

Article

Double-Cropped Winter Camelina with and without Added Nitrogen: Effects on Productivity and Soil Available Nitrogen

Stephen Gregg ¹, Jeffrey A. Coulter ¹, Jeffrey S. Strock ^{2,3}, Ronghao Liu ^{3,4} and Axel Garcia y Garcia ^{1,3,*}

¹ Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108, USA

² Department of Soil, Water, and Climate, University of Minnesota, Saint Paul, MN 55108, USA

³ Southwest Research and Outreach Center, University of Minnesota, Lamberton, MN 56152, USA

⁴ College of Water Resources Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China

* Correspondence: axel@umn.edu

Abstract: Double cropping winter camelina (*Camelina sativa* (L.) Crantz) with maize (*Zea mays* L.) and soybean (*Glycine max* L. (Merr.)) is a diversification strategy in northern regions. Winter camelina is reported to have low nutrient requirements, but its nitrogen (N) needs are not well understood. Studies on winter camelina without (Study 1) and with (Study 2) N fertilization were used to compare growth, seed yield and quality, and effects on soil N. Study 1 was conducted from 2015 to 2017 at one location and Study 2 was conducted from 2018 to 2020 at two locations. Grain yield was as much as six times higher in Study 2 compared with Study 1; averaged across treatments, winter camelina yielded 1157 kg ha⁻¹ in Study 2 and 556 kg ha⁻¹ without N. Oil and protein content ranged from 26.4 to 27.2% and 19.4 to 27.1%, respectively, in Study 1 and from 31.7 to 35.9% and 14.9 to 20.8%, respectively, in Study 2. N fertilizer increased winter camelina biomass and grain yield and soil N when double cropped with maize and soybean. Our study indicates that grain yield of winter camelina respond positively to N fertilization in a northern location. The drawback of this is the increase in residual soil N, which suggests the need for further research to balance agronomic practices with environmental outcomes.

Keywords: nitrate; relay cropping; sequential cropping; winter oilseed crop; water quality; biofuel crops



Citation: Gregg, S.; Coulter, J.A.; Strock, J.S.; Liu, R.; Garcia y Garcia, A. Double-Cropped Winter Camelina with and without Added Nitrogen: Effects on Productivity and Soil Available Nitrogen. *Agriculture* **2022**, *12*, 1477. <https://doi.org/10.3390/agriculture12091477>

Academic Editor: Lorenzo Barbanti

Received: 27 July 2022

Accepted: 7 September 2022

Published: 15 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The winter fallow period in the dominant maize (*Zea mays* L.)–soybean (*Glycine max* L. (Merr.)) rotation in the U.S. upper Midwest has contributed to significant soil erosion and water quality decline [1–3]. The most vulnerable time for erosion and nutrient loss is typically in the spring or early summer, when there is no or little crop biomass on the landscape and when high precipitation is common [4–6]. One strategy that is gaining traction for reducing nutrient loss and soil erosion is to add a winter annual crop to summer annual crop rotations. This is because winter annual crops are uniquely suited to summer cropping systems in the short growing season of the upper Midwest due to their ability to grow, overwinter, and yield before or soon after planting a summer annual [7–10].

Double cropping maize or soybean with a winter annual crop can increase ground cover and reduce nutrient loss and soil erosion from the fallow period [1,11]. This system could provide additional incentive to farmers due to the potential income from growing three crops in two growing seasons. A lack of economic return on time and money invested is a primary reason why farmers do not adopt cover crops [12]. Double cropping can be relay or sequential. Relay cropping refers to planting a second crop into an established first crop, which allows for longer-maturing summer annuals but also increases crop competition due the period of overlapping growth, e.g., maize or soybean interseeded into a winter annual approaching maturity [13,14].

In a relay system, for example, a winter annual like camelina (*Camelina sativa* (L.) Crantz) is harvested over the top of the growing summer annual, which then has full access to nutrients, sunlight, and water. Sequential cropping refers to planting a second crop soon after the harvest of the first, e.g., maize or soybean after the harvest of winter camelina. Sequential cropping eliminates the overlapping period but often requires a shorter-season second crop [11], a strategy that can result in low yield of the summer crop but high total yield [15]. Relay cropping typically results in higher total yield than sequential cropping because full-season crops produce more than short-season crops [13,15].

Winter camelina is one of the winter annual oilseeds that has shown promise in double-cropping systems in the U.S. upper Midwest due to its extreme cold tolerance. The crop has a long history in regions with similar weather such as Ukraine and Russia [16–18] and central Canada [19]. Winter camelina seed has high oil content, which provides production flexibility due to its potential use as a heart-healthy edible oil or as feedstock for biofuel [20,21].

Winter camelina is considered a low-input crop [22], but evidence shows that it responds to N fertilization [10]. Fertilization at 70–90 kg N ha^{−1} is usually reported in research trials [1,7,22] as yields have been low in non-fertilized studies [11,15,23]. Yet current research has not determined the N fertilizer requirement of winter camelina for conditions in the upper Midwest. In agricultural fields in the region with a high potential for NO₃–N leaching due to precipitation, the use of tile drainage, and low spring biomass levels [1,24], careful consideration of N fertilization is needed to increase environmental benefits in agroecosystems. That is, N fertilization of winter camelina could further compromise, from an environmental perspective, the sustainability of corn and soybean production practices in the region.

However, winter camelina is reported to reduce N losses in cropping systems. In a 2014 to 2015 study, for example, non-N-fertilized winter camelina double-cropped with soybean was found to reduce soil NO₃–N concentrations down to 60 cm by 53 to 72% in the fall and 18 to 19% in the spring compared with monocrop soybean [9]. Similarly, a study of relay soybean with winter camelina fertilized at 90 kg N ha^{−1} is reported to have significantly reduced soil NO₃–N concentration (by roughly 93–95%) compared with monocrop soybean throughout the growing season, except after camelina harvest [1].

