DIFFERENT BRASSICACEAE SPECIES IN ROTATION WITH PULSE CROPS AND WHEAT: EFFECTS ON BIOLOGICAL NITROGEN FIXATION, SELECTED SOIL PROPERTIES AND WHEAT PRODUCTIVITY

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By

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ABSTRACT

Brassicaceae crops produce glucosinolate (GLS)-degradation products with antimicrobial properties. Amounts and compositions of GLS vary among Brassicaceae species. Considering that biological nitrogen fixation (BNF) is microbially mediated, different Brassicaceae species may affect BNF in rotational pulse crops differently. To improve cropping system sustainability, diversifying rotations with lesser-grown crops is essential. Although previous studies have focused on N benefits of pulse crops, the impact of Brassicaceae crops on BNF in subsequent pulse crops and overall crop performance remains unclear. A 4-year field study was conducted at three test sites (Swift Current and Scott in SK, and Brooks in AB) to evaluate the effect of different Brassicaceae crops [Argentine canola (*Brassica napus* L.), camelina (*Camelina sativa* L. Crantz), industrial mustard (*Brassica carinata* L.), oriental mustard (*Brassica juncea* L.) and yellow mustard (*Sinapis alba* L.)] on BNF of field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medikus), soil properties and productivity of wheat grown as the fourth crop in the cycle. Overall, the percentage of N derived from atmosphere (%Ndfa) for field pea grown after Argentine canola was 5.3-34.6 % higher than the average of other Brassicaceae crops at all test sites. The increased *nif*H gene concentration and root nodule dry weight in field pea on Argentine canola stubble may contribute to enhanced BNF. In contrast, BNF in lentil was not affected by the preceding Brassicaceae crop species at all test sites, except at Brooks. Lentil grown on Argentine canola stubble showed 17.9 % higher %Ndfa and 26.6 % higher fixed N content than other Brassicaceae crops at Brooks, potentially attributable to higher root nodule dry weights. Thus, the impact of Brassicaceae crops on BNF of pulse crops may vary depending on the pulse crop species and test site. In addition, a controlled environment study with field pea showed that high GLS content (6.49 μmol g^{-1} of tissue) suppressed effective root nodulation by 14.6 % and soil nitrification by 16.2 % compared to zero GLS. Negligible GLS content in Argentine canola residues (0.02 μmol g^{-1} of tissue) was likely non-suppressive for beneficial interactions with diazotrophs, leading to increased BNF in pulse crops compared to other Brassicaceae crop residues. The field study further revealed that Brassicaceae and pulse crops in wheat-based crop sequences did not influence the selected soil properties within the 4-yr time frame. On average, crop sequences with Argentine canola consistently had higher amounts of light fraction organic matter than other Brassicaceae crops. Nonetheless, crop sequences with Brassicaceae and pulse crops increased seed yield of subsequent

wheat by 57 % and 1000-seed weight by 7.2-9.7 % compared to continuous wheat at test sites in the Brown soil zone. Argentine canola followed by either field pea or lentil increased subsequent wheat production by over 30 % compared to other Brassicaceae-pulse combinations at Swift Current. Overall, the study suggests that the inclusion of Brassicaceae oilseed and pulse crops in wheat rotations offers advantages over continuous wheat, particularly in the Brown soil zone. Moreover, Argentine canola appears to provide more benefits than other Brassicaceae oilseed crops, potentially enhancing cropping system performance.

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Sri Lanka is one of the few countries that provide free education from primary school through university degrees. Without the gift of this free education system, I would not be where I am today. My journey has been possible because of the generosity of every Sri Lankan who has paid taxes and contributed to my free education, knowingly or unknowingly. I owe a deep gratitude to them, and I dedicate my Ph.D. dissertation to each of them.

මගේම ආදරණීය අම්මාට, තත්තාට සහ අමෝද්ට. මගේ ගමනේ පදනම වූ ඔබේ කොන්දේසි විරහිත ආදරයට මම ඔබ සැමට සදාකාලිකව ස්තූතිවන්ත වෙමි.

ශ්‍රී ලංකාව ප්‍රාථමික අධ්‍යාපනයේ සිට විශ්වවිද්‍යාල උපාධිය දක්වා නොමිලේ අධ්‍යාපනය ලබාදෙන රටවල් අතලොස්සෙන් එකකි. ශ්‍රී ලංකාවේ නිදහස් අධ්‍යාපන ක්‍රමය නොවන්නට මට මාගේ අධ්‍යාපන ගමනේ මේ දුරට පැමිණීමට නොහැකි විය හැක. ඒ නිසාම, බදු ගෙවා, දැන හෝ නොදැන, මගේ නිදහස් අධ්‍යාපනයට දායක වූ සෑම ශ්‍රී ලාංකිකයකුටම මම ආදරයෙන් ණයගැති අතර මගේ ආචාර්ය උපාධි නිබන්ධනය උපහාරයක් වශයෙන් ඔවුන්ට පිරිනමමි.

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LIST OF ABBREVIATIONS

AC	Argentine canola
AGRes	Above-ground residue
AMF	Arbuscular mycorrhizal fungi
*amo*A	Gene encoding for ammonia monooxygenase enzyme
ANOVA	Analysis of variance
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
BGRes	Below-ground residue
BNF	Biological N_2 fixation
bp	Base pair
CL	Camelina
FP	Field pea
GLS	Glucosinolate
HFOM	Heavy fraction organic matter
HSD	Honestly significant difference
IM	Industrial mustard
ITC	Isothiocyanate
L	Lentil
LFOM	Light fraction organic matter
*nif*H	Gene encoding the molybdenum-iron protein subunit of nitrogen reductase
%Ndfa	Percentage of nitrogen derived from atmosphere
OrM	Oriental mustard
qPCR	Quantitative polymerase chain reaction
PCR	Polymerase chain reaction
SOC	Soil organic carbon
SOM	Soil organic matter
YM	Yellow mustard
W	Wheat

1. GENERAL INTRODUCTION

Agricultural crop diversification is a key management practice involving crop rotation, multiple cropping, such as intercropping, mixed cropping and relay cropping. Diversification improves crop productivity and delivers multiple ecosystem services (Francaviglia et al., 2022). In the Canadian prairies, incorporating alternative crops such as pulse and Brassicaceae oilseed crops into cereal-dominated systems has provided economic and environmental benefits (Wezel et al., 2014; Martens et al., 2015), while fostering production stability (Khakbazan et al., 2009; Cutforth et al., 2013). Studies have shown that diversified cropping systems with cereals, pulse and oilseed crops are generally more profitable than continuous cereal or crop-fallow cropping systems in rain-fed regions (Gan et al., 2017; Niu et al., 2017).

Pulse crops have been a mainstay of diversified crop rotations across the Canadian prairies for over 30 years. They are beneficial in improving soil structure (Lal, 2017), availability of soil water (Miller et al., 2003a; Gan et al., 2017), availability of soil nitrogen (N) (Lal, 2017), phosphorus mobilization (Hegewald et al., 2018), microbial community structure (Zander et al., 2016) and consequently increasing yield of the succeeding crop (Miller et al., 2003b; Angus et al., 2015). One of the most significant contributions of pulse crops is their role in biological N fixation (BNF) (Hossain et al., 2016). In general, pulse crops acquire a high proportion, approximately 60 % of their total N, from the BNF process (Walley et al., 2007). Crop rotations that include pulse crops may supply N-rich residues to subsequent crops depending on their BNF capacity, potentially reducing fertilizer inputs (Gan et al., 2011a) and decreasing the carbon (C) footprint of the agricultural system (Lemke et al., 2007; Gan et al., 2011b). However, the BNF capacity mainly relies on formation of root nodules initiated by specific rhizobial strains (Abdellatif et al., 2016). The effectiveness of these strains varies depending on the pulse crop species, cropping history and soil conditions (Evans et al., 2001; Hossain et al., 2016).

Incorporation of Brassicaceae oilseed crops in rotations with pulse crops has become popular in Canadian prairies due to their agronomic benefits (Gill, 2018). Canola, camelina and mustard are members of the Brassicaceae family and are well adapted to the cool and short-season conditions on the Canadian prairies. The inclusion of Brassicaceae oilseed crops into cropping systems improves N availability, nutrient supply, water use efficiency and provides allelopathic control functions (Kirkegaard et al., 2008; Angus et al., 2015). The allelopathic effect of

Brassicaceae crops is mainly due to the production of a secondary metabolite group called glucosinolate (GLS) (Nguyen et al., 2020). Glucosinolates themselves are relatively inactive (Borgen et al., 2010). However, upon tissue damage, the enzyme myrosinase catalyzes their breakdown into a range of potent compounds, including iso-thiocyanates (ITCs), thiocyanates, nitriles, goitrin and epithionitriles (Chhajed et al., 2019). These GLS hydrolysis products play a key role in plant defense against insects, bacteria and fungi (Singh, 2017). The composition and contents of GLSs are influenced by the plant genotype, growing conditions, cultivation conditions (fertilization and harvest time) and plant part (Rangkadilok et al., 2002; Tripathi and Mishra 2007; Verkerk et al., 2009). Different Brassicaceae oilseed crops contain various levels of GLSs with diverse compositions resulting in different biocidal activities (Clarke, 2010). However, this defense mechanism is not entirely selective. Thus, the resulting GLS hydrolysis products can also impact non-target soil microorganisms, which play a vital role in soil health, nutrient cycling, and ultimately, crop production (Bending and Lincoln, 2000; Zuluaga et al., 2015; Lemanceau and Alabouvette, 1993).

Previous research studies have primarily focused on benefits that pulse crops can confer to succeeding crops within rotations. However, the impact of preceding crops on BNF of pulse crops remains unclear, particularly the influence of Brassicaceae crops on BNF in following pulse crops. In addition, there is limited information on the sustainability and impacts of including different Brassicaceae oilseed crops into cropping systems with major field crops. Therefore, this study aimed to fill the knowledge gap on how different Brassicaceae oilseed crops (Argentine canola, yellow mustard, oriental mustard, industrial mustard and camelina) influence BNF of two selected succeeding pulse crops, field pea and lentil, in a wheat-based cropping system under different growing environments in the semi-arid Canadian Prairies.

In the current study, the following hypotheses were tested.

1. Brassicaceae oilseed crops with relatively high GLS levels (yellow, oriental and industrial mustard crops, and camelina) will reduce the ability of subsequent pulse crops (field pea and lentil) to fix N compared to those following Argentine canola, which contains low GLS. This is because residual GLS will negatively impact the diazotroph abundance and root nodulation in the pulse crops (Chapter 3).
2. Diversifying wheat-based cropping systems by introducing pulse crops (field pea and lentil) and Brassicaceae oilseeds (Argentine canola, mustards and camelina) will improve soil

physical, chemical, and biological properties and wheat crop productivity compared to continuous wheat. The incorporation of Argentine canola, a low level of GLS containing Brassicaceae oilseed species will enhance soil nutrient levels more effectively than other high-GLS containing Brassicaceae crops such as yellow, oriental and industrial mustard crops and camelina (Chapter 4).

3. The differences in C, N, C:N ratio, lignin, lignin:N ratio and GLS contents in different Brassicaceae oilseeds and wheat will be associated with residual N recovery levels and consequently influence soil N availability (Chapter 5).
4. The amount of soil available N and GLS content in oilseed crop residues will negatively affect the effective root nodulation in field pea grown on Brassicaceae stubble. Residues of high GLS content containing Brassicaceae species, yellow mustard will suppress soil nitrifying microbe abundance (ammonia-oxidizing microbes) compared to Argentine canola residues with low GLS content. The reduced abundance of nitrifying microbes will lead to lower soil nitrification, resulting in lower nitrate (NO_2^-) and nitrite (NO_3^-) levels and higher ammonium (NH_4^+) levels in the soil. The presence of high NH_4^+-N levels will adversely impact nodulation in subsequently grown field pea (Chapter 6).

The general objective of this study was to evaluate the impact of incorporating different Brassicaceae oilseed crops on BNF of subsequent pulse crops and the overall performance of cropping systems. This dissertation was organized in manuscript format with a general introduction (Chapter 1) followed by a literature review (Chapter 2) and four research chapters (Chapters 3-6) covering studies undertaken in the field and under controlled environmental conditions. Chapter 7 synthesizes the key findings (main results and conclusion) from the research chapters and identifies future research needs.

Chapter 1 (Introduction) provides background and research justification and explains how the study fills the knowledge gap.

Chapter 2 reviews the relevant literature on crop diversification using pulse and Brassicaceae oilseed crops in Canadian prairies highlighting the benefits of including those crops, the role of pulse crop contribution to BNF, the potential impacts of Brassicaceae crops on soil N transformation (Literature Review).

Chapter 3 documents a 4-yr crop rotation field experiment investigating the impact of preceding Brassicaceae oilseed crops on BNF in subsequent pulse crops considering their GLS profiles. The

main objectives of this experiment were to measure the total GLS content in each Brassicaceae crop residue and evaluate BNF ability of field pea and lentil grown after different Brassicaceae crops under three growing environments. This field study was conducted using ^{15}N isotope dilution technique.

Chapter 4 compares different combinations of Brassicaceae oilseed-pulse crops in wheat-based cropping systems that affect major soil properties and cropping system performance using the same field study in Chapter 3. This experiment was aimed to determine the effect of growing field pea and lentil after different Brassicaceae oilseed crops on selected soil properties (total soil N, organic C, soil organic matter, C:N ratio and moisture content), and subsequent wheat yield in the cropping system.

Chapter 5 describes an incubation experiment analyzing how the biochemical makeup of various crop residues influences decomposition and soil available N. This study was performed using ^{15}N isotope labeled crop residues and diffusion disk method to identify N released from crop residues (N recovery percentage) over a 120-d period. The key objectives were to quantify C, N, C:N ratio, lignin, lignin:N ratio, to gain a better understanding of the mechanisms that govern residue decomposition and to determine the most important biochemical properties of crop residues involved.

Chapter 6 provides an understanding of the effect of preceding Brassicaceae oilseeds at two extremes of GLS contents on subsequent pulse crop root nodulation. This experiment was mainly focused on evaluating the effect of preceding crop residue from yellow mustard (the highest GLS), Argentine canola (the lowest GLS) and wheat (zero GLS) on soil nitrifying microbial abundance, available N content and effective root nodulation in subsequent field pea.

Chapter 7 synthesizes and integrates the key findings of the individual experiments reported in this dissertation and concludes with suggestions for future research work.

2. LITERATURE REVIEW

2.1. Crop Diversification Practices on the Canadian Prairies

Canadian prairie agricultural production systems have historically relied on monoculture cereal cropping, with frequent summer-fallowing for moisture retention and extensive use of tillage for weed control and seed-bed preparation (Fan et al., 2020). Over the last two decades, crop producers have increasingly replaced summer-fallow and conventional tillage practices with crop diversification and conservation tillage in their production systems. Crop diversification is an attempt to expand agricultural diversity in space and/or time through crop rotation or multiple cropping, such as intercropping, mixed cropping and relay cropping (Kremen and Miles, 2012; Wezel et al., 2014). These practices have the common goal of adding variability in agro-ecosystem structure and function in areas, such as resource use, break pest and disease cycles (Wezel et al., 2014; Martens et al., 2015), improve productivity and sustainability and reduce economic risk of farm income relying on a single crop (Gan et al., 2002; Johnston et al., 2007; Smith et al., 2015). However, the requirement for specific knowledge on growth and development and management, as well as pest and diseases of different crop species within a cropping system highlights the challenges associated with crop diversification (Saskatchewan Pulse Growers, 2023).

The inclusion of alternative crops into wheat-based crop rotations has provided economic and environmental benefits to agricultural production systems on the Canadian prairies while reducing risk through improved production stability (Khakbazan et al., 2009; Cutforth et al., 2013; Luce et al., 2015). Many of these new production systems are considered more environmentally sustainable than mono-cropping systems; however, there is often a conflict between achieving the long-term goal of resource sustainability and the short-term goal of economic viability. Nonetheless, many studies have revealed that the profitability of diversified cropping systems with cereal, oilseed and pulse crops is higher than continuous cereal or fallow cropping systems in rain-fed regions (Niu et al., 2017; Smith et al., 2017; Khakbazan et al., 2020). Therefore, the inclusion of pulse and oilseed crops into cereal- and fallow-based cropping systems has been widely recognized for its rotational benefits.

2.2. Intensifying Crop Rotations with Pulse Crops

Pulse crops have been an important part of crop rotations across the Canadian prairies for more than 30 years. Pulse crops belong to the Fabaceae (Leguminosae) family and produce protein-rich dry seeds (Balasubramanian, 2015). Field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medikus), dry bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) are the dominant pulse crops produced in Canada, whereas faba bean (*Vicia faba* L.) and lupin (*Lupinus angustifolius* L.) are grown on a smaller scale (Balasubramanian, 2015; Government Canada, 2024). Globally, pulse crops are grown for their protein-rich seeds destined for human consumption, animal feeds, or industrial products (Siddique et al., 2012). In terms of annual production, pulse crops were the fifth largest crop group grown in Canada, making Canada a world leader in the pulse crop trade (Pulse Canada, 2023). Lentil and field pea are the prominent pulse crops grown in Canadian prairie cropping systems. In 2023, lentil and field pea production were 1.7 MT and 2.6 MT, respectively (Agriculture and Agri-Food Canada, 2024).

The benefits of pulse crops in production systems are well documented for atmospheric nitrogen (N) fixation (Hossain et al., 2016), pest and disease cycle disruption (Zander et al., 2016), water conservation (Gan et al., 2017), reduction in nitrous oxide (N_2O) emissions (Lemke et al., 2007) and improved soil health (Layek et al., 2018). In contrast, frequent inclusion of some pulse crops, such as chickpea, lentil and field pea growing in cropping systems are potentially susceptible to diseases, such as Ascochyta blight, Anthracnose and root rot diseases (Gan et al., 2006; Banniza et al., 2018), which could lead to yield losses resulting in reduced on-farm profitability.

2.2.1. Biological nitrogen fixation

Nitrogen is essential for plants for photosynthesis, growth and development (Sulieman, 2011). However, bioavailable N is considered one of the most limiting nutrients for crop production (Seitzinger et al., 2010). Approximately 90 % of the atmospheric N enters the biosphere through biological nitrogen fixation (BNF) because of diazotroph activity (Britannica, 2020) and it was discovered by Beijerinck in 1901 (Beijerinck, 1901). Thus, except for anthropic N inputs, BNF is the principal way the N supply is maintained and increased. Annual BNF accounts for 50-70 Tg of N, which is responsible for 16 % of the annual global N contribution for crop production (Herridge et al., 2008; Liu et al., 2010).

About a quarter of the total N-fixed in the global ecosystem is contributed by rhizobia–legume symbiosis (Singh and Varma, 2018; Wang et al., 2023). This process is mediated only by

N-fixing rhizobia bacteria (*Rhizobiaceae, α-Proteobacteria*), which develop endosymbiotic interactions with legume plants (Oldroyd and Downie, 2008). The rhizobia belong to five genera namely, *Rhizobium, Azorhizobium, Mesorhizobium, Sinorhizobium* and *Bradyrhizobium* (Andrews and Andrews, 2017). During highly specific interactions with legumes, these bacteria enter root tissues via root hairs or directly via wounded tissues and induce the formation of specialized root nodules (Dommergues et al., 1999). Biological nitrogen fixation in pulse crops is largely dependent on the formation of root nodules initiated by rhizobial strains (Abdellatif et al., 2016). Parameters such as the earliness of nodulation, nodule number, mass and colour, distribution of the root systems and longevity of the nodules are important for evaluating BNF (Goh et al., 2016; Hossain et al., 2017).

All diazotrophs encode nitrogenase, the enzyme complex that catalyzes the conversion of N_2 gas to ammonium (NH_4^+). The nitrogenase complex is highly conserved in all free-living and symbiotic diazotrophs (Koirala and Brözel, 2021). Nitrogenase enzyme has two oxygen-sensitive metalloprotein components: Molybdenum-iron protein (dinitrogenase) and iron protein (dinitrogenase reductase). The structural subunit of dinitrogenase reductase is encoded by the *nif*D and *nif*K genes. The 2 sub-units of dinitrogenase protein are encoded by the *nif*H gene, which is a universal marker for BNF (Wartiainen et al., 2008; Hu and Ribbe, 2015). This gene is the most sequenced and studied of the three core nitrogenase components. Transcription of *nif*H is strictly regulated by the level of molecular oxygen and fixed N, to minimize unnecessary energy consumption (Hu and Ribbe, 2015). Subsequently, *nif*H gene transcription is strongly related to the N-fixing activity of the plant (Hurek et al., 2002). Therefore, *nif*H is an ideal genetic marker for N-fixing microorganisms and can be used to verify the existence of N-fixing bacteria in samples as well as provide evidence of BNF in plants (Terakado-Tonooka et al., 2008; Lin et al., 2021).

In general, pulse crops acquire a high proportion, approximately 60 % or more of their total N through the BNF process. However, the BNF capacity of pulse crops is varied and dependent on complex interactions among plant genotypes, soil N levels and physical conditions, and success of inoculation (Fageria et al., 2014). The percentage of N derived from atmosphere (%Ndfa) by legume plants can be precisely quantified using stable isotopes of N (Rennie et al., 1982). Nitrogen is naturally available in two isotopic forms namely ^{15}N and ^{14}N, and ^{14}N being the dominant form. The ^{15}N isotope dilution technique can provide direct estimates of the quantity of biologically fixed N incorporated into plant tissues. In N-fixing plant tissue, ^{15}N content is the sum of ^{15}N content in

native N acquired from the soil, N fixed from the atmosphere, and N from the applied N fertilizer. The isotope dilution technique efficiently discriminates the N derived from atmosphere through fixation and the plant available N from soil or other growing medium when an appropriate non-fixing reference plant is used in the study (Danso, 1986) as the estimate varies depending on the non-fixing control crop (Wagner and Zapata, 1982).

2.3. Intensifying Crop Rotations with Oilseed Crops

Oilseed crops are the cornerstone of prairie agriculture, with a market value of more than 4.8 billion dollars annually (Statistics Canada, 2023). Oilseed crops grown in Canada include canola, soybeans, sunflowers, mustard, flax and camelina. Canola, camelina and mustard are well adapted to the cool, short-season conditions on the Canadian prairies. These three oilseed crops belong to the Brassicaceae family. Brassicaceae crops are widely adapted and cultivated around the world for human consumption and livestock feed.

Argentine canola (*Brassica napus* L.)

Canola was developed through an interspecific cross between *B. oleracea* and *B. rapa*, which contain high levels of erucic acid and glucosinolate (GLS) compounds (Kaur et al., 2022). Erucic acid is not metabolized properly in human or animal diets and is nutritionally undesirable. Glucosinolate reduces palatability and feeding efficiency in rapeseed meal. Consequently, consumers had a negative perception of the use of rapeseed oil in the household. Plant breeding efforts in Canada improved the fatty acid composition of rapeseed by reducing erucic acid contents to < 2 %, and markedly reducing total GLS contents to less than 30 μmoles g^{-1} of defatted rapeseed meal (Johnston et al., 2002). Therefore, after soybean [*Glycine max* (L.) Merr.], modified rapeseed has become the world's second most important vegetable oil due to these two significant improvements (Raymer, 2002). These modifications to the oil and meal of rapeseed led to the development of the name 'canola' to distinguish edible oil quality rapeseed from industrial quality oil (Johnston et al., 2002).

Canada is the largest single producer of Argentine canola, which is the number one cash crop and also the most economically important oilseed crop in Canada. Since the early 1940s, canola acreage has expanded, reaching 21.9 million acres by 2023 (Statistics Canada, 2023). Canola requires higher soil moisture for satisfactory establishment and emergence than wheat or barley

(Kephart and Murray, 1990). Thus, canola production is often constrained by drought and heat stresses during flowering on the semi-arid Canadian Prairies (Gan et al., 2004a).

Mustard species (*Sinapis alba* L., *Brassica juncea* L., *Brassica carinata* L. and *Brassica nigra* L.)

Mustard is a broad-leaf and cool-season annual oilseed crop produced primarily for the condiment market (Saskatchewan Mustard Development Commission, 2011). Studies on the Canadian prairies have demonstrated that mustard species have higher drought tolerance than other oilseed crops, particularly during the flowering stage (Angadi et al., 2000; Blackshaw et al., 2011).

Mustard is a significant oilseed crop grown in Canada with a farm cash receipt value of $ Can. 64.1 million in 2023 (Statistics Canada, 2023). Saskatchewan is the world's largest mustard exporter (Government of Saskatchewan, 2020). The three main types of mustard grown in western Canada are yellow mustard (*Sinapis alba* L.), brown mustard (*B. juncea* L.).) and oriental mustard (*B. juncea* L.). The different types of mustard vary in physical appearance and use (Government of Saskatchewan, 2020). Yellow mustard has a yellow seed coat and is primarily grown for the North American condiment industry (Saskatchewan Mustard Development Commission, 2023). The seed of yellow mustard also contains a water-binding mucilage that has been used as a binding agent and protein extender in prepared meats. Brown mustard has a reddish brown to dark brown seed coat, whereas oriental mustard seeds are primarily yellow to dark yellow. Both brown and Oriental mustards are used to develop products that are spicier than yellow mustard condiments (Saskatchewan Mustard Development Commission, 2023). Oriental mustard is primarily grown for export to Asian countries, where it is used to produce condiments. In addition, oriental mustard oil is used as a spicy cooking oil in some Asian countries, however, it is not used as a cooking oil in North America (Krstić et al., 2010).

Another species of mustard, *B. carinata* A. Braun, commonly called 'carinata' or 'Ethiopian mustard', has been grown in Saskatchewan for bioplastic and biofuel industries. Due to changing markets, however, there has been little to no Ethiopian mustard production in the province for several years (Government of Saskatchewan, 2020).

Black mustard (*B. nigra*) is predominant in some parts of Europe, Western Asia, North Africa and North and South America (Khaliq et al., 2017). However, in the United States and Europe, difficulties in harvesting reduced its popularity due to late maturity (Peter, 2012).

Camelina [*Camelina sativa* (L.) Crantz]

Camelina or false flax is a short-season annual or winter annual oilseed crop that is suitable for most soil types in rain-fed growing environments across the Great Plains (Gugel and Falk, 2006; Blackshaw et al., 2011). It is a new oilseed crop for the prairies that has been garnering significant attention over the past several years due to global interest in its agronomic benefits and industrial applications. Camelina has a unique oil profile making it suitable for a multitude of bio-based applications, including biofuel, equine and fish feed, bio-lubricants and healthy dietary oil (Berti et al., 2016, Zanetti et al., 2017).

Camelina is an attractive oilseed crop alternative for semi-arid prairies due to its environmental adaptability combined with satisfactory seed yields (Zanetti et al., 2017). Seedlings of camelina possess outstanding frost tolerance and full-grown plants exhibit good drought tolerance (Hunsaker et al., 2013). In addition, camelina is resistant to some of the disease common to Brassicaceae, such as Alternaria blackspot caused by *Alternaria brassicae*/ *A. alternata*/ *A. raphani* and blackleg disease caused by *Leptosphaeria maculans* (Deng et al., 2004; Vollmann and Eynck, 2015). Thus, camelina has been identified as a potential oilseed crop to intensify wheat-fallow rotations with relatively low agricultural input requirements (Obour et al., 2015).

2.3.1. Glucosinolates in Brassicaceae crops

Brassicaceae plants are a rich source of GLSs, which impart a characteristic spicy flavor profile to these plants (Nguyen et al., 2020). Glucosinolates are amino acid-derived, sulfur-containing secondary metabolites found not only in Brassicaceae plant species, but also in a few other plant families. The chemical structure of GLSs has three moieties: a β-thioglucose moiety, a sulfonated oxime moiety, and a variable side chain derived from amino acids (Fig. 2.1). Glucosinolates can be broadly classified as aliphatic, aromatic and indole according to the precursor amino acid (Lv et al., 2022). To date, nearly more than 200 different GLS structures have been identified and their distribution varies from species to species (Chhajed et al., 2019). Glucosinolates are synthesized from only eight amino acids (alanine, methionine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan) and several chain-elongated homologues (Chen and Andreasson, 2001; Fahey et al., 2001; Windsor et al., 2005). Secondary modifications of the GLS side chain (oxidation, hydroxylation and esterification) also contribute to the enormous diversity of this class of phytochemicals (Pfalz et al., 2011; Pfalz et al., 2016).

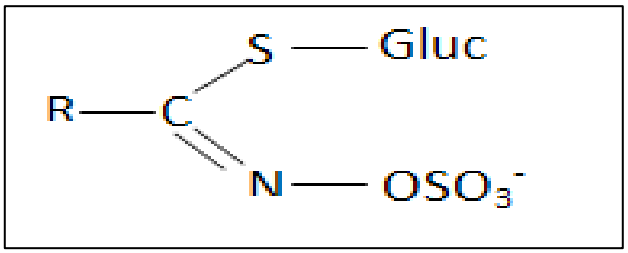

Fig. 2.1. General structure of glucosinolate (Lv et al., 2022)

Generally, GLSs are biologically inactive as thioglucoside glucohydrolase (myrosinase), the enzyme that facilitates hydrolysis, is segregated within plants (Borgen et al., 2010). The enzyme contacts with GLS upon tissue disruption and consequently, GLSs are hydrolyzed into a wide range of biologically active compounds, such as iso-thiocyanates (ITC), thiocyanates, nitriles, goitrin and epithionitriles (Chhajed et al., 2019). Glucosinolate hydrolysis products play a key role in plant defense against insects, bacteria, and fungi. Some hydrolysis products, such as ITCs', can be hydrolyzed further to generate toxic compounds that can be injurious to certain pathogens (Singh, 2017). The ultimate hydrolyzed product can vary depending upon many factors, including pH, the presence of ferrous ions and myrosinase interacting proteins. At neutral pH, ITC is the dominant product while nitrile derivatives are more prominent at acidic pH (Chen and Andreasson, 2001). Glucosinolates, which contain terminal double bonds produce an epithionitrile when they are degraded in the presence of epithio-specifier proteins and ferrous ions (Chen and Andreasson, 2001; Rouzaud et al., 2004).

The composition and contents of GLSs are influenced by the genotype, climatic conditions and crop management practices, including fertilization, irrigation, harvest time and plant part (Rangkadilok et al., 2002; Tripathi and Mishra 2007; Verkerk et al., 2009). They differ completely among plant genera and different parts (Table 2.1).

Table 2.1. Major type of glucosinolates in main Brassicaceae oilseed crops

Oilseed crop	Type of glucosinolate
Industrial mustard (carinata)	Sinigrin (Cartea and Velasc, 2008)
Oriental mustard	Sinigrin (Cartea and Velasco, 2008)
Yellow mustard	Sinalbin (Kirkegaard and Sarwar, 1999)
Camelina	Glucocamelinin (Schuster and Fried, 1998)
Canola	Progotrin (Cartea and Velasco, 2008)

2.3.2. Brassicaceae crops as a bio-fumigant

Creating a pest-free soil environment before replanting crops at the same site has become a major concern for growers (Winkelman et al., 2021). Some synthetic pesticides, such as methyl bromide have been withdrawn from the market due to their harmful effects on human health and the environment (Park et al., 2020). Therefore, replacing synthetic pesticides with natural compounds with anti-biological properties will be environmentally and economically beneficial (Adhikari et al., 2020).

The use of Brassicaceous plant species has been suggested as a means of mitigating the effects of replant disease through bio-fumigation (Ren et al., 2018; Hanschen and Winkelmann, 2020; Liu et al., 2021). The term 'bio-fumigation' was originally coined by J. A. Kirkegaard in 1993 (Matthiessen et al., 2006) and refers to the suppression of various soil-borne pests and diseases through naturally occurring compounds (Srivastava and Ghatak, 2017). The effect of bio-fumigation in soils results from the action of volatile substances, which are degradation products from Brassicaceae plant secondary metabolites, particularly ITC (Liu et al., 2021). Several plant pathogenic nematodes, such as *Pratylenchus penetrans* (Mazzola et al., 2009), *Globodera rostochiensis* (Bhatta et al., 2022), and *Meloidogyne incognita* (Dutta et al., 2019; Dutta et al., 2021) were suppressed by bio-fumigation using Brassicaceae plants, including black mustard, oriental mustard, yellow mustard and canola. In addition, multiple fungal soil-borne pathogens present in potato crops, including *Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Fusarium sambucinum* were successfully controlled by Brassicaceae crops planted as green manures (Larkin and Griffin, 2007). Volatile ITCs from a mixture of rapeseed and canola reduced the pathogens, *Alternaria alternata*, *Colletotrichum dematium*, *Fusarium oxysporum* and *Pythium ultimum* as well as weeds in strawberries (Mattner et al., 2008). Thus, all these results suggest that enzymatic GLS breakdown products in Brassicaceae crop residues have the potential to control a wide range of phytopathogens, which are vital in agricultural crop production.

Different Brassicaceae oilseed crops contain various levels of GLSs with diverse compositions resulting in different biocidal activities (Clarke, 2010). However, in the natural environment, pathogens co-exist with a diverse microbial community. Due to the anti-microbial activity in GLS hydrolysis products, Brassicaceous plant material may affect non-target micro-organisms, which may benefit plants (Lemanceau and Alabouvette, 1993). Once the pathogen is

suppressed due to the Brassicaceous materials, plant-beneficial microbes, may obtain more space and resources, leading to rapid proliferation. Therefore, plant disease control not only indicates pathogen suppression, but also alters available resources for microbial growth, which leads to changes in microbial communities (Ren et al., 2018). Thus, understanding the impact of Brassicaceae bio-fumigation on non-target soil micro-organisms with a central role in soil quality, nutrient cycling and crop production is vital (Astudillo-García et al., 2019).

2.4. Impact of Brassicaceae Oilseed and Pulse Crops in Cropping Systems

2.4.1. Impact on nitrogen cycle

There is increasing evidence that microbial abundance and diversity, and particularly that of N-cycling communities, is critical to soil nutrient transformations, plant nutrient uptake, protection against abiotic and biotic stresses, recycling of wastes, and detoxification of environmental pollutants (Maron et al., 2018; Chen et al., 2020). Plants have a large requirement for N and contain more atoms of N than any other soil-derived element, except hydrogen (Wagner, 2011). The fixation of atmospheric N_2, either through biological or industrial processes, is key to the N cycle (Stein and Klotz, 2016). Once this 'fixed' N enters the soil ecosystem, it undergoes a variety of microbially-mediated transformations, changing from one reactive form of N to another, and eventually being released back into the atmosphere, usually as N_2. Rochette et al. (2006) described the N-cycle as a 'loop within a loop system' (Fig. 2.2).

In that system, the outer loop (Fig. 2.2X) describes how N_2 originating in the atmosphere moves into the soil-plant system via N-fixation (industrial + biological) and is ultimately returned to the atmosphere as ammonia gas (NH_3) through volatilization, nitric oxide (NO) and N_2O through denitrification, and N_2 through both denitrification and anammox processes. The inner loop (Fig. 2.2Y) describes how N moves through the soil-plant system via mineralization, nitrification and immobilization, and is lost from the system through nitrate (NO_3^-) leaching (Fig. 2.2Z).

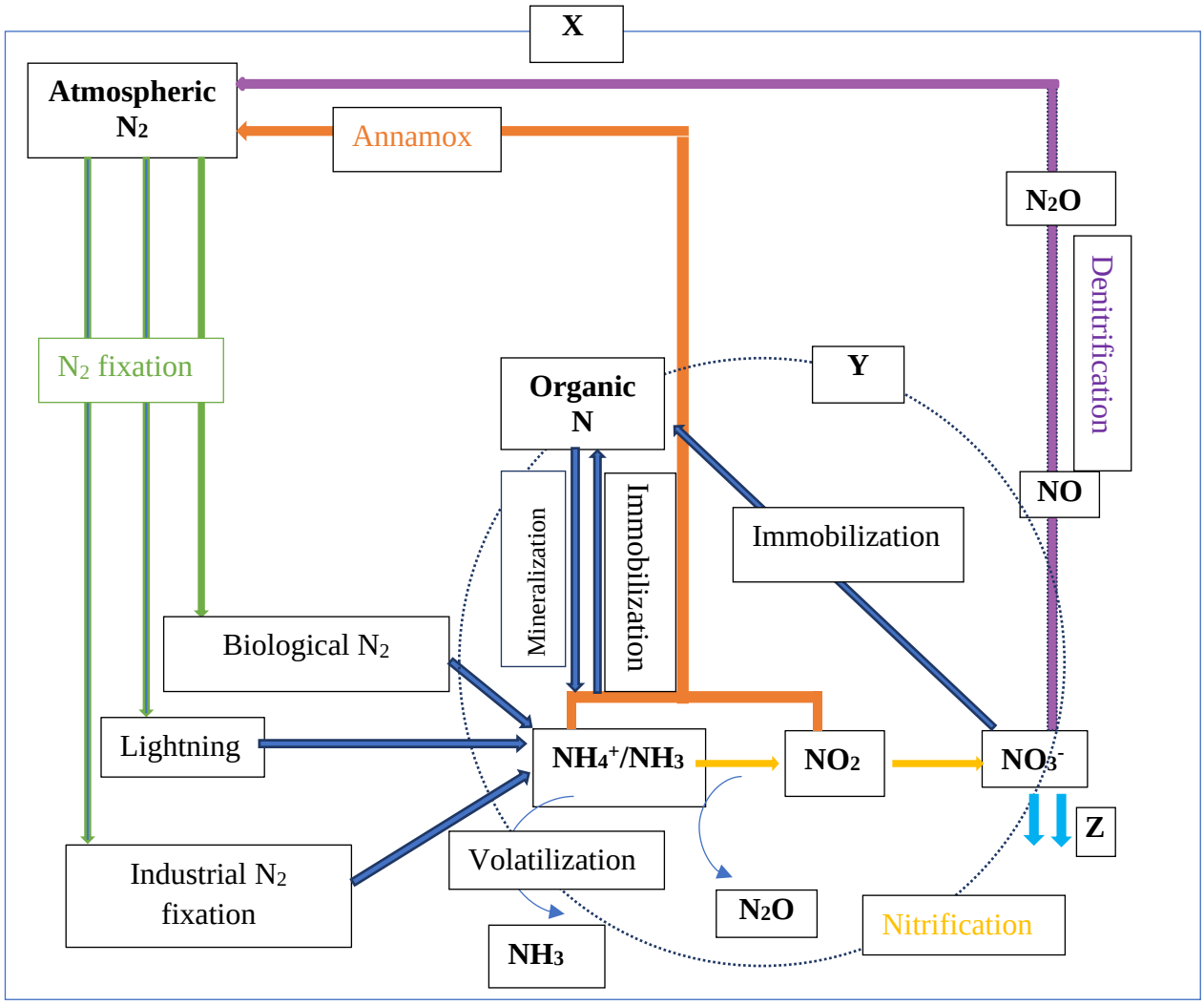

Fig. 2.2. Schematic representation of the nitrogen cycle as a 'loop within a loop' (modified from Rochette et al., 2006; Bernhard, 2010).

Mineralization in soil

Nutrient cycling within soil is a continuous process of competing mineralization and immobilization reactions, mediated by heterotrophic soil microorganisms. The principal products of the soil organic matter (SOM) mineralization process are carbon dioxide, methane, NH_4^+ (ammonium) and NO_3^- (Hopkins, 2008). In crop production, synchronizing nutrient release from organic residues with plant demand is vital throughout the growing season (Whalen, 2015). Soil nutrient status is related to both the quality and quantity of soil organic matter (SOM) and the organic materials added (Abbasi et al, 2015; Bashir et al., 2021). Generally, SOM plays a key role in soil physical, chemical and biological characteristics, including soil surface structure, porosity,

water infiltration, water and nutrient holding capacity, buffering capacity, soil faunal and microbial diversity and activity, nutrient availability and surface runoff. Therefore, elevated levels of high-quality SOM are important for maintaining a sustainable agricultural system (Janzen, 2006; Weil and Brady, 2017).

The quality of residue influences decomposition and N mineralization. Residues that have low lignin and cellulose contents with low carbon: nitrogen ratio (C:N) are considered high quality residues, which have high decomposition rates (Chaves et al., 2004; Manzoni et al., 2008; Gentile et al., 2009). Many studies investigated the biochemical composition of different Brassicaceae and pulse crop residues. Paul and Solaiman (2004) revealed that Brassicaceae crop residues had higher concentrations of C and N (44.6 % and 5.5 %, respectively), narrower C:N (8:1), and lower lignin concentration (5.3 %) when compared with sugarcane trash, press mud and cow dung. Moreover, the same study found that N released from the soil amended with Brassicaceae crop residues produced the highest amount of mineral N (ranging from 106 to 170 mg N kg^{-1} soil) throughout the 84-d incubation period. Snyder et al. (2009) characterized different Brassicaceae oilseed crop residues as averaging 50 % C and 5.9 % N by weight. The C:N of the reported crop residues (white mustard, 8.2:1; rapeseed, 8.7:1; Oriental mustard, 8.2:1) were similar and averaged 8.4:1.

Generally, pulse crop residues also have lower C:N ratios ranging from 25:1 to 40:1 (Stevenson and van Kessel, 1996) compared to cereal crops (80:1-127:1) and are also considered high quality residues (Gan et al., 2010). These low C:N maintain high C mineralization rates. Gan et al. (2010) reported that under low soil moisture, the N mineralization rate in soil under lentil (2.96 kg ha^{-1} day^{-1}) and dry pea (2.54 kg ha^{-1} day^{-1}) were greater than soil under wheat (2.12 kg ha^{-1} day^{-1}). Therefore, crop residues of Brassicaceae oilseed plants and pulse crops may have a potential to be utilized as organic sources of N and other nutrients in agricultural production systems and reduce the use of mineral fertilizers. However, studies revealed that soil erosion is accentuated after the harvest of oilseed and pulse crops compared to wheat due to accelerated crop residue decomposition (Soon and Arshad, 2002; Lupwayi et al., 2004; Sharratt and Schillinger, 2016).

Nitrification in soil

Nitrification is the biological process of oxidizing NH_4^+ to NO_3^-, which is a crucial step in soil N removal. This process has a profound influence on N cycling since it mediates the mineral

N uptake by plants, N retained in the soil or lost to the environment (Subbarao et al., 2015). Nitrification includes two steps; 1) oxidation of NH_4^+ to nitrite (NO_2^-) catalyzed by ammonia-oxidizing bacteria (AOB) and archaea (AOA) via the action of two enzymes ammonia monooxygenase and hydroxylamine oxidoreductase and 2) oxidation of NO_2^- to NO_3^- by nitrite-oxidizing bacteria via the enzyme nitrite oxidoreductase (Könneke et al., 2005; Prosser and Nicol, 2008). Researchers have indicated that the relationship between an increase in the population of AOB and AOA and nitrification activity is exponential (Di et al., 2009; Bock and Wagner, 2013). The diversity and ecology of AOB and AOA have been intensively studied relying mainly on analysis of *amoA* gene, which encodes the key metabolic enzyme ammonia monooxygenase (Alves et al., 2018; Aigle et al., 2019). Since the *amoA* gene is well conserved and present in all AOB and AOA, it has become a popular target for cultivation-independent molecular studies to evaluate ammonia-oxidizing microbes.

Suppression of soil nitrification, termed biological nitrification inhibition, occurs naturally in some ecosystems (Subbarao et al., 2006). Brassicaceae plants exhibit this process due to various GLS hydrolysis products (Bending and Lincoln, 2000; Subbarao et al., 2015). Brassicaceae oilseed crops, including canola, oriental mustard and yellow mustard with different GLS types, had a higher accumulation of NH_4^+-N in soils compared to soils amended with tissues containing no or low GLS concentrations (Brown and Morra, 2009). Field studies indicate that the abundance of microorganisms responsible for NH_4^+ oxidation is lower in soils after a canola crop than wheat (Kirkegaard et al., 1998). This further supports the possibility of delayed nitrification because of the compounds produced by Brassicaceae tissues. In addition, Bending and Lincoln (2000) demonstrated the specific inhibition of soil nitrifying bacterial community size and their activity by these GLS hydrolysis products, indicating that particularly, nitrification may be inhibited.

Several studies revealed that legume plants also have the ability to inhibit nitrification, due to the presence of phytochemicals in their root exudates. A field study of Paungfoo-Lonhienne et al. (2017) showed that peanut (*Arachis hypogaea* L.) and soybean in sugarcane farming systems had 30-35 % fewer ammonia-oxidizing microbes compared to a fallow system. Furthermore, another study with sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.) and peanut showed a detectable biological nitrification inhibition in root exudates (Subbarao et al., 2007). However, the mechanisms in driving responses of ammonia-oxidizing communities and their nitrification capacity in soil during legume cropping are still unknown.

Denitrification in soil

Denitrification is mediated through a sequence of enzyme-catalyzed reactions, in which NO_3^- is reduced to NO_2^- and NO to N_2O or N_2 under anoxic conditions by a diverse group of microorganisms. Nitrous oxide is a powerful greenhouse gas that contributes to ozone depletion (Hayashi and Itsubo, 2023). It is estimated that 70 % of global anthropogenic N_2O emissions are attributable to agricultural soils (Charles et al., 2017). Soil mineral N, applied fertilizer N, crop type and maturity level of crop residue are important factors influencing N_2O emissions (Abalos et al., 2022; Janz et al., 2022). By performing meta-analysis by Liu et al. (2016) concluded that N_2O emissions were positively correlated with crop residue-C inputs and soil respiration. This demonstrates the potential of crop residues to provide sufficient substrate for denitrifier populations, together with enhanced N availability, it could result in increased N_2O production.

Despite the prevalence of Brassicaceae oilseed crops in crop sequences on the prairies, information of how oilseed crops influence N_2O emissions compared with other crops is limited. In a meta-analysis study of oilseed rape, Walter et al. (2015) reported that annual N_2O emissions from winter oilseed rape were 22 % higher than those from winter cereals fertilized at the same rate. The accumulations of higher soil mineral N during the growing season, particularly in the post-harvest period under winter oilseed rape compared with winter cereals may have been partially responsible for the increased N_2O loss. In addition, Kirkegaard et al. (1998) suggested that biocidal compounds were released during the decay of canola residues resulting in a flush of microbial N, which stimulated activity and N transformations in the remaining microbial populations. Nevertheless, further research is needed to identify the pertinent biochemical characteristics of Brassicaceae crop residues to develop residue-specific N_2O emissions.

Pulse crop incorporation in crop rotations is one of the key strategies to mitigate synthetic N inputs while sustaining grain yields (Jensen et al., 2012). Grain legumes can reduce N demand of the subsequent crop, and consequently decrease N_2O emissions associated with synthetic N fertilizers. In an extensive review of the use of legumes to mitigate climate change, Jensen et al. (2012) concluded that N_2O emissions during the legume growing season did not differ substantially from unplanted or unfertilized soils. In contrast, some studies reported elevated N_2O losses after the termination of a legume crop, when plant residues were incorporated into the soil (Gomes et al., 2009, Pappa et al., 2011). The rapid mineralization of soil-incorporated legume crop residues resulted in substantial amounts of soil-accumulated N, which increased the potential of N as N_2O

via denitrification (Jensen et al., 2012). However, data for grain legumes on denitrification is extremely limited in agroecosystems.

Anammox produces N_2 by oxidizing NH_4^+ with NO_2^- reduction (Wang et al., 2019). Anammox has been detected in several aquatic ecosystems (Kuypers et al., 2005; Hietanen and Kuparinen, 2008; Stevens and Ulloa, 2008) and found in various soil types (Hu et al., 2011a; Zhu et al., 2011). However, the importance of anammox in soil N cycling has not been fully explored.

Biological nitrogen fixation

Génard et al. (2017) observed that lupin, clover and vetch grown with rapeseed in intercropping systems had 34 %, 140 % and 290 %, respectively higher BNF than in their respective mono-cropping systems). Another study of soybean in relay cropping with camelina showed a 44 % reduction in nodule number and 38 % reduction in ureide-N, indicating lower BNF compared to a soybean monocropping system. The researchers assumed BNF reduction in soybean occurred due to the presence of GLS hydrolysis products in camelina residues remained in soil (Mohammed et al., 2022). Studies also revealed that Brassicaceae oilseed crops require higher amounts of NO_3^- for their growth than cereals. Consequently, the high NO_3^- requirements of Brassicaceae plants decreased the NO_3^- concentration in the soil and in turn increased the BNF of pulse crops (Waterer et al., 1994; Macduff et al., 1996). However, information regarding studies that evaluated the impact of GLS contents in Brassicaceae crops on BNF capacity in pulse crops in crop rotations is not available on the Canadian prairies.

2.4.2. Impact on soil water uptake

Soil water availability is the most limiting factor for crop production in the Canadian semi-arid prairie (Cutforth et al., 2007). Potential evaporative demand in the Brown soil zone is the highest among the agro eco-regions on the Prairies (Cutforth et al., 1997). For successful production in semi-arid regions, a crop must be of high value and/or very efficient at extracting water from the soil and utilizing that water during the growth process. A well-distributed smaller root system with fewer roots at a shallow depth and high root biomass in deeper soil horizons is more efficient in using soil moisture (O'toole and Bland, 1987).

Brassicaceae spp. have taproot systems facilitating crop access to water and nutrients deep in the soil profile. Cutforth et al. (2013) reported that wheat and canola withdrew water from deeper soil profiles (120–130 cm), than pulse crops (100–110 cm). In addition, pulse crops withdrew

substantially less water than oilseed crops and wheat below about the 80-cm depth, increasing deep soil water reserves potentially accessible by deeper rooting crops planted in subsequent growing seasons. The oilseed crops withdrew less water from the upper regions of the soil profile than wheat. Thus, compared with wheat, oilseed crops leave a higher amount of water available to the following crop (Cutforth et al., 2013). Several studies revealed that *B. napus* and *B. juncea* had high yields under moisture stress conditions (Miller et al., 2003c; Gan et al., 2004a; Gan et al., 2007).

Root architecture is also an important physiological component for crop plants as it is responsible for water and nutrient uptake (Zobel et al., 2007). Rooting depths and root morphological characteristics of some oilseed crops have been studied extensively (Merrill et al., 2005; Liu et al., 2010). Liu et al. (2010) described *B. napus*, *B. juncea* and wheat had the greatest proportion (~85 %) of total root length as 'extra fine' roots (< 0.4-mm diameter), while they comprised only 15 % of 'fine roots' (0.4- to 2.0-mm diameter). For pulse crops, the contributions of extra fine roots to the total root length accounted for about 50 %, and the rest as fine roots. This result suggests that oilseed species and wheat have a stronger ability for water uptake and nutrient acquisition than pulse crops, as the former had more and finer roots than the latter. Therefore, producers on Canadian semi-arid prairie can increase the overall performance of the cropping system by improving water uptake by including shallow-rooted crops with Brassicaceae oilseed crops containing deep and fine rooting systems (Fan et al., 2016; Chen et al., 2022).

2.4.3. Impact on crop productivity and contribution to the Canadian economy

Crop productivity is the quantitative measurement of crop yield produced within a known area. The primary objective of crop production is to maximize the transformation of resources into useful products (Pelletier et al., 2011). Crops, generally, are more productive when grown after unrelated species. This benefit has come to be known as the rotation or 'break-crop' effect (Angus et al., 2015).

Many studies have documented favorable rotation benefits of Brassicaceae crops on the subsequent yield of wheat in the Canadian prairie region of Saskatchewan and Alberta, and in the US Northern Great Plains. The magnitude of a positive wheat yield response to previous Brassicaceae crops is in general consistent in these three geographic regions (Kirkegaard et al., 2008; Kirkegaard and Ryan, 2014). Much of the benefit of growing a Brassicaceae oilseed crop is

due to the control of root diseases (Angus et al., 1994). Some studies have attributed crop yield increases following canola to suppression of arbuscular mycorrhiza fungi (AMF) as canola is not a host of AMF. Arbuscular mycorrhizal fungi can act as a sink for photosynthates in a wheat crop that does not need the benefit of increased phosphorus uptake (Harris et al., 2002). Contradictory, Angus et al. (2015) suggested that the mean additional wheat yield after canola and mustard was independent of the yield level of the following wheat crop. Furthermore, a six-year study by Schillinger and Paulitz (2018) reported that the average spring wheat seed yield following canola was 3,292 kg ha^{-1} compared to 3,897 kg ha^{-1} following wheat representing a 17 % yield reduction.

Many studies demonstrated that the inclusion of grain legumes in crop rotations resulted in greater yields of subsequent crops in cropping systems (Zentner et al., 2004; Miller et al., 2006). Growing field pea or lentil before wheat or canola mostly increased seed yields in many rotational studies (Burgess et al., 2012; Campbell et al., 2011; Williams et al., 2014). For example, Burgess et al. (2012) showed that pulse crops produced positive rotational benefits on subsequent wheat yield compared to wheat following wheat, even though there was no difference in energy inputs and chemical usage between the two cropping systems. In that study, a higher wheat yield (28 %) was observed in a pea-wheat rotation (2.15 Mg ha^{-1}), as compared to continuous wheat (1.68 Mg ha^{-1}). Furthermore, a 3-yr cropping sequence study, repeated for five cycles in Saskatchewan involving pulse crops (field pea, lentil and chickpea), cereals (wheat, barley and durum) and summer-fallow showed that grain production and protein yield of subsequent wheat in the cereal-pulse system increased by more than 35 % and nearly 50 %, respectively compared to the summer-fallow system (Gan et al., 2015). Another study by O'Donovan et al. (2014) showed that on average, canola seed yield increased by 10 % when field pea or lentil was the preceding crop compared with spring wheat.

The incorporation of pulse crops and oilseed crops in cropping systems offers a multitude of agronomic and economic advantages, including enhanced water utilization, improved soil quality, and disrupt disease, pest and weed life cycles (Moussart et al., 2013; Gan et al., 2015; Gustafson and Yildiz, 2017). Pulse and Brassicaceae oilseeds have become dominant crops in Canadian agriculture over the last three decades, contributing to a yearly market worth approximately $3.4 billion and $14 billion throughout the period of 2021-2022 (Agriculture and Agri-Food Canada, 2024). One of the most highlighted benefits of grain legumes is their potential to reduce the use of crop inputs, such as fertilizer and irrigation. The BNF potential of pulse crops mitigated the

requirement of supplementary N for the subsequent non-N-fixing crops in cropping systems. Reduction of plant N requirement leads to both economic (Cox et al., 2010) and energy use benefits since over 80 % of total energy inputs from traditional grain production systems are made up of N fertilizer and fuel (Zentner et al., 2004). Thus, it consequently reduced associated costs and increased net revenue in the cropping system than traditional cereal cropping systems (Liu et al., 2016; Khakbazan et al., 2020).

Considering the available information, many studies illustrated the solitary impacts of including Brassicaceae oilseed and pulse crops in different cropping systems. The information addressing cumulative impacts of Brassicaceae oilseed and pulse crops in cropping systems on agronomically beneficial aspects is extremely limited, especially in the Canadian prairies. Thus, more research is required to evaluate the potential benefits that are associated specifically with oilseed and pulse crops in wheat-based cropping systems. The current research information will contribute to developing a comprehensive understanding to optimize the use of these alternative crops in diversified cropping systems.

