# CORN-ALFALFA INTERCROPPING WITH DIFFERENT ROW SPACINGS

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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# ABSTRACT

Alfalfa (*Medicago sativa* L.) is a staple crop grown mainly by dairy and beef farmers in the Midwest. To determine if seeding alfalfa with corn (*Zea mays* L.) could increase alfalfa forage yield and nutritive value in the second year, and provide a corn crop during the first year, an experiment was conducted in Prosper and Hickson, ND in 2020 and 2021. Corn grain yield in 2020 was negatively impacted by 152-cm corn row spacing compared with 76-cm row spacing. However, there was no significant difference in corn grain yield with or without an alfalfa intercrop for the same row spacing although it did trend lower in treatments with alfalfa intercropped. Intercropping corn and alfalfa with 76-cm corn row spacing was more profitable than conventional seeding. Optimizing this specific cropping system for growers in the Midwest could increase profitability as well as forage nutritive value and crop efficiency.

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# 1. INTRODUCTION

Alfalfa (*Medicago sativa* L.)-corn (*Zea mays* L.) intercropping for the purpose of additional alfalfa forage yield in the second year has been studied as an alternative to corn silage monocultures in the US Midwest (Osterholz et al., 2020). However, there is limited research on the forage yield and economic implications of this alternative cropping system in a corn for grain operation.

Despite being ranked second and third in total alfalfa acreage, South Dakota and North Dakota are ranked 5th and 11th respectively, in total alfalfa forage production in the U.S. (NASS, 2020). This is likely due to the colder climate and lower precipitation in these states than in other parts of the U.S. Total alfalfa hectares throughout the U.S. have declined by 37% since 1979, likely due to an increase in corn grain yield and prices (Zulauf, 2018). Alfalfa almost always yields considerably less in the seeding year which reduces average yield over the life of the stand (Aponte et al., 2019).

By reducing alfalfa hectares, we are losing some key economic and environmental benefits provided by alfalfa. Alfalfa is an excellent scavenger off soil $NO_3$-N and the alfalfa root system can reach depths of over 177-cm to reduce soil nitrogen by up to 64% (Randall et al., 1997; Fan et al., 2016). Reducing excess soil NO3-N, especially at depth, is key in preventing contamination of drinking water. Better known is alfalfa's ability to fix atmospheric $N_2$ efficiently which provides nitrogen credits for the following crop and reduces the need for synthetic fertilizer (Russel et al., 2001; Yost et al., 2013). Lesser-known benefits of growing alfalfa are many, but include sequestration of carbon in the soil, increased water infiltration, reduced compaction, and weed suppression (Meek et al., 1989; Mitchell et al., 1995; Belinder et al., 2004; Meyer-Aurich et al., 2008; Guo et al., 2019).

The most effective way to increase alfalfa forage yield and make it a more profitable crop is to focus on reducing the forage yield drag in the seeding year of alfalfa. Corn-alfalfa intercropping has been shown to compensate for the low-yielding alfalfa establishment year by increasing profitability through corn production and harvest as a forage or grain during alfalfa establishment (Osterholz et al., 2020; Berti et al., 2021). Osterholz et al. (2020) evaluated different silage corn/alfalfa sequences in an intercropped trial and a conventionally seeded trial. They found the most profitable conventional sequence to be four years of corn and four years of alfalfa whereas when alfalfa was intercropped the most profitable sequence was three years of corn and three years of alfalfa. These shorter duration alfalfa stands made possible by intercropping are likely more lucrative for farmers who wish to take advantage of the nitrogen alfalfa fixes for following crops.

This research project explored corn-alfalfa intercropping at two row spacings, 76 cm and 152 cm, to understand the potential for better alfalfa establishment as well as the effect that different corn row spacing has on grain yield. The aim of this project is to define a specific corn-alfalfa intercropping system that will minimize loss to corn grain yield, provide adequate establishment of alfalfa, and increase long-term grower profitability. This could lead to increased average alfalfa forage yield and total alfalfa acreage along with the benefits associated with alfalfa cultivation. Intercropping corn and alfalfa also provides specific benefits such as reduced nitrogen, phosphorus, and suspended solids in runoff compared with corn planted alone (Osterholz et al., 2019).

## 1.1. Objectives

Main Objective: ***Compare alfalfa plants grown in intercropping systems with corn at two row spacings, with spring-seeded alfalfa alone***.

Specific objectives

- To determine if alfalfa can be established while growing corn to skip the low yielding seeding year of alfalfa and to have a profitable year of corn production
- To determine corn yield losses due to alfalfa competition and intra-specific competition at 76- and 152- cm corn row spacings
- To determine the yield and forage nutritive value of the intercropped alfalfa in subsequent years
- To determine the effect of intercropping corn and alfalfa at 76- and 152- cm row spacings on alfalfa stand compared to alfalfa established alone

# 2. LITERATURE REVIEW

## 2.1. Alfalfa origin, history, and importance

Purple-flowered alfalfa (*Medicago sativa* L.) is native to northern Iran and surrounding areas, while yellow-flowered alfalfa (*M. falcata* L.) has a broader native range reaching as far north as Siberia (Hanson et al., 1988). Both varieties naturally hybridize, and it is believed this likely played a positive role in the spread of alfalfa as the yellow-flowered variety carries desirable genes such as winter hardiness, drought tolerance, and disease resistance (Hanson et al., 1988). The first documentation of alfalfa comes from Turkey and dates back to 1300 B.C., while the first record of its cultivation as forage is from Greece between 440 and 322 B.C. (Singh, 2009). The crop later spread throughout the Roman Empire and into China. By the beginning of the Christian era, alfalfa had spread throughout Europe, the Middle East, and eastern Asia. Although alfalfa almost entirely disappeared in Europe during the Dark Ages, it was subsequently reintroduced sometime later around the 16th century (Singh, 2009).

Alfalfa was first introduced to the New World in Mexico, Chile, and Peru in the 16th century. The earliest mention of alfalfa within the current United States boundaries occurred in 1736, in what is now the state of Georgia and is noted shortly thereafter in several other New England colonies as well. The alfalfa introduced by the English colonists did not survive well on the East coast. Alfalfa was then reintroduced to the southwestern U.S. around 100 years later mainly from alfalfa brought from Chile and Peru; it was favored by many California stockmen and gained popularity. Alfalfa continued to spread throughout the southwest as many Mormons in Utah grew alfalfa for seed. The most important introduction of alfalfa for the Upper Midwest and northern Great Plains likely came from a German who brought seed from Germany to Minnesota. This cultivar, known later as Grimm, underwent substantial natural selection during its initial plantings but eventually became one of the most notable original winter-hardy varieties of the time (Hanson et al., 1988).

Alfalfa is the oldest cultivated crop (USDA NRCS Plant Materials Program, 2006) and is the fourth most grown crop in the U.S. (National Agriculture Statistics Service, 2020). In 2020, growers in the U.S. harvested 6.6 million ha of alfalfa or mixtures including alfalfa. (National Agriculture Statistics Service, 2020). However, alfalfa acres in the U.S. have been steadily declining over the last 20 years likely due to lack of gain in alfalfa forage yield and continued yield increases in grain crops (Zulauf, 2018).

## 2.2. Alfalfa morphology and physiology

Alfalfa is part of the genus *Medicago,* which is comprised of 66 annual and 33 perennial species, and it belongs to the Fabaceae family. It belongs to the subfamily Faboideae and the tribe Trifolieae (Singh, 2009). Out of the 99 species that make up the genus *Medicago*, 17 are commonly cultivated as forage crops and used for soil improvement, human food-sprouts, and as companion crops (Singh, 2009). Alfalfa is the most cultivated species within the genus *Medicago*. More than 440 individual alfalfa cultivars have been developed since 1962 in the U.S. (USDA NRCS Plant Materials Program, 2006).

Alfalfa is a cool-season perennial legume. It is an autotetraploid with 32 chromosomes, $n$=8 (Singh, 2009). Alfalfa develops a deep taproot and a woody crown. Flowers come in many colors but are commonly purple and yellow. Alfalfa seeds are kidney shaped and are found in pods which can appear sickle shaped or twisted. Alfalfa thrives in well-drained soils with a pH over 6.7 (McLean and Brown, 1984).

## 2.3. Nitrogen fixation

Non-natural sources of nitrogen are now estimated at 210 Tg of N per year, primarily generated by cultivated legumes, fossil fuel use, and synthesized ammonia from the Haber-Bosch process. Biological nitrogen fixation as a direct reaction to human activities across the globe was estimated at 40 Tg of N per year with legumes as the main contributors (Galloway et al., 1995).

Alfalfa is one of the most efficient nitrogen fixing crops in large scale agriculture today. Total dinitrogen symbiotic fixation by alfalfa in the Mississippi River Basin was estimated at 152 kg N $ha^{-1}$ on an estimated 2.6 million hectares of alfalfa (Russelle and Birr, 2004). The same study found variation in alfalfa nitrogen fixation rates across the Mississippi River Basin from 43 to 471 kg N $ha^{-1}$, and this high variability can make predicting nitrogen credits for corn planted after alfalfa difficult. Under irrigation, Yang et al. (2011) found alfalfa to fix $N_2$ at a rate of 311 kg $ha^{-1}$ $yr^{-1}$. The amount of nitrogen alfalfa can fix and subsequently make available for the following crops varies depending on stand age, regrowth, soil conditions and environmental factors. Boawn et al., (1963) estimated that a three-year stand of alfalfa, including spring regrowth, when terminated with tillage could provide as much as 381 kg $ha^{-1}$ of N over five years after termination. Walker et al. (2017) found pre-plant soil $NO_3$-N tests in combination with alfalfa stand age to be the most accurate way of predicting corn yield response or lack of yield response to N fertilizer; these two factors accurately predicted corn yield response to N fertilizer 87% of the time.

Tillage as a termination method for alfalfa stands provides the most mineralized $NO_3$-N available for the following crop (Malhi et al., 2007). However, Yost et al., (2013) found no corn grain yield response to fertilizer N in no-till corn following alfalfa. They also examined previous literature, in which they found a grain yield response to N after no-till corn 29% of the time, which is comparable with 32% of the time with conventional tillage. This suggests tillage may not play a large role in N availability to corn following alfalfa (Yost et al., 2013).

## 2.4. $NO_3$-N scavenging

Alfalfa is well known as an efficient nitrogen-fixing legume; however, it does not expend energy on fixation unnecessarily. The alfalfa root system facilitates nitrogen uptake from the soil before it begins to use symbiotic fixation as its nitrogen source. Alfalfa can take up large amounts of excess nitrogen from the soil and store it in the alfalfa aboveground biomass, which is harvested. This means it can reduce $NO_3$-N leaching and preserve groundwater quality. In a study by Randall et al. (1997) it was shown that during a six-year period, alfalfa took up an average of 260 kg N $ha^{-1}$ $year^{-1}$ into its aboveground biomass compared with 114 kg N $ha^{-1}$ in the aboveground biomass of corn in a continuous corn system. Randall et al. (1997) also found alfalfa to reduce residual soil nitrogen by 64% when compared with a continuous corn system. In a remediation experiment by Russelle et al. (2001), a non-$N_2$-fixing alfalfa removed 972 kg $ha^{-1}$ of N over three years when irrigated with N contaminated water from the site.

Alfalfa can also access nitrogen much deeper in the soil profile than other crops. In a six-year study, Entz et al. (2001) showed that alfalfa increased the depth at which it could extract $NO_3$-N for the first four years until it reached a maximum depth around 270 cm. During the first four years of the study alfalfa reduced the nitrogen concentration in the 30- to 240-cm soil depth range by 3.8 mg $kg^{-1}$. During year 5 and 6 of continuous alfalfa, $NO_3$-N levels increased rather than decreased presumably because as the alfalfa stand aged many plants died leaving behind N that was either previously scavenged or fixed. The best cropping system for lowering subsoil $NO_3$-N concentrations in the experiment was four years of alfalfa followed by two years of wheat (*Triticum aestivum* L.).

Alfalfa can reduce tile line flow substantially by utilizing excess water. In a study conducted by Randall et al. (1997) continuous corn, corn-soybean [*Glycine max* (L.) Merr.] rotation, established alfalfa, and conservation reserve program (CRP) systems were all compared to show the differences in tile line drainage volume as well as $NO_3$-N concentrations. Row crop treatments let approximately 1.6 times as much water drain from the tile lines than the alfalfa and CRP treatments did. Concentrations of $NO_3$-N in tile line water was reduced in a perennial alfalfa cropping system. Alfalfa reduced tile line $NO_3$-N concentrations by 97% compared with continuous corn. These results are supported by Syswerda et al. (2012) who showed alfalfa reduced $NO_3$-N leaching losses by 80% compared with a conventionally tilled and fertilized row crop system.

## 2.5. Greenhouse gas emissions

Climate change is an increasingly common topic, especially in agriculture. Several companies are paying farmers for adding a measured amount of carbon to the soil. As a result, carbon sequestration is becoming incorporated into many farms' financial plans. During a 20-year study based in Ontario, Canada, it was determined that a corn-alfalfa rotation sequestered a significantly higher amount of soil carbon than a corn-soybean rotation (Meyer-Aurich et al., 2008). Corn-alfalfa rotations sequestered 1060 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ compared with a corn-soybean rotation releasing 268 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$. When factoring in $N_2O$ emissions from the soil and emissions from indirect and direct energy use such as fuel, fertilizer manufacturing, crop drying etc., net emissions were lower in the corn-alfalfa rotation at 1229 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ compared with the corn-soybean rotation at 2631 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (Meyer-Aurich et al., 2008). Continuous alfalfa sequestered the highest amount of carbon and released the lowest net emissions of greenhouse gases. Continuous alfalfa for more than four years is not an economical rotation due to reduced stands and less forage yield after several years (Meyer-Aurich et al., 2008).

A study conducted by Sainju and Lenssen (2011) also determined that alfalfa can sequester carbon, although this study only showed an increase of soil carbon by 325 kg $ha^{-1}$ $year^{-1}$. The lower rate of carbon storage could be attributed to the short length of the study (two seasons), or the difference in soil conditions and climate of eastern Montana in this study compared with Ontario, Canada in the study by Meyer-Aurich et al., (2008). Although alfalfa may sequester more carbon and increase organic matter it can also increase mineralization of soil organic carbon due to its higher biomass C:N ratio and additional nitrogen added to the soil (Aher et al., 2017). In some cases, this may be a reason for the lower-than-expected alfalfa carbon sequestration.

Not only does alfalfa sequester soil organic carbon but growing alfalfa produces less greenhouse gas (GHG) emissions than common row crops. Hoffman et al. (2018) found an organic rotation of corn-soybean-wheat-alfalfa-alfalfa-alfalfa to reduce total greenhouse gases emissions by 14% when compared with an organic rotation of corn-soybean-wheat. This is mainly due to less tillage during the alfalfa years. Tillage requires high fuel consumption increasing total GHG emissions.

### 2.6. Water infiltration

Alfalfa can significantly increase water infiltration. A slow infiltration can be a problem in shrink-swell clays such as those found in the Red River Valley of the North and other areas of the continental U.S. Mitchell et al. (1995) compared the ability of different types of macropores in the soil to conduct water during a heavy rainfall. In this study, they used flood irrigation and a blue methylene solution to track preferential water flow. In wheat stubble, the only type of preferential water flow was that which flowed through the cracks in the soil. The wheat did not produce any root channels capable of water flow after three months of the stubble lying fallow.

In the same study by Mitchell et al. (1995), the soil under alfalfa was able to conduct preferential water flow through macropores of three types: cracks in the soil, earthworm channels, and alfalfa root channels. The cracks were found to close after 10 min of ponding like those in the wheat stubble. The earthworm channels were not stable enough to survive the swelling of the soil during the influx of water. Living alfalfa root channels carried dye and therefore water down to a depth of 16 cm during the initial flooding but after 30 minutes, alfalfa living root channels did not carry water past 5 cm. This is likely due to the shrink-swell characteristics of the clay soil in which the experiment was conducted. However, root channels formed by decaying and dead alfalfa roots were found to carry water effectively, unlike the smaller roots from the wheat. The decaying alfalfa roots were able to carry dye and water past 55 cm. These root channels continued to carry water and dye to this depth even after 30 minutes of ponding, which demonstrates the root channel's ability to survive the swelling of the soil during heavy rain events. It should be noted this study took place on a low organic matter soil, 0.9% in the top 0.5-m of soil (Mitchell et al. 1995).

These findings are supported by a study done by Guo et al. (2019) which focused on comparing infiltration rate and cumulative infiltration in alfalfa and bare soil. Alfalfa was shown to increase infiltration rate by 27.7%, which in large rain events could substantially reduce soil erosion and nutrient run-off. Soils having alfalfa plants were also able to take up 1.13 times more water during a rain event than bare soil.

Alfalfa could increase infiltration even in environments with high soil compaction. Three years after seeding, under repeated heavy compaction on 100% of the soil surface, alfalfa was able to increase infiltration in the plot by 120% compared with the first-year post-seeding (Meek et al., 1989). In the same study, within a different treatment, they simulated the compaction caused by a grower planting and harvesting alfalfa which resulted in 48% of the area receiving wheel traffic. In this treatment, alfalfa increased infiltration by 160%. In plots with no compaction, infiltration was increased by 260%. Infiltration increased steadily throughout the three-year experiment and is mainly attributed to preferential water flow through macropores provided by an increasing number of dead alfalfa root channels.

## 2.7. Weed suppression

Alfalfa interacts with weeds in several ways. Alfalfa expresses allelopathy as well as autotoxicity, a specific type of allelopathy, as a natural means of competing against neighboring weeds and its own progeny, respectively (Chonet al., 2006). Established alfalfa is relatively resilient when competing with weeds due to its extensive root system and large tap root. Alfalfa is known to be highly competitive for water as well. The harvest practices common to alfalfa involve cutting it two to four times per year in the Midwest, which reduces weed pressure and limits the ability for weeds to go to seed.