As of 2022, studies comparing the production of non-N- versus N-fertilized winter camelina and their effects on environmental quality have not been conducted in the upper Midwest. The objectives of this study were to compare (i) growth and seed yield and quality and (ii) effects on soil N of winter camelina with and without N-fertilization when double cropped with maize and soybean. The hypothesis was that N fertilization would have a positive effect on the growth and grain yield and quality of winter camelina with no effect on soil N.

2. Materials and Methods

2.1. Experimental Sites

Experiments with winter camelina double cropped with maize and soybean were conducted from 2015 to 2017 without N fertilization (Study 1) at one location and 2018 to 2020 with N fertilization (Study 2) at two locations. 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 are characterized as Normania loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Amiret loam (fine-loamy, mixed, superactive, mesic Calcic Hapludolls) [25]. Study 2 was conducted at SWROC and at 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 are characterized as Sandberg sandy loam (sandy, mixed, frigid Calcic Hapludolls) (Table 1) [25]. The long-term average (LTA; 1994–2019) annual air temperature and precipitation are 10.8 °C and 716 mm at SWROC and 9.5 °C and 676 mm at WCROC,

respectively (Table 2) [25]. The winter hardiness zone is 4b for SWROC and 4a for WCROC, with the latter denoting slightly cooler air temperatures [26].

2.2. Experimental Design

Winter camelina was relay and sequential cropped with maize and soybean in rotation; both phases of the rotation were present in all site years. Within a major crop, experiments were conducted as randomized complete block design with four replications. Plot sizes were 20 m × 6 m in Study 1 and 6 m × 9 m in Study 2. Treatments in both studies included relay (rly), sequential (seq), control–relay (ctr), and control–sequential (cts) maize and soybean. Additional information about the maize phase in Study 1 can be found in Liu, et al. 2020 [23].

2.3. Agronomic Management

Study 1

This study was initiated in the fall of 2015 following oat (*Avena sativa* L.). Winter camelina (var. Joelle) was hand broadcast and then raked to enhance 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 [10,15,27]. Winter camelina was seeded at a rate of 13 kg ha^{−1} and harvested in the second half of June in both years, when more than 90% of silicles were brown and dry. No N fertilizer was applied to winter camelina to enhance agroecosystem benefits; soil P and K levels were both considered high (averages of 20 and 167, respectively) in the soil test category. Both maize and soybean were relay planted when winter camelina was at the BBCH50 (inflorescence still enclosed by leaves) stage of development [28].

Maize cultivars DKC49–72RIB (99 relative maturity) and DKC31–10RIB (81 relative maturity) were planted at 86,500 seeds ha^{−1} using a four-row (76 cm) John Deere 1100 Max-Emerge planter with row cleaners on 19 May 2016 and 12 May 2017 and in mid-June of both years in the relay (and control) and sequential (and control) treatments, respectively. The seedbed for the sequential treatments was prepared with a disc harrow. Maize was fertilized with urea at 135 kg N ha^{−1} in mid-June in 2016 in the relay treatments and mid-July in 2017 in the sequential treatments and was harvested in early October to late November during both years.

Soybean cultivar Stine 20RD20 (2.0 maturity group) was planted using a four-row (76 cm) John Deere 1100 MaxEmerge planter with row cleaners at 373,000 plants ha^{−1} on 19 May 2016 and 16 May 2017 and 22 June 2016 and 21 June 2017 on the relay and sequential treatments, respectively, on seedbeds prepared with a disc harrow. Soybean received no fertilizer and was harvested in early October to late November during both years.

Table 1. Average properties of the top 30 cm of soil at the SWROC and WCROC.

Study	Site–Year ‡	Soil Textural Class	OM [†] (%)	pH	CEC (meq 100 g ^{−1})	NO ₃ –N ----- ppm -----	Bray P	K	Ca	Mg
Study 1	SWROC 2015	Loam	3.9	5.2	27	10.7	20	168	2333	507
Study 2	SWROC 2018	Loam	3.6	5.5	20	1.5	10	95	1908	386
	WCROC 2018	Sandy loam	6.4	6.2	26	10.5	18	171	3115	630

‡ SWROC = Southwest Research and Outreach Center near Lamberton, MN; WCROC = West Central Research and Outreach Center in Morris, MN. † OM = organic matter; CEC = cation exchange capacity; NO₃–N = nitrate nitrogen; P = phosphorous; K = potassium; Ca = calcium; Mg = magnesium.

Table 2. Deviations from the long-term averages (LTA) of monthly precipitation and average air temperature (AT) at the experimental locations. Gray values denote periods of no field studies.

Month	Southwest Research and Outreach Center						West Central Research and Outreach Center		
	----- Study 1 -----						----- Study 2 -----		
	LTA (1994–2019)	2015	2016	2017	2019	2020	LTA (1994–2019)	2018	2019
	Precipitation (mm)	Deviation (mm) from LTA					Precipitation (mm)	Deviation (mm) from LTA	
January	14	−2	−6	−2	−3	−2	19	−16	−12
February	15	−11	3	−13	30	4	19	3	1
March	35	−25	16	−25	33	38	30	−3	18
April	75	−43	10	2	83	−41	60	−43	−3
May	99	39	42	53	15	−11	79	−25	24
June	111	18	−45	−42	6	−4	109	108	9
July	96	0	80	6	21	48	102	66	13
August	88	25	47	37	−32	9	92	−11	48
September	87	0	47	−32	68	27	72	−25	97
October	60	−19	12	89	42	−35	69	0	8
November	29	55	18	−27	−1	10	24	−2	−15
December	21	13	8	−11	18	−10	20	6	6
Average Air Temperature (°C)		Deviation (°C) from LTA					Average Air Temperature (°C)	Deviation (°C) from LTA	
January	−9	2	0	1	−3	0	−11	0	−3
February	−6	−6	2	5	−9	−2	−9	−4	−8
March	2	0	2	−2	−6	−1	0	−3	−6
April	10	−1	−1	−2	−3	−4	10	−10	−5
May	18	−4	−3	−4	−6	−5	18	−1	−7
June	24	−4	−3	−3	−3	−1	23	−2	−4
July	25	−3	−3	−3	−3	−2	25	−4	−3
August	23	−3	−2	−4	−3	−2	22	−2	−3
September	18	1	0	0	0	−2	16	0	0
October	10	0	0	−1	−3	−5	8	−4	−2
November	1	3	4	−1	−3	2	−1	−8	−2
December	−6	3	−2	−2	−1	2	−8	−3	−1

