TWO PULSE-OILSEED INTERCROP COMBINATIONS TO ENHANCE YIELD AND NUTRIENT AVAILABLILTY IN SASKATCHEWAN

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By

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ABSTRACT

The interest in growing pulse-oilseeds together as intercrop combinations in western Canada is increasing, but little is known about the operative nutrient dynamics that drive the observed synergies. Two promising pulse-oilseed combinations (kabuli chickpea-brown flax and dry pea-white mustard) were grown as intercrops in mixed and alternate row configurations in two contrasting soil zones (Brown Chernozem and Black Chernozem) in southern Saskatchewan in 2019 and 2020 without added fertilizer along with their corresponding traditional monocrop systems. Comparison was made of grain and straw yields and nitrogen (N) and phosphorus (P) uptake, proportion and amount of biologically fixed N contributed from the pulse crops, and the transfer of fixed N to the oilseed crops. The two pulse-oilseed intercrops grain yield land equivalent ratios (LER) values, N uptake LER values, and P uptake LER values were at or above 1 for the four site-years, indicating benefit from intercropping in increasing total yield, N and P uptake from a land area. The proportion of N derived from biological N fixation (BNF) was not enhanced in the two intercrop combinations, but significant biologically fixed N (9% - 41%) was transferred from the pulse crops to the oilseed crops that reduced depletion of soil N and contributed additional N in the intercrops compared to monocrops.

Supporting evidence for synergy in nutrient availability in the intercropping systems was found in consistently greater concentrations of water extractable organic carbon (WEOC) and total dissolved N (TDN) found in the root zone of the pulse crop partners (kabuli chickpea, dry pea) alone and in the mixes with non-pulse partners (brown flax, white mustard) at both Redvers and Central Butte sites in both years. Enhanced nutrient availability was provided by the pulse crop partner with greater Plant Root Simulator (PRS™) nitrate (NO_3-N) supply rates observed in the root zone of the pulse crop mixes over the season.

Overall, the results of this study show benefits can be realized from intercropping of kabuli chickpea-brown flax and dry pea-white mustard in yield and nutrient utilization efficiency from a land area, improved nutrient use efficiency, and synergies.

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DEDICATION

I would like to thank my friends, William May and Orla Willoughby, who introduced me to the world of agricultural research and provided the inspiration to begin a new career. I could not have started this journey nor completed this project without the support of my mother, Mary Ann Slonowski, my husband, Jay Reid, and my children, Sam Reid, William Reid, and Ruth Reid. It was their belief in me that allowed me to see this project through to the end.

I dedicate this thesis in memory of my father, John Slonowski, and my grandmother, Bessie Melnyk. They valued and encouraged the pursuit of higher education, science education, and the application of science to commercial farming and domestic horticulture production.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%^{15}N a.e.	percent of ^{15}N atom excess over the natural abundance in atmospheric N_2
%Ndfa	percentage of fixed N derived from the atmosphere
%Ntransfer	percentage of fixed N transferred to non-fixing companion plant
AAFC	Agriculture and Agri-Food Canada
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
BNF	biological nitrogen fixation
cal BP	calendar years before present
CBE	chlorazole black E
CDC	Crop Development Centre
CL	Clearfield®
CMN	common mycorrhizal network
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
EC	electrical conductivity
FAME	fatty acid methyl ester
ICDC	Irrigation Crop Diversification Corporation
ICY_a	yield of *crop-a* as a component of the intercrop from a defined area
ICY_b	yield of *crop-b* as a component of the intercrop from a defined area
IHARF	Indian Head Agricultural Research Foundation
LER	land equivalency ratio
MY_a	yield of *crop-a* as a monocrop from the same defined area
MY_b	yield of *crop-b* as a monocrop from the same defined area
NDIR	non-dispersive infrared
NHI	nitrogen harvest index
$NICY_a$	nitrogen yield of *crop-a* as a component of the intercrop from a defined area
$NICY_b$	nitrogen yield of *crop-b* as a component of the intercrop from a defined area
N*inc*	potential addition or removal of N from the cropping system
NLER	nitrogen uptake land equivalent ratio
NLFA	neutral lipid fatty acid

NMY_a	nitrogen yield of *crop-a* as monocrop from the same defined area
NMY_b	nitrogen yield of *crop-b* as monocrop from the same defined area
NP LER	nitrogen or P uptake land equivalent ratio
NUE	nutrient utilization efficiency
NUE-N	nitrogen utilization efficiency
NUE-P	phosphorus utilization efficiency
OC	organic carbon
OM	organic matter
OP	open pollinated
PLER	phosphorus uptake land equivalent ratio
PLFA	phospholipid fatty acid
PRS™	Plant Root Simulator™
SERF	South East Research Farm
SOM	soil organic matter
TDN	total dissolved N
WARC	Western Applied Research Corporation
WEOC	water extractable organic C

1. INTRODUCTION

Intercropping is when two or more crops are grown together in the same field within the same growing season (Gaudio et al., 2019; Hauggaard-Nielsen & Jensen, 2005). There are numerous benefits to intercropping, some of which are increased yield of one or both crops, yield stability or reduced risk of crop failure, and lower input costs of commercial fertilizer and pesticide applications (Orrell & Bennet, 2013; Pappa et al., 2012). Intercropping is of interest to organic producers, conventional producers, and a growing number of producers who wish to change their mode of production from conventional to low-input agriculture (Hummel et al., 2009; Nelson et al., 2012).

The most popular intercrop combination in temperate growing zones is a legume with a non-legume (Fletcher et al., 2016). Legumes (including perennial forages and annual pulse crops) are considered a good choice for intercropping due to their ability to biologically fix N (Ehrmann & Ritz, 2014; Hauggaard-Nielsen & Jensen, 2005). Biological N fixation is an external N input which could reduce competition for N in the system (Ehrmann & Ritz, 2014). Intercropping with legumes could also enable improved nutrient recovery from existing soil reserves (Ehrmann & Ritz, 2014). Legumes use two methods to enhance soil nutrient availability and uptake: acidification of the rhizosphere soil with root exudates; and development of strong mycorrhizal relationships (Hauggaard-Nielsen & Jensen 2005). Lowering the rhizosphere pH in calcareous soils can facilitate P uptake in the non-legume companion crop (Hinsinger, 2001; Hauggaard-Nielsen & Jensen, 2005). The synergies achieved in intercropping are often expressed as LER values, which are defined as the land area of a monocrop needed to generate the same yield as the intercrop components (Fletcher et al., 2016). A LER value above 1 indicates that the intercrop uses the land area more efficiently (Bybee-Finley & Ryan, 2018; Lithourgidis et al., 2011).

At the Southeast Research Farm (SERF) near Redvers SK, intercropping has been conducted for a number of years. The field research has enabled identification of optimum seeding rates, agronomic benefits, logistical considerations, and best crop combinations for this region of Saskatchewan (Shaw, 2018; Shaw 2019). The most promising combination identified to date is growing a pulse crop together with an oilseed (L. Shaw, personal communication, November 2018). There are currently few published studies on nutrient dynamics in pulse-oilseed intercrops in western Canadian soils. More information is needed to document their impact and to make selections of nutrient efficient pulse-oilseed intercrop systems for western Canada. My research addresses this need through an exploration of the impact of pulse crops and oilseed crops grown together in mixed and alternate rows compared to when grown separately as monocrops. Assessments of crop yield, N and P uptake and soil availability and uptake, BNF, and labile pools of N and carbon (C) in the soil are made in this thesis research. The goal is to provide a better understanding of how intercropping of pulse crops with oilseeds may influence crop yield and associated nutrient uptake and removal along with the processes involved.

In this thesis research, three main hypotheses are tested.

1) There will be greater yield and N and P uptake within a pulse-oilseed intercrop system than when the crops are grown as a monoculture. This will be expressed in higher LER values.
2) Biological N fixation will be enhanced in intercrop systems. The competitive stress of intercropping will increase the percent fixed N per plant.
3) Intercropping and the seeding arrangement (alternate rows or mixed rows) used will influence nutrient supply and availability for plant and microbial utilization. In-season transfer of fixed N from the pulse crop to the oilseed crop will occur and be related to root system proximity and contact.

To test these hypotheses, yield was determined for two promising pulse-oilseed intercrop combinations: kabuli chickpea-brown flax and dry pea-white mustard, which were grown in two contrasting soil climatic zones in Saskatchewan: Brown Chernozem (Central Butte, SK) and Black Chernozem (Redvers, SK). Above-ground biomass, seed yield, and calculations of LER were used to compare the performance of mixed row and alternate row configurations to their respective crops grown as monocultures. Nitrogen and P uptake in straw and seed and efficiency of uptake in producing yield was determined for the intercrop systems. The ^{15}N isotope dilution method was used to assess the BNF of the pulse crop in the intercrop mixes and transfer to the non-fixing species. To better understand soil nutrient cycling processes in intercropping systems, N and P availability and mobility in the seed-row was assessed *in situ* using PRS™ anion probes to determine NO_3-N and PO_4-P supply rates; measurement of WEOC and TDN in the seed-row and mid-row; and colonization of roots by arbuscular mycorrhizal fungi (AMF).

This thesis is comprised of an introduction, literature review, two main research chapters, a synthesis and conclusion, reference listing and appendices. The two main research chapters were written in manuscript style for publication. The chapters are interlinked and overlapping, with each addressing more than one of the above hypotheses. Chapter 3 describes the crop yields, grain N and P uptake, and BNF in kabuli chickpea-brown flax and dry pea-white mustard intercropping systems in the 2019 and 2020 growing seasons at Redvers and Central Butte, Saskatchewan. In Chapter 4, the seed-row and mid-row soil nutrient amounts and supply rates, WEOC and TDN concentrations, and root colonization by AMF is covered. These chapters are followed by a synthesis of the individual research studies (Chapter 5), and includes the overall conclusions of the study as a whole and recommendations for future work. Literature cited throughout the thesis are compiled in the Reference section that follows immediately after Chapter 5. Ancillary data from the trial sites are included in Appendix A.

2. LITERATURE REVIEW

2.1 Intercropping: General Introduction, Background and History

Intercropping is when two or more crop species are grown at the same time in the same place (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; Gaudio et al., 2019). Intercropping systems that are used worldwide are diverse. The crops can be annuals with similar life cycle (phenology) but different plant architecture (morphology), such as dry pea and spring barley, or with similar phenology and morphology, such as midge tolerant and non-tolerant blended varieties of wheats (Fletcher et al., 2016; Gaudio et al., 2019; Saskatchewan Wheat Development Commission, 2020; Saskatchewan Seed Growers Association, 2016). Annual intercrops may also be composed of crops with different phenology but with similar morphology, such as winter wheat and spring barley (Gaudio et al., 2019). Perennial intercropping can include two different perennials such as banana and cocoa trees (Brooker et al., 2015) as well as mixes of perennial and annual crops, such as orchards underplanted with herbs (agroforestry) (Brooker et al., 2015; Carrubba & Catalano, 2009), or red clover and corn (Bybee-Finley & Ryan, 2018).

There are a variety of ways to arrange the crops in time and space. In mixed intercropping species are seeded together, either broadcast or in seeded rows (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; Gaudio et al., 2019; Iqbal et al., 2019). Crops may be seeded together in the same row or separately in alternating rows (Iqbal et al., 2019). Crops that are seeded in alternate rows but are harvested in one operation is also called mixed intercropping (Gaudio et al., 2019). Crops that are seeded in alternate rows that are far enough apart to allow for separate harvest operations, or with several rows of one crop alternating with several rows of another is referred to as strip cropping (Bybee-Finley & Ryan, 2019; Fletcher et al., 2016; Gaudio et al., 2019; Knörzer et al., 2009). Strip cropping can further separate the crops in time with different seeding and harvest dates, to limit competition for water, nutrient, and light resources. This is called relay cropping (Bybee-Finley & Ryan, 2019; Dedio, 1994; Fletcher et al, 2016; Gaudio et al., 2019; Knörzer et al., 2009). In some intercropping systems not all of the crop components are harvested, such as those with a main cash crop and companion crop. The companion crop may be grown for ecosystem services such as weed suppression, pest control, BNF (green manure), and soil erosion control (cover crop) (Bybee-Finley & Ryan, 2018; Pridham & Entz, 2008), whereas the main cash crop is grown to harvest.

Mixed cropping, or polyculture as it is sometimes termed, has been practiced for thousands of years (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; Ehrmann and Ritz, 2014; Hauggaard-Nielsen & Jensen, 2005). There is a pollen record of maize (*Zea mays*) and sweet potato (*Ipomoea batatas*) being grown together in the Eastern Amazon Amazonian Dark Earths soils (formerly known as *Terras Pretas de Indio*) going back to ~3,200 calendar years before present (cal BP). The inhabitants added manioc (*Manihot esculenta*) and squash (*Curcurbita* sp.) when these crops arrived via trade ~2,250 and ~600 cal BP, respectively (Maezumi et al., 2018). Modern farmers in Latin America continue to grow the majority of their beans with maize, potatoes and other crops (Brooker et al., 2015; Bybee-Finley & Ryan, 2018).

Intercropping was regarded as a special form of crop rotation in the Dong Zhou and Qin dynasties in China (2790-2226 cal BP) (Knörzer et al., 2009). Their intercropping began as grains or cereals planted in forests, to which hemp, soybean, mung bean, rice, and cotton were added. These farmers also cropped grains using a green manure intercropping system. The possibility of improving soil quality by intercropping red bean, mung bean and flax is mentioned in "Important Means of Subsistence for Common People" from the Wei and Jin dynasties (1820-1440 cal BP), an early document about crop rotations and intercropping, with other ancient Chinese documents that pertain to polyculture and intercropping spanning from 1420 to ~420 cal BP (Knörzer et al., 2009).

The accounts from English medieval demesnes record that various intercrops were regularly sown (Stone, 2005). Dredge was a mixture of spring barley and oat, mixtill was a mixture of winter wheat and winter barley, and maslin was a mixture of winter wheat and winter rye. These cereal intercrops would not have been separated after harvest and were sold as mixed grain. For a short period after the Black Death there is also record of faba bean and pea grown as an intercrop mix. Pulse crops were usually grown as animal feed. Dredge was more commonly grown than monocrop spring barley, perhaps due to the greater yield stability of oat. Mixtill had better yield stability than monocrop wheat, which may have been driven by high yielding winter barley. The number of acres planted with intercrop mixed grains versus monocrop grain varied according to local market prices and demand. The number of acres sown to pulse crops was dependant on the price of wheat. More acres of pulse crops were grown as a preceding crop to wheat when the price of wheat was high (Stone, 2005).

In addition to written treatises, detailed proto-scientific knowledge for intercropping *Cunninghamia lanceolate* with diverse crops (a process called *shamu jianzhong*) has been passed down orally in families that cultivate this important timber species in northeastern villages in Fujian Province, China (Chandler, 1994). Chandler (1994) reported that families who had practiced *shamu jianzhong* for thirty generations had completely adapted the intercropping system to match their environment (Chandler, 1994). These examples illustrate how ancient peoples were able to sustain highly productive agroecosystems for thousands of years (Chandler, 1994; Knörzer et al., 2009; Maezumi et al., 2018).

2.2 Relevance of Intercropping

Industrial mechanized intensive agriculture has reduced the complexity of natural systems and simplified them to large areas of monoculture crops, composed of a single species of a single genotype (Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016). The ecosystem services found in natural systems, such as nutrient cycling or water and microclimate regulation, have been replaced with external chemical and fossil fuel inputs (Erhmann & Ritz, 2014; Gaudio et al., 2019; Weil & Brady, 2017c). Breeding programs for industrial agriculture may have eliminated root traits that were beneficial in intercropping systems (Thorup-Kristensen et al., 2020; White et al., 2013). The simplified production system with externally regulated nutrient cycles is inherently less resilient, because loss of redundancy can lead to loss of ecosystem function (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014). This lack of resiliency may be seen in yield reduction, increased pest and pathogen pressures, and depletion of natural soil resources (Brooker et al., 2015; Erhmann & Ritz, 2014; Harker et al., 2018).

Labor and time are resources that are expensive and scarce in intensive agriculture systems. Economies of scale require that decision making, field management, and physical handling of inputs and harvest are as efficient as possible (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014; Fletcher et al., 2016; Stone, 2005). The increased complexity of intercropping may be seen as undesirable in mechanized agriculture, and any benefits or ecosystem services that can be provided from intercropping are not viewed as being equal to the cost of reducing harvest efficiency (Amicheu et al., 2020; Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016; Lithourgidis et al., 2011; Orrell & Bennett, 2013).

Bybee-Finley and Ryan (2018) suggest that farmers, researchers, and other stakeholders need to work together during the entire intercropping research process with field-scale equipment to reduce the perceived management barriers to using intercropping (Bybee-Finley & Ryan, 2018). However, until there are several drastic changes to how the current industrial agriculture system operates (MacRae et al., 1993; Vandermeer, 1995) farmers will not be prepared to increase the diversity of their agroecosystems or change their management style to use intercropping for more resilient and sustainable production (Antón et al., 2011; Gaudio et al., 2019). Examples of changes to the current industrial system that could promote resilient and sustainable production methods, such as intercropping, include increased cost of chemical inputs (Orrell & Bennett, 2013); reduced access to components of chemical inputs, such as P (Orrell & Bennett, 2013; Vandermeer, 1995); government payment programmes that encourage farmers to take responsibility for management of normal farming risk by rewarding diversification as a risk management strategy (Antón et al., 2011); reduction in industry demand for a single standardized product (Brooker et al., 2015; MacRae et al., 1993); increase in purchasing power of consumers who are willing to pay for more sustainable agriculture practices and their associated ecosystem services (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; MacRae et al., 1993; Orrell & Bennett, 2013); and change in legal restrictions on non-commercial, decentralized breeding programmes for development of crop cultivars suitable for intercropping in each ecozone (Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016; MacRae et al., 1993; Orrell & Bennett, 2013).

2.3 Intercropping Systems in Western Canada

Crop choices for intercropping in western Canada are limited by the environment. The growing season of approximately 100 to 110 frost-free days and semi-arid climate (Saskatchewan Crop Insurance Corporation, 2020a, 2020b) limits utilization of relay and cover crops (Bybee-Finley & Ryan, 2019; Fletcher et al, 2016; Gaudio et al., 2019; Knörzer et al., 2009). Varietal blends (crops with same morphology and phenology) (Fletcher et al., 2016; Gaudio et al., 2019), are currently restricted to midge-tolerant wheats (Government of Canada, 2014; Saskatchewan Wheat Development Commission, 2020; Saskatchewan Seed Growers

Association, 2016). Growers lose marketability of their grains when they are composed of different genotypes and species (Brooker et al., 2015; Government of Canada, 2013).

Growers in western Canada currently use crop rotations (temporal diversification) (i.e., wheat-pea-canola) with different rotations on different fields (spatial diversification) to regain some of the ecosystem services of complex natural systems (pest suppression, water regulation, yield stability) that were absent in the cereal–based production systems employed in western Canada prior to the 1980's (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; Gaudio et al., 2019; Khakbazan et al., 2018; Xie et al., 2018). Yield benefits may arise from a peaola (pea-canola intercrop)-wheat rotation but there is concern that it will be more difficult to break disease cycles if crop species frequency increases in this two-year intercrop system compared to a three-year canola-pea-wheat monocrop rotation (Fletcher et al., 2016; Shaw, 2019).

Intercropping may be more attractive in prairie production systems that have fewer marketing restrictions, such as forage, seeded pastures, and livestock operations that grow their own feed (Alemu et al., 2019; Fletcher et al., 2016; Jedel & Helm, 1993; Smith et al., 1997). Agroforestry in western Canada is limited to silvopastures in the aspen parkland ecoregion and aging shelterbelts (Amicheu et al., 2020; Baah-Acheamfour et al., 2015; Government of Canada, 2021). Perceived yield losses and economies of scale outweigh the ecosystem services and compensation provided for wildlife habitat retention, carbon sequestration and greenhouse gas emission reductions provided by shelterbelts, so aging shelterbelts and windrows are commonly being removed by grain producers (Amicheu et al., 2020; Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016; Orrell & Bennett, 2013).

Many common western Canadian crops have been grown as intercrops over the recent decades. Crop combinations that have been studied include competitive cereals, competitive cereals with competitive oilseeds, competitive cereals with pulse crops, competitive oilseeds with pulse crops, and non-competitive oilseeds with pulse crops (**Table 2.1**). Categorization of oilseeds into competitive and non-competitive species is somewhat arbitrary, but is based here on hybrid canola being considered highly competitive, while flax, mustard, carinata and rapeseed (non-hybrid) are not.

Table 2.1: Intercrop combinations studied in western Canada.

Category	Crops	Location	References
Competitive cereals	Wheat + barley	Alberta Manitoba	Nelson et al. (2012), Pridham & Entz (2008)
	Wheat + oat	Manitoba	Pridham & Entz (2008)
	Wheat + spring rye	Manitoba	Pridham & Entz (2008)
Competitive cereal + competitive oilseed	Wheat + CL canola	Alberta Manitoba	Nelson et al. (2012) Szumigalski & Van Acker (2006, 2008)
	Wheat + canola	Alberta	Hummel et al. (2009)
Competitive cereal + non-competitive oilseed	Wheat + flax Wheat + oriental mustard	Manitoba	Pridham & Entz (2008)
Competitive cereal + pulse crop	Oat + yellow pea	Saskatchewan	Cowell et al. (1989)
	Oat + field pea	Saskatchewan	Nybo & Sluth (2015)
	Barley + dry bean	Saskatchewan	Nleya et al. (1999)
	Barley + field pea	Saskatchewan	Nybo & Sluth (2015) Malhi (2012)
	Wheat + yellow pea	Manitoba Manitoba	Pridham & Entz (2008) Szumigalski & Van Acker (2006, 2008)
	Wheat + green pea	Alberta	Nelson et al. (2012)
	Wheat + field pea	Saskatchewan	Nybo & Sluth (2015)
Competitive cereal + cover crop	Wheat + red clover Wheat + hairy vetch Wheat + annual ryegrass	Manitoba	Pridham & Entz (2008)
Competitive oilseed + pulse crop	Hybrid CL canola + yellow pea	Manitoba	Szumigalski & Van Acker (2006, 2008) Chalmers (2014) VanKoughnet (2015) VanKoughnet (2016)
	Hybrid CL canola + green pea	Manitoba Saskatchewan	Chalmers (2014) Shaw (2019)
	Canola + field pea	Saskatchewan	Nybo & Sluth (2015) IHARF (2013) Malhi (2012)
	Hybrid sunflower + garden pea	Manitoba	Dedio (1994)

Non-competitive oilseed + pulse crop	Flax + large green lentil Rapeseed + yellow pea	Saskatchewan	Cowell et al. (1989)
	Mustard + yellow pea	Saskatchewan Saskatchewan Manitoba	Cowell et al. (1989) Shaw (2018) Waterer et al. (1994)
	Mustard + green pea Mustard + large green lentil Mustard + small red lentil	Saskatchewan	SERF (2017) Shaw (2018)
	Mustard + maple pea	Saskatchewan	SERF (2017)
	Mustard + marrowfat pea	Saskatchewan	ICDC (2017)
	OP CL canola + green pea	Saskatchewan	Shaw (2019)
	Carinata + faba bean Carinata + dry bean Carinata + maple pea	Saskatchewan	SERF (2017)
	Chickpea + flax	Saskatchewan	SERF (2015) SERF (2017) WARC (2016) IHARF (2015)
Three or more species	Wheat + canola + pea	Manitoba	Szumigalski & Van Acker (2006, 2008)
	Wheat + barley + canola Wheat + barley + pea Wheat + canola + pea Wheat + barley + canola + pea	Alberta	Nelson et al. (2012)

CL = Clearfield
OP = open pollinated;

Intercropping trials that compare effects of seeding arrangement are rare in western Canada. Malhi (2012) used alternate and mixed rows in a study of barley-pea and canola-pea in a trial at Star City, Saskatchewan. The rational for studying two seeding arrangements was not given, however, the result was that higher seed yield LER values and higher seed protein concentrations were observed in mixed rows for both intercrop combinations. Malhi (2012) attributes this to higher interspecies competition and more available soil N in the mixed rows (Malhi, 2012).

Indian Head Agricultural Research Foundation (IHARF) (2013) used alternate and mixed rows in their study of canola-pea intercropping at two sites near Indian Head, SK, and near Melita, MB. The purpose of the two seeding arrangements was to compare the performance of the intercrops to monocrop canola, and to develop agronomic recommendations for producers. Mixed rows yielded higher than alternate rows, but only significantly in the 2011 heavy clay site. Canola had higher plant counts, biomass, and grain yields in alternate rows than in mixed rows, but pea was significantly higher in the mixed rows than in alternate rows for the same parameters (IHARF, 2013).

Chalmers (2014) followed the above study with a trial near Melita and Elva, MB, to further investigate the impact of seeding arrangement for canola-pea intercrops on light, water use and nutrient use, in addition to the yield parameters used in the previous study. Alternating double and triple rows were added to mixed rows and single alternate rows with the hypothesis that alternate double rows would have the better N use efficiency by keeping the additional N fertilizer applied to the canola rows away from pea to encourage higher BNF. The results were that pea performed best and canola worst when in alternate rows. Pea had high partial LERs for all intercrop treatments, indicating that they were the more competitive partner in this study. Chalmers (2014) found that yields and LER values increased as the proximity of the two crops became closer together, and that yields and LER values decreased when the intercrop had more resemblance to monocrops. Mixed row canola used more soil water due to the proximity of excess soil N from pea (Chalmers, 2014).

IHARF (2015) used mixed and alternate rows for their chickpea-flax study near Indian Head, SK to determine if flax would provide the moisture stress and N deficiency stress to induce early seed maturation in chickpea to expand chickpea production to new parts of the province. The results show that higher yields were achieved in mixed rows with added N fertilizer than in alternate rows where added N fertilizer was only applied to the flax rows. Chickpea yield in all the treatments was low, which may be due to the very wet growing conditions in the year of the study.

