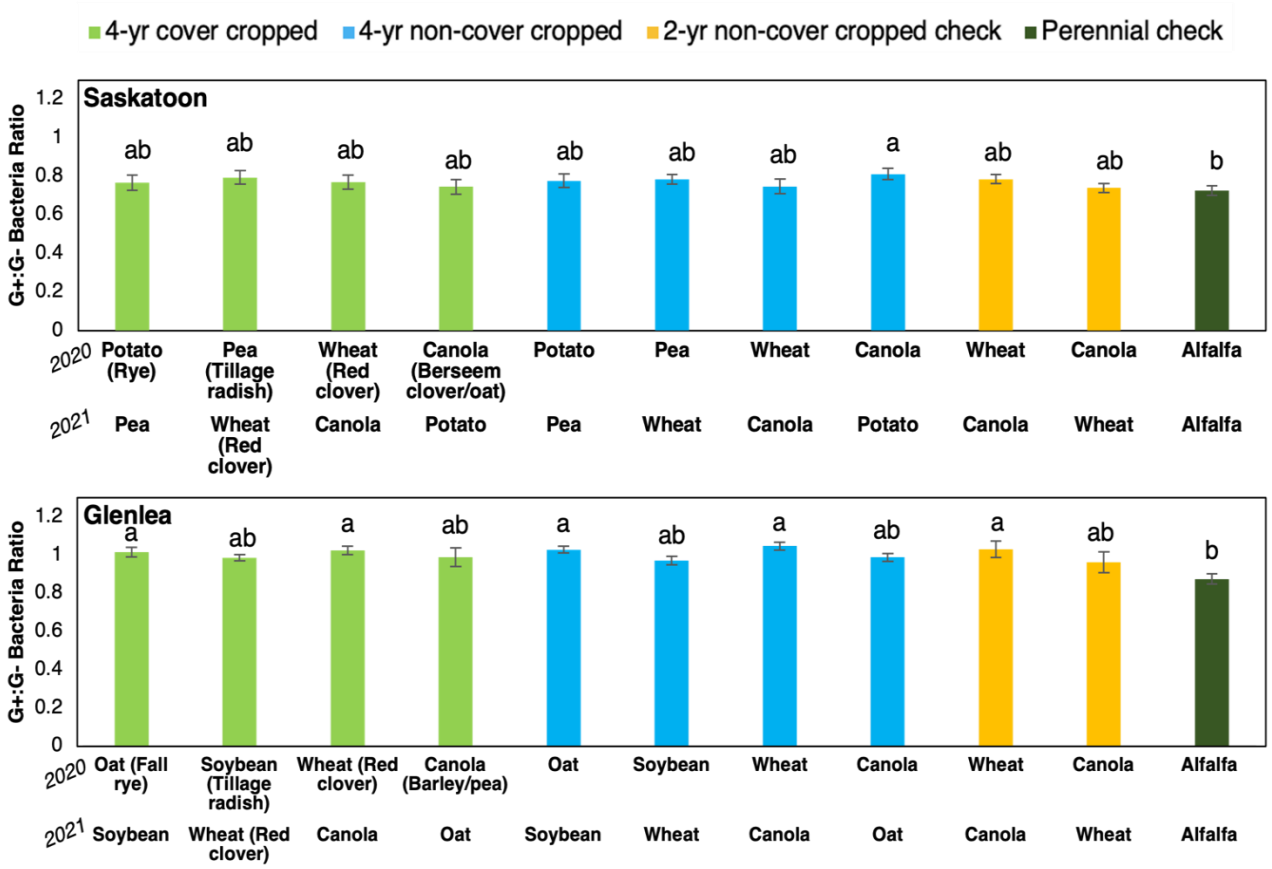

Fig 4.8: Gram positive to Gram negative bacteria ratio at the Saskatoon and Glenlea sites by treatment, averaged across sampling times. Crops listed were grown during the 2020 and 2021 growing seasons; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

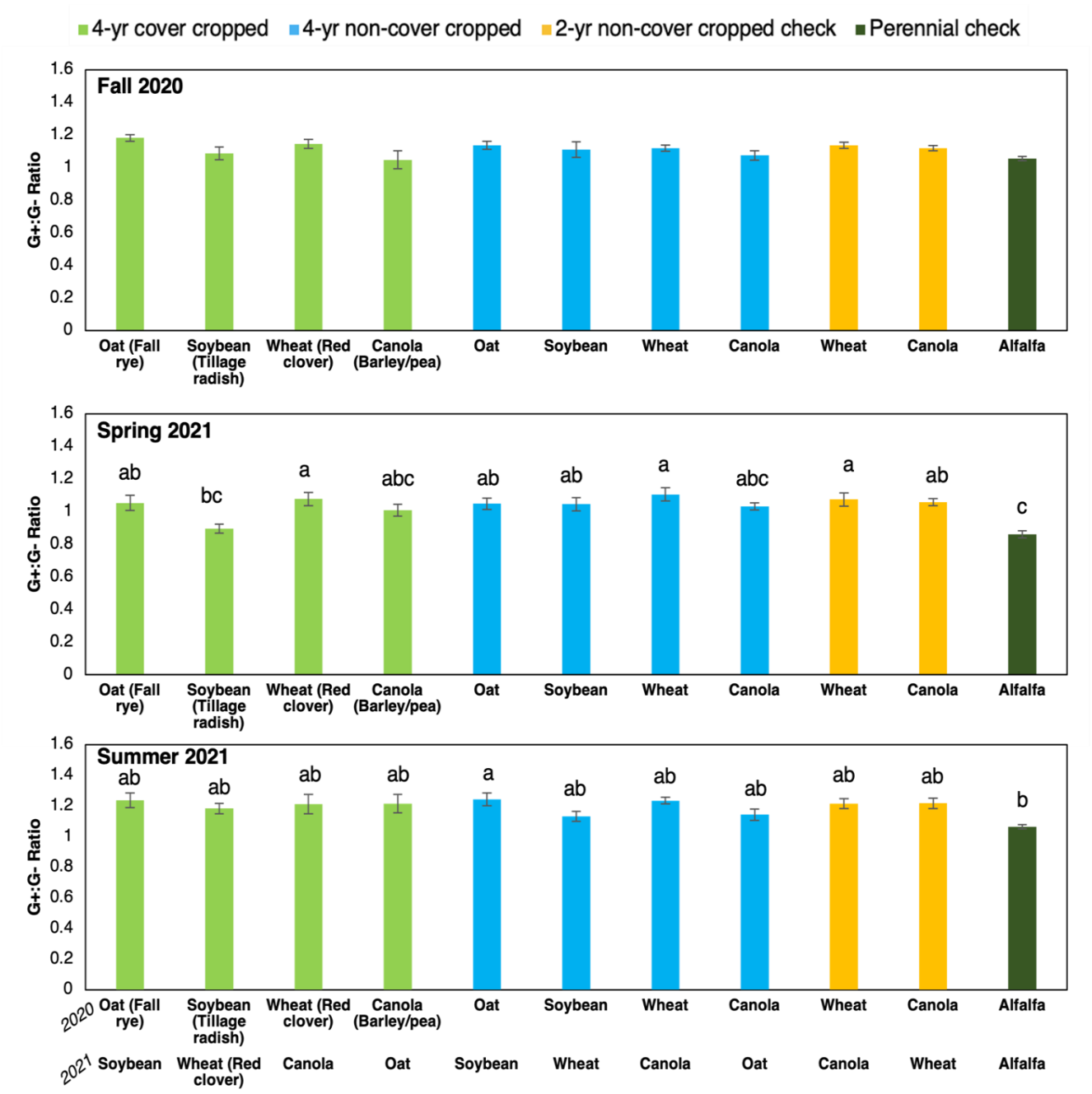

Fig 4.9: Gram positive to gram negative bacteria ratio at the Carman site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

4.2 Extracellular Enzyme Analysis

The extracellular activity of three enzymes were tested: alkaline phosphatase (AP), β glucosidase (βG), and N-acetyl-β-D-glucosaminidase (NAG). The activities of all three enzymes were affected by crop rotation treatment, sampling time, and their interaction at the Saskatoon and Glenlea sites (Table 4.3). At the Carman site, sampling time affected all three enzymes, and while there was a significant interaction between treatment and sampling time for AP activity at Carman, treatment on its own had no effect on enzyme activity in Carman (Table 4.3). However, when summer 2021 was analyzed independently from the other sampling times—taking the living crop into account—AP activity was affected by treatment in Carman (p=0.0015) (Table 4.4). In summer 2021, treatment significantly impacted the enzyme activity of all three enzymes tested at the Saskatoon site, whereas in Glenlea, treatment had no effect on the activity of any of the enzymes during summer 2021 (Table 4.4).

Table 4.3: Two-way ANOVA F-test results for the effect of crop rotation treatment (trt) and sampling time on extracellular enzyme activity at the Saskatoon, Carman, and Glenlea research sites at fall 2020, spring 2021, and summer 2021 sampling times; values are F-statistics and values in brackets are p values.

Location	Factor	df	AP[a]	βG[b]	NAG[c]
Saskatoon	Trt	10	3.279 (0.0048)**[d]	7.829 (<0.0001)***	6.532 (<0.0001)***
	Sample time	2	75.523 (<0.0001)***	90.470 (<0.0001)***	44.295 (<0.0001)***
	Trt × Sample time	20	9.838 (<0.0001)***	5.024 (<0.0001)***	4.881 (<0.0001)***
Carman	Trt	10	1.5968 (0.1511)	0.7641 (0.6612)	0.704 (0.7143)
	Sample time	2	60.2893 (<0.0001)***	5.6486 (0.0054)**	3.173 (0.0483)*
	Trt × Sample time	20	4.1168 (<0.0001)***	1.1737 (0.3045)	1.424 (0.1429)
Glenlea	Trt	10	2.1968 (0.0437)*	2.604 (0.0188)*	1.122 (0.3758)
	Sample time	2	2.5243 (0.0878)	32.764 (<0.0001)***	37.416 (<0.0001)***
	Trt × Sample time	20	2.9372 (0.0005)***	3.046 (0.0004)***	2.297 (0.0061)**

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[d] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table 4.4: One-way ANOVA F-test results for the impact of treatment on extracellular enzyme activity at the Saskatoon, Carman, and Glenlea research sites at the summer 2021 sampling time; values are F-statistics and values in brackets are p values.

Location	Factor	Df	AP[a]	βG[b]	NAG[c]
Saskatoon	Trt	10	11.005 (<0.0001)***[d]	4.7832 (0.0003)***	2.9286 (0.0100)**
Carman	Trt	10	3.896 (0.0015)**	0.8286 (0.6047)	0.8927 (0.5499)
Glenlea	Trt	10	1.0915 (0.3966)	0.7431 (0.6796)	0.7431 (0.6796)

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[d] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

In Saskatoon, the greatest differences between treatments in AP and βG enzyme activity occurred in summer 2021 compared to fall and spring sampling time (Figs. 4.10, 4.11). In summer 2021, AP activity was higher in the perennial alfalfa treatment and the short rotation that was planted into wheat in summer 2021 and canola the previous year compared to all of the non-cover cropped long-rotations, but not compared to any of the cover cropped rotations (Fig. 4.10). However, there was no similarly consistent positive effect of cover crops to AP in the fall or spring, indicating that the cover crop effect took time. The non-cover cropped pea plots, which had previously been in potato, had significantly lower AP activity than the other treatments (Fig. 4.10). Although not statistically significant, it is interesting to note that the legacy of disturbance from the 2020 cover-cropped potato treatment appears to have waned by the summer 2021 sampling time (Fig. 4.10). Treatment differences in βG enzyme activity were most apparent in spring 2021 compared to the other sampling times, when perennial alfalfa supported the highest activity (Fig. 4.11). By summer (July) 2021 when all crops were actively growing, treatment differences were not as strong. In summer 2021, βG enzyme activity was lowest in the plots that were planted into potatoes in summer 2021—in both cover cropped and no cover crop treatments (Fig. 4.11). Potato planting, cultivation, and harvest is highly disruptive to the soil environment and is a likely

contributor to the low enzyme activity in these plots. In spring and summer 2021 in Saskatoon, perennial alfalfa plots had the highest NAG enzyme activity (Fig. 4.12).

In Carman, AP activity was not significantly different between treatments in fall 2020 or summer 2021, but AP activity was highest and most differentiated between treatments in spring 2021 (Fig. 4.13). Activity for AP was higher for the perennial alfalfa treatment compared to the short rotation non-cover cropped treatments. βG enzyme activity was lowest in summer 2021 and highest in spring 2021 (Fig. 4.14). NAG enzyme activity in Carman was sensitive to sampling time, but not to treatment (Table 4.3). Overall, seasonal variability appears to be less in Carman compared to Saskatoon. Additionally, the magnitude of EEA at Carman was substantially less than at Saskatoon and Glenlea for all enzymes tested.

Alkaline phosphatase EEA was most different between treatments in Glenlea in fall 2020. In the fall at Glenlea the wheat plot cover cropped with red clover had especially low AP EEA (Fig. 4.15). Perennial alfalfa had the highest enzyme activity for all three enzymes in the fall (Fig. 4.15, 4.16, 4.17). There were no treatment effects in spring 2021 or summer 2021 for any of the three enzymes tested in Glenlea (Fig. 4.15, 4.16, 4.17).

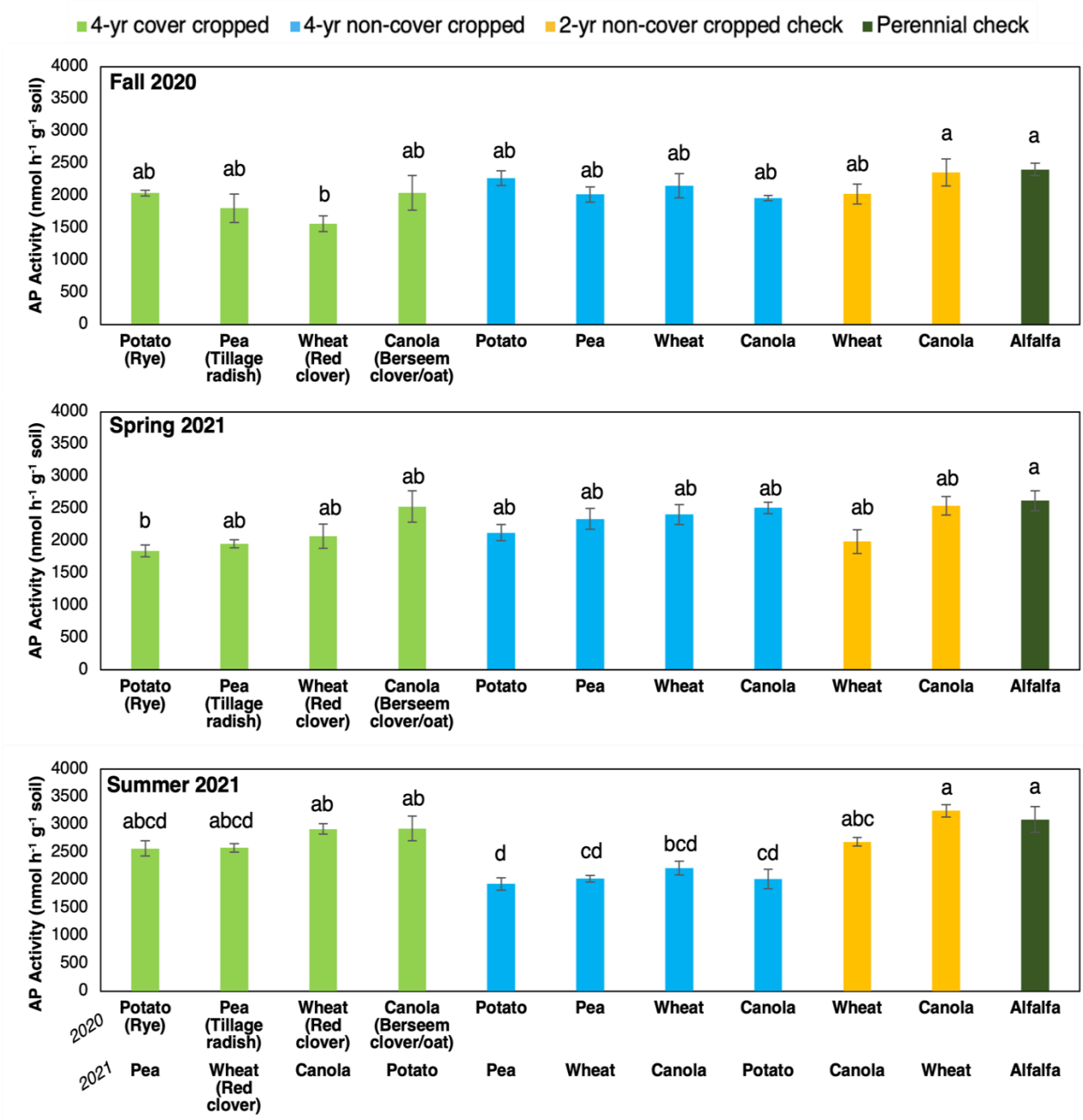

Fig 4.10: Alkaline phosphatase enzyme activity at the Saskatoon site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

