WINTER CAMELINA RESPONSE TO NITROGEN FOR DOUBLE CROPPING WITH MAIZE AND SOYBEAN IN THE UPPER MIDWEST

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List of Abbreviations

C, carbon

ctr, control relay

cts, control sequence

EPA, environmental protection agency

FA, fatty acids

GDD, growing degree days

GS, glucosinolates

LAI, leaf area index

N, nitrogen

P, phosphorous

rly, relay

seq, sequential

SLRF, Swan Lake Research Farm at USDA Agricultural Research Service in Morris, MN

SWROC, Southwest Research and Outreach Center near Lamberton, MN

UMN, University of Minnesota

USD, United States Dollar

WCROC, West Central Research and Outreach Center in Morris, MN

1. Winter camelina: A promising crop for double cropping systems in the upper Midwest

1.1 Conventional Agriculture in the Upper Midwest

The Midwestern United States is one of the most intensively farmed landscapes in the world (USDA 2017). In 2007 alone the region produced USD 76 billion worth of maize [*Zea mays* L.], soybean [*Glycine max* (L.) Merr.], and a variety of other crops; around 75% of this productive land was in maize and soybean (USDA 2017). The upper Midwest is a sub-region of the upper Mississippi River Basin that includes the states of Illinois, Iowa, Michigan, Minnesota, Missouri, North Dakota, South Dakota, and Wisconsin. In 2017 to 2019, the upper Midwest produced roughly 60% of the total maize and soybean in the U.S. (USDA-NASS, 2020). While the upper Midwest is generally colder than the remainder of the Midwest, maize and soybean yields have improved greatly through the 20th century due to breeding and technological advances in management (Galzki, Olmanson, & Mulla, 2018). For instance, Minnesota's maize yields have increased six-fold since the 1930's (USDA-NASS 2019).

In the region, as is true of much of the Midwest, tile drainage use in agriculture is widespread to provide suitable conditions for crop growth. This is largely due to the glacial origins of the soil that make it slow to drain and to the cool and humid weather (Madramootoo et al., 2007). While drainage improves crop productivity by avoiding excess water stress, it also facilitates leaching of nitrate-N (NO_3^--N) into surface and ground water (Kladivko et al., 1991). The intensity of tile drainage has increased along with the use of fertilizers, a combination that is reported to have allowed for expanding the yield potential of corn (Hart, 1986). Improvements from breeding have also helped boost yield potential of both maize and soybean, but over the latter half of the 20th century, the United States has also seen a nearly four-fold increase for N use in maize (little N has been used for soybean) and a two-fold and seventeen-fold increase in phosphorous (P) for maize and soybean respectively from 1964 to 2018 (USDA-ERS, 2019). This has led to serious long-term environmental consequences due to leaching of NO_3-N (and to a lesser extent, P) into waterways (Alexander et al., 2008). Nutrient leaching in maize-soybean rotations mostly occurs in spring and fall before planting and after harvest, when residual fertilizers in fallow soil can percolate into ground water (Heggenstaller, Anex, Liebman, Sundberg, & Gibson, 2008).

In addition to negatively affecting water quality downstream, loss of fertilizer comes at significant monetary and health costs. For example, Midwestern farmers are reported to lose almost U.S. dollar (USD) 500 million year^{-1} from N leaching in maize and soybean rotations (Basso, Shuai, Zhang, & Robertson, 2019). Health effects linked to N in drinking water include increased cancer risk (Ward et al., 1996; Weyer et al., 2001), birth defects, and premature birth of infants (Brender et al., 2013; Bukowski et al., 2001). For a typically small rural community of 3,000 inhabitants, the community could expect to pay between USD 140,000 to 1.1 million for treatment for safe drinking water (Schechinger & Cox 2018). In 2019 in Minnesota, 10% of wells exceeded the 10 mg L^{-1} nitrate level threshold set by the Environmental Protection Agency (EPA) (Minnesota Department of Health 2019). In some regions in Minnesota such as in Dakota County, the number of wells exceeding that threshold is much higher at 27% with some individual communities reaching above 50% (Dakota County 2020). According to Juntakut et al. (2020), who studied two agricultural regions in Nebraska, 17% of wells exceeded EPA thresholds for safe drinking water in one region while 82% of wells exceeded the EPA threshold in the other. These communities could expect to pay up to USD 25 per household for the first region and up to USD 508 per household for the second for remediating drinking water to levels considered safe. Holding onto this reactive N in the agroecosystems could greatly improve environmental quality and save money for farmers and communities. With these health, economic, and environmental issues from current crop production practices, there is a need for alternative crop production practices that achieve a high level of agricultural production while reducing societal costs.

1.2 Sustainable Production Practices

There are myriad ways farmers and researchers can approach the environmental degradation caused by N and other nutrient loss on the landscape. Options are typically variations of (i) moderating N type and application method on the distribution end and (ii) increasing diversity or production time so more N can be used in the soil.

For (i), precision agriculture, an umbrella term that applies to techniques that vary the agronomic treatment of a field with the use of a large amount of data and application of technology, is one possible option that focuses on the N application end. In this instance, techniques used include variable rate to correspond with production levels and auto-steer technology to decrease fertilizer overlap (Basso et al., 2019; Galzki et al., 2018). Other sustainable practices employ urease and/or nitrification inhibitors, sometimes referred to as enhanced efficiency nitrogen fertilizers (EENF), which slow the breakdown of N into reactive forms and may reduce its loss and increase crop absorption (Thapa, et al., 2015; Zaman et al., 2008).

For (ii), sustainable practices regarding increasing or diversifying plants, cover cropping and double cropping are two such practices, although their use is still limited in the region. Cover cropping is a technique that involves growing additional crops (usually annuals) in addition to production crops. Cover crops can be interseeded into a standing major crop or seeded after harvest of the major crop (CTIC, SARE, & ASTA, 2020). Double cropping involves producing two crops in one season, which keeps vegetation on the soil for a longer period of time. This practice is used more typically in the southern U.S. with small grains and soybean (Berti et al., 2015). Relay cropping is a double cropping method that involves planting the second crop into the first crop as it is maturing (Chan, Johnson, & Brown, 1980; Gesch, Archer, & Berti, 2014). Relay cropping results in harvest of the first crop over the second. Sequential cropping is another method of double cropping and involves growing two crops in sequence, planting the second after the harvest of the first (Gesch & Archer, 2013). Double cropping will be discussed in greater detail throughout this thesis as it relates to techniques relevant to incorporating winter camelina [Camelina *sativa* (L.) Crantz] into the maize and soybean production system, the topic of this thesis.

1.3 Cover Crop and Double Cropping in the Upper Midwest

Cover crops have shown promise in alleviating some of the issues caused by intensive maize and soybean rotations such as reducing NO_3-N leaching (Strock, Porter, & Russelle, 2004; Thapa, Mirsky, & Tully, 2018), improving soil quality (Fortuna et al., 2008), and increasing weed suppression (Finney, White, & Kaye, 2016). With these benefits, there is still low cover crop adoption in the upper Midwest. According to the most recent USDA NASS Census of Agriculture data, 4% of cropland planted in the region was planted to cover crops in 2017 (Zulauf & Brown, 2019). This low rate of adoption seems to be due to farmers concerns with time and labor costs and the lack of economic return upon termination of cover crops (CTIC & SARE, 2017). These concerns are exacerbated by the bio-physical constraints of the upper Midwest from the relatively shorter growing season (Rusch et al., 2020; Wilson, Baker, & Allan, 2013) and lower average USDA plant hardiness zone of this region, as many cover crops are effective in USDA zone 6a and above, depending on seasonal soil moisture and soil type (Appelgate et al., 2017).

These concerns could be addressed by employing double cropping instead of cover cropping. A winter annual could theoretically be incorporated into the existing summer annual maize-soybean rotation, even in the short growing season of the upper Midwest (Berti et al., 2015; Gesch & Archer, 2013; Johnson et al., 2017; Liu, Wells, & Garcia y Garcia, 2020). Winter annual oilseeds are considered to be a viable option for such a strategy in the region for the production of oil that could be used for biofuels or human consumption. Winter camelina is considered a low-input, cold-hardy oilseed crop that could be double cropped with maize, soybean, and other summer annuals (Gesch & Archer, 2013; Gesch, Archer, & Berti, 2014; Liu, Wells, & Garcia y Garcia, 2020; Ott et al., 2019).

In the upper Midwest, double-cropped winter camelina is reported to produce as much as 1745 kg ha^{-1} of grain yield (Berti et al., 2015). Studies have also shown 20 to 40% penalty on yield of soybean in relay with winter camelina; the total yield (winter camelina + soybean) often equals or is greater than monocropped soybean and net return either did not differ or was higher in the relay system (Gesch & Archer, 2013; Gesch et al., 2014; Ott et al., 2019). Such results suggest that double cropping is a strategy with potential to be economically viable, but improvements are still needed. The studies presented in this thesis focus on winter camelina for double-cropping systems in the maize-soybean rotation in the upper Midwest.

2. Winter camelina yield response to nitrogen

Summary

Winter camelina [*Camelina sativa* (L.) Crantz] is a potential third crop that could be used to intensify maize [*Zea mays* L.]-soybean [*Glycine max* (L.) Merr.] rotations. It is considered a low-input crop, but previous studies have shown that it responds to added N. Yet, no formal fertilization studies have been conducted to determine optimum N levels for conditions in the upper Midwest. A study on camelina response to fertilizer N was conducted from fall 2018 to fall 2020 at three locations in Minnesota. The objectives were to: (i) determine the response of winter camelina to N and (ii) assess the effects of N fertilization strategy (fall-spring split or spring only application) on the productivity and quality of winter camelina. Data collected included grain yield, biomass, grain quality, and yield components. Grain yield and biomass were both affected by N in all locations and years, and both were higher in 2019 compared to 2020; among N rates, grain yield

was significantly different, while no differences were found for biomass. Both, oil and protein content in grain were affected by N, with oil content generally declining with N rates increasing beyond 67 or 100 kg N ha^{-1}, depending on location and year. Among yield components, branches and silicles per plant were significantly different among N rates; the former, along with the seed:shell ratio were significantly different in all years and locations. Based on the results of this study, a fertilization rate of 97 kg N ha^{-1} was found to maximize grain and oil yield of winter camelina in southwest Minnesota.

2.1 Introduction

Sustainable intensification of the roughly 18 million ha of maize and soybean in the upper Midwest can help decrease negative environmental impacts of conventional agriculture while increasing farmer profitability (Gesch & Archer, 2013; Heaton et al., 2013; Johnson et al., 2015). It is well documented that conventional maize and soybean production practices leave soil fallow from early fall to late spring, a period of roughly 6 months in a year. The fallow period in this enormous agroecosystem contributes to significant NO_3-N leaching, decreased biodiversity, and loss of soil and other nutrients due to erosion (Basso et al., 2019; Davis et al.,2000; Fortuna et al., 2008; Syswerda & Robertson, 2014; Thom et al.,2018). These problems exist both within the farming communities of the upper Midwest and compound for communities further downstream (Basso et al., 2019; Strock et al., 2004).

In the upper Midwest, temporal intensification, or increasing the number of crops grown in a season (Heaton et al., 2013), can be applied to summer annual cropping systems through the use of winter annuals, which are planted in the fall after summer annual harvest and harvested the following spring before summer annual planting. This intensification strategy can contribute some of the well documented ecological benefits of a cover crop, such as decreased weed pressure and soil erosion with improved soil health (Appelgate et al., 2017; Strock et al., 2004; Thapa et al., 2018), while also offering financial return when the crop is harvested instead of terminated (Berti et al., 2017; Gesch & Archer, 2013). This could further increase diversity on the landscape when, despite decades of promising cover crop research, only 4% of cropland used cover crops in the upper Midwest (Zulauf & Brown, 2019), mainly due to concerns with the costs, planting difficulties, and lack of financial return (CTIC & SARE, 2017).

Winter camelina [*Camelina sativa* (L.) Crantz], a hardy oilseed crop in the Brassicaceae family, is a promising winter annual for the sustainable intensification of crop production in the region. This winter oilseed crop produces both oil- and protein-rich seeds and has shown encouraging results for double-cropping in the shorter growing season of the upper Midwest (Gesch & Archer, 2013; Pilgeram et al., 2007; Zubr, 1997). Camelina is an ancient crop comparable or superior to canola in many ways: similar edible oil, but higher omega-3 fatty acid levels; higher resistance to common pests and diseases; and higher resistance to lodging (Berti et al., 2016; Gesch, 2014; Séguin-Swartz et al., 2009). Modern breeding techniques are now being used to shorten the camelina lifecycle and improve seed yield—traits that will increase its double-cropping suitability in a variety of cropping rotations in the U.S. Corn Belt (Berti et al., 2016; Eynck & Falk, 2016).

Camelina has spring and winter biotypes with much of the research focusing on the former, mostly in the arid western United States or in Canada (Allen, Vigil, & Jabro, 2014; Enjalbert & Johnson, 2011; Malhi et al., 2014; McVay & Khan, 2011; Sintim et al., 2016; Wysocki et al., 2013). Due to its winter hardiness and short-season characteristics, winter camelina is considered more suitable to intensification efforts in the upper Midwest (Berti et al., 2015; Gesch & Archer, 2013). Winter camelina has similar oil content, but is generally expected to yield less than spring cultivars, although some studies have only differentiated between planting dates rather than established winter and spring cultivars (Guy et al., 2014; Prakhova, Prakhov, & Danilov, 2018; Sintim et al., 2016). Camelina is considered a low-input crop in a cover crop setting, but if used as a cash crop for its oil, fertilization is likely required to increase yield (Berti et al., 2016; Gesch & Archer, 2013). Spring camelina fertilization studies have shown positive response to N at rates ranging from 130 to 200 kg ha^{-1} (Jiang & Caldwell, 2016; Johnson, Gesch, & Barbour, 2019). Johnson et al. (2019) reported that spring camelina was relatively efficient at taking up N at moderate levels of fertilization, but higher levels do not necessarily translate to higher yields (Johnson, Gesch, & Barbour, 2019). Such results could support the use of camelina as a cover crop to use residual N rather than as a cash crop in the upper Midwest, although little research has been conducted in this realm. While fertilization studies have focused on spring biotypes (Jiang & Caldwell, 2016; Johnson et al., 2019; Malhi et al., 2014), recent winter camelina research report the use of N fertilizer, often between 70 to 90 kg N ha^{-1} due to low yields reported in studies without N fertilization (Gesch & Archer, 2013; Liu, Wells, & Garcia y Garcia, 2020).

To date, most winter camelina studies in the region have focused on cropping systems in maize-soybean rotation practices with little attention to its requirements for N. This research was designed to determine the maximum rate and timing of N fertilization for winter camelina grown in Minnesota. The hypothesis was that N fertilizer will have a positive effect on winter camelina yield, biomass, and yield components. The specific objectives of this study were to (i) determine the response of winter camelina to N, and (ii) assess the effects N fertilization strategy (fall-spring split or spring only application) on productivity and quality of winter camelina.

2.2 Materials and Methods

2.2.1 Experimental Sites

Field experiments were conducted at three Minnesota locations from fall 2018 to fall 2020. Locations included the University of Minnesota Southwest Research and Outreach Center near Lamberton, MN (SWROC; 44°14'02.20"N 95°18'6.87"W), the University of Minnesota West Central Research and Outreach Center (WCROC; 45°35'37.17"N 95°52'42.63"W), and the Swan Lake Research Farm (SLRF; 45°36'4.30"N 95°54'11.09"W) near Morris MN. Dominant soils were characterized as Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls) at SWROC, Nutley Flom clay loam (fine, smectitic, frigid chromic Hapluclerts and fine-loamy, mixed, superactive, frigid Typic Encloaquolls) at WCROC, and Barnes clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) at SLRF (Soil Survey Staff, 2020).

The climate of the region is characterized as continental with long, cold winters and short, wet springs and summers. The 25-year (1994 to 2019) long-term average (LTA) for annual temperature and rainfall are 7°C and 737 mm at SWROC and 5.8°C and 670 mm at WCROC. The SWROC is located within winter hardiness zone 4b while SLRF and WCROC are both located within winter hardiness a 4a; the latter slightly colder (USDA-ARS). Long-term air temperature and precipitation data were obtained from the National Oceanic and Atmospheric Administration (www.ncdc.noaa.gov/).

2.2.2 Experimental Design

The experiment was set up as a completely randomized design in a 2 × 5 factorial arrangement. Factor A contained two N fertilizer application strategies: spring-only application (Strat 1) and fall-spring 33 to 67 % split application (Strat 2). Factor B consisted of five N fertilizer rates: 0, 33, 67, 100, and 135 kg ha^{-1}. Each treatment had four replications and plots were 3 m × 5 m.

2.2.3 Agronomic Management

At all three sites, the study was conducted in different field each year and followed small grains to avoid residual N effects from year one to year two: oats [*Avena sativa* L.] at SWROC and spring wheat (*Triticum aestivum* L.) at WCROC and SLRF. Trifluralin N-dipropyl-4-(trifluromerhyl) aniline and glyphosate [N-(phosphonomehtyl) glycine] at 2.0 kg a.e. ha^{-1} were used as preplant herbicides for weeds at SLRF and SWROC, respectively in fall 2018 and 2019. No preplant herbicides were used at WCROC in fall 2018 or 2019 due to planting of winter camelina within a few days of harvesting and discing the previous crop. Seedbeds were prepared with a tandem disc at

SWROC and a field cultivator at WCROC and SLRF. Winter camelina cultivar Joelle (USDA), obtained through the Forever Green program of the University of Minnesota, was seeded at a rate of 9 kg ha^{-1} at SWROC and WCROC and 8 kg ha^{-1} at SLRF at 1 to 2 cm depth at all locations. Rows were planted 19 cm apart using a Great Plains Seeder (model 3P1006NT, Great Plains, Salina, KS) at SWROC and SLRF and a Case IH 4.3 m drill (Model 5100; Case IH, Racine, WI, USA) at WCROC. Winter camelina was planted in mid-September to early October at SWROC and WCROC, and in mid-September in 2018 and early September 2019 at SLRF. At SLRF in the fall of 2018, camelina was mistakenly over-seeded, which did not seem to have affected the results, probably due to the phenotypic plasticity that has been noted in camelina in response to planting density (Gesch et al., 2018; Turina et al., 2020).

At all sites a preplant application of fertilizer was broadcast in the fall (Strat 2) and incorporated to 10 cm using a Kuhn power tiller at SWROC and WCROC and a disc harrow at SLRF. Spring-applied fertilizer was broadcast in all plots at the rosette stage in late April to mid-May in 2019 and in late April 2020, close to inflorescence emergence, which has been reported as a time that maximizes seed yield in the field and greenhouse (Gesch & Cermak, 2011; Johnson & Gesch, 2013; Ott et al., 2019). Fertilizer applied was N_{trt}-30-30, (where N_{trt} represents the N treatment amount) as urea (NH_2-CO-NH_2) triple superphosphate (P_2O_5) and Muriate of potash (K_2O). Camelina was harvested when > 90% of silicles were brown and dry.

2.2.4 Data Collection

Weather data during the experimental years were collected from automated weather stations located within 1 km, 1.5 km, and 0.5 km at SWROC, WCROC and SLRF. Canopy coverage of winter camelina was obtained every two weeks starting in late-April using the Canopeo App v 2.0 (Patrignani & Ochsner, 2015) to estimate fractional green canopy cover using a 0.5 m x 0.5 m quadrat. Above ground biomass within the quadrat was collected at anthesis (BBCH65; Martinelli & Galasso, 2011) at WCROC and SLRF 2019, at inflorescence emergence (BBCH50) at SLRF in 2020 and at harvest (Martinelli & Galasso, 2011). Biomass was dried in a forced air oven at 60°C to a constant weight. Biomass and grain samples were then ground with a Thomas Wiley Mill Model 4 to pass through a 1-mm screen. Biomass and grain samples at 5 to 15 mg and 10 to 15 mg were analyzed for carbon (C) and N analysis, respectively, by combustion using a Elemento Vario EL Cube (Elementar Americas Inc., Ronkonkoma, NY). Additionally, leaf area index (LAI) was obtained by measuring photosynthetically active radiation (PAR) using an AccuPAR LP-80 ceptometer (Meter Group, Inc. USA) every 2 weeks starting in late April. Readings in each plot were taken between 1000 and 1400 h on days when ambient PAR readings were above 600 μmols m^{-1} s^{-1}. Below canopy PAR was taken with the ceptometer probe placed perpendicular to the planting rows in the center of the plot and three readings were averaged to generate one reading. Above canopy PAR was simultaneously taken using a sensor on a 1 m pole. Tau, the ratio of below to above canopy PAR, was then calculated and used for comparing canopy cover between treatments.

Yield was harvested by hand and with a 2-row Kincaid 8-XP plot combine (Kincaid Equipment Manufacturing) at SWROC and a HEGE combine (Model 160) at SLRF Morris. Combine yield was not obtained at WCROC due to excess rain that limited field access after the time of hand harvest. Hand harvest was completed cutting at ground level all plants within a 1 m^2 quadrat at SWROC and WCROC; at SLRF, hand harvest was < 1 m^2. After oven-dried, seeds were separated from the biomass using a belt thresher (Almaco, BT14 Belt Thresher). Following hand-harvest, all plots were combine-harvested, and grain seed processed to determine yield loss as compared to hand harvest yield. Oil and protein content in seeds were obtained through Nuclear Magnetic Resonance Spectrometry (NMR) at the Seed Laboratory of Oregon State University with a flow cytometer (CyFlow®Space; Partec, Görlitz, Germany). Harvest index (HI) was calculated as the ratio of grain yield (GY; kg ha^{-1}) to total aboveground biomass (TBM; kg ha^{-1}) (eq. 1) at physiological maturity.

$$\mathrm{HI} = \frac{GY}{TBM} \tag{1}$$

Yield components and seed:shell ratio were obtained using three-plant subsamples representative of each plot from the 1 m^2 hand-harvested sample. Subsamples were dried at 60°C to a constant weight and weighed. Four yield components were obtained in 2019: number of silicles per plant, number of seeds per silicle, weight of one silicle, and 1000-seed weight. In 2020, silicle-bearing branches per plant were also measured. Subsample processing contained the following steps: (i) count the number of silicle-bearing branches per plant and record the average; (ii) set aside 10 representative silicles, taking three to four per plant; (iii) count the number of seeds in the 10 silicles and record the average per silicle; (iv) record weight of 10 empty silicles; record the weight of the seeds in 10 silicles; (v) separate the remaining silicles and record the total weight, seed weight, and empty silicle weight, including the 10 silicles from the step (ii). The 1000-seed weight was determined using a seed counter (Seedburo, 801-Count-A-Pak) from the 1 m^2 hand-harvested sample.

For each year, growing degree days (GDD) were calculated from planting to harvest following the procedure by Mcmaster and Wilhelm (1997). Base temperature (T_{base}) was 4°C and absolute maximum (T_x) was 28°C (Tribouillois et al., 2016).

$$GDD = \Sigma(\frac{Tmax + Tmin}{2} - Tbase) \tag{2}$$

where T_{max} = maximum daily air temperature and T_{min} = minimum daily air temperature. The following thresholds were used to limit the temperature within the range of T_{base} and T_x:

$if\ T_{max} > T_x\ then\ T_{max} = T_x$

$if\ T_{max} < T_{base}\ then\ T_{max} = T_{base}$

$if\ T_{min} > T_x\ then\ T_{min} = T_x$

$if\ T_{min} < T_{base}\ then\ T_{min} = T_{base}$

2.2.5 Statistical Analysis

All data were analyzed using R statistical software (version 4.0.3; R Core Team 2020). Grain yield, oil and protein content, oil and protein yield, biomass, LAI, Tau (the ratio of below to above canopy PAR), percent cover, C, and N were analyzed using the linear mixed effects model analysis of variance (ANOVA) to determine significant effects and interactions. Locations were analyzed independently due to the differing amounts and types of data from each site. Regression analysis using the least squares method was performed to find functional relationships between selected variables and N. Models that minimized the sum of the squared errors and showed best r^2 values were selected. Year, fertilization strategy, and N rate were considered fixed effects. Assumptions of normality and constant variance of the model residuals were assessed visually. If the combined analysis showed significant interactions, separate ANOVA were then run on the response variable. Post hoc analysis was conducted using Tukey's Honest Significant Difference (HSD) with the '*agricolae*' package at $P \leq 0.05$ to determine means separation for variables by treatment. The Pearson coefficient of correlation was used to determine the strength of association between yield component and yield.

2.3 Results and Discussion

2.3.1 Weather Conditions

In 2018 at all three locations monthly average air temperatures were below the long-term averages (LTA); 2°C at SWROC and SLRF and 3°C at WCROC. At all three locations, October and November in 2019 were also colder than the LTA: 4°C and 5°C at SWROC, 4°C and 8°C degrees at WCROC, and 3°C and 4°C at SLRF. In 2019, monthly average temperatures were lower than the LTA for January to August; all three locations were 8° to 9°C colder in February and 6° to 7°C colder in March and May. In 2020, temperatures returned to within 2°C of the LTA with the exception of April and May, which were 4 to 5°C and 5 and 7°C colder than the LTA at all locations (Table 2.1).

The 2019 year was wetter than the LTA at all locations while the 2020 year was wetter at SWROC and drier in both Morris locations. SWROC received > 280 mm more precipitation than the LTA in 2019, with 70 mm above the LTA falling during both April and July coinciding with the BBCH50 (inflorescence emergence) and BBCH89 (ripening) stages of winter camelina (Martinelli & Galasso, 2011). Studies at WCROC and SLRF, each received roughly 190 mm of rain above the LTA in 2019. In 2019, September and October were more than 100 mm wetter than the LTA at SWROC, saturating the soil of the newly planted winter camelina before the ground froze. Saturated soils in spring of 2020 may have affected productivity in 2020, as it has been reported that camelina yield is negatively affected by waterlogging stress in early spring, especially in conventionally tilled plots (Gesch & Archer, 2013; Gesch & Cermak, 2011). In 2020 until harvest in July, SWROC was 41 mm wetter than the LTA, while WCROC and SLRF were 187 and 119 mm drier respectively. The most significant dry period in 2020 in Morris was from February through May at WCROC with 133 mm less than the LTA and January to May at SLRF with 119 mm less (Table 2.1).

2.3.2 Winter Camelina Growth and Development

Total biomass was significantly affected by fertilizer N in all locations, year at SWROC and SLRF, and strategy at SLRF. Because of crop failure due to weather at WCROC in spring 2020, no data were collected that year. At SWROC, 67 and 33 kg N ha^{-1} were the lowest N rates that provided the highest statistically significant biomass yield in 2019 and 2020 respectively. In 2020 all of N rates above were significantly different from the control but did not differ from each other. At WCROC in 2019, biomass was significantly affected by N rates in both locations and application strategy at SLRF, where Strat 1 yielded higher than Strategy 2. In 2019 for both strategies and in 2020 for Strat 2, fertilizer N did not affect biomass production, although increasing N rates provided higher average biomass. In 2020, 67 kg N ha^{-1} provided the highest biomass yield at WCROC. (Figure 2.3).

In 2019, mild temperatures and average precipitation in the fall combined with higher-than-average precipitation in spring and a cooler summer during flowering and pod fill could have contributed to higher production that year compared to 2020 (Gugel & Falk, 2006; Walia et al., 2018) (Table 2.1). Precipitation in May in particular was 30%, 34%, and 22% higher than the LTA conditions at WCROC, SWROC, and SLRF respectively. Temperatures between January and August of 2019 averaged 4°C cooler at SWROC and SLRF and 5°C cooler in WCROC. In 2020, biomass was lower by an average of 12% and 50% at SWROC and SLRF respectively but response to N was similar in both 2019 and 2020.