2.4 Reported Benefits of Intercropping

Benefits realized from intercropping arise over the short-term (current growing season) and can also occur beyond and over several years of employment of the system.

2.4.1 Short-term

Over-yielding, in which intercrop yields surpass the sum of intercrop components grown alone, is one of the most important considerations related to within-season benefits of intercropping with species-diverse systems (Brooker et al., 2015; Fletcher et al., 2016; Hauggaard-Nielsen & Jensen, 2005). This is commonly assessed and reported as LER values (Mead & Willey, 1980). The equation for LER (**Eq. 2.1**) represents the land area of a monocrop needed to generate the same yield as the intercrop component

$$LER = \frac{ICY_a}{MY_a} + \frac{ICY_b}{MY_b} \quad (2.1)$$

where ICY_a= yield of *crop-a* as a component of the intercrop from a defined area, My_a= yield of *crop-a* as monocrop from the same defined area, ICY_b= yield of *crop-b* as a component of the intercrops from the same defined area, MY_b= yield of *crop-b* as a monocrop from the same defined area (Fletcher et al., 2016).

Yield components, such as N and P content can be calculated with the same equation (**Eq. 2.2**)

$$NLER = \frac{NICY_a}{NMY_a} + \frac{NICY_b}{NMY_b} \quad (2.2)$$

where $NICY_a$= nitrogen yield of *crop-a* as a component of the intercrop from a defined area, NMy_a= nitrogen yield of *crop-a* as monocrop from the same defined area, $NICY_b$= nitrogen yield of *crop-b* as a component of the intercrops from the same defined area, NMY_b= nitrogen yield of *crop-b* as a monocrop from the same defined area (Szumigalski & Van Acker, 2006).

Efficient land use by intercrops is indicated with LER values above 1 (Bybee-Finley & Ryan, 2018; Lithourgidis et al., 2011). For example, an LER of 1.3 means that 30% more land area is needed to produce the same yield in a monocrop compared to the total yield from the intercrop (Hauggaard-Nielsen & Jensen, 2005). Greater LER values are seen in intercrop mixtures that do not have a dominant cereal crop (Fletcher et al., 2016).

A partial LER, a single term in the LER equation, provides an indication of competitive interactions in the intercrop system (Bybee-Finley & Ryan, 2018). When a legume partial LER is greater than 0.2, the total LER will be greater than 1 (Fletcher et al., 2016). Visualization of intercrop competitive and complementary interactions are often shown with partial LERs in stacked columns or radar plots (Bybee-Finley & Ryan, 2018; Szumigalski & Van Acker, 2008) (**Figure 2.1**).

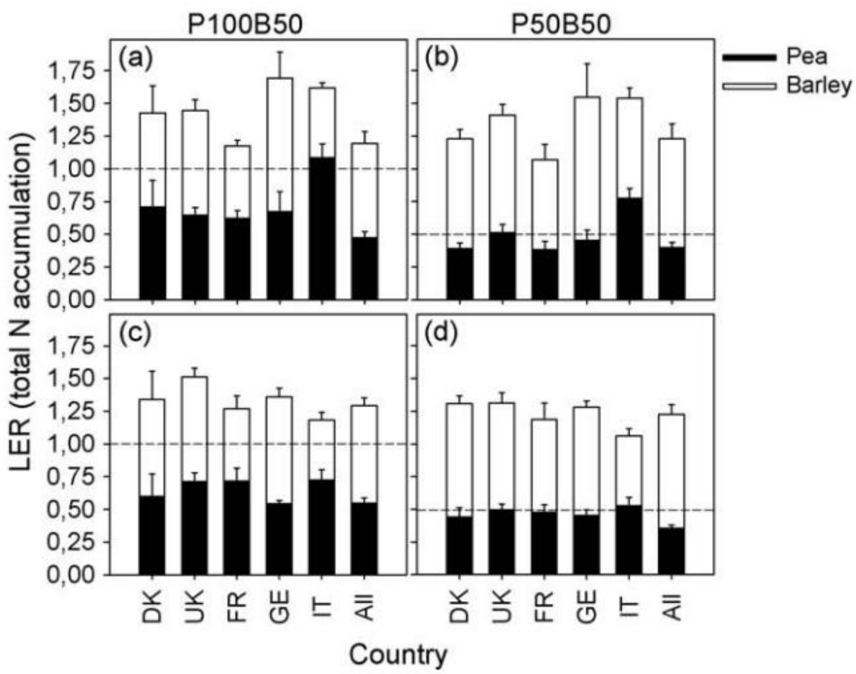

Figure 2.1. Pea (P) and barley (B) partial Land Equivalent Ratio (LER) using final total shoot N accumulation at the flowering (a and b) and maturity growth stages (c and d) when intercropped in either the additive (P100B50) or replacement (P50B50) design in Denmark (DK), United Kingdom (UK), France (FR), Germany (GE), Italy (IT), and as a mean of countries (All). The values are the mean (n=12)$\pm$SE. The values for the overall mean across countries are the mean (n=60)$\pm$SE. The horizontal vertical dotted line indicates initial pea sowing proportion. Reproduced with permission from Hauggaard-Nielson et al., 2009.

Indirect within-season benefits can contribute to higher productivity in intercrop systems (Dowling et al., 2021; Fletcher et al., 2016). These benefits can be from reduced lodging and improved harvestability (Cowell et al., 1989; Dowling et al., 2021; Fletcher et al., 2016; Shaw, 2018; Shaw, 2019); reduced inputs, such as pesticides, because of lower disease and insect pressure (Brooker et al., 2015; Bybee-Finley & Ryan, 2018; Dowling et al., 2021; Fletcher et al., 2016; Hauggaard-Nielsen & Jensen, 2005; Hummel et al., 2009; Orrell & Bennett, 2013; Pridham & Entz, 2008); and reduced competition for resources from weeds (Bybee-Finley & Ryan, 2018; Fletcher et al., 2016; Nelson et al., 2012; Pridham & Entz, 2008; Shaw, 2019; Szumigalski & Van Acker, 2008).

There are two mechanisms at work that lead to overyielding in intercropping systems: resource (or niche) partitioning and facilitation (Bybee-Finley & Ryan, 2018; Fletcher et al., 2016; Orrell & Bennett, 2013). Niche partitioning is when the intercrop components use the same resources without competition (Chalmers, 2014; Dowling et al., 2021; Ehrmann & Ritz, 2014; Fletcher et al., 2016). This can be achieved by partitioning the resources in time, as in relay cropping (Dedio, 1994; Fletcher et al., 2016), but usually the partitioning is in space. Examples are light interception differences between horizontal dicotyledonous leaves (e.g., faba bean) and vertical monocotyledonous leaves (e.g., wheat) that result in more efficient use of solar radiation (Eskandari & Ghanbari, 2010), improved access to water and nutrients within the soil profile arising from a combination of shallow and deep root architectures (Ehrmann & Ritz, 2014; Thorup-Kristensen et al., 2020), preference for different N forms (NO_3-N or ammonium (NH_4-N)) enabling use of both forms (Chalmers, 2014; Gaudio et al, 2019), and reduced competition for soil-available N due to the biologically fixed N from the pulse crops (Brooker et al., 2015; Chalmers, 2014; Dowling et al., 2021; Ehermann & Ritz, 2014; Gaudio et al., 2019).

Facilitation in intercrop systems occurs when one crop component causes resources to become more available to the other intercrop component(s) (Chalmers, 2014; Dowling et al., 2021; Fletcher et al., 2016). Indirect, or asymmetric, facilitation (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014) examples are crop canopy microclimate regulation in the "three sisters" polyculture of maize, bean and squash (Brooker et al., 2015), and hydraulic lift of soil water by deep-rooted intercrop components which can benefit shallow-rooted intercrop components (Brooker et al., 2015; Erhmann & Ritz, 2014). Direct, or symmetric facilitation occurs within the rhizosphere interface (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014) and includes water and nutrient redistribution by AMF or common mycorrhizal networks (CMN) which benefit shallow-rooted intercrop components (Brooker et al., 2015; Hauggaard-Nielsen & Jensen, 2005), and acidification of rhizosphere soil (a by-product of BNF in pulse crops, or from organic acid root exudates) which can increase the availability of limiting nutrients such as P and iron (Fe) when the roots of the intercrops are within the 0.5 mm zone of root exudates of each other (Brooker et al., 2015; Dowling et al., 2021; Erhmann & Ritz, 2014; Fletcher et al., 2016; Gaudio et al, 2019; Hauggaard-Nielsen & Jensen, 2005; Knörzer et al., 2009; Li et al., 2007).

2.4.2 Long-term

Carry-over or long-term benefits of intercropping that can be seen in the following year's crop yield are higher soil available N from pulse crop residues (Fletcher et al., 2016; Iqbal et al., 2018; Miheguli et al., 2018; Pappa et al., 2012), reduced N leaching from deep-rooted crop components and/or more efficient uptake by the intercrop components (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014; Fletcher et al., 2016; Thorup-Kristensen et al., 2020), and reduced weed seed bank due to weed suppression (Bybee-Finley & Ryan, 2018; Dhima et al., 2018; Ehermann & Ritz, 2014; Fletcher et al., 2016; Nelson et al., 2012; Reis et al., 2019). Within-year and year-to-year yield stability are additional intercropping benefits (Anil et al., 1998; Bybee-Finley & Ryan, 2018; Fletcher et al., 2016; Iqbal et al., 2018; Stone, 2005). Since intercrop systems are able to more fully exploit existing resources (Brooker et al., 2015) they may contribute to depletion of soil nutrient levels in low-input farming systems in the long-term (Erhmann & Ritz, 2014; Thorup-Kristensen et al., 2020).

Long-term benefits to soil health from intercropping may not be seen immediately after switching to an intercropping system (Iqbal et al., 2018). Farming systems that use high levels of nutrient inputs often support a single dominant AMF species (Erhmann & Ritz, 2014; Orrell & Bennett, 2013). Routine applications of fungicides, soil disturbances, and low host diversity can reduce indigenous populations of beneficial AMF and other fungal endophytes (Erhmann & Ritz, 2014; Kramer & Gleixner, 2008; Orrell & Bennett, 2013). Increasing crop diversity, both spatially and temporally, with intercropping systems will positively influence soil microbial diversity and function (Erhmann & Ritz, 2014; Tian et al., 2019). Higher above-ground biomass production in intercrop systems will necessitate greater root biomass within the surface and deeper layers of the soil profile, and produce more root exudates (Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016; Kaiser & Kalbitz, 2012; Thorup-Kristensen et al., 2020).

This will, in turn, support more soil microbial activity, higher nutrient cycling, greater redundancy and resiliency in soil functions (Bybee-Finley & Ryan, 2018; Erhmann & Ritz, 2014; Kuzyakov & Blagodatskaya, 2015; Moreno et al., 2019; Thorup-Kristensen et al., 2020), more soil organic matter (SOM) stabilization and improved soil structure (Brooker et al., 2015; Cotrufo et al., 2013; Dijkstra et al., 2010; Erhmann & Ritz, 2014; Sokol et al., 2019; Tian et al., 2019). Higher microbial abundance and activity will suppress soil disease and pests, and support general soil health (Brooker et al., 2015; Erhmann & Ritz, 2014; Fletcher et al., 2016; Hauggaard-Nielsen & Jensen, 2005; Weil & Brady, 2017b).

2.5 Important Nutrient Cycling Processes in Intercropping Systems

Biological N fixation is a process performed by some bacteria and archaea in which the triple bonds of atmospheric N_2 are broken through a complex series of enzymatic steps. Once the bonds are broken, the N atoms are then combined with protons to produce reactive nitrogen as ammonia (NH_3) which is then incorporated into organic forms in the plant (Bottomley & Myrold, 2015; Weil and Brady, 2017a). The BNF process is energetically expensive, requiring 16 adenosine triphosphate (ATP) molecules for every one molecule of N_2. As a consequence, the majority of BNF prokaryotes form associations with plants to access photosynthetically derived C from the plant for substrate and energy, or are free-living photosynthetic diazotrophs. Legumes form a symbiotic relationship with numerous genera and species of alpha- and beta-protobacteria (Bottomley & Myrold, 2015; Weil and Brady, 2017a). This symbiosis gives legumes the capacity to supply some of their N needs without relying on soil mineral N (Dowling et al., 2021; Fletcher et al., 2016). Inclusion of perennial forages and pulse crops in intercrop systems can reduce the reliance on existing soil N pools and external mineral N inputs (Erhmann & Ritz, 2014; Fletcher et al., 2016). Higher rates of BNF will occur in intercrop systems where the non-fixing crop is more competitive for soil N resources (Chalmers, 2014; Erhmann & Ritz, 2014). Furthermore, higher LER values should be obtained in pulse/non-pulse intercrops where existing soil N pools are low (Chalmers, 2014; Davis et al., 1986; Dowling et al., 2021; Fletcher et al., 2016; Irrigation Crop Diversification Corporation (ICDC), 2017).

Nitrogen exists in two stable isotopes, ^{15}N and ^{14}N (Bottomley & Myrold, 2015). Soils often have a higher ratio of ^{15}N to ^{14}N than the atmosphere (Rose et al., 2018). Application of a small amount of ^{15}N-enriched inorganic fertilizer can be used to trace the uptake of N (Bottomley &

Myrold, 2015). The uptake of ^{15}N-enriched soil N will give the plant a greater $^{15}N/^{14}N$ ratio than found within a plant with natural abundance (Hardarson & Danso, 1990). The amount of ^{15}N above natural abundance is termed %^{15}N atom excess (%^{15}N a.e.). The relative amount of N that a plant assimilated from the applied fertilizer and the amount that was obtained from soil N can then be calculated. Leguminous plants that can biologically fix N can access N from the atmosphere, adding a third pool of N. To determine how much atmospheric N was biologically fixed, a crop that does not carry out fixation is compared with one that does. An assumption that both crops will take up soil N in the same manner must be used (Hardarson & Danso, 1990). This difference allows soil microbiologists and field scientists to quantify BNF in natural and agricultural ecosystems (Bottomley & Myrold, 2015).

Numerous biotic and abiotic factors influence the contribution of BNF to a system (Bottomley & Myrold, 2015; Erhmann & Ritz, 2014). High amounts of available soil N can suppress BNF organisms (Erhmann & Ritz, 2014; Fletcher et al., 2016). Adequate available soil P is needed for the ATP used in BNF. Access to available soil P is gained from acidification of the rhizosphere from two separate processes: 1) as a by-product of the BNF processes (Erhmann & Ritz, 2014; Fletcher et al., 2016); and 2) from organic acid root exudates which are not part of the BNF process (Erhmann & Ritz, 2014). Another source of solubilized P is from AMF that colonize legume plants along with the BNF rhizobia (Antunes & Goss, 2005; Wright, 2005). Legumes can fail to form nodules for BNF without AMF colonization, and this three-way association is referred to as the tripartite symbiosis (Antunes & Goss, 2005).

Assessment of AMF colonization is done by clearing root segments and then staining them with dyes that adhere to any fungal structures the root segments contain (Giovannetti & Mosse, 1980). Trained observers are able to identify AMF hyphae, arbuscules, and vesicles in stained root segments (Wright, 2005). There are numerous ways to examine active soil bacterial and fungal communities (Kandeler, 2015). Common C substrates can be added to field-moist soil samples, incubated, and the amount of respired carbon dioxide (CO_2) measured to determine substrate preferences of the soil microbial communities. Soil samples can be fumigated, then incubated, and respired CO_2 measured to give an indication of amount of soil microbial biomass in the sample. Membrane phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) can be extracted from living cells and compared to widely accepted biomarkers to differentiate Gram positive bacteria, Gram negative bacteria, and fungi. Enzymes can be extracted from soils to give indications of energy transfer and nutrient cycling. However, the source of the enzymes can be from living cells, dormant cells, dead cells, or complexed to mineral and organic soil particles, so may not accurately represent the soil microbial community functions at the time of sampling (Kandeler, 2015).

Intraspecific competition in monocrops can result in reduced root growth to avoid competition with neighboring plants of the same kind (Erhmann & Ritz, 2014; Tosti & Throup-Kristensen, 2010). When different crops are placed together in intercrop systems, interspecific competition will alter the crop's rooting morphology to either avoid the neighbor, to avoid the nutrient depletion zone caused by the neighbour, or to out-compete their neighbour for resources (Dowling et al., 2021; Erhmann & Ritz, 2014; Hauggaard-Nielsen & Jensen, 2005; Tosti & Thorup-Kristensen, 2010). Root competition is symmetric, and larger plants do not have a disproportionate advantage like they do for light competition (E. Lamb, personal communication, September, 2016). Differences in rooting depth, root lengths, or different occupied soil volumes will lead to niche differentiation (Dowling et al., 2021; Erhmann & Ritz, 2014; Hauggaard-Nielsen & Jensen, 2005; Thorup-Kristensen et al., 2020). Increased root growth and densities may enhance facilitation if the roots are within each other's rhizosphere to benefit from differences in nutrient solubilization and availability (Chalmers, 2014; Dowling et al., 2021; Erhmann & Ritz, 2014). Competitive or niche partitioning of root distributions in intercrops can be measured by tracing the movement of stable isotopes, either in pots or field situations with ^{15}N-labeled fertilizer (Bremer & Greer, 2021; Dowling et al., 2021) or ^{32}P gelatine capsules (Hauggaard-Nielsen & Jensen, 2005), and associated effects by assessing soil nutrient concentrations and supply rates. Facilitative root interactions can be measured in pot studies where roots of intercropped species are prevented from inter-growing using solid or mesh barriers and compared to species growing with no barriers between the plants (Li et al., 2009; Xiao et al., 2004).

As revealed in this literature review, intercropping systems have a long history of use and benefit in crop production. However, the systems are complex and varied, with limited utilization and investigation in western Canada. Important synergies may be realized in soil resource acquisition and efficiency of use, especially pertaining to nutrients, that have not been well documented in prairie soils. My thesis research addresses these gaps.

3. YIELD, NITROGEN AND PHOSPHORUS UPTAKE, AND BIOLOGICAL NITROGEN FIXATION INPUTS IN TWO PULSE-OILSEED INTERCROPPING SYSTEMS IN SOUTHERN SASKATCHEWAN

3.1 Preface

The potential to increase total yield from a land area and improve yield stability are common observed benefits of intercropping. Intercrop combinations in western Canada have been grown with a variety of small grains with similar phenology and with seeds that are easily separated after harvest. However, the balance between competition and facilitation in these intercrop combinations has been difficult to achieve without an understanding of the mechanisms that operate within intercrop systems. In this study, I investigated two promising pulse-oilseed combinations to observe and document the operative processes that underlie enhancements in yield and nutrient availability in pulse-oilseed intercrops in western Canadian soils. In this chapter, the yield parameters of grain and straw yield, grain and straw N and P uptake, and BNF are examined for kabuli chickpea-brown flax and dry pea-white mustard intercrops grown in Saskatchewan.

3.2 Abstract

There is a growing interest in pulse-oilseed intercrop combinations, but little is known about what drives the observed synergies in them. To address this, two promising pulse-oilseed combinations for western Canada (kabuli chickpea-brown flax and dry pea-white mustard) were grown as intercrops in mixed and alternate row configurations in two contrasting soil zones (Brown Chernozem and Black Chernozem) in southern Saskatchewan in 2019 and 2020 without added fertilizer. Comparison was made between the intercrop combinations and their corresponding traditional monocrops for grain and straw yields, grain N and P uptake, proportion and amount of biologically fixed N contributed from the pulse crops, and the transfer of fixed N to the oilseed intercrops. Total LER values were calculated for grain yield and grain N and P uptake for the intercrops to determine benefit to intercropping versus monocrops. Partial LER values for each crop of the intercrops were calculated to evaluate dominance of each crop within the intercrop system. Benefit from intercropping by way of increased total yield, and N and P uptake for the two pulse-oilseed intercrop studied was seen in LER values at or above 1 for grain yield, grain N uptake, and grain P uptake. The proportion of N derived from BNF and the BNF in the pulse crop itself was not enhanced in the two intercrop combinations. The significant ($p \leq 0.05$) reduction of soil N depletion in oilseed intercrops compared to oilseed monocrops could be attributable to the amount of biologically fixed N (9% - 41%) that was transferred to the oilseed partner. The partial LER values showed that brown flax was dominant and kabuli chickpea subordinate in kabuli chickpea-brown flax intercrops at all four site-years. In contrast, dry pea was dominant and white mustard subordinate for the two Redvers, SK site-years while white mustard was more competitive at the two Central Butte, SK site-years. White mustard's higher competitiveness at the Central Butte sites was attributed to higher available soil N and P concentrations at those trial sites compared to the Redvers sites. Early establishment of kabuli chickpea appeared to be an important factor in preventing brown flax dominance.

3.3 Introduction

Grain producers in western Canada are beginning to take an interest in adding intercrops to their crop rotations (Bird, 2020). Some are seeking the direct intercrop benefits of higher total yields from their land area and yield stability (Bybee-Finley & Ryan, 2018). Others use intercrops for the indirect benefits of reduced lodging to increase crop harvestability; reduced weed, disease, and pest pressure to limit amount and frequency of pesticide applications; and some seek to improve nutrient availability (Fletcher et al., 2016; Orrell & Bennet, 2012; Pappa et al., 2012). Others see intercropping as an opportunity to introduce new or specialty crops into their crop rotations (SERF, 2017; Western Applied Research Corporation (WARC), 2016).

Intercrops in western Canada are generally annual grains with similar phenology, using crops with seeds of different size that are easily separated after harvest (Shaw, 2018; Shaw, 2019). Various combinations of cereals, oilseeds, and pulse crops have been grown as intercrops in western Canada (Dowling et al., 2021). Considerations and questions have arisen in the pursuit of the above direct and indirect intercropping benefits, and these are covered in detail in the literature review of this thesis. The search for the optimum balance of competition and facilitation in intercropping is hampered by a lack of understanding of intercrop dynamics (Dowling et al., 2021) and the highly variable environmental conditions commonly found in the northern Great Plains (Cutforth et al., 2007; Lenssen et al., 2007). This often results in one crop in the intercrop to dominate at the expense of the other intercrop companions and a failure to achieve a co-crop equilibrium (Chalmers, 2014; Irrigation Crop Diversification Corporation (ICDC), 2017). However, mechanisms surrounding interactions, associated benefits as well as negative impacts, are poorly understood.

Pulse crops form a symbiotic relationship with *Rhizobium* spp. of bacteria in nodules on roots that are able to break the triple bonds of atmospheric N_2 to produce useable form of N (Bottomley & Myrold, 2015). This symbiosis allows pulse crops to utilize atmospheric N_2 in addition to soil available N (Dowling et al., 2021; Fletcher et al., 2016). Inclusion of a pulse crop in intercrop systems is a way to use the indirect benefit of increased nutrient availability for the direct benefit of increased yields (Dowling et al., 2021; Fletcher et al., 2016). Documentation and understanding of the operative processes and mechanisms in intercrops that include pulse crops is needed for successful introduction and adoption of these intercrop systems by grain producers (Gaudio et al., 2019).

Field research on intercropping has been conducted at the Southeast Research Farm (SERF) near Redvers, SK, for a number of years. Pulse-oilseed intercrops were identified as the most promising intercrop combination to date (L. Shaw, personal communication, November 2018). Past studies conducted in western Canada compared pulse (dry pea, lentil) in intercrop combination with oilseed (canola, rapeseed, flax, white mustard) and cereals (oats, barley) with N fertilizer added have shown synergy in LER values greater than 1 (Cowell et al., 1989; Malhi, 2012; Waterer et al., 1994), and increased proportion of N derived from biological fixation in intercropping compared to pulse monocrops (Cowell et al., 1989). Under N fertilization, lower total BNF from intercropping versus monocropping and minimal transfer of N from pulse crop to non-pulse crop was reported (Cowell et al., 1989; Waterer et al., 1994). However, very few recent western Canadian pulse-oilseed intercrops studies have been published that have investigated nutrient dynamics and synergies in unfertilized pulse-oilseed intercrops or considered P along with N. Therefore, a two-year field trial at two sites in contrasting soil zones in southeastern and southwestern Saskatchewan was conducted to examine the nutrient dynamics of two pulse-oilseed intercrops. In this chapter yield indices of straw and grain yield, grain N and P uptake, and BNF were measured in four seeding arrangements (monocrop pulse crop, monocrop oilseed, pulse-oilseed in mixed rows, pulse-oilseed in alternate rows) to understand the above-ground synergies at work in pulse-oilseed intercrops.

3.4 Materials and Methods

3.4.1 Site description

The project involved field trials conducted over two growing seasons (2019 and 2020) at two main locations: Central Butte, SK (Orthic Brown Chernozem of the Kettlehut-Ardill soil association) and Redvers, SK (Orthic Black Chernozem of the Oxbow soil association) for a total of four site-years. A third location at AAFC Indian Head, SK (Calcareous Black Chernozem of the Indian Head soil association) was used in 2019 as a supplemental site at which only BNF was assessed. The site at Indian Head was an on-going intercropping trial with fertilized kabuli chickpea-brown flax treatments belonging to AAFC researcher William May. Its location between Central Butte and Redvers provided a convenient means to provide additional information on intercropping and biological nitrogen fixation. Data from this site is provided in Appendix A.

3.4.2 Experimental design and field operations

Two intercropping combinations at the two main locations were grown: dry pea-white mustard and kabuli chickpea-brown flax. For each combination there were four treatments: pulse monocrop, oilseed monocrop, pulse and oilseed in alternate rows, pulse and oilseed mixed in the same rows (**Fig. 3.1**).

