

FOUR COVER CROPS DUAL-CROPPED WITH SOYBEAN:  
AGRONOMICS, INCOME, AND NUTRIENT UPTAKE ACROSS  
MINNESOTA

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## Abstract

Many agricultural watersheds in Minnesota have toxic levels of phosphorus and nitrogen, much of which originates in agricultural fields that are fallowed from October through May. Autumn-sown winter cover crops can be used to retain these nutrients. Soil NO<sub>3</sub>-N levels and quantities of N sequestered by winter rye (*Secale cereale*), Tillage Radish® (*Raphanus sativus*), and the oilseed crops, winter camelina (*Camelina sativa*), and pennycress (*Thlaspi arvense*) were evaluated in a relayed cover crop/soybean production system at three sites spanning the north-south climatic gradient of Minnesota. Tillage Radish® sequestered the most N in autumn, but winter-killed and had high soil NO<sub>3</sub>-N levels in spring. Winter rye was terminated chemically by early May at each site, whereas the oilseed crops were allowed to grow into June to full maturity and their seeds were harvested. In autumn through early May, winter camelina and pennycress sequestered about 25% less N than winter rye. However, they often sequestered  $\geq 2.5$  times more N than winter rye when compared at maximum seasonal biomass (up to 130 kg N ha<sup>-1</sup>), with some of this N coming from spring fertilizer application. The relative amount of applied N captured by oilseeds, defined here as applied N sequestration efficiency, was 95% and 68% for winter camelina and pennycress, respectively. Winter camelina yields ranged from 600 to 1100 kg ha<sup>-1</sup>, while pennycress yields ranged from 900 kg ha<sup>-1</sup> to 1550 kg ha<sup>-1</sup>. When combined with yields of relay-cropped soybean, net income for relay-crop systems was generally equivalent to mono-cropped soybean.

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Author's Note: This thesis has been formatted in compliance with the Agronomy Journal standards for the purpose of efficient publication.

# Chapter 1

## Winter Oilseed and Soybean Yields, Agronomics, and Income

### Summary

Cover crops have been shown to reduce water pollution by  $\text{NO}_3\text{-N}$ , but only 2% of Minnesota cropland is planted with cover crops each year. It is expected that improving the net financial return from cover crops could facilitate more widespread adoption of them among growers. Here we have evaluated the yields and economics of four cover crops and two winter fallow treatments in a spring wheat and soybean rotation. The four cover crop treatments were winter rye, an improved forage radish variety called Tillage Radish®, winter camelina, and pennycress, all of which were sown in autumn. The winter fallow treatments were a no-tilled, spring wheat stubble treatment, and a fall and spring tilled treatment. Tillage Radish® winter killed, allowing for soybean planting that required little field preparation. Winter rye was terminated chemically by early May, then soybean was subsequently planted. Soybean was planted by early May between rows of the bolting oilseed crops (i.e. relay-cropped), which were allowed to grow into June to full maturity and their seeds were harvested. Winter camelina yields ranged from 600 to 1100  $\text{kg ha}^{-1}$ , while pennycress yields ranged from 900  $\text{kg ha}^{-1}$  to 1550  $\text{kg ha}^{-1}$ . Mono-cropped soybean averaged 1819, 3510, and 4180  $\text{kg ha}^{-1}$  in Roseau, Morris, and Waseca, respectively, which matched the yield expectations for climates of these regions. Soybean seedlings under the oilseed cover crop canopy exhibited symptoms of light stress, which likely affected soybean yield in these treatments. Averaging across sites and years, soybean yields within the oilseed treatments ranged from 66% to 79% of those within



fallow treatments. When oilseed and relay-cropped soybean yields were combined, total seed yields generally were equal to or exceeded those of mono-cropped soybean, whereas net income for relay-crop systems were typically equivalent to mono-cropped soybean. Lastly, there was often less weed pressure in the oilseed treatments compared to all other treatments.

## Introduction

Summer annual grain crops only protect the soil for a few months of each year. Soils in these systems erode at much higher rates than soils under perennial grassland or woodland vegetation (Lubowski et al., 2006; Tilman et al., 2002). Exposed soils facilitate runoff and leaching of nutrients, which are major causes of water pollution (Randall et al., 1997). A conservative estimate of external costs solely related to damage to soil and water resources in the United States from conventional agriculture is \$2.6 billion annually (Tegtmeier and Duffy, 2004).

Much of the cover crop research in the Upper Midwest has been on winter cereals (Kladivko et al. 2004, Kaspar et al. 2012, Strock et al. 2004, McCracken et al. 1994). Winter rye, winter wheat, and similar crops performed well as cover crops in the Central and Southern US (McKibben and Pendleton 1968, McCracken et al., 1994), but Minnesota farmers have been reluctant to adopt cover cropping (USDA Census of Agriculture, 2012). Presently, only 2% of Minnesota cropland is planted with any type of cover crop, of which winter rye is the most common (USDA Census of Agriculture 2012). Likely reasons for this are: 1) winter rye matures too slowly to be harvested for grain in Minnesota before the summer annual crops must be planted, and 2) winter rye

substantially depletes soil water for the following crop (Wyse, 1994, Krueger et al., 2012). Since most commonly used cover crops including winter rye provide little, if any, direct income to growers, there is need to investigate other winter annual crops that offer potential financial returns for northern US growers.

Batho (1939) reported that a field with abundant wild pennycress could yield up to 1,345 kg seeds ha<sup>-1</sup>. Interest in pennycress as an oil source within the United States began in 1944 due to a sharp drop in vegetable oil supply, as previously 90% of rapeseed (*Brassica* spp.) oil had been imported from Japan (Clopton and Triebold 1944). Interest waned after World War II, but was renewed when the importance of cover crops for soil health and water quality was demonstrated (Best and McIntyre 1975, Mitich 1996).

Camelina has been grown for centuries starting in Eastern Europe and Western Asia, and as a result, it is more domesticated than pennycress as an oilseed crop (Vollmann and Eynck 2015). The oil profiles from both pennycress and camelina seeds have been analyzed for industrial and food quality (Clopton and Triebold 1944, Moser 2009, Moser 2010). Due to high glucosinolate and erucic acid contents, pennycress oil is not USDA-approved for human or livestock consumption, while the high omega-3 fatty acid and tocopherol content in winter camelina facilitated USDA approval for both food and feed (Food and Drug Administration, 2016).

Both spring and winter biotypes of camelina have been described (Mirek 1980). To date, most agronomic studies as well as breeding have involved the use of spring biotypes. Both pennycress and winter camelina have high rates of winter survival in the Upper Midwest and mature in mid-to-late June (Gesch and Cermak, 2011; Johnson et al., 2015). These traits permit soybean and other short-season crops to follow them, i.e.,

double-cropping with its attendant net economic benefits (Phippen and Phippen, 2012; Gesch et al., 2013). So far, most of the research on double-cropping with pennycress has used the sequential planting method, where soybean is planted after pennycress harvest (Phippen and Phippen, 2012; Johnson et al., 2015). For winter camelina, however, relay planting is more productive. In this system, soybean is interseeded into the standing cover crop at about the planting time recommended for the region (Gesch et al., 2014; Berti et al., 2015). The relay planting method was used for both pennycress and winter camelina for the research discussed here.

With the increasing number of herbicide-resistant weed species, and due to several issues related herbicide drift (Bohnenblust et al. 2016), alternative approaches to managing weeds are becoming relevant again. Potential crop yield losses due to weeds are estimated to be between 20 and 40%, depending on crop type (Oerke 2006). There is a substantial amount of research showing that winter rye suppresses weeds (Barnes et al. 1983, Weston 1996, Teasdale 1996, Leavitt et al. 2011 etc.), but there have been relatively few investigations into the weed suppression potential of mustard-type cover crops (forage radish: Gieske et al. 2016, Lawley et al. 2012, camelina: Gesch et al. 2011, Sauke et al. 2006), with none on pennycress of which the authors are aware. Thus, the weed suppression effect of four cover crop species was quantified in this study.

Use of these oilseeds as cover crops is a relatively recent innovation, which has been examined experimentally within limited areas of Minnesota (Gesch et al., 2014; Johnson et al, 2017), thereby necessitating fine-tuning of agronomic practices at heterogeneous locations. Thus, the objective of this study was to evaluate the productivity, economics,

and weed suppression potential of relay cropping winter oilseeds with soybean across three diverse environments (plant hardiness zones) in Minnesota.

## **Materials and Methods**

### **Experimental Sites and Crop Operations**

The study was conducted from August 2014 to October 2016, spanning two complete growing seasons for both the winter and summer annual crops, and at three research sites: the Magnusson Research Farm, 7 km NW of Roseau, MN (48°52' N, 95°50' W); the Swan Lake Research Farm, 24 km NE of Morris, MN (45°35'N 95°54' W); and the Southern Research and Outreach Center, 1 km SW of Waseca, MN (44°04' N 93°31' W). Soils at these sites were a Bearden-Colvin-Fargo complex (fine-silty, mixed, superactive, frigid Aeric Calciaquoll; fine-silty, mixed, superactive, frigid Typic Calciaquoll; fine, smectitic, frigid Typic Calciaquet); a Barnes loam soil (fine-loamy, mixed, superactive, frigid Calcic Hapludoll); and a Clarion-Nicollet-Webster complex (fine-loamy, mixed, superactive, mesic Typic Hapludoll; fine-loamy, mixed, superactive, mesic Aquic Hapludoll; fine-loamy, mixed, superactive, mesic Typic Endoaquoll), respectively. These sites represented a gradient in temperature and precipitation across the state of Minnesota, with annual averages for Roseau, Morris and Waseca of 2.5, 5.8, and 7.1°C, 530, 670, and 910 mm, and plant hardiness zones 3a, 4a, and 4b (Anon. 2012), respectively.

Weather data were collected from databases at websites maintained by the University of Minnesota's Southern Research and Outreach Center, the USDA-ARS Swan Lake

Research weather station, and the Minnesota State Climatology Office (Roseau Soil and Water Conservation District).

There were four autumn-planted cover crop treatments, a tilled winter fallow control (autumn and spring chisel plowed to a 15 cm depth), and a no-tilled fallow control with spring wheat stubble (referred to as stubble hereafter) placed in a randomized complete block experimental design that included four blocks for each site-year. The four cover crop treatments were winter rye (variety not specified), an improved forage radish variety called Tillage Radish® (hereafter, radish), winter camelina ('Joelle'), and pennycress ('Beecher Farms'). Plots were 3 m by 9.1 m, in which 12 rows (25 cm spacing) of cover crops and later 4 rows (76 cm spacing) of soybean were planted.

### Crop Analyses

Cover crops were sown in early autumn into spring wheat stubble with a no-till drill. Seeding rates and dates are given in Table 1. Winter camelina and pennycress at all sites were fertilized by broadcasting 80-30-30 kg ha<sup>-1</sup> N-P-K after the late April or early May soil sampling date. This fertility regime maximizes winter oilseed development and yield in a relay cropping system (Gesch and Cermak 2011; Gesch et al. 2014). Soybeans were sown into growing winter camelina and pennycress that were beginning to bolt, into standing winter rye that was killed with glyphosate (1.1 kg a.e. ha<sup>-1</sup>), and into fallow plots for the other treatments (Table 1). The soybean varieties used were Pioneer 91Y70 (RM 1.0) in Waseca, Pioneer P09T74R2 (RM 0.9) in Morris, and Pioneer P01T06R (RM 0.1) in Roseau.

Soybean biomass was sampled near its peak in mid to late August, near the R6 stage of development. These samples were 0.5 m in length and were taken from the two central

rows of each plot (0.25 m<sup>2</sup>). Within these samples, the number of pods of three plants in each row were recorded. Both cover crop and soybean biomass samples were weighed as soon as possible to determine fresh weight and then oven-dried for 48 h at 65°C before determining dry weight.