At SLRF, N rate, year and the strategy × year and N rate × year interactions significantly affected canopy cover at anthesis. In 2019 at SWROC, canopy cover at anthesis was significantly different among N rate treatments with higher ground cover at 135 kg N ha^{-1}. Canopy cover at SWROC and WCROC showed a quadratic curve response to N rate in all years of measurements. At WCROC in 2019, and regardless of the fertilization strategy, canopy cover at anthesis was higher at 100 kg N ha^{-1}, but differences were not significant among N fertilized treatments, averaging 73% canopy cover (Table 2.6). Canopy cover at SLRF showed a quadratic response to N rate in 2019;

in 2020, however, no clear pattern was found. In 2019, canopy cover from Strategy 1 was highest at 100 kg ha^{-1}; yet, differences among N fertilized treatments were not significant, averaging 18.5% canopy cover. Canopy cover at anthesis was higher in 2019 than in 2020, likely due to better growth in 2019 compared to 2020 (Figure 2.5). Worth noting is that canopy cover reported here, which were obtained mid-May to mid-June, represent the time when a relay crop could be planted (Berti et al., 2015; Liu, Wells, & Garcia y Garcia 2020; Ott et al., 2019). Adequate PAR levels under winter camelina during the relay phase is important for the timely growth of the relayed crop, suggesting that management practices like high N rates could be counterproductive as may promote extra growth and block PAR, delaying the growth of the relayed crop.

In fact, the ratio of lower to upper PAR (Tau), was significantly affected by N rate at SWROC; therefore, the pooled average from 2019 and 2020 results was used for analysis. The lowest Tau was observed at 135 kg N ha^{-1} rate, which averaged roughly one-fift light transmission to below canopy. By contrast, the highest Tau was observed in the control treatment (0 kg N ha^{-1}), averaging roughly half light transmission to below canopy. At WCROC, Tau was not affected by year, N rate, or fertilization strategy. At SLRF, Tau was only measured in 2020 and was affected by fertilizer N rates only, Tau was lowest (0.25)in the 100 kg N ha^{-1} rate and highest (0.60) in the highest control treatment (0 kg N ha^{-1}) (Figure 2.6).

2.3.3 Winter Camelina Grain Yield Response to N

Grain yield was affected by year and N rate. The response of grain yield to N was quadratic in each location and year. At SWROC, N rates did not affect grain yield above the 67 kg N ha^{-1} in 2019 and 100 kg N ha^{-1} in 2020. At WCROC, rates were not significantly different beyond the 33 kg N ha^{-1} in 2019. At SLRF, grain yield was highest at 67 kg N ha^{-1} in 2019 while grain yield in 2020 was not significantly different beyond 33 kg N ha^{-1}. At SLRF, grain yield was highest at 67 kg N ha^{-1} in 2019 while no significant differences were observed beyond 33 kg N ha^{-1}in 2020. Yield at SWROC and SLRF averaged 26% and 56% less in 2020 than 2019 respectively. Yield at SLRF for both years was lower than SWROC and WCROC (Figure 2.2), mainly due to harvest losses.. Average yields at SWROC in 2019 for the 67 to 135 kg N ha^{-1} were, higher than the 1900 and 1932 kg ha^{-1} reported by Berti et al. (2015), which were obtained in the region and under similar conditions of weather and management as they also harvested later than usual.

In all three locations, the highest N rates often resulted in high canopy cover and low Tau, but this was not translated into highest grain yield. These results suggest that winter camelina allocated more N to vegetative mass rather than seed mass. This has also been reported in *Arabidopsis thaliana* (L.) Heynh, a relative of camelina (Yan et al., 2019; Zhang, Niu, Liu, Jia, & Du, 2014). Zhang et al., (2014), reported that some forbs delay flowering in response to N, which could be caused by a delay in changing from vegetative to reproductive phases of development, depending on species. Yan et al., 2019, reported that increases in N relative to P generally increased leaf biomass allocation in *Arabidopsis* and decreased fruit biomass allocation, but had no effect on flowering date.

Nitrogen rate affected HI in two of three locations, but significant differences were only observed in two of six instances within Strat 2 at WCROC in 2019 and both fertilization strategies at SLRF in 2019. Harvest index ranged from 0.17 to 0.40 across all locations and years, with the lowest at SWROC and SLRF and the highest at WCROC. Harvest index was significantly different in only two of six instances at the Morris locations; increased HI with increased N rates at WCROC for Strat 2 and decrease of HI with increase N rates in both fertilization strategies at SLRF in 2019 (Figure 2.4). This range and response is similar to results found in spring camelina grown previously at SLRF, where camelina HI did not respond to N rate (Johnson et al., 2019).

2.3.4 Effect of N on Winter Camelina Grain Quality

Oil content was affected by year, N rate, and their interaction at SWROC and SLRF (Table 2.2). Fertilization strategy significantly affect seed oil content at SWROC only. Fixed effects and their interactions had no effect on seed oil content at WCROC. In 2019 at SWROC and SLRF, seed oil content showed a declining tendency with increased fertilizer N after peaking at 33 or 0 kg N ha^{-1} at SWROC and SLRF respectively. At SWROC and SLRF, oil content was lowest at 135 kg N ha^{-1} and 100 kg N ha^{-1}, respectively. The oil difference between the control (0 N) and the highest rate (135 kg N ha^{-1}) treatments was 4.9% at SLRF and 3.2% at SWROC for Strat 1 while differences were minimum for Strat 2. Oil content at SWROC and SLRF did not vary significantly among treatments in 2020. Oil content varied from 34%–40%, 40.5%–42.3%, and 34%–43.5% at SWROC, WCROC, and SLRF respectively. Results from this study are within the range (31.4% to 43.8%) reported from studies conducted in the upper Midwest (Berti et al., 2015; Gesch & Archer, 2013; Gesch et al., 2014). In 2020, oil content was lower than in 2019 by an average of 3.5% at SWROC and 6.6% at SLRF (Figure 2.7); most likely due to waterlogging stress experienced in the spring of 2020. Excess water in spring has been reported to have a likely negatively affect oil content in the region (Gesch & Archer, 2013; Gesch & Cermak, 2011).

Protein content was significantly affected by year at SWROC and SLRF; N rate and the year by N rate interaction significantly affected protein content at the latter. At WCROC, protein content was not affected by year, N rate, or fertilization strategy (Table 2.2). At SWROC, protein content averaged 1.6% higher across treatments in 2019 compared to 2020 (Figure 2.8). In 2019, protein content in grain of N-fertilized treatments was 2.5% higher than that from the non-fertilized treatment. In 2020 at SWROC, protein content was within 0.8% across N rates. At SLRF, grain had a higher protein content in 2020 compared to 2019, averaging 20% with no response to N rate. In 2019, protein content increased from 12 to 17.3% across N rates, with the highest N rate (135 kg N ha^{-1}) producing the highest protein content. The range of 12 to 20.1% for protein content found in this study are lower than the range of 23.0 to 27.9% typically reported for winter camelina cultivar Joelle (Gesch et al., 2014, 2018; Walia et al., 2018). Low protein content in soybean in central Europe has been found when excessive precipitation occurs during the seed filling stage (Vollmann et al., 2000). High yield and low protein content was also found in rainfed wheat under Mediterranean conditions during growing seasons with excessive rainfall, which is reported to be due to N dilution in plant tissue, which could explain the protein content in 2019 in the current study (López-Bellido et al., 1998).

Another reason results from this research differ from previous research might be the method of extraction. We used NMR while most studies use 6.25 as a constant to convert N in grain to protein; the latter is a standard method in use since the 19th century based on the assumption that the N content of proteins is 16% (Mariotti, Tomé, & Mirand, 2008). However, different proteins have been found to have different N content due to the amino acids present (Mariotti, Tomé, & Mirand, 2008; Jones, 1941).

2.3.5 The Effect of N Rate on Winter Camelina Yield Components and Seed:Shell Ratio

Yield components were measured in 2019 and 2020 at SWROC and SLRF; complete results were obtained for the former but incomplete for the latter (only 1000-seed weight in 2019). Due to crop failure, no data was obtained at WCROC in 2020. Additionally for WCROC, silicles per plant are not reported due to unreliable data from this location. Branches per plant were affected by N rate at SWROC and SLRF in 2020. Silicles per plant were affected by year, fertilization strategy, and the year × fertilization strategy, year × N rate, and year × fertilization strategy × N rate at SWROC interactions. At SLRF, silicles per plant was only affected by N rate. Seeds per silicle was affected by year and N rate, only in SWROC. Seed weight was affected by year and N rate at SWROC and SLRF and at SWROC the year × N rate interaction was also significant. At WCROC, seed weight was significantly affected by N rate.

The average 1000-seed weight was lower by 27 % in 2019 in SWROC compared to the average of other locations and years which averaged roughly 0.94 g. Seeds per silicle averaged highest in SWROC in both years. Fertilization Strat 1 increased yield components in all cases, except for silicles per plant and seeds per silicle at 100–135 kg N ha^{-1} in 2020 at SWROC. Of note, silicles per plant at SWROC were higher in 2019 than 2020, which could possibly also be due to "poor" winter camelina growth in 2020 due to excessive precipitation in the spring. These numbers varied even further from those found at SLRF in 2020, with similar poor growing conditions present there. Yield component values generally increased with increasing N rates, except in 2019 with 1000-seed weight at SWROC in Strat 1 and at SLRF across fertilization strategies, when 1000-seed weight values declined with increasing N application. A similar tendency for declining 1000-seed weight values has been observed across spring camelina yield components with increasing N rates in a study conducted in Lithuania (Končius & Karčauskiene, 2010).

Strong positive association ($r = 0.77$–0.80; $p < 0.001$) was found between grain yield and silicles per plant at SWROC and WCROC. This was most significant in Strat 1 in 2019 at WCROC and SWROC and 2020 Strat 2 at SWROC. These results support the findings on spring camelina cultivars from a two-year fertilization study in Lithuania reporting that branches per plant and number of seeds per silicle increased with increasing N rate (Končius & Karčauskiene, 2010). Results from a two-year study on spring camelina genotypes and N conducted in Nova Scotia, Canada show increase in number of branches and silicles per plant with increased N (Jiang & Caldwell, 2016). In the current study, a strong and highly significant negative correlation (r = -0.72; $p <$ 0.001) was found between 1000-seed weight and yield with Strat 1 in 2019 at SWROC, while no association was found at SLRF in 2019. Significant positive correlations (r = 0.45; $p < 0.01$) were found between 1000-seed weight and yield in 2020 at SWROC and at the other locations and years. Results from previous research report no association between seed weight and yield (Agegnehu & Honermeier, 1997; Jiang & Caldwell, 2016; Ku Končius & Karčauskiene, 2010). Končius & Karčauskiene (2010) for example, reports around 6.7% significant increase in 1000-seed weight as N rate increased from 0 to 120 kg N ha^{-1} in one of two experimental years; opposite significant results were found in year two. In the current study, seeds per silicle (r = 0.54; $p < 0.001$) and branches per plant (r = 0.73; $p < 0.001$) showed strong correlation with grain yield in 2019 and 2020, respectively at SWROC. These results suggest that the increase in yield component values may result in significant associated to yield, opening the opportunity for specific research aiming at identifying traits for breeding efforts to improve the productivity potential of winter camelina.

Seed:shell ratio was affected by N rate at all locations and by the year × N rate interaction at SWROC. Seed:shell ratio was lowest at SLRF, mainly due to high 1000-seed weight.

2.4 Tables and Figures

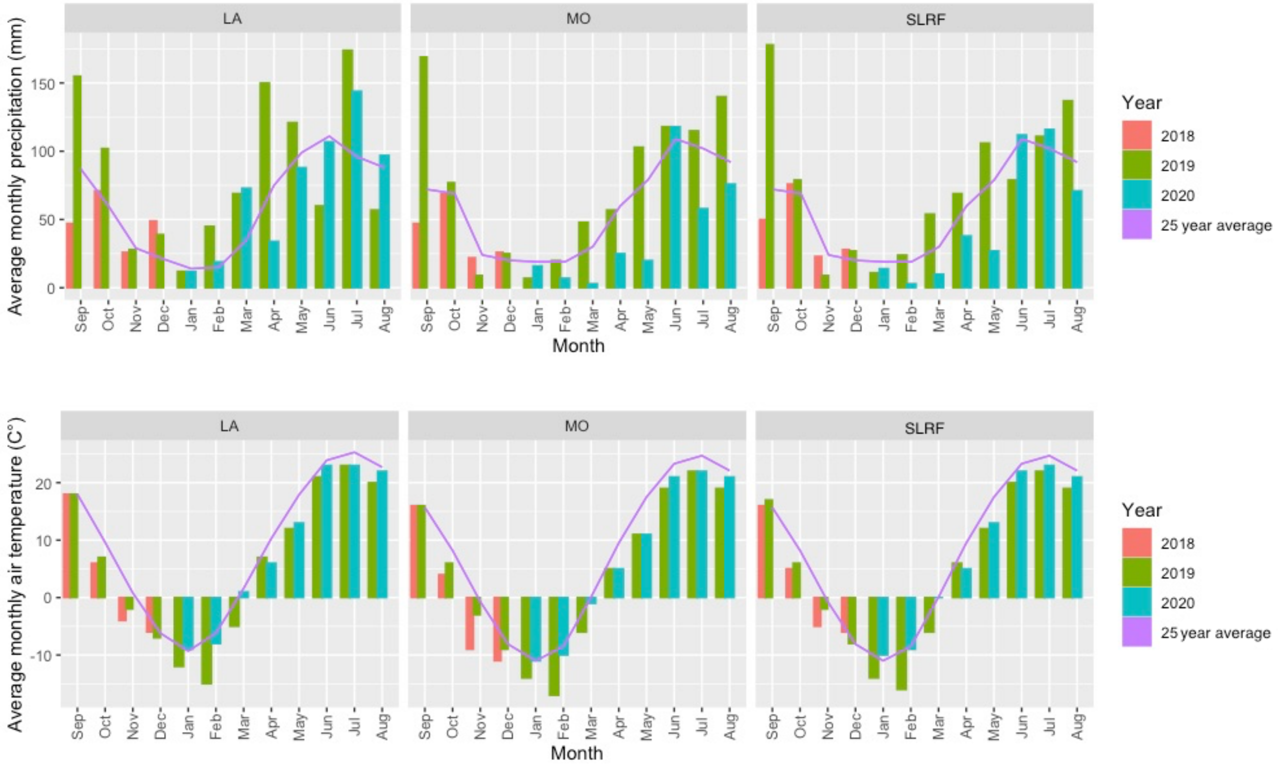

Figure 2.1 Average monthly precipitation and temperature as compared to long-term average (1994 to 2019) at the Southwest Research and Outreach Center near Lamberton (SWROC), West Central Research and Outreach Center in Morris (WCROC), and the USDA-ARS Swan Lake Research Farm near Morris (SLRF).

Table 2.1 Deviation of monthly precipitation (mm) and average air temperature (°C) of experimental years from the long-term average (LTA, 1994 to 2019) conditions at the Southwest Research and Outreach Center near Lamberton (SWROC), West Central Research and Outreach Center in Morris (WCROC), and the USDA-ARS Swan Lake Research Farm near Morris (SLRF).

Month	SWROC near Lamberton				Morris WCROC Morris			SLRF Morris			
	LTA	Deviation from LTA			LTA	Deviation from LTA		LTA	Deviation from LTA		
		2018	2019	2020		2018	2019		2018	2019	2020
Monthly precipitation (mm)											
January	14		-2	-2	19		-12	19		-8	-5
February	15		+30	+4	19		+1	19		+5	-16
March	35		+34	+38	30		+18	30		+24	-20
April	75		+75	-41	60		-3	60		+9	-22
May	99		+22	-11	79		+24	79		+27	-52
June	111		-51	-4	109		+9	109		-30	+3
July	96		+78	+78	102		+13	102		+9	+14
August	88		-31	+9	92		+48	92		+45	-21
September	87	-40	+68		72	-25	+97	72	-22	+106	
October	60	-11	+42		69	0	+8	69	+7	+10	
November	29	+28	+1		24	-2	-15	24	-1	-15	
December	21	+46	+18		20	+6	+5	20	+8	+7	
Average air temperature (°C)											
January	-9		-3	0	-11		-3	-11		-3	-1
February	-6		-9	-2	-9		-8	-9		-7	0
March	2		-7	-1	0		-6	0		-6	0
April	10		-3	-4	10		-5	10		-4	-5
May	18		-6	-5	18		-7	18		-6	-5
June	24		-3	-1	23		-4	23		-3	-1
July	25		-2	-2	25		-3	25		-3	-2
August	23		-3	-2	22		-3	22		-3	-1
September	18	0	0		16	0	0	16	0	-1	
October	10	-4	-3		8	-4	-2	8	-3	-2	
November	1	-5	-3		-1	-8	-2	-1	-4	-1	
December	-6	0	-1		-8	-3	-1	-8	-2	0	

Table 2.2 Effect of fixed effects on winter camelina grain yield (Yg), total biomass weight (TB), harvest index (HI), Tau (Tau), canopy cover (CC), oil content (Oil), and protein content (Protein) for conditions at three locations during the 2018-2019 and 2019-2020 growing seasons.

Location	Source of variation	Yg	TB	HI	CC	Tau[†]	Oil	Protein
SWROC	Year (Y)	***[§]	***	***	ns	ns	***	***
	Fertilization strategy (S)	ns	ns	ns	ns	*	**	ns
	Nitrogen rate (R)	***	***	ns	***	ns	***	ns
	Y x S	ns	ns	ns	ns	ns	**	ns
	Y x R	ns	ns	ns	ns	**	***	ns
	S x R	ns	ns	ns	ns	ns	ns	ns
	Y x S x R	ns	ns	ns	ns	ns	ns	ns
WCROC	Year (Y)	-	-	-	-	-	-	-
	Fertilization strategy (S)	ns	ns	ns	ns	ns	ns	ns
	Nitrogen rate (R)	***	***	**	***	ns	ns	ns
	Y x S	-	-	-		-	-	-
	Y x R	-	-	-		-	-	-
	S x R	ns	ns	*	ns	ns	ns	ns
	Y x S x R	-	-	-	-	-	-	-
SLRF	Year (Y)	***	***	ns	***	-	***	***
	Fertilization strategy (S)	ns	ns	ns	ns	ns	ns	ns
	Nitrogen rate (R)	**	**	ns	***	***	***	***
	Y x S	ns	ns	ns	**	-	ns	ns
	Y x R	ns	*	**	*	-	***	**
	S x R	ns	**	ns	ns	ns	ns	ns
	Y x S x R	ns	ns	ns	ns	-	ns	ns

[†] Tau = ratio of below to above canopy PAR

§Variables with ***, **, * are significant at the 0.001, 0.01, and 0.05 levels. ns denotes not significant

Table 2.3 Significance of F value for fixed effects sources of variation for yield components [branches per plant (BP), silicles per plant (SP), seeds per silicle (SS), 1000-seed weight (SW)] and seed:shell ratio (SSR) for conditions at three location during 2018-2019 to 2019-2020 growing seasons.

Location	Source of variation	BP	SP	SS	SW	SSR
SWROC	Year (Y)	***§	***	***	ns	ns
	Fertilization strategy (S)	ns	ns	ns	ns	*
	Nitrogen rate (R)	***	***	ns	***	ns
	Y x S	ns	ns	ns	ns	ns
	Y x R	ns	ns	ns	ns	**
	S x R	ns	ns	ns	ns	ns
	Y x S x R	ns	ns	ns	ns	ns
WCROC	Year (Y)	-	-	-	-	-
	Fertilization strategy (S)	ns	-	ns	ns	ns
	Nitrogen rate (R)	***	-	**	***	ns
	Y x S	-	-	-		-
	Y x R	-	-	-		-
	S x R	ns	-	*	ns	ns
	Y x S x R	-	-	-	-	-
SLRF	Year (Y)	***	***	ns	***	-
	Fertilization strategy (S)	ns	ns	ns	ns	ns
	Nitrogen rate (R)	**	**	ns	***	***
	Y x S	ns	ns	ns	**	-
	Y x R	ns	*	**	*	-
	S x R	ns	**	ns	ns	ns
	Y x S x R	ns	ns	ns	ns	-

§ Variables with ***, **, * are significant at the 0.001, 0.01, and 0.05 levels. ns denotes not significant and - denotes not available

Table 2.4 Effect of fertilizer N on yield components (branches per plant (BP); silicles per plant (SP); seeds per silicles (SS); 1000-seed weight (SW); and seed:shell ratio (SSR) of winter camelina grown by location during two growing seasons.

Growing season	N rate (kg ha^{-1})	BP		SP		SS		SW		SSR	
		S1†	S2	S1	S2	S1	S2	S1	S2	S1	S2
SWROC											
2018-2019	0	-	-	247c[§]	247a	12b		0.70a	0.70b	1.60a	
	33	-	-	278b	239b	14b		0.69a	0.78a	1.48c	
	67	-	-	317a	250ab	14b		0.64b	0.77a	1.54b	
	100	-	-	299ab	244ab	14b		0.60c	0.67b	1.62a	
	135	-	-	277b	276ab	14b		0.61c	0.65c	1.55b	
	r[‡]	-	-	0.80***[±]	0.24	0.54***		-0.72***	-0.09	0.16	
2019-2020	0	2.0b		85d	85d	12ab		0.89a	0.89a	156bc	
	33	3.6a		91c	117c	11b		0.95a	0.95a	1.49c	
	67	4.5a		149a	146b	14ab		0.96a	0.96a	1.55bc	
	100	5.1a		113b	205a	13ab		0.92a	0.98a	1.69a	
	135	4.4a		132a	204a	12ab		0.91a	0.95a	1.62ab	
	r	0.73***		0.34	0.77***	0.14		0.23	0.45*	0.55***	
WCROC											
2018-2019	0	-	-	-	-	11a		0.91a		1.67b	
	33	-	-	-	-	13a		0.92a		1.85ab	
	67	-	-	-	-	12a		0.92a		1.85ab	
	100	-	-	-	-	12a		1.00a		2.00a	
	135	-	-	-	-	13a		0.96a		1.94ab	
	r	-	-	-	-	0.08		0.26		0.53***	
SLRF											
2018-2019	0	-		-		-		1.04a		-	
	33	-		-		-		1.00ab		-	
	67	-		-		-		0.98bc		-	
	100	-		-		-		0.95cd		-	
	135	-		-		-		0.91d		-	
	r	-		-		-		0.00		-	
2019-2020	0	3c		15b		8a		0.94a		1.11a	
	33	3bc		23a		10a		0.96a		1.44a	
	67	5ab		33a		11a		0.99a		1.43a	
	100	4abc		23a		11a		0.99a		1.50a	
	135	6a		34a		10a		0.66a		1.32a	
	r	0.29		0.29		0.30		0.24		0.39*	

---

† S1 denotes fertilization strategy 1, one application in spring; S2 denotes fertilization strategy 2, split (33%) fall and (67%) spring. Data centered between strategies represents that strategy was not significant

± *** denotes significance at 0.001, ** denotes significance at 0.01, * denotes significance at 0.05, and - denotes not available

‡ r denotes Pearson coefficient of correlation for yield component and yield

§ In a column, within a year, values followed by the same lowercase letter are not significantly different at $P < 0.05$.

Table 2.5 Quadratic ($Y = \beta_1 + \beta_2x + \beta_3x^2$) and linear ($Y = \beta_1 + \beta_2x$) equations for yield components of winter camelina grown at three Minnesota locations during two growing seasons.

Growing Season	Coeff	BP‡		SP		SS		SW		SSR	
		S1 †	S2	S1	S2	S1	S2	S1	S2	S1	S2
					SWROC						
2018-2019	β1			231	248	12.26		0.70	0.71	-	
	β2			338	0.34	0.0553		0.0012	0.0020	-	
	β3			0.022	0.0039	-0.00032		0.0000034	0.000019	-	
	r^2			0.65	0.43	0.42		0.49	0.36	-	
2019-2020	β1	1.99		81.10	80.85	11		-	-	1.52	
	β2	0.058		0.91	1.31	0.045		-	-	0.00047	
	β3	0.00030		0.0041	0.00258	0.00028		-	-	0.0000037	
	r^2	*0.58*		*0.22*	*0.57*	*0.16*		-	-	*0.24*	
					WCROC						
2018-2019	β1			222.11		-		0.90		1.68	
	β2			2.794		-		0.000483		0.0048	
	β3			-0.0133		-		0.00000051		0.000020	
	r^2			0.74		-		0.12		0.20	
					SLRF						
2018-2019	β1							1.04			
	β2							-0.00091			
	β3							-0.0000008			
	r^2							*0.66*			
2019-2020	β1	2.66			15.75			1.039	0.94	1.13	
	β2	0.027			0.235			-0.00091	0.0013	0.00945	
	β3	-0.00006			-0.0009			-0.00000026	-0.0000078	0.00006	
	r^2	0.21			0.16			0.66	0.16	0.16	

Blank cells show that data was unavailable and - denotes no significance. Data centered between strategies represents that strategy was not significant

† S1 denotes fertilization strategy 1, one application in spring; S2 denotes fertilization strategy 2, split (33%) fall and (67%) spring.

‡ BP = branches per plant, SP = silicles per plant, SS = seeds per silicle, SW = 1000-seed weight, and SSR = seed:shell ratio

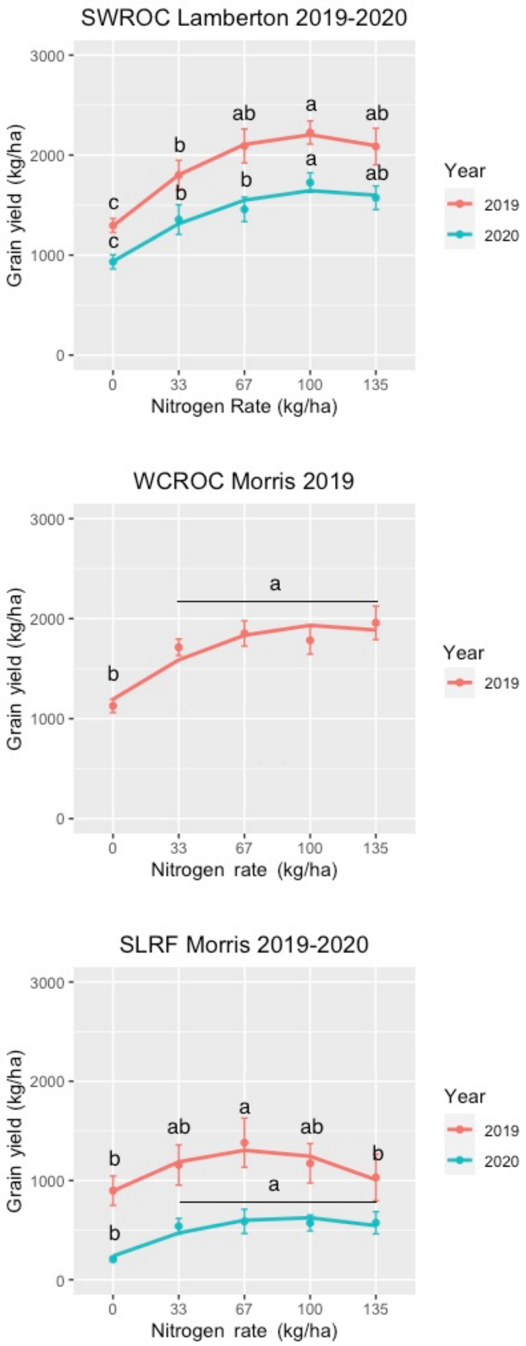

Figure 2.2 Effect of fertilizer N on grain yield (kg/ha) of winter camelina grown at three Minnesota locations during two growing seasons. Error bars represent SE (n=8). Values of N rates with different letters above differ significantly at $P \leq 0.05$.