Dry pea (*Pisum sativum* L. cv. CDC Inca) was seeded at 209 kg ha^{-1} as a monocrop [P100], 104 kg ha^{-1} as alternate row intercrop [P50] , and 180 kg ha^{-1} as mixed row intercrop [P86]. White mustard (*Sinapsis alba* L. cv. Andante) was seeded at 8 kg ha^{-1} as a monocrop [M100], 4 kg ha^{-1} as alternate row intercrop [M50], and 4 kg ha^{-1} as mixed row intercrop [M50]. The four dry pea-white mustard treatments were dry pea monocrop [P100], dry pea-white mustard alternate row [P50M50], dry pea-white mustard mixed row [P86M50], and white mustard monocrop [M100].

Kabuli chickpea (*Cicer arietinum* L. cv. CDC Orion) was seeded at 231 kg ha^{-1} as monocrop [C100], 115 kg ha^{-1} as alternate row intercrop [C50], and 205 kg ha^{-1} as mixed row intercrops [C89]. Brown flax (*Linum usitatissimum* L. cv. CDC Glas) was seeded at 56 kg ha^{-1} as a monocrop [F100], 27 kg ha^{-1} as alternate row intercrop [F50], and 12 kg ha^{-1} as mixed row intercrop [F21]. The four kabuli chickpea-brown flax treatments were kabuli chickpea monocrop [C100], kabuli chickpea-brown flax alternate rows [C50F50], kabuli chickpea-brown flax mixed row [C89F21], and brown flax monocrop [F100].

Seeding rates were based on previous studies conducted by Lana Shaw in her work on determining optimum seeding rates for intercropping systems at the SERF at Redvers, SK. All test plots were seeded into wheat stubble from the previous cropping season, except for the Central Butte 2020 site. The Central Butte 2020 site was seeded into herbicide fallow from the previous cropping season.

There were four replicates of each treatment, for a total of 32 plots, arranged in a randomized split-block design with four blocks of replicates (**Table 3.1**). Due to a seeding error, there were five replicates of kabuli chickpea monocrop at the Redvers 2019 site (**Figure 3.3**). The row spacing was 25cm for all treatments, as this is a common row spacing used by producers in Saskatchewan. The Central Butte sites were planted with a double-disk single row

Table 3.1. Seeding arrangements and crop combinations used in pulse-oilseed intercropping trials at two field research sites in Saskatchewan.

Site/ Soil Type	Seeding arrangement	Crop combination
Redvers/ Black Chernozem	Pulse monocrop	Kabuli chickpea/ brown flax
Central Butte/ Brown Chernozem	Oilseed monocrop	Dry Pea/ white mustard
	Pulse/oilseed in alternate rows	
	Pulse/oilseed in mixed rows	

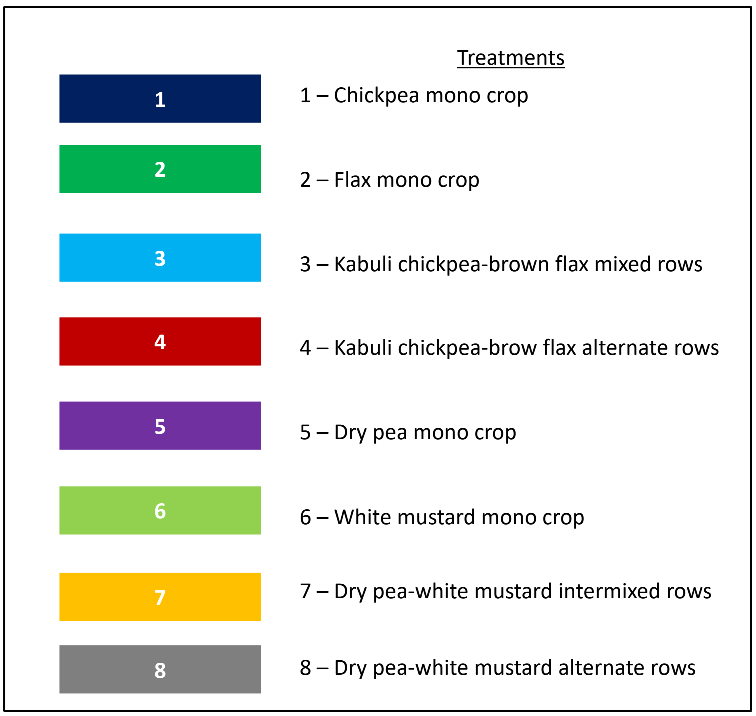

Fig. 3.1. Key to plot maps for pulse-oilseed intercropping trial in Saskatchewan.

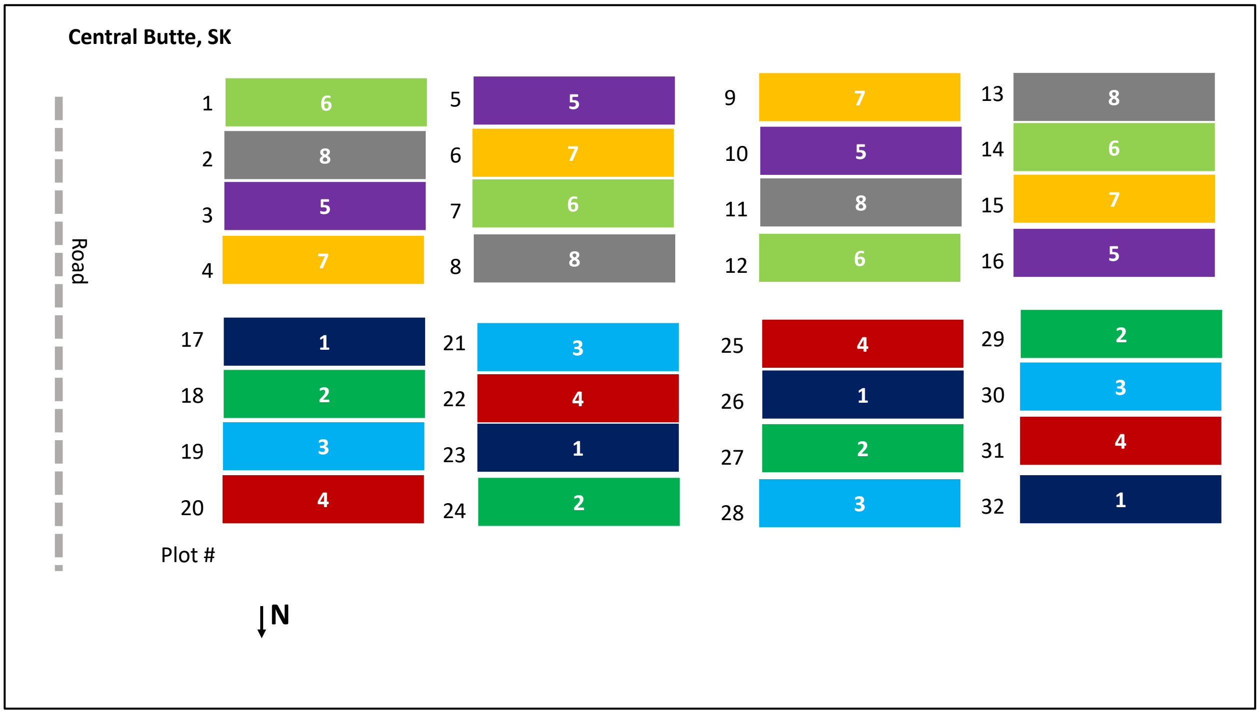

Fig. 3.2. Plot map of pulse-oilseed intercropping trial at Central Butte, SK site in 2019.

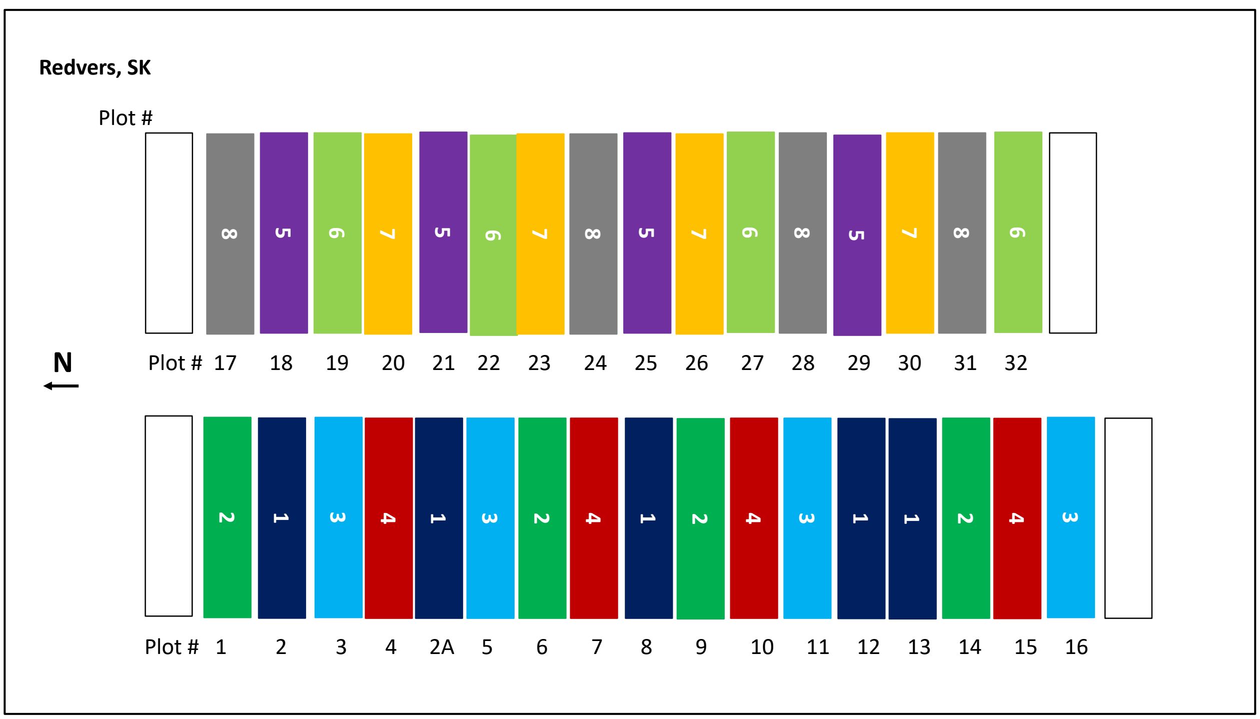

Fig. 3.3. Plot map of pulse-oilseed intercropping trial at Redvers, SK site in 2019.

press drill which distributed the seed via rotating cones. The Redvers plots were planted with a 10-foot wide Seedmaster™ plot drill. The plot size at Central Butte was 1m x 3m and each plot had four rows (**Fig. 3.2**). The plot size in Redvers was 3m x 9m in 2019 and 3m x 6m in 2020. Each plot in Redvers had 12 rows (**Fig. 3.3**). No fertilizer was applied. The pulse crops were inoculated with either granular or peat-based or both forms of *Rhizobium* inoculants, depending on availability at each site each year. Anchor® seed treatment was used to coat chickpea seed at recommended rate of application in Central Butte in 2019 and 2020 due to known elevated levels of root disease at these sites.

^{15}N-labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) was applied to subplots within the main plots for the purpose of calculating the BNF of the pulse crops as influenced by intercrop verses monocrop treatment. It was applied to all plots of all treatments, with the assumption that the monocrop oilseed crops would not biologically fix N, and so could be used as the reference crop for calculating BNF. Prior to application of ^{15}N, a stock solution was made by dissolving 144.1g of 10 atom% excess $^{15}NH_4{}^{15}NO_3$ fertilizer in 450mL deionized water. When the pulse crop plants were in the first true leaf stage, a 1m x 1m subplot was marked in each of the plots (**Figs. 3.4, 3.5**). A plastic frame was placed on the ground to confine the fertilizer solution within the plot area. For application of ^{15}N to the subplots, 5mL of the stock solution was dissolved in 4L of deionized water to achieve an application rate of 5kg N ha^{-1} per subplot. This was followed with application of an additional 4L of water over the subplot area to rinse off the residual fertilizer from the surface of the leaves into the soil.

In addition to Central Butte and Redvers, a third site at Indian Head, SK in 2019 also had $^{15}NH_4{}^{15}NO_3$ added to 1m x 1m subplots in a similar chickpea/flax intercropping trial at that location. Due to the poor germination at the Central Butte 2019 site, subplots were not installed at that location in 2019.

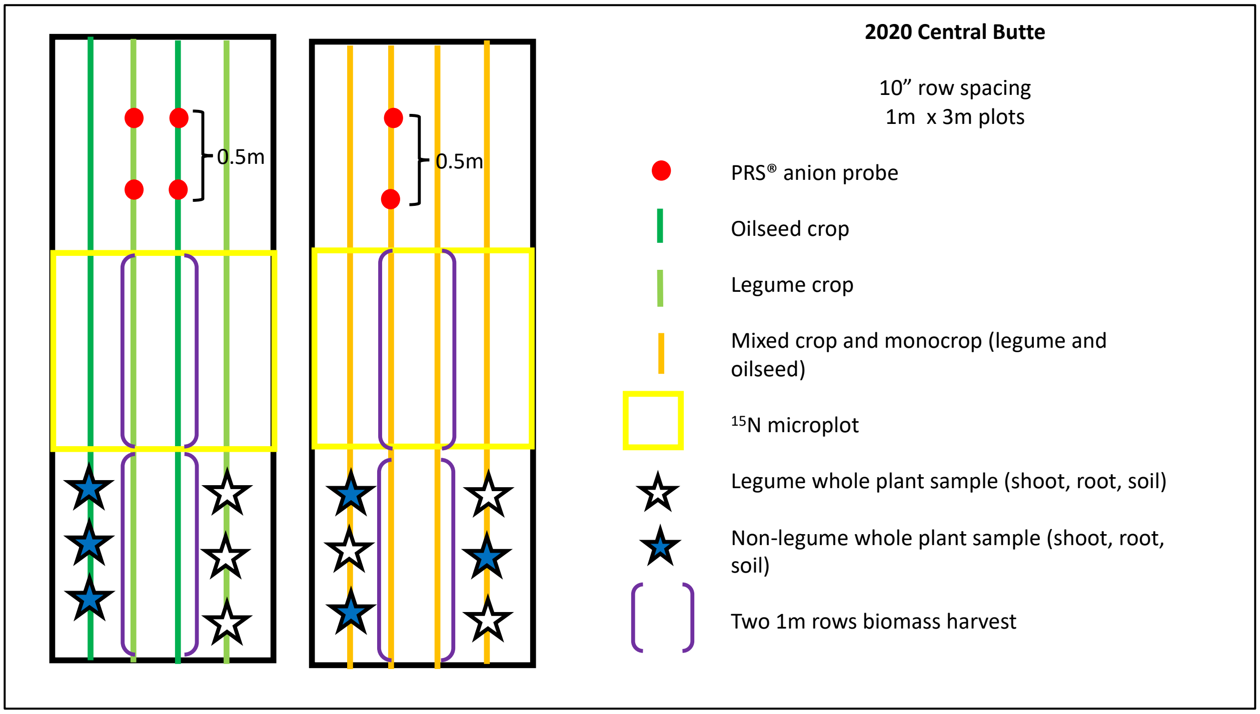

Fig. 3.4. Plot map showing locations of ^{15}N microplots and Plant Root Simulator (PRS™) probes in Central Butte, SK site in 2020.

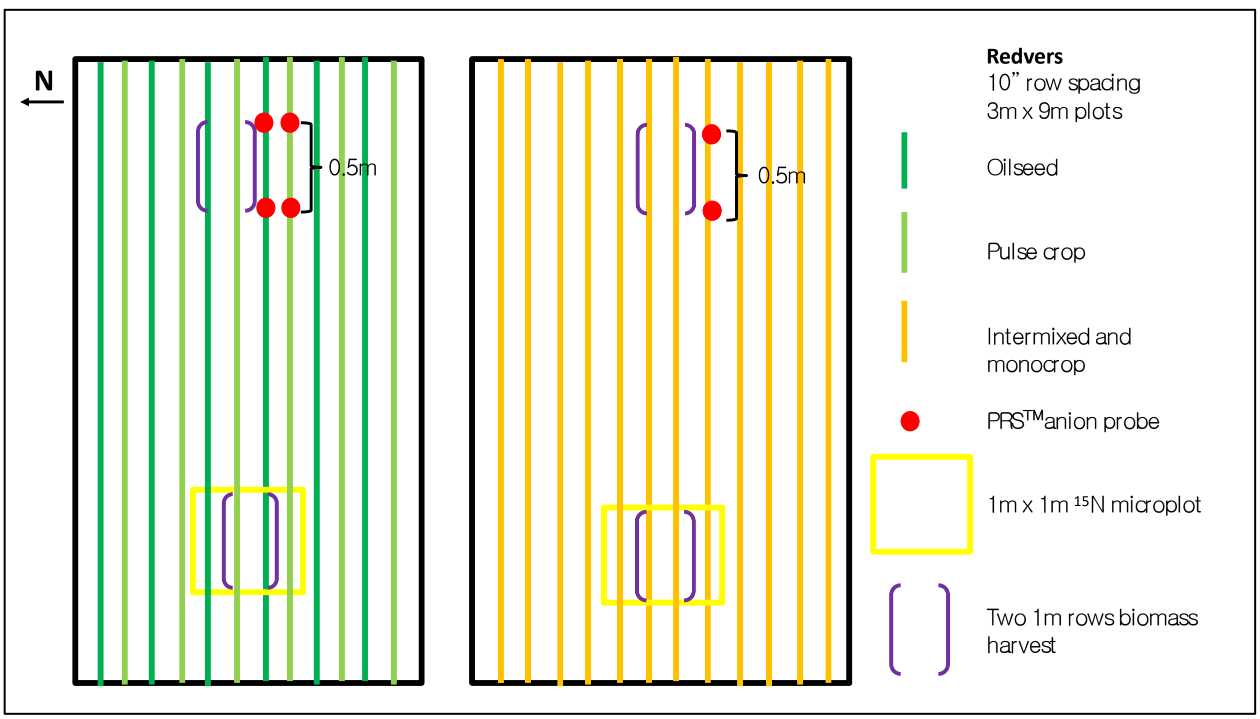

Fig. 3.5. Plot map showing locations of ^{15}N microplots and Plant Root Simulator (PRS™) probes in Redvers, SK site in 2019.

3.4.3 Weed control

For weed control, ethafluralin was applied at 0.8 kg ethafluralin per hectare as 10% A.I. clay impregnated granules pre-seeding to the pea/mustard plots in Redvers and sulfentrazone was applied at 88 mL/acre pre-seeding to the chickpea/flax plots in Redvers and Central Butte in 2019 and 2020. Glyphosate was applied at 0.8 L/acre pre-seeding to all plots in Central Butte in 2020. Fungicide and hand weeding was applied as required to maintain good pest control.

3.4.4 Climate data

The conditions experienced at the two sites in 2019 and 2020 seasons may be generally characterized as drier and warmer than normal. The Central Butte weather in 2019 began with May rainfall below the 30-year average (**Table 3.2**). Rain did not arrive until the third week of June. In July rainfall was above the 30-year average, while August was below the 30-year average rainfall. In 2020 there was below seasonal rainfall in May, which was accompanied by warmer than average temperatures. June and July of 2020 at the Central Butte site were close to historical averages for rain and temperature, however, August was dry and hot which was associated with early plant senescence.

The growing season at Redvers in 2019 began with below the 30-year average rainfall in May, but the remainder of the growing season had generally average rainfall amounts and distribution, and temperatures (**Table 3.3**). The spring of 2020 also began with dry conditions, which were followed by July and August having below 30-year average rainfall.

Table 3.2. Weather data from MET weather station at the Central Butte trial sites and Environment Canada weather station (Elbow, SK) 40km from Central Butte, SK.

	Precipitation mm			Daily mean temperature ºC		
	Central Butte 2019	Central Butte 2020	Elbow 2 NE Historic Average 1981-2010	Central Butte 2019	Central Bute 2020	Elbow 2 NE Historic Average 1981-2010
May	3.6	10.6	51.2	10.5	12.1	10.4
June	92.1	48.1	78.9	16.7	16.6	15.2
July	22.0	17.2	53.4	18.8	19.0	18.3
August	34.4	3.0	45.2	16.4	19.3	17.6
Total precipitation	152.1	78.9	228.7			

Table 3.3. Weather data from weather station at the Redvers trial sites and Environment Canada weather station (Maryfield, SK) 40km from Redvers, SK.

	Precipitation mm			Daily mean temperature ℃		
	Redvers 2019	Redvers 2020	Maryfield Historic Average 1981-2010	Redvers 2019	Redvers 2020	Maryfield Historic Average 1981-2010
May	18.0	22.9	58.9	8.7	10.5	11.3
June	79.0	59.7	86.2	16.7	16.8	16.0
July	54.0	47.8	73.0	18.3	19.2	18.6
August	88.0	36.1	58.0	16.5	18.5	18.0
Total precipitation	239.0	166.5	276.1			

3.4.5 Spring soil sampling and analysis

Spring soil samples were taken at 0-15cm, 15-30cm, and 30-60cm depths with a 5cm diameter coring device at ten points along a transect across the experimental area to characterize the site for initial soil properties and soil fertility. Soil samples were kept frozen until air dried at 35℃, sieved (<2mm) and analyzed. Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water suspension (Nelson & Sommers, 1982). Soil NO_3-N was extracted with 0.01M calcium chloride ($CaCl_2$) (Houba et al., 2000). Automated colorimetry (SEAL™) was used to analyze the extracts for concentrations of NO_3-N. Available soil P and potassium (K) were measured on the soil depth samples of 0-15cm and 15-30cm using a modified Kelowna extraction procedure (Qian et al., 1994). Extracts were then analyzed for P using a SEAL™ segmented flow automated system (Technicon Industrial Systems, Tarrytown, NY, USA). Potassium concentration in the extracts was analyzed using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer, Varian Inc., Palo Alto, CA, USA). The spring soil analyses at the Central Butte sites show that both site years were comparable in macronutrient availability (**Table 3.4**). The spring soil analyses at the Redvers sites show that soils at both site years had low available P, and that the 2020 site had elevated sulfate (SO_4^{2-}) levels.

Table 3.4. Physical and chemical characteristics in spring soils at the Central Butte and Redvers, SK sites from the 0-15cm depth.

Soil Property (*n*=10)	Central Butte 2019	Central Butte 2020	Redvers 2019	Redvers 2020
NO_3 (mg N kg^{-1})	6.6	10.2	7.2	14.6
SO_4 (mg S kg^{-1})	6.1	4.6	7.9	44.7
P (mg P kg^{-1})	14.3	15	6.5	5.9
K (mg K kg^{-1})	606	406	248	234
OC (%)	1.9	1.77	2.34	2.77
pH	7.5	7.3	7.9	7.6
EC (mS cm^{-1})	0.183	0.164	0.211	0.448

(*n*=10) for all the measurements except for Redvers 2020 which came from a single composite sample.
total organic carbon (OC) measured by LECO™ automated combustion analyzer
EC = electrical conductivity

3.4.6 Fall harvest

To determine harvest yield components, two 1m rows from each plot were hand harvested outside the ^{15}N microplots; and two 1m rows were hand harvested within each subplot (**Figs. 3.4, 3.5**). The crop samples were air dried, weighed, then threshed using a stationary mechanical thresher to provide grain and straw yield. Subsamples of grain and straw were then taken and ground using a grinding mill prior to analysis for N and P. An acid digest of ground grain and straw was conducted according to the method of Thomas et al. (1967). Briefly, 0.25g ($\pm$ 0.001g) of finely ground plant grain or straw was weighed into glass digestion tubes and 5mL of concentrated sulfuric acid (H_2SO_4) was added. Samples were placed on a digestion block at 360℃ for 30 minutes. Following this, samples were removed from the digestion block, allowed to cool, and 0.5mL hydrogen peroxide (H_2O_2) was added. Samples were then placed on the digestion block an additional three times for 30 minutes, adding H_2O_2 after each heating period. Finally, samples were placed on the digestion block for 1 hour. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample to 75mL to achieve a final concentration within the detection limit of the instrumentation. Samples were placed in a refrigerator until analysis for N and P by SEAL™ automated colorimetry. For ^{15}N analysis, the finely ground grain and straw sub-samples were weighed (grain ~2.0 mg and straw ~4.0 mg) and used to produce encapsulated samples. The encapsulated grain and straw samples were then analyzed for percent N and atom%^{15}N using a Costech® ECS4010 elemental analyzer coupled to a Delta V Advantage® Mass Spectrometer with a standard for the spectrometry measurement.

Post-harvest soil samples at 0-15cm, 15-30cm, and 30-60cm depths were taken from each plot with a punch truck, frozen until they could be air-dried and sieved, and the <2mm fraction was used to determine residual available macronutrients.

3.4.7 Calculations

The isotopic dilution method was used to determine N_2 fixed (kg N ha^{-1}) by calculating percentage of N derived from atmosphere (%Ndfa) and multiplying by total plant N calculated for the grain and straw separately. The BNF crop (BNF plant) is the pulse crop in the intercrop (dry pea or kabuli chickpea). The non-fixing crop (non-BNF plant) is the non-pulse crop (white mustard or brown flax) in the intercrop partnership grown in separate unlabelled plots.

Quantification of BNF was determined using the following equation **(Eq. 3.1)**:

$$\%Ndfa = \left[1 - \frac{\%^{15}N\ a.e._{(BNF\ plant)}}{\%^{15}N\ a.e._{(non-BNF\ plant)}}\right] \times 100 \tag{3.1}$$

where %Ndfa = percentage of fixed N derived from the atmosphere , $\%^{15}N$ a.e.$_{(BNF\ plant)}$ = percent of ^{15}N atom excess over the natural abundance in atmospheric N_2 (0.36637) in the BNF plant, and $\%^{15}N$ a.e.$_{(non\text{-}BNF\ plant)}$ = percent of ^{15}N atom excess over the natural abundance in atmospheric N in the non-BNF plant (Bottomley & Myrold, 2015).