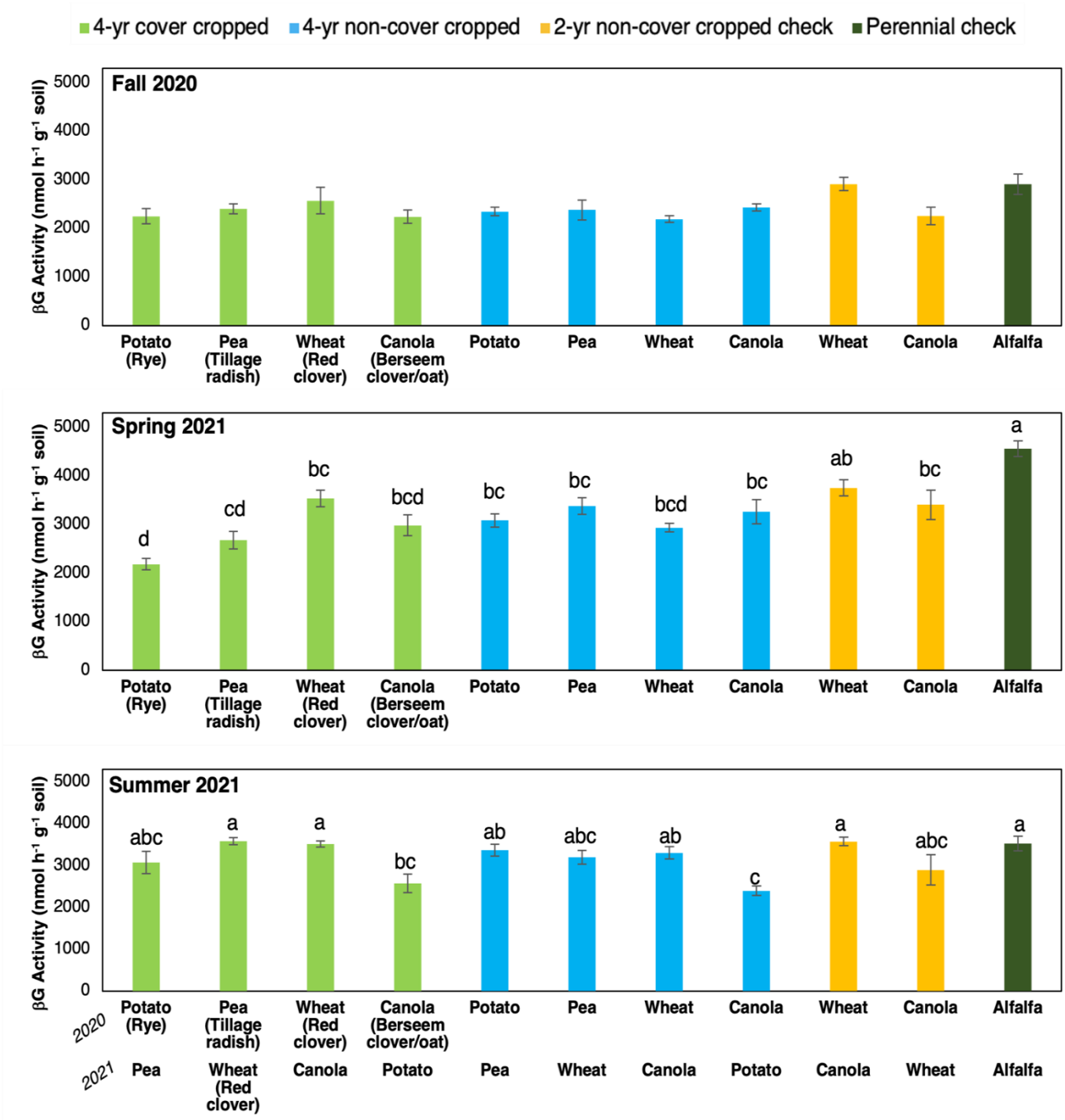

Fig 4.11: βG enzyme activity at the Saskatoon site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

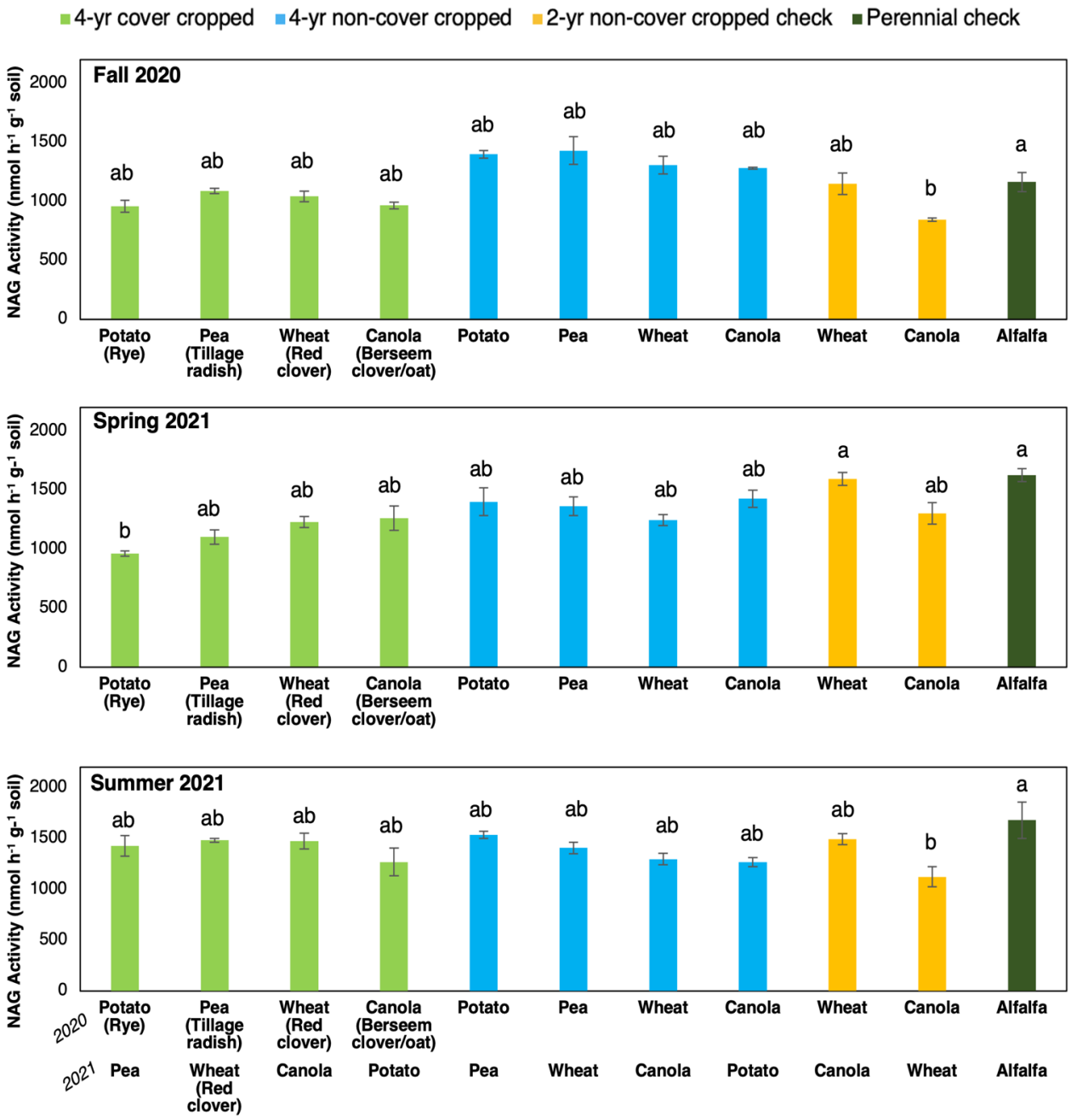

Fig 4.12: NAG enzyme activity at the Saskatoon site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, o or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

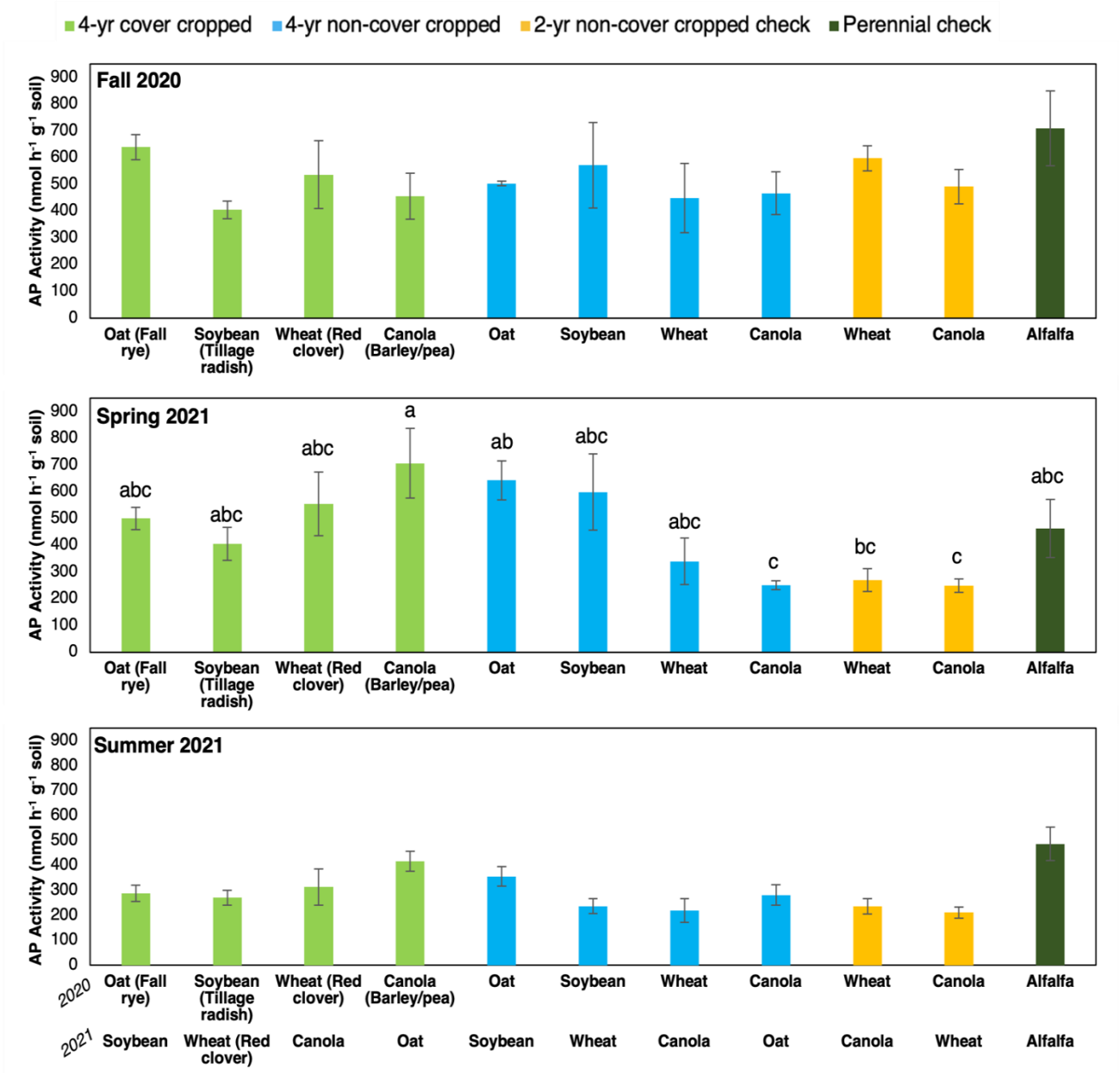

Fig 4.13: Alkaline phosphatase enzyme activity at the Carman site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

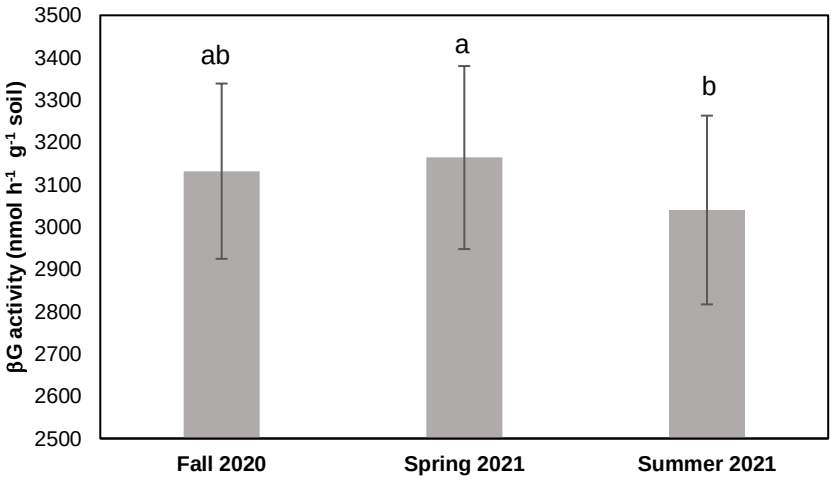

Fig 4.14: βG enzyme activity at the Carman site at each sampling time. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$.

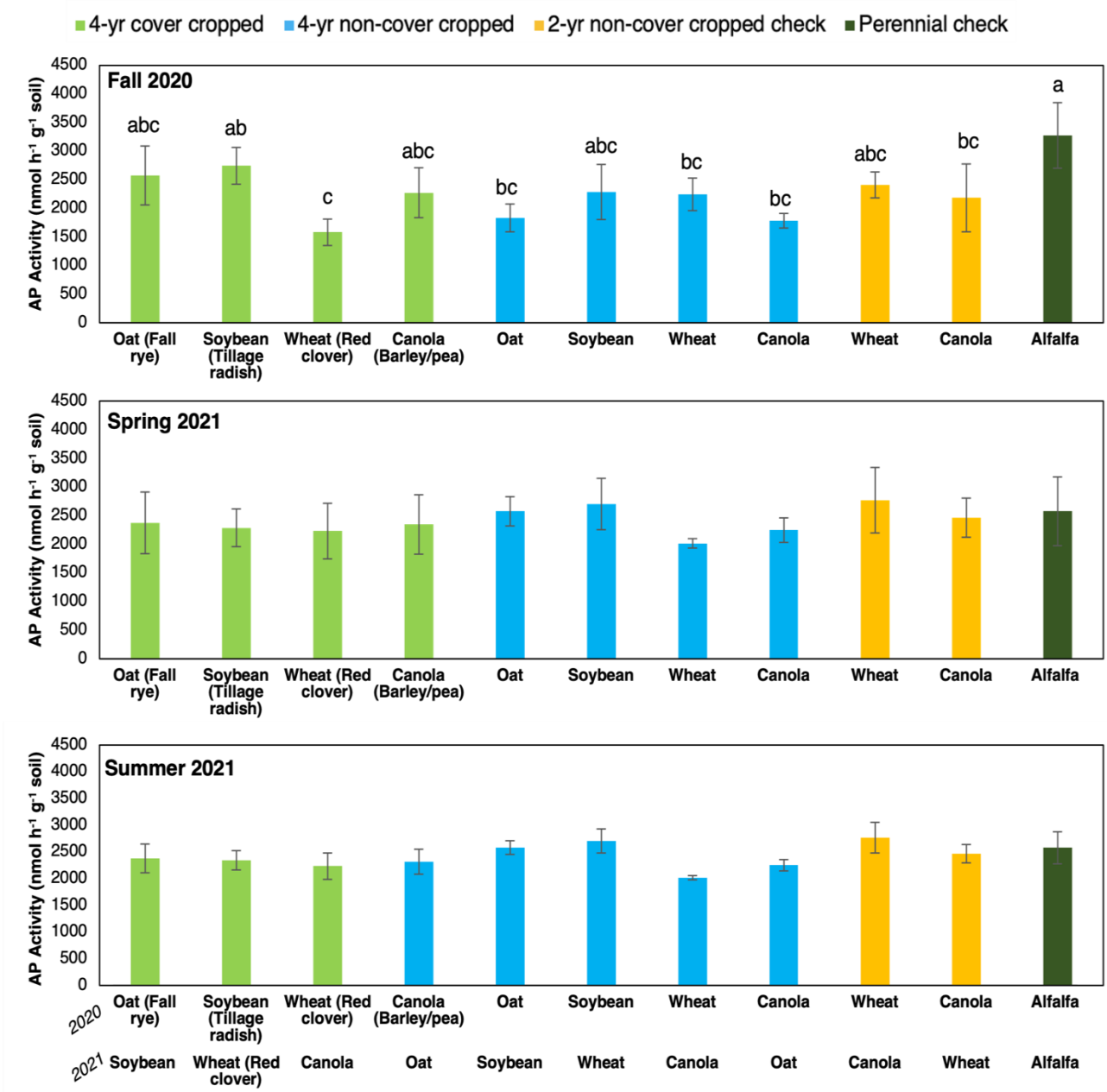

Fig 4.15: Alkaline phosphatase enzyme activity at the Glenlea site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