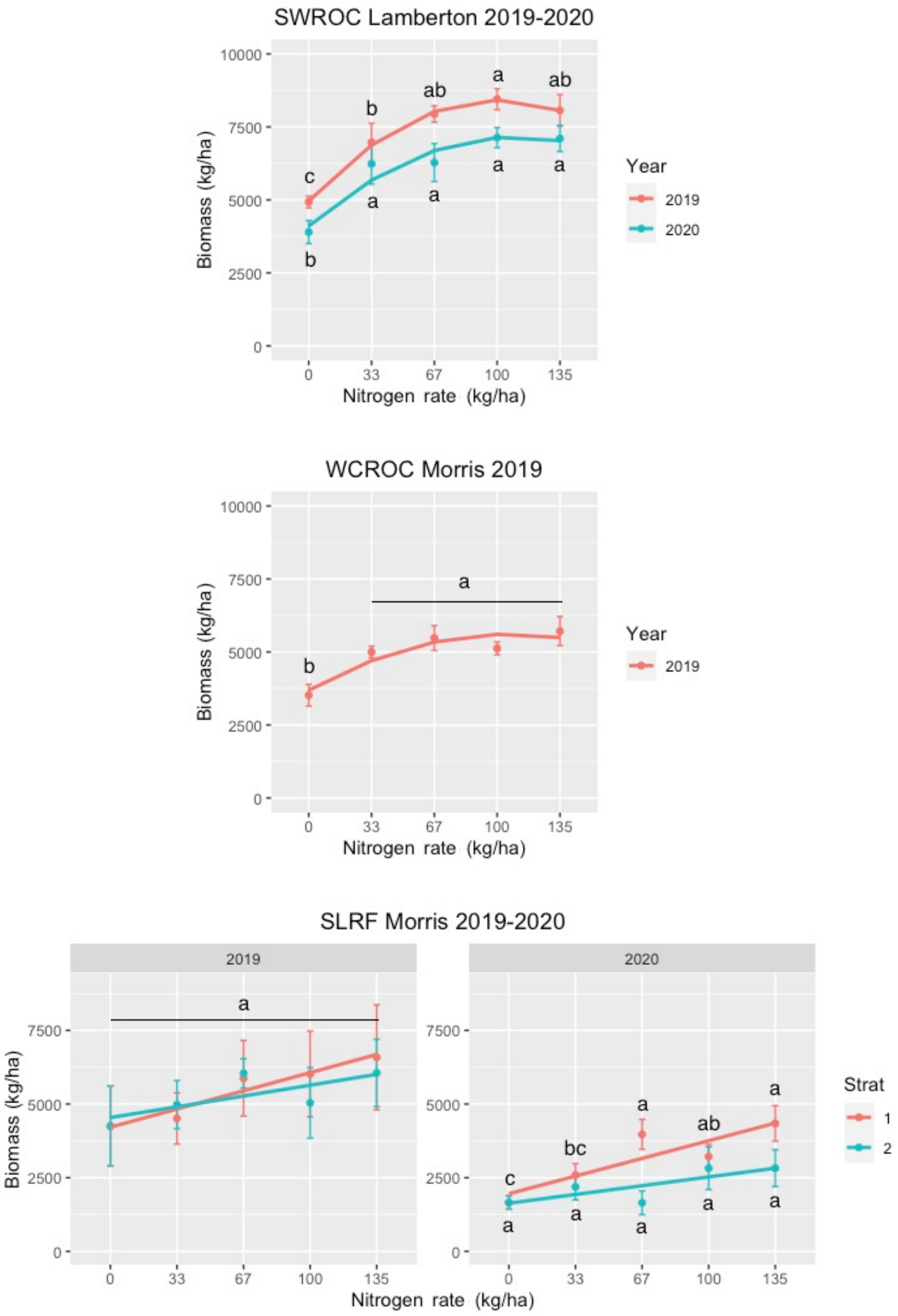

Figure 2.3 Effect of fertilizer N on biomass weight (kg/ha) at maturity of winter camelina grown at three Minnesota locations. At SLRF, strategy was significant so each strategy is shown. Error bars represent SE (n=8). Values of N rates with different letters above differ significantly at $P \leq 0.05$.

Table 2.6 Effect of fertilizer N on winter camelina harvest index (HI), canopy cover percentage (CC), ratio of below to above canopy PAR (Tau), oil content (Oil), and protein content (Protein) at three Minnesota locations during two growing seasons.

Growing season	N rate (kg ha^{-1})	HI		CC		Tau		Oil		Protein	
		S1†	S2	S1	S2	S1	S2	S1	S2	S1	S2
SWROC											
2018-2019	0			66.6c§‡		0.45a‡ (0)		37.9ab	37.9ab		
	33			83.0b				38.6a	40.0a		
	67	0.26		86.9ab		0.36ab (33)		36.9ab	38.5abc	17.5	
	100			90.5ab				35.1ab	37.7bc		
	135			93.2a		0.26bc (67)		34.7b	36.5c		
2019-2020	0			66.6c							
	33			83.0b		0.24c (100)					
	67	0.23		86.9ab				33.9		15.9	
	100			90.5ab		0.21c (135)					
	135			93.2a							
WCROC											
2018-2019	0		0.35b	52.3b							
	33		0.34b	71.0a							
	67	0.33	0.36b	67.8a		0.58		41.3		16.5	
	100		0.35b	79.4a							
	135		0.40a	77.0a							
SLRF											
2018-2019	0	0.24a		37.4b	37.4b	-		43.5a		12.0b	
	33	0.23ab		63.5ab	51.7ab	-		43.1a		13.8b	
	67	0.21ab		74.5a	68.2a	-		42.1a		14.1ab	
	100	0.20ab		79.7a	67.0a	-		39.8b		14.6ab	
	135	0.19b		76.3a	71.9a	-		38.6b		17.2a	
2019-2020	0				12.0c	0.60a					
	33				19.5bc	0.42b					
	67	0.20		18.5	27.7ab	0.36bc		16.5		20.0	
	100				34.8ab	0.27c					
	135				40.8a	0.25c					

† S1 denotes fertilization strategy 1, one application in spring; S2 denotes fertilization strategy 2, split (33%) fall and (67%) spring. Data centered between strategies represents that strategy was not significant. Data with only one entry per N rates or strategy represents that strategy and/or N rate were not significant

§ In a column, within a yield component, within a year, values followed by the same lowercase letter are not significantly different at $P < 0.05$

‡ denotes instances where year was not significant for the variable. Both years show the same data and letters. Numbers in parentheses represent N rates

Table 2.7 Quadratic and linear equations (Linear: $Y = \beta_1 + \beta_2x$; Quadratic: $Y = \beta_1 + \beta_2x + \beta_3x^2$) for winter camelina harvest index (HI), canopy cover percentage (CC), ratio of below to above canopy PAR (Tau), oil content (Oil), and protein content (Protein) by location.

Year	Coeff	HI		CC		Tau		Oil		Protein	
		S1†	S2	S1	S2	S1	S2	S1	S2	S1	S2
SWROC											
2019	β_1	-		68.1‡		0.45‡		38.3	38.3	-	
	β_2	-		0.41		-0.003		0.01	0.03	-	
	β_3	-		-0.002		0.00001		0.0001	0.0004	-	
	r^2	*0.04*		*0.56*		*0.40*		*0.52*	*0.39*	*0.11*	
2020	β_1	-		-		-		-	-	-	
	β_2	-		-		-		-	-	-	
	β_3	-		-		-		-	-	-	
	r^2	-		-		-		-	-	-	
WCROC											
2019	β_1	-	0.35	54.1		-		-		-	
	β_2	-	-0.0005	0.41		-		-		-	
	β_3	-	0.000006	-0.002		-		-		-	
	r^2	-	*0.68*	*0.2*		*-0.03*		-		-	
SLRF											
2019	β_1	0.24		39.7	37.2			43.7		12.4	
	β_2	-0.0006		0.72	0.56			-0.02		0.002	
	β_3	0.000001		-0.003	-0.002			-0.0002		0.0001	
	r^2	*0.27*		*0.62*	*0.53*			0.71		*0.30*	
2020	β_1	-		-	11.8	0.59		1.039	-	-	
	β_2	-		-	0.26	-0.005		-0.00091	-	-	
	β_3	-		-	-0.0003	0.00002		-0.00000026	-	-	
	r^2	-		-	*0.63*	*0.71*		*0.66*	-	-	

Blank cells show that data was unavailable and - denotes no significance. Data centered between strategies represents that strategy was not significant

† S1 denotes fertilization strategy 1, one application in spring; S2 denotes fertilization strategy 2, split (33%) fall and (67%) spring

‡ Denotes instances where year was not significant for the variable. Coefficients represent a model with both years combined

3. Chapter 3 – Nitrogen use efficiency in winter camelina in relation to N availability

Summary

Maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] in the upper Midwest are productive, but decades of these monocultures with winter fallow and late spring planting are in part responsible for loss in agroecological functioning as well as nitrogen (N) pollution in the agricultural communities and downstream. Winter camelina [*Camelina sativa* (L.) Crantz] is a third crop that could grow during this fallow period, but the environmental impacts of its N requirements are not well known. A study was conducted at three locations in Minnesota to determine the response of winter camelina do N. Five N rates (0, 33, 67, 100, 135 kg N ha^{-1}) and two application timings (spring, and fall-spring split) were used to assess the N use efficiency (NUE) and residual N in winter camelina grown for grain yield from fall 2018 to fall 2020.

Results showed higher NUE for fall-spring split application compared to spring only application. The agronomic efficiency (AE), internal efficiency (IE), and nitrogen recovery efficiency (NRE) tended to decrease with increasing N rates; AE generally decreased beyond 67 kg N ha^{-1} in most instances. Total N uptake ranged from 34 to 176 kg ha-1 across N rates. Residual soil N increased with increasing N rates, especially at the 15 cm depth. Based on declining NUE and increasing residual soil N with increasing N rates, an N rate between 33 to 67 kg N ha^{-1} could balance an efficient use of fertilizer with less environmental risk of higher N rates.

3.1 Introduction

Upper Midwestern agriculture is dominated by maize and soybean. In 2019, Minnesota alone planted crops on over 50% of its 20 million total hectares, roughly 60% of which was planted in maize or soybean, producing harvest values over 7 billion USD (USDA-NASS 2019). This productive system largely built on two crops has resulted in diversity loss with negative agroecological effects such as increased nitrate nitrogen (N) leaching, greenhouse gas emissions, and soil erosion (Finney et al., 2016; Syswerda & Robertson, 2014). Part of the negative environmental impacts are due to the fallow period from early fall to late spring. Nitrate-N leaching from the U.S. Corn Belt during the spring has for decades contributed to the formation of the hypoxic zone in the Gulf of Mexico, which in the past five years averaged almost 14,000 km^2 (EPA 2020). This lost nitrate comes at a significant cost to farmers in the form of a lost input, estimated at 500 million USD (Basso et al., 2019) and to their communities in the form of nitrate treatment of drinking water, costing an estimated 141,000 to 1.1 million USD, depending on U.S. region and other variables (Schechinger and Cox 2018). In addition to the environmental and economic costs, the fallow period results in a lost opportunity for growing additional crops to produce additional biomass or grain, which could provide some economic and environmental benefit for farmers and society as a whole.

Cover crops are an old technology that fell out of favor with the advent of chemical fertilizers but are getting renewed attention from farmers and researchers due to the environmental effects of fertilizer. Cover crops can reduce environmental harm by reducing nitrate leaching and soil erosion, and can contribute to production benefits like soil health and weed suppression (Finney et al., 2016; Peterson et al., 2019; Strock et al., 2004). For instance, Strock et al. (2004) reported that a cereal rye cover crop following maize or soybean reduced NO_3-N by 13% over a three-year period compared to no cover crop. Finney et al. (2016) found in a two-year study of 18 cover crop combinations that high C/N ratios correlated to a reduction of NO_3-N leaching but were also found to limit inorganic N availability and reduce yield in the following maize crop. Farmer concerns over cost and potential complications suggest an explanation why cover crop adoption is growing slowly, averaging around 4% in the upper Midwest as of 2017 (CTIC & SARE, 2017; CTIC et al., 2020; Zulauf & Brown, 2019).

Double cropping, a form of sustainable temporal intensification where two crops are harvested in a single season, is a method practiced that may help farmers adopt more diverse and intense rotations that provide ecological benefit, while also providing an economic return (Berti et al., 2015; Gesch & Archer, 2013; Gesch et al., 2014). Summer annuals typically grown in the upper Midwest leave a long fallow period and a short season winter annual can fit into this system. Crops that can be used to produce biofuel are of growing interest to the U.S. commercial aviation industry, Navy, and Airforce; winter annual oilseeds could meet some of that demand (Berti et al., 2015; Sindelar et al., 2017).

Winter camelina is an annual oilseed crop with high grain oil and protein content that has potential for incorporation into maize and soybean in the upper Midwest, due to its short relative maturity and winter hardiness (Berti et al., 2015; Gesch & Archer, 2013). Camelina can be used to produce biodiesel similar to the more common and more expensive soybean and can fit into current production systems with existing machinery (Moser & Vaughn, 2010).

Winter camelina has also demonstrated many positive environmental effects associated with cover crops including reduced nitrate nitrogen (NO_3^--N) loss (Strock et al., 2004; Weyers et al., 2019), increased weed suppression (Hoerning et al., 2020; Saucke & Ackermann, 2006), and pollinator benefits (Eberle et al., 2015; M. D. Thom et al., 2018; Matthew D. Thom et al., 2016).

Research on weed suppression with camelina has been conducted primarily in organic systems. Camelina has been found to reduce weed cover in peas and spring barley at 63% and 56% respectively (Raslavicius & Povilaitis, 2013; Saucke & Ackermann, 2006). In Minnesota, N uptake of winter camelina matched winter rye in in spring, accumulating between 28 to 49 kg N ha^{-1}, and reducing N in the soil solution by half compared to tilled or no-till winter fallow soybean (Johnson et al., 2017; Weyers et al., 2019). In a two-year Minnesota study on sowing date and tillage effects, fertilized winter camelina yield (744 kg ha^{-1}) was around 40% higher than the non-fertilized treatment (Gesch & Cermak, 2011), evidencing the positive effect of N on yield production. Other studies have also shown the positive effect of N fertilization on winter camelina (Johnson et al., 2019; Wysocki et al., 2013).

Nitrogen is one of the most important macronutrients in crop production. Knowledge about crop requirements and application strategies is expected to result in better growth and higher yield. The nitrogen use efficiency (NUE) indices provide various ways to understand N uptake by the crop to produce a desired output. This helps not only with production, but also with minimizing the environmental consequences of agronomic decisions (Cassman, Dobermann, Walters, & Yang, 2003; Liang & MacKenzie, 1994). For instance, in a spring camelina fertilization study in Minnesota, the agronomic efficiency of N is reported to have decreased from 4.28 to 1.29 kg kg^{-1} as fertilizer N increased from 34 kg ha^{-1} to 202 kg ha^{-1} and showed that N beyond moderate fertilization rates had no effect in in either shoot or grain N (Johnson et al., 2019). A study with non-fertilized winter camelina double-cropped with maize demonstrates the ability of winter camelina to take up residual soil N, with total N uptake ranging from 36 to 57 kg ha^{-1} (Liu, Wells, & Garcia y Garcia, 2020). Such studies demonstrate the necessity to balance the need of winter camelina for fertilizer N and its potential environmental impacts.

Much of the research on camelina has focused on the spring biotype, which provides a yield later in the season therefore making it difficult, if not impossible to fit into the dominant cropping systems in the upper Midwest. This research was designed to determine how N fertilization affects winter camelina and residual soil N. The hypothesis was that N fertilization affects the NUE of winter camelina and the residual soil N. The objectives of this study were to determine the (i) NUE of winter camelina in response to N availability, and (ii) soil residual N from N-fertilized winter camelina.

3.2 Materials and Methods

3.2.1 Experimental Sites

Field experiments were conducted at three Minnesota locations from fall 2018 to fall 2020. Locations included the University of Minnesota Southwest Research and

Outreach Center (SWROC; 44°14’02.20”N 95°18’6.87”W) near Lamberton, MN, University of Minnesota West Central Research and Outreach Center (WCROC; 45°35’37.17”N 95°52’42.63”W) near Morris, MN, and the Swan Lake Research Farm (SLRF; 45°36’4.30”N 95°54’11.09”W) of the USDA-ARS near Morris, MN. Dominant soils were characterized as Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls) at SWROC, Nutley Flom clay loam (fine, smectitic, frigid chromic Hapluclerts and fine-loamy, mixed, superactive, frigid Typic Encloaquolls) at WCROC, and Barnes clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) at SLRF (Soil Survey Staff, 2020).

The region has a continental climate with long, cold winters with short, wet springs and summers. The 25-year long term average (LTA) temperatures and rainfall are 7°C and 737 mm at SWROC and 5.8°C and 670 mm at WCROC and SLRF. The USDA climate hardiness zones are 4b at SWROC and 4a at WCROC and SLRF (USDA-ARS). Long-term air temperature and precipitation data was obtained from the National Oceanic and Atmospheric Administration (www.ncdc.noaa.gov/).

3.2.2 Experimental Design

This experiment was a completely randomized design in a 2 × 5 factorial. Factor A consisted of two N-fertilization strategies: a spring application (Strat 1) and a fall-spring split application (Strat 2, 33% fall and 67% spring). Factor B consisted of five N fertilizer rates (0, 33, 67, 100, 135 kg ha^{-1}); plots were 3 m × 5 m and there were four replications.

3.2.3 Agronomic Management

Agronomic management was described in depth previously in section 2.2.3. At each of the three sites, small grains were previously grown in the field before winter camelina to reduce residual soil N. Herbicides trifluralin N-dipropyl-4-(trifluromerhyl) aniline and glyphosate [N-(phosphonomehtyl) glycine] at 2.0 kg a.e. ha^{-1} were used at SWROC and SLRF respectively, with no preplant herbicide used at WCROC, due to planting soon after harvest and discing of previous crop. Winter camelina cultivar, Joelle (USDA), was drill-seeded at a rate of 9 kg ha^{-1} at SWROC and WCROC and 8 kg ha^{-1} at SLRF. Rows were planted 19 cm apart and camelina was planted in mid-September to early October in SWROC and WCROC and early September to mid-September at SLRF.

The fall portion of the fall-spring split N application (Strat 2) was applied in corresponding plots at preplanting and were incorporated. The spring N application was applied in all plots at the inflorescence emergence (BBCH50) stage. Fertilizer used was N_{trt}-30-30, (where N_{trt} represents the N treatment amount) as urea (NH_2-CO-NH_2) triple superphosphate (P_2O_5) and Muriate of potash (K_2O). Camelina was harvested when > 90% of silicles were brown and dry. Camelina was harvested when > 90% of silicles were brown and dry.

3.2.4 Data Collection

Soil samples were collected before winter camelina planting in the fall and after harvest in July at four points per plot in a diagonal pattern at two depths: 15 cm and 30 cm. The sub-samples from each depth were mixed to make a composite sample and allowed to air dry before being ground using a Dynacrush soil crusher to pass through a 2 mm sieve (Custom Laboratory Equipment Inc., Holden, MO, USA). Samples were analyzed for NO_3^--N, pH, organic matter (OM), Bray-1 phosphorous (Bray P), cation exchange capacity (CEC), K^+, Ca^+, and Mg^{+3}.

Nitrogen use efficiency indices included nitrogen recovery efficiency (NRE), agronomic efficiency (AE), and internal efficiency (IE); additionally, the nitrogen exported in grain (NE) and total N uptake (NU) was calculated. Nitrogen uptake is the total N uptake of the biomass at physiological maturity (eq. 1, kg ha^{-1}), NUE is the ability of mature above ground biomass to capture N compared to the non-fertilized control (eq. 2, kg kg^{-1}), AE is the increase in seed yield per N rate compared to the non-fertilized control per unit N applied (eq. 3, kg kg^{-1}), IE is a measure of how efficiently the plant converts biomass N into grain yield (eq. 4, kg kg^{-1}), and NE is the amount of N removed from the system in the grain harvested (eq. 5, kg ha^{-1}) (Moll, Kamprath, & Jackson, 1982; Woli et al., 2016).

$$\text{NU} = BM * BM_{N\%} \tag{1}$$

$$\text{NRE} = \frac{BM_{Nuptake} - BM_{N0}}{N_{applied}} \tag{2}$$

$$\text{AE} = \frac{GY_N - GY_0}{N_{applied}} \tag{3}$$

$$\text{IE} = \frac{GY_N}{BM_{Nuptake}} \tag{4}$$

$$\text{NE} = GY * GY_{N\%} \tag{5}$$

where BM = total biomass (kg ha^{-1}) and $BM_{N\%}$ = N content (%) of biomass, $BM_{Nuptake}$ = N uptake in biomass of fertilized treatment (kg ha^{-1}), BM_{NO} = N uptake in biomass of non-fertilized treatment (kg ha^{-1}), $N_{applied}$ = N rate (kg ha^{-1}), GY_N = grain yield of fertilized treatment (kg ha^{-1}), GY_0 = grain yield of non-fertilized treatment (kg ha^{-1}), and $GY_{N\%}$ = N content (%) of grain yield.

3.2.5 Statistical Analysis

All data were analyzed using R statistical software (version 4.0.3; R Core Team 2020). Nitrogen exported, NU, AE, NRE, IE, and residual soil N were analyzed using the linear mixed effects model ANOVA to determine significant effects and interactions. Year, fertilization strategy, and N rate were considered fixed effects. Regression analysis was performed to find the functional relationship between a given variable and N rate using the least squares method. Models were used that minimized the sum of the squared errors and showed the highest r^2 values. Locations were analyzed independently due to the differing varieties of data per location. Assumptions of normality and constant variance of model residuals were visually assessed. If a combined analysis showed significant interactions, response variables were separated for ANOVA. Post hoc analysis was conducted using Tukey's Honest Significant Difference (HSD) with the '*agricolae*' package at $P \leq 0.05$ to determine means separation for variables by treatment.

3.3 Results

3.3.1 Weather Conditions

Weather conditions were described in depth previously in 2.3.1. At all three locations, monthly average air temperatures were below the LTA by 2 to 3°C in 2018. The 2019 year was consistently colder than the LTA. In 2020, the temperatures were closer to the LTA than previous years, but during the inflorescence emergence and flowering stages for camelina (April and May) air temperatures were 4 to 7°C colder. Precipitation was higher than the LTA at all locations in 2019 and at SWROC in 2020, with a drier-than-average year in 2020 in both Morris locations. At SWROC in 2019, 70 mm more precipitation fell than the LTA in April and July, with 100 mm more in the fall after planting, which potentially caused spring waterlogging stress of the 2020 crop, possibly contributing to lower grain and biomass yields (Gesch & Cermak, 2011). The sites at WCROC and SLRF experienced similar high rainfall in 2019, receiving 190 mm above the LTA. Precipitation in 2020 at SWROC in 2020 was 41 mm higher than the LTA from January to July. Precipitation in 2020 at the Morris locations was lower than the LTA, with over 100 mm less from late-winter to late-spring (Table 3.2).

3.3.2 Nitrogen Use Efficiency of Winter Camelina

Agronomic efficiency was affected by year only at SWROC and N rate at all locations. Internal efficiency was affected by N rate and year × N rate interaction, except in fertilization strategy 2 in 2019 at SLRF. The NRE, IE, and total N uptake were obtained at SLRF in both years and SWROC in 2020 only, due to inconsistencies with results from SWROC and WCROC in 2019, as a result of intense precipitation following fertilization. Nitrogen recovery efficiency was significantly affected by N rate × strategy interaction in SWROC 2020 only. At SLRF, NRE increased in 2019 with increasing N rates, but decreased in 2020, with no significant differences either year. Significant differences in NRE were observed in 2020 among Strat 1 treatments at SWROC, where NRE decreased sharply beyond the 33 kg ha^{-1} rate, with significant differences found only in Strat 1 between the 33 and 67 kg N ha^{-1} rates (Table 3.4). The NRE ranged from

6% to 78%, with higher values at SWROC in 2020 compared to SLRF in 2019 and 2020. The low NRE value of 6% was due to little difference in biomass weight and N content between the 33 kg ha^{-1} rate and the non-fertilized (control) treatments at SLRF in 2019. These results are similar to those reported by Mahli et al. (2014) for spring camelina grown in Saskatchewan and Alberta, Canada, which averaged 35% NRE across N rates and declined 21% between the lowest and highest N rates (25 to 200 kg N ha^{-1}).

Results from this study indicate that winter camelina was effective at capturing N, but this did not necessarily translate into grain production. The AE declined at N rates beyond 67 kg N ha^{-1}, with significant differences found in all instances; this trend was better explained by a quadratic response. Similarly, the quadratic regression model best described the IE response to N rate at SWROC and SLRF, with significant differences among N rates only at SLRF in Strat 2 in 2020 (Table 3.4). Nitrogen use efficiency indices typically declined with N rates above 67 kg N ha^{-1} (Table 3.4). For instance, AE decreased as much as 60% from 33 to 67 kg N ha^{-1} and from 67 to 100 kg N ha^{-1}; IE showed no significant differences across N rates, except for Strat 2 in 2020 at SLRF; and NRE decreased as much as 66% between 33 and 67 kg N ha^{-1} and 30% from 67 to 100 kg N ha^{-1} at SLRF, although at SWROC, NRE increased 30% from the 67 to 100 kg ha^{-1} rate in both Strat 1 and 2. These results evidence that winter camelina uses excess N for biomass rather than grain production (section 2.3.3, Figure 2.2, Figure 2.3). The AE from this study vary from 1.0 to 19.0 while most results for spring camelina range from 1.9 to 10.8 (Bronson et al., 2019; Johnson et al., 2019). In this study, AE increased with increased N rate up to 67 kg ha^{-1} compared to the control. The IE of winter camelina from this study ranged from as little as 6.2 to as high as 18.8 and were close to the 12.4 to 20.9 range reported by Bronson et al. (2019). For comparison and context, current and historical values of maize NUE can be found in Figure 3.1, adapted from Mueller, Messina, and Vyn (2019).

3.3.3 Nitrogen in Biomass and Camelina Grain

Little information currently exists on winter camelina response to N, and most studies have been in spring camelina. In the current study, field experiments on winter camelina response to N were established at SWROC, SLRF, and WCROC. Due to weather conditions, trials at SWROC and WCROC in 2019 produced limited data, and due to COVID-19 the latter site was not considered in year 2020. As a result, two years (2019 and 2020) of data from SLRF and one year (2020) from SWROC are presented. Total N was affected by year and N rate at SLRF and by N rate at SWROC. Significant differences between N rates were found in both locations in 2020; N uptake was highest at 100 kg N ha^{-1} rate. At SLRF in 2019, total N uptake increased with an increase of fertilizer N, but differences were not significantly different beyond the 33 kg ha^{-1} rate. At all locations and years, a quadratic regression model best described the response of winter camelina to fertilizer N. At SLRF, total N uptake increased 52% across N rates in 2019 and 97% in 2020. At SWROC, total N uptake increased 89% across N rates. Results from SWROC showed higher total N uptake compared to SLRF in either year. In this study, total N uptake across fertilizer N rates varied from 34 to 176 kg ha^{-1}. These results are close to the 55 to 176 kg ha^{-1} N uptake reported by Johnson, Gesch, and Barbour (2019) in spring camelina, but slightly wider than the 10 to 132 kg N ha^{-1} reported by Mahli et al.

(2014) for spring camelina. The lower N uptake in the current study were obtained in 2020 at SLRF while the highest were reported in 2020 at SWROC. At SWROC, total N uptake was higher than applied N in all N rates, suggesting that N mineralization during the season may have supplied additional N to winter camelina, a possibility also reported by Johnson et al. (2019). Similarly, canola, a close relative of camelina, is reported to have uptake nearly double the N applied (Riar, Gill, & McDonald, 2020). In a double cropping non-fertilized winter camelina study, total N uptake was found to range from 36 to 57 kg N ha^{-1}, an indication of the ability of winter camelina to scavenge soil available N (Liu, Wells, & Garcia y Garcia, 2020).