Percentage of fixed N transferred from the intercropped pulse crops to the intercropped oilseed crops was calculated according to Farnham and George (1993) (**Eq. 3.2**).

$$\%Ntransfer = \left[1 - \frac{\%N\ a.e._{(intercrop\ oilseed)}}{\%N\ a.e._{(monocrop\ oilseed)}}\right] \times 100 \tag{3.2}$$

Amount of N fixed was calculated according to Hardarson and Danso (1990) (**Eq. 3.3**).

$$Nfixed = \left[\frac{\%Ndfa \times total\ N_{fixing}}{100}\right] \tag{3.3}$$

The potential addition or removal of N from the cropping system (N*inc*) was calculated according to Evans et al., (2001) (**Eq. 3.4**).

$$Ninc = total\ N\ input\ from\ fixation\ of\ straw\ and\ grain - N\ uptake\ grain \tag{3.4}$$

The land equivalency ratio LER for yield was calculated with the following equation **(Eq. 3.5**)

$$LER = \frac{ICY_a}{MY_a} + \frac{ICY_b}{MY_b} \tag{3.5}$$

where ICY_a= yield of *crop-a* as a component of the intercrop from a defined area, My_a= yield of *crop-a* as monocrop from the same defined area, ICY_b= yield of *crop-b* as a component of the intercrops from the same defined area, MY_b= yield of *crop-b* as a monocrop from the same defined area (Fletcher et al., 2016).

The LER was calculated for grain and straw N and P uptake as shown below (**Eq.3.6**):

$$NP\ LER = \frac{NP\ ICY_a}{NP\ MY_a} + \frac{NP\ ICY_b}{NP\ MY_b} \tag{3.6}$$

where *NP ICY*$_a$= N or P yield of *crop-a* as a component of the intercrop from a defined area, *NP My*$_a$= N or P yield of *crop-a* as monocrop from the same defined area, *NP ICY*$_b$= N or P yield of *crop-b* as a component of the intercrops from the same defined area, *NP MY*$_b$= N or P yield of *crop-b* as a monocrop from the same defined area (Szumigalski & Van Acker, 2006).

The N harvest index (NHI) was calculated for N uptake (**Eq. 3.7**).

$$NHI = \frac{N\ uptake_{grain}}{(N\ uptake_{grain} + N\ uptake_{straw})} \tag{3.7}$$

The Nutrient Utilization Efficiency (NUE) was calculated with the equation found in Weih et al. (2011) (**Eq. 3.8**)

$$NUE = \frac{grain\ yield\ kg/ha}{straw\ nutrient\ uptake + grain\ nutrient\ uptake\ kg\ nutrient/ha} \tag{3.8}$$

3.4.8 Statistical analysis

The kabuli chickpea-brown flax treatments and the dry pea-white mustard treatments were considered separate intercrop systems. Statistical analysis was performed on treatments within each intercrop system and not between intercrop systems. Statistical analysis by ANOVA was done using PROC GLIMMIX with SAS 9.4 software (SAS Institute, 2012) to determine treatment effects and interactions for each parameter measured. Refer to Stroup (2014) for discussion regarding use of generalized linear mixed model (GLMM) in plant and soil science. The two sites were analyzed together for each growing season. The SLICE statement was used to facilitate comparisons for interactions. The LINES option with the LSMEANS and SLICE statement was used to compute means and comparisons of treatments for each site separately. Analysis of variance tables that are presented are for the combined analysis. Statistical differences indicated in tables are for individual sites, treatments, and year. Arcsine-square root transformations were done for percentage and proportion data prior to analyses and they were backtransformed for presentation. The significance of treatments was assessed with Tukey's Studentized range test using $p \leq 0.05$ to determine if a main effect or interaction was significant. Data was checked for outliers using Grubb's test, however, due to the ability of PROC GLIMMIX to handle unbalanced and variable data, the identified outliers were removed from only a few data sets. Normal distribution was checked using Shapiro-Wilk values at $p \leq 0.05$.

3.5 Results

3.5.1 Kabuli chickpea-brown flax combinations

3.5.1.1 Yield

Kabuli chickpea straw and grain yield followed similar trends despite year to year and site variability. Kabuli chickpea as an intercrop yielded significantly lower ($p \leq 0.05$) than as a monocrop (**Table 3.5**). There were no significant differences ($p \leq 0.05$) in yields between the two seeding arrangements of intercrops.

While consistent trends were seen in kabuli chickpea yields, this was not mirrored in brown flax yields. At the Central Butte site in 2019, the alternate row brown flax significantly ($p \leq 0.05$) outyielded monocrop brown flax (**Table 3.5**). The Central Butte 2020 site mixed row brown flax yield was comparable to brown flax monocrop yield, and both were significantly ($p \leq 0.05$) higher than the alternate row brown flax yields (**Table 3.5**). There were no significant differences ($p \leq 0.05$) in brown flax yields at the Redvers 2019 site. In 2020, the Redvers monocrop brown flax yields were significantly higher ($p \leq 0.05$) than intercrop brown flax yields. The Redvers 2020 mixed row brown flax yielded significantly higher ($p \leq 0.05$) than alternate row brown flax.

The grain yield LER values for the kabuli chickpea-brown flax intercrops were above 1 in 2019 at both sites and in 2020 at Central Butte. The grain LERs were ~1 at Redvers in 2020 (**Table 3.6**).

Table 3.5. Kabuli chickpea and brown flax straw and grain yields from monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in mixed and alternate rows with flax at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

CHICKPEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
				- - - kg ha^{-1} - - -				
Monocrop	2709 a	1788 a	3180 a	1395 a	2129 a	3133 a	2068 a	1751 a
Alternate Row	903 b	735 b	1864 ab	767 ab	767 b	1232 b	470 b	313 b
Mixed Row	511 b	613 b	685 b	609 b	401 c	567 c	95 c	31 b

Source of variation	Probability (p) - 2019 - Straw	- 2019 - Grain	- 2020 - Straw	- 2020 - Grain
Site	0.0662	0.6298	0.1144	**0.0031**
Treatment	**0.0010**	**0.0010**	**<0.0001**	**<0.0001**
Site*Treatment	0.7415	0.5035	0.3431	0.1177

FLAX

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
				- - - kg ha^{-1} - - -				
Monocrop	2094 a	1558 a	3209 b	1396 b	2159 a	1467 a	4252 a	2187 ab
Alternate Row	1723 a	1364 a	4673 a	2638 a	1162 b	802 c	3144 b	2010 b
Mixed Row	2200 a	1380 a	2976 b	1446 b	1764 a	1143 b	4026 a	2344 a

Source of variation	Probability (p) - 2019 - Straw	- 2019 - Grain	- 2020 - Straw	- 2020 - Grain
Site	**0.0011**	0.1227	**<0.0001**	**0.0012**
Treatment	0.3973	0.1240	**0.0002**	**0.0004**
Site*treatment	0.0859	0.0609	0.7252	**0.0130**

Values in a column followed by same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

Table 3.6. Kabuli chickpea-brown flax intercrop total land equivalent ratios (LER) for grain and straw yield at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Alternate Row	1.4 a	1.2 a	2.6 a	2.3 a	1.0 a	0.9 a	1.1 a	1.0 a
Mixed Row	1.3 a	1.2 a	1.3 b	0.9 b	1.0 a	1.0 a	1.1 a	1.0 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	Grain	Straw	Grain	Straw
Site	0.2279	0.2940	0.1215	0.7677
Treatment	0.0509	0.0605	0.7858	0.1432
Site*Treatment	0.0908	**0.0419**	0.9802	0.4009

Total LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

3.5.1.2 Grain N and P uptake

Kabuli chickpea grain N and P uptake followed the same trends as kabuli chickpea grain yields. Kabuli chickpea as an intercrop had significantly lower grain N and P uptake ($p \leq 0.05$) than as a monocrop (**Table 3.7**). There were no significant differences ($p \leq 0.05$) in grain N and P uptake between the two seeding arrangements of intercrops (**Table 3.7**).

The nutrient utilization efficiency (NUE) values (**Table 3.8**) indicate that mixed row kabuli chickpea had a significantly higher ($p \leq 0.05$) NUE for N (NUE-N) than monocrop kabuli chickpea in Redvers 2019, but there were no differences between treatments at the Redvers 2020 site. Intercrop kabuli chickpea had significantly higher ($p \leq 0.05$) NUE for P (NUE-P) than monocrop kabuli chickpea at the Redvers 2019 site. In 2020 the Redvers site mixed row kabuli chickpea NUE-P was significantly ($p \leq 0.05$) higher than alternate row kabuli chickpea, which was significantly higher than for monocrop kabuli chickpea. The Central Butte 2019 site revealed mixed row kabuli chickpea to have significantly higher ($p \leq 0.05$) NUE-N and NUE-P than the other two treatments. This was not repeated in 2020 at Central Butte, with monocrop kabuli chickpea having significantly higher ($p \leq 0.05$) NUE-N than the other two treatments, and no significant differences ($p \leq 0.05$) between treatments for NUE-P.

Table 3.7. Kabuli chickpea and brown flax grain N and P uptake for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

CHICKPEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	N	P	N	P	N	P	N	P
				- - - kg nutrient uptake ha^{-1} - - -				
Monocrop	51.0 a	5.1 a	46.1 a	5.8 a	90.1 a	9.3 a	58.6 a	6.0 a
Alternate row	23.6 b	1.6 b	24.9 ab	2.6 b	34.8 b	2.9 b	10.7 b	1.0 b
Mixed row	19.4 b	1.3 b	17.1 b	1.4 b	15.5 b	1.6 b	1.0 b	0.1 b

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	N	P	N	P
Site	0.7227	0.3351	**0.0199**	**0.0334**
Treatment	**0.0029**	**0.0002**	**<0.0001**	**<0.0001**
Site*treatment	0.9161	0.8105	0.4893	0.5174

FLAX

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	N	P	N	P	N	P	N	P
				- - - kg nutrient uptake ha^{-1} - - -				
Monocrop	51.7 a	8.4 a	38.9 b	9.2 b	34.3 a	8.4 a	79.3 a	10.2 a
Alternate row	49.8 a	7.6 a	82.0 a	16.9 a	23.0 b	5.2 b	71.0 a	9.3 a
Mixed row	45.5 a	7.4 a	45.9 b	10.0 b	31.1 ab	5.9 b	82.0 a	10.6 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	N	P	N	P
Site	0.4757	**0.0191**	**<0.0001**	**0.0043**
Treatment	0.0863	0.1175	**0.0312**	**0.0130**
Site*treatment	0.0805	0.0807	0.7285	0.0633

Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table 3.8. Kabuli chickpea and brown flax grain N and P nutrient utilization efficiency (NUE) for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

CHICKPEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P
	- - - kg grain yield / [kg straw nutrient uptake + kg grain nutrient uptake] - - -							
Monocrop	20.1 b	210.2 b	19.4 b	155.9 b	30.8 a	321.5 c	22.2 a	237.0 a
Alternate row	24.7 ab	346.8 a	21.1 b	232.2 b	32.2 a	419.7 b	18.5 b	227.7 a
Mixed row	27.6 a	427.1 a	28.1 a	353.6 a	33.8 a	470.8 a	17.2 b	228.8 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	NUE-N	NUE-P	NUE-N	NUE-P
Site	0.5128	**0.0226**	**<0.0001**	**0.0010**
Treatment	**0.0050**	**0.0001**	0.5480	**0.0015**
Site*treatment	0.4980	0.6391	**0.0185**	**0.0006**

FLAX

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P
	- - - kg grain yield / [kg nutrient uptake straw + kg nutrient uptake grain] - - -							
Monocrop	26.8 a	170.1 a	30.2 a	136.2 a	40.7 a	173.1 a	25.2 a	217.0 a
Alternate row	24.8 a	166.4 a	27.1 ab	137.7 a	31.3 b	145.2 a	26.0 a	218.1 a
Mixed row	25.1 a	168.9 a	25.9 b	124.9 a	32.6 b	240.7 a	25.7 a	218.6 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	NUE-N	NUE-P	NUE-N	NUE-P
Site	0.1696	**0.0008**	0.4641	**0.0106**
Treatment	**0.0387**	0.6947	0.3236	0.1347
Site*treatment	0.5270	0.6163	0.3352	0.0775

Values in a column followed by the same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

The alternate row brown flax at the Central Butte 2019 site significantly ($p \leq 0.05$) outyielded monocrop flax in grain N and P uptake (**Table 3.7**). There were no significant differences ($p \leq 0.05$) between treatments for grain N and P uptake at Central Butte 2020 site (**Table 3.7**). There were no significant differences ($p \leq 0.05$) among treatments in brown flax grain N and P uptake at Redvers 2019 site. The Redvers 2020 site monocrop brown flax grain N and P uptakes were significantly higher ($p \leq 0.05$) than intercrop treatment brown flax uptake. There were no significant differences between the intercrop treatments for grain N and P grain uptake at the Redvers site in 2020.

There were no differences between brown flax treatments for NUE-P at Central Butte 2019 and 2020 sites (**Table 3.8**). The NUE-N was significantly higher ($p<0.05$) in monocrop brown flax than in mixed row brown flax at Central Butte 2019 site, but there were no differences between treatments in 2020 (**Table 3.8**).

Table 3.9. Kabuli chickpea-brown flax intercrop grain N and P uptake total land equivalent ratios (NLER and PLER) at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	NLER	PLER	NLER	PLER	NLER	PLER	NLER	PLER
Alternate row	1.5 a	1.3 a	2.9 a	2.5 a	1.1 a	0.9 a	1.1 a	1.1 a
Mixed row	1.3 a	1.1 a	1.3 b	1.0 b	1.2 a	0.9 a	1.1 a	1.1 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	NLER	PLER	NLER	PLER
Site	0.2407	0.2072	0.6260	**0.0455**
Treatment	**0.0381**	0.0716	0.7529	0.7788
Site*treatment	0.0962	0.1156	0.7964	0.5579

Total LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Yield components, such as grain N and P uptake, may also be assessed with LER. The high alternate row brown flax yield at Central Butte site in 2019 resulted in significantly higher grain N uptake land equivalent ratio (NLER) and grain P uptake land equivalent ratio (PLER) values for kabuli chickpea-brown flax in alternate rows than in mixed rows. There were no significant differences between seeding arrangements for brown flax grain NLER and PLER uptake values in Central Butte 2020, and Redvers 2019 and 2020 sites (**Table 3.9**). The kabuli chickpea-brown flax grain NLER and PLER uptake values were above 1 for Central Butte in 2019 and 2020 sites and Redvers 2019 site. The Redvers 2020 site kabuli chickpea-brown flax grain N uptake LER value above 1, but was below 1 for grain P uptake.

3.5.1.3 Biological N fixation

Monocrop kabuli chickpea had significantly (p≤0.05) higher amounts of biologically fixed N in aboveground biomass (**Table 3.11**) at all site-years except Redvers 2019. There were no differences in %Ndfa between the kabuli chickpea treatments (**Table 3.10**). There were no differences in the %Ntransfer to the intercropped brown flax between the two intercropping seeding arrangements in the four site-years, nor were there differences in the amount of fixed N in the aboveground biomass between the two intercrop seeding arrangements, however, some N transfer from the kabuli chickpea to the brown flax is evident when they are grown together (**Tables 3.10**, **3.11**). Nitrogen was removed from the system (N*inc*) in all treatments (**Table 3.12**). The intercropped brown flax removed significantly (p≤0.05) less N from the system than

Table 3.10. Kabuli chickpea-brown flax intercrop grain percent N fixed from atmosphere (% Ndfa) and percent fixed N transferred to non-fixing crop (%Ntransfer) at Redvers, Indian Head (IH), and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	**Redvers 2019**	**IH 2019**	**Redvers 2020**	**CB 2020**
		- - - %Ndfa - - -		
Chickpea mono	58.2 a	67.8 a	69.4 a	67.4 a
Chickpea alt row	59.7 a	65.1 a	68.3 a	46.8 a
Chickpea mix row	63.1 a	64.0 a	74.2 a	45.0 a
		- - - %Ntransfer - - -		
Flax alt row	21.8 b	26.6 b	8.0 b	19.4 b
Flax mix row	19.7 b	30.3 b	11.3 b	11.5 b

Source of variability	Probability (p)	
	2019	2020
Site	0.2404	0.1811
Treatment	**<0.0001**	**<0.0001**
Site*Treatment	0.8157	**0.0143**

Arcsine-square root transformations were done for percentage data prior to analyses and backtransformed for presentation.
Values in a column followed by same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

Table 3.11. Amount of biologically fixed N in shoot in monocrop kabuli chickpea and kabuli chickpea-brown flax intercrops in mixed and alternate rows at Redvers, Indian Head (IH), and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019	IH 2019	Redvers 2020	CB 2020
		- - - kg N ha^{-1} - - -		
Chickpea mono	48.2 a	88.4 a	66.7 a	45.4 a
Chickpea alt row	26.8 ab	47.7 b	29.8 b	6.7 b
Chickpea mix row	13.3 b	50.0 b	19.2 bc	0.7 b
Flax alt row	12.7 b	7.5 c	1.7 c	15.8 b
Flax mix row	13.0 b	5.5 c	4.1 c	10.3 b

Source of variation	Probability (p)	
	2019	2020
Site	**0.0059**	0.1271
Treatment	**<0.0001**	**<0.0001**
Site*treatment	**0.0085**	**0.0487**

Values in a column followed by the same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

Table 3.12. Potential addition (positive value) or removal (negative value) of N from the cropping system (N*inc*) for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in mixed and alternate rows at Redvers, Indian Head (IH), and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019	IH 2019	Redvers 2020	CB 2020
		- - - kg N ha^{-1} - - -		
Chickpea mono	-8.21 a	-26.51 c	-22.75 bc	-13.15 a
Chickpea alt row	-2.10 a	-21.89 bc	-10.43 ab	-3.98 a
Chickpea mix row	-6.10 a	-25.09 c	-5.45 a	-0.19
Flax monocrop	-51.60 c	-51.06 d	-44.42 d	-79.33 c
Flax alt row	-37.13 b	-13.66 ab	-16.46 abc	-55.22 b
Flax mix row	-34.52 b	-6.52 a	-28.71 c	-71.63 c

Source of variation	Probability (p)	
	2019	2020
Site	0.9646	**0.0010**
Treatment	**<0.0001**	**<0.0001**
Site*treatment	**<0.0001**	**<0.0001**

N*inc* = total N input from fixation of straw and grain – N $uptake_{grain}$
Values in a column followed by the same letter are not significantly different.
Bolded values indicate significance at the p≤0.05 level of probability.

monocrop brown flax in all the site-years except Central Butte 2020.

3.5.2 Dry pea-white mustard combinations

3.5.2.1 Yield

There were no grain or straw yield differences among dry pea seeding treatments across the four site-years, with the exception of significantly ($p \leq 0.05$) lower straw yield at Central Butte 2020 site in the alternate row dry pea (**Table 3.13**). The Redvers 2019 site monocrop white mustard straw and grain yields were significantly higher ($p \leq 0.05$) than the intercrop white mustard yields, but there were no yield differences among white mustard seeding treatments in 2020 (**Table 3.13**). The Central Butte 2019 and 2020 sites monocrop white mustard yields were significantly higher ($p \leq 0.05$) than mixed row mixed mustard yields (**Table 3.13**).

Overall, dry pea yields on per ha basis were generally not reduced by growing dry pea in combination with white mustard, Reflecting this, dry pea-white mustard intercrop grain yield LER values were consistently above 1 for all intercrop seeding arrangements, and there were no significant differences between seeding arrangements (**Table 3.14**).

Table 3.13. Dry pea and white mustard straw and grain yields for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

PEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
				- - - kg ha^{-1} - - -				
Monocrop	3236 a	2206 a	3207 a	1499 a	3367 a	2950 a	1985 a	866 a
Alternate Row	2293 a	2112 a	2636 a	737 a	2449 a	2269 a	686 b	646 a
Mixed Row	2270 a	2019 a	2525 a	1708 a	2745 a	2445 a	1401 ab	833 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	Straw	Grain	Straw	Grain
Site	0.5246	**0.0081**	**0.0052**	**0.0043**
Treatment	0.2292	0.5609	**0.0189**	0.2740
Site*Treatment	0.9276	0.5165	0.8140	0.6121

MUSTARD

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
				- - - kg ha^{-1} - - -				
Monocrop	2851 a	685 a	4443 a	1751 a	2031 a	644 a	4572 a	1798 a
Alternate Row	554 b	243 b	3790 a	1495 a	638 b	189 b	3815 ab	1502 ab
Mixed Row	886 b	256 b	2812 a	983 a	582 b	240 b	2122 b	789 b

Source of Variation	Probability (p)			
	- 2019 -		**- 2020 -**	
	Straw	Grain	Straw	Grain
Site	**0.0001**	**0.0003**	**0.0010**	**0.0023**
Treatment	**0.0017**	**0.0029**	**0.0178**	0.0519
Site*Treatment	0.0929	0.2081	0.3880	0.3310

Values in a column followed by same letter not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table 3.14. Dry pea-white mustard intercrop total land equivalent ratios (LER) for grain and straw yield at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Alternate Row	1.5 a	1.0 a	1.6 a	1.8 a	1.2 a	1.1 a	2.0 a	1.6 a
Mixed Row	1.4 a	1.2 a	2.8 a	1.8 a	1.2 a	1.2 a	1.9 a	1.5 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	Grain	Straw	Grain	Straw
Site	0.2315	0.1012	0.1374	0.2180
Treatment	0.2190	0.9460	0.9626	0.7277
Site*treatment	0.1657	0.6321	0.6591	0.3551

Total LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probablility.

3.5.2.2 Grain N and P uptake

There were no grain N and P uptake differences among dry pea treatments across the four site-years (**Table 3.15**). There were no differences between dry pea treatments across the four site-years for grain NUE, with the exception of significantly (p≤0.05) lower grain NUE-P at the Central Butte 2020 site alternate row dry pea treatment (**Table 3.16**).

The Redvers 2019 site monocrop white mustard grain N and P uptakes were significantly higher (p≤0.05) than in the intercrop white mustard treatments (**Table 3.15**). The Redvers 2020 site mixed row white mustard grain P uptake was significantly lower (p≤0.05) than the other two treatments. At the Central Butte site in 2019, the monocrop white mustard grain N and P uptake was significantly higher (p≤0.05) than intercropped white mustard, with mixed row white mustard P uptake significantly lower (p≤0.05) than alternate row white mustard. In 2020, the Central Butte site mixed row white mustard N and P uptake was significantly lower (p≤0.05) than the other white mustard treatments. Overall, the white mustard yields and uptake of nutrients were reduced when grown together with the pea, especially in the mixed row arrangement.

There were no differences among white mustard treatments for grain NUE-N and NUE-P at Redvers and Central Butte sites in 2019 (**Table 3.16**). The Redvers 2020 site monocrop white

Table 3.15. Dry pea and white mustard grain N and P uptake for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

PEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	N	P	N	P	N	P	N	P
			- - - kg nutrient uptake ha^{-1} - - -					
Monocrop	90.4 a	7.0 a	56.7 a	5.6 a	100.8 a	7.9 a	30.0 a	2.2 a
Alternate Row	75.4 a	5.4 a	25.9 a	2.6 a	77.8 a	5.9 a	24.7 a	2.2 a
Mixed Row	74.1 a	5.5 a	58.2 a	5.4 a	85.3 a	6.2 a	31.3 a	2.6 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	N	P	N	P
Site	**0.0017**	**0.0087**	**0.0127**	**0.0115**
Treatment	0.3514	0.2653	0.3469	0.4788
Site*treatment	0.5951	0.6633	0.5806	0.3372

MUSTARD

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	N	P	N	P	N	P	N	P
			- - - kg nutrient uptake ha^{-1} - - -					
Monocrop	29.1 a	5.3 a	72.4 a	16.0 a	26.6 a	5.3 a	88.2 a	11.4 a
Alternate row	10.5 b	1.7 b	55.3 b	12.0 b	8.4 b	1.5 a	78.9 a	8.6 ab
Mixed row	11.6 b	1.8 b	43.4 b	7.3 c	9.2 b	1.3 b	37.8 b	4.6 b

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	N	P	N	P
Site	**0.0004**	**0.0002**	**0.0034**	**0.0019**
Treatment	**0.0003**	**0.0001**	0.0626	**0.0120**
Site*treatment	0.3803	**0.0383**	0.2545	0.4432

Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table 3.16. Dry pea and white mustard grain N and P nutrient utilization efficiency (NUE) for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

PEA

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P
	kg ha^{-1} grain yield / [kg ha^{-1} straw nutrient uptake + kg ha^{-1} grain nutrient uptake]							
Monocrop	19.6 a	272.6 a	16.2 a	189.3 a	23.4 a	342.8 a	15.9 a	284.5 a
Alternate row	23.0 a	330.2 a	12.2 a	146.5 a	24.8 a	364.6 a	19.1 a	215.4 b
Mixed row	23.0 a	322.0 a	18.2 a	214.0 a	23.4 a	374.5 a	18.1 a	253.2 ab

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	NUE-N	NUE-P	NUE-N	NUE-P
Site	**0.0392**	**0.0151**	**0.0026**	**0.0011**
Treatment	0.3507	0.5090	0.1461	0.2457
Site*treatment	0.2543	0.3075	0.5917	**0.0408**

MUSTARD

	Redvers 2019		**CB 2019**		**Redvers 2020**		**CB 2020**	
Treatment	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P	NUE-N	NUE-P
	kg ha^{-1} grain yield / [kg ha^{-1} straw nutrient uptake + kg ha^{-1} grain nutrient uptake]							
Monocrop	18.5 a	115.3 a	20.4	96.5	20.7 a	115.0 ab	18.5 a	153.4 b
Alternate row	19.2 a	128.4 a	19.4	93.0	16.6 b	110.1 b	16.6 a	172.7 a
Mixed row	15.9 a	115.9 a	17.4	113.9	15.3 b	126.9 a	17.1 a	169.6 ab

Source of variation	Probability (p)[d]			
	- 2019 -		- 2020 -	
	NUE-N	NUE-P	NUE-N	NUE-P
Site	0.1963	**0.0192**	0.9012	**0.0002**
Treatment	0.0640	0.3760	**0.0016**	0.0688
Site*treatment	0.7644	0.0664	0.0759	0.0973

Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
2019 CB mustard alt row NUE-N and NUE-P (*n*=2), therefore, statistical comparison of the treatments was not included.
Bolded values indicate significancy at the $p \leq 0.05$ level of probability.