Study 2

This study was initiated in the fall of 2018 following annual ryegrass (*Lolium multiflorum* L.) at SWROC and spring wheat (*Triticum aestivum* L.) at WCROC. Due to an excessively wet early spring at SWROC in 2019 and fall at WCROC in 2019, winter camelina did not establish, and no data were collected from either location. 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 on 23 September 2019 at SWROC. At both sites, fertilizer for camelina was surface applied and non-incorporated in all plots at the BBCH 12 to 20 stages (rosette to single true leaves developing) in mid-May, a strategy reported to maximize seed yield in the field and greenhouse [7,27,29]. The fertilizer was a 6.5–14–27 (N–P–K) blend of monoammonium phosphate and potash with urea added to reach 100 kg N ha⁻¹ at WCROC in 2019 and 78 kg N ha⁻¹ at SWROC in 2020. Winter camelina was harvested when >90% of silicles were brown and dry, first using 1 m² quadrats for a hand harvest in both locations and then by combine in mid-July at WCROC in 2019 and in early July at SWROC in 2020.

Maize cultivar 2417 VT2P RIB (85 d relative maturity) 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) John Deere 1100 MaxEmerge planter with row cleaners, which was used for all maize and soybean planting. Fertilization for the maize consisted of 157 kg N ha⁻¹ at planting with no P or K. No fertilizer was applied for soybean. Maize and soybean were sequentially planted in mid-July following the harvest of camelina. Glyphosate [N-(phosphonomehtyl)glycine] at 1.28 kg a.e. ha⁻¹ was applied on 19 July 2019; then, eventual weeds were removed periodically by hand. Late harvest of winter camelina due to unfavorable weather conditions delayed planting of both relay maize and soybean, neither of which reached physiological maturity. Consequently, only total biomass was collected.

At SWROC, maize DKC47–54RIB (97 d relative maturity) was used in the relay treatment, while DKC29–89RIB (79 d relative maturity) was used in the sequential treatment. Maize and soybean were planted on 2 June 2020 and 13 July 2020 for the relay and sequential systems, respectively. Soybean AG06X8 (0.6 maturity group) was planted in both relay and sequential treatments 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 (NH₂–CO–NH₂), P₂O₅ as triple superphosphate [Ca(H₂PO₄)₂ · H₂O], and K₂O as muriate of potash (KCl): 40% N at planting and the remaining at V5; 2 July for relay and 4 August for sequential. Glyphosate at 2.8 kg a.e. ha⁻¹ was applied for weeds 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 applied for control of soybean 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.

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 all treatments (including controls) except in spring 2016, when samples were only 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 using a push probe (1.7-cm diameter, JMC Soil Samplers, Newton, IA, USA) and separated into 0–15 cm and 15–30 cm layers. Subsamples from each layer were mixed to make a composite sample and allowed to air dry before being ground to pass a 2-mm screen using a Dynacrush soil crusher (Custom Laboratory Equipment Inc., Holden, MO, USA). Samples were analyzed for NO₃–N, pH, organic matter (OM), Bray–1 P, cation exchange capacity (CEC), K, Ca, and Mg [30].

Biomass was collected for winter camelina, maize, and soybean at maturity in both studies and all locations. Biomass for winter camelina was obtained by harvesting 0.5 m² in 2016 to 2017 and 1 m² in 2019 and 2020. Winter camelina seed was adjusted to 10% moisture. Biomass for maize was obtained by harvesting the two central rows in 2016 and 2017 and six plants per plot in 2019 and 2020. Biomass for soybean was obtained by harvesting 0.5 m² in all studies. Biomass from all crops 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 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, USA). For winter camelina, protein and oil content were obtained by pulsed nuclear magnetic resonance (NMR) in both studies (Minisper MQ20; Bruker, Ettlingen, Germany for Study 1 and CyFlow Space; Partec, Görlitz, Germany for Study 2). Five grams of harvested seed was dried at 130 °C for 4 h and cooled for 15 min in a desiccator before measuring protein and oil content. The machine was calibrated using pure camelina oil [31].

Historical (1994–2009) and experimental-year weather data collected from weather stations located at both study sites were obtained, and experimental-year conditions are presented as deviations from LTA (Table 2).

2.5. Statistical Analysis

Data were subjected to ANOVA using R version 4.0.3 (R Core Team 2020), with each study analyzed independently due to differences in timing, space, and management. 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. For soil NO₃–N sampling dates, years and depths were analyzed separately. If any combined analysis showed significant interactions, a separate ANOVA was then run on the response variable. Fisher's least significant difference (LSD) was used for post hoc analysis at $p \leq 0.05$ using the 'agricolae' package to determine means separation within treatments.

3. Results and Discussion

3.1. Weather Conditions

During Study 1, the fall of 2015 was warmer, while the 2016 growing season was slightly cooler than the LTA. The fall of 2016 and the growing season of 2017 were both slightly cooler than the LTA. Year 2016 was 32% wetter than the LTA, while 2017 was about the same with the notable exception of a 54% wetter May. In 2016, March through December were notably wetter than the LTA with the exception of June. In 2017, May and August and March and June were notably wetter and drier, respectively (Table 2).