Nitrogen exported represents N removed from the system in grain yield. Nitrogen exported was affected by N rate in all instances except in Strat 2 at WCROC in 2019. Nitrogen exported was significantly affected by year at SWROC and SLRF, with significant effect of strategy and the N rate × strategy interaction at WCROC. Significant differences between N rates were observed in every instance and a quadratic regression model best described the response of winter camelina grain N to fertilizer N. At all locations, the N uptake from most N treatments was significantly higher than the N uptake from the control treatment. Among locations, N exported was highest at WCROC in 2019, where it ranged from 98 to 122 kg ha^{-1}, followed by SWROC from 40 to 99 kg ha^{-1}, and SLRF from 11 to 68 kg ha^{-1}. Nitrogen exported in 2019 was higher than 2020, mostly due to higher grain yield as a consequence of better growing conditions (mainly rainfall) at around N fertilization. Most results from this study were within the 23 to 72 kg ha^{-1} reported for irrigated spring camelina for conditions in Arizona (Bronson, Hunsaker, & Thorp, 2019) and the 50-67 kg ha^{-1} reported by (Johnson et al., 2019), with results from 2020 at SLRF lower due to low yields.

3.3.4 Residual Soil NO_3-N

Residual soil N was affected by N rate and depth at all locations. At SWROC, year and the interactions of N rate × year, N rate × strategy, and N rate × strategy × year were also significant. At SLRF, residual soil N was affected by the interactions of N rate × strategy and N rate × depth. Significant differences among treatments were primarily found within the top 15 cm of soil, except at SWROC in 2020 for Strat 2 and SLRF in 2019 for Strat 2. Compared to Strat 2, Strat 1 resulted in higher residual N at fertilization rates above 67 kg ha^{-1} (Figure 3.3). Among treatments at either depth, the top N rate produced significantly higher residual soil N than the control in 10 of 16 instances. At all three locations, residual N at the 33 and 67 kg ha^{-1} rates were not significantly different than the non-fertilized (control) treatment. At SWROC, residual soil N in 2019 was significantly higher than in 2020. These findings are similar to those reported by Johnson et al. (2019) for a spring camelina N rate study conducted at SLRF in which the 134 kg N ha^{-1} rate produced significantly higher residual soil N than the 0, 34 and 67 kg N ha^{-1} rates; the lowest 34-67 kg N ha^{-1} rates did not produce significantly different soil N from the non-fertilized. Results from this study suggests that rates of N above 67 kg N ha^{-1} are more likely to cause loss of N from the system due to leaching, something that has also been observed in previous studies in the region (Johnson et al., 2019; Robertson & Vitousek, 2009; Strock et al., 2004).

3.4 Tables and Figures

Table 3.1 Average properties in the 0 to 30 cm depths of soil at the three experimental sites in 2018.

Site	Textural class	OM	pH	CEC	NO_3-N	Bray P	K	Ca	Mg
		%		meq 100 g^{-1}	----------------------------------	----------------------------------	--ppm--	----------------------------------	----------------------------------
SWROC	Clay loam	3.5	6.4	19	3.8	8	116	2402	581
WCROC	Clay loam	6.5	5.8	27	5.5	13	149	3002	594
SLRF	Clay loam	3.0	7.4	20	5.9	11	166	3095	467

Table 3.2 Deviation of monthly precipitation (mm) and average air temperature (°C) of weather conditions during the experimental years as compared to long-term average (LTA, 1994 to 2019) conditions at SWROC near Lamberton and WCROC and SLRF in Morris, MN.

Month	SWROC near Lamberton				Morris WCROC Morris			SLRF Morris			
	LTA	Deviation from LTA			LTA	Deviation from LTA		LTA	Deviation from LTA		
		2018	2019	2020		2018	2019		2018	2019	2020
Monthly precipitation (mm)											
January	14		-2	-2	19		-12	19		-8	-5
February	15		+30	+4	19		+1	19		+5	-16
March	35		+34	+38	30		+18	30		+24	-20
April	75		+75	-41	60		-3	60		+9	-22
May	99		+22	-11	79		+24	79		+27	-52
June	111		-51	-4	109		+9	109		-30	+3
July	96		+78	+78	102		+13	102		+9	+14
August	88		-31	+9	92		+48	92		+45	-21
September	87	-40	+68		72	-25	+97	72	-22	+106	
October	60	-11	+42		69	0	+8	69	+7	+10	
November	29	+28	+1		24	-2	-15	24	-1	-15	
December	21	+46	+18		20	+6	+5	20	+8	+7	
Average air temperature (°C)											
January	-9		-3	0	-11		-3	-11		-3	-1
February	-6		-9	-2	-9		-8	-9		-7	0
March	2		-7	-1	0		-6	0		-6	0
April	10		-3	-4	10		-5	10		-4	-5
May	18		-6	-5	18		-7	18		-6	-5
June	24		-3	-1	23		-4	23		-3	-1
July	25		-2	-2	25		-3	25		-3	-2
August	23		-3	-2	22		-3	22		-3	-1
September	18	0	0		16	0	0	16	0	-1	
October	10	-4	-3		8	-4	-2	8	-3	-2	
November	1	-5	-3		-1	-8	-2	-1	-4	-1	
December	-6	0	-1		-8	-3	-1	-8	-2	0	

Table 3.3 Significance of F value for fixed effect sources of variation for total N uptake, N exported in grain, agronomic efficiency (AE), nitrogen recovery efficiency (NRE), and internal efficiency (IE) by nitrogen rate (kg N ha^{-1}) of winter camelina grown at three locations from 2018 to 2019 and 2019 to 2020.

Location	Source of variation	N exported	N uptake	AE	NRE	IE	Residual Soil N
SWROC	Year (Y)	***§	-	***	-	-	***
	Fertilization strategy (S)	ns	ns	ns	ns	ns	ns
	Nitrogen rate (R)	***	***	***	**	*	***
	Y x S	ns	-	ns	-	-	ns
	Y x R	ns	-	ns	-	-	***
	S x R	ns	ns	ns	ns	ns	**
	Y x S x R	ns	-	**	-	-	**
WCROC	Year (Y)	-	-	-	-	-	-
	Fertilization strategy (S)	**	-	ns	ns	-	ns
	Nitrogen rate (R)	***	-	***	ns	-	**
	Y x S	-	-	-	-	-	-
	Y x R	-	-	-	-	-	-
	S x R	*	-	*	ns	-	ns
	Y x S x R	-	-	-	-	-	-
SLRF	Year (Y)	***	***	ns	ns	***	ns
	Fertilization strategy (S)	ns	ns	ns	ns	ns	***
	Nitrogen rate (R)	***	***	***	ns	ns	***
	Y x S	ns	ns	ns	ns	*	ns
	Y x R	ns	*	ns	ns	*	ns
	S x R	ns	**	ns	ns	ns	***
	Y x S x R	ns	ns	ns	ns	ns	ns

§ Variables with ***, **, * are significant at the 0.001, 0.01, and 0.05 levels. ns denotes not significant and - denotes not available

Table 3.4 Effect of fertilizer N on total N uptake, N exported in grain, agronomic efficiency (AE), nitrogen recovery efficiency (NRE), and internal efficiency (IE) by N rate (kg N ha^{-1}) of winter camelina grown at three Minnesota locations from 2018 to 2019 and 2019 to 2020.

Location	**Year**	**N Rate**	**N Uptake**	**N Exported**	**AE§**		**NRE**		**IE**	
			(kg ha^{-1})		*S1*†	*S2*	*S1*	*S2*	*S1*	*S2*
SWROC	2019	0	-	61c	-	-	-	-	-	-
		33	-	76bc	11.4ab	19.0a	-	-	-	-
		67	-	93ab	13.8a	9.9b	-	-	-	-
		100	-	99a	9.4Aab	9.2b	-	-	-	-
		135	-	89ab	4.8b	6.9b	-	-	-	-
	2020	0	93c	40c	-	-	-	-	13.7a	
		33	138b	56b	15.7a	10.0a	0.78a	0.33a	13.0a	
		67	147b	61b	6.3b	9.4a	0.26b	0.47a	13.2a	
		100	175a	74a	7.8b	8.0ab	0.34ab	0.61a	13.1a	
		135	176a	67ab	4.1b	5.4b	0.37ab	0.43a	11.5a	
WCROC	2019	0	-	98b	-		-	-	-	-
		33	-	108ab	19.6a		-	-	-	-
		67	-	109ab	11.0b		-	-	-	-
		100	-	105ab	6.3b		-	-	-	-
		135	-	122a	7.3b		-	-	-	-
SLRF	2019	0	75a	50b	-		-		13.4a	13.4a
		33	78a	64ab	9.0a±		0.06a		18.8a	14.0a
		67	98a	80a	6.5a		0.33a		15.2a	14.6a
		100	98a	60ab	3.2b		0.23a		13.0a	11.0a
		135	115a	68ab	1.8b		0.29a		11.1a	10.0a
	2020	0	34c	11b			-		6.2a	6.2b
		33	50bc	27a			0.49a		9.7a	12.2ab
		67	57ab	29a			0.33a		7.7a	14.0a
		100	60ab	28a			0.27a		9.7a	9.7ab
		135	68a	28a			0.27a		7.8a	9.8ab

† S1 denotes fertilization strategy 1, one application in spring; S2 denotes fertilization strategy 2, split (33%) fall and (67%) spring. Data centered between strategies represents that strategy was not significant For a given location and year, means followed by different letters within a column are significantly different at $P \leq 0.05$

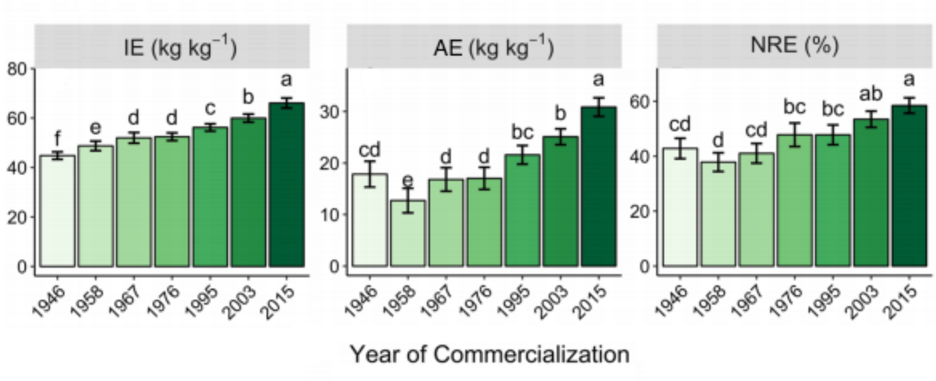

Figure 3.1 Historical nitrogen use efficiency (NUE) measures for maize at 220 kg N ha^{-1}: internal efficiency (IE), agronomic efficiency (AE), and nitrogen recovery efficiency (NRE). (Mueller, Messina, & Vyn, 2019)

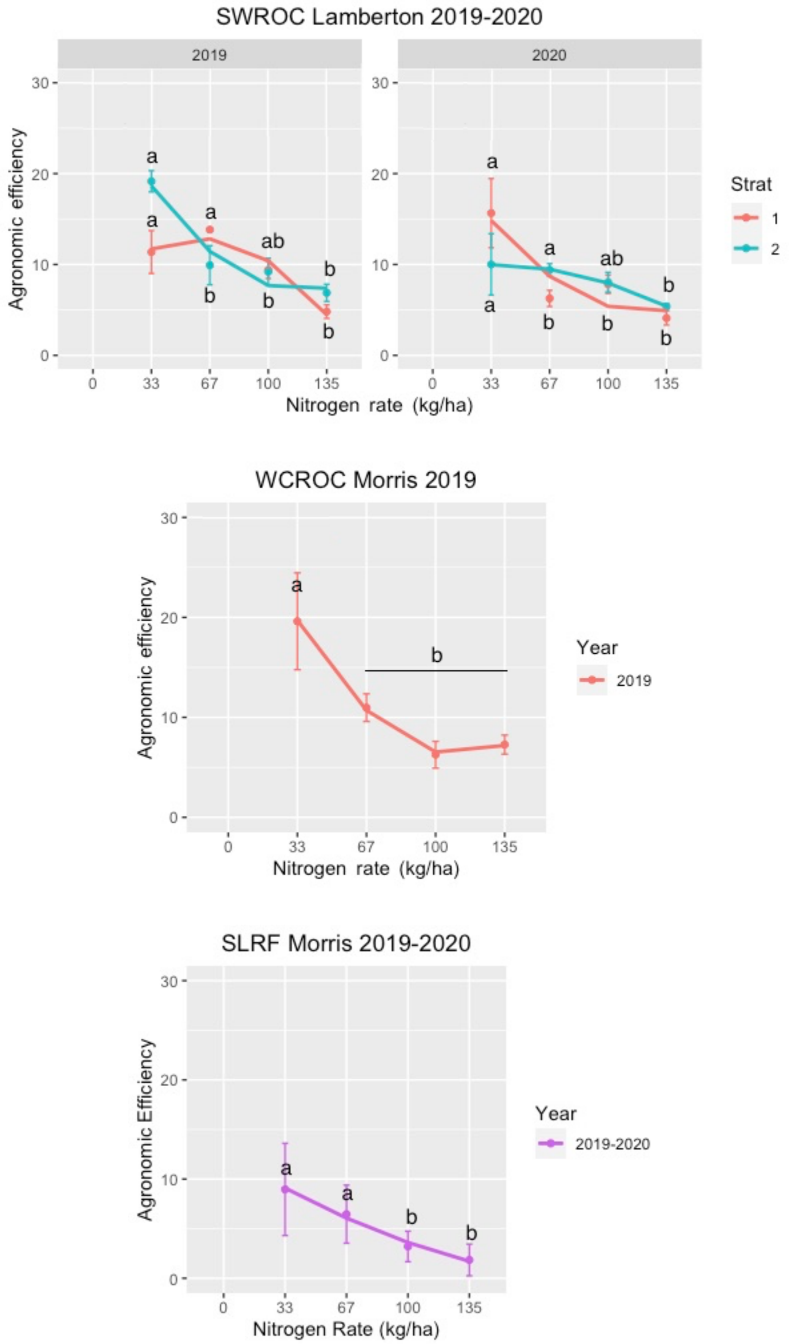

Figure 3.2. Effect of fertilizer N on agronomic efficiency of winter camelina grown at three Minnesota locations during two growing seasons. In WCROC Morris, 2020 data was not available. Error bars represent SE (n=4 for strategy, n=8 for year). Values of N rates with different letters above differ at $P \leq 0.05$.

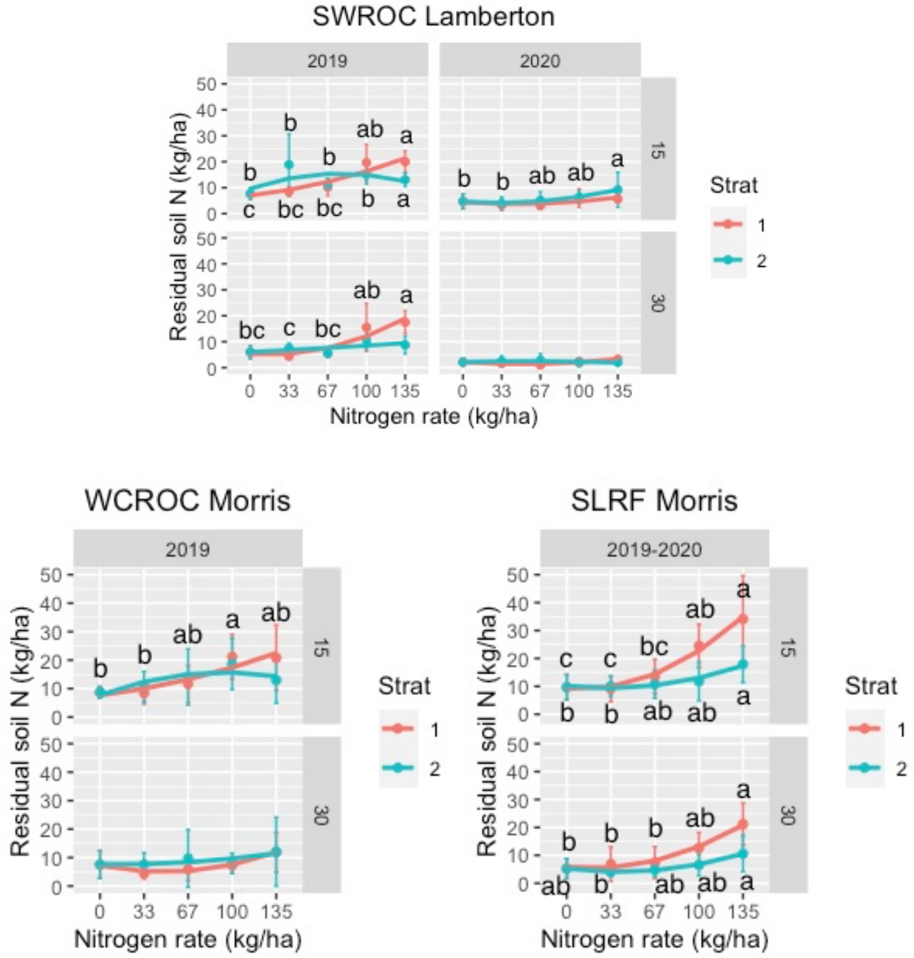

Figure 3.3. Effect of N rate on residual NO_3-N in the 0–15 cm and 15–30 soil layers following winter camelina grown at three Minnesota locations and two growing seasons. Error bars represent SE (SWROC and WCROC n=4; at SLRF n=8). Within a depth and year at a location, N rates with different letters above differ at $P \leq 0.05$. When only one set of letters is shown for a depth, strategies were not significantly different.

4. Chapter 4 – Winter camelina double cropped with maize and soybean: N-fertilized versus non-fertilized trials

Summary

Double cropping with winter camelina is a diversification option for the typical maize-soybean rotation in the upper Midwest, a strategy that promises environmental and economic benefits. Studies on double-cropping maize and soybean with non-fertilized- (Study 1) and nitrogen (N)-fertilized (Study 2) winter camelina were compared to assess the growth and yield, N, water use (WU), and water productivity (WP) of winter camelina in two locations in Minnesota. Study 1 was conducted from 2015–2017 in one location and the Study 2 was conducted from 2018–2020 in two locations, both studies in Minnesota. Yield of winter camelina was as much as six times higher in Study 2 compared to Study 1; averaged across treatments, Study 2 yielded 1157 kg ha^{-1} compared to 556 kg ha^{-1} from Study 2. In Study 1, oil and protein content ranged from 26.4 to 27.2% and 19.4 to 27.1% respectively. In Study 2, oil and protein content ranged from 31.7 to 35.9% and 14.9 to 20.8% respectively. Water use tended to follow similar trends between studies. Winter camelina average WU across cropping systems was similar between Study 1 and Study 2 (165 compared to 168 mm). Camelina WP was higher in Study 2 compared to Study 1, and ranged from 0.60 to 0.84 and 0.20 to 0.42 respectively. Fertilizer N was generally found to increase biomass, yield, WU, WP, and residual soil N in winter camelina double cropped with maize and soybean.

4.1 Introduction

The winter fallow period in the dominant maize-soybean rotation of the U.S. upper Midwest has contributed to significant soil erosion and water quality decline (Basso et al., 2019; Robertson & Vitousek, 2009; Weyers et al., 2019). The most vulnerable time for erosion and nutrient loss is often in the spring before planting, due to high precipitation at a time when there are no active roots in the soil (Randall et al., 1997). One strategy that is gaining traction is adding a winter annual to summer annual crop rotations. Winter annuals are uniquely suited to placement within summer annual cropping and to the short growing season of the region due to their ability to grow, overwinter, and yield before or soon after planting a summer annual (Gesch & Archer, 2013; G. A. Johnson et al., 2017; Ott et al., 2019; Sindelar et al., 2017).

Double cropping maize or soybean with a winter annual could increase ground cover and reduce the negative environmental impacts from the fallow period, while also providing additional incentive to farmers due to the potential additional income provided from growing three crops instead of two in two seasons, an opportunity to overcome the primary reasons given that farmers do not currently adopt such practice (CTIC & SARE, 2017; CTIC et al., 2020). Double cropping can be relay or sequential. Relay cropping involves an overlapping growth period; e.g., maize or soybean interseeded into the winter annual approaching maturity (Berti et al., 2015; G. A. Johnson et al., 2015). Sequential cropping refers to planting a second crop soon after harvest of the first; e.g., maize or soybean after harvest of winter camelina. Sequential cropping often requires a shorter-season second crop and eliminates the competition of the two crops by seeding one after the other (Moore & Karlen, 2013), but can result in a lower summer annual yield and a higher yield overall (Gesch et al., 2014). In a relay system, the winter annual is harvested over the top of the growing summer annual, which then has full access to nutrients, sunlight, and water. Relay cropping typically results in total yields higher than sequential cropping because full-season crop produce more than short season crops (Berti et al., 2015; Gesch et al., 2014). Winter camelina [*Camelina sativa* (L.) Crantz] is one of the winter annual oilseeds that has shown promise in double cropping systems in the colder climates of the U.S. upper Midwest due to its extreme cold tolerance. Winter camelina grain has high oil content, which provides production flexibility due to its potential use as a heart-healthy edible oil or as feedstock for biofuel (Berti et al., 2016, 2015; Heggenstaller et al., 2008).

Winter camelina is considered low input, but there are some evidences that it responds well to N (Gesch & Archer, 2013). Yet, current research has not determined the N needs of winter camelina for conditions in the upper Midwest, although fertilization at 70–90 kg N ha^{-1} is usually reported in research trials (Gesch et al., 2014; Gesch, Dose, & Forcella, 2017; Gesch & Johnson, 2015; Ott et al., 2019; Walia et al., 2018; Weyers et al., 2019), likely to boost low yields found in early non-fertilized studies (Berti et al., 2016, 2015; Gesch & Cermak, 2011). In an agroecosystem with a high potential for NO_3-N leaching, careful consideration for N fertilization is needed to increase environmental benefits, a premise that could compromise the possibility of double cropping maize with winter camelina due to the N needs of both crops. In a 2014 to 2015 study, non-fertilized winter camelina was found to reduce soil NO_3-N at 60 cm by 53 to 72% in the fall and 18 to 19% in the spring in a double cropping system with soybean compared to monocrop soybean (Johnson et al., 2017). In a double-cropping study with winter camelina fertilized at 90 kg N ha^{-1}, soil NO_3-N was significantly lower in the camelina treatment compared to monocrop soybean in all sampling dates except after the harvest of camelina (Weyers et al., 2019). By 2018, specific studies comparing the production and environmental quality of N fertilized versus non-fertilized winter camelina have not been conducted in the region. This research was designed to determine the differences between non-fertilized and N-fertilized winter camelina double cropped with maize and soybean to determine the effect of N in the soil and crop water use and water productivity of winter camelina. The hypothesis was that N fertilization will have a positive effect on yield and growth of winter camelina, soil N, and crop water use. The objectives of this study were to assess the effect of N fertilization on (i) growth and yield of double cropped winter camelina, (ii) soil and soil-solution nitrate-N, and (iii) the water use and water productivity of double cropped winter camelina.

4.2 Materials and Methods

4.2.1 Experimental Sites

Experiments were conducted at one location from fall 2015 to fall 2017 (Study1) and 2 locations from fall 2018 to fall 2020 (Study 2). Study 1 was conducted at the University of Minnesota Southwest Research and Outreach Center near Lamberton, MN (SWROC; 44°14’02.20”N 95°18’6.87”W). The dominant soils at SWROC were characterized as Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls)

(Soil Survey Staff, 2020). Study 2 was conducted at SWROC and the University of Minnesota West Central Research and Outreach Center near Morris, MN (WCROC; 45°35’37.17”N 95°52’42.63”W). The dominant soils at WCROC were characterized as Nutley Flom clay loam (fine, smectitic, frigid chromic Hapluclerts and fine-loamy, mixed, superactive, frigid Typic Encloaquolls). The 25-year long term average (LTA) temperatures were 7°C for SWROC and 5.8°C for WCROC with yearly precipitation averaging 754 mm and 693 mm, respectively. The winter hardiness zone for SWROC is 4b and WCROC at 4a, the latter slightly cooler (USDA-ARS).

4.2.2 Experimental Design

Double cropping rotations experiments with winter camelina and maize/soybean were conducted from 2015 to 2017 and from 2018 to 2020; both maize and soybean phases of the rotation were present in all site-years. Plots were 20 m x 6 m in the 2015-2017 study and 6 m x 9 m in the 2018-2020 study. Treatments for both studies contained controls with no winter camelina for relay and sequentially seeded maize or soybean. Both studies were set as a randomized complete block design with four replications.

4.2.3 Agronomic Management

Study 1

Study 1 was initiated in the fall of 2015 at SWROC, where the previous crop was oats (*Avena sativa* L.) to reduce residual NO_3-N in the soil. Winter camelina (var. Joelle), along with other winter-hardy crops (Liu et al., 2020), was hand-broadcast and then raked to provide better seed-to-soil contact on 31 August 2015 and 14 September 2017. The cultivar Joelle has been used in foundational double cropping research in the upper

Midwest (Gesch & Archer, 2013; Gesch et al., 2014; Gesch & Cermak, 2011). Winter camelina was seeded at 13 kg ha^{-1} without N fertilizer in order to enhance agroecosystem benefits and was harvested when > 90% of silicles were brown and dry, around mid-June for both years.

Maize cultivar DKC49-72RIB with a relative maturity of 99 d was used in the relay plots and DKC31-10RIB with a relative maturity of 90 d was used in the sequential plots. Relay maize was seeded at 86,500 plants ha^{-1} on 19 May 2016 and 12 May 2017, when winter camelina was at the BBCH50 (inflorescence emergence) stage of development. Maize was seeded using a four-row (76 cm) John Deere 1700 planter with row cleaners. Sequential maize was seeded at 86,500 plants ha-1 in mid-June on both years on a seedbed prepared with a disc harrow. All maize was fertilized in mid-June in 2016 and mid-July in 2017. Relay and sequential maize were harvested late October and early November respectively in 2016 and both treatments were harvested in late October in 2017.

Soybean cultivar Stine 20RD20 was seeded at 373,000 plants ha^{-1} on May 19, 2016 and May 16, 2017 for relay plots, and June 22, 2016 and June 21, 2017 for sequential plots beds were prepared with a disc harrow. Soybean received no fertilizer and was harvested in early November and late October in 2016 and 2017 respectively.

Study 2

Study 2 was initiated in the fall of 2018 at WCROC Morris and SWROC Lamberton where the previous crop was spring wheat (*Triticum aestivum* L.) and annual ryegrass (*Lolium multiflorum* L.), respectively, to reduce residual NO_3-N in the soil. Due to extreme weather conditions, winter camelina did not establish in fall of 2018 at SWROC and 2019 at WCROC, so no data were collected in spring at both locations. Seedbeds were prepared for camelina with a field cultivator and seeded using an InterSeeder (InterSeeder Technologies, Woodward, PA, USA) and a Case IH 4.2 m grain drill (Model 5100; Case IH, Racine, WI, USA) at SWROC and WCROC, respectively, at rate of 9 kg ha^{-1}. Winter camelina was seeded on 3 October 2018 at WCROC and 23 September 2019 at SWROC. At both sites fertilizer for camelina was broadcast on the soil surface in all plots at the BBCH 12 to 20 stages (rosette; single true leaves developing) in mid-May, which has been reported to maximize seed yield in the field and greenhouse (Gesch & Cermak, 2011; Johnson & Gesch, 2013; Ott et al., 2019). Fertilizer was a 6.5-33-33 blend of monoammonium phosphate and potash with urea added to attain 100 kg N ha^{-1} for WCROC in 2019 and 78 kg N ha^{-1} for SWROC in 2020 due to preliminary evidences that 100 kg N ha^{-1} may be unnecessary to achieve satisfactory yield. Winter camelina was harvested when > 90% of silicles were brown and dry, first using 1 m^2 quadrats for a hand harvest in both locations and then by combine mid-July at WCROC in 2019 and early July at SWROC in 2020.