Crop heights were recorded weekly until the oilseeds were harvested, and then every other week throughout the rest of the growing season for soybean. One cover crop plant was measured from each of the ten central rows of the cover crop treatments, and two soybean plants were measured from the two central rows for all plots.

Photosynthetically active radiation (PAR) available to soybean underneath the winter camelina and pennycress canopies was quantified weekly in spring using a ceptometer (LP-80, Decagon Devices, Pullman, WA). The center of the ceptometer was placed under the oilseed cover crop canopy and directly over and perpendicular to a soybean row. Five readings were taken in this manner at 10 cm intervals, and the mean was recorded. Immediately following the fifth PAR reading, the ceptometer was held above the canopy and three readings were taken and averaged to determine above-canopy PAR.

Winter camelina and pennycress seed yields for this study were determined by collecting 0.25 m<sup>2</sup> samples from two central rows of each plot. Soybean seed yields were determined by collecting 0.76 m<sup>2</sup> samples from two central rows of each plot. Samples were collected when >90% of the crop senesced. The samples were threshed with a Wintersteiger Model 160 plot combine when available, otherwise they were threshed by hand using a Seedburo No. 8Y 6/64" round seed sieve. Seed weights were standardized for moisture content: 100 g kg<sup>-1</sup> for winter camelina and pennycress and 130 g kg<sup>-1</sup> for soybean. When available, a grain moisture meter (Dickey-John GAC 2100AG) was used

to determine seed moisture content, otherwise seeds were weighed and then oven-dried at 80°C for 48 h to determine dry weight.

Growing degree days (GDD) were calculated for all crops. Base and ceiling temperatures were 4° and 30°C for the cover crops and 10° and 30°C for soybean.

The methods and approach of Gesch et al. (2014), including cropping system expense estimates that accounted for materials, fuel, labor, repairs, interest payments, depreciation, and overhead, were used to calculate gross (Table 3) and net (Tables 4-8) income for all treatments. The 2016 mean market price of canola (\$352 Mg<sup>-1</sup>) was used to estimate the market price of pennycress and winter camelina. Likewise, the 2016 mean market price for soybean (\$345 Mg<sup>-1</sup>) was used for economic analysis. The costs associated with each treatment were estimated as \$360 ha<sup>-1</sup> for tilled, \$355 ha<sup>-1</sup> for stubble, \$494 ha<sup>-1</sup> for radish, \$425 ha<sup>-1</sup> for winter rye, and \$680 ha<sup>-1</sup> for the oilseed treatments.

### **Weed Abundance Analyses**

In 2015, weed populations were quantified in each plot by counting weeds within one 10 by 50 cm quadrat. In 2016, above ground weed biomass was collected within a 10 by 50 cm quadrat in three areas within each plot. Comparisons between the 2015 and 2016 data were made by converting the observations to relative weed abundance, which was done by scoring the plot within a block that had the greatest weed count or biomass as one, and all other plots within that block being proportionally assigned a value less than one.

## Statistical Analyses

Statistical analyses for oilseed and soybean yield included a standard ANOVA test of significance at the  $P = 0.05$  level unless otherwise noted. Tukey's Honest Significant Difference was used to compare treatment means. Treatment means for soybean biomass, soybean pod counts, and for the amount of PAR underneath oilseed crop canopies were compared using standard errors. Due to non-constant variance between years for all of the data included here, each year was analyzed separately. These procedures were performed using R version 3.3.2. Regressions were performed using the "data analysis toolpak" in Microsoft® Excel® 2016.

## Results and Discussion

### Weather

Waseca was wetter for both years of the study compared to the 30 year normal (Table 2), and set a state record for total annual precipitation in 2016, punctuated by an intense, two-day precipitation event of 254 mm in September. Morris suffered a very dry autumn in 2014 and below normal rainfall in June for both years (37% of normal in 2015 and 53% of normal in 2016), which likely impacted the growth of the crops, especially soybean. Precipitation in Roseau was near average in both years of the study except for April 2015, in which there was less than half of the normal precipitation. In general, Waseca had the wettest environment followed by Roseau and then Morris.

As expected, GDD accumulation during combined autumn, spring, and summer seasons was highest for Waseca both years. GDD accumulation in Morris represented



about 88 to 99% of that in Waseca, whereas GDD in Roseau was only 66 to 83% of that in Waseca.

### Winter Oilseed Yields

The range in camelina yield across locations and years was 593 kg ha<sup>-1</sup> (Roseau 2015) to 1126 kg ha<sup>-1</sup> (Waseca 2015), while pennycress yield ranged from 929 kg ha<sup>-1</sup> (Morris 2015) to 1547 kg ha<sup>-1</sup> (Roseau 2015). The locations with the highest mean yields across years were Morris for camelina and Roseau for pennycress (Figure 1). The camelina seed yields fit within the reported range for the area surrounding Morris (Gesch et al., 2013, 2014; Berti et al., 2015), which has ranged from 200 to 1900 kg ha<sup>-1</sup>, and Waseca (Johnson et al., 2017), which has ranged from 150 to 900 kg ha<sup>-1</sup>. The pennycress seed yields in this study were higher than those observed in Rosemount, MN, by Johnson et al. (2015), which ranged from 1100 to 1400 kg ha<sup>-1</sup>. From the time pennycress was planted in Morris in the autumn of 2014 until the time it flowered on 9 May 2015, only 64 mm of precipitation fell, which was far below the minimum requirement of 100 mm recommended for canola (for lack of a direct comparison) for the period between planting and flowering (McKenzie and Woods 2011). This likely reduced pennycress yield in Morris in both years. Precipitation explained over 31% of the variation in pennycress yield across all site-years ( $P=0.006$ , Figure 2). Camelina has been shown to maintain yields under drought conditions in Arizona (Hunsaker et al. 2011), and though the water use efficiency of pennycress relative to camelina is not yet known, it is conceivable that it would be relatively lower given the high baseline for camelina. This is a question that is worth investigating, as the findings would perhaps help explain why pennycress was more affected by the lack of precipitation observed in Morris during this study. Another

factor that may at least partially explain this is that camelina flowered 11 days later than pennycress in Morris in 2015, and over 100 mm of precipitation was observed there within these 11 days. Thus, camelina yield was likely less adversely affected by the 2015 dry period in Morris because less water stress occurred during its reproductive phase.

### **Soybean Yield**

The range in soybean yield across all site years was 443 kg ha<sup>-1</sup> (Roseau 2015 relayed into pennycress) to 4596 kg ha<sup>-1</sup> (Waseca 2016 relayed into camelina) (Figure 1). Mono-cropped soybean averaged 1819, 3510, and 4180 kg ha<sup>-1</sup> in Roseau, Morris, and Waseca, respectively, which matched the yield expectations for climates of these regions.

Averaging across sites and years, soybean yields within the camelina treatment were 79% and 76% of those within the stubble and tilled treatments, respectively. Soybean yields within the pennycress treatment were 74% and 66% of those within the stubble and tilled treatments, respectively. This observed yield penalty for soybean in a relay-cropping system was similar to that observed in a Kansas wheat and soybean relay-cropping system, where relay-cropped soybean yielded 72% of mono-cropped soybean (Duncan et al. 1990); as well as southeastern Minnesota where yield of relayed soybean was reduced up to 30% by camelina and pennycress cover crops (Johnson et al. 2017).

In half of the site-years, soybean relay-cropped with pennycress had significantly lower yields than soybean in the fallow, radish, and rye treatments. For two site-years, soybean relay-cropped into camelina had significantly lower yields than soybean in the fallow treatments, but they were never significantly different from those in rye (Figure 1). Competition between oilseeds and soybean for light in all locations appeared to be a factor contributing to the observed soybean yield penalties in the relay-planted system

(Figures 3). In the second year of the study in Morris, there was also a longer overlap (4-6 days) between pennycress and soybean crops compared to that in Roseau and Waseca, respectively. Similarly, the overlap between camelina and soybean was longer (5-8 days in year one and 11 days in year two) in Morris than in Roseau and Waseca, respectively. Additionally, the aforementioned low levels of precipitation in Morris, especially during June, likely prevented maximum development of both oilseeds and soybean at that location. Relatively low plant productivity of the oilseed cover crops in Waseca during the 2016 growing season, as indicated by relatively low biomass production (Table 3), likely led to less competition of light and soil resources, which may explain why there were no significant differences in soybean yields among treatments at that location.

### **Combined Oilseed and Soybean Yield**

When winter oilseed and soybean yields were aggregated, the total seed yield was often greater compared to yields of mono-cropped soybean in Waseca and Roseau, but statistical significance varied (Figure 1). The most pronounced instances where combined seed yields were greater than soybean alone were in Roseau in 2015 and Waseca in 2016, where pennycress plus soybean yielded 1990 and 5393 kg ha<sup>-1</sup>, respectively, which was significantly more than the 1111 and 4147 kg ha<sup>-1</sup> for the mono-cropped tilled treatment at the same sites. Camelina plus soybean in Waseca in 2016 yielded 5349 kg ha<sup>-1</sup>, which was also significantly more than the tilled treatment there. Aggregate yields in Morris were, at best, equivalent to those of mono-cropped soybean, and significantly less than mono-cropped soybean for the 2016 pennycress treatment. The low aggregate yields resulted from atypically poor soybean yields, which likely reflected intense crop-crop competition for water during June in Morris. The absolute oilseed and soybean yields

were greatest in Waseca, likely due to this southern MN location having the longer growing season than the central and northern MN locations in this study. There also appeared to be less competition between the oilseed cover crops and soybean in Waseca, which will be discussed in more detail in the section related to soybean development.

### **Gross and Net Income**

New crops such as camelina and pennycress do not have fully developed markets and reliable price estimates. Consequently, the 2016 mean market price of canola (\$352 Mg<sup>-1</sup>) was used to estimate prices of these crops. Likewise, the 2016 mean market price for soybean (\$345 Mg<sup>-1</sup>) was used for economic analysis. The prices for these crops have been highly correlated historically (correlation of 0.83 over the 84 months spanning 2010 to 2016), which is a factor to consider if different crop price scenarios are simulated. In a similar study (Gesch et al. 2014), the agricultural economist, D. Archer, included different price scenarios for canola (with the soybean price held fixed) in an attempt to account for potential price fluctuations that may be stronger for one of the two crops. Simulating different prices scenarios was beyond the scope of this manuscript, but will be included in a journal article that is in progress. Gross and net income for all treatments are presented in Table 3 and Table 4, respectively.

Relay-cropping systems with oilseeds and soybean, which result in significantly higher yields, do not automatically equate to significantly higher gross incomes because the commodity price of soybean is often slightly higher than that for canola and, by extension, winter camelina and pennycress. Few studies have attempted to quantify potential net income of cover crops in Minnesota. Feyereisen et al. (2013) evaluated winter rye as a potential cellulosic ethanol feedstock in the Upper Midwest, but did not

analyze economics. Given that only about 2% of Minnesota cropland is cover-cropped, efforts to make winter rye profitable for growers have yet to be successful. The net income from the oilseed cover crop-soybean system was never greater than that of the mono-cropped soybean in this study when the 2016 mean canola price was used (Table 4). However, income-neutrality with oilseed cover crops may actually be a better starting point for further development than with cover crops that cost a similar amount, but have yet to result in new markets or substantial direct income to growers. Net income ranged from \$47 to \$1453 ha<sup>-1</sup> for pennycress plus soybean; -\$160 to \$1451 ha<sup>-1</sup> for camelina plus soybean; -\$28 to \$1299 ha<sup>-1</sup> for soybean alone in the radish treatment; \$49 to \$1338 ha<sup>-1</sup> for soybean alone in the winter rye treatment; \$444 to \$1436 ha<sup>-1</sup> for soybean alone in the stubble treatment; and \$91 to \$1323 ha<sup>-1</sup> for soybean alone in the tilled treatment, all in Roseau and Waseca, respectively (Table 4). Since the earliest winter rye matures in the Upper Midwest is not until the third week in July (Oelke et al. 1990), the only income it can provide in a double-cropping system with soybean is to harvest it much earlier as a forage or cellulosic ethanol feedstock, which are less lucrative commodities compared to oilseeds.