A study by Clay and Aguilar (1998) compared the effect of alfalfa in a corn rotation for reducing weeds. Treatments consisted of continuous corn and alfalfa-alfalfa-corn. Each treatment was divided into 1) high input, with recommended fertilizer application, pre- and post-emergence herbicide and insecticide; 2) medium input, with half the recommended fertilizer application rate and post-emergence herbicide and insecticide; 3) low input, with no herbicide, insecticide, or fertilizer. In the low input sub-treatment, the alfalfa-alfalfa-corn rotation reduced broadleaf weed densities by 60-80%, depending on the year, compared with the continuous corn, which can likely be attributed to the reduction of weed seed bank during the two years of alfalfa before planting the corn.

## 2.8. Annual companion crops

Alfalfa can often be found growing in conjunction with other crops such as annual cereal grains for establishment, other perennial forages, and under a row crop during the establishment year. There are many methods for establishing alfalfa. Companion crops, usually cereal grains, seeded with alfalfa may provide several benefits and some disadvantages for growers. Effective alfalfa establishment is critical as very low plant densities cause lower forage yields (Berti and Samarappuli, 2018).

Oat (*Avena sativa* L.) as a companion crop may reduce alfalfa stand density in the first and second harvests in the first year (Hoy et al., 2002) especially through competition for light (Janson and Knight, 2012). A study in Iowa by Hoy et al., (2002) showed that even though stand density (stems $m^{-2}$) was low during the first harvest in the first year when seeded with oats, by the time of the second harvest, alfalfa stem density had recovered to a level comparable to alfalfa which was directly seeded. This may be less evident in more northern climates where only two harvests in the first year are possible. When harvested for forage, an oat companion crop can provide higher total forage yield in the alfalfa seeding year than alfalfa seeded alone. Oat as a companion crop may also reduce weed densities in the seeding year (Hoy et al., 2002).

Forage barley (*Hordeum vulgare* L.) is another companion crop often seeded with alfalfa to suppress weeds and protect alfalfa during its establishment. Alfalfa stand density is affected by the date at which companion crops are harvested, presumably due to increased canopy cover as the companion crop continues to grow. Tan and Serin (2004) showed harvesting barley for grain reduced stand density by 10 plants $m^{-2}$ compared with harvesting barley at the milk-dough stage for forage. A barley companion crop, with a seeding rate of 180 kg $ha^{-1}$, was shown to reduce the weed content of the total forage by up to 94%. Weed biomass was also reduced in the second year of alfalfa in the treatments where 180 kg $ha^{-1}$ of barley was seeded compared with alfalfa seeded without a companion crop (Tan and Serin, 2004). They also found that while increasing the seeding rate of barley from 60 to 180 kg $ha^{-1}$ did reduce weed biomass, it did not have any significant impact on alfalfa stand density although all seeding rates of barley provided denser alfalfa stands than seeding alfalfa alone. Zaman et al., (2003) found that weeds in alfalfa forage in the establishment year did cause significant economic impact, which matches the findings of a previous study by Smith et al. (1997). However, some mixes of weeds, barley companion crop, and alfalfa were shown to be more digestible than pure alfalfa. This indicates that depending on the specific weed problems in a field, weed control may not need to be of high concern when establishing alfalfa. Palatability and toxicity of weeds present should be considered when deciding on an establishment method (Zaman et al., 2003).

Wiersma et al. (1999) compared four different establishment methods for alfalfa as well as other legumes. The companion crops they compared were oat, oat + field pea (*Pisum sativum* L.), annual ryegrass (*Lolium multiflorum* L.), and festulolium (*Festulolium braunii* K.A.). They found annual ryegrass seeded with alfalfa provided substantially higher relative feed values than oat and oat + field pea seeded with alfalfa in the first year. Annual ryegrass seeded as a companion crop also proved to yield just as much as the oat + field pea in the first year. Festulolium seeded with alfalfa had a slightly higher relative feed value than annual ryegrass but also had a slightly lower forage yield. Annual ryegrass and festulolium both provided better overall performance in the first year than oat or oat + field pea. However, where annual ryegrass was used as a companion crop in the first year, there was a reduction of alfalfa forage yield of

0.67 Mg $ha^{-1}$ compared with all other treatments. Wiersma et al., (1999) note that using annual ryegrass as a companion crop during a year that is exceptionally favorable to the growth of annual ryegrass could lead to a substantial reduction in alfalfa stand density.

## 2.9. Alfalfa-grass mixtures

Alfalfa-grass mixtures have been shown to increase forage yield compared with alfalfa or grass grown alone (Malhi et al., 2002). Nevertheless, other researchers have not found a significant yield difference between alfalfa grown alone and alfalfa grown with a grass (Aponte et al., 2019; Foster et al., 2014). Fiber digestibility was higher in certain alfalfa-grass mixtures than alfalfa grown alone (Veiral et al., 2003). The grasses used in mixtures include, but are not limited to, smooth bromegrass (*Bromus inermis* L.), meadow bromegrass (*Bromus riparius* Rehmann), crested wheatgrass [*A. desertorum x A. cristatum* (L.) Gaertn], tall wheatgrass [*Thinopyrum ponticum* (Podp.) Z.-W.Liu & R.-C.Wang], intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], western wheatgrass [*Pascopyrum smithii* (Rydb.) A.], reed canarygrass (*Phalaris arundinacea* L.), orchardgrass (*Dactylis glomerata* L.), tall fescue [*Schenodorus arundinaceus* (Schreb.) Dumort.], meadow fescue (*Schedonorus pratensis* Huds.), Kentucky bluegrass (*Poa pratensis* L.), and timothy (*Phleum pratense* L.) (Aponte et al., 2019; Bélanger et al., 2017). Adding alfalfa to grasses provided a more consistent forage yield throughout the year (Aponte et al., 2019).

## 2.10. Corn-alfalfa intercropping

Corn-alfalfa intercropping is a popular practice and research topic in China, especially in the North China Plain (Xu, et al. 2021), the Corn Belt of northeast China (Sun et al., 2018) and the Loess Plateau (Wu, et al. 2022). Intercropping systems in China vary considerably in row spacing, harvest timing, and end goal.

In the northern Great Plain of China the weather patterns prevent a profitable alfalfa harvest in the summer months although corn does quite well. Because the alfalfa grows better in the spring and corn grows better in the summer, Xu. (2021) found that a hybrid relay/intercropping system was more efficient than a monocropping system of either crop. The relay cropping system involves establishing alfalfa with 20-cm row spacing and leaving gaps or removing an alfalfa row every 60-cm. After the first alfalfa harvest corn is planted into these gaps and after maturing corn and alfalfa are harvested for biomass. The alfalfa was harvested twice before corn planting in the following years and the plots lasted three years in total. Although alfalfa yield and corn yield were both reduced when intercropped, together they yielded more over the course of the cropping sequence with high cropping efficiency.

In the Corn Belt of northeast China, Sun et al. (2018) found an intercropping system more related to strip cropping to be most profitable. In this trial after every three rows of corn, one row was replaced by either three rows of alfalfa with 30-cm row spacing or five rows of alfalfa with 20-cm row spacing. Monocrop check treatments of corn alone and alfalfa alone were also planted. Intercropping with five rows of alfalfa produced more total biomass than intercropping with three rows of alfalfa or corn planted alone.

In the Loess Plateau, Wu et al. (2022) compared three intercropping treatments, one row of corn and two rows of alfalfa, two rows of corn and two rows of alfalfa, and two rows of corn and four rows of alfalfa. They found biomass yield of corn to increase when intercropped as compared to corn alone and alfalfa yield from intercrop treatments varied relative to alfalfa alone over two years. They found intercrop land use efficiency to be higher in the intercrop treatments compared with the alfalfa alone. The treatment with two rows of corn and four rows of alfalfa was considered to have the highest light use efficiency and total productivity.

Corn-alfalfa intercropping is relatively unheard of in the U.S. and has only become a viable practice since the final approval of glyphosate-tolerant alfalfa. The practice involves planting corn and alfalfa together in the spring and allowing the alfalfa to establish itself while the corn is grown for harvest. In most cases the goal of this practice is to avoid the low yielding establishment year of alfalfa. By using environmental resources more efficiently and completely, intercropping systems are often able to yield more in total profit than mono-cropping systems (Mousavi and Eskandari, 2011).

Several factors determine the success of corn-alfalfa intercropping for alfalfa establishment. These are mainly corn yield in year one, alfalfa stand following year one, and alfalfa yield following year one. Berti et al. (2021a) found alfalfa, when intercropped with corn, to reduce corn grain yield by 14-19% and Patel et al. (2021) found a reduction of 23-26%. When considering corn biomass Grabber et al. (2016) found an average reduction of 10% when corn was intercropped with alfalfa. Berti et al. (2021b) found no corn biomass yield reduction when corn and alfalfa were intercropped at 76-cm corn row spacing although there was one instance of corn yield reduction at a narrower row spacing. In contrast, a different study by Berti et al. (2021a) found a corn biomass yield reduction of 16-26%.

Alfalfa establishment and forage yield following establishment are key to a productive, profitable multi-year alfalfa stand. In a study spread out across three states Berti et al. (2021a) found that alfalfa intercropped with corn yielded less in the second year than alfalfa established alone. This reduction varied by location, but the total range was 14% to 47%. The reduction was mostly from a difference in the first cut in the second year indicating that the difference in treatments may not be present in the third year. These results were supported by Patel et al. (2021) who also observed lower alfalfa stem density and biomass yield in the first alfalfa production year of intercropped alfalfa compared with alfalfa established alone. Patel et al. (2021) found that there was not a significant difference in stem density or biomass yield in the second production year between intercropped alfalfa and alfalfa established alone.

In a field trial by Grabber (2016), intercropping of alfalfa with corn for silage was studied to determine the effect of plant growth retardants on alfalfa establishment. They found that alfalfa when intercropped with corn for silage produced 85% more forage yield in the first harvest year than alfalfa which was seeded alone in the spring. Applying plant growth retardants slowed the growth of alfalfa during the periods of high growth of corn and subsequently allowed for increased alfalfa stand survival and growth after corn was harvested for silage. The plant growth retardants increased alfalfa yield by 15% compared with intercropped alfalfa without growth retardants. In later studies by Berti et al. (2021a,b), growth retardants did not increase alfalfa stand or alfalfa forage yield in the second year.

### 2.11. Economics of corn-alfalfa intercropping

Grower success and profitability with this new cropping system will determine its rate of adoption by farmers in the Midwest. Profitability will change as the prices of corn and alfalfa fluctuate independently of each other. However, by using average prices and expenses we can get an idea of the economies which affect this intercropping system.

Using prices and expenses reported by growers from 2008 to 2018 the average gross return from corn in northwest Minnesota was \$1566 ha$^{-1}$ at an average corn price of \$0.17 kg$^{-1}$. The average gross return on alfalfa acreage across the whole state of Minnesota was \$1608 ha$^{-1}$ at an alfalfa price of \$124 Mg$^{-1}$. While the gross income of both crops was very similar, the return on labor and management was much greater for alfalfa at \$506 ha$^{-1}$ compared with corn which was \$133 $ha^{-1}$ (University of Minnesota Extension, 2020). This provides evidence that alfalfa can be a profitable crop in this region.

Osterholz et al. (2020) used extensive forage yield data from several years to create a model to predict the profitability of intercropping alfalfa with corn silage. They analyzed the net returns of intercropping alfalfa with corn for silage in many crop sequences. They found that intercropping alfalfa with corn for silage provided an increase in predicted net returns of 7-33%. Establishment success of alfalfa was built into the model as well as corn yield drop due to stress from intercropping with alfalfa. The most profitable intercropping crop sequence was found to have an advantage over the most profitable conventional sequence at all alfalfa establishment success rates above 33%. The most profitable intercropping sequence was also found to have an advantage at all corn yields in the seeding year with a yield reduction of less than 20%.

When alfalfa establishment was assumed to be 90% successful and corn yield loss was held at 5% or less the most profitable intercropped sequence out yielded the most profitable conventionally seeded sequence by 15% (Osterholz et al., 2020). Intercropping also allowed for a shorter alfalfa stand life of three harvest years, compared with four harvest years for conventionally seeded alfalfa (Osterholz et al., 2020). This means growers who intercrop alfalfa can rotate alfalfa across more hectares in a shorter time, thereby obtaining the ecological and economic benefits of alfalfa on more acreage.

In an economic analysis, Berti et al. (2021b) compared changes in price rather than changes in yield across corn alfalfa intercropping and continuous corn treatments. Intercropping silage corn with alfalfa was found to be a more profitable two-year sequence compared with continuous corn for silage at all but the lowest alfalfa prices and highest corn prices. When alfalfa was priced at \$166 Mg $ha^{-1}$ and corn silage was priced under \$36.6 Mg $ha^{-1}$ intercropping corn and alfalfa was still more profitable than a continuous corn sequence - even when assuming a corn biomass yield reduction of 30% when intercropping corn and alfalfa.

# 3. MATERIALS AND METHODS

## 3.1. Experimental approach

The experiment was planted at two locations, one near Prosper, ND (46°59’56.1” N, -97°06’53.0” W, elevation 280 m), and a second near Hickson, ND (46°38’13.2” N, -96°49’25.0” W, 280 m elevation), in 2020 and 2021. The soil type at the Prosper location is a Kindred-Bearden silty clay loam and the soil type at the Hickson location is Fargo-Hegne silty clay (Kindred: Fine-silty, mixed, superactive, frigid Typic Endoaquolls; Bearden: Fine-silty, mixed, superactive, frigid Aeric Calciaquolls; Fargo: fine, montmorillonitic, frigid, Vertic Haplaquol; Hegne: fine, smectitic, frigid Typic Calciaquerts) (Soil Survey, 2016).

Two experiments were established in 2020 and two more were established in 2021. Each experiment was laid out in a randomized complete block design consisting of four replicates and five treatments. The five treatments in Year 1 corresponds to five specific crop sequences. Treatments in Year 1 were as follows: 1) corn (76-cm row spacing), 2) corn (76-cm row spacing) + alfalfa intercrop, 3) alfalfa alone, 4) corn (152-cm row spacing), 5) corn (152-cm row spacing) + alfalfa intercrop. In the second year, Treatments 1 and 4 were seeded with alfalfa in the spring whereas Treatments 2, 3, and 5 had alfalfa established in Year 1. Corn was harvested in Treatments 1, 2, 4, and 5 in Year 1 only. Treatment 3, alfalfa alone, was harvested twice in Year 1 in 2020, and three times in 2021. Every experimental unit with corn included four total rows of corn, two rows in the middle of each plot for harvest and one row on each side as a border. In Year 2, established alfalfa was harvested four times, and the new seeding was harvested three times in 2021.

In 2020, corn and alfalfa were planted on 21 May. In 2021, corn and alfalfa were planted between May 11 and May 13. The corn was an 85-day VT double pro hybrid 75k85, produced by Peterson Farm Seeds. All corn was planted as closely as possible to 86,485 pure live seeds (PLS) ha$^{-1}$ in both 76-cm and 152-cm row spacing treatments. This means in the 152-cm row spacing treatments there was double the number of corn plants per linear unit of measure in the corn row. Corn was planted with a 4-row plot planter (Almaco, Nevada, IA). The alfalfa seed was Cropland brand glyphosate-resistant and potato leafhopper-(*Empoasca fabae*) resistant cultivar, RR Vamoose. Alfalfa PLS seeding rate was 11.2 kg ha$^{-1}$. Alfalfa was seeded immediately after corn with a plot drill (Wintersteiger, Austria) with 15-cm spacing.

### 3.2. Weather and growing degree days

The North Dakota Agricultural Weather Network (NDAWN, 2020) weather station in Prosper, ND was used to measure minimum/maximum temperature and rainfall for the experiments located in Prosper, ND. The NDAWN weather station in Sabin, MN was used to record minimum/maximum temperature for the experiments located in Hickson, ND. Rainfall for experiments in Hickson, ND was measured on site by KayJay Ag Services. A base temperature of 10□ and maximum temperature of 30°C was used for determining corn growing degree days (GDD) (Badh and Akyüz, 2010). For alfalfa 5□ was used as the base temperature and no maximum temperature was used (Sanderson, 1992). When calculating corn GDD, if the minimum or maximum temperature was lower than 10□, it was set equal to 10□ and any minimum or maximum temperature higher than 30□ was set equal to 30□ When calculating alfalfa GDD, if the minimum or maximum temperature was lower than 5□, it was set equal to 5□ (Akyüz and Ransom, 2015). To calculate growing degree days on a daily basis we used the following equation.

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

### 3.3. Soil sampling and fertility

Soil samples were collected before planting from every experimental unit at 0-15 cm and 15-61 cm. Soil collected from 0-15 cm was analyzed for $NO_3$-N, P, K, pH, and organic matter. Soil from 15-61 cm was analyzed for $NO_3$-N only. Soils were then sampled again in the fall after harvest at 0-15 cm and 15-61 cm for $NO_3$-N. Soils were also sampled in the spring after Year 1 establishment in the same manner as the first year for N, P, K, pH, and organic matter at 0-15 cm and $NO_3$-N only at 15-61 cm.

In 2020, the soil test results showed an average P concentration from 0-15 cm of 12.5 mg $kg^{-1}$ in Hickson, and 29.3 mg $kg^{-1}$ in Prosper. Taking this into consideration, the experiment in Hickson was fertilized with 56 kg $ha^{-1}$ of $P_2O_5$ using mono ammonium phosphate (MAP) (11:52:0) as the source of $P_2O_5$. This was broadcast on the entire experiment in Hickson. Soil samples had an average of 95 kg $ha^{-1}$ of $NO_3$-N in Hickson, and 91 kg $ha^{-1}$ of $NO_3$-N in Prosper. Hickson experiments were fertilized with 112 kg $ha^{-1}$ of N and Prosper experiments, with 134.5 kg $ha^{-1}$ of N in the form of urea. The amount of urea was adjusted for the Hickson location based on the amount of N from MAP.