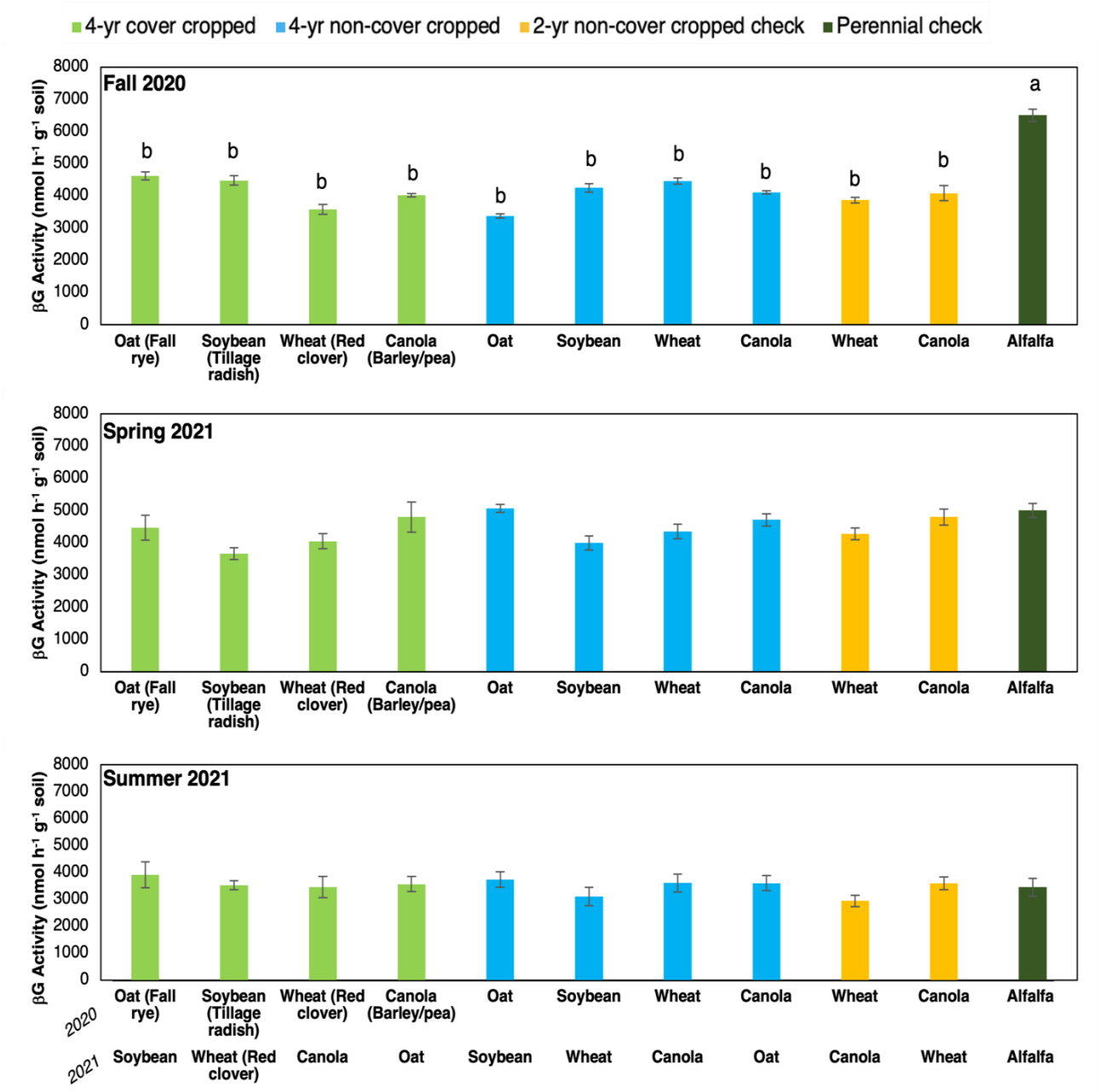

Fig 4.16: βG enzyme activity at the Glenlea site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

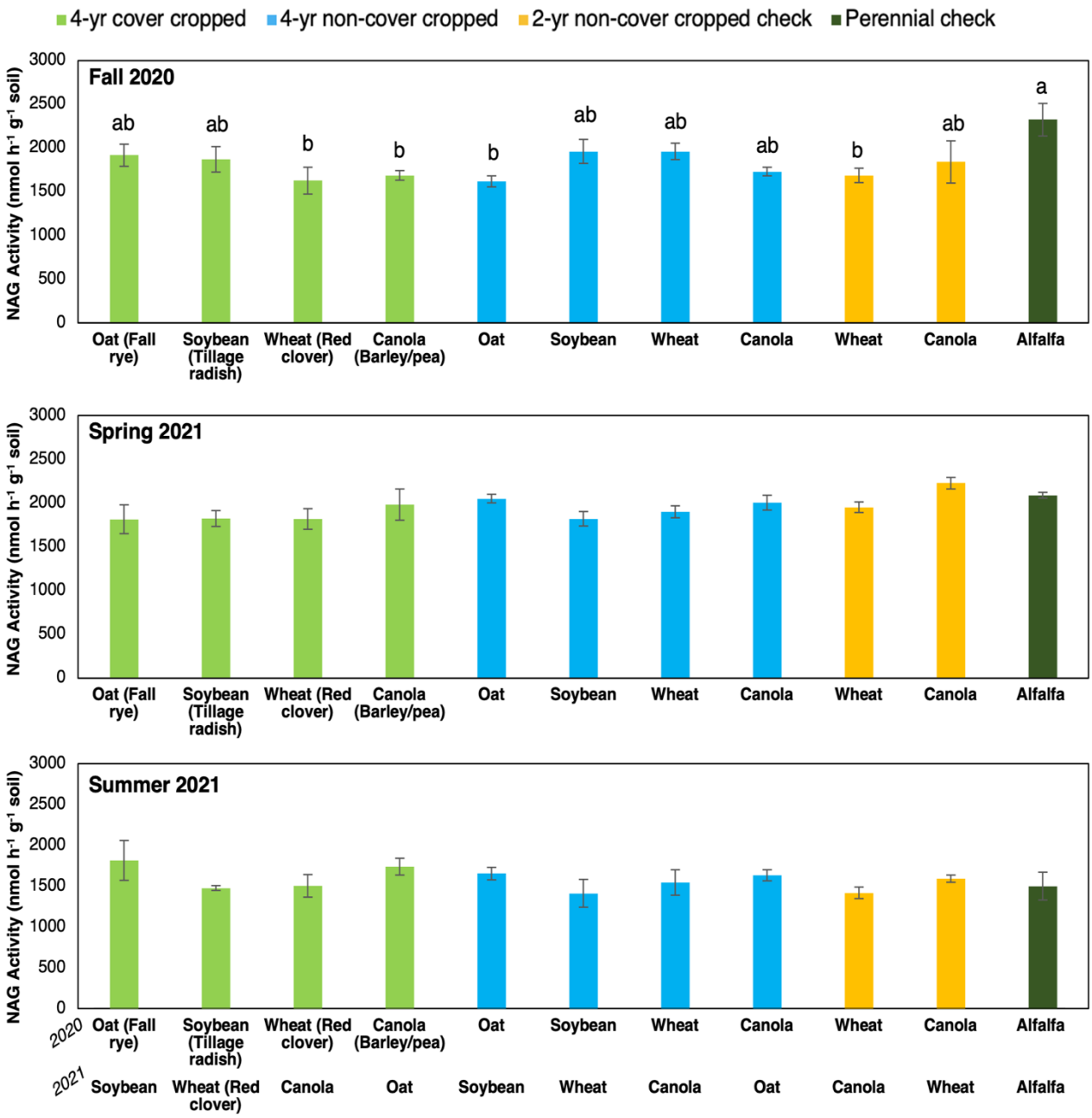

Fig 4.17: NAG enzyme activity at the Glenlea site across sampling times. Crops listed were grown during the 2020 growing season and the 2020 and 2021 growing seasons for summer 2021; crops in brackets are the associated cover crops grown in select rotations, or growing at the time of sampling. Different letters above bars indicates significant differences between means according to Tukey's HSD, $p<0.05$. No letters indicates no significant differences.

4.3 Correlation Analysis

Correlations between PLFA biomarkers (total PLFA, general bacteria, and fungi) and extracellular enzyme activities (βG, NAG, and AP) at all locations were low, where low is considered any relationship with an r value of < ±0.5 (see **Appendix E**). There were no significant correlations between NAG enzyme activities and PLFA biomarkers tested, whereas almost all AP correlations with PLFA biomarkers—while low—were significant (Table A14). βG correlations were consistently low and sometimes insignificant (Table A14). There was a consistent and significant low negative correlation between the stress ratio and fungal abundance at Saskatoon (r = -0.642), Carman (r = -0.383), and Glenlea (r = -0.293) at $p < 0.0001$. Plots with high fungal abundance tended to have lower stress 2 ratios and vice versa. βG and NAG enzymes followed similar trends to each other. The activities of these two enzymes had a positive correlation as confirmed with a Spearman's rank correlation test, with r values of 0.720, 0.754, and 0.880 at the Saskatoon, Carman, and Glenlea sites respectively (Table 4.5). AP activities were not strongly correlated with βG or NAG activities (Table 4.5). This suggests that nitrogen and labile organic matter cycle similarly, and that phosphorous cycling behaves differently.

Table 4.5: Spearman's rank correlation analysis between the activities of each enzyme against each other at the Saskatoon, Carman, and Glenlea research sites across all sampling times; values are Spearman correlation coefficients (R-values) and values in brackets are p values.

Location	βG[a] and NAG[b]	βG and AP[c]	NAG and AP
Saskatoon	0.720 (<0.0001)***[d]	0.429 (<0.0001)***	0.294 (0.0006)***
Carman	0.754 (<0.0001)***	0.465 (<0.0001)***	0.248 (0.0042)**
Glenlea	0.880 (<0.0001)***	0.248 (0.0042)**	0.164 (0.0606)

[a] βG = β-glucosidase
[b] NAG = N-acetyl-β-D-glucosaminidase
[c] AP = alkaline phosphatase
[d] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

4.4 General Trends

In general, perennial alfalfa plots had higher/highest F:B PLFA ratios and low/lowest stress 2 ratios, as well as tended to have the highest fungal PLFA abundance. Perennial alfalfa also tended to have higher enzyme activity than the other treatments. In some cases, canola and wheat performed oppositely, wherein canola had the highest PLFA abundance or enzyme activity for some biomarker categories, and wheat plots in the same crop rotation had the lowest recorded PLFA abundance or enzyme activity. Additionally, plots inclusive of canola in the 2020 or 2021 seasons tended to have higher stress 2 indices, however this was generally not statistically significant. Stress 2 ratios tended to be higher and more differentiated in the summer season across research locations when compared to fall and spring sampling times. G+:G- ratios did not reveal consistent trends across treatments, with the exception of the perennial alfalfa which tended to have low G+:G- bacteria ratio values. Plots with higher fungal abundance tended to have lower stress 2 ratio levels. βG and NAG enzyme activities were closely correlated with each other at all research locations. Sampling time was consistently a significant factor for both PLFA and EEA results at all research sites and across almost all parameters measured. However, differences attributed to seasonality were not the same across sites. The cover cropped treatments did not consistently perform differently than the non-cover cropped treatments for both PLFA analysis and EEA analysis.

5.0 DISCUSSION

5.1 Cover crops had no effect on soil microbial communities in the semi-arid prairies in the short-term

Cover cropping is a core soil health practice (Blanco-Canqui et al., 2015; Van Eerd et al., 2023) and has also been touted as an important nature-based climate solution that can mitigate CO_2 emissions, especially in the Canadian prairies (Drever et al., 2021). My research investigated these predictions by understanding soil microbial communities and the processes underpinning soil C and biogeochemical dynamics. However, cover crops had no influence on the abundance or composition of soil microbial communities nor on biogeochemical cycling based on extracellular enzyme activities (refer to **Appendix E**). The findings from my research are in opposition to a meta-analysis conducted by Muhammad et al., (2019), which found that cover cropping increased total PLFA abundance by 24% compared to not cover cropping. While microbial parameters related to soil C and soil N were found to vary with soil and climate conditions, this was not true of PLFA abundance (Muhammad et al., 2019). Interestingly, the meta-analysis also revealed that microbial community abundance and diversity decreased with increasing levels of precipitation (Muhammad et al., 2019). The meta-analysis analyzed findings from 21 different countries, however, it is perhaps important to note that there were no Canadian sites, let alone sites in the Canadian prairies. While the limited sampling timepoints in my experiment do not allow me to explore the impact of precipitation on microbial communities in depth, I can speculate that we would see different results in the Canadian prairies. Since water is the main limiting factor to crop production in semi-arid environments such as the prairies, perhaps the lower soil water content in semi-arid environments also limits microbial abundance. This could also be indirectly related to lower levels of cover crop plant biomass that we tend to experience in the Canadian prairies compared to more humid agroecosystems (Otchere et al., 2022), since higher cover crop biomass

enhances the associated effects that can be measured belowground (Strickland et al., 2019). At the Saskatoon site, cover crop biomass amongst all plots in the 4-year cover crop phase of the experiment in spring 2020 ranged from 90 – 428 kg ha^{-1} (Otchere et al., 2022). This is far below the target springtime aboveground cover crop biomass of >1000 kg ha^{-1} at which Hively et al. (2009) suggest ecosystem services can be expected to be imparted on the soil environment from the cover crops.

While the amount of research on cover crops in semi-arid environments is limited, where they have occurred, studies found no differences in soil health measurements between cover cropped and non-cover cropped plots in the short term (Bielenberg et al., 2023) nor in the long-term (Singh & Kumar, 2021). I observed similar results, wherein cover cropped treatments did not perform differently than annual rotation treatments that did not include cover crops. As such, prairie growers might not expect to see changes in biological soil health metrics within the first couple years of growing cover crops as a result of the practice. Climatic conditions and hence length of cover crop adoption play an important role when it comes to the impact that cover cropping has on soil health (Bielenberg et al., 2023; Van Eerd et al., 2023; Peng et al., 2023). Growers in more temperate environments may expect to see changes in soil health caused by cover crops within a shorter timeframe (McDaniel et al., 2014; Muhammad et al., 2019). This being said, anecdotally, 70% of prairie growers surveyed reported positive impacts from growing cover crops within three years of implementing the practice – although it should be noted these benefits are not restricted to soil health parameters (Morrison, 2021).

Similar to perennials, cover crops encourage growth and shifts in soil microbial communities through their increased inputs to the soil compared to annuals, such as through root exudates, which act as an energy source for soil microbes (Finney et al., 2017). As such, it is likely

that the benefits expected from cover cropping in my study were constrained by the short period of time with cover crops implemented, limited biomass accumulation (Otchere et al., 2022; Strickland et al., 2019), and related restrictions in belowground inputs that would have otherwise supported microbial growth and activity (Strickland et al., 2019). Future projects may find it insightful to look closely at fungi, F:B ratios, and stress 2 ratios to understand the effects of short-term management changes on soil microbial communities in semi-arid environments since these are the parameters in which I noticed the greatest changes in the short-term. Further, this study serves as a benchmark to compare results to in a longer-term cover cropping study at the same sites.

While it remains unclear whether cover cropping will increase cash crop productivity in prairie Canada, it is important to note the value of other cover cropping benefits including mitigating erosion, and enhancing biodiversity, which occur regardless of climatic ecozone (Van Eerd et al., 2023).

