INTEGRATING ALFALFA AND WINTER CAMELINA INTO WHEAT-SUNFLOWER-SOYBEAN ROTATIONS ENHANCES BIODIVERSITY AND CROPPING SYSTEM RESILIENCE

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ABSTRACT

Annual cropping systems offer many challenges in terms of biodiversity and environmental impact, especially with respect to shifting climate and the increased demand for food and fuel. This study was conducted to determine if the addition of alfalfa (*Medicago sativa* L.) and/or winter camelina (*Camelina sativa* (L.) Crantz) to annual crop rotations including sunflower (*Helianthus annus* L.), wheat (*Triticum aestivum* L.), or soybean [*Glycine max* (L.) Merr.] would increase cropping system agronomic resiliency and arthropod biodiversity. Evaluations of ten crop sequences at Prosper and Hickson, ND took place in 2022-2023. Analysis through life cycle assessment (LCA) was completed to quantify the carbon intensity (CI). Relaying soybean into winter camelina achieved lower total oilseed yield; however, the double crop with sunflower and winter camelina yielded more oil than the respective monocrops. Systems including alfalfa had the lowest CI value. Winter camelina introduced beneficial and pollinator arthropods early in the season.

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DEDICATION

I dedicate this thesis to my paternal grandparents, Lee and Judy Kurth, who have passed and were unable to see me achieve this dream and many others. I know they would be proud.

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1. INTRODUCTION

As demand for food, fuel, and fiber continue to rise, resiliency in agriculture is an increasingly important concept in order to maintain healthy and sustainable cropping systems and soils. Resiliency is defined as an agricultural system's ability to withstand change before a critical threshold of negative impact is reached (University of Nebraska-Lincoln, 2024). There are many tactics for increasing resiliency in cropping systems; however, climate-smart crop rotations and incorporating winter cover and perennial species are key concepts that can increase biodiversity and cropping system resilience. Winter annual species have the ability to provide winter and early spring cover, but also be harvested as a cash crop. Many producers have concerns regarding yield loss due to cover crops; however, yield impacts are considered minor when compared with the agronomic benefits. In a majority of the cases, subsequent crop yield is not increased, but is also not decreased (Hendrickson et al., 2021). Monetary value of soil protection afforded by cover and winter annual crops can be difficult to estimate. Few winter annual crop options are available, but winter camelina in particular has shown success in the Northern Great Plains region.

Winter camelina is a winter annual oilseed crop of the Brassicaceae family that has multiple uses as a feedstock for biofuel, oil for human consumption, and animal feed (Berti et al., 2016). Renewed interest in camelina has occurred due to its desirable traits such as long-chain unsaturated fatty acid content as a sustainable feedstock for the aviation biofuel industry and high omega-3 content for dietary oils (Mach, 2019). Winter camelina's short life cycle and early maturity (~70 days from consistent 5 °C temperatures in spring) promises relay or double-cropping potential with certain crops common to the northern Great Plains (Mohammed et al., 2022). Relay-cropping is the practice of planting a secondary crop into an established primary crop. Double-cropping is the practice of harvesting two monocrops in the same year. Due to its early maturity and flowering period, winter camelina provides the earliest floral resources available in the northern Great Plains region, and is often categorized as having a high agroecosystem value because of this (Eberle et al., 2015). Winter camelina uniquely has the ability to provide the ecosystem services of a cover crop with fall and early spring growth, but can be harvested as an oilseed crop.

Alfalfa is a highly important perennial species that is vital to maintaining livestock production. Alfalfa's ability to stay-green late in the season and provide continuous cover offers refuge for beneficial insects and other small mammals. An insect population in alfalfa was typically 99% beneficial and 1% pest (Putnam et al., 2001). Alfalfa suppresses many problematic annual and perennial weed species with early and competitive growth making weed populations in following rotations minimal. Glyphosate-resistant cultivars have improved weed control in the crop, especially during establishment year, when weed control is most critical (Berti & Samarappuli, 2018).

One method to quantify the resiliency of a cropping system is to examine the global warming potential (GWP) via life cycle assessment (LCA). Dual or relay cropping systems have the potential to intensify production while providing enhanced ecosystem benefits. High input cropping systems have higher GWP due to increased fertilizer, fuel, and other chemical use (Nemecek et al., 2015). Winter camelina, a relatively low input crop, has the potential to reduce greenhouse gas (GHG) emissions when cropped as a biodiesel or jet fuel feedstock (Li & Mupondwa, 2014). One conflict of double or relay cropping systems is the increased need for field activity, therefore increasing fossil fuel-related CO_2 emissions. Berti et al. (2017) reported that N_2O emissions were greater in soybean-camelina relay systems due to increased N fertilizer additions for camelina in comparison with a soybean monocrop. Alfalfa, a perennial crop, has the potential to reduce GWP in crop rotations. Despite increased field activity associated with multiple harvests each growing season, annual planting is not required, and because alfalfa is a legume, no N fertilizer is required, while providing year-round soil cover. When compared with a corn (*Zea mays* L.) for silage system, alfalfa produced 2.24 kg ha^{-1} of N_2O where silage corn produced 5.38 kg ha^{-1} of N_2O annually (Fathollahi et al., 2018).

Practicality of a cropping system is not only important in terms of agronomics but also profitability to the grower. Adding alfalfa to a crop rotation has been shown to increase potential profit. Average net return of an alfalfa-alfalfa-corn rotation was found to be \$919 ha yr^{-1} where a soybean-wheat-corn rotation was only \$11 ha yr^{-1} (Goplen et al., 2018). The difference in net return was attributed to alfalfa's ability to suppress weeds and withstand environmental change more efficiently compared with annual crops like soybean, wheat, and corn. Winter camelina markets are not as strong as other oilseed markets such as soybean and sunflower. Gesch et al. (2014) reported that net economic returns from a winter camelina-soybean relay trial were comparable with monocrop soybean.

This study aims to assess the improvements in biodiversity and soil benefits of traditional annual cropping systems by incorporating winter camelina and alfalfa into wheat-sunflower-soybean rotations at two locations in North Dakota. Assessments were made by various methods including: soil sampling, yield comparisons, economic benefits, forage nutritive value analysis, life cycle assessment, and arthropod trap sampling to determine impacts of the rotations on cropping system resilience and arthropod biodiversity.

1.1. Objectives

Main objective: Assess the changes in the productivity, soil health, and arthropod biodiversity of traditional annual cropping systems by incorporating winter camelina and/or alfalfa into wheat-sunflower-soybean sequences.

Specific objectives:

- To determine the grain and/or oil yield of wheat, sunflower, soybean, and winter camelina.
- To determine the forage nutritive value of harvested alfalfa.
- To determine the quality of wheat, sunflower, soybean, and camelina grain and/or oil after harvest.
- To assess soil nutrients and properties and their variations between cropping systems.
- To conduct a cost analysis of each system to determine economic benefits.
- To determine the global warming potential (GWP) of each cropping sequence via life cycle assessment.
- To determine biodiversity impacts of different crop sequences by counting and assessing arthropod populations.

2. LITERATURE REVIEW

2.1. Cover crops and relay cropping

In recent years, much attention has been placed on the importance of maintaining soil cover year-round to conserve soil nutrients and protect against erosion. One method of maintaining year-round soil cover is growing a cover crop such as winter camelina. Increased costs associated with implementing cover crops have led to hesitation among farmers. Conservation practices, such as cover crops, do not always have direct quantifiable monetary benefits; however, producers are likely to see ecosystem benefits within several years of implementing a cover crop plan. In a recent national survey about cover crops, farmers estimated that they had a 3.6% yield increase in soybean yield following an average of 4.9 years of cover crop use (Sustainable Agriculture Research & Education, 2023). Producers are more likely to plant a fall cover crop if there is an increase in cash crop yield the subsequent year, or if harvesting the cover crop is an option, such as in the case of winter camelina. In most cases, subsequent crop yield is not increased, but is also not negatively impacted (Hendrickson et al., 2021). Cover crops can change soil chemistry, physical properties, and increase insect populations, which can affect yields in following seasons (Crotty & Stoate, 2019). Although many of these effects are minimal on their own, when combined, they have the ability to greatly enhance a cropping system through increased plant and arthropod biodiversity and improved soil health. Soil health is defined as the capacity of soil to function as a living ecosystem to support plants, animals, and humans (NRCS, 2024). Lengthening rotations by including more crops in a longer rotation schedule and implementing cover crops can enhance soil nutrient cycling and soil organic matter (Sprunger et al., 2020). Especially in semiarid environments, cover crop impacts on crop yield and available water vary year-to-year based on climate and precipitation

(Hendrickson et al., 2021). Winter camelina offers the benefits of a brassica cover crop with harvest potential as an oilseed monocrop or in relay. Unlike winter rye (*Secale cereale* L.), it will not immobilize nitrogen, which makes it desirable in rotation before a N fertilizer-requiring crop (Forever Green Initiative, 2023).

Relay-cropping, which uses ideas of cover cropping, has the potential to sustainably increase crop production. Relay-cropping is a system where a second cash crop is planted into an actively growing first cash crop. Timing and weather are major factors in the effectiveness of relay-cropping. Although relay cropping can have positive soil and biodiversity effects, environmental impacts can be negative if compared with soybean alone. Global warming potential (GWP) of a soybean-camelina relay system was approximately two times higher than a soybean monocrop due to increased inputs and field activities; however, the study did not include non-N fertilizer N_2O field emissions in soybean (Berti et al., 2017).

2.2. Arthropod biodiversity

Traditional agricultural systems lack biodiversity and are some of the most intensely managed ecosystems on the planet. Biodiversity in agriculture includes the animals, plants, insects, and microorganisms that have direct and indirect impacts to food, fuel, and fiber production and agriculture. These living organisms provide many ecosystem services, or the benefits humans derive from ecosystem functions (Huang et al., 2015). Specifically, arthropod diversity is indicative of healthy ecosystems. Increasing cropping system diversity, through plants and arthropods, can address many of the challenges faced by modern agriculture (Carof et al., 2022).

One of the most insightful and easily conducted methods to sample for arthropod biodiversity is to record changes in arthropod populations throughout the growing season and between crop species. A study sampled ground-dwelling Carabidae arthropods using pitfall traps to determine if differences occurred between plant species. Beetle populations peaked in fallow ground later in the season when plant material in plots became less available and beetles migrated to field margins in order to find groundcover (DuPrem et al., 2021). This study highlights the importance that ground beetles, natural predators of insect pests, need groundcover to thrive. Another study noted that ground beetles are highly sensitive to their agricultural environment, including tillage and pesticide use (Gayer et al., 2019). Arthropods of the Carabidae family are natural predators and their presence indicates greater ecosystem resilience (Gonzalez del Portillo et al., 2022). Alfalfa can provide a space for carabids to overwinter and remain in the ecosystem. By maintaining small uncut patches in alfalfa throughout the growing season, Carabid and Coccinellid species can be retained as effective aphid predators (Gonzalez del Portillo et al., 2022).

Pollinator species are important in agricultural systems, even for self-pollinated species like camelina. The USDA (2024) reports that about 35% of the world's food crops rely on pollinators for reproduction. A high-density population of honey bees (*Apis mellifera* L.) in camelina fields was found to enhance seed yield by allowing pollen transfer to occur 10- to 15-m away from the source (Zhang et al., 2021). Although much research focuses on bee (*Apidae spp.*) populations, many arthropod pollinator species belonging to multiple orders provide ecosystem services. Such orders include: Diptera, Lepidoptera, Coleoptera, and Hymenoptera (Katumo et al., 2022). Sunflower is another crop that has been selected from its native relatives to be self-compatible; however, it benefits from insect pollination. It has been reported that oil yield could increase 6.4% in hybrids exposed to a honey bee population with a density of 20 bees per 100 heads (Kandel, 2020).

2.3. Wheat history and cropping systems

Wheat contributes substantially to North Dakota's crop production and economy. Originally gathered in oak savannahs by the earliest *Homo sapiens*, wheat started its path to domestication nearly 13,000 years ago at the site of Abu Hureyra in modern day Syria (Zabinski, 2020). Wheat has one of the largest genomes in comparison to other grains and has been constantly improved with traditional breeding techniques. The Great Plains region of the United States grows around 16 million ha^{-1} wheat, over 60% of the U.S. total wheat production, every year (Paulsen & Shroyer, 2008). Hard-red spring wheat (HRSW) was a critical crop grown by early settlers to the Great Plains region as a productive food source. Winter wheat cultivars are not as common in North Dakota due to high risk of winterkill. After initial objection by bakers not accustomed to spring wheat, 'Red Fife' HRSW swept the Great Plains and has been bred since to the hard red spring wheat grown today (Paulsen & Shroyer, 2008). North Dakota produced 275 million bushels (9.7 billion kg^{-1}) of wheat, including durum (*Triticum turgidum* L.), spring, and winter cultivars between 2001-2003 and had direct economic impacts of $1.4 billion during those years (Bangsund & Leistritz, 2005). A recent survey by the USDA-NASS stated that North Dakota producers planted 2.25 million ha^{-1} of spring wheat that was valued over $1.5 billion (2022). Wheat, specifically spring wheat, continues to be an increasingly important crop for producers and the food industry. Production of HRSW is concentrated in the Red River Valley and the northern portion of North Dakota (Bangsund & Leistritz, 2005). A limiting factor in wheat production and quality is nitrogen. Optimal nitrogen rates must consider yield potential and cost of the fertilizer addition. To achieve the highest yield in medium-sized production systems and ideal protein content, the optimal nitrogen rate for HRSW wheat is 140 to 160 kg N ha^{-1} (Baker et al., 2004). Harvested HRSW must contain at least 140 g kg^{-1} protein content to avoid deductions upon sale (Franzen, 2022a). Kernel protein content can be enhanced with properly timed nitrogen applications.

Weed control is a major issue in wheat systems, especially in the Great Plains, due to similarities in weed growth cycles to wheat. At high densities of greater than 100 shoots m^{-2}, annual grass weeds such as green foxtail (*Setaria viridis* L.) and yellow foxtail (*Setaria pumila* L.) can severely reduce wheat yield (Khan et al., 1996). Proper crop management is key to protect wheat yield and reduce herbicide input. Early seeding dates, in mid-May, and seeding rates of 270 kg ha^{-1} are vital components of reducing weed pressure. Seeding date was found to be more important than seeding rate for North Dakota (Khan et al., 1996). Double-cropping spring wheat can be challenging in northern climates due to a lack of suitable weather after wheat is harvested. One study determined that fall planted alfalfa following spring wheat contained a weed biomass of 18% in comparison to only 1% when alfalfa followed soybean, which was due to a poor stand (Anderson, 2017).

2.4. Sunflower history and cropping systems

Sunflower is one of the most aesthetically appealing and economically important oilseed crops. A member of the Asteraceae family, the genus *Helianthus* contains 67 species (Kandel et al., 2020). Native to North America, wild types were domesticated and eventually transformed into the hybrids grown today. Oilseed sunflower was developed in Russia and soon spread as a popular crop throughout Europe. It has been an economically important crop in the United States since 1966 (Kandel et al., 2020). North Dakota is a top producer in the United States of oilseed sunflower. In 2021, North Dakota farmers harvested over 195,000 ha of sunflower that contributed to just under $2.5 million in production value (USDA-NASS, 2022). Sunflower production is concentrated in the Great Plains region of the United States. With many hybrids available, choosing the right hybrid for individual growing needs is important. Breeding efforts have focused on yield, oil content, fatty acid profile, and maturity of hybrids. Maturity is an important characteristic to consider because of climate, specific crop rotation, and drying costs (Kandel et al., 2020).

Northern climates have shorter growing seasons and require crops with earlier maturity groups. Early-maturing hybrids are typically reserved for late-planting, replanting, or double-cropping. Intercepted solar radiation and temperature during grain fill period in sunflower, which is from the end of anthesis to physiological maturity, has the greatest impact on yield (Kaya et al., 2004). Late planting dates can impact sunflower’s ability to capture the light and temperature needed to produce quality grain. The study by Kaya et al. (2004) noted that later-maturing, late-planted hybrids did not accumulate enough heat units to reach physiological maturity until two weeks after the typical frost date. Yield, oil content, and test weight can all be reduced if a frost occurs before maturity (Kandel et al., 2020). For a full season hybrid, the average accumulated growing degree days (°C) is 1,266, which occurs approximately 119 days after planting (Kandel et al., 2020).

Plant density and nitrogen application are key factors in sunflower achene yield. Yield increases are typically seen at plant densities up to 85,000 plants ha^{-1} (Ali et al., 2013). Nitrogen is an important factor in rapid leaf area development and increased photosynthetic rates. Nitrogen applications of 100 kg N ha^{-1} are typically the most economical for increased sunflower yield (Ali et al., 2013). North Dakota recommendations for nitrogen applications are based on nitrogen cost and sunflower market price. Recommendations are generally designed to ensure that approximately 160 kg ha^{-1} total N is available; however, recommendations should be made on the basis of N input price per kilogram and sunflower price per kilogram which can lower N recommendations down to 100 to 150 kg N ha^{-1} (Kandel et al, 2002; Franzen, 2022b). Phosphorus fertilization is generally not needed to achieve high sunflower yield.

Pests are a major challenge for sunflower production. One of the largest issues is bird damage, most commonly from blackbirds (*Turdus merula*) and pheasants (*Phasianinae*). One method of controlling bird damage is to apply a non-lethal repellent as a seed treatment and as the crop is maturing. One study tested anthraquinone-treated seed (Avipel) and found no negative sunflower yield effects, and both pheasants and grackles (*Quiscalus quiscula*) left newly planted seed alone. When Avipel was applied to maturing sunflower in the field, 18% damage was recorded compared with 64% of the untreated sunflower (Werner et al., 2011). Challenges faced with application of bird repellents include equipment, time, and cost of repellent. Bird damage continues to be a concern of sunflower producers, which is typically justified by large acreage, with damage being a lesser fraction amongst a large planting.

2.5. Soybean history and cropping systems

Accounting for over 32 million ha^{-1} in the United States, soybean is one of the most widely grown annual crops (USDA-ERS, 2022). Soybean is a legume in the Fabaceae family with a broad range of uses from human consumption to biofuel. Through breeding and domestication over many years, current soybean was bred from wild relatives *Glycine soja* Sieb. and Zucc. (Young Kim et al., 2012). Soybean is native to Asia. According to Chinese literature, soybean was first domestically cultivated during the Shang Dynasty from 1,700 to 1,100 BC (Young Kim et al., 2012). Soybean was introduced to North America in 1765 to the British colony of Georgia before being widely distributed to farmers in the Corn Belt in 1851 (North Carolina Soybean Producers Association, 2019). Since the mid-1800's soybean popularity has grown exponentially among U.S. farmers. North Dakota farmers harvested over 2.8 million ha^{-1}

of soybean in 2021 (USDA-NASS, 2022). Due to genetic improvements, climate shifts, and agronomic advancements, average soybean yield has increased linearly by 23.4 kg ha^{-1} yr^{-1} from 1924 to 2010 (Fox et al., 2013). Increasing population demand and a search for alternative fuel sources have allowed soybean to remain an important crop for the U.S. Soybean is most often grown in rotation with corn because of soybean's ability to provide nitrogen credits to corn the following growing season and provide a break to corn and grass crop disease and pest concerns. Nitrogen credits from soybean are highly dependent on soil type, and the best indicators for specific nitrogen credits are yield and plant nitrogen uptake. Bundy et al. (1993) examined various rotations including corn-corn, soybean-corn, and soybean-corn-corn to assess N-credits in these rotations. On all soil types studied except irrigated sandy soil, mean yields in the soybean-corn rotation were 1.4-2.2 Mg ha^{-1} larger than corn-corn and soybean-corn-corn rotations. Mean N uptake was also much higher in the corn of the soybean-corn rotation (Bundy et al., 1993). Nitrogen credits from previous crops, such as soybean, are important to reduce fertilizer inputs. Any nitrogen-requiring crop planted after soybean can benefit from nitrogen credits from the previous growing season.

2.6. Winter camelina history and cropping systems

Camelina is a short-season annual crop in the Brassicaceae family cultivated since 4000 BCE for oil and meal (Berti et al., 2016). Europe has been producing camelina oil in limited amounts for over 3,000 years, and camelina was possibly brought to the United States as a contaminant in flax (Schillinger et al., 2012). A majority of the camelina produced in the United States occurs in states with limited precipitation such as Montana, Minnesota, North Dakota, and South Dakota (Wright et al., 2022). Camelina has two biotypes, winter and spring (Anderson et al., 2018). The winter biotype, known for its ability to produce as an oilseed crop, has an outstanding resiliency to harsh, cold winters such as those that occur in North Dakota (Wittenberg et al., 2020). Camelina contains on average 35% oil between winter and spring biotypes (Anderson et al., 2019). Freezing tolerance of the winter biotype is associated with a greater ratio of a single base frameshift mutation at *FLOWERING LOCUS C* on chromosome 20 (Anderson et al., 2018; Soorni et al., 2019). Renewed interest in camelina has occurred due to its desirable traits such as long-chain unsaturated fatty acid content for aviation fuel, sustainable feedstock, and high omega-3 content for dietary oils (Berti et al., 2016). An isoparaffin-rich jet fuel created using camelina oil was shown to meet aviation fuel standards. Use of hydrotreated renewable jet fuel would save an estimated 75% in greenhouse gas emissions when compared with traditional petroleum-based fuel; however, the study assumes seed yields of 3,000 kg ha^{-1} of camelina, a yield unlikely to be obtained in the Northern Great Plains (Shonnard et al., 2010). Winter camelina has the ability to serve as a cover crop and also as a secondary cash crop. Relay potential is possible with shorter-season soybean, sunflower, and other annual crops (Gesch & Archer, 2013). Adding winter camelina to traditional annual cropping systems such as corn or soybean is an excellent method of enhancing crop diversity to ensure sustainable cropping systems in the Great Plains (Mohammed et al., 2022). Ideal planting dates for winter camelina in North Dakota are late September through the first week of October, with no differences observed in crop yield between this period (Wittenberg et al., 2020). Double-cropping an early-maturing sunflower with winter camelina has potential in various climates, including North Dakota. Double-cropping generally decreased subsequent sunflower yield. When double-cropped with winter camelina (cv. Joelle), total oilseed yield was 1.5 times that of a traditional sunflower monocrop (Gesch et al., 2022). A study revealed the benefits of relay cropping soybean following winter camelina in northern climates. Although the soybean growing season is shortened, overall oilseed yield between soybean and camelina was found to be 43% greater than full-season monocrop soybean, and seed protein and oil content of soybean were comparable in the relay system to the soybean monocrop (Mohammed et al., 2022). Camelina can provide the benefits of a cover crop with the additional benefit of a secondary cash crop harvest. Intensification with relay- or double-cropping can increase crop diversity and provide more floral resources for pollinators and beneficial insects (Berti et al., 2017). Because of a lack of chemical weed control options available for most winter camelina cultivars, planting into a weed-free seedbed and using tillage are vital for weed control (Wright et al., 2022). Camelina is very susceptible to sclerotinia stem rot (*Sclerotinia sclerotiorum* Lib. De Bary) and downy mildew (*Hyaloperonospora camelinae)*, similar to soybean and sunflower. Due to this susceptibility, camelina should only be planted in the same field one out of every three years, especially when grown as a monocrop (Wright et al., 2022).

2.7. Alfalfa history and cropping systems

Alfalfa is a popular cool-season perennial legume, member of the Fabaceae family, and used for hay and haylage in livestock production. In 2021, North Dakota harvested over 372,000 ha^{-1} of alfalfa monoculture (USDA-NASS, 2022). North Dakota ranks low for overall alfalfa production compared with its Midwest neighbors, making the addition of more alfalfa into North Dakota cropping systems a good opportunity for increasing crop diversity and ecosystem benefits. Increased alfalfa production is also necessary to meet rising demand for livestock. From 2022-2023, alfalfa hay production in most states remained the same or minimally increased (USDA-NASS, 2023). Alfalfa nutritive value is a key characteristic for determining forage and animal feed quality. Specific cultivars have traits associated with forage nutritive value. Some lab assessments that can be made to assess forage quality include: dry matter yield (DMY), leaf/stem ratio (LSR), crude protein (CP), acid detergent fiber (ADF), natural detergent fiber (NDF), digestible dry matter (DDM), and relative feed value (RFV). High ADF and NDF content is negatively associated with digestibility. According to the University of Minnesota (2021), 'Supreme' forage quality is designated by: an ADF under 27%, NDF under 34%, RFV over 185, total digestible nutrients (TDN) over 62%, and CP over 22%. Alfalfa's perennial nature allows for multiple harvests within a growing season. Although many producers practice a three-cut system, a four-cut system can be beneficial to overall yield. Following a fourth cutting, yield of the following year's first harvest was reduced; however, overall seasonal yield increased 1.0 to 3.9 Mg ha^{-1} according to a study conducted in Fargo, ND (Berti et al., 2012). When considering a four-cut system, it is important to remember that fall growth needs to be adequate to replenish root reserves for overwintering and provide some plant growth to maintain stubble all winter (McDonald et al., 2021). Winter-kill is a major concern due to harsh North Dakota winters, especially for cultivars that lack extreme winterhardiness.

Alfalfa-based cropping systems, when compared with grain-based systems, have higher total nitrogen, total organic carbon, and permanganate oxidizable carbon (POXC) in the soil (Jokela et al., 2011). Total nitrogen is typically higher in the top 30-cm in alfalfa-based systems. When comparing corn and alfalfa systems for dairy forage, alfalfa increased soil bulk density, soil potassium, and soil pH slowly over time, as shown in an 18-year study (Jokela et al., 2011). Nitrogen applications are not recommended for alfalfa because of its ability to fix atmospheric nitrogen, and if additions are made, nitrates toxic to livestock can build in harvested hay (Franzen & Berti, 2017). Potassium recommendations in North Dakota are based on smectite-to-illite clay ratios regionally. Alfalfa is a high user of potassium, so it is crucial to make applications according to clay ratios. Smectite-to-illite ratios of 3.5 or greater indicate that soils can draw potassium back into clay layers rendering some potassium unavailable, thus higher rates are recommended in this soil type (Franzen & Berti, 2017). Alfalfa is a model crop in terms of exhibiting the principles to manage soil health, as defined by the NRCS (2024): minimize disturbance, maximize living roots, maximize soil cover, and maximize biodiversity. Alfalfa's perenniality allows for continuous living roots and soil cover, therefore giving it the ability to enhance soil and arthropod biodiversity. Because it does not need to be planted annually, soil disturbance is minimized.

In addition, diversity of insects in alfalfa can be affected by crop management and management of surrounding crops. A study found that neighboring fields of orchards, intensely managed corn, and forest can reduce beneficial predatory insects in alfalfa stands (Madiera et al., 2021). The presence of orchards or fruit trees, which typically require chemical input to manage pests, had a negative impact on alfalfa's most common generalist predator, *Orius* spp. which is predatory to spider mites (*Tetranychidae* spp.), aphids (*Aphidae* spp.), leafhoppers (*Cicadellidae* spp.), and thrips (*Thripidae* spp.). Use of insecticides in or around alfalfa fields has impacts to biodiversity.

2.8. Life cycle assessment (LCA)

As environmental impact remains of top interest for scientists, methods to quantify the greenhouse gas (GHG) emissions of crops and products are being utilized. An LCA is the compilation and evaluation of the GHG emissions to the environment (air, water, and soil) caused by inputs, outputs, and potential environmental impacts of a crop or product through its life cycle from 'cradle to grave' (ISO 14040, 2006). Life cycle assessments are often used in industry to demonstrate the environmental impact of a specific product or processes, including agricultural production, manufacturing, and transportation. For agricultural production, inputs include: seed, fertilizers, chemicals, and field activities. Output for crops is measured in crop yield or oil yield depending on the final use of the crop. Soil GHG emissions, such as nitrous oxide (N_2O) from denitrification processes, also play a large role. Global warming potential (GWP) is a common way to express results of an LCA. This metric provides a way to compare the global warming impacts of different greenhouse gasses. Most important GHGs include carbon dioxide (CO_2), methane (CH_4), and N_2O. Carbon dioxide accounted for almost 80% of total U.S. GHG emissions in 2021, where N_2O accounted for 6.2% (U.S. EPA, 2023). Although N_2O emissions were significantly lower than CO_2 emissions, N_2O has an impact factor of 298, meaning that one metric ton of N_2O is equivalent to 298 tons of CO_2, because it has 298 times greater ability to retain heat in the atmosphere (U.S. EPA, 2020). A major source of N_2O emissions is agricultural soil management, particularly fertilization. Agricultural soil management accounted for 73% of all U.S. N_2O emissions from 1990-2021 (U.S. EPA, 2023). Synthetic fertilizers, manure management, and burning of crop residues are all contributing factors. High input cropping systems have a higher GWP due to increased risk of N_2O losses due to fertilization (Berti et al., 2017). An LCA provides a way for agricultural scientists to compare cropping systems' carbon intensity, the amount of CO_2 released for producing a crop or product, during their production and identify ways that producers can lessen their GHG emissions by altering farming practices.

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3. ALFALFA IS KEY TO CROPPING SYSTEM RESILIENCE IN CROP ROTATIONS OF THE NORTHERN GREAT PLAINS

3.1. Abstract

Annual cropping systems pose many challenges in terms of agronomics and environmental impact with respect to shifting climate and increased demand for food and fuel. This study was conducted to determine if the addition of alfalfa (*Medicago sativa* L.) and/or winter camelina (*Camelina sativa* (L.) Crantz) to more traditional annual crop rotations including sunflower (*Helianthus annus* L.), wheat (*Triticum aestivum* L.), or soybean [*Glycine max* (L.) Merr.] would offer solutions to agronomic challenges. Evaluations of agronomics and economics were investigated for ten crop sequences in Hickson and Prosper, ND in 2022 and 2023. Analysis through life cycle assessment (LCA) was completed to quantify each systems' carbon intensity (CI). Alfalfa had consistently high yields, while grain crops performed poorly due to weed pressure and environmental conditions. Two crop sequences including alfalfa had a positive net economic return. Systems including alfalfa had the lowest CI value, 64 to 80 g CO_{2e} MJ of net energy for lactation (NEL) per year^{-1}.