3. THE IMPACT OF SELECTED BRASSICACEAE OILSEED CROPS ON THE BIOLOGICAL NITROGEN FIXATION IN SUBSEQUENT PULSE CROPS

3.1. Preface

Grain legumes are widely recognized for their ability to fix atmospheric di-nitrogen, which offers a sustainable alternative to synthetic nitrogen (N) fertilizers. Thus, incorporation of pulse crops into cereal cropping systems is beneficial. However, understanding how a preceding crop impacts biological nitrogen fixation (BNF) in pulse crops in agricultural crop rotations is limited. This is especially relevant to Western Canada, where Brassicaceae oilseed crops are frequently rotated with grain legumes. These oilseed crops are known to contain bioactive chemical compounds mainly glucosinolates (GLSs), which are potentially toxic to non-targeted soil microorganisms. This study, therefore, investigated the BNF in pulse crops grown on stubble of various Brassicaceae oilseed species, each with distinct GLS profiles.

3.2. Abstract

Glucosinolates (GLS) in Brassicaceae crops have antimicrobial properties, which may impact nitrogen (N) fixing microbes, crucial for biological nitrogen fixation (BNF). Despite the widespread cultivation of Brassicaceae crops in rotations, their impact on the BNF capacity of succeeding pulse crops in cropping systems remains unclear. Thus, a field experiment was designed to evaluate how different Brassicaceae oilseed crop species affect N fixation parameters in two pulse crops grown in the semi-arid Canadian Prairies. This field study was conducted from 2018 to 2020 using five different Brassicaceae crop species [Argentine canola (*Brassica napus* L.), camelina (*Camelina sativa* L. Crantz), industrial mustard (*Brassica carinata* L.), oriental mustard (*Brassica juncea* L.) and yellow mustard (*Sinapis alba* L.)]. In the following cropping year, field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medikus) were planted on each Brassicaceae crop stubble, except lentil on yellow mustard stubble. The percentage of N derived from the atmosphere (%Ndfa), fixed N amount, root nodule dry weight and root diazotroph concentration (*nif*H gene copy concentration) were quantified in the pulse crop phase. On average, field pea grown on Argentine canola stubble consistently had a 5.3-34.6 % higher %Ndfa for total above-ground biomass than the average of field pea on other Brassicaceae crop stubbles at all test sites. The higher *nif*H gene concentration and root nodule dry weight in field pea on Argentine canola stubble may

contribute to enhanced BNF. In contrast, lentil had a uniform BNF capacity regardless of the different Brassicaceae oilseed crop stubbles at Swift Current and Scott. At Brooks, lentil grown on Argentine canola stubble showed 17.9 % higher %Ndfa and 26.6 % higher fixed N content than lentil on other Brassicaceae stubbles potentially attributable to higher root nodule dry weights. Brassicaceae seed GLS analysis indicated that yellow mustard seeds had the highest GLS concentration (163.1-138.4 μmol g^{-1} of seed**)** and Argentine canola had the lowest (6.10-9.58 μmol g^{-1} of seed). However, there was no apparent correlation between Brassicaceae seed GLS content and root nodulation, *nif*H gene copy concentration or BNF capacities in subsequent pulse crops. In addition, the fixed N amount and total soil available N (0-30 cm) had a negative correlation for field pea (r = - 0.65, $P<0.0001$) and lentil (r = - 0.73, $P<0.0001$). The study indicated that the impact of Brassicaceae residues on BNF in subsequent pulse crops varies with the pulse crop species and growing environment. In addition, Argentine canola stubble had a lesser suppressive effect on plant diazotrophs in subsequent pulse crops than other Brassicaceae crop stubbles. Further research and analyses would deepen the understanding of the complex interactions between the chemical composition of Brassicaceae plant tissue and subsequent pulse crop BNF for sustainable agricultural practices.

3.3. Introduction

Nitrogen is a crucial plant nutrient that originates solely from the atmosphere, and its transformation and movement within an ecosystem are predominantly mediated by biological processes and the hydrologic cycle (Griffiths et al., 2016). The atmosphere contains a vast amount of di-nitrogen (N_2) gas (4×10^9 Tg N), which is biologically unavailable. A small portion of N_2 (ca. 473 Tg N) is converted annually into biologically active forms (Fowler et al., 2015) with an estimated 120 Tg N transformed through industrial fixation of ammonia, 40 Tg N through fossil fuel combustion, 5 Tg N through natural non-biological processes (i.e., lightning) and 128 Tg N through biological N_2 fixation (BNF) in natural terrestrial ecosystems, 120 Tg N in marine and aquatic ecosystems and 60 Tg N in agricultural ecosystems.

In agricultural cropping systems, the capability of grain legumes to biologically fix atmospheric N_2 and supply N for subsequent non-legume crops is widely recognized (Stagnari et al., 2017; Jensen et al., 2020; Costa et al., 2021). Rhizobia-legume symbiosis is an alternative to reduce reliance on synthetic N fertilizers and a vital component that facilitates establishing

sustainable cropping systems. While it is widely known that the inclusion of pulse crops in cereal-based cropping systems has many benefits (Kumar et al., 2023; Lasisi and Liu, 2023), the understanding of how a preceding crop impacts BNF in grain legumes is limited.

Inclusion of Brassicaceae crops, especially canola, every other year in crop rotations has become increasingly common in the Canadian prairies (Gill, 2018). In contrast, other Brassicaceae species, such as condiment mustard and camelina are less frequently utilized. Understanding how these lesser-grown Brassicaceae crops impact pulse-wheat cropping system performance is important for further diversifying cropping systems and enhancing sustainability in prairie agriculture. Thus, it is important to examine the impact of Brassicaceae oilseed crops on pulses in rotations to develop crop management recommendations and mitigate agronomic risk with respect to cropping system performance. Most Brassicaceae crops can act as bio-fumigants because they contain glucosinolate (GLS) hydrolysis products, such as thiocyanates, isothiocyanates (ITSs), epithionitriles, nitriles, indoles and oxazolidine-2-thiones (Hu et al., 2011b). Glucosinolate hydrolysis products are toxic to plant pathogens including, *Rhizoctonia solani* (Ren et al., 2018), *Sclerotinia sclerotiorum* (Chen et al., 2020), *Alternaria brassicicola* (Tao et al., 2022) and *Fusarium oxysporum* (Meng et al., 2018). The toxicity of these compounds on microbes includes inhibition of growth and germination of sclerotia or spores.

Research on the allelopathic effects of Brassicaceae crops on the performance of other crops within production systems is sparse and presents conflicting results. For instance, Pellerin et al. (2007) found incorporation of canola (*Brassica napus*) residues had a neutral effect on mycorrhizal colonization of corn (*Zea mays*) crop. In contrast, some studies showed allelopathic effects of synthesized GLS hydrolysis products on plant-beneficial endophytes (Nongbri et al., 2012), arbuscular mycorrhizal (Stinson et al., 2006; Cantor et al., 2011) and ectomycorrhizal fungal colonization (Cantor et al., 2011). Portales-Reyes et al. (2015) revealed that leaf extracts and synthetic compounds resembling key GLS hydrolysis products present in leaf extract (allyl ITC) and benzyl ITC) of garlic mustard (*Alliaria petiolata*) prevented the formation of nodules in the annual legume, *Amphicarpaea bracteate in vitro*. In addition, application of these chemicals reduced plant growth and disrupted legume-rhizobia mutualism. Conversely, incubation studies indicated that application of *Brassica juncea* seed meal increased soil diazotrophs, specifically *Sinorhizobium* and *Bradyrhizobium* abundance (Hollister et al., 2013; Siebers et al., 2018). These

findings suggest that the impacts of GLS hydrolysis products vary with the type of chemical compound added and the type of soil or plant micro-organism targeted (Hu et al., 2011b).

Most studies investigating how GLSs affect N_2-fixing microbes have used pure strains and *in vitro* approaches, instead of natural or field environments. The impact of the various GLS hydrolysis products may vary within the soil environment due to complex interactions with soil solids (Matthiessen and Shackleton, 2005). In addition, most of the studies focused on the chemical compounds present in Argentine canola (*B. napus*) and brown mustard (*B. juncea*) plants (Hollister et al., 2013; Siebers et al., 2018). However, condiment mustards (yellow, oriental and industrial mustards) have different compositions of GLS, which can potentially influence BNF of succeeding pulse crops differently than *B. napus* and *B. juncea*. Moreover, studies adding GLS hydrolysis products in a pure chemical form rather than plant residue do not resemble natural strategies that occur during the decomposition process of plant residues (Baldrian et al., 2011; Hollister et al., 2012). Thus, there is a dearth of information on how BNF ability in pulse crops is affected by high GLS containing Brassicaceae oilseed crop stubble in their natural environment despite the use of canola with cereal and pulse crops in crop rotations. This field experiment was conducted to assess BNF ability of field pea and lentil grown on stubble of selected Brassicaceae oilseed crop species, including, Argentine canola (*B. napus* L.), industrial mustard (*B. carinata* L.), oriental mustard (*B. juncea* L.), yellow mustard (*Sinapis alba* L.) and camelina [*Camelina sativa* (L.) Crantz], which contain different profiles of GLS (Kirkegaard and Sarwar, 1999; Sun et al., 2019), after developing the following hypotheses:

1) Glucosinolate profiles will vary quantitatively and qualitatively in seeds of canola, yellow mustard, oriental mustard, industrial mustard and camelina crops;

2) Lentil and field pea grown on the stubbles of preceding yellow, oriental and industrial mustard crops, and camelina will have lower BNF ability than when grown on canola stubble. BNF ability will correlate with the abundance of diazotrophs, which is influenced by the plant GLS content.

The objectives of the experiment were to:

1) Evaluate the GLS content in seeds of canola, yellow mustard, oriental mustard, industrial mustard and camelina.

2) Quantify the BNF ability of succeeding field pea and lentil grown on canola, yellow mustard, oriental mustard, industrial mustard and camelina stubble.

3) Compare the effect of preceding canola, yellow mustard, oriental mustard, industrial mustard and camelina on the root nodulation and the abundance of diazotrophs in subsequent field pea and lentil.

3.4. Materials and Methods

3.4.1. Experiment sites, design and management

The field experiment was established in 2018 at three test sites located near Brooks, AB; Scott, SK and Swift Current, SK (Table 3.1). This experiment is a component of a larger study. For the current study, nine rotation treatments and continuous wheat (as a control) were selected (Table 3.2). Data from years 2 and 3 of the cycle were reported here. To simplify, rotations are abbreviated considering the final three years (2019, 2020 and 2021).

Table 3.1. Global Positioning System (GPS) coordinates and dominant soil types of three test sites

Information type	Brooks, AB	Scott, SK	Swift Current, SK
GPS coordinates	Lat. 50° 33′ 51″ N; Long. 111° 53′ 56″ W; Elev. 747 m	Lat. 52° 17′ 34″ N; Long. 108° 57′ 3″ W; Elev. 660 m	Lat. 50° 17′ 16″ N; Long. 107° 47′ 38″ W; Elev. 825 m
Dominant soil type	Orthic Brown Chernozem with a Maleb sandy loam	Orthic Dark Brown with an Elstow loam	Orthic Brown Chernozem with a Swinton silt loam

Three major crop groups were used in this experiment: (i) cereal [spring wheat (*Triticum aestivum* L.) cultivar AAC Brandon], (ii) oilseeds [Argentine canola (*Brassica napus* L.) cultivar L233P, camelina [*Camelina sativa* (L.) Crantz] cultivar SES0787LS 'Cypress', industrial mustard (*B. carinata* L.) cultivar A 120, oriental condiment mustard (*B. juncea* L.) cultivar AC Cutlass, yellow condiment mustard (*Sinapis alba* L.) cultivar Andante] and (iii) pulse crops [yellow pea (*Pisum sativum* L.) cultivar CDC Meadow and red lentil (*Lens culinaris* Medikus) CLEARFIELD type cultivar CDC Maxim]. Argentine canola was used to establish a baseline for GLS contents, as the crop contains low amounts of the compound (Government Canada, 2017).

Table 3.2. Crop species grown in the four-year rotation treatments at three test sites from 2018 to 2021 cropping seasons

Treatment[l]	Treatment Abbreviation	Year 1 2018	Year 2 2019[‡]	Year 3 2020	Year 4 2021
1. W-YM-FP-W	YM-FP-W	W	**YM**	**FP**	W
2. W-IM-FP-W	IM-FP-W	W	**IM**	**FP**	W
3. W-AC-FP-W	AC-FP-W	W	**AC**	**FP**	W
4. W-OrM-FP-W	OrM-FP-W	W	**OrM**	**FP**	W
5. W-CL-FP-W	CL-FP-W	W	**CL**	**FP**	W
6. W-IM-L-W	IM-L-W	W	**IM**	**L**	W
7. W-AC-L-W	AC-L-W	W	**AC**	**L**	W
8. W-OrM-L-W	OrM-L-W	W	**OrM**	**L**	W
9. W-CL-L-W	CL-L-W	W	**CL**	**L**	W
10. W-W-W-W	W-W-W	W	**W**	**W**	W

[l]W=Wheat; YM=Yellow mustard; FP=Field pea; IM=Industrial mustard; AC=Argentine canola; OrM=Oriental mustard; CL=Camelina; L=Lentil.
[‡] The years of columns with bolded letters correspond to the years included in this experiment.

Treatments were arranged in a randomized complete block design (RCBD) with four replicates at each test site. Dimensions of the experimental unit (plot) varied with test site (12-m long × 3-m wide plots at Brooks; 10-m long × 4-m wide plots at Swift Current; and 10-m long × 3.6-m wide plots at Scott). Crops were seeded at 22.5-30.0 cm row spacing, using recommended seeding rates (Appendices A.1-A.3).

Agronomic practices

Cultural practices, including seeding, weed control, and pests and disease control were carried out based on recommended practices for each crop. Seed treatments and rates and plant densities for different crops at each site are given in Appendices A.1-A.3. *Rhizobium leguminosarum* (Nodulator® Duo SCG, Saskatoon, SK) for field pea and lentil was applied with seed at 4 kg ha^{-1}, as per manufacturer's recommendation. Fertilizer application and dates in each year at each site are reported in Appendices A.1-A.3. As needed, appropriate post-emergent herbicides were applied at recommended stages and rates for broad-leaf and/or grassy weed control for each crop species. A summary of dates and treatment rates of herbicides and fungicides at the test sites was reported in Appendices A.4-A.10.

The grain yield and biomass data for the Brassicaceae (2019) and pulse (2020) crop phases were collected by staff at each site and are reported in Appendices A.11-A.13.

3.4.2 Glucosinolate analysis

Ten to 15 plants from each Brassicaceae plot at each site were randomly selected and above-ground plant parts were hand-harvested at physiological maturity in 2019. During the harvesting process, careful handling was ensured to minimize damage to plant tissues, as external damage can alter plant GLS content (Rhee et al., 2020). Seeds were separated from residue and seeds within the same plot were combined and placed in polyethylene freezer bags. Seed samples were flash-frozen using liquid N_2 in the field and then stored at -80°C to prevent enzyme degradation until further processing.

Processing and analysis of Brassicaceae seed samples were performed at the Agriculture and Agri-Food Canada (AAFC) research facility in Saskatoon. Frozen samples were lyophilized for 48 h at -80 °C and stored until analysis. The seeds were analyzed for GLSs using the official method of the Canadian Grain Commission for the determination of GLS content in rapeseed/canola as given by Heaney and Fenwick (1980) and modified by Daun and McGregor (1981). In this method, trimethylsilyl (TMS) derivatives of desulfo-GLSs are determined by gas chromatography. All samples were analyzed in triplicate.

3.4.3. Quantification of total soil available nitrogen

Prior to seeding of pulse crops in early spring of 2020, three soil cores (2-cm i.d.) were collected from three random places in each plot to a depth of 30 cm. The triplicate soil cores were pooled to form one composite sample for each soil depth per plot. All soil samples were stored at 4 °C until further analysis. The soil was sieved through a 2-mm screen and extracted with 2 M KCl (Bundy and Meisinger, 1994). The extracts were stored at -20 °C until analyzed. Before analysis, the frozen samples were thawed at room temperature for 24 h and thawed samples were analyzed for NH_4^+-N and NO_3^--N contents using a SEAL Autoanalyzer 3HR (Seal Analytical Inc., Mequon, Wisconsin, USA).

3.4.4. Estimation of biological nitrogen fixation (BNF) ability using ^{15}N-enriched isotope dilution

^{15}N-fertilizer application

The BNF ability was assessed in the pulse crop phase at all three sites in 2020, using the ^{15}N-enriched isotope dilution technique (Hardarson and Danso, 1990). Approximately four weeks after seeding (at the 3-4-leaf-stage of pulse crops), a solution containing ^{15}N-NH_4NO_3 solution was applied to the soil surface of a 1 m^2 micro-plot marked within each plot at a rate equivalent to 5.6 kg N ha^{-1} (Hossain et al., 2016). A stock solution was prepared by dissolving 80.04 g of double-labeled, 10 atom % ^{15}N-NH_4NO_3 in 250 mL of deionized water. Five mL of the stock solution was dispensed into 5 L of water and evenly applied to the soil surface of the micro-plot area using a watering can. The micro-plot was irrigated with an additional 8 L m^{-2} water to leach the ^{15}N-NH_4NO_3 into the soil profile (Unkovich et al., 2008). Wheat was used as the non-N_2 fixing reference crop for BNF measurements and received the same ^{15}N-NH_4NO_3 application.

Sample processing and analysis

At physiological maturity (reached ca. 98 d after the seeding) above-ground plant parts in the micro-plots were hand harvested at 0.5 cm above the soil surface and placed in a cloth bag for drying. Plant materials were dried at 60 ºC in a forced-air oven to stable dry weight (ca. 48 h). The dried plants in each sample were separated into seed containing pods and a mixture of leaves and stems. The dry weight of each component was recorded. Samples were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ), and then re-ground using a ball grinder (8000D Mixer/Mill, SPEXSamplePerp® LLC., Metuchen, NJ, USA) to the consistency of fine powder. The ground plant samples from each plot were encapsulated in 8.5 mm tin capsules for mass spectrometry analysis. The sample size for seeds of all plant species was 1.8 ± 0.4 mg and all the other plant parts were 3.5 ± 0.3 mg. The samples were analyzed for total N concentration (%) and atom % ^{15}N on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany). Pea grain flour (0.3673 atom% ^{15}N) was used as an internal standard.

Atom % excess in the plant parts was calculated by subtracting the average natural abundance level of plant material (i.e., 0.36637) from the measured atom% ^{15}N content of the plant part (Habinshuti et al., 2021).

The percentage of N derived from the atmosphere (%Ndfa) per plant part was estimated using the following equation (Eq. 3.1) according to Hardarson and Danso (1990):

$$\%Ndfa\ in\ plant\ part = [1- (\frac{atom\ \%15N\ excess\ in\ plant\ part\ fixing\ crop}{atom\ \%15N\ excess\ in\ plant\ part\ of\ non\ fixing\ crop}) \times 100] \quad \text{[Eq. 3.1]}$$

Total N in each plant part namely seed containing pods and leaves with stems using the following equation:

$$Total\ N\ in\ plant\ part\ of\ fixing\ crop = \frac{(\%N\ in\ each\ plant\ part\ ) \times dry\ biomass\ of\ each\ plant\ part}{100} \quad \text{[Eq. 3.2]}$$

The amount of the BNF per plant part (Hardarson and Danso, 1990) was determined as:

$$BNF\ in\ plant\ part = \frac{\%Ndfa\ in\ plant\ part \times total\ N\ in\ plant\ part\ of\ fixing\ crop}{100} \quad \text{[Eq. 3.3]}$$

The amount of BNF in above-ground plant is the sum of BNF in seed containing pods and the mixture of stems and leaves (straw).

3.4.5. Evaluation of diazotroph abundance and root nodulation

Plant sample collection

Root nodule samples from lentil and field pea were collected at 50-60 % of the flowering stage on June 6, 2020 at the Brooks site and on June 10, 2020 at the Swift Current site. COVID-19 restrictions prevented access to the Scott site for root sampling. Consequently, root nodulation data was limited to Brooks and Swift Current only. Twelve plants were randomly selected in each plot from the area outside of the ^{15}N microplots. These plants were excavated to a depth of 30 cm and a diameter of 15 cm with minimal disturbance to the root nodules. The above-ground plant part was excised at ground level. The roots were washed with water through a 0.2 mm mesh to remove soil debris. The roots from the 12 plants in each plot were separated into two groups of six, stored in separate sterile polyethylene bags and transported in an ice-filled cooler. One sample was used to evaluate nodule dry weight, and the second sample was used for DNA extraction.

The sample used for the estimation of nodule dry weight was stored at 4 ºC for a maximum 48 h (Gan et al., 2004b). All the root nodules from the roots within a plot at each site were combined. The nodule samples were oven-dried at 60-70 ºC for 2-3 days to constant weight and dry weight recorded.

For DNA extraction, root nodules were surface sterilized as follows. Root nodules were detached carefully from the roots using curve-tipped forceps. Two g of nodules were placed in a 500-mL Erlenmeyer flask containing 200 mL of sterile phosphate-buffered saline (PBS; 1.2 g L^{-1} Na_2HPO_4, 0.18 g L^{-1} NaH_2PO_4, 8.5 g L^{-1} NaCl; pH 7.6) and placed on a rotary shaker (150 rpm) at 22°C for 25 min. Root nodules were transferred into a 300-mL Erlenmeyer flask containing 100 mL of NaClO (1.05 % $v.v^{-1}$) in sterile PBS and placed on a rotary shaker (150 rpm) at 28°C for 15 min. Root nodules were rinsed 10 times with 100 mL of sterile tap water (Siciliano and Germida, 1999). Surface sterilized nodules were stored at -80 ºC until DNA extraction.

Quantification of the abundance of diazotrophs (*nif*H gene) in pulse crop phase by real time-PCR

DNeasy Plant Pro kits were used to extract the DNA from root nodules according to the manufacturer protocols (QIAGEN, Hilden, Germany). To assess DNA concentration and purity, the DNA extracts were run on 1 % agarose gels at 110 V for 30 min and quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). The extracted DNA solutions were stored at −80°C until further analysis.

Prior to real-time PCR analysis, DNA concentration was optimized to mitigate inhibitor effects. qPCR master mix components and primers were also optimized to achieve a reaction efficiency between 90-110 % and correlation coefficients (r) of 0.99 for the standard curve. Once an acceptable reaction efficiency was achieved, samples were analyzed. Each plate contained duplicate samples for the standard curve as well as an internal control sample to ensure reaction reproducibility. The standard curve was generated from a 10-fold serial dilution (10^2 -10^9 copies per µL) of gene Block (gBlock; the sequence verified double-stranded DNA fragments) containing the target functional gene fragment.

The *nif*H gene abundance was measured using qPCR on C1000 Touch Thermal Cycler system (Applied Biosystems, Foster City, CA, USA). The absolute qPCR correlates the PCR signal to input copy numbers using a calibration curve, and neither comparisons nor references are needed

(Pfaffl, 2004). The primers *nif*H-Forward (5'- AAAGGYGGWATCGGYAARTCCACCAC-3') and *nif*H-Reverse (5'-TTGTTSGCSGCRTACATSGCCATCAT-3') were used during qPCR (Rösch et al., 2002). A 96-well plate (Thermo Fisher Scientific Inc. Canada) was used, with each well containing 10 µL of iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, United States), 1.5 µL of each primer (10 mmol L^{-1}; Invitrogen Life Technologies, Canada), 0.4 µL of $MgCl_2$, 3.6 µL of UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen Life Technologies, Canada) and 3 µL of DNA template. The following two-step amplification protocol was used for quantification: one cycle at 95°C for 30 s, and 40 cycles of 5 s at 95°C, 34 s at 55°C and 1 min at 72°C. The number of gene copies was directly calculated from gBlock gene fragments (Integrated DNA Technologies Inc, Canada) concentration and presented as functional gene abundance. Standards were prepared by mixing 2.5 µL each of 30 different gBlock solutions (2×10^9 copies μL^{-1}). The PCR efficiency was 90-110 % and r for standard curves was 0.999.

3.5. Statistical Analysis

Data analyses were performed for the rotations with field pea and lentil separately using SAS 9.4 (SAS Institute, 2017). Prior to analysis, data were tested for normality using the Shapiro-Wilk test ($P \geq 0.05$) and homogeneity of variance using Levene's test ($P \geq 0.05$). Data from each test site was analyzed separately using the mixed model in a randomized complete block design by considering crop sequence (treatment) as a fixed factor, and block as a random factor. Overall treatment means were declared significant at $P \leq 0.05$. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test.

In addition, the following contrasts were carried out for each test site to answer the following pre-planned specific questions on selected plant traits (ex. N_2 fixation ability, root nodule dry weight and *nif*H gene copy number) of lentil and field pea after growing on stubble of different Brassicaceae and non-Brassicaceae oilseed crop species in rotations:

1. Does the performance of field pea grown on Argentine canola (with low levels of total GLSs) stubble differ from field pea grown on stubbles of other Brassicaceae crop species (with high levels of total GLSs) in rotation?

Field pea grown on Argentina canola vs. average of other Brassicaceae crop stubble = Treatment 3 vs. Treatments (1 + 2 + 4 + 5) / 4

2. Does the performance of field pea grown on non-mustard oilseed crop (i.e., camelina) stubble differ from field pea grown on mustard species (yellow, industrial and oriental mustards) in rotations?

Field pea grown on camelina (non-mustard) vs. average of mustard crop stubble = Treatment 5 vs. Treatments (1 + 2 + 4) / 3

3. Does the performance of field pea grown on non-brassica genus (*Sinapis alba*; yellow mustard) stubble differ from that grown on Brassicaceae genus mustard crop (industrial and oriental mustards) stubbles in rotation?

Field pea grown on yellow mustard vs. average of oriental and industrial mustard stubble = Treatment 1 vs. Treatments (2 + 4) / 2

4. Does the performance of lentil grown on Argentine canola (with low levels of total GLSs) stubble differ from that grown on stubbles of other Brassicaceae species (with high levels of total GLS) in rotation?

Lentil grown on Argentina canola vs. average of other Brassicaceae crop stubble = Treatment 7 vs. Treatments (6 + 8 + 9) / 3

5. Does the performance of lentil grown on non-mustard oilseed crop (i.e., Camelina) stubble differ from that grown on mustard species (yellow, industrial and oriental mustards) in rotations?

Lentil grown on camelina (non-mustard) vs. average of mustard crop stubble = Treatment 9 vs. Treatments (6 + 8) / 2

(Note: 1= YM-FP-W, 2= IM-FP-W, 3=AC-FP-W, 4= OrM-FP-W, 5= CL-FP-W, 6= IM-L-W, 7= AC-L-W, 8= OrM-L-W, 9= CL-L-W)

Pearson's correlation analysis was performed to determine the significance and nature (positive or negative) of the association between BNF parameters, root nodule dry weight, *nif*H gene copy number and total soil mineral N content. All tests were declared significant at $P \leq 0.05$.

3.6. Results

3.6.1. Total seed glucosinolate content in different Brassicaceae species

Yellow mustard seeds consistently showed the highest total GLS content across all three test sites (163.1-155.8 µmol g^{-1}; Table 3.3). In contrast, seeds of Argentine canola had the lowest total GLS contents, ranging from 6.10- 9.58 µmol g^{-1} among the three test sites. Notably, total seed GLS contents of individual Brassicaceae species remained consistent across all three test sites (Table 3.3).

Pre-planned comparison indicated that, on average, seeds of selected Brassicaceae species contained higher total GLS content than Argentine canola at all three test sites, and the difference was more prominent at Brooks (Table 3.3). On average, the total GLS content of yellow, industrial and oriental mustard seeds was higher than that of camelina at all three sites. Seed of yellow mustard had a higher total GLS content than the average of oriental mustard and industrial mustard at all three sites, and the difference was more pronounced at Swift Current (Table 3.3). Based on these comparisons the Brassicaceae species ranking from low to high total GLS content is AC <CL< IM < OM < YM.

Table 3.3. Total seed glucosinolate content (µmol g^{-1} of seed) in different Brassicaceae species grown at three test sites.

Treatments (Trt.) with different Brassicaceae crops[l]	Total seed glucosinolate contents		
	Swift Current	**Scott**	**Brooks**
1 = **YM**-FP-W	155.8[a‡]	138.4[a]	163.1[a]
2 = **IM**-FP-W	79.7[c]	69.2[c]	107.7[c]
3 = **AC**-FP-W	6.10[e]	7.41[e]	9.58[e]
4 = **OrM**-FP-W	108.0[b]	103.9[b]	135.9[b]
5 = **CL**-FP-W	25.6[d]	22.8[d]	32.3[d]
6 = **IM**-L-W	77.7[c]	67.7[c]	110.7[c]
7 = **AC**-L-W	6.44[e]	7.10[e]	9.58[e]
8 = **OrM**-L-W	113.4[b]	102.9[b]	133.6[b]
9 = **CL**-L-W	27.4[d]	23.5[d]	32.6[d]
***P* value**	**<0.0001**[§]	**<0.0001**	**<0.0001**
	Contrasts and corresponding *P* values		
Field pea rotations[¶]:			
Argentine canola vs. the average of other Brassicaceae crops	-77.7[#] **<0.0001**	-68.2 **<0.0001**	-92.8 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crops	-80.4 **<0.0001**	-73.3 **<0.0001**	-97.8 **<0.0001**
Yellow mustard vs. the average of oriental and industrial mustard	+61.1 **<0.0001**	+52.5 **<0.0001**	+41.1 **<0.0001**
Lentil rotations;			
Argentine canola vs. the average of other Brassicaceae crops	-66.4 **<0.0001**	-57.6 **<0.0001**	-82.8 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crops	-107.0 **<0.0001**	-61.8 **<0.0001**	-89.55 **<0.0001**

[l] YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil, bold letters indicate oilseed crops within the specified rotation.
[‡] The comparisons were made for each test sites. Values with different letters within each site are significantly different at $P \leq 0.05$ (n=4).
[§] Bolded *P* values indicate significant differences at $P \leq 0.05$.
[¶] Other Brassicaceae crop stubble includes camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard
Contrast value = value of the left side - value of the right in the comparison with *P* value following the contrast value.

3.6.2. Atmospheric nitrogen acquisition by pulse crops

%Ndfa in field pea

The percentage of N derived from the atmosphere by field pea and lentil crops was estimated using straw, seed+pods and total above-ground biomass (combined straw and seed+pod).

Straw

At Swift Current, straw of field pea grown on Argentine canola stubble had higher %Ndfa than field pea grown on oriental and yellow mustard stubble, but was statistically comparable with that grown on the stubbles of industrial mustard and camelina (Table 3.4). In contrast, field pea on oriental mustard stubble had the highest %Ndfa for straw at Scott, approximately 54.9 % higher than the average of other field pea crop sequences. At Brooks, straw in all field pea treatments showed similar %Ndfa. The pre-planned comparison of %Ndfa for field pea straw further showed that significant differences in comparisons were not prominent at Brooks (Table 3.5). At Swift Current, the %Ndfa of straw field pea on Argentine canola stubble was higher than the average %Ndfa in field pea treatments preceded by other Brassicaceae crops and this pattern was reversed at Scott. However, at Scott, the average of %Ndfa straw was higher in field pea in mustard crop sequences compared to field pea on camelina stubble and this difference was not significant at other two test sites. Moreover, the average %Ndfa for straw in field pea on oriental mustard and industrial stubble was higher than field pea on yellow mustard stubble at Scott (Table 3.5).

Seed+pod

Field pea grown on Argentine canola stubble had the highest %Ndfa (80.2 %) for seed+pod at Swift Current (Table 3.4). At Scott, field pea grown on Argentine canola, yellow, industrial and oriental mustard stubbles had statistically comparable %Ndfa for seed+pod. At Brooks, field pea on yellow mustard stubble had the highest %Ndfa for seed+pod portion, which was 37.7 % higher than the average of other treatments. On average, the %Ndfa in lentil seed+pod portion was higher than field pea at Brooks, but statistically comparable with those at Swift Current and Scott. (Table 3.5). At Scott and Brooks, the %Ndfa of field pea seed+pod portion in field pea grown on Argentine canola stubble was higher than the average of the treatments with field pea

preceded by other Brassicaceae crops. However, this impact was statistically comparable at Swift Current. The %Ndfa for seed+pod portion in field pea grown on camelina stubble was lower than the average of that of grown on yellow, industrial and oriental mustard stubbles at Swift Current and Brooks. However, this difference was not significant at Scott. In addition, at Brooks, the %Ndfa for pea seed+pod portion in field pea grown on yellow mustard stubble was higher than the average of field pea on industrial and oriental mustard stubble.

Total above-ground biomass

At Swift Current, field pea grown on Argentine canola had the highest %Ndfa for total above-ground biomass, which was approximately 34.7 % higher than the average of other treatments (Table 3.4). In contrast, at Scott and Brooks, %Ndfa in total above-ground biomass of field pea grown on Argentine canola, yellow, industrial and oriental mustard stubble was statistically comparable. The %Ndfa for total above-ground biomass of field pea grown on Argentine canola stubble was increased by 38.3 % at Swift Current, 8.6 % at Scott and 4.5 % at Brooks than the average of that in field pea preceded by other Brassicaceae crops (Table 3.5). The %Ndfa in field pea total above-ground biomass in field pea on camelina stubble was lower than the average of the field pea treatments with three mustard species at Swift Current and Brooks. In addition, the %Ndfa in total above-ground biomass of field pea grown on yellow mustard stubble was higher than the average of field pea on industrial and oriental mustard stubble at Scott. However, this effect was statistically comparable at Swift Current and Brooks (Table 3.5).

%Ndfa in lentil

Analysis of variance did not reveal any differences among the crop rotations for %Ndfa in any of the different plant parts of lentil (Table 3.4).

Straw

Pre-planned comparisons revealed different response patterns for different sites. At Swift Current, the %Ndfa for straw of lentil grown on Argentine canola was lower than the average of the crop rotations with oriental, industrial mustards and camelina (Table 3.5). This effect was reversed at Scott, where Argentine canola had the highest straw %Ndfa. There were no differences among the stubble treatments at Brooks.

At Swift Current, the straw %Ndfa for lentil on camelina stubble was lower than the average of the two rotations with industrial and oriental mustard. There was no difference at Scott and Brooks.

Seed+pod

Except Swift Current, where there was no difference in %Ndfa in the seed+pod of Argentine canola compared to the other Brassicaceae crops, at Scott and Brooks, lentil grown on Argentine canola stubble had higher %Ndfa for seed+pod (Table 3.5). In contrast, the %Ndfa for seed+pod in lentil grown on camelina stubble was higher than the average of rotations including industrial and oriental mustards at Swift Current, but no difference was observed at Scott and Brooks (Table 3.5).

Total above-ground biomass

As was observed for straw at Swift Current the %Ndfa for total above-ground biomass in lentil grown on Argentine canola stubble was lower than the average of rotations with the other Brassicaceae crops (Table 3.5). At Scott and Brooks, the total above-ground biomass %Ndfa mirrored seed %Ndfa, in that Argentine canola had the highest %Ndfa compared to the other rotation crops.

Total above-ground %Ndfa of lentil grown on the non-mustard camelina compared to the mustards was entirely site dependent. Camelina was not different from the mustards at Swift, was lower at Scott and was higher at Brooks (Table 3.5).

Table 3.4. Percentage of nitrogen derived from atmosphere (%Ndfa) in different plant parts of field pea and lentil grown in different crop rotations at three test sites.

Treatments[1]	Percentage of nitrogen derived from atmosphere								
	Straw			Seed+pods			Total above-ground biomass		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Rotations with field pea									
1 = YM-FP-W	$33.3^{B‡}$	29.1^{C}	58.4^{A}	58.4^{B}	72.5^{AB}	77.0^{A}	47.4^{B}	54.7^{AB}	67.8^{A}
2 = IM-FP-W	42.9^{AB}	54.4^{B}	43.3^{A}	64.7^{B}	71.7^{AB}	50.1^{B}	54.9^{B}	64.3^{AB}	46.9^{AB}
3 = AC-FP-W	66.4^{A}	50.1^{B}	56.5^{A}	82.2^{A}	76.0^{AB}	52.8^{B}	75.3^{A}	64.5^{AB}	55.2^{AB}
4 = OrM-FP-W	22.2^{B}	90.3^{A}	66.3^{A}	60.4^{B}	94.1^{A}	60.1^{B}	43.6^{B}	73.3^{A}	62.9^{A}
5 = CL-FP-W	38.6^{AB}	25.3^{C}	34.8^{A}	60.6^{B}	50.3^{B}	28.7^{C}	50.9^{B}	40.1^{C}	31.3^{B}
***P* value**	**0.0049**[§]	**<0.0001**	0.1359	**0.0012**	**0.0015**	**<0.0001**	**0.0006**	**0.0004**	**0.0025**
Rotations with lentil									
6 = IM-L-W	59.1^{a}	69.2^{a}	46.8^{a}	67.4^{a}	81.4^{a}	41.8^{a}	63.8^{a}	68.0^{a}	44.2^{a}
7 = AC-L-W	51.6^{a}	82.4^{a}	57.4^{a}	61.4^{a}	84.3^{a}	64.3^{a}	57.7^{a}	71.6^{a}	59.9^{a}
8 = OrM-L-W	61.6^{a}	72.3^{a}	54.8^{a}	71.4^{a}	79.3^{a}	47.0^{a}	68.1^{a}	75.8^{a}	51.8^{a}
9 = CL-L-W	56.4^{a}	78.6^{a}	49.6^{a}	60.2^{a}	77.2^{a}	46.3^{a}	56.3^{a}	72.7^{a}	51.4^{a}
***P* value**	0.0923	0.2675	0.5414	0.1269	0.9103	0.0811	0.0864	0.4048	0.075

[1] YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil

‡ The comparisons were made for each plant part among all test sites. Lentil and field pea rotations were analyzed separately, with significant differences among treatments ($P \leq 0.05$) indicated by uppercase letters for field pea and lowercase letters for lentil (n=4).

§ Bolded *P* values indicate significant differences ($P \leq 0.05$).

Table 3.5. Summary of contrasts and corresponding *P* values from pre-planned comparisons for percentage of nitrogen derived from atmosphere (%Ndfa) in different plant parts of field pea and lentil grown in different crop sequences.

Treatments (Trt.) comparison	Contrasts and corresponding *P* values								
	Straw			Seed+pods			Total above-ground biomass		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Field pea grown on[§];									
Argentine canola vs. the average of other Brassicaceae crop stubble	+32.1 **0.0081**	-3.46 **<0.0001**	+5.75 0.0854	+21.2 0.1137	+3.83 **0.0008**	+0.620 **0.0043**	+26.1 **0.0101**	+6.36 **0.0012**	+2.93 **0.0500**
Camelina (non-mustard) vs. the average of mustard crop stubble	+5.75 0.0587	-37.6 **0.0107**	+21.2 0.8272	-0.610 **0.0134**	-29.17 0.334	-31.25 **<0.0001**	-2.22 **0.0155**	-23.9 0.7134	-27.8 **0.0024**
Yellow mustard vs. the average of oriental and industrial mustard stubble	+0.750 0.266	-50.8 **0.0058**	+3.58 0.1015	-4.10 0.1141	-10.38 0.2354	+25.5 **0.0331**	-1.87 0.1278	+3.58 **0.0208**	+12.9 0.5941
Lentil grown on;									
Argentine canola vs. the average of other Brassicaceae crop stubble	-7.44 **0.003**	+9.08 **<0.0001**	+7.01 0.0696	+3.16 0.0966	+4.99 **0.0008**	+11.09 **0.0242**	-5.02 **0.0074**	+7.01 **<0.0001**	+10.8 **0.0496**
Camelina (non-mustard) vs. the average of mustard crop stubble	-3.90 **0.0203**	+7.82 0.3566	-1.19 0.1772	+3.01 **0.0043**	-3.13 0.2407	-1.20 0.5956	-1.19 0.1772	-10.3 **<0.0001**	+3.40 **0.0003**

[†] Contrast value = value on the left side - value on the right side in the comparison with *P* value following the contrast value.
[‡] Bolded *P* values indicate significant differences ($P \leq 0.05$).
[§] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard.

Amounts of biologically fixed nitrogen

Amounts of biologically fixed nitrogen in field pea

Straw

At both Swift Current and Brooks, field pea straw produced on Argentine canola had the most fixed N (2.51, 8.76 kg N ha^{-1}, Table 3.6) compared to the other rotations. At Scott, pea in rotation with oriental mustard had the highest fixed N in straw (10.1 kg N ha^{-1}).

Amounts of fixed N in straw tended to be opposite at Swift Current and Scott (Table 3.7). In contrast to %Ndfa, amounts of fixed N in pea straw were higher when grown after Argentine canola than the other oilseed crops at Swift Current but at Scott, the reverse was observed. Similarly, pea grown after Camelina had more fixed N than after mustard crops at Swift Current, but less fixed N after Camelina at Scott. In general, there were few differences among the rotations at Brooks (Table 3.7).

Seed+pod

Field pea grown on Argentine canola at Swift Current had the highest fixed N amounts in seed+pod portion, which was 33.5 % higher than the other crop sequences (Table 3.6). At both Scott and Brooks, fixed N amounts for seed+pod of field pea grown on stubble of Argentine canola and the three mustard species were statistically comparable and higher than that grown on camelina stubble. Field pea grown on oriental mustard fixed the highest amount of N in seed+pod at Scott (32.6 kg N ha^{-1}) and yellow mustard at Brooks (29.6 kg N ha^{-1}). Field pea grown after camelina had consistently low amounts of fixed N compared to the mustard crops at Scott and Brooks (Table 3.7).

Total above-ground biomass

Scott was the only site, where the amount of fixed N in total above-ground field pea biomass was not the highest when grown after Argentine canola (Table 3.6) instead at Scott, field pea grown after oriental mustard had the most fixed N (42.7 kg N ha^{-1}). At Swift Current, field pea grown on Argentine canola had the highest fixed N amounts in total above-ground biomass of field pea (19.7 kg N ha^{-1}; Table 3.6) whereas at Brooks, except for pea grown after camelina, pea grown after all

of the other oilseeds had comparable amounts of fixed N (Table 3.6). Pea grown after camelina consistently had the lowest amounts of fixed N.

Amounts of fixed nitrogen in lentil

Straw

Irrespective of differences in preceding Brassicaceae oilseed crop species in the rotation, the fixed N amounts in lentil straw were comparable at all three test sites (Table 3.6).

Seed+pod

The amounts of fixed N in lentil seed+pod portion in all the crop sequences were statistically comparable at Swift Current and Scott (Table 3.6). In contrast, lentil grown on Argentine canola stubble had the highest fixed N amount in seed+pod at Brooks.

Total above-ground biomass

Similar to seed+pod all crop sequences showed similar fixed N contents at both Swift Current and Scott sites (Table 3.6) and lentil grown on Argentine canola stubble had the highest fixed N amount at Brooks. Pre-planned comparisons confirmed that at Scott, lentil grown after Argentine canola had higher amounts of fixed N than lentil grown after the other Brassicaceae crops.

Table 3.6. Biologically fixed nitrogen (N) content (kg N ha^{-1}) in different plant parts of field pea and lentil in different crop sequences at different test sites

Treatments[1]	Biologically fixed N contents of different plant parts of field pea and lentil in different crop sequences								
	Straw			Seed+pods			Total above-ground biomass		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Rotations with field pea									
1 = YM-FP-W	0.93^{B‡}	2.32^{C}	5.83^{B}	12.6^{B}	26.3^{AB}	29.6^{A}	13.5^{B}	28.6^{B}	37.2^{A}
2 = IM-FP-W	1.31^{AB}	5.65^{B}	3.76^{B}	12.1^{B}	24.5^{AB}	22.7^{A}	13.4^{B}	30.2^{B}	26.5^{B}
3 = AC-FP-W	2.51^{A}	4.55^{B}	8.76^{A}	17.2^{A}	24.3^{AB}	24.5^{A}	19.7^{A}	28.8^{B}	33.3^{AB}
4 = OrM-FP-W	0.61^{B}	10.1^{A}	5.47^{B}	11.1^{B}	32.6^{A}	23.1^{A}	11.7^{B}	42.7^{A}	28.5^{AB}
5 = CL-FP-W	1.28^{AB}	1.77^{C}	3.43^{B}	9.94^{B}	15.6^{B}	12.9^{B}	11.2^{B}	17.4^{C}	16.6^{C}
***P* value**	**0.0045**	**<0.0001**	**<0.0001**	**0.0006**	**0.0003**	**0.001**	**0.0003**	**<0.0001**	**<0.0001**
Rotations with lentil									
6 = IM-L-W	1.29^{a}	6.07^{a}	4.97^{a}	7.59^{a}	21.8^{a}	15.8^{b}	8.88^{a}	27.8^{a}	20.8^{b}
7 = AC-L-W	0.67^{a}	6.50^{a}	4.17^{a}	8.09^{a}	22.0^{a}	23.5^{a}	8.76^{a}	28.5^{a}	27.9^{a}
8 = OrM-L-W	0.67^{a}	5.06^{a}	5.87^{a}	8.92^{a}	20.7^{a}	13.9^{b}	9.59^{a}	25.8^{a}	19.8^{b}
9 = CL-L-W	0.83^{a}	5.63^{a}	4.83^{a}	8.10^{a}	21.4^{a}	15.5^{b}	8.93^{a}	27.1^{a}	20.8^{b}
***P* value**	0.3034	0.835	0.1545	0.5627	0.9323	**<0.0001**	0.7622	0.5018	**<0.0001**

[1] YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine Canola, OrM=Oriental mustard, CL=Camelina, L=Lentil

‡ The comparisons were made for each plant part within a test site. Lentil and field pea rotations were analyzed separately, with significant differences among treatments ($P \leq 0.05$) indicated by uppercase letters for field pea and lowercase letters for lentil (n=4).

§ Bolded *P* values indicate significant differences ($P \leq 0.05$).

Table 3.7. Summary of contrasts and corresponding *P* values from pre-planned comparisons of biological nitrogen fixation capacity of different plant parts of field pea and lentil in different crop sequences at different test sites

Treatments (Trt.) comparison	Summary contrasts and corresponding *P* values								
	Straw			Seed+pods			Total above-ground biomass		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Field pea grown on[§];									
Argentine canola vs. the average of other Brassicaceae crop stubble	+1.48 **0.0158**	-0.41 <**0.0001**	+4.14 0.9777	+5.77 0.0900	-0.49 0.7967	+2.42 0.7967	+7.25 **0.0340**	-0.90 <**0.0001**	+6.62 0.7403
Camelina (non-mustard) vs. the average of mustard crop stubble	+0.33 **0.0385**	-4.25 **0.0023**	-1.59 0.4786	-1.97 0.6266	-12.18 **0.0014**	-12.18 **0.0014**	-1.63 0.3020	-16.42 0.1378	-13.70 **0.0001**
Yellow mustard vs. the average of oriental and industrial mustard stubble	-0.03 0.1298	-5.55 **0.0002**	+1.22 **0.0071**	+0.97 0.1989	-2.25 0.1452	+6.73 0.1452	+0.92 0.1275	-7.80 **0.0061**	+7.75 0.4395
Lentil grown on;									
Argentine canola vs. the average of other Brassicaceae crop stubble	-0.26 0.3871	+0.91 0.5122	-1.05 0.0837	-0.11 0.8825	+0.69 0.6951	+8.45 <**0.0001**	-0.37 0.6004	+1.60 0.3056	+7.40 <**0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.15 0.6382	+0.07 0.9646	-0.59 0.3371	-0.15 0.8523	+0.19 0.9211	-0.60 0.5710	-0.31 0.6907	+0.25 0.8764	+0.01 0.9935

[†] Contrast value = value on the left side - value on the right side in the comparison with *P* value following the contrast value.

[‡] Bolded *P* values indicate significant differences ($P \leq 0.05$).

[§] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard

3.6.3. Root nodule analysis in field pea and lentil crops in different crop sequences

Dry weight of root nodules

Root nodule analysis for field pea and lentil was performed only at Swift Current and Brooks. At Swift Current, field pea grown on oriental mustard stubble had over 2-fold total weight of root nodules (0.319 g plant^{-1}) than in field pea preceded by Argentine canola (0.147 g plant^{-1}) and camelina (0.129 g plant^{-1}), but the effect was reversed at Brooks (Table 3.8). Field pea grown on yellow mustard stubble had the lowest root nodule dry weight (0.083 g plant^{-1}) at Swift Current and on industrial mustard stubble at Brooks (0.065 g plant^{-1}). On average, field pea grown on Argentine canola stubble had higher nodule dry weights than field pea grown on the other oilseed stubble at both test sites (Table 3.8). In addition, field pea grown on yellow mustard stubble had a lower nodule dry weight than the average of field pea grown on oriental and industrial mustard stubble at Swift Current, but the effect was reversed at Brooks (Table 3.8).

Lentil grown on stubble of industrial mustard, oriental mustard and camelina had statistically comparable and higher nodule dry weight than field pea grown on the stubble of Argentine canola (Table 3.8). However, all the treatments with lentil at Brooks had similar nodule dry weights (P=0.4312). Similar to field pea crop sequences, lentil grown on Argentine canola stubble also had higher nodule dry weights than lentil grown on the other oilseed stubble at Brooks, whereas Swift Current had a reversed trend (Table 3.8). In addition, lentil nodule dry weights were higher when they grew on non-mustard (camelina) stubble than on mustard stubble at both test sites.

Table 3.8. Dry weight of root nodules (g plant^{-1}) in field pea and lentil in different crop sequences at two test sites.

Treatments (Trt.)[l]	The dry weight of root nodules at two test sites	
	Swift Current	**Brooks**
Rotations with field pea		
1 = YM-FP-W	0.083^{D‡}	0.128^{B}
2 = IM-FP-W	0.136^{C}	0.065^{D}
3 = AC-FP-W	0.189^{B}	0.147^{A}
4 = OrM-FP-W	0.319^{A}	0.094^{C}
5 = CL-FP-W	0.134^{C}	0.129^{B}
***P* value**	**<0.0001**[§]	**<0.0001**
Rotations with lentil		
6 = IM-L-W	0.029^{a}	0.018^{a}
7 = AC-L-W	0.014^{b}	0.021^{a}
8 = OrM-L-W	0.027^{a}	0.022^{a}
9 = CL-L-W	0.034^{a}	0.021^{a}
***P* value**	**<0.0001**	0.4312
Contrast and corresponding *P* values		
Field pea grown on[#]		
Argentine canola vs. the average of other Brassicaceae crop stubble	+0.02 **<0.0001**	+0.04 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.05 0.7392	+0.03 **0.0027**
Yellow mustard vs. the average of oriental and industrial mustard stubble	-0.14 **<0.0001**	+0.05 **0.0003**
Lentil grown on		
Argentine canola vs. the average of other Brassicaceae crop stubble	-0.02 **<0.0001**	+0.001 **<0.0001**
Camelina (non-mustard) vs. the average of mustard stubble	+0.002 **<0.0001**	+0.002 **<0.0001**

[l] YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil, bold letters indicate oilseed crops within the specified rotation.

‡ The comparisons were made within a test site. Lentil and field pea rotations were analyzed separately, with significant differences among treatments ($P \leq 0.05$) indicated by uppercase letters for field pea and lowercase letters for lentil (n=4).

§ Bolded *P* values indicate significant differences ($P \leq 0.05$).

¶ Contrast value = value of the left side - value of the right in the comparison with *P* value following the contrast value.

Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard.

3.6.4. *nif*H gene copy concentration in field pea and lentil root nodules

Field pea grown on yellow mustard and Argentine canola stubbles had the highest *nif*H gene copy concentration at Brooks (11.9-12.2 log copy number g^{-1} root nodules, Table 3.9). In contrast, all the crop sequences with field pea had statistically comparable *nif*H gene copy concentration at Swift Current. The treatment with lentil grown on camelina stubble (11.7 log copy number g^{-1} root nodules), and on industrial mustard stubble (11.6 log copy number g^{-1} root nodules) had the highest *nif*H gene copy concentration at Brooks. In contrast, lentil grown on camelina stubble had the highest *nif*H gene copy concentration (11.9 log copy number g^{-1} root nodules) at Swift Current (Table 3.9).

Pre-planned comparison further revealed that on average, field pea grown on different Brassicaceae stubble had higher *nif*H gene copy concentration than lentil in rotation at both test sites (Table 3.9). In addition, the average *nif*H gene copy concentration in field pea grown on Argentine canola stubble was higher than the average of field pea grown on other Brassicaceae crop stubble at both Swift Current and Brooks. The same trend was noticed in lentil rotations, only at Swift Current. In addition, lentil cultivated on non-mustard crop, camelina stubble consistently had elevated concentrations of *nif*H gene copies than those grown on mustard stubbles at Swift Current and Brooks. However, a reversed trend was observed in field pea rotations at Swift Current. At Swift Current, field pea preceded by yellow mustard showed higher *nif*H gene copy concentration than field pea preceded by oriental and industrial mustard, while they showed statistically comparable *nif*H gene copy concentration at Brooks (Table 3.9).

Table 3.9. *nif*H gene copy concentration (log gene copy number g^{-1} root nodules) in root nodules of field pea and lentil in different crop sequences at two test sites.

Treatments[l]	*nif*H gene copy concentration	
	Swift Current	**Brooks**
Rotations with field pea		
1 = YM-FP-W	12.0[A‡]	11.9[AB]
2 = IM-FP-W	11.9[A]	11.6[B]
3 = AC-FP-W	12.2[A]	12.2[B]
4 = OrM-FP-W	11.9[A]	11.7[B]
5 = CL-FP-W	11.9[A]	11.7[B]
***P* value**	0.0711	**0.0306**[§]
Rotations with lentil		
6 = IM-L-W	11.5[c]	11.6[a]
7 = AC-L-W	11.7[b]	11.4[b]
8 = OrM-L-W	11.5[c]	11.4[b]
9 = CL-L-W	11.9[a]	11.7[a]
***P* value**	**<0.0001**	**<0.0001**
Contrasts and corresponding *P* values		
Field pea grown on[#];		
Argentine canola vs. the average of other Brassicaceae crop stubble	+0.25 **<0.0001**	+0.45 **0.0048**
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.06 **<0.0001**	-0.09 0.5325
Yellow mustard vs. the average of oriental and industrial mustard stubble	+0.19 **<0.0001**	+0.24 0.1166
Lentil grown on;		
Argentine canola vs. the average of other Brassicaceae crop stubble	+0.10 **0.0263**	-0.22 **<0.0001**
Camelina (non-mustard) vs. the average of mustard stubble	+0.45 **<0.0001**	+0.22 **0.0002**

[l] YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil, bold letters indicate oilseed crops within the specified rotation.

[‡] The comparisons were made within a test site. Lentil and field pea rotations were analyzed separately, with significant differences among treatments ($P \leq 0.05$) indicated by uppercase letters for field pea and lowercase letters for lentil (n=4).