5.2 Perennialization had the strongest effect on soil biological properties

Cover crops extend the time that a living root system is in the ground—which is important for building soil health (Van Eerd et al., 2021)—and more closely approximates a perennial system (Otchere et al., 2022). In my study, perennial alfalfa was included as a check treatment—anticipated to have the strongest effects on biological properties—against the annual cover cropped treatments. Indeed, the perennial treatment outperformed annual treatments, including those that had cover crops in rotation, for a number of PLFA biomarker groups and EEAs. Most notably, perennial plots in my study tended to have the highest fungal PLFA abundance compared to the rotation treatments.

Significant positive relationships have been observed between increased soil fungi and the quantity and quality of SOM (Six et al., 2006). These relationships, specifically the tendency towards fungal dominance, tends to be stronger in less disturbed soils (Six et al., 2006), such as perennial systems. Essentially, higher fungal abundance in perennial systems (Six et al., 2006) is at least partially driven by the increased time with living roots in the soil which provide a constant energy input, in the form of C, via root exudates and plant litter decomposition (Van Eerd et al., 2021; Arcand et al., 2016). This suggests that, in my study, perennial treatments may have the capacity to develop more SOM, and SOM of a higher quality, than annual treatments. Further, fungi are slower-growing than bacteria (Taylor & Sinsabaugh, 2015) and thus fungi have a greater chance for growth in perennial versus annual systems.

Perennial cropping systems are less intensively managed than annual systems (Mann et al., 2019), and tend to have higher PLFA abundance in the fungal, AMF, and G- biomarker groups, higher soil microbial biomass, and higher SOC compared to more intensively managed plots, including rotation and row crop treatments (Mann et al., 2019; Alagele et al., 2020). Specifically, soil disturbance is known to decrease F:B ratios: for example, Stevenson et al. (2014) observed lower F:B ratios in plots eight years after a single tillage event, versus plots that had not been tilled. The potato plots in Saskatoon were the most intensively managed due to planting, hilling, and harvest requirements and had the lowest fungal PLFA abundance. There is an inverse relationship between soil disturbance and fungal abundance, where fungal abundance deceases with increasing disturbance (Martensson & Olsson, 2012). This is likely due to the physical interruption of fungal hyphae caused by soil disturbance (Martensson & Olsson, 2012), such as that which occurs during potato hilling. Overall, soils growing perennial crops tend to support greater soil microbial biomass than those with annual crops (Mann et al., 2019; Milne & Haynes, 2004; Alagele et al., 2020),

likely due in part to the less intensive management (Stevenson et al., 2014; Mann et al., 2019). These findings suggest improved resilience and more active microbial communities in perennial treatments compared to annual rotation treatments.

Indeed, perennialization can buffer against abiotic stress (Yang et al., 2014; Eziz et al., 2021). In my study, stress 2 ratios—commonly associated with abiotic stresses (Grogan & Cronan, 1997)—tended to be lowest in perennial treatments. Stress 2 is referred to as the water stress indicator by some researchers (Guckert et al., 1986; Lundquist et al., 1999; Diedhiou-Sall et al., 2021). Established fungi produce hyphal networks that extend beyond the rooting zone of the plants and enable the plants to scavenge nutrients and water from a larger soil volume, improving their ability to cope with stress, including water stress (Taylor & Sinsabaugh, 2015; Diedhiou-Sall et al., 2021). Indeed, I found that fungal PLFA abundance and stress 2 ratios were negatively correlated at all research locations. Thus, greater fungal abundance in the soil may also, theoretically, increase the ability of a soil ecosystem to cope with environmental stresses—including water stress (Guckert et al., 1986; Lundquist et al., 1999, Diedhiou-Sall et al., 2021), specifically drought which was prominent in summer 2021 at all research locations.

Gram positive to G- bacteria ratios can give an indication as to the relative C available in the soils for bacterial communities to use (Fanin et al., 2019). G- bacteria use simpler, more labile C sources that are plant-derived as their primary energy source, whereas G+ bacteria is more stress-resistant and uses more recalcitrant C sources that are derived from SOM (De Vries & Shade, 2013; Fanin et al., 2019). Fanin et al. (2019) found that when tree roots and shrubs were removed from their study location, the G+:G- ratio increased, indicating that the G+ bacteria thrived in that environment. They determined that the removal of the plant matter limited the plant-derived C input into the soil, depriving the G- bacteria from its primary energy source (Fanin et al., 2019).

In my research, G+:G- ratios were higher in perennial treatments (fig. 4.8, 4.9) and also tended to be highest in summer (fig. 4.1). These findings, in combination with stress ratio results and fungal abundance results, suggest that the perennial treatments are more resilient than the annual rotation treatments, including those that had cover crops. Seeing as summer 2021 was a drought year at all sampling locations, the higher G+:G- ratios in summer supports the idea that G+ bacteria out-perform G- bacteria in harsh environments (De Vries & Shade, 2013).

While cover crops, as an integral component of a rotation in the long-term, may emulate perennial treatments more than non-cover cropped annual treatments, the cover cropped treatments in this study did not achieve the same superior performance as the perennial crop. Some studies categorize five years of cover cropping as short-term, and expect to see treatment effects in much longer timeframes—sometimes closer to 20 years (Blanco-Canqui, 2022). With only two to three years of cover crop inclusion in the annual rotations at the time of sampling at the Saskatoon and Carman sites, and only one to two years at the Glenlea site, this study is considered a short-term cover cropping project; stronger treatment effects may be expected over a longer timescale and support the need for long-term rotation trials (Norris, Gorzalek et al., 2023). Currently, long-term cover crop studies are severely limited in number (Van Eerd et al., 2023), and tend to report differing results depending on location. While some studies show positive responses in soil health parameters as a result of cover cropping within as little as one year (Mukherjee & Lal, 2015; Strickland et al., 2019), others take much longer, or simply do not find significant differences attributed to cover cropping (Singh & Kumar, 2021; Bielenberg et al., 2023). In their meta-analysis, Bai et al. (2019) found increasing responsiveness of SOC content to cover cropping with increasing time in cover crops. My results align with the former groups, where cover cropped treatments did not show consistent differences compared to their non-cover cropped counterparts.

5.3 Seasonality affected soil biological activity and abundance in the semi-arid growing environments

Seasonal shifts in soil biology are often attributed to changes in abundance of root exudates and the seasonality of available soil nutrients as a function of climate (Stevenson et al., 2014; Zuo, et al., 2023), which is closely linked with the soil health principle of living roots in the ground (Van Eerd et al., 2021). Specifically, several researcher groups have hypothesized that seasonal shifts in soil bacterial community structure are due to the changes in C compounds produced by plant roots in the rhizosphere over the course of the year, as well as temporal changes in plant litter deposition (Bardgett et al., 1997; Grayston et al., 2001; Kennedy et al., 2005). In some cases, seasonality affected the structure of soil bacterial communities, but the effects were inconsistent (Kennedy et al., 2005). Some studies that looked more broadly at soil microbial biomass found microbial activity and abundance to be highest in summer and lowest in winter (Bardgett et al., 1997), while others found microbial abundance to be unresponsive to seasonality, but found microbial activity to be responsive and highest in the spring (Grayston et al., 2001). Bell et al. (2010) found microbial biomass and enzyme activity to be highly responsive to seasonality, but enzyme activity was not responsive to treatment, which is similar to results found in my study.

In my study, plots were sampled over a full growing season arc: fall 2020 post-harvest, spring 2021 pre-plant, and summer 2021 at mid growing season. Similar to Kennedy et al. (2005), I found that, while sampling time was always a significant factor ($p<0.0001$) for total PLFA and EEA for all enzymes (except AP EEA in Glenlea), the patterns in seasonal shifts were not consistent across locations. Total PLFA abundance was greatest in spring in Saskatoon, fall in Carman, and summer in Glenlea (Fig. 4.1), with bacteria following the same trends. Fungal PLFA abundance tended to be lower in the summer than in fall and spring (Fig. 4.1). Microbial activity,

as approximated through EEA, was highly inconsistent and generally not statistically significantly different but appeared to be higher in summer in Saskatoon, and lower in summer in Carman and Glenlea.

When considering climatic reasons that may help to explain the seasonal differences at my three research locations I thought about precipitation, seeing as this is a limiting factor in prairie agroecosystems. Carman had substantially more rainfall in June 2021 compared to Saskatoon and Glenlea (Figs. 3.1, 3.2, 3.3). Some studies suggest a positive correlation between soil moisture and PLFA abundance (Wu et al., 2016), so we might expect to see an increase in total PLFA in Carman because of the increased rainfall in June. However, this difference is not reflected in the PLFA or EEA data for the Carman site – in fact, summer PLFA and EEA was never higher than other sampling times in Carman. This suggests that the higher June rainfall in Carman did not lead to increased soil microbial abundance nor activity, or that perhaps the post-rainfall flush in microbial activity was not captured since soil sampling occurred several weeks later in July. Perhaps changes in soil microbiology would have been observed had a similar situation occurred in a more temperate ecozone (Wu et al., 2016).

Aside from precipitation, factors related to seasonality that may have impacted microbial abundance and activity in my research was the availability of plant residue and root exudates. In Saskatoon, AP activity appeared to be highest and most differentiated between cover cropped versus non-cover cropped treatments in summer 2021 (Fig 4.10). This would have been when plant roots were largest and most active, and thus root exudates are likely to have been most available. However, the most AP EEA differentiation in Carman and Glenlea occurred in spring 2021 and fall 2020 respectively, which is not when I would expect root exudates to be most available.

It is also interesting to note the differences in magnitude of EEA between sites: EEA activity in Carman tended to be much lower than in Saskatoon and Glenlea. Soils in Saskatoon and Glenlea had near-neutral pH levels of 7.13 and 7.05 respectively, while Carman had acidic soils of pH 4.15 (Table 3.4). According to Sinsabaugh et al. (2008), there is a significant relationship between EEA and the pH of the soil. Since EEA was analyzed at the same pH for all locations, and since the pH of Saskatoon and Glenlea soils was similar, while the pH of soils at Carman were much lower, it is likely that the differences in magnitude of EEA between sites was at least partly due to the effect of soil pH.

5.4 Soil microbial abundance was not associated with biochemical cycles

In general, soil microbial biomass is positively related to soil biochemical nutrient cycling (McDaniel et al., 2014; Otchere et al., 2022). This relationship is potentially driven by increased diversity in rotation and increased soil cover, both strategies to which cover cropping is, theoretically, a positive contributor (McDaniel et al., 2014; King & Hofmockel, 2017; Otchere et al., 2022). In a meta-analysis, McDaniel et al. (2014) found that more diverse cropping systems, especially those which included cover crops in rotation, had superior soil C and N pools compared to more simple rotations.

However, in my study, this relationship between microbial biomass and nutrient cycling potential—as determined by extracellular enzyme activity—was not observed. PLFA abundance and EEAs were not strongly or significantly correlated in most cases. This suggests that, in my study, soil microbial abundance—represented by PLFA data—and nutrient cycling in the soil, which was proxied by EEA data (Joergensen et al., 1995), were governed by different factors. Otchere et al. (2022) proposed that, overtime, cover cropped and perennial systems would improve mineralizable N pools in soil. Perhaps, then, the limited duration of my study constrained the potential for such relationships between biomass and nutrient cycling to be realized.

Looking specifically at EEA, I found that βG and NAG EEAs, which represent C and N cycling respectively (Table 3.5), were moderately to strongly correlated with each other. The activity of AP, however, which is a proxy indicator for soil P cycling, was not strongly correlated with the activities of βG nor NAG. This data suggests that P cycles differently than C and N do in soils. A study involving soil health indicators in a dryland agriculture environment found similar results: they concluded that βG and NAG EEAs were both positively related to crop yield (and

thus each other), but that P-cycling EEA's – although related to some other soil health indicators – were not correlated with crop yield nor with βG or NAG activity (Sainju et al., 2022).

5.5 Soil health parameters were site dependent

Differences in climate and soil physico-chemical properties have a significant impact on soil microbial diversity and soil health parameters (Griffiths & Philippot, 2013). Some studies concluded that soil texture, specifically, has a significant impact on soil pH and total PLFA abundance, among other parameters (Zhu et al., 2021). In some studies, researchers found that soils with higher clay content tend to have higher total PLFA abundance (Muhammad et al., 2021; Zhu et al., 2021). There is also evidence to support the claim that PLFA composition was impacted by the pH of the soil but that PLFA abundance was not affected (Rousk et al., 2010). In the case of Rousk et al. (2010), AMF and G- bacteria abundance increased with increasing pH and G+ bacteria decreased with increasing pH (Rousk et al., 2010). Clearly, it is important to be cautious when making comparisons in soil microbial data between sites since site-specific properties, such as soil pH, texture, and climate, can be confounding.

I had similar findings in my research at the summer sampling time, wherein Glenlea, the research site with the highest clay content (Table 3.4), tended to have the highest total PLFA abundance compared to the other sites (Fig. 4.1). Not surprisingly, SOM content appeared to be a key driver in relation to total PLFA abundance (Fig 4.1). Higher SOM was correlated to higher total PLFA abundance. Glenlea, was the research site with the highest SOM content and it also had the highest PLFA abundance compared to the Saskatoon and Carman sites, which had significantly lower SOM (Table 3.4, Fig. 4.1).

However, the pH trends related to soil pH that were observed by Rousk et al. (2010) were dissimilar to my findings. Glenlea soils had a neutral pH of 7.05 and the Carman site, which had the most acidic soils in the experiment, had PLFA composition values similar to the Saskatoon and Carman sites. That is to say that the acidity of the soil was not correlated to PLFA composition. However, my study did not focus on soil pH as a factor affecting microbial communities and thus did not incorporate enough sites of varying pH to draw conclusions in this area.

6.0 SUMMARY AND CONCLUSION

This research contributes to the understanding of the relationship between cover crops and soil health through a biological lens. Specifically, it provides insight into potential differences in biological indicators of soil health, including extracellular enzymatic activity and PLFAs between cover cropped and non-cover cropped plots in the Canadian Prairies. It addresses the question of whether growing cover crops is a worthwhile option for prairie producers and helps them to make more informed management decisions in relation to cover cropping.

Overall, findings suggest greater soil microbial activity in perennial soils compared to soils with annual crops, and limited consistent differences between cover cropped and non-cover cropped soils. The magnitude of enzyme activity in soils tended to vary by season, but this variation was different between locations. As such, future research should make a point of selecting one consistent sampling timepoint for ease of comparison across years and between locations. Additionally, trends in PLFA data tended to differ temporally and between locations as well. Differences in PLFA and EEA data between locations may be related to soil properties such as pH or soil texture. Climatic differences may also have been an important factor, especially in their role in the ability of the cover crop to become established and accumulate sufficient biomass to be able to impart benefits to the soil microbial community. Factors such as crop yield, cover crop biomass, and soil nutrient profiles should also be considered when interpreting the potential of cover crops to benefit prairie agroecosystems in a biological capacity. As such, care should be taken not to make broad comparisons in biological indicators of soil health between locations, but rather compare a single location to itself over time.

While it remains unclear whether cover cropping in the Prairies will increase cash crop productivity, it is exciting to see changes in the perennial treatment so quickly following cover

crop adoption. Part of the idea of cover cropping is to emulate perennial systems by increasing time with living roots in the ground (Otchere et al., 2022), so perhaps we could expect to see similar results in the cover cropped treatments in the future as we are starting to see in the perennial treatments in the short-term. It should be noted that the potential benefits of cover crops in rotation may take numerous years to be realized, where the effects of cover crop biomass inputs might accumulate year after year if cover crops become an integral component of the cropping system. In terms of regenerative agricultural practices, this study is based on short-term implementation. Future research should focus on longer-term cover cropping studies, especially in non-drought years. Additional soil properties, such as temperature, gravimetric water content, and pH, should be included in the experimental design process to help account for a greater number of potential variables. Trends from this study should be monitored continuously in the coming years to improve our overall understanding of the impacts of cover cropping on biological indicators of soil health in the prairies.

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8.0 APPENDICES

8.1 Appendix A: Agronomic details of the Saskatoon, Carman and Glenlea sites

Table A1: Agronomic details of the cash crops at the Saskatoon site from the 2020 growing season.