Pennycress and camelina are still being domesticated and refined, and there have been several recent advances in the pennycress germplasm (Dorn et al., 2015), which suggests there is a great potential for improvement in pennycress and camelina. Since pennycress has a relatively high baseline yield across Minnesota and camelina does so in the central and southern regions of the state, gains from domestication may confer great potential for these cover crops to change the Upper Midwestern landscape.

Although soybean was the summer annual that was the focus of this study, there are other summer annuals that conceivably could perform well in a relay- or perhaps even double-cropping system with pennycress in northern Minnesota and elsewhere. Examples include sunflower or dry beans. Other studies have found that dual cropping camelina and soybean can yield more seed and oil than mono-cropped soybean in central Minnesota (Morris region), especially when a skip-row planting system is used (Gesch et al. 2013, 2014, Berti et al. 2015). Higher total yields in these cases did not translate into significantly higher net returns for the dual cropping systems with camelina due to lower seed prices for camelina than for soybean and the extra costs required for its production, although the relay system was found to be economically competitive with a sole full-season soybean crop (Gesch et al. 2014). However, as pointed out by Gesch et al. (2014), who were the first to perform an in-depth economic analysis of winter camelina-soybean dual cropping systems, more research is needed on these systems across a range of environments to evaluate management inputs (e.g., soybean cultivar selection and planting methods) to improve yields and bolster economics. Additionally, both camelina and pennycress provide important agroecosystem services such as sequestration of labile soil chemicals and pollinator forage resources. However, values of these services are difficult to assess economically.

### **Canopy Light Penetration and Soybean Development**

Soybean under the winter camelina canopy consistently received more light than soybean under the pennycress canopy (Figure 3). This was due to a denser pennycress canopy and, in part, to differences in infructescence morphology between camelina and pennycress. Pennycress canopies in late May and June were comprised largely of 1- to 2-

cm diameter silicles that are disk-shaped and have a greater surface area than the smaller (< 1 cm) and pear-shaped silicles that characterize camelina.

With less light reaching soybean seedlings under the pennycress and winter camelina canopies early in the primary growing season compared to mono-cropped soybean, the potential for soybean etiolation and lodging increased, which may have resulted in reduced yields. Varying degrees of soybean etiolation and instances of soybean yield penalties associated with the oilseed cover crop treatments were observed. For example, in Waseca and in Roseau for both years of the study, the soybean seedlings in pennycress and winter camelina plots were taller than those of other treatments until after the winter oilseeds were harvested (Figure 4). The first two weeks in June appeared to be the period in which soybean seedlings etiolated in Waseca, which was more pronounced under the pennycress canopy. This window of etiolation in Roseau was approximately the last two weeks in June and into the first week of July. Etiolation of soybean ceased after removing the winter oilseeds, after which time their rate of height increase lagged behind that of soybean from other treatments. The reduced vegetative growth of soybean relayed into pennycress and winter camelina likely led to reduced grain yields in four of the six site-years (see below).

In South Carolina, Wallace et al. (1992 and 1996) conducted relay-cropping experiments with winter wheat and soybean. In 1988, they planted soybean between rows of winter wheat, which was harvested 19 days later, and did likewise in 1989 with winter wheat harvest occurring 14 days later. Their control treatment for this study was soybean planted on the same date as the relay-cropped soybean, but planted into winter wheat stubble after flail mowing. They observed soybean etiolation in both years of their study,

but did not observe differences in yield between relay and mono-cropped soybean. Similarly, in 1991 and 1992, they relay-planted soybean into winter wheat, which was harvested 19 and 27 days later, respectively. However, in this second experiment, their control treatment was sequentially-planted winter wheat and soybean, so soybean was planted immediately after winter wheat harvest about three weeks after relay-cropped soybean. In addition to observing etiolation as before, they also observed lodging in relay-cropped soybean. There was no mono-cropped soybean treatment with which to compare in this later experiment, but yields between relay-cropped and sequentially-cropped soybean were not different.

In Kansas, irrigation was necessary to support relay-cropping of winter wheat and soybean, and soybean etiolation and lodging were also observed, contributing to a 28% yield reduction there (Duncan et al. 1990). These observations of light and water stress in a relay-cropping system with soybean are similar to what we observed in this study, and estimating the effect of these different factors on soybean yield remains a challenge. Although disentangling these stressors is difficult, evidence shows that a 3-4 week period of overlap between a winter crop and soybean may not adversely affect soybean yield. The overlap period in this study was about six weeks for pennycress/soybean and eight weeks for camelina/soybean. The site that had the least amount of overlap between the oilseed and soybean crops was Waseca. Thus, it is unlikely a coincidence that this site exhibited the least amount of soybean yield reduction in the oilseed treatments: 1% and 10% on average for soybean in camelina and pennycress treatments, respectively, relative to averaged winter fallow treatments versus the Roseau site with 34% and 36% reductions on average for soybean in camelina and pennycress, respectively, relative to



averaged winter fallow treatments. Also, optimal planting date studies in Minnesota have shown that delaying planting of soybean to May 10 will cause little to no yield losses (Hardman et al. 1988), which may be important for growers who are interested in relay-cropping oilseeds with soybean. The 3-4 week overlap that has been shown to have little adverse effect on soybean provides a definitive goal for breeders working to develop early maturing varieties of winter camelina and pennycress.

The row spacing for oilseeds used in this study (25 cm) was relatively wide and chosen due to limitations in equipment availability, though this wider row spacing was expected to allow ample light to reach the soybean seedlings underneath the oilseed canopy in spring. Since there were signs of light stress in soybean in Waseca and Roseau for both years, it seems likely that more cultural practices will be necessary to improve the micro-environment underneath the oilseed canopy for soybean. Skip-row planting with alternating sections of wider and narrower row spacings may be a viable approach to optimize yields of both the summer and winter crops. Additionally, orienting the oilseed rows in the north-south direction may also reduce competition for light between oilseeds and soybean (Borger et al. 2010).

Peak soybean biomass ranged from about 6 to 9, 2 to 9, and 1 to 3 Mg ha<sup>-1</sup> in Waseca, Morris, and Roseau, respectively (Figure 5A). These values mirror the differences in weather and maturity groups planted at each site. Additionally, at each site, soybean biomass was lower in camelina and pennycress treatments than in the stubble and tilled treatments, reflecting the crop-crop competition between soybean and cover crop in the relay system.

The number of pods per soybean plant differed between years, but ranged from 20 to 60, 20 to 40, and 10 to 70 for Waseca, Morris, and Roseau, respectively (Figure 5B). Interestingly, the number of pods per plant was relatively consistent across treatments for a given year and location. Soybean in the pennycress and winter camelina treatments appeared to have partially compensated for their reduced biomass by producing similar numbers of pods as the control treatments. Since pod number was similar among treatments, reduced pod fill or fewer seeds per pod likely explains the observed soybean yield penalty in the pennycress and winter camelina treatments. Perhaps a longer maturity soybean would allow greater soybean pod fill in the relay-cropping system (Figure 5B).

### **Relative Weed Abundance in Cover Crop and Winter Fallow Treatments**

There was a general pattern of winter rye, pennycress, and camelina suppressing weeds (Table 5). Winter rye demonstrated the most aggressive weed suppression and radish the least, as there were four sampling dates in which winter rye had significantly less relative weed abundance (RWA) than radish. This finding corresponds with a pattern of weed suppression by winter rye that many others have observed (Barnes et al. 1983, Weston 1996, Teasdale 1996, and Leavitt et al. 2011). Pennycress never differed from winter rye and had a lesser RWA than radish on two sampling dates. Camelina differed from winter rye on only one occasion, and had a lesser RWA than radish on two sampling dates. A weed suppression effect exhibited by camelina was also observed by Gesch et al. (2011). Because radish winter kills, it is logical that little weed suppression was associated with this crop. Gieske et al., (2016) also observed a similar lack of weed suppression when radish was used as a cover crop. Since chemical approaches to weed management are becoming more challenging, systems such as relay-cropping with

oilseeds and soybean may have even more benefits besides those typically associated with cover crops.

## Conclusions

Two requirements likely exist for a cover crop to be considered a viable option for growers in the Upper Midwest. The cover crop must provide: 1) enough of a financial incentive to be considered worth the time and effort to grow, and 2) a substantial service that improves, biodiversity, soil, and water quality characteristics. With the cost of all inputs, including labor, the revenue generated from the winter oilseeds was at least enough to be equivalent to mono-cropped soybean. Though higher net yields would be preferable for the oilseed-soybean relay cropping system, the high baseline level of oilseed yield is expected to be sufficient for plant breeding to make necessary gains to achieve a profitable system. Skip-row planting and orienting oilseed rows in a north-south direction may also allow for more light to penetrate the canopy and reduce light stress on soybean seedlings in a relay cropping system. Shade tolerant varieties of soybean may also improve yields in this system. Additionally, each crop may have its own niche based upon climate, soil, and economics. For instance, it appears that pennycress thrives in Roseau (plant hardiness zone 3a), while winter camelina thrives in Waseca (plant hardiness zone 4b). As breeding for each of these crops continues at the University of Minnesota and elsewhere, further improvements in their deliverables are expected.

**Table 1.** Summary of field-related experimental procedures performed in Waseca, Morris, and Roseau, MN from 2014-16.

| Crop                    | Year  | Seeding Rate                   | Planting Depth | Planting/Removal Date  |                        |                  | Removal Method         |
|-------------------------|-------|--------------------------------|----------------|------------------------|------------------------|------------------|------------------------|
|                         |       |                                |                | Waseca, MN             | Morris, MN             | Roseau, MN       |                        |
| Radish                  | 14/15 | 11 kg ha <sup>-1</sup>         | 1.3 cm         | 5 Sep/NA               | 2 Sep/NA               | 28 Aug/NA        | Winter Killed          |
|                         | 15/16 |                                |                | 22 Sep/NA              | 31 Aug/NA              | 3 Sep/NA         |                        |
| W. Rye                  | 14/15 | 7.6 kg ha <sup>-1</sup>        | 1.3 cm         | 5 Sept/27 Apr          | 2 Sep/1 May            | 28 Aug/4 Jun     | Glyphosate Application |
|                         | 15/16 |                                |                | 22 Sep/5 May           | 31 Aug/22 Apr, 16 May  | 3 Sep/17 May     |                        |
| W. Camelina             | 14/15 | 6.7 kg ha <sup>-1</sup>        | 0.6 cm         | 5 Sep/18 Jun           | 2 Sep/2 Jul            | 28 Aug/2 Jul     | Harvested              |
|                         | 15/16 |                                |                | 22 Sep/23 Jun          | 31 Aug/23 Jun          | 3 Sep/7 Jul      |                        |
| Pennycress              | 14/15 | 6.7 kg ha <sup>-1</sup>        | 0.6 cm         | 5 Sep/18 Jun           | 2 Sep/23 Jun           | 28 Aug/1 Jul     | Harvested              |
|                         | 15/16 |                                |                | 22 Sep/21 Jun          | 31 Aug/16 Jun          | 3 Sep/7 Jul      |                        |
| Soybean                 | 15    | 444,800 seeds ha <sup>-1</sup> | 2.5 cm         | 24 Apr/2 Oct           | 30 Apr/15 Sep          | 5 May/6 Oct      | Harvested              |
|                         | 16    |                                |                | 3 May/6 Oct†           | 22 Apr/19 Sep‡         | 17 May/3 Oct     |                        |
| <u>Operation</u>        | 15    |                                |                | 27 Apr§/5 Jun¶/mid Jul | 1 May§/7 Jul           | 4 Jun§/early Jul |                        |
| Glyphosate Applications | 16    |                                |                | 5 May§/28 Jun          | 22 Apr§/16 May§/21 Aug | 17 May§/15 Jul   |                        |
| Tillage Treatment       | 14    |                                |                | 8 Sep/~23 Apr          | 9 Sep/~29 Apr          | 28 Aug/5 May     |                        |
| Weed Sampling           | 15    |                                |                | late Sep/~2 May        | late Aug/22 Apr        | 14 Sep/6 May     |                        |
|                         | 15    |                                |                | 1 Jun                  | 28 May/22 Jun          | 10 Jun/2 Jul     |                        |
|                         | 16    |                                |                | 16 Apr/6 Jun           | 31 May/24 Jun          | 17 May           |                        |