3.2. Introduction

As challenges in agriculture are exacerbated by a changing climate and an increasing global population, managing crop rotations with resilience in mind is key. Diversity in crop rotations can improve system resilience to withstand environmental stressors, volatile markets, and other challenges. One such method is to diversify crop rotations. From 1987 to 2012, crop diversity significantly decreased in all regions of the United States (Aguilar et al., 2015). Crop homogeneity introduces challenges and risks in agriculture.

Degani et al. (2019) defined diverse rotations for their study as those containing an intercrop system, followed by a brassica winter cover crop, and completed with a spring legume. Their study found that the diverse rotations outperformed simple rotations by containing higher soil moisture and having higher yield performance under stress (Degani et al., 2019). Diversifying crop rotations can refer to the specific crops grown or how they are grown. The northern Great Plains has seen a great increase of corn and soybean crop rotations, which has impacted ecosystem services with corn being a high input crop and soybean leaving minimal residue (Liebig et al., 2024). A crop like winter camelina is not as widely grown in the northern Great Plains as corn or soybean, but has promise in relay- or double-crop scenarios.

Winter camelina (*Camelina sativa* (L.) Crantz) is a hardy crop that can survive winters in the northern Great Plains and the droughts that may accompany the growing season (Anderson et al., 2018). Camelina is desirable for its use as a biofuel crop. It is often grown as a relay-crop with soybean or as a double-crop with early-maturing sunflower. Winter camelina-soybean relay trials have concluded that the net income from the relay system is equivalent or slightly higher to that of monocrop soybean (Gesch et al., 2014; Ott et al., 2019). It was noted, however, that soybean yield was reduced up to 30% due to shading from the winter camelina canopy (Ott et al., 2019). Biofuel crops are often characterized through life cycle assessment (LCA) to determine their global warming potential (GWP) and compatibility as a sustainable fuel. Double- or relay-cropping systems increase the need for field activity, which can increase GWP from fuel emissions. Berti et al., (2017) reported that winter camelina had lower carbon intensity when grown as a monocrop compared with monocrop soybean and corn (*Zea mays* L.).

Diversified crop rotations often include perennial crops. Perennial crops, such as alfalfa, are shown to increase soil organic carbon sequestration and reduce soil erosion when included in rotation (Berti & Cecchin, 2023). Alfalfa is a N_2-fixing legume that provides a nitrogen benefit to subsequent crops. Farmers in Canada reported 71% positive yield response in annual crops following alfalfa in comparison to following another annual crop (Franco et al., 2021). Environmental impact of alfalfa varies based on practice, but its GWP benefits from not requiring N fertilizer, which accounts for over 70% of GHG emissions by agriculture (Nemecek et al., 2015). Due to alfalfa's low input requirement, yield consistency, and ability to suppress weeds, crop rotations that include alfalfa have higher net returns than rotations of strictly corn, soybean, and wheat (Goplen et al., 2018).

This study aimed to assess the agronomic, economic, and environmental impacts to traditional annual cropping systems by incorporating winter camelina and/or alfalfa into wheat-sunflower-soybean sequences at two locations in North Dakota.

3.3. Materials and methods

3.3.1. Experimental design

The experiment was organized in a randomized block design and planted at two locations in ND, Prosper and Hickson. Prosper (46°59'56.1" N, -97°06'53.0" W) contains Bearden silty clay as a soil type (Bearden: fine-silty, mixed, superactive, frigid Aeric Calciaquoll) (Soil Survey Staff, 2005; Soil Survey Staff 2014) and Hickson (46°38'13.2" N, 96°49'25.0" W) contains Fargo silty clay as a soil type (Fargo: fine, smectitic, frigid Typic Epiaquert) (Soil Survey Staff, 2016; Soil Survey Staff 2015).

Each location had ten treatments and four replicates established in 2022. Each treatment had a corresponding rotation schedule. The cultivar of hard red spring wheat (HRSW) planted was Glenn. The sunflower hybrid planted was N4H161 CL, which is part of the Clearfield™ system and is tolerant to imazamox [(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1Himidazol-2-yl]-5-(methoxymethl)-3-pyridinecarboxylic acid] herbicides. The early maturity N4H161 CL hybrid was planted both early and late in the season depending on treatment (Fig. 3.1). The alfalfa cultivar was glyphosate-resistant and potato leafhopper-(*Empoasca fabae)*-resistant, RR Vamoose. Alfalfa was planted in the spring and the late summer to compare dates of establishment. Once planted with alfalfa, the plot remained alfalfa for the duration of the experiment. The soybean cultivar planted was ND2108GT73, a cultivar developed by North Dakota State University (NDSU) with glyphosate-resistance and a maturity rating of 0.8. The winter camelina cultivar planted was Joelle, a cultivar with obligate vernalization for bolting and high winter survival. Seeding rates (pure live seed) for each crop are as follows: wheat 3,255,385 seeds ha^{-1}, sunflower 64,276 seeds ha^{-1}, soybean 420,000 seeds ha^{-1}, alfalfa 11.2 kg ha^{-1}, and winter camelina 11.2 kg ha^{-1}. The treatment schedule for 2022-2023 is as follows (Fig. 3.1; Table 3.1):

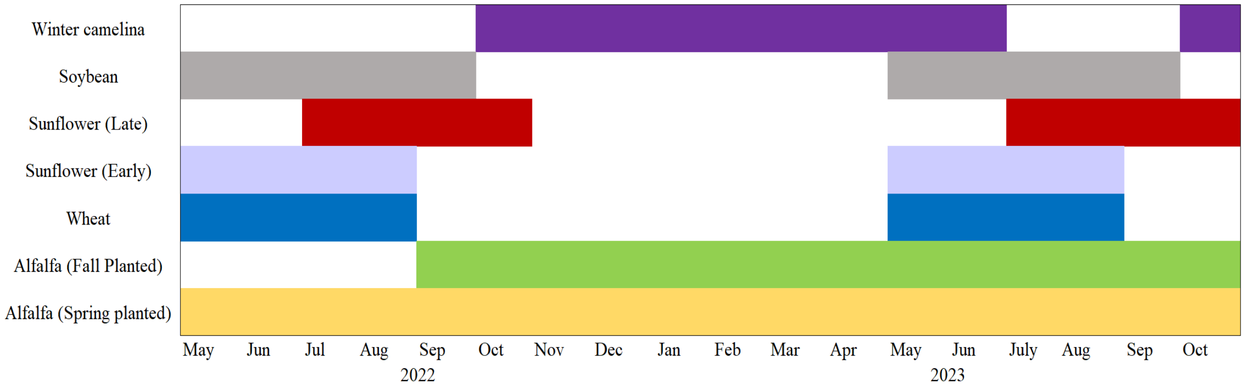

Figure 3.1. Experiment calendar depicts when alfalfa (spring-planted), alfalfa (fall-planted), sunflower (early-planted), sunflower (late-planted), soybean, and winter camelina are in the field for all seasons of 2022-2023.

Table 3.1. Specific crop sequence for each treatment throughout the duration of the experiment. Sunflower (E) refers to early-planted sunflower. Sunflower (L) refers to late-planted sunflower. Treatments 6 and 2 are soybean relay-cropped into winter camelina.

Treatment	Spring 2022	Fall 2022	Spring 2023	Fall 2023	Spring 2024
1	Wheat	Winter camelina	Sunflower (L)	Fallow	Soybean
2	Wheat	Fallow	Sunflower (E)	Winter camelina	Soybean
3	Wheat	Alfalfa	Alfalfa	Alfalfa	Alfalfa
4	Sunflower (E)	Alfalfa	Alfalfa	Alfalfa	Alfalfa
5	Sunflower (E)	Winter camelina	Alfalfa	Alfalfa	Alfalfa
6	Sunflower (E)	Winter camelina	Soybean	Fallow	Wheat
7	Sunflower (L)	Fallow	Wheat	Fallow	Alfalfa
8	Soybean	Fallow	Wheat	Winter camelina	Sunflower (L)
9	Fallow	Winter camelina	Fallow	Fallow	Sunflower (E)
10	Alfalfa	Alfalfa	Alfalfa	Alfalfa	Alfalfa

In Year 1, all treatments, except for the late sunflower, fall-planted alfalfa, and fall-planted winter camelina were planted on 26 May, 2022. Early-planted sunflower was planted on 26 May, 2022. Treatment 7, late-planted sunflower, was planted on 6 July in Prosper and 7 July, 2022 in Hickson. Fall-planted alfalfa was planted on 1 September, 2022. Treatments with winter camelina were planted on 26 and 28 September, 2022 at Prosper and Hickson, respectively. Sunflower was planted with a four-row plot planter (Almaco, Nevada, IA), and alfalfa, soybean, wheat and winter camelina were planted with a plot drill (Wintersteiger, Austria).

In Year 2, wheat and alfalfa (replant due to poor germination) were planted on 17 and 23 of May, 2023 in Prosper and Hickson, respectively. Soybean relay into standing winter camelina was planted on 23 May, 2023 in Hickson and 26 May, 2023 in Prosper. Early-planted sunflower was planted on 25 May, 2023 in both Hickson and Prosper. Late-planted sunflower was planted on 24 July, 2023 in both Hickson and Prosper. Late-planted alfalfa was planted on 10 August, 2023 in both Hickson and Prosper. Winter camelina was planted on 2 October, 2023 in Prosper and 3 October, 2023 Hickson.

3.3.2. Rainfall and temperature

Climate data was analyzed for each growing season using data from the North Dakota Agricultural Weather Network (NDAWN, 2022; 2023). Rainfall and temperature data are most critical to this experiment. The NDAWN weather station in Prosper, ND measured daily minimum and maximum temperature and rainfall for the Prosper location. The NDAWN station in Sabin, MN measured daily minimum and maximum temperature for the Hickson location. On-site precipitation measurements were collected at Hickson by KayJay Ag Services (KestrelMet 6000, Kestrel Instruments, Boothwyn, PA). Growing degree days (GDD) were calculated with the following equation and minimum and maximum daily temperatures. Base temperature for each crop was: 6.7°C for sunflower (NDAWN, 2023), 0°C for wheat (NDAWN, 2023), 10°C for soybean (NDAWN, 2023), 5°C for alfalfa (Sanderson, 1992), and 4°C for winter camelina in the fall after planting until a hard freeze below -7°C (Wittenberg et al., 2020). No upper threshold was used for sunflower, alfalfa, or winter camelina; however, 30°C for soybean, 21°C and 35°C for wheat, dependent on growth stage, were considered. The following equation was used to calculate GDD with assistance from the GDD tool provided by NDAWN for sunflower, wheat, and soybean:

$$\text{GDD} = \sum \left[\frac{(\text{maximum temperature} + \text{minimum temperature})}{2} - \text{Base temperature} \right] \quad (1)$$

3.3.3. Soil sampling

Initial soil samples were collected in June 2022 to determine baseline bulk density of the soil. Two samples were collected from each of the four replicates at both locations for bulk density. One sample was between 0- and 15-cm depth and the other sample between 15-and 30-cm. Bulk density was calculated based on the total mass of the dried sample divided by the given volume of the collection ring, 90.59 cm^3. Protocol written by Purdue University (2014) was followed for collection and calculation. Bulk density samples were then collected on a per-plot basis in October 2023 for specific treatment comparison and to quantify soil carbon stock.

Soil samples from the top 15-cm of soil were taken on a bi-weekly basis during the 2022 and 2023 growing seasons via a soil probe to determine gravimetric water content variations between treatments. Each treatment per experimental location was sampled on this schedule for a total of eight measurements in Year 1 and five measurements in Year 2. A wet weight was recorded, soil samples were placed in an oven at 105° C for a minimum of 24 h, and a dry weight was recorded in order to determine gravimetric water content. The formula for gravimetric water content is:

$$\% \text{ Gravimetric water } (\Theta g) = (\text{Wet weight} - \text{Dry weight}) / (\text{Dry weight}) \times 100 \qquad (2)$$

3.3.4. Soil fertility

Soil samples were collected at depths of 0-15-cm, and 15-60-cm in the spring of 2022 to analyze for soil physicochemical characteristics. These samples were sent to Agvise Laboratories for analysis of pH (1:1 in H_2O; Peters et al., 2015), organic matter (loss on ignition; Combs & Nathan, 2015), total N (Kjeldahl; Bremner, 1996), P (Olsen; Frank et al., 2015), and K (1.0 M ammonium acetate; Warnacke & Brown, 2015). Samples from the same 0-15-cm and 15-60-cm depth were analyzed for total C (combustion; Nelson & Sommers, 1996), and total organic C (total C – inorganic C; inorganic carbonates 15% HCl; Loeppert & Suarez, 1996) (Agvise Laboratories, 2023). Samples from 0-15-cm depth of select treatments were also sent to Brookside Laboratory in Ohio for analysis of soil protein (autoclaved citrate extractable; Hurisso et al., 2018) and permanganate oxidizable carbon (POXC; 0.02 M $KMnO_4$; Weil et al., 2003). Wheat-fallow-sunflower-winter camelina, wheat-alfalfa, soybean-wheat-winter camelina, and continuous alfalfa treatments were all sampled for protein and POXC. Upon return of results, and based on North Dakota Extension recommendations for each crop, N and P amendments were added to standing crops in July during Year 1. No K was required per soil analysis (Table 3.2). Hickson was deficient in both N and P, where Prosper was only deficient in N. Plots containing wheat in Hickson received 22.4 kg ha^{-1} N and 33.6 kg ha^{-1} P_2O_5 in the forms of urea (46-0-0) and monoammonium phosphate (MAP) (11-52-0), respectively. Plots containing alfalfa received 33.6 kg ha^{-1} P_2O_5 in the form of MAP, and plots containing soybean also received 33.6 kg ha^{-1} P_2O_5 in the form of MAP. Plots containing sunflower at Hickson received 89.7 kg ha^{-1} N in the form of urea. At the Prosper location, wheat and sunflower plots received 39.2 and 112 kg ha^{-1} N in the form of urea, respectively. Late-planted sunflower at Hickson and Prosper received 89.7

kg ha^{-1} N in the form of urea. All fertilizer additions were broadcast to standing crops approximately six weeks after planting. The same rates of N and P were applied in the 2023 growing season regardless of soil test results to maintain uniformity from Year 1 to Year 2. Winter camelina was fertilized with 23 kg ha^{-1} N in the form of urea at both Hickson and Prosper as well as 33.6 kg ha^{-1} P_2O_5 in the form of MAP in Hickson. Fertilization took place in mid-June 2023 for all crops. This is considered late for fertilizing winter camelina. Fall soil sampling to 60-cm was done to determine NO_3-N content using a hydraulic soil probe. Spring and fall soil sampling were repeated in Year 2 for NO_3-N only. Total C, total N, and soil organic C were evaluated again in fall of Year 2.

Table 3.2. Spring 2022 soil baseline test results that were averaged across four replicates at Hickson and Prosper, ND to calculate fertilizer amendments for sunflower, wheat, alfalfa, and soybean.

Location	pH	OM[a]	N (total)[b]	P	K	TC[c]	TOC[d]	CCE[e]
		g kg^{-1}	kg ha^{-1}	----mg kg^{-1}----		--------%--------		kg ha^{-1}
Hickson	8.0	39	63	9	321	3.0	2.7	38
Prosper	7.6	31	46	29	350	2.0	1.7	45

[a] Organic matter
[b] Refers to 0-60 cm depth
[c] Total carbon
[d] Total organic carbon
[e] Calcium carbonate equivalent

3.3.5. Weed control

Weed pressure was a constant issue during Years 1 and 2, with the most severe weed pressure occurring in Year 2. Both chemical and physical methods of control were used to decrease weed pressure. Hand weeding and tillage were used for physical weed control. A rototiller implement was used in between individual plots and alleys. Sunflower plots at each experimental location received multiple applications of imazamox (specifically, Beyond) (BASF, Ludwigshafen, Germany) at a rate of 0.29 L ha^{-1} during Year 1 and only in Hickson in Year 2.

Soybean, alfalfa, and fallow ground at each experimental location received 2.24 L ha^{-1} glyphosate (N-phosphono methyl glycine) (specifically, Roundup Power Max) (Bayer Crop Science, Leverkusen, Germany) in Years 1 and 2. Wheat at each experimental location received one 0.58 L ha^{-1} application of 2,4-D amine (dimethylamine salt of 2,4-dichlorophenoxyacetic acid). An additional application of 2,4-D to wheat was made to control weeds present. In Year 2, pinoxaden (Propanoic acid, 2,2-dimethyl-, 8-(2,6- diethyl-4-methylphenyl)-1,2,4,5-tetrahydro7-oxo-7H-pyrazolo[1,2- d][1,4,5]oxadiazepin-9-yl ester) (specifically, Axial) at 0.5 L ha^{-1} was applied to wheat in Hickson and Prosper to control grass weed species present. Winter camelina is not tolerant to broadleaf herbicides, so only physical weed control was used.

3.3.6. Insect control

Soybean in Hickson initially showed a concerning level of foliar damage caused by bean leaf beetle (*Cerotoma trifurcate*) in Year 1. In order to prevent damage to new foliar growth, lambda-cyhalothrin ([1a(S*),3a(z)]-cyano(3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate), (specifically, Warrior II with Zeon Technology) (Syngenta, Basel, Switzerland) was applied at a rate of 0.11 L ha^{-1}. This treatment showed success within a week and no further issues were detected later in the growing season. Bean leaf beetle was not an issue in Year 2. To control excessive levels of common field grasshoppers (*Chorthippus brunneus*) at Prosper during Year 1, an aerial application of chlorantraniliprole (3-bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide) (specifically, Vantacor) (FMC, Philadelphia, Pennsylvania) at a rate of 0.07 L ha^{-1} was applied to a majority of the research station. Insect control measures were not utilized in Year 2 to not influence arthropod biodiversity sampling.

3.3.7. In-season plant measures

Plant counts in each crop were taken at both experimental locations approximately four weeks after plant emergence and again after harvest. Plant count locations were determined by placing markers along a 2-m length between the two-middle rows of crop and counting the number of plants in the two-rows on either side of the markers. Plant height measurements were started simultaneously with plant counts and repeated weekly until plant maturity. This allowed for weekly staging and growth tracking in each crop. To determine intercepted photosynthetically active radiation (PAR) by crop, biweekly measurements with a ceptometer (LP-80, Decagon Devices, INC., Pullman, WA) were taken. Three measurements per plot containing a crop cover were collected by placing the ceptometer as close to the soil as possible underneath the two-middle rows in each plot. Three readings were taken per plot and averaged. These measurements were also collected in Year 2; however, readings were taken much less frequently, approximately every four weeks. The formula for intercepted PAR is:

$$\text{Intercepted PAR (\%)} = \frac{(\text{Light above canopy} - \text{Light below canopy})}{(\text{Light above canopy})} \text{ x } 100 \qquad (3)$$

3.3.8. Harvest and quality measures

3.3.8.1. Alfalfa

Alfalfa planting and harvest dates are indicated in Table 3.3. In Hickson only, two plots of alfalfa following wheat were harvested at the same time as the first harvest of the alfalfa monocrop treatment.

Table 3.3. Planting and harvest dates of treatments including alfalfa at Hickson and Prosper, ND in 2022 and 2023.

Treatment	2022			2023			
	Planting date	Harvest date 1	Harvest date 2	Harvest date 1	Harvest date 2	Harvest date 3	Harvest date 4
				Hickson			
Alfalfa monocrop	26 May	21 July	31 Aug	14 Jun	11 July	9 Aug	12 Oct
Alfalfa following wheat	1 Sep	--	--	14 Jun	11 July	9 Aug	12 Oct
Alfalfa following sunflower	1 Sep	--	--	11 July	9 Aug	12 Oct	--
				Prosper			
Alfalfa monocrop	26 May	25 July	30 Aug	13 Jun	10 July	16 Aug	16 Oct
Alfalfa following wheat	1 Sep	--	--	10 July	16 Aug	16 Oct	--
Alfalfa following sunflower	1 Sep	--	--	10 July	16 Aug	16 Oct	--

Alfalfa biomass was harvested with a flail forage harvester (Carter, Brookston, IN) in one pass along a 0.9-m x 6.1-m strip in each plot, where edges were discarded prior to sample collection to remove edge effect. Biomass yield was collected from the scale on the harvester in-field. Biomass wet weight was recorded, and the biomass was placed in burlap sacks to completely dry in the oven at 50°C. Dried alfalfa biomass was ground through a 1-mm mesh using a Model 4 cutting mill (Eberbach Corporation, Ann Arbor, MI, US). Biomass was then analyzed for crude protein (CP), ash, K, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber digestibility (NDFD), and ether extract (EE). Based on this analysis, dry matter intake (DMI), non-fiber carbohydrates (NFC), total digestible nutrients (TDN), and relative forage quality (RFQ) were calculated based on the following equations (Undersander & Moore, 2001):

$$\mathrm{DMI_{legume}} = \left(\frac{120}{NDF}\right) + (\mathrm{NDFD} - 45) \text{ x } \left(\frac{0.374}{1350}\right) \text{ x } 100 \qquad (4)$$

$$\mathrm{NFC} = 100 - \mathrm{Ash} - \mathrm{CP} - \mathrm{EE} - \mathrm{NDF} \qquad (5)$$

$$\mathrm{TDN_{legume}} = (\mathrm{NFC} \text{ x } 0.98) + (\mathrm{CP} \text{ x } 0.93) + (\mathrm{FA} \text{ x } 0.97 \text{ x } 2.25) + \left(\frac{NDF \text{ x } NDFD}{100}\right) - 7 \qquad (6)$$

*FA denotes fatty acid

$$\mathrm{RFQ} = \frac{DMI \text{ x } TDN}{1.23} \qquad (7)$$

Alfalfa and other crops analyzed via near infrared spectroscopy (NIRS) were analyzed using an XDS Near Infrared Rapid Content Analyzer calibrated with software from Foss (Foss, Copenhagen, Denmark).

3.3.8.2. Wheat

Wheat was harvested at each location in Year 1 on 25 August along a 0.9-m x 6.1-m strip in each plot. Initial harvest was done with a Hege 125B plot combine (Hans-Ulrich Hege, Waldenberg, Germany). Due to poor performance by harvest equipment, wheat was run through another combine (Wintersteiger, Austria) to eliminate chaff and ensure grain was separated from the hull. Wheat grain was then cleaned a second time using a model Office Clipper seed cleaner (Clipper Separation Technologies, Bluffton, IN) to rid the samples of weed seeds. Wheat was harvested on 21 August in Prosper and 22 August in Hickson during Year 2 only with the Hege plot combine and later cleaned with the Office Clipper seed cleaner. Whole wheat kernels were analyzed for protein and moisture content using the XDS NIRS analyzer.

3.3.8.3. Sunflower

Sunflower (early) heads were harvested prematurely during the R8 stage on 28 August 2022 in Hickson and 30 August 2022 in Prosper by hand due to bird damage. In Year 2, mesh netting was placed over the two middle rows of each plot as heads developed in attempt to minimize bird damage. Late-planted sunflowers were harvested on 3 October 2022 in Hickson and Prosper. Heads were placed in burlap sacks to dry in the oven until seed was dry enough to thresh and clean with a seed cleaner. Two entire sunflower plants from each plot were harvested to determine height, harvest index, and biomass N content. Early-planted sunflower was harvested on 13 September in Prosper and 16 September in Hickson at the R9 stage during 2023. Late-planted sunflower was not harvested in 2023 at either location due to severe deer damage in Hickson and plants not achieving a growth stage suitable for harvest in Prosper. Sunflower biomass was ground using the same mill as alfalfa. Ground sunflower biomass was sent to AgVise Laboratory for analysis of total N (Dumas: combustion; Jones Jr. & Case, 1990) (Agvise Laboratories, 2023). Sunflower achenes were analyzed at the USDA lab for fatty acids profile using analysis of fatty acid methyl esters (FAME). The FAME analysis is based on procedure by Zheljazkov et al. (2009) and uses a model Trace 1310 gas chromatograph (Thermo Scientific, Waltham, MA) with a 30 m x 0.25 mm DB-23 capillary column (J&W Scientific, Folsom, CA).

Standards 17A, 21A, and 68B (Nu-Chek-Prep, Inc., Elysian, MN) were used as references to represent a mixture of fatty acid methyl esters. The software used to determine fatty acid concentrations (Chromeleon v7.2, Thermo Scientific, Waltham, MA) is based on the means of three replicates. To determine total oil content in sunflower achenes, a nuclear magnetic resonance (NMR) analyzer with proprietary software (Model MQC+, Oxford Instruments, United Kingdom) located in the USDA/ARS lab in Fargo was used. An 80-mL sample from each plot was analyzed after thorough seed cleaning with a fraction aspirator (Carterday International, Minneapolis, MN).

3.3.8.4. Soybean

Soybean was harvested by hand in a 6.1-m x 0.9-m strip in each plot on 3 October 2022 in Hickson and Prosper. Harvested soybean was passed through a combine to separate grain before cleaning on the seed cleaner. Soybean was harvested by hand in 0.09 m^2 areas in Hickson on 11 October 2023, and hand shucked from pods. No harvest occurred in Prosper during 2023 due to complete soybean death, likely from an herbicide toxicity issue. Soybean seed was confirmed to have the CP4 EPSPS (Roundup Ready ®) gene by an immunochromatographic GMO strip test (specifically, QuickStix) (Envirologix, Portland, ME). Whole soybean seed was analyzed using XDS NIRS analyzer.

3.3.8.5. Winter camelina

Winter camelina treatments, including soybean relay treatment were harvested on 11 July and 18 July 2023 in Prosper and Hickson, respectively using the same Hege 125B plot combine as wheat. Winter camelina seed was hand-sifted to clean, and a 5 g portion of fully cleaned seed was selected for NIRS analysis for oil content and fatty acid profile, using calibration developed for camelina by Anderson et al. (2019).

3.3.8.6. Crop sequences

To compare crop sequence yields, yield was classified four different ways. Yield was quantified in terms of grain, forage, oil, and protein dependent on the measurable qualities per crop. Land equivalent ratios (LER) were also calculated to quantify the productivity of certain sequences. Land equivalent ratios are useful for comparison of relay- or double-cropping systems to monocrop systems. Values over 1.0 indicate yield advantages on the same amount of land. The following formula was used to calculate LER:

$$\text{LER} = \sum\left(\frac{Yield\ cropping\ sequence\ 1}{Yield\ cropping\ sequence\ 2}\right) \tag{8}$$

3.3.9. Statistical analyses

Statistical analyses using SAS 9.4 (SAS Institute., Cary, NC, 2023) with PROC MIXED procedure were performed to detect significant interaction of collected data. Data was combined for location when variances were homogenous. A mixed model was performed for all analyses. Locations were considered random effects and crop and cropping sequence treatments as fixed effects. Least square means were estimated (LS means). For LS means, least significant difference (LSD) values at the 95% level of confidence were calculated by multiplying the standard error for the pair of differences (p-diff) by the *t*-table value for the degrees of freedom (df) of the corresponding error that was used to calculate the *F*-value for individual sources of variation. Alfalfa was analyzed by cut as a repeated measure.

3.3.10. Cost and return analysis

A cost and return analysis was performed for each cropping sequence based on the first two years of the sequences: i) wheat, winter camelina, late-planted early-maturing sunflower ii) wheat, fallow, early-planted early-maturing sunflower, winter camelina; iii) wheat, alfalfa; iv) early-planted early-maturing sunflower, alfalfa; v) early-planted early-maturing sunflower, winter camelina, alfalfa; vi) early-planted early-maturing sunflower, winter camelina, soybean; vii) late-planted early-maturing sunflower, fallow, wheat; viii) soybean, fallow, wheat, winter camelina; ix) winter camelina; and x) alfalfa.

Each component of production including: seed cost, herbicide, fertilizer, machinery, and labor were used as metrics to determine cost of each sequence. Total calculated cost was then compared with yield multiplied by average price for each commodity to determine net revenue. Basic costs for inputs were utilized to better generalize the cropping system based on realistic farmer scenarios. Projected 2023 Crop Budgets developed by NDSU (Haugen, 2023) provided many of the estimates for wheat, sunflower, and soybean production costs. Alfalfa production costs as well as specific machinery for each cropping system were adapted from Estimated Costs of Crop Production in Iowa 2023 (Plastina, 2023). Machinery cost only includes field equipment and does not factor in transportation or drying. Fixed machinery costs refer to ownership costs and include: depreciation, interest, taxes, insurance, housing, and maintenance (Edwards, 2015). Variable machinery costs refer to specific crop production. Labor costs were based on a rate of \$18 h^{-1}. Labor estimates for wheat and sunflower cropping systems were adapted from University of Nebraska-Lincoln Crop Budgets (Klein & McClure, 2023). Little specific information exists about the production costs of winter camelina, so values were adapted from soybean production and the particular equipment used in planting, fertilizing, and harvesting winter camelina. Each value provided from the various sources was on a per acre basis and was converted into a per hectare equivalent. Commodity prices for market value were averaged from FINBIN (2022) for each crop except for winter camelina. Price for winter camelina contracts was determined from the University of Minnesota (Forever Green Initiative, 2023).