[§] Bolded *P* values indicate significant differences ($P \leq 0.05$).

[¶] Contrast value = value of the left side - value of the right in the comparison with *P* value following the contrast value.

Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard.

3.6.5. Total soil available nitrogen

Total available N concentrations at 0-30 cm soil depth were similar among different crop sequences with field pea at Swift Current (*P*= 0.2025, Table 3.10) and Brooks (*P*=0.3085) and with lentil at Swift Current (*P*=0.1224). In contrast, lentil on industrial mustard stubble showed the highest soil available N concentration (33.2 mg N kg^{-1} of soil) at Brooks. None of the differences was significant in the pre-planned comparisons at $P \leq 0.05$ neither at Swift Current nor Brooks.

Table 3.10. Soil available nitrogen (N) concentration (mg N kg^{-1} of soil) in 0-30 cm soil depth before seeding of field pea and lentil in different rotations at two test sites

Treatments[l]	Soil available N	
	Swift Current	**Brooks**
Rotations with field pea		
1 = YM-FP-W	11.8[A‡]	23.2[A]
2 = IM-FP-W	13.1[A]	31.4[A]
3 = AC-FP-W	10.7[A]	24.7[A]
4 = OrM-FP-W	13.1[A]	18.6[A]
5 = CL-FP-W	11.0[A]	18.8[A]
***P* value**	0.2025	0.1224
Rotations with lentil		
6 = IM-L-W	10.8[a]	33.2[a]
7 = AC-L-W	13.5[a]	22.4[b]
8 = OrM-L-W	11.6[a]	22.7[b]
9 = CL-L-W	11.3[a]	19.3[b]
***P* value**	0.3085	**0.0054**[§]
Contrast and *P* values for pre-planned comparison		
Field pea grown on[¶]		
Argentine canola vs. the average of other Brassicaceae crop stubble	-1.55[#] 0.6901	+1.70 0.1717
Camelina (non-mustard) vs. the average of mustard crop stubble	-1.66 0.2113	-5.60 0.1507
Yellow mustard vs. the average of oriental and industrial mustard stubble	-1.30 0.6603	-1.80 0.2586
Lentil grown on		
Argentine canola vs. the average of other Brassicaceae crop stubble	+2.27 0.3020	-2.67 0.0852
Camelina (non-mustard) vs. the average of mustard stubble	+0.10 **0.0068**	-8.65 0.8900

[l]YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil, bold letters indicate oilseed crops within the specified rotation. ‡ Lentil and field pea rotations were analyzed separately, with significant differences among treatments ($P \leq 0.05$) indicated by uppercase letters for field pea and lowercase letters for lentil (n=4). § Bolded *P* values indicate significant differences ($P \leq 0.05$). ¶ Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. # Contrast value = value of the left side - value of the right in the comparison with *P* value following the contrast value.

3.6.6. Correlation between root nodule parameters and total biological nitrogen fixation content

Pearson correlation analysis showed that seed GLS concentration was not significantly correlated with root nodule dry weight, *nif*H gene copy concentration, or BNF parameters in either field pea or lentil (Table 3.11). However, within field pea, root nodule dry weight and *nif*H gene copy concentration had a weak positive correlation (r= +0.32, *P*=0.0413). In contrast, *nif*H gene copy concentration and %Ndfa showed a positive correlation (r= +0.56, *P*<0.0001). Soil mineral N content was strongly and negatively correlated with biologically fixed N content in both field pea (r= -0.65, *P*<0.0001, Table 3.11) and lentil (r= -0.73, *P*<0.0001). Furthermore, soil mineral N had a negative correlation with root nodule dry weight in field pea (r = -0.40, *P*=0.01) and lentil (r = -0.48, *P*=0.01).

Table 3.11. Pearson's correlation coefficients (r) between root nodule parameters and plant biological nitrogen fixation

	Root nodule dry weight		***nif*H gene copy concentration**		**%Ndfa**		**Biologically fixed N content**	
	Field pea	**Lentil**	**Field pea**	**Lentil**	**Field pea**	**Lentil**	**Field pea**	**Lentil**
Seed glucosinolate concentration	-0.13	-0.17	-0.14	**-0.40**[†]	-0.04	-0.13	+0.18	-0.12
Root nodule dry weight	-	-	**+0.32**	+0.005	+0.02	+0.35	+0.33	+0.33
*nif*H gene copy concentration	-	-	-	-	**+0.56**	+0.16	+0.17	+0.39
Soil mineral N concentration	**-0.40**	**-0.48**	**-0.53**	-0.12	-0.18	-0.47	**-0.65**	**-0.73**

[†]Bolded *P* values indicate the significance at *P*<0.05.

3.7. Discussion

The BNF capacities of field pea and lentil were determined by the relative dependence of the crop on BNF (%Ndfa) and the amount of N fixed by the crop over the growing season. Overall, field pea grown on Argentine canola stubble had a higher %Ndfa for total above-ground biomass compared to the treatments with other Brassicaceae crops at all the test sites. This suggests that the residue or conditions following Argentine canola harvest might enhance N-fixing potential in field pea. This can be partially attributed to the differences observed in *nif*H gene copy concentration and nodule dry weight. A higher *nif*H gene copy concentration in root nodules indicates a larger population of N_2-fixing bacteria, suggesting an increased potential for BNF performance (Cheng

et al., 2023). This was further shown by the significant correlation between *nif*H gene copy concentration and %Ndfa (r = +0.56, Table 3.11). Previous research indicated that root nodule dry weight also plays a vital role in BNF (Martins et al., 2022; Cheng et al., 2023). However, the differences in root nodule dry weight did not correlate with %Ndfa in this study. All nodules on a plant may not be equally effective in fixing N, whereas some nodules may be inactive or inefficient, possibly limiting their contribution to BNF. However, field pea grown on Argentine canola residue showed a higher %Ndfa accompanied by higher *nif*H gene copy concentration and nodule dry weight compared to the average of field pea grown on other Brassicaceae oilseed crop residues (Tables 3.5, 3.8 and 3.9). This was consistent at both Swift Current and Brooks.

The *nif*H gene concentration and root nodule dry weight of subsequent pulse crops may rely on GLS levels released from the roots and residue of preceding Brassicaceae crops to the soil. Glucosinolate contents correspond to the potential amounts of their biologically active hydrolysis products, including ITCs, thiocyanates and nitriles, which have toxic effects on soil microbes (Barba et al., 2016; Hanschen et al., 2017). This study aimed to assess the GLS levels in various tissues of Brassicaceae crop species included in the study. Due to technical limitations, only seed GLS content was evaluated. The total seed GLS concentrations varied among the Brassicaceae crop species used in the current study (Table 3.4). Yellow mustard had the highest GLS content, whereas Argentine canola seeds consistently showed the lowest among the crop species, and these results align with previous studies, which further showed that canola crop residue also contained lower amounts of total GLSs than the other Brassicaceae species (Lee et al., 2020; Lietzow, 2021; Mocniak et al., 2023). Consequently, Argentine canola residue may potentially release lower amount of GLS hydrolysis products and develop a more favorable environment for nitrogen-fixing bacteria than other Brassicaceae crop residues. This could lead to higher *nif*H gene copy concentration and root nodule dry weights in field pea grown on Argentine canola residue compared to other Brassicaceae oilseed crop residues. Although studies conducting a comparative evaluation of the effect of multiple Brassicaceae crops on root nodulation are limited, Muehlchen et al. (1990) found a diminution in the field pea root nodule number when yellow mustard residue was incorporated into soil before sowing field pea, as compared to those of cabbage (*B. oleracea*), oil radish (*Raphanus sativus*) and rapeseed (*B. compestris*). Moreover, some pot experiments indicated that ground root tissue of Brassicaceae was toxic to *Bradyrhizobium* pure strains due to the presence of ITCs (Trinick and Hadobas, 1995; O'Callaghan et al., 2000). However, the impact

of these GLS derivatives on diazotroph abundance was not extensively explored under field conditions. Even though the seed GLS content in this study provided valuable insights, it is important to understand that it does not guarantee or directly reflect GLS levels in other plant tissues in the same crop. This is mainly because biosynthesis, distribution, and accumulation of GLSs can differ between seeds and other plant parts (Sun et al., 2019). Therefore, it is essential to exercise caution when extrapolating these findings to predict GLS concentrations in the entire plant and correlate the results with BNF-related parameters in this study.

The study further showed that the fixed N amount for total above-ground biomass had a strong negative correlation with total soil available N content within 0-30 cm soil depth in field pea ($r = -0.65$, Table 3.11). This also possibly contributed to the observed %Ndfa and fixed N contents in the rotations of field pea grown on different Brassicaceae crop stubbles, which were dependent on the test site (Tables 3.4 and 3.6). Field pea grown on Argentine canola and other mustard crop (yellow, industrial and oriental) stubbles showed similar Ndfa% and fixed N contents at the Scott and Brooks sites. In contrast, field pea grown on Argentine canola stubble at Swift Current showed the highest %Ndfa and fixed N content (Tables 3.4 and 3.6). This correlation indicates the field pea grown on Argentine canola compensated for marginally reduced soil N availability through slightly increased BNF (Reinprecht et al., 2020; Ladha et al., 2022). Despite the presence of similar soil N levels, field pea grown on camelina and mustard stubble had lower levels of fixed N and %Ndfa for total above-ground biomass than field pea on Argentine canola stubble. Camelina stubble might lead to short-term soil N immobilization at the soil sampling stage (Cao et al., 2021). However, with the microbial decomposition processes, the immobilized N can be mineralized and released back into the soil, making the soil available N content similar to the field pea treatments with oriental, industrial and yellow mustard crops. This may lead to the observed amount of fixed N and %Ndfa for total above-ground biomass in field pea grown on camelina stubble comparable with field pea grown on the three condiment mustard crop stubbles. The same trend was observed at the Scott and Brooks test sites. Field pea grown on Argentine canola, yellow mustard and industrial mustard had higher total soil N at Brooks and this variation was slightly different than Swift Current. Alkaline ($pH = 8.0$) soil conditions at Brooks can lead to a shift in microbial community composition, alter enzyme activities and nutrient cycling processes, and consequently affect the availability of soil N compared to approximately neutral pH soils ($pH = 6.5$) at Swift Current (Neina, 2019). However, the total soil available N did not align with the

%Ndfa and amount of N fixed for total above-ground biomass at Brooks. Despite having lower total soil available N in field pea treatments established on oriental mustard stubble, field pea grown on Argentine canola, yellow mustard and industrial mustard stubble had a comparable %Ndfa for total above-ground biomass. This also may be attributed to the temporary soil N immobilization, which releases N immediately after the decomposition of microbial biomass. However, %Ndfa in both pulse crops had non-significant correlations with total soil available N. The difference in the relationships between %Ndfa and fixed N for total above-ground biomass with total soil available N may be related to the biomass of the pulse crops (Collino et al., 2015). %Ndfa focuses on relative proportions of N, which does not change significantly with variations in plant biomass. The biologically fixed N amount considers the absolute amount of N incorporated into the biomass, making it more sensitive to changes in plant biomass driven by soil N levels.

Moreover, the lentil treatments showed uniform BNF capacity across all treatments, despite the presence of different Brassicaceae oilseed crop stubble at Swift Current and Scott. This suggests that the soil microbial communities in the lentil treatments at Swift Current and Scott might be less affected by the changes induced by different Brassicaceae residues, compared to field pea treatments due to species-specific sensitivity (Zhang et al., 2021). At Brooks, the lentil treatment with Argentine canola showed the highest amount of fixed N and %Ndfa for total above-ground biomass than lentil on other Brassicaceae stubble, potentially attributable to higher root nodule dry weight. This further highlights the positive impact of growing Argentine before pulse crops than growing other Brassicaceae crops.

The amount of N fixed by the symbiotic relationship in pulse crops is determined by the relative dependence of the crop on BNF for growth and the amount of N accumulated by the crop over the growing season (Jensen et al., 2010). Biologically fixed N amount ranges in field pea (14-28 kg N ha^{-1}) and lentil (9.1-22 kg N ha^{-1}) in the current study were relatively lower than prior experiments. Field pea can potentially fix 165 kg N ha^{-1}, but the usual range of N_2 fixation under field conditions is 40–60 kg ha^{-1} (Bourion et al., 2007). In the semi-arid Canadian prairies, the total amount of N fixed is about 70 kg ha^{-1} (Hossain et al., 2016, Liu et al., 2019). Total biologically fixed N amount for lentil ranged from about 50 kg N ha^{-1} to 75 kg N ha^{-1} (Cowell et al., 1989; Matus et al., 1997) with a median value of 72 kg N ha^{-1} (Walley et al., 2007). Several factors may have contributed to differences between the current results and those reported in other studies. One potential factor is the choice of crop cultivars, which may differ from those in the aforementioned

studies (Walley et al.,2007; Hossain et al., 2016). Additionally, the "Birch effect," in which mineralizing N when dry soil is rewetted (Birch, 1958), may have reduced N_2-fixation. This could have reduced N fixation in the current study by making soil mineral N readily available during the growth phase, potentially suppressing the requirement of the plants to fix atmospheric N (Ladha et al., 2022). However, the absence of a wheat-pulse system makes it difficult to isolate the specific effect of the presence of Brassicaceae crops on N fixation.

The seed+pod portion of field pea and lentil grown on stubble of different Brassicaceae species contained a relatively higher %Ndfa and fixed N content than the straw. The N amount in the preceding crop straw (leaf+stem) plays a key role in returning residue to the soil, which can effectively contribute to the soil fertility through decomposition and its beneficial for the long-term sustainability of agricultural output. Thus, field pea grown on oriental mustard at Scott and field pea grown on Argentine canola at Brooks had the highest amount of fixed N. In contrast, field pea straw at Swift Current had comparable amounts of fixed N regardless of the preceding Brassicaceae crop. Across all three test sites, lentil grown on different Brassicaceae oilseed crops had similar amounts of fixed N in their straw. This consistency implies that the type of preceding Brassicaceae oilseed crop does not influence the N content of lentil straw produced in rotations.

3.8. Conclusion

The impact of Brassicaceae residues on BNF in subsequent pulse crop varied depending on the pulse crop species and the growing environment. For higher and consistent BNF capacity in straw and seeds+pod portions, it is desirable to grow field pea on Argentine canola stubble rather than on yellow mustard, industrial mustard, oriental mustard or camelina stubble. In addition, Argentine canola leave less available N post-harvest and stimulates BNF, which consequently impacts the performance of the subsequent pulse crops. Argentine canola with less GLS content potentially has a lesser suppressive effect on plant diazotrophs in subsequent pulse crops and stimulates BNF than other Brassicaceae crop stubbles. However, it is important to note that the current study focused only on seed GLS content, not on the GLS levels in crop residues. Therefore, this limits the generalization of the current results, as GLS distribution throughout the plant may vary. Thus, more research is required to evaluate the BNF ability and GLS content of preceding crop residue, particularly straw, which plays a significant role in the soil in the rotation system. The results of the current study suggest that in lentil, %Ndfa and the amount of fixed N amount remain constant

or slightly varied when growing on stubbles of different preceding Brassicaceae crop species, in contrast to field pea.

4. THE IMPACT OF BRASSICACEAE OILSEED AND PULSE CROPS ON SELECTED SOIL PROPERTIES AND CROP PRODUCTIVITY IN WHEAT-BASED CROPPING SYSTEMS

4.1. Preface

Diversification of wheat-based crop rotations by including different Brassicaceae oilseed and pulse crops has many agronomic benefits. Chapter 3 showed that Brassicaceae species, characterized by varying levels and types of glucosinolates (GLSs), influenced the nitrogen fixation capacity of subsequently grown field peas in wheat-based cropping systems. However, information is scarce regarding the effect of Brassicaceae oilseed and pulse crops on soil and crop performance in wheat-based cropping systems under different climatic, environmental and soil conditions. Therefore, this study was conducted to assess the overall impact of different rotations involving Brassicaceae oilseeds and grain legumes on selected soil properties and subsequent wheat grain yield.

4.2. Abstract

Conserving soil quality while improving crop productivity is essential for agricultural sustainability. How cropping systems with different Brassicaceae crop species affect soil properties and cropping system performance remains less clear, particularly in the context of their allelopathic effects and the influence of glucosinolates on soil microorganisms. This understanding is crucial for crop growers to make informed decisions about crop selection. A field study was conducted to investigate how different Brassicaceae oilseed crops in rotations with pulse crops and wheat affect soil properties and wheat crop performance. The study compared ten crop sequences as replicated treatments arranged in a randomized complete block design at three test sites, Swift Current and Scott in Saskatchewan, and Brooks in Alberta, Canada. Brassicaceae crops [Argentine canola (*Brassica napus* L.), industrial mustard (*B. carinata* L.), oriental mustard (*B. juncea* L.), camelina (*Camelina sativa* L. Crantz) and yellow mustard (*Sinapis alba* L.)] were planted into wheat (*Triticum aestivum* L.) stubble in 2019 and followed by the pulse crops [field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medikus)] in 2020 and wheat in 2021. A continuous wheat treatment was included for comparison purposes. Soils were evaluated for changes in pH, electrical conductivity, soil organic carbon (SOC), total nitrogen (N) content, SOC:N ratio, moisture and soil organic matter (SOM) fractions over the study. Wheat productivity of different crop rotation

systems was assessed in 2021. Compared to the continuous wheat, the Brassicaceae containing rotations increased LFOM accumulation in the 0-15 cm soil depth by 47.7-56.3 % at Swift Current, 27.7-28.2 % at Scott and 20.5-22.0 % at Brooks in 2020 and 2021. In addition, from spring 2019 to 2021, the Brassicaceae-pulse treatments increased SOC 59.6 % at the 0-15 cm and 49.7 % at the 30-60 cm soil depths compared to the continuous wheat at Swift Current. Crop sequences with Argentine canola consistently increased LFOM content more than the average of the other Brassicaceae crops at each test site. Furthermore, growing yellow mustard before field pea improved SOC (49.6- 62.2 %), total soil N (50.0- 77.7 %) and LFOM (36.7 %) compared to industrial and oriental mustard crops at Swift Current. Compared to the continuous wheat, rotations with Brassicaceae and pulse crops increased wheat yield (52.7 %) and 1000-seed weight (9.7 %) at Brooks, and only 1000-seed weight (7.2 %) at Swift Current. Argentine canola followed by either field pea or lentil increased subsequent wheat yield by over 30 % compared to the crop sequences with other Brassicaceae species. Moreover, yellow mustard in crop sequences increased the 1000-seed weight of subsequent wheat by 4.6 % compared to oriental and industrial mustard crops. Overall, the study suggests that the inclusion of Brassicaceae oilseed and pulse crops in wheat rotations offers advantages over continuous wheat systems, improving soil properties. Argentine canola and yellow mustard were particularly beneficial preceding crops, enhancing subsequent wheat productivity.

4.2. Introduction

Brassicaceae oilseed crop production has increased in recent decades due to their suitability for the semi-arid in the Canadian Prairies (Gan et al., 2015). The inclusion of Brassicaceae oilseed, especially canola and pulse crops in traditional cereal-based cropping systems has been shown to improve overall productivity (Liu et al., 2019 and 2020) and economic profitability (Khakbazan et al., 2020). Despite crop production increases, concerns remain regarding sustainable soil management (van der Sloot et al., 2022). Soil is a critical, multifaceted resource for agriculture, acting as a water and nutrient reservoir (Gavrilescu, 2021), serving as a significant carbon (C) sink sequestering atmospheric C (Paustian et al., 2019), harboring a diverse and beneficial microbial community (Singavarapu et al., 2023), and functioning as a natural filter and buffer (Dvořáčková et al., 2022). Thus, conserving soil while improving crop productivity is essential for agricultural sustainability.

Soil moisture is one of the most important parameters that regulates soil temperature (Haskell et al., 2012), microbial activity, availability of nutrients (Bian et al., 2022) and soil structure (Xia et al., 2018). In arid and semi-arid areas, soil moisture status is the main resource limiting crop productivity (Gan et al., 2017; Allen and Kirhner, 2021). Rotating Brassicaceae, pulse and wheat crops, which have contrasting rooting systems can mitigate the adverse effect of water deficit on crop growth (Gan et al., 2009a; Miller et al., 2003a). Both wheat and Brassicaceae crops have deep rooting systems spanning up to 100-120 cm while most pulse crops have only 4 to 7 % of the root mass beyond the 60 cm depth (Gan et al., 2009a; Ding et al., 2018). This allows wheat and Brassicaceae crops to access water and nutrients in deep soil layers. Nielsen (1998) reported that canola can extract moisture down to 165 cm and 95 % of its water use comes from the upper 119 cm of the soil profile. In contrast, most pulse crops primarily rely on water within the top 60 cm of the soil profile (Gan et al., 2009a). Additionally, pulse crops help to improve water use efficiency of a cropping system due to their lower water requirements during their growth cycle than wheat and Brassicaceae crops (Ding et al., 2018) leaving relatively high amounts of post-harvest residual soil moisture for succeeding crops with deep rooting systems (Cutforth et al., 2009; Bahl, 2015). Among oilseeds, mustard was reported to be more tolerant to water stress than canola (Ding et al., 2018). Thus, these complementary water-use characteristics of Brassicaceae, pulse and wheat crops, potentially improve the overall water use efficiency of cropping systems in semi-arid environments.

Soil nutrient content is critical for sustainable crop production. Availability of nutrients is mainly influenced by pH and electrical conductivity (EC). Soil pH plays a key role in soil nutrient availability, toxicities (e.g. Al and Mn) and deficiencies (e.g. Mg, Fe and Zn), and soil processes such as ammonification, nitrification, and other microbially mediated processes (Heiniger et al., 2003; Hartemink and Barrow, 2023). Soil organic matter (SOM) is a major component in agricultural systems, providing nutrients to crops upon decomposition and increasing nutrient-holding capacity and water-holding capacity (Lal, 2004). However, SOM is not a uniform substance; it is a balance of SOM fractions, including active and passive pools (Gosling and Parsons, 2013). Density separation separates the fast-cycling SOM fraction (i.e., light fraction organic matter: LFOM; <1.3–1.8 g cm^3) from the slow-cycling passive fraction (i.e., heavy fraction: HFOM; >1.3–1.8 g cm^3). Light fraction organic matter generally serves as a source of nutrients for plants and microorganisms whereas HFOM acts as a long-term storehouse of carbon

in soil (Schmidt et al., 2011). Light fraction organic matter is more sensitive to changes in crop management than total SOM. Understanding the changes in LFOM and HFOM fractions can provide valuable insights into the long-term sustainability of cropping systems (Tan et al., 2007). How rotations with Brassicaceae oilseed and pulse crops influence SOM fractions in western Canadian soils is largely unknown.

Soil organic carbon (SOC) is crucial for increased soil fertility (Bolinder et al., 2010), improved biological and physical soil characteristics (Hati et al., 2007) via a reduction in bulk density, improved water-holding capacity and enhanced activity of soil microbes (Yang et al., 2012; Zhou et al., 2020). Agricultural practices significantly influence SOC by affecting the amount of C entering soil through crop residues and rates of decomposition. Crop residue composition, particularly its carbon to nitrogen ratio (C:N) strongly influences decomposition and mineralization processes. Crop residues of wheat (C:N 83:1- 139:1) and canola/mustard (C:N 46:1-86:1) have higher C:N ratios than pulse crop residues (C:N 14:1- 60:1) (Stevenson and van Kessel, 1996; Gan et al., 2010). Presence of high N content in pulse crop residues enables them to mineralize rapidly and fertilize the soil without contributing to passive soil organic matter (SOM) accumulation (Brady and Weil, 2008). In contrast, Brassicaceae oilseed crop residues with low N content facilitate the accumulation of passive SOM. However, the combined effects of including these two crop types in wheat-based cropping systems on SOC remain unclear.

Nitrogen (N) is commonly the most limiting nutrient element in agricultural production and is also an important component of SOM. Biological N fixation in pulse crops results in relatively high amounts of N in biomass and the potential transfer of N to the soil with crop residue degradation. A significant portion, 18-49 % of the N fixed by pulse crops is retained in the below-ground residues, representing a major pathway of N transfer from pulses to subsequent crops via mineralization (Lupwayi and Soon, 2016). Brassicaceae oilseed crops contain glucosinolates (GLSs), which could have allelopathic effects on soil microbial communities and their functions. These effects may slow down the decomposition of Brassicaceae residues and potentially reduce mineralization, hindering N availability for subsequent crops (Snyder et al., 2010; Wang et al., 2015). On the other hand, this reduced mineralization rate leads to a slow release of nutrients from SOM into plant-available forms, thereby reducing nutrient losses (e.g. through nitrate leaching, soil run-off, and/or denitrification) and thereby meeting the crop nutrient requirements along their growth cycle (Moore et al., 2010). However, allelopathic effects of GLSs depend on the

Brassicaceae species, as the amount and type of GLSs vary considerably (Mocali et al., 2015). For instance, canola has a relatively low total GLS content (less than 30 µmol g^{-1} in seeds) compared to other Brassicaceae oilseed crops (Government Canada, 2017). However, studies comparing the effect of different Brassicaceae oilseed crops with different GLS profiles on soil N availability are extremely limited.

The inclusion of pulse and Brassicaceae oilseed crops as break crops, which disrupt pest and disease cycles, and increase diversity in cereal-based systems has conferred significant yield benefits on subsequent cereal crops (Malik et al., 2015; Liu et al., 2020). This has been well documented around the world with grain legumes (Miller et al., 2003b; Krupinsky et al., 2006; Williams et al., 2014a) and canola (Kirkegaard et al., 2010; Bushong et al., 2012; Angus et al., 2015). A 6-year study conducted in the southeast Peace region of Alberta revealed that wheat yield was significantly greater in rotations with field pea and canola than continuous wheat (Gill, 2018). Moreover, wheat yield tended to increase more following field pea than canola in this study. However, there is a dearth of information on how variations in climatic, environmental and soil factors affect the benefits of including pulses and oilseed crops in wheat cropping systems.

To address this knowledge gap, a four-year field study with different combinations of different Brassicaceae and pulse crop species was conducted at three test sites in the Canadian prairies. The central hypothesis of this study described in this chapter was that crop rotations with different Brassicaceae oilseed crop species (canola, camelina and mustards) in pulse- and wheat-based cropping systems will improve soil properties and crop productivity in the system compared to continuous wheat. Furthermore, it was hypothesized that Argentine canola, which contains low GLS levels will increase the soil nutrient content more than that of high level of GLS containing Brassicaceae oilseed crops, such as camelina, yellow mustard, oriental mustard and industrial mustard. Thus, the overall objective of this study was to determine the effect of growing different combinations of pulse and Brassicaceae oilseed crops on selected soil properties, including SOM fractions, SOC, total soil N content, soil C:N ratio, and soil moisture content and subsequent wheat yield in the cropping system.

4.3. Materials and Methods

This field study was established at three test sites (Brooks, AB; Scott, SK and Swift Current, SK). The experimental design is described in detail in Chapter 3 (section 3.4.1). This study was

established on spring wheat [(*Triticum aestivum* L.) (W)] stubble, with Brassicaceae crops followed by pulse crops followed by spring wheat. Five Brassicaceae crops; Argentine canola (*Brassica napus* L.) (AC)], industrial mustard [(*B. carinata* L.) (IM)], oriental mustard [(*B. juncea* L.) (OrM)], camelina [(*Camelina sativa* L. Crantz) (CL)] and yellow mustard [(*Sinapis alba* L.) (YM)] and two pulse crops; field pea [(*Pisum sativum* L.) (FP)] and lentil [(*Lens culinaris* Medikus) (L)] were grown (Table 4.1). The crop sequences are outlined in Table 4.1.

A summary of agronomic practices including seeding, weed control, pests and disease control and fertilizer application in each year at each test site are reported in Appendices A.1-A.10.

Table 4.1. An overview of treatments in the study

Treatment description (crop sequence)	Abbreviation	Treatment number
Field pea (FP) on yellow mustard (YM) stubble followed by wheat (W)	YM-FP-W	1
FP on industrial mustard (IM) stubble followed by W	IM-FP-W	2
FP on Argentine canola (AC) stubble followed by W	AC-FP-W	3
FP on oriental mustard (OrM) stubble followed by W	OrM-FP-W	4
FP on camelina (CL) stubble followed by W	CL-FP-W	5
Lentil (L) on IM stubble followed by W	IM-L-W	6
L on AC stubble followed by W	AC-L-W	7
L on OrM stubble followed by W	OrM-L-W	8
L on CL stubble followed by W	CL-L-W	9
Continuous wheat (W)	W-W-W	10

4.3.1. Soil sampling and analysis

Soil samples were collected in spring of 2019 (before seeding oilseed crops), spring of 2020 (before seeding pulse crops), fall of 2020 (after harvesting pulse crops), spring of 2021 (before seeding 2nd spring wheat crop) and fall of 2021 (after harvesting spring wheat) to analyze different soil properties (Table 4.2). Three, 4.5-cm dia. soil cores were sampled randomly to a 60-cm depth in each plot. Each core was divided into three segments based on depth (0-15 cm, 15-30 cm and 30-60 cm). Soil samples at the same depth within a plot were combined, resulting in three composite samples per plot.

Table 4.2. Different soil sampling times and depths for analyzed soil properties

Soil property	Time	Soil depth (cm)
pH	Spring 2019 and 2021	0-15
EC	Spring 2019 and 2021	0-15
Soil organic carbon	Spring 2019 and 2021	0-15, 15-30 and 30-60
Total soil nitrogen	Spring 2019 and 2021	0-15, 15-30 and 30-60
Organic matter fractions	Spring 2020 and 2021	0-15
Gravimetric moisture content	Spring and Fall 2020 and 2021	0-15, 15-30 and 30-60

An approximately 100 g sub-sample of wet soil from each depth was stored at 4°C to estimate the gravimetric soil moisture content. The remaining soil from each plot was air-dried for 4-5 days and sieved through a 2-mm mesh sieve. After air drying and sieving, another approximately 100-g sub-samples from each 0-15 cm composite sample was stored at room temperature for SOM analysis. After removing visible plant materials, the remaining soil from each depth was ground separately using a coffee grinder and re-ground using a ball grinder (8000D Mixer/Mill, SPEX SamplePrep® LLC., Metuchen, NJ, USA) into a fine powder. These samples were used for determining soil pH, electrical conductivity (EC), SOC and total N.

Soil pH and electrical conductivity (EC)

Soil pH and EC were measured in soils collected at 0-15 cm depths in spring of 2019 and 2021. Twenty-five g of dried, ground soil was suspended in 50 mL of deionized water. The soil pH (Hendershot et al., 2006) and EC (Miller and Curtin, 2006) were measured using a pH and conductivity meter (PC700, Oakton, Canada), after thorough stirring of the suspension.

Soil organic carbon and total soil nitrogen

Soil organic carbon (SOC) and total N were measured on soils collected in spring of 2019 and 2021 from all three depths. A 1.0-g sample of dried and ground soil was analyzed for total soil N content using a LECO TruMac CNS analyzer (630-300-400, LECO Corporation, Saint Joseph, Michigan, USA).

For SOC analysis, 1.0 g of soil sample was weighed into a nickel-lined ceramic combustion boat. Samples were moistened with distilled water and acidified with 6 % (w/v) sulfurous acid (H_2SO_3) to remove inorganic carbonates while keeping the samples at 70 °C. In the presence of inorganic carbonates, the acid reacts with carbonates to form CO_2. Sulfurous acid was added

repeatedly until no more CO_2 was produced. The soil samples were oven-dried overnight and analyzed for SOC using a Leco C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA).

Soil gravimetric moisture content

Gravimetric based soil moisture content was determined at three depths (0-15 cm, 15-30 cm and 30-60 cm) in the early spring and fall of 2020 and 2021 g. Twenty-five g of fresh soil per plot was oven-dried at 105 °C (±5 °C) until the dry weight of the sample became constant. Soils were re-weighed, and the percentage moisture contents was determined.

Soil organic matter fractionation

Air-dried, sieved soils were divided into heavy fraction organic matter (HFOM) and light fraction organic matter (LFOM) using sodium iodide (NaI) (Gregorich and Beare, 2006). A 50-g soil sample was mixed with 100 mL of NaI in a clean, disposable plastic vial. The vials were capped, placed upright and shaken on a rotary shaker for 1 h at 160 rpm and then maintained at room temperature (ca. 22 ± 2 °C) for 48 h. The LFOM floating on the surface of the NaI was decanted under vacuum through a 0.4 μm nitrocellulose membrane filter. The HF remained in the beaker. The LFOM and HFOM were washed with approximately 75 mL of 0.01 M calcium chloride ($CaCl_2$) followed by further washing with 75 mL of deionized water. The washed LFOM and HFOM were separately re-filtered and the materials were dried at 60 °C and weighed. This LFOM material was retained for quantification of total C (LFOM-C) and total N (LFOM-N) using Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA).

4.3.2. Wheat productivity, yield components and harvest index

Wheat plant density (number of plants m^{-2}) was estimated by counting plants in 1 m lengths of four plant rows in a plot (two front and two back rows). Six plant rows in the center of each wheat plot were harvested with a plot combine (Delta, Wintersteiger, Austria) to determine seed yield and expressed as kg ha^{-1}.

In addition, 10 plants per plot were randomly selected and harvested separately. These wheat samples were placed in a forced-air drier at 39-42 °C for about one week until the dry weight stabilized. After drying, total above-ground dry biomass was recorded. Then, seeds were separated

from the straw and seed dry biomass was recorded. The information was used to calculate the harvest index (HI) as follows.

$$Harvest\ index = \frac{total\ seed\ weight}{total\ aboveground\ biomass\ weight} \times 100\% \quad [4.1]$$

Thousand-seed weight of wheat was determined by weighing a sub-sample of 250 seeds and multiplying the weight by 4.

4.4. Data Analysis

To assess changes in soil properties over time due to the crop sequence treatments, the difference between initial and final sampling year values for each soil response variable was calculated. The change in soil properties (pH, EC, SOC, total N and SOC:N ratio) between the initial sampling year (spring 2019) and the last sampling year (spring 2021) was calculated by taking the difference of each parameter. Spring of 2019 was just prior to the implementation of the different crop sequence treatment. Data for fall soil moisture and OM fractions in 2020 and 2021 and wheat crop productivity and seed yield components in 2021 are reported directly without any modification (transformations).

Data analysis was performed using SAS 9.4 (SAS Institute, Inc, Cary, NC, USA, 2017) for each test site independently. Prior to analysis, all data were tested for normality using the Shapiro-Wilk test ($P \geq 0.05$) and homogeneity of variance using Levene's test ($P \geq 0.05$). The data at each test site were analyzed using mixed model in a randomized complete block design (RCBD). Soil properties in each year were statistically analyzed separately while considering the treatment and soil depth as fixed factors, and block as a random factor. Wheat crop productivity and seed yield component data were analyzed in the RCBD mixed model as mentioned above. Overall treatment means were declared significant at $P \leq 0.05$. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test.

In addition, the following comparisons were carried out for each test site to answer the following pre-planned specific questions on selected soil properties and crop productivity parameters of treatments with different combinations of Brassicaceae and pulse crop species;

1. Does the performance of continuous wheat differ from the crop sequences with Brassicaceae crops and pulse crops (field pea or lentil) in wheat-based cropping systems?

Continuous wheat vs. the average of Brassicaceae rotations and pulse rotations = Treatment 10 vs. Treatments (1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9) / 9 (Table 4.1)

2. Does the performance of crop sequences with field pea on Argentine canola stubble (with low levels of total GLSs) differ from field pea grown on other Brassicaceae crop stubble (with high levels of total GLSs)?
Field pea grown on Argentine canola vs. the average of other Brassicaceae crop stubble = Treatment 3 vs. Treatments (1 + 2 + 4 + 5) / 4

3. Does the performance of field pea grown on non-mustard oilseed crop (i.e., camelina) stubble differ from field pea grown on mustard species (yellow, industrial and oriental mustards) stubble in rotations?
Field pea grown on camelina (non-mustard) vs. mustard crop stubble = Treatment 5 vs. Treatments (1 + 2 + 4) / 3

4. Does the performance of field pea grown on non-brassica genus (yellow mustard; *Sinapis alba*;) stubble differ from field pea grown on Brassicaceae genus mustard crop (industrial and oriental mustards) stubble in rotation?
Field pea grown on yellow mustard vs. the average of industrial and oriental mustard stubble = Treatment 1 vs. Treatments (2 + 4) / 2

5. Does the performance of crop sequences with lentil on Argentine canola stubble (with low levels of total GLSs) differ from lentil grown on other Brassicaceae crop stubble (with high levels of total GLSs)?
Lentil grown on Argentine canola vs. the average of other Brassicaceae crop stubble = Treatment 7 vs. Treatments (6 + 8 + 9) / 3

6. Does the performance of lentil grown on non-mustard oilseed crop (i.e., camelina) stubble differ from lentil grown on mustard species (industrial and oriental mustards) stubble in rotations?
Lentil grown on camelina (non-mustard) vs. mustard crop stubble =Treatment 9 vs. Treatments (6 + 8) / 2

4.5. Results

Soil pH and EC

Prior to planting in 2019, soil pH at the test sites ranged from neutral (~ pH 7.0 at Swift Current and ~ pH 6.4 at Scott) to slightly alkaline (~ pH 8.0 at Brooks) (data not shown). Different crop sequences generally lowered pH at Swift Current (P=0.0002, Table 4.3) with some sequences causing pH decrease compared to others. Regardless of the legume in the sequence, soils on which yellow mustard, industrial mustard and Argentine canola were grown had lower pH values over the two years, than the sequence with field pea growing after oriental mustard, or either lentil or field pea growing after camelina. At Scott, on average, the rotation treatments reduced soil pH, with no differences among the treatments (P=0.1942, Table 4.3). In contrast, at Brooks, on average, rotation treatments increased mean soil pH slightly with no differences among the treatments (P= 0.9740, Table 4.3). Furthermore, pre-planned comparison revealed the treatment with Argentine canola (low GLS containing Brassicaceae oilseed species) followed by field pea had lowered pH than the treatments with other Brassicaceae crops (P=0.0078, Table 4.3). In addition, treatments with camelina increased soil pH compared to the treatments with mustard crops regardless of succeeding pulse crop species at Swift Current.

From 2019 to 2021, soil EC remained relatively consistent at each site (Appendix B.1).

Table 4.3. Change in soil pH units (0-15 cm depth) due to crop sequences at three test sites

Crop sequence[†]	Swift Current[‡]	Scott	Brooks
	----------Soil pH----------		
1 = **YM**-FP-**W**	-0.40[cd§]	-0.52[a]	+0.20[a]
2 = **IM**-FP-**W**	-0.63[a]	-1.12[a]	+0.15[a]
3 = **AC**-FP-**W**	-0.56[ab]	-0.68[a]	+0.22[a]
4 = **OrM**-FP-**W**	-0.11[d]	-0.88[a]	+0.27[a]
5 = **CL**-FP-**W**	-0.01[d]	-0.80[a]	+0.25[a]
6 = **IM**-L-**W**	-0.43[cd]	-1.00[a]	+0.25[a]
7 = **AC**-L-**W**	-0.41[cd]	-0.88[a]	+0.05[a]
8 = **OrM**-L-**W**	-0.34[cd]	-0.70[a]	+0.22[a]
9 = **CL**-L-**W**	-0.11[d]	-0.72[a]	+0.22[a]
10 = **W**-W-**W**	-0.19[d]	-0.82[a]	+0.25[a]
***P* value**	**0.0002**[¶]	0.1942	0.9740
	----------Contrast and *P* values----------		
Continuous wheat vs. the average of Brassicaceae and pulse rotations	+0.12[#]	-0.01	+0.04
	0.2612	0.9214	0.7363
Field pea grown on[††];			
Argentine canola vs. the average of other Brassicaceae crop stubble	-0.33	+0.15	+0.01
	0.0078	0.3316	0.9643
Camelina (non-mustard) vs. the average of mustard crop stubble	+0.30	+0.04	+0.04
	0.015	0.8106	0.7731
Yellow mustard vs. the average of oriental and industrial mustard stubble	-0.14	+0.08	-0.01
	0.2816	0.0991	0.935
Lentil grown on;			
Argentine canola vs. the average of other Brassicaceae crop stubble	-0.12	-0.07	-0.18
	0.3316	0.6836	0.2109
Camelina (non-mustard) vs. the average of mustard crop stubble	+0.28	+0.13	-0.01
	0.0368	0.4726	0.9350

[†]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Bolded letters represent the soil sampling phases. [‡] Treatment values are soil pH in spring 2021, before planting wheat - soil pH in spring 2019, before oilseed crops. [§]Values with different letters within each test site are significantly different ($P \leq 0.05$). [¶]Bolded *P* values indicate significant differences ($P \leq 0.05$). [#]Contrast value = value on the left - value on the right in the comparison with *P* value following the contrast value. [††]Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard.

Soil organic carbon (SOC)

In 2019, the mean SOC concentration at 0-60 cm soil depth was 8.1-8.5 g C kg^{-1} of soil for Swift Current, 11.3-12.2 g C kg^{-1} of soil for Scott and 8.4-8.5 g C kg^{-1} of soil for Brooks (Appendices B.2-B.4). In general, the measured SOC concentration increased at all the test sites during spring 2019 to spring 2021 period (Fig. 4.1). At Swift Current, soil with yellow mustard followed by field pea had the highest average gain in SOC at 0-60 cm (4.25 g C kg^{-1} of soil, Fig. 4.1A), representing a 2.7-fold increase compared to other treatments (P<0.0001, Table 4.4). Soil organic C changes across the three soil depths at Swift Current were not significantly different (P=0.3263). In contrast, treatments at Scott (P=0.1215, Table 4.4) and Brooks (P=0.0867, Table 4.4) had no significant differences in SOC content changes, but did differ with depth (Table 4.4). At Scott, SOC concentration increased more in the upper soil layers (0-15 cm and 15-30 cm) compared to the subsoil (30-60 cm) (Fig. 4.1B). Conversely, Brooks showed the opposite trend, with a higher SOC gain at 0-15 cm and 30-60 cm depths compared to the 15-30 cm depth (Fig. 4.1C).

In general, SOC content changes were more pronounced at Swift Current than the other two test sites (Table 4.5). At Swift Current, on average, crop rotations with Brassicaceae and pulse crops showed higher gains in SOC than continuous wheat at 0-15 and 30-60 cm soil depths (Table 4.5). In contrast, Brooks had the opposite trend at the 0-15 cm soil depth with continuous wheat increasing SOC more than the treatments with Brassicaceae and pulses. Crop rotations with mustard crops followed by field pea had a higher increase in average SOC concentration than camelina at all soil depths at Swift Current. Furthermore, yellow mustard followed by field pea grown soil had a higher increase in SOC content than the average of the rotations with oriental and industrial mustard at all depths (Table 4.5).

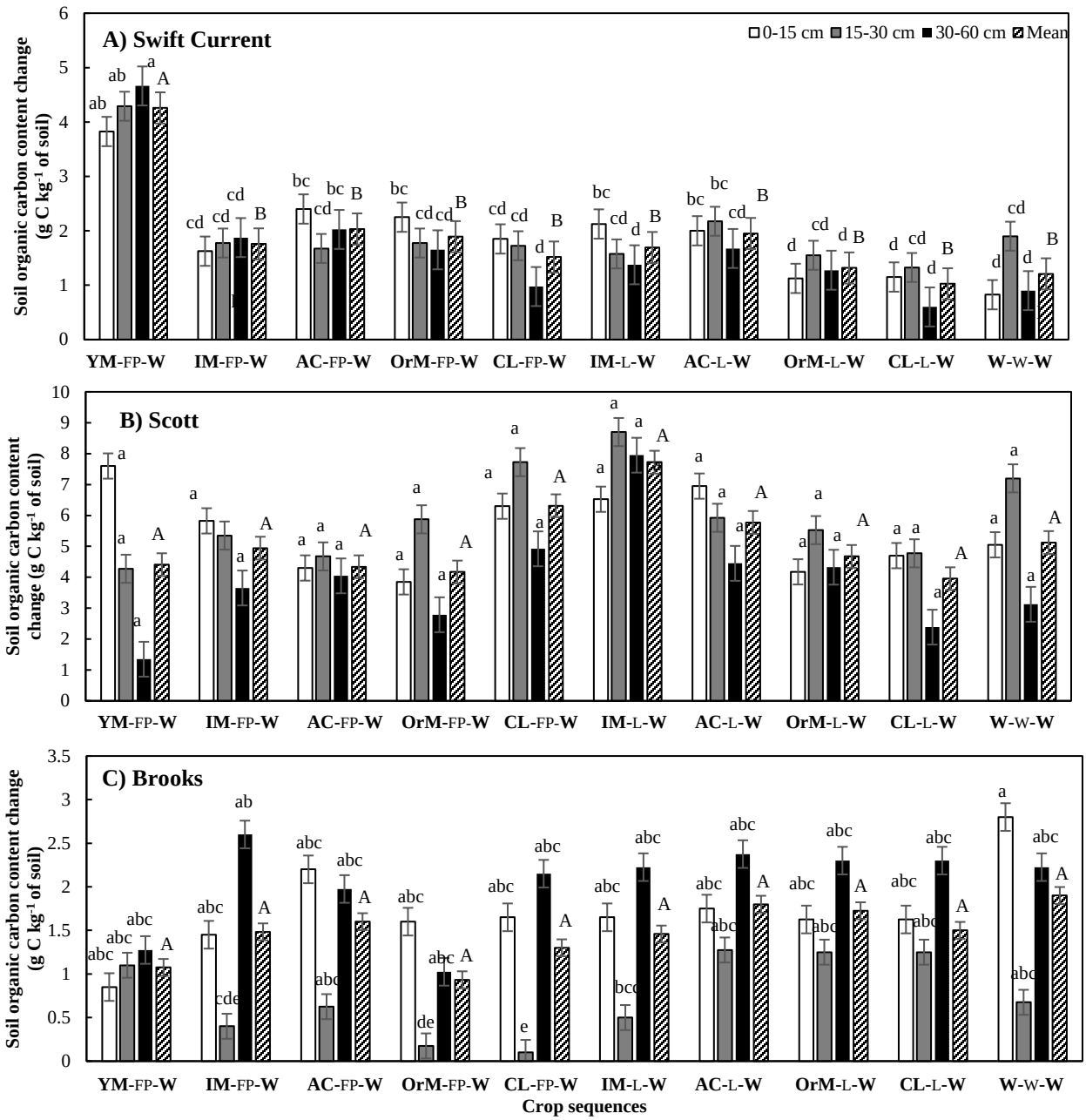

Fig. 4.1. Soil organic carbon (SOC) concentration change (g C kg^{-1} of soil) after growing different crop sequences at three test sites from spring 2019 (before planting oilseed crops) to spring 2021 (before planting wheat). W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Bolded letters in a sequence represent soil sampling phases. Within a site, lower case letters indicate significant differences among crop sequences across all soil depths and upper-case letter indicate differences between means for each crop sequence ($P \leq 0.05$).
Note: The y-axis in different graphs are in different scales. Bars indicate standard error of means.

Table 4.4. Summary of *P* values for main and interaction effects of treatments (T) and soil depth (D) for soil organic carbon concentration change at three test sites.

Effect	Swift Current	Scott	Brooks
T	**<0.0001** [†]	0.1215	0.0867
D	0.3263	**0.0102**	**<0.0001**
T×D	0.7802	0.9153	0.2640

[†] Bolded *P* values indicate significant differences ($P \leq 0.05$).

Table 4.5. Summary of contrast and *P* values from pre-planned comparisons of the soil organic carbon concentration change (g C kg^{-1} of soil) at three soil depths at three test sites.

Crop sequence description[†]	Swift Current			Scott			Brooks		
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm
Continuous wheat vs. the average of	-1.21[‡]	-0.09	-0.89	-0.53	+1.33	-0.86	+1.20	-0.07	+0.20
Brassicaceae and pulse rotations	**0.0112**[¶]	0.8246	**0.0126**	0.7842	0.4467	0.5242	**0.0032**	0.9779	0.6229
Field pea grown on;									
Argentine canola vs. the average of	+0.01	-0.72	-0.27	-1.59	-1.13	+0.87	+0.81	+0.18	+0.21
other Brassicaceae crop stubble	0.9791	0.0859	0.4589	0.4399	0.5411	0.5442	**0.0474**	0.5709	0.6317
Camelina (non-mustard) vs. the	-0.72	-0.89	-1.75	+0.54	+2.56	+2.32	+0.35	-0.46	+0.52
average of mustard crop stubble	**0.0038**	**0.0412**	**<0.0001**	0.7985	0.1865	0.1230	0.3939	0.1715	0.2633
Yellow mustard vs. the average of	+1.89	+2.52	+2.90	+2.76	-1.34	-1.87	-0.68	+0.81	-0.54
oriental and industrial mustard	**0.0011**	**<0.0001**	**<0.0001**	0.2252	0.5097	0.2370	0.1268	0.0565	0.2724
stubble									
Lentil grown on;									
Argentine canola vs. the average of	+0.53	+0.69	+0.59	+1.82	-0.41	-0.44	+0.10	+0.50	+0.12
other Brassicaceae crop stubble	0.2845	0.1072	0.1163	0.3945	0.8304	0.7671	0.8063	0.1371	0.7986
Camelina (non-mustard) vs. the	-0.48	-0.24	-0.73	-0.65	-2.34	-3.75	+0.04	-0.30	-0.01
average of mustard crop stubble	0.3673	0.5948	0.0717	0.7728	0.2532	0.0223	0.9309	0.3937	0.9794

[†] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡] Contrast value = value on the left - value on the right in comparison with *P* value following the contrast value. [¶]Bolded *P* values indicate significant differences ($P \leq 0.05$). [§]

Note: Soils were sampled in spring 2019 before planting oilseed crops and in spring 2021 before planting wheat.

Total soil nitrogen content

Prior to seeding the plots in 2019, the total soil N concentration was 0.88-1.62 g N kg^{-1} of soil at Swift Current, 1.21-1.33 g N kg^{-1} of soil for Scott and 0.84-0.88 g N kg^{-1} of soil for Brooks (Appendices B.5-B.7). On average, the total soil N concentration changes (gains and losses) in all treatments were not affected by crop sequence treatments at Swift Current (Fig. 4.2A; *P*=0.9778, Table 4.6) and Scott (Fig 4.2B; *P*=0.0888, Table 4.6). In contrast, at Brooks, treatment had a significant effect on the total soil N concentration (*P*=0.0187, Table 4.6). Specifically, when lentil was grown after oriental mustard the soil gained 72.3 % more total soil N than when pea was grown after oriental mustard. In addition, the OrM-L-W treatment increased mean total soil N by 62.7 % than CL-L-W (Fig. 4.2C). At Swift Current, there was a reduction in total N at the 15-30 cm depth, but not at the other depths. Only very slight changes in total N were observed at the 0-15 and 30-60 cm depths. In contrast to Swift Current, at the Brooks site, soils in the 15-30 cm depth changed negligibly (very slight gains or losses), whereas the soils at the 0-15 and 30-60 cm depths consistently gained very small amounts of N. Soils at Scott showed similar soil N concentration changes across the soil depths (*P*=0.0577, Table 4.6).

The pre-planned comparisons revealed that crop sequences with camelina, a non-mustard crop followed by field pea had lower total soil N concentration change than the average of mustard crops followed by field pea at all the depths at Swift Current (Table 4.7). The same trend was observed at 15-30 cm and 30-60 cm soil depths at Brooks. In addition, growing yellow mustard before field pea in the sequence increased total soil N more than treatments with oriental and industrial mustards across all the soil depths at Swift Current (Table 4.7). The same trend was observed at the 30-60 cm soil depth at Scott.

Soil organic carbon: total nitrogen (C:N) ratio

On average, crop rotation had no significant effect on soil C:N ratio changes at all three test sites (Appendices Fig. B.1 and Table B.8).

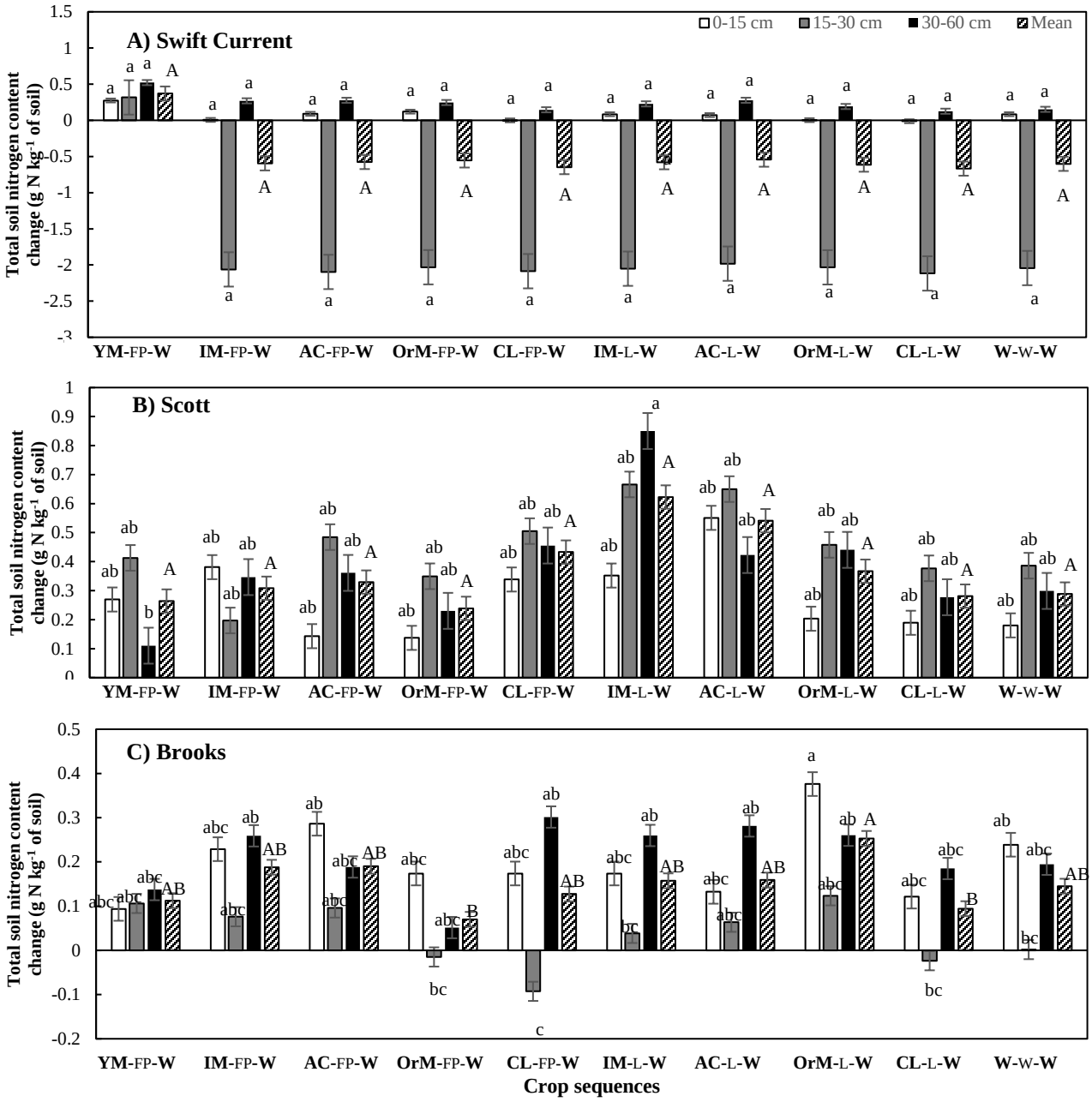

Fig. 4.2. Total soil nitrogen concentration change (g N kg^{-1} of soil) after growing different crop sequences at three test sites from spring 2019 (before planting oilseed crops) to spring 2021 (before planting wheat). W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Bolded letters in a crop sequence represent soil sampling phases. Within a site, lower case letters indicate significant differences among crop sequences across all soil depths and upper-case letter indicate differences between means for each crop sequence ($P \leq 0.05$). **Note**: The y-axis in different graphs are in different scales. Bars indicate standard error of means.