†For one block, soybean was harvested on 13 Oct for these treatments: No-Till, Tilled, W. Rye, and Radish

‡For all blocks, soybean was harvested on 29 Sep for the Pennycress and W. Camelina treatments

§Applies to W. Rye only

¶Applies to non-oilseed treatments only

**Table 2.** Temperature and precipitation observations in Waseca, Morris, and Roseau, MN from 2014-16.

| Month          | Mean Air Temperature (°C) |            |                   | Accumulated GDD (4/30 °C d) |            | Accumulated GDD (10/30 °C d) |            | Total Precipitation (mm) |            |                   |
|----------------|---------------------------|------------|-------------------|-----------------------------|------------|------------------------------|------------|--------------------------|------------|-------------------|
|                | '14<br>'15                | '15<br>'16 | 30 Year<br>Normal | Oils<br>eed                 |            | Soybean                      |            | '14<br>'15<br>'16        | '15<br>'16 | 30 Year<br>Normal |
|                |                           |            |                   | '14<br>'15                  | '15<br>'16 | '14<br>'15                   | '15<br>'16 |                          |            |                   |
| Waseca         |                           |            |                   |                             |            |                              |            |                          |            |                   |
| Sept.          | 16                        | 20         | 16                | 359                         | 476        | 187                          | 296        | 59                       | 149        | 93                |
| Oct.           | 9                         | 11         | 9                 | 149                         | 211        | 24                           | 59         | 35                       | 31         | 68                |
| Apr.           | 9                         | 9          | 8                 | 150                         | 169        | 41                           | 53         | 70                       | 50         | 82                |
| May            | 14                        | 15         | 15                | 323                         | 342        | 149                          | 172        | 121                      | 95         | 100               |
| Jun.           | 20                        | 21         | 20                | 483                         | 513        | 303                          | 333        | 194                      | 121        | 119               |
| Jul.           | 21                        | 23         | 22                | 539                         | 572        | 353                          | 386        | 188                      | 227        | 112               |
| Aug.           | 20                        | 22         | 21                | 489                         | 560        | 303                          | 374        | 152                      | 297        | 121               |
| Sept.          | 20                        | 19         | 16                | 476                         | 456        | 296                          | 276        | 149                      | 376        | 93                |
| Mean/<br>Total | 16                        | 18         | 16                | 2968                        | 3299       | 1656                         | 1949       | 968                      | 1346       | 788               |
| Morris         |                           |            |                   |                             |            |                              |            |                          |            |                   |
| Sept.          | 16                        | 19         | 15                | 347                         | 449        | 172                          | 269        | 17                       | 32         | 74                |
| Oct.           | 9                         | 11         | 7                 | 155                         | 204        | 37                           | 50         | 9                        | 38         | 64                |
| Apr.           | 8                         | 7          | 7                 | 146                         | 133        | 42                           | 43         | 20                       | 52         | 59                |
| May            | 14                        | 15         | 14                | 309                         | 340        | 143                          | 167        | 149                      | 43         | 72                |
| Jun.           | 20                        | 20         | 19                | 488                         | 481        | 308                          | 301        | 38                       | 54         | 102               |
| Jul.           | 22                        | 21         | 21                | 550                         | 535        | 364                          | 349        | 74                       | 184        | 99                |
| Aug.           | 20                        | 21         | 20                | 498                         | 521        | 312                          | 335        | 85                       | 94         | 85                |
| Sept.          | 19                        | 17         | 15                | 449                         | 380        | 269                          | 201        | 32                       | 43         | 74                |
| Mean/<br>Total | 16                        | 16         | 15                | 2942                        | 3043       | 1647                         | 1715       | 424                      | 540        | 629               |
| Roseau         |                           |            |                   |                             |            |                              |            |                          |            |                   |
| Sept.          | 14                        | 16         | 13                | 284                         | 358        | 119                          | 184        | 59                       | 45         | 62                |
| Oct.           | 7                         | 8          | 5                 | 116                         | 120        | 29                           | 20         | 34                       | 58         | 46                |
| Apr.           | 6                         | 3          | 4                 | 103                         | 58         | 15                           | 5          | 12                       | 34         | 35                |
| May            | 11                        | 14         | 12                | 228                         | 304        | 93                           | 140        | 108                      | 94         | 70                |
| Jun.           | 18                        | 18         | 17                | 408                         | 405        | 228                          | 225        | 97                       | 126        | 107               |
| Jul.           | 21                        | 20         | 20                | 516                         | 497        | 330                          | 311        | 130                      | 116        | 84                |
| Aug.           | 19                        | 19         | 19                | 445                         | 462        | 259                          | 276        | 115                      | 48         | 78                |
| Sept.          | 16                        | 14         | 13                | 358                         | 58         | 184                          | 135        | 45                       | 115        | 62                |
| Mean/<br>Total | 14                        | 14         | 13                | 2458                        | 2262       | 1257                         | 1296       | 600                      | 636        | 544               |

**Table 3.** Gross revenue of the oilseed-soybean relay cropping system compared to the double and mono-cropped soybean systems. Based upon yields observed in Waseca, Morris, and Roseau, MN in 2015 and 2016. Values are means with  $n=4$ .

| Cover Crop Treatment  | Waseca              |         | Morris |        | Roseau |       |
|-----------------------|---------------------|---------|--------|--------|--------|-------|
|                       | 2015                | 2016    | 2015   | 2016   | 2015   | 2016  |
|                       | \$ ha <sup>-1</sup> |         |        |        |        |       |
| None: Till + Soybean  | 1413a               | 1431c   | 1189a  | 1251ab | 383b   | 820a  |
| Stubble + Soybean     | 1403a               | 1523abc | 1142a  | 1263a  | NA     | 679a  |
| Radish + Soybean      | 1482a               | 1525abc | 1114a  | 1179ab | 396b   | 723a  |
| W. Rye + Soybean      | 1425a               | 1499bc  | 1115a  | 1077ab | 402a   | 817a  |
| W. Camelina + Soybean | 1669a               | 1851ab  | 1105a  | 1066ab | 472b   | 756a  |
| Pennycress + Soybean  | 1632a               | 1868a   | 1157a  | 845b   | 697a   | 1091a |

2016 Calendar Year Mean Soybean Price: \$345 Mg<sup>-1</sup>

2016 Calendar Year Mean Canola Price: \$352 Mg<sup>-1</sup>

Values within columns followed by the same letter are not significantly different.

Analyzed using Tukey's HSD.

**Table 4.** Net revenue of the oilseed-soybean relay cropping system compared to the double and mono-cropped soybean systems. Based upon yields observed in Waseca, Morris, and Roseau, MN in 2015 and 2016. Values are means with  $n=4$ . Input costs are from Gesch et al. 2014, adjusted for 2014-15 fuel prices.

| Cover Crop Treatment  | Waseca              |       | Morris |       | Roseau |      |
|-----------------------|---------------------|-------|--------|-------|--------|------|
|                       | 2015                | 2016  | 2015   | 2016  | 2015   | 2016 |
|                       | \$ ha <sup>-1</sup> |       |        |       |        |      |
| None: Till + Soybean  | 1054a               | 1071a | 829a   | 891a  | 24a    | 461a |
| Stubble + Soybean     | 1048a               | 1168a | 787a   | 909a  | NA     | 325a |
| Radish + Soybean      | 988a                | 1031a | 620ab  | 685ab | -97ab  | 230a |
| W. Rye + Soybean      | 1001a               | 1075a | 690ab  | 652ab | -22a   | 392a |
| W. Camelina + Soybean | 990a                | 1171a | 425b   | 387bc | -207b  | 77a  |
| Pennycress + Soybean  | 953a                | 1189a | 477ab  | 166c  | 18a    | 412a |

2016 Calendar Year Mean Soybean Price: \$345 Mg<sup>-1</sup>

2016 Calendar Year Mean Canola Price: \$352 Mg<sup>-1</sup>

Values within columns followed by the same letter are not significantly different.

Analyzed using Tukey's HSD.

**Table 5.** Relative weed abundance among relay, double, and mono-cropped systems. Based upon counts or biomass made within a 0.05 m<sup>2</sup> quadrat in 2015 and 2016, respectively. Transformed data are presented in transformed units. Conducted in Waseca, Morris, and Roseau, MN.

Values are means with  $n=4$ .

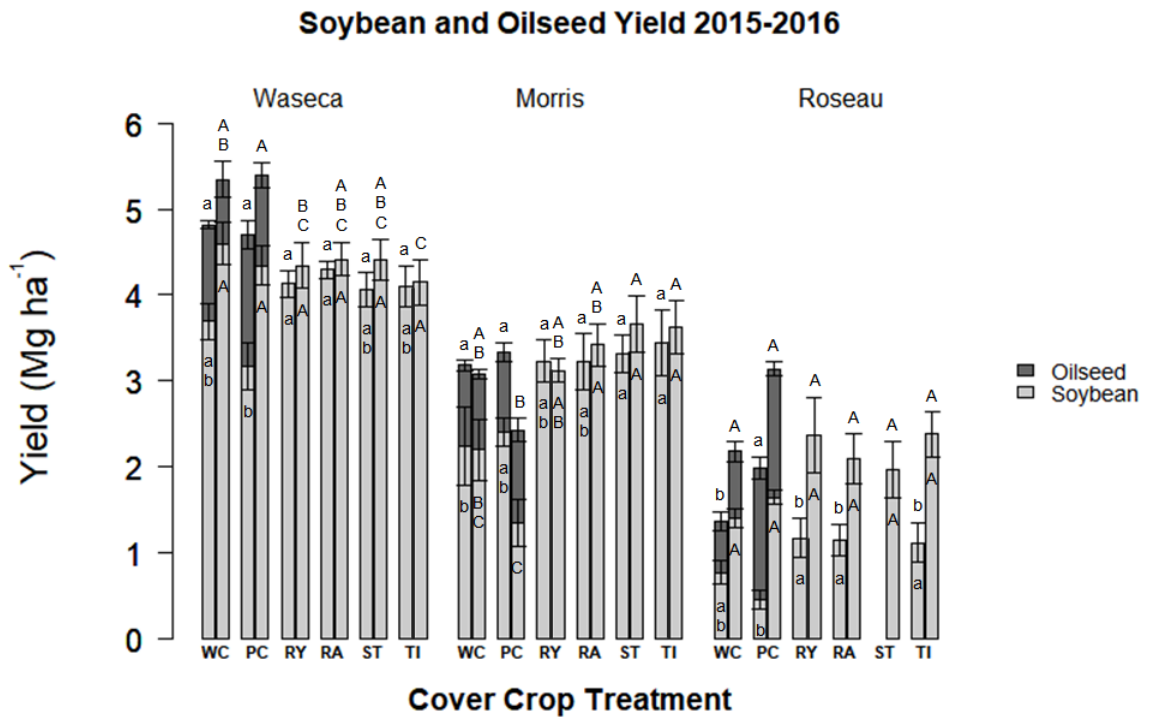
| Sampling Date  | Cover Crop Treatment | Waseca |    | Morris |      |    | Roseau |    |
|----------------|----------------------|--------|----|--------|------|----|--------|----|
| Spring<br>2015 | Tilled               | 0.75   | NS | 0.13   | a    | †  | 1.00   | a  |
|                | Stubble              | 0.35   |    | 0.08   | ab   |    | NA     |    |
|                | Radish               | 0.50   |    | 0.08   | ab   |    | 0.49   | b  |
|                | W. Rye               | 0.34   |    | 0.02   | c    |    | 0.06   | c  |
|                | W. Camelina          | 0.21   |    | 0.05   | bc   |    | 0.22   | bc |
|                | Pennycress           | 0.29   |    | 0.04   | bc   |    | 0.27   | bc |
| Summer<br>2015 | Tilled               | NA     |    | 0.58   | NS   |    | 0.51   | bc |
|                | Stubble              |        |    | 0.30   |      |    | NA     |    |
|                | Radish               |        |    | 0.35   |      |    | 0.90   | a  |
|                | W. Rye               |        |    | 0.24   |      |    | 0.20   | c  |
|                | W. Camelina          |        |    | 0.63   |      |    | 0.87   | ab |
|                | Pennycress           |        |    | 0.36   |      |    | 0.35   | c  |
| Spring<br>2016 | Tilled               | 0.72   | NS | 0.08   | ab   | †‡ | NA     |    |
|                | Stubble              | 0.6    |    | 0.37   | a    |    | 0.89   | a  |
|                | Radish               | 0.48   |    | 0      | b    |    | 0.66   | ab |
|                | W. Rye               | 0.18   |    | 0      | b    |    | 0.29   | b  |
|                | W. Camelina          | 0.17   |    | 0      | b    |    | 0.51   | ab |
|                | Pennycress           | 0.37   |    | 0      | b    |    | 0.18   | b  |
| Summer<br>2016 | Tilled               | 0.19   | ab | †‡     | 0.02 | ab | †§     | NA |
|                | Stubble              | 0.23   | ab |        | 0.01 | ab |        |    |
|                | Radish               | 0.76   | a  |        | 0.08 | a  |        |    |
|                | W. Rye               | 0.00   | b  |        | 0.04 | ab |        |    |
|                | W. Camelina          | 0.00   | b  |        | 0.00 | b  |        |    |
|                | Pennycress           | 0.03   | b  |        | 0.01 | ab |        |    |