3.3.11. Life cycle assessment

To determine the environmental impact of each cropping sequence, a life cycle assessment of each cropping system was completed. The boundary set for the LCA of this study was ‘cradle to farm gate’ indicating that strictly agricultural production is considered. Based on published values for N, P, K, herbicide, insecticide, seed, fuel, and electricity, all inputs and field activities are accounted for in the life cycle inventory (LCI) to determine total GHG emissions and global warming potential (GWP) (Tables 3.4, 3.5). Typically, a simulation software would be used for analysis; however, a spreadsheet of gathered coefficients and applicable formulas was sufficient for this study. Actual calculations reflect specific practices, products, and rates used in this study with the exception of K fertilizer in alfalfa, which was not applied in this study but is typically applied in alfalfa production systems (Franzen & Berti, 2017). Actual yields from the experiment were considered in the calculations as well. Global warming potential was expressed in more than one functional unit: kg carbon dioxide equivalent per kilogram of seed (kg CO_{2e} kg^{-1}) or as carbon intensity (CI) in g CO_{2e} MJ^{-1} and refers to the amount of CO_2 released to produce one MJ of energy produced by the crop. Low heating values (LHV), the theoretical quantity of heat released by total combustion, are expressed in MJ kg^{-1}. It is important to note that this study does not consider emissions due to land use change (LUC) or indirect land use change (ILUC). Field emissions of nitrous oxide (N_2O) were estimated based on fertilizer applications and general crop emissions by residue decomposition, N_2-fixation, etc. Emission of N_2O from general crop emissions are based on previous annual and perennial crop research by Berti & Cecchin (2023). Emission of N_2O from fertilizer applications containing N were calculated with the following formula:

$$CO_{2e} \text{ from } N_2O \text{ emission} = (N \times 0.0133 \times 298) \qquad (9)$$

N = Actual N applied (kg ha^{-1})
Average N_2O losses from kg of N fertilizer applied (Berti & Cecchin, 2023) = 0.0133
Impact factor to convert N_2O to CO_{2e} = 298

The impact factor, as noted in the above equation, is 298 for converting N_2O into CO_{2e}. Nitrous oxide losses from the soil were based on research by Berti & Cecchin (2023), who calculated residual losses for alfalfa and soybean. Soybean's N_2O loss value was used to represent all other annual crops in this study. Soil organic carbon decomposition plays a large role in the GWP of cropping systems; however, a majority of publications exclude this portion, so losses of CO_2 by soil respiration were not included in this study. Each rotation's overall impact is reported in functional units per hectare and per energy equivalent (MJ). For alfalfa specifically, the functional unit, net energy for lactation (NEL) was calculated. This value refers to the amount of energy from alfalfa hay that is available to animals for milk production, maintenance, and growth. The formula to calculate NEL is (Belyea et al., 1993):

$$\text{NEL (Mcal kg}^{-1}\text{)} = 1.037 - 0.0124 \times \text{ADF} \tag{10}$$

The constant to convert from Mcal kg^{-1} to MJ kg^{-1} is 4.184. The average NEL from each year and cropping system was used in this experiment. Alfalfa hay at an average of 85% dry matter has an energy equivalent of 14.7 MJ kg $forage^{-1}$ (Asgharipour et al., 2016). This value was used for calculation of NEL. Fallow ground in absence of the winter camelina monocrop treatment for this study was considered as chemical and mechanical fallow. One application of glyphosate (Table 3.4) and one pass with a tandem disk (Table 3.5) were accounted for. Fallow ground also has residual N_2O losses from soil. The value for fallow ground emissions was derived from the average of seasonal data reported by Wagner-Riddle et al. (1997). Emissions were calculated from May through September and applied on a per month basis to sequences where fallow ground existed. The tables below demonstrate known values for inputs to the LCI.

Table 3.4. Seeding rates, reference energy coefficients, and reference greenhouse gas (GHG) emission factors for each crop and input used to calculate life cycle assessment.

	Seeding rate	Energy	CI
Crop	kg ha^{-1}	MJ kg^{-1}	kg CO_{2e} kg^{-1}
Soybean	62	33[a]	0.25[b]
W. camelina	11.2	25[a]	0.84[a]
Sunflower	3.4	67[c]	0.28[c]
Alfalfa	20.4	28[d]	2.63[b]
Wheat	104	13[e]	0.13[b]
Pesticide			
Glyphosate		267[a]	23.3[a]
Imazamox		267[a]	23.3[a]
Lambda-cyhalothrin		320[a]	27.1[a]
Fertilizer			
Urea		49.5[a]	5.2[a]
MAP		14.1[a]	1.1[a]
Potash		8.8[a]	0.55[a]

[a] Berti et al. (2017); [b] West & Marland (2002); [c] Pimentel & Patzek (2005); [d] Fathollahi et al. (2018); [e] Jekayinfka et al. (2015)

MAP refers to monoammonium phosphate fertilizer (11-52-0)

Table 3.5. Life cycle inventory of inputs via field practices utilizing low-sulfur diesel.

Input	Rate	Energy	GHG emission
	L ha^{-1}	MJ kg^{-1}	kg CO_{2e} MJ^{-1}
Tillage - tandem disk [a]	8	45.3	0.087[c]
Harrowing - tine harrow [a]	2.5	45.3	0.087
Sowing [b]	10	45.3	0.087
Chemical application	4	45.3	0.087
Fertilizer application	14	45.3	0.087
Harvest - combine	25	45.3	0.087
Harvest - forage chopper	25	45.3	0.087
Seed transportation	8	45.3	0.087

[a] Zentner et al. (2004)

[b] Inputs, rate, and LHV values from Berti et al. (2017)

[c] GHG emission values from FootprintCalc (2023)

3.4. Results and discussion

3.4.1. Rainfall and GDD

Growing degree days were calculated based on specific planting and harvest dates of the crop each year (Table 3.6). Alfalfa was separated by treatment according to the different planting dates used. The lack of accumulated GDD for late-planted sunflower in 2023 is attributed to the plants not reaching full maturity. Compared with full-season sunflower, early-maturing hybrids such as the one used in this study accumulated significantly fewer GDD. According to the NDSU Sunflower Production guide, full-season sunflowers will reach 1,266 GDD (°C) by physiological maturity (Kandel et al., 2020). Early-maturing sunflower physiological maturity was about 538 less GDD (°C) in this experiment. Winter camelina's GDD were calculated on the first harvested crop only and does not consider the fall 2023 GDD of winter camelina following early-planted, early-maturing sunflower and winter camelina following wheat. In fall of 2022, a hard freeze (-7°C) occurred on 16 October, which corresponds to the date that winter camelina GDD calculations extend for that year.

Table 3.6. Accumulated growing degree days (GDD) during the 2022 and 2023 growing season for all crops and planting times averaged across both locations, Hickson and Prosper, ND.

Crop	2022	2023
	GDD (°C)[a]	
Alfalfa (Trt 10[b])	2180	2412
Alfalfa (Trt 3,4[c])	551	2225
Alfalfa (Trt 5[d])	--	2412
Wheat	1871	1881
Sunflower (E)[e]	1335	1561
Sunflower (L)[f]	1125	1015
Soybean	1168	1185
Winter camelina	154	1104

[a] GDD base temperature is 5°C for alfalfa, 0°C for wheat, 6.7°C for sunflower, 10°C for soybean, and 4°C for winter camelina.
[b] Trt 10 refers to alfalfa sown in May 2022.
[c] Trt 3,4 refers to alfalfa sown in September 2022.
[d] Trt 5 refers to alfalfa sown in May 2023.
[e] (E) refers to early-planted sunflower May 2022 and 2023.
[f] (L) refers to late-planted sunflower July 2022 and 2023.

When averaged across dates, 2023 received less rainfall at 38-cm compared with 45-cm in 2022 (Fig. 3.2). The fall of 2022 was dry, which likely impacted fall-seeded crops emergence such as winter camelina and alfalfa. Spring-seeded alfalfa tends to have less weed competition and moisture stress than fall-seeded, as success is dependent on moisture and sufficient growth before a killing frost (Undersander et al., 2015). April through October of 2022 saw high-intensity rainfall events, where 2023 saw more frequent, smaller quantities of rainfall (Fig. 3.3). Average minimum and maximum temperatures were very similar for both years. The fall of 2023 was warmer compared with the fall of 2022 (frost date, 28 September 2022) and had a later first frost date of 7 October 2023. The last frost of the spring of 2023 was 3 May 2023, which is earlier when compared with 2022 when the last frost occurred on 21 May 2022.

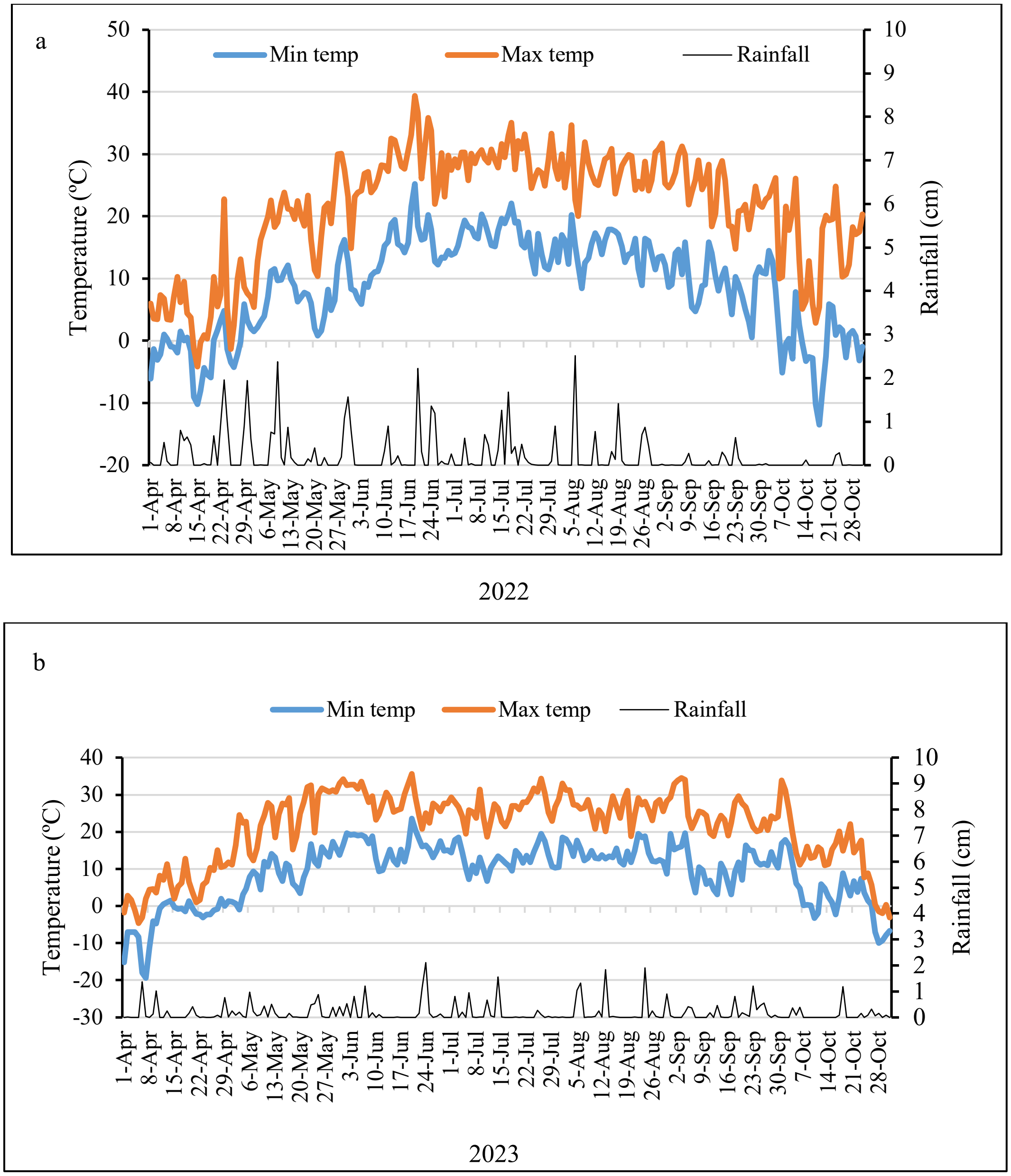

Figure 3.2. Daily rainfall, maximum temperature, and minimum temperature for a) 2022 and b) 2023 growing seasons averaged across two locations, Hickson and Prosper, ND.

3.4.2. Soil measurements

3.4.2.1. Soil gravimetric water

In 2022, location by date was significant; however, there was no difference in treatment (Table 3.7). The same sources of variation were significant in 2023 along with treatment and date ($P \leq 0.05$). One treatment that was consistently low in gravimetric water during Year 2 was established alfalfa (Fig. 3.3). Alfalfa, being a high-yielding perennial, has high water consumption, especially due to its deep root system. A study found that one-year-old alfalfa showed the highest water consumption compared with several annual crops due to its rigorous, perennial growth (Huang et al., 2018). Because of its high-water use, older alfalfa stands could deplete soil water quickly in comparison to newer stands. Despite the different crops grown in the experiment, gravimetric water was well correlated to rainfall and overall soil water content, and it was not necessarily crop-dependent.

Table 3.7. Combined analysis of variance and mean square (MS) values of soil gravimetric water content at two locations (Loc), Hickson and Prosper ND, ten treatments (Trt), and eight sampling dates (Date) in 2022 and five sampling dates in 2023.

	2022		2023	
SOV	df	MS	df	MS
Trt	9	10786	9	23383*
Date x trt	63	5625	36	9104
Date	7	110949	4	345684*
Loc	1	106617	1	73508
Loc(rep)	6	21049*	6	17263*
Loc x date	7	33094*	5	23633*
Loc x trt	9	5216	9	1471
Loc x date x trt	63	6223	27	5255
Residual	474	5125	263	8020
CV, %		32		48

* Significant at $P \leq 0.05$, level of probability

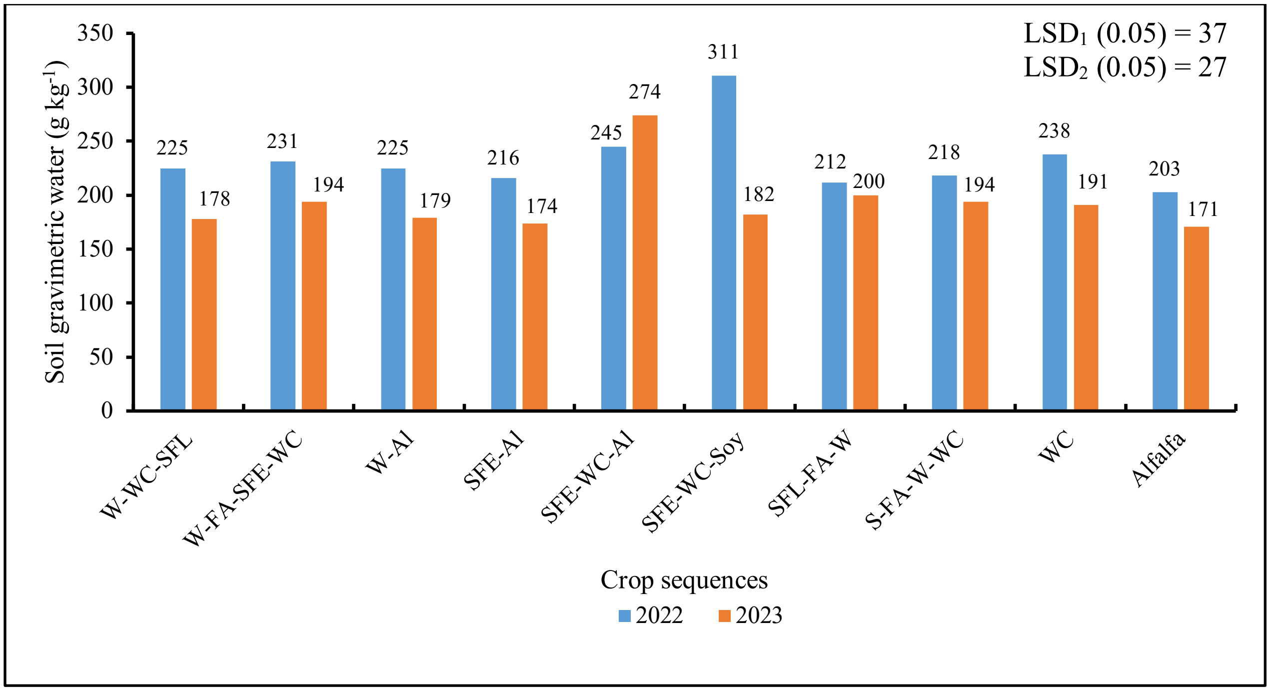

Figure 3.3. Mean values for seasonal soil gravimetric water per crop sequence combined for two locations, Hickson and Prosper, ND in 2022 and 2023 (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, Soy-soybean). LSD_1= to compare crop sequences in 2022; LSD_2= to compare crop sequences in 2023.

3.4.2.2. Soil carbon and nitrogen

There were no significant differences in soil organic carbon, total carbon, soil bulk density, or soil nitrate among the ten treatments (Table 3.8). Location by treatment was significant for nitrate only ($P \leq 0.05$). Differences in crop rotations may be more pronounced in subsequent years; however, a two-year rotation is not enough time for significant changes in soil carbon to occur (Table 3.9). The only significant difference ($P \leq 0.05$) detected within the four treatments sampled for POXC and soil protein was by location (Table 3.8). Although treatment was not significant, treatment values are displayed by location (Table 3.10).

Table 3.8. Combined analysis of variance and mean square values for soil organic carbon (SOC), total carbon (TC), soil bulk density (BD), nitrate (NO_3-N), permanganate oxidizable carbon (POXC), and soil protein from soil samples taken in fall 2023 for carbon; fall 2022, spring 2023, and fall 2023 for nitrate; and fall 2023 for POXC and protein at two locations (Loc), Hickson and Prosper, ND and ten treatments (Trt).

		SOC	TC	Soil BD		NO_3-N		POXC	Protein
SOV	df	MS	MS	MS	df	MS	df	MS	MS
Trt	9	34	101	0.01	9	2076	3	3005	0.32
Loc	1	3322	16768	2.5	1	189	1	117007	4.3*
Loc(rep)	6	836*	4616	0.9*	6	3787*	3	609	0.26
Loc x trt	9	21	282	0.01	9	1450*	3	7019	0.88
Residual	134	64	240	0.01	454	751	18	3337	0.12
CV, %		24	36	7		49		16	15

*Significant at $P \leq 0.05$, level of probability

Alfalfa has the potential to increase total carbon in soil, especially soil organic carbon; however, findings are inconsistent. Increased carbon was observed in continuous alfalfa systems after four years in a study performed in the Loess Plateau in China (Niu et al., 2020). A study conducted on forage systems in Minnesota indicated that alfalfa reduced C losses by 23% compared with continuous silage corn, but still had a net loss of 3.8 Mg C ha yr^{-1} (Gamble et al., 2021). Alfalfa is often thought to be a model crop for C sequestration with great potential as a carbon-sink. However, the annual N_2O emissions from mineralization, nitrification, and denitrification processes related to N_2 fixed by alfalfa are significant, therefore offsetting the C-sink by up to 14% annually (Anthony et al., 2023). Reduced tillage in alfalfa systems could potentially help build carbon, but the overall C-balance of the forage legume may not be as positive as initially thought. Crops like wheat, with a high residue carbon to nitrogen ratio, could contribute more carbon to soil; however, how long the C will remain in the soil is unknown. A study conducted in the Northern Great Plains indicated that soil organic carbon only increased under a spring wheat-corn-soybean rotation after six years (Liebig et al., 2024).

Table 3.9. Average total carbon (TC) stock, total soil organic carbon (SOC), and soil bulk density (BD) at 0-15 and 15-30-cm depth of each treatment (W-wheat, WC-winter camelina, SFL-late-planted sunflower, FA-fallow, SFE-early-planed sunflower, Al-alfalfa, Soy-soybean) by location, Hickson and Prosper, ND. Baseline samples were taken in spring of 2022 and are reported as the average of four replicates. Carbon stock calculated based on soil bulk density, total carbon content, and soil organic carbon content from soil analysis sampled in fall 2023.

Rotation	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
	Hickson		Prosper		Hickson		Prosper		Hickson		Prosper	
	------------Mg TC ha^{-1}-----------				-------------Mg SOC ha^{-1}-----------				----------g cm^{-3} Soil BD----------			
Baseline	54	58	35	31	46	29	30	30	1.2	1.3	1.2	1.4
W-WC-SFL	45	63	35	22	41	34	34	18	1.1	1.2	1.3	1.4
W-FA-SFE-WC	50	51	39	42	45	34	38	24	1.1	1.1	1.3	1.4
W-Al	53	56	35	31	49	36	35	23	1.2	1.2	1.3	1.3
SFE-Al	50	55	39	34	44	34	38	24	1.1	1.1	1.4	1.3
SFE-WC-Al	43	49	36	32	41	32	34	22	1.0	1.1	1.2	1.4
SFE-WC-Soy	51	56	39	33	46	35	36	20	1.1	1.1	1.3	1.4
SFL-FA-W	45	54	39	34	42	32	38	23	1.1	1.0	1.3	1.4
S-FA-W-WC	47	52	37	35	43	28	35	22	1.1	1.1	1.3	1.4
WC	47	48	37	42	42	32	35	24	1.0	1.1	1.3	1.3
Alfalfa	46	43	37	38	42	31	35	23	1.1	1.1	1.3	1.4
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Nitrate was highly variable in the rotations, likely due to its mobility in the soil and conversion through biological activity (Table 3.10). In Fall 2022 and Spring 2023, Prosper soils contained more NO_3-N in 0-15-cm soil, likely residual from the previous soybean crop. Despite Hickson soil having a higher clay content and less leaching potential, shallow depth NO_3-N was not observed until Fall 2023. The late-planted sunflower treatment was an outlier with a high NO_3-N value in Fall 2022, likely due to the later season fertilization of 89.7 kg ha^{-1} N on 23 August 2022 and 24 August 2022 in Prosper and Hickson, respectively. Nitrate tends to be higher in the 15-60-cm depth due to the mobility of the nutrient in water and rainfall received (NDAWN, 2022; 2023). Spring 2023 to Fall 2023 indicated most treatments increased in NO_3-N concentration in the 0-15-cm depth in Hickson and Prosper. Following crop rotations with consistently high N-fertilization, NO_3-N was likely building up in the soil and the warmer temperature and low rainfall at the time did not mobilize the nutrient deeper in the soil. Treatment 10, which was planted as alfalfa in Spring 2022, gradually increased in NO_3-N concentration from Fall 2022 to Fall 2023 in the 0-15-cm depth. Alfalfa's ability to increase residual soil NO_3-N makes it valuable in rotation as it can supply some of the needed N for the subsequent crop. Silage corn was found to not need any additional N fertilizer when planted after alfalfa because the N from mineralization after terminating alfalfa was sufficient for first-year silage corn (Clark et al., 2021). Any N fertilizer-requiring crop will greatly benefit from being grown after alfalfa as alfalfa's biomass returned to the soil will release N as they decay; however, the potential for leaching and denitrification is present.

Table 3.10. Mean values for NO_3-N from baseline spring 2022 (average of four replicates), fall 2022, spring 2023, and fall 2023 for ten treatments (Trt) (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, S-soybean) at two depths and at two locations, Hickson and Prosper, ND.

Treatment	Hickson		Prosper	
	0-15 cm	15-60 cm	0-15 cm	15-60 cm
	------------------------kg ha^{-1} NO_3-N------------------------			
Baseline	11	46	13	28
		Fall 2022		
1 (W-WC-SFL)	5	28	18	9
2 (W-FA-SFE-WC)	9	29	17	8
3 (W-Al)	6	27	13	7
4 (SFE-Al)	8	25	25	97
5 (SFE-WC-Al)	8	35	21	11
6 (SFE-WC-Soy)	9	32	18	8
7 (SFL-FA-W)	48	26	18	18
8 (Soy-FA-W-WC)	3	19	10	36
9 (WC)	30	51	49	18
10 (Al)	5	10	4	8
LSD (0.05)	18	NS	12	NS
		Spring 2023		
1 (W-WC-SFL)	2	8	5	7
2 (W-FA-SFE-WC)	3	19	9	19
3 (W-Al)	2	9	5	17
4 (SFE-Al)	1	23	13	52
5 (SFE-WC-Al)	1	15	10	28
6 (SFE-WC-Soy)	3	10	6	18
7 (SFL-FA-W)	4	8	8	37
8 (Soy-FA-W-WC)	4	9	13	70
9 (WC)	4	12	11	35
10 (Al)	3	4	8	12
LSD (0.05)	NS	NS	NS	NS
		Fall 2023		
1 (W-WC-SFL)	22	60	20	4
2 (W-FA-SFE-WC)	16	5	6	3
3 (W-Al)	25	6	7	4
4 (SFE-Al)	13	5	13	30
5 (SFE-WC-Al)	19	62	19	9
6 (SFE-WC-Soy)	23	89	29	10
7 (SFL-F-W)	19	52	20	8
8 (Soy-FA-W-WC)	24	65	18	3
9 (WC)	18	45	32	19
10 (Al)	16	5	17	4
LSD (0.05)	NS	48	NS	NS

Although POXC and soil protein are considered better short-term measurements for soil health indicators due to management changes, no differences were seen in the selected treatments for this study (Table 3.11). Permanganate oxidizable carbon measures a smaller fraction of carbon in the soil, thought to be more recently processed C, which is why changes in this measurement could be seen more quickly compared with TC or SOC. Malone et al. (2023) determined that inherent factors such as location, soil order, texture, and drainage class played a larger role in POXC differences than management practices. This likely explains the differences in location and lack of differences between treatments. Most agricultural soils in the northern Great Plains will range from 400-900 mg kg soil^{-1} POXC (Breker, 2020). Soil protein has shown high responsiveness to cropping system and field practices compared with NH_4^+ and NO_3-N (Naasko et al., 2023). Practices such as reduced tillage, cover cropping, and growing perennial legumes promote higher levels of soil protein. Soil protein was highest in mowed grasslands and lowest in a conventional soybean system (Naasko et al., 2023). The treatment differences detected in the Naasko et al. (2023) study among other studies would lead to the belief that treatment differences, specifically with alfalfa, could be seen in this study; however, no differences were detected.

Table 3.11. Mean values for soil protein and soil permanganate oxidizable carbon (POXC) from 2022 baseline and fall 2023 for four treatments at one depth (0-15-cm) and at two locations, Hickson and Prosper, ND.

	Protein		POXC	
Treatment[a]	Hickson	Prosper	Hickson	Prosper
	-------g kg soil^{-1}-------		-------mg kg soil^{-1}-------	
Baseline	2.1	2.8	474	351
W-FA-SFE-WC	2.0	3.2	446	326
W-Al	1.9	2.8	368	330
Soy-FA-W-WC	2.0	2.4	477	304
Alfalfa	1.9	2.4	441	288
LSD (0.05)	NS	NS	NS	NS

[a] W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, S-soybean

3.4.3. Photosynthetically active radiation (PAR)

Based on measurements with the ceptometer, alfalfa achieved the highest amount of intercepted PAR first in the growing season compared with the other crops seeded at the same time (Fig. 3.4). Alfalfa maintained significant intercepted PAR ($P \leq 0.05$) throughout the season and only dropped after harvest occurred when most of the biomass is removed. Alfalfa's morphology, adaptation to grow with cold temperatures in the spring, and close 15-cm row spacing helped it to achieve a full cover sooner than other crops. Annual crops such as wheat, sunflower, and soybean gradually increased intercepted PAR, plateaued, decreased slightly as the crop senesced, then reached zero after harvest. Due to the differences in growth and harvest patterns of crops measured, date was significant (Table 3.12). Alfalfa will never reach zero PAR interception during its planting year or following production years because the entirety of the plant is not harvested. Typically, 7.5-cm of stubble is left after harvest in North Dakota as that is the stubble height that compromises for maximum yield and nutritive value (Meyer & Larson, 1975). Because of this, alfalfa maximizes intercepted PAR and provides continuous living cover, one of the five principles of soil health (USDA NRCS, 2024). Measurements of PAR were taken sporadically in 2023 due to time constraints and cloud cover during sampling times. Data from 2023 is not displayed.

Table 3.12. Combined analysis of variance and mean square values for photosynthetically active radiation (PAR) of four crops: alfalfa, wheat, sunflower, and soybean averaged across two locations, Hickson and Prosper, ND in 2022.

SOV	df	MS
Crop	3	2056
Date	7	14165*
Date x crop	18	1153
Loc	1	4513
Loc(rep)	6	348
Loc x crop	3	3403
Date x loc x crop	21	1353*
Residual	314	127
CV, %		15

* Significant at $P \leq 0.05$, level of probability

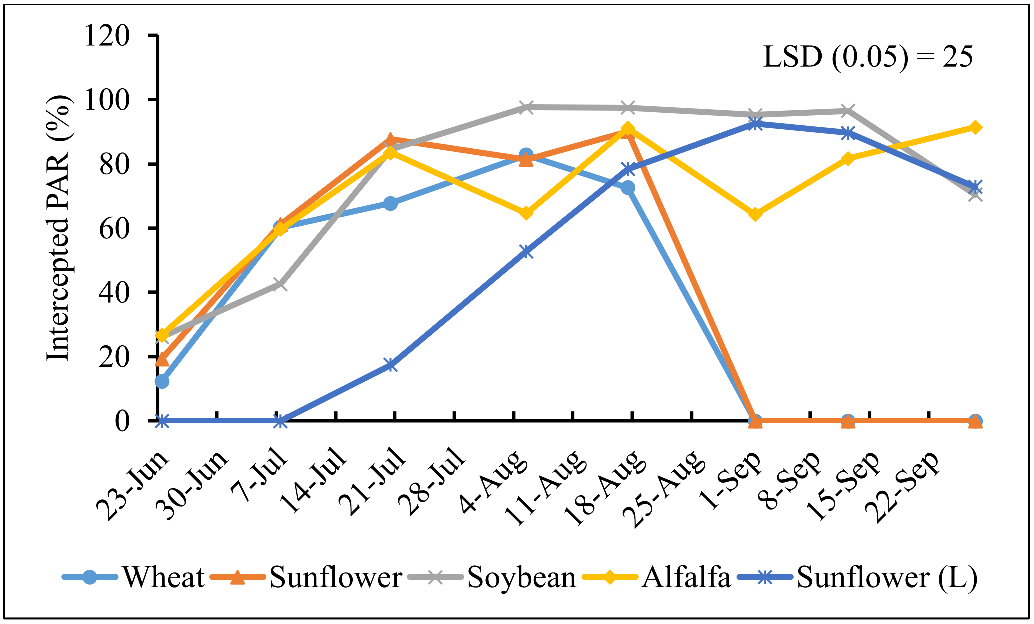

Figure 3.4. Date by crop interaction for photosynthetically active radiation (PAR) interception of wheat, early-maturing sunflower, soybean, alfalfa, and late planted, early-maturing sunflower (L) averaged across two locations, Hickson and Prosper, ND in 2022.