Table 4.6. Summary of *P* values for main and interaction effects of treatments (T) and soil depth (D) for total soil nitrogen concentration change at three test sites

Effect	Swift Current	Scott	Brooks
T	0.9778	0.0888	**0.0187**
D	**<0.0001**[†]	0.0577	**<0.0001**
T × D	1.00	0.7654	0.1979

[†] Bolded *P* values indicate significant differences ($P \leq 0.05$). **Note**: Soils were sampled in spring 2019 before planting oilseed crops and in spring 2021 before planting wheat.

Table 4.7. Summary of contrast and *P* values from pre-planned comparisons of total soil nitrogen concentration change (g N kg^{-1} of soil) at three soil depths at three test sites

Crop sequence contrast description[†]	Swift Current			Scott			Brooks		
	0-15 cm	**15-30 cm**	**30-60 cm**	**0-15 cm**	**15-30 cm**	**30-60 cm**	**0-15 cm**	**15-30 cm**	**30-60 cm**
Continuous wheat vs. the average of	+0.014[‡]	-0.248	-0.101	-0.104	-0.069	+0.330	+0.043	-0.039	-0.019
Brassicaceae and pulse rotations	0.7667	0.7385	**0.0226**[¶]	0.4737	0.6674	0.6639	0.4414	0.4453	0.7579
Field pea grown on[§];									
Argentine canola vs. the average of other	-0.01	-0.630	-0.02	-0.14	+0.12	+1.02	+0.12	+0.08	0.00
Brassicaceae crop stubble	0.8450	0.4275	0.6734	0.3736	0.4914	0.2116	0.0530	0.1653	0.9863
Camelina (non-mustard) vs. the average	-0.13	-0.32	-0.03	+0.08	+0.14	+0.54	+0.01	-0.15	-0.15
of mustard crop stubble	**0.0156**	**0.0315**	**0.0002**	0.6347	0.2998	0.8190	0.8916	**0.0131**	**0.0314**
Yellow mustard vs. the average of	+0.21	+0.36	+0.26	+0.01	+0.02	+0.73	-0.11	+0.08	-0.02
oriental and industrial mustard stubble	**0.0007**	**0.0104**	**<0.0001**	0.9515	0.4592	**<0.0001**	0.1060	0.2137	0.8048
Lentil grown on;									
Argentine canola vs. the average of other	+0.05	+0.09	+0.10	+0.30	0.00	-4.14	-0.09	+0.02	+0.05
Brassicaceae crop stubble	0.3681	0.9166	0.4640	0.6590	0.4001	0.9045	0.1427	0.7566	0.4962
Camelina (non-mustard) vs. the average	-0.06	-0.02	+0.03	-0.09	+0.03	+0.26	-0.15	-0.10	-0.07
of mustard crop stubble	0.3178	0.9306	0.0980	0.6022	0.3283	0.6772	0.0543	0.0900	0.3010

[†]Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡] Contrast value = value on the left - value on the right in the comparison with *P* value following the contrast value. [¶]Bolded *P* values indicate significant differences ($P \leq 0.05$).

Note: Soils were sampled in spring 2019 before planting oilseed crops and in spring 2021 before planting wheat.

Gravimetric soil moisture content

The total precipitations at Swift Current and Brooks for the April-September period in 2020 was lower than the normal or 30-yr average (Appendices Figs. B.2A and B.2C), whereas Scott showed higher precipitation (Appendix Fig. B.2B). However, all test sites had lower precipitation in 2021 than the 30-yr average.

In fall 2020 after harvesting pulse crops, yellow mustard followed by field pea had the lowest average soil moisture across all test sites (Figs. 4.4, 4.5 and 4.6). At Swift Current, the treatment × depth interaction for soil moisture content was significant in 2020 (P<0.0001, Table 4.8). This was primarily due to soil moisture content being 59.5 % higher at 30-60 cm soil depth than the average soil moisture at 0-15 cm and 15-30 cm in the yellow mustard followed by field pea (Fig. 4.4). The average soil moisture content in yellow mustard followed by field pea was 43.6 % lower at Scott (P<0.0001, Table 4.8) and by 32.5 % at Brooks in 2020 compared to the other treatments (P=0.0446). Moreover, all the other treatments had comparable soil moisture contents at each test site in 2020. However, the average fall soil moisture in 2021 after harvesting wheat crop did not vary among the treatments at all test sites (Figs 4.4, 4.5 and 4.6).

The pre-planned comparison suggests that soil moisture content at 0-15 cm and 15-30 cm soil depths in continuous wheat was higher than the average of the treatments with Brassicaceae and pulse crops across three sites in fall of 2020 (Table 4.9). The same trend was observed in the fall 2021 but only at 0-15 cm soil depth at Scott and Brooks. In addition, growing Argentine canola before field pea increased soil moisture content at 15-30 cm soil depths more than the average of the treatments with other Brassicaceae crops across all three test sites in 2020. The same trend was observed in camelina followed by field pea in sequences, which increased fall soil moisture content at 15-30 cm soil depth than the average of treatments with mustard crops at all the test sites in 2020. The rotations with yellow mustard crops had lower soil moisture content at 0-15 cm and 15-30 cm soil depths than the average of the treatments with oriental and industrial mustard followed by field pea at all test sites in 2020. However, no such differences among treatments were noted in the fall 2021 (Table 4.9).

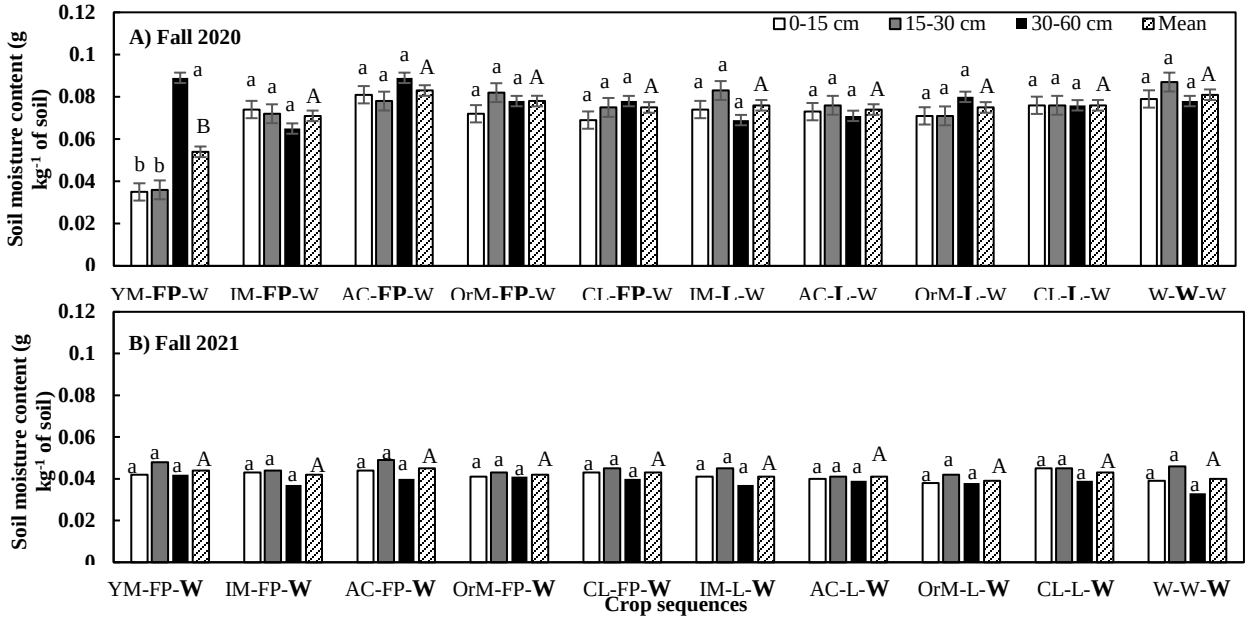

Fig. 4. 3. Fall gravimetric soil moisture content (g kg^{-1} of soil) of different crop sequences at three soil depths at Swift Current across two crop phases A) Fall 2020 (after harvesting pulse or continuous wheat crops) and B) Fall 2021 (after harvesting wheat crop). Bolded letters in crop sequences represent sampling phases. W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Within a sampling year, lower case letters indicate significant differences among crop sequences across all soil depths and uppercase letter indicate differences between means for each crop sequence ($P \leq 0.05$) phase. **Note**: Bars indicate standard error of means.

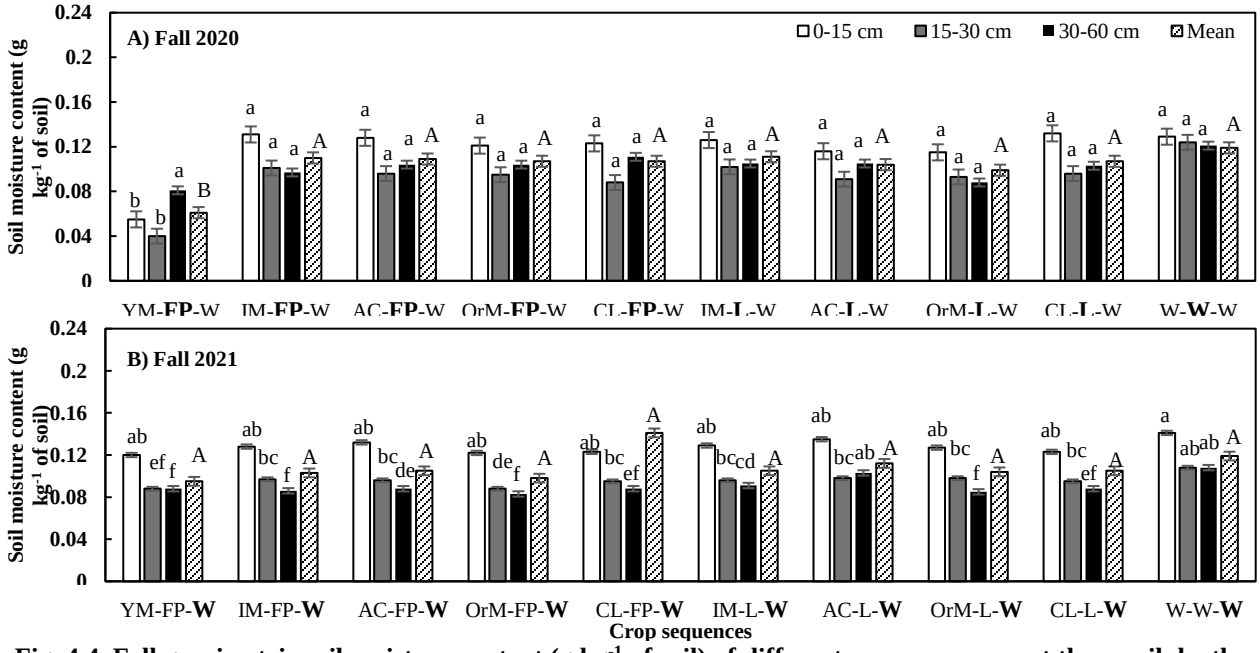

Fig. 4.4. Fall gravimetric soil moisture content (g kg^{-1} of soil) of different crop sequences at three soil depths at Scott across two crop phases A) Fall 2020 (after harvesting pulse or continuous wheat crops) and B) Fall 2021 (after harvesting wheat crop). Bolded letters in crop sequences represent sampling phases. W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Within a sampling year, lower case letters indicate significant differences among crop sequences across all soil depths and uppercase letter indicate differences between means for each crop sequence ($P \leq 0.05$) phase. **Note**: Bars indicate standard error of means.

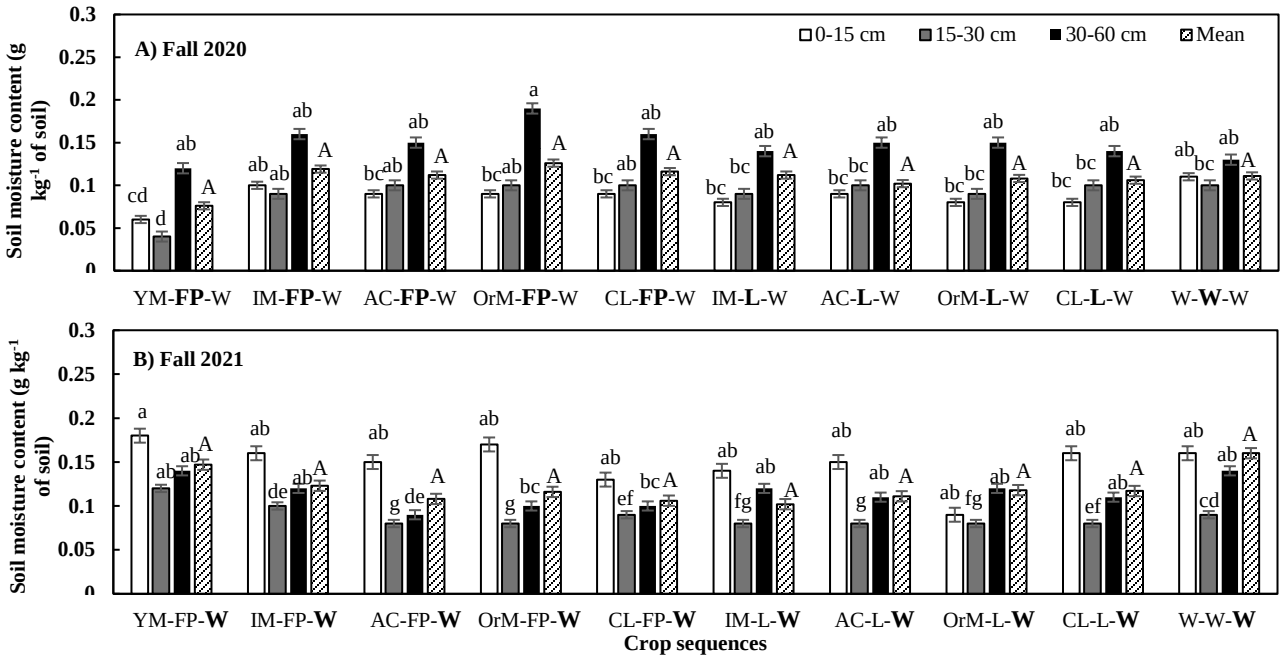

Fig. 4.5. Fall gravimetric soil moisture content (g kg^{-1} of soil) of different crop sequences at three soil depths at Brooks across two crop phases A) Fall 2020 (after harvesting pulse or continuous wheat crops) and B) Fall 2021 (after harvesting wheat crop). Bolded letters in crop sequences represent sampling phases. W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Within a sampling year, lower case letters indicate significant differences among crop sequences across all soil depths and uppercase letter indicate differences between means for each crop sequence ($P \leq 0.05$) phase. **Note**: Bars indicate standard error of means.

Table 4.8. The summary of *P* values for main and interaction effects of treatments (T) and soil depth (D) in fall gravimetric soil moisture contents at three test sites.

Effect	Swift Current		Scott		Brooks	
	2020	**2021**	**2020**	**2021**	**2020**	**2021**
T	**<0.0001**[1]	0.2754	**<0.0001**	0.4706	**0.0446**	0.0665
D	**0.0042**	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
T× D	**<0.0001**	0.8131	0.3727	0.9999	0.9634	0.8882

[1] Bolded *P* values indicate significant differences ($P \leq 0.05$).

Note: Soils were samples in fall 2020 after harvesting pulse or continuous wheat crops and in fall 2021 after harvesting wheat.

Table 4.9. Summary of contrast and *P* values from pre-planned mean comparisons of fall gravimetric soil moisture content (g kg^{-1} of soil) at three test sites

Crop sequence contrast description[l]	Fall 2020								
	Swift Current			Scott			Brooks		
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm
Continuous wheat vs. the average of	+0.020[‡]	+0.020	+0.010	+0.010	+0.010	+0.020	+0.020	+0.020	-0.02
Brassicaceae and pulse rotations	**0.010**[¶]	**0.002**	0.9400	**0.0400**	**0.0269**	0.1462	**0.0463**	**0.0226**	0.4503
Field pea grown on;									
Argentine canola vs. the average of other	+0.018	+0.011	+0.011	+0.021	+0.015	+0.004	+0.008	+0.013	-0.012
Brassicaceae crop stubble	**<0.0001**	**0.0167**	0.1047	**0.039**	**0.0391**	0.8015	0.4630	**0.0003**	0.6782
Camelina (non-mustard) vs. the average of	+0.009	+0.012	+0.001	+0.020	+0.009	+0.014	+0.005	+0.022	+0.001
mustard crop stubble	0.275[0]	**0.0175**	0.9244	**0.0064**	0.2221	0.4224	0.6318	**<0.0001**	0.9736
Yellow mustard vs. the average of oriental and	-0.039	-0.041	+0.018	-0.071	-0.058	-0.013	-0.033	-0.058	-0.048
industrial mustard stubble	**<0.0001**	**<0.0001**	**0.021**	**<0.0001**	**<0.0001**	0.4819	**0.0102**	**<0.0001**	0.1252
Lentil grown on;									
Argentine canola vs. the average of other	-0.0001	0.000	-0.0003	-0.005	-0.006	+0.006	-0.001	+0.003	+0.006
Brassicaceae crop stubble	0.7681	0.9470	0.6132	0.4702	0.3938	0.7329	0.9607	0.3058	0.8329
Camelina (non-mustard) vs. the average of	+0.004	+0.001	-0.012	+0.003	+0.007	+0.005	-0.003	-0.003	-0.020
mustard crop stubble	0.3545	0.7093	0.8125	0.6884	0.8520	0.7503	0.8138	0.0524	0.9591
					Fall 2021				
Continuous wheat vs. the average of	+0.030	0.000	-0.020	+0.010	0.000	+0.020	+0.100	0.000	+0.030
Brassicaceae and pulse rotations	0.1867	0.5878	0.0716	**0.006**	0.9917	0.0536	**0.0079**	0.6155	0.1483
Field pea grown on;									
Argentine canola vs. the average of other	+0.002	+0.004	+0.001	+0.009	-0.025	+0.004	-0.007	-0.011	-0.025
Brassicaceae crop stubble	0.3617	0.1944	0.7981	0.0594	0.5377	0.7113	0.8390	0.0585	0.1970
Camelina (non-mustard) vs. the average of	+0.002	0.00	0.00	+0.001	+0.120	+0.006	-0.037	-0.09	-0.021
mustard crop stubble	0.5398	0.9311	0.8936	0.8348	**0.0076**	0.6172	0.3203	0.1227	0.2927
Yellow mustard vs. the average of oriental and	0.00	+0.004	+0.002	-0.008	-0.005	-0.002	+0.011	+0.036	+0.034
industrial mustard stubble	0.9580	0.2098	0.4817	0.1261	0.9172	0.8527	0.7743	**<0.0001**	0.1128
Lentil grown on;									
Argentine canola vs. the average of other	-0.001	-0.002	+0.001	+0.005	+0.002	+0.014	-0.004	-0.004	-0.006
Brassicaceae crop stubble	0.6585	0.4250	0.7219	0.2910	0.9700	0.2252	0.9209	0.5353	0.7752
Camelina (non-mustard) vs. the average of	+0.006	+0.001	+0.002	+0.005	-0.002	0.00	+0.010	+0.001	-0.005
mustard crop stubble	0.0553	0.7257	0.5108	0.3959	0.9630	0.9933	0.8104	0.8459	0.7998

[l]Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡] Contrast value =value on the left - value on the right in the comparison and the *P* value following the contrast value. [¶]Bolded *P* values indicate significant differences ($P \leq 0.05$). **Note:** Soils were samples in fall 2020 after harvesting pulse or continuous wheat crops and in fall 2021 after harvesting wheat crops.

Organic matter fractions

Soil organic matter (SOM) was fractionated into light fraction organic matter (LFOM) and heavy fraction organic matter (HFOM). On a mass basis, the LFOM accounted for approximately 0.2-2.0 % of the soil across three test sites (Table 4.10). Lentil grown on Argentine canola stubble had the highest LFOM and the lowest HFOM at Swift Current, whereas field pea grown on Argentina canola stubble had the highest LFOM and lowest HFOM at Scott and Brooks sites, consistently in 2020 and 2021.

Pre-planned mean comparisons revealed that on average, the treatments with Brassicaceae and pulse crops had higher LFOM (P<0.0001, Table 4.11) with lower HFOM (P<0.0001) compared to those of continuous wheat at all test sites in 2020 and 2021. In addition, regardless of the pulse species in the sequence, growing Argentine canola as a preceding crop consistently increased LFOM and decreased HFOM than the average of other Brassicaceae crops at all three test sites in 2020 and 2021. Moreover, the treatment with yellow mustard followed by field pea consistently had higher LFOM than the average of the treatments with other mustard crops followed by field pea at all test sites (Table 4.11).

Table 4.10. Light fraction and heavy fraction concentrations at 0-15 cm depth of different crop sequences (g kg^{-1} of soil) at three test sites.

Crop sequence[l]	Light fraction concentration					
	Swift Current		Scott		Brooks	
	2020	2021	2020	2021	2020	2021
YM-FP-W	10.8^{bc}	12.1^{b}	10.4^{b}	11.1^{b}	13.0^{b}	14.7^{ab}
IM-FP-W	9.70^{bcd}	10.5^{bc}	4.15^{de}	4.50^{ef}	5.70^{cd}	6.05^{d}
AC-FP-W	11.6^{b}	13.2^{b}	12.1^{a}	13.1^{a}	15.9^{a}	16.2^{a}
OrM-FP-W	3.95^{e}	5.45^{d}	4.00^{de}	4.35^{ef}	6.25^{cd}	5.65^{d}
CL-FP-W	7.60^{bcde}	7.75^{cd}	7.30^{de}	7.75^{c}	7.90^{c}	9.05^{c}
IM-L-W	6.10^{def}	7.65^{cd}	5.60^{cd}	6.45^{d}	5.75^{cd}	5.45^{d}
AC-L-W	20.2^{a}	20.9^{a}	9.65^{b}	10.2^{b}	11.6^{b}	13.5^{b}
OrM-L-W	6.50^{cde}	6.70^{cd}	5.20^{d}	6.05^{d}	5.15^{d}	5.75^{d}
CL-L-W	6.35^{cde}	6.55^{cd}	4.10^{de}	4.55^{e}	6.95^{cd}	7.85^{c}
W-W-W	4.80^{de}	4.40^{d}	2.85^{e}	3.30^{f}	5.00^{d}	5.55^{d}
***P* value**	**<0.0001**[§]	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Heavy fraction concentration					
YM-FP-W	989^{cd}	988^{c}	990^{e}	989^{cd}	987^{c}	985^{cd}
IM-FP-W	990^{bcd}	989^{bc}	996^{ab}	995^{bcd}	994^{ab}	994^{ab}
AC-FP-W	988^{d}	987^{c}	988^{f}	986^{f}	984^{d}	984^{d}
OrM-FP-W	996^{a}	995^{a}	996^{ab}	996^{a}	994^{ab}	994^{a}
CL-FP-W	992^{abcd}	992^{ab}	993^{d}	992^{abcd}	992^{b}	991^{b}
IM-L-W	994^{abc}	992^{ab}	994^{c}	994^{abc}	994^{ab}	995^{a}
AC-L-W	980^{e}	979^{d}	990^{e}	990^{e}	988^{c}	986^{c}
OrM-L-W	993^{abc}	993^{ab}	995^{c}	994^{abc}	995^{a}	994^{a}
CL-L-W	994^{abc}	993^{ab}	996^{b}	995^{abc}	993^{ab}	992^{b}
W-W-W	995^{ab}	996^{a}	997^{a}	997^{ab}	995^{a}	994^{a}
***P* value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

[l]W=Wheat, YM=Yellow mustard, FP=Field pea IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each test site for each parameter. Thus, values with different letters within each column are significantly different at $P \leq 0.05$. [§] Bolded *P* values indicate significant differences ($P \leq 0.05$. **Note**: Soils were sampled in spring 2020 after growing oilseed and continuous wheat crops and in spring 2021 after growing the pulse and continuous wheat crops

Table 4.11. Summary of contrast and *P* values from pre-planned mean comparison of concentration of light (LFOM) and heavy (HFOM) fraction organic matter at different sites.

Crop sequence contrast description[1]	for soil LFOM					
	Swift Current		Scott		Brooks	
	2020	2021	2020	2021	2020	2021
Continuous wheat vs. the average of Brassicaceae and pulse rotations	-4.39‡ **0.0005**¶	-5.68 **<0.0001**	-4.09 **<0.0001**	-4.26 **<0.0001**	-3.67 **<0.0001**	-3.81 **<0.0001**
Field pea grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	+3.58 **0.0048**	+4.25 **<0.0001**	+5.63 **<0.0001**	+6.16 **<0.0001**	+7.68 **<0.0001**	+7.33 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.550 0.6628	-1.60 0.1057	+1.11 **0.0111**	+1.10 **0.0007**	-0.417 0.5297	+0.25 0.5199
Yellow mustard vs. the average of oriental and industrial mustard stubble	+3.97 **0.0050**	+4.12 **0.0010**	+6.32 **<0.0001**	+6.67 **<0.0001**	+7.02 **<0.0001**	+8.85 **<0.0001**
Lentil grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	+13.8 **<0.0001**	+13.93 **<0.0001**	+4.68 **<0.0001**	+4.51 **<0.0001**	+5.65 **<0.0001**	+7.15 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	+0.500 0.9692	-0.625 0.5395	-1.30 **0.0059**	-1.70 **<0.0001**	+1.50 **0.0327**	+2.25 **<0.0001**
	for soil HFOM					
Continuous wheat vs. the average of Brassicaceae and pulse rotations	+4.33 **0.0005**	+6.22 **<0.0001**	+3.89 **<0.0001**	+5.78 **<0.0001**	+3.78 **<0.0001**	+3.44 **<0.0001**
Field pea grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	-3.75 **0.0048**	-4.00 **<0.0001**	-5.75 **<0.0001**	-7.00 **<0.0001**	-7.75 **<0.0001**	-7.00 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	0.33 0.6628	+1.33 **<0.0001**	-1.00 **0.0111**	-1.33 **0.0007**	+0.33 0.5297	-0.26 0.5199
Yellow mustard vs. the average of oriental and industrial mustard stubble	-4.00 **0.0050**	-4.00 **<0.0001**	-6.00 **<0.0001**	-6.50 **<0.0001**	-7.00 **<0.0001**	-9.00 **<0.0001**
Lentil grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	-13.6 **<0.0001**	-13.6 **<0.0001**	-5.00 **<0.0001**	-4.33 **<0.0001**	-6.00 **<0.0001**	-7.66 **<0.0001**
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.50 0.9692	-0.50 0.5395	+1.50 **0.0059**	-1.00 **<0.0001**	-1.50 **0.0327**	-2.25 **<0.0001**

[1] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. ‡ Contrast value = value on the left- value on the right in the comparison and the *P* value following the contrast value. ¶Bolded *P* values indicate significant differences ($P \leq 0.05$). §.

Note: Soils were sampled in spring 2020 after growing oilseed and continuous wheat crops and in spring 2021 after growing the pulse and continuous wheat crops**.**

Light fraction carbon (LFOM-C) and nitrogen (LFOM-N) concentration

The treatment with lentil grown after Argentine canola consistently had the highest amount of LFOM-C at Swift Current in 2020 (4.65 g kg^{-1} of soil, Fig. 4.7A) and 2021 (3.13 g kg^{-1} of soil, Fig. 4.7A). At Scott, soils with Argentine canola stubble generally had the highest LFOM-C in 2020, regardless of the subsequent pulse species (Fig. 4.7B). However, in 2021, all three treatments AC-L-W, AC-FP-W and IM-L-W had the highest LFOM-C content. At Brooks, treatment had no significant effect on LFOM-C in 2020, but the Argentine canola followed by lentil treatment had the highest value in 2021 (Fig. 4.7C).

Similar to LFOM-C, Argentine canola followed by lentil had the highest LFOM-N at Swift Current in 2020 (Fig. 4.8 A). In contrast, all the treatments had comparable LFOM-N in 2021. At Scott, rotation treatments including Argentine canola consistently had higher LFOM-N than other treatments in both sampling years (Fig. 4.8B). At Brooks, all crop sequences had comparable LFOM-N in 2020, but in 2021, AC-LW had the highest LFOM-N (Fig. 4.8C).

On average, continuous wheat cultivation consistently resulted in lower LFOM-C and LFOM-N content compared to treatments with Brassicaceae and pulse crops. This trend was predominantly noted at all the test sites in both sampling years except at Swift Current in 2021 (Table 4.12). Crop sequences with Argentine canola generally increased both LFOM-C and LFOM-N content compared to other Brassicaceae crops, regardless of the following grain legume species. This trend was observed at each site in both sampling years. Furthermore, crop sequence with yellow mustard followed by field pea showed higher LFOM-C and LFOM-N than the average of field pea crop sequences with other mustards at Swift Current and Scott in 2020 and 2021 and Brooks in 2021(Table 4.12).

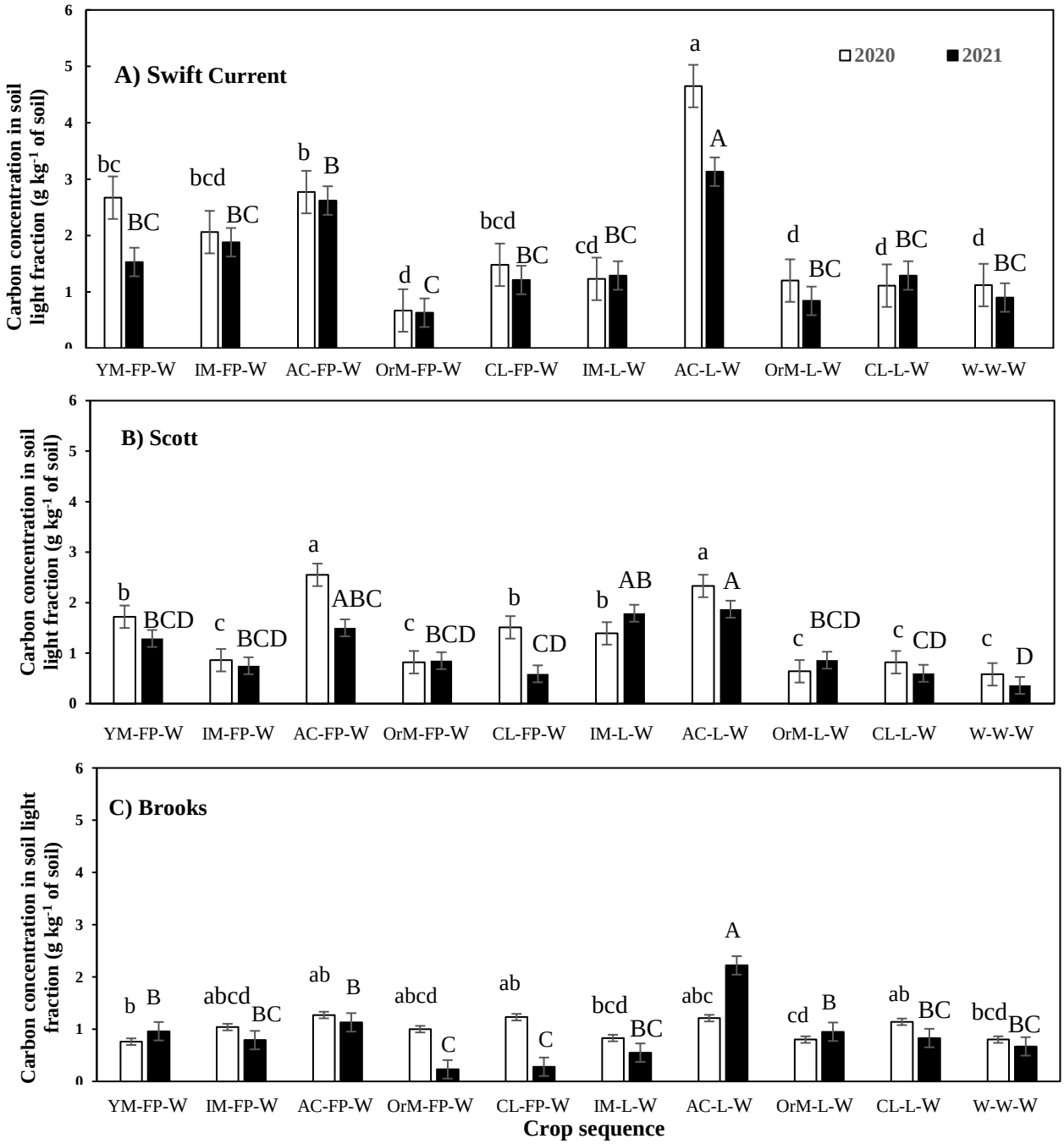

Fig. 4.6. The concentration of carbon in soil light fraction (LFOM-C) of different rotation sequences sampled at 0-15 cm soil depth at three test sites, A) Swift Current B) Scott and C) Brooks in the springs of 2020 (after growing oilseed and continuous wheat crops) and spring 2021 (after growing pulse and continuous wheat crops). W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. White and black colors represent the concentrations of LFOM-C in 2020 and 2021, respectively. Lowercase letters indicate significant differences among crop sequences in spring 2020 and upper-case letter indicate significant differences between among crop sequences in spring 2021 at (P≤0.05). **Note**: Bars indicate the standard errors of means.

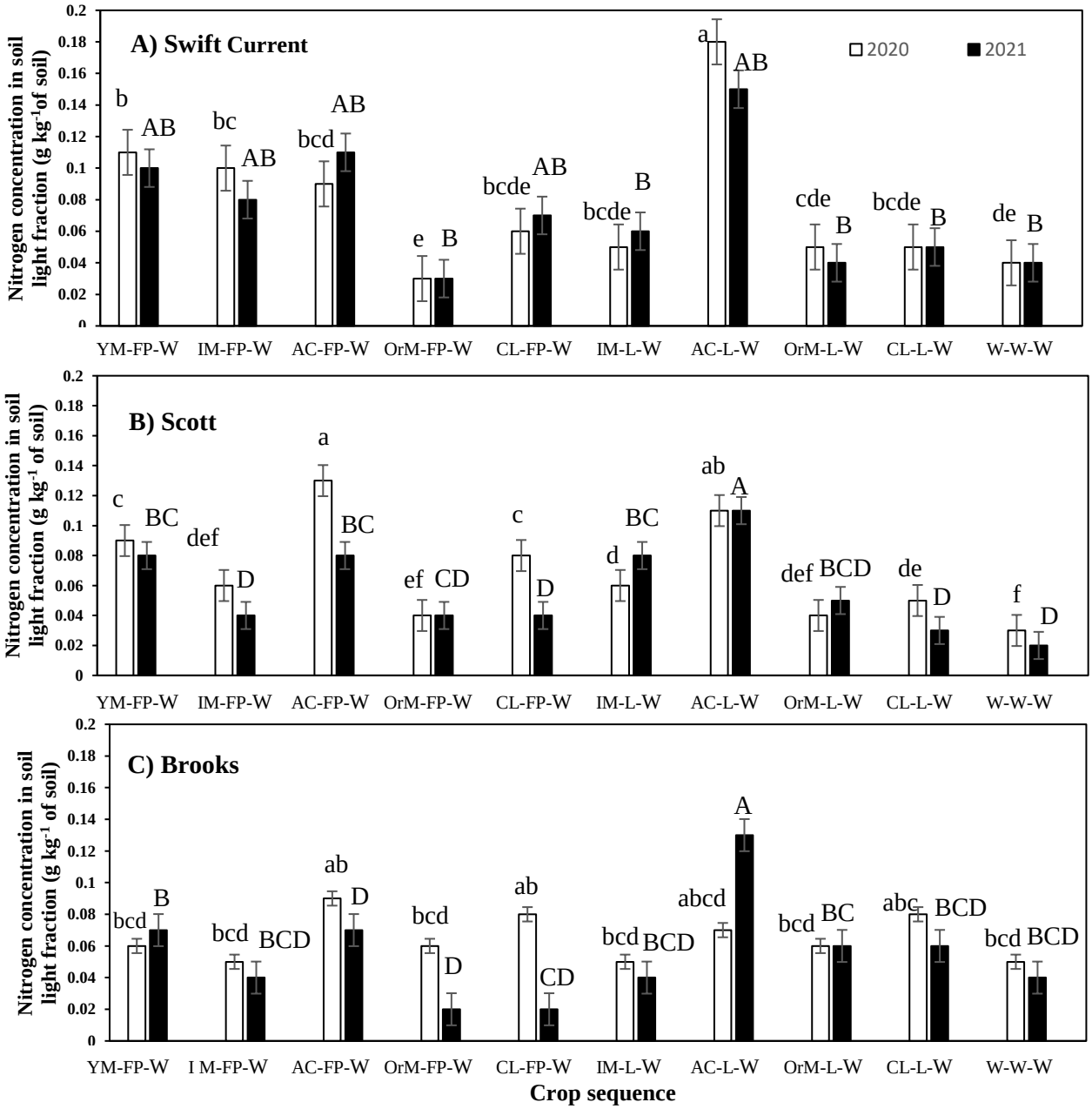

Fig. 4.7. The concentration of carbon in soil light fraction (LFOM-C) of different rotation sequences sampled at 0-15 cm soil depth at three test sites, A) Swift Current B) Scott and C) Brooks in the spring of 2020 (after growing oilseed and continuous wheat crops) and spring 2021 (after growing pulse and continuous wheat crops). W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. White and black colors represent the abundance of LFOM-C in 2020 and 2021, respectively. Lowercase letters indicate significant differences among crop sequences in spring 2020 and upper-case letter indicate significant differences between among crop sequences in spring 2021 at (P≤0.05). **Note**: Bars indicate the standard errors of means.

Table 4.12. Summary of contrast and *P* values from pre-planned mean comparison of light fraction carbon (LFOM-C) and nitrogen (LFOM-N) concentration at 0-15 cm soil depth at three test sites in the spring of 2020 and 2021

Crop sequence contrast description[l]	for LFOM-C					
	Swift Current		Scott		Brooks	
	2020	2021	2020	2021	2020	2021
Continuous wheat vs. the average of	-0.86[‡]	-0.70	-0.82	-0.76	-0.23	-0.21
Brassicaceae and pulse rotations	**0.0103**[§]	0.0967	**<0.0001**	**0.0027**	**0.0053**	**0.0496**
Field pea grown on;						
Argentine canola vs. the average of other	+1.05	+1.31	+1.322	+0.633	+0.261	+0.569
Brassicaceae crop stubble	**0.0036**	**0.0057**	**<0.0001**	**0.0197**	**0.022**	**0.0007**
Camelina (non-mustard) vs. the average	-0.317	-0.136	+0.383	-0.370	+0.301	-0.380
of mustard crop stubble	0.3630	0.7658	**0.0001**	0.1716	**0.0008**	0.0204
Yellow mustard vs. the average of	+1.29	+0.276	+0.878	+0.496	-0.261	+0.452
oriental and industrial mustard stubble	**0.0014**	**0.5690**	**<0.0001**	**0.0475**	**0.0045**	**0.0101**
Lentil grown on;						
Argentine canola vs. the average of other	+3.47	+1.98	-1.38	+1.139	+0.187	+1.09
Brassicaceae crop stubble	**<0.0001**	**0.0002**	**<0.0001**	**0.0002**	**0.0260**	**0.0001**
Camelina (non-mustard) vs. the average	-0.098	+0.224	-0.194	-0.729	+0.323	+0.074
of mustard crop stubble	0.7889	0.6436	**0.0452**	**0.0148**	**0.0007**	0.6554
			for LFOM-N			
Continuous wheat vs. the average of	-0.040	-0.040	-0.04	+0.08	-0.020	-0.020
Brassicaceae and pulse rotations	**0.0082**	0.0511	**<0.0001**	**<0.0001**	**0.0072**	**0.0210**
Field pea grown on;						
Argentine canola vs. the average of other	+0.012	+0.042	+0.073	+0.034	+0.026	+0.032
Brassicaceae crop stubble	0.4086	**0.0500**	**<0.0001**	**0.0004**	**<0.0001**	**0.0022**
Camelina (non-mustard) vs. the average	-0.016	-0.005	+0.024	-0.014	+0.019	-0.024
of mustard crop stubble	0.2846	0.8168	**<0.0001**	0.1106	**0.0023**	**0.0194**
Yellow mustard vs. the average of	+0.044	+0.039	+0.045	+0.035	-0.005	+0.035
oriental and industrial mustard stubble	**0.0082**	**0.0025**	**<0.0001**	**0.0007**	0.3846	**0.0021**
Lentil grown on;						
Argentine canola vs. the average of other	+0.131	+0.104	+0.062	+0.060	+0.006	+0.080
Brassicaceae crop stubble	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.3240	**0.0001**
Camelina (non-mustard) vs. the average	+0.002	-0.008	-0.001	-0.031	+0.023	+0.005
of mustard crop stubble	0.8941	0.7316	0.9131	**0.0021**	**0.0008**	0.6240

***P* values for treatment effect**

LFOM-C				LFOM-N			
	Test site				Test site		
	Swift Current	Scott	Brooks		Swift Current	Scott	Brooks
2020	**<0.0001**	**<0.0001**	**<0.0001**	2020	**<0.0001**	**<0.0001**	**<0.0001**
2021	**0.026**	**0.001**	**0.0004**	2021	0.821	**0.001**	**0.033**

[l] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡] Contrast value = value on the left- value on the right in the comparison and the *P* value following the contrast value. [§]Bolded *P* values indicate significant differences ($P \leq 0.05$).

Wheat yield components, grain productivity and harvest index

The cropping sequences had no significant effect on the plant density of subsequent wheat grown in 2021 across all three test sites (Tables 4.13). However, the pre-planned mean comparison indicated that wheat plant density was 18 % lower, the number of seeds per plant was 46 % higher and 1000-seed weight was 9.6 % lower in continuous wheat than the average of wheat preceded by Brassicaceae and pulse crops at Swift Current (Tables 4.13). In addition, growing yellow mustard followed by field pea increased subsequent wheat 1000-seed weight by 4.3 % compared to the average of crop sequences with industrial and oriental mustard crops followed by field pea. At Brooks, continuous wheat decreased 1000-seed weight of 2021 grown wheat by 10.5 % than wheat preceded by Brassicaceae and pulse crops (Table 4.13).

Different crop sequences did not affect wheat grain yield in 2021 (Table 4.14). However, pre-planned mean comparison showed that the inclusion of Argentine canola in crop sequence increased the subsequent wheat grain yield by 31.8-32.1 % than the other Brassicaceae crops followed by field pea or lentil at Swift Current. In addition, the grain yield of continuous wheat was 52.7 % lower than the average of wheat preceded by Brassicaceae and pulse crops at Brooks (Table 4.14).

Within each test site, the harvest index values remained consistent among the different crop sequences except at Swift Current (P=0.0142, Table 4.14). At Swift Current, growing camelina before lentil resulted in a 10.7% decrease in subsequent wheat harvest index compared to the average of oriental and industrial mustard.

Table 4.13. Plant density, number of seeds per plant and 1000-seed weight of wheat in different crop sequences at three test sites in the 2021 crop phase

Crop sequence[†]	Yield component								
	Plant density (number of plants m^2)			Number of seeds per plant			1000-seed weight (g)		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
1= YM-FP-W	130.9[a]	196.0[a]	267.2[a]	20.2[ab]	37.9[a]	19.3[a]	33.1[a‡]	31.7[a]	32.4[ab]
2= IM-FP-W	160.9[a]	182.6[a]	240.0[a]	20.6[ab]	37.2[a]	26.7[a]	31.5[ab]	33.2[a]	31.7[ab]
3= AC-FP-W	161.4[a]	187.3[a]	223.3[a]	25.8[ab]	38.1[a]	25.8[a]	31.3[ab]	32.2[a]	31.7[ab]
4= OrM-FP-W	145.2[a]	193.0[a]	229.8[a]	23.3[ab]	33.4[a]	29.2[a]	31.8[ab]	31.6[a]	31.0[ab]
5= CL-FP-W	157.2[a]	205.3[a]	227.8[a]	24.9[ab]	32.3[a]	23.1[a]	31.9[ab]	32.9[a]	33.7[a]
6= IM-L-W	155.5[a]	189.7[a]	244.7[a]	23.2[ab]	38.8[a]	31.1[a]	31.2[ab]	32.2[a]	31.6[ab]
7= AC-L-W	162.4[a]	200.9[a]	238.6[a]	23.2[ab]	33.8[a]	22.9[a]	31.7[ab]	32.7[a]	32.2[ab]
8= OrM-L-W	144.7[a]	178.0[a]	235.0[a]	17.0[b]	27.2[a]	25.5[a]	31.8[ab]	31.2[a]	33.5[a]
9= CL-L-W	143.0[a]	216.8[a]	229.7[a]	13.3[b]	32.3[a]	20.4[a]	31.8[ab]	32.6[a]	31.7[ab]
10= W-W-W	123.5[a]	196.0[a]	215.7[a]	38.5[a]	36.8[a]	23.9[a]	29.5[b]	32.3[a]	29.1[b]
***P* value**	0.1137[§]	0.1605	0.2124	**0.0256**	0.6265	0.8006	**0.0120**	0.9162	**0.0162**
Contrast and *P* values from pre-planned mean comparisons									
Continuous wheat vs. the average of Brassicaceae and pulse rotations	-27.7[#] **0.0141**	+1.60 0.8650	-21.0 0.09	+17.8 **0.0005**	+2.26 0.6055	-0.97 0.95	-2.28 **0.0002**	+0.03 0.9746	-3.12 **0.0006**
Wheat preceded by field pea grown on;									
Argentine canola vs. the average of other Brassicaceae crop stubble	+12.85 0.2610	-6.92 0.4894	-17.90 0.1869	+3.55 0.4428	+2.85 0.5379	+1.27 0.4617	-0.77 0.1879	-0.150 0.8634	+0.50 0.5591
Camelina (non-mustard) vs. the average of mustard crop stubble	-11.53 0.3261	+14.76 0.1622	-17.86 0.2104	+3.53 0.4627	-3.92 0.4137	-1.99 0.7399	-0.233 0.7012	+0.733 0.5068	+2.00 **0.031**
Yellow mustard vs. the average of oriental and industrial mustard stubble	-22.15 0.0822	+8.20 0.4581	+32.30 **0.0308**	-1.75 0.7226	+2.62 0.6049	-8.68 0.1791	+1.45 **0.0333**	-0.700 0.5507	+1.05 0.2715
Wheat preceded by lentil grown on;									
Argentine canola vs. the average of other Brassicaceae crop stubble	+14.66 0.2151	+6.06 0.5572	+2.13 0.8759	+5.36 0.2623	+0.977 0.8381	-2.77 0.6440	+0.10 0.8834	+0.700 0.5298	-0.07 0.9762
Camelina (non-mustard) vs. the average of mustard crop stubble	-7.10 0.5656	+32.95 **0.0054**	-10.15 0.4851	-6.80 0.1867	-0.74 0.8821	-7.95 0.2177	+0.30 0.6786	+0.900 0.4622	-0.85 0.3981

[†]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each site for each parameter. Values with different letters within each column are significantly different at $P>0.05$. [§] Bolded *P* values indicate significant differences ($P\leq0.05$). [#]Contrast value = value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value.

Table 4.14. Grain yield and harvest index (HI) of wheat in different crop sequences at three test sites in the 2021 crop phase

Crop sequence[ł]	Grain yield (kg ha^{-1})			HI		
	Swift Current	**Scott**	**Brooks**	**Swift Current**	**Scott**	**Brooks**
1= YM-FP-W	935[a]	2621[a]	1103[a]	0.498[a‡]	0.573[a]	0.460[a]
2= IM-FP-W	1371[a]	2647[a]	1210[a]	0.498[a]	0.577[a]	0.424[a]
3= AC-FP-W	1905[a]	2384[a]	1104[a]	0.465[ab]	0.574[a]	0.399[a]
4= OrM-FP-W	1475[a]	2298[a]	866[a]	0.485[ab]	0.558[a]	0.420[a]
5= CL-FP-W	1410[a]	2688[a]	1166[a]	0.492[a]	0.562[a]	0.448[a]
6= IM-L-W	1399[a]	2382[a]	1177[a]	0.461[ab]	0.550[a]	0.438[a]
7= AC-L-W	1604[a]	2496[a]	1014[a]	0.457[ab]	0.571[a]	0.400[a]
8= OrM-L-W	994[a]	1726[a]	968[a]	0.455[ab]	0.568[a]	0.415[a]
9= CL-L-W	872[a]	2415[a]	1093[a]	0.409[b]	0.542[a]	0.432[a]
10= W-W-W	1636[a]	2011[a]	509[a]	0.469[ab]	0.563[a]	0.373[a]
***P* value**	0.1696[§]	0.2027	0.2419	**0.0142**	0.4809	0.4991
Contrast and *P* values from pre-planned comparisons						
Continuous wheat vs. the average of Brassicaceae and pulse rotations	+306[¶] 0.2823	-395 0.1390	-568 **0.005**	0.00 0.9780	0.00 0.96	-0.05 0.0655
Wheat preceded by field pea grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	+607 **0.0500**	-179 0.5188	+17.8 0.9299	-0.03 0.1187	+0.18 0.6318	-0.039 0.1947
Camelina (non-mustard) vs. the average of mustard crop stubble	+149 0.628	+166 0.5629	+106 0.6073	+0.02 0.9441	+0.007 0.5835	+0.013 0.6534
Yellow mustard vs. the average of oriental and industrial mustard stubble	-488 0.1438	+148 0.6255	+65 0.7687	+0.007 0.7236	+0.005 0.6780	+0.038 0.2558
Wheat preceded by lentil grown on;						
Argentine canola vs. the average of other Brassicaceae crop stubble	+515 **0.0338**	+321 0.3242	-65.3 0.9273	+0.015 0.4102	+0.018 0.1966	-0.028 0.3601
Camelina (non-mustard) vs. the average of mustard crop stubble	-324 0.3261	+361 0.2403	+20.5 0.4083	-0.049 0.0167	-0.017 0.2437	+0.006 0.8635

[ł]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each test site for each parameter. Thus, values with different letters within each column are significantly different at $P \leq 0.05$, according to Tukey's HSD test. [§] Bolded *P* values indicate significant differences ($P \leq 0.05$). [¶]Contrast value = the value on the left - the value on the right in the comparison and the *P* value was mentioned following the contrast value.

4.6. Discussion

4.6.1. Selected soil properties of the cropping system

Understanding the effect of rotation sequence on fall soil moisture (post-harvest residual soil moisture) content at different soil depths is vital to developing crop rotation systems with efficient water utilization. Similar soil moisture contents in both spring (before seeding) and fall (after harvest) indicated that all of the crops in a given cropping season including continuous wheat had equivalent soil moisture usage from within the 0-60 cm soil depth. This aligns with Gan et al. (2015), which described that field pea and lentil in wheat-based cropping systems had similar post-harvest residual soil moisture contents at 0-60 cm soil depth. In contrast, other studies reported that pulse crops in wheat-based crop rotations used less water from deeper soil profiles, leaving more water for the next crop in a rotation compared to deeper rooting crops, such as wheat (Gan et al., 2007; Wang et al., 2013). Field pea, lentil and wheat had a larger proportion of total root biomass (61-67 %) in the 0-20 cm depth (Gan et al., 2009b). In addition, 77- 85 % of the roots of field pea and lentil crops are distributed within the 0-40 cm soil profile (Liu et al., 2010). Low precipitation at all test sites in 2020 and 2021 (Fig. 4.6) probably caused water deficit conditions, which can hinder the differences in water uptake differences due to all available water uptake despite differences in rooting depth. In 2020, continuous wheat showed higher post-harvest residual soil moisture content compared to all the diversified crop rotations. Evapotranspiration also plays a key role in soil water storage capacity (Gan et al., 2015). Evapotranspiration rates are usually greater in annual legumes than in other grain crops, such as spring wheat under semi-arid conditions (Thomson et al., 1997). Slow development of canopy coverage in annual grain legumes (Merrill et al., 2002) can increase evaporation and result in low amount of soil water.

In the present study, similar changes in SOC contents among all the treatments may be the result of an inadequate length of rotation history in this study (only 2 years) to induce measurable changes (Fig. 4.1; Table 4.4). Similar results were shown in other crop-rotational studies (King and Bleash, 2018; Kostensalo et al., 2023). The accumulation of SOC is a slow process, which generally takes around 10-20 years (Kern and Johnson, 1993), thus, the time duration of the study was insufficient to identify significant SOC changes among crop rotation treatments. In addition, soil moisture content is a key factor influencing microbial activity and SOC processing (Schimel et al., 2012; Kerr and Ochsner, 2022). The lack of variation in moisture contents (Figs. 4.3, 4.4 and 4.5)

might have further limited the potential for observing differences in SOC among treatments. However, changes in SOC content induced by crop rotations are complex because of the variations in climatic factors, initial soil properties, and agronomic practices. A meta-analysis by Liu et al. (2022) found no significant differences in SOC between continuous cropping and rotational cropping systems when the mean annual precipitation was less than 600 mm. Mean annual precipitation at test sites in this study was 244-285 mm. This lack of difference could be attributed to less favorable conditions for soil microbes to grow (Li et al., 2018), as well as low gross primary production and reduced biomass inputs (above- and below-ground), which collectively limit SOC accumulation (Barman et al., 2014). In the current study, crop rotations increased SOC concentration by 1.1-50.7% across three test sites over the two years. Initial SOC concentration can influence the observed percentage increases in SOC in the current study. In plots with relatively low initial SOC (4.5-5.9 g C kg^{-1} soil at Swift Current), even small organic matter inputs or crop management improvements can result in substantial percentage increases (Oldfield et al., 2019). In contrast, previous studies suggest that crop diversification can increase SOC by varying percentages, ranging from 1.1- 29.0 % (Maiga et al., 2019; González-Rosado et al., 2022; Rui et al., 2022). However, the substantial increase observed from 2019 to 2020 in the current study can partly be due to sampling or analytical errors, which influenced SOC measurements and account for such variations.