† Indicates that the data was transformed with the inverse hyperbolic sine function to meet assumptions of ANOVA.

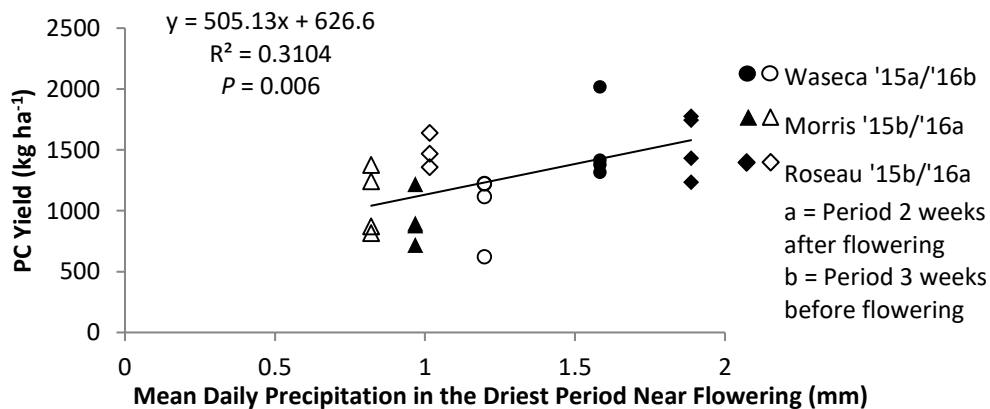
‡ Indicates that the data did not meet the assumptions of ANOVA.

§ Indicates ANOVA  $P < 0.1$  and  $\alpha$  for Tukey's HSD set to 0.1.

**Figure 1.** Oilseed yields from two cover crop treatments and soybean yields from four cover crop and two winter fallow treatments. For each pair of bars, left is 2015, right is 2016. Tukey's honest significant difference was used for mean separations. Lower case letters are for 2015, capital letters are for 2016, letters above bars compare total seed yield among all treatments (soybean and, if present, oilseed), and letters within bars compare soybean yields among all treatments. Observations made in Waseca, Morris, and Roseau, MN. WC=Winter Camelina, PC=Pennycress, RY=Winter Rye, RA=Radish, ST=Stubble, TI=Till. Values are means  $\pm$  SE,  $n=4$ .

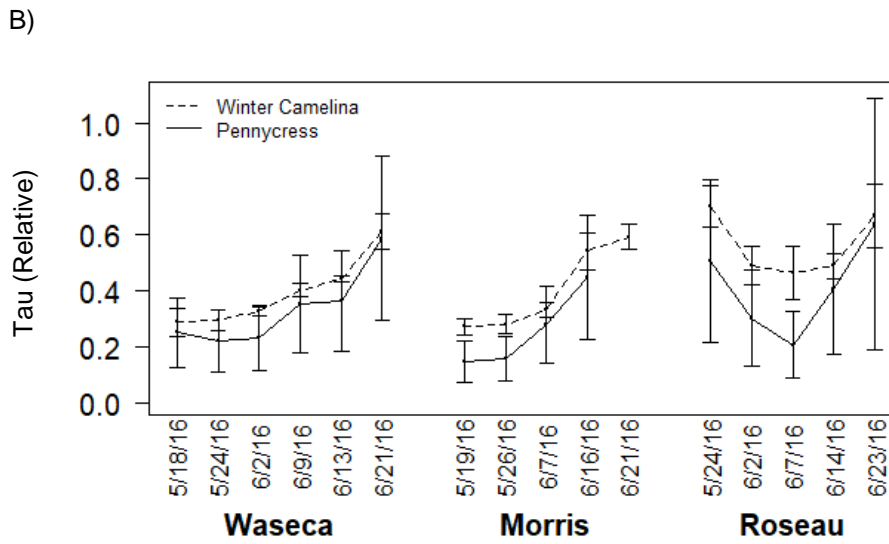
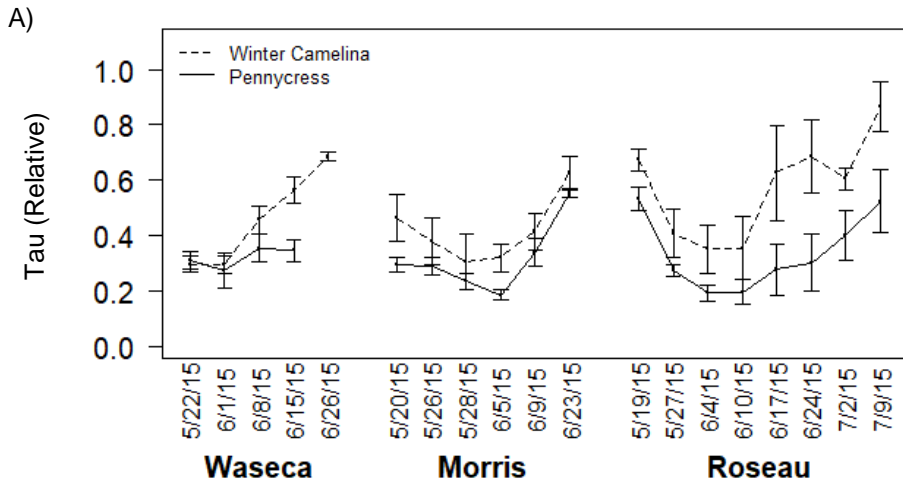


**Figure 2.** Regression of pennycress yield as a function of mean daily precipitation in the driest period near pennycress flowering in Waseca, Morris, and Roseau, MN in 2015 and 2016.

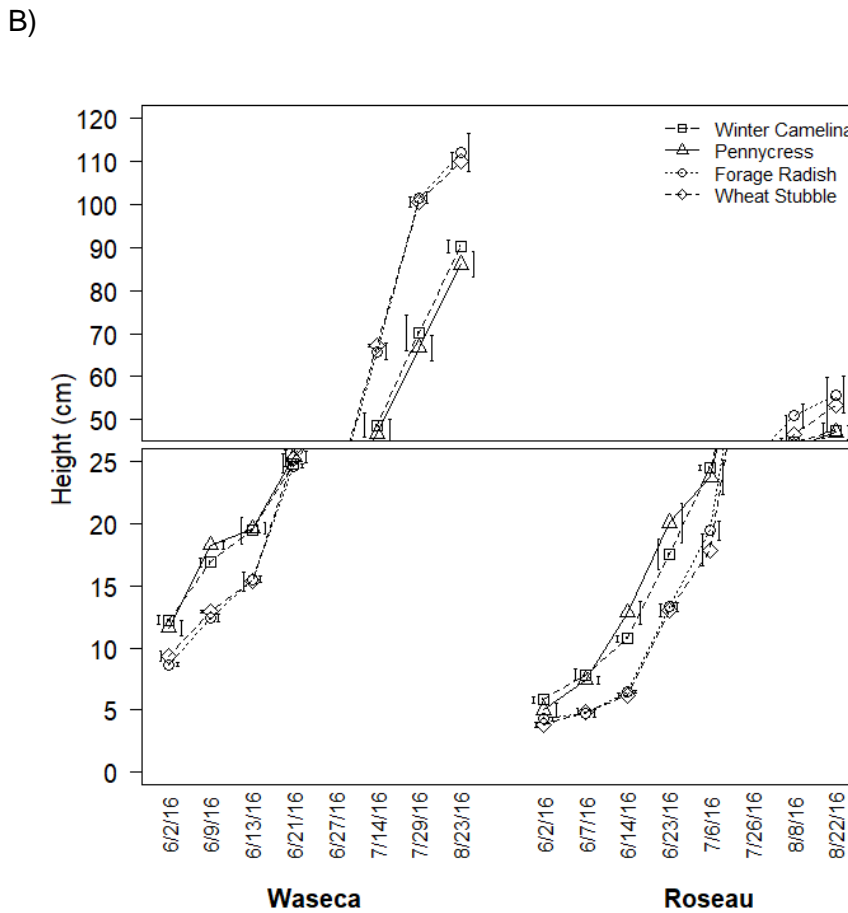
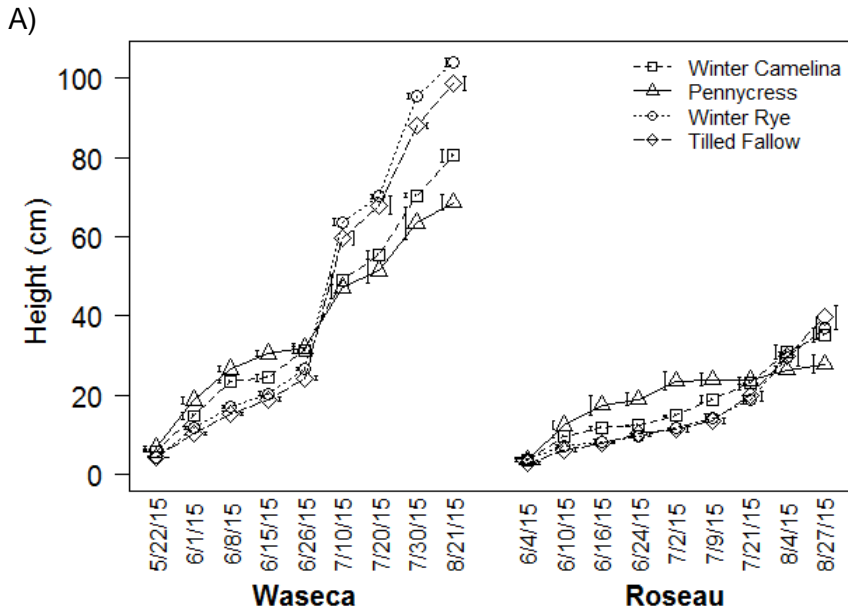