3.4.4. Harvest and quality measures

3.4.4.1. Alfalfa

Alfalfa yields differed between treatments ($P \leq 0.05$) due to different planting dates and environmental conditions. Treatment, cut, and treatment by cut were all significant in 2023 (Table 3.13). Notably, alfalfa planted following sunflower on 1 September, 2022 had less yield than that of alfalfa following wheat planted on 1 September, 2022 (Table 3.15). It is possible that the crop that preceded the alfalfa made a difference. Wheat preceding alfalfa received one application of 2,4-D, with minimal residual effect, while sunflower preceding alfalfa was sprayed with imazamox. Plant back interval is three months, which is just within the window of when the alfalfa was planted (BASF, 2024). Residual effect from the herbicide may have hindered seedling development and fall growth. Alfalfa is tolerant to imazamox sprayed post-emergence after the third trifoliolate, but it has not been reported if this herbicide could affect emerging alfalfa seedlings (Ikley et al., 2024). Plant stand was determined to compare treatment establishment (Table 3.16). Although not statistically significant by treatment, location was significant. Prosper alfalfa established after sunflower only contained 26 plants m^{-2}, whereas monocrop alfalfa contained 39 m^{-2}. In contrast, Hickson monocrop alfalfa had the lowest plant count with 65 plants m^{-2}. Alfalfa established after sunflower contained 81 plants m^{-2}, and alfalfa established after wheat contained 75 plants m^{-2} in Hickson. Alfalfa stands were denser in Hickson. Poor stand is considered fewer than 44 plants m^{-2} according to Schroeder (2015). Fall 2022 was dry in comparison to other years, meaning that alfalfa had little moisture to grow. It is only recommended to seed late-summer or fall alfalfa if good soil moisture is present and at least six weeks of growth can occur after germination, to ensure contractile growth is completed and plants can survive the winter (Undersander et al., 2015). According to NDSU alfalfa production guides, planting alfalfa after 15 August is not recommended (Brummer, 2014). Despite variable establishment compared with spring-seeded alfalfa in 2023, a significant difference was only observed in the first harvest of 2023 ($P \leq 0.05$) (Table 3.15). The fall-seeded alfalfa achieved a similar forage yield to spring-seeded alfalfa in subsequent harvests. Alfalfa planted in spring 2022 was harvested four times in 2023. The fall harvest (fourth) can reduce the forage yield in the first harvest of the following year, but the seasonal yield increases (Berti et al., 2012). It is recommended to make a fourth harvest of alfalfa between 30 September and 15 October to reduce the probability of winter injury (McDonald et al., 2021). The fourth harvest of this experiment was made on 12 and 16 October, which approaches the later part of the deadline.

Table 3.13. Combined analysis of variance and mean square values for alfalfa forage yield in 2022 for two locations, Hickson and Prosper, ND and separated by location in 2023.

	2022		2023 Hickson		2023 Prosper	
SOV	df	MS	df	MS	df	MS
Loc	1	703444	--	--	--	--
Loc(rep) or rep	1	1458310	3	641936	3	11964
Trt	1	--	2	3622021*	2	9034777*
Cut	1	12260	3	5019989*	3	3636094*
Cut x loc	1	263792	--	--	--	--
Trt x cut	6	330515	5	2133123*	4	2053030*
Trt x cut x loc	6	1458310	--	--	--	--
Residual	6	500467	28	440629	27	390111
CV, %		26		30		24

* Significant at $P \leq 0.05$, level of probability

Table 3.14. Mean values for alfalfa forage yield by cut and total at Hickson and Prosper, ND during the 2022 growing season.

	----------------------Forage yield (kg ha^{-1})--------------------			
Alfalfa	Cut 1	Cut 2	LSD (0.05)	Total
Hickson	2299	2847	1123	5146
Prosper	3159	2500	1951	5659

LSD$_1$= to compare cut 1 and cut 2 in Hickson

LSD$_2$= to compare cut 1 and cut 2 in Prosper

Table 3.15. Mean values for alfalfa yield by cut and combined total for three planting times at Hickson and Prosper, ND during the 2023 growing season.

	Cut 1	Cut 2	Cut 3	Cut 4	Total
Treatment			Hickson		
	------------------------	------------	kg ha^{-1}	------------	------------------------
Planted fall 2022[a]	2573	1363	1428	1943	7307
Planted fall 2022[b]	1997	1502	1821	--	5320
Planted spring 2022	4531	2743	1421	1953	10648
LSD (0.05)	961	555	1201		1407
			Prosper		
Planted fall 2022[a]	2768	2293	2127	--	7188
Planted fall 2022[b]	1452	2635	2108	--	6195
Planted spring 2022	4608	2999	3511	1879	12997
LSD (0.05)	523	906			1852

[a] Refers to alfalfa seeded in fall 2022 following wheat

[b] Refers to alfalfa seeded in fall 2022 following early-planted, early-maturing sunflower

Table 3.16. Combined analysis of variance and mean square values for alfalfa plant counts taken for three treatments (Trt) in spring 2023 at Hickson and Prosper, ND.

SOV	df	MS
Trt	2	7
Loc	1	10417*
Loc(rep)	6	1069*
Loc x trt	2	446
Residual	12	162
CV, %		24

* Significant at $P \le 0.05$, level of probability

Location by cut was significant ($P \le 0.05$) for ADF, ash, NDFD, TDN, and RFQ in 2022 (Table 3.17). The first harvest at Hickson had an ADF value of 273 g kg^{-1} whereas Prosper was 297 g kg^{-1}. Ash content was lowest for the first harvest at Hickson and increased for the second harvest. The opposite occurred at Prosper. Both locations saw lower NDFD values for the second harvest, but the second harvest at Hickson was significantly ($P \le 0.05$) lower than Prosper. Total digestible nutrient content was similar for the first harvest at both locations; however, the second harvest in Hickson was only 695 g kg^{-1} and Prosper was 727 g kg^{-1}. Again, the second harvest at Hickson saw a significantly ($P \leq 0.05$) lower RFQ, of only 152. Other harvests ranged from 173 to 188.

In 2023, ash, CP, NDFD, TDN, and RFQ were all significant for location by cut by treatment. Specifically, CP and NDFD were significant ($P \leq 0.05$) by cut in 2023 (Table 3.17). Crude protein is likely to differ by cut and the growth stage at which harvest occurred. The first harvest of 2023 occurred when alfalfa was 40-100% bloom at both locations, hence the reason it contained the lowest CP of each treatment and cut (Table 3.18). First cut is recommended to be harvested at 10% bloom but timely harvest was not possible. The measured indicators are all important in determining quality for animal feed. Although RFQ is not utilized to develop a ration, it is a reasonable estimate of a forage's ability to provide a quality and cost-effective nutrition to an animal. A dairy calf and a dairy cow in the first 120 days of milk production require the highest quality feed compared with other livestock and a minimum RFQ of 140 (Hancock, 2011). Premium hay is denoted by an RFQ of 151 or greater. Alfalfa planted in spring 2022 failed to reach the premium rank on the third harvest of 2023 in Prosper. Relative forage quality is generally highest in alfalfa and other legumes compared with grasses (Hancock, 2011). The IVDMD was not influenced by treatment factors. Nutritive value parameters were all directly related to the maturity and quality of the plant harvested. The oldest portion of alfalfa stems typically contain 10% CP where newer alfalfa leaves contain 24% (Ball et al., 2001). It has been established that CP and IVDMD amongst other nutritive value parameters are higher in the first cut or in newly established alfalfa compared with subsequent cuts (Undersander & Moore, 2001). In the first harvest of treatment ten, alfalfa plants were the most mature and contained a lower leaf-to-stem ratio.

Table 3.17. Combined analysis of variance and mean square values for alfalfa nutritive value indicators from three treatments (Trt) and two harvest dates (Cut) combined across two locations in Hickson and Prosper, ND in 2022 and four cuts combined across two locations in Hickson and Prosper, ND in 2023.

SOV	df	ADF	Ash	ADL	CP	IVDMD	NDFD	TDN	RFQ
	2022								
Cut	1	964	1.4	119	10	7639	1847	1096	1448
Loc	1	0.7	69	27	150	212	1838	766	71
Loc(rep)	6	291	12*	8	137	156	82	194	160
Loc x cut	1	2299*	154*	68	493	2079	492*	1230*	1149*
Residual	6	369	0.3	23	137	373	72	190	171
CV, %		7	0.6	8	5	2	2	2	8
	2023								
Trt	2	14838	673	578	716	2137	829	3059	3304
Cut	3	3509	360	169	18022*	6443	35805*	3395	728
Trt x cut	5	1915	454	96	2582	3816	3051	2292	1882
Loc	1	3416	1970	49	8927	4428	251	1956	1243
Loc(rep)	6	356	59	40	219	1825	1222*	656	510
Loc x trt	2	33	10	121	359	914	2786	1345	173
Loc x cut	3	828	259	48	1694	209	492	1607	13
Loc x trt x cut	4	928	207*	37	1465*	1389	1666*		
								3318*	859*
Residual	55	461	51	30	168	743	536	540	259
CV, %		7	8	9	5	4	5	3	10

* Significant at $P \leq 0.05$, level of probability

[a] Acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), in vitro dry matter digestibility in 48 h (IVDMD), neutral detergent fiber digestibility in 48 h (NDFD), total digestible nutrients (TDN), relative feed quality (RFQ)

Table 3.18. Mean values for select alfalfa forage nutritive value parameters for three treatments and harvest dates (Cut) in Hickson and Prosper, ND in 2023.

Alfalfa	CP	NDFD	RFQ
	-----------------g kg^{-1}-----------------		
Cut 1	199	466	164
Cut 2	258	435	167
Cut 3	255	381	155
Cut 4	244	383	164
LSD (0.05)	31	39	26

3.4.4.2. Wheat

In Year 1, yield was influenced by a treatment by location interaction ($P \leq 0.05$) (Table 3.19). Yields were overall higher in Hickson in 2022 due to less weed pressure and better spring establishment. No differences were detected for protein content. In trials during development of the 'Glenn' cultivar, average yield is listed as 3,632 kg ha^{-1}, whereas this experiment only achieved an average of 1,345 kg ha^{-1} considering Years 1 and 2 (Glenn Hard Red Spring Wheat, 2005; Table 3.20). Yields were lower most likely due to weed pressure with the most significant weed pressure caused by foxtail (*Setaria* spp.) and seed shattering at harvest.

Table 3.19. Combined analysis of variance and mean square values of wheat grain yield and grain protein content at two locations (Loc), Hickson and Prosper, ND and three treatments (Trt) in 2022 and two treatments (Trt) in 2023.

	2022			2023		
SOV	df	Yield	Protein	df	Yield	Protein
Trt	2	131480	26	1	179354	4
Loc	1	835266	298	1	3965850	689
Loc(rep)	6	739607*	179*	6	449460	266
Loc x trt	2	428496*	16	1	1047391	89
Residual	12	58844	19	4	389742	62
CV, %		16	2		39	3

*Significant at $P \leq 0.05$, level of probability

Table 3.20. Mean values for wheat grain yield and grain protein content at two locations, Hickson and Prosper, ND and three treatments in 2022 and two treatments in 2023.

2022			2023		
Treatment	Yield	Protein	Treatment	Yield	Protein
	kg ha^{-1}	g kg^{-1}		kg ha^{-1}	g kg^{-1}
Wheat preceding w. camelina[a]	1426	164	Wheat following sunflower (L)[b]	1564	158
Wheat preceding fallow	1647	162	Wheat following soybean	1539	159
Wheat preceding alfalfa	1424	166			
LSD (0.05)	1408	9		734	68

[a] Winter camelina

[b] Late-planted, early-maturing sunflower

3.4.4.3. Sunflower

Sunflower biomass yield was influenced by treatment ($P \leq 0.05$) (Table 3.21). Biomass yield was significantly lower in the 2023 growing season as sunflower plants did not grow as tall or as vigorously. In 2022, the average biomass of sunflower plants, both early- and late-planted was 11,798 kg ha^{-1} and in 2023, it was only 5,218 kg ha^{-1}, which included only early-planted sunflower (Table 3.22). Poor establishment due to lack of rainfall, disease presence, and insect damage likely played roles causing low biomass yield. Sunflowers at each location in 2022 were impacted by red sunflower weevil (*Smicronyx fulvus*) and minor levels of sclerotinia stem and head rot (*Sclerotinia sclerotiorum* Lib. de Bary). In 2023, sunflowers were severely impacted by red sunflower weevil, sunflower midge (*Contarinia schulzi*), and sclerotinia head rot. The economic threshold for red sunflower weevil is 4 to 8 adult weevils per head, which was observed in plots; however, the plots were not sprayed due to additional arthropod biodiversity objectives described in chapter 4 (Kandel et al., 2020). Achene yield was also reduced due to bird damage, especially in Year 1. During Year 2, protective netting was placed on harvest area of each experimental unit; however, birds had already reached the area and caused minor damage. Although yield was not statistically significant, it was variable between planting times and year (Table 3.21). Treatment and location by treatment were significant ($P \leq 0.05$) when comparing the double cropping system to sunflower and winter camelina monocrops (Table 3.21). Overall oilseed yield of double cropping systems with winter camelina was higher in both locations (Fig. 3.5), although closer to monocrop sunflower yield in Prosper. This is likely due to the low winter camelina yields in Prosper. A study by Gesch et al. (2022) achieved similar results and found that the success of the early-maturing sunflower-winter camelina double-crop system is highly dependent on weather. They found that in one year, the double-crop system never outproduced the monocrop sunflower. The only information obtained by this study was winter camelina following early-maturing sunflower. No information was reported on early-maturing sunflower planted after winter camelina due to the sunflower not reaching maturity. This is an important point to consider as farmers can be taking a major risk with a similar double-cropping system in North Dakota if the weather is not favorable for two crops. Another idea to consider is that early-maturing sunflower hybrids are lower-yielding than full season. Early hybrids produced an average of 1,460 kg ha^{-1} where full-season hybrids produced an average of 3,029 kg ha^{-1} (Gesch et al., 2022). No comparison could be made in 2023 with late-planted sunflower. Harvest indices were similar between treatments and years meaning that biomass to achene ratios were comparable (Tables 3.21, 3.22).

Table 3.21. Combined analysis of variance and mean square values for sunflower achene yield, biomass yield (BM), and harvest index (HI) of five treatments (Trt) and two locations in Hickson and Prosper, ND in 2022 and 2023.

SOV	df	Yield	BM	HI	DC yield[a]
Trt	4	2825574	116350644*	50.0	675793*
Loc	1	3330028	1983589	8.2	517173
Loc(rep)	3	64241	27843768*	4.7	20557
Loc x trt	4	1035145	17189718	60.0	148633*
Residual	24	210302	11059038	27.0	35570
CV, %		2.3	2.7	2.0	26

* Significant at $P \leq 0.05$, level of probability.

[a] DC = double crop

Table 3.22. Mean values for sunflower achene yield, biomass yield (BM), and harvest index (HI) of five treatments (Trt) and averaged across two locations in Hickson and Prosper, ND in 2022 and 2023.

Treatment	Yield	BM	HI
	----------kg ha^{-1}-----------		%
Sunflower (E)[a] following wheat (2023)	1194	5218	29
Sunflower (E) preceding alfalfa (2022)	2338	12028	24
Sunflower (E) preceding winter camelina (2022)	2568	6893	24
Sunflower (E) preceding winter camelina (2022)	2219	13490	23
Sunflower (L)[b] (2022)	1464	14779	27
LSD $_{1,2}$ (0.05)	NS	7326[c], 10448[d]	NS

[a] Refers to early-planted, early-maturing sunflower

[b] Refers to late-planted, early-maturing sunflower

[c] LSD_1= to compare between Trt 2 to Trts 4, 5, and 6

[d] LSD_2= to compare Trt 2 to Trt 7, Trt 4 to Trt 7, and Trt 2 to Trts 5, 6, and 7

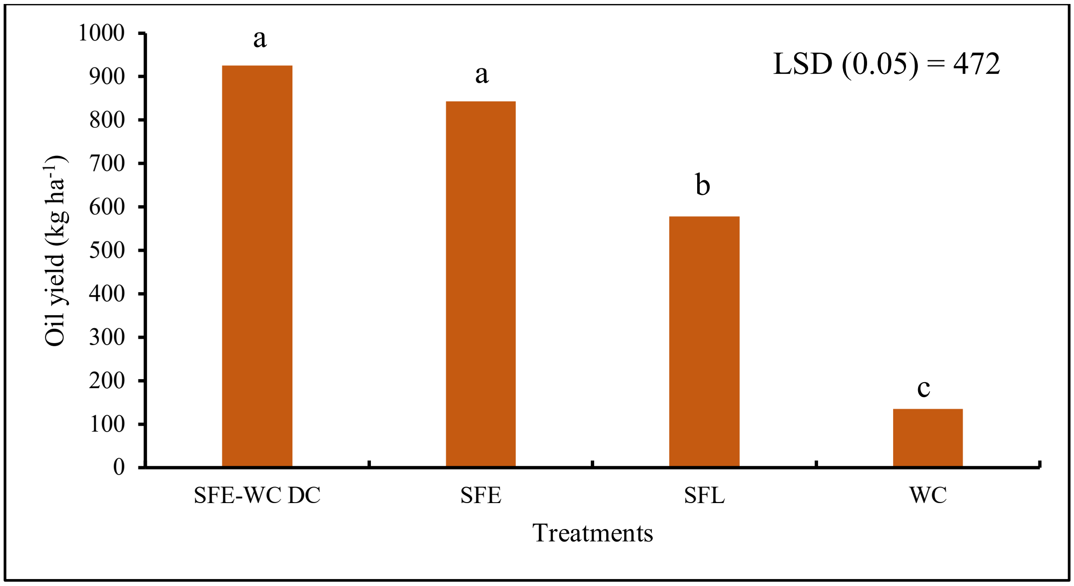

Figure 3.5. Average oil yield from various treatments averaged across Hickson and Prosper, ND in 2022 for sunflower and 2023 for winter camelina to compare double-crop sunflower and winter-camelina yield with their respective monocrops. SFE-WC DC= early-planted sunflower and winter camelina double crop combined oil yield; SFE= early-planted sunflower monocrop oil yield. SFL= late-planted sunflower monocrop oil yield; and WC= winter camelina monocrop oil yield.

Results for achene oil content and biomass N concentration were consistent throughout the experiment (Tables 3.23, 3.24). The lowest yielding oil content treatment, sunflower preceding alfalfa, was one of the higher achene-yielding treatments (achene yield not displayed; Table 3.24). This could be due to red sunflower weevil damage affecting oil content within the achene. Visible holes in seeds made by the pest were indicative of a reduction in oil content and seed weight.

Location was significant for both oleic and linoleic acids ($P \leq 0.05$). Sunflower must contain at least 82% oleic acid to be considered high-oleic (Kandel et al., 2020). Most samples in this study were above 85%. There was variability in samples which could stem from environmental conditions or presence of sunflower pests that affected achene quality. Planting dates and growing conditions are related to oil content and composition. Oleic fatty acid content was found to be the greatest ($P \leq 0.05$) when planting occurred around 23 May and showed significant decline when planting occurred after 10 June (Kandel et al., 2020). Water stress during achene filling period can cause the oleic/linoleic acid ratio to increase according to Flagella et al. (2002).

Table 3.23. Combined analysis of variance and mean square values for sunflower achene oil concentration, fatty acid profile, and biomass nitrogen concentration of five treatments (Trt) and two locations (Loc) in Hickson and Prosper, ND during the 2022 and 2023 growing seasons.

SOV	df	Oil	Biomass N	16:0[a]	18:0	18:1	18:2
Trt	4	2847	0.18	0.44	0.38	20.0	27
Loc	1	1274	0.53	0.0003	0.03	18.0*	14*
Loc(rep)	6	702	0.57*	0.09	0.22	22.0	19
Loc x trt	4	3483	0.18	0.01	0.38	8.8	5
Residual	24	1323	0.15	0.07	0.15	31.0	27
CV, %		1	2.90	0.78	1.34	0.6	13

* Significant at $P \leq 0.05$, level of probability.

[a] 16:0 = palmitic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid

Table 3.24. Mean values for sunflower achene oil content fatty acid profile, and sunflower biomass nitrogen concentration of five treatments (Trt) and two locations in Hickson and Prosper, ND during the 2022 and 2023 growing seasons.

Treatment	Oil	16:0[a]	18:0	18:1	18:2	Biomass N
	g kg^{-1}	---------------%---------------				kg^{-1}
Sunflower (E)[b] following wheat	373	3.8	2.7	85.6	7.3	11.7
Sunflower (E) preceding alfalfa	343	3.2	2.9	88.1	3.7	14.2
Sunflower (E) preceding winter camelina	355	3.2	2.7	88.6	3.6	14.8
Sunflower (E) preceding winter camelina	360	3.2	2.8	89.8	2.3	15.1
Sunflower (L)[c]	393	3.5	3.2	87.1	4.5	12.4
LSD (0.05)	NS	NS	NS	86.4	4.7	2.9

[a] 16:0 = palmitic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid

[b] Refers to early-planted, early-maturing sunflower

[c] Refers to late-planted, early-maturing sunflower

3.4.4.4. Soybean

Seed composition was significant ($P \leq 0.05$) when comparing soybean grown as a monocrop with a relay-crop system in terms of oil and protein content (Table 3.25). Protein content averaged 35 g kg^{-1} more in the relay system with winter camelina compared with the monocrop soybean in Hickson (Table 3.26). Prosper plots were lost. The increase of protein could be due to an early-season application of N for the winter camelina, or the fact that lower yield results in greater protein content. Gesch et al. (2014) reported higher protein content in soybean grain grown in a relay system with winter camelina compared with a monocrop system. Monocrop soybean did not receive any N fertilizer. Nitrogen plays a major role in seed protein formation.

Soybean did not appear as healthy or as vigorous in the relay system. The canopy of winter camelina impacted the amount of sunlight the soybean received and created a more closed canopy environment, but yield was not significantly different. Gesch et al. (2014) saw similar results in their study; soybean relay-cropped with winter camelina always had a lower oil yield than monocrop soybean, sometimes a yield reduction of 50%. Soybean plant height was noticeably different in the field and significant ($P \leq 0.05$). Monocrop soybean in Hickson had an average height of 75-cm and relay crop soybean in Hickson only had an average height of 46-cm. Weed pressure present from the winter camelina during establishment and plant competition are possible factors as to why the soybean lacked in growth and yield. Soybean could not receive a typical early-season glyphosate application with the winter camelina present.

Table 3.25. Combined analysis of variance and mean square values for soybean yield, plant height (Hickson only), oil, and protein concentration from two locations, Hickson and Prosper, ND in 2022 and soybean relay (with winter camelina) average is from one location, Hickson, ND in 2023.

SOV	df	Yield	Plant height	Oil	Protein
Trt	1	1254356	1355*	2.20*	18.0*
Rep	3	93586	111	0.05	0.1
Residual	2	507459	28	0.01	0.03
CV, %		22	8	0.50	0.5

* Significant at $P \leq 0.05$, level of probability.

Table 3.26. Mean yield, plant height (Hickson only), oil, and protein concentration of soybean seed. Soybean monocrop average is from two locations, Hickson and Prosper, ND in 2022 and soybean relay (with winter camelina) average is from one location, Hickson, ND in 2023.

	Yield	Plant height	Oil	Protein
	kg ha^{-1}	cm	----------g kg^{-1}----------	
Soybean monocrop	3301	75	205	362
Soybean relay	2265	46	192	397
LSD (0.05)	NS	18	3	6

3.4.4.5. Winter camelina

Winter camelina seed quality was significant by location for oil and crude protein (Table 3.27). Average oil concentration in Hickson was 395 g kg^{-1} and 350 g kg^{-1} in Prosper (Table 3.28). The soybean relay treatment saw significantly lower values in Prosper. The soybean relay treatment in Hickson was comparable to the other winter camelina cropping systems (Table 3.28). Results from the double-crop study by Gesch et al. (2014) showed no differences in oil or crude protein between soybean-winter camelina double and relay systems; however, they did not have a winter camelina monocrop for comparison. Despite winter camelina being a low-input crop, it has been noted that N fertilizer is necessary to achieve both grain yield and oil yield of winter camelina (Gregg et al., 2022). Relay-cropping with N_2-fixing soybean or following sunflower, a heavily N-fertilized crop, is not enough to achieve a desirable yield. Yield of relay-cropped winter camelina with soybean that did not receive N fertilizer had about 50% of the yield compared with the same relay treatment that received N fertilizer (Gregg et al., 2022). The significant differences ($P \leq 0.05$) in fatty acid composition of harvested winter camelina seed was by location (Table 3.27). According to Berti et al. (2016), camelina seed oil composition varies with location, cultivar, and environment. Treatment was not significant for either location meaning that previous crop or presence of soybean in the relay system did not impact fatty acid composition.

Table 3.27. Combined analysis of variance and mean square (MS) values for oil content, fatty acid profile (16:0 palmitic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 20:1 eicosenoic, 22:1 erucic), and crude protein (CP) in winter camelina seed at two locations (Loc), Hickson and Prosper, ND and four treatments (Trt) in 2023.

SOV	df	Oil	16:0	18:1	18:2	18:3	20:1	22:1	CP
Trt	3	6	0.06	0.58	0.64	0.50	0.43	0.06	3
Loc	1	130*	3.80*	2.70	42.0*	48.0	12.0	2.70*	45*
Loc(rep)	6	3	0.06	1.60	2.80	2.50	0.51	0.09*	2
Loc x trt	3	5	0.13*	4.30	2.30	0.30	0.13	0.18	2
Residual	16	3	0.04	0.78	1.20	1.60	0.44	0.03	1
CV, %		5	3.28	8.13	4.58	3.53	4.30	4.98	3

* Significant at $P \leq 0.05$, level of probability.

Table 3.28. Mean values for, oil concentration, fatty acid concentration (16:0 palmitic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 20:1 eicosenoic, 22:1 erucic), and crude protein (CP) in winter camelina (WC) seed at two locations, Hickson and Prosper, ND and four treatments (Trt) in 2023.

Trt	Oil	16:0	18:1	18:2	18:3	20:1	22:1	CP
	g kg^{-1}	---------------------%------------------------						g kg^{-1}
WC following wheat	370	6.1	11.2	23.1	35.6	15.2	3.5	290
WC following sunflower	390	5.8	10.8	23.8	36.2	15.6	3.7	300
WC-soybean relay crop	370	6.0	10.6	23.7	35.3	15.3	3.7	300
WC monocrop	360	6.0	11.1	23.1	35.3	15.7	3.7	310
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Locations were analyzed separately for winter camelina yield due to lack of homogeneity of variances. No significance in treatment was noted in either location (Table 3.29). Yields were highly variable in each location due to specific conditions of each plot, most notably weed pressure and winter camelina establishment, especially in Prosper (Table 3.30). No winter survival rate could be determined due to a lack of plant emergence after planting in fall 2022. Winter camelina plants only demonstrated spring growth. Below average rainfall occurred shortly after planting led to lower-than-expected stands. Fall 2022 was the dryest August through October with 8.6-cm rainfall since 2012 and the second dryest since 1994 according to measurements collected at the Prosper, ND weather station (NDAWN, 2024). Weed pressure was intense at both locations in May through harvest, particularly at Prosper. There are no herbicides labeled for broadleaf weed control in winter camelina. Winter camelina was planted after early-maturing sunflower for two treatments. Early-maturing sunflower received an application of imazamox in summer 2022 before winter camelina was sown in fall 2022. Imazamox was chosen because the sunflower cultivar is part of the Clearfield® system. It was not realized until after application that this herbicide has an 18–26-month plant-back interval for other brassica crops including canola (*Brassica napus* L.) and mustard (*Brassica juncea* L.)

(BASF, 2024). A study was conducted to determine the effects of residual herbicides used on corn had implications to interseeded cover crops. Brooker et al. (2020) determined that Group 2 herbicides, the mode of action of imazamox, reduced oilseed radish (*Raphanus sativus* L.) establishment by 70% or more. Radish, a brassica, like winter camelina is very sensitive to the residual effects of Group 2 herbicides. This residual herbicide activity likely contributed to poor establishment of winter camelina; however, no treatment differences denoted significance. Likely this factor, in combination with weather and weed pressure contributed to minimal yields. In the double-crop study done by Gesch et al. (2022), no in-season application of herbicide was used for sunflower, only an application of trifluralin incorporated in the soil before sowing camelina; however, this study sequence was winter camelina preceding early-maturing sunflower. It is advisable to use a different early-maturing sunflower cultivar that utilizes a different herbicide system with no residual effects on brassica crops.

Table 3.29. Analysis of variance and mean square values for seed yield of winter camelina for four treatments (Trt) at Hickson and Prosper, ND in 2023.

SOV	df	Hickson	df	Prosper
Trt	3	71484	3	2211
Rep	3	1208712	3	1120
Residual	7	352408	9	4512
CV, %		78		104

Table 3.30. Mean values for seed yield of winter camelina (WC) for four treatments (Trt) and two locations, Hickson and Prosper, ND in 2023.

Treatment	Hickson	Prosper
	------------kg ha^{-1}------------	
WC following wheat	766	87
WC following sunflower	590	53
WC-soybean relay crop	845	37
WC monocrop	622	80
LSD (0.05)	NS	NS

3.4.5. Crop sequence cumulative productivity

The crop sequence yield assessment was divided into four different ways to classify crop yield (Table 3.31). When comparing crops like alfalfa to sunflower, it is difficult to compare a specific unit as one is a forage and the other is an oilseed. An oilseed producer is looking for yield in kg oil ha^{-1}, where a forage producer is looking for yield in terms of kg forage or protein ha^{-1}. For oil production, early-maturing sunflower followed by winter camelina-soybean relay had the highest yield ($P \leq 0.05$). Seasonal forage yield of a four-cut alfalfa system is consistent with the yield achieved with including a fourth fall cut by Berti et al. (2012), who notes that an additional 1,000 to 3,000 kg ha^{-1} yield can be achieved with a fourth harvest at the end of the season.

Table 3.31. Crop sequences cumulative grain, forage, oil, and protein yield of ten crop rotations (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, Soy-soybean) combined for two locations, Hickson and Prosper, ND in 2022 and 2023.