Table 3.17. Dry pea-white mustard intercrop grain N and P uptake total land equivalent ratios (NLER and PLER) at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		**CB 2020**		**Redvers 2020**		**CB 2020**	
Treatment	NLER	PLER	NLER	PLER	NLER	PLER	NLER	PLER
Alternate row	1.3	1.2	1.6 a	1.5 a	1.2 a	1.1 a	2.2 a	2.3 a
Mixed row	1.4	1.2	2.7 a	2.5 a	1.3 a	1.1 a	2.0 a	2.1 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	NLER	PLER	NLER	PLER
Site	0.3788	0.3269	0.1410	0.0789
Treatment	0.3050	0.3790	0.9452	0.6849
Site*treatment	0.3428	0.3884	0.4800	0.6559

Total LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
2019 Redvers mix row N and P (*n*=2), therefore, statistical comparison of treatments was not included.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

mustard had significantly higher ($p \leq 0.05$) grain NUE-N than the other two white mustard treatments, and mixed row white mustard had significantly higher ($p \leq 0.05$) NUE-P than alternate row white mustard. The Central Butte 2020 site had no differences between white mustard treatments for NUE-N, and alternate row white mustard had significantly higher ($p \leq 0.05$) NUE-P than monocrop white mustard. Dry pea-white mustard intercrop grain NLER and PLER values for nutrient uptakes show that there were no differences among intercrop seeding arrangements across the four site-years, and that all the LER values were consistently above 1 (**Table 3.17**). As for the chickpea-flax intercrops, the LER nutrient uptake values above 1 for the dry pea-white mustard intercropping arrangements for all the site-years indicates a nutrient uptake and efficiency enhancement in both mixed and alternate row intercropping compared to monocropping.

3.5.2.3 Biological N fixation

As observed for the kabuli chickpea in the kabuli chickpea-brown flax intercrops, there were no differences in %Ndfa or amount of fixed N in aboveground shoot biomass among the Redvers 2019 dry pea treatments or %N transfer or amount of fixed N in aboveground shoot biomass between the Redvers 2019 intercropped white mustard treatments (**Tables 3.18**, **3.19**). Percent N

Table 3.18. Dry pea-white mustard intercropping grain percent N fixed from atmosphere (%Ndfa) and percent fixed N transferred to non-fixing crop (%Ntransfer) at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	**Redvers 2019**	**Redvers 2020**	**CB 2020**
		- - - %Ndfa - - -	
Dry pea monocrop	51.1 a	68.3 ab	16.3 a
Dry pea alternate row	69.6 a	73.3 a	16.6 a
Dry pea mixed row	67.9 a	69.9 ab	23.8 a
		- - - %Ntransfer - - -	
White mustard alt row	9.3 b	14.6 c	22.5 a
White mustard mix row	17.5 b	44.1 b	16.9 a
Source of variation	Probability (p)		
	2019	2020	
Site	na	**0.0205**	
Treatment	**<0.0001**	0.0539	
Site*treatment	na	**0.0074**	

Arcsine-square root transformations were done for percentage data prior to analyses and backtransformed for presentation.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.
na = not applicable.

Table 3.19. Amount of biologically fixed N in shoot in monocrop dry pea and dry pea-white mustard intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	**Redvers 2019**	**Redvers 2020**	**CB 2020**
		- - - kg N ha^{-1} - - -	
Dry pea monocrop	63.7 a	87.4 a	5.8 a
Dry pea alternate row	56.5 a	61.9 b	2.6 a
Dry pea mixed row	63.7 a	66.3 ab	14.2 a
White mustard alt row	1.6 b	1.8 c	17.1 a
White mustard mix row	4.8 b	4.8 c	5.3 a
Source of variation	Probability (p)		
	2019	2020	
Site	na	**0.0029**	
Treatment	**0.0001**	**<0.0001**	
Site*treatment	na	**<0.0001**	

Values in a column followed by same letter are not significantly different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.
na = not applicable

Table 3.20. Potential addition (positive value) or removal (negative value) of N from the cropping system (N*inc*) for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in mixed and alternate rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (n=4).

Treatment	Redvers 2019	Redvers 2020	CB 2020
		- - - kg N ha^{-1} - - -	
Dry pea monocrop	-18.3 ab	-16.7 a	-24.2 a
Dry pea alternate row	-18.9 a	-10.1 a	-22.1 a
Dry pea mixed row	-10.4 a	-14.0 a	-17.1 a
White mustard monocrop	-35.9 b	-33.2 a	-88.2 b
White mustard alt row	-8.9 a	-9.3 a	-61.9 b
White mustard mixed row	-6.9 a	-5.6 a	-32.5 a
Source of variation	Probability (p)		
	2019	2020	
Site	na	**0.0385**	
Treatment	**0.0070**	**0.0004**	
Site*treatment	na	**0.0397**	

N*inc* = total N from fixation of straw and grain – N $uptake_{grain}$
Values in a column followed by same letter are not significantly different.
Bolded values indicate significance at the p≤0.05 level of probability.
na = not applicable

transfer was significantly (p≤0.05) higher in mixed row white mustard than in alternate row white mustard at the Redvers 2020 site. There was no difference in amount of fixed N in aboveground shoot biomass in the two dry pea intercrop treatments, but the amount of fixed N in the aboveground shoot biomass of dry pea in alternate rows was significantly (p≤0.05) lower than monocrop dry pea (**Table 3.19**) The four dry pea-white mustard treatments showed a net removal of N from the cropping system (N*inc*) (**Table 3.20**). The Redvers 2019 intercropped white mustard treatment had significantly (p≤0.05) lower N removal than monocrop white mustard, but this was not repeated in 2020. The Central Butte 2020 mixed row white mustard treatment had significantly (p≤0.05) lower N removal than the other white mustard treatments.

3.6 Discussion

Dry seeding conditions in Central Butte in 2019 suppressed germination, resulting in a thin stand. Rain arrived in the third week of June which promoted a second stage of brown flax germination (**Fig. 3.6**). Above historical average rain received in July 2019 supported disease-free chickpeas through a dry August. Similar conditions were reported by Lenssen et al. (2007) in their Montana trial of various pulse crops and oilseeds. The Central Butte 2020 kabuli chickpeas were setback by disease at flowering as a resulting from onset of rain in later June and early July. Fungicide was applied to the kabuli chickpeas and they recovered. However, by the time re-growth and flowering resumed it was August. The brown flax had finished blooming, closed the canopy, and was setting seed (**Fig. 3.7**). In addition, August had below historical average rainfall coupled with above historical average daytime temperatures (**Table 3.2**). These combined factors limited kabuli chickpea yield in Central Butte in 2020 (**Fig. 3.8**). Kabuli chickpea grows best with daytime temperatures between 21℃ and 30℃, and is more tolerant to high temperatures during inflorescence compared to dry pea (Cutforth et al., 2007). However, when daytime temperatures rise above this range, early senescence will limit yield

Fig. 3.6. Two stages of emergence at the Central Butte, SK site in 2019. Photo taken on July 3, 2019.

Fig. 3.7. Disease setback of kabuli chickpea at the Central Butte, SK site in 2020. On the left kabuli chickpeas prior to spraying with fungicide on July 16, 2020. On the right kabuli chickpeas with re-growth and re-start of blooming on August 5, 2020. The flax had finished blooming, closed the canopy and was setting seed.

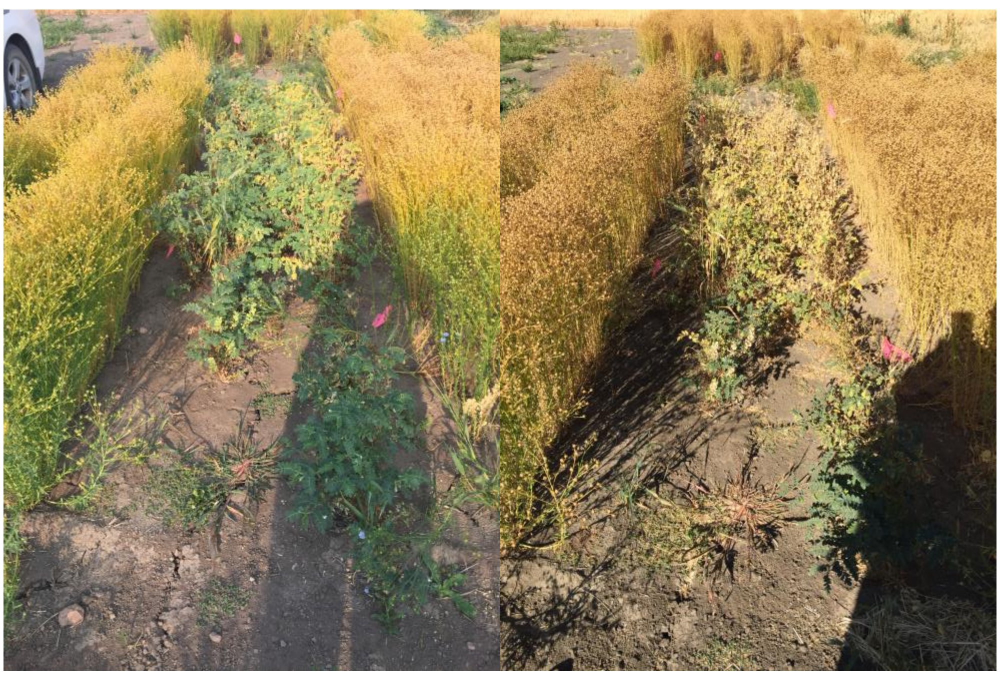

Fig. 3.8. July and August drought combined with above historic average daytime temperatures initiated early senescence, limiting kabuli chickpea yield at the Central Butte, SK site in 2020. Photo on left taken on August 20, 2020. Photo on right taken on August 30, 2020.

(Cutforth et al., 2007). The Environment Canada Elbow CS weather station (40km from Central Butte, SK) recorded three days at the end of July and ten days in August of daily maximum temperatures ≥30℃ (>32℃ two days in July and five days in August) in 2020.

The efficiency of producing yield and grain N and P uptake and removal per unit area of land were greater within the pulse-oilseed intercrop systems tested in the trial compared to their corresponding monocrops. Overall, the predominance of LER values for N and P uptake above 1 indicate synergy in nutrient uptake from the kabuli chickpea-brown flax intercropping arrangements versus monoculture. The kabuli chickpea-brown flax LER (except Redvers 2020), NLER, and PLER values (except Redvers 2020) were above 1 indicating benefit from intercropping (**Tables 3.6**, **3.9**). Hauggaard-Nielsen et al. (2009) also reported LER values greater than 1 for aboveground biomass P, K, and S uptake in their European organic system dry pea-barley intercropping trial. A similar observation was made by Malhi (2012) in their dry pea-canola intercrop study in Saskatchewan. This positive interaction is indicated in many cases in higher NUE values for intercropping. Intercropped kabuli chickpea, regardless of seeding arrangement, had significantly higher NUE-P than monocrop kabuli chickpea in Redvers 2019. Greater crop uptake and removal of P in harvest associated with greater yield and nutrient demand in these pulse-oilseed intercrops will require greater attention to replacing P removed with P fertilizer or manure in order to maintain fertility, similar to that reported by Miheguli et al. (2018) when forage legumes are used in short rotation with cereals or oilseeds without added fertilizer. Presence of pulse crops may enhance soil P scavenging (Miheguli et al., 2018).

The observed high LER values and intercrop nutrient efficiencies correspond with previously reported results of other pulse-oilseed trials (Dowling et al., 2021). In the review by Dowling et al. (2021), they postulate that in low-input systems, it is low soil N values that promote a higher reliance on BNF to give higher yields in pulse-oilseed intercrops (Dowling et al., 2021). This was observed by Waterer et al. (1994) in the low N fertilizer treatment in their dry pea-white mustard trial during the site-year of average precipitation and warmer temperatures, but not in the site-year of above average precipitation and normal temperatures (Waterer et al., 1994). However, in the trial of Cowell et al. (1989), the higher %Ndfa in the low N fertilizer treatment did not have a corresponding higher yield LER in their dry pea-rapeseed and dry pea-white mustard trial sites (Cowell et al., 1989). In both of their studies the depletion of the available N by the non-pulse crop could stimulate onset of N fixation. In the current study, no N fertilizer was added and available N was low at time of seeding, so N fixation would be maximized in all arrangements.

Similar to that observed by Cowell et al. (1989), there were significantly lower amounts of N derived from fixation in the kabuli chickpea in the intercropping systems compared to monoculture. Amount of N derived from fixation is dependent on yield (van Kessel & Hartley, 2000), so it is expected that the lower intercrop chickpea yields have a corresponding lower amount of fixed N. Hauggaard-Nielsen et al. (2009) found that in dry pea-barley intercrops seeded in an additive design (100 percent pea-50 percent barley) resulted in a 25% reduction in BNF, but a replacement intercrop design (50 percent pea-50 percent barley) the reduction in pea BNF was a result of the lower plant density which was compounded by high available soil N values at some of their sites (Hauggaard-Nielsen et al., 2009). This is different from Waterer et al. (1994) who observed no differences in amount of N fixed by monocrop and intercropped dry pea.

Although the %Ndfa values and proportions of N derived from fixation were not significantly enhanced by intercropping in this study, the in-season transfer of fixed N from the kabuli chickpea to the brown flax observed may be responsible for higher yields. This was similar to the observed results in the study of Waterer et al. (1994) in dry pea-white mustard intercrops. Cowell et al. (1989) did not find evidence for N transfer between species in their pulse-oilseed intercropping study, however, their reported %Ntransfer values were means of all applied fertilizer rates.

There were no differences in the %Ndfa of kabuli chickpea and dry pea as monocrops or as intercrop components for each site-year (**Tables 3.10**, **3.18**). However, the lower %Ndfa at the Redvers 2019 site in kabuli chickpeas compared to that found at Redvers in 2020 site may be attributable to some light disease pressure noted in 2019 compared to no disease pressure in 2020 (**Table 3.10**). Therefore, %Ndfa may be more sensitive to environmental conditions and pest pressure than seeding arrangement in soils that are N limited (Ehrmann & Ritz, 2014). Hauggaard-Nielsen et al. (2009) suggest that a strong N sink and/or strong photosynthate production of the pulse crop component of an intercrop are required to increase nitrogen fixation in the intercrop system (Hauggaard-Nielsen et al., 2009). The low available soil P combined with drought conditions may have limited both of these requirements for an increase of BNF in intercropped kabuli chickpea at the Redvers sites in 2019 and 2020. This is in agreement with the pot study by Betencourt et al. (2012) in which low %Ndfa was observed in the treatments with low soil available P in chickpea-durum wheat intercrops. Intercropped dry pea had slightly higher %Ndfa at the Redvers 2019 site, and alternate row dry pea had slightly higher %Ndfa at the Redvers and Central Butte 2020 sites, but the differences were not significant. However, the %Ndfa in intercropped kabuli chickpea was lower than monocrop kabuli chickpea at the Central Butte 2020 site.

The reduced proportion and amount of N derived from fixation in intercropped brown flax at the Central Butte site in 2020 may reflect loss of photosynthetic chickpea biomass from disease (Hossain et al., 2017; Knight, 2012; López-Bellido et al., 2011). Late season drought and heat stress during inflorescence and grain filling (Cutforth et al., 2007; Hossain et al, 2017; Hocking et al., 2002; Lenssen et al., 2007; Sinclair & Weiss, 2010) may have shifted the interspecific competition to favor the brown flax intercrop component, allowing it to dominate and suppress BNF in kabuli chickpea (Dowling et al., 2021; Hauggaard-Nielsen et al., 2001; Hauggaard-Nielsen et al., 2009; Malhi, 2012; SERF, 2017). Shade from the dominant brown flax may have further reduced photosynthesis in kabuli chickpea, which would impact N transfer and uptake in the above-ground biomass (Barker & Francis, 1986). Rates of BNF are influenced by both abiotic and biotic factors, which are then compounded in an intercrop system by interplant competition and their associated biological and chemical interactions (Erhmann & Ritz, 2014). Amount and quality of light interception by the companion pulse crop along with shade tolerance of the pulse crop needs to be considered when selecting species and cultivars for pulse-oilseed intercropping systems to ensure optimal BNF and N transfer (Davis et al., 1986; Gliessman, 1986; Sinclair & Weiss, 2010).

Biologically fixed N from the pulse crops was transferred (%Ntransfer) to the intercropped oilseeds. There were no differences among seeding arrangements for %Ntransfer in intercropped brown flax (**Table 3.10**). Mixed row white mustard had significantly ($p \leq 0.05$) higher %Ntransfer in Redvers 2020 than alternate row white mustard (**Table 3.18**). Similar %Ntransfer values were observed in a dry pea-white mustard trial in Manitoba (Waterer et al., 1994). However, due to the low grain yield and N uptake of Redvers 2020 mixed row white mustard, the significantly higher %Ntransfer resulted in only a slightly higher (but not significantly) partial NLER value.

Fig. 3.9. Patches of poor dry pea-white mustard growth in south east corner of plot area at the Central Butte, SK site in 2020. Top photo is of dry pea-white mustard plots: block 2 in the foreground, block 1 in the background (red square). Bottom photo is of all Central Butte 2020 plots. Visible surface salt crusts in wheat downslope of block 1 and 2 dry pea-white mustard plots (red square). Block 4 of the kabuli chickpea-brown flax and dry pea-white mustard (orange square) both had high clay soil content which was discovered during root washing. High clay content soils en route to previous long-term test plots may have experienced compaction limiting nodule formation in pulse crops. Top photo taken July 19, 2020. Bottom photo taken July 16, 2020 by Mingxuan Shau.

The amount of biologically fixed N in aboveground biomass was significantly ($p \leq 0.05$) lower in intercropped kabuli chickpea than in monocrop kabuli chickpea at all four site-years (**Table 3.11**). This is, in part, due to the lower plant density in intercrops versus monocrops (Ehrmann & Ritz, 2014). The effect of year on yield and amount of biologically fixed N can be attributed to variations in agronomic practices as well as environmental conditions (López-Bellido et al., 2011). The Redvers 2019 kabuli chickpeas had light disease pressure while Redvers 2020 kabuli chickpeas had very little disease.

The amount of biologically fixed N in dry pea varied, but generally the differences were not significant among treatments. However, the Central Butte 2020 peas in all treatments had very low %Ndfa, with zero %Ndfa in blocks 1 and 4. Dry pea roots were very small, thin, and slightly caramel-colored in block 1, and the roots in both blocks had very few nodules. Poor nodulation is attributed to the upward movement of salts and nitrates as a result of the strong evaporative conditions during the hot dry July and August (**Figure 3.9**). High levels of soil available N have been shown to inhibit nodule formation in pulse crops (Chalmers, 2014; Dayoub et al., 2016; Hossain et al., 2017; Knight, 2012). Malhi (2012) and ICDC (2017) both observed that high levels of available soil NO_3-N favors competition of canola and white mustard over dry pea and marrowfat pea (ICDC, 2017; Malhi, 2012). In accordance with this, at the Central Butte 2020 site higher available soil NO_3-N supply rates were observed in dry pea-white mustard plots than in kabuli chickpea-brown flax plots when measured *in situ* using PRS™ probes (see chapter 4).

Nitrogen was removed from the cropping system (negative N*inc* values) for all crops in all seeding arrangements, in all site-years. The intercropped oilseeds had significantly ($p \leq 0.05$) lower N*inc* values compared to monocrop oilseeds (**Tables 3.12**, **3.20**). In addition to lower plant production and N demand when grown in intercrop configuration, these smaller negative N*inc* values can also be attributed to biologically fixed N that is transferred from the pulse crops to the oilseed crops (**Tables 3.10**, **3.18**). Walley et al. (2007) found that under normal growing conditions, monocrop dry pea could achieve positive N*inc* values but that monocrop chickpea could not. As part of intercrop systems, and especially combined with soil moisture deficits, pulse-oilseed intercrops cannot be expected to ameliorate soil N when used in low or no-input cropping systems (Arcand et al., 2013; Bottomley & Myrold, 2015; Ehrmann & Ritz, 2014; Walley et al., 2007). However, the benefit of in-season cross species N transfer (Tomm et al.,

1994) could be a valuable asset under N deficiency and dry conditions, as was experienced and observed in the current study, due to limited N mineralization and N mobility in the soil.

Seeding arrangement of the two pulse-oilseed intercrops appeared to have little influence on the yield or BNF parameters measured in this study. However, it was obvious that each crop exerted different competitive pressures within each intercrop system when partial LER values were examined (Bybee-Finley & Ryan, 2018). At the Redvers 2019 site where the target population of kabuli chickpea was reduced, the intercrop brown flax partial grain LER, NLER, and PLER values were near 1 while the intercrop kabuli chickpea partial grain LER and NLER values were 0.4-0.5 (**Tables A.3**, **A.6**). In comparison, Redvers 2020 site kabuli chickpea treatments were at target plant density and alternate row kabuli chickpea and brown flax partial grain LER values were 0.4 and 0.5, respectively, while the mixed row kabuli chickpea and brown flax partial grain LER values were 0.2 and 0.8, respectively. The difference from seeding arrangement, in this case, was significant. Similar significant differences were seen between seeding arrangements for the partial NLER value.

The higher kabuli chickpea partial LER and NLER values in alternate rows versus mixed rows indicates that kabuli chickpea is the subordinate crop in this intercrop species combination (Dowling et al., 2021). This is reflected in the kabuli chickpea-brown flax mixed row seeding rate of [C89F21] developed by Lana Shaw. Brown flax, as the dominant crop, is suggested to suppress growth and therefore BNF in kabuli chickpea (IHARF, 2015; SERF, 2017; Tosti & Thorup-Kristensen, 2010). This was seen in this study at the Central Butte 2019 and 2020 sites which had poor kabuli chickpea establishment in 2019 and disease setback in 2020. The subordinate crop within a intercropping system determines the yield efficiency (Trydeman Knudsen et al., 2004), and as a consequence, the weak competitiveness of kabuli chickpea (Tosti & Thorup-Kristensen, 2010) at the Central Butte sites is reflected in their low partial LER values.

A reverse situation was noted for the dry pea-white mustard intercrop combination, where there were also site driven effects. At the Redvers 2019 and 2020 sites, the dry pea partial grain LER, NLER, and PLER values were all in the 0.8 to 1.0 range, with the corresponding white mustard partial grain LER, NLER and PLER values in the 0.3 to 0.4 range (**Tables A.7**, **A.11**). There were no significant differences between seeding arrangements, but high partial LER values for dry pea compared to white mustard point to dry pea being the dominant crop in this intercrop combination at the Redvers sites. This is reflected in the dry pea-white mustard mixed row seeding rate of [P86M50] developed by Lana Shaw. Similar results were observed in other pea-canola/mustard trials (Chalmers, 2014; IHARF, 2013; Waterer et al., 1994). Ilgen & Stamp (1992) found that the large seed reserves and rapid establishment of seedling roots is what gives an early season competitive advantage to dry pea, whereas white mustard's competitive advantage is later in the season with the development of many short lateral roots (Ilgen & Stamp, 1992). The shorter life-cycle of dry pea may also be an advantage to dry pea in this intercrop combination for drought avoidance (Cutforth et al., 2007). White mustard is noted to be poorly adapted to drought conditions, and high daytime temperatures at inflorescence limits yield (Lenssen et al., 2007). The late season high daytime temperatures coupled with low precipitation and low available soil P values at the Redvers 2019 and 2020 sites were likely contributing factors to the low white mustard yields (**Table 3.13**).

White mustard was a more competitive partner in the dry pea-white mustard intercrops at the Central Butte 2019 and 2020 sites. The partial grain LER, NLER, and PLER values for white mustard were in the 0.5 to 1.0 range, with higher values observed in alternate rows (**Tables A.7**, **A.11**). Dry pea partial grain LER, NLER, and PLER values were in the 0.7 to 1.8 range, suggesting that it is the dominant crop in this intercrop combination (Chalmers, 2014; IHARF, 2013). It is notable that the %Ntransfer and amount of biologically fixed N in aboveground biomass in alternate row white mustard was higher than the %Ndfa and amount of biologically fixed N in aboveground biomass in alternate row pea (**Tables 3.18**, **3.19**) which may be an indication that white mustard to become parasitic for N acquisition at the expense of dry pea (Chalmers, 2014). It has been observed in other trials that poor establishment of dry pea and dominance of canola or mustards occurs when soil moisture and/or soil N levels are high (ICDC, 2017; Malhi, 2012; SERF, 2019; VanKoughnet, 2015; VanKoughnet, 2016). Rapid root development during early growth stages may be one of the factors that determines the competitiveness of a crop in an intercrop system (Ehrmann & Ritz, 2014). Hauggaard-Nielsen et al. (2009) recommend regulation of the competitiveness of each intercrop component when designing an intercrop system, taking into account soil available N and other factors that may limit plant growth and seed production. All the intercrops in our study followed a replacement design (Hauggaard-Nielsen et al., 2009). Barker and Francis (1986) suggest that intercrop systems be treated unique "species" or as complete units when it comes to fertility management.