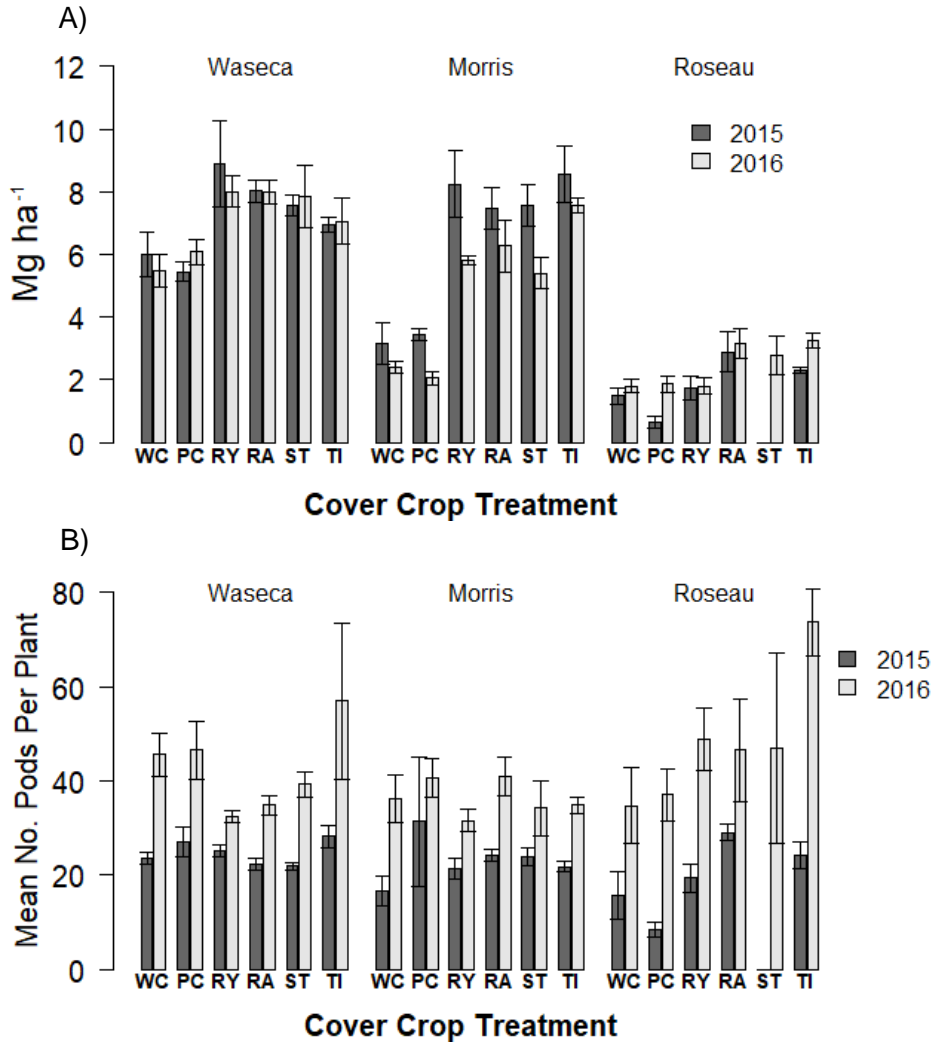
**Figure 3.** Photosynthetically Active Radiation (PAR) available to soybean under the canopy of winter camelina and pennycress observed in Waseca, Morris, and Roseau, MN in 2015 (A) and 2016 (B). Tau (Relative) = (PAR below oilseed canopy)/(PAR above oilseed canopy). Values are means  $\pm$  SE,  $n=4$ .



**Figure 4.** A) Soybean heights measured in four cover crop treatments and two winter fallow control treatments in 2015 and B) 2016 in Waseca, Morris, and Roseau, MN. Values are means  $\pm$  SE,  $n=4$ .



**Figure 5.** A) Soybean above-ground biomass from four cover crop treatments and two winter fallow treatments. B) The number of pods per plant observed from the biomass samples. Observations made in Waseca, Morris and Roseau in 2015 and 2016. WC=Winter Camelina, PC=Pennycress, RY=Winter Rye, RA=Radish, ST=Stubble, TI=Till. Values are means  $\pm$  SE,  $n=4$ .



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## Chapter 2

# Nitrogen and Phosphorus: Amounts Sequestered by Cover Crops and Levels in the Soil

### Summary

Many agricultural watersheds in Minnesota have toxic levels of phosphorus and nitrogen, which largely originate from agricultural fields that are fallowed from October through May. Autumn-sown winter cover crops can be used to retain these nutrients. Soil NO<sub>3</sub>-N levels and quantities of N sequestered by winter rye (*Secale cereale*), radish (*Raphanus sativus*), and the oilseed crops, winter camelina (*Camelina sativa*), and pennycress (*Thlaspi arvense*) were evaluated in species-specific, dual-cropping systems with soybean at three sites spanning the north-south climatic gradient of Minnesota. Radish sequestered the most N in autumn, but winter-killed and had high soil NO<sub>3</sub>-N levels in spring. Winter rye was terminated chemically by early May at each site, whereas the oilseed crops were allowed to grow into June to full maturity and their seeds were harvested. In autumn through early May, winter camelina and pennycress sequestered about 25% less N than winter rye. However, they often sequestered  $\geq 2.5$  times more N than winter rye when compared at maximum seasonal biomass (up to 130 kg N ha<sup>-1</sup>), with some of this N coming from spring fertilizer application. The relative amount of applied N captured by oilseeds, defined here as applied N sequestration efficiency, was 95% and 68% for winter camelina and pennycress, respectively.



## Introduction

The most severe water pollution in Minnesota is in the southern half of the state, and the primary cause is contamination from croplands (MPCA, 2014). In the central region of Minnesota, 94% of shallow wells (<15 m depth) had levels of NO<sub>3</sub>-N exceeding the safe drinking water limit of 10 mg L<sup>-1</sup>, and 75% of deep wells (>15 m depth) exceeded this limit (MDA 2014). The MPCA also determined that 75,000 MT of nitrogen (N) annually flow into the Mississippi River in Minnesota. The fate of much of this N is in the Gulf of Mexico where it is a primary cause of algal blooms (Rabalais et al., 2007). The MPCA's goal is to reduce nutrient loading by 20% by 2025 and 45% by 2040 (MPCA, 2014). To reach these goals, improvements in fertilizer efficiency and drainage tile line management may not be sufficient; widespread adoption of cover crops, which sequester soil nutrients, also may be necessary (MPCA, 2014).

Little is known about how much N and P is sequestered by winter camelina and pennycress (Johnson et al., 2017). Thus, the objective of this study was to determine to what extent these oilseed cover crops remove N and P from soil and sequester them in above ground biomass.

## Materials and Methods

### Soil Nutrient Analyses

All research was performed in the same experimental plots and timeframe as described in Chapter 1. Soil samples at depths of 0-30 cm and 30-60 cm were collected five times during each growing season: 1) before planting cover crops in early autumn, 2) after planting cover crops in the last two weeks of October, 3) before planting soybean in mid April to early May, 4) after the harvest of winter camelina and pennycress in late June to early July, and 5) after soybean harvest in late September to early October. Soil cores were 3.2 cm in diameter, four cores were taken in each plot (two for N and P analysis and two for gravimetric water balance), cores were taken approximately 2 meters from the ends of each plot, and they were aggregated subsequently within plots prior to chemical analyses. Soil nutrient samples were dried, ground to pass through a 500  $\mu\text{m}$  mesh sieve, and subsamples were analyzed for  $\text{NO}_3\text{-N}$  (2 g subsample) and  $\text{PO}_4\text{-P}$  (1 g subsample) using the procedures outlined by Mulvaney (1996) for N and Olsen and Sommers (1982) for P.

### Crop Analyses

Cover crops were sown as described in Chapter 1, and related crop operations can also be found there.

Cover crop above-ground biomass was sampled three times each year: 1) after cover crop establishment in the last two weeks of October, 2) while winter annual cover crops were bolting in late April to mid-May, and 3) during anthesis of the oilseed cover crops in late May to early June. Cover crop above-ground biomass samples were 0.5 m in length from two central rows (0.25  $\text{m}^2$ ). In addition to dry matter, the samples were

analyzed for percent N and C using a Leco CN-2000 combustion analyzer (Leco Corporation, St. Joseph, MI). Samples were dried to a constant weight at 65°C, ground to pass through a 420 µm mesh sieve, and a 200 mg portion was analyzed. The amount of N sequestered was calculated by multiplying above-ground dry biomass by the percentage N. Applied N sequestration efficiency (ANSE) of the oilseed cover crops was calculated as follows:

$$\frac{((\text{amount of oilseed cover crop N sequestration in May or June}) - (\text{amount of oilseed cover crop sequestration in April}))}{(\text{amount of N applied})}$$

### Statistical Analyses

Statistical analyses for cover crop biomass and soil NO<sub>3</sub>-N levels included a standard ANOVA test of significance at the  $P = 0.05$  level unless otherwise noted. Tukey's Honest Significant Difference was used to compare treatment means. The only data set that contained heteroscedasticity was for the soil NO<sub>3</sub>-N. Due to heterogeneity of error variances among site-years, each site-year was analyzed separately, following the guidelines described in Gomez 1984. Some site-years in this data set did not meet the assumptions of ANOVA, thus log transformations were used for these data. If ANOVA assumptions were still unmet, square-root and Box-Cox transformations were also attempted. Due to non-constant variance between years for all of the data included here, each year was analyzed separately. These procedures were performed using R version 3.3.2. Regressions were performed using the "data analysis toolpak" in Microsoft® Excel® 2016.

# Results and Discussion

## Weather

Weather data are presented in Chapter 1.

### Cover Crop Biomass Production and N and P Content

The cover crop biomass samples were analyzed for dry weight, percent N, and N uptake per unit area (Table 1). Although percent N varied widely across crops and time periods (from 1.4 to 5.5%), cover crop biomass alone explained 88 percent of the variation in the amount of N sequestered (Figure 1). In autumn, radish consistently produced significantly more biomass than winter camelina and pennycress. It accumulated up to 2500 kg ha<sup>-1</sup> of biomass, and it sequestered up to 100 kg N ha<sup>-1</sup>. Winter rye also produced high levels of biomass (up to 1400 kg ha<sup>-1</sup>) in autumn and sequestered up to 57 kg N ha<sup>-1</sup>, significantly more than pennycress, but not different than camelina. Radish winter-killed, thus it provided no living biomass in spring; and winter rye sequestered N until it was killed chemically in late April to early May. Biomass of winter rye before it was terminated in spring was as high as 1900 kg ha<sup>-1</sup> with sequestered N levels of up to 50 kg ha<sup>-1</sup>, which was often equivalent to the oilseed cover crops (Table 1).

The percentage of N in the aboveground biomass of the remaining cover crops had shifted appreciably by April. Winter camelina had a significantly higher percentage of N (often  $\geq 4\%$ ) in all six-site years compared to winter rye, while for pennycress, five site-years showed a higher percentage than winter rye (Table 1). These percentages were manifested in the amount of N sequestered by each cover crop in early spring, which ranged from 20 to 62 kg N ha<sup>-1</sup>. Though winter rye biomass production was significantly

greater than that for winter camelina in Waseca and Roseau, there were no differences for any site-year with regard to the amount of N sequestered. Biomass production compensated for nitrogen concentration in the case of winter rye versus pennycress in Waseca in 2015, as the amount of N sequestered was significantly greater for winter rye there. However, this was not the case in Waseca in 2016, where there was no significant difference between pennycress and winter rye in the amount of N sequestered. Since winter rye did not reach peak biomass before soybean planting, and radish did not survive the winter, the total amount of N that winter camelina and pennycress sequestered was often 2-3 times greater than these other covers, with some of this N coming from the 80 kg ha<sup>-1</sup> spring N application following the April biomass sampling (Table 1). The relative amount of this applied N that was captured in the above ground biomass by the oilseeds when they reached their peak biomass by the May/June biomass sampling date was termed “applied N sequestration efficiency” (ANSE). Across sites and years, the mean ANSE was 95% for camelina and 68% for pennycress.