In 2021, the established experiments carried over from 2020 did not receive any fertilizer. In 2021, the new experiment in Hickson received 67.3 kg $ha^{-1}$ of $P_2O_5$ in the form of MAP based on an average soil test of 6.45 mg $kg^{-1}$ of P. Hickson also received 134.5 kg $ha^{-1}$ of N in the form of urea. The 14.2 kg $ha^{-1}$ of N from the MAP was considered. In 2021, in Prosper, the new experiment only received 134.5 kg $ha^{-1}$ of N in the form of urea.

In both years, urea was broadcast on Treatments 1, 2, 4, and 5; no urea was spread on alfalfa alone. In Treatments 4 and 5, urea was spread on a 76-cm width centered over each corn row. This was to simulate how a grower would sidedress N in a field at 152-cm corn row spacing to avoid spreading N too far from the corn.

### 3.4. Weed control

All treatments were sprayed with only glyphosate (N-phosphono methyl glycine) (specifically, Roundup Power Max) at a rate of 0.438 L $ha^{-1}$ as the corn hybrid and alfalfa cultivar were both glyphosate resistant.  Glyphosate was generally sprayed twice on each experiment in Year 1. In Year 2, glyphosate was sprayed twice on the newly seeded alfalfa only. Clethodim ((E)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one)(specifically, Select Max) was sprayed on Year 2 experiments twice to kill volunteer corn from the previous season. Glyphosate was also used around the experiments to keep alleys and small spaces between experimental units clean.

### 3.5. Other in-season measures

Stand counts of corn were evaluated between two to four weeks after emergence. Emergence was calculated by measuring and flagging 1-m in both center corn rows and counting plants in those 2-m within each treatment. End-of-year corn stand counts were calculated during the corn harvest procedures. In 2020, alfalfa emergence was calculated on 15 July in both locations. In 2021, new seeding alfalfa emergence and established alfalfa stand counts were taken on 19 July in Prosper and 23 July in Hickson. In both years, emergence was calculated later than expected; this delay was due to slow emergence of alfalfa likely due to light soil crusting in 2020 and lack of water in 2021. To measure alfalfa emergence, we measured 0.61 m near the middle of the plot and counted plants in each of the two rows bordering the measurement. This gave us the number of plants in 0.19 $m^2$. Emergence and stand counts were then calculated for plants per $m^2$. Alfalfa stand counts were measured at the beginning and end of each subsequent growing season.

Gravimetric water content was collected from the first-year experiments three times in 2020 and six times in 2021 throughout the growing seasons. One soil sample, 0-15 cm, was collected in every experimental unit. In treatments with corn the soil sample was taken approximately 30-38-cm from the corn row. A wet weight was acquired from each sample and after 24 h of drying at 105□ a dry weight was measured. Wet and dry weights were then used to determine soil water content using methods from Black (1965).

Normalized difference vegetation index (NDVI) and red edge normalized difference vegetation index (RENDVI) was measured using a DJI "Matrice 100" unmanned aerial vehicle (UAV) in 2020 and a DJI "Matrice 300 RTK" UAV in 2021 (DJI, Nanshan, Shenzhen, China) with a Red Edge MX multispectral camera (MicaSense, Seattle, WA, USA) and a DJI Zenmuse X3 color camera in 2020. In 2020, aerial imagery was collected every two weeks starting 22 June 2020 and ending 2 November 2020. In 2021, NDVI and RENDVI were collected using the same Red Edge MX multispectral camera, but color imagery and thermal imagery was collected with a DJI Zenmuse XT2 camera. In 2020 and 2021, Pix-4D (Prilly, Switzerland) was used to create orthomosaic whole field images. These orthomosaic images were then processed in ArcGIS version 2.9 (Redlands, CA, US) to measure variables of interest.

Photosynthetic activity and thereby plant health can be estimated using NDVI and RENDVI (Tucker, 1979). Values are expressed as a decimal between zero and one with higher values indicating more photosynthetic activity and better plant health. Our goal was to find differences between treatments in Year 1 of the study using these indicators. We also collected thermal imagery in the hope of identifying water stress; we assumed leaves which were transpiring less due to water stress would be higher in temperature than leaves transpiring greater amounts of water.

Photosynthetically active radiation (PAR) and leaf area index (LAI) were measured four times throughout the 2020 growing season and six times throughout the 2021 growing season. These readings were only taken from experiments in Year 1. This was done by positioning a ceptometer (LP-80, Decagon Devices, INC., Pullman, WA) between the two-center rows of corn just above the soil. In the alfalfa alone treatment, the below canopy reading was taken as low to the ground as possible. A reading from above and below the canopy was taken simultaneously to calculate light intercepted by the crop canopy. Three readings were taken in different locations within each experimental unit; these three readings were then averaged. The formula for intercepted PAR is:

$$\text{Intercepted PAR (\%)} = (\text{Light readings above the canopy} - \text{Light readings below the canopy})/(\text{Light readings above the canopy}) \times 100$$

The formula for LAI is:

$$\text{LAI} = (\text{Leaf area (m}^2\text{)})/(\text{Ground area (m}^2\text{)})$$

Plant height and reproductive stage of alfalfa plants was measured at the time of each harvest for Treatment 3 in Year 1 and for all Treatments in Year 2. Plant height of corn was measured at harvest and averaged across two plants in each experimental unit.

### 3.6. Harvest measures

Corn grain was harvested on 13 October in 2020 and on 24 September in 2021. Corn grain was harvested by collecting corn cobs from two linear meters from each of the two-center rows in each experimental unit for a total of four linear meters of corn row. The corn cobs were then placed in burlap sacks and dried at 50°C for 14 days or more. Corn was then shelled using a

Agriculex SCS-2 corn sheller (Guelph, Ontario, Canada) in 2020 and an Almaco MAZ corn sheller (Nevada,IA) in 2021. During the shelling process the number of corn cobs were counted to calculate the number of plants in four linear meters of row in each plot and a stand count in plants $ha^{-1}$ was calculated. Shelled grain was then weighed and that mass in combination with the moisture content was used to calculate a yield in Mg $ha^{-1}$. Two whole corn plants were collected from each experimental unit at the same time as grain harvest. These two plants were collected from outside the grain harvest sample area. The height of each plant was recorded, and the plant was placed in a burlap sack and dried at 50°C for 14 days or more. The sack with two whole corn plants was then weighed. The corn grain on the cob of each of these plants was then shelled and weighed as well. Corn biomass without grain was calculated and analyzed. We also used these measurements to calculate harvest index with the following equation:

$$\text{Harvest index} = (\text{Corn grain}) / (\text{Whole corn plant} - \text{corn grain})$$

Alfalfa from Treatment 3, alfalfa alone, was harvested twice in 2020 and three times in 2021 in the seeding year. Alfalfa from intercropped Treatments 2 and 5 was harvested by hand at the end of the seeding year. The alfalfa sample was taken from two adjacent alfalfa rows 0.6 linear meters in length for a total area of 0.18 $m^2$. In 2021, alfalfa established in the previous year was harvested four times and the new seeding alfalfa in Year 2 was harvested three times. Alfalfa harvest was done with a flail forage harvester (Carter, Brookston, IN) by harvesting one swath 0.9-m wide by 7.6-m long, the length of one plot, in the first year. Plots were trimmed down to 6.1-m long in the second year before each harvest to remove any edge effect. Biomass yield with the flail forage harvester was taken by using an onboard scale. This yield was corrected for dry matter by extracting a sample from the cutting of each plot of approximately 0.5 kg wet weight. This sample was stored in a burlap sack and a wet weight was taken immediately upon harvesting of the sample. The sample was then dried at approximately 50°C for 14 days or longer. The dry weight of the sample was then collected, and the weight of the bag subtracted to calculate the moisture of the alfalfa during harvest. This moisture calculation was then used to determine the dry matter yield of each experimental unit in combination with the weight recorded from the flail forage harvester.

The same dried alfalfa sample used to calculate moisture content at harvest was also used in the chemical analysis of the alfalfa biomass. The sample, once dried and weighed, was ground through a 1-mm mesh with a Model 4 cutting mill (Eberbach Corporation, Ann Arbor, MI, US). The ground samples were analyzed using an XDS Near Infrared Rapid Content Analyzer calibrated with software purchased from Foss (Foss, Copenhagen, Denmark). Alfalfa biomass samples were analyzed for crude protein (CP), ash, K, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber digestibility (NDFD), ether extract (EE). From these components, dry matter intake (DMI), non-fiber carbohydrates (NFC), total digestible nutrients (TDN), and relative forage quality (RFQ) were calculated (Schroeder, 2004):

$$DMI = \left(\frac{120}{NDF}\right) + (NDFD - 45)\ x \left(\frac{0.374}{1350}\right) x\ 100$$

$$NFC = 100 - Ash - CP - EE - NDF$$

$$TDN = (NFC\ x\ 0.98) + (CP\ x\ 0.93) + (FA\ x\ 0.97\ x\ 2.25) + \frac{NDF\ x\ NDFD}{100} - 7$$

$$RFQ = \frac{DMI\ x\ TDN}{1.23}$$

Corn grain was analyzed for quality using the same XDS Near Infrared Rapid Content Analyzer with a calibration purchased from the Foss corporation for corn whole grain. Corn grain was analyzed for ash, moisture, fat, CP, and fiber. Corn biomass from the same samples used to collect height, biomass yield, and harvest index was ground through a 1-mm mesh with the same Model 4 cutting mill and analyzed for ADF, starch, dry matter (DM), NDF, ADL, CP, phosphorus (P), and ash. Only significant effects are shown in the results.

### 3.7. Statistical analysis

Year one of the cropping sequence was evaluated with four total experiments. Two experiments from 2020 and two experiments from 2021 with two locations in each year, Prosper and Hickson, ND. Year two of the cropping sequence was evaluated in 2021 using the two experiments established in 2020.

The MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary NC) was used to analyze our data and predict significant interactions. Data collected consistently over time and throughout the growing season was analyzed as repeated measures; this included all UAV collected data, gravimetric water, LAI, and intercepted PAR in Year 1 and alfalfa harvests (Cut) in Year 2. It is important to note that alfalfa forage yield and nutritive value for Cut 1 was analyzed separately as a single measure because we could not analyze all four cuts of the established alfalfa and include the three cuts from the newly seeded alfalfa together without experiencing errors due to the missing data due to the difference in the number of cuts. For this reason, we split off data from the first cut of the established alfalfa and analyzed it separately. We decided the three cuts for the newly seeded alfalfa aligned best with the second, third, and fourth cuts of the established alfalfa due to them being close in physical harvest date. We then analyzed them as Cuts 2, 3, and 4 (corresponding to Cut 1, 2, and 3 for the established alfalfa) as repeated measures.

Data collected was analyzed separately by location and by year and the data was combined when variances were homogeneous. The combination of location and year was called hence forth “environment”. For metrics collected only in one year, we use the term “location” in the analysis. For metrics in which two locations from each year were combined we use the term "environment". Location, replicate, and environment were analyzed as random effects; treatment, sampling or harvest date, and treatment by sampling or harvest date were analyzed as fixed effects. For mean separation the least significant differences (LSD) at 95% confidence interval was used. The appropriate LSD values were calculated by multiplying the standard error for the pair differences (pdiff) in PROC MIXED by the *t*-table value for the degrees of freedom of the corresponding error used to calculate the *F*-value of an individual source of variation.

### 3.8. Cost analysis

A cost analysis was conducted for each of our two year cropping sequences: i) Year 1- corn alone at 76-cm row spacing, Year 2- spring-seeded alfalfa; ii) Year 1- corn intercropped with alfalfa at 76-cm row spacing, Year 2- established alfalfa; iii) Year 1- spring-seeded alfalfa, Year 2- established alfalfa; : iv) Year 1- corn alone at 152-cm row spacing, Year 2- spring seeded alfalfa; vi) Year 1- corn intercropped with alfalfa at 152-cm row spacing, Year 2- established alfalfa.

The metrics to evaluate these cropping sequences came from several sources which we found to be the most robust and up to date. Costs associated with alfalfa production were taken from Iowa State Extension (2022). Several costs from this budget were modified to fit our specific cropping sequence.

Herbicide cost for intercropping corn and alfalfa or spring seeded alfalfa alone was assumed to require two applications of glyphosate. For this reason, a quote was obtained from an agronomic supply company (Allied Cooperative, Galesville, Wisconsin) for \$15.9 $L^{-1}$ of Roundup PowerMAX 3 herbicide. Assuming an application rate of 2.33 L $ha^{-1}$ our cost per hectare per application was estimated at \$37 $ha^{-1}$.

Alfalfa seed was another cost we priced separately from the budget because we used a glyphosate-resistant variety and specifically RR Vamoose which was priced by the same agronomic supply company at $412 per 22.7 kg bag of seed. After correcting for germination and inert material there is 13.2 kg of PLS in each bag. Therefore, the price per kg of PLS is $31.3 and with our seeding rate of 11.2 kg ha$^{-1}$ of PLS the cost of alfalfa seed was estimated at $351 ha$^{-1}$.

Fertilizer cost for alfalfa from the budget by Iowa State Extension (2022) changed based on sequence. In the seeding year, we assumed an application rate of 39 and 140 kg ha$^{-1}$ P and K, respectively based on average application according to soil test and removal of nutrients in the seeding year. When alfalfa was established, we based fertilizer cost on a removal basis assuming a removal rate of 13 and 50 kg ha$^{-1}$ P and K per Mg of dry matter harvested, respectively. Therefore, in each sequence our fertilizer cost when alfalfa was alone varied based on the actual average forage yield observed for that treatment.

Production costs for alfalfa from the budget by Iowa State Extension (2022) also were modified to account for the cost each cut for alfalfa adds to the operation. Fixed costs per cut included mowing, raking, and baling, and they totaled $43.97 ha$^{-1}$ per cut. Variable costs for the same activities totaled $29.89 ha$^{-1}$ per cutting. Trucking costs were based on forage yield and therefore also changed with our specific results, but they were $1.80 and $2.76 for fixed and variable costs respectively per Mg of dry matter produced.

Labor costs for alfalfa harvest were also based on the number of cuts in each harvest. Labor required for alfalfa production in the seeding year was assumed to be 2.5 h ha$^{-1}$ before harvest and 3.7 h ha$^{-1}$ per seeding year harvest. In the production years it is assumed to take 3.3 h

$ha^{-1}$. The labor cost is estimated at \$17 $h^{-1}$ for alfalfa production. For corn production labor is a fixed average cost of \$107 $ha^{-1}$ from NDSU Extension (2021).

Costs associated with growing corn were all from a three-year average (2019-2021) of corn production cost estimates for the southern Red River Valley released by the North Dakota State University Extension program (NDSU Extension, 2021). Land charge or rental equivalent cost was taken from these estimates as well since it likely best represents the cost of land where the research was conducted. Fertilizer for corn production was given as a total average cost in these budgets from the North Dakota University Extension program and was not therefore partitioned to specific rates. The costs for corn production did not change based on our treatments except for herbicide which was assumed to be two applications of glyphosate if intercropped with alfalfa. Otherwise, the average values from NDSU Extension (2021) were used.

Total revenue was based on the price of corn from NDSU Extension (2021) at \$168.5 $Mg^{-1}$ (\$3.86 $bu^{-1}$) and the price of alfalfa from Iowa State Extension (2022) of \$136 $Mg^{-1}$ (\$150 $ton^{-1}$). The yield from each treatment was multiplied by the price to find the total estimated revenue from each treatment and costs were subtracted to show net revenue.

A sensitivity analysis was conducted to show the interaction in net revenue of each treatment and the price of each commodity. All costs were kept the same as the projected two-year sequence budget for each treatment. Prices of alfalfa ranged from \$91 to \$182 $Mg^{-1}$ (\$100 to \$200 $ton^{-1}$) and prices for corn ranged from \$138 to \$321 $Mg^{-1}$ (\$3 to \$7 $bu^{-1}$) to reflect current prices of alfalfa and corn.

# 4. RESULTS AND DISCUSSION

## 4.1. Rainfall and GDD

Total rainfall, between 1 April and 31 October was similar between the 2020 and 2021 growing seasons at 41- and 38-cm respectively. However, rainfall from 1 May to 31 August 2021 was less during the hottest part of the year. During this timeframe, 2020 received 29 cm of rainfall while 2021 only received 18 cm of rainfall. The average maximum temperature during this time was higher in 2021 at 27.1 °C than in 2020 at 25.2 °C. Corn GDD were similar between years; when measured between planting and harvest corn accumulated 1326 and 1372 GDD in 2020 and 2021, respectively. Alfalfa GDD for the new seeded alfalfa in 2020 were measured between planting on 21 May and 31 October. Newly seeded alfalfa in 2020 accumulated 2014 GDD. In 2021, newly seeded alfalfa accumulated 2344 GDD between planting on 12 May and 31 October. Established alfalfa accumulated 2522 GDD between 1 April and 31 October.