Therefore, it may be necessary to reduce the seeding rate of the oilseed component of pulse-oilseed intercrop systems under high available soil N conditions (Hauggaard-Nielsen et al., 2009). Conversely, an increase in the seeding rate of the pulse crop component of the intercrop under low available soil N conditions while ensuring that the available soil P levels are adequate for optimum BNF to may be required to maintain the balance between the intercrop components for resource acquisition (Betencourt et al., 2012; Hauggaard-Nielsen et al., 2009; Rao, 1986).

Overall, the two pulse-oilseed intercrop systems showed synergy in yield and nutrient efficiency which may be attributed to the observed in-season transfer of soluble N from the pulse crop to the oilseed partner. Greater crop uptake and removal of soil P in the pulse-oilseed intercrop systems will need to be integrated into nutrient management decisions. Attention will need to be made to provide adequate available soil P to support BNF in companion pulse crops.

4. SOIL NUTRIENT DYNAMICS IN TWO PULSE-OILSEED INTERCROPPING SYSTEMS IN SOUTHERN SASKATCHEWAN

4.1 Preface

Intercropping is a means to incorporate some of the beneficial interactions that are found in natural complex systems into current cropping systems. However, the mechanisms associated with synergies of intercrops and the nature of the nutrient inputs, outputs and transformations require further investigation. In this thesis research, I investigated two promising pulse-oilseed intercrop combinations for western Canada: kabuli chickpea-flax and dry pea-white mustard. In chapter 3, the above-ground effects on crop yield and nutrient uptake, LER values, and BNF and N transfer in pulse-oilseed intercrop systems were described. In this chapter, the emphasis is on the below-ground impacts of pulse-oilseed intercrop systems, including midseason WEOC and TDN in the root zone, *in situ* supply rates of NO_3-N and PO_4-P, percent root colonization by AMF, and soil available N and P drawdown from spring to fall are examined for two pulse-oilseed intercrop combinations grown in Saskatchewan.

4.2 Abstract

There is an increasing interest in growing intercrop combinations that include oilseeds with pulse crops in western Canada, but little is known about the soil nutrient dynamics that operate when pulse crops and oilseeds are grown in close association. Two promising pulse-oilseed combinations (kabuli chickpea-brown flax and dry pea-white mustard) were grown as intercrops in mixed and alternate row configurations in two contrasting soil zones (Brown Chernozem and Black Chernozem) in southern Saskatchewan in 2019 and 2020 without added fertilizer, and compared to traditional single species monocrop systems.

Water extractable organic C and TDN in the seed-row and mid-row were assessed in the intercrop arrangements at mid-season (flowering). Root zone supply rates of NO_3-N and PO_4-P during the growing season were measured *in situ* using PRS™ probes, and the percent root colonization by AMF was assessed in plant samples removed mid-season from the field. Available soil N and P drawdown over the season were calculated through comparison of spring pre-seeding and fall post-harvest soil available nutrient levels.

The seed-row and mid-row WEOC and TDN concentrations were generally higher in the pulse monocrop and the intercrop alternate row configuration compared to the oilseed monocrops and intercrop mixed rows at the two Redvers sites. Conversely, the seed-row and mid-row WEOC concentrations were generally higher in the oilseed monocrops and intercrop alternate rows compared to the pulse monocrops and intercrop mixed rows at the two Central Butte sites. The root zone supply rates of NO_3-N were often significantly higher in the treatments that contained a pulse crop, while supply rates of PO_4-P were higher only at the Redvers 2019 site. Overall, the percent root colonization of AMF showed little difference among treatments. Higher concentrations and supplies of soluble soil plant available nutrient observed in treatments where the pulse crop was present is consistent with the important contribution made by the pulse crops to enhance soil fertility and nutrition, particularly for soluble N production and transfer. Less soil N and P drawdown was observed in treatments with pulse crop partners, especially in kabuli chickpea, supporting the ability of the pulse crop to help maintain soil N and P supplies and provide some additional nutrient to the companion oilseed.

4.3 Introduction

The improved plant diversity found in intercrop systems, especially those that include N fixing pulse crops, has been shown to positively affect the abundance, activity and composition of soil microbial communities (Bybee-Finley & Ryan, 2018; Ehrmann & Ritz, 2014). These below-ground interactions impact plant development by increasing the availability of soil nutrients for uptake by plants (Bybee-Finley & Ryan, 2018; Dowling et al., 2021; Ehrmann & Ritz. 2014; Fletcher et al., 2016). There are three ways in which the soil nutrient dynamics in intercrops differ from monocrops: 1) complementary resource use spurred by interplant competitive interactions (Ehrmann & Ritz, 2014); 2) nutrient facilitation, either due to indirect positive changes in rhizosphere processes (Bybee-Finley & Ryan, 2018; Ehrmann & Ritz, 2014; Jamont et al., 2013), or direct transfer of nutrients from one crop to another via AMF and CMN (Ehrmann & Ritz, 2014; Hauggaard-Nielsen & Jensen, 2005; Simard et al., 2012); and 3) differences in SOM inputs from rhizodeposition, soil enzyme activity, and decomposer communities (Ehrmann & Ritz, 2014).

In addition to the below-ground indirect transfer of biologically fixed N to non-fixing crops via NH_4-N, amino acids, or sloughed-off root cells (Ehrmann & Ritz, 2014), the ability of pulse crops to more effectively utilize insoluble soil P reserves through rhizodeposition of carboxylates and phosphatases (Veneklaas et al., 2003; Wang et al., 2007) may be another facet of indirect intercrop nutrient facilitation. Direct P transfer via AMF associations may also contribute to nutrient facilitation benefits realized in intercropping (Eason et al., 1991). Rhizosphere acidification is a by-product of BNF (Hinsinger, 2001) which may assist with solubilizing P in calcareous soils, however, pulse crops rely on other root-induced processes for P solubilization when nodulation and BNF is hindered (Betencourt et al., 2012; Pearse et al., 2007). While it has been shown that inclusion of pulse crops in traditional monocrop rotations will facilitate the availability and uptake of P in the following crop in western Canada (Miheguli et al., 2018), it is unknown if this long-term benefit would be seen within the growing season of a pulse-oilseed intercrop.

Very few recent western Canadian studies have been published that have investigated nutrient dynamics and synergies in non-fertilized pulse-oilseed intercrops along with seeding arrangement. To test the hypothesis that intercropping (versus monoculture) and intercrop seeding arrangement (alternate rows or mixed rows) will influence nutrient supply and availability for plant and microbial utilization in the seed-row, a two-year field trial at two sites in contrasting soil zones (Brown Chernozem and Black Chernozem) in southwestern and southeastern Saskatchewan in 2019 and 2020 was conducted to examine the nutrient dynamics of two promising pulse-oilseed intercrops: kabuli chickpea-brown flax and dry pea-white mustard). In-season soil nutrient cycling impacts were assessed to understand the below-ground synergies at work in pulse-oilseed intercrops. This was accomplished through measurement of mid-season WEOC and TDN concentrations in soil samples from the seed-row and mid-row, *in situ* root zone supply rates of NO_3-N and PO_4-P determined over the growing season, percent root colonization by AMF, and soil available N and P drawdown.

4.4 Materials and Methods

4.4.1 Site description

The project involved field trials conducted over two growing seasons (2019 and 2020) at two main locations: Central Butte, SK (Orthic Brown Chernozem of the Kettlehut-Ardill soil association) and Redvers, SK (Orthic Black Chernozem of the Oxbow soil association) for a total of four site-years.

4.4.2 Experimental design and field operations

Two intercropping combinations at the two main locations were grown: dry pea-white mustard and kabuli chickpea-brown flax. For each combination there were four treatments: pulse monocrop, oilseed monocrop, pulse and oilseed seeded in alternate rows, and pulse and oilseed seeded mixed in the same rows. Refer to chapter 3 for the specifics of cultivars used, seeding rates, treatments, field plot arrangement, seeding method, seed treatment, weed control, climate data, and spring and fall soil sampling and analysis.

4.4.3 Growing season data collection

After seeding of the crops in May, soil available N and P supply rates were measured using PRS™ anion exchange resin membrane probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) using the technique described by Xie et al. (2018). The PRS™ probes were installed at Redvers in 2019 and Central Butte in 2020 in the seed rows in each plot to track supply rates of NO_3-N and PO_4-P during the growing season. The first burial took place after seeding by inserting PRS™ probes vertically into the 0-15cm soil depth in the seeded row. Two anion-exchange PRS™ probes were inserted in each plot, 0.5m apart, except for the alternate row intercrop treatments, in which two probes were installed in the pulse crop row, and two probes were installed in the oilseed row (**Figs. 3.3**, **3.4**). The probes were replaced with a new set of probes in the same insertion slot at the end of each measurement interval. The probe measurements were made over two 3.5-week intervals (total of 7 weeks) in 2019 and three 2.5-week intervals (total of 7.5 weeks) in 2020. Probes were replaced by inserting a set of newly-regenerated probes in the same soil slots as the previous probes. Replacement of the probes proved to be difficult because the soil became hard and dry as the season progressed. In 2019 the last sets of probes were removed at time of dry pea-white mustard harvest in Redvers. In 2020 the last set of probes were removed two weeks prior to dry pea-white mustard harvest in Central Butte. The PRS™ probes were chilled in a cooler and transported promptly back to the laboratory after each removal.

The analysis and regeneration of PRS™ probes followed the protocol of Hangs et al. (2004). Briefly, probes were washed using deionized water until they were free of soil and then eluted with a 0.5M HCl solution. The eluant was analyzed for NO_3-N and PO_4-P colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA) and the resultant values were used to calculate the supply rate or flux of ion per unit area of membrane surface basis. The PRS™ probes were regenerated by shaking three times in a 0.5M sodium bicarbonate ($NaHCO_3$) solution for 4h, and between each shaking deionized water was used to thoroughly rinse the probes. Cumulative supply rates of these nutrients were calculated over the measurement period by multiplying the calculated rate by the time period (Western Ag Innovations Inc., 2006).

At mid-season at each location soil samples were taken with a 1.8cm diameter modified Backsaver™ probe from the seed row from the 0-10cm depth for the purpose of determining the level of soluble N and C in the seeded row. Soil samples were measured for WEOC and TDN as detailed by Chantigny et al. (2008). In brief, 30ml of 5mM $CaCl_2$ was added to 15g of air-dry soil. The suspension was stirred gently for one minute with a glass rod to make a homogeneous slurry. The slurry was filtered through a vacuum filter unit equipped with a 0.4 μm polycarbonate filter into a glass vial. The filtrate was stored at -20°C, then thawed (Kalbitz et al., 2003) prior to analysis. The filtrate was analyzed for its WEOC and TDN concentration by combustion using non-dispersive infrared (NDIR) detection with a Shimadzu TOC-VCPN analyzer (Shimadzu Corporation, Kyoto, Japan). While dissolved organic C (DOC) refers to any organic matter (OM) dissolved in the soil solution as it exists in the field, the WEOC measured in this study relates to soluble SOM extracted from a soil sample with low ionic strength aqueous solution that passes through a 0.4 μm filter. Therefore, WEOC is considered an acceptable proxy for DOC in soil solution that is collected *in situ* (Kalbitz et al., 2003).

For AMF root colonization counts, three plants of each crop type from each plot were removed from a row with a spade at mid-flowering (**Fig. 3.4**). Soil and roots were removed from the 0-15cm depth. The plants with their intact roots and clinging soil were chilled and transported from the field to the laboratory at the University of Saskatchewan in a cooler. The roots from these plants were immediately washed, then stored in a 50:50 (vol:vol) solution of distilled water and ethanol in a refrigerator (ca. 4℃) for the purpose of determining the AMF colonization in the roots. The roots retained their integrity when stored for about six months, after which they began to deteriorate and/or become infected with saprotrophic fungi. This was observed in a few samples that were stored for longer than six months. The deteriorated samples were discarded and not included in the data set. The root preparation and staining procedure used was an adaptation of the procedure outlined by Brundrett et al. (1994). Root segments were cut into longer segments (1-2cm) and enclosed in the staining cassettes (VWR Premium Biopsy Cassette, #18000-032). Root lengths were longer than reported by Brundrett et al. (1994) to ensure that they did not migrate out of the cassette during clearing and staining. Roots were cleared for 15 min by submersing them in a 10% potassium hydroxide (KOH) solution (wt/vol) maintained at 90℃ on a hotplate in a fume hood. It was important to not let the solution boil as boiling caused the roots to disintegrate. A lower temperature prolonged the clearing time considerably. A clearing time of 15 minutes was sufficient for most root segments. The root staining solution was prepared by dissolving 0.03% (wt/vol) chlorazole black E (CBE) in lactoglycerol. The CBE solution was kept near 90℃ on a hotplate in a fume hood, again ensuring that it did not boil. A lower temperature did not stain the fungal structures well. Roots were submerged in the CBE solution for 3h and then destained in a 1:1 gylcerol:distilled water solution overnight at room temperature. The roots in their cassettes were transferred to zip-top plastic bags containing distilled water. The bags were sealed and refrigerated until analysis.

The stained roots were examined with a dissecting microscope (Zeisse Stemi SV6) at 40x magnification. The procedure used to quantify root colonization by AMF was the gridline intersect method as described by Giovannetti & Mosse (1980), in which vertical and horizontal gridlines were scanned and the presence or absence of AMF was recorded at each point where the roots intersected a line. Each sample was only counted once.

4.4.4 Calculations

Calculation of percent root colonization was done with the modified frequency distribution method of Biermann & Lindermann (1983) in which the number of intersections with AMF is divided by the total number of root-intersections (**Eq. 4.1**):

$$Root\ colonization\ \% = \left[\frac{number\ of\ colonized\ root\ segments}{overall\ number\ of\ root\ segents\ evaluated}\right] \times 100 \qquad (4.1)$$

Amount of soil N and P drawdown was calculated using the following equation (**Eq. 4.2**) for the depth increments of interest.

$$soil\ nutrient\ drawdown = fall\ soil\ nutrient\ (kg\ ha^{-1}) - spring\ soil\ nutrient\ (kg\ ha^{-1}) \qquad (4.2)$$

4.4.5 Statistical analysis

The kabuli chickpea-brown flax treatments and the dry pea-white mustard treatments were considered separate intercrop systems. Statistical analysis was performed on treatments within each intercrop system and not between intercrop systems. Statistical analysis by ANOVA was done using PROC GLIMMIX with SAS 9.4 software (SAS Institute, 2012) to determine treatment effects and interactions for each parameter measured. Refer to Stroup (2014) for discussion regarding use of generalized linear mixed model (GLMM) in plant and soil science. The two sites were analyzed together for each growing season. The SLICE statement was used to facilitate comparisons for interactions. The LINES option with the LSMEANS and SLICE statement was used to compute means and comparisons of treatments for each site separately. Analysis of variance tables that are presented are for the combined analysis. Statistical differences indicated in tables are for individual sites, treatments, and year. Arcsine-square root transformations were done for percentage and proportion data prior to analyses and they were backtransformed for presentation. The significance of treatments was assessed with Tukey's Studentized range test using $p \leq 0.10$ to determine if a main effect or interaction was significant. Data was checked for outliers using Grubb's test, however, due to the ability of PROC

GLIMMIX to handle unbalanced and variable data, the identified outliers were removed from only a few data sets. Normal distribution was checked using Shapiro-Wilk values at p≤0.10. Due to the more variable nature of the soil measurements presented in this chapter an alpha level of 0.1 was used. The alpha level was set at 0.10 and means declared significantly different at p≤0.10 to minimize the chance of making a type II error.

4.5 Results

4.5.1 Mid-season soluble soil C and N

There were no differences in WEOC concentrations among treatments nor between mid-row and seed-row samples taken at mid-flowering in the kabuli chickpea-brown flax intercrop treatments at the Redvers 2019 site (**Table 4.1**). The WEOC concentrations in the seed-row in the kabuli chickpea alternate row plots was significantly (p≤0.1) higher than levels in both the brown flax alternate row and mixed row kabuli chickpea-brown flax, however there were no differences among treatments in the mid-row, nor between sampling locations at the Redvers 2020 site. The WEOC concentrations, on average, were lower (~6%) at the Redvers 2020 site compared to the Redvers 2019 site.

Table 4.1. Mid-season water extractable organic carbon (WEOC) in seed-row and mid-row for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row
	- - - mg C / kg soil - - -							
Chickpea mono	116.57 a	105.59 a	112.75 ab	102.3 a	104.78 ab	94.20 a	72.07 b	67.41 a
Chickpea alt row	114.59 a	107.29 a	99.15 c	96.29 a	111.35 a	98.98 a	71.44 b	62.97 a
Mixed row	103.07 a	107.47 a	104.96 bc	102.31 a	95.51 b	103.07 a	69.00 b	66.12 a
Flax alt row	95.84 a	103.73 a	107.89 bc	108.76 a	95 28.b	103.96 a	83.59 a	67.30 a
Flax monocrop	110.47 a	107.25 a	121.14 a	106.55 a	99.39 ab	98.26 a	83.23 a	64.26 a

Source of variation	Probability (p)			
	Redvers 2019	CB 2019	Redvers 2020	CB 2020
Treatment	0.7526	**0.0772**	0.8312	0.1186
Location	0.7828	**0.0988**	0.6911	**0.0001**
Treatment*location	0.8792	0.6181	0.3065	0.1457

Values in a column followed by same letter are not significantly (p≤0.1) different.
Bolded values indicate significance at the p≤0.1 level of probability.

The seed-row brown flax monocrop had significantly (p≤0.1) higher WEOC concentrations than brown flax in alternate rows, kabuli chickpea in alternate rows, and kabuli chickpea-brown flax in mixed rows at the Central Butte 2019 site (**Table 4.1**). This was slightly different in 2020, where brow flax monocrop and brown flax alternate rows had significantly (p≤0.1) higher WEOC concentrations than the three treatments with kabuli chickpea. For both Central Butte site-years there was no difference among treatments for the mid-row WEOC concentrations. However, there were significant (p≤0.1) differences between the seed-row and mid-row in both site-years. The soluble WEOC concentrations were lower (~30% to 36%) at the Central Butte 2020 site compared to the Central Butte 2019 site.

The seed-row TDN concentration was significantly (p≤0.1) higher in chickpea monocrop than in the three treatments with flax at the Redvers 2019 site (**Table 4.2**). At the Redvers 2020 site both the kabuli chickpea monocrop and kabuli chickpea alternate row arrangements had significantly (p≤0.1) higher TDN concentrations than the three treatments with flax. For both Redvers site-years there was no difference among treatments at the mid-row location, nor were there differences between sampling locations. The TDN concentrations were lower (~18% to 26%) at the Redvers 2020 site compared to the Redvers 2019 site, and the difference was greater than what was observed in the corresponding WEOC concentrations.

Table 4.2. Mid-season total dissolved N (TDN) in seed-row and mid-row for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row
	- - - mg N kg^{-1} soil - - -							
Chickpea mono	16.27 a	14.32 a	12.22 a	11.40 a	13.45 a	11.02 a	9.13 ab	11.69 a
Chickpea alt row	13.07 ab	12.93 a	9.56 b	8.15 b	12.81 a	9.39 a	9.83 a	7.11 b
Mixed row	11.49 b	13.48 a	7.97 bc	7.90 b	8.52 b	8.98 a	7.53 bc	6.94 b
Flax alt row	10.89 b	13.05 a	7.57 c	8.09 b	8.09 b	9.60 a	6.76 c	6.63 b
Flax monocrop	10.47 b	11.31 a	8.25 bc	7.67 b	7.93 b	8.95 a	6.79 c	5.76 b

Source of variation	Probability (p)			
	Redvers 2019	CB 2019	Redvers 2020	CB 2020
Treatment	**0.0751**	**<0.0001**	**0.0076**	**0.0003**
Location	0.5605	0.3200	0.4363	0.4915
Treatment*location	0.6337	0.7377	0.1482	0.0687

Values in a column followed by same letter are not significantly (p≤0.1) different.
Bolded values indicate significance at the p≤0.1 level of probability.

Trends similar to those observed at Redvers over the two years of the study were also observed at the Central Butte sites. The seed-row TDN concentration was significantly (p≤0.1) higher in the kabuli chickpea monocrop compared to all other treatments at the Central Butte 2019 site (**Table 4.2**). In 2020, the kabuli chickpea alternate row had significantly (p≤0.1) higher seed-row TDN concentration than the three treatments with brown flax. Interestingly, there was a difference among treatments for the mid-row TDN concentrations. The kabuli chickpea monocrop had significantly (p≤0.1) higher mid-row TDN concentration than the all the other treatments at Central Butte 2019 and 2020 sites. However, while there were no differences between sampling locations for both years, the TDN concentrations at the Central Butte 2020 site were lower (~11% to 12%) than the Central Butte 2019 site.

Table 4.3. Mid-season water extractable organic carbon (WEOC) in seed-row and mid-row for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row
				- - - mg C kg^{-1} soil - - -				
Pea monocrop	143.72 a	123.29 b	120.29 a	102.65 ab	98.46 a	108.06 ab	60.96 b	62.32 b
Pea alt row	135.47 ab	143.05 a	116.22 a	105.25 ab	96.96 a	112.76 a	64.13 ab	66.78 ab
Mixed row	121.96 bc	119.49 b	115.33 a	110.77 b	97.00 a	105.85 ab	68.48 a	64.53 ab
Mustard alt row	130.15 ab	134.17 ab	103.07 a	111.44 ab	94.35 a	112.67 a	67.50 a	69.17 a
Mustard monocrop	108.13 c	118.08 b	112.74 a	119.24 a	93.27 a	101.49 b	65.82 ab	63.12 ab

Source of variation	Probability (p)			
	Redvers 2019	CB 2019	Redvers 2020	CB 2020
Treatment	**0.0053**	0.7636	0.3815	0.1388
Location	0.9507	0.2229	**<0.0001**	0.9053
Treatment*location	0.2143	0.2458	0.6116	0.6259

Values in a column followed by same letter are not significantly (p≤0.1) different.
Bolded values indicate significance at the p≤0.1 level of probability.

The effects on WEOC and TDN in the soil mid-season with dry pea-white mustard followed similar trends to kabuli chickpea-brown flax. The dry pea monocrop seed-row WEOC concentrations were significantly (p≤0.1) higher than white mustard monocrop and dry pea-white mustard mixed rows at the Redvers 2019 site (**Table 4.3**). This was nearly mirrored in the mid-row, with dry pea alternate rows having significantly (p≤0.1) higher WEOC concentration than dry pea monocrop, white mustard monocrop, and dry pea-white mustard mixed row. There were no differences between sampling locations at the Redvers 2019 site. In contrast, there were no differences among dry pea-white mustard treatments for seed-row WEOC concentrations at the Redvers 2020 site, however, differences remained in the mid-row with dry pea and white mustard in alternate rows soluble having WEOC concentrations that were significantly ($p \leq 0.1$) higher than white mustard monocrop. Overall, differences between seed-row and mid-row WEOC concentrations were less with the dry pea-white mustard than kabuli chickpea-brown flax systems. In 2020, there were significant ($p \leq 0.1$) differences between sampling locations at the Redvers site. The WEOC concentrations at the Redvers 2020 site were lower (~15% to 24%) than the Redvers 2019 values.

There were no differences among dry pea-white mustard treatments for seed-row WEOC concentrations at the Central Butte 2019 site, although, mid-row WEOC concentrations were significantly ($p \leq 0.1$) higher in white mustard monocrop than dry pea-white mustard mixed rows (**Table 4.3**). At the 2020 Central Butte site, the WEOC concentrations in the seed-row of white mustard in alternate rows and dry pea-white mustard in mixed rows was significantly ($p \leq 0.1$) higher than dry pea monocrop. There was a similar effect in the mid-row with white mustard alternate row having significantly ($p \leq 0.1$) higher WEOC concentrations than dry pea monocrop. There were no differences between sampling locations at Central Butte 2019 and 2020 sites. As observed in the Central Butte kabuli chickpea-brown flax treatments, the WEOC concentrations in 2020 were noticeably lower (~40% to 42%) than the Central Butte 2019 values.

Following a pattern similar to that observed in the kabuli chickpea-brown flax monocrop and intercrop arrangements, the seed-row TDN was significantly ($p \leq 0.1$) higher in dry pea monocrop than in all the other dry pea-white mustard treatments at the Redvers 2019 site (**Table 4.4**). However, there were no differences among treatments in the mid-row, nor were there differences between sampling locations (seed-row versus mid-row). At the Redvers 2020 site the seed-row TDN was significantly ($p \leq 0.1$) higher in dry pea monocrop and dry pea alternate row than in the corresponding white mustard treatments. This was also observed in the mid-row with dry pea alternate row TDN concentration significantly ($p \leq 0.1$) higher than white mustard monocrop and dry pea-white mustard mixed row. There were no differences between sampling locations at Redvers 2020 site. The TDN concentrations were lower (~19%) at the Redvers

2020 site compared to the Redvers 2019 site, but the difference was consistent with what was observed in the WEOC concentrations for Redvers sites dry pea-white mustard treatments.

As at Redvers, the Central Butte 2019 seed-row TDN concentrations were significantly ($p \leq 0.1$) higher in dry pea monocrop than white mustard alternate row, but there were no differences among treatments in the mid-row, nor were there differences between sampling locations (**Table 4.4**). The Central Butte 2020 TDN concentration for dry pea monocrop was significantly ($p \leq 0.1$) higher than all the other treatments both in the seed-row and mid-row soils, however, there were no differences between sampling locations. Interestingly, while there were observed lower WEOC concentrations in the at both the Redvers and Central Butte 2020 sites in the kabuli chickpea-brown flax and dry pea-white mustard treatments, and for TDN concentration at the Redvers 2020 site, this trend was not continued for TDN at the Central Butte 2020 site. There the seed-row TDN concentration was ~7% higher in 2020 compared to 2019, and the mid-row TDN concentration was ~12% lower than 2019.