For the May/June winter camelina and pennycress biomass samples, there were generally no significant differences among treatments in dry weight, percentage N, and sequestered N. However, the extent of N sequestered by the two winter oilseed crops is notable, with ranges of 67 to 129 kg ha<sup>-1</sup> for winter camelina and 55 to 131 kg ha<sup>-1</sup> for pennycress (Table 1). These values represent appreciable levels of sequestration during a time of year when N is vulnerable to loss by erosion and leaching. Direct sequestration of P by the oilseed crops was not as notable (up to 21 kg ha<sup>-1</sup>), but others have demonstrated that cover crops significantly reduce P movement by preventing erosion (Kovar et al. 2011).

## Soil Nitrogen and Phosphorus Levels

Soil NO<sub>3</sub>-N levels in the 0-30 cm and 30-60 cm depths are presented in Table 2.

There was a significant, negative correlation between cover crop N sequestration and soil NO<sub>3</sub>-N levels (Table 3) indicating that cover crop biomass production was an effective proxy for identifying trends in soil NO<sub>3</sub>-N levels. By the October soil sampling dates for both years, all of the cover crop treatments had been well established except at Waseca in 2015, for which inclement weather delayed cover crop planting. In autumn, radish produced more biomass than the other cover crop treatments, and as expected, mean soil NO<sub>3</sub>-N was generally lower in the radish treatment than in the fallow treatments. In half of the site-years and mostly in spring and summer, mean soil NO<sub>3</sub>-N was lower in the camelina treatment than in the fallow treatments and there were few differences detected in the pennycress and winter rye treatments.

By the mid-April to early May soil sampling date, the winter hardy cover crops were beginning stem elongation and, as expected, soil NO<sub>3</sub>-N levels in these treatments were often lower than in the fallow treatments and winter killed radish. Averaged over sites and years and considering only the autumn and early spring dates for which living winter rye was present, this treatment had 55% less soil NO<sub>3</sub>-N at a depth of 0-30 cm than the stubble treatment (11 vs. 24 kg ha<sup>-1</sup>, respectively), pennycress had 46% less (13 vs. 24 kg ha<sup>-1</sup>, respectively), and camelina had 49% less (12 vs. 24 kg ha<sup>-1</sup>, respectively). At a depth of 30-60 cm, the winter rye treatment had 51% less than the stubble treatment (4 vs. 10 kg ha<sup>-1</sup>, respectively), and pennycress had 18% less (8 vs. 10 kg ha<sup>-1</sup>, respectively), and camelina had 42% less (5.5 vs. 10 kg ha<sup>-1</sup>, respectively). For comparison, in another study, Kaspar et al. (2007) observed that a winter rye cover crop reduced the NO<sub>3</sub>-N load

in water drained from tile lines by 61% (31 kg N ha<sup>-1</sup>) compared to a fallow control. Other researchers have also observed a similar pattern of winter rye cover crops reducing NO<sub>3</sub>-N concentration in ground water (Strock et al. 2004, Qi et al. 2011, Kaspar et al. 2012). The present study, however, is the first of its kind to report soil NO<sub>3</sub>-N reductions as influenced by winter camelina and pennycress grown as cover crops.

After cover crop harvest in late June/early July, mean soil NO<sub>3</sub>-N levels tended to be similar between the winter fallow and cover crop treatments. This was not surprising as the winter camelina and pennycress treatments each were fertilized with 80 kg ha<sup>-1</sup> of N immediately following the April soil sampling date, but also indicated, as did our estimates of applied N sequestration efficiency, that the winter oilseeds effectively used the applied N for their growth and seed production. This rate of N application was found to be within a range that optimizes oilseed growth and yield response (Johnson and Gesch 2013).

Following soybean harvest in late September/early October, all treatments generally had similar NO<sub>3</sub>-N levels in the 0-30 cm soil profile. Trends in soil NO<sub>3</sub>-N by cropping treatment at the 30-60 cm depth were generally similar to those for the 0-30 cm depth. In half of the site-years, the radish treatment had a lower mean soil NO<sub>3</sub>-N content than the fallow treatments. The winter camelina and the winter rye treatments seldom had a lower mean soil NO<sub>3</sub>-N content than the fallow treatments, while the pennycress treatment was never significantly higher in NO<sub>3</sub>-N content than the fallow treatments. This agrees with logic, as all treatments by this time had gone through a growing season with a legume.

In April, the winter rye treatment often had a significantly lower mean soil NO<sub>3</sub>-N content than the fallow treatments at a depth of 0-30 cm. The winter camelina treatment

had a significantly lower mean soil NO<sub>3</sub>-N content than the fallow treatments in half of the site-years, while the radish and pennycress treatments seldom had NO<sub>3</sub>-N levels that were significantly different from the fallow treatments.

After oilseed harvest in late June/early July, the pennycress treatment often had a significantly lower mean soil NO<sub>3</sub>-N content than the fallow treatments at the 0-30 cm depth, the winter camelina and winter rye treatments did so in half of the site-years, while the radish treatment seldom did so.

Spring through early summer is a critical window of time in which the potential for N leaching is higher than almost any other time throughout the year. Though pennycress soil NO<sub>3</sub>-N levels at a depth of 30-60 cm were equivalent to those of the fallow treatments in April, by late June/early July these levels had fallen significantly below those of the fallow treatments. This decrease in soil NO<sub>3</sub>-N levels from April through early summer was not observed for radish, where there was no change in differences between spring and summer sampling dates. A likely explanation for this is that pennycress scavenged N during this time, while winter-killed radish could not. Results indicate there was a general pattern of elevated NO<sub>3</sub>-N levels in soils sampled in spring or early summer in which radish had been planted the previous autumn. This pattern is discernable for 7 of the 12 dates/depths of observation.

Dean and Weil (2009) demonstrated that forage radish releases part of the N it sequesters during autumn to ground water at depths below annual crops' root zones in spring on coarser textured soils. Their research also showed that in the spring, even on finer textured soils, N levels were elevated in shallow depths of these soils in plots where forage radish had been grown relative to other cover crops such as winter rye and rape.



Some of this N could also potentially escape the root zone of summer annual crops as their root systems are not fully established by this time.

For the post soybean harvest in late September/early October, there were generally no differences among treatments in terms of soil NO<sub>3</sub>-N, and again this agrees with logic considering all treatments had gone through a growing season with a legume.

Levels of soil PO<sub>4</sub>-P were never different among treatments of the same sampling date and depth, which was to be expected given what we observed with the sequestration of P in cover crop biomass. For the 0-30 cm sample depth, soil PO<sub>4</sub>-P in winter rye ranged from 9 kg ha<sup>-1</sup> (Morris, spring 2015) to 82 kg ha<sup>-1</sup> (Roseau, autumn 2014). For the 30-60 cm sample depth, soil PO<sub>4</sub>-P ranged from 0 kg ha<sup>-1</sup> in several site years (three observations for camelina and winter rye treatments and one observation for radish and tilled treatments), to 56 kg ha<sup>-1</sup> in the Waseca summer 2015 wheat stubble treatment.

## Conclusions

Winter camelina and pennycress sequester substantial amounts of NO<sub>3</sub>-N and thus can help address the issue of water pollution in the Upper Midwest if used as cover crops. This finding will support work by growers and policy experts to reduce N contamination of rural drinking water and to achieve the MPCA goal of a 20% reduction in nutrient loading of the Mississippi River by 2025 and a 45% reduction by 2040. Pennycress sequestered relatively less of the applied N compared to camelina, while producing similar seed yields (Chapter 1), which is an indicator that it may have a greater nitrogen use efficiency than camelina. Future research to determine if a spring N application is necessary for either oilseed may be worthwhile. As the agronomics and genetics of the winter oilseed cover crops continue to be improved at the University of Minnesota and elsewhere, further acceleration toward these goals is expected.

**Table 1.** Cover crop above ground biomass, percentage N, and sequestered N (biomass x %N). Above ground biomass was collected in Waseca, Morris, and Roseau, MN from 2014-16. Values are means with n=4.

| Month/<br>Year     | Cover Crop<br>Treatment | Waseca   |        |  | Morris   |        |  | Roseau   |        |  |
|--------------------|-------------------------|--|--------|--|--|--------|--|--|--------|--|
|                    |                         | Above<br>Ground<br>Biomass<br>(kg ha <sup>-1</sup> ) | %N     | Sequest.<br>ered N<br>(kg ha <sup>-1</sup> ) | Above<br>Ground<br>Biomass<br>(kg ha <sup>-1</sup> ) | %N     | Sequest.<br>ered N<br>(kg ha <sup>-1</sup> ) | Above<br>Ground<br>Biomass<br>(kg ha <sup>-1</sup> ) | %N     | Sequest.<br>ered N<br>(kg ha <sup>-1</sup> ) |
| Sept./Oct.<br>2014 | Radish                  | 1579 a   | 2.6 a  | 41 a   | 1032 a   | 3.1 NS | 32 a   | 2529 a   | 4.2 a§ | 104 a†                                       |
|                    | W. Rye                  | 1413 a   | 2.9 a  | 41 a   | 829 ab   | 3.1    | 26 ab  | 1319 b   | 4.4 a  | 57 b   |
|                    | W. Camelina             | 1045 ab  | 2.5 a  | 24 b   | 488 bc   | 3.4    | 16 bc  | 1030 bc  | 3.5 a  | 36 c   |
|                    | Pennycress              | 631 b  | 2.3 a  | 14 b   | 237 c  | 3      | 7 c  | 684 c  | 4 a    | 26 c   |
| Apr. 2015          | W. Rye                  | 1083 a   | 3.6 c  | 39 a   | 770 a§   | 3.3 b  | 26 a†  | 1810 a   | 2.8 b  | 50 a†  |
|                    | W. Camelina             | 669 b  | 4.5 a  | 30 ab  | 525 a  | 5.5 a  | 29 a   | 1095 a   | 4.2 a  | 41 a   |
|                    | Pennycress              | 492 b  | 4.1 b  | 20 b   | 1015 a   | 4.7 a  | 48 a   | 1442 a   | 4.4 a  | 62 a   |
| May/Jun.<br>2015   | W. Camelina             | 4348 a   | 3 a§   | 129 a  | 3260 a   | 3.5    | 115 a  | 3501 a   | 3.4    | 123 a  |
|                    | Pennycress              | 3985 a   | 2.4 a  | 94 a   | 3617 a   | 2.9    | 104 a  | 3957 a   | 3.4    | 131 a  |
| Sept./Oct.<br>2015 | Radish                  | 105 a  | 3.3 b  | 3 a  | 1396 a   | 2.7 b  | 38 a   | 381 a  | 3.4 NS | 12 a†  |
|                    | W. Rye                  | 63 ab  | 3.7 ab | 2 ab   | 673 b  | 4.1 a  | 27 ab  | 293 ab   | 4      | 11 ab  |
|                    | W. Camelina             | 40 b   | 4 a    | 2 b  | 302 bc   | 4.7 a  | 14 bc  | 121 b  | 4.1    | 8 b  |
|                    | Pennycress              | 27 b   | 4 a    | 1 b  | 123 c  | 4.4 a  | 6 c  | 270 ab   | 3.2    | 5 ab   |
| Apr. 2016          | W. Rye                  | 901 a  | 3 b    | 27 NS  | 1776 a§  | 2.4 b  | 41 b   | 1909 a   | 1.4 b  | 27 NS  |
|                    | W. Camelina             | 526 b  | 4.6 a  | 24   | 1147 a   | 3.5 a  | 37 b   | 342 b  | 4.9 a  | 17   |
|                    | Pennycress              | 429 b  | 4.8 a  | 20   | 1983 a   | 2.7 ab | 57 a   | 1045 ab  | 3.8 a  | 35   |
| May/Jun.<br>2016   | W. Camelina             | 3407 a   | 2.4 a  | 81 a   | 4422 a   | 2.7    | 120 a  | 3393 a   | 2 b    | 67 a   |
|                    | Pennycress              | 2161 a   | 2.5 a  | 55 a   | 3906 a   | 2.5    | 99 a   | 3112 a   | 2.7 c  | 83 a   |

† Indicates log transformation to meet assumptions of ANOVA.