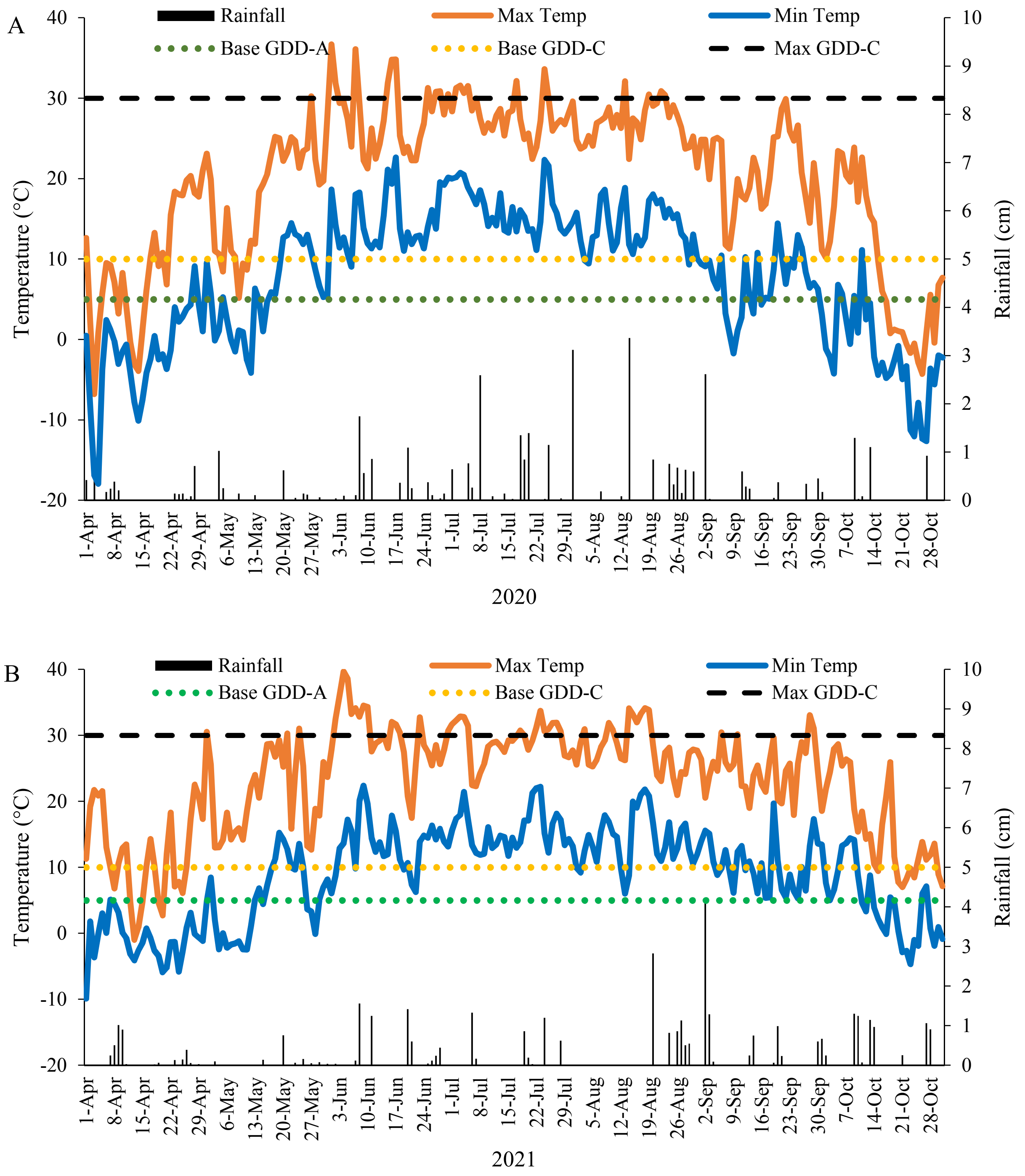


Fig. 1. Daily rainfall (bars above X-axis), base growing degree days for alfalfa (base GDD-A), base growing degree days for corn (Base GDD-C), maximum growing degree days for corn (Max GDD-C), maximum temperature, and minimum temperature of A) 2020 and B) 2021 growing seasons averaged across two locations, Prosper and Hickson, ND.

### 4.2. Soil $NO_3$-N

The decrease in soil $NO_3$-N between the spring and fall in 2021was not significant for the treatment or treatment by environment interaction (Table 1). However, the data on average suggests soil $NO_3$-N may decrease more in plots with alfalfa than those without (Fig. 2). Our experiments averaged 86 kg $ha^{-1}$ of $NO_3$-N before planting.

Patel et al. (2021) did not find a significant difference in fall soil $NO_3$-N between corn-alfalfa intercropped treatments and corn alone. However, they did find alfalfa alone plots to have significantly lower $NO_3$-N than all other treatments in one year of the study. Conversely, Osterholz et al. (2021) found alfalfa intercropped with corn could reduce the soil $NO_3$-N especially between corn harvest in the first year and spring of the second year. Additionally, during this time period significant N stratification occurred resulting in the reduction of soil $NO_3$-N at depth and an increase in soil $NO_3$-N near the soil surface. This is attributed to the alfalfa plant gathering $NO_3$-N at depth and when vegetative growth at the surface dies at the end of the growing season N is then deposited at the surface. This means alfalfa intercropped with corn can help to prevent soil $NO_3$-N losses and eventual ground water contamination. Osterholz et al. (2021) also found alfalfa intercropped with corn to reduce soil, N, and P runoff losses. Although the $NO_3$-N reduction losses in our study were not significant; the average values we observed align well with the results from Osterholz et al. (2021). High variability in soil $NO_3$-N (CV, 114%, Table 1) across sampled area and differences in weather conditions between years likely caused the lack of significance. In 2021, we experienced an extended period of no rain directly after the application of N fertilizer which likely led to severe N losses to volatilization in our high pH soils (Fowler and Brydon, 1989). The lack of rain in 2021 could have caused a lower total N uptake especially in intercropped treatments, which had significantly lower water content in the first 15-cm of soil. Soil water availability has a direct impact on nitrogen uptake (Gheysari et al., 2009).

Table 1. Combined analysis of variance and mean square values for the decrease in soil $NO_3$-N from spring to fall for five treatments (Trt) and four environments (Env) in Prosper and Hickson ND, in 2020 and 2021.

| SOV | % of total variation | df | Decrease in $NO_3$-N† |
|---|---|---|---|
| Env | 19 | 3 | 12315* |
| Trt | 9 | 4 | 4477 |
| Env x trt | 12 | 12 | 1852 |
| Env(rep) | 15 | 12 | 2449 |
| Residual | 44 | 48 | 1762 |
| CV, % | | | 114 |

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

†Soil was sampled to a depth of 61 cm

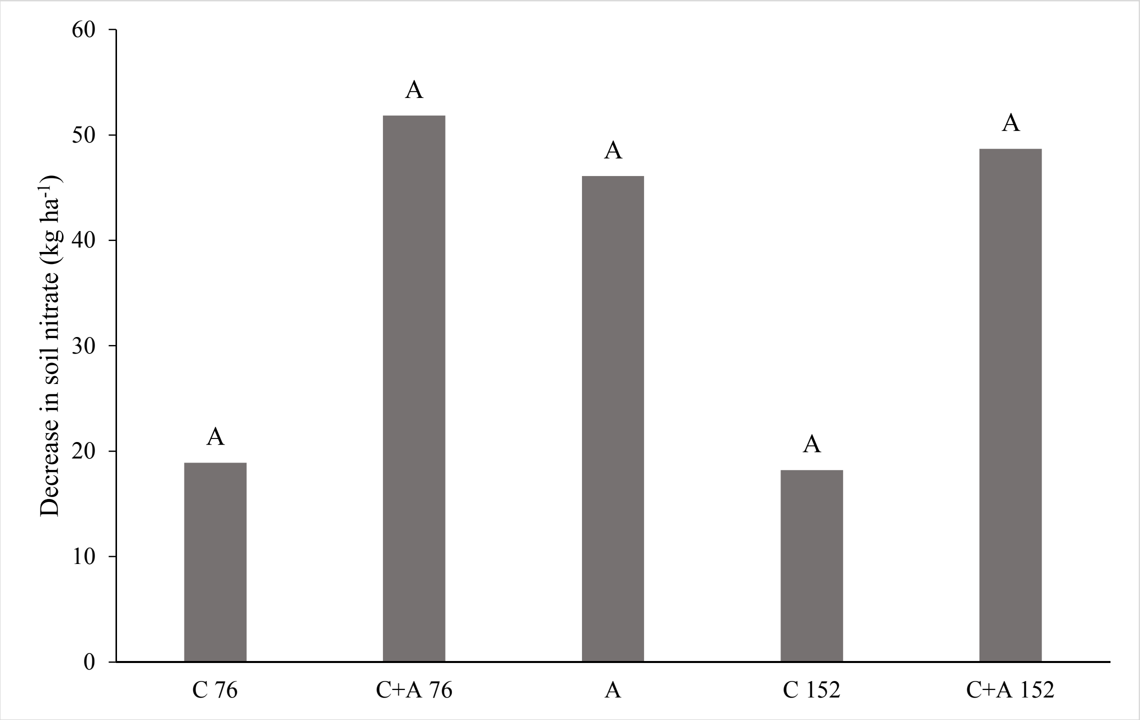


Fig. 2. Decrease in soil $NO_3$-N from spring to fall averaged across two locations, Prosper and Hickson, ND and two years 2020 and 2021. Soil was sampled to a depth of 61 cm. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; A= spring-seeded alfalfa; C 152= corn with 152-cm row spacing; C+A 152= Corn-alfalfa intercrop with 152-cm corn row spacing. Different letters indicate a significant difference between treatments at $P \leq 0.05$.

### 4.3. Soil gravimetric water

In 2020, location, location by treatment and location by date all significantly affected soil gravimetric water concentration (Table 2). This suggests location and possibly weather in each location played a larger role in gravimetric water content than treatment alone. In 2021, treatment, date, location, location by date, location by treatment, and location by date by treatment all significantly affected soil gravimetric water content.

Table 2. Combined analysis of variance and mean square values (MS) of soil gravimetric water content for two locations (Loc), Prosper and Hickson ND, five treatments (Trt), and three sampling dates (Date) in 2020 and six sampling dates in 2021.

| | 2020 | | 2021 | |
|---|---|---|---|---|
| SOV | df | MS | df | MS |
| Trt | 4 | 102 | 4 | 2771* |
| Date x trt | 8 | 240 | 20 | 1165 |
| Date | 2 | 5635 | 5 | 47690* |
| Loc | 1 | 13446* | 1 | 51598* |
| Loc(rep) | 6 | 116 | 6 | 1436* |
| Loc x date | 2 | 441* | 5 | 5960* |
| Loc x trt | 4 | 505* | 4 | 293 |
| Loc x date x trt | 8 | 89 | 20 | 1254* |
| Residual | 84 | 174 | 174 | 316 |
| CV, % | | 6.09 | | 9.39 |

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

In 2021, a steady decline in soil gravimetric water content until the end of August due to a lack of rainfall was observed (Fig. 3). If we assume a bulk density for our soil of 1.10 g cm$^{-3}$ (USDA NRCS, 2008) we find that our experiments were at or under the permanent wilting point of 20% water by volume from 22 July to 18 August 2021 (Huffman et al., 2013). It is important to note that we only measured to 15 cm whereas a deeper sample would have likely measured more soil moisture. Regardless, this likely affected plant growth and increased competition for water in intercropped plots.

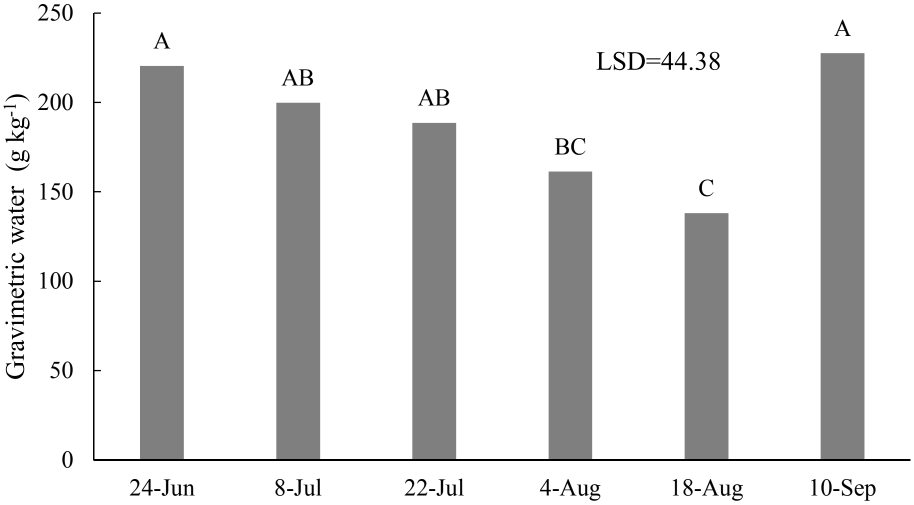


Fig. 3. Soil gravimetric water content measured from 0- to 15-cm on different sampling dates averaged across all treatments, and two locations, Prosper and Hickson, ND, in 2021. LSD= to compare gravimetric water between dates. Different letters between columns indicate significant differences at $P \leq 0.05$.

In 2021, both treatments with corn alone had significantly higher gravimetric water than treatments with the same row spacing with intercropped alfalfa (Fig. 4.). This suggests that adding intercropped alfalfa to either 76- or 152-cm corn row spacing may significantly reduce soil gravimetric water during a dry year but changing corn row spacing may not. Corn alone at 76-cm row spacing was statistically similar to alfalfa alone, intercropped corn, and corn-alfalfa intercrop on 152-cm row spacing. This indicates that widening corn rows from 76 to 152 cm could counteract the reduction in soil gravimetric water content caused by the addition of alfalfa in a corn-alfalfa intercrop.

These results confirm our hypothesis that alfalfa can compete with corn for soil water. We expected intercrop treatments to have lower gravimetric water content due to a higher combined plant density and therefore more water use. Alfalfa can be a strong competitor for water and when intercropping with corn; soil water is generally lower than with corn alone (Sun et al., 2018). Sun et al. (2018) found that when one of every four corn rows was replaced with three rows of alfalfa soil water was lower than corn alone. When one of every four corn rows was replaced by five alfalfa rows soil water was even lower, indicating alfalfa presence and density had a significant impact on soil water content. This could translate to a lower corn grain and biomass yield when intercropped during a dry year as it did in an experiment by Patel et al. (2021).

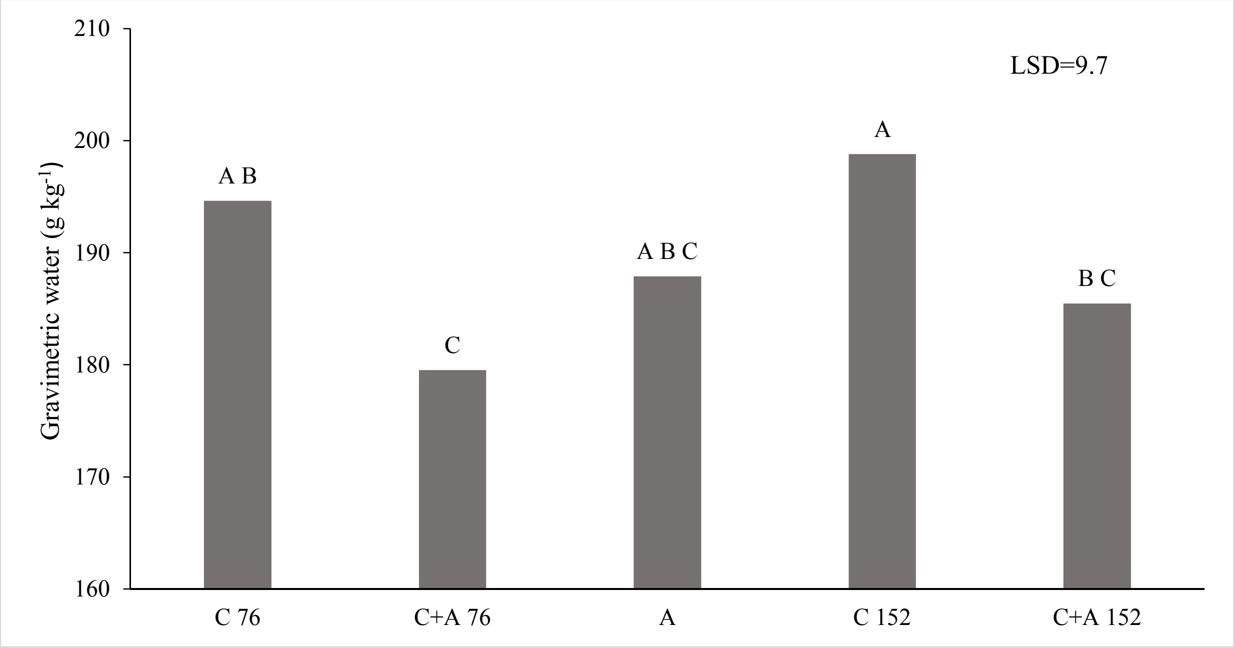


Fig. 4. Soil gravimetric water content measured from 0- to 15-cm averaged across two locations, Prosper and Hickson, ND and six sampling dates in 2021. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; A= spring-seeded alfalfa; C 152= corn with 152-cm row spacing; C+A 152= corn-alfalfa intercrop with 152-cm corn row spacing. LSD= to compare gravimetric water between dates. Treatments with different letters are significantly different at $P \leq 0.05$.

### 4.4. Stand counts

Stand counts were not different ($P \leq 0.05$) between any of the three treatments with alfalfa established in Year 1 or the decrease in alfalfa stand from the fall of Year 1 to the spring of Year 2 (Table 3). Alfalfa intercropped with corn at 76-cm row spacing stand counts in the fall of Year

1 and the spring of Year 2 were on average 74 and 63 plants $m^{-2}$, respectively. Alfalfa alone and alfalfa intercropped at 152-cm row spacing averaged 80 and 77 plants $m^{-2}$ in the fall of Year 1 and 69 and 74 plants $m^{-2}$ in the spring of Year 2, respectively. During the growing season of Year 2 new seeding alfalfa stand counts averaged 111 plants $m^{-2}$.

Berti et al. (2021a) also reported no significant differences between alfalfa alone compared with alfalfa intercropped with corn at 76-cm row spacing in experiments conducted over 2 years in Minnesota and North Dakota. However, in Iowa experiments there were significantly less stems per $m^{-2}$ in the intercropped treatment in the fall of the Year 1 and the spring of Year 2. Results from Patel et al. (2021) also show a lower stem count in intercropping treatments in Year 1 and the spring of Year 2 in 2017 compared with intercropping treatments. However, during 2018 in Year 2 there was no difference in stem counts between intercropping treatments and alfalfa established alone (Patel et al., 2021). Grabber et al (2021) found alfalfa stand reduction to be greater than in our study when comparing our alfalfa intercropped at 76-cm corn rows and their alfalfa intercropped in the same way without the prohexadione-calcium (PHD) treatment they were testing. They found without a PHD treatment in Year 1 their Year 2 stand counts averaged 48 plants $m^{-2}$. These results in combination with the results from our study indicate there is a potential for stand reduction and the extent of stand reduction is variable. We believe the reason for the severe reduction of stands in more southerly experiments, Iowa and Wisconsin, compared with experiments in North Dakota is due to the shorter corn maturity used in North Dakota. Shorter maturity corn is not in full canopy as long as longer maturity corn and does not grow as tall allowing for more light to reach intercropped alfalfa. In addition, Grabber et al., (2021) indicated that the humid conditions under the canopy in Wisconsin also leads to root and leaf diseases and increases death of alfalfa plants without the application of agri-chemicals.