Sequence	Grain/seed	Forage	Oil	Protein [a]
	----------------------------	-kg ha^{-1} -	----------------------------	
W-WC-SFL	1853	--	172	367
W-FA-SFE-WC	2841	--	458	268
W-Al	1424	6752	--	1761
SFE-Al	2338	6006	825	1412
SFE-WC-Al	2910	--	1027	97
SFE-WC-Soy	4984	--	1302	963
SFL-FA-W	3101	--	577	250
Soy-FA-W-WC	4863	--	704	1572
WC	351	--	135	109
Alfalfa	--	16625	--	4051

[a] Sunflower achene protein was not measured in this study

Land equivalent ratios give insight as to how productive a system is, in terms of yield, compared with another on the same amount of land. This ratio is beneficial to compare monocrops with other systems such as relay and double crops. A value over one indicates that the system is more productive than its comparison. For oilseed yield, the winter camelina-soybean relay system following sunflower is more productive than the winter camelina double crop following sunflower, as demonstrated by a LER of 1.7 (Table 3.32). The soybean monocrop yielded 1,000 kg ha^{-1} more than the relay system. Including the winter camelina in the relay system and comparing it with soybean monocrop, the LER was ≤ 1 meaning the soybean monocrop was still more productive than both together. Ideally, relay and double crop systems should have a LER ≥ 1. One study calculated the LER of soybean-winter camelina relay systems and found the LER to range between 1.3 and 1.8. Each relay system outperformed the monocrop in this scenario, which is an expected outcome (Mohammed et al., 2022). Winter camelina seed yields achieved in the study in Minnesota were significantly higher than in this experiment. If winter camelina yields could be improved, a LER ≥ 1 could easily be achieved making the relay system worthwhile. The sunflower double-crop system compared with monocrop sunflower LER was greater than one. When winter camelina is planted after early-maturing sunflower, the yield results are greater than that of the early-maturing sunflower monocrop. In the studies done with the double crop system, researchers did not compare specific LER values, but double-cropping increased total oilseed yield, meaning the LER is greater than one (Gesch et al., 2022).

Table 3.32. Land equivalent ratio (LER) comparisons for selected treatments (Trt) from two-year cumulative oilseed yields (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, Soy-soybean) in Hickson and Prosper, ND in 2022 and 2023.

Treatment	Yield Trt 1	Yield Trt 2	LER
	-----kg ha^{-1}-----		
SFE-WC-Soy vs SFE-WC-Al	5003	2910	1.7
SFE-WC double crop vs SFE monocrop	2910	2590	1.1
WC-Soy relay vs Soy monocrop	2716	3324	0.82
WC vs WC in double crop with SFE	135	319	0.42
WC vs WC in relay with Soy	135	392	0.34
Soy monocrop vs Soy relay	3324	2324	1.43

3.4.6. Cost and return analysis

The cumulative economic analysis shows that only two of the crop sequences were profitable over a two-year period (Table 3.33). Early-maturing sunflower followed by alfalfa and alfalfa monocrop had positive net returns. The significant negative net returns are likely due to overall low yields for wheat, sunflower, soybean, and winter camelina and the inclusion of land and machinery costs. These aspects were included because they are true costs for farmers. Alfalfa saw much consistency with growth and little weed or pest issues, therefore making it a profitable enterprise. Labor cost greatly contributed to the total production cost of alfalfa due to multiple harvests each growing season. Fertilizer and herbicide accounted for major input costs across all rotations. Because alfalfa is a relatively low-input perennial, it was found that in the corn belt of China, three years of continuous alfalfa followed by two years of corn increased economic return by \$2,360 ha^{-1} compared with continuous corn, and corn yield was greater in the two years following alfalfa (Sun & Li, 2019). Alfalfa production value totaled \$281 million in North Dakota alone in 2023 (USDA-NASS, 2023). Net return was exceedingly negative for late-planted sunflower because there was no harvestable crop in 2023. Double- and relay-cropping systems with winter camelina have been found to be more economically favorable than a monocrop system; however, the low yields in this study for soybean-winter camelina systems do not support that idea (Gesch et al., 2014). The soybean-relay system had negative profits, but loss was less than that of the sunflower-winter camelina double-crop system.

Table 3.33. Two-year cost and return analysis of ten crop sequences (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, Soy-soybean) for two locations, Hickson and Prosper, ND in 2022 and 2023.

Rotations [a]	W WC SFL	W FA SFE WC	W Al	SFE Al	SFE WC Al	SFE WC Soy	SFL FA W	Soy FA W WC	WC	Al
	------------------------------$ ha^{-1}----------------------------------									
Inputs										
Fertilizer	865	865	409	283	586	521	562	658	303	130
Seed	264	264	461	483	567	348	180	326	84	382
Herbicide	229	229	140	158	227	158	159	303	69	69
Fixed machinery	223	176	193	197	180	212	155	175	69	221
Variable machinery	159	134	161	156	130	143	113	136	47	211
Crop insurance	40	40	14	26	26	42	40	30	--	--
Labor	622	569	525	622	453	507	525	336	98	534
Land rental	272	272	272	272	272	272	272	272	272	272
Total Costs	**2674**	**2549**	**2175**	**2197**	**2441**	**2203**	**2006**	**2236**	**942**	**2819**
Yield kg ha^{-1}										
Wheat	1426	1647	1424	--	--	--	1637	1539	--	--
Alfalfa	--	--	6752	6006	--	--	--	--	--	16625
Sunflower	--	1194	--	2338	2590	2287	1464	--	--	--
Soybean	--	--	--	--	--	2325	--	3324	--	--
Winter camelina	427	--	--	--	320	392	--	--	351	--
Price \$ kg^{-1}										
Wheat	0.32	0.32	0.32	--	--	--	0.32	0.32	--	--
Alfalfa	--	--	0.17	0.17	--	--	--	--	--	0.17
Sunflower	--	0.62	--	0.62	0.62	0.62	0.62	--	--	--
Soybean	--	--	--	--	--	0.50	--	0.50	--	--
Winter camelina	0.55	0.55	--	--	0.55	0.55	--	--	0.55	--
Gross Revenue	691	1267	1604	2471	1782	2796	1432	2154	193	2826
Net Return (\$/ha)	-1983	-1282	-571	274	-659	-593	-574	-82	-749	43

[a] Rotations represent two years of the study, thus, some crops in the rotations are not accounted for in the net return, but are for input costs.

3.4.7. Life cycle assessment

Cropping system was significant (*P* ≤ 0.05) for CI, but individual crops were not, although individual crops displayed wide ranges of CI values (Table 3.34).

Table 3.34. Combined analysis of variance and mean square (MS) values for average total carbon intensity (CI) and global warming potential (GWP) for each crop, CI for ten crop sequences (CS), and net energy of lactation (NEL) for crop sequences including alfalfa combined for two locations, Hickson and Prosper, ND in 2022 and 2023.

SOV	df	CI	GWP	SOV	df	CI	SOV	df	NEL
Crop	6	2457748	1549	CS	9	2318933*	Trt	2	642*
Loc	1	380051	240	Loc	1	2493639	Loc	1	389
Loc(rep)	6	183506	111	Loc(rep)	6	340000	Loc(rep)	6	117
Loc x crop	6	750584*	469*	Loc x CS	9	515132*	Loc x trt	2	5
Residual	132	123418	77	Residual	54	224095	Residual	20	544
CV, %		184	188	CV, %		112			38

* Significant at *P* ≤ 0.05, level of probability.

In comparison with the other crops studied, alfalfa, especially in the production year, or second year, has a low GWP (Table 3.35). In the production year, less inputs are utilized despite the increase in harvest activity, and forage yield is significantly higher increasing energy output. The increase in harvest activity is displayed through having one of the highest energy inputs of all the sequences.

Table 3.35. Carbon intensity (CI) and global warming potential (GWP) equivalent of crops in experiment averaged across two locations, Hickson and Prosper, ND in 2022 and 2023.

Crop	CI	GWP
	g CO_{2e} MJ^{-1}	kg CO_{2e} kg $seed^{-1}$ or $forage^{-1}$
Alfalfa – Year 1[a]	14	0.21
Alfalfa – Year 2[b]	6	0.09
Soybean – Monocrop	12	0.41
Soybean – Relay [c]	14	0.67
Winter camelina	814	21
Wheat	71	0.93
Sunflower – Early-planted	12	0.77
Sunflower – Late-planted	15	0.99
Fallow – May-October	583	NS
LSD (0.05)	691	17

[a] Refers to alfalfa in establishment year
[b] Refers to alfalfa in full production year
[c] Refers to soybean relay-cropped with winter camelina

Since alfalfa is an N_2-fixing legume and does not require a N fertilizer input, CI and GWP are reduced. The impact of N fertilizer on GWP is greater than that of P fertilizer, and suggestions to farmers include avoiding excess N application to reduce CI (Taki et al., 2018). Previous research indicates that N_2O fluxes from soil increase immediately after N fertilizer additions (Berti & Cecchin, 2023). Crop rotations that include alfalfa will see positive environmental benefits and lower GWP. The first production year of alfalfa also saw the lowest emissions per NEL value. This refers to a lower CO_2 emission per energy unit beneficially consumed by livestock. When referring to alfalfa's CI, this is the CO_2 released to obtain the energy in the forage. Silage corn is a prominent forage crop in the dairy and livestock industry; however, it has lower net energy (MJ ha^{-1}) and higher non-renewable energy (MJ ha^{-1}) and GWP than alfalfa (Fathollahi et al., 2018). This is partly due to the need for nitrogen fertilizer input. Fathollahi et al. (2018) reported that the climate change impact category of alfalfa was 282 kg CO_{2e} Mg^{-1}, where silage corn was 329 kg CO_{2e} Mg^{-1}. Bacenetti et al. (2018) found the climate change impact category of alfalfa to be 80 kg CO_{2e} Mg^{-1}. A recent study out of Iran quantified a no-till silage corn as having an impact of 120 kg CO_{2e} Mg^{-1} (Mirzaei et al., 2022). Values obtained in other studies are agreeable with this study. Trends in alfalfa climate impact are similar throughout alfalfa-producing regions of the world. Slight differences occur in terms of management strategies and field activity. Alfalfa, in terms of environmental impact, is a superior forage crop.

Sunflower and soybean have relatively low CI. According to the European Union Renewable Energy Directive (2023), it is desirable for soybean to have a CI less than 18 g CO_{2e} MJ^{-1}, which was achieved in this study (Table 3.35). When comparing the cradle to farm gate portion of the LCA of winter camelina and soybean for biofuel production, soybean is superior in terms of a lower GWP, even in the lower-yielding relay system. In an LCA of camelina and soybean biodiesel production, soybean biodiesel reduced emissions 63-85% where camelina reduced emissions by 37-73% compared with petroleum diesel (Krohn and Fripp, 2012). This study and the results of this experiment indicate that soybean is more environmentally favorable option for biodiesel production than winter camelina. When considering hydrotreated vegetable oil production (HVO), winter camelina’s GWP was found to be 27 g CO_{2e} MJ^{-1} with a yield of 1,560 kg ha^{-1} (Karlsson Potter et al., 2023). This value was calculated excluding the impacts of soil organic carbon (SOC). If SOC effects were included, the GWP was reduced to 15 g CO_{2e} MJ^{-1} (Karlsson Potter et al., 2023). Including soil carbon increases from crop residues, if seen, can reduce CI. Soybean has a lower seed oil content; however, the yield of soybean is consistently higher than winter camelina. Soybean CI remains low, but increases in the relay system where yields lacked. Soybean is a low-input crop in terms of fertilization. Berti et al. (2017) saw similar trends with soybean in their study, which had higher yields. The yield of sunflower lowered the CI in relation to the high amount of N fertilizer applied. Mineral fertilizers had the greatest impact to sunflower GWP also in a study by Iriarte et al. (2010) who noted that raw material extraction and production of fertilizers were major contributors. Iriarte et al. (2010) noted an even larger impact of N fertilizer on sunflower's N_2O emissions than that calculated for this study.

Wheat's CI was relatively high in comparison to sunflower, alfalfa, and soybean due to its low yield and high inputs. Wheat was harvested in mid- to late-August in 2022 and 2023 so fallow period in September and October was considered in the calculation for the W-FA-SFE-WC sequence (Table 3.36). A majority of the inventory items, such as N fertilizer, P fertilizer, and herbicide application contributed to a higher CI and GWP. Wheat's reliance on fertilizer and herbicide inputs relative to achievable yields made these values increase. This was also noted in a study by Li et al. (2021), where fertilizers contributed to over 30% of the GWP of wheat. A study conducted on dryland cropping systems of the northern Great Plains indicated that the average GWP of winter wheat grain was 0.27 kg CO_{2eq} kg seed^{-1}, but this value was calculated from yields of 2,550 to 2,820 kg ha^{-1} (Shrestha et al., 2020). A continuous winter wheat-fallow rotation was compared with several other diverse rotations suitable for the northern Great Plains. The study stated that increased rotational diversity increased net crop productivity by two to four times (Shrestha et al., 2020). This highlights the importance of smart-crop rotations in terms of GWP. While the goal is to reduce CI and GWP, including a high impact crop can be less impactful in rotation with other low impact crops given the long-term analysis of a crop rotation. Carbon intensities and GWP can be lower in rainfed regions like the northern Great Plains because no irrigation is used, given yields are equivalent to those in irrigated regions. Many studies include the impacts of irrigation to environmental values, which have the potential to greatly increase such numbers.

Figure 3.6 shows how each crop is impacted differently by inputs, such as fertilizer, and field activities, such as harvest. Alfalfa has a noticeably high GWP per hectare, particularly in Year 2, or the production year, due to the increase in field activities with multiple harvests (Table 3.36).

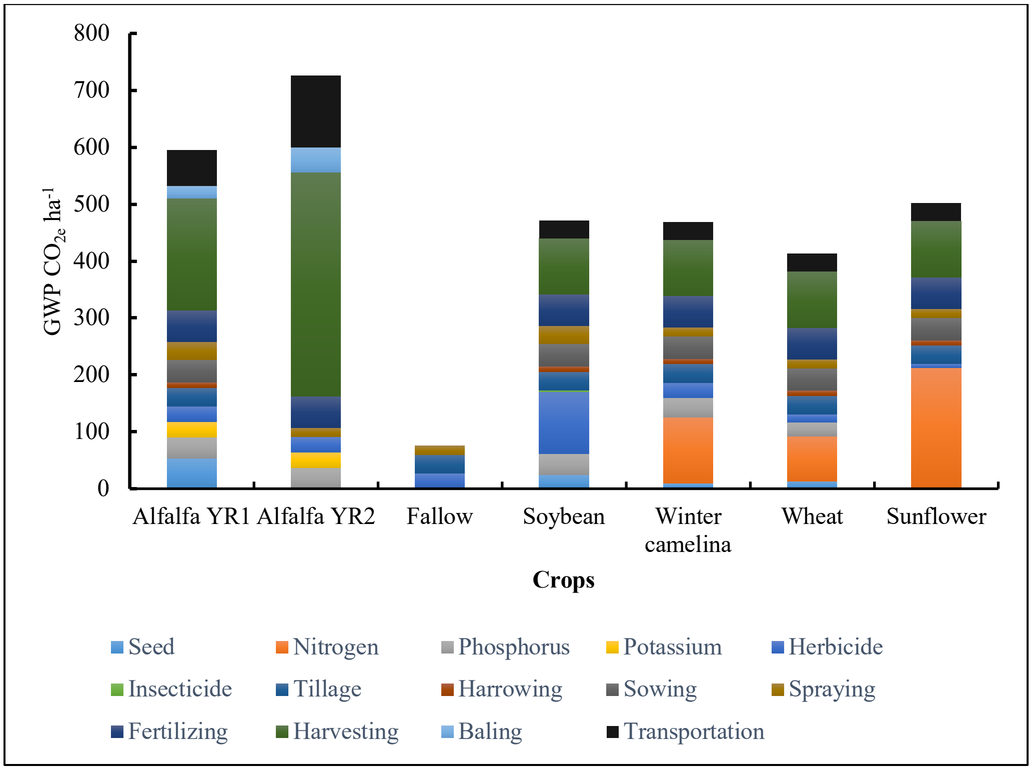

Figure 3.6. Total global warming potential (GWP) per hectare of each crop studied divided into specific input categories to show impact differences between crops. Values are averaged across both locations and years. Inputs are based on actual field practices from experiments in Hickson and Prosper, ND in 2022 and 2023. Values only include fertilizer production and not field N_2O emissions from fertilizer.

Field activities require considerable fuel, and therefore, energy. Bacenetti et al. (2018) noted that field operations contributed the most to alfalfa's GWP, with turning of hay being the most consumptive. Turning was not considered in this study. Total harvesting process of alfalfa contributed 83% to the GWP of alfalfa systems (Bacenetti et al., 2018). Despite this high GWP per hectare, alfalfa's consistently high forage yield offsets this number to have the overall lowest CI per MJ energy produced. The NEL of each sequence including alfalfa was also significant.

Figure 3.7 shows the distribution of N_2O emission sources for each crop. Nitrous oxide emissions from soil, especially for fallow ground and annual crops is notable. From inputs alone, fallow ground has the lowest GWP; however, fallow ground has similar N_2O emissions to annual crops, so it would be advisable to maintain vegetation on fallow ground. Figure 3.8 shows the combined total GWP of each crop.

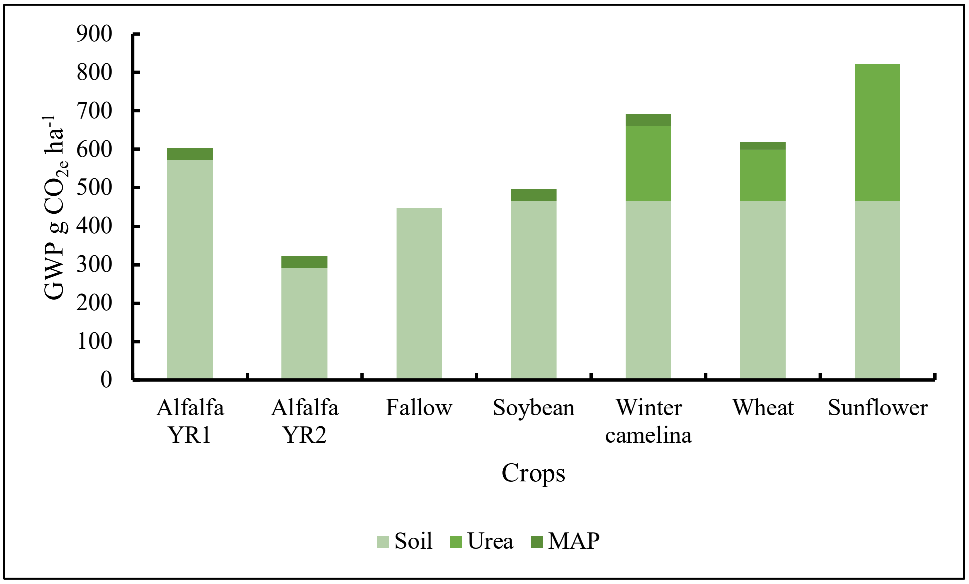

Figure 3.7. Global warming potential (GWP) of emissions produced by fertilizer application in g CO_{2e} ha^{-1} for each crop studied averaged across experiments in Hickson and Prosper, ND in 2022 and 2023. Nitrous oxide emission sources include residual soil emissions (Soil) (Berti & Cecchin, 2023), emissions from urea nitrogen (Urea) fertilizer, and emissions from fraction of N in monoammonium phosphate (MAP) fertilizer.

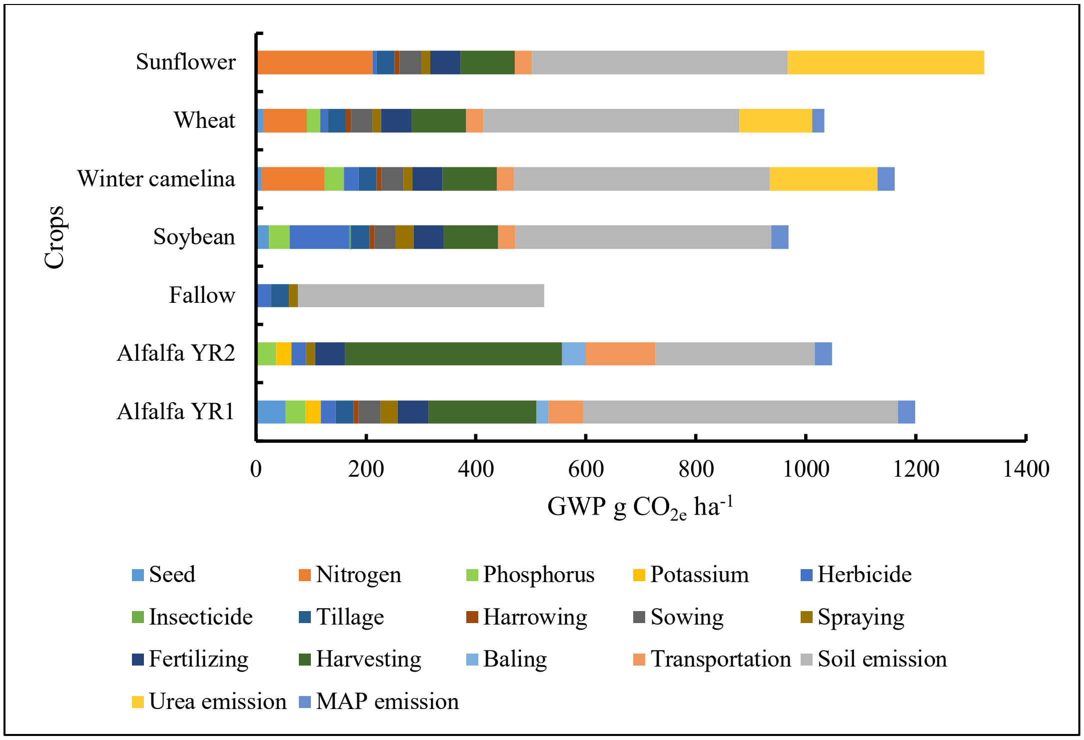

Figure 3.8. Total global warming potential (GWP) per hectare of each crop studied divided into specific input categories to show impact differences between crops. Values are averaged across both locations and years. Inputs are based on actual field practices from experiments in Hickson and Prosper, ND in 2022 and 2023. Values include every emission contributing to GWP considered in the life cycle inventory (LCI). Soil, urea, and monoammonium phosphate (MAP) emissions are from N_2O emissions multiplied by 298 to convert them to CO_{2e}.

The LCA of this experiment gave insight into the CI and GWP of individual crops as well as how they perform together in rotation. Cropping sequence was significant ($P \leq 0.05$) for CI, and specifically, sunflower preceding winter camelina-soybean relay and winter camelina monocrop were significant (Tables 3.34, 3.36). The winter camelina-fallow sequence had a great portion of the two-year rotation as fallow ground. It is important to note that the fallow ground in this experiment consisted of chemical and mechanical fallow. Glyphosate was sprayed and the ground was tilled before planting winter camelina in order to keep the ground clear of weeds. Fallow ground also releases N_2O as an intermediate of denitrification and as microbes break down organic matter (Wagner-Riddle et al., 1997). With no seed or grain return on that land while it is fallow, the CI remains very high. Fallow ground was considered for periods from May to October and allocated to rotations based on when they contained fallow land.

Continuous alfalfa had the greatest environmental impact in terms of NEL; however, this is considering two years of alfalfa harvest for a total of five to six cuts (Table 3.36). The other sequences are only for one year, which included to three to four harvests. In crops requiring N fertilization, N contributes significantly to the GWP per hectare, especially in a crop like sunflower. Nitrogen fertilizer production requires significant amounts of energy, about 64 MJ kg^{-1} fertilizer (urea), which can contribute to over 50% of certain crops' energy demand and also increases field N_2O emissions (Moeller et al., 2017).

Yield plays a large role in the CI and GWP. Crops that are low-yielding have notably higher CIs and GWPs (Table 3.36). In this experiment, winter camelina low seed yield was the greatest contributing factor to an increase in CI in crop sequences that included winter camelina (Table 3.36). From preparing land, N fertilizer, to extremely low yields, the environmental impact of this crop is great in comparison to the other crops studied. In a study by Berti et al. (2017), values for GWP of winter camelina seed ranged from 0.53-0.84 kg CO_{2e} kg $seed^{-1}$. This value is significantly lower than obtained from our experiment likely because yields in the study by Berti et al. (2017) were much higher (~1,000 kg ha^{-1}), and also, N_2O emissions from the field were not considered. Many studies of the LCA of camelina use yield values that are not likely achievable in northern growing regions of the U.S., which leads to false positive results. Krohn and Fripp (2012) determined that winter camelina yield needs to be greater than 800 kg ha^{-1} for biodiesel production to be an environmentally viable option, but only saw small improvements when theoretical yield was increased to 3,000 kg ha^{-1} in their model. Dangol et al. (2020) used an achievable yield of 1,570 kg ha^{-1} in their study of camelina-based biofuel production. They also determined that the total MJ ha^{-1} input of a camelina monocrop was 5,677 MJ ha^{-1}, which was lower than this experiment's calculation which was 6,749 MJ ha^{-1}, excluding the fallow ground period (Dangol et al., 2020). Karlsson Potter et al. (2023) concluded that adding winter camelina in a cereal-based crop rotation can overall increase yield, but climate impact is very similar to rotations without winter camelina. Including winter camelina increased emissions, but the increase in SOC from the crop rotation compensated for the input (Karlsson Potter et al., 2023). Differences in values can be accounted for in field practices, products and machinery used, and variances in coefficients used. Comparisons with other research are relative due to the nature of variation based on soil type and region specificity. Life cycle assessment values are only as valid as the assumptions used in the analysis thus providing clear documentation of the assumptions is important.

Table 3.36. Cumulative sequential assessment of average total carbon intensity (CI) and energy input of ten crop sequence (CS) (W-wheat, WC-winter camelina, SFL-late-planted sunflower, SFE-early-planted sunflower, FA-fallow, Al-alfalfa, Soy-soybean) and net energy for lactation (NEL) for alfalfa, averaged across two locations, Hickson and Prosper, ND in 2022 and 2023.

CS	CI	Energy Input	NEL
	g CO_{2e} MJ^{-1}	MJ ha^{-1}	g CO_{2e} MJ^{-1} lactation
W-WC-SFL	418	13480	--
W-FA-SFE-WC	81	14733	--
W-Al	98	14007	64
SFE-Al	24	15278	72
SFE-WC-Al	736	14751	--
SFE-WC-Soy	1155	21984	--
SFL-FA-W	94	14733	--
Soy-FA-W-WC	93	13964	--
WC	1525	7925	--
Alfalfa	22	16101	80
LSD (0.05)	812	0	14

3.5. Conclusion

Integrating alfalfa or winter camelina into wheat-sunflower-soybean annual rotations may be a beneficial way to enhance cropping system agronomic resilience.

Although the soybean-winter camelina relay was not as productive in terms of oilseed yield as previous studies had achieved, the early-maturing sunflower and winter camelina double-crop yielded more than the early-maturing sunflower monocrop. However, early-maturing, late-planted sunflower following winter camelina was not able to be harvested due to lack of maturity. This is a major consideration for growers given the uniqueness of each year's weather patterns in the northern Great Plains. If winter camelina can be harvested by late June and the season remains feasible for sunflower maturity through early fall, then a late-planted, early-maturing sunflower crop may be valuable in terms of increasing LER and economic return. This study did not indicate any benefits in the life cycle analysis of using winter camelina as a biofuel source. Future research should indicate more reliable ways to incorporate winter camelina into crop rotation to make the crop economically and environmentally viable.

Alfalfa demonstrated its ability to outperform the other crops studied in terms of yield consistency, economic benefit, and importance to nutrient cycling in crop rotations. Despite variable weather patterns, alfalfa continued to be high-yielding and maintained high forage nutritive value. Alfalfa's low CI was attributed to less inputs and yield return. Soil NO_3-N slowly increased throughout the two-year period without N fertilization, meaning that subsequent crops in the rotation could positively benefit from alfalfa's leguminous nature. Further research should be conducted on alfalfa's contribution to ecosystem services and the various benefits associated with perennial legume production demonstrated to growers to increase alfalfa production in the northern Great Plains.

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4. CROPPING SYSTEMS OF THE NORTHERN GREAT PLAINS INFLUENCE ARTHROPOD BIODIVERSITY

4.1. Abstract

The loss of arthropod biodiversity has been attributed to intensification of agricultural practice. This study was to determine if the addition of alfalfa (*Medicago sativa* L.) and/or winter camelina (*Camelina sativa* (L.) Crantz) to annual crop rotations would increase cropping system arthropod biodiversity associated with crops including sunflower (*Helianthus annus* L.), wheat (*Triticum aestivum* L.), or soybean [*Glycine max* (L.) Merr.]. Evaluation of biodiversity was conducted through weekly sampling with sticky and pitfall traps in selected treatments at two locations in North Dakota in 2022 and 2023. Diversity and abundance of arthropods were tracked during both growing seasons, weekly. Ground and canopy arthropods differed in crops and crop sequences both years and at both locations. Alfalfa had the highest Shannon Diversity index. Winter camelina acted as a mass-flowering crop and introduced mostly pollinator species in the orders Diptera and Hymenoptera early in the growing season. Arthropod communities were influenced by crop morphology, weather, and management.

4.2. Introduction

Biodiversity is of renewed interest as modern, intensive agriculture is expected to meet challenging population growth demands in the near future. The world population is predicted to peak at 10.4 billion people by 2086, which means agriculture is required to meet that growing food, fuel, and fiber demand (United Nations, 2022). In order to meet growing population demands, intensification, or the process of increasing agricultural production on the same unit of land, will continue, possibly at higher rates than seen in the recent past. Intensification implies reduced crop diversity. Conversely, extensification is the practice of converting natural landscapes into arable land. Land conversion and reduced crop diversity have exceedingly negative impacts on arthropod biodiversity with the threat of extinction of over 40% of the world's insect species (Sanchez-Bayo & Wyckhuys, 2019). Reduced crop diversity has been noted in the northern Great Plains with the conversion of grasslands to cropland. In 2007, ten million acres of land in the northern Great Plains was designated as Conservation Reserve Program (CRP) land; however, over 60% of this land was returned to crop production with the expiration of contracts, therefore losing the ecosystem services and biodiversity provided by grasslands (Morefield et al., 2016).

Modern agriculture relies on insecticides to control insect pests, which are necessary in certain scenarios; however, insecticides can disrupt the natural predator-prey relationship in agricultural ecosystems. Thus, more effort has been placed on practicing integrated pest management (IPM), which combines cultural practices with minimal insecticide use. Insecticides have repercussions to mammals and aquatic life as well; however, insect declines are substantially worse than those monitored for birds or plants (Sanchez-Bayo & Wyckhuys, 2019). Insecticide use is conflicting with the desire for high arthropod diversity, and fertilizers can alter a crop canopy, by increasing growth, in such a way that it is unsuitable for certain arthropod communities (Gonzalez del Portillo et al., 2022). Insecticides used to control crop pests often kill beneficial insects. Each insecticide has varying impacts on the naturally-occurring insect population. Insect populations were studied after an insecticide application in alfalfa to control alfalfa weevil (*Hypera postica*) and found that the indoxacarb insecticide was the only tested product that did not reduce beneficial Coccinellidae species, but all insecticides used reduced the population of beneficial Hymenoptera parasitoids (Michaud & Bain, 2016).