During Study 2 at WCROC and compared with the LTA, October and November of 2018 were 6 °C cooler on average and September of 2018 was 25 mm drier. Year 2019 was cooler and wetter than the LTA. At SWROC and compared with the LTA, the fall of 2019 was slightly cooler and 127 mm wetter. This saturated the soil of the newly planted winter camelina in the fall, a condition that has been reported to negatively impact spring growth and grain yield [20,32,33]. In 2020, at SWROC, air temperature was about 2 °C below the LTA with the exception of April and May, which were both about 5 °C cooler. Compared with the LTA, March was wetter by 38 mm and April and May were drier by 41 and 11 mm, respectively (Table 2).

3.2. Growth and Development of Winter Camelina with and without Added N Fertilizer

Despite the different planting methods between Study 1 and Study 2, the stands were visually similar, and there was no concern about stand quality in the broadcast camelina in Study 1. In Study 1, winter camelina produced more biomass in relay with maize compared with soybean but was similar in sequential cropping with both main crops during both years. In 2016, the biomass of winter camelina was as low as 1531 kg ha^{−1} and as high

as 2871 kg ha⁻¹ in relay and sequential cropping with soybean, respectively. In 2017, the biomass of winter camelina was higher in both relay and sequential cropping with maize and soybean compared with 2016.

All treatments produced comparable amounts of biomass, which ranged from 3106 kg ha⁻¹ in relay cropping with maize to 3840 kg ha⁻¹ in sequential cropping with soybean (Table 3). Differences in productivity across years was partly due to a colder, wetter spring in 2016 compared with 2017 [23]. In 2016, grain yield of winter camelina ranged from 247 to 494 kg ha⁻¹ in relay cropping with soybean and maize, respectively. In 2017, ranged from 609 to 786 kg ha⁻¹ in relay and sequential cropping with maize and soybean, respectively. These results are within the range reported previously in winter camelina studies without N fertilization in the region [9,20].

In Study 2 at WCROC in 2019, the total biomass and grain yield of winter camelina were both significantly higher in sequential than in relay cropping with both maize and soybean. Biomass was as low as 2965 kg ha⁻¹ in relay cropping with maize and as high as 4134 kg ha⁻¹ in sequential cropping with soybean. Grain yield was as low as 851 kg ha⁻¹ and as high as 1461 kg ha⁻¹ in relay and sequential cropping with soybean, respectively. At SWROC in 2020, the total biomass of winter camelina ranged from 3840 to 4488 kg ha⁻¹ with no difference between relay and sequential cropping in either maize or soybean. Grain yield was significantly different between relay and sequential cropping in soybean only. However, overall grain yield tended to be higher in sequential than in relay cropping with soybean.

Biomass was higher in Study 2 than in Study 1, as much as 4488 kg ha⁻¹ in the former and 3840 kg ha⁻¹ in the latter. Similarly, the grain yield of winter camelina was higher when N fertilized, as much as 1461 kg ha⁻¹ in Study 2 and 786 kg ha⁻¹ in Study 1 (Table 3). These differences in biomass and grain yield from both studies are strong indicative of the response of winter camelina to N fertilizer. The yield and biomass of winter camelina from Study 2 are comparable to those reported in similar studies in the region [7,10,11,21,22,33,34].

This study suggests that yield of winter camelina increases with N application, but the profitability of such technology was not determined. A study reports that winter camelina relayed with soybean generally provided equivalent net incomes to that from monocropped soybean [7]. It could be expected that farmers would need a higher net income for the double cropping system to be worth the additional labor.

3.3. Oil and Protein of Winter Camelina

In Study 1, the oil content of winter camelina seed was similar among cropping systems and ranged from 26.4 to 27.2%. The protein content of winter camelina seed was higher in sequential than in relay cropping with soybean; results from sequential and relay cropping were significantly different in soybean but not maize. The protein content among cropping systems ranged from 19.4 to 27.2%, the former from the relay cropping with soybean and the latter from the relay cropping with maize (Table 3). Our results were below the average of 35.0% oil content and close to the average of 27.6% protein content reported in previous studies conducted in the region [11,20,29,34–36]. Winter camelina is reported to be stressed by waterlogged soils [27]. Copious rainfall, totaling 111 mm over a 9 d period in mid-May of 2017 temporarily waterlogged the experiment at the time of pod fill, which might have caused excess water stress that resulted in low oil content. To the best of our knowledge, the protein content of winter camelina seed without N fertilization had not been reported at the time these studies were conducted.

Table 3. Performance of winter camelina with and without N-fertilization double cropped with maize and soybean at two locations in Minnesota.

Year	Cropping System ‡	Followed by Maize				Followed by Soybean			
		Biomass	Grain			Yield	Grain		
			Yield	Oil	Protein		Oil	Protein	
		(kg ha ^{−1})	(%)			(kg ha ^{−1})	(%)		
Study 1—Broadcast Seeded, non-N–fertilized									
Southwest Research and Outreach Center									
2016	Relay	2553 a ± 51	494 b [§] ± 28	—	—	1531 b ± 367	247 b ± 147	—	—
	Sequential	2808 a ± 65	394 b ± 21	—	—	2871 b ± 352	480 b ± 244	—	—
2017	Relay	3106 a ± 83	609 a ± 18	27.2 a ± 0.3	21.5 a ± 3.6	3760 a ± 332	735 a ± 140	26.7 a ± 1.4	19.4 b ± 2.3
	Sequential	3505 a ± 54	703 a ± 22	26.4 a ± 1.5	27.1 a ± 4.2	3840 a ± 1261	786 a ± 491	26.7 a ± 1.8	25.6 a ± 3.6
Study 2—Drill Seeded, N–fertilized									
West Central Research and Outreach Center									
2019	Relay	2965 b ± 49	875 b ± 15	33.3 b ± 1.3	20.8 a ± 1.4	3028 b ± 182	851 b ± 64	35.1 a ± 1.2	17.9 a ± 1.8
	Sequential	3943 a ± 61	1393 a ± 31	35.9 a ± 1.0	17.5 a ± 2.8	4134 a ± 735	1461 a ± 273	35.9 a ± 0.93	17.4 a ± 0.9
Southwest Research and Outreach Center									
2020	Relay	4171 a ± 38	1170 a ± 91	31.7 a ± 2.1	16.3 a ± 2.0	3949 a ± 309	1061 b ± 168	32.7 a ± 1.1	18.1 a ± 0.9
	Sequential	3840 a ± 36	1159 a ± 11	32.7 a ± 0.9	16.4 a ± 2.2	4488 a ± 286	1288 a ± 24	32.6 a ± 2.1	14.9 a ± 2.5

‡ Relay: maize and soybean were planted into standing winter camelina; Sequential: maize and soybean were planted after the harvest of winter camelina. § In a column, within a year, values followed by the same letter are not significantly different at $p \leq 0.05$; — denotes data not available.