	Wheat	**Canola**	**Potato**	**Pea**	**Alfalfa**
Crop variety	CDC Abound	LL Canola	Norland Red	CDC Meadow	Equinox
Seeding date	May 7/20	May 7/20	May 12/20	May 7/20	2018
Seeder	Drill	Drill	Potato planter	Drill	Drill
Inoculant (rate, name)	-[a]	-	-	Nodulator, 2.7 lb/ac	-
Seed trt	-	-	-	-	-
Row spacing (cm)	30	30	100 x 30	30	30
Seeding rate (kg/ha)	83	13	1520	110	12
Soil moisture at planting	Dry	Dry	Dry	Dry	-
			Fertilizers (lb/ac)		
N	166	82	117	0	-
P	0	0	n/a[b]	0	-
K	0	0	n/a	0	-
S	0	0	n/a	0	-
Fertilizer name	Urea 46-0-0	Urea 46-0-0	Urea, MAP, K_2SO_4	n/a	-
			Herbicides, Fungicides, Insecticides		
Product applied	Roundup Glyphosate	Roundup Glyphosate	Roundup Glyphosate	Roundup Glyphosate	-
Application date	May 5/20 (pre-plant)	May 5/20 (pre-plant)	May 5/20 (pre-plant)	May 5/20 (pre-plant)	-
Application rate (lb/ac)	1.34	1.34	1.34	1.34	-
Product applied	-	-	Decis	Vertisan	-
Application date	-	-	July 30/20 (in-season)	July 3/20 (in-season)	-
Application rate (mL/ha)	-	-	150	600	-

[a] - = Not applicable to the given crop
[b] n/a = Information not available

Table A2: Agronomic details of the cash crops at the Saskatoon site from the 2021 growing season.

	Wheat	Canola	Potato	Pea	Alfalfa
Crop variety	CDC Abound	LL Canola	Norland Red	CDC Meadow	Equinox
Seeding date	May 13/21	May 13/21	May 18/21	May 13/21	2018
Seeder	Drill	Drill	Potato planter	Drill	Drill
Inoculant (rate, name)	-[a]	-	-	Nodulator, 2.7 lb/ac	-
Seed trt	-	-	-	-	-
Row spacing (cm)	30	30	100 x 30	30	30
Seeding rate (kg/ha)	83	13	1520	110	12
Soil moisture at planting	Dry	Dry	Dry	Dry	-
			Fertilizers (lb/ac)		
N	211	186	143.6	0	-
P	21	0	0	0	-
K	17	0	0	0	-
S	0	138	0	0	-
Fertilizer name	n/a[b]	n/a	n/a	n/a	-
			Herbicides, Fungicides, Insecticides		
Product applied	Roundup Glyphosate	Roundup Glyphosate	Roundup Glyphosate	Roundup Glyphosate	-
Application date	May 5/21 (pre-plant)	May 5/21 (pre-plant)	May 5/21 (pre-plant)	May 5/21 (pre-plant)	-
Application rate (lb/ac)	1.34	1.34	1.34	1.34	-
Product applied	-	-	Decis	-	-
Application date	-	-	n/a	-	-
Application rate (L/ha)	-	-	0.15	-	-

[a] - = Not applicable to the given crop
[b] n/a = Information not available

Table A3: Agronomic details of the cover crops at the Saskatoon site from the 2020 growing season.

	Red clover	**Oat/Berseem**	**Rye**	**Tillage radish/Mustard**
Seeding date	May 12/20, July 2/20, August 19/20	August 19/20	August 19/20	August 19/20
Seeder	Drill/Broadcast	Drill	Drill	Drill
Row spacing (cm)	30	30	30	30
Seeding rate (kg/ha)	12	Oat: 67 Berseem: 11	80	Radish: 11 Mustard: 11
Soil moisture at planting	Dry	Normal	Normal	Normal

Table A4: Agronomic details of the cover crops at the Saskatoon site from the 2021 growing season.

	Red clover	**Oat/Berseem**	**Rye**	**Tillage radish**
Seeding date	May 13/21	Sept 9/21	Sept 9/21	Sept 9/21
Seeder	Broadcast	Drill	Drill	Drill
Row spacing (cm)	30	30	30	30
Seeding rate (kg/ha)	12	Oat: 67 Berseem: 11	80	11
Soil moisture at planting	Dry	Normal	Normal	Normal

Table A5: Agronomic details of the cash crops at the Carman site from the 2020 growing season.

	Wheat	Canola	Oat	Soybean	Alfalfa
Crop variety	n/a[a]	n/a	n/a	n/a	n/a
Seeding date	May 8/20	May 11/20	May 8/20	May 18/20	n/a
Seeder	Drill	Drill	Drill	Drill	Drill
Inoculant (rate, name)	-[b]	-	-	n/a	-
Seed trt	-	-	-	-	-
Row spacing (cm)	19	19	19	76	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a	n/a
Soil moisture at planting	Normal	Normal	Normal	Normal	-
			Fertilizers (lb/ac)		
N	95.75	120	62	0	-
P	18.75	53.5	17	0	-
K	0	0	0	0	-
S	0	30	0	0	-
Fertilizer name	Urea, MAP	Urea, MAP, AMS	Urea, MAP	n/a	-
			In-Crop Herbicides, Fungicides, Insecticides		
Product applied	Buctril M	Roundup WeatherMax	Buctril M	Roundup WeatherMax	-
Application date	June 2/20	May 22/20	June 2/20	May 26/20	-
Application rate (L/ac)	0.40	0.67	0.40	1.00	-
Product applied	-	Roundup WeatherMax	-	Roundup WeatherMax	-
Application date	-	June 11/20	-	June 11/20	-
Application rate (L/ac)	-	1.00	-	1.00	-

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A6: Agronomic details of the cash crops at the Carman site from the 2021 growing season.

	Wheat	Canola	Oat	Soybean	Alfalfa
Crop variety	n/a[a]	n/a	n/a	n/a	n/a
Seeding date	May 7/21	May 7/21	May 7/21	May 14/21	n/a
Seeder	Drill	Drill	Drill	Drill	Drill
Inoculant (rate, name)	-[b]	-	-	n/a	-
Seed trt	-	-	-	-	-
Row spacing (cm)	19	19	19	76	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a	n/a
Soil moisture at planting	Dry	Dry	Dry	Dry	-
			Fertilizers (lb/ac)		
N	71	86	41	0	-
P	35	49	31	0	-
K	0	0	0	0	-
S	43	27	16	0	-
Fertilizer name	n/a	n/a	n/a	-	-
			In-Crop Herbicides, Fungicides, Insecticides		
Product applied	Buctril M	Roundup WeatherMax	Buctril M	Roundup WeatherMax	-
Application date	May 31/21	May 31/21	May 31/21	May 31/21	-
Application rate (L/ac)	0.40	0.70	0.40	0.70	-
Product applied	Liquid Achieve	Roundup WeatherMax	Caramba	Roundup Transorb	-
Application date	June 14/21	May 14/21	July 5/21	June 24/21	-
Application rate (L/ac)	0.20	1.00	0.25	0.70	-
Product applied	Caramba	-	Coragen	-	-
Application date	July 5/21	-	July 5/21	-	-
Application rate (L/ac)	0.25	-	0.50	-	-
Product applied	Coragen	-	-	-	-
Application date	July 5/21	-	-	-	-
Application rate (L/ac)	0.50	-	-	-	-

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A7: Agronomic details of the cover crops at the Carman site from the 2020 growing season.

	Red clover	Barley/Pea	Fall Rye	Tillage radish
Seeding date	July 22/20	n/a[a]	n/a	July 22/20 (re-seeded Sept 11/20)
Seeder	Broadcast	n/a	n/a	One-row seeder
Row spacing (cm)	-[b]	19	19	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a
Soil moisture at planting	Dry	n/a	n/a	Dry

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A8: Agronomic details of the cover crops at the Carman site from the 2021 growing season.

	Red clover	Barley/Pea	Fall Rye	Tillage radish
Seeding date	May 7/21 (re-seeded Aug 17/21)	Sept 9/20	Sept 9/20	July 20/21 (re-seeded Aug 31/21)
Seeder	Broadcast	n/a[a]	n/a	One-row seeder
Row spacing (cm)	-[b]	19	19	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a
Soil moisture at planting	Dry	n/a	n/a	Dry

Table A9: Agronomic details of the cash crops at the Glenlea site from the 2020 growing season.

	Wheat	Canola	Oat	Soybean	Alfalfa
Crop variety	n/a[a]	n/a	n/a	n/a	n/a
Seeding date	May 11/20	May 11/20 (re-seeded June 4/20)	May 11/20	May 19/20	n/a
Seeder	Drill	Drill	Drill	n/a	Drill
Inoculant (rate, name)	-[b]	-	-	n/a	-
Seed trt	-	-	-	-	-
Row spacing (cm)	19	19	19	76	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a	n/a
Soil moisture at planting	Normal	Normal	Normal	Normal	-
		Fertilizers (lb/ac)			
N	0	0	0	0	-
P	0	0	0	0	-
K	0	0	0	0	-
S	0	0	0	0	-
Fertilizer name	-	-	-	-	-
		In-Crop Herbicides, Fungicides, Insecticides			
Product applied	-	Roundup WeatherMax	-	Roundup WeatherMax	-
Application date	-	May 29/20	-	May 29/20	-
Application rate (L/ac)	-	1.00	-	1.00	-
Product applied	-	Decis	-	-	-
Application date	-	May 29/20	-	-	-
Application rate (L/ac)	-	n/a	-	-	-
Product applied	-	Reglone	-	-	-
Application date	-	June 5/20	-	-	-
Application rate (L/ac)	-	n/a	-	-	-

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A10: Agronomic details of the cash crops at the Glenlea site from the 2021 growing season.

	Wheat	Canola	Oat	Soybean	Alfalfa
Crop variety	n/a[a]	n/a	n/a	n/a	n/a
Seeding date	May 12/21	May 12/21	May 12/21	May 17/21	n/a
Seeder	Drill	Drill	Drill	n/a	Drill
Inoculant (rate, name)	-[b]	-	-	n/a	-
Seed trt	-	-	-	-	-
Row spacing (cm)	19	19	19	76	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a	n/a
Soil moisture at planting	Dry	Dry	Dry	Dry	-
			Fertilizers (lb/ac)		
N	55	92	50	0	-
P	15	0	15	0	-
K	0	24	0	0	-
S	11	0	9	0	-
Fertilizer name	-	-	-	-	-
			In-Crop Herbicides, Fungicides, Insecticides		
Product applied	Buctril M	Roundup Transorb	Buctril M	Roundup Transorb	-
Application date	June 8/21	June 8/21	June 8/21	June 8/21	-
Application rate (L/ac)	0.40	0.70	0.40	0.70	-
Product applied	-	Liberty	-	Roundup Transorb	-
Application date	-	June 8/21 (select plots)	-	July 6/21	-
Application rate (L/ac)	-	0.54	-	0.70	-
Product applied	-	Liberty	-	-	-
Application date	-	June 21/21 (select plots)	-	-	-
Application rate (L/ac)	-	1.00	-	-	-

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A11: Agronomic details of the cover crops at the Glenlea site from the 2020 growing season.

	Red clover	Barley/Pea	Fall Rye	Tillage radish
Seeding date	June 17/20 (re-seeded Aug 28/20)	n/a[a]	n/a	July 22/20 (re-seeded Aug 28/20 and Sept 11/20)
Seeder	Broadcast	n/a	n/a	One-row seeder
Row spacing (cm)	-[b]	19	19	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a
Soil moisture at planting	Dry	n/a	n/a	Dry

[a] n/a = Information not available
[b] - = Not applicable to the given crop

Table A12: Agronomic details of the cover crops at the Glenlea site from the 2021 growing season.

	Red clover	Barley/Pea	Fall Rye	Tillage radish
Seeding date	May 12/21 (re-seeded Aug 18/21)	Sept 10/20	Sept 10/20	July 22/21 (re-seeded Sept 1/21)
Seeder	n/a[a]	n/a	n/a	One-row seeder
Row spacing (cm)	-[b]	19	19	n/a
Seeding rate (kg/ha)	n/a	n/a	n/a	n/a
Soil moisture at planting	Normal	n/a	n/a	Dry

[a] n/a = Information not available
[b] - = Not applicable to the given crop

8.2 Appendix B: NMDS Plots

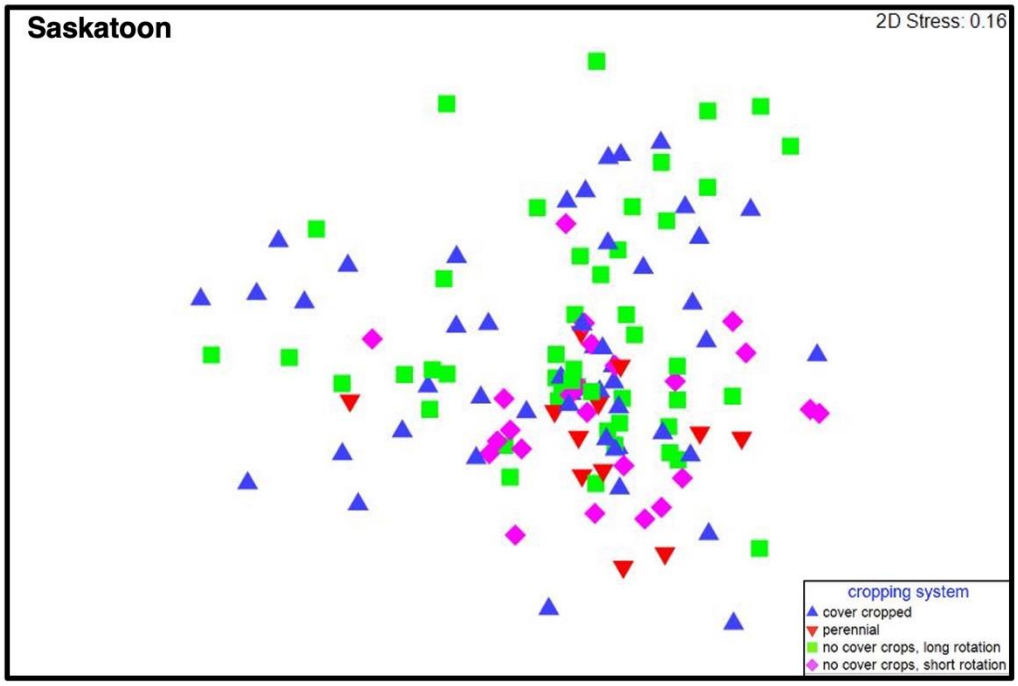

Fig. A1: Non-metric multi-dimensional scaling (NMDS) plot for phospholipid fatty acid (PLFA) abundance at the Saskatoon research site.

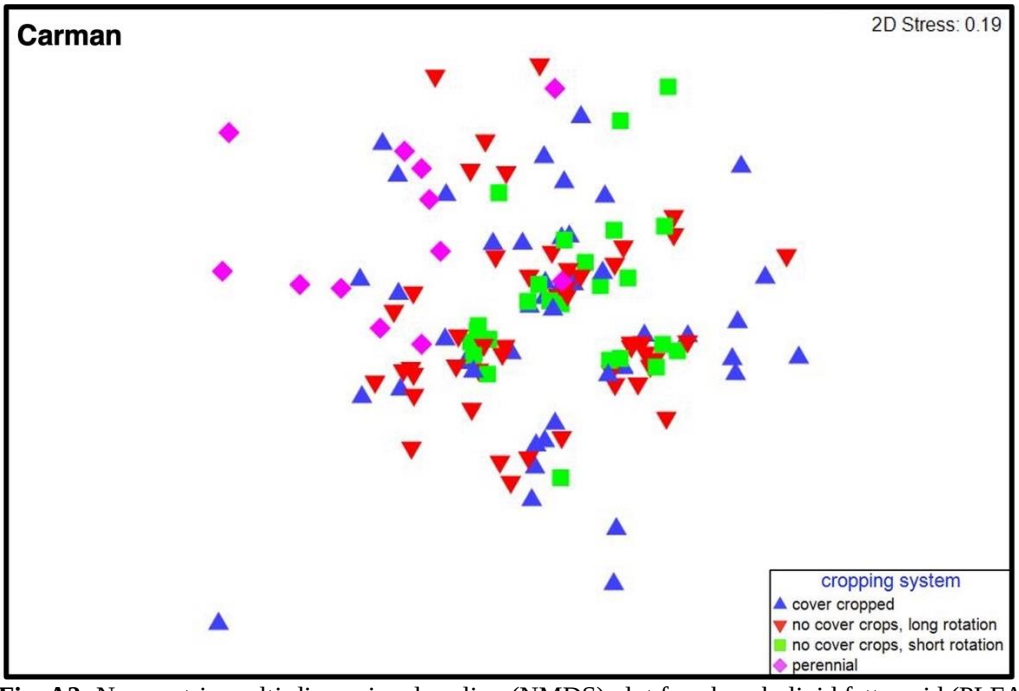

Fig. A2: Non-metric multi-dimensional scaling (NMDS) plot for phospholipid fatty acid (PLFA) abundance at the Carman research site.