Efforts to remediate lost biodiversity include addition of hedgerow strips surrounding agricultural fields. In Europe, hedgerows have been noted as one of the most important non-crop habitats for arthropods that are typically found surrounding farmland. Hedgerows located on field edges have high alpha-biodiversity, or high species richness within the sample area, and serve as ecological corridors (Precigout & Robert, 2022). Such areas can increase natural predator and pollinator populations in nearby agricultural fields. Including trees in hedgerows can increase soil litter, which encourages populations of predatory arthropods of the Staphylinidae and Lycosidae families (Precigout & Robert, 2022).

Crop diversification can introduce different plant and arthropod species to agricultural systems by attracting different arthropods and altering field practice. Diversifying crops can also increase abundance of pollinator species. Annual crops such as corn (*Zea mays* L.), soybean, and wheat are undesirable for pollinator species (Forcella et al., 2020). A survey of the species of Coleoptera residing in alfalfa fields was conducted and determined that alfalfa fields have high diversity with over eight arthropod families regularly associated with alfalfa fields (Augul & Al-Saffar, 2016). Alfalfa was also found to be a favored environment for bee groups when flowering according to Rollin et al. (2013); however, frequent cutting reduces the presence of flowers and therefore bee populations. Management of alfalfa stands can impact arthropod communities. Honeybees (*Apis mellifera* L.) prefer mass-flowering crops such as sunflower or canola (*Brassica napus* L.), where wild bees prefer a crop like alfalfa and other natural habitats (Rollin et al., 2013). Alfalfa tends to support many types of beneficial insects. Species of *Orius*, ladybugs, carabids, and spiders, which are all effective pest control species, have been found in high abundance in alfalfa fields (Gonzalez del Portillo et al., 2022). Ladybugs and carabids are efficient at controlling aphid populations in alfalfa. Mass-flowering crops such as sunflower rely on insect pollination for yield in addition to self-pollination. Honeybees were found to have a large impact on yield. Fields with high honeybee abundance saw a 41% yield increase from fields with low abundance (Perrot et al., 2019). Including a crop such as sunflower in rotation can attract beneficial pollinator species to agricultural land.

This study aimed to assess the arthropod biodiversity of traditional annual cropping systems of the northern Great Plains by incorporating winter camelina and/or alfalfa into wheat-sunflower-soybean sequences.

4.3. Materials and methods

4.3.1. Field collection and identification

To compare arthropod biodiversity between crops and crop rotations, two measurements of arthropod diversity and abundance were taken at experiment sites in Hickson and Prosper, ND in 2022 and 2023. The Hickson, ND site was surrounded by other experiment plots and a residential home. The Prosper, ND site was surrounded by plots and was located at an experiment station. These measurements started in mid-August during Year 1. Sampling in Year 2 started in mid-June. Experiment sites contained ten treatments and four replicates of which five plots per replicate were chosen to represent the various crops and sequences. The following crop sequences were sampled: i) wheat, winter camelina, late-planted early-maturing sunflower; ii) early-planted early-maturing sunflower, winter camelina, alfalfa; iii) soybean, fallow, wheat; iv) fallow, winter camelina, fallow; v) alfalfa. Planting and harvest dates are indicated in Table 4.1.

Table 4.1. Crop sequence planting and harvest dates at Hickson and Prosper, ND in 2022 and 2023. 2023 alfalfa monocrop harvest dates are indicated on a separate line.

Crop sequence[a]	Planting date 1	Harvest date 1	Planting date 2	Harvest date 2	Planting date 3
	Hickson				
W-WC-SFL	26 May 2022	25 Aug 2022	28 Sep 2022	18 July 2023	24 July 2023
SFE-WC-Al	26 May 2022	28 Aug 2022	28 Sep 2022	18 July 2023	10 Aug 2023
Soy-FA-W	26 May 2022	3 Oct 2022	23 May 2023	22 Aug 2023	--
WC	28 Sep 2022	18 July 2023	--	--	--
Alfalfa	26 May 2022	21 July 2022	--	31 Aug 2022	--
	Harvest date 3	Harvest date 4	Harvest date 5	Harvest date 6	
Alfalfa	14 June 2023	11 July 2023	9 Aug 2023	12 Oct 2023	
	Prosper				
W-WC-SFL	26 May 2022	25 Aug 2022	26 Sep 2022	11 July 2023	24 July 2023
SFE-WC-Al	26 May 2022	30 Aug 2022	26 Sep 2022	11 July 2023	10 Aug 2023
Soy-FA-W	26 May 2022	3 Oct 2022	17 May 2023	21 May 2023	--
WC	26 Sep 2022	11 July 2023	--	--	--
Alfalfa	26 May 2022	25 July 2022	--	30 Aug 2022	--
	Harvest date 3	Harvest date 4	Harvest date 5	Harvest date 6	
Alfalfa	13 June 2023	10 July 2023	16 Aug 2023	16 Oct 2023	

[a] W = wheat; WC = winter camelina; SFL = late-planted, early-maturing sunflower; SFE = early-planted, early-maturing sunflower; Al = alfalfa; Soy = soybean; FA = fallow

In 2023, after winter camelina was harvested in Hickson, sticky and pitfall traps were moved from one plot in each replicate to the winter camelina-soybean relay system to sample remaining soybean. This was for comparison with soybean monocrop from 2022, which was sprayed with insecticide. In June 2022, soybean monocrop was sprayed with lambda-cyhalothrin ([1a(S*),3a(z)]-cyano(3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate), (specifically, Warrior II with Zeon Technology) (Syngenta, Basel, Switzerland) at a rate of 0.11 L ha^{-1} to control bean leaf beetle (*Cerotoma trifurcata*). No other insecticides were applied in 2022 or 2023.

Pitfall traps were installed at ground-level in selected plots using 0.5 L plastic cups and 60 mL of propylene glycol ($CH_3CH(OH)CH_2OH$) in a secondary cup. The propylene glycol acted as an attractive and sticky substance to trap ground and low-flying insects. In Year 2, pitfall trap substance was switched to common anti-freeze, which was more cost effective. A metal cover fabricated by the NDSU mechanical shop was placed approximately 2.5-cm over each trap in an effort to keep small mammals, amphibians, and debris from entering the traps. Yellow sticky traps, 7.6 x 12.7 cm (Pherocon Predator, Trece), were also installed in the same selected plots higher in the plant canopy attached to a wooden dowel rod (Fig. 4.1).

Figure 4.1. Example of sticky trap (left) and pitfall trap with swivel cover off to the side for easy access (right).

Deploying both pitfall and sticky traps allowed for comparison of ground and canopy arthropods. Traps were replaced every week. Pitfall traps were drained through a mesh screen and mixed with alcohol to ensure insects were killed. Insects collected from pitfall traps were counted in the field. Sticky traps were frozen to preserve insects for later analysis under a microscope. Insects were identified using insect guides at the family level and counted (Insect Identification, 2024; BugGuide, 2024; Triplehorn & Johnson, 2004). Additional identification assistance was provided by the NDSU Entomology Department. Species in the order Diptera were difficult to identify to family level, so a majority of Diptera species were classified as

Diptera only (i.e. to order). Field sampling and identification procedures were developed by Dr. William Lamp entomology laboratory at University of Maryland.

4.3.2. Statistical analysis

Arthropods from each sampling were summed for total number and families with the highest frequency were analyzed. To meet assumptions of normality, arthropod abundance was log-transformed before analysis. The Shannon Diversity index (H-index) was calculated per crop species to measure diversity of arthropod species within the crop using the following equation:

$$H = -\Sigma p_{\mathrm{i}} \text{ x } ln \ (p_{\mathrm{i}}) \qquad (1)$$

$$p_{\mathrm{i}} = \text{ the proportion of the entire community made up of species i}$$

A negative binomial generalized mixed linear model was performed using SAS 9.4 (SAS Institute., Cary, NC, 2023) to detect significant effects ($P \leq 0.05$) among crops and sampling dates for total insects, total families, specific families, and Shannon Diversity index. Locations and replicates were considered random effects and treatments (crop and crop sequence) and sampling dates as fixed effects. Sampling dates were analyzed as repeated measures. Least square means (LS means) were estimated. For LS means, least significant difference (LSD) values at the 95% level of confidence were calculated by multiplying the standard error for the pair of differences (p-diff) by the t-table value for the degrees of freedom (df) of the corresponding error that was used to calculate the F-value for individual sources of variation.

4.4. Results and discussion

4.4.1. Pitfall traps year 1

Crop and crop sequence were significant ($P \leq 0.05$) for arthropod abundance, family diversity, Shannon Diversity index, and the families Gryllidae, Carabidae, and Pholcidae at both locations sampled via pitfall traps (Tables 4.2, 4.3). Pitfall traps also included the occasional collection of field mice (*Apodemus sylvaticus*) and toads (*Bufo bufo*), despite having covers on the traps. Mice favored alfalfa plots as they provided the most consistent groundcover. Treatment by date was significant ($P \leq 0.05$) for arthropod abundance in Hickson only (Table 4.2). Plots containing wheat generally had the highest arthropod abundance.

Table 4.2. Analysis of variance and mean square values for total arthropods, total number of families, and Shannon Diversity index (H-index) for pitfall traps of five treatments (Trt) and seven sample dates (Date) in Hickson and Prosper, ND in 2022.

		Hickson			Prosper		
SOV	df	Arthropods	Families	H-index	Arthropods	Families	H-index
Rep	3	1343*	0.6*	0.1	112	2	0.06
Trt	4	2411*	4*	0.2*	4072*	4*	0.4*
Date	6	3422*	13*	0.8*	681	17*	1.0*
Trt x Date	24	265*	0.8	0.05	311	1	0.06
Residual	101	274	0.8	0.05	392[a]	1	0.07
CV, %		48	20	20	41	21	22

* Significant at $P \leq 0.05$, level of probability

[a] Residual MS df Prosper = 102

Table 4.3. Analysis of variance and mean square values for select ground-dwelling arthropod families collected in pitfall traps of five treatments (Trt) and seven sampling dates (Date) in Hickson and Prosper, ND in 2022.

SOV	df[a]	Gryllidae	Carabidae	Pholcidae	Lycosidae
			Hickson		
Rep	3	140	444*	24	0.9
Trt	4	393*	476*	87*	2.0
Date	6	2559*	544*	80*	0.4
Trt x date	17-24	138	161*	23*	0.5
Residual	21-92	132	119	12	2.3
CV, %		98	109	45	68
			Prosper		
Rep	3	273	20	17	9
Trt	4	3015*	350*	266*	8
Date	6	850*	149	419*	2
Trt x date	14-24	119	39	47	9
Residual	29-92	248	69	36	10
CV, %		81	66	54	101

[a] df for Trt x date and residual varied by family if specimens from each family were present or not in each trap at each date, treatment, and replicate

In 2022, pitfall traps captured large numbers of field crickets (*Gryllidae*) which could be attributed to wheat providing a prime habitat (Fig. 4.2). Crickets favor low-growing plants that have a lower degree of shading (Gawalek et al., 2014). Notably, field cricket populations in wheat peaked early to mid-August, which was approximately one to two weeks before crop harvest (Fig. 4.3). At this point, wheat was senescing and more light was able to reach through the wheat canopy to create a favorable environment for the crickets. Gawalek et al. (2014) noted that vegetation for crickets must be sparse enough to allow communication with other crickets, but not be open enough for predators, like birds, to easily find them. Alfalfa did not contain as many field crickets as wheat, likely due to its thick foliage. Soybean monocrop arthropod diversity and abundance was low in both locations, especially in Hickson. This can be attributed to an application of lambda-cyhalothrin insecticide to control bean leaf beetle (*Cerotoma* *trifurcata*). Earlier season application of lambda-cyhalothrin insecticide in May to alfalfa reduced negative impacts on lady beetle and spider populations (McClure et al., 2023). However, in this study, species in the Pholcidae family (Aranae) were unaffected. Lambda-cyhalothrin insecticide targets a family member of the class Arachnida, spider mites (*Tetranychidae* spp.) (Syngenta, 2024), but there is no report of effect on beneficial arachnids. Pholcidae, being a mostly ground-dwelling species, may be less affected by this insecticide. With the exception of Pholcidae, the application of insecticide in mid-June to soybean produced season-long detrimental effects to the arthropod community.

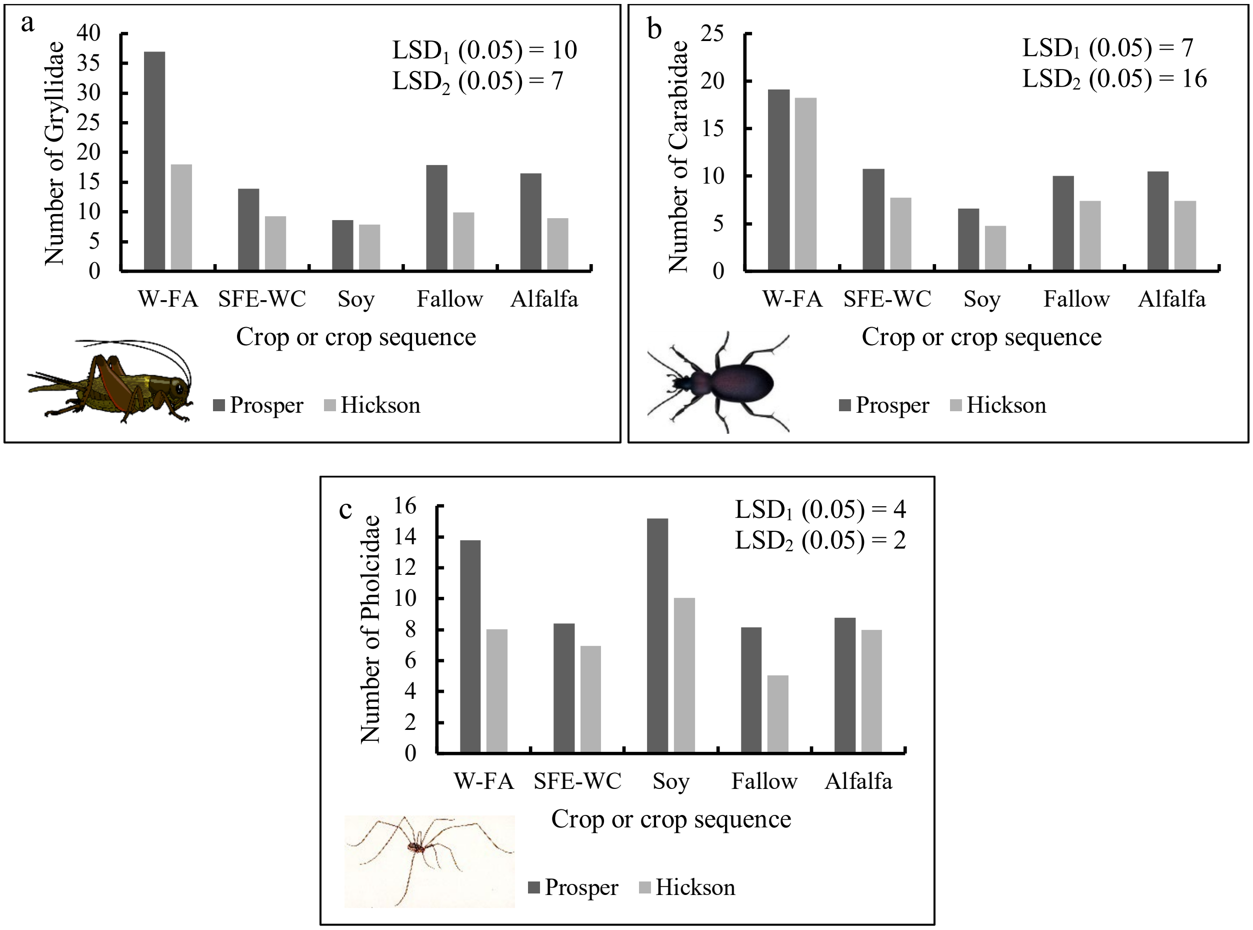

Figure 4.2. Seasonal average arthropod abundance by family: a) Gryllidae, b) Carabidae, c) Pholcidae, and location collected in pitfall traps at Hickson and Prosper, ND in 2022. LSD$_1$ = to compare treatments at Prosper; LSD$_2$ = to compare treatments at Hickson. W = wheat; FA = fallow; SFE = early-planted, early-maturing sunflower; WC = winter camelina.

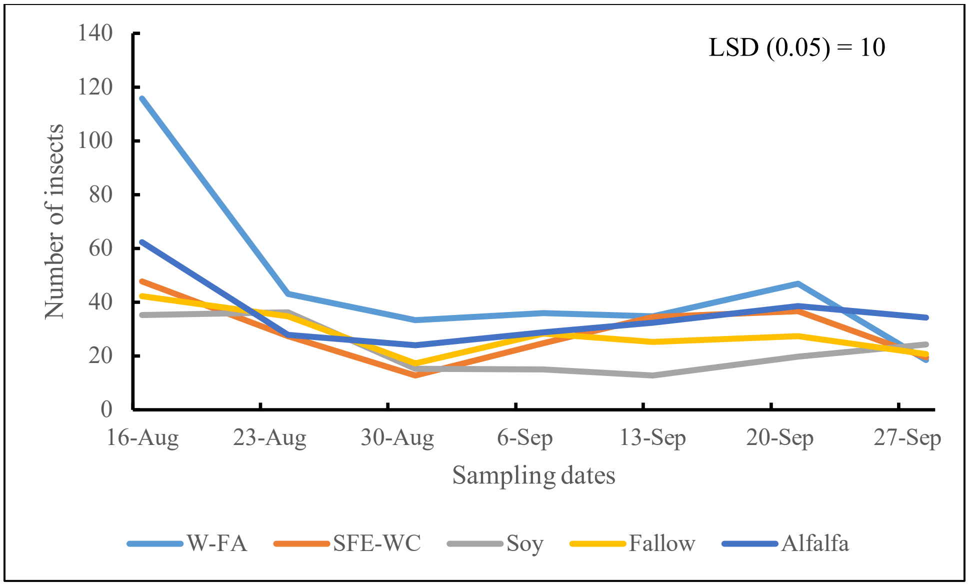

Figure 4.3. Arthropod abundance from pitfall traps collected at Hickson, ND during seven sample dates in 2022. W= wheat; FA = fallow; SFE= early-planted, early maturing sunflower; WC = winter camelina

Alfalfa and early-planted sunflower preceding winter camelina had the greatest abundance of arthropod families (Fig. 4.4). Prosper had greater family diversity when compared with Hickson. Alfalfa is known to support diverse ground insects. Research conducted in California identified over 1,000 arthropod species in alfalfa fields (Putnam et al., 2001). In this study, alfalfa plots contained the second-most species of Carabidae, or ground beetles, with wheat plots containing the most. Presence of carabids often is synonymous with ecosystem biodiversity. Despite soybean's lack of diversity, plots still contained large numbers of spiders in the Pholcidae family. Although there is no specific research done on this family in the presence of insecticides, McClure et al. (2023) found decreases in other families of Araneae populations after application of lambda-cyhalothrin insecticide. The evidence from this study suggests that Pholcidae populations might not be as affected by insecticide use.

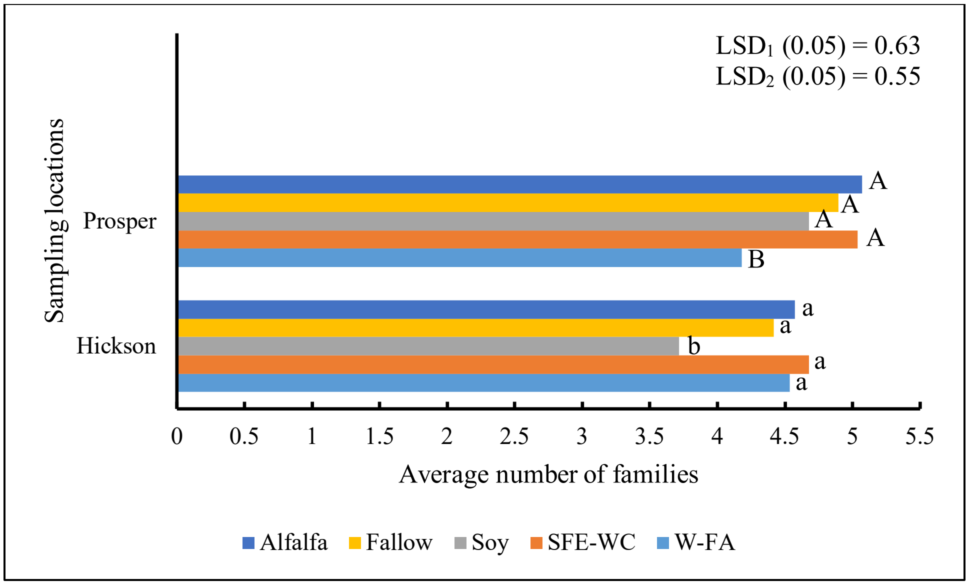

Figure 4.4. Seasonal average number of arthropod families collected in pitfall traps at Hickson and Prosper, ND in 2022. SFE = early-planted, early-maturing sunflower; WC = winter camelina; W = wheat; FA = fallow. LSD_1 = to compare crops and crop sequences in Prosper; LSD_2 = to compare crops and crop sequences in Hickson. Different letters within a same location indicate means are significantly different ($P \leq 0.05$).

Shannon Diversity index (H-index) values were similar among locations and significant by treatment ($P \leq 0.05$) (Table 4.1). Early-planted sunflower preceding winter camelina in Prosper had an H-index of 1.32 but was not different from the other crops except wheat-fallow (Fig. 4.5). Alfalfa and fallow ground had similar diversity. Fallow ground biodiversity is attributed to the presence of weed species. The ground was not considered bare soil until September 2022 when it was tilled and sprayed with glyphosate (N-phosphono methyl glycine) (specifically, Roundup Power Max) (Bayer Crop Science, Leverkusen, Germany) before winter camelina was planted. Although weeds are not desirable for crop production, weed patches have been shown to increase biodiversity by providing environment for carabid species (Kulkarni et al., 2017). Carabid species are highly important in agricultural systems. Not only are they predacious to certain insect pests, but they also consume weed seeds to reduce weed seedbank population (Kulkarni et al., 2017).

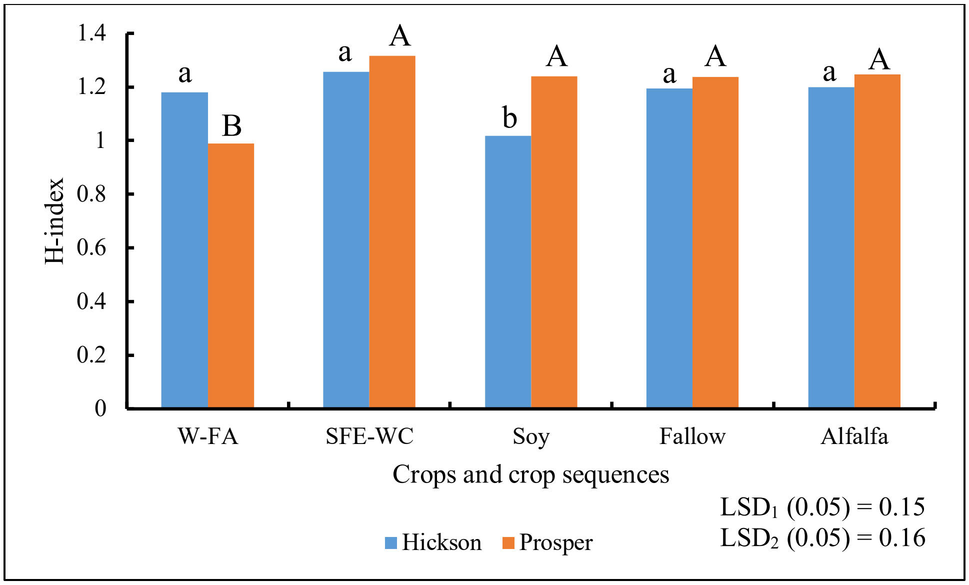

Figure 4.5. Seasonal average Shannon Diversity index (H-index) for arthropods collected in pitfall traps at Hickson and Prosper, ND in 2022. W = wheat; FA = fallow; SFE = early-planted, early-maturing sunflower; WC = winter camelina. LSD_1 = to compare crops and crop sequences in Hickson; LSD_2 = to compare crops and crop sequences in Prosper. Different letters within a same location indicate means are significantly different ($P \leq 0.05$).

4.4.2. Pitfall traps year 2

In 2023, treatment and treatment by date were significant ($P \leq 0.05$) for arthropod abundance, family diversity, and Shannon Diversity index at both locations (Table 4.4). In Hickson, the families Gryllidae, Carabidae, and Pholcidae were significant ($P \leq 0.05$) for sampling date, and Pholcidae for treatment by date (Table 4.5). In Prosper, the Gryllidae family was significant ($P \leq 0.05$) for treatment, date, and treatment by date, while Carabidae was only significant ($P \leq 0.05$) for treatment, and Pholcidae for date and treatment by date ($P \leq 0.05$) (Table 4.5). Variation among specific species was high, as noted by large coefficient of variation values (Table 4.5).

Table 4.4. Analysis of variance and mean square values for total arthropods, total number of families, and Shannon Diversity index (H-index) for pitfall traps of six or five treatments in Hickson and Prosper, ND in 2023.

	Hickson				Prosper			
SOV	df	Arthropods	Families	H-index	df	Arthropods	Families	H-index
Rep	3	33	2.0	0.1	3	211	6.0*	0.2*
Trt	5	431*	11.0*	0.5*	4	6645*	14.0*	0.5*
Date	8	1634*	18.0*	1.0*	8	2431*	11.0*	0.8*
Trt x Date	35	334*	4.0*	0.3*	32	598*	4.0*	0.4*
Residual	127	189	1.4	0.08	131	254	1.4	0.1
CV, %		56	30	27		53	31	31

* Significant at $P \leq 0.05$, level of probability

Table 4.5. Analysis of variance and mean square values for select ground-dwelling arthropod families collected in pitfall traps of five treatments in Hickson and Prosper, ND in 2023.

SOV	df[a]	Gryllidae	Carabidae	Pholcidae
		Hickson		
Rep	3	16	37	22
Trt	5	91	30	37
Date	8	545*	84*	148*
Trt x date	26-31	55	23	35*
Residual	58-104	92	39	21
CV, %		96	76	57
		Prosper		
Rep	3	119	96	85
Trt	4	1628*	355*	65
Date	8	636*	91	1507*
Trt x date	22-29	210*	82	96*
Residual	47-107	84	76	59
CV, %		68	104	71

[a] df for Date, Trt x date and residual varied by family if specimens from each family were present in each trap at each date, treatment, and replicate

Again, wheat contained the highest number of crickets, similar to 2022 (Fig. 4.6). Alfalfa contained the highest abundance of Carabidae species (Fig. 4.6). Abundance of arthropods was highest in winter camelina in Hickson at the beginning of sampling in June (Fig. 4.7). This is due to winter camelina being the earliest flowering and maturing crop compared with any other crop grown in the study. As visualized in Figure 4.7, arthropod abundance experienced many peaks and valleys. Several of the sampling dates, especially those in June and July, occurred right after rainfall events (NDAWN, 2023). This could explain why abundance dropped, as arthropods were seeking shelter under plant material and not traveling around areas of the pitfall traps. The Hickson location also had more disturbance in the area with nearby experiment plots and mowing of residential lawn. These undocumented events could also explain the peaks and valleys that occurred in Hickson only.

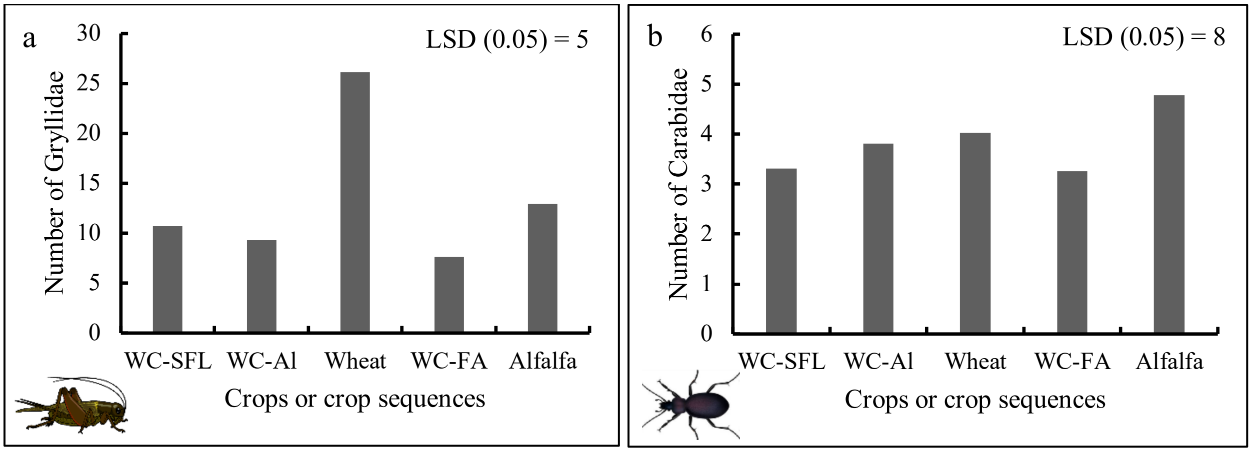

Figure 4.6. Seasonal average arthropod abundance by family; a) Gryllidae and b) Carabidae; by treatment collected in pitfall traps at Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow.