Maize cultivar 2417 VT2P RIB with a relative maturity rating of 85 and soybean cultivar AG07X9 (0.7 maturity group) were relay planted into camelina on 13 June 2018 at WCROC using a four-row (76 cm) planter. Fertilization for maize consisted of 157 kg N ha^{-1} at planting with no P or K. No fertilizer was used for soybean. Sequence maize and soybean was planted mid-July following harvest of camelina. Glyphosate [N-(phosphonomehtyl) glycine] at 2.8 kg a.e. ha^{-1} was used with 141 g of InterLock adjuvant on 19 July 2019. Other weeds were removed periodically by shovel. Due to late planting, both relay maize and soybean did not reach physiological maturity; total biomass was collected.

At SWROC, maize DKC47-54RIB with a relative maturity rating of 97 was used in the relay treatment while DKC29-89RIB with a relative maturity rating of 79 was used in the sequential treatment. Maize and soybean were planted on 2 June 2020 and 13 July 2020 for relay and sequential systems, respectively. Soybean AG06x8 (0.6 maturity group) was used in both relay and sequential planting using a four-row (76 cm) planter. Fertilizer was applied for maize at a rate of 112 kg N ha^{-1}, 67 kg P ha^{-1}, and 67 kg K ha^{-1}; N as urea (NH_2-CO-NH_2), P_2O_5 as triple superphosphate [Ca(H2PO4)2. H2O], and K_2O as Muriate of potash (KCl): 40% N at planting per treatment and the remaining N at V5 on 2 July for relay and 4 August for sequential. Glyphosate [N-(phosphonomehtyl) glycine] at 2.8 kg a.e. ha^{-1} was used with Interlock adjuvant at 141 g and applied 12 August 2020 on sequential crops. Chlorpyrifos (diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ5-phosphane, 48% w/v) at 1.1 L ha^{-1} was used for aphids on 24 August 2020. Relay maize was terminated by hand-cutting at camelina harvest due to maize height interfering with camelina combining. Control relay maize was harvested on 15 October. Relay and sequential soybean were harvested 13 and 19 October, respectively.

4.2.4 Data Collection

In Study 1, soil samples were collected in the spring of 2016 and 2017, and in the fall after maize and soybean harvest. In spring 2016, soil samples were collected in the relay and its control. In Study 2, additional soil samples were taken after camelina harvest. Soil samples were taken at four points per plot in a diagonal pattern at 15 cm and 30 cm depth. Subsamples from each depth were mixed to make a composite sample and allowed to air dry before being ground using a Dynacrush soil crusher (Custom Laboratory Equipment Inc., Holden, MO, USA). Samples were analyzed for NO_3^--N, pH, organic matter (OM), Bray-1 phosphorous (Bray P), cation exchange capacity (CEC), K^+, Ca^+, and Mg^{+3}. Nitrate-N concentration in the soil solution was obtained from samples collected at a 1 m depth in relay and control relay plots at SWROC using ceramic suction cups placed in holes made with a Giddings probe (Giddings Machine Company; Windsor, CO, USA) in the center of each plot. Volumetric soil moisture was measured using a PR2/6 moisture probe Delta-T Devices, Cambridge, UK) at 10, 20, 30, 40, 60, and 100 cm. Access tubes for the PR2/6 soil moisture probe were installed in the center of each plot and readings were taken with a handheld readout device HH2 (Delta-T Devices, Cambridge, UK). Both, soil solution samples and moisture readings were taken every 7–10 days. For the former, a manual pump was used to apply vacuum to ceramic cup samples, extract the solution 48 to 72 h later, and placed in 50 mL centrifuge tubes, which were frozen until analysis. Samples were processed for NO_3-N concentration with Vanadium (III) reduction using the manual spectrophotometric procedure (Doane & Horwáth, 2003). Ammonium-N (NH4-N) was determined from the same solution with the salicylate microplate method using the manual spectrophotometric procedure (APHA, 1992).

Biomass was collected for each crop at maturity in both studies and all locations. Biomass at maturity for winter camelina was obtained by harvesting 0.5 m^2 in 2016 to 2017 and 1 m^2 in 2019 and 2020. Winter camelina grain was adjusted to 10% moisture. Maize biomass at maturity was collected by harvesting the two central rows in 2016 to 2017 and harvesting six plants per plot in 2019 and 2020, with moisture adjusted to 15.5%. Soybean above ground biomass at maturity was harvested using 0.5 m^2 quadrats in all studies and processed for dry matter. Biomass was dried in a forced air oven at 60°C to a constant weight. Biomass and grain samples were ground with a Thomas Wiley Mill Model 4 to pass through a 1-mm screen for carbon (C) and N analysis, testing 10–15 mg grain and 5–10 mg tissue samples by combustion using a Vario EL Cube (Elementar Americas Inc., Ronkonkoma, NY). For winter camelina, oil and protein-rich seed meal are the primary products; protein and oil content were measured by nuclear magnetic resonance (NMR) in both studies (Minisper MQ20; Bruker, Ettlingen, Germany for Study 1 and CyFlow®Space; Partec, Görlitz, Germany for Study 2).

Long-term (1994–2009) averages and experimental-year weather data were collected from weather stations located at both sites. Soil samples were collected using a push probe (1.7-cm diameter, JMC Soil Samplers, Newton, IA, USA) at 15 cm and 30 cm depths. More information on soil sampling can be found in Section 3.2.4 of this thesis.

Growing degree days (GDD) for each crop were calculated each year from planting to harvest. For winter camelina, base temperature (T_{base}) was set at 4°C and absolute maximum (T_x) at 28°C (Tribouillois et al., 2016). For maize and soybean, base temperature was set at 10°C and absolute maximum (T_x) at 30°C (Akyuz, Kandel, &

Morlock, 2017; Russelle et al., 1984). Growing degree days were calculated following the procedure by Mcmaster and Wilhelm (1997) (eq. 1):

$$GDD = \Sigma(\frac{Tmax + Tmin}{2} - Tbase) \tag{1}$$

where T_{max} = maximum daily air temperature and T_{min} = minimum daily air temperature. The following thresholds were used to limit the temperature within the range of T_{base} and T_x:

$$if\ T_{max} > T_x\ then\ T_{max} = T_x$$

$$if\ T_{max} < T_{base}\ then\ T_{max} = T_{base}$$

$$if\ T_{min} > T_x\ then\ T_{min} = T_x$$

$$if\ T_{min} < T_{base}\ then\ T_{min} = T_{base}$$

Water use (WU, mm) was calculated for winter camelina, soybean, maize, and for the full season of winter camelina with maize or soybean. A simplified water balance (Sintim, Zheljazkov, Obour, & Garcia y Garcia, 2016) was used as follows:

$$\text{WU} = P \pm \Delta SW \tag{2}$$

where P is precipitation (mm), ΔSW is change in soil water (mm) in the top 60 cm because soil moisture at 100 cm did not change. It should be noted that winter camelina roots in Minnesota have been found to typically go to less than 1 m, with half of the roots occurring within the top 10 cm (Zanetti et al., 2020). Changes in soil water were calculated using volumetric soil moisture readings between sampling dates within a given growing season. Water for the top 60 cm was calculated using the following equation:

$$S = \frac{(\bar{\theta}_{t1} - \bar{\theta}_{t2})}{100} z \qquad (3)$$

where S = water storage (mm), t1 and t2 = volumetric water content readings at time 1 and time 2, z = the active root zone (mm) set at 60 cm depth, and 100 a constant to convert values from percentage to fraction. Daily water use corresponded to S divided by the number of days between the two reading dates. Water use was then added together for each crop period and for the full season. Due to its marginal growth in fall, the WU of winter camelina corresponds to the spring growth only; for maize and soybean, water productivity (WP, kg m^{-3}) was calculated as the ratio of yield to the amount of water needed to produce that yield, as follows:

$$WP = \frac{Yield}{WU} \qquad (4)$$

where Yield (kg ha^{-1}) is the crop yield or the total seasonal yield. Water productivity was calculated for camelina in maize, camelina in soybean, soybean after camelina harvest, maize after camelina harvest and the full season.

4.2.5 Statistical Analysis

All data were subjected to ANOVA using R version 3.5.0 and 4.0.3 (R Core Team 2020), with each study analyzed independently due to differing planting methods and fertilization. Normality was assessed with the Shapiro-Wilk test of residuals in Study 1 and visually in Study 2. Year and cropping system were treated as fixed effects, and replication was treated as a random effect. Soil depth was considered a fixed effect in the analysis. For fertility sampling, years and depths were analyzed separately. Soil solution NO_3-N was grouped into three biologically significant periods: the camelina phase (from the beginning of spring regrowth to planting of the relay crops), the intercrop phase (from relay planting of maize or soybean to camelina harvest), and the main crop phase (from camelina harvest to main crop harvest). Soil solution NO_3-N had non-normal distribution but did not respond to transformation, so ANOVA could not be performed. If any combined analysis showed significant interactions, separate ANOVA were then run on the response variable. Tukey's Honest Significant Difference (HSD) was used for post hoc analysis at $P \leq 0.05$ using the 'agricolae' package to determine means separation within treatments.

4.3 Results and Discussion

4.3.1 Weather Conditions

Study 1

Compared to the long-term average (LTA 1999 to 2019), fall of 2015 was warmer and the 2016 growing season, from April to August was slightly cooler. Fall of 2016 and the growing season of 2017 were both slightly cooler than the LTA. Years 2016 and 2017 were wetter by 34% and 20% compared to the 730 mm LTA. In 2016, March through December were notably wetter than the LTA with the exception of June. In 2017, May and August were notably wetter, while March and June were notably drier (Table 4.3).

Study 2

In 2018 at WCROC, monthly average air temperatures were cooler in October and November. September received 25 mm less precipitation than the LTA. In 2019, monthly average temperatures were lower from January to August and the growing season was wetter than the LTA. At SWROC, 2019 fall was slightly cooler than the LTA, and precipitation was 127 mm higher than the LTA. This saturated the soil of the newly planted camelina crop in the fall and throughout the winter, a condition that has been reported to negatively impact the spring growth and final yield of winter camelina (Gesch & Archer, 2013; Gesch & Cermak, 2011). In 2020 at SWROC, average air temperatures were within 2°C of the LTA with the exception of April and May, which were both cooler. March was wetter by 38 mm than the LTA and April and May were both drier by 41 and 11 mm respectively (Fig 4.2).

4.3.2 Growth and Development of Non-Fertilized Winter Camelina

Winter camelina total biomass at SWROC in 2016 and 2017 was affected by year and cropping system in both the maize and soybean. Winter camelina produced more biomass in relay with maize compared to relay with soybean (Table 4.4). Winter camelina in relay with soybean produced the least biomass at 1531 kg ha^{-1} in 2016 while camelina in relay with maize and sequential cropping with maize and soybean produced similar amounts of biomass, ranging from 2553 to 2871 kg ha^{-1} (Table 4.4). Winter camelina biomass was higher in 2017, with all treatments producing comparable amounts, ranging from 3106 to 3840 kg ha^{-1}. The difference between years was reported previously by Liu, Wells, & Garcia y Garcia (2020) as partly due to a colder, wetter spring in 2016 compared to 2017. Grain yield was affected by year but not cropping system. Grain yield in relay and sequential treatments ranged from 247 to 494 kg ha^{-1} in 2016 and from 609 to 786 kg ha^{-1} in 2017. These biomass and grain yield results are within the range reported previously in non-fertilized winter camelina studies in the region (Gesch & Cermak, 2011; Johnson et al., 2017).

Yield quality was measured only in 2017 in Study 1. Oil content was similar among cropping system treatments and ranged from 26.4 to 27.1 %. Protein content was significantly higher in sequential cropping with soybean but results from both relay and sequential cropping systems were not significantly different in maize. Protein content among cropping systems ranged from 19.4 to 27.1%; the lowest came from the relay with soybean (Table 4.3). Oil and protein content found in these two studies support previous findings in the region, which range from 29.4 to 42.1% and 24.5 to 25.9%, respectively (Gesch et al., 2014; Gesch & Cermak, 2011). Previous results for protein content from non-fertilized winter camelina have not been reported at the time these studies were conducted.

4.3.3 Growth and Development of N-Fertilized Winter Camelina

In Study 2, total biomass of winter camelina was affected by cropping system in maize only at WCROC in 2019, and grain yield was affected by cropping system in maize and soybean at SWROC in 2020 (Table 4.3). Percent canopy cover during the ripening stage (BBCH 81) was not significant at either location in any treatment. At WCROC in 2019, total biomass ranged from 2965 to 4134 kg ha^{-1} and was significantly higher in sequential cropping of both maize and soybean, compared to relay treatments (Table 4.4). At SWROC in 2020, total biomass ranged from 3840 to 4488 kg ha^{-1} with no significant difference between relay and sequential cropping in both maize or soybean (Table 4.4). Biomass was higher in Study 2 than in Study 1; from 2665 to 4488 kg ha^{-1} in the former and from 1531 to 3840 kg ha^{-1} in the latter (Figure 4.3). Similarly, grain yield of winter camelina varied from 247 to 786 kg ha^{-1} in Study 1 and from 851 to 1461 kg ha^{-1} in Study 2. Such differences in biomass and grain yield from both studies is an indicative of the response of winter camelina to the addition of N fertilizer in Study 2.

In Study 2, oil content of winter camelina was similar in maize and soybean systems at WCROC, ranged from 33.3 to 35.9% and were significantly higher in sequential compared to relay in maize. Oil content was not different between cropping systems in 2019 but was lowest inform the relay with maize (Table 4.4). In 2020 at SWROC, oil content ranged from 31.7 to 32.7%, with no significant differences between cropping systems. Oil content between maize and soybean systems was similar. Protein in grain of winter camelina ranged from 17.4 to 20.8% and 14.9 to 18.1 % at WCROC in 2019 and SWROC in 2020, respectively, with no significant differences between cropping systems in a given year. (Table 4.4). Oil content at WCROC 2019 and SWROC 2020 were higher than SWROC in 2017, but protein content was generally lower.

Yield, biomass, and oil content of winter camelina found in Study 2 are comparable to those reported in similar studies in the region (Gesch & Archer, 2013; Gesch et al., 2014; Ott et al., 2019). However, protein content was lower than the typical range of 23 to 27.9% reported in N-fertilized winter camelina experiments in the upper Midwest (Gesch et al., 2014, 2018; Walia et al., 2018). This is possibly due to laboratory method differences; results from Study 2 were obtained using NMR technology rather than the 6.25 constant, a standard to estimate protein in foods. The constant is based on the assumption that N in protein is 16%, which is generally accurate; however, different proteins have been found to have different N content due to the amino acids present (Mariotti, Tomé, & Mirand, 2008; Jones, 1941).

4.3.4 Soil Moisture, Water Use, and Water Productivity of Crops in Non-fertilized Winter Camelina Double Cropped with Maize and Soybean

Volumetric soil water content was analyzed at maize/soybean relay planting and after camelina harvest in the Study 1. The volumetric soil water content was significantly affected by cropping system, depth, and the cropping system × depth interaction, mostly in the relay system with both maize and soybean. Soil in maize relay plots was significantly drier than the control at the 20 and 30 cm depths, but wetter at the 10 and 60 cm depths. After camelina harvest, soil in the top 20 cm was significantly drier in the control compared to the relay treatment. This is likely due to the better growth in the maize control (17,460 kg DM ha^{-1} at harvest) compared to the relay treatment (13563 kg DM ha^{-1}) in 2017 (Figure 4.3, Figure 4.5). In soybean, soil was significantly drier in the control at 10 cm and 40 cm depths at relay planting, but similar at the 20 and 30 cm depths. After winter camelina harvest, the soil was drier at the 10 and 30 cm depths in the control compared to the relay, but similar in the 20 to 60 cm layer. Similar to maize, this may be the consequence of higher biomass produced (therefore more water is used) in the control compared to the relay.

Water use for maize and soybean tended to be higher in 2016 compared to 2017, and for camelina the opposite was observed (Table 4.5). Water use of crops was affected by year and cropping system in all instances, and by cropping system × year interaction in soybean. The combined WU of crops in relay was significantly higher than the WU of monocrops (control) in 2016 and 2017, averaging 95 and 118 mm more than the controls (537 and 544 mm) during both the 2016 and 2017 growing seasons for the winter camelina-maize and -soybean relay systems, respectively. Maize in relay used significantly less water than its control 2016 and 2017, an indication of limitations on plant available water in the soil due to winter camelina WU. In fact, WU of winter camelina in relay with maize was 117 and 201 mm in 2016 and 2017, respectively. Maize WU in relay was 572 and 342 mm in 2016 and 2017, respectively, versus the control WU of 594 and 356 mm in 2016 and 2017, respectively. Winter camelina WU in relay with soybean was 126 and 219 mm for 2016 and 2017 respectively. The WU of soybean in relay was similar to the control: 585 and 358 mm compared to the control WU of 584 and 364 for 2016 and 2017, respectively. The WU of winter camelina in the relay treatment includes some overlap of the interseeded main crops, which was neglected since WU of main crops during this period is minimal, an approach that has been used in previous studies (Gesch & Johnson, 2015).

Water productivity was affected by cropping system in both maize and soybean monocrops, and by year in maize. The WP of the relay system for maize and soybean tended to be lower than in the control in all instances in 2016 and 2017, except for soybean in 2017. The WP for the winter camelina-maize relay was 1.74 and 1.46 kg m^{-3} compared to 2.22 and 2.13 kg m^{-3} in the control for 2016 and 2017, respectively; the low WP for maize in 2017 is largely due to low maize yield. Findings of WP for maize in this study are lower than those reported for continuous maize by Copeland et al. (1993) at SWROC, mainly due to lower WU estimations, which averaged 278 mm compared to

468 mm reported here. Results from this study, however, support those obtained in Michigan from a maize-soybean rotation by Hussain et al. (2019), reported an average WP of 2.01 kg m^{-3} WU of 469 mm.

Water productivity of winter camelina in relay with soybean was 0.48 and 0.58 kg m^{-3} in 2016 and 2017, respectively, and 0.62 kg m^{-3} in the control during both years. Previous studies have reported lower WP of monocrop compared to relay soybean with winter camelina (Gesch and Johnson, 2015), largely due to lower yields of the oilseed crop in the latter. In this study, the WP of winter camelina was higher in relay with maize in 2016 compared to 2017 while the opposite was observed when in relay with soybean. The WP of winter camelina was 0.42 and 0.31 kg m^{-3} when in relay with maize and 0.20 and 0.34 kg m^{-3} when in relay with soybean in 2016 and 2017, respectively. The WP of winter camelina from this study are similar to those found by Sintim et al (2016) for fertilized winter camelina but are lower than the 0.58 to 1.58 kg m^{-3} reported by Gesch and Johnson (2015) for N-fertilized winter camelina.

4.3.5 Soil Moisture, Water Use, and Water Productivity of Crops in N-Fertilized Winter Camelina Double Cropped with Maize and Soybean

Volumetric soil water content was only measured in 2020 at SWROC soon after maize/soybean relay planting and after camelina harvest. Volumetric soil water was affected by depth for all monitoring dates in maize and soybean systems, and by cropping system at relay planting of maize and soybean. For instance, relay plots in both maize and soybean were significantly drier than the control, an indication of the winter camelina effect on soil moisture. Significant differences in soil water were observed between treatments after planting (8 June 2020) and harvesting (10 July 2020) (Fig 4.5 and Fig 4.6), except at planting in soybean in 2020, where differences were not significant, mainly due to large variation among replications (Figure 4.6). After relay planting maize, soil moisture in relay and control plots was not significantly different in the top 20 cm of soil, but differences were significant in the remaining soil profile. After winter camelina harvest, the soil at 20 and 30 cm depths in the relay plots was significantly wetter compared to the control. This is likely due to higher water use of maize in the control, which is supported by higher biomass production as well. Soybean showed similar trends, except that the soil water content at relay planting was not significantly different among depths due to the high variability between replications.

Water use of crops was similar within each phase (camelina, maize/soybean, full season) and cropping systems (Table 4.5). Water use was not significantly affected by cropping system except in the soybean phase, where the relay used less water than the control treatment, likely due to reduced growth which was consequence of limited light for the relayed crop. The WU was generally higher in Study 1 than Study 2, except for the lower WU of camelina in maize and soybean in 2016. This is in part due to more available water (wetter May) in both years of Study 1, with precipitation in 2020 closer to the LTA. The WP from Study 2 was not significantly different between treatments, although WP in camelina averaged higher in the sequence compared to the relay. Camelina WP in Study 2 was much higher than in Study 1, mainly due higher yields in the N fertilized study and the lower WU obtained in the non-fertilized study. The soybean phase and system WP were much higher in Study 2 compared to Study 1 due to lower

WU in the former (Table 4.5). Camelina WP reported here are nearly double those reported for fertilized winter camelina grown in Wyoming in 2013 and 2014 (Sintim et al., 2016) due to higher WU than the average reported in Study 2. The WP results from this study are similar to those reported by Gesch and Johnson (2015) and reaffirm that winter camelina is a low-water-use crop in double cropping systems and consistently uses less water than soybean and maize.

4.3.6 Soil N Dynamics in Non-fertilized Winter Camelina Double Cropped with Maize and Soybean

In Study 1, NO_3-N concentration in the soil solution averaged lower in the relay compared to the control, except in the winter camelina in relay with maize in 2017. In 2016, the average NO_3-N in the soil solution was higher than in 2017, likely due to more precipitation in spring and midsummer compared to for the same periods in 2017 (except in May when the SWROC site received over 50 mm more precipitation than the LTA). In 2017, NO_3-N concentration in winter camelina relayed with maize was higher than the levels observed in 2016, when they were negligible. The wetter May in 2017 might have increased drainage and been the cause of the highest levels of NO_3-N concentration of 2017, which occurred during the intercrop phase in both maize and soybean. The NO_3-N concentration in the soil solution was higher in soybean compared to maize, especially in the relay, which may be due to excess N from the previous maize crop and residue mineralization. The winter camelina phase in the soybean relay and its control had higher levels of NO_3-N than in maize in both years. In both situations, the average NO_3-N concentration was generally higher in the control than the relay, suggesting that winter camelina used some of the excess N in the system. Across years, winter camelina reduced the concentration of NO_3-N in the soil solution by an average of 31% in both maize and soybean compared to the control. Across cropping systems, the average concentration of NO_3-N in the soil solution never exceeded 18 mg L^{-1}. Results from this study are much lower than those from a similar study conducted at SLRF, which reports between 45 and 27 mg L^{-1} in till and no till treatments, respectively for both the winter camelina and the winter camelina-soybean intercrop period (Weyers et al., 2019).

Residual NO_3-N in the soil was affected by cropping system at the 15 cm depth in maize in spring and fall of 2017 and at the 30 cm depth in soybean in spring only. In almost all instances, the highest average concentration of residual NO_3-N occurred in the control treatment. These results were significantly different in soybean only (Figure 4.9, Figure 4.10). In most instances, the average concentration of NO_3-N was higher at 15 cm than at 30 cm depth in 2017, except during fall for maize in the sequential treatment and its control. Across soil depths and dates in 2017, the sequential and control averages in soybean ranged from 3 to 8 kg N ha^{-1} and 3 to 17 kg N ha^{-1}, respectively, and in maize from 3 to 13 kg N ha^{-1} and 6 to 14 kg N ha^{-1}, respectively, while the relay and control relay averaged below 11 kg N ha^{-1} across all dates.

4.3.7 Soil N Dynamics in N-fertilized Winter Camelina Double Cropped with Maize and Soybean

In this experiment at SWROC in 2020, NO_3-N concentration in the soil solution remained relatively low and did not exceed 13 mg L^{-1} (Figure 4.7, Figure 4.8). In maize, NO_3-N concentration in the intercrop phase was reduced by 57 and 78% in the sequential and relay treatments, respectively compared to their control. In soybean, NO_3-N increased 11% in the relay and was reduced 70% in the sequential, both compared to their control. This is supported by the significantly higher biomass and grain yield of winter camelina in the sequential treatment compared to its control.

Soil residual NO_3-N in the fall was affected by cropping system at WCROC only, where the relay and sequential treatments in maize reduced NO_3-N by 47 and 33%, respectively, and in soybean by approximately 53% in both systems. In spring at SWROC, before fertilization of winter camelina, residual NO_3-N was slightly higher compared to fall levels in soybean and in maize, except in the 0–15 cm soil layer in relay and sequential maize where soil NO_3-N decreased and was significantly lower than the control. Soil residual NO_3-N after winter camelina harvest in the relay and sequential systems at WCROC was not analyzed due to quality issues with results. At SWROC, residual NO_3-N was significantly affected by cropping system in 2020 in the 0–15 cm soil layer in maize. Residual NO_3-N in the sequential treatment was significantly lower than the other treatments (Figure 4.9). During summer at SWROC, the average residual NO_3^--N in maize and soybean systems was 47, 34, and 31 kg N ha^{-1} in the relay, control relay and control sequential treatments, respectively; the lowest residual NO_3-N was observed in the sequential at 16 kg N ha^{-1}, which may have been due to less fertilizer-N applied to maize in the sequential system relative to the relay. This is notable compared to Study 1, where residual NO_3-N levels varied more narrowly between seasons.

4.3.8 Ecological Services

Winter camelina shows promise to provide numerous ecological services, depending on N fertilization strategy and levels. It is well known that overwintering crops such as cereal rye (*Secale cereale* L.) can reduce NO_3 -N vulnerable to leaching during the spring in the upper Midwest (Strock et al., 2004; Thom et al., 2018; Weyers et al., 2019). Findings from this study show that residual soil NO_3-N can also be reduced by winter camelina when grown without N fertilizer in double cropping systems. Moreover, compared to the non-fertilized study, N-fertilized winter camelina can dramatically reduce residual soil NO_3-N during the intercrop period of a rely with maize system. Soybean in Study 2 showed more plant available nitrogen (PAN) in the soil solution during the intercrop phase of the relay system in relation to its control and to Study. Residual soil NO_3-N showed large differences between Study 1 and Study 2 than soil solution PAN. For instance, in Study 2 at SWROC in 2020, the average residual NO_3-N in maize during the summer exceeded 45 kg ha^{-1} at the 15 cm depth compared to a pooled average over site-years of 22 kg ha^{-1} in Study 1 during the same period. Residual NO_3-N in soybean was less dramatic at both SWROC and WCROC locations, yet the latter exceeded 25 kg N ha^{-1} in fall at 15 and 30 cm depths in the control treatments. Overall, the lower NO_3-N in the Study 1 system may lead to reduced leaching potential.

Soil cover during spring in a maize and soybean system can reduce soil erosion (Thom et al., 2018) and suppress weed populations (Gesch et al., 2018; Saucke & Ackermann, 2006). Study 1 clearly produced less biomass and soil cover than Study 2, an indication that the positive response of winter camelina to N, supported by the almost double biomass weight produced, may result in reduced soil erosion and weed growth suppression due to a higher percentage of ground coverage. In fact, biomass in Study 2 (N-fertilized) was as high as 5000 kg DM ha^{-1}, compared to around 2500 kg DM ha^{-1} in Study 1 (non-fertilized). The average percent cover of winter camelina at maturity was 58% in Study 2 compared to 40 in Study 1 (Figure 4.4); the lowest percent cover was observed in the relay system of Study 1 at 28%.