Crop sequences with Brassicaceae and pulse crops at Swift Current showed higher gains in SOC at 0-30 cm and higher LFOM contents at 0-15 cm soil depth than continuous wheat (Table 4.5 and 4.11). The magnitude of the LFOM and HFOM pools indicates the balance between residual addition and decomposition (Murindangabo et al., 2023). While above-ground biomass production in continuous wheat was higher than the average of Brassicaceae oilseeds in 2019 (5593 kg ha^{-1} vs. 4806 kg ha^{-1}, P=0.05, Appendix Table B.5), biomass production was similar in the pulse crop phase in 2020 (P=0.60), which suggests that the quality of the residue and its composition affecting decomposition rate of crop residues possibly plays a more significant role than the total amount of residue input. This may explain the higher LFOM accumulation observed in the diversified cropping sequences compared to continuous wheat. The crop residues of Brassicaceae and pulse crops tend to have lower C:N ratios, leading to accelerated mineralization rate and potentially reduced LFOM contents compared to cereals (Gan et al., 2011a; Rezgui et al., 2021). However, the presence of high amounts of phytochemicals in legumes (phenol and alkaloid) and

in Brassicaceae crops (phenol, alkaloid and GLS) diminishes mineralization rates (Kaale et al., 2023; Malka et al., 2023). Slower decomposition leads to a higher accumulation of LFOM observed in the rotations with Brassicaceae and pulse crops than continuous wheat. The importance of LFOM is widely recognized for its role in the formation and stability of soil structure, particularly in promoting the formation and stabilization of soil macro-aggregates (>250 μm) (Qu et al., 2019). Guest et al. (2022) reported soil macro-aggregation drives SOC sequestration. Thus, the presence of higher LFOM in crop sequences with Brassicaceae and pulse crops possibly contributes ultimately to their higher SOC contents than continuous wheat. The neutral soil pH (pH 7.0) at Swift Current might be more conducive to microbial activity and crop residue decomposition than Scott (pH 5.8-6.4) and Brooks (pH 8.0-8.2). This may allow changes in SOC among the treatments to be more noticeable at Swift Current than the other two test sites (Malik et al., 2018). However, diversified crop sequences had no prominent impact on total soil N content change during the study time compared to continuous wheat. Many studies have shown, the inclusion of pulse crops in rotations to be an effective strategy for improving total soil N through N-rich residual input and root depositions (Gylfadottir et al., 2007; Rasmussen et al., 2007; Gan et al., 2015). However, incorporation of N-rich pulse crop residues might not result in high soil N since a considerable fraction of N in pulse crops is stored in seeds removed from the field at harvest. Thus, the residual contribution for the soil N has a relatively minor impact (Peoples et al., 2009).

On average, growing condiment mustard crops before field pea increased SOC and total soil N compared to camelina at Swift Current. Among the condiment mustards, yellow mustard appears to be the most effective at increasing SOC, total soil N, and LFOM compared to oriental and industrial mustard. This positive impact of yellow mustard crop sequences on soil properties might be attributed to the presence of higher levels of GLSs and polyphenols in their residues than other mustard crops (Dubie et al., 2013; Harbaum et al., 2008). These compounds potentially lead to a higher accumulation of LFOM associated with improved soil macro-aggregation, which can further contribute to increased SOC and total soil N.

Regardless of the pulse species in sequence, growing Argentine canola in rotations had the highest LFOM and the lowest HFOM at 0-15 cm soil depth at each site in 2020 and 2021. This might be partially explained by higher residual input associated with Argentine canola than other crops. However, it is important to note that the above-ground biomass in 2019 did not follow the same pattern as the LFOM concentration, suggesting other factors may also be influencing LFOM

levels. Gan et al. (2009b) showed that root mass was highest for canola (1470 kg ha^{-1}) and wheat (1311 kg ha^{-1}), followed by mustard (893 kg ha^{-1}) and pulse crops (848 -524 kg ha^{-1}). Moreover, prior research indicates that a significant portion, ranging from 59 % to 80 % of total root biomass for pulse, cereal, and oilseed crops is concentrated within the upper 20-cm soil layer (Pietola and Alakukku, 2005; Williams et al., 2013). This underscores that root biomass plays a key role in residual input in topsoil layers.

4.6.2. Wheat productivity in the cropping system

One of the major goals of farming is to increase crop yield per unit of input. In semi-arid areas, soil moisture is the key to crop yield due to uneven distribution and limited precipitation in this region (Gan et al., 2009a; Wang et al., 2013). Compared to the 30-yr historical average, precipitation levels at all test sites were lower in both 2020 and 2021 (Fig. 4.6). This may have limited the potential impact of different crop sequences on subsequent wheat yield. In addition, the lack of significant differences in wheat grain yield across treatments may be due to the absence of apparent variation in soil moisture among the treatments (fall soil moisture- Figs. 4.3, 4.4 and 4.5; Table 4.9 and spring soil moisture- Figs. B.2, B.3 and B.4; Appendix Table B.4). Water stored in the soil profile is vital as it is used for carbohydrate synthesis and transport in the grain-filling stage, which maximizes yield (EI Habti et al., 2020). Thus, the comparable moisture contents among the treatments may hinder the effect of different crop rotations on subsequent wheat yield.

On average, at Brooks, diversifying crop sequences with Brassicaceae oilseed and pulse crops increased subsequent wheat yield by 52.7 % (Table 4.15) and 1000-seed weight by 19.0 % (Table 4.14) compared to the continuous wheat. This yield improvement aligns with the observed significant increase in SOC content in the diversified cropping systems from 2019 to 2021 (Fig. 4.1). Similarly, previous studies in most intensive agricultural regions showed that SOC contents were positively correlated with crop yield (Lal, 2006; Pan et al., 2023). Studies further reported that an increase of 1 ton of SOC increased wheat grain yield by 27- 40 kg ha^{-1} (Edmeades, 2003; Pan et al., 2023). Moreover, wheat grown in the diversified rotations showed elevated levels of LFOM, particularly LFOM-C and LFOM-N at 0-15 cm soil depth compared to continuous wheat (Table 4.11). The increase in LFOM-C and LFOM-N favor enhanced nutrient availability for the subsequent wheat crops.

At Swift Current, growing Argentine canola followed by field pea and or lentil in rotations resulted in a substantial increase in subsequent wheat yield (> 30 %) compared to the crop sequences with other Brassicaceae species (Table 4.14). This yield benefit could be linked to improved soil fertility, potentially driven by the higher accumulation of LFOM, specifically LFOM-C and LFOM-N in rotations with Argentine canola than other Brassicaceae crops (Table 4.12). In addition, the 1000-seed weight of the subsequent wheat crop was higher in the crop sequences involving Brassicaceae and pulse crops than continuous wheat at Swift Current without affecting the grain yield. This could be primarily attributed to lower number of seeds per wheat plant in the treatments, where Brassicaceae and pulse crops were grown than continuous wheat (Table 4.13). The fewer seeds per plant tend to receive more nutrients and resources, leading to large and heavy seeds. This finding highlights that crop sequences can influence not only total crop yield but also the quality of the harvested seeds, such as their weight and size (Theimer, 2003). In addition, higher levels of SOC, total soil N and LFOM in wheat preceded by Brassicaceae and pulse crops may also be attributed to larger seeds than continuous wheat. Improved SOC content combined with optimal N management can result in higher grain quality in a cropping system (Wang et al., 2023). Many studies have shown, including Brassicaceae and pulse crops in rotations to be an effective strategy for improving total SOC and N through residual input and root deposition (Kirkegaard et al., 2000; Gan et al., 2015; Peoples et al., 2017). Furthermore, wheat preceded by yellow mustard and field pea had a higher 1000-seed weight than wheat grown on other mustard-field pea crop stubble at Swift Current. This may be attributed to the higher total soil N content in yellow mustard-field pea rotation than the rotations with other mustard crops in both sampling years (Table 4.7). The elevated N might be due to the presence of a larger LFOM in yellow mustard followed by field pea rotation compared to the other Brassicaceae-field pea rotation (Haynes, 2005).

4.7. Conclusion

This study gives an insight into the potential role of incorporation of Brassicaceae oilseed and pulse crops into wheat-based cropping systems in enhancing the formation of labile soil organic matter fraction and SOC compared to the continuous wheat system. Argentine canola caused a gradual increase in LFOM at all three test sites. This indicates that Argentine canola could be an effective preceding crop with higher amount of dry matter than wheat and other Brassicaceae

oilseed crops. Moreover, growing condiment mustards, especially yellow mustard before field pea could be a promising strategy for improving soil organic matter, total soil N and potentially soil fertility, specifically at Swift Current. However, overall treatments provided similar total SOC and N benefits at all the soil depths that demonstrated total SOC, and N may not be responsive to preceding crops in the short-term under no-till cropping systems. Therefore, results of this study suggest that the combination of Brassicaceae oilseed and pulse crops in wheat-based cropping systems may require a longer time, more than one cycle) to occur significant changes in soil properties. Diversification of wheat-based cropping systems with Brassicaceae and pulse crops was beneficial in producing higher quality and quantity of grain yield than traditional wheat systems in Brown soil. However, the most pronounced yield benefits were observed at Swift Current. Among Brassicaceae oilseed crops, Argentine canola and yellow mustard were beneficial preceding crops to improve both the quality and quantity of subsequent wheat yield. Moreover, crop yield performance potentially can improve by providing/maintaining an appropriate amount of soil moisture. performance.

5. NITROGEN MINERALIZATION IN SOILS INCUBATED WITH ^{15}N-ISOTOPE LABELED BRASSICACEAE CROP RESIDUES

5.1. Preface

Root nodulation is an essential process for effective biological nitrogen fixation (BNF) in pulse crops. Chapter 3 showed that growing Brassicaceae species with varying levels of glucosinolates (GLS) influence root nodule weight of subsequent pulse crops grown in wheat-based cropping systems. Since root nodulation is dependent on N availability in the soil, this finding suggests a potential impact of Brassicaceae residues on available soil N. The differences in N availability are speculated to result from a combination of factors, including differences in mineralization and immobilization associated with Brassicaceae residues and soil nitrification compared to non-Brassicaceae crop residues. However, it is uncertain as to which mechanism might be most important for soil N dynamics and root nodulation. Thus, this experiment was designed to evaluate how various Brassicaceae oilseed crop residues influence soil N availability.

5.2. Abstract

Brassicaceae oilseed crop residues contain diverse chemical compositions. However, information on how these variations among Brassicaceae species influence N mineralization and N recovery in soil is lacking. This study investigated the impact of distinct chemical properties [glucosinolate content (GLS), carbon: nitrogen ratio (C:N) lignin content, and lignin:N] of crop residues on their N recovery potential, ultimately affecting soil N availability. A laboratory incubation was performed using ^{15}N-labeled crop residues of spring wheat (*Triticum aestivum* L.) and five oilseed crops; Argentine canola (*Brassica napus* L.), industrial mustard (*B. carinata* L.), oriental mustard (*B. juncea* L.), camelina (*Camelina sativa* L. Crantz) and yellow mustard (*Sinapis alba* L.). Yellow mustard crop residues contained the highest amount of GLS (6.49 μmol g^{-1} of tissue, 89.3 % higher than other Brassicaceae crop residues) and Argentine canola contained the least amount (0.02 μmol g^{-1} of tissue). Crop residues of the various plant species had a narrow variation in carbon (C) content, ranging from 442 to 495 g kg^{-1} of crop residue. Both Argentine canola (17.8:1) and oriental mustard (19.5:1) residues had the lowest lignin:N due to their high N content. During the 120-d incubation, 47.1-53.7 % of N in the residues was mineralized and recovered as ammonium and nitrate. However, Brassicaceae residues initially suppressed microbial

N mineralization and N recovery compared to wheat. The study identified a strong negative correlation between N recovery and GLS content from day 3 to day 28 (r= - 0.737 to - 0.846), which weakened by day 56. Similarly, the negative correlation between N recovery percentage and lignin:N was significant (r= - 0.631 to - 0.552) from day 3 to day 56, and weakened over time. These findings suggest that mainly high GLS content and lignin:N of plant tissues hinder N recovery percentages. GLS breakdown products may temporarily inhibit microbes involved in N cycling, explaining the observed short-term suppression.

5.3. Introduction

Growing Brassicaceae oilseed crops can increase mineral N accumulation in topsoil compared to non-Brassicaceae crops (Kirkegaard et al., 1999). This enhanced N availability may be due to a combination of factors associated with Brassicaceae residue decomposition, including accelerated N mineralization and reduced microbial N immobilization (Ryan et al., 2006). The quality and quantity of the plant material largely influence the N mineralization/immobilization kinetics of crop residues (Abbasi et al., 2015).

Among the residue quality parameters, C:N is critical in determining how fast organic N transforms to plant-available mineral N (Vahdat et al., 2011). During decomposition, soil microbes use C mainly for energy and N for growth and enzyme production. When the C:N of the residues is high (>25), microbes may immobilize available soil N to meet their requirements, leading to a temporary reduction in plant-available N (Flavel and Murphy, 2006). Crop residues with low C:N (<25) contain more N than the soil microbes require and therefore, increase soil mineral N availability through net N mineralization (Leifeld et al., 2020; Geisseler et al., 2021; Rummel et al., 2021). Crop residues of canola and mustard generally have C:N ratios ranging from 46:1 to 86:1, while wheat residues have higher C:N ratios between 83:1 and 139:1 (Stevenson and van Kessel, 1996; Gan et al., 2010). The high C:N ratios suggest that these crop residues may require additional soil N for microbial decomposition, potentially leading to a temporary decrease (immobilization) of plant-available N.

Lignin content and lignin:N ratio in crop residues also play a key role in N mineralization. High lignin content hinders N mineralization due to its complex chemical structure, making crop residues resistant to microbial decomposition (Austin and Ballare, 2010). A high lignin:N ratio indicates a relative scarcity of N to the microbes. To access the limited N, microbes may

immobilize available soil N and slow down the overall N release process. Thus, plant residues with high N concentrations, low lignin, low C:N and low lignin:N ratios often result in high N mineralization rates (Chaves et al., 2004; Manzoni et al., 2008; Gentile et al., 2009). However, information on the impact of the chemical composition of different Brassicaceae crop residues on N mineralization is limited.

One unique property of many plants in the Brassicaceae family is the presence of GLSs, which hydrolyze into biocidal chemicals, such as isothiocyanates (ITSs), ionic thiocyanate, organic cyanides, and oxazolidinethiones (Abdel-Massih et al., 2023). A limited number of studies have reported that ITCs and related compounds may affect soil bacterial and eukaryotic community structure (Smith and Kirkegaard, 2002; Rumberger and Marschner, 2003; Siebers et al., 2018). Alteration of soil microbial communities may directly impact soil N mineralization and N cycling. Moreover, GLS profiles widely vary depending on plant species (Morra, 2004). The GLS types and their concentration often determine the level of their toxicity or species of organism affected by biologically active GLS-degradation products (Brown and Morra, 1997; Vaughn, 1999). Canola was developed from rapeseed through breeding, and it contains lower total GLS contents (< 30 μmol g^{-1} in seed meal) than other Brassicaceae crops (Mejicanos et al., 2016). Despite existing research on GLS content in Brassicaceae seeds and seed meals, limited data is available on quantification of GLSs in crop residues (Cools and Terry, 2018; Lietzow, 2021). Furthermore, studies evaluating how GLS contents impact residue N mineralization are scarce. Comprehensive understanding of the impact of GLSs on N release from crop residues is necessary to optimize the use of Brassicaceae crops in rotations.

A laboratory incubation experiment was conducted using ^{15}N-labeled residues of five Brassicaceae oilseed species and wheat. This main objective was to characterize distinct chemical properties; residual GLS content, C:N ratio, lignin content, and lignin:N ratio and evaluate their impact on residual N recovery potential, ultimately affecting soil N availability. The changes in ammonium (NH_4^+) and nitrate (NO_3^-) content were monitored over 120 days. Nitrogen recovery percentage was used to evaluate how efficiently the residual N is converted to plant-available forms through N mineralization. It was hypothesized that distinct residue properties of Brassicaceae oilseed and wheat crops will differentially influence their N recovery percentage.

5.4. Materials and Methods

This experiment included three major components; 1) labeling the crop residues with ^{15}N, 2) characterizing the residues for GLSs C, N, C:N, lignin and lignin:N and, 3) applying the ^{15}N labeled residues to soil to evaluate mineralization/immobilization.

5.4.1. ^{15}N-labeled crop residue production

This experiment was conducted in a growth chamber in the phytotron facility at the University of Saskatchewan in 2021. Approximately 100 kg of soil was collected at a depth of 0-15 cm from an agricultural field with cereal stubble located near Saskatoon, SK (Orthic Dark Brown Chernozem). Soil was air-dried, sieved (2 mm) to remove any rocks, and mixed with silica sand in a 4:1 ratio (soil:sand) by weight to facilitate drainage. The resulting soil-sand mixture had a pH of 6.8 (1:2 soil:H_2O). Field capacity of the soil mixture was estimated by saturating a soil sample with water, letting it drain for 24-48 hours, then weighing the soil sample for gravimetric moisture sample. Field capacity was calculated using moisture retained relative to the dry soil weight.

Spring wheat (*Triticum aestivum* L.), Argentine canola (*Brassica napus* L.), industrial mustard (*B. carinata* L.), oriental mustard (*B. juncea* L.), camelina (*Camelina sativa* L. Crantz) and yellow mustard (*Sinapis alba* L.) were grown in pots considering each pot as a replicate. Eight replicates per crop species were maintained. Forty-eight 3 L plastic pots were filled with 3 kg of the air-dried soil-sand mixture and packed to a bulk density of approximately 1.3 g cm^{-3}. Pots were watered to 75 ± 5 % field capacity based on weight and left to stabilize for one week before planting.

After one week, pots were fertilized with mono-ammonium phosphate ($NH_4H_2PO_4$, 11:51:0) at a rate of 18.6 mg kg^{-1} soil (43 kg of product ha^{-1}) and urea (46:0:0) at a rate of 47.1 mg kg^{-1} soil (109 kg of product ha^{-1}). The required amount of fertilizer per pot was dissolved in water and applied to the soil, before bringing the soil to field capacity. Seeds were treated with fungicides at the recommended rates to control seed-borne pathogens and insect pests (Appendices A.1-A.3). In each treatment pot, 10 seeds were sown: wheat at a 3-cm depth and Brassicaceae at a 1-cm depth. The seeded pots were maintained under light intensity of approximately 600 μmol m^{-2} s^{-1}, and 16 h day (22 °C) and 8 h dark (15 °C) growing conditions in a growth chamber (Jones-Baumgardt et al., 2019). Ten days after emergence, seedlings were thinned down to two plants per pot.

Nitrogen-15 labeled ammonium nitrate ($^{15}NH_4$-$^{15}NO_3$) solution (10 atom % excess) was applied to the soil surface of each pot (0.017 m^2 area) at a rate equivalent to 5 kg N ha^{-1} (Hossain et al., 2017). The ^{15}N enriched solution was prepared by dissolving 0.16 g ^{15}N-NH_4NO_3 in 2,400 mL of distilled water. Fifty mL of the solution was applied directly to the soil around the rooting zone of each pot using a syringe. The plants were watered based on soil weight to maintain ~80% field capacity. Pots were arranged in a randomized complete block design (RCBD). All the soil pots were regularly irrigated to maintain ~80% field capacity by adding water at 2-3 d intervals.

Crop residue collection

Above-ground (leaves, stems, seeds and seed pods) and below-ground (roots) materials were collected at physiological maturity of each crop species. Individual 5-g sub-samples of seeds, pods, straw (leaves and stems combined) and roots of each crop species were collected for GLS analyses. These samples were immediately stored in separate polythene bags at -80 °C to prevent enzyme degradation until further processing. Frozen samples were lyophilized for 48 h and stored at -80 °C again until analysis. This detailed GLS analysis served as a supportive evaluation for Chapter 3, where limitations prevented GLS analysis of all plant parts. After sub-sampling was complete, the remaining leaves, stems and roots (referred to as residue) were mixed to achieve a homogenized mixture. From each homogenized mixture, a sub-sample (5 g) was ground to pass through a 2-mm sieve and stored at room temperature (ca. 20 °C) for the analysis of total lignin, C, N and atom % ^{15}N contents and residue GLS content. Wheat was not analyzed for GLS content.

Crop residue characterization

Glucosinolate analysis

Analysis of Brassicaceae samples was performed in collaboration with the Department of Food and Bioproduct Sciences at the University of Saskatchewan. Seeds, pods without seed, straw and roots and each homogenized residue mixture of each Brassicaceae species were analyzed for the concentration of primary GLS. It was assumed that primary GLS accounts for 90% of the total GLS abundance (He et al., 2002; Agneta et al., 2014). The analysis was performed according to Heaney and Fenwick (1980), which was modified by Daun and McGregor (1981). The procedure is described in detail in Chapter 3 (section 3.4.2).

Total carbon, nitrogen and atom% ^{15}N

Total C and N content in crop residues was determined by dry combustion and C:N ratio was calculated. Details are provided in Chapter 4 (section 4.3.1). The samples were analyzed for atom % ^{15}N on a Costech ECS4010 elemental analyzer (Costech Analytical Technologies., Valencia, CA, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany). Pea grain flour (0.3663 atom% ^{15}N) was used as an internal standard.

Lignin analysis

Lignin analysis was conducted in collaboration with the Department of Animal Science at the University of Saskatchewan. Plant tissues were sequentially digested for hemicellulose, cellulose, and lignin content with the ANKOM filter bag method using an ANKOM-200 Fiber Analyzer (ANKOM Technology, Macedon, NY). This method sequentially extracts neutral detergent fiber, acid detergent fiber and acid detergent lignin using a 0.5 g oven-dried sample (AOAC, 2005; ANKOM Technology, 2024). Then, the lignin:N ratio of each crop residue was calculated.

5.4.2. Soil incubation

Soil was collected to a 15-cm soil depth from a research field at Agriculture Agri-Food Canada, Swift Current, SK (Orthic Brown Chernozem). Soil was air-dried and sieved (2 mm). Soil pH was 6.7 (1:2 soil:H_2O) and the texture was loam (29 % sand, 45 % silt, and 26 % clay).

The laboratory incubation experiment was conducted using 50 mL-plastic snap vials and each vial was considered as an experimental unit. This experiment contained seven treatments: soil amended with residues from 1) yellow mustard, 2) industrial mustard, 3) Argentine canola, 4) oriental mustard, 5) camelina, 6) wheat, and 7) un-amended control. Forty vials per treatment were maintained for destructive sampling across ten sampling days, with each day involving four replicate vials (4 replicates × 10 sampling days = 40 vials per treatment).

Each vial received 30 g of pre-processed air-dry soil. Deionized water was added to adjust the soil moisture content to 60 ± 5 % of field capacity. Following a 5-day pre-incubation period in darkness, ^{15}N-labeled crop residues were incorporated into the corresponding vial at a rate of 2 % (w/w; weight of residue/weight of soil, Snyder et al., 2010). The mixtures were then thoroughly homogenized to ensure an even distribution of the residue within the soil matrix. The control

contained soil without any amendment. Each vial, containing a specific soil and residue mixture, was weighed to determine the precise amount of deionized water required to adjust the soil moisture content to 60 ± 5 % of its field capacity. After adding water, the vials were sealed with Parafilm. A small opening was made in the Parafilm of each vial using a 20 µL pipette tip to allow for adequate gas exchange while minimizing evaporation. The vials were then incubated in the dark at a constant temperature of 25 °C. Deionized water was added at regular intervals (approximately 5-6 d) to maintain the soil moisture content (60 ± 5 % of field capacity) throughout the experiment (Snyder et al., 2010).

5.4.3. Soil inorganic N and ^{15}N sampling and analysis

At each sampling date (3-, 7-, 14-, 21-, 28-, 42-, 56-, 70-, 90- and 120-d) following crop residue application, four vials per treatment were randomly selected. Soil available N [extractable ammonium (NH_4^+–N) and nitrate (NO_3^-–N)] was extracted from the soil with 2M KCl. Ten g of soil from each replicate was added to 100 mL of 2M KCl and 40 mL of the extract was analyzed for NH_4^+ and NO_3^- concentration using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA). The remaining 60 mL of the KCl extract was used to quantify the ^{15}N-labeled nitrate and ammonium using the diffusion disk method adapted from Stark and Hart (1996) as follows. All the extracts were transferred into clean 100 mL centrifuge tubes. One diffusion disc was added to each vial to collect $^{15}NH_4^+$ and $^{14}NH_4^+$ in the extracts. Diffusion discs were made from 7 mm dia. discs of filter paper. The discs were pre-rinsed with 2 M KCl solution followed by rinsing with deionized water. The discs were placed on strips of polytetrafluoroethylene (PTFE) tape to dry. Discs were acidified with 10 µL 2.5 M potassium bisulfate ($KHSO_4$). A second strip of PTFE tape was placed over top so that the discs were enveloped between the two pieces of tape. Using a punch, the tape around the disc was sealed, releasing acidified filter paper discs sealed between two pieces of Teflon tape. Then, 0.48 g of MgO was added to each extract containing a prepared diffusion disc to raise the pH up to ≥12 (Bedard-Haughn et al., 2004; Stewart et al., 2013) and the tubes were immediately capped. Diffusion samples were incubated at room temperature for 7 d on a rotary shaker at 120 rpm. Bottles were checked daily to ensure the diffusion disc was not sticking to the side of the tubes. On the eighth day, diffusion discs were removed from the extract and taken apart and the filter disc rinsed in 0.5 M HCl followed by deionized water. The discs were dried at room temperature (ca.

20 °C) for approximately 24 h. After removing diffusion discs for NH_4^+ analysis, tubes were left uncapped for 4 d, swirling each day, to eliminate any residual NH_4^+.

Nitrate (NO_3^-) content was determined using the same KCl extracted samples. The procedure involved adding new diffusion discs to the vials and adding approximately 0.4 g of Devarda's alloy to each vial. These vials were then incubated for 6 d. After the incubation, the extracted discs were rinsed in 0.5 M HCl followed by deionized water and dried at room temperatures for about 24 h. Standards for NH_4^+ and NO_3^- were prepared separately for each diffusion step at concentrations of 0.5, 1, 2, 3, 5, 7.5 and 10 ppm. Dried diffusion disks were encapsulated in tin and analyzed using an ECS4010 elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) coupled to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, Germany) to determine atom % ^{15}N NH_4^+ and NO_3^- contents.

Total soil inorganic N was calculated by combining NH_4^+–N and NO_3^-–N concentrations.

The amounts of total soil inorganic N derived from the crop residues (Ncrop residue) (µg inorganic N g^{-1} soil) were calculated using the following equation:

$$Ncrop\ residue = \frac{c-b}{a-b} \times TNinorg \quad [5.1]$$

where a = atom% ^{15}N in the crop residue, b = natural abundance of atom% ^{15}N in soil and unlabeled-crop residue (assumed as 0.36637), c = atom % ^{15}N of total soil inorganic N in soils amended with labeled-crop residue, and TN_{inorg} = total soil inorganic N (µg inorganic N g^{-1} soil). The percentage of crop residue N recovered as total soil inorganic N (N recovery percentage) was calculated using the following equation:

$$N\ recovery\ percentage = \frac{Ncrop\ residue}{AN} \times 100 \quad [5.2]$$

where AN = the amount of total crop residual N applied (µg total crop residual N applied g^{-1} soil). The percentage of crop residual N recovered in total soil inorganic N (N recovery percentage) was used as an estimate of the percentage of total crop residual N mineralized and present in total soil inorganic N on a given day.

5.5. Statistical Analysis

Data analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2017). All data were tested for normality using the Shapiro-Wilk test ($P \geq 0.05$) and homogeneity of variance using Levene's test ($P \geq 0.05$) before analysis. To determine differences in selected biochemical parameters of different crop residues, ANOVA was conducted considering crop species as a fixed

factor and block as a random factor. Mean comparisons were performed using Tukey's Honestly Significant Different (HSD) test.

In the incubation study, soils sampled from different treatments at different sampling times were analyzed using factorial arrangement in a RCBD. In this analysis, the treatment and sampling time were considered as fixed factors, whereas block was considered as a random factor. Overall treatment means were declared significant at $P \leq 0.05$. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test. Pearson's correlation analyses were performed to determine the significance of the association between N recovery percentage and selected biochemical parameters. For the correlation analysis between N recovery percentage and GLS contents, the GLS content in wheat was considered negligible.

Furthermore, several pre-planned group comparisons were performed to answer the following questions (Appendix C.2).

1. Does the N recovery percentage of wheat crop residues differ from Brassicaceae crop residues?

Wheat vs. the average of Brassicaceae crop residues = Treatments 6 vs. Treatments (1 +2+ 3 + 4 + 5) / 5

2. Does the N recovery percentage in Argentine canola (the crop species with low level of GLSs) crop residues differ from other Brassicaceae crop residues (crop species with high levels of GLSs)?

Argentine canola vs. the average of other Brassicaceae crop residues = Treatment 3 vs. Treatment (1 + 2 + 4 + 5) / 4

3. Does the N recovery percentage of non-mustard oilseed crop (i.e. camelina) residue differ from mustard species (yellow, industrial and oriental mustards) residues?

Camelina (non-mustard) vs. the average of mustard crop residues = Treatment 5 vs. Treatment (1 + 2 + 4) / 3

4. Does the N recovery percentage of non-brassica genus (yellow mustard; *Sinapis alba*) residue differ from Brassicaceae genus mustard crop (industrial and oriental mustards) residues?

Yellow mustard vs. the average of industrial and oriental mustard crop residues = Treatment 1 vs. Treatment (2 + 4) / 2

(**Note**: Treatment 1 = yellow mustard crop residues amended soil; Treatment 2 = industrial mustard crop residues amended soil; Treatment 3 = Argentine canola crop residues amended soil; Treatment 4 = oriental mustard crop residues amended soil; Treatment 5 = camelina crop residues amended soil; 6 = wheat crop residues amended soil).

5.6. Results

Glucosinolate content

The primary (major) GLS type varied among the different Brassicaceae species (Fig. 5.1). The homogenized plant residue mixture (roots+leaves+stems) in yellow mustard mainly contained sinalbin (4-hydroxybenzyl GLS) and both industrial and oriental mustard plant tissues contained sinigrin (2-propenyl GLS). The primary GLS type in camelina was glucocamelinin (Potassium 10-methylsulfinyldecyl GLS) and Argentine canola was neoglucobrassicin (1-methoxy-3-indolylmethyl GLS). Among crop species, yellow mustard had the highest GLS content (6.49 µmol g^{-1} of tissue), exceeding the average of other Brassicaceae residues by 89.3%. Argentine canola contained the lowest amount of GLSs (0.02 µmol g^{-1} of tissue) among Brassicaceae crops. Moreover, it was assumed that wheat residues did not contain GLSs.

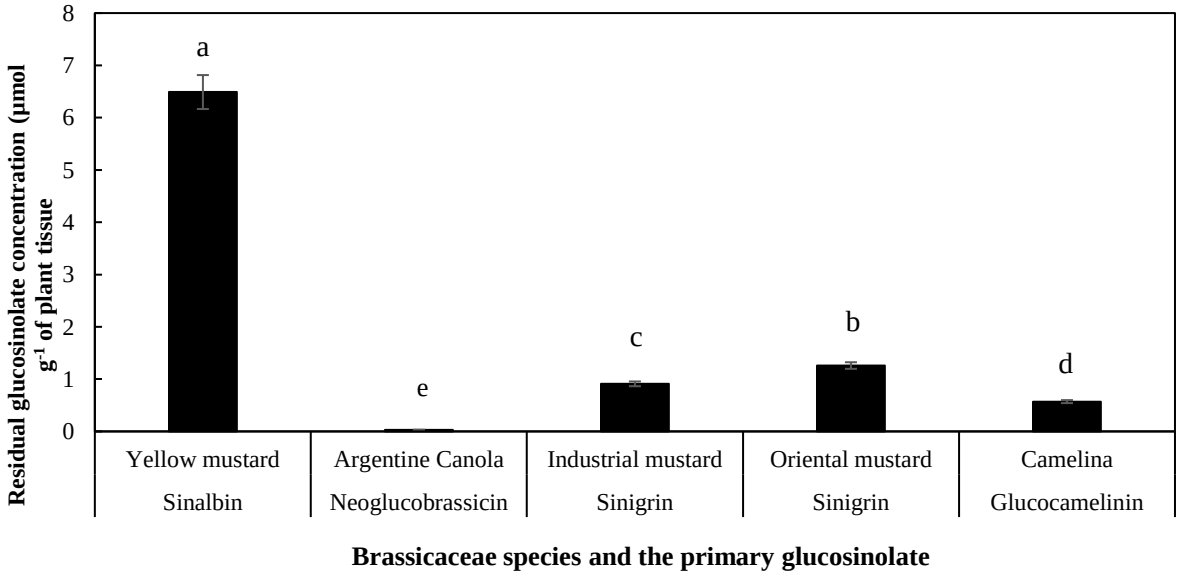

Fig. 5.1. The primary glucosinolate and its concentration in Brassicaceae crop residue mixtures (roots+leaves+stems) used in the study.
Note: Letters indicate significant differences among different mixtures at $P \leq 0.05$. The bars indicate the standard error of the means (n=3)

Crop residue carbon, nitrogen and lignin content

Crop residues of the various plant species had a narrow variation in C content, ranging from 442 to 495 g kg^{-1} of crop residue (*P*=0.0270, Table 5.1). Industrial mustard residue, with the lowest N content (3.86 g kg^{-1}), had the highest C:N ratio (126:1) while Argentine canola had the lowest ratio (58:1) attributable to its high N content (7.70 g kg^{-1}). Lignin content also varied among the crop residues (*P*<0.0001, Table 5.1). Industrial mustard residue had the highest lignin content (163 g kg^{-1} of crop residue), and consequently, the highest lignin:N ratio. Conversely, wheat residue had the lowest lignin content (114 g kg^{-1} of crop residue), whereas Argentine canola (17.8:1) and oriental mustard (19.5:1) residues had the lowest lignin:N ratios attributable mainly to their high N contents.

Table 5.1. Chemical composition of the plant residues used in the experiment

Plant residue	Carbon (g kg^{-1} residue)	Nitrogen (g kg^{-1} residue)	Lignin (g kg^{-1} residue)	C:N ratio	Lignin:N ratio
Yellow mustard	495[a†]	5.07[c]	146[c]	98[b]	28.8[c]
Industrial mustard	485[ab]	3.86[e]	163[a]	126[a]	42.2[a]
Argentine canola	442[b]	7.70[a]	137[d]	58[d]	17.8[e]
Oriental mustard	479[ab]	6.36[b]	124[e]	75[c]	19.5[e]
Camelina	474[ab]	4.40[d]	152[b]	108[b]	34.5[b]
Wheat	443[b]	4.62[cd]	114[f]	96[b]	24.7[d]
***P* value**	**0.0270**[‡]	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

[†]Values with different letters within each column are significantly different at $P \leq 0.05$ (n=3). [‡]Bolded *P* values indicate significant levels.

Soil nitrogen mineralization and nitrification

There was a period of N immobilization of 7 d after all the residues were applied to the soil (Fig. 5.2, The significant differences among treatments are reported in Appendix Table C.1. This immobilization period was evident for NH_4^+-N (Fig. 5.2A), NO_3^- -N (Fig. 5.2B) and total inorganic N (NH_4^+-N + NO_3^- -N, Fig. 5.2C) in all the treatments, except the unamended soil immediately after the crop residue application. Following the immobilization period, a progressive increase in NH_4^+-N + NO_3^- -N and total N contents was observed in all the treatments from day 14 (Figs. 5.2A and 5.2C). Soil amended with wheat and Argentine canola crop residues showed the highest cumulative NH_4^+-N and total N contents throughout the incubation. In contrast, yellow mustard residue initially showed the lowest cumulative NH_4^+-N and total N levels (from day 14 to day 21). Nevertheless, cumulative NH_4^+-N and total N levels in all treatments containing camelina and all

three mustard species (yellow, industrial and oriental) residues were comparable from day 28 to the end of incubation.

Soil amended with wheat and Argentine canola residues had the highest cumulative NO_3^--N contents in the early stages (from day 14 to day 56). Similar to NH_4^+-N, yellow mustard residue initially showed the lowest NO_3^--N contents (from day 14 to day 28). However, by day 77 cumulative NO_3^--N contents in soil with all Brassicaceae and wheat residues were comparable, and it persisted until the end of incubation (Fig. 5.2B).

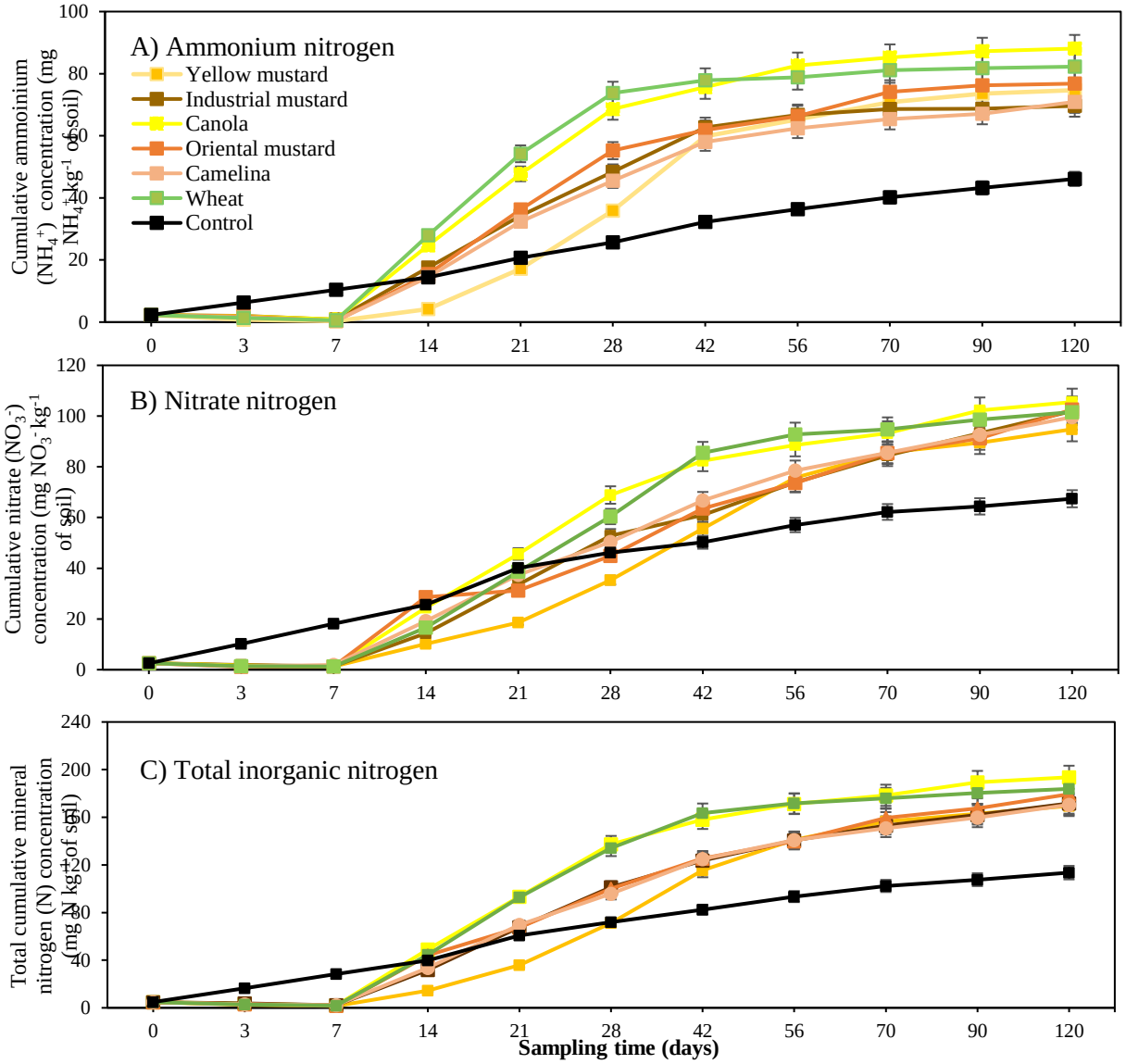

Fig. 5.2. Change in cumulative mineral nitrogen concentrations during 120-d incubation.
Note: The y-axis in different graphs is in different scales. Bars indicate standard error of means (n=4). The significant differences among different treatments are in Appendix Table C.1.

Percentage of N recovered from different crop residues

The percentage of N recovered from residues in the soil inorganic N pool varied significantly among treatments from day 3 to day 28 in the incubation (Fig. 5.3). Yellow mustard crop residues consistently had the lowest N recovery from day 14 to day 28. However, all the other crop residues had comparable N recovery percentages during that time. Starting on day 42, all crop residues had similar percentages of N recovered.

Pre-planned tests revealed that significant differences in N recovery occurred primarily during the early stages of incubation. Wheat residues had a higher N recovery percentage than the average of Brassicaceae crop residues at day 3 and from day 21 to 28. Among the Brassicaceae crops, Argentine canola residues had higher N recovery percentages than the average of the other Brassicaceae residues from day 14 to day 42. In contrast, yellow mustard residues showed lower N recovery percentages compared to industrial and oriental mustard residues from day 7 to day 28.

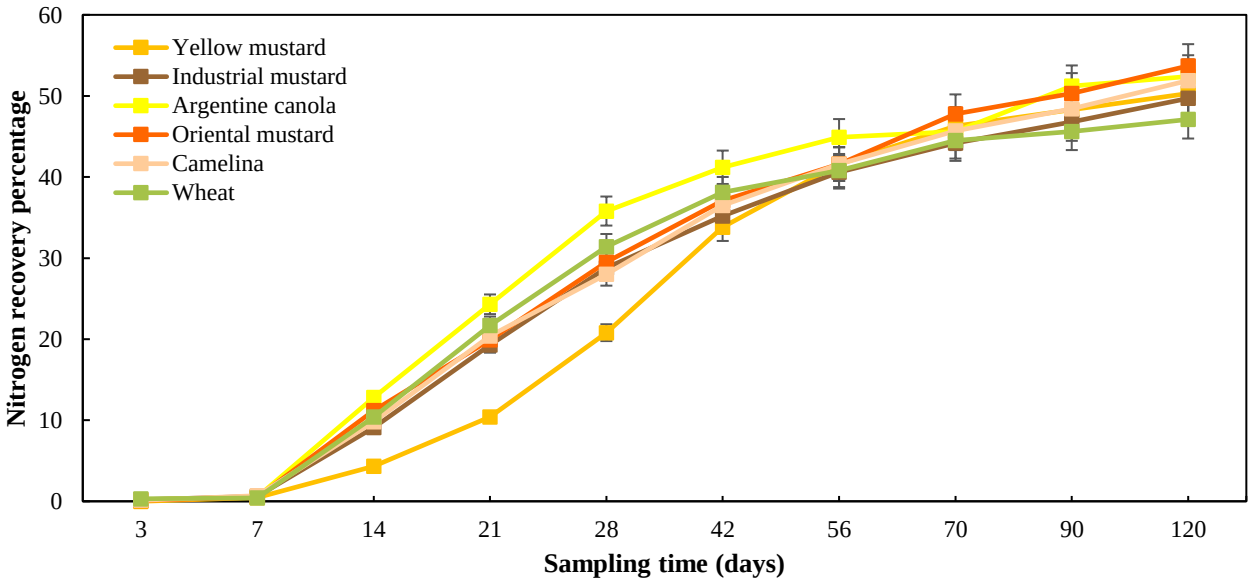

Fig. 5.3. Nitrogen recovery percentages (%) from different crop residues over the 120-d of incubation
Note: Bars indicate standard error of means (n=4). The significant differences among different treatments are in Appendix Table C.2.

Table 5.2. Summary of Contrast and *P* values from pre-planned comparisons in nitrogen (N) recovery percentages (%) from different crop residues over the 120-d of incubation

Comparison[†]	N recovery percentage in different sampling days									
	03	**07**	**14**	**21**	**28**	**42**	**56**	**70**	**90**	**120**
Wheat vs. the average of Brassicaceae crop residues	+0.159[‡]	-0.121	+0.95	+2.84	+2.82	+1.32	-1.26	-1.42	-3.40	-4.50
	0.002[§]	**0.002**	0.376	**0.009**	**0.002**	0.465	0.531	0.579	0.220	0.062
Argentine canola vs. the average of other Brassicaceae crop residues	+0.047	-0.034	+4.19	+6.80	+9.02	+5.55	+3.55	-0.40	+2.75	+1.00
	0.122	0.315	**0.003**	**<0.0001**	**0.001**	**0.014**	0.104	0.860	0.308	0.652
Camelina vs. the average of mustard crop residues	-0.024	+0.151	+1.59	+3.86	+1.63	+1.33	+0.33	-0.40	-0.66	+0.66
	0.411	**0.001**	0.180	**0.002**	0.323	0.578	0.878	0.887	0.983	0.816
Yellow mustard vs. the average of industrial and oriental mustard crop residues	-0.02	-0.095	-5.84	-9.20	-8.35	-2.35	+0.50	+0.30	-0.25	-1.40
	0.536	**0.023**	**0.001**	**<0.0001**	**0.001**	0.265	0.811	0.904	0.928	0.579

[†]Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡] Contrast value = value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value. [§]Bolded *P* values indicate significant differences ($P \leq 0.05$).

Correlation between nitrogen recovery percentage and selected biochemical parameters

Higher GLS content and lignin:N ratio in plant tissue were associated with lower N recovery percentages. The negative correlation between N recovery and GLSs was strong from day 3 to day 28 (r = -0.737 to -0.846, $P < 0.05$), then weakened by day 56 (Table 5.3). A similar trend was shown by the association of N recovery percentage with lignin:N ratio. The negative correlation between N recovery percentage and lignin:N ratio was significant from day 3 to day 56 (r = -0.631 to -0.552), and then weakened over time. In contrast, the correlation of C:N ratio and lignin content in plant residues with N recovery percentage was not significant during the incubation time (Table 5.3).

Table 5.3. Pearson correlation coefficients between the nitrogen recovery percentage and selected biochemical parameters of different crop residues over the 120 days

Parameter	Correlation coefficients with recovery percentage on different sampling days									
	03	**07**	**14**	**21**	**28**	**42**	**56**	**70**	**90**	**120**
Glucosinolate content	**-0.737**[†]	**-0.655**	**-0.788**	**-0.902**	**-0.846**	**-0.523**	**-0.300**	-0.126	+0.010	**-0.417**
Carbon:nitrogen	0.080	0.335	-0.445	-0.368	**-0.481**	**-0.517**	-0.381	-0.209	-0.381	0.270
Lignin content	-0.379	**-0.632**	-0.335	-0.287	-0.322	-0.315	-0.035	-0.099	-0.017	0.090
Lignin:nitrogen	**-0.631**	**-0.732**	**-0.377**	**-0.674**	**-0.584**	**-0.421**	**-0.552**	-0.577	-0.264	-0.292

[†]Bolded *P* values denote significance of the correlation ($P \leq 0.05$).

5.7. Discussion

Total soil mineral N contents (NH_4^+-N and NO_3^--N) decreased immediately following crop residue application in all soils compared to the unamended control (Fig. 5.2). This initial drop reflects a temporary immobilization of soil N by microbes decomposing the residues. Crop residues have a complex biochemical composition with different C:N ratios (Grzyb et al., 2020). It has been reported that crop residues with C:N ratios higher than 25:1 are immobilized faster than they are mineralized (Mubarak et al., 2010; Grzyb et al., 2020). The high C:N ratio of the crop residues ranging from 75:1 to 126:1 used in this study probably caused this initial net immobilization (Table 5.1). In addition, immobilization persisted for about a week in this study (Fig. 5.2). Similarly, in previous studies, incorporating crop residues often leads to a temporary net N immobilization during the initial decomposition stages, followed by net N mineralization within days or weeks depending on the specific chemical composition of the crop residues and environmental conditions (Reddy et al., 2008). Immobilization of inorganic N can be detrimental if plant N requirement exceeds available soil inorganic N (Coyne, 1999). However, immobilized microbial biomass N is less susceptible to volatilization or leaching losses and thus, serves as a protected source of N that can become available to plants during microbial biomass turnover (Brookes, 2001).

After the immobilization period, all the treatments showed higher NH_4^+ and NO_3^- contents than the unamended control, indicating relatively rapid residue mineralization percentages than the control (Mirzaei et al., 2022). Nitrogen recovery percentages in wheat residues were higher than the average of all Brassicaceae crop residues only early in the incubation on days 03, 21 and 28 (Table 5.2). Argentine canola, the crop species with the lowest GLS content, C:N and lignin:N consistently showed higher N recovery percentages than the other Brassicaceae residues at the early stages of incubation (from day 14 to day 42). This suggests a potential negative impact of GLS hydrolyzation products on N mineralization. Furthermore, a strong negative correlation between N recovery percentage and GLS content ($r = -0.737$ to -0.846) from day 03 to day 28 of this study further suggested that GLSs may inhibit microbial activity and N recovery from the residues (Fig. 5.3). The differences in N recovery percentages among different crop residues observed in this study may align with the presence of different types of released GLS hydrolysis products (Fig. 5.1). as well as residue quality as depicted in C;N and lignin:N ratio (Table 5.1). Previous studies revealed that the level of toxicity depends on the type of GLS hydrolyzed products. Benzyl ITC

derived from sinalbin (4-hydroxybenzyl GLS) in yellow mustard was reported to be more inhibitory to soil microbes than allyl ITCs derived from sinigrin (2-propenyl GLS) in industrial and oriental mustards, and glucocamelinin (potassium 10-methylsulfinyldecyl GLS) in camelina (Nowicki et al., 2016; Wang et al., 2020). This explains the observed stronger initial suppression of N recovery in yellow mustard residues (with high benzyl ITC potential) compared to other Brassicaceae residues (Fig. 5.1). However, the inhibition of N release due to the presence of GLS appeared to be short-lived. By day 42, similar NH_4^+-N, NO_3^--N contents and N recovery percentages were observed in all the treatments with amended soil (Table 5.3). The short half-life of GLS hydrolysis products could be a major factor contributing to the short-term effect on microbes (Ntalli and Caboni, 2017).

The N recovery percentages of crop residues were negatively correlated with the initial lignin:N ratio of crop residues. Several mechanisms have been used to explain how lignin and N interact to influence residue mineralization. Talbot et al. (2012) suggested that lignin may form cross-linkages to the more labile N thereby chemically protecting N from hydrolysis during litter decay. Alternatively, lignin may physically protect the more labile cell wall components (e.g. structural polysaccharides and N) from microbial attack during litter decomposition when lignin is deposited in cell walls within the hemicellulose-protein matrices (Boerjan et al., 2003). However, the relative influence of lignin:N on N recovery percentage decreased over time. The absence of correlation between N recovery percentage and lignin: N was consistent with Oglesby and Fownes (1992). This could be related to a decrease in the relatively labile fraction of crop residue N concentration over time.

The negative correlation between N recovery percentage and C:N was significant only on days 28 and 42. Previous studies revealed C:N ratio was adequate for predicting the N release from crop residue. However, presence of lignin or inhibitory compounds can mask the influence of C:N ratio. Several studies highlighted the role of lignin and lignin:N that affects N release from plant residues compared to C:N ratio (Vahdat et al., 2011; Abbasi, 2015). However, N mineralization from organic residues is variable and controlled by many site-specific factors, including soil temperature and moisture, drying and rewetting events, and physical and biochemical properties of the soil (Cabrera et al., 2005) beside the residue composition. This experiment serves as a general estimate of N recovery from crop residues under controlled environment, thus, results may not be indicative of what would occur under field conditions. Furthermore, potential N losses from the

soil inorganic N pool through volatilization, denitrification or immobilization were not measured. It can be presumed that these loss mechanisms were present to some degree during this experiment, thus the mineralization estimates may be underestimates of the percent of N recovery.

Similar to the mineralization suppression, nitrification inhibition was observed in the presence of Brassicaceae crop residues. Yellow mustard showed a pronounced suppression of nitrification from day 14 to day 42 in the incubation. Nitrifiers are one of the most sensitive groups of micro-organisms and are commonly inhibited by naturally occurring or applied chemicals, including pesticides. Previous studies showed the toxicity of GLS hydrolysis products on soil nitrification microbes (Subbarao et al., 2013; Fang et al., 2019). Consequently, reducing nitrification process in agricultural systems increases N retention time in the root zone as NH_4^+ is much less mobile than NO_3^-, providing additional time for the plants to absorb N. This in turn reduces the amount of N lost through leaching and denitrification (Hodge et al., 2000; Subbarao et al., 2012).

5.8. Conclusion

Nitrogen recovery of crop residues is mainly dependent upon their biochemical composition. All crop residues, including wheat and Brassicaceae species, caused a temporary decrease in soil mineral N content. This reflects N immobilization by microbes decomposing the residues, particularly those with high C:N ratios. However, GLS content and lignin:N ratio of residues appeared to be significant predictors in predicting the cumulative N recovery percentage of the crop residues used in this study. The findings are consistent with presence of GLS in crop residues that can initially suppress N mineralization and reduce N recovery percentage, and this effect diminishes over time. It is uncertain whether this suppression would be sustained under field conditions, where GLS hydrolyzed products could more readily dissipate. Over 45 % of crop residue N was mineralized within 120-d incubation period, suggesting that wheat and Brassicaceae crop residues potentially can be used as a source of rapidly mineralized N. However, the mineralization can be influenced by field conditions, including temperature and moisture, which may deviate from the optimal conditions maintained during incubation. Furthermore, Brassicaceae crop residues can inhibit the soil nitrification process. Nitrification inhibition may be desirable to retain inorganic N in the less mobile NH_4^+ species, but long-term effects on the nitrifier community may be undesirable and should be considered.

6. THE IMPACT OF BRASSICACEAE CROP RESIDUES ON SOIL NITRIFICATION AND EFFECTIVE NODULATION IN SUBSEQUENT FIELD PEA

6.1. Preface

During the microbial degradation of Brassicaceae residues in soil, a range of low molecular weight, volatile sulfur-rich compounds, predominantly isothiocyanates (ITCs), are released upon glucosinolate (GLS) hydrolysis. ITCs have bio-fumigation properties, which consequently affect soil nitrification. In Chapter 5, the total amount of GLS varied, not only among different Brassicaceae species, but also among different plant parts of the same species. Consequently, various Brassicaceae species and plant parts release different quantities and types of GLS hydrolysis products. An experiment was conducted to examine the influence of these GLS hydrolysis products on abundance and activity of ammonia-oxidizing microbes, as well as nodulation of field pea grown in soils amended with different Brassicaceae residues. For this comparative analysis, Brassicaceae crops were selected based on their GLS content in above-ground (AGRes) and below-ground residue (BGRes) in Chapter 5, representing two extremes in GLS levels. Yellow mustard, with the highest total GLS content, and Argentine canola, with the lowest, were chosen as the representatives of Brassicaceae species for this experiment.