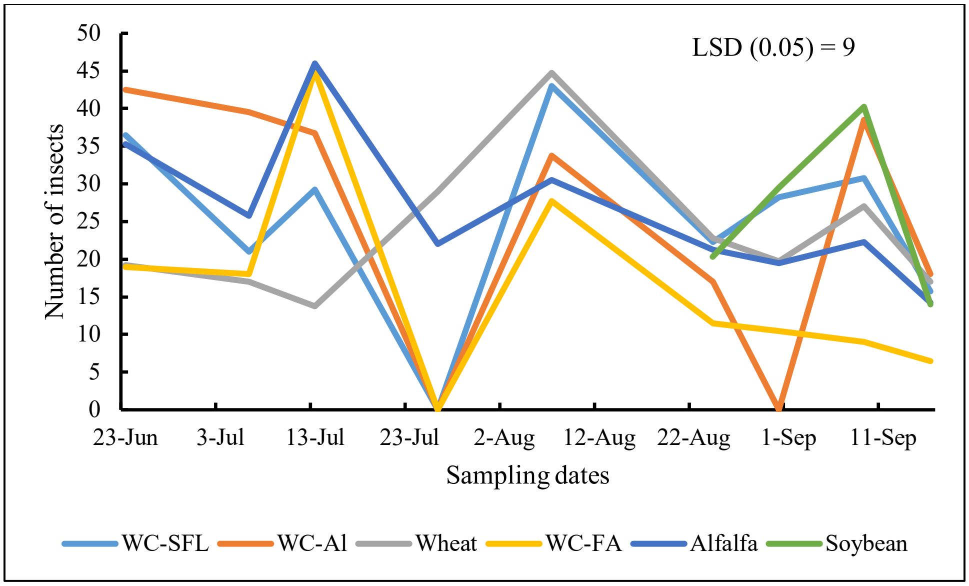

Figure 4.7. Arthropod abundance from pitfall traps collected at Hickson, ND during nine sample dates in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow. Number of arthropods being at zero indicates no sample was collected on that date.

Winter camelina was able to provide early cover for ground-dwelling arthropods in Hickson, but this was not observed in Prosper. Winter camelina had the lowest abundance at the start of the season (Fig. 4.8). This could be attributed to poor winter camelina emergence and stand. Wheat and alfalfa had the highest abundance at the beginning of the sampling period. As previously discussed, crickets and ground beetles prefer a moderate amount of crop cover, which the wheat and alfalfa are able to provide early in the growing season. Values of zero represent a missing sample due to a recent harvest or ground transitioning to fallow before the next crop in the sequence. Overall, abundance was lower, particularly in Hickson, compared with 2022 pitfall samples. This could be due to the change from propylene glycol to antifreeze. Antifreeze was of lower quality and tended to evaporate over the course of a week and not preserve arthropods as well. Solution can have a significant impact on arthropod attraction. Kwon et al. (2022) found that when 70% ethyl alcohol solution was used in pitfall traps, abundance was greatest compared with salt, ethylene glycol, and bleach solutions. Each solution attracted a different diversity of arthropods, which is likely the case in this experiment.

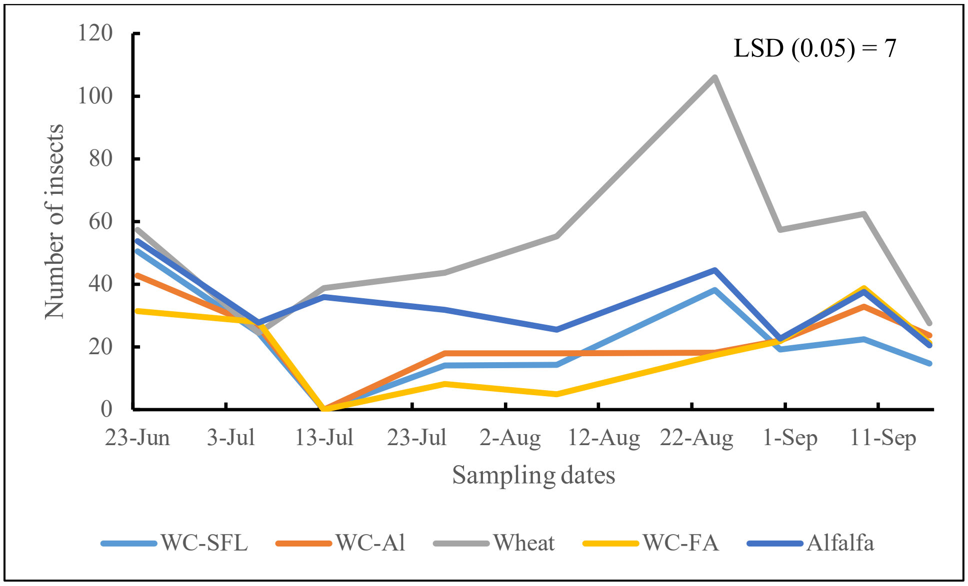

Figure 4.8. Arthropod abundance from pitfall traps collected at Prosper, ND during nine sample dates in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow. Number of arthropods being at zero indicates no sample was collected on that date.

Alfalfa had the greatest and most consistent family diversity throughout the sampling period (Fig. 4.9, 4.10). In Hickson, late-planted sunflower increased later-season family diversity by providing plant cover after wheat was harvested and before alfalfa was well-established (Fig. 4.9). Ellsbury et al. (1998) suggested that low-management, or low field activity, and low-input cropping systems consistently have higher arthropod family diversity.

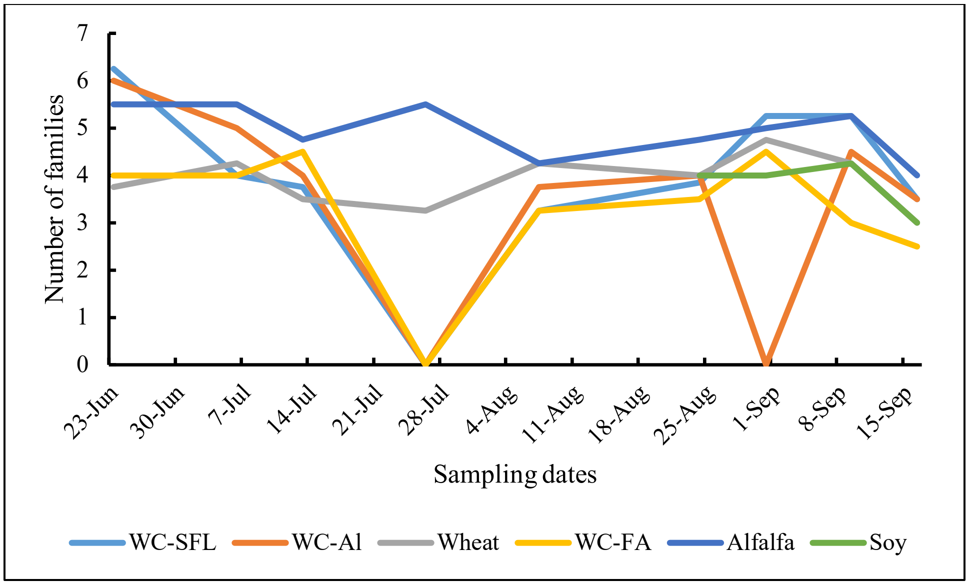

Figure 4.9. Abundance of arthropod families collected in pitfall traps by sampling date at Hickson, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow. Number of families being at zero indicates no sample was collected on that date.

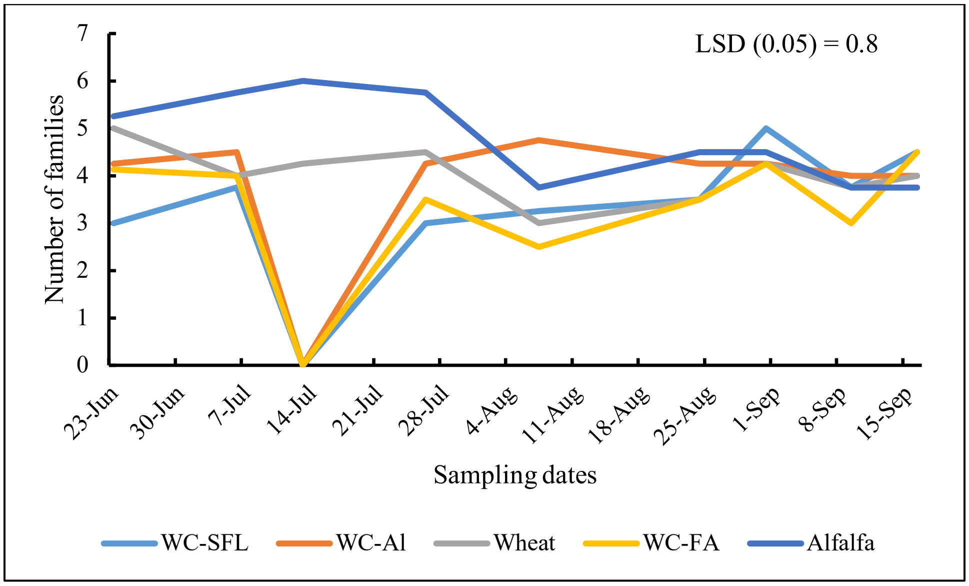

Figure 4.10. Abundance of arthropod families collected in pitfall traps by sampling date at Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow. Number of families being at zero indicates no sample was collected on that date.

The Shannon Diversity index was similar between Hickson and Prosper in 2023 (Fig. 4.11). Continuous alfalfa had the highest H-index both in Hickson and Prosper ($P \leq 0.05$), meaning alfalfa had the greatest arthropod diversity in 2023 (Fig. 4.11). Alfalfa was found to have the lowest H-index in terms of Carabidae species specifically in the study by Ellsbury et al. (1998). Soybean had a similar family diversity and H-index to other crops, indicating that effects from insecticide in 2022 did have an impact as results were not hindered in 2023. Shannon Diversity index differed by sampling date in each location ($P \leq 0.05$) (Fig. 4.12). This could be attributed to relative plant cover at the time or slight differences in temperature or weather.

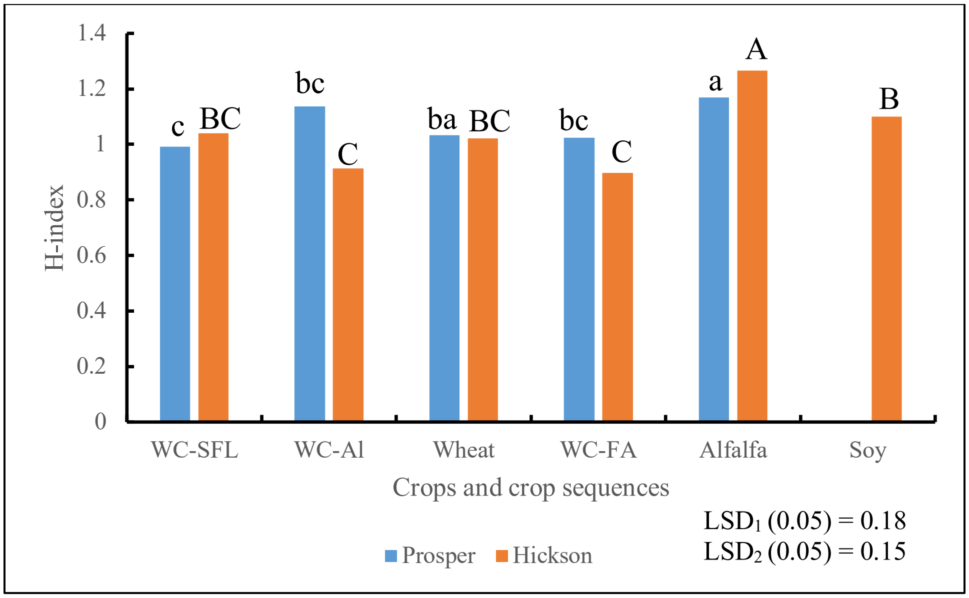

Figure 4.11. Seasonal Shannon Diversity index (H-index) for crops and crop sequences sampled in 2023. LSD_1 = to compare crops and crop sequences in Hickson; LSD_2 = to compare crops and crop sequences in Prosper. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow. Soybean data was only collected in Hickson. Different letters within a same location indicate means are significantly different ($P \leq 0.05$).

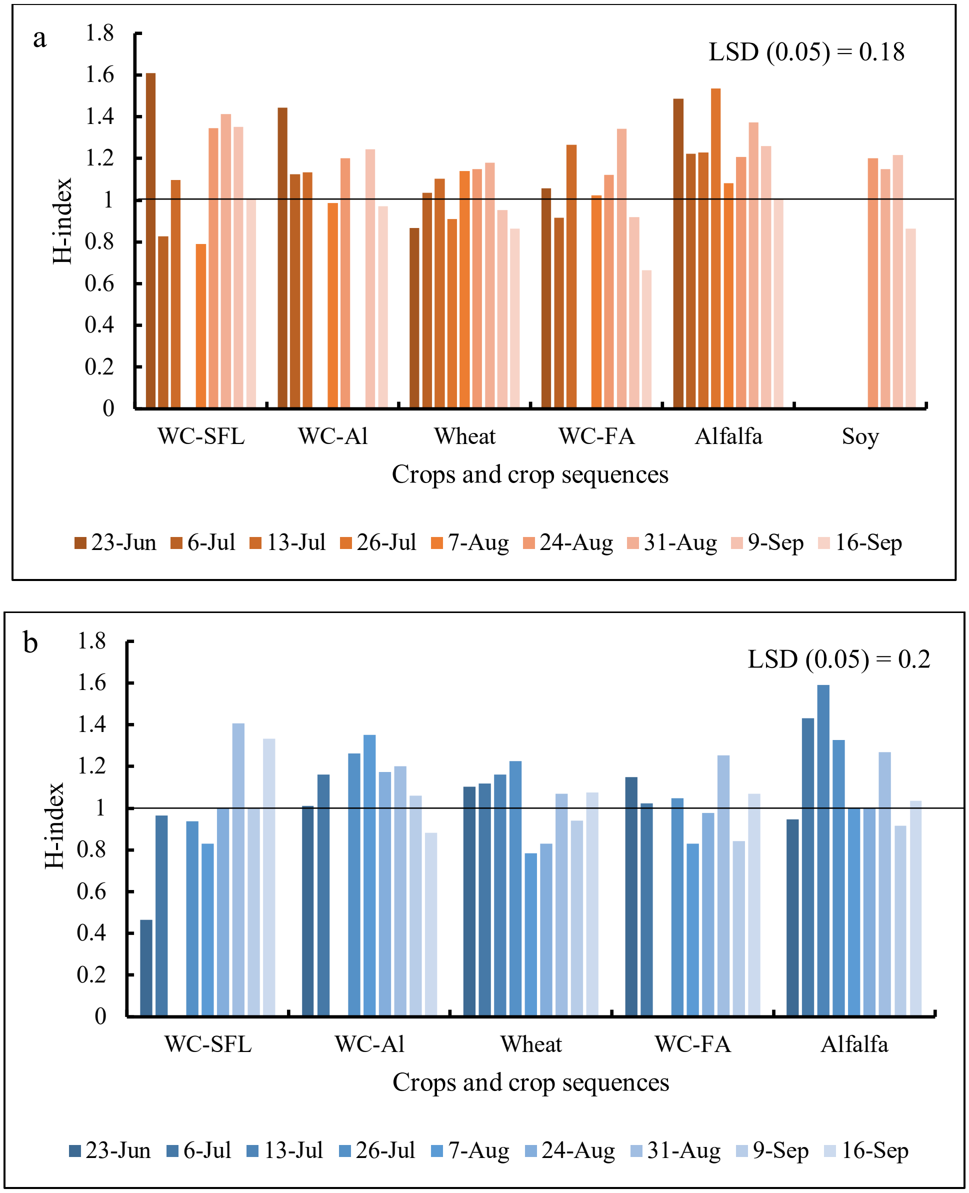

Figure 4.12. Shannon diversity index (H-index) by crop or crop sequence and sampling date for pitfall traps collected in a) Hickson, ND and b) Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow. Missing bar indicates no sample was collected on that date.

4.4.3. Sticky traps year 1

Treatment was significant ($P \leq 0.05$) for arthropod abundance, family diversity, and Shannon Diversity index at both locations (Table 4.6). Arthropod abundance and H-index were also significant ($P \leq 0.05$) for treatment by date in Hickson. Treatment by date was significant ($P \leq 0.05$) for insect abundance only in Prosper. In Hickson, all selected arthropod families with the exception of Chalcidae were significant ($P \leq 0.05$) for treatment and date, with the order Diptera and families Aphelinidae, Apidae and Thripidae being significant ($P \leq 0.05$) for the treatment by date interaction (Table 4.7). In Prosper, all selected arthropod families with the exceptions of Chalcidae and Apidae were significant for treatment, but all were significant ($P \leq 0.05$) for date. Only Diptera and Syrphidae were significant ($P \leq 0.05$) for treatment by date interaction (Table 4.7).

Table 4.6. Analysis of variance and mean square values for total insects, total number of families, and Shannon Diversity index (H-index) for sticky traps of five treatments in Hickson and Prosper, ND in 2022.

		Hickson			Prosper		
SOV	df	Insects	Families	H-index	Insects	Families	H-index[a]
Rep	3	2621	2	0.06	11289*	6	0.3
Trt	4	79150*	124*	1.4*	118689*	166*	2.6*
Date	7	28424*	18*	0.4*	43025*	26*	0.3
Trt x Date	28	6741*	6	0.1*	6551*	7	0.1
Residual	115	1752	3.6	0.04[a]	3122	6	0.1
CV, %		27	21	13	32	27	25

* Significant at $P \leq 0.05$, level of probability

[a] H-index residual MS df = 1

Table 4.7. Analysis of variance and mean square values for select arthropod families collected with sticky traps at Hickson and Prosper, ND in 2022.

				Hickson				
SOV	df[a]	Diptera	Aphelinidae	Sciaridae	Syrphidae	Chalcidae	Apidae	Thripidae
Rep	3	204	25	1509*	49	180	6	65
Trt	3-4	5111*	783*	1117*	188*	199	10*	3636*
Date	5-7	9275*	1808*	6350*	448*	211	15*	1595*
Trt x date	15-28	525*	88*	389	31	82	10*	845*
Residual	37-110	256	51	283	34	103	4	276
CV, %		33	32	43	53	54	210	47
				Prosper				
Rep	3	394	93	1293*	184	201	12	3509*
Trt	3-4	2177*	259*	6905*	526*	406	42	8784*
Date	5-7	4359*	801*	25052*	1530*	1076*	142*	1127*
Trt x date	15-28	517*	79	621	133*	265	16	372
Residual	36-107	283	59	463	74	210	18	498
CV, %		44	43	37	54	58	52	60

* Significant at $P \leq 0.05$, level of probability

[a] Df for Trt, Date, and Trt x date varied by family if specimens from each family were present or not in each trap at each date, treatment, and replicate

Fallow ground contained an abundance of the selected families, except for thrips, likely due to not having a plant canopy (Fig. 4.13). Fallow ground sticky traps were in the open and not surrounded by growing crops. Flying insects passed through fallow ground easily to get to areas with growing crops and therefore were collected on sticky traps. One study noted that fallow ground was dominated by Hymenoptera species (Mhlanga et al., 2022), which is what was observed in this experiment with Aphelinidae and Syrphidae species. Syrphidae species serve as pollinators, which is noted as the population was greater ($P \leq 0.05$) in sunflower compared with any other crop (Fig. 4.13). Fungus gnat species in the family Sciaridae were most abundant in fallow plots, but almost equally abundant among sunflower, alfalfa, and wheat plots. Larvae of Sciaridae are phytosaprophagous, meaning they feed on decaying plant material and soil fungi, and are considered a predominant taxon in arable lands (Nielsen & Nielsen, 2004). Fallow plots contained the least amount of decaying plant material, which contradicts what is known about the patterns of Sciaridae. However, soil fungi were not evaluated and could have contributed to the thriving Sciaridae population in fallow plots.

Alfalfa plots contained the greatest abundance of thrips (*Thripidae*) (Fig. 4.13). Thrips are considered a pest of alfalfa as they can transmit viruses. Madiera et al. (2021) found that thrip species *Frankliniella occidentalis* (*Thripidae*) was the most abundant herbivore insect in alfalfa crops representing 80 to 88% of the total herbivore population. It is known that thrips feed on alfalfa pollen and the canopy of alfalfa is attractive to species in the Thripidae family (Terry & Alston, 2011).

The average number of potato leafhoppers (*Empoasca fabae*) collected on sticky traps was 5.6, which is important to note for comparison with 2023 as the cultivar of alfalfa used was

Vamoose, which is leafhopper resistant. Common pests found in alfalfa plots include: flea beetle (*Altica spp.*), leafhopper, and aphids (*Aphidae spp.*).

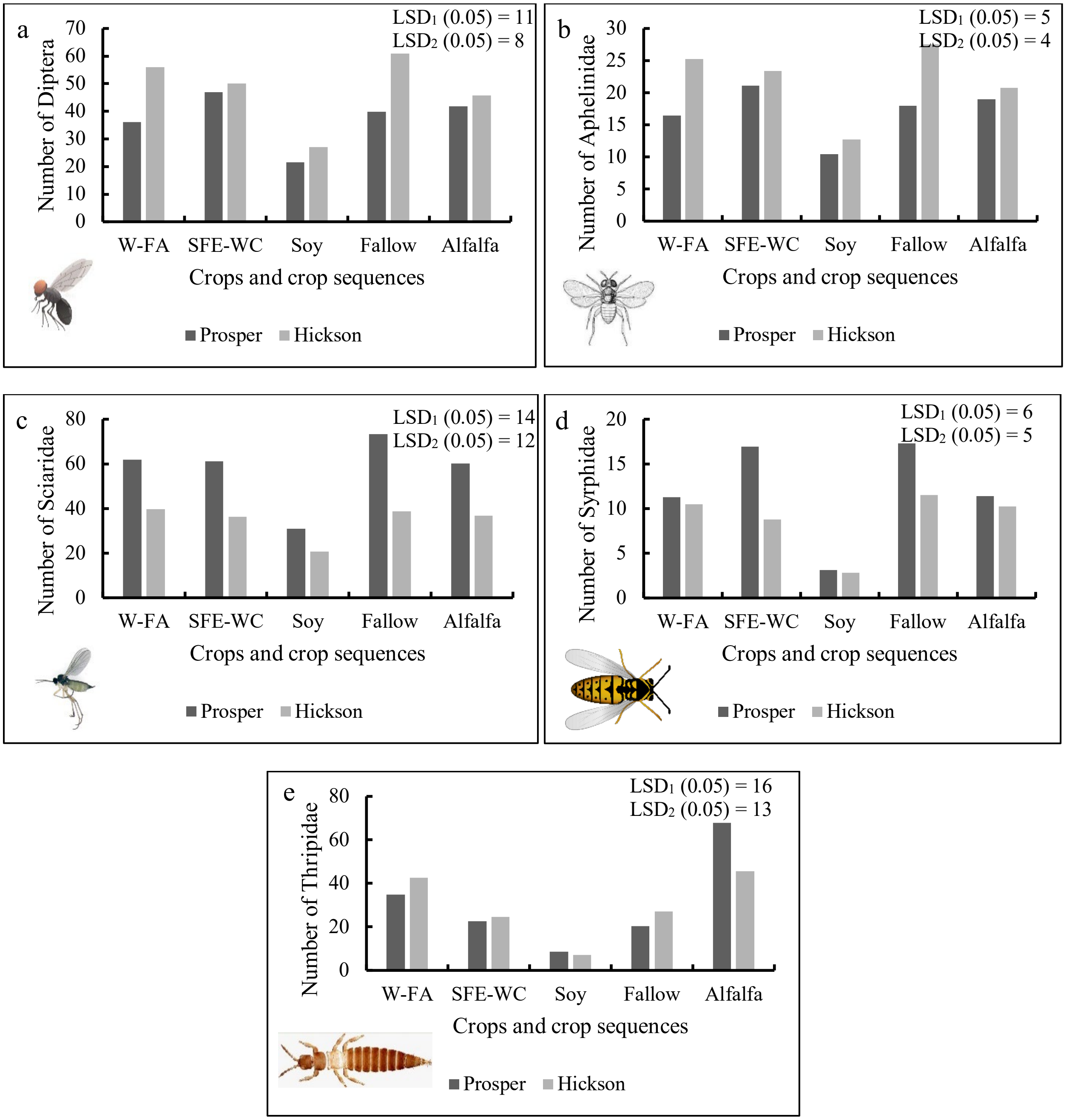

Figure 4.13. Seasonal average abundance of a) Diptera, b) Aphelinidae, c) Sciaridae, d) Syrphidae, and e) Thripidae families collected on sticky traps by treatment in Hickson and Prosper, ND in 2022. LSD_1 = to compare crops and crop sequences at Prosper; LSD_2 = to compare crops and crop sequences at Hickson. W = wheat; FA = fallow; SFE = early-planted, early-maturing sunflower.

Alfalfa's abundance peaked around mid-September, when in vegetative growth stages (Fig. 4.14, 4.15). This is due to alfalfa being one of the only green crop canopies remaining at the time. Soybean and late-planted sunflower were both well into reproductive stages and starting to desiccate. Similar to pitfall results, soybean monocrop lacked in abundance and diversity due to the insecticide application. The effects of the insecticide are more prominent in canopy insects because that is where the chemical was applied (Fig. 4.14, 4.15).

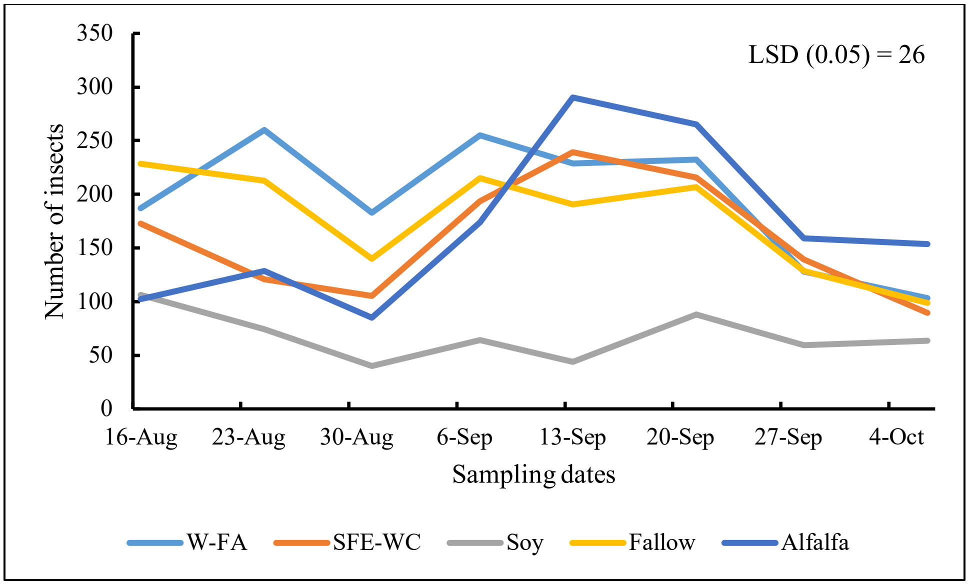

Figure 4.14. Arthropod abundance from sticky traps collected at Hickson, ND during eight sample dates in 2022. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow.

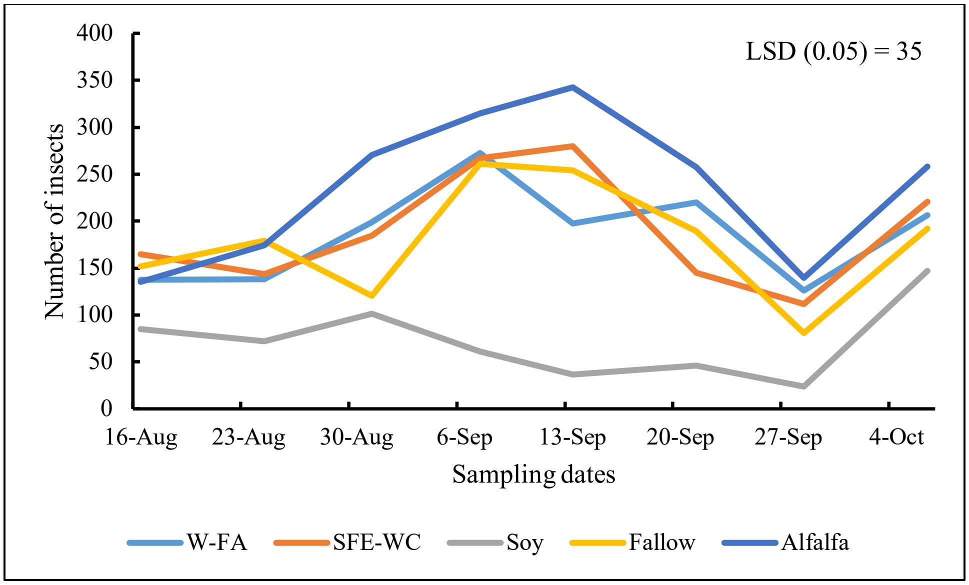

Figure 4.15. Arthropod abundance from sticky traps collected at Prosper, ND during eight sample dates in 2022. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA= fallow.

Soybean had the lowest family diversity compared with the other crops and sequences ($P \leq 0.05$), consistent with previous discussion regarding insecticide (Fig. 4.16). Alfalfa in Prosper had the greatest family diversity with an average of 11 families ($P \leq 0.05$). Alfalfa in Hickson was slightly lower with an average of 9.8 families but not significantly different from wheat-fallow.

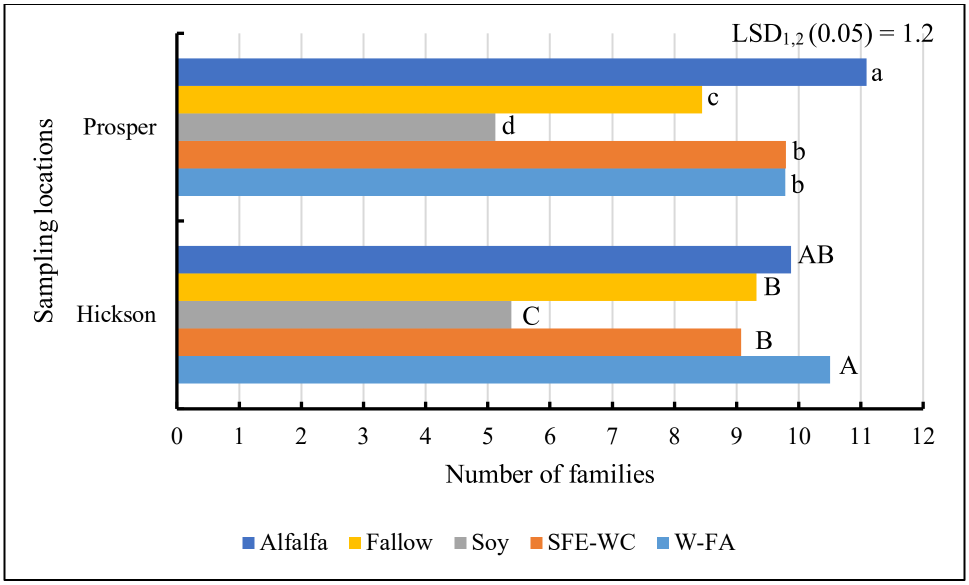

Figure 4.16. Seasonal average abundance of arthropod families collected in sticky traps at Hickson and Prosper, ND in 2022. SFE = early-planted, early-maturing sunflower; WC = winter camelina; W = wheat; FA = fallow. Different letters within a same location indicate means are significantly different ($P \leq 0.05$).