Increased soil C stock is another ecological service that can be provided over time from diversified cropping systems and with continuous living cover. Biomass C, especially in low or no-till systems, can add to soil organic C which is an important indicator of soil quality (Dabney, Delgado, & Reeves, 2001; Reeves, 1997). Increasing soil C has the potential to help create climate smart cropping systems (Johnson et al., 2007). Due to time (two-year studies), soil C was not monitored in these studies; still, C in the biomass (without grain) of winter camelina averaged 1059 and 992 and 1600 kg ha^{-1} at WCROC in 2019 and SWROC in 2020, respectively in Study 2 (Figure 4.11). Also, the C:N ratio average was higher in Study 1 compared to Study 2 (43% compared to 11%). This impacts the stability of the C and also affects N immobilization from decaying winter camelina biomass. Ratios of 25 or below are desirable as ratios above 25 increase N immobilization. Low ratios lead to faster decay which could lead to decreased carbon sequestration (Dabney et al., 2001; Gill et al., 2006; Sainju, Whitehead, & Singh, 2005).

4.4 Tables and Figures

Table 4.1 Average properties in the 0 to 30 cm depths of soil at the two experimental sites.

Study	Site-Year	Textural class	OM (%)	pH	CEC (meq 100 g^{-1})	NO_3-N	Bray P	K	Ca	Mg
						ppm				
Study 1	SWROC 2015	Fine loam	3.9	5.2	27	10.7	20	168	2333	507
Study 2	SWROC 2018	Fine loam	3.6	5.5	20	1.5	10	95	1908	386
	WCROC 2018	Clay loam	6.4	6.2	26	10.5	18	171	3115	630

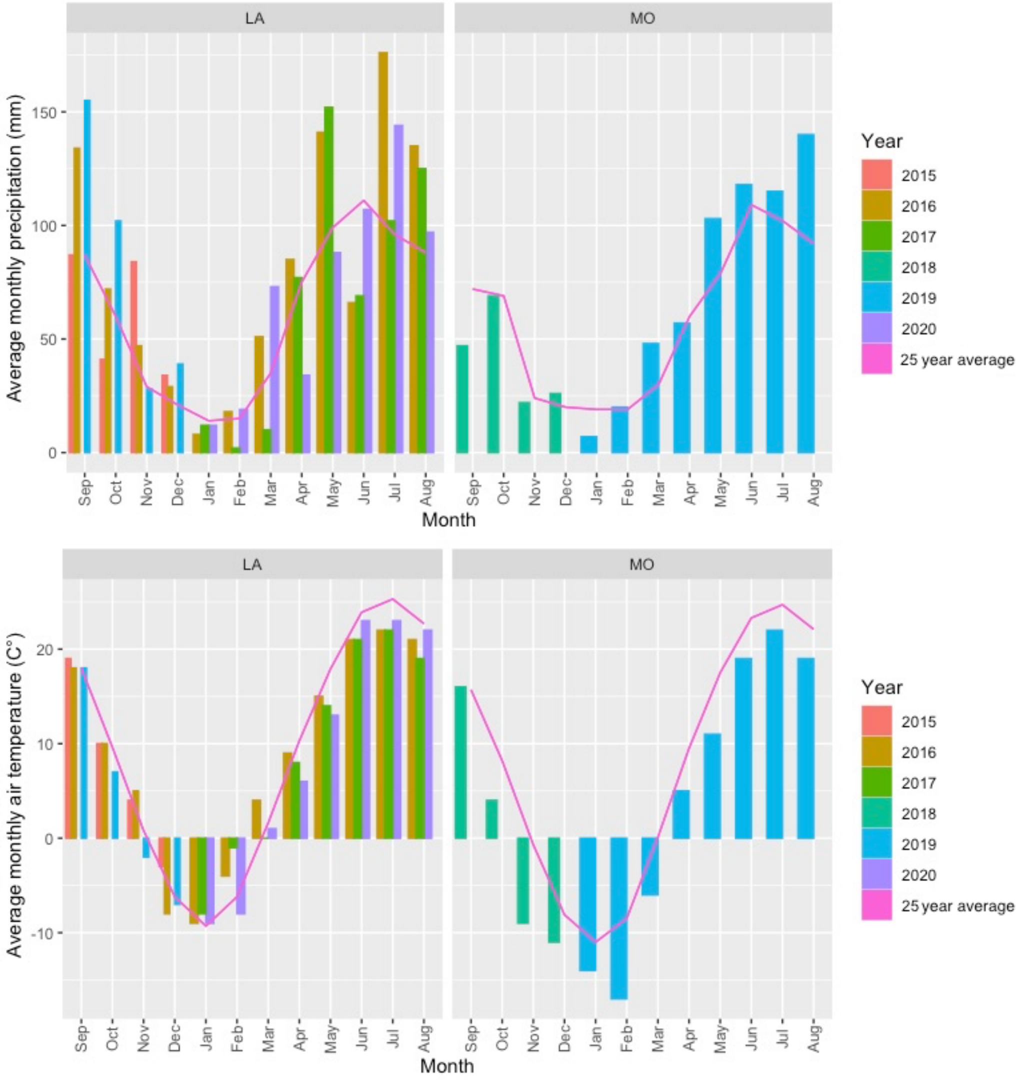

Figure 4.1 Average monthly precipitation and temperature as compared to long-term average (1994-2019) conditions at SWROC and WCROC.

Table 4.2 Deviation of monthly precipitation (mm) and average air temperature (°C) of experimental years from the long-term average (LTA, 1994-2019) conditions at SWROC and WCROC.

Month	SWROC near Lamberton						WCROC Morris		
	LTA	Deviation from LTA					LTA	Deviation from LTA	
		2015	2016	2017	2019	2020		2018	2019
Monthly precipitation (mm)									
January	14		-6	-2		-2	19		-12
February	15		+3	-13		+4	19		+1
March	35		+16	-25		+38	30		+18
April	75		+10	+2		-41	60		-3
May	99		+42	+53		-11	79		+24
June	111		-45	-42		-4	109		+9
July	96		+80	+6		+48	102		+13
August	88		+47	+37		+9	92		+48
September	87	0	+47		+68		72	-25	
October	60	-19	+12		+42		69	0	
November	29	+55	+18		-1		24	-2	
December	21	+13	+8		+18		20	+6	
Average air temperature (°C)									
January	-9		0	1		0	-11		-3
February	-6		+2	5		-2	-9		-8
March	2		+2	-2		-1	0		-6
April	10		-1	-2		-4	10		-5
May	18		-3	-4		-5	18		-7
June	24		-3	-3		-1	23		-4
July	25		-3	-3		-2	25		-3
August	23		-2	-4		-2	22		-3
September	18	+1	0		0		16	0	
October	10	0	0		-3		8	-4	
November	1	+3	+4		-3		-1	-8	
December	-6	+3	-2		-1		-8	-3	

Table 4.3 Effect of fixed effects on grain yield and biomass of maize and soybean at two locations in Minnesota. Study 1 was conducted at SWROC from 2015-2017 with non-fertilized winter camelina and Study 2 was at WCROC and SWROC from 2018-2020 with N-fertilized winter camelina.

Year	**Cropping System**[‡]	**Maize**		**Soybean**	
		Yield (kg ha^{-1})	*Bio (kg ha^{-1})*	*Yield (kg ha^{-1})*	*Bio (kg ha^{-1})*
Study 1: SWROC near Lamberton, MN – Broadcast, non-fertilized winter camelina					
2016	Relay	11853b[§]	16975a	3220b	11303a
	Sequential	8069c	16621a	2205c	6526b
	Control relay	13608a	17382a	3640a	12109a
	Control sequential	8392c	17635a	2370c	7618b
2017	Relay	7531b	13563b	2748ab	8691b
	Sequential	6969b	15311ab	2677ab	7477b
	Control relay	9791a	17460a	3016a	11447a
	Control sequential	5118c	12093b	2311b	7382b
Year (Y)		**	***	ns	***
Cropping system (CS)		***	**	***	ns
Y x CS		ns	**	***	ns
Study 2: WCROC at Morris, MN – Drilled, N-fertilized winter camelina[†]					
2019	Relay	-	1199b	-	3106a
	Sequential	-	-	-	-
	Control relay	-	2110a	-	2122a
	Control sequential	-	-	-	-
Study 2: SWROC near Lamberton, MN – Drilled, N-fertilized winter camelina[†]					
2020	Relay	-	-	2633b	4615a
	Sequential	-	10458b	-	2590b
	Control relay	13440 (± 973)	22216a	3091a	5050a
	Control sequential	-	10418b	-	2410b

Study 1 and Study 2 were analyzed independently, with locations in Study 2 also analyzed independently. Direct comparisons between studies are limited

‡ Relay cropping refers to planting a second crop (maize or soybean) into a standing first crop (winter camelina); Sequential cropping refers to planting a second crop (maize or soybean) after the harvest of the first crop (winter camelina).

§ In a column, within a year, values followed by the same letter are not significantly different at $P \leq 0.05$; *** denotes significance at 0.001, ** denotes at 0.01, and * denotes at 0.05, - denotes not available

† Relay-maize developed to R4 only; biomass of relay and control was collected on 22 July 2019. Relay-soybean developed to R7 only; biomass of relay and control was collected on 21 August 2019. The 2020 relay-maize grew weak and tall, hindering winter camelina harvest and injuring maize beyond use, leading to abandonment of that portion. The 2020 sequential-maize and its control reached R3/R4 stages while sequential-soybean and its control reached R6.

Table 4.4 Performance of N- and non-fertilized winter camelina double cropped with maize and soybean at two locations in Minnesota. Bio = biomass yield, BioC = biomass carbon, Oil = oil in grain, and Protein = protein in grain.

Year	Cropping System[‡]	**Winter camelina followed by maize**					**Winter camelina followed by soybean**				
		Yield	*Bio*	*BioC*	*Oil*	*Protein*	*Yield*	*Bio*	*BioC*	*Oil*	*Protein*
		(kg ha^{-1})			*(%)*		*(kg ha^{-1})*			*(%)*	
		Study 1: SWROC – Broadcast, non-fertilized winter camelina									
2016	Relay	494b[§]	2553a	895a	-	-	247b	1531b	556b	-	-
	Sequential	394b	2808a	1065a	-	-	480b	2871b	1024a	-	-
2017	Relay	609a	3106a	-	27.2a	21.5a	735a	3760a	-	26.7a	19.4b
	Sequential	703a	3505a	-	26.4a	27.1a	786a	3840a	-	26.7a	25.6a
		Study 2: WCROC – Drilled, N-fertilized winter camelina									
2019	Relay	875b	2965b	2476b	33.3b	20.8a	851b	3028b	2605a	35.1a	17.9a
	Sequential	1393a	3943a	3483a	35.9a	17.5a	1461a	4134a	3436a	35.9a	17.4a
		Study 2: SWROC – Drilled, N-fertilized winter camelina									
2020	Relay	1170a	4171a	1617a	31.7a	16.3a	1061b	3949a	1585a	32.7a	18.1a
	Sequential	1159a	3840a	1446a	32.7a	16.4a	1288a	4488a	1749a	32.6a	14.9a

Study 1 and 2 were analyzed independently; locations in Study 2 were also analyzed independently. Direct comparisons between studies are limited

‡ Relay cropping refers to planting a second crop (maize or soybean) into standing first crop (winter camelina); Sequential cropping refers to planting a second crop (maize or soybean) after the harvest of the first crop (winter camelina).

§ In a column, within a year, values followed by the same letter are not significantly different at $P \leq 0.05$; - denotes not available

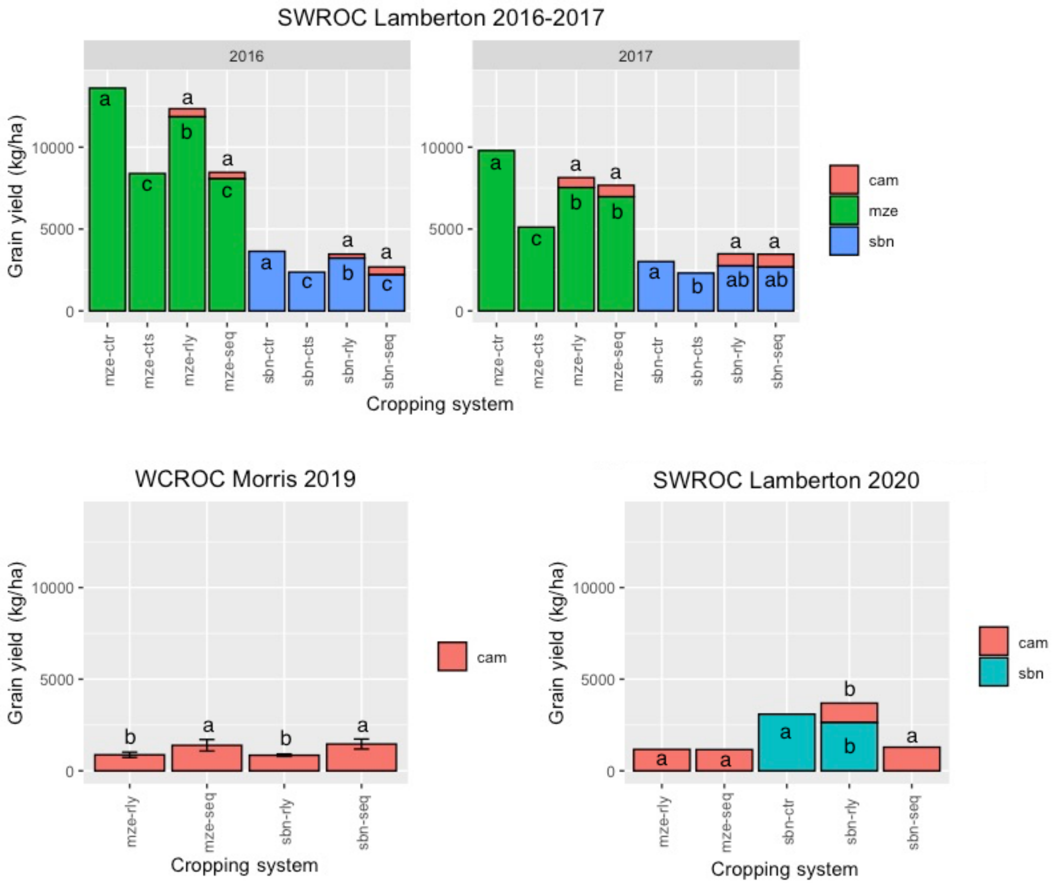

Figure 4.2 E Effect of cropping system on grain yield of winter camelina (cam), maize (mze), and soybean (sbn) at two locations in Minnesota. The SWROC 2016-217 corresponds to non-fertilized winter camelina. The WCROC 2019 and SWROC 2020 correspond to N-fertilized winter camelina. rly = relay cropping, seq = sequential cropping, ctr = monocrop control relay cropping, cts = monocrop control sequential cropping. In a given year and location, mean grain yield followed by different letters are significantly different at $P \leq 0.05$

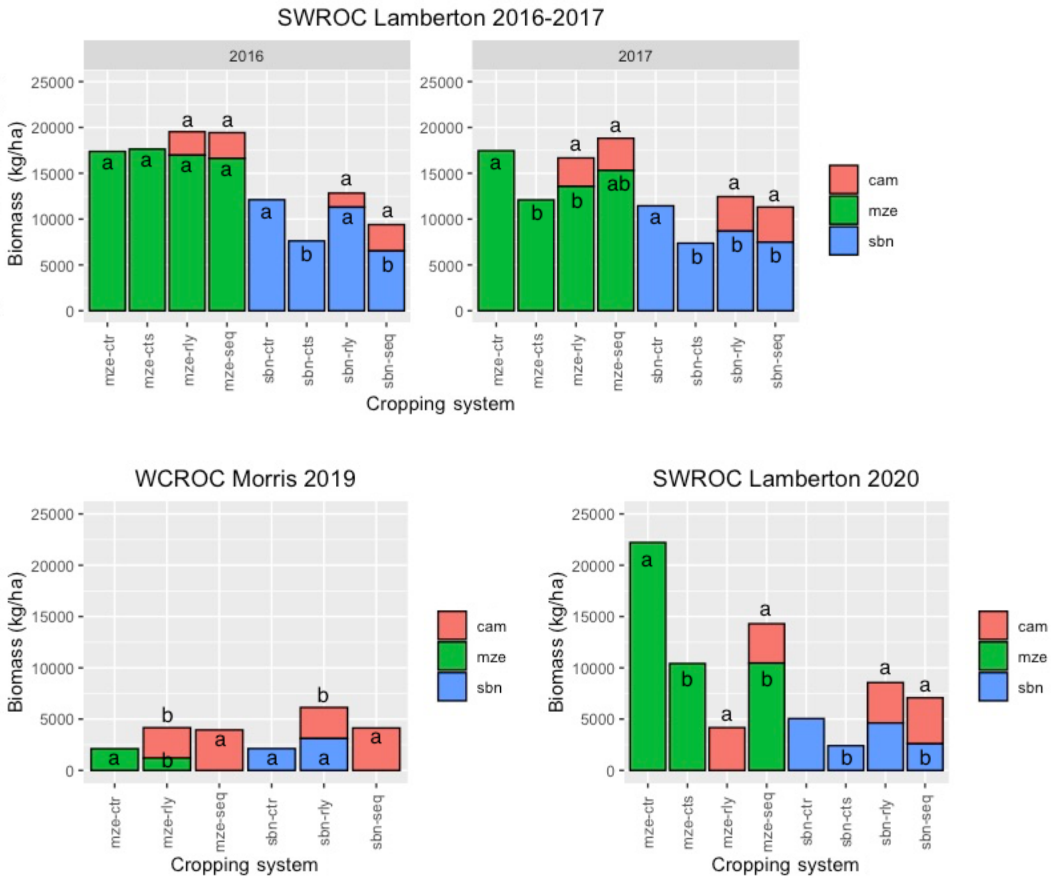

Figure 4.3 Effect of cropping system on biomass yield of winter camelina (cam), maize (mze), and soybean (sbn) at two locations in Minnesota. The SWROC Lamberton 2016-2017 corresponds to non-fertilized winter camelina, with percent cover representing maize or soybean with camelina. The WCROC 2019 and SWROC 2020 figures correspond to N-fertilized winter camelina, with percent cover only representing camelina. rly = relay cropping, seq = sequential cropping, ctr = monocrop control relay cropping, cts = monocrop control sequential cropping. In a given year and location, mean grain yield followed by different letters are significantly differences at $P \leq 0.05$.

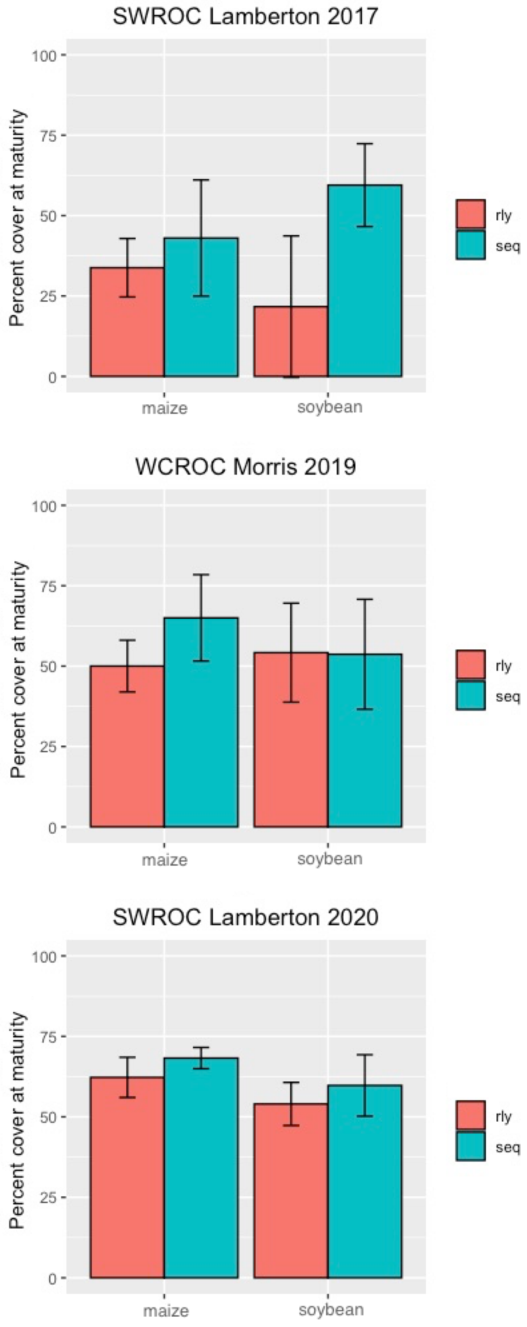

Figure 4.4 Effect of cropping system on percent cover of winter camelina in relay with maize and soybean in two locations in Minnesota. The SWROC 2016 corresponds to non-fertilized and WCROC 2019 and SWROC 2020 to N- fertilized winter camelina. rly = relay and seq = sequential cropping. Within location and year, differences were no significantly different at *P* ≤ 0.05.

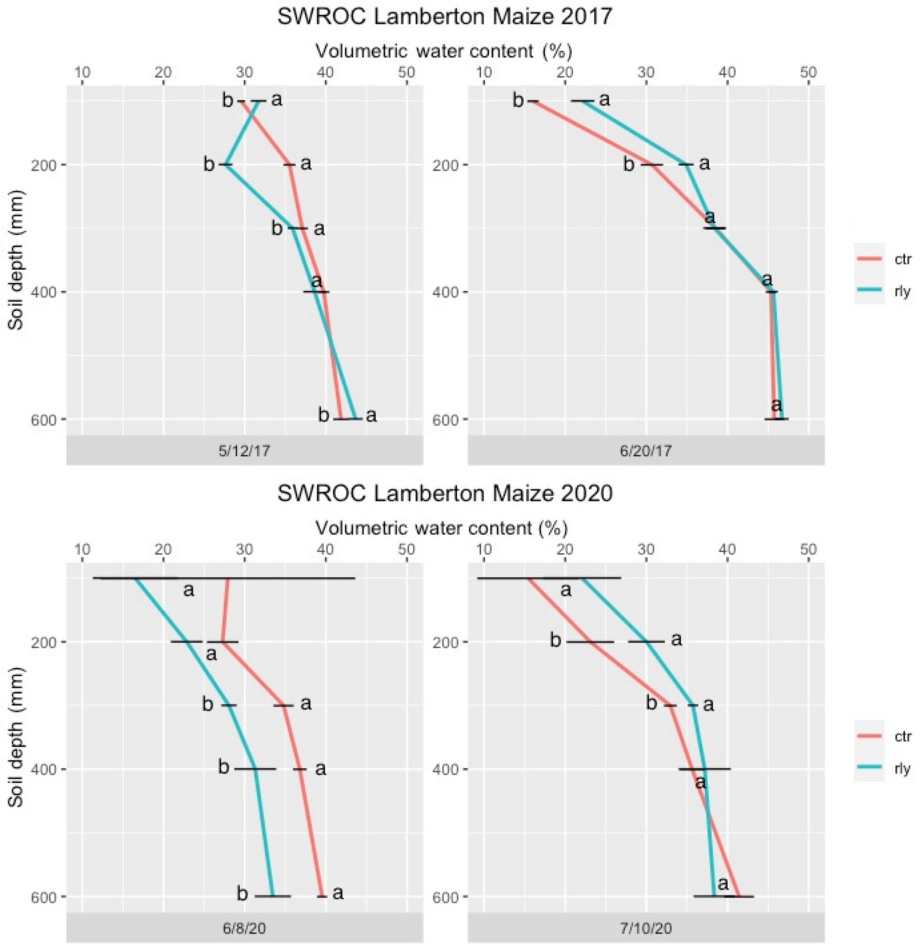

Figure 4.5 Volumetric water content in the soil profile of plots after planting and after harvesting non-fertilized (2017) and N-fertilized (2020) winter camelina in relay with maize at SWROC. ctr = control and rly = relay cropping. Within a year and date, soil volumetric water content at a given depth followed by a different letter is significantly different at $P \leq 0.05$.

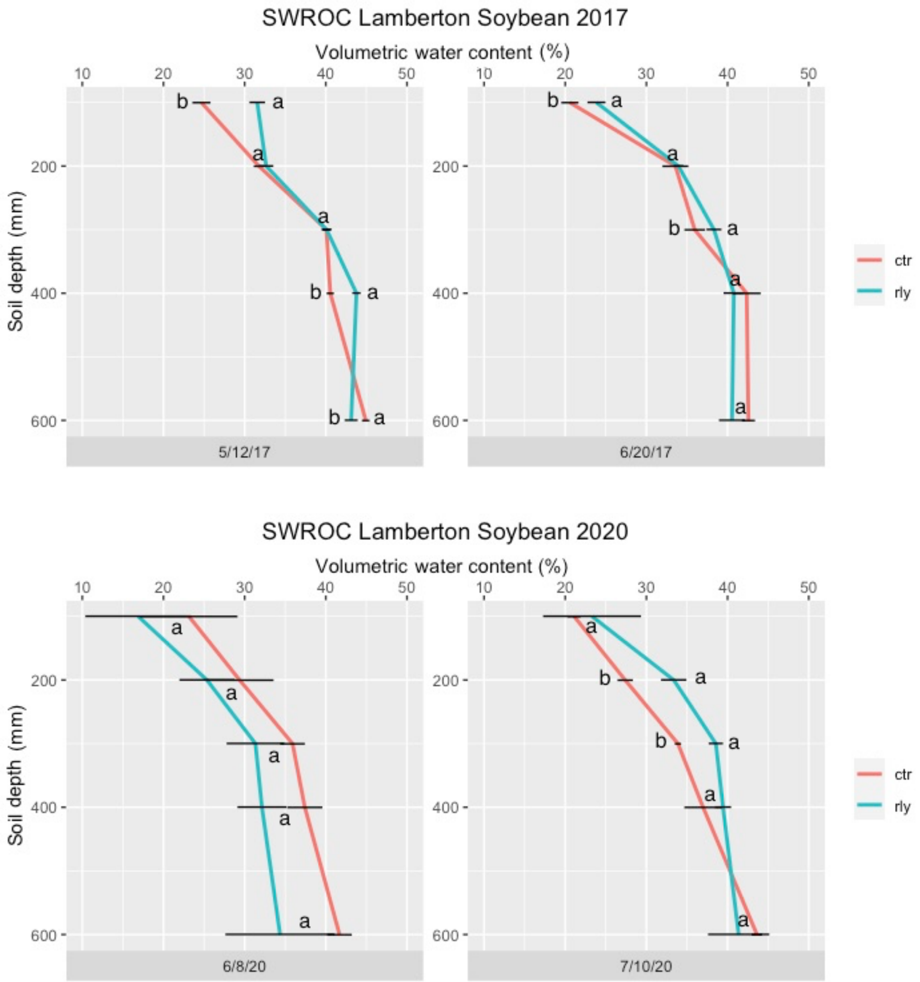

Figure 4.6 Volumetric water content in the soil profile of plots after planting and after harvesting non-fertilized (2017) and N-fertilized (2020) winter camelina in relay with soybean at SWROC. ctr = control and rly = relay cropping. Within a year and date, soil volumetric water content at a given depth followed by a different letter is significantly different at $P \leq 0.05$.

Table 4.5 Water use (WU) and water productivity (WP) of mono and double cropped crops for conditions at SWROC near Lamberton, MN. Study 1 was from 2015-2017 and Study 2 was from 2018-2020.