6.2. Abstract

Brassicaceae crops contain glucosinolates (GLSs) that have been shown to inhibit soil nitrification. Despite the widespread use of Brassicaceae crops in rotations with pulse crops in wheat-based cropping systems, comprehending of the specific effects of Brassicaceae residues on soil nitrification and root nodulation of subsequent pulse crops is limited. This controlled environment experiment examined the effect of crop residues of selected Brassicaceae species on the *amo*A gene (encodes the alpha subunit of ammonia monooxygenase in nitrifying microbes) and on effective root nodulation in field pea (*Pisum sativum* L.). The field pea crop was grown after spring wheat (*Triticum aestivum* L.) and Brassicaceae crops with contrasting contents of GLS: low-GLS containing Brassicaceae crop species, Argentine canola (*Brassica napus* L.) and high-GLS containing Brassicaceae crop species, yellow mustard (*Sinapis alba* L.). Nine treatments were established with field pea grown on: (treatments 1-3) BGRes of Argentine canola, yellow mustard, or spring wheat; (treatments 4-6) a combination of BGRes and AGRes from each crop residue;

(treatments 7-8) two chemical amendments,4-hydroxy benzyl isothiocyanate (ITC) and urea and (9) an unamended control. Soil nitrification was quantified by ammonia-oxidizing bacterial and archaeal *amo*A gene copy concentration and total soil-available N concentration (NO_2^- + NO_3^--N and NH_4^+-N) at pre-seeding, flowering and harvest of subsequent field pea. Nodulation was quantified at flowering and maturity. On average, Brassicaceae crops suppressed soil nitrification with a 16.2 % lower NO_2^- + NO_3^--N level, 80.2 % lower total *amo*A gene copy concentration, and at flowering, the subsequent field pea had 14.6 % fewer effective number of root nodules than field pea grown on wheat residues. At the flowering stage, yellow mustard suppressed nitrification and root nodulation of subsequent field pea to a greater degree than Argentine canola. Field pea grown on BGRes+AGRes had 49.0-57.2 % more effective root nodules than when grown on BGRes alone. However, the suppressive effects of Brassicaceae residues diminished as field pea matured. The treatments with different residue combinations had no effect on the nitrification process. Over time, the Brassicaceae residues decomposed and gradually increased soil available N regardless of the GLS presence in plant tissues.

6.3. Introduction

Microbial-mediated nitrification is a vital step in the N cycle as it controls the conversion of ammonium (NH_4^+) to nitrate (NO_3^-), which begins with the oxidation of ammonia to hydroxylamine, followed by oxidization of hydroxylamine to nitrite (NO_2^-) and NO_3^-. Ammonia oxidation is generally regarded as the nitrification rate-limiting step, mediated by ammonia-oxidizing bacteria (AOB) and archaea (AOA). Among the AOB, complete ammonia oxidizers (comammox) can perform the entire two-step nitrification process, making them important contributors to nitrification on a global scale (Lehtovirta-Morley, 2018; Xia et al., 2018). Most AOB belong to the β-proteobacteria (Mohanty, et al., 2018), while AOA are members of the phylum Thaumarchaeota (Gribaldo et al., 2010).

Ammonia oxidation is catalyzed by ammonia monooxygenase (AMO), a membrane-bound enzyme that is shared by all ammonia-oxidizing microorganisms. The gene *amo*A is responsible for encoding the alpha-subunit of the enzyme AMO and is the second most frequently sequenced marker gene in microbial ecology, after the 16S ribosomal RNA (rRNA) gene (Alves et al., 2018). Thus, *amo*A gene is extensively used as a molecular marker to study AOB and AOA in nitrification

under different environmental conditions, including in agricultural ecosystems (Mukhtar et al., 2019; Huang et al., 2021).

Brassicaceae crops are known for containing a major secondary metabolite group called glucosinolates (GLSs). Their hydrolysis products include isothiocyanates (ITCs), thiocyanates and nitriles. These compounds can inhibit nitrification in soil incubated with different synthetic products (Bending and Lincoln, 2000) and soil incubated with brassica seed meals (Reardon et al., 2013). Isothiocyanates in particular, are toxic to a range of soil organisms (Andini et al., 2020; Gao et al., 2021). Most studies evaluating ITCs have used pure forms instead of plant residues within their natural environment. Adding pure forms, instead of decomposable plant tissues does not resemble real-world strategies (Baldrian et al., 2011; Hollister et al., 2012). Moreover, the long-term effects of ITCs are uncertain due to their short half-life in the soil (Siebers et al., 2018). Hence, a comprehensive understanding of how GLS-containing plant residues impact soil processes is necessary to maximize rotational benefits of Brassicaceae plants with other plants.

The availability of soil N plays a vital role in crop production systems. When the nitrification process slows down, it causes denitrification to decelerate due to less substrate leading to an increase in available N in the system via elevated NH_4^+ content (Gupta et al., 2023). NH_4^+ adsorbs onto negatively charged soil particles making it less susceptible to leaching than NO_3^-. However, elevated soil NH_4^+ content can negatively affect pulse crop root nodulation (Xia et al., 2017). Moreover, the presence of GLS hydrolysis products in the soil, resulting from the decomposition of brassica residues can also hinder rhizobial activity and/or their interaction with legume roots, leading to reduced nodulation (Deakin and Broughton, 2009).

Thus, the objective of this study was to determine the effect of crop residues of selected Brassicaceae species on soil nitrifying microbial abundance, their activity, and effective root nodulation in field pea. Soil nitrifying microbial abundance was measured using *amo*A gene copy number in AOB and AOA and soil available N (NH_4^+-N and NO_2^- + NO_3^--N) was used to evaluate their activity. Two Brassicaceae species, yellow mustard (YM), a high level of total GLS containing Brassicaceae crop species and Argentine canola (AC), allow level of total GLS containing Brassicaceae crop species were compared to determine whether the hydrolysis products of GLSs from the crop residues reduce the abundance of soil nitrifying microbes in subsequent field pea. The hypotheses were:

1. Rhizosphere soil of field pea grown on Argentine canola residues (with low levels of total GLSs) will have higher abundance of ammonia-oxidizing microbes than yellow mustard (with high levels of total GLSs), yet lower than those present in the rhizosphere soil when they grow on wheat residues (with zero GLS).
2. Reduced abundance of ammonia-oxidizing microbes in soils of field pea grown on yellow mustard and canola residues will lead to decreased nitrification, resulting in lower concentrations of NO_2^- + NO_3^--N than when grown on wheat residue.
3. Decreased nitrification in field pea grown on yellow mustard and Argentine canola residues will result in higher NH_4^+ concentration than when grown on wheat residue.
4. Higher amounts of NH_4^+-N and ITCs associated with yellow mustard and Argentine canola residues compared to wheat residue will have an adverse impact on the nodulation of field pea.

6.4. Materials and Methods

6.4.1. Experimental design and general description

This experiment was conducted in a greenhouse at the University of Saskatchewan commencing on February 15, 2022. The Orthic Brown Chernozemic soil was collected at a 15-cm soil depth from a research field at Agriculture Agri-Food Canada, Swift Current, SK. Collected soil was air-dried, sieved (2 mm) to remove any rocks, and mixed with silica sand in a 4:1 ratio (soil: sand) by weight. The resulting soil-sand mixture had a pH of 6.6 (1:2, soil:H_2O) and was loam in texture (29 % sand, 45 % silt and 26 % clay). The experiment had two distinct crop phases, phase I with Brassicaceae/wheat and phase II with field pea (Table 6.1).

Phase I

This phase involved growing yellow condiment mustard (*Sinapis alba* L.) cultivar Andante, Argentine canola (*Brassica napus* L.) cultivar L233P and spring wheat (*Triticum aestivum* L.) cultivar AAC Brandon. Yellow mustard was selected due to the presence of the highest content of residual GLSs among the Brassicaceae oilseed species used in this research study (Chapter 5). Argentine canola was used as the low-GLSs containing crop and wheat was used as the zero-GLS containing crop.

The crops were grown in 3-L pots (13 cm bottom diam. × 16.5 cm top diam. × 17.8 cm height) and each pot was considered as a replicate. Pots were filled with 3 kg of the soil sand mixture and packed to a bulk density of approximately 1.3 g cm^{-3}. Twelve replicates per treatment were established to enable destructive sampling of 4 pots at three growth stages of field pea. Soil moisture levels were maintained at 75 ± 5 % field capacity based on weight and left to stabilize for one week before planting.

Table 6.1. Treatments and major crop species assigned in the controlled environment study.

Treatment number and description	Treatment abbreviation	Phase I crop	Phase II	
			Crop	Amendment
1. Yellow mustard (YM) followed by field pea (FP) grown on YM below-ground residue (BGRes)	FP on YM BGRes	YM	FP	YM BGRes
2. Argentine canola (AC) followed by FP grown on AC BGRes	FP on AC BGRes	AC	FP	AC BGRes
3. Wheat (W) followed by FP grown on W BGRes	FP on W BGRes	W	FP	W BGRes
4. YM followed by FP grown on YM BGRes and above-ground residue (AGRes)	FP on YM BGRes+AGRes	YM	FP	YM BGRes+AGRes
5. AC followed by FP on AC BGRes and AGRes	FP on AC BGRes+AGRes	AC	FP	AC BGRes+AGRes
6. W followed by FP grown on W BGRes and AGRes crop residue	FP on W BGRes+AGRes	W	FP	W BGRes+AGRes
7. Fallowed (F) soil followed by FP on 4-hydroxy benzyl isothiocyanate (ITC)	FP on ITC	F	FP	ITC
8. F soil followed by FP on urea	FP on urea	F	FP	Urea
9. F soil followed by FP on un-amended soil	FP on Un-A	F	FP	None

After one week, pots were fertilized with mono-ammonium phosphate ($NH_4H_2PO_4$, 11:51:0) at a rate of 18.6 mg kg^{-1} soil (43 kg product ha^{-1}) and urea (46:0:0) at a rate of 47.1 mg kg^{-1} soil (109 kg product ha^{-1}). The required amount of fertilizer per pot was dissolved in water and applied to the soil, before bringing the soil to field capacity. Seeds for phase I were treated with applicable fungicides at the recommended rates to control seed-borne pathogens and insect pests (Appendices A.1-A.3). For each treatment pot (Treatment 1-6), 10 seeds were sown: wheat at 3-cm depth and canola and mustard at 1-cm depth (Table 6.1). Ten days after emergence, seedlings were thinned down to two plants per pot. Treatments 7, 8 and 9 were unplanted. Treatments were arranged in a

randomized complete block design (RCBD). All the soil pots, including the unplanted pots were irrigated at 1-2d intervals to maintain the moisture level (75 ± 5 %) with a day/night temperature of 22 °C / 18 °C and day/night length of 17 h / 7 h.

Soil sampling before incorporation of crop residues

At harvest, above-ground plant parts from all pots were cut at the soil level, leaving roots in the soil. Seeds were removed from each plant sample. From each treatment, four of the 12 replicate pots were randomly selected for soil analysis. Soil core samples were taken randomly from experiment unit (pot) at a 15-cm deep (3-cm internal diam.). After sampling, these pots were discarded. Each core was divided into two sub-samples: each weighing 50 g for DNA analysis to determine *amo*A nitrifying microbe abundance and to quantify available N content. All the samples collected for soil available N were stored at 4 ºC, whereas those used for microbial DNA analysis were stored at -80 ºC until further analysis (Gimsing and Kirkegaard, 2006).

Crop residue incorporation

After removing the seeds, above-ground residues (AGRes) were separately ground to pass through a 2-mm sieve. Below-ground residues (BGRes) were left intact in soil in which they were grown. Treatments 4, 5 and 6 AGRes were returned to the specific pot they were harvested from. Soils in treatments 1, 2 and 3 contained only BGRes of the preceding crop (Table 6.1). Above-ground residues from these pots were discarded. Treatments 4, 5 and 6 contained AGRes + BGRes. The incorporated plant residues were thoroughly mixed with surface soil (0-5 cm).

Soils of treatment 7 were amended with a stock solution of 4-hydroxybenzyl ITC, HBITC (MuseChem, New Jersey, USA. ≥ 95 % purity). This was prepared by adding 10 mg of HBITC to 5 mL of distilled H_2O, and sonicating for 30 min (Williams et al., 1993). Stock solution was added to the soil at a concentration of 20 mL of stock solution g^{-1} of fresh soil samples before adding crop residues. Estimation of potential amounts of ITC that could be generated from brassica green manure was based on the equivalents of HBITC derived from HBITC forming GLS (Williams et al., 1993; Kirkegaard and Sarwar, 1998). The average recovered N content was calculated for yellow mustard using the incubation study data in Chapter 5 and soils in treatment 8 were amended with 50 μg N g^{-1} of soil.

Phase II

Each treatment pot was seeded with 8 field pea seeds (*Pisum sativum* L. cultivar CDC Meadow), inoculated with *Rhizobium leguminosarum* (Nodulator® sterilized peat-based, Becker Underwood, Saskatoon, SK) at the recommended rate. Seeds were planted at 2.5 cm depth. After seedling emergence, they were thinned to two plants per pot. Treatments were arranged in a RCBD with eight replicates for each treatment, which facilitated using four replicates for each sampling time (flowering and maturity). Pots were watered as needed to maintain approximately 75 ± 5 % field capacity.

Soil and root sampling after incorporation of residues

Soils were sampled for DNA analysis and N content at flowering and after harvesting field pea as described for Phase I. Field pea root nodule sampling was carried out at flowering and after harvesting of the crops. Four pots from each treatment were selected randomly and the two plants in each pot were excavated carefully, retaining as much root mass as possible. Plants were stored at 4 °C until nodulation assessments were completed. In the laboratory, roots were cut off from each plant sample. Then root samples with intact nodules were washed gently using tap water on a 100-mesh (150 µm pore size). Roots were washed and nodules were removed with forceps. The effective root nodule number of the plants was determined by visually assessing the percentage of nodules per plant, based on the presence of red pigmentation from leghemoglobin content (Gwata et al., 2003).

6.4.2. Soil analysis

Quantification of *amo*A gene copy concentration to estimate the abundance of ammonia-oxidizing microbes

Soil from both phases of the study was used to quantify *amo*A gene copy number concentration. Soil microbial DNA was extracted according to the manufacturer's protocol using a DNeasy Power Soil Pro Kit (QIAGEN, Hilden, Germany). To assess DNA concentration and purity, the DNA extracts were run on 1 % agarose gels at 110 V for 30 min and quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., United States). The extracted DNA solutions were stored at -80 °C.

Prior to real-time PCR analysis, DNA concentration was optimized to mitigate inhibitor effects. qPCR master mix components and primers were also optimized to achieve a reaction efficiency between 90-110 % and correlation coefficients (r) of 0.99 for the standard curve. Once an acceptable reaction efficiency was achieved, samples were analyzed. Each plate contained duplicate samples for the standard curve as well as an internal control sample to ensure reaction reproducibility. The standard curve was generated from a 10-fold serial dilution (10^2–10^9 copies per µL) of gene Block (gBlock; the sequence verified double-stranded DNA fragments) containing the target functional gene fragment.

The AOB and AOA *amo*A gene copy concentration was measured using the qPCR method on C1000 Touch Thermal Cycler system (Applied Biosystems, Foster City, CA, United States). The absolute qPCR correlates the PCR signal to input copy numbers using a calibration curve, and neither comparisons nor references are needed (Pfaffl, 2004). qPCR was performed for AOA and AOB separately using published primers (Appendix D.1). A 96-well plate (Thermo Fisher Scientific Inc. Canada) was used, with each well containing 10 µL of iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, United States), 1.5 µL of each primer for the selected microbial group (10 mmol L^{-1}; Invitrogen Life Technologies, Canada), 0.4 µL of $MgCl_2$, 3.6 µL of UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen Life Technologies, Canada) and 3 µL of DNA template. The following two-step amplification protocol was used for quantification: one cycle at 95 °C for 30 s, and 40 cycles of 5 s at 95 °C, 34 s at 55 °C and 1 min at 72 °C. The number of gene copies was directly calculated from gBlock gene fragments (Integrated DNA Technologies Inc, Canada) concentration and presented as functional gene concentration. Standards were prepared by mixing 2.5 µL each of 30 different gBlock solutions (2×10^9 copies μL^{-1}).

Soil available nitrogen

Following the protocol of Bundy and Meisinger (1994), 5 g of fresh soil, which had been stored at 4 °C, was weighed into a 50-mL disposable centrifuge vial. The soil was mixed with 50 mL of 2M potassium chloride (KCl). The suspension was centrifuged at 230 rpm and the supernatant was filtered through KCl pre-washed filter papers (Whatman No. 42). The extracts were stored at -20 °C until analyzed. Before analysis, the frozen samples were thawed at room temperature for 24 h and were analyzed for NH_4^+-N and NO_3^--N contents colorimetrically using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA).

6.4.2. Statistical Analysis

Data analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA, 2017). Prior to analysis, data were tested for normality using the Shapiro-Wilk test ($P \geq 0.05$) and homogeneity of variance using Levene's test ($P \geq 0.05$). Soil sampled from different treatments at different growth stages was analyzed using a factorial arrangement in a RCBD. In this analysis, the treatment and growth stages were considered as fixed factors, whereas block was considered as a random factor. Overall treatment means were declared significant at $P \leq 0.05$. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test. Pearson's correlation analyses were performed to determine the significance of the association between *amo*A gene copy concentration in archaea and bacteria and available soil NO_2^- + NO_3^--N concentrations in each sampling time and between available N and the number of effective root nodules. Furthermore, several pre-planned group comparisons were carried out to answer the following questions.

1. Are soil nitrification and effective root nodule numbers different in field pea cultivated after a crop compared to those cultivated after a fallow period?

Field pea cultivated after a crop vs. fallow period= Treatments (1 + 2 + 3 + 4 + 5 + 6) / 6 vs. Treatments (7 + 8 + 9) / 3

2. Are soil nitrification and effective root nodule numbers different in field pea grown without amendments compared to when grown with chemical amendments (ITC and urea)?

Field pea cultivated without amendments vs. chemical amendments= Treatment 9 vs. Treatments (7 + 8) / 2

3. Are soil nitrification and effective root nodule numbers different in field pea grown on BGRes compared to when grown on BGRes + AGRes?

Field pea grown on BGRes vs. on BGRes + AGRes = Treatments (1 + 2 + 3) / 3 vs. Treatments (4+5+6) / 3

4. Are soil nitrification and effective root nodule numbers different in field pea grown on BGRes + AGRes of wheat compared to grown on BGRres + AGRes of Brassicaceae?

Field pea grown on BGRes + AGRes of wheat vs. on BGRres + AGRes of Brassicaceae= Treatment 3 vs. Treatments (1 + 2) / 2

7. Are soil nitrification and effective root nodule numbers different in field pea grown on BGRes of wheat compared to BGRes of Brassicaceae?

Field pea grown on BGRes of wheat vs. BGRes of Brassicaceae = Treatment 6 vs. Treatments (4 + 5) / 2

6.5. Results

6.5.1. Soil available nitrogen

Treatment (preceding crop type or chemical amendment) and soil sampling time (field pea growth stage) had a significant impact on the total available N, NO_2^- + NO_3^--N and NH_4^+-N contents (Fig. 6.1, Table 6.2). The NO_2^- + NO_3^--N represented the majority (93-96 %) of total available soil N, with only a very small amount of NH_4^+-N contributing to total available N. Consequently, the fluctuation in total soil NO_2^- + NO_3^--N across soils treated with different amendments closely mirrored the fluctuation observed in the total N content.

The treatment × soil sampling time interaction for soil NO_2^- + NO_3^--N content was significant (P<0.0001, Table 6.2). This was mainly attributed to the gradual decrease in NO_2^- + NO_3^--N content in wheat-grown soil from seeding to harvest. However, no such decrease, but a marginal change in NO_2^-+NO_3^--N contents was measured at flowering and harvest of field pea on canola- and yellow mustard-grown soil regardless of the presence or absence of AGRes of the preceding crop. In contrast, over a 2.5-fold increase in NO_2^- + NO_3^--N contents was observed in both urea- and HBITC-amended soil from pre-seeding to harvest of field pea. However, NO_2^- + NO_3^--N contents in field pea on Un-A soil were unchanged (22.2-23.3 mg kg^{-1} of soil, Table 6.2). Treatment differences in NO_2^-+NO_3^--N were not evident at harvest.

Before seeding of field pea, un-amended soil had the lowest NO_2^- + NO_3^--N contents (Fig. 6.1, Appendix Table D.2). Wheat-grown soil had the highest NO_2^- + NO_3^--N contents (79.9-81.6 mg kg^{-1} of soil) followed by canola-grown soil (64.1 mg kg^{-1} of soil) and yellow mustard-grown soil as the lowest (45.6 mg kg^{-1} of soil). In general, field pea grown on wheat BGRes + AGRes was consistently among the treatments with the highest NO_2^- + NO_3^--N at all sampling times and field pea on un-amended soil (Un-A) had the lowest.

The amounts of NH_4^+-N in soils with different amendments were similar at all three growth stages of field pea (Fig. 6.1). Moreover, soil NH_4^+-N in each treatment was consistent over the growth of field pea.

Pre-planned comparison showed that on average, wheat-, Argentine canola- and yellow mustard-grown soils had higher NH_4^+-N and NO_2^- + NO_3^--N content than the average of the no-crop soil during phase 1 (field pea on HBITC, urea and Un-A) and this was consistent for all the sampling times (Table 6.2). However, the differences between the comparisons were gradually diminished over field pea growth. In addition, soil treatments with BGRes+AGRes showed higher NH_4^+-N and NO_2^- + NO_3^--N contents than the treatments with only BGRes at field pea harvest. Specifically, wheat-grown soil had higher NH_4^+-N and NO_2^- + NO_3^--N content compared to the average of Argentine canola- and yellow mustard-grown soil at the flowering stage irrespective of the presence of AGRes from the preceding crop (Table 6.2).

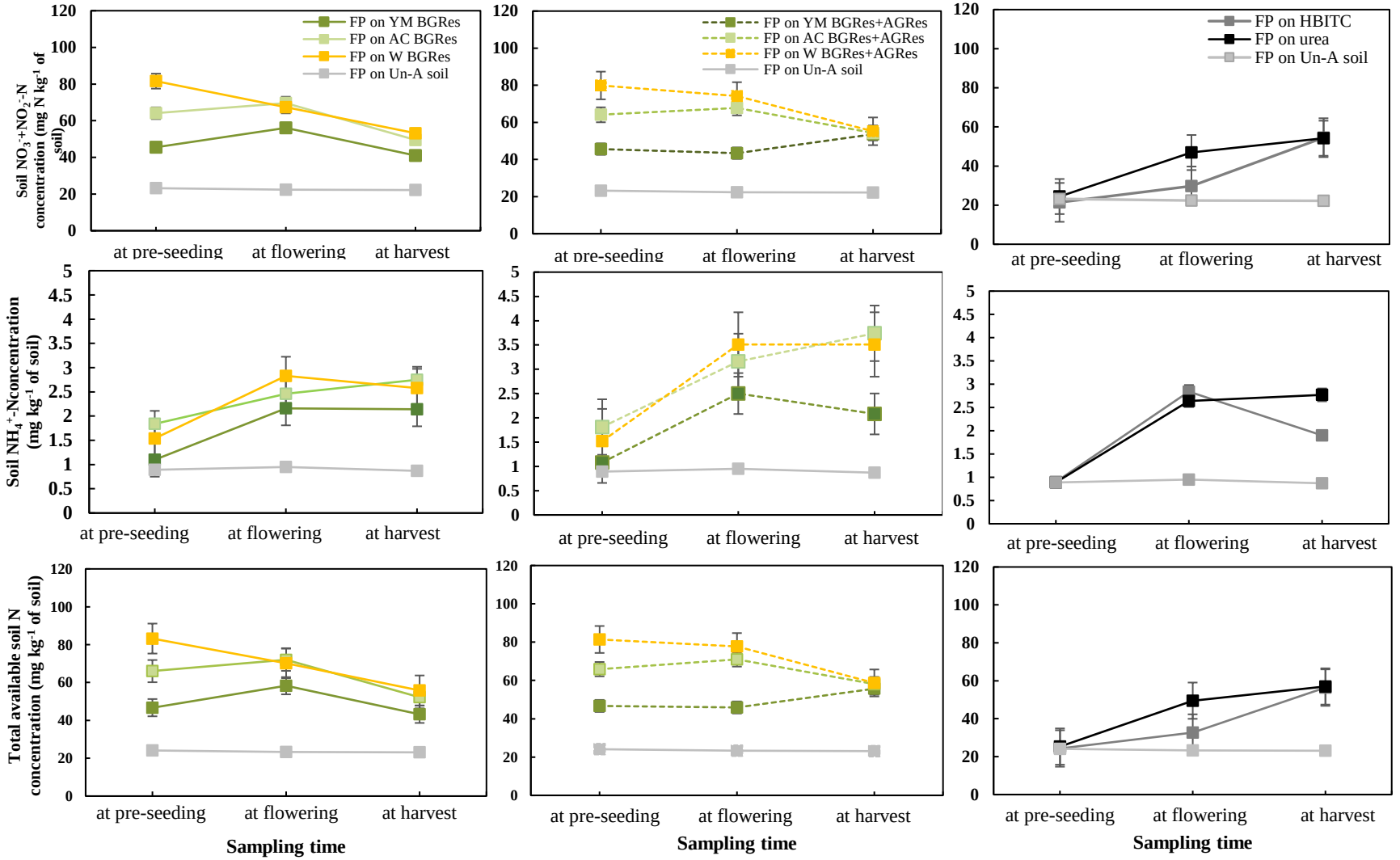

Fig. 6.1. The concentrations of soil available nitrogen in different treatments at different growth stages (sampling time) of field pea. Key-FP=Field Pea, BGRes=Below-Ground Residue, YM=Yellow Mustard, W=Wheat, Un-A=Un-Amended soil, AGRes=Above-Ground Residue, HBITC=4-hydroxybenzyl isothiocyanate. Note: FP on Un-A soil was included into each graph as a reference.
Note: Bars indicate standard error of means. The significant differences among different crop rotations based on the Tukey HSD test are in Appendix Table D.2.

Table 6.2. Summary of contrast and *P* values from pre-planned comparisons in the concentration of soil available nitrogen (mg N kg^{-1} of soil) in different treatments at different growth stages (sampling time) of field pea.

Treatment (Trt.)[1]	Soil total available nitrogen (N) concentration								
	Nitrite and nitrate nitrogen (NO_2^-+NO_3^--N)			Ammonium nitrogen (NH_4^+-N)			Total available N		
	Pre-seeding	Flowering	Harvesting	Pre-seeding	Flowering	Harvesting	Pre-seeding	Flowering	Harvesting
Field pea grown after a crop vs. fallow period	+40.5‡ **<0.0001**§	+30.0 **<0.0001**	+7.50 **0.0001**	+0.588 **<0.0001**	+0.626 **<0.0001**	+0.953 **0.0006**	+40.4 **<0.0001**	+30.6 **<0.0001**	+8.45 **<0.0001**
Field pea on unamended soil vs. chemical amendments	+0.300 0.8775	-16.0 **<0.0001**	-32.1 **0.0001**	-0.005 0.9789	-1.79 **<0.0001**	-1.46 **0.0019**	-0.700 0.8764	-17.8 **<0.0001**	-33.6 **<0.0001**
Field pea grown on BGRes vs. on BGRes + AGRes	-0.566 0.6811ns	-2.56 **0.0171**	+6.46 **0.0008**	-0.023 0.4300	+0.573 **<0.0001**	+0.620 **0.0365**	-0.633 0.6478	-1.96 0.0564	+7.10 **0.0002**
Field pea grown on BGRes + AGRes of wheat vs. Brassicaceae	+26.75 **0.0001**	+4.55 **0.0066**	+7.95 **0.0042**	+0.07 0.5332	+0.520 **0.0037**	+0.135 0.7509	-26.80 **<0.0001**	+5.05 **0.0026**	+8.10 **0.0023**
Field pea grown on BGRes of wheat vs. Brassicaceae	+25.0 **0.0001**	+18.60 **<0.0001**	+1.25 0.6399	-0.075 0.8100	+0.680 **0.0003**	+0.600 0.1673	+25.1 **<0.0001**	+19.2 **<0.0001**	+1.80 0.4582

***P* values for main and interaction effects of treatment (T) × Sampling time (S)**

	T	S	T × S
NO_2^- +NO_3^--N	**<0.0001**	**<0.0001**	**<0.0001**
NH_4^+-N	**<0.0001**	**<0.0001**	0.1373
Total available N	**<0.0001**	**<0.0001**	**<0.0001**

[1] BGR=Below-Ground Residue, AGR=Above-Ground Residue

‡ Contrast value = value on the left side - value on the right side in the comparison.

§ Bolded *P* values indicate significant differences ($P \leq 0.05$).

6.5.1. Ammonia oxidizing *amo*A gene copy concentration

Soil ammonia-oxidizing microbial concentration was estimated using bacterial and archaeal *amo*A gene concentration. The combination of bacterial and archaeal *amo*A gene copy concentration was considered as the total *amo*A gene copy concentration in soil (Fig. 6.2). In general, bacterial gene copy concentration was higher than archaea at the pre-seeding and harvest of field pea in contrast to the flowering stage (Appendix D.3).

The treatment × sampling time interaction was significant for the total *amo*A gene copy concentration (P<0.0001, Table 6.3). This was mainly attributed to the presence of a gradual decrease in *amo*A gene copy concentration in wheat-grown soil from pre-seeding to harvest regardless of the presence of wheat AGRes. However, soil total *amo*A gene copy concentration increased in canola-grown soil, yellow mustard-grown soil, ITC-treated soil, urea-treated soil and Un-A soil (Table 6.3, Appendix D.3).

The no-crop soil during phase 1 (field pea on HBITC, urea and Un-A) had the lowest total *amo*A gene copy concentration (9.4 lg gene copy number g^{-1} soil, Fig. 6.2) at pre-seeding of field pea. Wheat-grown soil had the highest total *amo*A gene copy concentration (12.4 lg gene copy number g^{-1} soil) followed by canola-grown soil (10.3 lg gene copy number g^{-1} soil) and yellow mustard-grown soil (10.0-10.1 lg gene copy number g^{-1} soil), while having comparable gene copy concentration at field pea pre-seeding (Fig.6.2). Field pea grown on wheat BGRes + AGRes consistently had the highest total *amo*A gene copy concentration. The same trend was shown in the *amo*A gene copy concentration in each bacterial and archaeal component. Moreover, a notable increase in *amo*A gene copy concentration was observed in field pea grown on canola BGRes + AGRes compared to those grown on yellow mustard BGRes + AGRes at the flowering stage, highlighting the impact of the combined application of BGRes and AGRes from preceding canola over yellow mustard on amoA gene copy concentration. In contrast, by the harvest stage of field pea, the differences in *amo*A gene copy concentration were not prominent across the presence of AGRes from different preceding crops.

On average, wheat-, Argentine canola- and yellow mustard-grown soils had higher *amo*A gene copy concentration than the average of the soil kept fallow during phase 1 and this was consistently observed at all the sampling times (Table 6.3). In addition, soil amended with combined BGRes + AGRes showed higher total *amo*A gene copy concentration at field pea flowering and harvest than the soil amended only with BGRes.

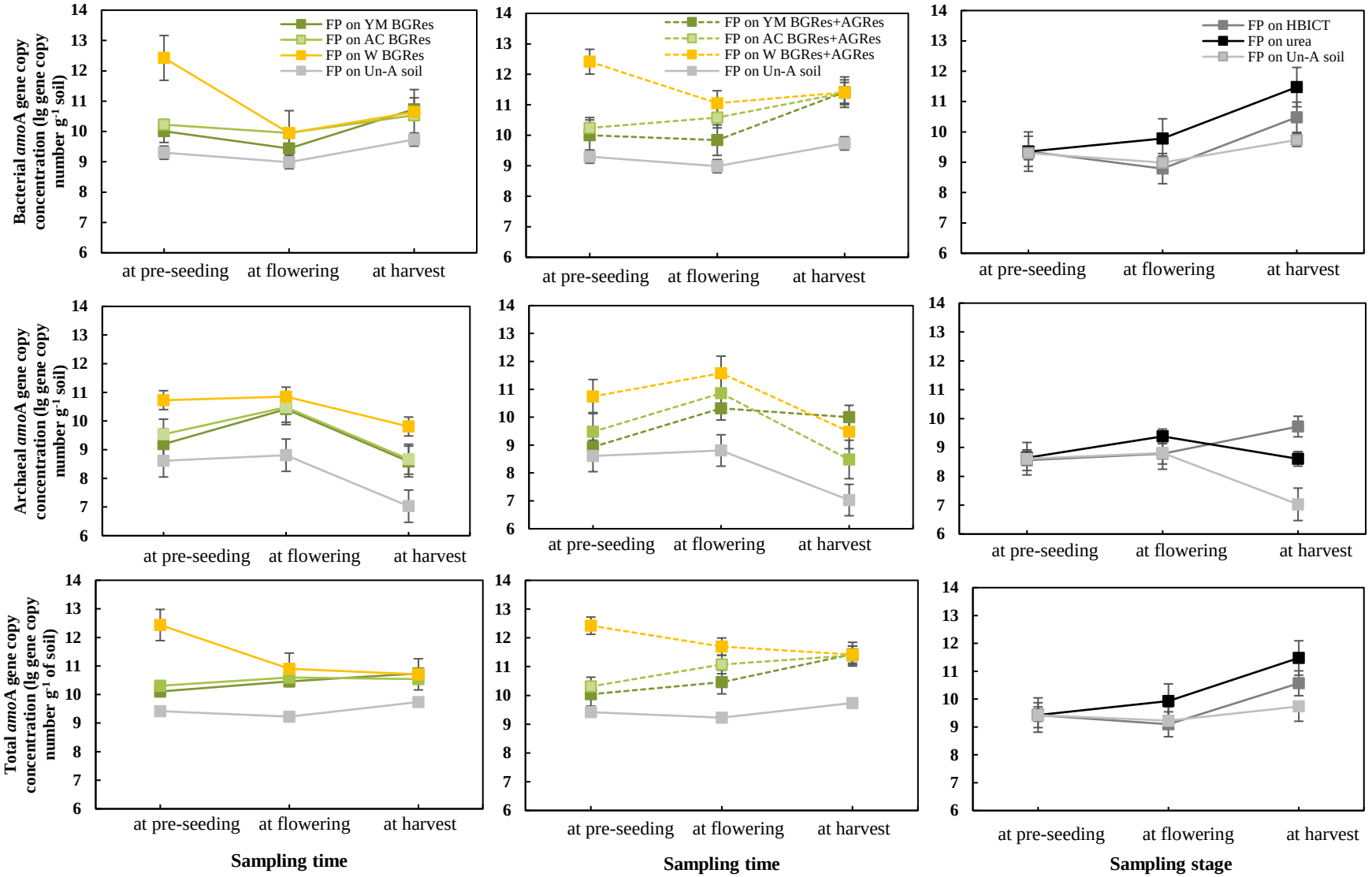

Fig. 6.2. The concentration of *amo*A gene copies in different treatments at different growth stages (sampling time) of field pea. Key-FP=Field Pea, BGR=Below-Ground Residue, YM=Yellow Mustard, W=Wheat, Un-A=Un-Amended soil, AGR=Above-Ground Residue, HBITC=4-hydroxybenzyl isothiocyanate. Note: FP on Un-A soil was included in each graph as a reference.

Note: Bars indicate standard error of means. The significant differences among different crop rotations are in the Appendix Table D.3.

Table 6.3. Summary of *P* values and contrast values from pre-planned comparisons in bacterial, archaeal and total (bacteria+archaea) *amo*A gene concentration (gene copy number g^{-1} soil) in different treatments at different growth stages of field pea

Treatment [I]	Ammonia-oxidizing *amo*A gene copy concentration								
	Bacterial *amo*A			Archaeal *amo*A			Total *amo*A		
	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest
Field pea grown after a crop vs. fallow period	$+8.8\times10^{11}$ ‡ **<0.0001** §	$+2.7\times10^{10}$ **<0.0001**	$+3.8\times10^{10}$ **<0.0001**	$+1.9\times10^{10}$ **0.0001**	$+9.9\times10^{10}$ **0.0001**	$+1.6\times10^{9}$ **0.0001**	$+9.0\times10^{11}$ **<0.0001**	$+1.2\times10^{11}$ **0.0001**	$+3.9\times10^{10}$ **<0.0001**
Field pea on unamended soil vs. chemical amendments	-2.6×10^{8} 0.1710	-2.4×10^{9} **0.0136**	-1.6×10^{11} **<0.0001**	$+7.4\times10^{6}$ 0.927	-8.7×10^{8} **0.0338**	-2.8×10^{9} **<0.0001**	-2.7×10^{7} **0.9123**	-3.2×10^{11} **0.0049**	-1.6×10^{11} **<0.0001**
Field pea grown on BGRes vs. on BGRes + AGRes	-2.3×10^{10} 0.971	$+4.6\times10^{10}$ **<0.0001**	$+2.1\times10^{11}$ **<0.0001**	$+9.5\times10^{7}$ 0.1652	$+1.2\times10^{11}$ **0.0003**	$+2.1\times10^{9}$ **0.0338**	-2.3×10^{10} 0.3629	1.5×10^{10} **<0.0001**	$+2.1\times10^{11}$ **<0.0001**
Field pea grown on BGRes + AGRes of wheat vs. Brassicaceae	$+2.6\times10^{12}$ **<0.0001**	$+3.01\times10^{9}$ **0.0348**	-2.6×10^{8} 0.9347	$+5.1\times10^{10}$ **<0.0001**	$+4.3\times10^{10}$ **0.0028**	$+6.0\times10^{12}$ **<0.0001**	$+2.7\times10^{12}$ **<0.0001**	$+4.6\times10^{10}$ **0.0004**	$+6.1\times10^{9}$ 0.5385
Field pea grown on BGRes of wheat vs. Brassicaceae	$+2.6\times10^{12}$ **<0.0001**	$+9.1\times10^{10}$ **<0.0001**	$+4.2\times10^{9}$ 0.9463	$+5.3\times10^{10}$ **<0.0001**	$+3.3\times10^{11}$ **0.0001**	-2.2×10^{9} 0.257	$+2.6\times10^{12}$ **<0.0001**	$+4.2\times10^{10}$ **<0.0001**	$+2.0\times10^{9}$ 0.9463

***P* values for main and interaction effects of treatment (T) × Sampling time (S)**

	T	S	T × S
Bacterial *amo*A	**<0.0001**	**<0.0001**	**0.0001**
Archaeal *amo*A	**<0.0001**	**0.0001**	**0.0001**
Total *amo*A	**<0.0001**	**0.0001**	**0.0001**

[I] BGR=Below-Ground Residue, AGR=Above-Ground Residue.

‡ Contrast value = value on the left side - value on the right side in the comparison.

§ Bolded *P* values indicate significant differences ($P \leq 0.05$).

6.5.3. Effective root nodule number

Field pea on wheat BGRes + AGRes had the highest number of effective root nodules both at flowering (60 nodules plant^{-1}) and harvest (30 nodules plant^{-1}) (Fig. 6.3). At flowering, the field pea on urea amendment had the lowest effective root nodule number (17 nodules plant^{-1}). However, multiple treatments including field pea on BGRes of preceding wheat, Argentine canola and yellow mustard and field pea on urea had the lowest effective nodule number at harvest. Field pea grown with HBITC application and un-amended soil showed comparable effective root nodule numbers in both sampling times. On average, soil with wheat and Brassicaceae crops grown during phase 1 had higher numbers of effective root nodules than the no-crop soil and this trend was consistent at both sampling times (Table 6.4). On average, field pea grown on preceding crop BGRes + AGRes had higher number of effective root nodules than field pea on preceding BGRes alone.

At maturity, the average number of effective nodules decreased by nearly 51% (16 nodules plant^{-1}) compared to flowering (35 nodules plant^{-1}) (Fig. 6.2). However, the reduction in the effective root nodule number in field pea grown on HBITC amendment was above the average (60.4 %) and field pea grown on urea amendment was below the average (41.1 %). Thus, this was mainly attributed to the treatment × the sampling stage interaction being significant (P<0.0001, Table 6.4).

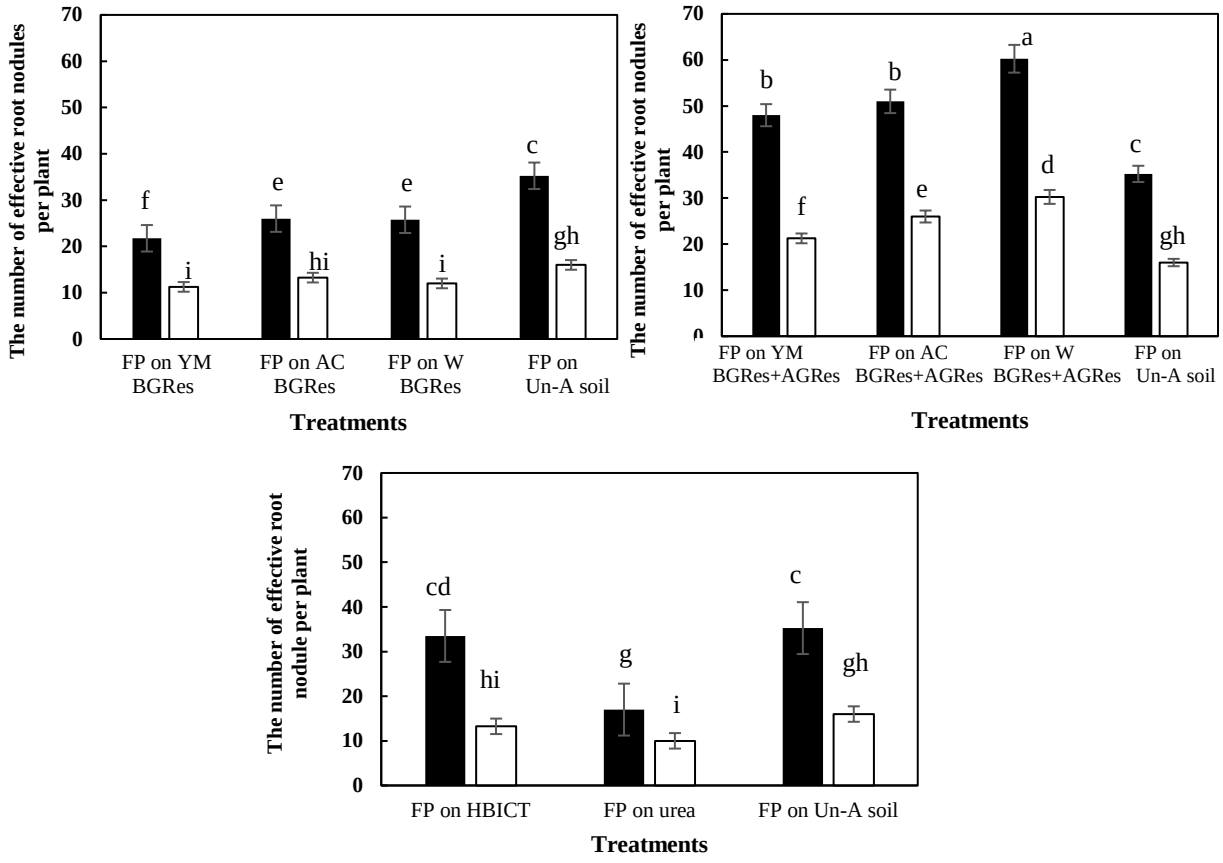

Fig. 6.3. The number of effective nodules in different treatments. Key: FP=Field Pea, BGR=Below-Ground Residue, YM= Yellow Mustard, AC-Argentine Canola, W= Wheat, AGR=Above-Ground Residue, HBITC- 4-hydroxybenzyl isothiocyanate application. Black and white colors represent the sampling times of field pea flowering and harvest stages, respectively. Comparisons are for both sampling stages of all the treatments. Different letters near bars correspond significantly different values ($P \leq 0.05$). **Note**: Bars indicate the standard errors of means.

Table 6.4. Summary of *P* values and contrast values from pre-planned comparisons in the number of effective root nodules (plant^{-1}) in different treatments at different growth stages (sampling times) of field pea

Treatment (Trt) comparisons [1]	Effective root nodulation at different growth stages of field pea	
	At flowering	At harvest
Field pea grown after a crop vs. fallow period	+10.2‡ **<0.0001**§	+5.91 **<0.0001**
Field pea on unamended soil vs. chemical amendments	+10.0 **<0.0001**	+4.37 **<0.0001**
Field pea grown on BGRes vs. on BGRes + AGRes	+28.6 **<0.0001**	+13.6 **<0.0001**
Field pea grown on BGRes + AGRes of wheat vs. Brassicaceae	+1.87 0.0539	-0.25 0.7004
Field pea grown on BGRes of wheat vs. Brassicaceae	+10.7 **<0.0001**	+6.6 **<0.0001**

***P* values for main and interaction effects of treatment (T) × Sampling time (S)**	
T	**<0.0001**
S	**<0.0001**
T × S	**<0.0001**

[1]1=FP on YM BGR, 2=FP on AC BGR, 3=FP on W BGR 4=FP on YM BGR+AGR, 5=FP on AC BGR+AGR, 6=FP on W BGR+AGR, , 7=FP on HBITC, 8=FP on urea, 9=FP on Un-A soil (FP=Field Pea, BGR=Below-Ground Residue, YM= Yellow Mustard, AC-Argentine Canola, W= Wheat, AGR=Above-Ground Residue, HBITC- 4-hydroxybenzyl Isothiocyanate application, Un-A= Un-Amended). ‡ Contrast value = value on the right side - value on the left side in the comparison. § Bolded *P* values indicate significant differences ($P \leq 0.05$).

6.5.4. Correlation between the parameters

Soil available NO_2^- +NO_3^--N had a strong and positive correlation with archaeal (r = 0.764, $P<0.0001$), bacterial (r = 0.700, $P<0$ .0001) and total (r = 0.821, $P<$ 0.0001) *amo*A gene copy concentration (Table 6.5). However, the number of effective nodules was positively and weakly correlated with the available N in soil (for NO_2^- +NO_3^--N – r = 0.25, $P=0.03$ and NH_4^+-N – r = 0.29, $P=0.01$).

Table 6.5. Pearson correlation coefficients (r) between the major parameters taken in the study.

Parameter	Archaeal *amo*A gene copy concentration	Bacterial *amo*A gene copy concentration	Total *amo*A gene copy concentration	Number of effective nodules per plant
Soil available NO_2^- +NO_3^--N concentration	**0.764**[1]	**0.700**	**0.821**	**0.250**
Soil available NH_4^+-N concentration	**0.339**	**0.306**	**0.413**	**0.290**
Total N concentration	**0.764**	**0.700**	**0.824**	**0.262**

[1] Bolded values denote the significance of the correlation at $P<0.05$.

6.6. Discussion

6.6.1. Brassicaceae crop effect on nitrification in subsequent field pea-grown soils

Brassicaceae crop-grown soils had lower NO_2^- + NO_3^--N content (Fig. 6.1, Table 6.2) compared to wheat-grown soils during the first two samplings, regardless of the crop residue application (Fig. 6.2, Table 6.3). This decrease in soil with Brassicaceae could be linked to its diminished concentration of *amo*A gene copies in AOA and AOB than soil with wheat. The association between NO_2^- + NO_3^--N content and *amo*A gene copy concentration was further supported by the strong positive correlation of the concentration of AOA *amo*A (r=0.764, Table 6.4) and AOB *amo*A (r = 0.700) gene copies with NO_2^- + NO_3^--N content as previously reported (Castellano-Hinojosa et al., 2018; Zilio et al., 2020). The reduced *amo*A gene copy concentration of nitrifying microbes may indicate their reduced populations, which consequently leads to slower nitrification in soil Brassicaceae crops grown soil than in wheat-grown soil (Sanders et al., 2014). The biochemical composition of plant residues can influence the abundance of soil microbial populations differently (Liu et al., 2020; Fan et al., 2020). Thus, this might be due to the suppressive effects of GLS and their breakdown products in Brassicaceae plant tissues, primarily ITC. Isothiocyanates and other GLS hydrolysis products released from decomposed crop residues and remaining root tissues might continue this inhibitory effect while field pea is growing (Schreiner et al., 2011; Yasumoto et al., 2011). This inhibition can extend to AOB and AOA, resulting in lower *amo*A gene copy concentration and subsequently reduced nitrification, leading to lower concentrations of NO_2^-+ NO_3^--N in soils with Brassicaceae residues. In addition, the measured soil NH_4^+ showed a similar trend with NO_2^- + NO_3^--N only at field pea flowering (Table 6.2). This might also be attributed to the suppressive effect of GLS hydrolysis products on saprophytic microbes, which can decelerate decomposition processes (Siebers, et al., 2018). The less substrate (NH_4^+) availability may also contribute to lower AOA and AOB abundance in Brassicaceae-grown soil than in wheat-grown soil.

On average, yellow mustard-grown soil had lower AOB *amo*A gene copy concentration and NO_2^- + NO_3^--N than Argentine canola grown-soil during the first two samplings regardless of the residue application (Fig. 6.1 and Table 6.4). Chapter 5 revealed that the total amount of GLS in yellow mustard residues was the highest among the crops used in this study leading to produce higher amounts of ITCs than Argentine canola. In yellow mustard, the main ITC forming GLS is

4-hydroxybenzyl (HB), while 4-pentenyl is the form found in Argentine canola (Bown and Morra, 2009). 4-HBITC has a strong antibacterial effect due to its aromatic nature with a phenyl group, compared to the aliphatic nature of 4-pentenyl ITC (Dias et al., 2014; Wang et al., 2017). However, the inhibitory effects of ITCs depend not only on their absolute concentration but also on the biological availability and toxicity level of the specific ITC-forming GLSs (Borek et al., 1994; Bown and Morra, 2009). In contrast, AOA *amo*A gene copy concentration remained consistent across treatments, regardless of whether they included preceding Brassicaceae crop residues. AOA seems to exhibit resilience or adaptability to ITCs and the environmental changes brought about by Brassicaceae residues unlike AOB (Yin et al., 2108; Lin et al., 2021).

Field pea grown on urea-amended and yellow mustard-grown soil had comparable NO_2^-+ NO_3^--N levels and AOB *amo*A gene copy concentrations, yet lower than the treatment with wheat. This was unexpected and disagreed with the previous studies (Quemada et al., 2019). Urea supplies NH_4^+, which generally speeds up the rate of nitrification. Field pea may be rapidly assimilating NH_4^+-N, resulting in a temporary, but significant reduction in available NH_4^+-N for ammonia-oxidizing microbes.

On average, the soil that was left fallow during Phase I had lower NO_2^-+ NO_3^--N contents and lower *amo*A gene copy concentrations in AOA and AOB compared to the cultivated soil and this trend was consistent at all sampling times (Fig. 6.1, Table 6.2). When a crop is present, soil contains root exudates, senescing plant parts and crop residues that release N back into soil through decomposition. Absence of root exudates and presence of senescing plant parts potentially at later decomposition stages in no-crop soil can lead to decreased *amo*A gene copy concentrations (Ma et al., 2020). Reduced substrate availability was also supported by the presence of lower NH_4^+ levels in all fallow soils than in cropped soils at all sampling times in this study (Table 6.2). In addition, fallow soil leads to greater N losses through leaching than soils with actively growing plants and that also might contribute to low NO_2^- + NO_3^--N content in fallow soil (Shakoor et al., 2023).

There was a significant decrease in NO_2^-+ NO_3^--N in field pea grown on yellow mustard BGRes + AGRres compared to yellow mustard BGRes alone only at flowering. However, *amo*A gene copy concentration remained the same at flowering regardless of adding AGRes. This may be due to suppressive effects of yellow mustard residues on ammonium-oxidizing microbial activity (Muehlchen et al.,1990). However, there was no apparent synergistic effect of applying BGRes + AGRes in wheat and Argentine canola on soil NO_2^- + NO_3^--N throughout field pea

growth. In contrast, total *amo*A gene copy concentration in field pea grown on wheat and Argentine canola with BGRes + AGRes was higher than their AGR-free counterparts at flowering and harvest. This could be due to the increased substrate availability provided by the residues, which facilitates the growth of AOA and AOB.

The AOA and AOB *amo*A gene copy concentrations in unamended, and soils amended with HBITC were comparable at the flowering stage of field pea. In contrast, HBITC-amended soil had higher total *amo*A gene copy concentration than unamended soil at maturity. The impact of chemical ITC on microbial communities is likely to be immediate and thus more apparent at earlier stages of plant growth. Over time, ITCs in the soil degrade and become less toxic, either through natural chemical breakdown or through microbial action. As the toxic effects of ITCs diminish, the soil environment may become more conducive to the growth and activity of ammonia-oxidizing microorganisms, leading to increased *amo*A gene copy concentration. This trend was further supported at the harvest of field pea, where the differences in microbial populations due to treatment were less prominent at harvest compared to earlier stages. As the field pea matures, the inhibitory effects of ITCs may diminish as these compounds degrade or are transformed in the soil. The continuous decomposition process of crop residues over the growth period of the field pea might result in a more sustained release of N, supporting higher populations of AOB towards the end of the growing season. The soil nitrifying microbes adapt and change in response to soil conditions and available nutrients. The presence of crop residues might have led to a microbial community more efficient in nitrification, reflected in higher AOB *amo*A gene copy concentration in these treatments. This indicates the beneficial effect of maintaining a crop residue over removing it for the ammonia-oxidizing bacterial population.

The current study showed that AOB was dominant compared to AOA. The composition of AOA and AOB communities in soil is largely influenced by soil N availability and pH. AOA prefers less NH_4^+ and acidic conditions, while AOB prefers N-rich neutral/alkaline conditions (Xiang et al., 2017; Prosser et al., 2020). For the experiment, soil from the Swift Current research field was used and the pH value was close to neutral (pH = 6.6). In addition, AOB have been shown to be generally more responsive than AOA to soil management practices (Ouyang et al., 2017).

6.6.2. The effect of Brassicaceae crop-induced soil nitrification changes on effective root nodulation in subsequent field pea

The correlation between effective root nodule number and total soil available N content was positive but weak (r = 0.262, *P*=0.0261). This observation contrasts with the previous studies, where root nodulation was found to be negatively correlated with soil available N (Ferguson et al. 2019; Alon et al., 2021). The positive correlation may potentially be due to the presence of low amount of soil N, which may not be high enough to suppress the root nodulation in the current study. In a hydroponic study, low concentrations of N (< 50 mg N kg^{-1} of water) enhanced weight and nodule number in soybeans, whereas higher concentrations of N (> 50 mg N kg^{-1} of water) significantly suppressed nodulation (Xia et al., 2017). The soil used in current study had 23.1-77.7 mg N kg^{-1}, which can be considered as low soil N and it might be below the threshold level for the inhibition of root nodulation of field pea.