In Study 2, the oil content of winter camelina seed ranged from 31.7 to 35.9% across locations, years, and treatments and was significantly different in maize only during 2019 at WCROC. Protein in seed ranged from 14.9 to 20.8% across locations, years, and treatments, with no differences between cropping systems in a given location or year. The oil content of winter camelina seed was higher in Study 2 than in Study 1, but protein content was generally lower in Study 2 (Table 3). The oil contents of N-fertilized winter camelina were comparable with those reported in similar studies in the region [7,10,15,31,37,38]. However, protein content was lower than the typical range of 23 to 31.5% reported in N-fertilized experiments in the upper Midwest [15,27,31,39]. Protein content in soybean is affected by environmental factors, including precipitation, temperature, nutrient levels, and combinations of these [40,41]. High levels of potassium in the soil, such as those found in Study 1 and Study 2 at WCROC, have also been found to contribute to lower protein levels [42,43].

3.4. Soil Available Nitrogen in Winter Camelina Production

In Study 1, soil available N was significantly different in the top 15 cm in spring and fall of 2017 and in the 15–30 cm layer in fall for maize and in the top 15 cm in fall and 15–30 cm layer in spring of 2017 for soybean. Similar results were found in a study of double-cropping winter camelina with soybean in which the former significantly reduce soil available N in the 0–30 cm layer in spring of both years compared with the tilled and no-till controls [1]. In most instances, soil available N in Study 1 followed a pattern of control treatments > sequential cropping > relay and was higher in sequential cropping and in the top 15 cm of soil (Figure 1), but most differences were not significant due to high variability. Across seasons and soil depths, soil available N in relay maize and soybean averaged around 5.1 kg ha⁻¹. The average soil available N was higher in the sequential and control treatments of both corn- and soybean-based cropping systems: respectively, 6.5 and 12.2 kg ha⁻¹ in soybean and 6.1 and 7.2 kg ha⁻¹ in maize. These results indicate that winter camelina in the maize–soybean rotation helped reduce soil available N that is vulnerable to leaching.

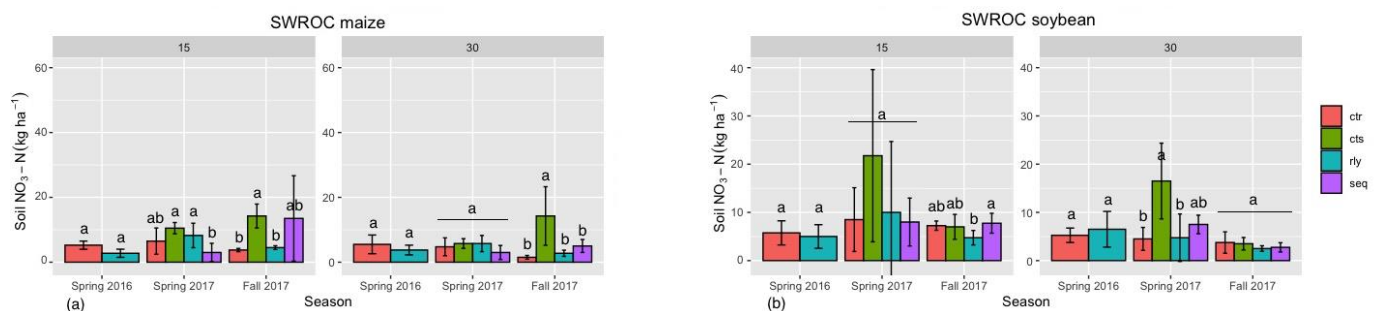


Figure 1. Effects of non-N-fertilized winter camelina double cropped with maize and soybean on soil available N in two soil layers: (a) 0–15 cm and (b) 15–30 cm. Vertical lines denote standard deviation. Southwest Research and Outreach Center (SWROC), 2016–2017. ctr = control relay, cts = control sequential, rly = relay cropping, and seq = sequential cropping. In a given season and depth within a location, bars followed by different letters are significantly different at $p \leq 0.05$.

In Study 2, soil available N was significantly different across seasons and depths within a location. At WCROC, soil available N was significantly different in both soil layers and crops in the fall; in most instances, it was higher in the control treatments than in the treatments with winter camelina. In the fall, soil NO₃-N was reduced by 47 and 33% under relay and sequential cropping with maize, respectively and by approximately 53% under both systems with soybean (Figure 2). At SWROC, soil available N was significantly different among the relay and sequential treatments with both maize and soybean. In spring, available N in the top 30 cm of soil was reduced to as much as 58% in the relay and 30% in the sequential cropping with maize and 33% in the top 15 cm of soil in the

soybean-based cropping systems (Figure 3). These results indicate that winter camelina can significantly reduce soil available N when fertilized, but the overall high residual N suggests an increased potential for losses.