In North Dakota, summer conditions are much drier and many of the diseases prevalent in Wisconsin are rare in North Dakota environments.

Table 3. Combined analysis of variance and mean square values (MS) of alfalfa stand counts in the fall of Year 1, the spring of Year 2 and the percent reduction between those times for two locations (Loc), Prosper and Hickson ND, five treatments (Trt) and three sampling dates (Date) in 2020 and six sampling dates in 2021.

| SOV | df | Fall 2020 | Spring 2021 | Reduction |
|---|---|---|---|---|
| Trt | 2 | 90 | 263 | 242 |
| Loc | 1 | 2665 | 585 | 858 |
| Loc(rep) | 6 | 535 | 301 | 151 |
| Locxtrt | 2 | 834 | 37 | 1962 |
| Residual | 12 | 355 | 201 | 360 |
| CV, % | | 24 | 21 | 247 |

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

## 4.5. Spectral indices

Date by treatment interactions for NDVI and RENDVI were significant in 2020 and 2021 (Table 4.). Treatment alone was only significant in 2020 but not in 2021 for both NDVI and RENDVI. Both NDVI and RENDVI are measures of crop health and aim to estimate photosynthetic activity based on the reflectance of the crop canopy (Tucker, 1979).

Table 4. Combined analysis of variance and mean square values for normalized difference vegetative index (NDVI), and red edge normalized difference vegetation index (RENDVI) for five treatments (Trt), two locations (Loc), Prosper and Hickson, ND, and eight sampling dates (Date) in 2020 and seven sampling dates in 2021.

| | | 2020 | | | 2021 | |
|---|---|---|---|---|---|---|
| SOV | df | NDVI | RENDVI | df | NDVI | RENDVI |
| Trt | 4 | 0.095* | 0.083* | 4 | 0.100 | 0.045 |
| Date x trt | 28 | 0.113* | 0.041* | 24 | 0.074* | 0.027* |
| Date | 7 | 1.247* | 0.584* | 6 | 0.241 | 0.211* |
| Loc | 1 | 0.002 | 0.009 | 1 | 0.003 | 0.042 |
| Loc(rep) | 6 | 0.023* | 0.024* | 6 | 0.013* | 0.010* |
| Loc x date | 7 | 0.007 | 0.006 | 6 | 0.062* | 0.020* |
| Loc x trt | 4 | 0.010 | 0.005 | 4 | 0.031* | 0.020* |
| Loc x date x trt | 28 | 0.006* | 0.003* | 24 | 0.003* | 0.001* |
| Residual | 234 | 0.002 | 0.001 | 204 | 0.001 | 0.001 |
| CV, % | | 6.45 | 7.79 | | 4.29 | 7.90 |

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

†Data collected using a drone mounted Red Edge MX multispectral camera (MicaSense, Seattle, WA, USA) flown at an altitude of 24 meters.

In 2020 and 2021, corn alone with 152-cm row spacing had lower NDVI than all other treatments with corn during the second and third sampling dates (Fig. 5). In 2020 and 2021, treatments with alfalfa had a higher NDVI and in 2020 a higher RENDVI during the last sampling date. This is likely because alfalfa continued to grow and remained green after the corn began to senesce. In our study, alfalfa alone exceeded all other treatments in 2020 and 2021 in NDVI and RENDVI at the end of the season. Alfalfa alone NDVI and RENDVI was affected by harvest which can be observed in both years by the drop in the reflectance of that treatment right after each cut.

We expected intercropped treatments to be significantly higher in both spectral indices compared with non-intercrop treatments because of the expected greater total biomass and groundcover. Sharaiha & Ziadat (2008) found a significant positive correlation between total biomass and NDVI in an intercropping study with barley (*Hordeum vulgare* L.) and common vetch (*Vicia sativa* L.). However, this was not the case in the 76-cm row corn because although we expected higher total biomass in the corn intercropped with alfalfa at 76-cm rows compared with corn alone at 76-cm rows, they were actually not significantly different (data not shown). The health and subsequent photosynthetic activity of the corn and alfalfa in intercrop treatment could have been affected by water stress which we know was more pronounced in intercrop treatments. This could have lowered NDVI and RENDVI in intercrop treatments and offset any increase due to additional green groundcover. As discussed, these values in the 152-cm corn were lower during the beginning of the year than with other treatments, but otherwise the differences between treatments with corn was not very clear until the last sampling date. Sharaiha and Ziadat (2008) also found a correlation between NDVI and runoff coefficient. This indicates that in treatments with higher NDVI such as treatments with alfalfa near the end of the year we could expect to see less runoff and in-turn potentially less soil erosion.

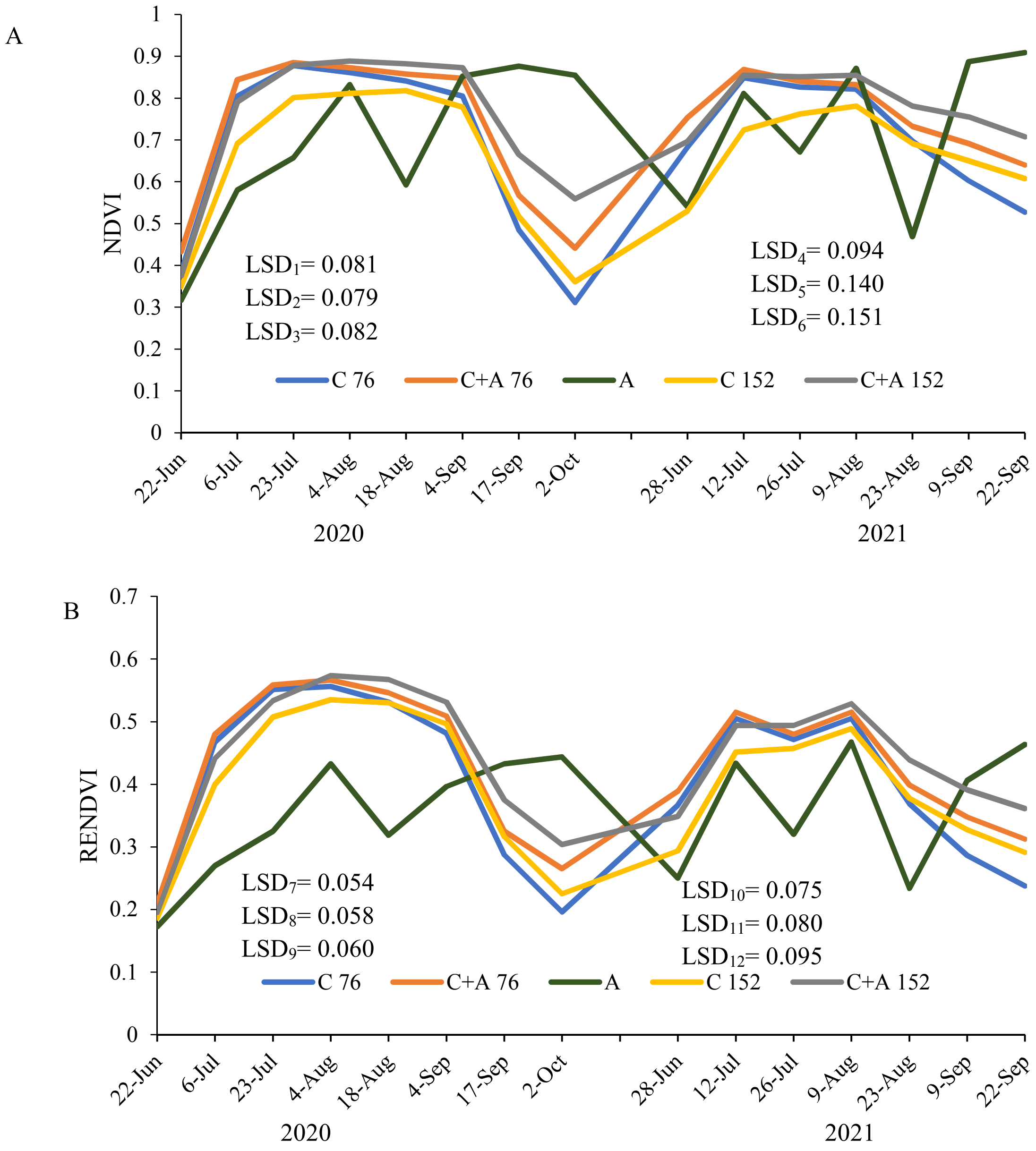


Fig. 5. A) Normalized difference vegetative index (NDVI) and B) Red edge normalized difference vegetative index (RENDVI) averaged across two locations Prosper and Hickson ND in the years 2020 and 2021. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; A= spring-seeded alfalfa; C 152= corn with 152 cm row spacing; C+A 152= Corn-alfalfa intercrop with 152-cm corn row spacing. LSD$_1$= to compare NDVI of different treatments on the same date in 2020; LSD$_2$= to compare NDVI of different dates in the same treatment in 2020; LSD$_3$= to compare NDVI across different dates and treatments in 2020; LSD$_4$= to compare NDVI of different treatments on the same date in 2021; LSD$_5$= to compare NDVI of different dates in the same treatment in 2021; LSD$_6$= to compare NDVI across different dates and treatments in 2021; LSD$_7$= to compare RENDVI of different treatments on the same date in 2020; LSD$_8$= to compare RENDVI of different dates in the same treatment in 2020; LSD$_9$= to compare RENDVI across different dates and treatments in 2020; LSD$_{10}$= to compare RENDVI of different treatments on the same date in 2021; LSD$_{11}$= to compare RENDVI of different dates in the same treatment in 2021; LSD$_{12}$= to compare RENDVI across different dates and treatments in 2021.

### 4.6. Photosynthetically active radiation and leaf area index

The percent of photosynthetically active radiation (PAR) intercepted by the crop canopy and the leaf area index (LAI) were both significantly affected by treatment, sampling date, and treatment by sampling date interaction (Table 5.). The PAR in 2020 and LAI in 2021 did not have significant treatment by sampling date interaction. Sampling date was expected to be significant as we assumed plant canopy light interception would increase throughout the growing season. Treatment was also expected to be significant as we expected 152-cm corn would be unable to fully cover the soil in between the rows.

Table 5. Analysis of variance and mean square values for PAR and LAI for five treatments (Trt), two locations (Loc), Prosper and Hickson, ND in 2020 and 2021 and four sampling dates in 2020 and six sampling dates in 2021.

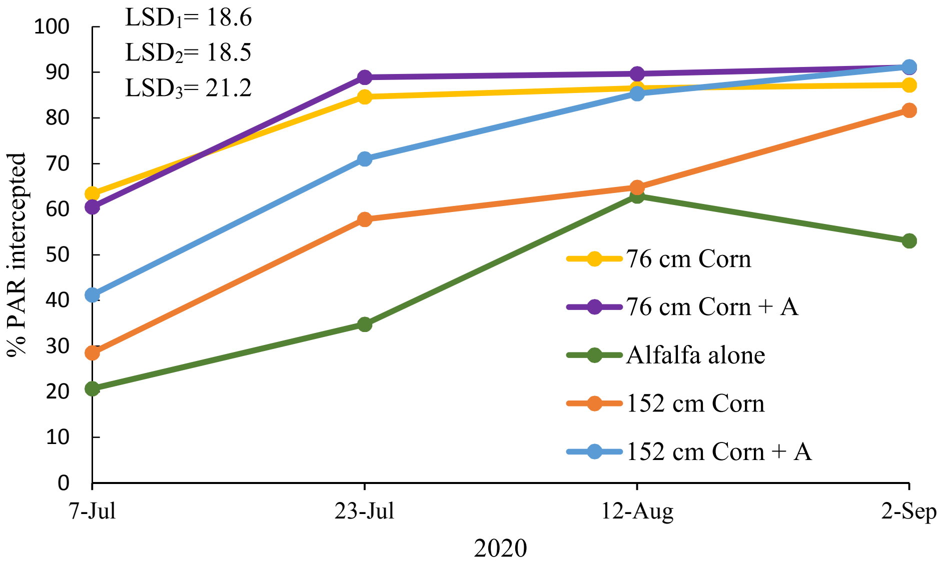
| | | 2020 | | | 2021 | |
|---|---|---|---|---|---|---|
| SOV | df | % PAR† | LAI | df | % PAR | LAI |
| Trt | 4 | 8840.06* | 33.28* | 4 | 5484.86* | 12.66 |
| Date x trt | 12 | 401.95 | 2.10* | 20 | 1134.42* | 1.87* |
| Date | 3 | 11702.00* | 24.42* | 5 | 18698.00* | 23.40* |
| Loc | 1 | 53.66 | 4.80 | 1 | 0.79 | 0.79 |
| Loc(rep) | 6 | 414.32* | 2.71* | 6 | 424.27* | 1.97* |
| Loc x date | 3 | 627.19 | 1.28 | 5 | 464.93* | 1.11 |
| Loc x trt | 4 | 573.91 | 2.06* | 4 | 436.36* | 2.13* |
| Loc x date x trt | 12 | 198.04 | 0.62 | 20 | 126.47* | 0.43* |
| Residual | 114 | 183.73 | 0.73 | 174 | 54.00 | 0.18 |
| CV, % | | 20.14 | 30.30 | | 11.08 | 19.28 |

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

†Canopy cover indices: percent photosynthetically active radiation intercepted by crop canopy (% PAR), and leaf area index (LAI).

The difference in PAR intercepted by an intercropped treatment and its respective corn alone control can be used to infer the amount of PAR available to alfalfa below the corn canopy at a given corn row spacing. For example, the average PAR intercepted by corn alone at 76-cm row spacing was 76.4% and in the 76-cm intercropped treatment it was 79.9%. Because we are measuring PAR at ground level the difference between these two indicates approximately how much light alfalfa was able to intercept in addition to corn. Although not always significant, when averaged across 2020 and 2021, alfalfa intercropped with 152-cm corn received 13.4% of total available PAR whereas alfalfa intercropped with 76-cm corn rows received just 3.6% of total PAR. Alfalfa alone intercepted an average of 47.3% PAR but it is important to consider alfalfa was cut twice during the sample period in both years and some of these cuts are evident in the lower PAR reading on certain dates in the alfalfa alone plots (Fig. 6). Regardless, it is clear alfalfa intercropped with corn received much less light than it can potentially use. Our results for corn alone and alfalfa alone are comparable to Berti et al. (2021a) where they found corn alone to intercept over 80% PAR during the growing season and alfalfa reached similar values between cuts. In 2020, our values for alfalfa alone did not get as high as Berti et al. (2021a) but this is likely due to the timing of cuts being shortly before several PAR sampling dates.

Reynolds et al. (1994) recorded light interception for wheat and barley intercropped with a variety of legumes. They found intercropping treatments to intercept less light than monocrops of wheat or barley alone. We did not find a significant difference between corn and alfalfa intercropped and corn alone at 76-cm row spacing at any date in 2020 or 2021. However, when considering corn and alfalfa intercropped and corn alone at 152-cm row spacing, the intercropping treatment intercepted significantly more light than the corn alone on 12 August in 2020 and 8 July, 22 July, and 4 August in 2021.



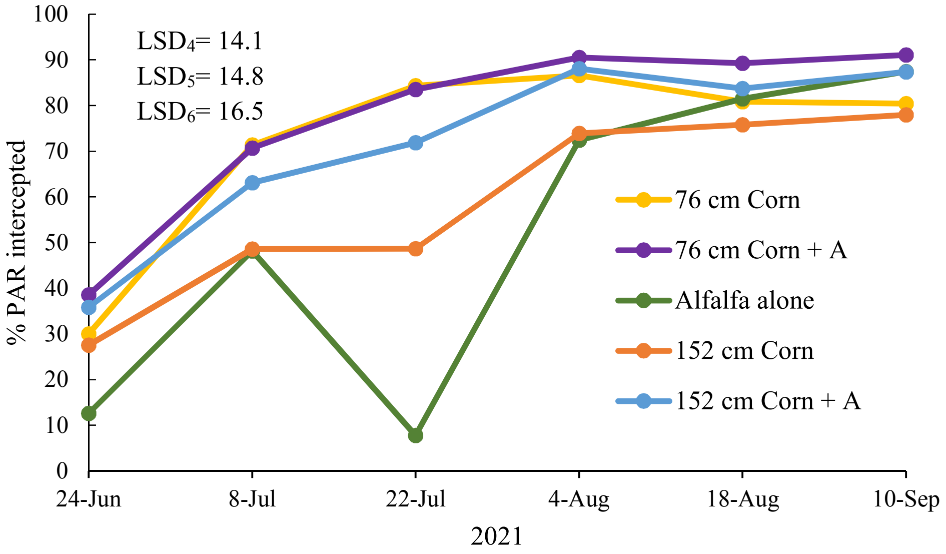
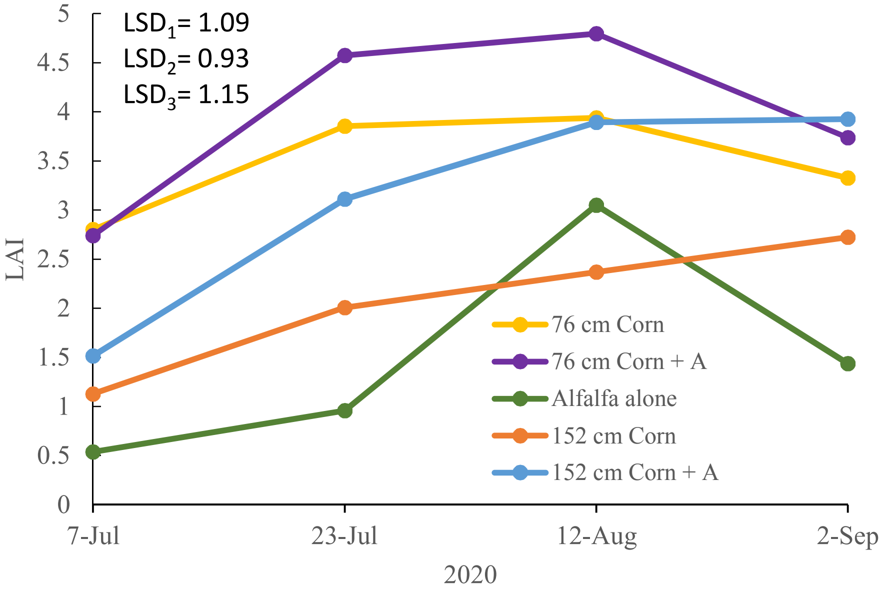


Fig. 6. Percent of photosynthetically active radiation (PAR) intercepted by the crop canopy averaged across two locations, Prosper and Hickson, ND in two years 2020 and 2021. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; A= spring-seeded alfalfa; C 152= corn with 152-cm row spacing; C+A 152= Corn-alfalfa intercrop with 152-cm corn row spacing. LSD$_1$= to compare PAR of different treatments on the same date in 2020; LSD$_2$= to compare PAR of different dates in the same treatment in 2020; LSD$_3$= to compare PAR across different dates and treatments in 2020; LSD$_4$= to compare PAR of different treatments on the same date in 2021; LSD$_5$= to compare PAR of different dates in the same treatment in 2021; LSD$_6$= to compare PAR across different dates and treatments in 2021.