Table 4.4. Mid-season total dissolved N (TDN) in seed-row and mid-row for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row	Seed-row	Mid-row
				- - - mg N kg^{-1} soil - - -				
Pea monocrop	18.47 a	15.41 a	12.14 a	9.87 a	13.08 a	13.09 ab	11.95 a	12.24 a
Pea alt row	15.07 b	16.20 a	11.52 ab	10.17 a	13.50 a	14.24 a	8.93 b	7.78 b
Mixed row	14.28 b	15.04 a	10.73 abc	9.26 a	12.26 ab	12.28 bc	8.66 b	7.63 b
Mustard alt row	14.63 b	16.50 a	9.43 c	9.94 a	11.32 b	13.10 ab	9.64 b	8.37 b
Mustard monocrop	13.08 b	16 18 a	9.61 bc	10.03 a	10.76 b	11.25 c	8.41 b	7.02 b

Source of variation	Probability (p)			
	Redvers 2019	CB 2019	Redvers 2020	CB 2020
Treatment	0.3121	0.4150	**0.0017**	**0.0002**
Location	0.3218	0.1353	0.1367	0.1109
Treatment*location	0.1404	0.4052	0.6156	0.8720

Values in a column followed by same letter are not significantly (≤0.1) different.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

The WEOC concentrations were lower at the 2020 sites compared to the 2019 sites. The Redvers 2020 kabuli chickpea-brown flax treatments, when averaged together, were 6% lower than in 2019. The Redvers 2020 dry pea-white mustard treatments were 15% to 24% lower than

2019. The Central Butte 2020 chickpea-flax treatments were 30% to 36% lower than 2019. The Central Butte 2020 dry pea-white mustard treatments were 40% to 42% lower than 2019.

As was observed in the WEOC concentrations, the TDN concentrations were lower at the 2020 sites compared to the 2019 sites. The Redvers 2020 kabuli chickpea-brown flax treatments, averaged together, had 18% to 26% lower TDN compared to 2019. The Redvers 2020 dry pea-white mustard treatments had 19% lower TDN compared to 2019. The Central Butte 2020 kabuli chickpea-brown flax treatments had 11% to 12% lower TDN compared to 2019. The exception to the above trend was the Central Butte 2020 dry pea-white mustard treatments with seed-row TDN concentrations that were 7% higher than in 2019, while the mid-row TDN concentrations were 12% lower than in 2019.

4.5.2 In-season supply rates of available soil N and P

The supply rates of NO_3-N as measured with PRS™ probes *in situ* in the root zone over the season were significantly ($p \leq 0.1$) higher in kabuli chickpea monocrop than in the three treatments with flax at Redvers 2019 site (**Table 4.5**). These findings are consistent with greater concentrations of TDN observed in the root zone during mid-season sampling as discussed in the previous section. Root zone supply rates of PO_4-P were significantly ($p \leq 0.1$) higher in kabuli chickpea monocrop than in brown flax monocrop at Redvers 2019 site.

Table 4.5. Root zone supply rates of NO_3-N and PO_4-P for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers, SK site in 2019 (cumulative over a 7 week period) and Central Butte (CB), SK site in 2020 (cumulative over a 7.5 week period) measured *in situ* using PRS™ probes (*n*=4).

	_- Redvers 2019 -		- CB 2020 -	
Treatment	NO_3-N	PO_4-P	NO_3-N	PO_4-P
		- - - μg anion cm^{-2} - - -		
Chickpea mono	17.33 a	1.49 a	27.84 b	2.47 a
Chickpea alt row	11.71 ab	1.13 ab	38.66 ab	2.31 a
Mixed row	7.24 b	1.39 ab	52.71 a	2.90 a
Flax alt row	4.01 b	1.11 ab	31.77 b	2.45 a
Flax monocrop	5.10 b	0.96 b	33.38 b	2.85 a
Source of variation		Probability (p)		
	- 2019 -		- 2020 -	
	NO_3-N	PO_4-P	NO_3-N	PO_4-P
Treatment	**0.0743**	0.3385	0.1722	0.9831

Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

The root zone supply rates of NO_3-N were significantly ($p \leq 0.1$) higher in kabuli chickpea-brown flax mixed row than in kabuli chickpea monocrop, brown flax alternate row, and brown flax monocrop at Central Butte 2020 site, however, there were no differences among treatments for root zone supply rate of PO_4-P (**Table 4.5**).

For the dry pea-white mustard system evaluated, dry pea tended to produce the highest supply rates of nutrient ion over the season (**Table 4.6**), similar to that observed for kabuli chickpea, and in agreement with patterns in soluble organic concentrations covered previously. The root zone supply rates of NO_3-N were significantly ($p \leq 0.1$) higher for dry pea monocrop than dry pea alternate row at Redvers 2019 site, however, there were no significant differences among treatments for root zone supply rates of PO_4-P (**Table 4.6**).

There were no differences among dry pea-white mustard treatments for root zone supply rates of NO_3-N and PO_4-P at Central Butte 2020 site (**Table 4.6**). However, it must be noted that the root zone supply rates of NO_3-N were quite high at the Central Butte 2020 site, and even higher in the dry pea-white mustard plots than in the kabuli chickpea-brown flax plots.

Table 4.6. Root zone supply rates of NO_3-N and PO_4-P for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers, SK site in 2019 (cumulative over a 7 week period) and Central Butte (CB), SK site in 2020 (cumulative over a 7.5 week period) measured *in situ* using PRS™ probes (*n*=4).

	- Redvers 2019 -		- CB 2020 -	
Treatment	NO_3-N	PO_4-P	NO_3-N	PO_4-P
		- - - μg anion cm^{-2} - - -		
Pea monocrop	6.14 a	1.83 a	63.05 a	2.72 a
Pea alt row	3.44 b	0.98 a	64.99 a	2.00 a
Mixed row	4.70 ab	0.92 a	46.93 a	2.57 a
Mustard alt row	4.09 ab	1.36 a	52.85 a	2.16 a
Mustard mono	5.32 ab	1.34 a	47.79 a	3.19 a
Source of variation		Probability (p)		
	- 2019 -		- 2020 -	
	NO_3-N	PO_4-P	NO_3-N	PO_4-P
Treatment	0.3518	0.4990	0.5346	0.4711

Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

4.5.3 Arbuscular mycorrhizal fungal colonization of roots

There were no differences among kabuli chickpea and dry pea treatments for percent AMF colonization at Redvers 2019 site (**Tables 4.7**, **4.8**). Brown flax in mixed rows did have slightly but significantly (p≤0.1) higher percent AMF colonization than the other two flax treatments at Redvers 2019 site. At the Redvers 2020 site there were no differences among kabuli chickpea, dry pea, or brown flax treatments for percent AMF colonization.

Table 4.7. Percent root colonization by arbuscular mycorrhizal fungi (AMF) for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax
				- - - % - - -				
Monocrop	98 a	86 b	94 a	81	97 a	98 a	94 b	94 a
Alt row	100 a	79 b	83 a	69	98 a	96 a	98 a	97 a
Mixed row	99 a	95 a	97 a	72	97 a	96 a	97 ab	98 a

Source of variation	Probability (p)			
	- 2019 -		- 2020 -	
	Chickpea	Flax	Chickpea	Flax
Site	**0.0680**	0.2103	0.7725	0.9612
Treatment	0.5992	0.3675	0.2251	0.9109
Site*treatment	0.4083	0.6301	0.4078	0.4457

Arcsine-square root transformations were done for percentage data prior to analyses and backtransformed for presentation.
Values in a column followed by same letter are not significantly (p≤0.1) different.
CB 2019 flax alt row (n=2) and CB 2019 flax monocrop (n=1), therefore, statistical comparison of treatments was not included.
Bolded values indicate significance at the p≤0.1 level of probability.

There were no differences among chickpea treatments for percent AMF colonization at the Central Butte 2019 site (**Table 4.7**). Unfortunately, there were not enough root samples of flax and dry pea to calculate statistical differences for Central Butte 2019 site due to deterioration of some samples during storage. The Central Butte 2020 chickpea alternate row had significantly (p≤0.1) higher percent root AMF colonization than chickpea monocrop, and dry pea monocrop and dry pea alternate row had significantly (p≤0.1) higher percent root AMF colonization than dry pea mixed row (**Tables 4.7**, **4.8**). However, as the percent root colonization values were high for all treatments at Redvers 2020 and Central Butte 2020 sites, these small statistical differences do not likely have large biological impact.

Table 4.8. Percent root colonization by arbuscular mycorrhizal fungi (AMF) for monocrop dry pea and dry pea intercropped with white mustard in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

Treatment	Redvers 2019	CB 2019	Redvers 2020	CB 2020
			- - - % - - -	
Monocrop	98 a	92	94 a	99 a
Alt row	100 a	92	97 a	99 a
Mixed row	99 a	98	94 a	93 b
Source of variation		Probability (p)		
		2019	2020	
Site		0.1199	0.3833	
Treatment		0.7255	**0.0124**	
Site*treatment		0.7014	0.1266	

Arcsine-square root transformations were done for percentage data prior to analyses and backtransformed for presentation.
Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.
CB 2019 pea mixed row (n=2), therefore, statistical comparison of treatments was not included.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

4.5.4 Soil available N and P drawdown

Soil available N and P drawdown over the season was assessed by comparing change in soil profile nutrient amounts (delta kg ha^{-1}) in the surface 0-15cm depth from spring to fall. There were no differences among treatments in Redvers 2019 soil available N and P drawdown in the root zone (0-15cm) in the kabuli chickpea-brown flax intercrop treatments (**Table 4.9**), with expected depletion observed in all treatments. The Central Butte 2019 site monocrop kabuli chickpea treatment had significantly less ($p \leq 0.1$) soil N drawdown than the other treatments, consistent with greater observed midseason soluble N concentrations. Soil P drawdown was significantly greater in the monocrop brown flax than in monocrop chickpea. The Redvers 2020 site showed no differences among treatments for soil P drawdown. Alternate row kabuli chickpea-brown flax had significantly ($p \leq 0.1$) less N drawdown than mixed row kabuli chickpea-brow flax and monocrop brown flax. There were no differences among treatments in Central Butte 2020 for soil N and P drawdown.

Table 4.9. Soil available N and P drawdown for 0-15cm depth for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brow flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
				--- mg kg^{-1} ---				
Chickpea monocrop	-3.2 a	-4.4 a	0.7 a	0.1 a	-8.0 ab	0.0 a	-6.1 a	-4.4 a
Alternate row	-4.0 a	-4.6 a	-4.3 b	-0.5 ab	-6.6 a	-0.5 a	-7.5 a	-2.7 a
Mixed row	-3.9 a	-5.1 a	-4.1 b	+0.5 ab	-10.2 b	-0.9 a	-8.1 a	-5.0 a
Flax monocrop	-3.7 a	-5.1 a	-4.2 b	-3.9 b	-10.2 b	-1.3 a	-8.7 a	-5.8 a

Source of variation	Probability (p)			
	2019		2020	
	N	P	N	P
Site	0.5281	**0.0048**	**0.0741**	0.3989
Treatment	**0.0939**	0.4068	0.1177	0.4741
Site*treatment	0.2680	0.5963	0.5908	0.7745

Drawdown is change in soil available N and P concentration from spring before seeding to fall after harvest.
Values in a column followed by same letter are not significantly (p≤0.1) different.
Bolded values indicate significance at the p≤0.1 level of probablility.

Table 4.10. Soil available N and P drawdown for 0-15cm depth for dry pea monocrop, white mustard monocrop, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
				--- mg kg^{-1} ---				
Pea monocrop	1.3 a	-2.9 a	1.0 a	0.2 a	-4.1 ab	-1.3 a	-6.8 a	-6.6 a
Alternate row	1.3 a	-3.2 a	-1.3 b	-1.2 a	-4.2 ab	-0.2 a	-7.6 a	-5.9 a
Mixed row	1.6 a	-4.3 a	0.2 ab	-1.1 a	-2.6 a	-1.3 a	-7.0 a	-7.1 a
Mustard monocrop	0.7 a	-2.3 a	1.3 a	1.8 a	-5.3 b	-1.5 a	-7.2 a	-6.6 a

Source of variation	Probability (p)			
	2019		**2020**	
	N	P	N	P
Site	0.2711	0.5577	**0.0029**	**0.0375**
Treatment	0.5655	0.5605	0.5288	0.7871
Site*treatment	0.2838	0.9490	0.6207	0.9921

Drawdown is change in soil available N and P concentration from spring before seeding to fall after harvest.
Values in a column followed by same letter are not significantly (p≤0.1) different.
Bolded values indicate significance at the p≤0.1 level of probability.

For the dry pea-white mustard intercropping system, there were no differences among dry pea-white mustard treatments in Redvers 2019 site for soil N and P drawdown and soil N had a small net gain over the growing season (**Table 4.10**). There were no differences among treatments in Central Butte 2019 site soil for the soil P drawdown. Soil N drawdown was slightly but significantly greater ($p \leq 0.1$) in alternate row dry pea-white mustard than in monocrop white mustard. There were no differences among treatments in Redvers and Central Butte 2020 sites for soil N and P drawdown, with the exception of Redvers 2020 mixed row dry pea-white mustard with significantly ($p \leq 0.1$) less soil N drawdown than monocrop white mustard. Overall, no large differences among treatments were observed in the two years at the two sites.

4.6 Discussion

Rhizosphere processes are driven by readily-available energy and nutrient sources for microorganisms originating from root-derived materials (Hinsinger, 2001; Horwath, 2015; Tang et al., 2014). These substrates include sloughed-off cells, senescing roots, and root exudates high in carbohydrates and amino compounds (Horwath, 2015). The activity of soil microorganisms and their effect on nutrient cycling and turnover is influenced by these rhizosphere substrates (Cheng & Kuzyakov, 2005) and the measurement of WEOC provides an indication of the amount of microbially-available C (Rees & Parker, 2005). At the Redvers sites there were very few differences among treatments for WEOC concentrations in the seed-row, with significantly ($p \leq 0.1$) higher concentrations only seen in dry pea monocrop in 2019 and kabuli chickpea alternate row in 2020. This was mirrored in the mid-row WEOC concentrations as well, with significantly ($p \leq 0.1$) higher concentrations observed in dry pea alternate rows at Redvers 2019 site and dry pea-white mustard alternate rows at Redvers 2020 site. The dry pea-white mustard treatments at the Redvers 2020 site was the only site-year that had significantly ($p \leq 0.1$) higher mid-row WEOC concentration than in the seed-row. Hinsinger (2001) suggests that this could be from the diffusion of root exudates away from the rhizosphere in to the bulk soil where the concentration of microbial activity is lower, but Arcand et al. (2013) provide the more likely suggestion that it simply represents the decomposition of roots (Arcand et al., 2013; Hinsinger, 2001). Another explanation of the higher WEOC concentration in the mid-row dry pea-white mustard treatments is that it may represent the lethal effects of the hot dry growing conditions of

2020 on the microbial biomass in the bulk soil (McGill et al., 1986). Cheng & Kuzyakov's (2005) hypothesis that plants will outcompete rhizosphere microorganisms for nutrient uptake under low nutrient concentrations is also a possibility (Cheng & Kuzyakov, 2005).

While pulse crops had higher WEOC concentrations in the seed-row and mid-row at the Redvers sites, it was the oilseeds that had higher WEOC concentrations at the Central Butte sites. Seed-row WEOC concentrations were significantly higher ($p \leq 0.1$) in brown flax monocrop in 2019; and seed-row WEOC concentrations were significantly higher ($p \leq 0.1$) in brown flax monocrop, brown flax alternate rows, white mustard alternate rows, and dry pea-white mustard mixed rows in 2020. This trend of oilseed significantly ($p \leq 0.1$) higher WEOC concentrations continued in the mid-row with white mustard monocrop in 2019 and white mustard alternate rows in 2020. Higher WEOC associated with the white mustard at the Central Butte sites may reflect the dominance of the white mustard at this site over the dry pea owing to hot, dry conditions and higher available P and N than at the Redvers sites.

It is interesting that the significantly higher WEOC concentrations were observed mainly in the monocrops or alternate rows. Tang et al. (2014) observed higher microbial biomass C values in their intercropped chickpea-wheat and lentil-wheat trials compared to the corresponding monocrops in southern France, however their trial site had higher soil available P (42mg kg^{-1} Olsen-P) and higher precipitation (538mm) than we had at our trial sites (Tang et al., 2014).

It is notable that the WEOC concentrations were lower at the 2020 sites compared to the 2019 sites. The most plausible explanation is the time of sampling, and the hot, dry mid-season growing conditions of 2020 limiting microbial activity (Cheng & Kuzyakov, 2005; McGill et al., 1986). The reason Redvers 2020 kabuli chickpea-brown flax WEOC concentrations were reduced to the least extent of the two intercrop systems at the two sites in 2020 could be the increase in chickpea plant density from the previous year. The Central Butte 2020 kabuli chickpea-brown flax and dry pea-white mustard treatments reduced WEOC concentrations could also be a result of being seeded on fallow land in 2020 versus on wheat stubble in 2019.

As expected, the seed-row TDN concentrations were higher in the pulse crops than in the oilseed crops for both intercropping systems in this study. There were no differences among treatments for mid-row TDN except for Central Butte 2019 and 2020 sites kabuli chickpea-brown flax and Central Butte 2020 dry pea-white mustard, and there were no differences between the mid-row TDN and seed-row TDN. At the flowering stage, when the samples were collected, the pulse crops were also likely already reducing the amount of rhizosphere deposited N and reallocating N back into the plant (Arcand et al., 2013), so it is expected that there would be no difference between the seed-row and mid-row TDN concentrations. However, as for the WEOC, the highest treatments were the monocrops and the alternate rows for all four site-years. This is different than what was observed by Tang et al. (2014) in their intercropping study. They found no differences in microbial biomass N among treatments within the rhizosphere, within the bulk soil, nor between the rhizosphere and bulk soil (Tang et al., 2014).

As for the WEOC, the hot dry growing season of 2020 could explain the observed reduction of TDN concentrations from 2019. Low soil moisture, high soil temperature, and low soil fertility can reduce microbial activity (Cheng & Kuzyakov, 2005; Paustian et al., 1997). The reduced change in TDN concentrations at the Central Butte 2020 site could also be a combination of increased root-shoot ratios and consequent turnover of fine roots as a response to low soil moisture and fertility (Arcand et al., 2013; López-Bellido et al., 2011), tillage effects that increase rate of nitrification (Cheng, & Kuzyakov, 2005; McGill et al., 1986; Paustian et al., 1997; Robertson & Groffman, 2015; Smith et al., 2015), and the observed high available NO_3-N supply rates measured *in situ* with PRS™ probes.

As expected, the observed root zone supply rates of NO_3-N were significantly ($p \leq 0.1$) higher in kabuli chickpea monocrop than in the treatments with brown flax. This corresponds with the observed TDN concentration data and supports the hypothesis that biologically fixed N from kabuli chickpea is released into the root zone over the course of the growing season (Arcand et al., 2013). The Redvers 2019 kabuli chickpea monocrop also had significantly ($p \leq 0.1$) higher root zone supply rates of PO_4-P than flax monocrop. Chickpea is known for its ability to hydrolyze organic P into plant available inorganic P by exuding acid phosphatases (Li et al., 2014; Xia et al., 2013). While chickpea root exudates also include malonate and carboxylate (Veneklass et al., 2003), Pearse et al. (2007) found that malonate does not enhance P acquisition in chickpea, and the rate of release of carboxylate along the entire chickpea root does not assist in transforming P into plant-available inorganic P (Pearse et al., 2007). However, it should be noted that in the pot study conducted by Pearse et al. (2007) the plants were not N deprived, and no nodules were observed. Chickpea also acidifies their rhizosphere with the net export of cations as a consequence of BNF, which could assist with P acquisition (Hinsinger, 2001). However, Betencourt et al. (2012) found no evidence of rhizosphere acidification when chickpeas have low nodulation and BNF rates in low available P soils. The increase in rhizosphere available P, and P uptake in chickpea-durum wheat intercrops observed in their pot study was attributed to other root-induced processes (Betencourt et al., 2012).

It is interesting to note that white mustard monocrop and white mustard alternate row root zone supply rates of PO_4-P were not significantly different from the other treatments at Redvers 2019. It is known that *Brassica napus* can efficiently access P from phosphate rocks by releasing H^+ (Hinsinger, 2001), malic acid and citric acids (Pearse et al., 2007; Zhang et al., 1997) from their roots. Pearse et al. (2007) observed that *B. napus* did not senesce and re-grow leaves under low-P conditions. They consider this a valuable trait to survive in P-limited environments (Pearse et al., 2007). Perhaps white mustard also possesses these traits of P acquisition and retention.

It is difficult to extrapolate further given only two site-years of data and especially in light of the high NO_3-N supply rates observed at the Central Butte 2020 site from accumulation of soil NO_3-N during the previous fallow year. The Central Butte 2020 spring soil samples didn't reveal elevated soil N levels at the 0-15cm increment, however, soil N concentration was consistent all the way down to 60cm depth. Perhaps the N increased at the surface during the season from evaporation and capillary rise (Scott, 2000).

The percent root colonization by AMF was, for the most part, fairly high and uniform. This is similar to the results reported by Wang et al. (2007) in their pot study of chickpea-wheat intercrops in low available P soils. Limitations of the root clearing, staining and identification technique and the deterioration in storage that was experienced in the current trial likely limited the utility of this procedure. While Li et al. (2009) reported in their pot study that intercropping improved AMF colonization in both components of their mung bean-rice intercrop system, differences between a glass house pot study in controlled conditions and a field study with adverse environmental effects may significantly alter AMF colonization patterns.

Some differences were observed in the roots segments for the degree of colonization by AMF. A portion of hyphae in a root segment is not the same as a root segment full of arbuscules or vesicles (see Kokkoris et al., 2019; Navarro-Fernández et al., 2016), but both would be recorded as "infected" in the gridline intersect method by Giovannetti & Mosse (1980), as used in this thesis research. A rating system, similar to cereal leaf disease ratings, was developed by Trouvelot et al. (1986). Adding the Trouvelot et al. (1986) rating system in future studies may give more information about when the plants become infected by AMF during the season, to what degree, if timing and degree of infection are linked to plant growth stage and/or environmental or soil conditions, and may reveal more about nutrient transfer between the plant and fungi. However, like all rating schemes, it is subjective.

The two main nutrients of interest in this study were N and P, both for supply and availability in the soil and ultimately their uptake by the plants. There have been numerous studies looking at root exudates and mechanisms of P uptake in commonly grown grain crops. Constituents and amounts of root exudates appear to not only vary from species to species (Zhang et al., 1997), but also from cultivar to cultivar (Cieslinski et al., 1997), from differing environmental conditions (Pearse et al., 2007), and with inter- and intraspecies competition (C. Li et al., 2016; L. Li et al., 2007; X. Li et al., 2018; Xia et al., 2013). Therefore, any results from a study on available soil P supply and plant uptake may only be applicable to the cultivar used in the study under the same conditions of the study.

The Central Butte 2019 monocrop kabuli chickpea had a net gain of soil N and P at the end of the growing season, reflecting the absence of the highly competitive non-N fixing brown flax crop. This agrees with the findings of Szumigalski and Van Acker (2006) who found less available N drawdown with dry pea alone compared to canola or canola-pea intercrop. Some mineralization and release of available N and P late season after harvest and before sampling may explain the net gain.

The Redvers 2019 site had a small net gain of soil N and P over the growing season that might reflect some post-harvest mineralization. This is different than that reported by Hocking et al. (2002) who observed large decreases in end of season soil N for oilseeds and wheat grown in Australia, with the exception of one site year that started with a low level of soil N (Hocking et al., 2002), and by Li et al. (2009) who observed greater soil N and P depletion in their mung bean-rice intercrop pot trial than in the corresponding monocrops, which they attributed to the greater N and P uptake in the intercropped plants. This is also different than the results of the study by Szumigalski & Van Acker (2006) in Manitoba who saw significantly lower soil available N in monocrop canola and dry pea-canola intercrops compared to monocrop dry pea soil N in three out of four site-years (Szumigalski & Van Acker, 2006). This could reflect a lower net N contribution and high N harvest index by dry pea (Arcand et al., 2013), and also reduced nutrient uptake and utilization by white mustard compared to canola.

Monocrop dry pea and monocrop white mustard ended the growing season with more soil available N and P than at the beginning of the season, and mixed row dry pea-white mustard ended the growing season with more soil N than at the beginning of the season. Similar results, though much larger differences, were reported by Gan et al. (2010) with monocrop pulse crops and monocrop oilseeds grown in Saskatchewan.

Overall, depletion of available NO3-N in the soil profile was lower in intercropped oilseed than in monocrop oilseeds, however, depletion was observed in all monocrop and intercrop treatments for all site-years. Starter N and P fertilizer may be helpful for early crop establishment but overall soil N rates need to be kept low to prevent intercrop oilseeds from dominating the companion pulse crops.

5. SYNTHESIS AND CONCLUSIONS

5.1 Overview

This study evaluated pulse monocrop, oilseed monocrop, pulse-oilseed alternate row intercrop, and pulse-oilseed mixed row intercrop treatments at two field site locations representing the Black and Brown soil-climatic zones in southern Saskatchewan in 2019 and 2020. This research emphasizes within-season soil fertility related aspects of growing pulse-oilseed intercrops in southern Saskatchewan. The information obtained on pulse-oilseed intercrop yields, nutrient uptake and removal, and the calculated total and partial LER values will be useful for growers choosing to include pulse-oilseed intercrops in their rotation, and for those interested in nutrient cycling in intercrop systems. The study documents the synergies and nutrient dynamics in two promising pulse-oilseed intercrop systems in Saskatchewan: kabuli chickpea-brown flax and dry pea-white mustard. More specifically, this research reports on straw and grain yield, N and P uptake in grain and straw, their related LER values, estimates of %Ndfa through BNF, mid-season WEOC and TDN in the seed-row and mid-row, root zone supply rates of NO_3-N and PO_4-P, percent root colonization by AMF, and seasonal soil nutrient drawdown as affected by seeding arrangement.