‡ Indicates significance at the P<0.05 level.

§ Indicates significance at the P<0.1 level.

**Table 2.** Soil NO<sub>3</sub>-N levels from soil samples taken at depths of 0-30 cm and 30-60 cm in four cover crop treatments and two winter fallow treatments. Based upon soil samples taken in Waseca, Morris, and Roseau, MN from 2014-2016. Units are kg ha<sup>-1</sup> unless a given site date required a transformation of the response to meet assumptions of ANOVA, in which case data are presented in transformed units. Values are means with *n*=4.

| Month/<br>Year           | Cover Crop<br>Treatment | Soil Core Depth: 0-30cm |         |        | Soil Core Depth: 30-60cm |         |        |
|--------------------------|-------------------------|-------------------------|---------|--------|--------------------------|---------|--------|
|                          |                         | Waseca                  | Morris  | Roseau | Waseca                   | Morris  | Roseau |
| Fall<br>2014<br>Year 1   | None: Tilled            | 0.8bc †                 | 8.7a ‡  | 7.4a § | 5.9bc                    | 1.1a †# | 28.4a  |
|                          | Stubble                 | 1.4a                    | 8.6a    | NA     | 11.2a                    | 1.0a    | NA     |
|                          | Radish                  | 0.6c                    | 3.1b    | 2.6b   | 4.1c                     | 0.6a    | 4.7b   |
|                          | Winter Rye              | 0.7c                    | 5.9ab   | 4.1ab  | 4.9c                     | 1.1a    | 8.8b   |
|                          | Winter Camelina         | 0.7c                    | 5.2ab   | 4.9b   | 5.0c                     | 0.9a    | 11.9b  |
|                          | Pennycress              | 1.1ab                   | 5.5ab   | 7.4a   | 10.3ab                   | 0.8a    | 15.8b  |
| Spring<br>2015<br>Year 1 | None: Tilled            | 14.2a                   | 1.4a †  | 1.9a † | 1ab †                    | 13.5a S | 25.0a  |
|                          | Stubble                 | 12.3ab                  | 1.5a    | NA     | 1.2a                     | 13.1a   | NA     |
|                          | Radish                  | 12.5ab                  | 1.3ab   | 1.5b   | 1ab                      | 5.8a    | 13.3ab |
|                          | Winter Rye              | 4.7c                    | 0.9c    | 1.1b   | 0.5c                     | 5.8a    | 7.0b   |
|                          | Winter Camelina         | 4.7c                    | 1.1bc   | 1.5b   | 0.7bc                    | 7.4a    | 15.4ab |
|                          | Pennycress              | 6.2bc                   | 1.1bc   | 1.4b   | 1ab                      | 5.6a    | 19.2ab |
| Summer<br>2015<br>Year 1 | None: Tilled            | 19.0ab                  | 1.3NS‡  | 69.8a  | 2.4NS‡                   | 1.9a †¶ | 8.7a § |
|                          | Stubble                 | 17.2ab                  | 1.4     | NA     | 2.2                      | 1.7ab   | NA     |
|                          | Radish                  | 13.7b                   | 1.4     | 51.0ab | 2.3                      | 1.7ab   | 5.2b   |
|                          | Winter Rye              | 12.4b                   | 1.4     | 20.8c  | 2.2                      | 1.4bc   | 1.7c   |
|                          | Winter Camelina         | 20.2ab                  | 1.4     | 26.0bc | 1.9                      | 1.5ab   | 4.2b   |
|                          | Pennycress              | 35.7a                   | 1.4     | 21.0c  | 2                        | 1.1c    | 4.0b   |
| Fall<br>2015<br>Year 2   | None: Tilled            | 1.0NS†                  | 1.5a †  | 12a ‡  | 0.5NS†                   | 10.2a   | 7.7NS  |
|                          | Stubble                 | 1.2                     | 1.3a    | 9.2ab  | 0.4                      | 7.7a    | 4.0    |
|                          | Radish                  | 1.0                     | 0.8b    | 4.8c   | 0.7                      | 2.8b    | 5.3    |
|                          | Winter Rye              | 1.0                     | 1.1ab   | 5bc    | 0.5                      | 8.1a    | 7.1    |
|                          | Winter Camelina         | 1.0                     | 1.4a    | 5.2bc  | 0.5                      | 10.1a   | 6.1    |
|                          | Pennycress              | 1.1                     | 1.2ab   | 6.8bc  | 0.6                      | 8.4a    | 10.0   |
| Spring<br>2016<br>Year 2 | None: Tilled            | 39.0NS                  | 37.4a   | 17.0a  | 20.0a                    | 1.2a †  | 0.8a † |
|                          | Stubble                 | 30.8                    | 33.8a   | 13.4ab | 19.4ab                   | 0.9abc  | 0.8a   |
|                          | Radish                  | 24.1                    | 26.3ab  | 12.3ab | 10.6bc                   | 0.7bc   | 0.5ab  |
|                          | Winter Rye              | 21.9                    | 19.7b   | 9.3b   | 6.6c                     | 0.5c    | 0.4b   |
|                          | Winter Camelina         | 21.7                    | 19.3b   | 7.9b   | 13.5abc                  | 0.6bc   | 0.4b   |
|                          | Pennycress              | 19.6                    | 25.1ab  | 6.6b   | 17.4ab                   | 0.9ab   | 0.6ab  |
| Summer<br>2016<br>Year 2 | None: Tilled            | 4.0NS§                  | 563a S‡ | 27.9NS | 1.4a †                   | 1.3a †¶ | 18.0a  |
|                          | Stubble                 | 3.3                     | 539a    | 22.3   | 1.1ab                    | 1.4a    | 11.8b  |
|                          | Radish                  | 4.5                     | 599a    | 24.7   | 1.1ab                    | 1.2ab   | 9.0bc  |
|                          | Winter Rye              | 3.7                     | 545a    | 26.5   | 0.9ab                    | 1ab     | 8.2bc  |
|                          | Winter Camelina         | 4.6                     | 224a    | 22.0   | 0.6b                     | 0.9ab   | 6.2c   |
|                          | Pennycress              | 6.2                     | 379a    | 28.1   | 0.8ab                    | 0.9b    | 9.2bc  |

† Indicates a log transformation was performed to meet assumptions of ANOVA.

‡ Indicates that a Box-Cox transformation was performed to meet assumptions of ANOVA.

§ Indicates that a square root transformation was performed to meet assumptions of ANOVA.

¶ Indicates assumptions of ANOVA were not met.

# Indicates significance at the P<0.1 level.

NS = not significant. S = significant. Means separation performed with Tukey's HSD.

**Table 3.** Regression results of soil NO<sub>3</sub>-N on N sequestered in cover crop biomass. Based upon soil and above ground biomass samples collected in Waseca, Morris, and Roseau, MN from 2014-16.

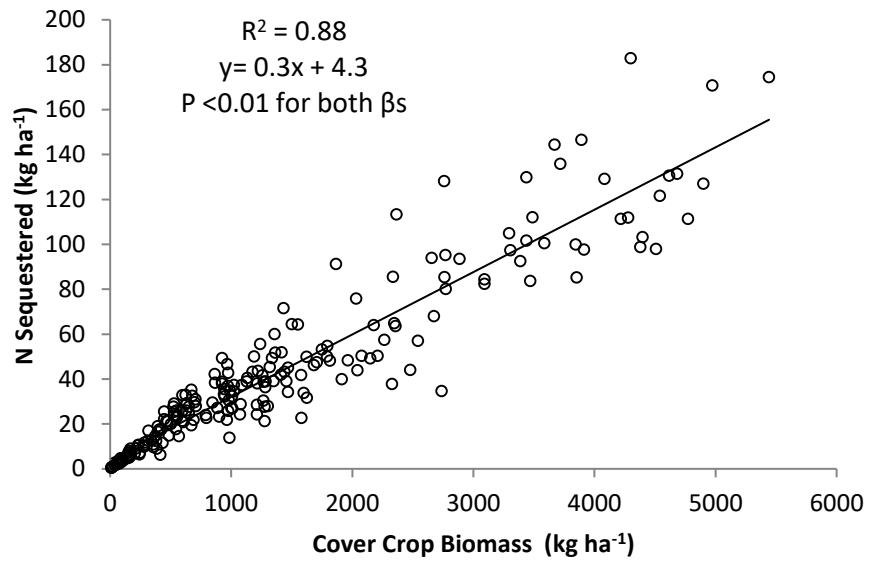
| Site   | Month/Year | Soil Depth (cm) | Correlation | P-value | Mean Crop Biomass |
|--------|------------|-----------------|-------------|---------|-------------------|
| Waseca | Oct-14     | 0-30            | -0.57       | <0.01   | 1556              |
| Morris | Oct-14     | 0-30            | -0.51       | 0.01    | 862               |
| Roseau | Oct-14     | 0-30            | -0.65       | <0.01 † | 1956              |
| Waseca | Oct-14     | 30-60           | -0.48       | 0.02    | 1556              |
| Morris | Oct-14     | 30-60           | -0.41       | 0.05 †  | 862               |
| Roseau | Oct-14     | 30-60           | -0.78       | <0.01   | 1956              |
| Waseca | Apr-15     | 0-30            | -0.76       | <0.01   | 997               |
| Morris | Apr-15     | 0-30            | -0.65       | <0.01   | 1026              |
| Roseau | May-15     | 0-30            | -0.84       | <0.01   | 1975              |
| Waseca | Apr-15     | 30-60           | -0.79       | <0.01   | 997               |
| Morris | Apr-15     | 30-60           | -0.48       | 0.03    | 1026              |
| Roseau | May-15     | 30-60           | -0.37       | 0.18    | 1975              |
| Waseca | Oct-15     | 0-30            | -0.04       | 0.87    | 78                |
| Morris | Oct-15     | 0-30            | -0.60       | <0.01   | 831               |
| Roseau | Oct-15     | 0-30            | -0.50       | 0.02    | 355               |
| Waseca | Oct-15     | 30-60           | 0.43        | 0.05 †  | 78                |
| Morris | Oct-15     | 30-60           | -0.58       | <0.01   | 831               |
| Roseau | Oct-15     | 30-60           | -0.32       | 0.14    | 355               |
| Waseca | Apr-16     | 0-30            | -0.47       | 0.041   | 825               |
| Morris | Apr-16     | 0-30            | -0.79       | <0.01   | 2180              |
| Roseau | May-16     | 0-30            | -0.44       | 0.06    | 1472              |
| Waseca | Apr-16     | 30-60           | -0.61       | 0.01    | 825               |
| Morris | Apr-16     | 30-60           | -0.74       | <0.01 ‡ | 2180              |
| Roseau | May-16     | 30-60           | -0.41       | 0.08    | 1472              |

Regression of above correlations and mean crop biomass. -0.62 <0.01

† Indicates one outlier (Bonferroni) was removed.

‡ Indicates two outliers (Bonferroni) were removed. Outlier from PC plot 126 had Bonferroni P-value of 0.0503.

**Figure 1.** Regression of amount of N sequestered as a function of cover crop biomass. Observations made in Waseca, Morris and Roseau in 2015 and 2016.



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