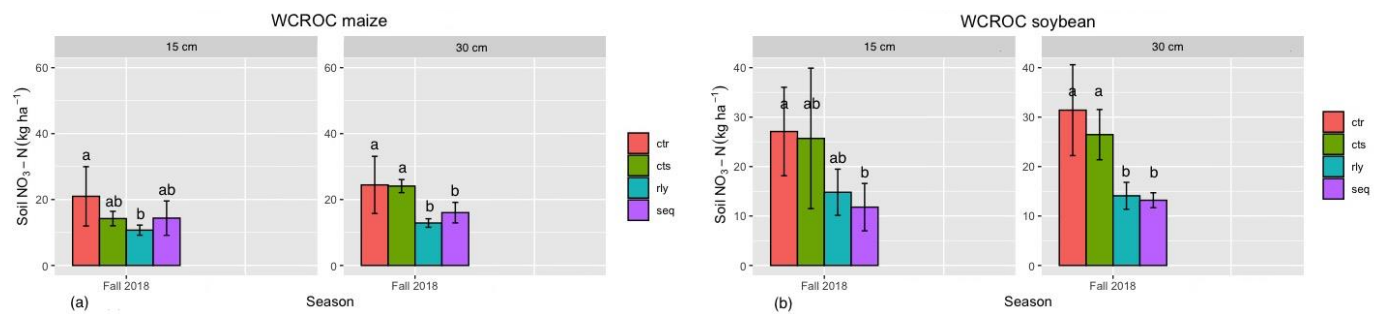


Figure 2. Effects of N-fertilized winter camelina double cropped with maize and soybean on soil available N at two soil layers: (a) 0–15 cm and (b) 15–30 cm. Vertical lines denote standard deviation. West Central Research and Outreach Center (WCROC) 2018–2019. ctr = control relay, cts = control sequential, rly = relay cropping, and seq = sequential cropping. In a given season and depth within a location, bars followed by different letters are significantly different at $p \leq 0.05$.

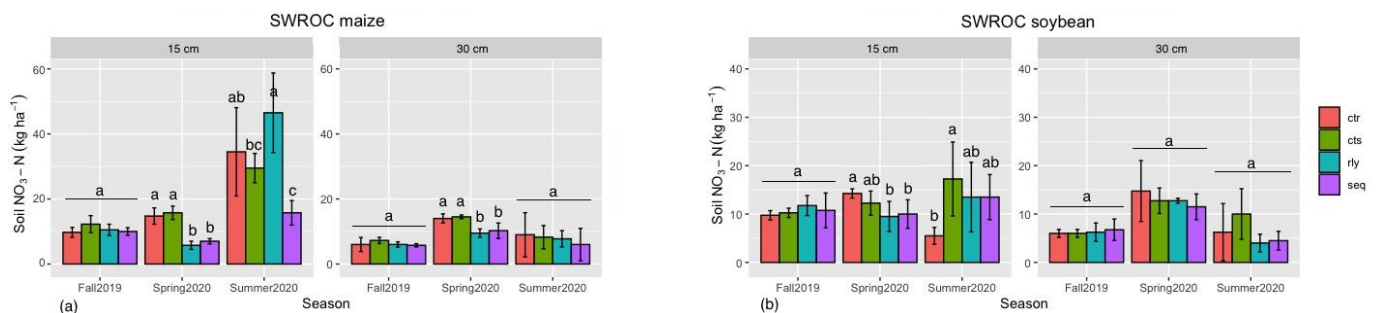


Figure 3. Effects of N-fertilized winter camelina double cropped with maize and soybean on soil available N at two soil layers: (a) 0–15 cm and (b) 15–30 cm with standard deviation. Southwest Research and Outreach Center (SWROC), 2019–2020. ctr = control relay, cts = control sequential, rly = relay cropping, and seq = sequential cropping. In a given season and depth within a location, bars followed by different letters are significantly different at $p \leq 0.05$.

In the fall, winter camelina in relay and sequential cropping with maize appears not to have affected soil available N, although double cropping with maize slightly reduced available soil N compared with controls. In spring, however, the average soil available N in the top 15 cm of soil in maize and soybean was 7 and 16 kg ha^{−1} in the winter camelina and control treatments, respectively, indicating that winter camelina reduced soil available N. This is notable compared with Study 1, in which residual N levels varied more narrowly between seasons. In most instances, soil available N followed a pattern of control treatments > sequential cropping > relay, and it was higher in sequential cropping and the top 15 cm of soil (Figures 1–3). In both studies, the available N in the top 15 cm of soil during fall and spring was typically lower than those reported in a winter camelina sowing date study in the upper Midwest [21], which could be due to the higher initial soil NO₃-N.

4. Conclusions

This study compared the growth, grain yield, and quality of winter camelina with and without N fertilization double cropped with maize and soybean and the resulting effects on soil available N. Winter camelina produced much greater biomass and grain yield when N fertilized. Averaged across treatments, winter camelina yielded 1157 and 556 kg ha^{−1} with and without fertilizer N, respectively. Similarly, oil content was higher in the N-fertilized winter camelina, averaging 34%, which was 20% more than in the non-N-fertilized. Protein content in the N fertilized treatments was 18%, around 21% less than in the non-N-fertilized;

weather and soil conditions might have contributed to this result. Average available soil N was also higher in all N-fertilized treatments.

Overall, our study indicates that grain yields of winter camelina respond positively to N fertilization in a northern location. It is expected, therefore, that N fertilization will be required for winter camelina to be profitable. The drawback of these findings is the increase in residual soil N, which suggests the need for further research to balance agronomic practices with environmental outcomes.

Double cropping winter camelina is a promising system for the region that could be agronomically and environmentally successful, but more research is needed to overcome the challenge of reliably harvesting two crops in a maize–soybean rotation. Additionally, integrating winter camelina with main crops other than maize and soybean, especially shorter-season crops like small grains and pulses, could help extend winter camelina viability in the region.

Author Contributions: Conceptualization, methodology, writing—review and editing S.G., J.A.C., J.S.S., R.L. and A.G.y.G.; validation, formal analysis, data curation, investigation S.G., R.L. and A.G.y.G.; resources A.G.y.G.; visualization, software, writing—original draft preparation S.G.; supervision, A.G.y.G., J.A.C. and J.S.S.; project administration and funding acquisition A.G.y.G. All authors have read and agreed to the published version of the manuscript.