Leaf area index generally followed the same trends as PAR during both years (Fig. 7). However, a decrease in leaf area in 2021 compared with 2020 is evident. We believe this was likely caused by a lack of rainfall and subsequent water stress. The field experiments endured six weeks of severe water stress when our gravimetric water content samples indicated the soil water was at or below wilting point. Near early to mid-September treatments with alfalfa were generally higher in LAI and PAR compared with corn alone treatments This is a trend also exhibited in NDVI and RENDVI (Fig. 5). This likely means alfalfa is actively growing later in the year than corn. This could have a positive effect on erosion prevention and soil health (Osterholz et al., 2021).



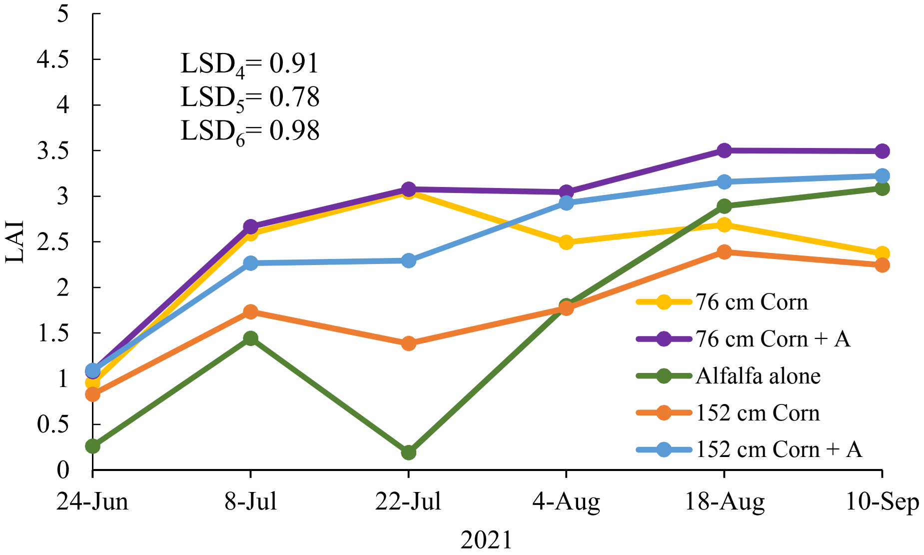


Fig. 7. Leaf area index (LAI) averaged across two locations, Prosper and Hickson, ND in two years 2020 and 2021. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; A= spring-seeded alfalfa; C 152= corn with 152-cm row spacing; C+A 152= Corn-alfalfa intercrop with 152-cm corn row spacing. LSD$_1$= to compare LAI of different treatments on the same date in 2020; LSD$_2$= to compare LAI of different dates in the same treatment in 2020; LSD$_3$= to compare LAI across different dates and treatments in 2020; LSD$_4$= to compare LAI of different treatments on the same date in 2021; LSD$_5$= to compare LAI of different dates in the same treatment in 2021; LSD$_6$= to compare LAI across different dates and treatments in 2021

## 4.7. Corn yield

Corn grain yield and biomass yield were both significantly impacted by treatment and corn grain yield also by the treatment by environment interaction (Table 6).

Table 6. Analysis of variance and mean square values for corn grain yield and corn biomass yield for five treatments (Trt) and four environments (Env), Prosper and Hickson, ND, in 2020 and 2021.

| SOV | df | Corn grain yield | Corn biomass yield |
|---|---|---|---|
| Env | 3 | 68.16* | 45.70* |
| Trt | 3 | 25.51* | 12.60* |
| Env x trt | 9 | 3.88* | 1.74 |
| Env(rep) | 12 | 4.48* | 2.70 |
| Residual | 36 | 1.34 | 1.70 |
| CV, % |  | 13.95 | 19.43 |

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

The presence of alfalfa at either row spacing did not significantly impact either corn grain yield or biomass yield (Fig. 8). However, there were notable numerical reductions to both corn grain and biomass yield. Corn intercropped with alfalfa yielded 11% and 17% less than corn alone at 76- and 152-cm row spacing respectively. Corn biomass at 76-cm row spacing shows a similar reduction of 12% from when intercropped with alfalfa. Corn biomass at 152-cm row spacing was very different than corn grain with a reduction of only 4% when intercropped with alfalfa.

Similar studies have reported significant reductions in corn grain and or corn biomass when intercropped with alfalfa (Berti et al., 2021a, Berti et al., 2021b, Patel et al., 2021, Sun et al., 2018, Grabber, 2016). Berti et al. (2021a) reported a corn grain yield reduction of 14% to 19% when alfalfa was intercropped with corn at 76-cm row spacing in a study that combined several experiments from North Dakota, Iowa, and Minnesota. Patel et al. (2021) found a more drastic reduction in corn grain yield of 23% to 26% in a study conducted in Iowa. These previous studies confirm that there is a potential for corn grain yield reduction and although our results were not statistically significant, it is likely that alfalfa will reduce corn grain yield.

Corn biomass yield is reported to be reduced due to alfalfa intercropping by 16% to 26% (Berti et al., 2021a). During an earlier study Berti et al. (2021b) found a non-significant corn biomass yield reduction of only 7% when alfalfa was intercropped with corn at 76-cm row spacing but found a significant yield reduction of 30% in one of two locations with corn at 61-cm row spacing. Grabber (2016) reported alfalfa intercropped with corn reduced corn biomass yield by about 3.2 Mg $ha^{-1}$ or 12% to 14%. In a different intercropping configuration involving every fourth row of corn replaced with three or five rows of alfalfa and corn planted in the same spot each year after alfalfa establishment, Sun et al. (2018) found the biomass of corn to be reduced significantly by the presence of alfalfa and the loss of a corn row. Our results for the corn and alfalfa intercropped at 76-cm row spacing, although not significant, align well with these previous studies. The reduction in corn grain yield from alfalfa intercropped with corn at 152-cm row spacing was expected, but we did not experience less total plant biomass. Because previous alfalfa intercropping studies have not considered row spacing as wide as 152-cm it is hard to draw on specific previous research. One possible explanation could be the presence of weeds in the corn alone at 152-cm row spacing. Although not quantified, it was observed that corn alone at 152-cm row spacing had more weeds present between the rows than any other treatment with corn during the mid- to late-growing season, probably due to less light interception than corn at 76-cm row spacing (Fig. 7). It is known that under severe stress, crop grain production is reduced more than crop biomass production (Sinclair et al. 1990). When the corn population is doubled in the row as it was for our experiment there is more intraspecific competition which is common at wider row spacing (Lutz et al., 1971)

Row spacing had a significant effect on corn grain and biomass yield. When compared with 76-cm corn row spacing, 152-cm row spacing reduced corn grain yield by 17% in corn alone treatments and 23% in intercropped treatments (Fig. 8). Using the same comparisons for corn biomass, yield was reduced in corn alone treatments by 22% and 15% in intercropped treatments. There is little previous work on corn with 152-cm row spacing. A study from Thapa et al. (2018) compared what they called a skip-row treatment to a standard row treatment at 76-cm. These would be comparable to our corn alone at 76- and 152-cm corn row spacing except they used a slightly lower corn population at 31,000 seeds ha$^{-1}$. In contrast to our results, they found corn row spacing did not significantly affect corn grain or biomass yield. In a study by Pavlista et al. (2010) they compared a system called "plant two-skip two" in reference to how the corn rows were planted. This meant they planted two corn rows and then skipped two corn rows; this was repeated across the field. They also doubled the population within the row to compensate for having half of the corn rows. Their results were mixed, showing a decrease in grain yield with this system in one location but an increase in another location as well as two locations with no significant difference when compared with a traditional system similar to our 76-cm corn alone treatment.

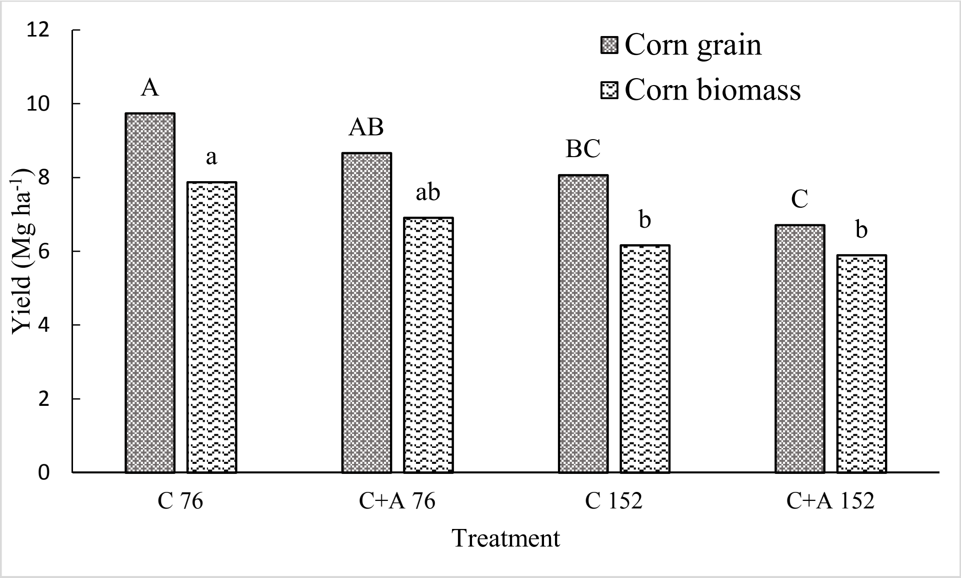


Fig. 8. Corn grain yield and corn biomass yield averaged across two locations, Prosper and Hickson, ND in 2020 and 2021. For treatments C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; C 152= corn with 152-cm row spacing; C+A 152= corn-alfalfa intercrop with 152-cm corn row spacing. Uppercase and lowercase letters represent a significant difference between treatments for corn grain and biomass yield respectively at $P \leq 0.05$, level of probability.

### 4.8. Alfalfa forage yield and plant height

In Year 1, alfalfa harvested from the alfalfa alone yielded an average of 5.1 Mg ha$^{-1}$ in 2020 when plots were harvested twice and 6.2 Mg ha$^{-1}$ in 2021 when plots were harvested three times.

When comparing yield and plant height of established alfalfa treatments from the first cutting in Year 2 there was no significant difference in forage yield, but we did observe a significant difference in alfalfa height in the first cutting (Table 7).

Table 7. Analysis of variance and mean square values for alfalfa yield and plant height from the first cut comparing three treatments (Trt) and two locations (Loc), Prosper and Hickson in 2021.

| SOV | df | Forage yield | Plant height |
|---|---|---|---|
| Loc | 1 | 0.45 | 645.4* |
| Loc(rep) | 6 | 2.94* | 32.9 |
| Trt | 2 | 3.56 | 175.3* |
| Loc x trt | 2 | 0.47 | 4.3 |
| Residual | 12 | 0.61 | 14.8 |
| CV, % | | 19.5 | 3.9 |

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

The alfalfa established alone was significantly taller than other established treatments during the first cut of Year 2. Alfalfa alone averaged 63 cm in height right before the first cut while alfalfa established from intercropping with corn at 76- and 152-cm corn rows averaged 54 and 56 cm in height, respectively. This could be because the alfalfa alone treatment established better in the first year and therefore grew faster in the spring of the second year. However, forage yield between these treatments was not significant. There are several potential explanations for the difference in plant height but lack of difference in forage yield. Most likely we just had more variation in forage yield data than plant height data (Table 7). This is confirmed by our coefficient of variation (CV) for both variables; the CV was 19.5% for alfalfa forage yield of the first cut of all established alfalfa treatments while the CV for plant height of the same treatments was 3.9% (Table 7). Also, it should be noted it was observed that some of the dry matter harvested from the previously intercropped treatments came from left over corn stalks as well as growing volunteer corn plants in V5 stage by the time of alfalfa first harvest. The extent to which this affected the data is unknown but presumed to be minor.

As for why we found alfalfa in Year 2 to be shorter when established with corn on 76-cm rows, we believe it is a combination of competition effects, mainly for light. Stress such as an untimely harvest in the fall can lead to lower root N reserves and storage proteins and consequently reduce or slowed spring regrowth (Dhont et al., 2006). We know alfalfa intercropped with corn had less PAR available during the growing season than alfalfa grown alone in Year 1. This most likely translated to less root reserves and slower growth before the first cut in Year 2.

Alfalfa harvested in Cuts 1-4 for the established alfalfa and Cuts 1-3 for the new seeding alfalfa were analyzed together and referred to Cuts-2-4 as the harvest times for these cuts of different treatments line up the best. When comparing all five alfalfa establishment methods we can see that there is no significant difference in forage yield between treatments which indicates that the only yield advantage from established alfalfa compared with newly spring-seeded alfalfa comes from the extra cut, in this case the first cut, that the established alfalfa provides (Table 8). However, we could probably find a significant difference between established alfalfa and spring-seeded alfalfa if we were to compare the three new seeding alfalfa harvests with the first three harvests from the established treatments as the first cut from the established treatments was higher in yield than cuts two, three, and four. Usually in the first production year (Year 2) alfalfa established the year prior has four cuts while spring-seeded alfalfa only two. However, in 2021 due to a mild fall we were able to take a third cut from the spring seeded alfalfa for a total of 3 cuts.

Although forage yield did not significantly differ for any treatment or any interaction, plant height was significantly affected by treatment and cut by treatment (Table 8).

Table 8. Analysis of variance and mean square values for alfalfa forage yield and plant height from three harvests (Cut), for five treatments (Trt) and two locations (Loc), Prosper and Hickson, in 2021.

| SOV | df | Forage yield | Plant height |
|---|---|---|---|
| Loc | 1 | 0.29 | 20.83 |
| Rep(loc) | 6 | 0.42 | 4.92 |
| Trt | 4 | 1.91 | 158.43* |
| Loc x trt | 4 | 0.82 | 11.27* |
| Cut | 2 | 1.69 | 301.23 |
| Loc x cut | 2 | 2.71* | 42.03* |
| Cut x trt | 8 | 0.30 | 28.44* |
| Loc x cut x trt | 8 | 0.31 | 2.41 |
| Residual | 84 | 0.25 | 2.33 |
| CV, % |  | 20.08 | 2.73 |

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

In Year 2, alfalfa intercropped with corn at 76-cm row spacing had an average Cut 1 forage yield of 3.22 Mg ha$^{-1}$. Alfalfa established alone had an average Cut 1 forage yield of 4.38 Mg ha$^{-1}$ and alfalfa established with corn at 152-cm row spacing had an average Cut 1 forage yield of 4.37 Mg ha$^{-1}$ (Fig. 9). Other experiments including alfalfa established alone and alfalfa established with intercropping on 76-cm rows have also found the first cut to show the most difference between intercropped treatments and alfalfa established alone (Berti et al. 2021a, Patel et al. 2021). Later cuts were found to be relatively similar in forage yield between alfalfa established alone and alfalfa established in intercrop. While corn row spacing from Berti et al. (2021a) and Patel et al. (2021) was 76-cm, our results indicate 152-cm corn row spacing may eliminate or reduce the yield loss in cut one of Year 2 due to intercropping. Furthermore, it is important to note our results showed little difference between established alfalfa yield and spring-seeded alfalfa yield in Cuts 2-4. This means any advantage from intercropping corn and alfalfa comes almost entirely from the additional harvest.