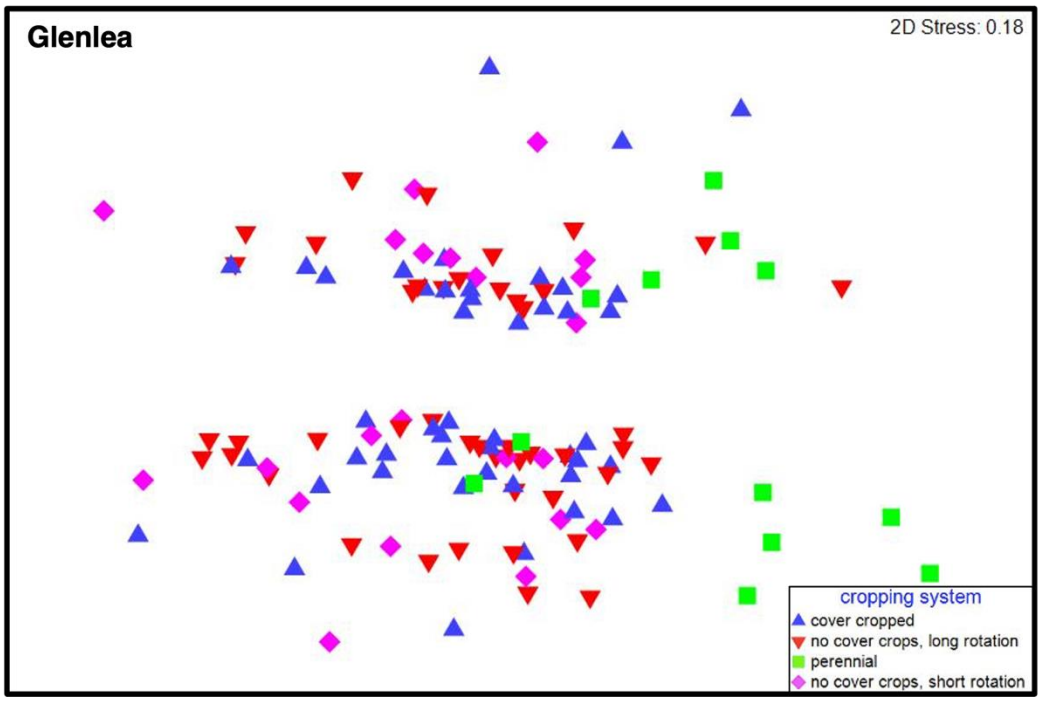

Fig. A3: Non-metric multi-dimensional scaling (NMDS) plot for phospholipid fatty acid (PLFA) abundance at the Glenlea research site.

8.3 Appendix C: Compositional PLFA data from the Saskatoon, Carman, and Glenlea research sites

Table A13: Two-way ANOVA F-test results for the effect of treatment and sampling time on phospholipid fatty acid (PLFA) compositional abundance at the Saskatoon, Carman, and Glenlea research sites at fall 2020, spring 2021, and summer 2021 sampling times; values are F-statistics and values in brackets are p values.

Location	Factor	Df	Gen. bac.[a]	Fungi	G+[b]	G-[c]	Actino[d]	AMF[e]
Saskatoon	Trt	10	1.1 (0.3607)	3.758 (0.0019)**[f]	1.17 (0.3458)	2.4 (0.0278)*	0.86 (0.5783)	2.59 (0.0192)*
	Sample time	2	107.2 (<0.0001)***	14.649 (<0.0001)***	86.22 (<0.0001)***	92.8 (<0.0001)***	49.70 (<0.0001)***	3.94 (0.0241)*
	Trt × Sample time	20	0.8 (0.6701)	1.679 (0.0602)	0.52 (0.9492)	0.8 (0.6782)	2.10 (0.0131)*	1.77 (0.0438)*
Carman	Trt	10	1.0 (0.4754)	7.1113 (<0.0001)***	4.8 (0.0003)***	1.55 (0.1666)	0.39 (0.9403)	7.635 (<0.0001)***
	Sample time	2	127.5 (<0.0001)***	8.9778 (0.0004)***	51.8 (<0.0001)***	147.73 (<0.0001)***	95.53 (<0.0001)***	41.341 (<0.0001)***
	Trt × Sample time	20	1.2 (0.3222)	1.2773 (0.2259)	1.4 (0.1431)	2.07 (0.0143)*	1.51 (0.1095)	3.000 (4e-04)***
Glenlea	Trt	10	2.4 (0.0307)*	11.611 (<0.0001)***	5.57 (0.0001)***	2.7 (0.0140)*	1.25 (0.2982)	6.55 (<0.0001)***
	Sample time	2	19.3 (<0.0001)***	13.789 (<0.0001)***	34.26 (<0.0001)***	78.1 (<0.0001)***	21.09 (<0.0001)***	30.71 (<0.0001)***
	Trt × Sample time	20	0.5 (0.9751)	1.958 (0.0231)*	1.02 (0.4525)	1.2 (0.2713)	0.59 (0.9083)	3.00 (5e-04)***

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

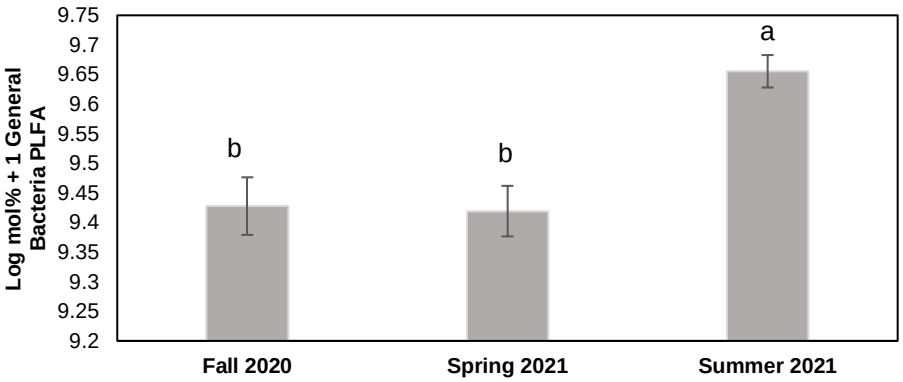

Fig. A4: General bacteria compositional phospholipid fatty acids (PLFA) at the Saskatoon site by sampling time, averaged across all treatments together. Different letters above bars represents significant differences between means.

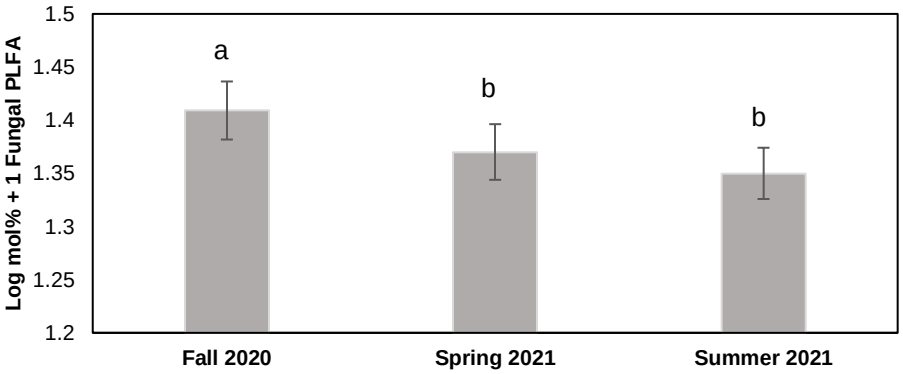

Fig. A5: Fungal compositional phospholipid fatty acids (PLFA) at the Saskatoon site by sampling time, averaged across all treatments together. Different letters above bars represents significant differences between means.

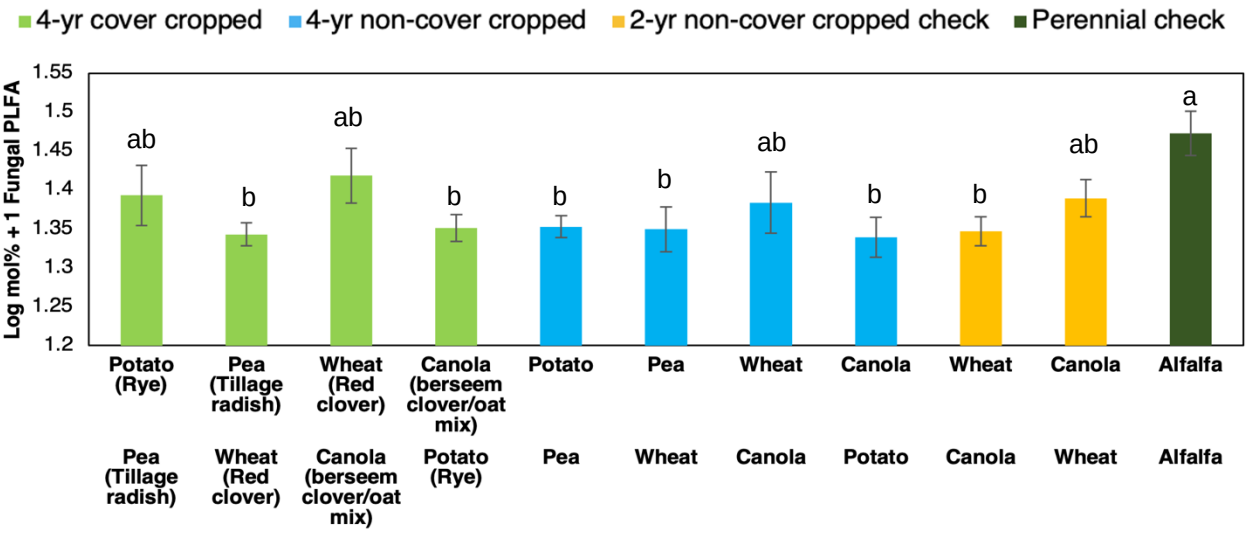

Fig. A6: Fungal compositional phospholipid fatty acids (PLFA) at the Saskatoon site by sampling time, averaged across all treatments together. Crops listed on top were grown during the 2020 growing season and crops listed on bottom were grown during the 2021 growing season; crops in brackets are the associated cover crops in select rotations. Different letters above bars represents significant differences between means.

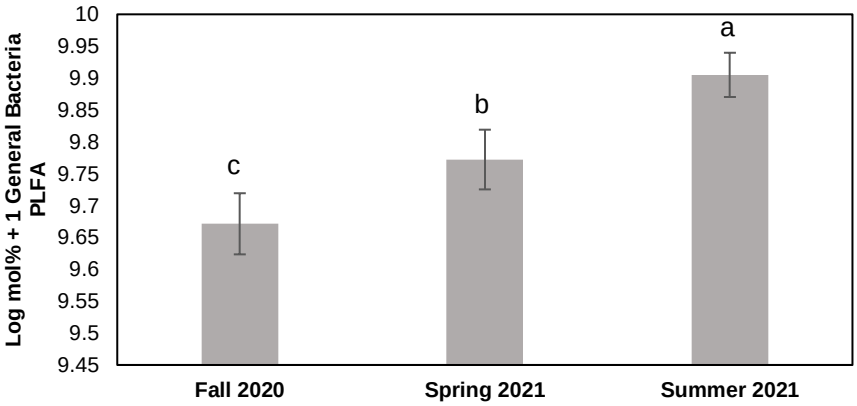

Fig. A7: General bacteria compositional phospholipid fatty acids (PLFA) at the Carman site by sampling time, averaged across all treatments together. Different letters above bars represents significant differences between means.

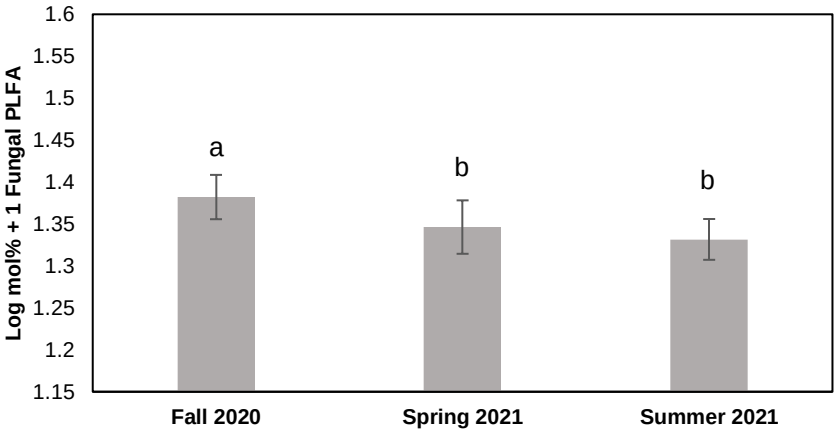

Fig. A8: Fungal compositional phospholipid fatty acids (PLFA) at the Carman site by sampling time, averaged across all treatments together. Different letters above bars represents significant differences between means.

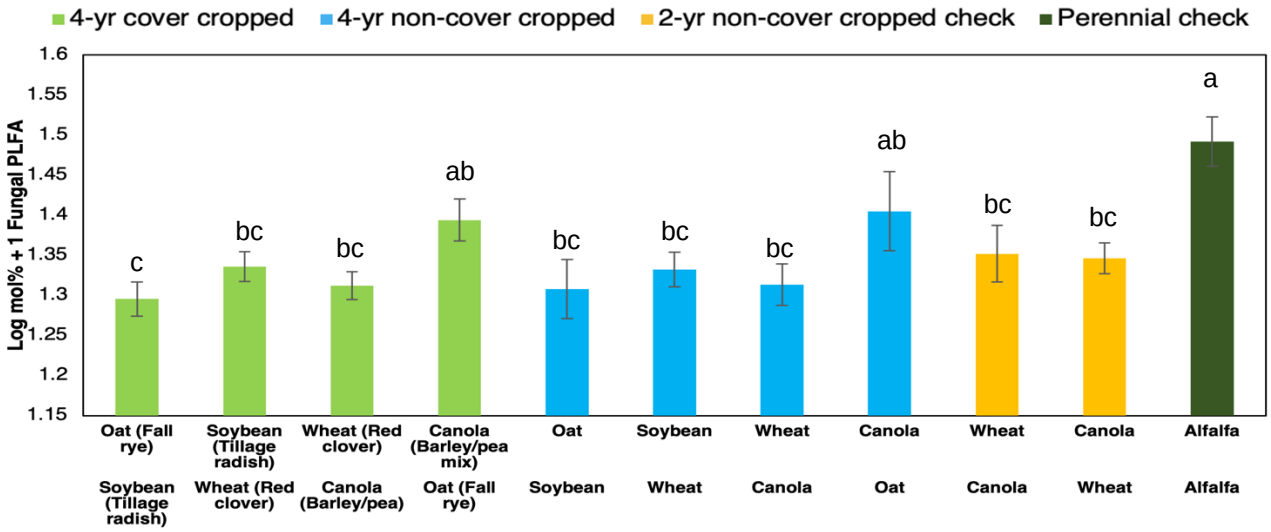

Fig. A9: Fungal compositional phospholipid fatty acids (PLFA) at the Carman site by sampling time, averaged across all treatments together. Crops listed on top were grown during the 2020 growing season and crops listed on bottom were grown during the 2021 growing season; crops in brackets are the associated cover crops in select rotations. Different letters above bars represents significant differences between means.

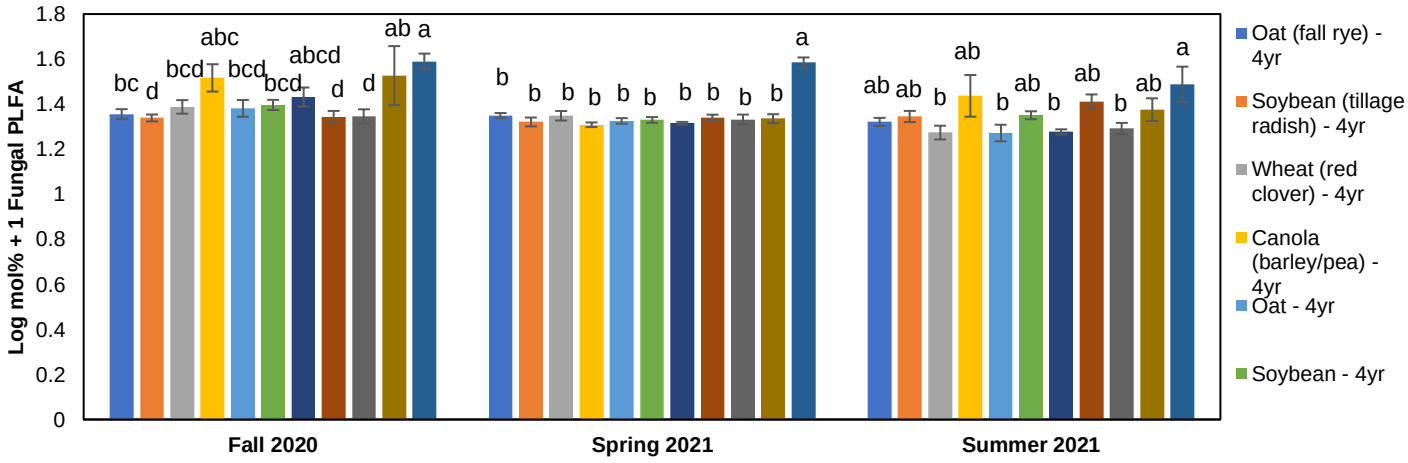

Fig. A10: Fungal compositional phospholipid fatty acids (PLFA) at the Glenlea site across sampling times. Crops listed were grown during the 2020 growing season; crops in brackets are the associated cover crops in select rotations. Different letters above bars represents significant differences between means.