5.2 Key Findings and Conclusions

Greater efficiency was observed in the production of yield and grain N and P uptake and their removal per unit area of land within the pulse-oilseed intercrop systems compared to the corresponding monocrops of the intercrop components. This positive interaction was also shown in higher NUE values in the pulse-oilseed intercrop systems compared to their corresponding monocrops. The higher seed-row TDN concentrations in the pulse crops compared to the oilseed crops reflects the observed transfer of biologically fixed N from the pulse crops to the intercropped oilseeds. This, in turn, would contribute to and explain the enhanced efficiency of yield production and plant N and P uptake in these pulse-oilseed intercrop systems.

Synergy in nutrient uptake in the kabuli chickpea-brown flax intercropping arrangements versus monoculture was expressed in LER values for N and P uptake above 1. The higher root zone supply rates of NO_3-N in kabuli chickpea versus brown flax combined with the in-season transfer of fixed N from the kabuli chickpea to the intercropped brown flax were evidence for this nutrient supply and uptake synergism.

Greater crop uptake and removal of P associated with the growth enhancement in pulse-oilseed intercrops will need to be taken into consideration in low-input production systems to prevent depletion of soil P reserves over many years of use of such systems. The similarity of root zone supply rates of PO_4-P in intercrop and monocrop white mustard indicate that the white mustard is capable of solubilizing and removing soil P independent from a companion pulse crop.

Over the growing season, available NO_3-N in the soil profile was depleted in all treatments, however, the depletion was lower in the intercrop oilseeds than in the monocrop oilseeds. Pulse-oilseed intercrop systems cannot be expected to completely maintain soil N when used in no-fertilizer input production systems. In-season cross species N transfer in pulse-oilseed intercrop systems may be of particular benefit in dry growing conditions with limited N mineralization and N mobility such as was experienced in these trials.

Other intercropping trials have reported higher %Ndfa in intercrop pulse crops versus monocrop pulse crops, however this was not observed in this trial. There were no differences in the %Ndfa between the monocrop and intercrop kabuli chickpeas, nor between the monocrop and intercrop dry pea in each site-year of this study. It is possible that environmental conditions had greater influence over %Ndfa than monocrop and intercrop seeding arrangements. This is supported by the observed lower seed-row WEOC and TDN concentrations at the 2020 sites compared to the 2019 sites. Dry conditions may have limited plant growth and subsequent BNF and N transfer in the pulse-oilseed intercrop systems studied in this trial. Other factors also come into play. All dry pea treatments at the Central Butte 2020 site had very low %Ndfa, with zero %Ndfa in blocks 1 and 4, which was explained by high root zone supply rates of NO_3-N measured over the growing season. Careful attention will need to be made when planning pulse-oilseed intercrops to ensure that low available soil N levels are balanced with adequate available soil P levels to facilitate optimum BNF and transfer in the intercrop system. Under conditions of high available N, a lower proportion of oilseed in the intercropping system would be desirable to maintain co-crop equilibrium.

Higher kabuli chickpea partial LER and NLER values in alternate rows versus mixed rows indicates that kabuli chickpea is the subordinate crop in a kabuli chickpea-brown flax intercrop system. Brown flax, as the dominate crop, may have suppressed growth and subsequent BNF in kabuli chickpea, as there were significantly lower amounts of N derived from fixation in intercrop kabuli chickpea compared to monoculture kabuli chickpea. Kabuli chickpea also had poor establishment at the two Central Butte sites, and disease pressure at the Redvers 2019 site and the Central Butte 2020 site which may have contributed to brown flax dominance in the kabuli chickpea-brown flax intercrop system. It will be important to ensure early kabuli chickpea establishment and to keep them disease-free to prevent brown flax dominance in kabuli chickpea-brown flax intercrop systems. It may also be prudent to either increase the kabuli chickpea seeding rate or decrease the brown flax seeding rate in situations where higher available soil N and/or moisture conditions are anticipated for the growing season.

High partial LER values for dry pea compared with white mustard indicate that dry pea is the dominate crop in dry pea-white mustard intercrop systems when grown in moisture deficit soils with low available N and P. Although the shorter life-cycle of dry pea may assist with drought avoidance, the dry pea-white mustard intercrop system did not appear to perform as well as the kabuli chickpea-brown flax intercrop system in the drought conditions experienced during this trial. Overall, the yields of white mustard were low compared to brown flax. However, in other studies white mustard and canola dominated dry pea under high available soil N and/or moisture conditions, in which case it would be necessary to reduce the white mustard seeding rate used in this trial to maintain a balance with dry pea yield.

5.3 Future Research

In future studies, it would be useful to include specific analyses of the microbial communities and species composition in the rhizosphere using molecular deoxyribonucleic acid (DNA), chloroform fumigation extraction, or fatty acid methyl ester (FAME) techniques as a complement to measuring percent root colonization by AMF. It would be important to continue to include percent root colonization by AMF in any future studies with flax, as AMF associations in the crop can affect uptake of heavy metals, such as cadmium, in flax (Grant et al., 2010).

One of the objectives in this study was to determine if there would be significant yield and soil nutrient differences between alternate row and mixed row intercrop seeding arrangements. Overall, no large differences were observed. However, the partial LER values from the two intercrop seeding arrangements highlighted which crop species was dominate or subordinate in each intercrop system investigated. It would be useful to continue to use mixed row and alternate row seeding arrangements for the purpose of comparing partial LER values in future intercrop trials when investigating optimal crop species combinations, cultivar selection for intercrop systems, additive or replacement designs for intercrop systems, and specific seeding rates required for best performance. This will allow each intercrop system to be optimized for particular growing conditions and locations.

This study examined some of the within-season processes that operate in two pulse-oilseed intercrop combinations in southern Saskatchewan. However, a complete understanding of the competitive and facilitative processes that operate in these intercrop systems has yet to be achieved. Future studies of kabuli chickpea-brown flax and dry pea-white mustard intercrop combinations will need more below-ground investigation, including rooting architecture and root exudates for nutrient solubilization, mobilization, and uptake.

To fully understand how these and other processes drive variations in intercrop yields, future pulse-oilseed intercrop studies need to move beyond investigating how within-season individual processes affect resource-use efficiency (Brooker et al., 2015). Examination of interactions at a wider long-term and systems-level will require a multi-disciplinary approach and include areas such as plant physiology, agronomy, ecology, and agricultural engineering and technology. Integration of crop production with ecosystem services and soil sustainability (Brooker et al., 2015) will contribute to making intercropping a viable option for western Canadian farmers.

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APPENDIX A: ANCILLERY DATA

Table A.1. Kabuli chickpea and brown flax straw and grain yields and grain N and P uptake for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Indian Head, SK site in 2019 (*n*=4).

CHICKPEA

	Yield - kg ha^{-1} -		N uptake - kg N ha^{-1} -		P uptake - kg P ha^{-1} -	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain
Monocrop	2956 a	3544 a	20.7 a	116.0 a	2.1 a	12.5 a
Alt row	1451 b	2137 b	7.2 b	68.4 b	0.5 b	6.5 b
Mixed row	1470 b	2460 b	6.3 b	79.2 b	0.5 b	7.7 b

Source of variation	Probability (p)					
	Yield		N uptake		P uptake	
	Straw	Grain	Straw	Grain	Straw	Grain
Treatment	**0.0007**	**0.0333**	**<0.0001**	**0.0258**	**<0.0001**	**0.0197**

FLAX

	Yield - kg ha^{-1} -		N uptake - kg N ha^{-1} -		P uptake - kg P ha^{-1} -	
Treatment	Straw	Grain	Straw	Grain	Straw	Grain
Monocrop	2299 a	1617 a	10.7 a	51.1 a	1.1 a	10.2 a
Alt row	863 b	681 b	4.1 b	23.2 b	0.5 b	4.5 b
Mixed row	741 b	464 b	3.6 c	15.2 b	0.4 b	3.3 b

Source of variation	Probability (p)					
	Yield		N uptake		P uptake	
	Straw	Grain	Straw	Grain	Straw	Grain
Treatment	**<0.0001**	**0.0003**	**<0.0001**	**0.0004**	**<0.0001**	**0.0006**

Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.2. Kabuli chickpea-brown flax intercrop land equivalent ratios (LER) for grain and straw yield and grain N and P uptake at Indian Head, SK site in 2019 (*n*=4).

	Yield		N uptake		P uptake	
Treatment	Grain	Straw	Grain	Straw	Grain	Straw
Alt row	1.0 a	0.9 a	1.1 a	0.7 a	1.0 a	0.7 a
Mixed row	1.0 a	0.8 a	1.0 a	0.6 a	1.0 a	0.6 a
Source of variation	Probability (p)					
	Yield		N uptake		P uptake	
	Grain	Straw	Grain	Straw	Grain	Straw
Treatment	0.8990	0.6741	0.7917	0.2070	0.9953	0.5830

Total LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

Table A.3. Kabuli chickpea-brown flax intercrop partial land equivalent ratios (LER) for grain yield at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax
Alt row	0.5 a	0.9 a	0.5 a	2.0 a	0.4 a	0.5 b	0.2 a	0.9 b
Mix row	0.4 a	0.9 a	0.6 a	1.1 a	0.2 b	0.8 a	0.0 b	1.1 a

Source of variation	Probability (p)			
	2019		2020	
	Chickpea	Flax	Chickpea	Flax
Site	0.2715	0.1994	**0.0006**	**<0.0001**
Treatment	0.9230	0.0521	**0.0010**	0.3480
Site*treatment	0.5650	0.0515	0.2058	**0.0019**

Total LER values (kabuli chickpea + brown flax) higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly (p≤0.05) different.
Bolded values indicate significance at the p≤0.05 level of probability.

Table A.4. Kabuli chickpea-brown flax straw N and P uptake for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

CHICKPEA

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
				- - - kg nutrient uptake ha^{-1} - - -				
Monocrop	25.0 a	2.2 a	32.0 a	4.2 a	11.6 a	0.6 a	18.1 a	1.4 a
Alt row	5.3 b	0.4 a	11.6 b	1.1 b	3.9 b	0.1 b	4.7 b	0.3 b
Mixed row	2.7 b	0.2 a	3.9 b	0.3 b	1.6 b	0.0 b	0.8 c	0.1 b

Source of variation	Probability (p)			
	2019		2020	
	N	P	N	P
Site	0.2485	0.1321	**0.0435**	**0.0165**
Treatment	**0.0023**	**0.0056**	**<0.0001**	**<0.0001**
Site*treatment	0.9098	0.4794	**0.0004**	**0.0020**

FLAX

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
				- - - kg nutrient uptake ha^{-1} - - -				
Monocrop	6.3 a	0.7 a	7.9 b	1.2 a	3.7 a	0.1 b	7.5 ab	0.1 a
Alt row	5.6 a	0.5 a	14.6 a	2.0 a	2.6 a	0.4 a	6.4 b	0.1 a
Mixed row	7.5 a	0.7 a	10.6 ab	1.8 a	4.0 a	0.3 a	8.6 a	0.0 a

Source of variation	Probability (p)			
	2019		**2020**	
	N	P	N	P
Site	**0.0122**	**0.0023**	**<0.0001**	**0.0340**
Treatment	0.2914	0.5113	0.0557	0.0650
Site*treatment	0.1347	0.2417	0.7828	0.1013

Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.5. Kabuli chickpea and brown flax N harvest index (NHI) for monocrop kabuli chickpea, monocrop brown flax, and kabuli chickpea-brown flax intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax
Monocrop	0.6 b	0.9 a	0.6 a	0.8 a	0.9 a	0.9 a	0.7 a	0.9 a
Alt row	0.8 a	0.9 a	0.7 a	0.8 a	0.9 a	0.9 a	0.6 b	0.9 a
Mix row	0.9 a	0.9 a	0.8 a	0.8 a	0.9 a	0.9 a	0.6 b	0.9 a

Source of variation	Probability (p)			
	2019		2020	
	Chickpea	Flax	Chickpea	Flax
Site	**0.0261**	**0.0042**	**0.0042**	**0.0203**
Treatment	**0.0059**	0.1805	0.2819	0.5990
Site*treatment	0.5135	0.8950	0.0847	0.9539

Nitrogen harvest index = N uptake seed / (N uptake seed + N uptake straw).
Values in a column followed by the same letter are not significantly different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.6. Kabuli chickpea-brown flax intercrop partial land equivalent ratios (LER) for grain nutrient uptake at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

GRAIN N UPTAKE

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax
Alt row	0.5 a	1.0 a	0.6 a	2.3 a	0.4 a	0.7 b	0.2 a	0.9 a
Mixed row	0.4 a	0.9 a	0.4 a	1.3 a	0.2 b	1.0 a	0.0 b	1.0 a

Source of variation	Probability (p)			
	2019		2020	
	Chickpea	Flax	Chickpea	Flax
Site	0.6812	0.1880	**0.0024**	**0.0033**
Treatment	0.4308	0.0525	**0.0021**	**0.0009**
Site*treatment	0.9560	0.0762	0.2086	0.2227

GRAIN P UPTAKE

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax	Chickpea	Flax
Alt row	0.3 a	0.9 a	0.5 a	2.0 a	0.3 a	0.6 a	0.1 a	0.9 a
Mixed row	0.3 a	0.8 a	0.3 a	1.2 b	0.2 b	0.7 a	0.0 b	1.1 a

Source of variation	Probability (p)			
	2019		2020	
	Chickpea	Flax	Chickpea	Flax
Site	0.5758	0.1385	**0.0126**	**0.0112**
Treatment	0.4062	0.0800	**0.0008**	0.2381
Site*treatment	0.6615	0.1576	0.7352	0.6371

Total LER values (kabuli chickpea + brown flax) higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.7. Dry pea-white mustard intercrop partial land equivalent ratios (LER) for grain yield at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Pea	Mustard	Pea	Mustard	Pea	Mustard	Pea	Mustard
Alt row	1.0 a	0.4 a	0.7 a	0.9 a	0.8 a	0.3 a	1.0 a	1.0 a
Mixed row	1.0 a	0.3 a	1.8 a	0.6 a	0.9 a	0.3 a	1.3 a	0.6 b

Source of variation	Probability (p)			
	2019		2020	
	Pea	Mustard	Pea	Mustard
Site	0.6473	**0.0008**	0.3879	0.0646
Treatment	0.2428	0.0931	0.4862	0.0615
Site*treatment	0.1851	0.5230	0.6766	0.0519

Total LER values (dry pea + white mustard) higher than 1 indicate benefit from the intercrop.
Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.8. Dry pea-white mustard straw N and P uptake for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

PEA

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
			- - - kg nutrient uptake ha^{-1} - - -					
Monocrop	28.9 a	2.3 a	34.2 a	2.2 a	27.2 a	0.8 a	19.4 a	0.7 a
Alt row	17.2 a	1.0 a	38.6 a	3.1 a	15.5 a	0.5 a	9.7 a	0.6 a
Mixed row	15.4 a	0.9 a	22.8 a	1.4 a	19.7 a	0.5 a	14.8 a	0.7 a

Source of variation	Probability (p)			
	2019		2020	
	N	P	N	P
Site	0.0742	0.1833	0.1634	0.6078
Treatment	0.2096	0.2748	0.0568	0.6021
Site*treatment	0.4589	0.2586	0.9332	0.7973

MUSTARD

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
			- - - kg nutrient uptake ha^{-1} - - -					
Monocrop	6.9 a	0.7 a	14.1 a	2.3 a	6.0 a	0.3 a	14.1 a	0.2 a
Alt row	2.3 b	0.2 b	11.7 a	1.7 b	2.8 b	0.2 a	11.7 ab	0.1 a
Mixed row	4.6 ab	0.4 ab	12.8 a	1.3 c	3.2 b	0.2 a	7.1 b	0.1 a

Source of variation	Probability (p)			
	2019		2020	
	N	P	N	P
Site	**0.0001**	**<0.0001**	**0.0026**	0.2928
Treatment	**0.0454**	**<0.0001**	**0.0240**	0.3371
Site*treatment	0.7620	**0.0098**	0.2510	0.6186

Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.9. Dry pea and white mustard N harvest index (NHI) for monocrop dry pea, monocrop white mustard, and dry pea-white mustard intercrops in alternate and mixed rows at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Pea	Mustard	Pea	Mustard	Pea	Mustard	Pea	Mustard
Monocrop	0.8 a	0.8 ab	0.6 a	0.8	0.8 a	0.8 a	0.6 b	0.9 a
Alt row	0.8 a	0.8 a	0.4 a	0.8	0.8 a	0.8 b	0.7 a	0.9 a
Mixed row	0.8 a	0.7 b	0.6 a	0.8	0.8 a	0.7 b	0.7 ab	0.8 a

Source of variation	Probability (p)			
	2019		2020	
	Pea	Mustard	Pea	Mustard
Site	**0.0138**	0.1457	**0.0200**	**0.0048**
Treatment	0.3139	0.0586	0.0623	0.0938
Site*treatment	0.2764	0.8864	0.3887	0.1557

Nitrogen harvest index = N uptake seed / (N uptake seed + N uptake straw).
Values in a column followed by same letter are not significantly ($p \leq 0.05$) different.
CB 2019 mustard alternate row (n=2).
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.10. Dry pea-white mustard intercrop land equivalent ratios (LER) for straw N and P uptake at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	N	P	N	P	N	P	N	P
Alt row	1.0 a	0.8 a	2.5 a	2.8 a	1.1 a	1.9 a	1.4 a	1.8 a
Mixed row	1.2 a	1.1 a	1.8 b	1.4 a	1.3 a	1.4 a	1.5 a	2.0 a

Source of variation	Probability (p)			
	2019		2020	
	N	P	N	P
Site	**0.0003**	**0.0313**	0.3377	0.6121
Treatment	0.1551	0.3256	0.6546	0.8265
Site*treatment	**0.0108**	0.1661	0.7744	0.4400

LER values higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.11. Dry pea-white mustard intercrop partial land equivalent ratios (LER) for grain nutrient uptake at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

GRAIN N UPTAKE

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Pea	Mustard	Pea	Mustard	Pea	Mustard	Pea	Mustard
Alt row	0.8 a	0.4 a	0.7 a	0.8 a	0.8 a	0.3 a	1.1 a	1.1 a
Mixed row	1.0 a	0.3 a	1.7 a	0.6 a	0.9 a	0.4 a	1.4 a	0.6 b

Source of variation	Probability (p)			
	2019		2020	
	Pea	Mustard	Pea	Mustard
Site	0.6561	**0.0049**	0.3260	0.1073
Treatment	0.2238	0.2023	0.4614	**0.0325**
Site*treatment	0.3157	0.9224	0.7486	**0.0213**

GRAIN P UPTAKE

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	Pea	Mustard	Pea	Mustard	Pea	Mustard	Pea	Mustard
Alt row	0.8 a	0.4 a	0.7 a	0.8 a	0.8 a	0.3 a	1.4 a	0.9 a
Mixed row	0.9 a	0.3 a	1.6 a	0.5 a	0.9 a	0.3 a	1.6 a	0.5 b

Source of variation	Probability (p)			
	2019		2020	
	Pea	Mustard	Pea	Mustard
Site	0.6082	**0.0071**	0.1659	0.0878
Treatment	0.2191	0.0957	0.7377	**0.0459**
Site*treatment	0.3350	0.4073	0.8530	0.0845

Total LER values (dry pea + white mustard) higher than 1 indicate benefit from the intercrop.
Values in a column followed by the same letter are not significantly ($p \leq 0.05$) different.
Bolded values indicate significance at the $p \leq 0.05$ level of probability.

Table A.12. Soil available K drawdown for 0-15cm depth in the kabuli chickpea-brown flax and dry pea-white mustard treatments at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

CHICKPEA-FLAX				
Treatment	Redvers 2019	CB 2019	Redvers 2020	CB 2020
	Fall soil residual K mg/kg soil – spring soil residual K mg/kg soil			
Chickpea mono	-70.2 a	-51.1 a	-41.5 a	-38.8 b
Alternate row	-53.4 a	-67.7 a	-8.0 a	35.3 a
Mixed row	-56.4 a	-72.0 a	-21.1 a	-48.1 b
Flax monocrop	-55.5 a	-83.3 a	-36.7 a	-14.4 b
Source of variation		Probability (p)		
		2019	**2020**	
Site		0.7214	0.7172	
Treatment		0.9814	**0.0411**	
Site*treatment		0.7777	0.3107	

PEA-MUSTARD				
Treatment	Redvers 2019	CB 2019	Redvers 2020	CB 2020
	Fall soil residual K mg/kg soil– spring soil residual K mg/kg soil			
Pea monocrop	-46.1 a	51.23 a	-36.8 b	-1.0 ab
Alternate row	-31.4 a	-24.0 a	3.44 ab	-12.7 b
Mixed row	-56.9 a	2.36 a	18.9 a	-15.8 b
Mustard mono	-33.9 a	44.9 a	-19.6 ab	40.9 a
Source of variation		Probability (p)		
		2019	**2020**	
Site		0.2104	0.8420	
Treatment		0.6645	0.4942	
Site*treatment		0.6202	**0.0805**	

Drawdown is change in soil available K concentration from spring before seeding to fall after seeding.

Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.

Bolded values indicate significance at the $p \leq 0.1$ level of probability

Table A.13. Soil available N drawdown for 15-30cm and 30-60cm depths in the kabuli chickpea-brown flax treatments at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	15-30cm	30-60cm	15-30cm	30-60cm	15-30cm	30-60cm	15-30cm	30-60cm
				--- mg kg^{-1} ---				
Chickpea mono	-3.8 ab	-1.4 ab	-3.7 a	-1.2 a	-4.9 b	-2.5 ab	-7.5 ab	-6.8 a
Alternate row	-4.0 b	-1.8 b	-3.9 a	-1.3 a	-4.4 a	-2.2 a	-7.7 ab	-7.2 a
Mixed row	-3.4 a	-1.3 ab	-4.0 a	-1.3 a	-4.8 b	-2.3 ab	-7.5 a	-7.3 a
Flax monocrop	-3.7 ab	-1.1 a	-3.6 a	-1.3 a	-4.7 ab	-2.9 b	-7.9 b	-7.4 a

Source of variation	Probability (p)			
	- 2019 -		- 2020-	
	15-30cm	30-60cm	15-30cm	30-60cm
Site	0.9299	0.8389	**<0.0001**	**<0.0001**
Treatment	0.4530	0.6513	0.5198	0.2270
Site*treatment	0.1520	0.6225	**0.0855**	0.5082

Drawdown is change in soil available N concentration from spring before seeding to fall after harvest.
Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

Table A.14. Soil available N drawdown for 15-30cm and 30-60cm depths in the dry pea-white mustard treatments at Redvers and Central Butte (CB), SK sites in 2019 and 2020 (*n*=4).

	Redvers 2019		CB 2019		Redvers 2020		CB 2020	
Treatment	15-30cm	30-60cm	15-30cm	30-60cm	15-30cm	30-60cm	15-30cm	30-60cm
				--- mg kg^{-1} ---				
Pea monocrop	0.0 a	-0.7 a	-3.0 a	-0.5 ab	-4.3 b	-0.5 a	-7.2 a	-6.5 a
Alternate row	-2.8 b	-1.8 a	-2.9 a	0.7 a	-3.5 a	-1.5 a	-7.3 a	-7.0 a
Mixed row	-3.6 b	-1.3 a	-2.5 a	-0.7 b	-3.8 ab	-1.1 a	-6.8 a	-6.4 a
Mustard mono	-3.2 b	-1.6 a	-2.9 a	-0.5 ab	-4.1 ab	-1.7 a	-7.3 a	-7.3 a

Source of variation	Probability (p)			
	2019		2020	
	15-30cm	30-60cm	15-30cm	30-60cm
Site	0.5147	**0.0808**	**0.0011**	**<0.0001**
Treatment	0.2393	0.5893	0.3091	0.2099
Site*treatment	0.1306	0.3340	0.4787	0.9291

Drawdown is change in soil available N concentration from spring before seeding to fall after harvest.
Values in a column followed by same letter are not significantly ($p \leq 0.1$) different.
Bolded values indicate significance at the $p \leq 0.1$ level of probability.

APPENDIX B: PERMISSION TO REPRODUCE

SV: permission to include figure in Master's thesis

Henrik Hauggaard-Nielsen <hnie@ruc.dk>
Thu 2022-03-03 1:59 AM
To: Reid, Melanie <melanie.reid@usask.ca>

CAUTION: External to USask. Verify sender and use caution with links and attachments. Forward suspicious emails to phishing@usask.ca

Dear Melanie

Indeed, it is my pleasure that you find this work relevant to be included in your thesis – full permission from me 😊

I wish you all the best of luck for the defense and final evaluation
Kind regards
Henrik Hauggaard-Nielsen
Professor

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Sendt: 2. marts 2022 18:38
Til: Henrik Hauggaard-Nielsen <hnie@ruc.dk>
Emne: permission to include figure in Master's thesis

Hello H. Hauggaard-Nielsen,

my name is Melanie Reid, and I am in the final stages of writing my Master's thesis on nutrient dynamics in pulse-oilseed intercrops in Saskatchewan, Canada. One of my supervisors recommended that I include in my literature review an example of a chart that shows the yields of each crop component as partial LER values. I thought your figure 2 from "Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems", Field Crops Research 113 (2009), 64-71, was an excellent example of displaying partial LER values.

May I have your permission to include your figure 2 from the above paper in my Master's thesis literature review? I do not intend to alter the figure.

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