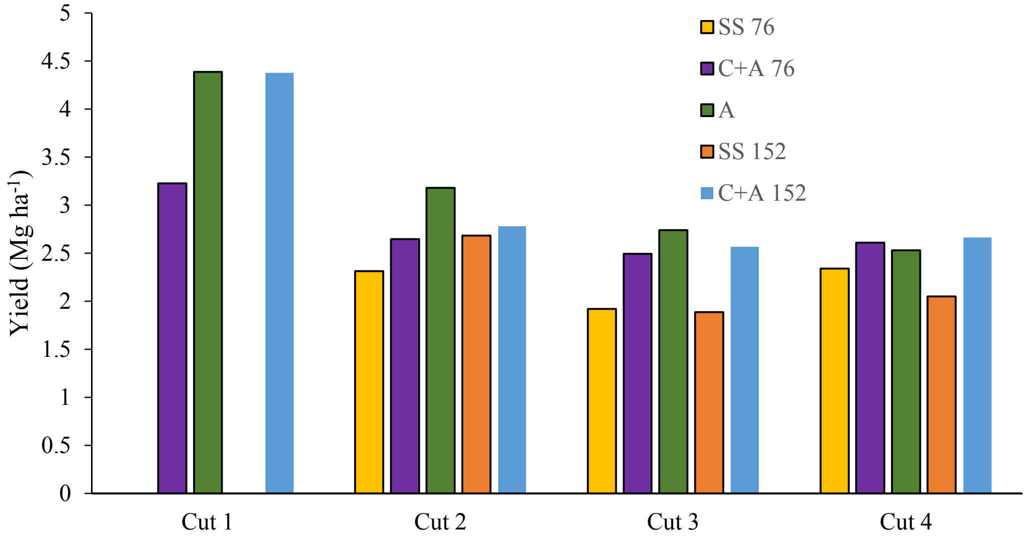


Fig. 9. Alfalfa biomass yield at harvest from five treatments in four consecutive cuttings in the second year averaged across two locations, Prosper and Hickson, ND in 2021. Treatment names refer to how alfalfa was established: SS 76= spring-seeded alfalfa after corn on 76-cm row spacing; C+A 76= established alfalfa from corn-alfalfa intercrop with 76-cm corn row spacing; SS 152= spring-seeded alfalfa after corn on 152-cm row spacing; C+A 152= established alfalfa from corn-alfalfa intercrop with 152 cm corn row spacing. The treatment by cut interaction was not significant at ($P \leq 0.05$) for cuts 2-5. Cut 1 was analyzed separately and there was no significance among treatments.

The spring-seeded alfalfa was significantly shorter in the first two cuts when comparing treatments in a specific cut which was expected as the spring-seeded alfalfa was still becoming established (Fig. 10). The newly seeded alfalfa had a higher plant density of 111 plants m$^{-2}$ compared with the average plant density of the established alfalfa, 71 plants m$^{-2}$. This could explain why it was able to yield as much as the established alfalfa in cuts two, three, and four even while being significantly shorter at harvest.

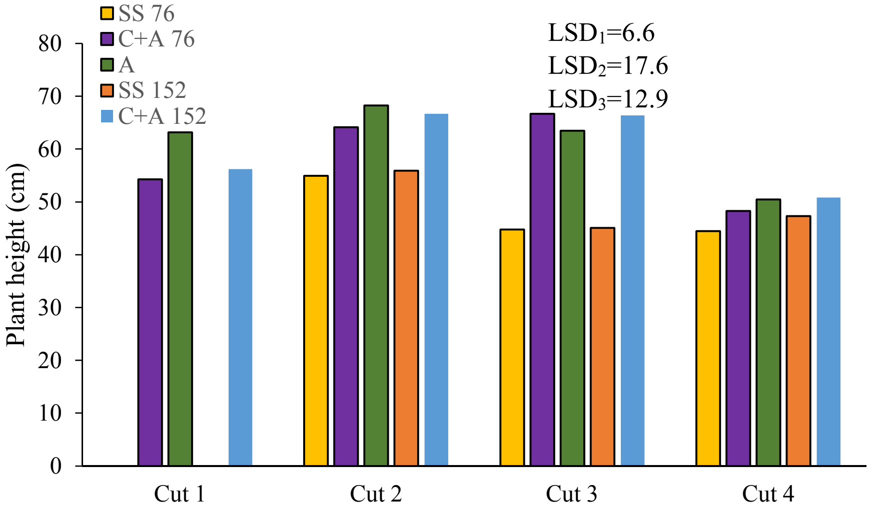


Fig. 10. Alfalfa plant height at harvest from five treatments in four consecutive cuttings in the second year averaged across two locations, Prosper and Hickson, ND in 2021. Treatment names refer to how alfalfa was established: SS 76= spring-seeded alfalfa after corn on 76-cm row spacing; C+A 76= established alfalfa from corn-alfalfa intercrop with 76-cm corn row spacing; SS 152= spring-seeded alfalfa after corn on 152-cm row spacing; C+A 152= established alfalfa from corn-alfalfa intercrop with 152-cm corn row spacing. LSD values apply to cuts 2, 3, and 4 only. $LSD_1$= to compare height of different treatments from the same cut; $LSD_2$= to compare height of different dates in the same treatment in 2020; $LSD_3$= to compare height across different dates and treatments in 2020.

Table 9. Analysis of variance and mean square values for alfalfa seasonal total forage yield for five treatments (Trt) and two locations (Loc), Prosper and Hickson, in 2021.

| SOV | df | Forage yield |
|---|---|---|
| Loc | 1 | 0.17 |
| Loc(rep) | 6 | 3.96 |
| Trt | 4 | 75.58* |
| Loc x trt | 4 | 1.70 |
| Residual | 24 | 1.76 |
| CV, % | | 13.43 |

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

Total seasonal alfalfa forage yield was significantly affected by treatment (Table 9). As expected, the spring-seeded alfalfa had a lower total seasonal forage yield than alfalfa established the previous year alone or in intercropping with corn (Fig. 11). This aligns with our hypothesis that the additional forage from established alfalfa treamtmets would significantly increase total seasonal forage yield of alfalfa established through intercropping with corn. When comparing established alfalfa treatments, alfalfa established alone yielded significantly more than alfalfa established in intercropping under 76-cm corn rows. Alfalfa established under 152-cm corn rows was not different from alfalfa alone or established in intercropping with corn at 76-cm row spacing the previous season.

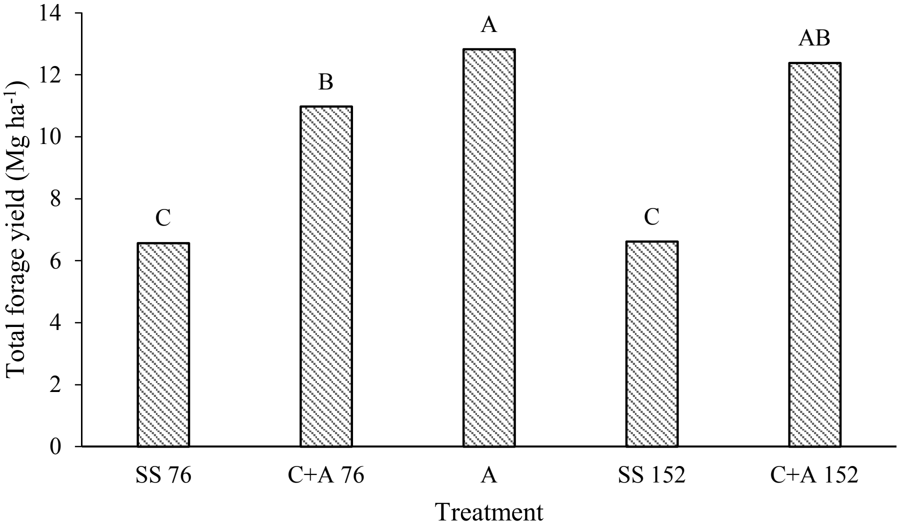


Fig. 11. Alfalfa total seasonal forage yield from five treatments in the second year averaged across two locations, Prosper and Hickson, ND, in 2021. Treatment names refer to how alfalfa was established: SS 76= spring-seeded alfalfa after corn on 76-cm row spacing; C+A 76= established alfalfa from corn-alfalfa intercrop with 76 cm corn row spacing; SS 152= spring-seeded alfalfa after corn on 152-cm row spacing; C+A 152= established alfalfa from corn-alfalfa intercrop with 152 cm corn row spacing. Letters represent a significant difference between treatments at $P \leq 0.05$, level of probability.

### 4.9. Alfalfa nutritive value

Treatment had a significant effect on acid detergent fiber (ADF), potassium (K), Acid detergent lignin (ADL), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ) when comparing biomass in Year 1 from alfalfa intercropped in 76-cm corn and alfalfa intercropped in 152-cm corn harvested at the end of the growing season (Table 10). Non-significant chemical components evaluated are not shown.

Table 10. Analysis of variance and mean square values for alfalfa forage nutritive value for alfalfa intercropped with corn at 76- and 152-cm row spacing sampled in October in four environments (Env), Prosper and Hickson, ND, in 2020 and 2021.

| SOV | df | ADF | K | ADL | NDF | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|
| Env | 3 | 93.9 | 0.15 | 7.86 | 77.6 | 36.07 | 103.1 | 2741.5 |
| Trt | 1 | 285.3* | 0.36* | 19.44* | 212.2* | 77.59* | 189.1* | 6647.1* |
| Env x trt | 3 | 13.6 | 0.01 | 0.52 | 13.0 | 4.53 | 16.8 | 570.6 |
| Env(rep) | 12 | 46.0 | 0.06 | 2.35 | 37.7* | 11.57 | 31.8* | 973.1* |
| Residual | 12 | 17.5 | 0.03 | 0.89 | 12.3 | 6.82 | 11.3 | 350.3 |
| CV, % | | 12.3 | 7.91 | 11.97 | 7.3 | 6.69 | 5.3 | 15.1 |

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

† acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative feed quality (RFQ).

Alfalfa intercropped with corn in 76-cm rows exhibited significantly lower ADF, ADL, NDF, and higher K, NDFD, TDN, and RFQ compared with alfalfa intercropped under 152-cm corn (Table 11). This could be associated with a response to shade. Alfalfa under 152-cm corn rows received 10 percentage points more total PAR than alfalfa under 76-cm corn rows. More shading could have caused the plant to grow more slowly. Fonseca et al. (1999) found alfalfa growing slowly with less vigor to have lower NDF, ADF, and lignin. This hypothesis is backed by a study done with soybeans by Shan et al. (2022). When shading was increased, they observed an increase in internode length and a decrease in lignin content in the cell walls. This was caused by changes in gibberellic acid ($GA_3$) in the internodes. This response would reduce ADL and the reduction in ADL would also decrease ADF and NDF in turn increasing NDFD, TDN and RFQ.

Table 11. Mean values for alfalfa nutritive value of alfalfa intercropped with 76- and 152-cm corn rows sampled in October in two environments, Prosper and Hickson, ND in 2020 and 2021.

| Treatment | ADF† | K | ADL | NDF | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|
| | ----------------------------- | | | g $kg^{-1}$ | ------------------------------ | | |
| C+A 76 | 309b | 24.5a | 71.0b | 454b | 406a | 659a | 138.8a |
| C+A 152 | 369a | 22.4b | 86.6a | 505a | 375b | 610b | 109.9b |

† acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ).
* Significant at $P \leq 0.05$, level of probability

Ash and NDFD of Cut 1 of established alfalfa in the second year of the cropping system was significantly affected by treatment (Table 12.). The main difference between these treatments during this time was the presence of corn residue in the intercropped treatments from the previous year. There was also a considerable amount of volunteer corn at V5 stage, some of which undoubtedly escaped our applications of clethodim and could have been harvested.

Table 12. Analysis of variance and mean square values for alfalfa nutritive value parameters in the first cut of Year 2 for alfalfa established alone and alfalfa intercropped with 76- and 152-cm corn rows averaged across two locations, Prosper and Hickson, in 2021.

| SOV | df | Ash | NDFD† |
|---|---|---|---|
| Loc | 1 | 0.84 | 23.64 |
| Trt | 2 | 1.14* | 11.08* |
| Loc x trt | 2 | <0.01 | 0.06 |
| Loc(rep) | 6 | 0.28 | 0.80 |
| Residual | 12 | 0.52 | 1.99 |
| CV, % | | 7.85 | 3.27 |

† neutral detergent fiber digestibility (NDFD)
* Significant at $P \leq 0.05$, level of probability

Alfalfa established alone had significantly higher ash than either intercropped alfalfa treatment (Table 13). This could be because there was more bare ground in this treatment whereas the intercropping treatments had corn residue covering some of the soil surface. With uncovered soil more splashing of soil in rain event can contaminate the biomass sample. Although other digestibility related parameters were not significant alfalfa established in 76-cm corn rows had significantly higher NDFD than the other two established alfalfa treatments (Table 13.). This is the same result when comparing NDFD of both intercropping treatments at the end of the establishment year. However, it is likely this result is not related to shading. Instead, because we observed a shorter height in alfalfa established in 76-cm corn rows compared to the other treatments which increases leaf: stem ratio increasing NDFD. Because lignin makes fiber less digestible in the rumen (Albrecht et al., 1987) a shorter, slower growing alfalfa plant is likely to have a higher NDFD (Fonseca et al., 1999).

Table 13. Mean values for alfalfa forage nutritive value parameters from the first cut of the year in Year 2 for alfalfa established alone and alfalfa intercropped with 76- and 152-cm corn rows averaged across two locations Prosper and Hickson in 2021.

| Treatment | Ash | NDFD‡ |
|---|---|---|
| | ------------------------------g $kg^{-1}$ | ------------------------------- |
| C+A 76†† | 89b | 445a |
| A | 96a | 423b |
| C+A 152 | 89b | 427b |

†Letters indicate a significant difference between treatments at (*P*≤0.05)
‡ Neutral detergent fiber digestibility (NDFD).
†Treatments were: C+A 76= Corn-alfalfa intercrop with 76 cm corn row spacing; A= Alfalfa alone C+A 152= Corn-alfalfa intercrop with 152 cm corn row spacing.

When comparing alfalfa nutritive value of all treatments across harvests two, three, and four in Year 2 we found treatment had a significant effect on ADF, ADL, NDF P, NDFD, TDN, and RFQ. Harvest date only affected CP and NDFD (Table 14).

Table 14. Analysis of variance and mean square values for alfalfa forage nutritive value parameters from five treatments (Trt) and three harvest dates (Cut) combined across two locations (Loc), Prosper and Hickson, ND in 2021.

| SOV | df | ADF | ADL | NDF | P | CP | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|---|
| Loc | 1 | 131.44 | 0.903 | 49.19 | 0.028 | 28.538 | 73.21 | 156.87 | 5211 |
| Trt | 4 | 51.71* | 2.985* | 33.38* | 0.001* | 0.381 | 22.64* | 26.58* | 1735* |
| Loc x trt | 4 | 1.11 | 0.108 | 0.97 | <.001 | 1.431 | 2.68 | 2.13 | 96 |
| Cut | 2 | 44.82 | 0.676 | 2.41 | 0.026 | 44.919* | 379.06* | 27.99 | 1720 |
| Loc x cut | 2 | 31.59 | 0.605 | 34.78 | 0.002 | 0.149 | 8.02 | 6.10 | 1236 |
| Cut x trt | 8 | 2.55 | 0.110 | 1.67 | <.001 | 4.34* | 7.60 | 10.42 | 215 |
| Loc x cut x trt | 8 | 5.98 | 0.418* | 9.32* | <.001 | 1.082 | 12.00 | 8.37 | 562 |
| Rep(loc) | 6 | 1.53 | 0.187 | 2.33 | <.001 | 0.934 | 1.23 | 1.46 | 85 |
| Residual | 84 | 3.32 | 0.134 | 3.00 | <.001 | 1.243 | 2.50 | 3.03 | 143 |
| CV, % |  | 6.05 | 5.442 | 2.21 | 5.21 | 4.51 | 3.70 | 2.59 | 7.7 |

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

† Acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ).

When comparing average treatment quality over all three cuts we can see the spring-seeded alfalfa was higher in nutritive value than established alfalfa in every significant parameter except NDFD where it was higher than the alfalfa alone and alfalfa intercropped in 152-cm corn rows but statistically similar to alfalfa intercropped in 76-cm corn rows (Table 15.). This is likely because the spring-seeded alfalfa was shorter with greater leaf: stem ratio and less lignified than the taller established alfalfa (Buxton, 1996). Because we analyzed the first cut of the established alfalfa separately the differences here are probably not due to time of cut, as the second, third, and fourth cuts of the established alfalfa were not very different in timing to the cuts one, two and three of the spring-seeded alfalfa. When comparing average cut nutritive value, we can see Cut 4 had higher crude protein and phosphorus than Cuts 2 and 3. This is due to cooler temperatures and therefore slower growth during the fourth cut which increases the leaf to stem ratio and reduces the lignin content (Buxton, 1996)

Table 15. Mean values for alfalfa forage nutritive value parameters for five treatments averaged across three harvest dates and two locations, Prosper and Hickson in 2021 and for three harvest dates averaged across five treatments and two locations.

| | ADF† | ADL | NDF | P | CP | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|
| | ------------------------------------ | | | --g kg$^{-1}$-- | ------------------------------------ | | | |
| SS 76† | 284.5 | 63.0 | 399.1 | 2.7 | 246.7 | 436.9 | 684.2 | 166 |
| C+A 76 | 314.6 | 70.0 | 421.8 | 2.8 | 246.3 | 418.0 | 663.1 | 149 |
| A | 305.9 | 69.4 | 412.7 | 2.8 | 248.4 | 416.4 | 663.8 | 152 |
| SS 152 | 286.7 | 63.8 | 399.5 | 2.7 | 246.5 | 431.0 | 680.1 | 164 |
| C+A 152 | 314.3 | 70.0 | 423.8 | 2.9 | 249.1 | 415.6 | 662.5 | 147 |
| LSD (0.05) | 8.4 | 2.6 | 7.9 | 0.1 | 9.6 | 13.1 | 11.7 | 8 |
| Cut 2 | 311.7 | 66.3 | 413.0 | 2.8 | 242.1 | 454.7 | 680.2 | 162 |
| Cut 3 | 301.4 | 68.7 | 408.6 | 2.6 | 240.5 | 423.0 | 664.2 | 155 |
| Cut 4 | 290.5 | 66.7 | 412.6 | 3.1 | 259.6 | 393.1 | 667.8 | 149 |
| LSD (0.05) | 54.1 | 7.5 | 56.7 | 0.5 | 3.7 | 27.3 | 23.8 | 33 |

† Acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative feed quality (RFQ).
‡Treatment names refer to how alfalfa was established: SS 76= spring seeded alfalfa after corn on 76-cm row spacing; C+A 76= established alfalfa from corn-alfalfa intercrop with 76-cm corn row spacing; SS 152= spring seeded alfalfa after corn on 152-cm row spacing; C+A 152= established alfalfa from corn-alfalfa intercrop with 152-cm corn row spacing. LSD indicates significance at $P \leq 0.05$.