8.4 Appendix D: Stress 2 PLFA data from the Glenlea research site

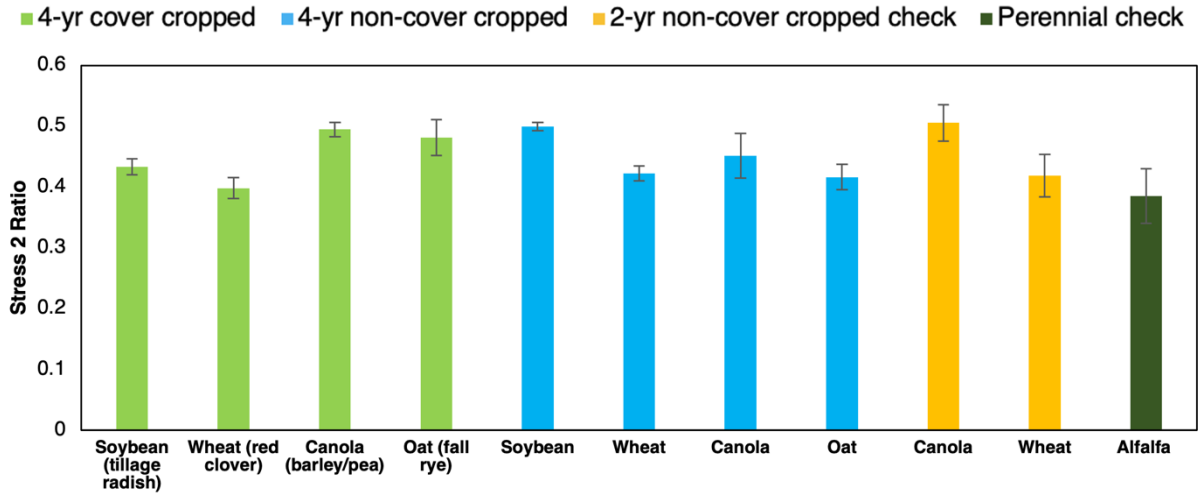

Fig. A11: Stress 2 ratio at the Glenlea site during summer 2021. Crops listed were grown during the 2021 growing season; crops in brackets are the associated cover crops in select rotations. Different letters above bars represents significant differences between means. No letters indicates no significant differences between means.

8.5 Appendix E: Correlation analysis between PLFA and EEA data

Table A14: Spearman's correlation matrix between phospholipid fatty acids (PLFA) biomarkers and extracellular enzyme activity at the Saskatoon, Carman, and Glenlea research sites at; values are r-values and values in brackets are p values.

Location	Biomarker	βG[a]	NAG[b]	AP[c]
Saskatoon	Total PLFA	0.186 (0.03326)*[d]	0.068 (0.4371)	0.208 (0.017)*
	General bacteria	0.198 (0.02325)*	0.082 (0.3498)	0.224 (0.009891)**
	Fungi	0.119 (0.1729)	-0.047 (0.5919)	0.149 (0.08825)
Carman	Total PLFA	0.306 (0.003838)**	0.100 (0.2521)	0.417 (8.617e-07)***
	General bacteria	0.280 (0.001198)**	0.086 (0.3249)	0.384 (6.523e-06)***
	Fungi	0.263 (0.002344)**	0.098 (0.261)	0.381 (7.624e-06)***
Glenlea	Total PLFA	0.075 (0.3974)	-0.081 (0.3617)	0.247 (0.004974)**
	General bacteria	0.036 (0.6876)	-0.112 (0.207)	0.227 (0.009846)**
	Fungi	0.231 (0.008822)**	0.096 (0.2823)	0.218 (0.0136)*

[a] βG = β-glucosidase
[b] NAG = N-acetyl-β-D-glucosaminidase
[c] AP = alkaline phosphatase
[d] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance. Note that correlations were considered meaningful at an r-value of ± 0.5, but due to high sample size (n), there are significant p-values associated with weak r-values in this table.

8.6 Appendix F: ANOVA tables from PLFA and EEA data at the Saskatoon, Carman, and Glenlea research sites

Table A15: Mean phospholipid fatty acids (PLFA) abundance ± standard errors (n=4) for cover cropped versus non cover cropped long rotation plots at the Saskatoon research site averaged across sampling times. Two-way ANOVA F-test results for the impact of cropping system and cash crop species on PLFA abundance averaged across sampling times; values are F-statistics and values in brackets are p values.

Cropping system	Cover crop '20	Cash crop '21	Total PLFA nmol g^{-1}	Gen. bac.[a] nmol g^{-1}	Fungi nmol g^{-1}	F:B ratio	G+[b] nmol g^{-1}	G-[c] nmol g^{-1}	Actino[d] nmol g^{-1}	AMF[e] nmol g^{-1}	G+:G- ratio	Stress 1 ratio	Stress 2 ratio
Cover crops, 4-yr	Red clover	Canola	28.3 ± 6.58	15.6 ± 3.67	0.460 ± 0.0826	0.0291 ± 0.00334	6.42 ± 1.55	8.60 ± 1.98	2.76 ± 0.645	1.120 ± 0.283	0.750 ± 0.0382	0.254 ± 0.0155	0.210 ± 0.0154
	Berseem clover/oat	Potato	26.3 ± 6.58	14.7 ± 3.67	0.302 ± 0.0826	0.0236 ± 0.00334	5.95 ± 1.55	8.04 ± 1.98	2.76 ± 0.645	1.141 ± 0.283	0.709 ± 0.0382	0.272 ± 0.0155	0.233 ± 0.0154
	Rye	Pea	23.6 ± 6.58	13.2 ± 3.67	0.308 ± 0.0826	0.0262 ± 0.00334	5.35 ± 1.55	7.08 ± 1.98	2.51 ± 0.645	0.959 ± 0.283	0.737 ± 0.0382	0.269 ± 0.0155	0.226 ± 0.0154
	Tillage radish	Wheat	24.5 ± 6.58	13.5 ± 3.67	0.280 ± 0.0826	0.0212 ± 0.00334	5.52 ± 1.55	7.16 ± 1.98	2.68 ± 0.645	0.999 ± 0.283	0.760 ± 0.0382	0.289 ± 0.0155	0.246 ± 0.0154
No cover crops, 4-yr		Canola	21.4 ± 5.55	11.7 ± 3.11	0.295 ± 0.0665	0.0279 ± 0.00326	4.68 ± 1.26	6.44 ± 1.71	2.28 ± 0.583	0.881 ± 0.251	0.710 ± 0.0314	0.290 ± 0.0143	0.243 ± 0.0152
		Potato	41.3 ± 5.55	23.1 ± 3.11	0.473 ± 0.0665	0.0204 ± 0.00326	9.45 ± 1.26	12.11 ± 1.71	4.59 ± 0.583	1.625 ± 0.251	0.792 ± 0.0314	0.257 ± 0.0143	0.242 ± 0.0152
		Pea	22.7 ± 5.55	12.6 ± 3.11	0.262 ± 0.0665	0.0222 ± 0.00326	5.14 ± 1.26	6.75 ± 1.71	2.45 ± 0.583	0.980 ± 0.251	0.738 ± 0.0314	0.272 ± 0.0143	0.235 ± 0.0152
		Wheat	24.0 ± 5.55	13.2 ± 3.11	0.286 ± 0.0665	0.0210 ± 0.00326	5.35 ± 1.26	7.06 ± 1.71	2.66 ± 0.583	0.981 ± 0.251	0.754 ± 0.0314	0.285 ± 0.0143	0.252 ± 0.0152
						Analysis of variance results							
	Factor	df											
	Cropping system	1	0.1580 (0.6946)	0.1391 (0.7124)	0.0271 (0.8706)	0.8430 (0.3677)	0.1191 (0.7330)	0.0799 (0.7798)	0.4718 (0.4988)	0.1085 (0.7448)	0.1530 (0.6993)	0.2246 (0.6398)	1.7215 (0.2019)
	Cash crop '21	3	1.2984 (0.2978)	1.3626 (0.2780)	1.1608 (0.3452)	1.9980 (0.1412)	1.3313 (0.2875)	1.2545 (0.3122)	1.7944 (0.1751)	1.1036 (0.3670)	0.2450 (0.8639)	0.8321 (0.4894)	0.8546 (0.4780)
	Crop sys: crop '21	3	1.1880 (0.3353)	1.1782 (0.3388)	1.7233 (0.1889)	0.1475 (0.903)	1.2439 (0.3158)	1.0088 (0.4060)	1.3027 (0.2965)	0.6452 (0.5935)	1.1050 (0.3665)	1.0729 (0.3792)	0.3165 (0.8133)

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A16: Mean phospholipid fatty acids (PLFA) abundance ± standard errors (n=4) for cover cropped versus non cover cropped long rotation plots at the Carman research site averaged across sampling times. Two-way ANOVA F-test results for the impact of cropping system and cash crop species on PLFA abundance averaged across sampling times; values are F-statistics and values in brackets are p values.

Cropping system	Cash crop '20		Total PLFA nmol g^{-1}	Gen. bac.[a] nmol g^{-1}	Fungi nmol g^{-1}	F:B ratio	G+[b] nmol g^{-1}	G-[c] nmol g^{-1}	Actino[d] nmol g^{-1}	AMF[e] nmol g^{-1}	G+:G- ratio	Stress 1 ratio	Stress 2 ratio
Cover crops, 4-yr	Wheat		29.4 ± 2.98	16.8 ± 1.81	0.343 ± 0.0412	0.0207 ± 0.0016	7.96 ± 0.817	6.98 ± 0.788	3.34 ± 0.332	0.957 ± 0.111	1.14 ± 0.0378	0.656 ± 0.0203	0.666 ± 0.0527
	Canola		33.9 ± 2.98	19.0 ± 1.81	0.557 ± 0.0412	0.0297 ± 0.0016	8.92 ± 0.817	8.54 ± 0.788	3.63 ± 0.332	0.843 ± 0.111	1.05 ± 0.0378	0.652 ± 0.0203	0.677 ± 0.0527
	Oat		31.1 ± 2.98	17.8 ± 1.81	0.325 ± 0.0412	0.0183 ± 0.0016	8.57 ± 0.817	7.24 ± 0.788	3.45 ± 0.332	0.913 ± 0.111	1.18 ± 0.0378	0.625 ± 0.0203	0.727 ± 0.0527
	Soybean		30.2 ± 2.98	17.2 ± 1.81	0.369 ± 0.0412	0.0212 ± 0.0016	8.11 ± 0.817	7.53 ± 0.788	3.13 ± 0.332	0.998 ± 0.111	1.09 ± 0.0378	0.639 ± 0.0203	0.610 ± 0.0527
No cover crops, 4-yr	Wheat		32.0 ± 3.12	18.2 ± 1.76	0.392 ± 0.148	0.0212 ± 0.0063	8.72 ± 0.856	7.78 ± 0.804	3.37 ± 0.327	0.951 ± 0.114	1.12 ± 0.0319	0.657 ± 0.0321	0.691 ± 0.0578
	Canola		35.5 ± 3.12	20.0 ± 1.76	0.704 ± 0.148	0.0341 ± 0.0063	9.33 ± 0.856	8.72 ± 0.804	3.99 ± 0.327	0.882 ± 0.114	1.07 ± 0.0319	0.647 ± 0.0321	0.719 ± 0.0578
	Oat		33.6 ± 3.12	19.1 ± 1.76	0.435 ± 0.148	0.0223 ± 0.0063	9.10 ± 0.856	8.04 ± 0.804	3.68 ± 0.327	0.975 ± 0.114	1.14 ± 0.0319	0.619 ± 0.0321	0.647 ± 0.0578
	Soybean		33.3 ± 3.12	19.0 ± 1.76	0.438 ± 0.148	0.0223 ± 0.0063	8.96 ± 0.856	8.09 ± 0.804	3.63 ± 0.327	1.086 ± 0.114	1.11 ± 0.0319	0.632 ± 0.0321	0.663 ± 0.0578
	Analysis of variance results												
	Factor	**df**											
	Cropping system	3	1.3171 (0.2624)	1.1698 (0.2902)	1.4924 (0.2337)	0.6010 (0.4485)	1.1592 (0.2923)	1.0835 (0.3083)	1.4708 (0.2370)	0.3287 (0.5718)	0.032 (0.8596)	0.049 (0.8269)	0.0697 (0.7940)
	Cash crop '20		0.6260 (0.6052)	0.4515 (0.7186)	2.6207 (0.0739)	2.8469 (0.0588)	0.3370 (0.7987)	0.9220 (0.4452)	0.8226 (0.4942)	0.8572 (0.4766)	2.935 (0.0538)	0.622 (0.6074)	0.4788 (0.7000)
	Crop sys: crop '20 interaction		0.0204 (0.9959)	0.0139 (0.9977)	0.0790 (0.9708)	0.0937 (0.9628)	0.0296 (0.9930)	0.0676 (0.9766)	0.1881 (0.9034)	0.0634 (0.9787)	0.531 (0.6655)	0.009 (0.9987)	0.6106 (0.6147)

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A17: Mean phospholipid fatty acids (PLFA) abundance ± standard errors (n=4) for cover cropped versus non cover cropped long rotation plots at the Glenlea research site averaged across sampling times. Different letters indicate significant differences between means ($p < 0.05$). Two-way ANOVA F-test results for the impact of cropping system and cash crop species on PLFA abundance averaged across sampling times; values are F-statistics and values in brackets are p values.

Cropping system	Cash crop '20	Total PLFA nmol g^{-1}	Gen. bac.[a] nmol g^{-1}	Fungi nmol g^{-1}	F:B ratio	G+[b] nmol g^{-1}	G-[c] nmol g^{-1}	Actino[d] nmol g^{-1}	AMF[e] nmol g^{-1}	G+:G- ratio	Stress 1 ratio	Stress 2 ratio
Cover crops, 4-yr	Wheat	24.1 ± 5.15	14.2 ± 2.88	0.379 ± 0.154	0.0248 ± 0.00496ab	6.44 ± 1.31	6.43 ± 1.51	2.81 ± 0.522	0.968 ± 0.232	1.013 ± 0.0369	0.446 ± 0.0176	0.408 ± 0.0216a
	Canola	25.0 ± 5.15	14.2 ± 2.88	0.652 ± 0.154	0.0420 ± 0.00496a	6.27 ± 1.31	7.27 ± 1.51	2.87 ± 0.522	0.854 ± 0.232	0.897 ± 0.0369	0.407 ± 0.0176	0.353 ± 0.0216a
	Oat	33.8 ± 5.15	19.9 ± 2.88	0.432 ± 0.154	0.0216 ± 0.00496ab	8.94 ± 1.31	9.09 ± 1.51	3.90 ± 0.522	1.438 ± 0.232	0.986 ± 0.0369	0.432 ± 0.0176	0.395 ± 0.0216a
	Soybean	25.3 ± 5.15	15.3 ± 2.88	0.292 ± 0.154	0.0197 ± 0.00496b	6.70 ± 1.31	7.05 ± 1.51	3.04 ± 0.522	1.044 ± 0.232	0.950 ± 0.0369	0.412 ± 0.0176	0.335 ± 0.0216a
No cover crops, 4-yr	Wheat	24.8 ± 5.44	14.6 ± 3.29	0.429 ± 0.0682	0.0297 ± 0.0034b	6.58 ± 1.43	6.55 ± 1.48	3.01 ± 0.708	1.003 ± 0.222	1.000 ± 0.212	0.441 ± 0.0175	0.410 ± 0.0266a
	Canola	25.5 ± 5.44	15.2 ± 3.29	0.293 ± 0.0682	0.0204 ± 0.0034b	6.80 ± 1.43	6.99 ± 1.48	3.04 ± 0.708	0.923 ± 0.222	0.983 ± 0.212	0.407 ± 0.0175	0.359 ± 0.0266a
	Oat	29.0 ± 5.44	17.2 ± 3.29	0.395 ± 0.0682	0.0242 ± 0.0034b	7.61 ± 1.43	7.86 ± 1.48	3.56 ± 0.708	1.258 ± 0.222	0.963 ± 0.212	0.410 ± 0.0175	0.367 ± 0.0266a
	Soybean	21.4 ± 5.44	12.8 ± 3.29	0.320 ± 0.0682	0.0252 ± 0.0034b	5.56 ± 1.43	5.93 ± 1.48	2.55 ± 0.708	0.881 ± 0.222	0.935 ± 0.212	0.425 ± 0.0175	0.329 ± 0.0266a
						Analysis of variance results						
	Factor	**df**										
	Cropping system	3	0.2531 (0.6195)	0.1844 (0.6714)	0.8858 (0.3560)	0.5099 (0.4821)	0.2143 (0.6476)	0.3527 (0.5582)	0.0689 (0.7953)	0.1373 (0.7143)	0.152 (0.7005)	0.084 (0.7748)	0.1375 (0.7140)
	Cash crop '20		0.9251 (0.4437)	0.9467 (0.4337)	0.6680 (0.5799)	1.8791 (0.1601)	0.9777 (0.4197)	0.7822 (0.5155)	0.9397 (0.4369)	1.6348 (0.2076)	2.160 (0.1190)	1.514 (0.2364)	3.7449 (0.0244)*
	Crop sys: crop '20 interaction		0.1452 (0.9318)	0.1956 (0.8983)	1.2636 (0.3092)	4.6884 (0.0103)*[f]	0.2268 (0.8768)	0.0966 (0.9612)	0.1588 (0.9230)	0.1638 (0.9197)	1.470 (0.2476)	0.350 (0.7894)	0.1898 (0.9023)

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A18: Mean phospholipid fatty acids (PLFA) abundance ± standard errors (n=4) by treatment at the Saskatoon research site in fall 2020. Different letters indicate significant differences between means ($p < 0.05$). One-way ANOVA F-test results for the impact of treatment on PLFA abundance at post-harvest sampling; values are F-statistics and values in brackets are p values.