Funding: Study 1 was funded by the Minnesota Soybean Research & Promotion Council, grant number 509-7522-534. Study 2 was funded by the National Science Foundation, grant number 1739191. Check carefully that the details given are accurate and use the standard spelling of funding agency names at <https://search.crossref.org/funding> (accessed on 12 September 2022). Any errors may affect your future funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data generated from this study are available upon request from the corresponding author.

Acknowledgments: The authors acknowledge the expert help with fieldwork and sample processing from Lindsey Englar, Nate Dalman, Alexis Giangrande, Steve Quiring, and Emily Evans at the Southwest Research and Outreach Center and Curt Reese at the West Central Research and Outreach Center, University of Minnesota.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Weyers, S.; Thom, M.; Forcella, F.; Eberle, C.; Matthees, H.; Gesch, R.; Ott, M.; Feyereisen, G.; Strock, J.; Wyse, D. Reduced Potential for Nitrogen Loss in Cover Crop-Soybean Relay Systems in a Cold Climate. *J. Environ. Qual.* **2019**, *48*, 660–669. [[CrossRef](#)] [[PubMed](#)]
2. Robertson, G.P.; Vitousek, P.M. Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. *Annu. Rev. Environ. Resour.* **2009**, *34*, 97–125. [[CrossRef](#)]
3. Basso, B.; Shuai, G.; Zhang, J.; Robertson, G.P. Yield stability analysis reveals sources of large-scale nitrogen loss from the US Midwest. *Sci. Rep.* **2019**, *9*, 5774. [[CrossRef](#)]
4. Randall, G.W.; Huggins, D.R.; Russelle, M.P.; Fuchs, D.J.; Nelson, W.W.; Anderson, J.L. Nitrate losses through subsurface tile drainage in conservation reserve program, alfalfa, and row crop systems. *J. Environ. Qual.* **1997**, *26*, 1240–1247. [[CrossRef](#)]
5. Peterson, A.T.; Berti, M.T.; Samarappuli, D. Intersowing cover crops into standing soybean in the US upper midwest. *Agronomy* **2019**, *9*, 264. [[CrossRef](#)]
6. Doran, J.W. Soil health and global sustainability: Translating science into practice. *Agric. Ecosyst. Environ.* **2002**, *88*, 119–127. [[CrossRef](#)]
7. Ott, M.A.; Eberle, C.A.; Thom, M.D.; Archer, D.W.; Forcella, F.; Gesch, R.W.; Wyse, D.L. Economics and agronomics of relay-cropping pennycress and Camelina with Soybean in Minnesota. *Agron. J.* **2019**, *111*, 1281–1292. [[CrossRef](#)]
8. Sindelar, A.J.; Schmer, M.R.; Gesch, R.W.; Forcella, F.; Eberle, C.A.; Thom, M.D.; Archer, D.W. Winter oilseed production for biofuel in the US Corn Belt: Opportunities and limitations. *GCB Bioenergy* **2017**, *9*, 508–524. [[CrossRef](#)]
9. Johnson, G.A.; Wells, M.S.; Anderson, K.; Gesch, R.W.; Forcella, F.; Wyse, D.L. Yield tradeoffs and nitrogen between pennycress, camelina, and soybean in relay- and double-crop systems. *Agron. J.* **2017**, *109*, 2128–2135. [[CrossRef](#)]