The weak association between the effective nodule number and the soil available N implies that other factor(s) may also have contributed to the variability in effective root nodulation. Effective root nodulation in this study was possibly related to phosphorus availability. The addition of P increases legume nodulation noticeably (Magadlela et al. 2016). Arbuscular mycorrhizal fungi (AMF) symbioses present in most terrestrial plants improve P efficiency (Hou et al., 2021). However, brassica plants are non-hosts for AMF due to the presence of ITC and other GLS hydrolysis products involved in inhibiting AMF symbiosis (Sharma et al., 2023). A previous study showed that AMF colonization of soybean decreased by 30 % when the preceding crop was rapeseed (Valetti et al., 2016). Thus, the lower effective nodule number in field pea grown on combined BGRes + AGRes of preceding Brassicaceae crops over wheat was likely to the ITCs and phytotoxic chemicals found in those crops. These compounds are released initially during the growth of Brassicaceae crops and following the incorporation of their residues. The highest number of effective nodules in field pea grown on wheat residue may be attributed to high P uptake of the plant due to the presence of an elevated abundance of AMF in field pea. However, GLS hydrolysis products were not measured directly in the current study. Furthermore, field pea preceded by Argentine canola had higher number of effective nodules than yellow mustard, regardless of the AGRes application during both sampling stages. This may be due to higher amount of ITC-forming GLSs in yellow mustard than Argentine canola, more effectively suppressing AMF colonization.

On average, the field pea grown on of BGRes + AGRes from preceding crops had higher numbers of effective root nodules than field pea grown on preceding crop BGRes alone at flowering and harvest stages. The additional nutrients from the AGRes possibly improved AMF symbiotic functioning over the treatment without residue (Ingraffia et al., 2021). AMF stimulates residue decomposition by promoting degradative enzymes, modifying root production and activity, and/or regulating the microbial community in the mycorrhizosphere and hyphosphere. Moreover, field pea grown on wheat crop BGRes + AGRes showed higher numbers of effective root nodules than when grown on Brassicaceae crop BGRes + AGRes, but no such difference was observed in the comparison of their AGRes-free counterparts, which contained only the preceding crop BGRes. Wheat AGRes residues, along with the beneficial effects of AMF, may have created a more conducive environment for nodulation in field pea. In treatments with only BGRes, the influence of the previous crop's root system and associated microbial communities is less pronounced than when AGRes are also present.

Field pea grown on HBITC-treated soil had lower effective root nodule numbers than field pea grown on soil with BGRes + AGRes of with either yellow mustard or Argentine canola. When ITC is applied in a pure form, it can immediately be available in the soil, potentially exerting a strong, direct bio-fumigant effect, effectively suppressing soil microbes. This aligns with the reduction in *amo*A gene copies in HBITC-treated soil than soil amended with BGRes + AGRes of yellow mustard or Argentine canola. The inhibitoryeffect could also extend to rhizobia, which are essential for nodulation in field pea. In contrast, ITCs released from the decomposing Brassicaceae crop residues are introduced into the soil more gradually. This slower release allows for a less abrupt change in the soil microbial environment, potentially giving rhizobia and other beneficial microbes time to adapt or recover, which could lead to better nodulation than HBITC-treated soil. In addition, the decomposition of Brassicaceae crop residues not only releases ITCs but also contributes additional organic matter and other nutrients to the soil, which can mitigate the potential negative effects of ITCs on nodulation, leading to higher nodulation compared to soils treated with pure HBITC. Field pea grown on urea had the lowest effective root nodule number. The availability of excessive N as a result of urea application may have discouraged the formation of effective root nodules in field pea, as the plants can more easily assimilate N directly from the soil (Du et al., 2020).

The 50 % decrease in nodule numbers from flowering to maturity of field pea could be due to the natural senescence of nodules. Nodule numbers generally peak during early- to mid-flowering stage (Voisin et al., 2003) and slow down with the onset of pod filling (Salon et al., 2001). As the plant reaches maturity, the demand for N through fixation decreases, and older nodules may start to senescence.

6.7. Conclusion

The type and species of the crop grown influenced the abundance of ammonia-oxidizing microbes and effective root nodulation of subsequent field pea differently, possibly due to the unique biochemical compositions of crop residues. Growing Brassicaceae crops and incorporating their BGRes and AGRes contained GLS, suppressed ammonia-oxidizing microbes, reduced NO_2^- + NO_3^--N content and effective root nodulation relative to wheat. Moreover, Brassicaceae crops containing higher GLS concentrations are more likely to suppress soil nitrification via decreasing soil available N, specifically NO_2- + NO_3- content and reducing effective root nodulation in field pea than the crops with less GLS content. The combined effect of BGRes and AGRes in yellow mustard synergistically suppressed soil nitrification and effective root nodulation in early growth stages of field pea. However, reduced nitrification did not increase the NH_4^+, possibly due to the negative impact of yellow mustard crops and their residues on soil saprophytic microbes decelerate decomposition processes. The less NH_4^+ availability also contributed to lower AOA and AOB abundance in Brassicaceae crop-grown soil than wheat. The suppressive effects of Brassicaceae residues gradually diminishes over time. Eventually, Brassicaceae residues decompose and increase soil available N over the subsequent crop growth regardless of the GLS amount of the presence in plant tissues. The continuous decomposition process of crop residues over the growth period of the field pea might result in a more sustained release of N, supporting higher populations of AOB towards the end of the growing season. Brassicaceae plant tissues are desirable in a crop production system where synchronizing N availability with GLS hydrolysis is important to maximize N use efficiency.

7. SYNTHESIS AND CONCLUSION

Overview

Growing pulse crops in cropping systems is known to improve soil N through biological nitrogen fixation (BNF) with the aid of rhizobia bacteria (Lal, 2017). This symbiotic process is estimated to contribute 200 million tonnes of nitrogen (N) annually to global ecosystem (Islam, 2017). Crop rotations that include pulse crops may supply N-rich residues to subsequent crops depending on their BNF capacity, potentially reducing fertilizer inputs (Gan et al., 2011a) and carbon (C) footprint of the agricultural system (Lemke et al., 2007; Gan et al., 2011b). However, the BNF capacity relies mainly on root nodule development initiated by specific rhizobial strains (Abdellatif et al., 2016). These strains vary in effectiveness depending on the pulse crop species, cropping history and environmental conditions (Hossain et al., 2016).

The current practice of incorporating Brassicaceae oilseed crops in rotations with pulse crops raises concerns about their potential impact on BNF (Gill, 2018). Most Brassicaceae crops can act as bio-fumigants as they contain GLSs, which can hydrolyze into a range of biocidal products, such as thiocyanates, ITCs, epithionitriles, nitriles, indoles and oxazolidine-2-thiones (Hu et al., 2011b). In general, these compounds play a key role in plant defense against soil pathogenic organisms (Singh, 2017). However, these released hydrolysis products can also impact non-target soil microorganisms beneficial in soil nutrient cycling (Astudillo-García et al., 2019). Brassicaceae oilseed crops varied in their GLS levels with diverse compositions resulting in different levels of biocidal activity (Clarke, 2010). Therefore, understanding the impact of Brassicaceae oilseed crops on beneficial microbes is crucial for sustainable agricultural practices to develop crop management recommendations and mitigate agronomic risk for cropping system performance.

Previous research studies have primarily focused on the N benefit a pulse crop can provide for subsequent crops and the impact of preceding crops on pulse BNF remains unclear. Results of the present study provide comprehensive knowledge on the impact of selected preceding Brassica crops on root nodulation and productivity of subsequent lentil and field pea, but such information are limited only one cycle crop rotation. Therefore, further investigations are necessary to evaluate the impact of Brassicaceae oilseed crops on BNF of subsequently grown pulse crops in western Canadian cropping systems, considering factors such as different Brassicaceae species, soil conditions, pulse crop species and crop rotation cycles.

The general goal of this study was to evaluate the impact of different Brassicaceae oilseed crops on BNF of subsequently growing pulse crops and the overall performance of cropping systems. A field experiment was performed to identify whether there are differences in BNF capacities of pulse crops (field pea and lentil) grown on different Brassicaceae oilseed (Argentine canola, camelina, oriental mustard, industrial mustard and yellow mustard) stubble in a 4-yr crop rotation study (oilseed-pulse-wheat-yellow mustard) in multiple locations (Swift Current and Scott in SK, and Brooks in AB) in western Canada from 2019 to 2022 (Chapter 3). The same field experiment was used to determine the changes in selected soil properties and subsequently grown wheat yield performance (2021) in the cropping systems (Chapter 4). Understanding the decomposition of Brassicaceae residues and their impact on soil N availability was crucial for assessing their influence on pulse crop nodulation. To explore this association, an incubation study was performed to analyze N recovery percentages of different Brassicaceae residues and the impact of their biochemical composition on N recovery (Chapter 5). Building on these findings, a controlled environment experiment was performed to examine the impact of released N and GLS hydrolysis products of Brassicaceae oilseed crops on effective nodulation in subsequently grown pulse crops (Chapter 6).

7.1. Summary and Synthesis of Findings

This study represents one of the first direct evaluations of the impact of different preceding Brassicaceae oilseed crops on BNF ability in subsequently grown pulse crops. The impact of Brassicaceae crops on BNF may vary depending on the specific pulse crop. Overall, growing Brassicaceae crops before lentil crops did not impact BNF ability (%Ndfa) and biologically fixed nitrogen (N) content of lentil (Chapter 3). This suggests that all Brassicaceae crops used in this study (Argentine canola, yellow mustard, oriental mustard, industrial mustard and camelina) could be suitable preceding crops for lentil rotations in the Brown and Dark Brown soil zones in the Canadian prairies allowing flexibility in rotation planning. In contrast, BNF ability and biologically fixed N contents in field pea were significantly influenced by the type of preceding Brassicaceae crop. This finding indicates that the preceding Brassicaceae crop is an important factor to consider when optimizing BNF in Brassicaceae oilseed-field pea crop rotations across these soil zones.

In general, field pea grown after Argentine canola consistently showed higher levels of plant diazotrophic abundance, root nodule weight and BNF ability in total above-ground biomass than

field pea grown after other Brassicaceae oilseed crops (yellow mustard, industrial mustard, oriental mustard, and camelina). This difference might be attributable to the lower suppressive effect of Argentine canola residues with the lowest GLS content (0.02 μmol g^{-1} of tissue) on plant diazotrophic microbes than other Brassicaceae crop residues (0.57-6.49 μmol g^{-1} of tissue, Chapter 5). Glucosinolate hydrolysis products are known for their antimicrobial properties. The observed difference between Argentine canola with very low GLS vs. other Brassicaceae crops with high GLSs at the two extremes may suggest a potential threshold effect of GLS content (minimum inhibitory concentration). This agrees with previous studies, which showed that GLS hydrolysis products, especially ITCs have minimum inhibitory concentrations when act as biocidal compounds (Kim et al., 2015; Krause et al., 2021). Negligible GLS content in Argentine canola residues possibly non-suppressive for pulse crop beneficial interactions with diazotrophs and other soil microbes, ultimately leading to increased BNF in field pea in contrast to other Brassicaceae crop residues. Thus, Argentine canola could be a favorable preceding crop for field pea than other Brassicaceae oilseed crops in the study.

There was no significant correlation between Brassicaceae crop residual GLS contents and diazotrophic abundance, root nodule weight, or BNF ability in subsequent field pea (Table 7.1, Chapters 3 and 5). The absence of a direct effect of GLS on BNF-related parameters possibly due to the level of toxicity depends on both the quantity and type of GLS hydrolyzed products (Nowicki et al., 2016; Wang et al., 2020). Chapter 5 further found that the Brassicaceae species used for this experiment had different quantities and types of GLSs. Yellow mustard residues had the highest GLS content (89.3 % higher than other Brassicaceae crop residues), which mainly contained sinalbin (4-hydroxybenzyl GLS, 6.49 μmol g^{-1} of tissue). Both industrial (0.91 μmol g^{-1} of tissue) and oriental (1.26 μmol g^{-1} of tissue) mustard plant tissues contained sinigrin (2-propenyl GLS). The primary GLS type in camelina (0.57 μmol g^{-1} of tissue) and Argentine canola were glucocamelinin (Potassium 10-methylsulfinyldecyl GLS) and neoglucobrassicin (1-methoxy-3-indolylmethyl GLS), respectively. However, GLS in crop residue had the ability to suppress pulse crop nodulation, which was further supported by the greenhouse experiment in Chapter 6. Growing Brassicaceae crops reduced effective root nodulation in field pea by 14.6 % relative to wheat with zero GLS. Moreover, Brassicaceae crops containing higher GLS concentrations had stronger suppression on effective root nodulation than the crops with less GLS. Among GLS hydrolysis products, ITCs have been recognized as the predominant compounds with biocidal activities. In

yellow mustard, the main ITC forming GLS is 4-hydroxybenzyl (HB), whereas 4-pentenyl is the form found in Argentine canola (Brown and Morra, 2009). 4-HBITC has a strong antibacterial effect due to its aromatic nature with a phenyl group, compared to the aliphatic nature of 4-pentenyl ITC (Dias et al., 2014; Wang et al., 2017). Thus, these findings suggest that GLS concentration and the type synergistically contribute to root nodule suppression.

Table 7.1. Pearson's correlation coefficients (r) for the association of residual glucosinolate levels with root nodule parameters and plant biological nitrogen fixation.

	Root nodule dry weight		***nif*H gene copy concentration**		**%Ndfa**		**Biologically fixed N content**	
	Field pea	**Lentil**	**Field pea**	**Lentil**	**Field pea**	**Lentil**	**Field pea**	**Lentil**
Residual glucosinolate content	-0.21[1]	-0.10	-0.14	-0.21	-0.22	-0.23	+0.15	-0.12

[1]non-bolded values denote absence of significance at $P<0.05$.

Chapter 6 further showed that crop residual GLS content influenced processes affecting soil available N composition. On average, field pea following Brassicaceae crops suppressed soil nitrification with 16.2 % lower NO_2^- + NO_3^--N, 80.2 % lower total *amo*A gene copy concentration. In addition, yellow mustard suppressed nitrification to a greater degree than Argentine canola and wheat. However, reduced nitrification did not increase NH_4^+, possibly due to the negative impact of yellow mustard crops and their residues on soil saprophytic microbes, which lead to decelerate decomposition processes. This further aligns with the findings in Chapter 5, which suggest that mainly high GLS content and lignin:N ratio of plant tissues hinder N recovery percentages. However, the suppressive effects of Brassicaceae residues diminished with time. Brassicaceae residues decomposed and gradually increased soil available N over time regardless of the GLS amount in plant tissues. The weak association between the effective root nodule number in field pea and the soil available N implies that other factor(s) may also have contributed to the variability in effective root nodulation. These GLS hydrolysis products suppress arbuscular mycorrhizal fungi (AMF), which facilitates plant phosphorus (P) uptake (Valetti et al., 2016; Sharma et al., 2023). Legumes rely on plant-mycorrhizal interactions to acquire especially sufficient P, a vital nutrient for root nodulation. Consequently, suppressing AMF leads to a scarcity of plant-available P and negatively impacting root nodule development and activity (Magadlela et al., 2016; Pérez-

Fernández et al., 2017). Some studies have suggested that low P concentration reduces the nitrogenase activity in nodules (Reed et al., 2007) and inhibits BNF (Divito et al., 2014). These findings suggest that minimizing these inhibitory compounds could be beneficial for field pea BNF. Overall, the current study highlights the potential complexity of the relationship of GLS type and quantity of preceding Brassicaceae crops with BNF of subsequent pulse crops.

In general, lower BNF ability and biologically fixed N contents were consistently observed in field pea grown on camelina stubble compared to other Brassicaceae stubble at both Scott and Brooks sites. It could be recommended to producers in this region to avoid growing pulse crops on camelina stubble; however, more site years in these soil zones are necessary to make a firm conclusion. Effects of stubble on pulse crop BNF were not consistent across the three test sites (Swift Current and Brooks with Brown soil and Scott with Dark Brown soil) investigated in the field study. The test site-dependent BNF suggests that multiple factors, including microbial communities, soil properties, and environmental conditions could be influencing the observed differences in diazotroph abundance, root nodulation, and BNF levels among the Brassicaceae species. Soil organic matter (SOM) acts as a buffer for soil microbial populations, making them more resilient to environmental fluctuations (Knight, 2012). In addition, SOM content influences the impact of GLS compounds in soil (Chen, 2016). Therefore, variations in SOM levels across the test sites can explain the differences in BNF response to preceding Brassicaceae crops.

The field experiment in Chapter 3 further revealed that growing field pea on different crop stubble affects the amount of biologically fixed N amount. However, this did not contribute to a change in total soil N among different crop sequences (Chapter 4). This was mainly because of a significant portion (73.5 % - 94.8 %) of the fixed N was accumulated in the seeds and pods, with only the remaining fraction present in the straw. Since most of the N is removed from the field during harvest in the form of seeds, only a small amount is returned to the soil as stubble and residue. In addition, different crop sequences had no impact on soil organic carbon (SOC) changes during 2 years (2019-2021). The time duration of the study was likely insufficient to identify the significant changes in SOC as the accumulation of SOC generally takes around 10-20 years (Kern and Johnson, 1993). Moreover, soil moisture contents were also comparable among the different treatments. The absence of significant changes in soil properties, such as total soil N, SOC, and soil moisture content likely contributed to the lack of variation in subsequent wheat grain yields

among the treatments. This suggests that, within the time frame of this study, the choice of the preceding crop had a limited impact on soil quality and subsequent crop performance.

Diversification of crop sequences with Brassicaceae and pulse crops had yield benefits over continuous wheat. However, the impact was dependent on the test site. Crop sequences diversified with Brassicaceae and pulse crops increased subsequent wheat yield by 52.7 % and elevated 1000-seed weight by 19.0 % compared to continuous wheat at Brooks. In contrast, the 1000-seed weight in subsequent wheat increased in Brassicaceae and pulse crop sequences compared to continuous wheat at Swift Current without affecting the grain yield. This yield improvement aligns with the observed significant increase in SOC and light fraction organic matter (LFOM) contents in the diversified cropping systems from 2019 to 2021 in both test sites. This result aligned with previous studies, which showed that SOC contents were positively correlated with crop yield (Lal et al., 2003; Pan et al., 2023). The increase in LFOM may favor enhanced soil nutrient availability for the subsequent wheat crops. This agrees with previous studies, which showed the inclusion of Brassicaceae and pulse crops in rotations to be an effective strategy for improving total SOC and N through residual input and root deposition (Kirkegaard et al., 2000; Gan et al., 2015; Peoples et al., 2017). In addition, the inclusion of Argentine canola into field pea or lentil rotations increased subsequent wheat yield by more than 30 % compared to the crop sequences with other Brassicaceae species at Swift Current. This yield benefit could be also linked to improved soil fertility, potentially driven by the higher accumulation of LFOM, specifically LFOM-C and LFOM-N contents in rotations with Argentine canola than other Brassicaceae crops. Furthermore, wheat preceded by yellow mustard and field pea had a higher 1000-seed weight than wheat grown on other mustard-field pea crop stubble at Swift Current. Overall, Brassicaceae oilseed and pulse crops are beneficial preceding crops not only to improve the quantity but also crop yield quality in wheat-based rotations. Among Brassicaceae crops, Argentine canola and yellow mustard were particularly beneficial in enhancing subsequent wheat productivity.

The field experiment was originally aimed to quantify GLS amounts in different crop residues. Due to the limited resources, only the seed GLS content of various Brassicaceae crops was analyzed. Even though seed GLS content in this study provided valuable insights, it is important to recognize that it does not directly reflect GLS levels of plant residues in the same crop. This is mainly because biosynthesis, distribution, and accumulation of GLSs can differ between seeds and other plant parts (Sun et al., 2019). However, the controlled environment

experiment in Chapter 5 presents data on GLS levels in different Brassicaceae crop residues. However, when comparing the results, the seed GLS results showed discrepancies between field and controlled environment studies. Under field conditions, the highest GLS level in seed was recorded in yellow mustard. In contrast, under controlled environment conditions, the highest GLS levels in seed were found in oriental mustard, with yellow mustard ranked third. These differences may be due to the different quantification methods used. The field experiment measured total GLS content, whereas the controlled environment experiment measured only primary GLS content, which accounts for more than 90 % of the total GLS content. In addition, environmental conditions can significantly influence GLS content in plants, contributing to the observed variations. Previous studies indicate that environmental factors, such as changes in light (Rosa and Rodrigues, 1998), temperature (Kissen et al., 2016), salinity (López-Berenguer et al., 2008) and drought (del Carmen Martínez-Ballesta et al., 2013) may modify GLS composition. While the GLS content measured in crop residues under controlled environment conditions may not directly mirror levels found in field conditions, this overall study was designed for comparative analysis. Therefore, the values offer valuable insights into the relative GLS content among Brassicaceae crops, serving as a basis for understanding their comparative profiles, despite not being exact representations of field residue levels.

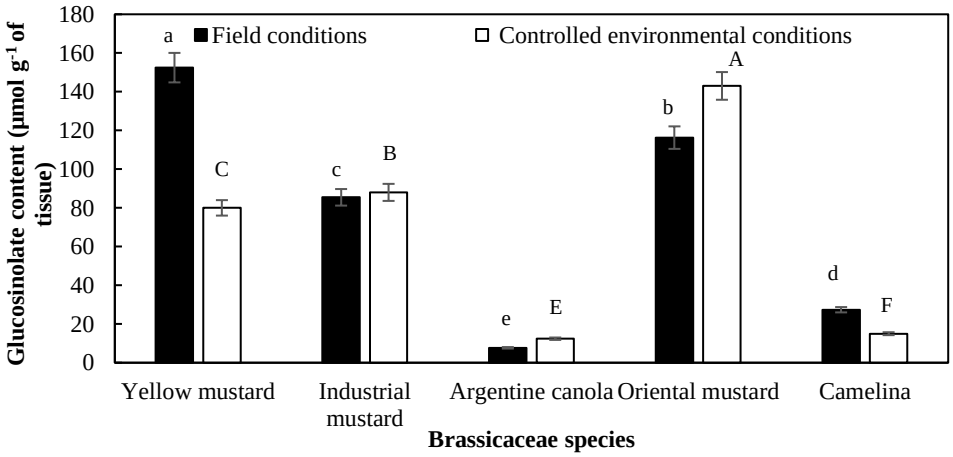

Fig. 7.1. Seed glucosinolate concentration in different Brassicaceae species under different growth conditions. Field condition data sourced from Chapter 4 and presented as mean values of three test sites (Swift Current, Scott and Brooks). Lower case and upper-case letters indicate significant differences under field conditions and controlled conditions, respectively ($P \leq 0.05$). **Note:** Bars indicate the standard errors of means.

7.2. Future Research

This study provided an understanding of the role of different Brassicaceae oilseed crops in influencing BNF ability of subsequently grown two pulse crops (field pea and lentil) and the overall cropping system performance. The continuous wheat, control treatment in the field study was not considered as the base treatment to the experiment assessing BNF capacities (Chapter 3) due to the absence of a pulse crop phase. Including additional treatments without incorporating a Brassicaceae crop or incorporating wheat followed by each pulse crop (field pea and lentil) would provide a baseline to BNF ability in field pea and lentil to compare with the treatments, with Brassicaceae crops preceding pulse crops. In addition, yellow mustard was only included in the field pea crop sequences. Inclusion of yellow mustard in lentil sequences would have provided a balanced experiment and a more comprehensive data set.

In the evaluation of soil quality of different treatments in the 4-year field study, most of the changes in soil properties were not significant across all test sites. Thus, soil quality needs to be further investigated using long-term rotation studies. Repeating this study at least for three cycles would allow to identify both potential drawbacks and the long-term benefits of these crop rotations on soil properties and overall cropping system productivity. In crop performance assessment, wheat grain yield itself could be a valuable predictor of nutrient removal and contribution. Future studies investigating the effect of treatments on N in wheat grain yield (harvest) will be important for a better understanding of the contribution of N by different treatments. Furthermore, quantifying C footprints of treatments will help identify the best treatment(s), which improve the environmental sustainability of the agricultural system. Moreover, evaluating the economic feasibility of incorporating specific Brassicaceae crops into rotations based on their impact on N fixation, potential yield improvements in subsequent crops, and overall cropping system profitability can provide comprehensive recommendations on the economic viability and agronomic benefits.

In this study, *nif*H, *amo*A and *amo*B gene copy concentrations were quantified to evaluate the diazotrophic and nitrifying microbial abundance. Gene copy number determines the number of copies in a particular gene present within a genome. It offers a measure of gene abundance, but it does not necessarily reflect the gene's expression. Presence of a high number of gene copies might suggest the potential for high protein production. However, regulatory mechanisms and epigenetic factors (DNA methylation, histone modifications, and chromatin structure) play crucial roles in controlling gene expression and influence the amount of protein made. For future studies aiming

for a more precise understanding of microbial activity, reverse transcription-quantitative PCR (qPCR) could be implemented to quantify functional genes. qPCR directly measures messenger RNA (mRNA) transcript levels, providing a more accurate representation of active gene expression and protein production potential. In the context of this comparative study, focusing mainly on gene copy number variations across different treatments can still be informative. However, future studies could consider employing the 16S rRNA gene as a reference for functional gene quantification to gain a more precise picture of microbial activity.

This study did not quantify residual GLS levels in the field experiment due to resource limitations. Since GLS content in Brassicaceae oilseed crops can vary depending on environmental factors, such as ambient temperature and precipitation, future studies that incorporate field-based measurements of residual GLS levels alongside BNF assessment would provide a more comprehensive picture of the relationship between preceding Brassicaceae oilseed crops and subsequent pulse crop BNF.

Evaluation of nitrifying microbial abundance and available soil N in Chapter 6 provided 'snapshots' at pre-seeding, flowering (peak nodulation) and harvest of field pea. Determining the parameters throughout the growth of field pea will provide a more accurate changing patterns for nitrifying microbial abundance and available soil N with time. In addition, validating these findings under field conditions with varying soil types and microbial communities will strengthen the generalizability of the results.

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APPENDIX A

Table A.1. Crop variety, seeding rate, seed treatment, and fertilizer rate used in the 4-year crop rotation at Swift Current

Crop variety	Cultivar	Seeding		Seed treatment		Fertilizer	
		Rate (kg ha^{-1})	Density (seed m^{-2})	Trade name	Rate	Nitrogen (kg N ha^{-1})	Phosphorus (kg P_2O_5 ha^{-1})
2018							
Spring wheat	AAC Brandon	111	300	None	-	80	0
2019							
Spring wheat	AAC Brandon	111	325	Brooks supplied seeds	-	50	22
Argentine canola	L233P	6.6	150	Brooks supplied seeds	-	50	22
Yellow mustard	Andante	9.2	150	Brooks supplied seeds	-	50	22
Oriental mustard	AC Cutlass	4.2	150	Brooks supplied seeds	-	50	22
Industrial mustard	A 120	6.4	150	Brooks supplied seeds	-	50	22
Camelina	SES0787LS 'Cypress'	9.8	500	Brooks supplied seeds	-	50	22
2020							
Spring wheat	AAC Brandon	117	300	Eco tea	7.7 mL kg^{-1} of seed	50	20
Field pea	CDC Meadow (Yellow)	229	100	Cruser max pulse	4.08 mL kg^{-1} of seed	0	20
Lentil	CDC Maxim CL (Red)	54.8	150	Cruser max pulse	4.08 mL kg^{-1} of seed	0	20
2021							
Spring wheat	AAC Brandon	118.5	300	Brooks supplied seeds	-	56	20

Table A.2. Crop variety, seeding rate, seed treatment, and fertilizer rate used in the 4-year crop rotation at Scott

Crop variety	Cultivar	Seeding		Seed treatment		Fertilizer	
		Rate (kg ha^{-1})	Density (seed m^{-2})	Trade name	Rate	Nitrogen (kg N ha^{-1})	Phosphorus (kg P_2O_5 ha^{-1})
2018							
Spring wheat	AAC Brandon	127	350	None	-	63	24
2019							
Spring wheat	AAC Brandon	127	325	Vibrance Quattro	3.25 mL kg^{-1} of seed	57	47
Argentine canola	L233P	6.8	150	Prosper EverGol+ Lumiderm	(0.35 mL+6.4 mL) kg^{-1} of seed	57	47
Yellow mustard	Andante	4.5	150	-	-	57	47
Oriental mustard	AC Cutlass	4.4	150	Helix Vibrance	15 mL kg^{-1} of seed	57	47
Industrial mustard	A 120	6.7	150	Helix Vibrance	15 mL kg^{-1} of seed	57	47
Camelina	SES0787LS 'Cypress'	9.3	500	-	-	57	47
2020							
Spring wheat	AAC Brandon	117	300	-	-	55	23
Field pea	CDC Meadow (Yellow)	229	100	-	-	5	23
Lentil	CDC Maxim CL (Red)	54.8	150	-	-	5	23
2021							
Spring wheat	AAC Brandon	118.5	300	-	-	8	20

Table A.3. Crop variety, seeding rate, seed treatment, and fertilizer rate used in the 4-year crop rotation at Brooks

Crop variety	Cultivar	Seeding rate		Seed treatment		Fertilizer	
		Rate (kg ha^{-1})	Density (seed m^{-2})	Trade name	Rate	Nitrogen (kg N ha^{-1})	Phosphorous (kg P_2O_5 ha^{-1})
2018							
Spring wheat	AAC Brandon	134	325	Vibrance Quattro	3.25 mL kg^{-1} of seed	55	20
2019							
Spring wheat	AAC Brandon	133	325	Vitaflo 280	3.30 mL kg^{-1} of seed	55	22
Argentine canola	L233P	7.8	150	-	-	55	22
Yellow mustard	Andante	4.5	150	-	-	55	22
Oriental mustard	AC Cutlass	4.6	150	-	-	55	22
Industrial mustard	A 120	7.1	150	-	-	55	22
Camelina	SES0787LS 'Cypress'	9.4	500	-	-	55	22
2020							
Spring wheat	AAC Brandon	117	300	-	-	55	22
Field pea	CDC Meadow (Yellow)	229	100	-	-	5	22
Lentil	CDC Maxim CL (Red)	54.8	150	-	-	5	22
2021							
Spring wheat	AAC Brandon	118.5	300	-	-	19	22

Table A.4. Dates of application of agro-chemicals in the 4-year crop rotation at Swift Current

Operation	Crops [1]	Fertilizer/Chemical	Application rate	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Pre-seeding burn off	W, AC. YM, OrM, IM, CL,	Roundup Weathermax	1655 mL ha^{-1}	-	11, May	-	May, 5
	W, AC. YM, OrM, IM, CL	Aim	71 mL ha^{-1}	-	11, May	-	May, 5
Pre-seeding chemical	W	Roundup Weathermax	1235 mL ha^{-1}	May, 24	-	-	May, 10
		Heat	54 mL ha-1				May, 10
Pre emergence chemical	All	Zidua	121 mL ha-1	-	-	May, 19	May, 10
				-	-	-	-
Post-seeding chemical	W	Horizon	929 mL ha^{-1}	June, 28	May, 30	-	-
		Buctril M	988 mL ha^{-1}	June, 28	-	-	-
	W, AC. YM, OrM, IM, CL	Matador	74 mL ha^{-1}	-		-	-
	W	Bison	494 mL ha^{-1}	-	-	June, 9	-
	W	Buctil	988 mL ha^{-1}	-	June, 10	June, 9	-
	AC, OrM, IM	Mustard toss	1.98 g ha^{-1}	-	June, 10	-	-
	W	Assure II	494 mL ha^{-1}	-	June, 12	-	-
	CL	Roundup Weathermax	1655 mL ha^{-1}	-	June, 26	-	-
		Solo ADV	803 mL ha-1	-	August, 27	-	-
	FP and L	Assure II	494 mL ha^{-1}	-	-	June, 11	-
		Matador	99 mL ha-1	-	-	June, 11	-
	L	Delaro	865 mL ha-1	-	-	July, 3	July, 13
	All			-	-	July, 3	-
Desiccation	L	Reglone	2051 mL ha-1	-	-	August,17	August, 5
Post harvest chemical	FP and L	Edge granular	21000 g ha-1	-	October, 21	-	-

[1]W=Wheat, AC= Argentine canola, YM=Yellow mustard, OrM=Oriental mustard, IM=Industrial mustard, CL=Camelina, FP=Field pea, L=Lentil

Table A. 5. Dates of application of agro-chemicals in the 4-year crop rotation at Scott

Operation	Crops[1]	Fertilizer/Chemical	Application rate	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Pre-seeding burn off	W	Roundup Transorb	1655 mL ha^{-1}	May, 21	-	-	May 16
		Aim	61 mL ha^{-1}	May, 21	-	-	-
		Merge	0.01 L L^{-1}	May, 21	-	-	May 16
		Zidua	121 mL ha^{-1}	-	-	-	May 16
	All	Roundup	1655 mL ha^{-1}	-	-	May 16	-
		Aim	61 mL ha^{-1}	-	-	May 16	-
	FP and L	Crush'R	1655 mL ha^{-1}	-	-	-	May 16
		Heat LQ	54.3 mL ha^{-1}	-	-	-	May 16
		Zidua SC	121 mL ha^{-1}	-	-	-	May 16
		Merge	494.2 mL ha^{-1}	-	-	-	May 16
Post-seeding chemical	W	Axial	1235 mL ha^{-1}	June 24	July 04	July 6	June 22
		Curtail M	2001 mL ha^{-1}	June 24	July 04	-	-
		Prestige XC-A	420 mL ha^{-1}	-	July 03	-	-
		Prestige XC-B	1976 mL ha^{-1}	-	July 03	-	-
		Enforcer	1260 mL ha^{-1}	-	-	July 6	-
		Frontline	1235 mL ha^{-1}	-	-	-	June 22
		MCPA	1186 mL ha^{-1}	-	-	-	June 22
	OrM and CL	Assure II	494 mL ha^{-1}	-	-	-	-
		Sure- Mix	1235 mL ha^{-1}	-	-	July 3	June 22
	AC	Liberty	3335 mL ha^{-1}	-	July 04	July 3	June 22
		Centurion	126 mL ha^{-1}	-	July 04	July	June 22
		Amigo	1260 mL ha^{-1}	-	July 02	-	-
	IM	Assure II	494 mL ha^{-1}	-	July 02	-	June 18
		Muster T & G	296 mL ha^{-1}	-	July 02	-	June 18

[1]W=Wheat, AC= Argentine canola, YM=Yellow mustard, OrM=Oriental mustard, IM=Industrial mustard, CL=Camelina, FP=Field pea, L=Lenti5

Table A.6. Dates of application of agro-chemicals and agro- fertilizers in the 4-year crop rotation at Scott

Operation	Crops	Fertilizer/Chemical	Application rate	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Post-seeding chemical	L[1]	Poast Ultra	380.5 mL ha^{-1}	-		July 6	-
		Solo Ultra	805.5 mL ha^{-1}	-		July 6	-
		Odyssey	43 mL ha^{-1}	-		-	-
		Post Ultra	98.8 mL ha^{-1}	-		-	-
		Merge	500 mL L^{-1}	-		-	-
	FP	Viper ADV	988 mL ha^{-1}	-		-	-
		UAN (28 %)	2001 ml ha^{-1}	-		-	-
		Assure II	494 mL ha^{-1}	-		-	-
Fungicide application	L and FP	Priaxor	444.7 mL ha^{-1}	-		June 21	-
Desiccation	L	Reglona	2051 mL ha^{-1}	-		September 17	-

[1]L=Lentil, FP=Field pea,

Table A.7. Dates of application of agro-chemicals and agro- fertilizers in the 4-year crop rotation at Brooks

Operation	Crops	Fertilizer/Chemical	Application rate	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Pre-seeding burn off	All	Bonanza	9.0 kg ha^{-1}	April, 26	-	-	-
		Round up	6.25 kg ha^{-1}	April, 26	April, 28	April, 28	April, 30
Pre-emergence burn off	All	Estaprop	1250 mL ha^{-1}	-	-	-	-
		Roundup	6.25 kg ha^{-1}	-	-	-	-
Pre-seeding chemical		Trifluralin	8500 mg ha^{-1}	-	-	-	-
Post-seeding chemical	All	Buctril M	1000 mL ł	June, 12	-	-	-
		Headline	500 mg ha	June, 26	-	-	-
		Roundup	1900 mL ha^{-1}	August, 15	-	-	-
		Muster Toss-N-Go	12g+ Ag-Surf 0.5% v/v	-	May, 28	-	-
						-	-
	W	Decis 5 EC	59 mL ha^{-1}	-	May, 29	-	-
		Matador	83 mL ha^{-1}	-	June, 5	-	-
		Buctril M	1000 mL ha^{-1}	-	-	June, 11	-
Desiccation	L and FP	Reglone	2050 mL ha^{-1}	-	-	August, 4 and 5	-

[1]W=Wheat, AC= Argentine canola, YM=Yellow mustard, OrM=Oriental mustard, IM=Industrial mustard, CL=Camelina, FP=Field pea, L=Lentil

Table A.8. Dates of different cultural operations and data collections in the 4-year crop rotation at Swift Current

Operation	Crop(s)	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Spring soil sampling	-	May, 31	April, 16	May, 15	April, 28
Seeding	All	NA[†]	May, 8	May, 12	May, 3 and 5
Plant density counts	All	NA	NA	June, 1	June, 4
Weed counts	All	NA	NA	June, 9	June, 10
Nodulation sampling	Field pea and lentils	-	-	June, 10	-
Weed biomass sampling	All	NA	NA	August, 4	July, 23
Harvest index sampling	Wheat	September, 10	NA	NA	-
Harvest date	Wheat	September 10-14	September, 4	August, 26	August, 16
	Argentine canola	-	September, 16	-	-
	Yellow mustard	-	August, 28	-	-
	Oriental mustard	-	September, 3	-	-
	Industrial mustard	-	September, 16	-	-
	Camelina	-	September, 6	-	-
	Field pea	-	-	August, 12	-
	Lentil	-	-	August, 21	-
Fall soil sampling	-	September 11	Within 36 hours of harvest	August, 27	August, 17

Table A.9. Dates of different cultural operations and data collections in the 4-year crop rotation at Scott

Operation	Crop(s)	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Spring soil sampling	-	May, 20	April, 16	May, 20	April, 28
Seeding	-	May, 25	May, 21- Canola May, 22 – All other crops	June, 2	May, 18 and 19
Plant density counts	-	June, 21	July, 2	Not collected	June, 21
Weed counts	-	NA[1]	NA	Not collected	June, 10
Nodulation sampling	-	-	-	Not collected	-
Weed biomass sampling	-	NA	August, 27	Not collected	August, 9
Harvest index sampling	Wheat	August, 29	October, 4	NA	August, 26
Harvest date	Wheat	September, 14	September, 4	September, 30	September, 1
	Argentine canola	-	October, 10	-	-
	Yellow mustard	-	October, 10	-	-
	Oriental mustard	-	October, 10	-	-
	Industrial mustard	-	October, 10	-	-
	Camelina	-	October, 4	-	-
	Field pea	-	-	September, 18	-
	Lentil	-	-	September, 24	-
Fall soil sampling		October, 4	November, 19	October, 20	October, 6 and 7

[1]NA-Data not available

Table A.10. Dates of different cultural operations and data collections in the 4-year crop rotation at Brooks

Operation	Crop(s)	Year 1 (2018)	Year 2 (2019)	Year 3 (2020)	Year 4 (2021)
Spring soil sampling	-	May, 20	May,1	May, 6	May, 14
Seeding	-	May, 25	May, 3	May, 7	May, 16
Re-seeding	-	-	-	-	-
Plant density counts	-	June, 18	July, 2	NA	NA
Weed counts	-	NA[1]	NA	NA	NA
Nodulation sampling	-	-	-	June, 6	-
Weed biomass sampling	-	NA	August, 27	NA	NA
Harvest index sampling	Wheat	NA	October, 4	NA	NA
Harvest date	Wheat	August, 22	August, 24	August, 20	August, 12
	Argentine canola	-	August, 21	-	-
	Yellow mustard	-	August, 21	-	-
	Oriental mustard	-	August, 21	-	-
	Industrial mustard	-	September, 4	-	-
	Camelina	-	August, 21	-	-
	Field pea	-	-	August, 6	-
	Lentil	-	-	August, 11	-
Fall soil sampling		October, 4	November, 19		NA

Table A.11. Plant density, number of seeds per plant and 1000-seed weight of field pea and lentil in the 2020 crop phase at three sites

Treatment[I]	Different yield components of wheat at different test sites								
	Plant density (number of plants m^2)			Number of seeds per plant			1000-seed weight (g)		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Rotations with field pea									
1= YM -FP -W	93[a]	62	74	22	45	38	187	178	198
2= IM -FP -W	65[b]	58	72	22	39	34	187	180	200
3= AC -FP -W	78[ab]	59	85	25	34	35	190	177	195
4= OrM -FP -W	85[a]	52	86	26	45	37	189	175	196
5= CL -FP -W	82[ab]	67	74	27	43	35	192	178	196
***P* value**	**0.0042**[§]	0.2701	0.2687	0.3176	0.1897	0.9165	0.1777	0.9065	0.5702
Rotations with lentil									
6= IM -L -W	122	117	132	81	89	90	31.2	31.0	32.4
7= AC -L -W	121	133	140	81	100	62	31.3	31.4	33.0
8= OrM -L -W	120	111	146	61	91	77	31.0	30.4	33.0
9= CL -L -W	128	116	125	72	74	72	31.2	30.5	32.9
***P* value**	0.6789	**0.0366**	0.1286	0.2061	0.0834	0.1746	0.8700	0.6607	0.4260
	***P* values and contrast values from pre-planned comparisons**								
Wheat preceded by field pea on;									
Argentine canola vs. the average of	-3.25	-0.75	+8.50	+0.75	-9.00	-1.00	+1.29	-0.75	-2.50
other Brassicaceae crop	0.47	0.89	0.21	0.92	**0.04**	0.72	0.57	0.93	0.30
Camelina (non-mustard) vs. the	+1.00	+9.66	-3.33	+3.66	0.00	-1.33	+6.33	+0.33	-2.00
average of mustard crop	0.89	0.10	0.65	0.14	0.94	0.71	**0.03**	0.96	0.52
Yellow mustard vs. the average of	+18.0	+7.00	-5.00	-2.00	+3.00	+2.50	-1.00	+0.50	0.00
industrial and oriental mustard crop	**0.003**	0.23	0.40	0.46	0.55	0.53	0.93	0.91	0.88
Wheat preceded by lentil on;									
Argentine canola vs. the average of	-2.33	+18.3	+5.66	+9.66	+15.33	-17.66	0.00	+0.63	-0.63
other Brassicaceae crop	0.70	**0.007**	0.45	0.24	0.06	0.09	0.98	0.67	0.11
Camelina (non-mustard) vs. the	+7.00	-2.00	-14.0.0	+1.00	-16.00	-11.50	-0.3	+0.05	-0.05
average of mustard crop	0.28	0.84	0.08	0.93	0.07	0.27	0.43	0.99	0.80

[I]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each site for each parameter. Values with different letters within each column are significantly different at $P>0.05$, according to Tukey's HSD test. [§ns]: Bolded *P* values denote significance at $P<0.05$, according to Tukey's HSD test. ‡ Contrast value = value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value

Table A.12. Grain yield and harvest index (HI) of field pea and lentil in the 2020 crop phase at three sites

Treatment	Grain yield			HI		
	Swift Current	Scott	Brooks	Swift Current	Scott	Brooks
Rotations with field pea						
1= YM-FP-W	4298	4368	2264	0.45	0.57	0.47
2= IM-FP-W	4126	4607	2488	0.44	0.56	0.44
3= AC-FP-W	4166	4383	2443	0.45	0.53	0.45
4= OrM-FP-W	4091	4369	2378	0.46	0.55	0.45
5= CL-FP-W	4416	4488	2441	0.47	0.57	0.44
***P* value**	0.1821	0.5691	0.3441	0.4283	0.0709	5071
Rotations with lentil						
6= IM-L-W	2563	1962	1357	0.44	0.41	0.39
7= AC-L-W	2594	2538	1293	0.47	0.44	0.42
8= OrM-L-W	2385	2354	1345	0.45	0.43	0.46
9= CL-L-W	2596	2384	1419	0.46	0.42	0.41
***P* value**	0.2481	0.3521	0.8028	0.5481	0.8569	0.3331
***P* values and contrast values from pre-planned comparisons**						
Field pea grown on;						
Argentine canola vs. the average of other	-66.8[¶]	-75	+50.2	-0.005	-0.03	0.00
Brassicaceae crop stubble	0.55	0.58	0.57	0.91	**0.02**[*]	0.83
Camelina (non-mustard) vs. the average of mustard	+244	+40	+64.3	+0.02	+0.01	-0.01
crop stubble	**0.06**	0.77	0.48	0.16	0.59	0.36
Yellow mustard vs. the average of industrial and	+189	-120	-169	0.00	+0.02	+0.02
oriental mustard crop stubble	0.14	0.42	0.10	0.64	0.10	0.19
Lentil grown on;						
Argentine canola vs. the average of other	-79.3	-304	-80.6	+0.02	+0.02	0.00
Brassicaceae crop stubble	0.40	0.26	0.45	0.21	0.49	0.96
Camelina (non-mustard) vs. the average of mustard	+122	+226	+68.0	+0.02	0.00	-0.02
crop stubble	0.23	0.42	0.55	0.55	0.89	0.51

[¶]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each site for each parameter. Values with different letters within each column are significantly different at $P>0.05$, according to Tukey's HSD test. [§ns]: Bolded *P* values denote significance at $P<0.05$, according to Tukey's HSD test. ‡ Contrast value = value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value

Table A.13. Above-ground biomass (kg ha^{-1}) of different crop sequences in 2019 and 2020 crop phase at three sites

Treatment[l]	Above-ground biomass in 2019 and 2020 crop phases at three test sites					
	Swift Current		Scott		Brooks	
	2019	2020	2019	2020	2019	2020
1= YM-P-W	4534^{bc‡}	5166^{ab}	6160^{bcd}	3249^{b}	3365	2555^{abcd}
2= IM-P-W	4906^{abc}	5268^{a}	7908^{a}	3692^{ab}	7628	3242^{a}
3= AC-P-W	5793^{ab}	5028^{ab}	6878^{ab}	3852^{ab}	4547	2944^{ab}
4= OrM-P-W	4293^{bc}	4855^{abc}	5099de	3570^{b}	3390	2849^{abc}
5= CL-P-W	4296^{bc}	5067^{ab}	4332^{e}	3437^{b}	8091	3159^{ab}
6= IM-L-W	4700^{bc}	3266^{def}	7771^{a}	2653^{b}	9438	2069^{bcd}
7= AC-L-W	6575^{a}	2882^{efgh}	6656^{abc}	3353^{b}	4394	1787^{cd}
8= OrM-L-W	4555^{bc}	2988^{defg}	5494^{cde}	3129^{b}	4159	1620^{d}
9= CL-L-W	3599^{c}	3113^{defg}	4294^{e}	3125^{b}	7544	2081^{bcd}
10= W-W-W	5593^{ab}	4310^{abc}	2149^{f}	4891^{a}	3107	3222^{a}
***P* values**	**0.0001**[§]	**<0.0001**	**<0.0001**	**0.0001**	0.0777	**<0.0001**
Contrast and *P* values from pre-planned comparison						
Continuous wheat vs. the average of Brassicaceae and pulse crop rotations	+787[¶]	+128	-3916	+1551	-2732	+743
	0.05	0.60	**<0.0001**	**<0.0001**	0.1288	**0.0043**
The average of rotations with field pea vs. lentil	-240	+1765	+802	+129	-324	+794
	0.9415	**<0.0001**	**<0.0001**	**0.005**	0.1258	**<0.0001**
The average of rotations with Argentine canola vs. other Brassicaceae crops	+1772	-291	+901	+337	-1760	-145
	0.10	0.13	**<0.0001**	0.10	0.0555	0.43
The average of rotations with camelina (non-mustard) vs. mustard crops	-650	+218	-2173	+22.4	2221	+153
	0.18	0.270	**0.0004**	0.91	0.9775	0.42
Rotation with yellow mustard vs. the average of industrial and oriental mustard	-79.5	+1071	-408	-12	-2788	+110
	0.69	**0.0003**	**<0.0001**	0.96	0.4295	0.66
The average of rotations with industrial mustard vs. oriental mustard	+379	+345	+2543	-177	+4758	+421
	<0.0001	0.15	**<0.0001**	0.48	0.0531	0.07

[l]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. [‡]Comparisons are for each site for each parameter. Values with different letters within each column are significantly different at $P>0.05$, according to Tukey's HSD test. [§]Bolded *P* values denote significance at $P<0.05$, according to Tukey's HSD test. [¶]Contrast value = value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value.

APPENDIX B

Table B.1. Change in soil electrical conductivity change (mS cm^{-1}) at 0-15 cm depth due to crop sequences at three test sites

Treatment[l]	Swift Current[‡]	Scott	Brooks
	----------	Soil EC	----------
1= **YM**-FP-**W**	-0.10[a§]	+0.62[a]	-0.22[a]
2= **IM**-FP-**W**	-0.75[a]	-0.15[a]	-0.07[a]
3= **AC**-FP-**W**	-0.67[a]	-0.25[a]	-0.12[a]
4= **OrM**-FP-**W**	+0.22[a]	-0.22[a]	+0.05[a]
5= **CL**-FP-**W**	-0.35[a]	-0.27[a]	+0.35[a]
6= **IM**-L-**W**	-0.67[a]	-0.15[a]	+0.52[a]
7= **AC**-L-**W**	-0.40[a]	-0.27[a]	+0.02[a]
8= **OrM**-L-**W**	-0.47[a]	-0.45[a]	-0.10[a]
9= **CL**-L-**W**	-0.27[a]	-0.07[a]	+0.27[a]
10= **W**-W-**W**	-0.42[a]	-0.17[a]	-0.15[a]
***P* value**	0.1574	0.0826	0.2678
	----------	Contrast and *P* values	----------
Continuous wheat vs. the average of Brassicaceae and pulse rotations	-0.04[¶]	-0.03	-0.22
	0.8740	0.8002	0.3227
Field pea grown on[#];			
Argentine canola vs. the average of other Brassicaceae crop stubble	-0.40	-0.20	-0.20
	0.1057	0.1426	0.5369
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.10	-040	+0.40
	0.5988	0.1407	0.916
Yellow mustard vs. the average of oriental and industrial mustard stubble	+0.20	+0.60	-0.20
	0.5695	0.1762	0.4257
Lentil grown on			
Argentine canola vs. the average of other Brassicaceae crop stubble	+0.10	-0.10	-0.20
	0.7802	0.7665	0.4077
Camelina (non-mustard) vs. the average of mustard crop stubble	-0.30	-0.20	+0.10
	0.2972	0.2140	0.8131

[l]W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Bolded letters in a sequence indicate soil sampling phases. [‡] Values are soil EC in spring 2021, before planting wheat - soil pH in spring 2019, before planting oilseed crops. [§]Treatment values with different letters within each test site are significantly different at $P \leq 0.05$. [¶] Contrast value = value on the left - value on the right in the comparison with *P* value following the contrast value. [#]Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard.

Table B.2. Soil organic carbon (SOC) concentration (g kg^{-1} of soil) in different crop sequences at Swift Current in the springs of 2019 and 2021

Treatment[l]	SOC content at different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	mean
YM-FP-W	12.2^{a}	16.0^{a}	7.60^{a}	12.0^{a}	4.53^{a}	9.19^{a}	8.10^{d}	12.4^{a}	10.2^{a}
IM-FP-W	11.8^{a}	13.4^{a}	7.83^{a}	9.60^{a}	5.90^{a}	7.78^{a}	8.51^{d}	10.3^{bc}	9.40^{b}
AC-FP-W	11.8^{a}	14.2^{a}	7.83^{a}	9.50^{a}	5.90^{a}	7.82^{a}	8.51^{d}	10.5^{bc}	9.52^{b}
OrM-FP-W	11.8^{a}	14.1^{a}	7.83^{a}	9.60^{a}	5.90^{a}	7.55^{a}	8.51^{d}	10.4^{bc}	9.46^{b}
CL-FP-W	11.8^{a}	13.7^{a}	7.83^{a}	9.55^{a}	5.90^{a}	6.88^{a}	8.51^{d}	10.0^{bc}	9.28^{b}
IM-L-W	11.8^{a}	14.0^{a}	7.83^{a}	9.40^{a}	5.90^{a}	7.28^{a}	8.51^{d}	10.2^{bc}	9.36^{b}
AC-L-W	11.8^{a}	13.8^{a}	7.83^{a}	10.0^{a}	5.90^{a}	7.58^{a}	8.51^{d}	10.5^{bc}	9.49^{b}
OrM-L-W	11.8^{a}	13.0^{a}	7.83^{a}	9.38^{a}	5.90^{a}	7.18^{a}	8.51^{d}	9.8^{bc}	9.18^{b}
CL-L-W	11.8^{a}	13.0^{a}	7.83^{a}	9.15^{a}	5.90^{a}	6.50^{a}	8.51^{d}	9.5^{bc}	9.03^{b}
W-W-W	11.8^{a}	12.6	7.83^{a}	9.72^{a}	5.90^{a}	6.80^{a}	8.51^{d}	9.7^{c}	9.12^{b}
Mean	12.8^{a}		8.79^{b}		6.60^{c}		8.47^{b}	10.33^{a}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	**<0.0001**[§]	T×D	0.3441	T×D×Y	0.8694
Y	**<0.0001**	T×Y	**<0.0001**		
D	**<0.0001**	D×Y	0.3388		

[l]W=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. [‡] Values with different letters in each category (treatment, depth and crop phase) are significantly different ($P \leq 0.05$). [§] Bolded *P* values denote significant ($P \leq 0.05$).

Table B.3. Soil organic carbon (SOC) concentration (g kg^{-1} of soil) in different crop sequences at Scott in the springs of 2019 and 2021

Treatment[l]	SOC content at different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	mean
YM-FP-W	17.9^{a}	25.5^{a}	8.85^{a}	13.1^{a}	7.10^{a}	8.45^{a}	11.3^{a}	15.7^{a}	13.5^{a}
IM-FP-W	19.5^{a}	25.3^{a}	9.88^{a}	15.2^{a}	7.15^{a}	10.8^{a}	12.2^{a}	17.1^{a}	14.6^{a}
AC-FP-W	19.5^{a}	23.8^{a}	9.88^{a}	14.6^{a}	7.15^{a}	11.2^{a}	12.2^{a}	16.5^{a}	14.3^{a}
OrM-FP-W	19.5^{a}	23.4^{a}	9.88^{a}	15.8^{a}	7.15^{a}	9.94^{a}	12.2^{a}	16.3^{a}	14.3^{a}
CL-FP-W	19.5^{a}	25.8^{a}	9.88^{a}	17.6^{a}	7.15^{a}	12.1^{a}	12.2^{a}	18.5^{a}	15.3^{a}
IM-L-W	19.5^{a}	26.0^{a}	9.88^{a}	18.6^{a}	7.15^{a}	15.1^{a}	12.2^{a}	19.9^{a}	16.0^{a}
AC-L-W	19.5^{a}	26.4^{a}	9.88^{a}	15.8^{a}	7.15^{a}	11.6^{a}	12.2^{a}	18.0^{a}	15.1^{a}
OrM-L-W	19.5^{a}	23.7^{a}	9.88^{a}	15.4^{a}	7.15^{a}	13.0^{a}	12.2^{a}	16.9^{a}	14.5^{a}
CL-L-W	19.5^{a}	24.2^{a}	9.88^{a}	16.5^{a}	7.15^{a}	9.53^{a}	12.2^{a}	16.1^{a}	14.2^{a}
W-W-W	19.5^{a}	24.6^{a}	9.88^{a}	17.1^{a}	7.15^{a}	10.3^{a}	12.2^{a}	17.3^{a}	14.7^{a}
Mean	22.1^{a}		12.8^{b}		9.1^{c}		12.1^{b}	17.2^{a}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	P value	Effect	*P* value	Effect	*P* value
T	0.0528[§]	T×D	0.9945	T×D×Y	0.9390
Y	**<0.0001**	T×Y	0.1394		
D	**<0.0001**	D×Y	**0.0116**		

[l]W=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. [‡] Values with different letters in each category (treatment, depth and crop phase) are significantly different at $P \leq 0.05$. [§] Bolded *P* values denote significant ($P \leq 0.05$).