Cash crop '20	Total PLFA nmol g^{-1}	Gen. bac.[a] nmol g^{-1}	Fungi nmol g^{-1}	F:B ratio	G+[b] nmol g^{-1}	G-[c] nmol g^{-1}	Actino[d] nmol g^{-1}	AMF[e] nmol g^{-1}	G+:G- ratio	Stress 1 ratio	Stress 2 ratio
Potato – 4yr (Rye)	16.7 ± 3.61	9.03 ± 1.95	0.257 ± 0.0731bc	0.0321 ± 0.0045	3.71 ± 0.821	4.79 ± 1.00	1.81 ± 0.397	0.634 ± 0.152	0.754 ± 0.0205	0.351 ± 0.011	0.264 ± 0.0108ab
Pea – 4yr (Tillage radish)	19.9 ± 3.61	10.80 ± 1.95	0.223 ± 0.0731c	0.0212 ± 0.0045	4.51 ± 0.821	5.53 ± 1.00	2.20 ± 0.397	0.812 ± 0.152	0.802 ± 0.0205	0.329 ± 0.011	0.281 ± 0.0108a
Wheat – 4yr (Red clover)	20.8 ± 3.61	11.15 ± 1.95	0.452 ± 0.0731abc	0.0401 ± 0.0045	4.51 ± 0.821	5.95 ± 1.00	2.32 ± 0.397	0.764 ± 0.152	0.760 ± 0.0205	0.314 ± 0.011	0.272 ± 0.0108ab
Canola – 4yr (berseem clover/oat mix)	20.4 ± 3.61	10.96 ± 1.95	0.327 ± 0.0731abc	0.0296 ± 0.0045	4.33 ± 0.821	5.86 ± 1.00	2.30 ± 0.397	0.824 ± 0.152	0.733 ± 0.0205	0.330 ± 0.011	0.250 ± 0.0108ab
Potato – 4yr	24.5 ± 3.61	13.24 ± 1.95	0.354 ± 0.0731abc	0.0268 ± 0.0045	5.41 ± 0.821	7.03 ± 1.00	2.60 ± 0.397	1.047 ± 0.152	0.770 ± 0.0205	0.310 ± 0.011	0.253 ± 0.0108ab
Pea – 4yr	24.5 ± 3.61	13.29 ± 1.95	0.314 ± 0.0731abc	0.0236 ± 0.0045	5.45 ± 0.821	6.94 ± 1.00	2.66 ± 0.397	0.998 ± 0.152	0.785 ± 0.0205	0.329 ± 0.011	0.270 ± 0.0108ab
Wheat – 4yr	17.2 ± 3.61	9.57 ± 1.95	0.223 ± 0.0731c	0.0258 ± 0.0045	3.78 ± 0.821	4.88 ± 1.00	2.11 ± 0.397	0.641 ± 0.152	0.754 ± 0.0205	0.344 ± 0.011	0.285 ± 0.0108a
Canola – 4yr	22.1 ± 3.61	11.78 ± 1.95	0.360 ± 0.0731abc	0.0299 ± 0.0045	4.89 ± 0.821	6.18 ± 1.00	2.36 ± 0.397	0.851 ± 0.152	0.788 ± 0.0205	0.315 ± 0.011	0.255 ± 0.0108ab
Wheat – 2yr	24.0 ± 3.61	13.08 ± 1.95	0.313 ± 0.0731abc	0.0246 ± 0.0045	5.38 ± 0.821	6.80 ± 1.00	2.65 ± 0.397	1.044 ± 0.152	0.788 ± 0.0205	0.323 ± 0.011	0.256 ± 0.0108ab
Canola – 2yr	28.3 ± 3.61	15.22 ± 1.95	0.581 ± 0.0731ab	0.0370 ± 0.0045	6.04 ± 0.821	8.44 ± 1.00	3.07 ± 0.397	1.230 ± 0.152	0.718 ± 0.0205	0.314 ± 0.011	0.238 ± 0.0108ab
Alfalfa	31.3 ± 3.61	16.96 ± 1.95	0.638 ± 0.0731a	0.0375 ± 0.0045	6.73 ± 0.821	9.17 ± 1.00	3.34 ± 0.397	1.260 ± 0.152	0.733 ± 0.0205	0.301 ± 0.011	0.227 ± 0.0108b
Analysis of variance results											
Factor	**df**										
Treatment	10	1.5117 (0.1792)	1.4865 (0.1884)	3.5149 (0.003)**[f]	1.9152 (0.0786)	1.2904 (0.2758)	1.8870 (0.0851)	1.2035 (0.3244)	1.9885 (0.0675)	1.738 (0.1132)	1.831 (0.0935)	2.636 (0.0176)*

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A19: Mean phospholipid fatty acids (PLFA) abundance ± standard errors (n=4) by treatment at the Glenlea research site averaged across sampling times. Different letters indicate significant differences between means ($p < 0.05$). One-way ANOVA F-test results for the impact of treatment on PLFA abundance averaged across sampling times; values are F-statistics and values in brackets are p values.

Treatment		Total PLFA nmol g^{-1}	Gen. bac.[a] nmol g^{-1}	Fungi nmol g^{-1}	F:B ratio	G+[b] nmol g^{-1}	G-[c] nmol g^{-1}	Actino[d] nmol g^{-1}	AMF[e] nmol g^{-1}	G+:G- ratio	Stress 1 ratio	Stress 2 ratio
Oat - 4yr		25.0	14.2	0.652	0.0420	6.27	7.27	2.87	0.854	0.897	0.407	0.353
(Fall rye)		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Soybean – 4yr		33.8	19.9	0.432	0.0216	8.94	9.09	3.90	1.438	0.986	0.432	0.395
(Tillage radish)		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Wheat – 4yr		25.3	15.3	0.292	0.0197	6.70	7.05	3.04	1.044	0.950	0.412	0.335
(Red clover)		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Canola – 4yr		24.1	14.2	0.379	0.0248	6.44	6.43	2.81	0.968	1.013	0.446	0.408
(Barley/pea)		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263a
Oat – 4yr		25.5	15.2	0.293	0.0204	6.80	6.99	3.04	0.923	0.983	0.407	0.359
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Soybean – 4yr		29.0	17.2	0.395	0.0242	7.61	7.86	3.56	1.1258	0.963	0.410	0.367
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Wheat – 4yr		21.4	12.8	0.320	0.0252	5.56	5.93	2.55	0.881	0.935	0.425	0.329
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Canola – 4yr		24.8	14.6	0.429	0.0297	6.58	6.55	3.01	1.003	1.000	0.441	0.410
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263a
Wheat – 2yr		27.5	14.6	1.110	0.0585	6.49	8.47	2.82	0.838	0.874	0.427	0.392
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Canola – 2yr		21.4	12.7	0.265	0.0209	5.70	5.79	2.52	0.850	1.018	0.419	0.398
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263ab
Alfalfa		36.4	20.7	1.061	0.0514	8.66	10.50	3.93	1.595	0.824	0.376	0.279
		± 5.31	± 2.93	± 0.264	± 0.0107	± 1.29	± 1.63	± 0.578	± 0.22	± 0.0439	± 0.0173	± 0.0263b
Factor	**df**											
Treatment	10	0.7885 (0.6397)	0.8172 (0.6145)	1.3157 (0.2629)	1.5997 (0.1502)	0.7172 (0.7024)	0.7729 (0.6534)	0.7209 (0.6991)	1.3840 (0.2305)	2.000 (0.0659)	1.250 (0.2977)	2.3626 (0.031)*[f]

[a] Gen. bac. = General Bacteria
[b] G+ = Gram positive bacteria
[c] G- = Gram negative bacteria
[d] Actino = Actinobacteria
[e] AMF = Arbuscular mycorrhizal fungi
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A20: Mean enzyme activity ± standard errors (n=4) by treatment at the Saskatoon research site in summer 2021 Different letters indicate significant differences between means ($p < 0.05$). One-way ANOVA F-test results for the impact of treatment on enzyme activity at mid-season sampling; values are F-statistics and values in brackets are p values.

Treatment	AP[a] nmol h^{-1} g^{-1} soil	βG[b] nmol h^{-1} g^{-1} soil	NAG[c] nmol h^{-1} g^{-1} soil
Pea – 4yr	2570	3073	1424
(Tillage radish)	± 139bcd	± 187abc	± 90.5ab
Wheat – 4yr	2580	3585	1482
(Red clover)	± 139abcd	± 187a	± 90.5ab
Canola – 4yr	2919	3514	1471
(berseem/oat mix)	± 139ab	± 187a	± 90.5ab
Potato – 4yr	2930	2574	1267
(Rye)	± 139ab	± 187bc	± 90.5ab
Pea – 4yr	1931	3367	1535
	± 139d	± 187ab	± 90.5ab
Wheat – 4yr	2028	3197	1406
	± 139cd	± 187abc	± 90.5ab
Canola – 4yr	2216	3301	1296
	± 139cd	± 187abc	± 90.5ab
Potato – 4yr	2019	2398	1266
	± 139cd	± 187c	± 90.5ab
Canola – 2yr	2691	3576	1492
	± 139abc	± 187a	± 90.5ab
Wheat – 2yr	3250	2897	1122
	± 139a	± 187abc	± 90.5b
Alfalfa	3093	3526	1678
	± 139ab	± 187a	± 90.5a
Analysis of variance results			
Factor **df**			
Treatment 10	11.005 (<0.0001)***[d]	4.783 (<0.0001)***	2.9286 (0.0097)**

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[d] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A21: Mean enzyme activity ± standard errors (n=4) by treatment at the Glenlea research site in summer 2021. Different letters indicate significant differences between means ($p < 0.05$). One-way ANOVA F-test results for the impact of treatment on enzyme activity at post-harvest sampling; values are F-statistics and values in brackets are p values.

Treatment		AP nmol h^{-1} g^{-1} soil	βG nmol h^{-1} g^{-1} soil	NAG nmol h^{-1} g^{-1} soil
Soybean – 4yr		2315	3561	1739
(Tillage radish)		± 214	± 314	± 131
Wheat – 4yr		2376	3908	1816
(Red clover)		± 214	± 314	± 131
Canola – 4yr		2340	3519	1476
(Barley/pea)		± 214	± 314	± 131
Oat – 4yr		2232	3460	1504
(Fall rye)		± 214	± 314	± 131
Soybean – 4yr		2249	3594	1631
		± 214	± 314	± 131
Wheat – 4yr		2577	3734	1654
		± 214	± 314	± 131
Canola – 4yr		2703	3101	1410
		± 214	± 314	± 131
Oat – 4yr		2017	3606	1543
		± 214	± 314	± 131
Canola – 2yr		2466	3591	1593
		± 214	± 314	± 131
Wheat – 2yr		2767	2937	1417
		± 214	± 314	± 131
Alfalfa		2579	3448	1500
		± 214	± 314	± 131
		Analysis of variance results		
Factor	**df**			
Treatment	10	1.0915 (0.3966)	0.7431 (0.6796)	0.9747 (0.4831)

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A22: Mean enzyme activity ± standard errors (n=4) for cover cropped versus non cover cropped long rotation plots at the Carman research site in summer 2021. Two-way ANOVA F-test results for the impact of cropping system and cash crop species on enzyme activity at mid-season sampling; values are F-statistics and values in brackets are p values.

Cropping system	Cash crop '20		AP[a] nmol h^{-1} g^{-1} soil	βG[b] nmol h^{-1} g^{-1} soil	NAG[c] nmol h^{-1} g^{-1} soil
Cover crop, 4-yr	Wheat		314 ± 47.3	2988 ± 257	1905 ± 97.8
	Canola		417 ± 47.3	2867 ± 257	2045 ± 97.8
	Oat		288 ± 47.3	3018 ± 257	1911 ± 97.8
	Soybean		271 ± 47.3	2702 ± 257	1908 ± 97.8
No cover crops, 4-yr	Wheat		220 ± 40	3015 ± 223	1861 ± 73.9
	Canola		281 ± 40	2886 ± 223	1861 ± 73.9
	Oat		356 ± 40	3049 ± 223	1949 ± 73.9
	Soybean		236 ± 40	2997 ± 223	1880 ± 73.9
			Analysis of variance results		
	Factor	**df**			
	Cropping system	3	2.4717 (0.1290)	0.2990 (0.5895)	0.794 (0.3816)
	Cash crop '20		2.1304 (0.1228)	0.2848 (0.8359)	0.278 (0.8408)
	Crop sys: crop '20 interaction		2.0421 (0.1347)	0.1574 (0.9238)	0.582 (0.6325)

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.

Table A23: Mean enzyme activity ± standard errors (n=4) for cover cropped versus non cover cropped long rotation plots at the Glenlea research site in summer 2021. Different letters indicate significant differences between means ($p < 0.05$). Two-way ANOVA F-test results for the impact of cropping system and cash crop species on enzyme activity at mid-season sampling; values are F-statistics and values in brackets are p values.

Cropping system	Cash crop '20	AP[a] nmol h^{-1} g^{-1} soil	βG[b] nmol h^{-1} g^{-1} soil	NAG[c] nmol h^{-1} g^{-1} soil
Cover crops, 4-yr	Wheat	2232 ± 235	3460 ± 352	1504 ± 151
	Canola	2315 ± 235	3561 ± 352	1476 ± 151
	Oat	2376 ± 235	3908 ± 352	1816 ± 151
	Soybean	2340 ± 235	3519 ± 352	1476 ± 151
No cover crops, 4-yr	Wheat	2017 ± 142	3606 ± 311	1543 ± 126
	Canola	2249 ± 142	3594 ± 311	1631 ± 126
	Oat	2577 ± 142	3734 ± 311	1654 ± 126
	Soybean	2703 ± 142	3101 ± 311	1410 ± 126
	Analysis of variance results			
	Factor	**df**		
	Cropping system	3	0.2662 (0.6106)	0.1931 (0.6643)	0.5756 (0.4554)
	Cash crop '20		1.7853 (0.1769)	0.7969 (0.5077)	1.9346 (0.1509)
	Crop sys: crop '20 interaction		0.9011 (0.4551)	0.2810 (0.8386)	0.1896 (0.9024)

[a] AP = alkaline phosphatase
[b] βG = β-glucosidase
[c] NAG = N-acetyl-β-D-glucosaminidase
[f] Numbers followed by *, **, or *** indicates significance at the 0.05, 0.01, and 0.001 levels of probability respectively. No asterisks represents no significance.