10. Gesch, R.W.; Archer, D.W. Double-cropping with winter camelina in the northern Corn Belt to produce fuel and food. *Ind. Crops Prod.* **2013**, *44*, 718–725. [CrossRef]
11. Moore, K.J.; Karlen, D.L. Double cropping opportunities for biomass crops in the North Central USA. *Biofuels* **2013**, *4*, 605–615. [CrossRef]
12. CTIC; SARE; ASTA. National Cover Crop Survey Annual Report 2019–2020. 2020. Available online: <https://www.sare.org/wp-content/uploads/2019-2020-National-Cover-Crop-Survey.pdf> (accessed on 12 September 2022).
13. Berti, M.; Gesch, R.; Johnson, B.; Ji, Y.; Seames, W.; Aponte, A. Double- and relay-cropping of energy crops in the northern Great Plains, USA. *Ind. Crops Prod.* **2015**, *75*, 26–34. [CrossRef]
14. Johnson, G.A.; Kantar, M.B.; Betts, K.J.; Wyse, D.L. Field pennycress production and weed control in a double crop system with soybean in minnesota. *Agron. J.* **2015**, *107*, 532–540. [CrossRef]
15. Gesch, R.W.; Archer, D.W.; Berti, M.T. Dual cropping winter camelina with soybean in the northern corn belt. *Crop. Econ. Prod. Manag.* **2014**, *106*, 1735–1745. [CrossRef]
16. Zubr, J. Oil-seed crop: Camelina sativa. *Ind. Crops Prod.* **1997**, *6*, 113–119. [CrossRef]
17. Ghamkhar, K.; Croser, J.; Aryanmanesh, N.; Campbell, M.; Kon'kova, N.; Francis, C. Camelina (*Camelina sativa* (L.) Crantz) as an alternative oilseed: Molecular and ecogeographical analyses. *Genome* **2010**, *53*, 558–567. [CrossRef]
18. Prakhova, T.Y.; Prakhov, V.A.; Danilov, M.V. Changes in the fat-acidic composition of Camelina Sativa oilseeds depending on hydrothermal conditions. *Russ. Agric. Sci.* **2018**, *44*, 221–223. [CrossRef]
19. Zanetti, F.; Eynck, C.; Christou, M.; Krzyżaniak, M.; Righini, D.; Alexopoulou, E.; Stolarski, M.J.; Van Loo, E.N.; Puttick, D.; Monti, A. Agronomic performance and seed quality attributes of Camelina (*Camelina sativa* L. crantz) in multi-environment trials across Europe and Canada. *Ind. Crops Prod.* **2017**, *107*, 602–608. [CrossRef]
20. Heggenstaller, A.H.; Anex, R.P.; Liebman, M.; Sundberg, D.N.; Gibson, L.R. Productivity and nutrient dynamics in bioenergy double-cropping systems. *Agron. J.* **2008**, *100*, 1740–1748. [CrossRef]
21. Wittenberg, A.; Anderson, J.V.; Berti, M.T. Crop growth and productivity of winter camelina in response to sowing date in the Northwestern Corn Belt of the USA. *Ind. Crops Prod.* **2020**, *158*, 113036. [CrossRef]
22. Gesch, R.W.; Dose, H.L.; Forcella, F. Camelina growth and yield response to sowing depth and rate in the northern Corn Belt USA. *Ind. Crops Prod.* **2017**, *95*, 416–421. [CrossRef]
23. Liu, R.; Wells, M.S.; Garcia y Garcia, A. Relay and sequential cropping corn with winter oilseed crops in northern climates. *Nutr. Cycl. Agroecosyst.* **2020**, *116*, 195–203. [CrossRef]
24. Strock, J.S.; Porter, P.M.; Russelle, M.P. Cover Cropping to Reduce Nitrate Loss through Subsurface Drainage in the Northern U.S. Corn Belt. *J. Environ. Qual.* **2004**, *33*, 1010. [CrossRef] [PubMed]
25. National Cooperative Soil Survey. National Cooperative Soil Characterization. Available online: <https://ncsslabdatamart.sc.egov.usda.gov/> (accessed on 12 September 2022).
26. U.S. Department of Agriculture, Agricultural Research Service. USDA Plant Hardiness Zone Map. 2012. Available online: <https://planthardiness.ars.usda.gov/> (accessed on 12 September 2022).
27. Gesch, R.W.; Cermak, S.C. Sowing date and tillage effects on fall-seeded Camelina in the Northern Corn Belt. *Agron. J.* **2011**, *103*, 980–987. [CrossRef]
28. Martinelli, T.; Galasso, I. Phenological growth stages of Camelina sativa according to the extended BBCH scale. *Ann. Appl. Biol.* **2011**, *158*, 87–94. [CrossRef]
29. Johnson, J.M.F.; Gesch, R.W. Calendula and camelina response to nitrogen fertility. *Ind. Crops Prod.* **2013**, *43*, 684–691. [CrossRef]
30. Doane, T.A.; Horwath, W.R. Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* **2003**, *36*, 2713–2722. [CrossRef]
31. Walia, M.K.; Wells, M.S.; Cubins, J.; Wyse, D.; Gardner, R.D.; Forcella, F.; Gesch, R. Winter camelina seed yield and quality responses to harvest time. *Ind. Crops Prod.* **2018**, *124*, 765–775. [CrossRef]
32. Berti, M.; Gesch, R.; Eynck, C.; Anderson, J.; Cermak, S. Camelina uses, genetics, genomics, production, and management. *Ind. Crops Prod.* **2016**, *94*, 690–710. [CrossRef]
33. Patel, S.; Lenssen, A.W.; Moore, K.J.; Mohammed, Y.A.; Gesch, R.W.; Wells, M.S.; Johnson, B.L.; Berti, M.T.; Matthees, H.L. Interseeded pennycress and camelina yield and influence on row crops. *Agron. J.* **2021**, *113*, 2629–2647. [CrossRef]
34. Zanetti, F.; Alberghini, B.; Marjanović Jeromela, A.; Grahovac, N.; Rajković, D.; Kiproviski, B.; Monti, A. Camelina, an ancient oilseed crop actively contributing to the rural renaissance in Europe. A review. *Agron. Sustain. Dev.* **2021**, *41*, 2. [CrossRef]
35. Gesch, R.W.; Wells, M.S.; Hard, A. Desiccation of corn allows earlier direct seeding of winter camleina in the Norhtern Corn Belt. *Crop Sci.* **2021**, *61*, 2787–2797. [CrossRef]
36. Gesch, R.W.; Johnson, J.M.F. Water use in camelina-soybean dual cropping systems. *Agron. J.* **2015**, *107*, 1098–1104. [CrossRef]
37. Wittenberg, A.; Anderson, J.V.; Berti, M.T. Winter and summer annual biotypes of camelina have different morphology and seed characteristics. *Ind. Crops Prod.* **2019**, *135*, 230–237. [CrossRef]
38. Gesch, R.W.; Mohammed, Y.A.; Walia, M.K.; Hulke, B.S.; Anderson, J.V. Double-cropping oilseed sunflower after winter camelina. *Ind. Crops Prod.* **2022**, *181*, 114811. [CrossRef]
39. Gesch, R.W.; Matthees, H.L.; Alvarez, A.L.; Gardner, R.D. Winter camelina: Crop growth, seed yield, and quality response to cultivar and seeding rate. *Crop Sci.* **2018**, *58*, 2089–2098. [CrossRef]

40. Assefa, Y.; Purcell, L.C.; Salmeron, M.; Naeve, S.; Casteel, S.N.; Kovács, P.; Archontoulis, S.; Licht, M.; Below, F.; Kandel, H.; et al. Assessing variation in US soybean seed composition (protein and oil). *Front. Plant Sci.* **2019**, *10*, 298. [[CrossRef](#)]
41. Rod, K.S.; Shockley, J.; Knott, C.A. Seed yield, seed quality, profitability, and risk analysis among double crop soybean maturity groups and seeding rates. *Agron. J.* **2021**, *113*, 1792–1802. [[CrossRef](#)]
42. Vyn, T.J.; Yin, X.; Bruulsema, T.W.; Jackson, C.J.C.; Rajcan, I.; Brouder, S.M. Potassium fertilization effects on isoflavone concentrations in soybean [*Glycine max* (L.) Merr.]. *Agric. Food Chem.* **2002**, *50*, 3501–3506. [[CrossRef](#)]
43. Krueger, K.; Goggi, A.S.; Mallarino, A.P.; Mullen, R.E. Phosphorus and potassium fertilization effects on soybean seed quality and composition. *Crop Sci.* **2013**, *53*, 602–610. [[CrossRef](#)]