Shannon diversity index varied by treatment and date in Hickson (Table 4.5). Soybean was likely an outlier in this experiment because the other crops and sequences followed similar patterns and values to each other (Fig. 4.17). In Prosper, treatment was significant ($P \leq 0.05$). Alfalfa had the greatest diversity but was not significantly different from other crops except for soybean. In an insect sampling study done on wheat in Pakistan via the sweeping method, wheat had an H-index ranging from 2.27 to 2.59 (Ghani & Maalik, 2020). This study considered insects to species level, which is why the value is much higher. Considering arthropods to only a family level means H-index will be lower. It is important to note that H-index indicates diversity; however, a large H-index can be harmful if your species population is a majority pest.

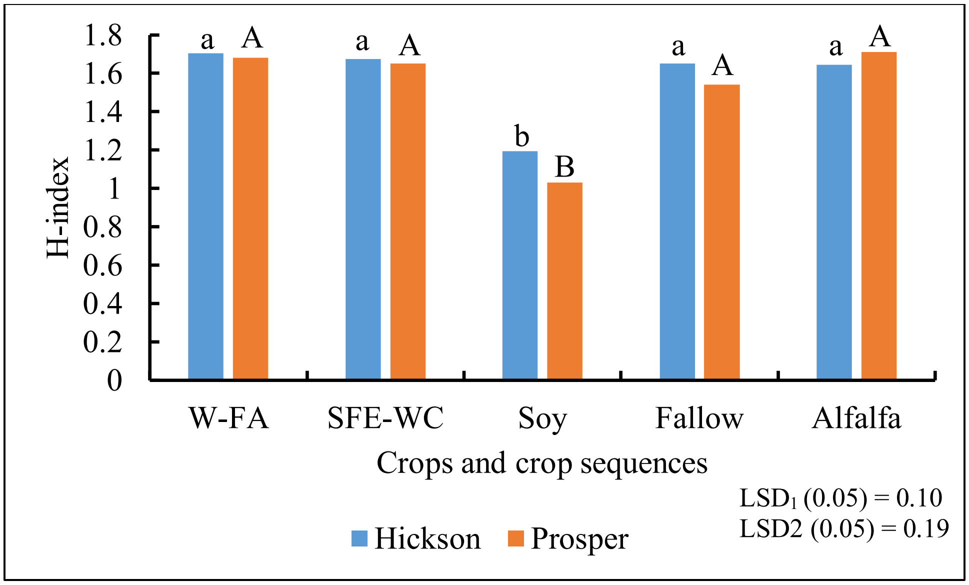

Figure 4.17. Shannon diversity index (H-index) by crop or crop sequence sticky traps collected in Hickson and Prosper, ND in 2022. W = wheat; FA = fallow; SFE = early-planted, early-maturing sunflower; WC = winter camelina. Different letters indicate means are significantly different ($P \leq 0.05$). LSD_1 = Hickson; LSD_2 = Prosper.

4.4.4. Sticky traps year 2

Treatment was again significant ($P \leq 0.05$) for arthropod abundance, family diversity, and Shannon diversity index at each location in 2023 (Table 4.8). The H-index was significant ($P \leq 0.05$) for treatment by date at both Hickson and Prosper, and abundance and family diversity were significant ($P \leq 0.05$) at Prosper only. Date was significant ($P \leq 0.05$) for each measure at both locations. Families Coccinellidae, Syrphidae, Chalcidae, Apidae, and Thripidae were all significant ($P \leq 0.05$) for treatment in Hickson (Table 4.9). Only Coccinellidae, Syrphidae, and Thripidae were significant ($P \leq 0.05$) for treatment in Prosper (Table 4.9). Thripidae was the only family to be significant for treatment by date in Prosper, where Coccinellidae and Chalcidae were significant in Hickson (Table 4.9).

Table 4.8. Analysis of variance and mean square values for total insects, total number of families, and Shannon Diversity index (H-index) for sticky traps of six or five treatments in Hickson and Prosper, ND in 2023.

	Hickson				Prosper			
SOV	df	Insects	Families	H-index	df	Insects	Families	H-index
Rep	3	113186	26	0.8*	3	67866	35*	0.4*
Trt	5	197250*	48*	0.7*	4	166053*	47*	0.8*
Date	10	650872*	76*	2*	11	1057362*	92*	2.0*
Trt x Date	44	54591	13	0.4*	44	59696*	19*	0.4*
Residual	153	44730	11	0.2[a]	175	41259	10	0.1[b]
CV, %		50	36	33		41	29	24

* Significant at $P \leq 0.05$, level of probability

[a] H-index residual MS df = 150

[b] H-index residual MS df = 173

Table 4.9. Analysis of variance and mean square values for select arthropod families collected with sticky traps at Hickson and Prosper, ND in 2023.

				Hickson				
SOV	df[a]	Diptera	Coccinellidae	Sciaridae	Syrphidae	Chalcidae	Apidae	Thripidae
Rep	3	6490*	3	7978*	3	667	0.2	6893
Trt	2-5	1043	29*	4828*	2	1362*	0.8*	31509*
Date	4-10	34915*	37*	2647	8*	3871*	0.3	326087*
Trt x date	8-41	2661	10*	1144	2	505*	0.2	8736
Residual	5-127	2433	6	1910	2	289	0.1	10205
CV, %		42	63	82	44	40	25	56
				Prosper				
Rep	3	2229	12	1291	9	469	0.5	11712
Trt	1-4	2779	20*	652	55*	949	0.4	30422*
Date	6-11	68836*	49*	7223*	103*	8598*	0.1	148932*
Trt x date	6-44	2325	6	717	25	860	0.9	13076*
Residual	7-156	2047	5	900	17	637	0.3	7520
CV, %		33	56	56	61	45	36	52

* Significant at $P \leq 0.05$, level of probability

[a] Df for Trt, Date, and Trt x date varied by family if specimens from each family were present or not in each trap at each date, treatment, and replicate

The abundance of arthropods overall was notably higher in 2023. Alfalfa averaged 537 arthropods per sticky trap in Hickson, where the abundance peaked at 290 arthropods per trap in 2022 (Fig. 4.18). It is possible that insecticide application to soybean played a role in all plots in 2022 considering their close proximity. Winter camelina-fallow sequence had significantly ($P \leq 0.05$) less abundance when compared with other crops and sequences. Abundance peaked at the beginning of sampling, in the middle, and started to increase toward the end of sampling in Prosper (Fig. 4.19). The end-of-season increase was also seen in 2022. Species of Diptera and Sciaridae peaked toward the end of the season with the increased presence of decaying plant matter. Diptera species were mostly considered beneficial as they function as scavengers, predators, and pollinators. In certain crops and scenarios, they can be considered pests. Diptera break down plant matter into nutrients for the soil and larvae serve as food for other species that make an agricultural system diverse (Oldroyd, 2023). Temperatures stayed above freezing (0° C) allowing arthropods to persist at the end of the sampling period. Winter camelina was the earliest flowering crop out of all sequences. Treatments containing winter camelina had abundant arthropod populations at the beginning of the season; however, those populations were similar to wheat and alfalfa (Fig. 4.19). A study conducted on the pollination characteristics of winter camelina noted that populations of Syrphidae were high and Apidae species were low (Forcella et al., 2020). Syrphidae played a role in pollination, even though winter camelina is a self-pollinated crop. Apidae species are found to be more abundant in August in the Northern Great Plains due to their transient nature (Forcella et al., 2020). Although not significant and therefore the data not displayed, minute pirate bugs (*Orius insidiosus*) were collected consistently in 2023 on sticky traps, most prominently in alfalfa and winter camelina plots. Minute pirate bugs are generalist predators toward mites, thrips, and aphids (Knodel et al., 2023). These predators are highly sensitive to insecticide use, particularly permethrin and cyfluthrin, in alfalfa and other crops, as discovered by Al-Deeb et al. (2001). Increased population of pests, particularly in alfalfa, may have increased the predatory population to balance the natural predator-prey relationship that exists in alfalfa fields. In 2022, potato leafhopper population averaged 5.6. In 2023, potato leafhopper population averaged 16.2. The potato leafhopper-resistant variety of alfalfa planted had minimal damage, but was present. Only thrips and ladybirds (*Coccinellidae spp.*) were significant by treatment in both Hickson and Prosper (Fig. 4.20). Alfalfa had the highest abundance of Thripidae species as in 2022 (Fig. 4.20). Soybean and wheat had the lowest abundance (Fig. 4.20). Ladybirds act as a predatory insect against aphid (*Aphidae spp.)* and thrips species. Aphid population was highest in continuous alfalfa plots. Hickson averaged 33 aphids and Prosper averaged 85 aphids per sticky trap seasonally. Ladybird population was higher in plots containing winter camelina. If ladybird population was higher in alfalfa, it is likely that aphid population would be lower.

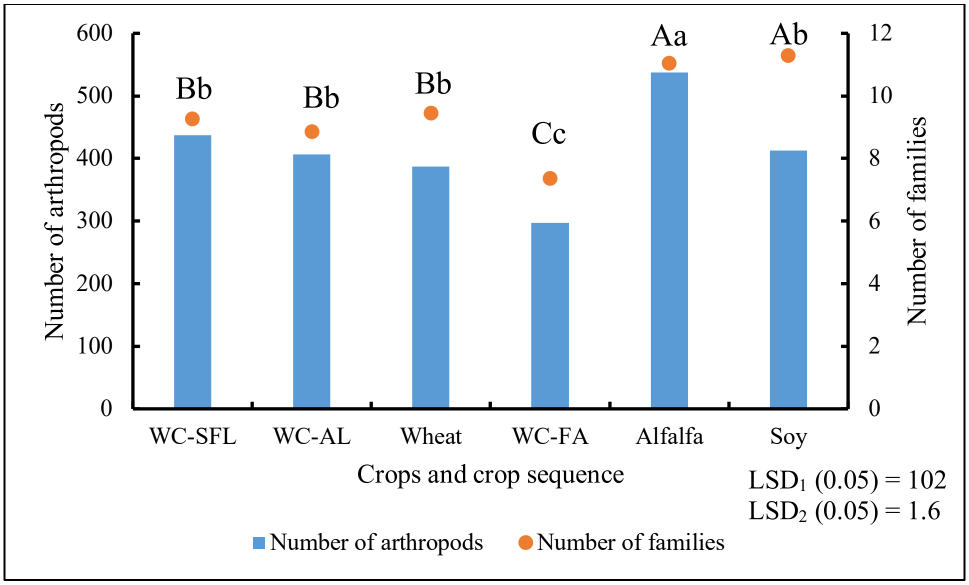

Figure 4.18. Average abundance of arthropods and arthropod families collected with sticky traps in Hickson, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; FA = fallow. Different letters indicate means are significantly different ($P \leq 0.05$). Lowercase letters indicate significance for number of arthropods and uppercase letters indicate significance for number of families. LSD$_1$ = number of arthropods; LSD$_2$ = number of families.

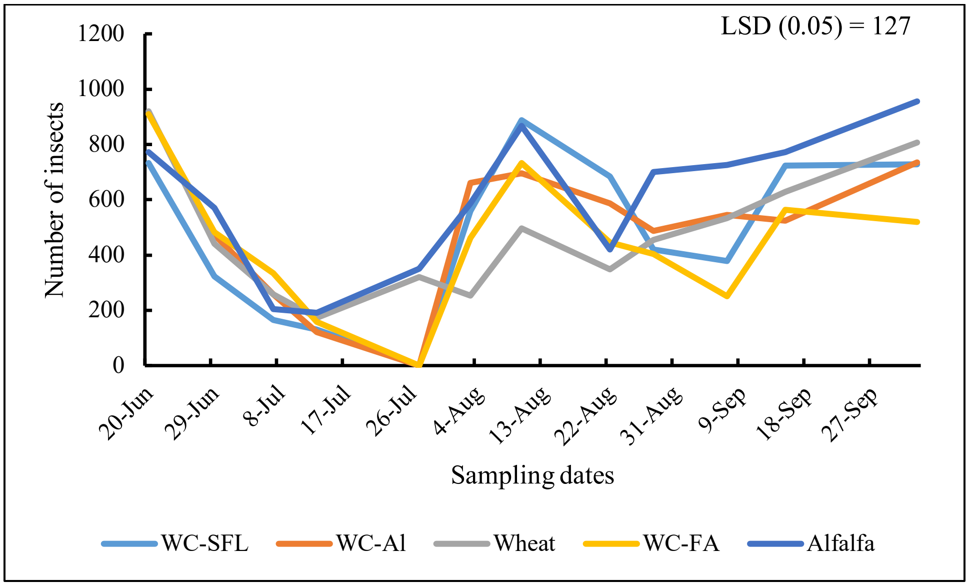

Figure 4.19. Average abundance of arthropods collected by date with sticky traps in Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; FA = fallow.

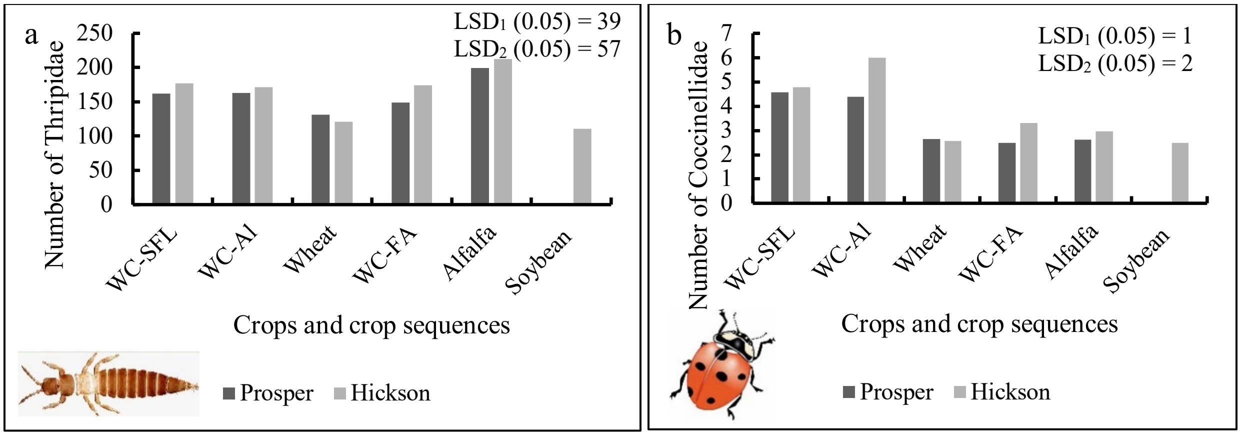

Figure 4.20. Seasonal average abundance of a) Thripidae and b) Coccinellidae collected on sticky traps by treatment in Hickson and Prosper, ND in 2023. LSD$_1$ = to compare crops and crop sequences at Prosper; LSD$_2$ = to compare crops and crop sequences at Hickson. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa, FA = fallow.

In Hickson, soybean family diversity was the same as alfalfa (Fig. 4.19). The soybean was grown in relay with winter camelina, so it is unknown if the preceding winter camelina contributed to the result as there was no strict soybean monocrop for comparison in 2023. The winter camelina-fallow sequence had the lowest ($P \leq 0.05$) family diversity. In Prosper, families varied by date ($P \leq 0.05$) (Fig. 4.21). Although fallow ground was efficient at maintaining an arthropod population, having a consistent crop growing can increase biodiversity. If land is to remain fallow for a significant period of time, adding vegetation can increase taxonomic diversity (Rischen et al., 2023). Alfalfa and wheat have similar trends by date in terms of abundance. However, aphids, leafhoppers, thrips and lygus bugs (*Lygus lineolaris*) were all very common in alfalfa plots. Presence of lygus bugs greatly increased in 2023. Statistically, crop or crop sequence was not significant for lygus bug populations; however, alfalfa serves as the preferred host. Lygus bugs are considered a devastating pest of alfalfa seed production as they feed on buds and flowers (Knodel et al., 2022). Considering biodiversity sampling measures, and that alfalfa was strictly cut as a forage, the decision was made to not treat with insecticide.

Increase in lygus bug presence could be attributed to the increase of alfalfa in the experimental units or increase in other host crops surrounding field sites.

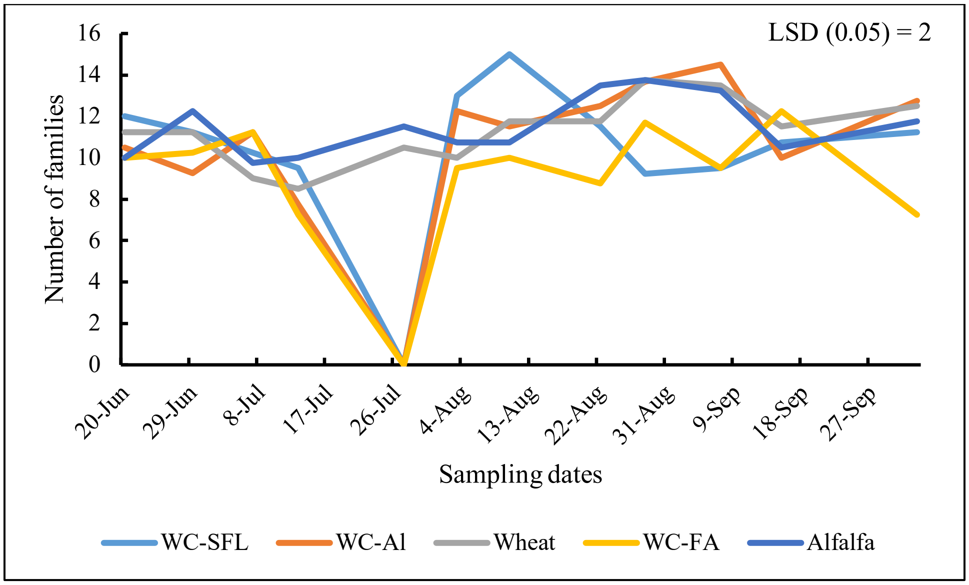

Figure 4.21. Average abundance of arthropod families by sampling date and crop or crop sequence collected with sticky traps in Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow. Number of arthropods being at zero indicates no sample was collected on that date.

Winter camelina H-indices were lowest compared with other crops at the beginning of the sampling period (Fig. 4.22, 4.23). The crop was starting to mature, which likely decreased the diversity of species present. Forcella et al. (2020) noted that species, particularly in the Diptera order, were highly dependent on flowering condition and wind speed at time of collection in camelina fields. Wind speed and direction measurements were not considered in this experiment; however, greater presence of arthropods on certain sides of the sticky trap were indicative of wind direction during the sample period. Wheat achieved two of the highest H-index values in Hickson and H-index of fallow ground increased during the fall with greater portions of Diptera and Hymenoptera species collected.

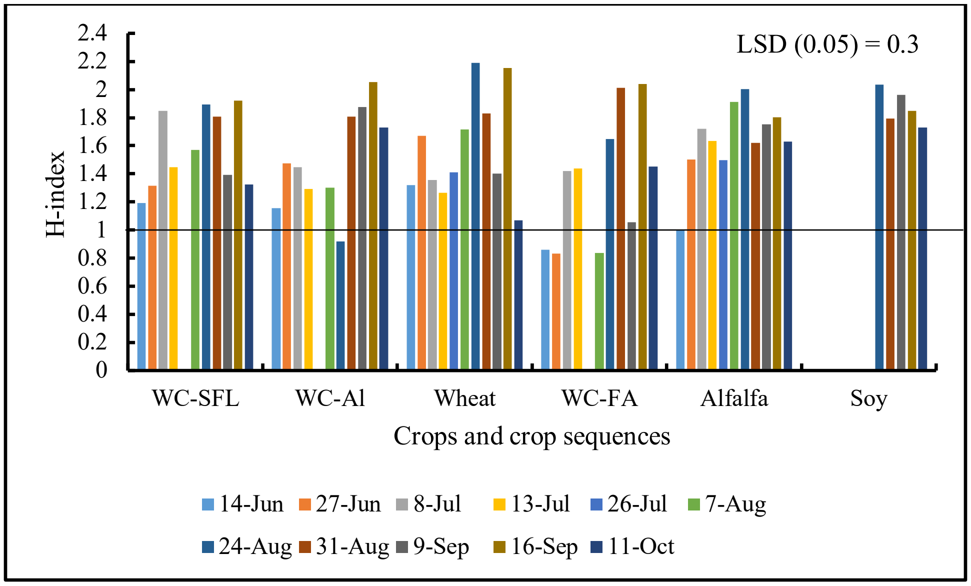

Figure 4.22. Shannon diversity index (H-index) by sampling date and crop or crop sequence for arthropods collected with sticky traps at Hickson, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow.

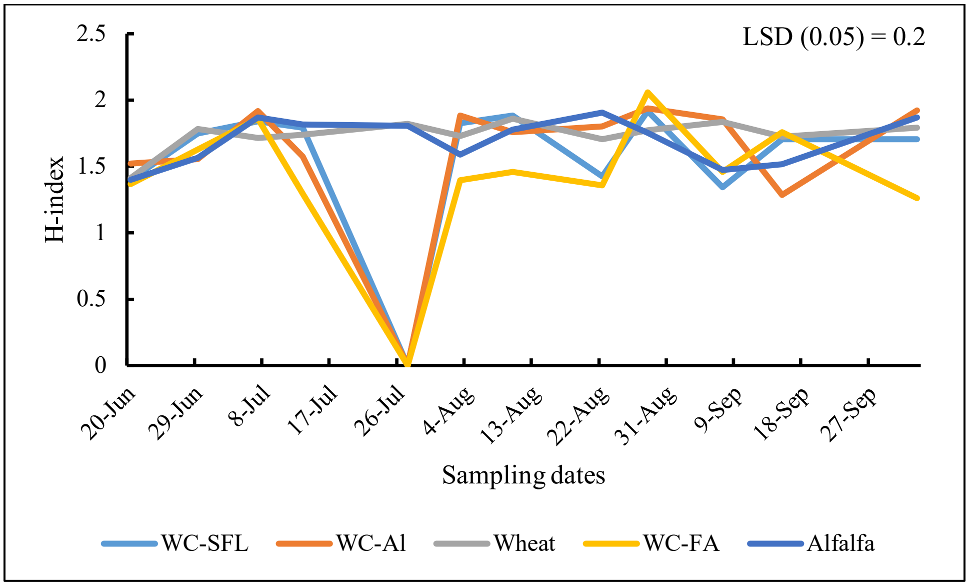

Figure 4.23. Shannon diversity index (H-index) by sampling date and crop or crop sequence for arthropods collected with sticky traps at Prosper, ND in 2023. WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow.

4.5. Conclusion

Incorporating winter camelina and/or alfalfa to crop rotations in the northern Great Plains may be a way to increase agricultural biodiversity in terms of plant and arthropod species. Crop and crop sequence had an impact on arthropods collected in pitfall and on sticky traps in both years and locations; crop presence plays a large role in arthropod abundance and diversity. Increased biodiversity indicates healthier agricultural systems and strengthens natural predator-prey relationships. Alfalfa and winter camelina were not always associated with the greatest arthropod diversity or abundance; however, each crop had positive contributions.

In Year 1, arthropod abundance and diversity of canopy arthropods was lower than Year 2. Year 1 ground insects were more abundant compared with Year 2. Soybean received an application of insecticide in Year 1, which likely contributed not only to the lack of abundance and diversity of arthropods in soybean plots, but also in surrounding plots. This had the greatest effect on canopy insects. Winter camelina acted as a mass-flowering crop and introduced mostly pollinator species in the orders Diptera and Hymenoptera early in the season. Alfalfa provided consistent groundcover as refuge for ground and canopy arthropods. Wheat harbored field crickets, as it provided an ideal habitat. Winter camelina introduced more beneficial and pollinator species to the landscape, especially early in the season. In the future, it would be beneficial to continue arthropod sampling to see long-term benefits of crop rotations and to use this information to improve IPM strategies. Quantifying the benefits of arthropod biodiversity further ecologically or economically will be important to encourage producers to implement crop diversification as a method to increase biodiversity in agricultural settings.

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5. CONCLUSION

Incorporating alfalfa and winter camelina into wheat, sunflower, and soybean rotations was impactful to agronomic and biodiversity characteristics of the cropping sequences studied.

Despite the yield deficiency introduced by winter camelina, the crop was able to increase beneficial arthropod populations. Early-season flowering attracted beneficial and predatory insects to the field before other crops could reach reproductive stage. Winter camelina's poor yield portrayed a crop that was not agronomically viable and a crop that was subpar for the environment; evidence that breeding and management efforts are needed in winter camelina. The soybean-winter camelina system did not perform as expected; however, early-maturing sunflower preceding winter camelina in the double-crop system improved yield compared with the sunflower monocrop. Yield discrepancy between previously conducted research is likely due to the environmental conditions of North Dakota in 2022 to 2023 and potential herbicide carryover.

When wheat and sunflower crops had unpredictable yields due to weed pressure, environment, and disease and pest presence, alfalfa remained high-yielding. They did, however, increase biodiversity in their respective plots. Sunflower especially increased pollinator families compared with soybean, wheat, and alfalfa. Alfalfa was able to withstand varying weather patterns and not succumb to disease or insect pressure. This study did not agree with previous findings stating that alfalfa can support a majority beneficial arthropods, however. Alfalfa plots housed many pest species, therefore reducing the ratio of beneficial to pest arthropods. The environmental impact of alfalfa was the least of all the crops studied despite the high field activity associated with multiple harvests per season. Overall, alfalfa was the only crop to see consistently positive agronomic, economic, and environmental results. Further work should be done to characterize the arthropod biodiversity impacts of alfalfa, as well as the associated soil biota.

Although much work is being conducted with winter camelina, this study struggled to find the environmental and agronomic benefits. Given ideal conditions, the crop would work well in a double-cropping system with early-maturing sunflower; however, the herbicide system must be adjusted and economics of fertilization studied. Alfalfa was consistent for many metrics, but other crops may introduce more biodiversity to the field. Longer crop sequences should be studied to see the long-term agronomic and biodiversity benefits of these crops in the northern Great Plains.

APPENDIX

Table A1. Mean values for select alfalfa forage nutritive value parameters for three treatments and harvest dates (Cut) in Hickson and Prosper, ND in 2023.

	Ash	CP[a]	NDFD	TDN	RFQ
			Hickson		
Alfalfa following wheat			g kg^{-1}		
Cut 1	98	212	500	700	168
Cut 2	94	267	440	708	182
Cut 3	101	267	363	674	152
Cut 4	99	267	384	709	178
Alfalfa following sunflower					
Cut 1	96	248	469	726	203
Cut 2	117	254	387	672	153
Cut 3	104	257	381	699	174
Alfalfa 2022 spring-planted					
Cut 1	101	194	442	636	131
Cut 2	97	252	463	708	180
Cut 3	93	276	381	681	149
Cut 4	95	245	379	685	160
LSD_1 (0.05)	16	26	50	45	32
LSD_2 (0.05)	13	20	40	36	26
			Prosper		
Alfalfa following wheat			g kg^{-1}		
Cut 1	64	150	441	621	147
Cut 2	88	279	419	711	157
Cut 3	99	231	384	675	154
Alfalfa following sunflower					
Cut 1	80	195	482	690	186
Cut 2	98	270	430	714	166
Cut 3	99	234	391	686	163
Alfalfa 2022 spring-planted					
Cut 1	86	192	463	668	149
Cut 2	80	228	470	696	161
Cut 3	78	263	384	670	135
Cut 4	89	221	386	672	154
LSD_1 (0.05)	7	17	25	32	21

LSD_1= to compare Trt 3 cut 4 with all cuts of Trt 10, all cuts of Trt 3 and cuts 1 and 2 of Trt 4 in Hickson

LSD_2=to compare all remaining Trts and cuts in Hickson

[a] Crude protein (CP), neutral detergent fiber digestibility in 48 h (NDFD), total digestible nutrients (TDN), relative feed quality (RFQ)

Table A2. Mean Shannon Diversity index (H-index) values for crops and nine sampling dates in pitfall traps at Hickson and Prosper, ND in 2023.

Date	WC-SFL[a]	WC-Al	Wheat	WC-FA	Alfalfa	Soy[b]
Hickson						
23-Jun	1.6	1.4	0.9	1.1	1.5	--
6-Jul	0.8	1.1	1.0	0.9	1.2	--
13-Jul	1.1	1.1	1.1	1.3	1.2	--
26-Jul	0	0	0.9	0	1.5	--
7-Aug	0.8	1.0	1.1	1.0	1.1	--
24-Aug	1.3	1.2	1.2	1.1	1.2	1.2
31-Aug	1.4	0	1.2	1.3	1.4	1.1
9-Sep	1.4	1.2	1.0	0.9	1.3	1.2
16-Sep	1.0	1.0	0.9	0.7	1.0	0.9
LSD (0.05)	0.2					
Prosper						
23-Jun	0.5	1.0	1.1	1.2	0.9	--
6-Jul	1.0	1.2	1.1	1.0	1.4	--
13-Jul	0	0	1.2	0	1.6	--
26-Jul	0.9	1.3	1.2	1.0	1.3	--
7-Aug	0.8	1.4	0.8	0.8	1.0	--
24-Aug	1.0	1.2	0.8	1.0	1.0	--
31-Aug	1.4	1.2	1.1	1.3	1.3	--
9-Sep	1.0	1.1	0.9	0.8	0.9	--
16-Sep	1.3	0.9	1.1	1.1	1.0	--
LSD (0.05)	0.2					

[a] WC = winter camelina; SFL = late-planted, early-maturing sunflower; Al = alfalfa; FA = fallow

[b] Soybean was only sampled during the last half of the dates at Hickson, and no samples were collected at Prosper

Values at zero indicate no sample was collected on that date