Year	**Cropping System**	**WU**						**WP**					
		CM‡	*M*	*C+M*	*CS*	*S*	*C+S*	*CM*	*M*	*C+M*	*CS*	*S*	*C+S*
					mm						*kg m^{-3}*		
				Study 1: SWROC – Broadcast, non-fertilized winter camelina									
2016	Relay	117	572b§	709a	126	585a	726a	0.42	-	1.74b	0.20	-	0.48b
	Control	-	594a	614b	-	584a	600b	-	2.29	2.22a	-	0.62	0.61a
	Sequential	-	-	-	-	-	-	-	-	-	-	-	-
	Control	-	-	-	-	-	-	-	-	-	-	-	-
2017	Relay	201	342b	557a	219	358b	599a	0.31	-	1.46b	0.34	-	0.58a
	Control	-	356a	460b	-	364a	489b	-	2.75	2.13a	-	0.83	0.62a
	Sequential	-	-	-	-	-	-	-	-	-	-	-	-
	Control	-	-	-	-	-	-	-	-	-	-	-	-
				Study 2: SWROC – Drilled, N-fertilized winter camelina									
2020	Relay	184a	-	-	163a	454b	497a	0.67a	-	-	0.77a	-	0.74a
	Control	-	-	-	-	479a	519a	-	-	-	-	0.63	0.59a
	Sequential	169a	-	-	158a	-	-	0.71a	-	-	0.84a	-	-
	Control	-	-	-	-	-	-	-	-	-	-	-	-

Study 1 and 2 were analyzed independently. Direct comparisons between studies are limited

‡ CM is winter camelina (C) double cropped with maize (M), C+M WU and WP of the system, CS is winter camelina double cropped with soybean (S), C + S is WU and WP of the whole system, and - denotes data not available

§ In a column, within a year, values followed by the same letter are not significantly different $P \leq 0.05$.

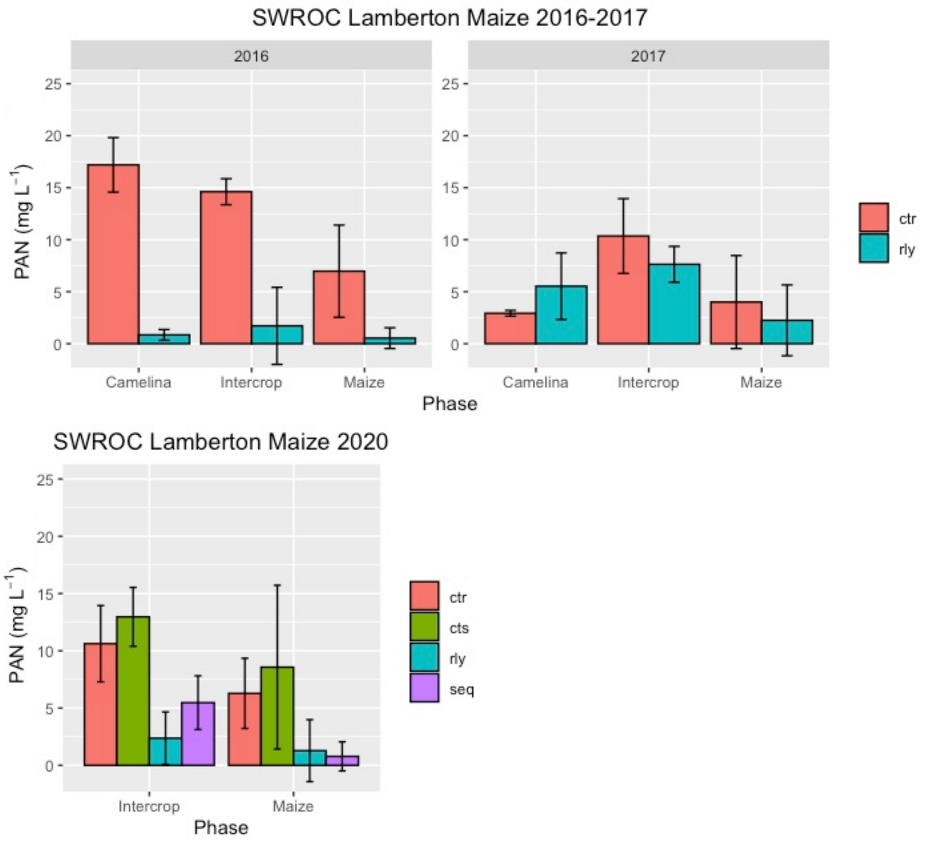

Figure 4.7 Plant available nitrogen (PAN; NO_3-N + NH_4) in mono and double cropped winter camelina with maize at SWROC in 2016 and 2017 (non-fertilized) and 2020 (N-fertilized) at different phases. Phases include camelina (from first spring regrowth to relay planting), intercrop (from relay planting to winter camelina harvest), and maize (from planting to physiological maturity of maize). ctr refers to control relay, cts refers to control sequence, rly = relay, and seq = sequential.

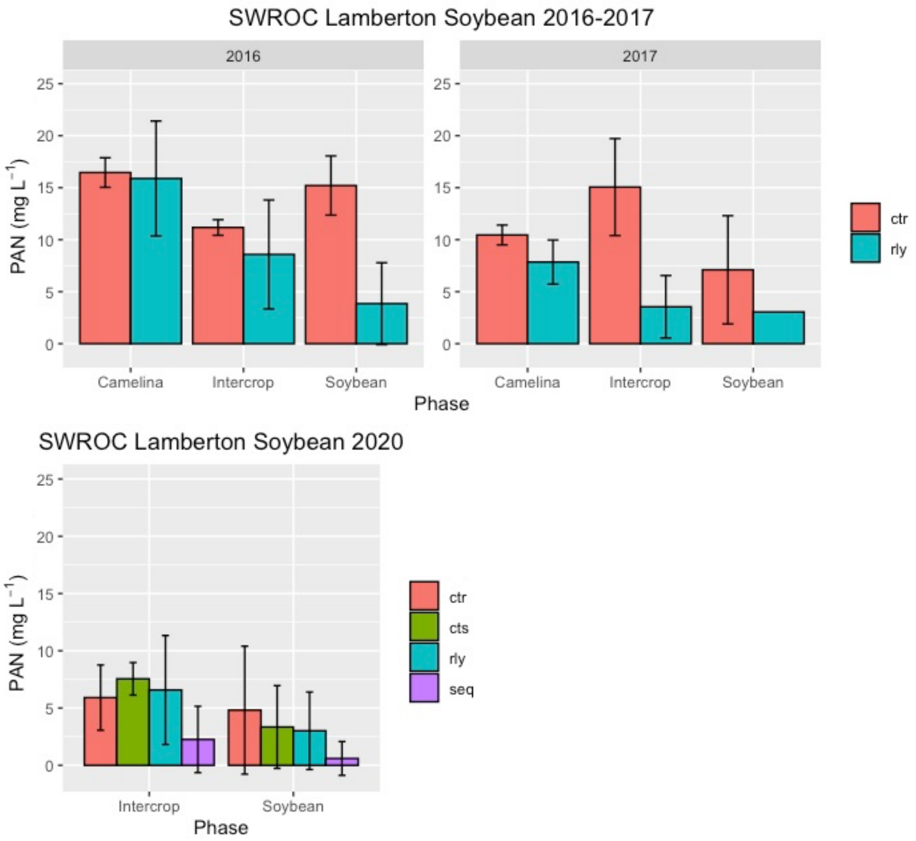

Figure 4.8 Plant available nitrogen (PAN; NO_3-N + NH_4) in mono and double cropped winter camelina with soybean at SWROC in 2016 and 2017 (non-fertilized), 2020 (N-fertilized) and WCROC 2019 (N-fertilized) at different phases. Phases include camelina (from first spring regrowth to relay planting), intercrop (from relay planting to winter camelina harvest), and soybean (from planting to physiological maturity of soybean). ctr refers to control relay, cts refers to control sequence, rly = relay, and seq = sequential.

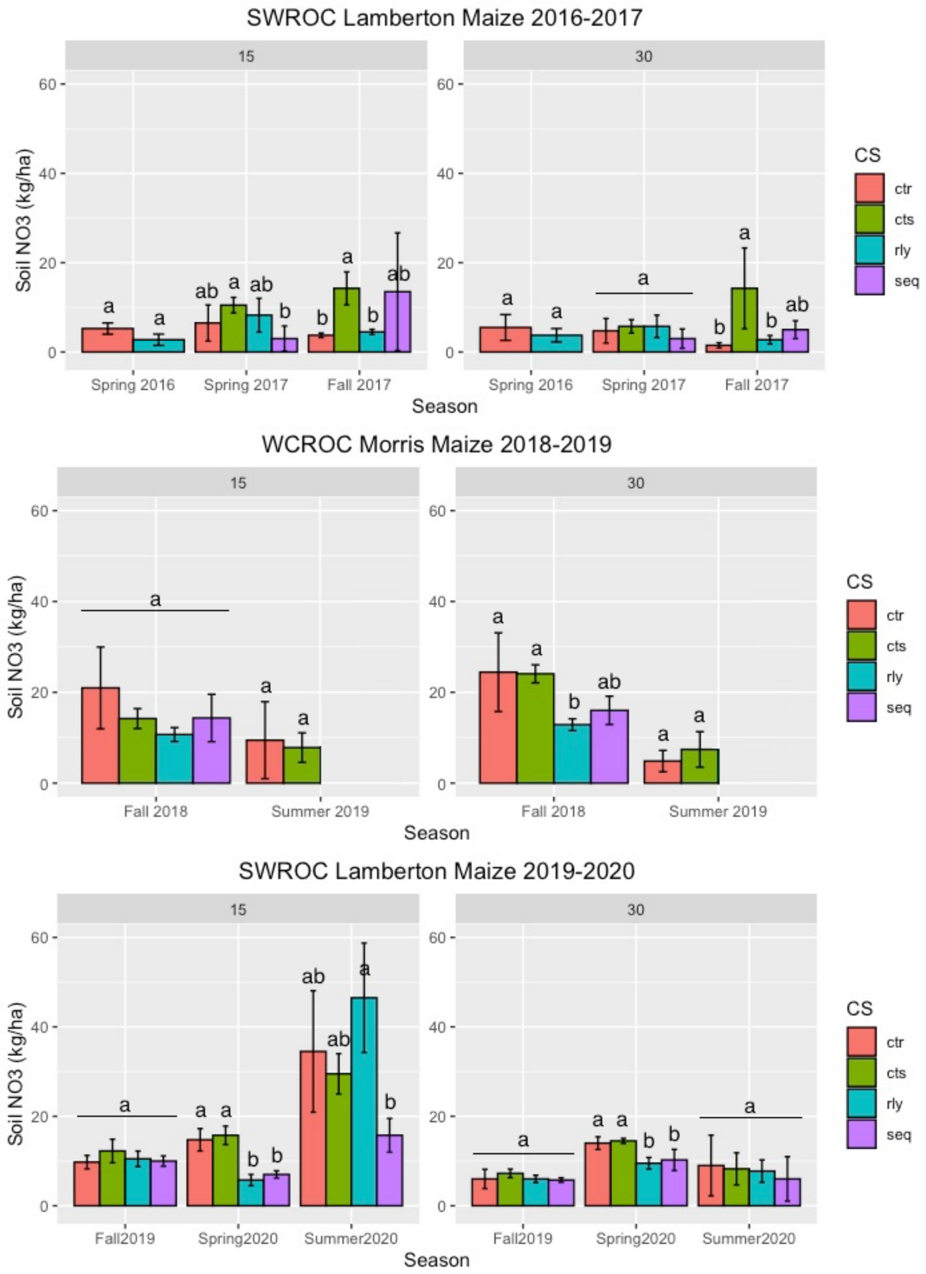

Figure 4.9 Effects of winter camelina double cropped with maize on residual soil NO$_3$-N at two depths during three seasons at SWROC in 2016 and 2017 (non-fertilized), 2019 and 2020 (N-fertilized), and WCROC (N-fertilized) in 2018 and 2019. ctr refers to relay (rly) control , cts = sequential (seq) control. In a given year and depth within a location, mean soil NO$_3$-N with different letters are significantly different at $P \leq 0.05$.

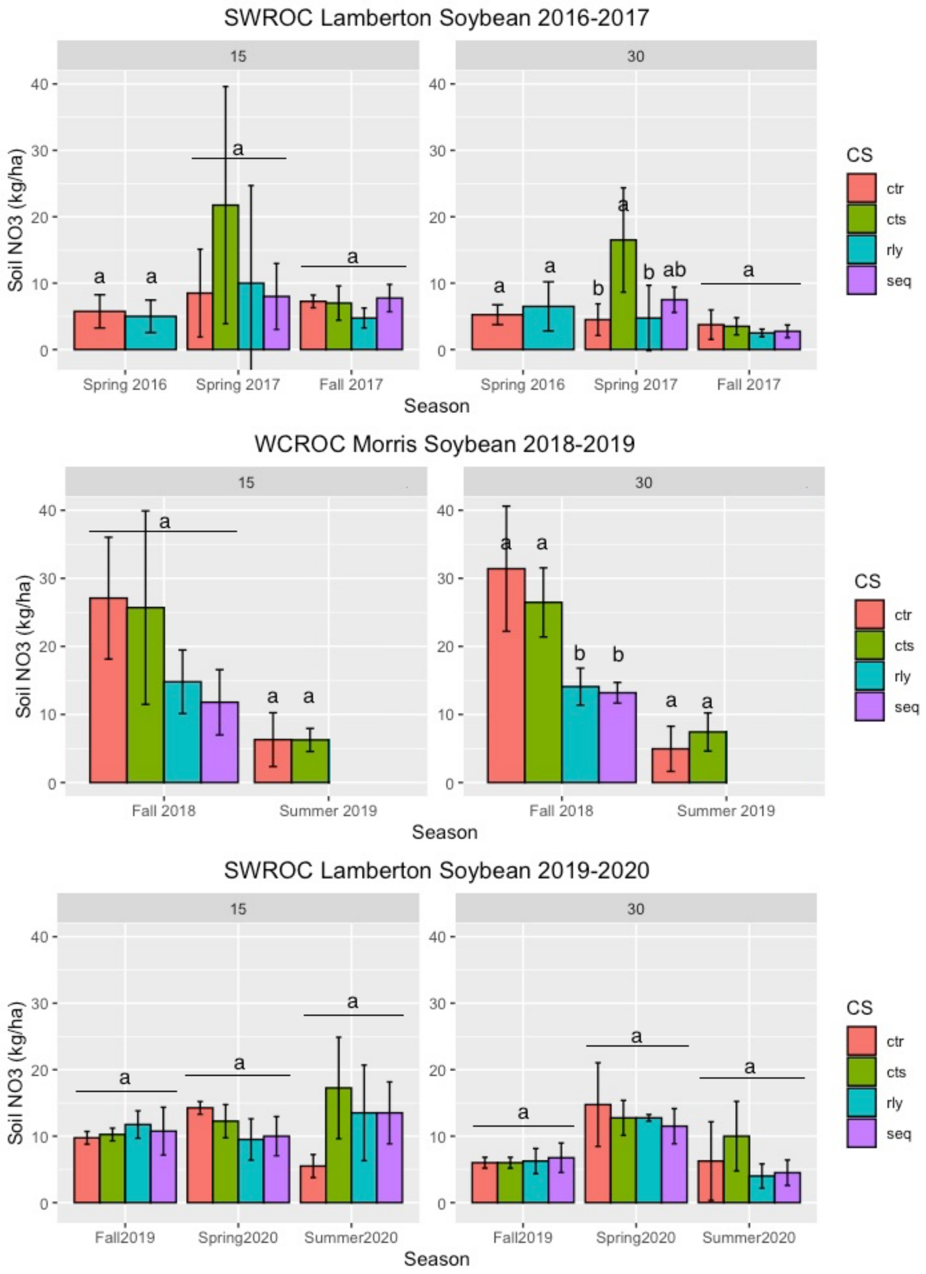

Figure 4.10 Effects of winter camelina double cropped with soybean on residual soil NO_3-N at two depths during three seasons at SWROC in 2016 and 2017 (non-fertilized), 2019 and 2020 (N-fertilized), and WCROC (N-fertilized) in 2018 and 2019. ctr refers to relay (rly) control , cts = sequential (seq) control. In a given year and depth within a location, mean soil NO_3-N with different letters are significantly different at $P \leq 0.05$.

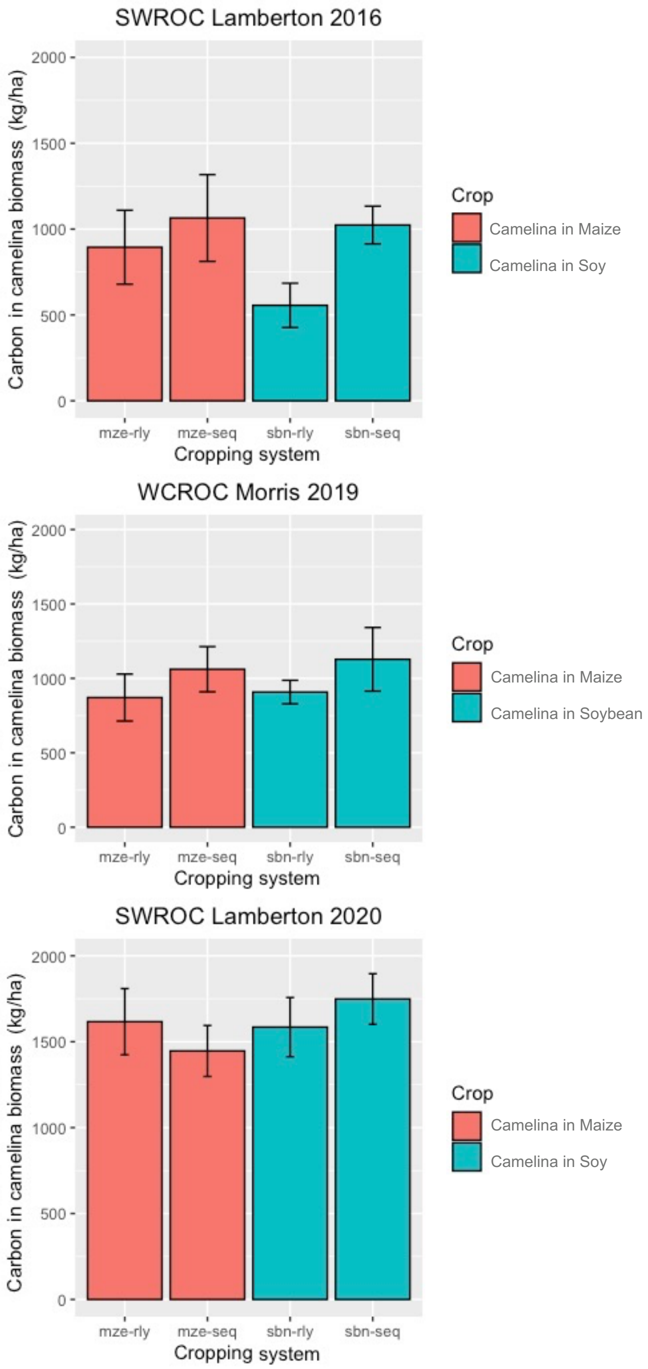

Figure 4.11 Effect of cropping system and N-fertilizer on biomass carbon of winter camelina double cropped with maize and soybean at harvest at SWROC in 2016 (non-fertilized), 2020 (N-fertilized) and WCROC 2019 (N-fertilized). Bars represent means (n=4). There were no significant differences among treatments at each site-year.

5. Chapter 5 – A practical guide to winter camelina in the upper Midwest

5.1 Introduction

Winter camelina (*Camelina sativa* (L.) Crantz) is an annual oilseed with agronomic advantages and flexibility in end-product uses from heart-healthy edible oils to biofuel. It produces seed with low resource requirements in a relatively short season (80 to 100 days), with some varieties exhibiting extreme cold tolerance comparable to that of other winter-hardy crops. It is also tolerant to some diseases that commonly affect crops in the Brassicaceae family.

Agriculture in the upper Midwest consists primarily of maize and soybean production, which compounds ecological issues and limits economic resiliency in difficult years. As agriculture in the Midwest diversifies and intensifies the current maize-soybean system to be more economically productive while being less negative to the environment, winter camelina is a crop that could result in economic and environmental benefits in production systems. Winter camelina seems to be an excellent candidate for double cropping systems in the short growing season of the region, an opportunity to produce three yields in two years while maintaining ground cover throughout the typically fallow October-May period of bare soil. This section intends to summarize the state of current management practices of winter camelina research.

5.1.1 Camelina Background and Biotypes

Camelina is thought to be native from northern and eastern Europe to northwestern Asia. Camelia and relatives (i.e., *Camelina sativa* L. and *C. linicola* K.F.Schimp. & Spenn) have an ancient history in Europe and Scandinavia, where they have been found at archaeological sites from the Bronze Age (1500–400 B.C.E). The grain from subspecies *linicola* mixed with flax (*Linum usitatissimum* L.) and other cereals is reported to provide a substantial portion of daily calories (Zubr, 1997). In ancient times, the seeds of camelina were boiled and crushed to produce oil that was consumed or used as lamp oil, among other uses. Camelina production continued in Europe and Russia into the 1940's but was replaced after World War II as higher-yielding crops like soybean became more widespread (Ehrensing & Guy, 2008). Traditional breeding techniques have been employed throughout history, but modern breeding with camelina is still limited.

Camelina can be split into two biotypes: spring and winter. While much of the research has been conducted on spring biotypes (George et al., 2017; Jiang et al., 2013; Jiang, Li, & Caldwell, 2016; Johnson et al., 2019; Malik et al., 2018), interest on its winter counterpart has been increasing in the Midwest (Berti et al., 2017; Eberle et al., 2015; Gesch & Archer, 2013; Liu, Wells, & Garcia y Garcia, 2020). Spring biotypes have been reported to produce yields ranging from 700 to 2400 kg ha^{-1} (Jiang & Caldwell, 2016; Mohammed, Chen, & Afshar, 2017; Solis et al.,2013; Wysocki et al., 2013a), while grain yield of winter camelina commonly ranges from 560 to 1700 kg ha^{-1} (Berti et al., 2015; Gesch & Archer, 2013; Gesch et al., 2018; Ott et al., 2019; Walia et al., 2018).

From planting to maturity, spring biotypes typically require 900 to 1300 degree-days to reach physiological maturity (Hunsaker, French, & Thorp, 2012; Sintim et al., 2016) while winter biotypes require 1200 to 1550 degree-days from fall planting to spring harvest (Walia et al., 2018). Both types are cold tolerant (Robinson, 1987; Karow et al. 2009), but winter camelina vernalizes during the winter before flowering and producing seed. Winter camelina has been reported to have similar winter hardiness to winter rye (Berti et al., 2016). It is this winter hardiness that has spurred interest for its use in double cropping systems in the upper Midwest.

Figure 5.1 Winter camelina growing in southwestern Minnesota on 23 May 2019

5.2 Ecological Benefits of Winter Camelina

5.2.1 Reduce Nitrate Loss

Basso, et al. (2019) estimated that in the upper Midwest 833,000 t of N and 2.98 billion USD are lost each year from fertilizing maize fields alone. This N loss causes innumerable water quality and environmental harm in farming communities and downstream (Strock et al., 2004; Turner & Rabalais, 1994). With the typical winter fallow in maize and soybean production, nitrate leaching in parts of the upper Midwest have been found to occur between April and June (Randall et al., 1997). Winter annuals are actively growing during this time and have been found to reduce N loss. For example, winter rye seeded after maize is reported to reduce nitrates in the leachate by an average of 13% in southwestern Minnesota (Strock et al., 2004). Similarly, winter camelina is reported to have reduced nitrate loss in relay with soybean compared to the till and no-till monocrop soybean(Weyers et al., 2019). Additionally, in Study 1 and Chapter 4 of this thesis, it is reported that non-fertilized winter camelina can reduce nitrate loss by roughly 30% compared to the control in maize and soybean systems across study-years, while N-fertilized camelina in sequential cropping with soybean was found to reduce nitrate loss by 70% compared to the control in one of two years (Figure 4.6, Figure 4.7). While such findings cannot be generalized yet, results are encouraging and suggest that winter camelina is indeed a promising candidate crop for conditions in the region.

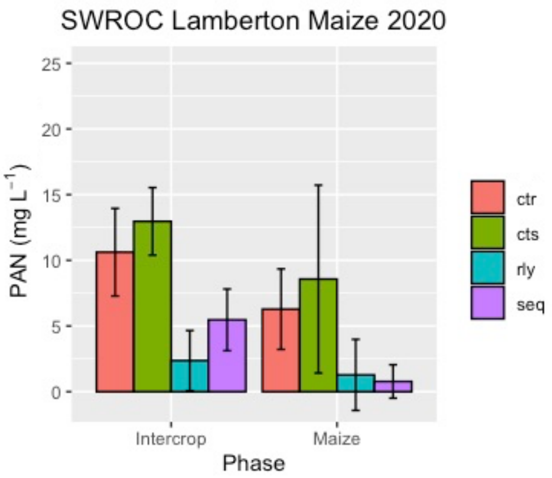

Figure 5.2 Winter camelina can reduce N in the soil solution that is vulnerable to leaching in double cropping with maize. ctr = control relay (rly), cts = control sequence (seq). “Intercrop” refers to the time when camelina and maize are growing together in a relay (~June) and “Maize” refers to maize after the camelina harvest (~July-October).

5.2.2 Soil Carbon

Soil C stock is of growing interest due to climate change. Soil has a greater ability to store carbon than any other possible sink; diversified, reduced or no-till agricultural systems have the ability to increase soil C pools. The contribution of winter camelina to soil carbon stock has not been well studied, perhaps because long-term research is needed to detect meaningful differences. It well known that the combination of sustainable practices like reduced till, cover crops, and fertility management could sequester 160 million tons of C in the soil per year in the U.S. (Post et al., 2004). Results from this thesis reported in Chapters 4 show that camelina can add over 1000 kg C ha^{-1} to the soil, depending on fertilization level and cropping system. These effects cannot be generalized but suggest the potential of the winter oilseed crop to sequester atmospheric C.

5.2.3 Pollinator Interactions

Honey bees (*Apis melifera* L.) and other pollinators are on the decline around the world, including in the U.S. (Potts et al., 2010). Part of this decline is linked to habitat loss caused by the monoculture agriculture. Diversifying maize-soybean rotations can provide additional pollen sources for insects. For example, winter camelina flowers throughout May can provide nectar for pollinators at a critical time when floral resources can be scarce (Berti, Johnson, et al., 2017; Eberle et al., 2015). Thom et al. (2018) reported that winter camelina produced more pollen than a spring biotype; pollen, protein content, and days of production were less than more nutritious flowers like borage (*Borago officinalis* L.) and echium (*Echium plantagineum* L.), but were produced at a much earlier and more critical time, attracting beneficial insects such as hoverflies. Similarly, Eberle et al. (2015) in a study conducted in Morris, MN reports that winter camelina had the highest agroecosystem value compared to field pennycress and winter canola (*Brassica napus* L.) when considering pollinator provisioning, spring cover, and final yield.

5.3 Growth and Development

5.3.1 Growth Requirements

Camelina can grow in marginal soils, including places where canola cannot grow (Malhi et al., 2014; Solis et al., 2013). Optimal planting date for winter camelina has been found to be mid-September (Walia et al., 2018) to early October (Gesch & Cermak, 2011). Camelina is able to germinate well at temperatures as low as 4°C, producing vigorous seedlings, but only spring camelina germination studies currently exist. For example, spring camelina emergence was found to be 100% at temperatures ranging from 4 to 27°C, declining to 80% at 32°C; emergence time varied from 9 days at 4°C to 2 days at 16°C and above (Russo, Bruton, & Sams, 2010).

Studies for the base, optimum, and maximum (cardinal) temperatures of winter camelina have not been conducted, but 0°C, 36°C, and 28°C, respectively are reported for spring biotypes (Tribouillois et al., 2016). Practically speaking, these temperatures appear to apply to winter camelina as well. Similarly, studies on winter camelina light requirement have not been conducted, but it is thought that low light conditions found under the canopy of early reproductive stages of maize may contribute to low stand establishment when winter camelina is late-interseeded into corn (Mohammed et al., 2020; Patel et al., 2021).