Table B.4. Soil organic carbon (SOC) concentration (g kg^{-1} of soil) in different crop sequences at Brooks in the springs of 2019 and 2021

Treatment[l]	SOC content in different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	mean
YM-FP-W	10.6[a]	11.4[a]	8.80[a]	9.90[a]	6.32[a]	7.60[a]	8.50[a]	9.64[a]	9.10[a]
IM-FP-W	10.1[a]	11.6[a]	9.25[a]	9.65[a]	6.10[a]	8.70[a]	8.48[a]	9.97[a]	9.23[a]
AC-FP-W	10.1[a]	12.3[a]	9.25[a]	9.88[a]	6.10[a]	8.08[a]	8.48[a]	10.1[a]	9.28[a]
OrM-FP-W	10.1[a]	11.7[a]	9.25[a]	9.42[a]	6.10[a]	7.12[a]	8.48[a]	9.42[a]	8.95[a]
CL-FP-W	10.1[a]	11.8[a]	9.25[a]	9.35[a]	6.10[a]	8.25[a]	8.48[a]	9.78[a]	9.13[a]
IM-L-W	10.1[a]	11.8[a]	9.25[a]	9.75[a]	6.10[a]	8.32[a]	8.48[a]	9.94[a]	9.21[a]
AC-L-W	10.1[a]	11.8[a]	9.25[a]	10.50[a]	6.10[a]	8.47[a]	8.48[a]	10.1[a]	9.38[a]
OrM-L-W	10.1[a]	11.7[a]	9.25[a]	10.51[a]	6.10[a]	8.40[a]	8.48[a]	10.2[a]	9.35[a]
CL-L-W	10.1[a]	11.8[a]	9.25[a]	9.82[a]	6.10[a]	8.35[a]	8.48[a]	9.98[a]	9.23[a]
W-W-W	10.1[a]	12.9[a]	9.25[a]	8.98[a]	6.10[a]	8.32[a]	8.48[a]	10.1[a]	9.28[a]
Mean	11.0[a]		9.49[b]		7.14[c]		8.49[b]	9.93[a]	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	0.4721[§]	T×D	0.4620	T×D×Y	0.1575
Y	**<0.0001**	T×Y	0.3446		
D	**<0.0001**	D×Y	**<0.0001**		

[l]W=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. [‡] Values with different letters in each category (treatment, depth and crop phase) are significantly different ($P \leq 0.05$). [§] Bolded *P* values denote significant ($P \leq 0.05$).

Table B.5. Total soil nitrogen (TSN) concentration (g kg^{-1} of soil) in different crop sequences at Swift Current in the springs of 2019 and 2021

Treatment[ł]	TSN content at different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	mean
YM-FP-W	1.35^{a}	1.62^{a}	0.89^{a}	1.20^{a}	0.40^{a}	0.92^{a}	0.88^{a}	1.25^{a}	1.06^{a}
IM-FP-W	1.31^{a}	1.34^{a}	3.03^{a}	0.97^{a}	0.51^{a}	0.78^{a}	1.62^{a}	1.03^{a}	1.33^{a}
AC-FP-W	1.33^{a}	1.42^{a}	3.03^{a}	0.93^{a}	0.51^{a}	0.79^{a}	1.62^{a}	1.05^{a}	1.34^{a}
OrM-FP-W	1.33^{a}	1.45^{a}	3.03^{a}	1.00^{a}	0.51^{a}	0.76^{a}	1.62^{a}	1.07^{a}	1.35^{a}
CL-FP-W	1.33^{a}	1.33^{a}	3.03^{a}	0.94^{a}	0.51^{a}	0.66^{a}	1.62^{a}	0.98^{a}	1.30^{a}
IM-L-W	1.33^{a}	1.41^{a}	3.03^{a}	0.98^{a}	0.51^{a}	0.74^{a}	1.62^{a}	1.04^{a}	1.33^{a}
AC-L-W	1.33^{a}	1.40^{a}	3.03^{a}	1.05^{a}	0.51^{a}	0.79^{a}	1.62^{a}	1.08^{a}	1.35^{a}
OrM-L-W	1.33^{a}	1.33^{a}	3.03^{a}	1.00^{a}	0.51^{a}	0.70^{a}	1.62^{a}	1.01^{a}	1.32^{a}
CL-L-W	1.27^{a}	1.32^{a}	3.03^{a}	0.91^{a}	0.51^{a}	0.64^{a}	1.62^{a}	0.96^{a}	1.29^{a}
W-W-W	1.33^{a}	1.42^{a}	3.03^{a}	0.99	0.51^{a}	0.66^{a}	1.62^{a}	1.02^{a}	1.32^{a}
Mean	1.36^{a}		1.91^{a}		0.62^{b}		1.55^{a}	1.04^{b}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	0.9999[§]	T×D	1.000	T×D×Y	1.000
Y	**0.0135**	T×Y	0.9887		
D	**<0.0001**	D×Y	**<0.0001**		

łW=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. ‡Values with different letters within each category (treatment, depth and crop phase) are significantly different (P>0.05). § Bolded P values denote significant (P≤0.05).

Table B.6. Total soil nitrogen (TSN) concentration (g kg^{-1} of soil) in different crop sequences at Scott in the springs of 2019 and 2021

Treatment[ł]	TSN content at different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall mean
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	
YM-FP-W	1.90^{bc}	2.17^{bc}	0.99^{bc}	1.41^{bc}	0.45^{b}	0.88^{c}	2.48^{a}	1.48^{b}	1.98^{a}
IM-FP-W	2.11^{bc}	2.49^{bc}	1.12^{bc}	1.32^{bc}	0.74^{a}	1.09^{bc}	1.33^{b}	1.63^{ab}	1.48^{a}
AC-FP-W	2.11^{bc}	2.26^{bc}	1.12^{bc}	1.61^{bc}	0.74^{a}	1.11^{bc}	1.33^{b}	1.66^{ab}	1.49^{a}
OrM-FP-W	2.11^{bc}	2.25^{bc}	1.12^{bc}	1.47^{bc}	0.74^{a}	0.98^{bc}	1.33^{b}	1.56^{b}	1.45^{a}
CL-FP-W	2.11^{bc}	2.45^{bc}	1.12^{bc}	1.63^{bc}	0.74^{a}	1.20^{bc}	1.33^{b}	1.76^{b}	1.54^{a}
IM-L-W	2.11^{bc}	2.46^{bc}	1.12^{bc}	1.79^{bc}	0.74^{a}	1.60^{bc}	1.33^{b}	1.95^{b}	1.64^{a}
AC-L-W	2.11^{bc}	2.66^{b}	1.12^{bc}	1.77^{bc}	0.74^{a}	1.17^{bc}	1.33^{b}	1.87^{b}	1.60^{a}
OrM-L-W	2.11^{bc}	2.32^{bc}	1.12^{bc}	1.58^{bc}	0.74^{a}	1.19^{bc}	1.33^{b}	1.70^{b}	1.51^{a}
CL-L-W	2.11^{bc}	2.30^{bc}	1.12^{bc}	1.50^{bc}	0.74^{a}	1.02^{bc}	1.33^{b}	1.61^{ab}	1.47^{a}
W-W-W	2.11^{bc}	2.29^{bc}	1.12^{bc}	1.51^{bc}	0.74^{a}	1.13^{bc}	1.33^{b}	1.61^{ab}	1.47^{a}
Mean	2.22^{a}		1.33^{b}		1.12^{b}		1.44^{b}	1.68^{a}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	0.1019[§]	T×D	**0.0003**	T×D×Y	**<0.0001**
Y	**0.0024**	T×Y	**0.0009**		
D	**<0.0001**	D×Y	0.0657		

łW=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. ‡ Values with different letters within each category (treatment, depth and crop phase) are significantly different (P>0.05). § Bolded P values denote significant (P≤0.05).

Table B.7. Total soil nitrogen (TSN) concentration (g kg^{-1} of soil) in different crop sequences at Brooks in the springs of 2019 and 2021

Treatment[ł]	TSN content in different soil depths in different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2019[‡]	2021	2019	2021	2019	2021	2019	2021	mean
YM-FP-W	1.15^{a}	1.24^{a}	0.942^{a}	1.05^{a}	0.560^{a}	0.698^{a}	0.845^{d}	1.00^{abc}	0.939^{ab}
IM-FP-W	1.04^{a}	1.27^{a}	0.957^{a}	1.03^{a}	0.536^{a}	0.795^{a}	0.883^{cd}	1.03^{ab}	0.940^{ab}
AC-FP-W	1.04^{a}	1.33^{a}	0.957^{a}	1.05^{a}	0.536^{a}	0.725^{a}	0.845^{d}	1.04^{ab}	0.941^{ab}
OrM-FP-W	1.04^{a}	1.22^{a}	0.957^{a}	0.94^{a}	0.536^{a}	0.588^{a}	0.845^{d}	0.92^{bcd}	0.881^{ab}
CL-FP-W	1.04^{a}	1.22^{a}	0.957^{a}	0.86^{a}	0.536^{a}	0.838^{a}	0.845^{d}	0.97^{abcd}	0.909^{ab}
IM-L-W	1.04^{a}	1.22^{a}	0.957^{a}	0.99^{a}	0.536^{a}	0.796^{a}	0.845^{d}	1.00^{abc}	0.924^{ab}
AC-L-W	1.04^{a}	1.18^{a}	0.957^{a}	1.02^{a}	0.536^{a}	0.817^{a}	0.845^{d}	1.00^{abc}	0.925^{ab}
OrM-L-W	1.04^{a}	1.42^{a}	0.957^{a}	1.08^{a}	0.536^{a}	0.796^{a}	0.845^{d}	1.10^{a}	0.972^{a}
CL-L-W	1.04^{a}	1.17^{a}	0.957^{a}	0.93^{a}	0.536^{a}	0.721^{a}	0.845^{d}	0.94^{bcd}	0.893^{ab}
W-W-W	1.04^{a}	1.28^{a}	0.957^{a}	0.96^{a}	0.536^{a}	0.731^{a}	0.845^{d}	1.00^{abc}	0.918^{ab}
Mean	1.15^{a}		0.97^{b}		0.64^{c}		0.84^{b}	0.99^{a}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × crop phase (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	**0.0316[§]**	T×D	0.4445	T×D×Y	0.2754
Y	**<0.0001**	T×Y	**0.0281**		
D	**<0.0001**	D×Y	**<0.0001**		

łW=Wheat, YM=Yellow Mustard, FP=Field Pea, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, L=Lentil. ‡ Values with different letters within each category (treatment, depth and crop phase) are significantly different (P>0.05). § Bolded P values denote significant (P≤0.05).

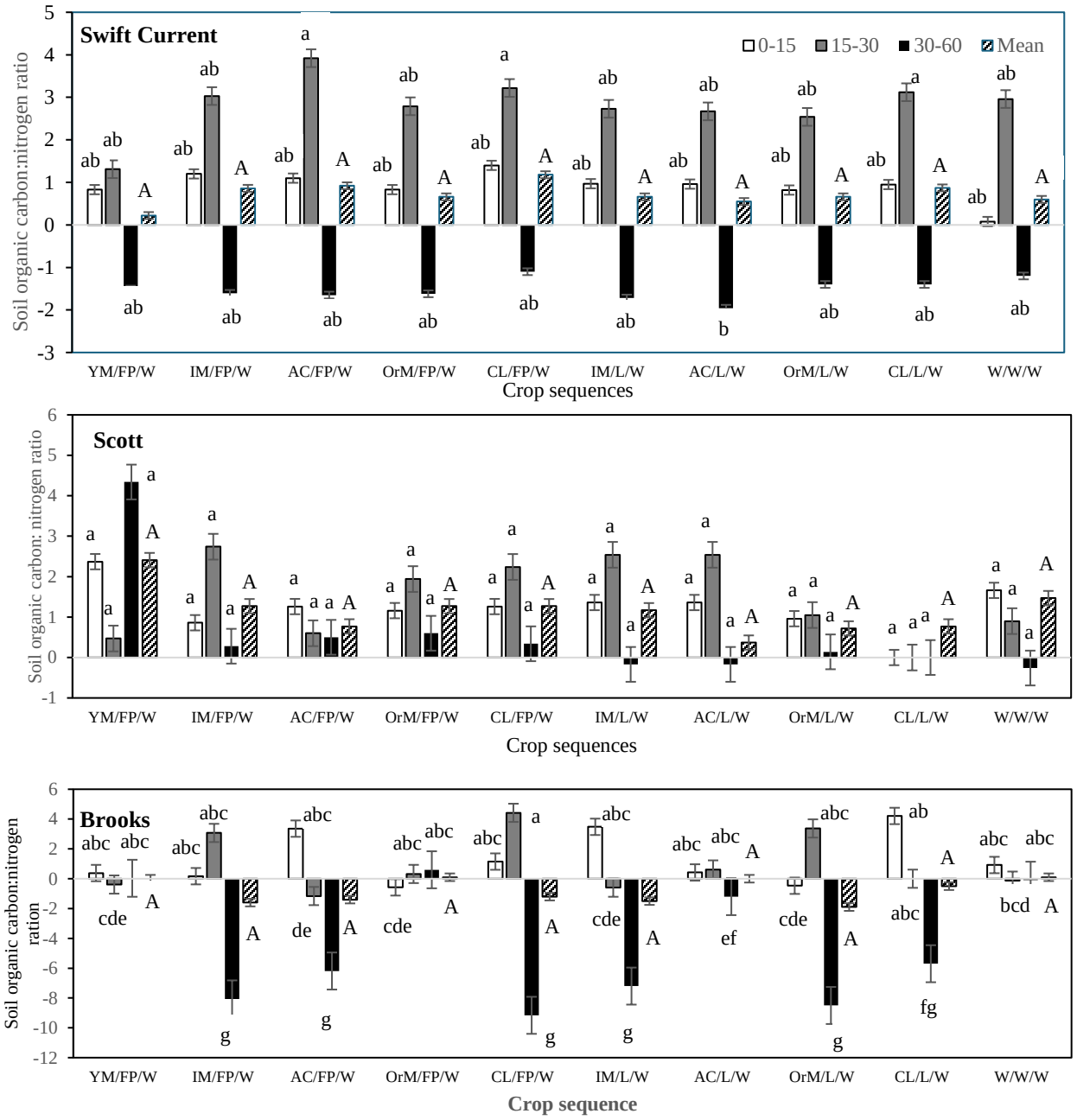

Fig. B.1. Soil organic carbon: nitrogen ratio changes after growing different crop sequences at three test sites from spring 2019 (before planting oilseed crops) to spring 2021 (before planting wheat). W=Wheat, YM=Yellow mustard, FP=Field pea, IM=Industrial mustard, AC=Argentine canola, OrM=Oriental mustard, CL=Camelina, L=Lentil. Within a site, lower-case letters indicate significant differences among crop sequences across all soil depths and upper-case letter indicate differences between means for each crop sequence ($P \leq 0.05$). Note: The y-axis in different graphs are in different scales. Bars indicate standard error of means

Table B.8. Summary of *P* values for main and interaction effects of treatments and soil depth for soil organic carbon:total soil nitrogen change at three test sites

Effect	Swift Current	Scott	Brooks
Treatment	0.9883	0.5674	0.0083
Depth	**<0.0001** [†]	0.1578	**<0.0001**
Treatment × Depth	0.9999	0.5347	**<0.0001**

[†] Bolded *P* values indicate significant differences ($P \leq 0.05$). Note: Soils were sampled in spring 2019 before planting oilseed crops and in spring 2021 before planting wheat.

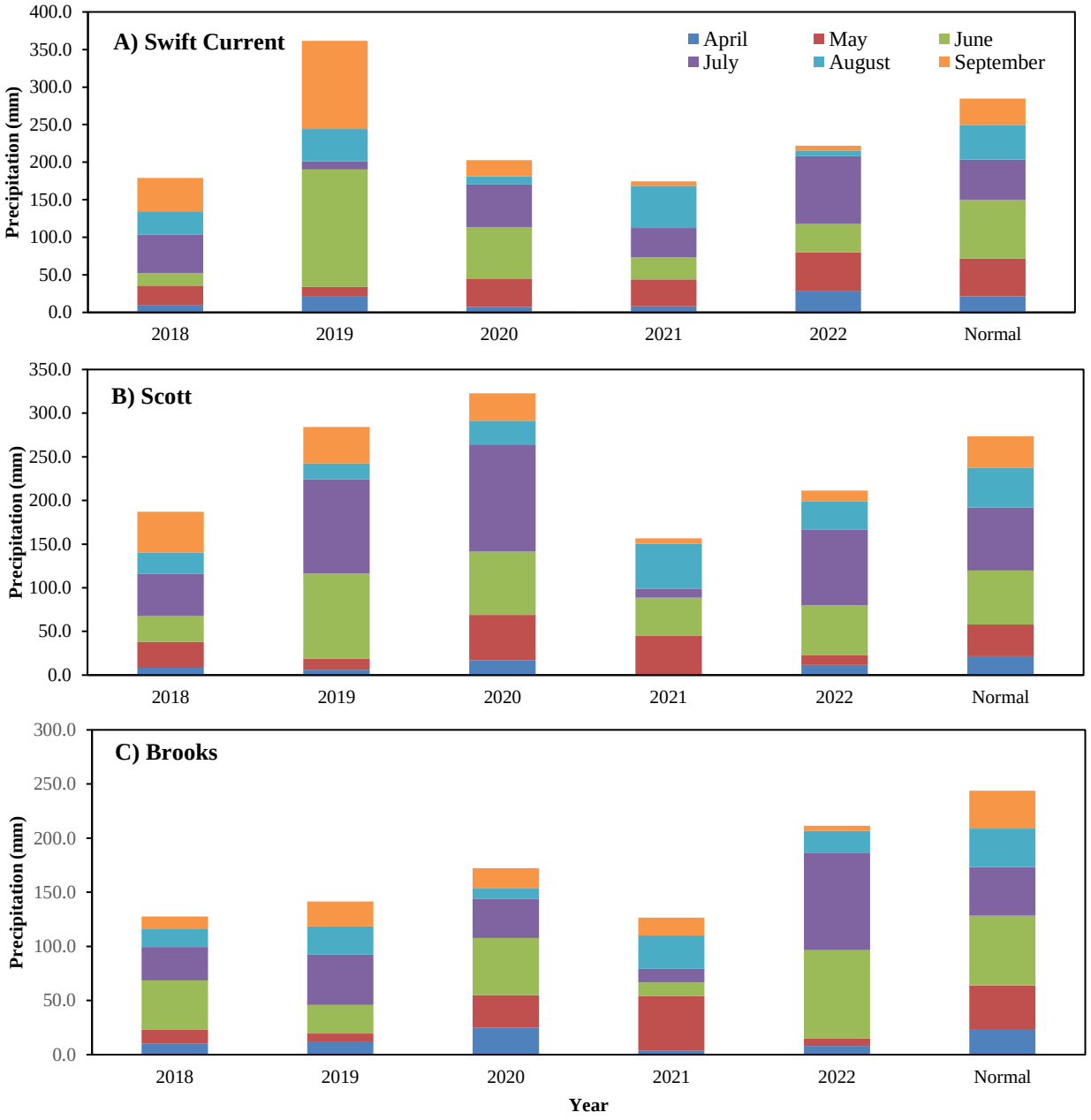

Fig. B.2. Precipitation (mm) from April to September at A) Swift Current B) Scott and C) Brooks from 2018 to 2022. Normal precipitation is the 30-year average precipitation (1991-2021)

Table B.9. Monthly temperature during the crop growing season at Swift Current from 2018-2020.

Month	Temperature (ºC)							
	2018		2019		2020		2021	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
April	-0.8	11.1	-4.3	6.5	-1.1	12.3	-3.8	6.4
May	5.0	19.7	7.3	21.9	2.1	16.9	6.2	15.2
June	8.9	22.4	10.2	24.0	9.4	22.2	9.3	21.3
July	12.5	28.7	11.5	26.1	10.8	24.6	11.6	23.8
August	10.9	25.8	10.9	26.4	10.0	23.5	10.3	28.1
September	6.3	20.3	4.1	14.4	7.0	17.7	5.3	20.7

Table B.10. Monthly temperature during the crop growing season at Scott from 2018-2020

Month	Temperature (ºC)							
	2018		2019		2020		2021	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
April	-2.1	8.3	-8.5	4.2	-2.9	11.3	-6.4	3.4
May	3.7	18.3	5.7	21.5	1.1	17	4.3	15.4
June	8.3	21.8	9.0	23.3	8.2	21.7	8.1	21.0
July	10.2	25.4	10.3	24.6	9.8	22.4	10.2	24.2
August	8.7	23.6	7.9	24.1	7.9	20.9	8.2	24.4
September	3.1	19.1	2.5	13.1	5.4	17.1	3.2	18.4

Table B.11. Monthly temperature during the crop growing season at Brooks from 2018-2020

Month	Temperature (ºC)							
	2018		2019		2020		2021	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
April	-1.7	12.9	-5.6	9.0	-2.1	14.5	-3.4	10.4
May	4.9	21.8	5.0	23.9	1.9	18.3	3.2	18.3
June	8.4	23.9	8.1	25.2	8.2	23.9	8.5	22.4
July	11.5	29.6	9.8	28.4	10.2	26.1	10.4	26.0
August	8.7	27.2	8.4	26.9	9.9	24.7	10.3	27.5
September	4.0	21.6	2.2	15.8	5.5	18.6	4.8	21.5

Table B.12. Soil moisture concentration (g kg^{-1} of soil) of different crop rotations at Swift Current in springs of 2020 and 2021

Treatment[l]	Spring soil moisture content at different depths at different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2020[‡]	2021	2020	2021	2020	2021	2020	2021	Mean
YM-FP-W	0.07^{lmnopqr}	0.088^{ijklmn}	0.06^{nopqr}	0.072^{lmnopqr}	0.11^{efghijk}	0.05^{opqr}	**0.08^{c}**	**0.07^{cd}**	0.075^{c}
IM-FP-W	0.15^{abcd}	0.091^{ijklmn}	0.12^{cdefghi}	0.067^{lmnopqr}	0.080^{klmnop}	0.044^{r}	**0.12^{b}**	**0.07^{cd}**	0.092^{b}
AC-FP-W	0.16^{a}	0.093^{hijlmn}	0.13^{abcde}	0.074^{lmnopqr}	0.11^{efghijk}	0.048^{pqr}	**0.13^{a}**	**0.07^{cd}**	0.103^{a}
OrM-FP-W	0.14^{abcd}	0.085^{klmn}	0.14^{abcd}	0.065^{lmnopqr}	0.10^{fghijkl}	0.049^{opqr}	**0.12^{ab}**	**0.07^{cd}**	0.096^{ab}
CL-FP-W	0.14^{abcd}	0.091^{ijklmn}	0.13^{abcde}	0.068^{lmnopqr}	0.10^{fghijkl}	0.048^{pqr}	**0.12^{ab}**	**0.07^{cd}**	0.095^{ab}
IM-L-W	0.15^{abcd}	0.086^{jklmn}	0.14^{abcd}	0.068^{lmnopqr}	0.084^{klmn}	0.043^{r}	**0.12^{ab}**	**0.07^{cd}**	0.095^{ab}
AC-L-W	0.15^{abcd}	0.085^{klmn}	0.13^{abcde}	0.062^{mnopqr}	0.087^{ijklmn}	0.047^{qr}	**0.12^{ab}**	**0.07^{cd}**	0.093^{ab}
OrM-L-W	0.14^{abcd}	0.080^{klmnopq}	0.12^{cdefghi}	0.062^{nopqr}	0.098^{fghijkl}	0.046^{r}	**0.12^{ab}**	**0.06^{d}**	0.092^{b}
CL-L-W	0.15^{abcd}	0.10^{fghijkl}	0.13^{abcde}	0.067^{lmnopqr}	0.093^{hijklmn}	0.047^{pqr}	**0.12^{ab}**	**0.07c^{d}**	**0.098^{ab}**
W-W-W	0.16^{ab}	0.082^{klmno}	0.14^{abcd}	0.069^{lmnopqr}	0.10^{ghijkl}	0.041^{r}	**0.13^{ab}**	**0.06^{d}**	**0.10^{ab}**
Mean	**0.11^{a}**		**0.09^{b}**		**0.07^{c}**		**0.12^{a}**	**0.07^{b}**	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × sampling year (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	<0.0001	T×D	<0.0001	T×D×Y	<0.0001
Y	<0.0001	T×Y	<0.0001		
D	<0.0001	D×Y	0.2091		

[l]W=Wheat, YM=Yellow Mustard, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, FP=Field Pea, L=Lentil. [‡]Values with different letters within each category (treatment, depth and crop phase) are significantly different ($P \leq 0.05$). [§] Bolded *P* values denote significant ($P \leq 0.05$).

Table B.13. Soil moisture concentration (g kg^{-1} of soil) of different crop rotations at Scott in the springs of 2020 and 2021

Treatment[l]	Spring soil moisture content at different depths at different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2020[‡]	2021	2020	2021	2020	2021	2020	2021	Mean
YM-FP-W	0.12	0.25	0.07	0.13	0.11	0.10	**0.10**	**0.16**	**0.13^{b}**
IM-FP-W	0.28	0.27	0.17	0.15	0.12	0.10	**0.19**	**0.17**	**0.18^{a}**
AC-FP-W	0.27	0.28	0.16	0.14	0.13	0.11	**0.19**	**0.18**	**0.18^{a}**
OrM-FP-W	0.26	0.26	0.16	0.13	0.13	0.10	**0.18**	**0.16**	**0.17^{ab}**
CL-FP-W	0.26	0.26	0.14	0.32	0.14	0.11	**0.18**	**0.23**	**0.20^{a}**
IM-L-W	0.27	0.27	0.17	0.14	0.13	0.11	**0.19**	**0.18**	**0.18^{a}**
AC-L-W	0.25	0.28	0.15	0.15	0.13	0.12	**0.18**	**0.18**	**0.18^{a}**
OrM-L-W	0.24	0.27	0.15	0.15	0.11	0.10	**0.17**	**0.17**	**0.17^{ab}**
CL-L-W	0.26	0.28	0.16	0.14	0.13	0.11	**0.18**	**0.18**	**0.18^{a}**
W-W-W	0.27	0.30	0.17	0.16	0.15	0.13	**0.20**	**0.20**	**0.20^{a}**
Mean	**0.26a**		**0.16b**		**0.12c**		**0.17**	**0.18**	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × sampling year (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	**0.0003**	T×D	0.3258	T×D×Y	0.2311
Y	0.383	T×Y	0.0906		
D	**<0.0001**	D×Y	**0.0264**		

[l]W=Wheat, YM=Yellow Mustard, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, FP=Field Pea, L=Lentil. [‡]Values with different letters within each category (treatment, depth and crop phase) are significantly different ($P \leq 0.05$). [§]Bolded *P* values denote significant ($P \leq 0.05$).

Table B.14. Soil moisture concentration (g kg^{-1} of soil) of different crop rotations at Brooks in the springs of 2020 and 2021

Treatment[l]	Spring soil moisture content at different depths at different crop phases								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2020[‡]	2021	2020	2021	2020	2021	2020	2021	Mean
YM-FP-W	0.13	0.37	0.07	0.18	0.15	0.17	**0.12^{c}**	**0.24^{ab}**	**0.18**
IM-FP-W	0.21	0.35	0.16	0.13	0.19	0.14	**0.19^{abc}**	**0.21^{abc}**	**0.20**
AC-FP-W	0.20	0.32	0.16	0.12	0.18	0.11	**0.18^{abc}**	**0.18^{abc}**	**0.18**
OrM-FP-W	0.19	0.35	0.16	0.12	0.23	0.12	**0.20^{abc}**	**0.20^{abc}**	**0.20**
CL-FP-W	0.19	0.28	0.17	0.13	0.19	0.12	**0.18^{abc}**	**0.18^{abc}**	**0.18**
IM-L-W	0.18	0.29	0.14	0.13	0.17	0.14	**0.16^{bc}**	**0.18^{abc}**	**0.17**
AC-L-W	0.18	0.31	0.15	0.12	0.18	0.13	**0.17^{bc}**	**0.19^{abc}**	**0.18**
OrM-L-W	0.19	0.33	0.15	0.12	0.18	0.14	**0.17^{bc}**	**0.20^{abc}**	**0.19**
CL-L-W	0.18	0.33	0.16	0.13	0.17	0.13	**0.17^{bc}**	**0.20^{abc}**	**0.18**
W-W-W	0.23	0.53	0.15	0.13	0.16	0.17	**0.18^{abc}**	**0.28^{a}**	**0.23**
Mean	**0.28^{a}**		**0.14^{b}**		**0.16^{b}**		0.17^{b}	0.20^{a}	

***P* values for main and interaction effects of treatment (T) × soil depth (D) × sampling year (Y)**

Effect	*P* value	Effect	*P* value	Effect	*P* value
T	0.1792	T×D	0.4198	T×D×Y	0.9500
Y	**0.0002**	T×Y	**0.0093**		
D	**<0.0001**	D×Y	**<0.0001**		

[l]W=Wheat, YM=Yellow Mustard, IM=Industrial Mustard, AC=Argentine Canola, OrM=Oriental Mustard, CL=Camelina, FP=Field Pea, L=Lentil. [‡]Values with different letters within each category (treatment, depth and crop phase) are significantly different ($P \leq 0.05$). [§] Bolded *P* values denote significance at ($P \leq 0.05$).

Table B.15. Summary of *P* values and contrast values from pre-planned comparison of soil moisture concentration at different test sites in the Springs of 2020 and 2021 crop phases

Treatment (Trt.) and site contrast description[1]	***P* values and contrast values in 2020 crop phase in different soil depths at different test sites**								
	Swift Current			**Scott**			**Brooks**		
	0-15 cm	**15-30 cm**	**30-60 cm**	**0-15 cm**	**15-30 cm**	**30-60 cm**	**0-15 cm**	**15-30 cm**	**30-60 cm**
Continuous wheat vs. the average of	+0.02[‡]	-0.02	0.00	+0.02	+0.02	+0.02	+0.05	0.00	-0.02
Brassicaceae and pulse crop rotations	**0.01**[§]	**0.002**	0.93	0.07	**0.05**	0.14	**0.05**	0.12	0.47
The average of rotations with field pea vs. lentil	-0.06	-0.01	+0.01	-0.02	-0.02	0.00	0.00	-0.01	+0.01
	0.001	**0.006**	0.12	0.08	**0.02**	0.97	0.90	0.08	0.85
The average of rotations with Argentine canola	+0.02	+0.01	+0.01	+0.02	+0.01	+0.01	+0.01	+0.01	0.00
vs. other Brassicaceae crops	**0.001**	0.06	0.45	0.12	0.34	0.67	0.61	**0.001**	0.98
The average of rotations with camelina (non-	+0.08	+0.07	+0.05	+0.14	+0.08	+0.08	+0.10	+0.10	+0.09
mustard) vs. mustard crops	**0.01**	0.07	0.88	0.03	0.35	0.42	0.84	**<0.0001**	0.62
Rotation with yellow mustard vs. the average of	-0.08	-0.07	+0.02	-0.14	-0.09	-0.01	-0.06	-0.08	-0.04
industrial and oriental mustard	**<0.0001**	**<0.0001**	**0.02**	**<0.0001**	**<0.0001**	0.50	**0.01**	**<0.0001**	0.46
The average of rotations with industrial mustard	-0.01	0.00	-0.02	+0.02	+0.02	+0.005	+0.005	-0.005	-0.02
vs. oriental mustard	0.40	0.79	**0.04**	0.15	0.30	0.73	0.80	0.28	0.53
	***P* values and contrast values in 2021 crop phase in different soil depths at different test sites**								
Continuous wheat vs. the average of	-0.01	0.00	-0.01	+0.03	0.00	+0.02	+0.20	0.00	+0.04
Brassicaceae and pulse crop rotations	0.19	0.60	0.08	**0.02**	0.99	0.05	**0.01**	0.62	0.62
The average of rotations with field pea vs. lentil	0.00	+0.01	0.00	-0.01	+0.03	-0.01	+0.02	+0.01	0.00
	0.33	0.09	0.46	0.07	0.44	0.33	0.62	**0.02**	**0.02**
The average of rotations with Argentine canola	0.00	0.00	0.00	+0.01	-0.02	+0.01	-0.01	-0.01	-0.02
vs. other Brassicaceae crops	0.77	0.79	0.71	0.06	0.64	0.24	0.81	0.06	0.06
The average of rotations with camelina (non-	+0.05	+0.04	+0.03	+0.14	+0.16	+0.06	+0.14	+0.06	+0.05
mustard) vs. mustard crops	0.08	0.83	0.64	0.43	0.06	0.69	0.55	0.22	0.22
Rotation with yellow mustard vs. the average of	0.00	0.00	+0.01	-0.02	-0.01	0.00	+0.04	+0.06	+0.04
industrial and oriental mustard	0.63	0.15	0.34	0.09	0.86	0.68	0.56	**<.0001**	**<.0001**
The average of rotations with industrial mustard	+0.005	+0.005	-0.01	+0.005	+0.005	+0.005	-0.02	+0.01	+0.01
vs. oriental mustard	0.21	0.31	0.33	0.38	0.93	0.79	0.70	0.27	0.27

[1] Other Brassicaceae crops include camelina, yellow mustard, oriental mustard and industrial mustard; mustard crops include yellow mustard, oriental mustard and industrial mustard. [‡]Contrast value =value on the left - value on the right in the comparison and the *P* value was mentioned following the contrast value.
[§] Bolded *P* values denote significance ($P<0.05$).

APPENDIX C

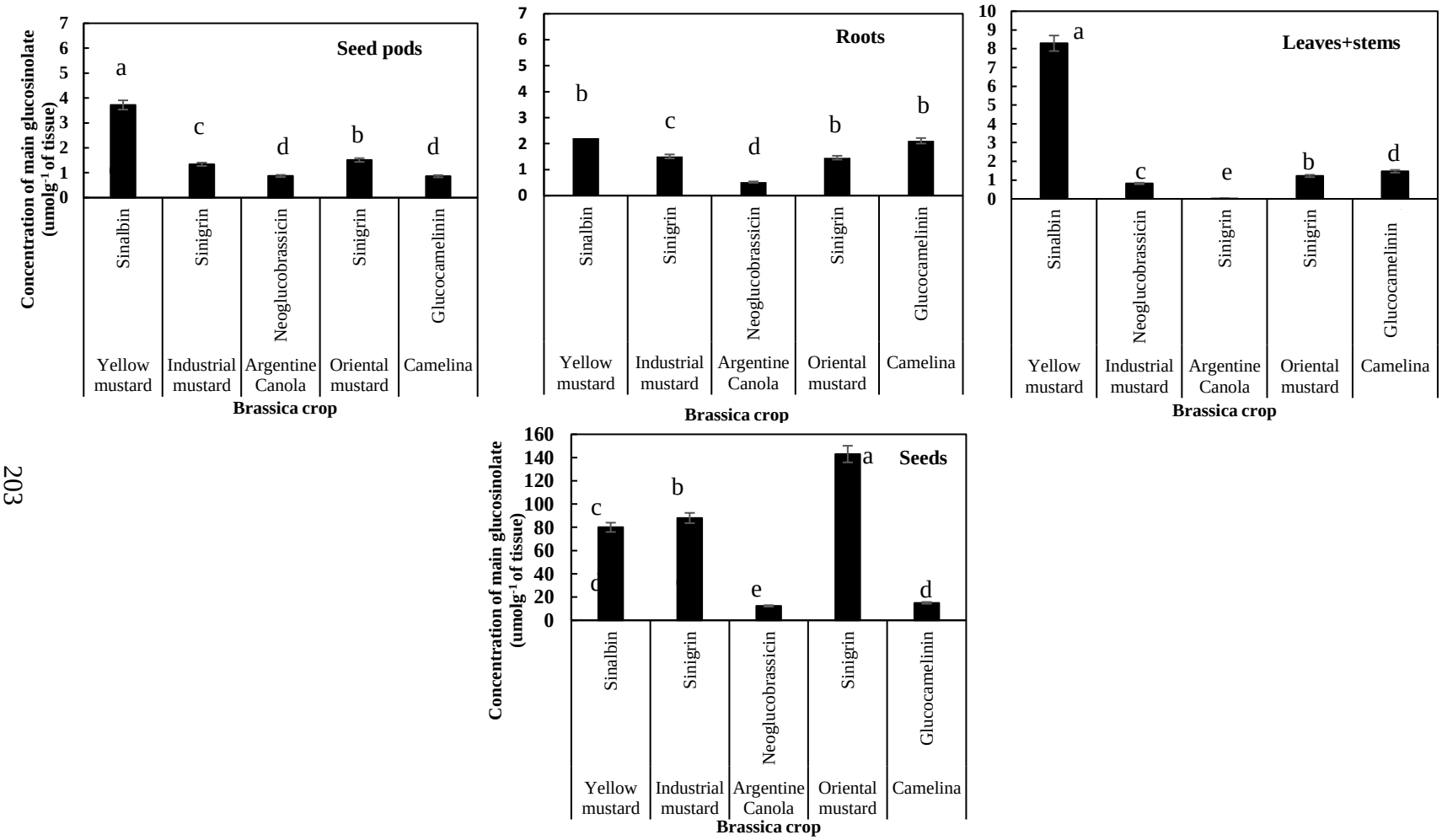

Fig. C.1. The primary glucosinolate and its concentration (µmol g^{-1} of tissue) in different plant parts from the Brassicaceae crops used in the study. The bars followed by the same letter within each plant part are not significantly different at $P \leq 0.05$. **Note:** The bars indicate the standard error of the means (n=3).

Table C.1. Change in cumulative mineral nitrogen (ammonium-nitrogen, nitrate-nitrogen and total mineral-nitrogen) concentration (mg N kg^{-1} of soil) during 120-d incubation.

Crop residue type amended in soil	Cumulative ammonium-nitrogen concentration in different sampling days										
	0	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**77**	**90**	**120**
Yellow mustard	2.32^{E}	0.791^{F}	0.317^{G}	4.21^{DE}	17.1^{B}	35.8^{vw}	59.8^{opqr}	65.3^{lmno}	70.8^{hijkl}	73.5^{ghijk}	74.7^{fghij}
Industrial mustard	2.32^{E}	1.964^{F}	0.834^{G}	17.5^{B}	34.2^{vwx}	48.4^{st}	62.7^{mnop}	66.7^{lmn}	68.6^{jklm}	68.7^{jklm}	69.6^{ikl}
Argentine canola	2.32^{E}	1.886^{F}	0.885^{G}	24.7^{A}	47.7^{st}	68.6^{jklm}	75.7^{efghi}	82.7^{abcd}	85.2^{abc}	87.2^{ab}	88.1^{a}
Oriental mustard	2.32^{E}	1.802^{EF}	0.373^{G}	15.4^{BC}	36.3^{vw}	55.2^{qr}	61.8^{nopq}	66.3^{lmno}	74.2^{ghi}	76.2^{defghi}	76.8^{defgh}
Camelina	2.32^{E}	1.552^{F}	0.438^{G}	14.5^{BC}	32.3^{wxy}	45.5^{tu}	58.1^{pqr}	62.4^{mnop}	65.3^{lmno}	67.0^{klmn}	70.9^{hijkl}
Wheat	2.32^{E}	1.354^{F}	0.565^{G}	27.9^{xyz}	54.2^{rs}	73.7^{ghij}	77.8^{defg}	78.8^{cdefg}	81.1^{bcdef}	81.7^{abcde}	82.3^{abcde}
Un-amended	2.32^{E}	6.262^{DE}	10.3^{CD}	14.4^{BC}	20.4^{A}	26.3^{yzA}	32.3^{wxy}	36.4^{vw}	40.1^{uv}	43.2^{tu}	46.1^{tu}
	Cumulative nitrate-nitrogen concentration in different sampling days										
Yellow mustard	2.53^{F}	1.71^{G}	1.27^{G}	10.2^{EF}	18.6^{BCD}	35.3^{wxy}	55.6^{qr}	75.7^{jkl}	85.7^{jfghi}	89.5^{defgh}	94.8^{abcde}
Industrial mustard	2.53^{F}	1.91^{G}	1.35^{G}	14.4^{D}	33.5^{wxyz}	52.8^{rst}	61.0^{nopq}	73.8^{klm}	84.5^{ghi}	93.2^{bcdef}	102.0^{ab}
Argentine canola	2.53^{F}	1.58^{G}	1.15^{G}	24.4^{BC}	45.6^{tu}	68.9^{lmno}	82.4^{hij}	88.5^{fgh}	83.3^{ghij}	102.2^{ab}	105.5^{a}
Oriental mustard	2.53^{F}	1.13^{G}	1.29^{G}	28.7^{yzA}	31.2^{xyzA}	44.9^{tuv}	63.6^{nopq}	73.6^{klm}	85.4^{fghi}	91.3^{defg}	102.6^{ab}
Camelina	2.53^{F}	1.33^{G}	1.86^{G}	19.1^{BCD}	37.3^{vwx}	50.3^{st}	66.7^{mno}	78.5^{ik}	85.5^{fghi}	92.6^{cdef}	99.5^{abc}
Wheat	2.53^{F}	1.38^{G}	1.20^{G}	16.6^{CD}	38.7^{vwx}	60.3^{opqr}	85.5^{fgh}	92.7^{cdef}	94.7^{bcdef}	98.5^{abcd}	101.5^{ab}
Un-amended	2.53^{F}	10.1^{E}	18.1^{BCDE}	25.5^{Zab}	40.1^{vw}	46.1^{tu}	50.2^{st}	57.1^{pqrs}	62.2^{nopq}	64.4^{nop}	67.4^{mno}
	Cumulative total mineral nitrogen concentration in different sampling days										
Yellow mustard	4.85^{BC}	2.50^{C}	1.58^{C}	14.4^{AB}	35.7^{xyz}	71.1^{u}	115.4^{opq}	141.0^{lm}	156.4^{jk}	163.0^{ghij}	169.4^{efgh}
Industrial mustard	4.85^{BC}	3.86^{C}	2.18^{C}	31.9^{yz}	67.7^{uv}	101.1^{rs}	123.7^{op}	140.5^{lm}	153.1^{jk}	161.9^{ghij}	171.6^{defg}
Argentine canola	4.85^{BC}	3.46^{C}	2.03^{C}	49.0^{w}	93.3^{s}	137.4^{m}	158.0^{ijk}	171.2^{defg}	168.4^{fgh}	189.4^{ab}	193.6^{a}
Oriental mustard	4.85^{BC}	2.93^{C}	1.65^{C}	44.1^{wx}	67.5^{uv}	100.1^{rs}	125.4^{no}	139.9^{m}	159.5^{hijk}	167.5^{fghi}	179.3^{bcde}
Camelina	4.85^{BC}	2.88^{C}	2.30^{C}	33.6^{yz}	69.7^{uv}	95.8^{s}	124.7^{no}	140.9^{lm}	150.8^{kl}	159.6^{hijk}	170.5^{defg}
Wheat	4.85^{BC}	2.73^{C}	1.81^{C}	44.5^{wx}	92.9^{s}	134.1^{mn}	163.3^{ghij}	171.5^{defg}	175.8^{cdef}	180.3^{bcd}	183.8^{abc}
Un-amended	4.85^{BC}	16.37^{A}	28.4^{z}	39.9^{wxy}	60.4^{v}	72.4^{tu}	82.4^{t}	93.4^{s}	102.3^{rs}	107.6^{qr}	113.5^{pq}

[l]Comparisons are for all sampling days within each nitrogen concentration category. Thus, values with different letters within each category are significantly different at $P \leq 0.05$. Note: Due to the extensive number of comparisons, both uppercase and lowercase letters were used to indicate statistically significant differences.

Table C.2. Nitrogen recovery percentages (%) from different crop residues over the 120-d of incubation

Plant residue	N recovery percentage in different sampling days									
	03	**07**	**14**	**21**	**28**	**42**	**56**	**70**	**90**	**120**
Yellow mustard	0.136[bł]	0.463[c]	4.31[c]	10.4[c]	20.8[c]	33.8[a]	41.6[a]	46.3[a]	48.3[a]	50.3[a]
Industrial mustard	0.197[ab]	0.623[ab]	9.1[ab]	19.3[ab]	28.8[ab]	35.2[a]	40.6[a]	44.2[a]	46.8[a]	49.7[a]
Argentine canola	0.190[ab]	0.530[bc]	12.8[a]	24.3[a]	35.8[a]	41.2[a]	44.9[a]	45.6[a]	51.2[a]	52.4[a]
Oriental mustard	0.114[b]	0.493[bc]	11.2[a]	19.9[ab]	29.5[ab]	37.1[a]	41.6[a]	47.8[a]	50.3[a]	53.7[a]
Camelina	0.125[b]	0.677[a]	9.8[a]	20.4[ab]	28.0[ab]	36.5[a]	41.6[a]	45.7[a]	48.4a	51.9[a]
Wheat	0.312[a]	0.426[c]	10.4[a]	21.7[ab]	31.4[ab]	38.1[a]	40.8[a]	44.5[a]	45.6[a]	47.1[a]
***P* value**	**0.002**[‡]	**0.0008**	**0.002**	**<0.0001**	**0.005**	0.116	0.590	0.871	0.575	0.300
	Contrast and *P* values from pre-planned comparisons									
Wheat vs. the average of Brassicaceae crops	+0.159[§]	-0.121	+0.95	+2.84	+2.82	+1.32	-1.26	-1.42	-3.40	-4.50
	0.002	**0.002**	0.376	**0.009**	**0.002**	0.465	0.531	0.579	0.220	0.062
Argentine canola vs. the average of other Brassicaceae crops	+0.047	-0.034	+4.19	+6.80	+9.02	+5.55	+3.55	-0.40	+2.75	+1.00
	0.122	0.315	**0.003**	**<0.0001**	**0.001**	**0.014**	0.104	0.860	0.308	0.652
Camelina (non-mustard) vs. the average of mustard crops	-0.024	+0.151	+1.59	+3.86	+1.63	+1.33	+0.33	-0.40	-0.66	+0.66
	0.411	**0.001**	0.180	**0.002**	0.323	0.578	0.878	0.887	0.983	0.816
Yellow mustard vs. the average of industrial and oriental mustard	-0.02	-0.095	-5.84	-9.20	-8.35	-2.35	+0.50	+0.30	-0.25	-1.40
	0.536	**0.023**	**0.001**	**<0.0001**	**0.001**	0.265	0.811	0.904	0.928	0.579

[ł]Comparisons are for each sampling day. Thus, values with different letters within each column are significantly different at $P \leq 0.05$. [‡]Bolded *P* values indicate significant differences ($P \leq 0.05$). § Contrast value = value on the left- value on the right in the comparison and the *P* value following the contrast value

APPENDIX D

Table D.1. qPCR target genes used to analyze nitrifying microbial gene copy concentration

Gene	Primer	Sequence (5'→3')	Product (base pairs)
Archaeal *amo*A	Crenamo23F (Tourna et al., 2008)	ATGGTCTGGCTWAGACG	629
	Crenamo616r48x (Nicol et al., 2008)	GCCATCCABCKRTANGTCCA	
Bacterial *amo*A	amoA1F	GGGGTTTCTACTGGTGGT	491
	amoA2R (Rotthauwe et al., 1997)	CCCCTCKGSAAAGCCTTCTTC	

Table D.2. The concentration of soil available nitrogen (mg N kg^{-1} of soil) in different treatments at different growth stages (sampling times) of field pea

Treatment [l]	Available inorganic nitrogen amounts at different growth stages of field pea								
	Ammonium nitrogen (NH_4^+-N)			Nitrite and nitrate nitrogen (NO_2^-+NO_3^--N)			Total available N		
	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest
FP on YM BGR	1.10^{bc}	2.16^{abc}	2.14^{abc}	45.6^{fg}	56.1^{de}	41.0^{g}	46.7^{gh}	58.3^{ef}	43.2^{h}
FP on AC BGR	1.84^{abc}	2.46^{abc}	2.75^{abc}	64.1^{cd}	69.6^{bc}	49.5^{efg}	66.0^{de}	72.0^{bcd}	52.2^{fgh}
FP on W BGR	1.54^{abc}	2.83^{abc}	2.58^{abc}	81.6^{a}	67.4^{bc}	53.2^{ef}	83.2^{a}	70.2^{cd}	55.8^{fg}
FP on YM BGR+AGR	$1.08^{c‡}$	2.50^{abc}	2.08^{abc}	45.6^{fg}	43.4^{g}	53.6^{ef}	46.7^{gh}	45.9^{h}	55.7^{fg}
FP on AC BGR+AGR	1.81^{abc}	3.16^{abc}	3.74^{a}	64.1^{cd}	67.8^{bc}	54.3^{ef}	65.9^{de}	71.0^{cd}	58.1^{ef}
FP on W on BGR+AGR	1.52^{abc}	3.51^{ab}	3.51^{ab}	79.9^{a}	74.2^{a}	55.2^{de}	81.4^{ab}	77.7^{abc}	58.7^{ef}
FP on HBITC	0.93^{c}	2.84^{abc}	1.90^{abc}	21.4^{h}	29.8^{h}	54.5^{ef}	24.3^{ij}	32.7^{i}	56.4^{fg}
FP on urea	0.89^{c}	2.64^{abc}	2.77^{abc}	24.4^{h}	46.9^{efg}	54.2^{ef}	25.3^{ij}	49.5^{fgh}	57.0^{ef}
FP on Un-A soil	0.89^{c}	0.95^{c}	0.87^{c}	23.2^{h}	22.4^{h}	22.2^{h}	24.1^{ij}	23.3^{i}	23.1^{i}
Mean	1.50^{b}	2.56^{a}	2.48^{a}	50.0^{b}	53.0^{a}	48.6^{b}	51.5^{b}	55.6^{a}	51.1^{b}

***P* values for main and interaction effects of treatment (T) × Sampling times (S)**

	T	S	T×S
NH_4^+-N	**<0.000** [§]	**<0.0001**	**0.1373**
NO_2^- +NO_3^--N	**<0.0001**	**0.0001**	**0.0001**
Total available N	**<0.0001**	**0.0001**	**0.0001**

[l] FP=Field Pea, BGR=Below-Ground Residue, YM=Yellow Mustard, W=Wheat, AGR=Above-Ground Residue, HBITC=4-hydroxybenzyl isothiocyanate, Un-A=Un-Amended soil.

[‡] The comparisons were made for each nitrogen type (ammonium, nitrite+nitrate and total). Values share the same letter within a type of nitrogen are not significantly different at $P>0.05$, according to Tukey's HSD test (n=4).

[§] Bolded *P* values denote significance at $P<0.05$.

Table D.3. The concentration of bacterial, archaeal and total (bacteria+archaea) *amo*A gene copies (lg gene copy numbers g^{-1} soil) in different treatments at different growth stages (sampling times) of field pea

Treatment[l]	Ammonia-oxidizing *amo*A gene copy concentration								
	Bacterial *amo*A			Archaeal *amo*A			Total *amo*A		
	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest	Prior to seeding	Flowering	Harvest
FP on YM BGR	10.0^{hi}	9.44^{jkl}	10.7^{de}	9.19^{ghijk}	10.4^{bcd}	8.58^{kl}	10.1^{hijk}	10.5^{fgh}	10.7^{def}
FP on AC BGR	10.2^{gh}	9.95^{hi}	10.5^{efg}	9.54^{fgh}	10.4^{bc}	8.66^{kl}	10.3^{ghij}	10.6^{efg}	10.5^{efg}
FP on W BGR	12.4^{a}	9.94^{hi}	10.6^{efg}	10.7^{b}	10.8^{b}	9.80^{defg}	12.4^{a}	10.9^{de}	10.7^{def}
FP on YM BGR+AGR	$10.0^{hi‡}$	9.84^{hij}	11.4^{bc}	8.93^{hijkl}	10.3^{bcde}	10.0^{cdef}	10.0^{ijk}	10.5^{fgh}	11.4^{bc}
FP on AC BGR+AGR	10.2^{fgh}	10.6^{efg}	11.4^{bc}	9.47^{fghi}	10.8^{b}	8.48^{l}	10.3^{ghi}	11.1^{cd}	11.4^{bc}
FP on W on BGR+AGR	12.4^{a}	11.0^{cd}	11.4^{bc}	10.7^{b}	11.6^{a}	9.48^{fgh}	12.4^{a}	11.7^{b}	11.4^{bc}
FP on HBITC	9.35^{klm}	8.79^{n}	10.5^{efg}	8.55^{kl}	8.78^{jkl}	9.72^{efg}	9.4^{lm}	9.1^{m}	10.6^{efg}
FP on urea	9.30^{klm}	9.78^{ij}	11.4^{bc}	8.64^{kl}	9.38^{fghij}	8.66^{kl}	9.4^{lm}	9.9^{jk}	11.5^{bc}
FP on Un-A soil	9.30^{lm}	8.98^{mn}	9.7^{ijk}	8.60^{kl}	8.80^{ijkl}	7.02^{m}	9.4^{lm}	9.2^{m}	9.7^{kl}
Mean	10.4^{b}	9.82^{c}	10.9^{a}	9.38^{b}	10.2^{a}	8.9^{c}	10.4^{b}	10.4^{b}	10.9^{a}

***P* values for main and interaction effects of treatment (T) × Sampling time (S)**

	T	S	T×S
Bacterial *amo*A	**<0.0001**[§]	**<0.0001**	**0.0001**
Archaeal *amo*A	**<0.0001**	**0.0001**	**0.0001**
Total *amo*A	**<0.0001**	**0.0001**	**0.0001**

[l] FP=Field Pea, BGR=Below-Ground Residue, YM=Yellow Mustard, W=Wheat, AGR=Above-Ground Residue, HBITC=4-hydroxybenzyl isothiocyanate, Un-A=Un-Amended soil,

[‡] The comparisons were made for each microbial group (bacterial, archaeal and total). Values share the same letter within a microbial group are not significantly different at $P>0.05$, according to Tukey's HSD test (n=4).

[§] Bolded *P* values denote significance at $P<0.05$.