Winter camelina has been found to respond positively to N, with rates varying between 70 and 90 kg ha^{-1}. Based on previous winter camelina and canola (a close relative of camelina) research, 34 kg ha^{-1} of phosphorous is sometimes recommended in the upper Midwest, while potassium is usually not applied (Gesch & Archer, 2013; Gesch et al., 2014; Knodel, 2011). In soils with low to very low potassium (0 to 80 ppm), however, 56 to 160 kg K ha^{-1} are used in canola production. Overall, the interest in N-fertilization of winter camelina has increased, but requirements are still not well known (Gesch & Archer, 2013; Liu, Wells, & Garcia y Garcia, 2020; Ott et al., 2019; Walia et al., 2018). To the best of our knowledge, there are no studies on winter camelina requirements for phosphorous, potassium, or sulfur. As discussed in Chapter 2 of this thesis, yield of winter camelina in Minnesota seems to be highest at N rates between 67 and 100 kg ha^{-1} (Figure 2.2).

Winter camelina does not grow well in saturated soils. Some research reports that yield is more negatively affected when soil is too wet in early spring rather than dry conditions during that period (Gesch & Archer, 2013; Gesch & Cermak, 2011; USDA, 1998). Spring camelina used in dryland cropping systems is reported to produce over 1000 kg grain ha^{-1} (McVay & Khan, 2011; Mohammed et al., 2017). However, complete crop failure has also been reported due to extreme drought (Wysocki et al., 2013). Winter camelina typically requires 1200 to 1300 degree-days to reach physiological maturity and an additional 150 to 250 degree-days for seed dry enough for harvest (Walia et al., 2018).

5.3.2 Physiology

Camelina seed does not exhibit dormancy, which allows for use in cropping systems without becoming a weed (Iskandarov, Cahoon, & Kim, 2014; Robinson, 1987). Winter camelina grows as a rosette in the fall, producing leaves and side shoots on 19 nodes before vernalization will occur (Martinelli & Galasso, 2011). Vernalization is gene-regulated and occurs at or below 4°C temperature for the commonly used Joelle cultivar, which requires around four weeks of vernalization (Anderson et al., 2018). Winter camelina plant height has been shown to fall within the range reported for spring camelina, approximately 60 to 80 cm, and is largely affected by cultivar. Greater height has been reported with low to medium seeding rates (~300 to 650 seeds m^{-2}) (Gesch et al., 2018; Vollmann, Moritz, Kargl, Baumgartner, & Wagentristl, 2007).

Stem elongation takes 7-10 days in Minnesota and is followed by anthesis, and flowering period occurring over roughly three weeks. Flowers bloom starting at the bottom of the plant and continue up the stem as the silicles (seed pods) form and develop, also from the bottom to the top. Timespan between full flower to full-sized silicle is around 5 days at 25°C (Martinelli & Galasso, 2011). After reaching final size, silicles take an additional 150 to 250 degree-days to dry enough for harvest. It was found in this study that both, branching and silicle formation respond positively to N and negatively to planting density (Table 2.10; Gesch et al., 2018).

Camelina has a relatively large tap root with smaller lateral roots which do not typically go deeper than 0.9 m, although milder climates than those found in the upper Midwest may provide conditions for deeper and more extensive roots (Zanetti et al., 2020). A study conducted in Morris, MN and Bologna, Italy reports that 50% of winter camelina roots were found in the top 10 cm of soil in the former location, but a higher proportion of roots was found deeper at the latter location; root biomass averaged 4.66 Mg ha^{-1} across treatments in Morris, MN and 7.75 Mg ha^{-1} in Bologna, Italy (Zanetti et al., 2020).

5.3.3 Grain Quality

Winter camelina seed contains relatively high levels of oil and protein. Oil yield is typically of the most interest because of its high-quality, which is relatively high in omega-3 fatty acids (FA) and low in saturated FAs (Ehrensing & Guy, 2008). This oil is suited for human consumption or for industrial uses such as fuel or lubricant. The FA makeup of winter camelina oil by highest to lowest proportion consists of linolenic and linoleic (omega-3 and -6 FA, respectively), followed by oleic, gondoic, and erucic acids. Erucic acid is a potentially harmful FA that is typically found in amounts under 5%. However, erucic acid levels in camelina vary between 1.1 to 2.2% (Gesch et al., 2018; Walia et al., 2018), which is close to the < 2% standard set for canola, and suggests that breeding will be needed to further reduce erucic acid content for widespread adoption of winter camelina oil as an edible oil. Oil yield typically varies from 27.1 to 42.5%, depending on weather, soil and cultivar (Figure 2.6; Table 4.6; Gesch et al., 2014; Gesch & Cermak, 2011; Walia et al., 2018; Zanetti et al., 2020). Oil content has been found to be negatively correlated to N and protein content, due to competition between the FA and protein synthesis pathways (Figure 2.6; Jiang, Caldwell, & Falk, 2014; Johnson et al., 2019).

The byproduct of camelina oil production is a seed meal that can be used as a protein supplement in animal feed. The seed meal of camelina contains lower levels of glucosinolates (GS), another potentially harmful compound typically found in brassicas that could limit both animal and human consumption (Jiang et al., 2016; Singh, Bala, & Rai, 2014; Tripathi & Mishra, 2007). In animals, high GS levels have been found to negatively affect growth and/or performance of some livestock: pigs have been found to be most consistently affected by high levels of GS, compared to ruminants, rabbits, poultry, and fish (Tripathi & Mishra, 2007). Levels of GS in spring camelina are reported between 11–44 μmol g^{-1} (Jiang et al., 2016; Lange et al., 1995; Singh et al., 2014) and are negative correlation with N (Jiang & Caldwell, 2016). As a comparison, *E. sativa* L., a relative of camelina that is used as a salad green or biofuel feedstock, is reported to have

GS at level of 115 μmol g^{-1} (Singh et al., 2014). Protein levels in winter camelina have been reported to range from 12 to 28% (Chapter 2, Gesch et al., 2014; Gesch et al., 2018; Walia et al., 2018; Zanetti et al., 2020).

5.3 Table: Winter Camelina Varieties with Sources

Cultivar	Notes
Joelle Origin: USDA Source: University of Minnesota	Most commonly used in Minnesota. Extremely winter hardy and adaptable. Greatest plant height, which is important for relaying. Greatest seed oil content. (Eberle et al., 2015; Gesch et al., 2018; Ott et al., 2019; Zanetti et al., 2020)
Bison Origin: Colorado Source: High Plains Crop Development, Torrington, WY	Greatest yield, similar flowering time to Joelle, not as winter hardy. Shorter than Joelle. Large seed size. (Gesch et al., 2018; Wittenberg et al., 2019)
BSX-WG1 Origin: Colorado Source: North Dakota State University Research and Extension Center	Lower winter hardiness, seed yield, and oil content compared to Joelle. (Gesch et al., 2018; Kurasiak-Popowska et al., 2018; Sintim et al., 2016)
HPX-WG1-35 Origin: Colorado Source: High Plains Crop Development, Torrington, WY	Lower winter hardiness, earlier flowering, lower oil content, greater seed protein content than Joelle and Bison. Low yield, lower plant height. (Gesch et al., 2018)
HPX-WG4-1	Less winter hardiness compared to Joelle. Earlier flowering and greater seed protein content than Joelle and Bison (Gesch et al., 2018)
Przybrodzka	Polish cultivar that many mutation lines were derived from. Unknown winter hardiness compared to Joelle. High levels of linolenic acid found (46.7%). Listed in some studies as winter cultivar, and in others as spring. (Kurasiak-Popowska, Graczyk, & Stuper-Szablewska, 2020; Kurasiak-Popowska et al., 2018; Wiwart et al., 2019)
Luna	Mutation line from Przybrodzka, with a similar, but potentially more consistent yield. Unknown winter hardiness compared to Joelle. Lower 1000-seed weight. (Wittenberg et al., 2019; Kurasiak-Popowska et al., 2018; Wiwart et al., 2019)
Maczuga	Mutation line of Przybrodzka, higher yielding than Przybrodzka. Unknown winter hardiness compared to Joelle. (Kurasiak-Popowska et al., 2018; Kwiatek et al., 2021)
Zolta	Mutation line of Przybrodzka, higher yielding than Przybrodzka. Unknown winter hardiness compared to Joelle. (Kurasiak-Popowska et al., 2018)
Kozyr	Russian cultivar with higher oil content than Russian spring cultivar. Unknown winter hardiness compared to Joelle. (Prakhova et al., 2018)

5.4 Management Practices

5.4.1 Preparation and Planting

Weed control is necessary in preparation for planting, and the most effective method is chemical control. However, long-lasting soil herbicides should be avoided because camelina is currently sensitive to many commonly used herbicides (USDA, 2012). Glyphosate [N-(phosphonomethyl)glycine] (0.75 to 1.1 kg a.e. ha^{-1}) and trifluralin [2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)aniline] (1.13 kg a.e. ha^{-1}) as a pre-emergent have been used successfully (Gesch et al., 2018; Robinson, 1987; Thom et al., 2018).

Seedbeds, if tilled, should be packed with a roller packer or empty seeder to create an even surface. Drill seeding is recommended and often results in suitable establishment, one necessary step to successful growth and yield (Berti et al., 2017; Gesch & Cermak, 2011; Liu, Wells, & Garcia y Garcia, 2020; Ott et al., 2019; Weyers et al., 2019).

Winter camelina seeding rate is somewhat flexible, depending on the desired outcome. It has been reported that seed rates as low as 3.4 kg ha^{-1} yield as well as higher rates (Gesch et al., 2018); however, 6 to 9 kg ha^{-1} seems to be the most common rate used to encourage strong stands (Berti et al., 2015; Berti et al., 2017; Walia et al., 2018; Zanetti et al., 2020).

Results from research conducted in the region show that seeding date affects emergence, flowering and maturity of winter camelina. For example, seeding 27 to 42 days apart is reported to promote early flowering, something within 10 to 11 d of each other (Gesch & Cermak, 2011; Sintim et al., 2016). If sown earlier than September, there is a very high probability of failure because it will not survive the winter (Wittenberg, Anderson, & Berti, 2020). Sowing in early October, seems to increase population density compared to mid-October and early to mid-September, though the reasons are unclear (Gesch & Cermak, 2011). Sowing early-September is reported to be key for biomass production and reduction soil residual N, both important features of cover cropping (Wittenberg, Anderson, & Berti, 2020). Overall, high seed yield is associated to winter camelina fall sowing as early as September and as late as early October (Gesch & Cermak, 2011; Wittenberg, Anderson, & Berti, 2020). Seeding winter camelina after maize or soybean harvest rather than into standing crops may be best for both crops, as competition is reduced and camelina has been found to reach maturity at a similar time as earlier seedings (Berti, Samarappuli, et al., 2017).

The recommended seeding depth is should be less than 1.5-cm; greater depths may reduce stand, and therefore yield (Gesch et al., 2017; Berti et al., 2017; Sintim et al., 2016; Zanetti et al., 2020). Row spacing ranges from 19 to 30 cm for either mono or double cropping systems (Gesch & Archer, 2013; Gesch et al., 2014, 2018; Ott et al., 2019; Zanetti et al., 2020; Liu, Wells, & Garcia y Garcia 2020)

Tillage practice has varying results winter camelina performance, which is often due to weather. No-till has shown to provide denser stands, higher winter survivability and earlier flowering, with no effect on yield. If excessive precipitation falls in spring, conventional till appear to drain more quickly and provide conditions for higher yields (Gesch & Archer, 2013; Gesch & Cermak, 2011).

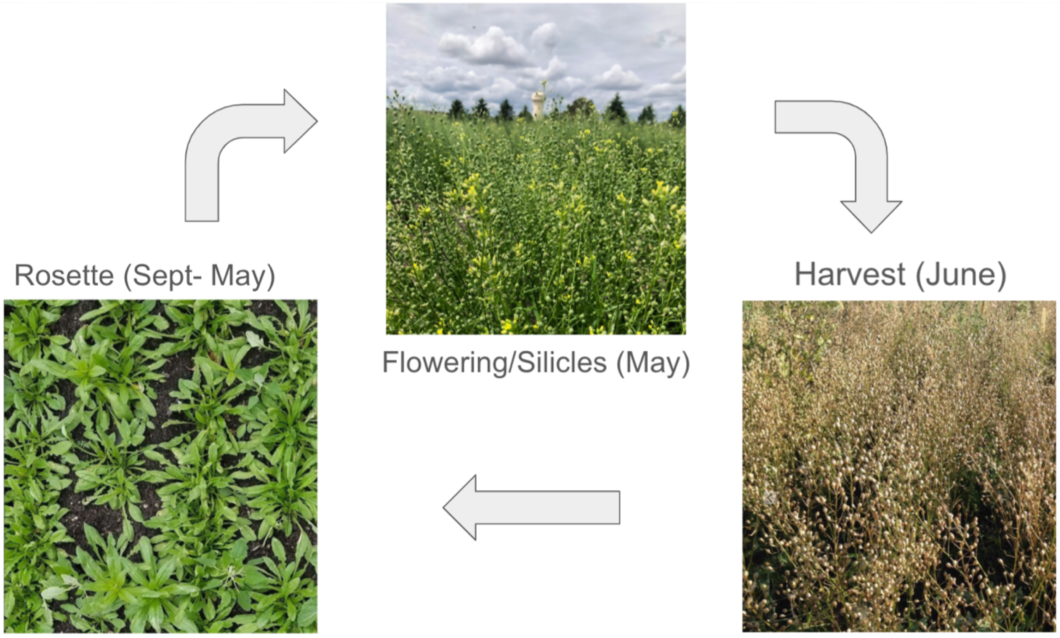

Figure 5.3 Life cycle of winter camelina in the upper Midwest

5.4.2 Harvest Timing, Techniques, and Seed Shatter

Winter camelina is typically harvested for grain yield when 80 to 90% of silicles are dry and yellowish-brown with reddish-brown color seeds, which typically occurs in mid-June to mid-July (Berti et al., 2016; Gesch & Cermak, 2011; Johnson & Gesch, 2013; Walia et al., 2018) and corresponds to phenological stages BBCH807-809 (Martinelli & Galasso, 2011). Yield can be harvested with a combine used with other oilseed brassicas such as flax, rape, mustard, and canola with the inclusion of a 3 mm lower sieve attached (Eynck & Falk, 2016; Robinson, 1987). Adjusting the header on the combine to the maximum height will reduce camelina stems plugging issues, and reducing airflow can minimize loss of the small seeds (Enjalbert & Johnson, 2011).

Seed shatter in the literature is not considered to be a problem (USDA, 2012; Zubr, 1997); however Sintim et al. (2016) found a 24% reduction in yield harvested when silicles were 90% dry due to the combine loss. Conversely, Johnson et al. (2017) on a study conducted in Minnesota, reported that harvesting late (90% of silicles dry) generally resulted in higher yields compared to harvesting 10–13 days earlier; the early stage is not described, but would be expected to be below 70% ripeness. In Walia et al. (2018), optimal yield of winter camelina was reported to have been reached at 1200–1300 accumulated degree-days, which corresponded to mid-June in Minnesota, grain moisture content to allow for mechanical harvesting was reached later. Swathing or desiccation are possible solutions for maximizing winter camelina yield while also shortening the time until harvest to better fit into double cropping systems in the upper Midwest. Walia et al. suggested 48% moisture content as an ideal time to swath or apply a shorten harvest time. An earlier study in Minnesota showed that swathing and desiccation added additional costs to double cropping and had no effect on camelina yield (Gesch et al., 2014). Once harvested, moisture levels of 8% are ideal for storing camelina seed (Enjalbert & Johnson, 2011).

5.4.3 Pests and Diseases

Camelina is considered to be resistant or tolerant to many pests and diseases that affect other brassicas. Weeds can be a complicating factor because many camelina varieties are susceptible to broadleaf herbicides (Sobiech et al., 2020), but dense fall seeding rates have shown to reduce weed stands the following year (Gesch & Cermak, 2011). Seeding above 3.4 kg ha^{-1} has shown to reduce weeds (Gesch et al., 2018), although yield may not increase at higher rates. Seeding rates as high as 11.2 kg ha^{-1} are reported to reduce cold season weeds through early summer by 88% (Hoerning et al., 2020).

Spring camelina has been found to be resistant to highly resistant to many diseases such as blackleg [*Leptosphaeria maculans* (Desmaz.) (Ces. & de Not.)] (Salisbury, 1987), a significant disease in canola, as well as alternaria blight [*Alternaria brassicae* (Berk.) Sacc.] (Eynck & Falk, 2016; Narasimhulu et al., 1994; Putnam et al., 1993). Robinson (1987) reported that camelina is susceptible to downy mildew [*Hyaloperonospora camelinae* Gäum] and aster yellows [*Candidatus Phytoplasma* asteris], both of which have the potential to lower yields. Camelina has been reported to transmit turnip yellow mosaic virus through its seeds (Hein, 1984). Camelina is susceptible to clubroot (*Plasmodiophora brassicae* Woronin), white rust (*Albugo candida*), sclerotinia stem rot (*Sclerotinia sclerotiorum* Lib. De Bary), brown girdling root rot (*Rhizoctonia solani* Kühn) (Eynck & Falk, 2016). Eynck and Falk (2016) reported that genotypes have been found with resistance to some of these diseases and that breeding work should be done to create resistant cultivars. It is worth mentioning that most disease-related studies have been conducted on spring camelina biotypes, and response differences between spring and winter biotype are still unknown.

Camelina has also been found to be tolerant to brassica insect pests like flea beetle [*Phyllotreta cruciferae* (Goeze)] (Robinson, 1987) and cabbage root fly [*Delia brassicae* Wiedemann (Diptera: Anthomyiidae)] (Finch, 1978).

Additionally, camelina growth may reduce beneficial and non-beneficial soil microbes. For example, an 8-year study on winter wheat and spring camelina followed by a fallow period showed reduced total microbial abundance, which returned to normal levels when practices returned to a winter wheat-fallow rotation (Hansen et al., 2020). Laboratory studies have shown that winter camelina to consistently reduce soil cyst nematode populations by an average of 48% (Acharya, Yan, & Berti, 2019).

5.4.4 Cropping Systems

Winter camelina research has focused s primarily in double cropping systems for grain production, but some results, including those in this thesis, suggest that winter camelina could be used as a cover crop in current or novel rotation practices. Double cropping can be relay or sequential cropping. The former refers to planting the second crop into the first crop before harvest with an overlapping phase, so the mature first crop is harvested over the second crop growing underneath. The latter refers to planting one crop after the other with no overlapping growth phase. For example, in sequential cropping winter camelina is planted after harvest of a summer annual then, winter camelina is harvested the following year in early summer before planting another summer annual. In upper Midwestern states like Minnesota, summer annuals like maize and soybean would have to be shorter season to allow for both crops in the sequence to produce a yield. In relay cropping for example, a summer annual like maize or soybean can be relayed into standing winter camelina in spring, when the latter is at the BBCH 71 stage (early maturing silicles) in the upper Midwest. Similarly, winter camelina can be relayed into a standing summer annual in late season, usually at maize R5-R6 and soybean R7-R8 stages of development. Great success has been shown in relay cropping winter camelina with soybean compared to maize, due to relative ease of harvesting camelina over soybean, and the higher level of light that can reach the relayed camelina through a soybean canopy compared to maize (Berti, Samarappuli, et al., 2017; Gesch & Archer, 2013; Liu et al., 2020).

Although limited, research on double cropping winter camelina with crops other than maize and soybean exist and include oilseed sunflower, forage or grain millet, and forage sorghum. Oilseed sunflower double cropped with winter camelina was found to produce higher net returns compared to forage/grain millet or soybean (Gesch and Archer, 2013). Berti et al. (2015) report that energy efficiency was highest for forage sorghum relayed with winter camelina when compared to 12 different mono- and sequential-cropping strategies.

Winter camelina has been studied much less without N-fertilization as either a grain crop or cover crop. The only study in the upper Midwest to date (Liu, Wells, & Garcia y Garcia, 2019)found that winter camelina as cover crop produced significantly less biomass than winter rye in a maize rotation. Winter camelina, even when fertilized, has been found to reduce soil available N similarly to winter rye, matching winter rye for spring N uptake (Liu, Wells, & Garcia y Garcia, 2019; Thom et al., 2018; Weyers et al., 2019).

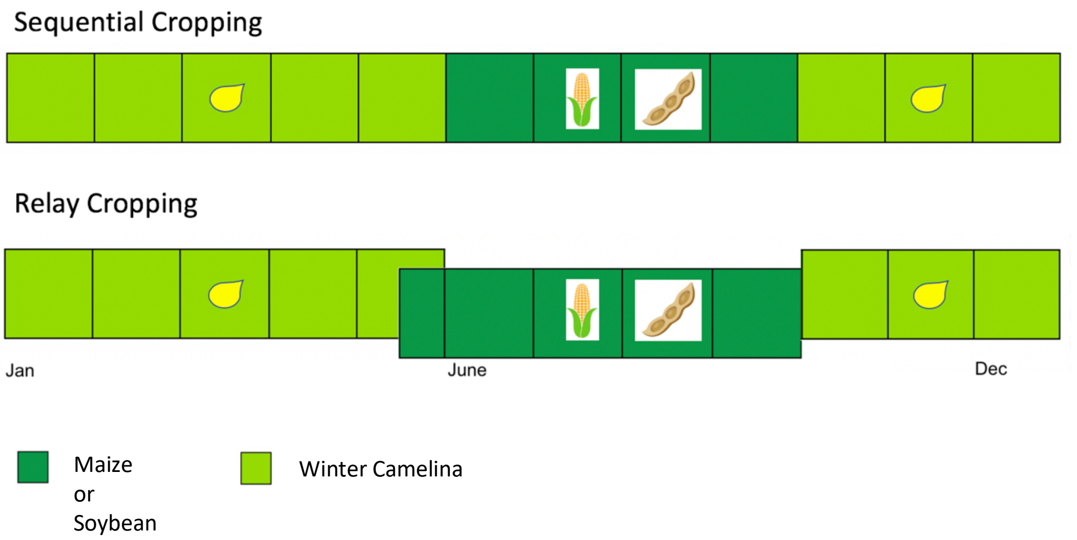

Figure 5.4 Double cropping with winter camelina and maize or soybean year schematic

5.5 Economics

Studies have shown that winter camelina in double cropping (relay) with other crops can produce more overall yield (camelina + relayed crop) than monocrops, and has the potential to lead to a higher economic return (Berti et al., 2015; Gesch et al., 2014; Ott et al., 2019). With demand increasing for alternative fuels, growing winter camelina with other oil-producing crops such as soybean could make a higher net return more likely.

No market currently exists for industrial scale camelina seed or meal, and contracts would be required to sell the final product. As a result, economic analyses often use the price of canola as representative (Gesch et al., 2014; Ott et al., 2019), so such results should be taken with caution. That said, it is reported that winter camelina double cropped with soybean produced 29 to 39% higher net returns, depending on tillage, compared to monocropped soybean in one of two experimental years (Gesch and Archer,

2013) and could produce 50% higher oil yield, although costs among double cropping systems averaged 186% higher per hectare than the monocropped soybean (Gesch et al., 2014).

In a more recent agronomic and economic study in Minnesota, researchers found that with 600-1100 kg ha^{-1} grain yield, camelina double cropped with soybean provided similar net returns to monocropped soybean (Ott et al., 2019). With more inputs required in a double cropping system, improvements on the agronomic, economic, or policy level may be needed for this system to be financially appealing for farmers in the near term.

In an economic study on the feasibility of a farmer growing spring camelina to produce on-farm fuel in the western US, the meal byproduct was found to have the greatest impact on profitability, until diesel prices reached USD 0.89 L^{-1} in 2013 dollars (Keske et al., 2013). Similar studies for conditions in the Midwest do not currently exist.

5.6 Future Research

Agronomic research for winter camelina has been increasing in the last 15 years, but many basic aspects of the crop need additional investigation. Far more spring camelina cultivars have been bred for adaptation to varying climates compared to winter camelina, where the winter cultivar most often used is Joelle. More research is needed on the outcomes and economics of winter camelina grown as a cover crop in order to expand near-term possibilities for winter camelina in the upper Midwest. Additional studies looking at NUE and N in the environment are needed. While pest and disease research on spring camelina exists and possibly could be extrapolated to winter camelina, specific research on the latter has not been conducted for conditions in the upper Midwest.

Similarly, the effect of winter camelina on soil microbial communities is needed, as little is known on the effect of winter camelina on soil microbial communities. For winter camelina to succeed in the region, breeding to develop shorter season, high yielding varieties is ultimately needed. Additionally, biotic and abiotic factors seem to affect the quality of winter camelina grain, which calls for specific studies to advance our understanding on this issue.

6. Chapter 6 – Conclusions

6.1 Chapter 2

This study determined the response of winter camelina yield, yield components, and yield quality, to N and fertilization timing application. Nitrogen significantly increased biomass and canopy cover. The derived maximum yield was found at 97 kg N ha^{-1}, however yield was generally not significantly higher beyond the 33 or 67 kg ha^{-1} rates. Yield at the highest rate of 135 kg N ha^{-1} was not significantly different to yield from other treatments in any instance. Results showed that a rate of 33 kg ha^{-1} would likely produce a similar yield to higher N rates and therefore would be expected to be more beneficial environmentally as well. Nitrogen did not affect harvest index in any location or year. Yield quality reported here supports previous research, showing that oil content decreased with an increase in N, while protein generally increased. Protein had a slight positive correlation with N but appears to be affected by more factors beyond N rate compared to oil content.

Grain yield was strongly correlated to silicles per plant, but branches per plant, and seed:shell ratio showed positive correlation as well. The spring-only fertilizer application tended to affect yield components. Branches per plant showed a strong significant correlation with yield, but results correspond to one year at two locations only. The 1000-seed weight was affected by N rate in all locations, but results were highly variable within a location, resulting in a weaker correlation to yield compared to other components.

6.2 Chapter 3

This study compared the effect of N-fertilizer rates and fertilizer application timing (fall/spring split and single spring application) on winter camelina N uptake, N use efficiency, and residual soil N. The fall/spring split application was found to reduce residual N in 3 of 5 site-years, but these results were not significantly different. Nitrogen use efficiency was decreased with N rates beyond 67 kg N ha^{-1}, especially agronomic efficiency. Nitrogen recovery in camelina decreased with increasing N application. Residual soil N increased with increasing N application, especially in the top 15 cm layer. N uptake in winter camelina increased with N application and was higher than applied N at SWROC, but not at SLRF, which reinforces research that shows winter camelina is quite capable of scavenging N. Fertilization rate should be balanced with yield to create a recommendation that takes agronomic and environmental effects into account.

6.3 Chapter 4

This study compared the growth and development of N-fertilized and non-fertilized winter camelina double cropped with maize and soybean as related to N, WU and WP in the system. Nitrogen-fertilized winter camelina produced much higher grain yield, biomass, and percent cover compared to non-fertilized winter camelina. Water use increased with the incorporation of winter camelina, when comparing double-cropped camelina to the control in both studies. Water productivity was typically higher in the fertilized than the non-fertilized winter camelina, mainly due to higher yields.

Plant available nitrogen in the soil solution was generally reduced in the relay plots compared to the control in the non-fertilized study, with low levels of residual soil NO_3^-N found as well. In the fertilized study, N in the soil solution was dramatically reduced in the relay and sequential plots compared to controls during the intercrop phase; in some instances, soil solution N was higher in the relay than the control. Residual soil NO_3-N was generally higher in the fertilized study, especially in the summer after harvest.

Double cropping winter camelina with maize was found to be difficult due to synchrony of crops development and timing of relay that resulted in mechanical injuries to winter camelina and a short growing season for the sequential crop to reach maturity, resulting in marginal to no yield. Across studies, soybean was harvested in three of four site-years, but with yield penalties likely due to resource competition between the two crops. Relayed summer annuals tended to produce higher yields due to the longer growing time from earlier planting.

Double cropping winter camelina is a promising system for the region, but more research is needed to overcome the challenge of reliably harvesting two crops in a single short season in the upper Midwest. Cropping systems beyond maize and soybean with winter camelina could also help extend winter camelina viability in the region.

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