## 4.10. Corn biomass chemical composition

Corn biomass crude protein (CP) was affected by treatment (Table 16). As the season progressed corn likely accumulated most of the nitrogen it would use before it had finished increasing in biomass. While we know biomass and grain yield are tightly tied to N supply and subsequent N uptake, grain yield is more responsive to these factors than biomass yield (Ciampitti, 2011). For this reason, it could be concluded that if stress, most likely water stress, was most prevalent after the majority of nitrogen was accumulated then a reduction in potential biomass at this time would essentially concentrate nitrogen in a lower quantity of biomass compared with treatments with low stress. This would then translate to a higher biomass crude protein content which is known to be directly correlated to total nitrogen content (Cox, 2001). This is supported by our corn biomass yield data. Both treatments with 152-cm corn rows had significantly lower biomass than corn alone at 76-cm row spacing (Fig. 12). Corn at 76-cm row spacing with alfalfa also had a lower corn biomass yield although not significantly. This means treatments with lower corn biomass yield had a higher concentration of CP. This interaction was also observed by Xu et al. (2022). They found when intercropping corn and alfalfa, corn biomass was reduced yet corn crude protein concentration was higher than corn alone.

Table 16. Analysis of variance and mean square values for corn biomass crude protein for five treatments (Trt) and four environments (Env), Prosper and Hickson, ND, in 2020 and 2021.

| SOV | df | CP |
|---|---|---|
| Trt | 3 | 95.9* |
| Env x trt | 3 | 9.9 |
| Env | 12 | 229.9 |
| Env(rep) | 9 | 49.1 |
| Residual | 36 | 16.5 |
| CV, % | | 16.1 |

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

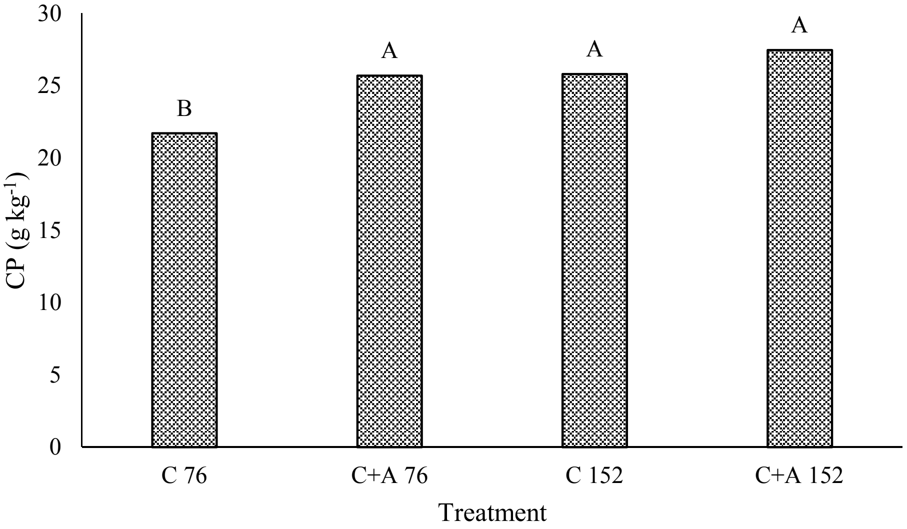


Fig. 12. Corn biomass crude protein (CP) averaged across two locations, Prosper and Hickson, ND in 2020 and 2021. For treatments: C 76= corn with 76-cm row spacing; C+A 76= corn-alfalfa intercrop with 76-cm corn row spacing; C 152= corn with 152-cm row spacing; C+A 152= corn-alfalfa intercrop with 152-cm corn row spacing. Different letters between columns indicate a significant difference at $P \leq 0.05$.

### 4.11. Economic Analysis

Using average prices for corn and alfalfa combined with the average yields from our experiments we found that the most cost-effective way to establish alfalfa is by intercropping it with corn at 76-cm row spacing (Table 17). When comparing two-year sequences by intercropping alfalfa in 76-cm corn rows we save an estimated \$148 ha$^{-1}$ compared with planting alfalfa alone. We save an estimated \$297 ha$^{-1}$ compared with seeding alfalfa in the spring after 76-cm corn. Establishing alfalfa by intercropping with 152-cm corn row spacing was the most expensive way to establish alfalfa. We can see that the difference in net return between the treamtments with corn alone in the first year, at 76- and 152-cm row spacing, represents the reduction in revenue from the lower yields in the wide row corn.

Table 17. Two-year cost and return analysis of five treatments combined across two locations Prosper and Hickson in 2021.

| 2-year cropping system | C 76 | A SS | C+A 76 | A Est. | A SS | A Est. | C 152 | A SS | C+A 152 | A Est. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yr-1 | Yr-2 | Yr-1 | Yr-2 | Yr-1 | Yr-2 | Yr-1 | Yr-2 | Yr-1 | Yr-2 |
| **Inputs** | ---$ ha$^{-1}$--- | | | | | | | | | |
| Fertilizer | 346 | 226 | 346 | 436 | 226 | 508 | 346 | 226 | 346 | 490 |
| Seed | | | | | | | | | | |
| Corn | 248 | | 248 | | | | 248 | | 248 | |
| Alfalfa | | 351 | 351 | | 351 | | | 351 | 351 | |
| Herbicide | 90 | 74 | 74 | | 74 | | 90 | 74 | 74 | |
| Fixed machinery costs | 185 | 199 | 185 | 204 | 199 | 204 | 185 | 199 | 185 | 204 |
| Variable machinery costs | 228 | 153 | 228 | 163 | 153 | 163 | 228 | 153 | 228 | 163 |
| Crop insurance | 27 | 12 | 27 | 12 | 12 | 12 | 27 | 12 | 27 | 12 |
| Labor/Management | 107 | 231 | 107 | 224 | 231 | 224 | 107 | 231 | 107 | 224 |
| Rent | 311 | 311 | 311 | 311 | 311 | 311 | 311 | 311 | 311 | 311 |
| **Total costs** | 1542 | 1558 | 1877 | 1350 | 1558 | 1421 | 1542 | 1558 | 1877 | 1404 |
| Yield Mg | 10 | 7 | 9 | 11 | 7 | 13 | 8 | 7 | 7 | 12 |
| Price Mg | 177 | 137 | 177 | 137 | 137 | 137 | 177 | 137 | 177 | 137 |
| †**Total revenue** | 1719 | 897 | 1542 | 1498 | 897 | 1747 | 1418 | 904 | 1188 | 1691 |
| **Net return per year** | 177 | -661 | -335 | 148 | -661 | 326 | -124 | -654 | -690 | 287 |
| **Net return of both years** | -484 | | -187 | | -335 | | -778 | | -403 | |

Treatments are: C 76= Corn with 76 cm row spacing, new seeding alfalfa in year two; C+A 76= Corn-alfalfa intercrop with 76 cm corn row spacing; C 152= Corn with 152 cm row spacing, new seeding alfalfa in year two; C+A 152= Corn-alfalfa intercrop with 152 cm corn row spacing; A SS= Spring-seeded alfalfa: A Est.= alfalfa alone already established. †Total revenue calculated assuming a corn grain price of \$168.5 Mg$^{-1}$ and an alfalfa forage price of \$136 Mg$^{-1}$

When comparing a range of corn and alfalfa prices we found that during times of high alfalfa prices and low corn prices alfalfa established alone after corn on 76-cm rows is more cost effective than intercropping in 76-cm corn rows (Table 18). However, intercropping on 76-cm corn rows is more cost effective at all other prices of corn and alfalfa. Growing corn alone at 152-cm rows and seeding alfalfa the following year is always worse than the same practice with 76-cm corn rows. This also holds true when comparing both intercropping treatments; 152-cm corn rows are always less effective.

Berti et al. (2021b) also found alfalfa corn intercropping to be the most profitable two-year sequence compared with continuous corn or alfalfa seeded after corn. Osterholz et al. (2020) found intercropping corn and alfalfa to be the more economical way of establishing alfalfa compared with seeding alfalfa after corn. However, both studies (Berti et al. 2021b and Osterholz et al. 2020) were done with corn for silage rather than corn for grain. Comparing the economic analysis from this study with previous studies, more negative total net returns were generally observed than in previous studies (Berti et al. 2021b and Osterholz et al. 2020). However, this is likely because we included all realized costs as well as unrealized costs such as labor and land rent equivalent.

Table 18. Net return of five different treatments at different prices of corn and alfalfa with data collected frow 2020 and 2021 in Prosper and Hickson ND. Matrices labeled with the first- and second-year treatment separated by a hyphen. For treatments: C 76= Corn with 76-cm row spacing, new seeding alfalfa in year two; C+A 76= Corn-alfalfa intercrop with 76-cm corn row spacing; C 152= Corn with 152-cm row spacing, new seeding alfalfa in year two; C+A 152= Corn-alfalfa intercrop with 152 cm corn row spacing; A NS= New seeding alfalfa: A Est.= Established alfalfa.

| Price of alfalfa | Price of corn grain | | | | |
|---|---|---|---|---|---|
| ($ Mg) | ($ Mg) | | | | |
| | 138 | 184 | 230 | 276 | 321 |
| | | | C 76 - A SS | | |
| | | ---------------------($ ha)------------------- | | | |
| 91 | -1166 | -720 | -275 | 170 | 616 |
| 114 | -1016 | -571 | -125 | 320 | 765 |
| 137 | -867 | -421 | 24 | 469 | 915 |
| 159 | -717 | -272 | 173 | 619 | 1064 |
| 182 | -568 | -122 | 323 | 768 | 1214 |
| | | | C+A 76 - Est. A | | |
| | | ---------------------($ ha)------------------- | | | |
| 91 | -1030 | -631 | -231 | 168 | 568 |
| 114 | -780 | -381 | 19 | 418 | 818 |
| 137 | -531 | -131 | 268 | 668 | 1067 |
| 159 | -281 | 118 | 518 | 918 | 1317 |
| 182 | -31 | 368 | 768 | 1167 | 1567 |
| | | | SS A - Est. A | | |
| | | ---------------------($ ha)------------------- | | | |
| 91 | -1217 | -1217 | -1217 | -1217 | -1217 |
| 114 | -776 | -776 | -776 | -776 | -776 |
| 137 | -335 | -335 | -335 | -335 | -335 |
| 159 | 105 | 105 | 105 | 105 | 105 |
| 182 | 546 | 546 | 546 | 546 | 546 |
| | | | C 152 - SS A | | |
| | | ---------------------($ ha)------------------- | | | |
| 91 | -1395 | -1028 | -661 | -293 | 74 |
| 114 | -1245 | -877 | -510 | -143 | 225 |
| 137 | -1094 | -727 | -359 | 8 | 375 |
| 159 | -944 | -576 | -209 | 158 | 526 |
| 182 | -793 | -426 | -58 | 309 | 676 |
| | | | C+A 152 - Est. A | | |
| | | ---------------------($ ha)------------------- | | | |
| 91 | -1231 | -923 | -615 | -308 | 0 |
| 114 | -949 | -641 | -334 | -26 | 282 |
| 137 | -667 | -360 | -52 | 256 | 563 |
| 159 | -385 | -78 | 230 | 538 | 845 |
| 182 | -104 | 204 | 512 | 819 | 1127 |

# 5. CONCLUSION

Intercropping corn and alfalfa can be a profitable and sustainable practice for growers in the Midwest. We demonstrated that treatments with corn and alfalfa intercropped had active growth and better living soil cover later in the year than corn grown alone. Although intercropped alfalfa did not reduce corn yield significantly it did trend lower in both row spacings compared with corn alone. The corn and alfalfa intercropped also averaged the lowest gravimetric water content over the course of the growing season. This could translate to a more serious yield loss in dryer conditions. Widening corn row spacing did reduce corn yield substantially however it allowed for good alfalfa establishment, and in more adverse growing conditions could represent a more consistent way to establish alfalfa with corn. This is in part due to alfalfa established with 152-cm corn rows intercepting approximately three times more PAR during the height of the growing season compared to alfalfa established with 76-cm corn rows. At the end of the growing season in Year 1, we observed all treatments with alfalfa maintained higher NDVI and RENDVI values compared to corn alone treatments. This means alfalfa intercropped with corn can extent the time in which there is growing plant material on the landscape which may have positive environmental impacts such as reduced erosion and nitrate leaching.

In Year 2, alfalfa intercropped with 76-cm row corn yielded less than alfalfa established alone. We expect most of this yield loss to have taken place during the first cut in the second year. Regardless, alfalfa was successfully established by intercropping in 76- and 152-cm corn rows and in Year 2 both intercropping treatments yielded significantly more than alfalfa seeded in Year 2 after corn. When corn was intercropped with alfalfa it did average less grain yield, but this was not significant at either row spacing. In Year 2 alfalfa that grew slower was generally higher in one or more forage quality parameters. This included alfalfa from cut one in Year 2 after being established with corn at 76-cm row spacing and spring seeded alfalfa in Year 2, which was generally slower growing than established alfalfa. Alfalfa stand was not significantly different between intercropping treatments compared with alfalfa established alone. Through economic analysis we found that price of alfalfa and corn impact which cropping system, alfalfa alone or alfalfa intercropped at 76-cm corn row pacing, is more cost effective. Of course, many other factors will affect this outcome as well such as weather, field conditions, and the specific operation of the grower. It is also important to consider the number of cuts allowed in the alfalfa seeding year. We were able to take three cuts in 2021 and therefore used that number of cuts in our calculations however this is not always the case either due to weather or alfalfa maturity. Regardless, corn alfalfa intercropping is a viable practice given the right conditions.

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## APPENDIX

Table A1. Analysis of variance and mean square values for alfalfa forage nutritive value for alfalfa intercropped with corn at 76- and 152-cm row spacing sampled in October in four environments (Env), Prosper and Hickson, ND, in 2020 and 2021.

| SOV | df | ADF | Ash | K | ADL | NDF | P | CP | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | 3 | 93.9 | 1.75 | 0.15 | 7.86 | 77.6 | 0.0116 | 70.04 | 36.07 | 103.1 | 2741 |
| Trt | 1 | 285.3* | 2.52 | 0.36* | 19.44* | 212.2* | 0.0036 | 60.00 | 77.59* | 189.1* | 6647* |
| Env x trt | 3 | 13.6 | 0.58 | 0.01 | 0.52 | 13.0 | 0.0007 | 7.12 | 4.53 | 16.8 | 571 |
| Env(rep) | 12 | 46.0 | 0.68 | 0.06 | 2.35 | 37.7* | 0.0009 | 6.40 | 11.57 | 31.8* | 973* |
| Residual | 12 | 17.5 | 0.12 | 0.03 | 0.89 | 12.4 | 0.0007 | 3.34 | 6.82 | 11.3 | 350 |
| CV, % | | 12.3 | 5.55 | 7.91 | 11.97 | 7.3 | 9.26 | 8.45 | 6.69 | 5.3 | 15 |

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

† acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ).

Table A2. Analysis of variance and mean square values for alfalfa nutritive value parameters in the first cut of Year 2 for alfalfa established alone and alfalfa intercropped with 76- and 152-cm corn rows averaged across two locations, Prosper and Hickson, in 2021.

| | df | ADF | Ash | K | ADL | NDF | P | CP | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trt | 2 | 12.5 | 1.13* | 0.01 | 0.87 | 32.8 | 0.0003 | 4.99 | 11.08* | 10.51 | 0.08 |
| Loc | 1 | 65.1 | 0.84 | 0.43 | 2.03 | 84 | 0.002 | 11.99 | 23.64 | 42.67 | 0.33 |
| Loc(rep) | 6 | 11.4 | 0.28 | 0.02 | 0.49 | 14.8 | 0.0006 | 4.77 | 0.8 | 6.59 | 0.04 |
| Loc x trt | 2 | 11.2 | <0.01 | 0.01 | 0.51 | 23.3 | 0.0004 | 5.45 | 0.06 | 6.89 | 0.06 |
| Residual | 12 | 4.6 | 0.52 | 0.06 | 0.20 | 9.3 | 0.0003 | 1.8 | 1.99 | 4.21 | 0.03 |
| CV, % | | 5.8 | 7.85 | 10.70 | 5.57 | 6.3 | 7.21 | 6.91 | 3.27 | 3.29 | 7 |

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

† Acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ)

Table A3. Analysis of variance and mean square values for alfalfa forage nutritive value parameters from five treatments (Trt) and three harvest dates (Cut) combined across two locations (Loc), Prosper and Hickson, ND in 2021

| SOV | df | ADF | Ash | K | ADL | NDF | P | CP | NDFD | TDN | RFQ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Loc | 1 | 131.44 | 5.38 | 0.08 | 0.90 | 49.19 | 0.028 | 28.53 | 73.2 | 156.9 | 5211 |
| Trt | 4 | 51.71* | 1.19 | 0.05 | 2.99* | 33.38* | 0.001* | 0.38 | 22.6* | 26.6* | 1735* |
| Loc x trt | 4 | 1.11 | 0.31 | 0.01 | 0.11 | 0.97 | <0.001 | 1.43 | 2.7 | 2.1 | 96 |
| Cut | 2 | 44.82 | 0.21 | 1.60 | 0.68 | 2.41 | 0.026 | 44.92* | 379.0* | 28.0 | 1720 |
| Loc x cut | 2 | 31.59 | 0.21 | 0.44 | 0.61 | 34.78 | 0.002 | 0.15 | 8.0 | 6.1 | 1236 |
| Cut x trt | 8 | 2.55 | 0.69 | 0.11 | 0.11 | 1.67 | <0.001 | 4.34* | 7.6 | 10.4 | 215 |
| Loc x cut x trt | 8 | 5.98 | 0.55 | <0.01 | 0.42* | 9.32* | <0.001 | 1.08 | 12.0 | 8.4 | 562 |
| Rep(loc) | 6 | 1.53 | 0.29 | 0.02 | 0.19 | 2.33 | <0.001 | 0.93 | 1.2 | 1.5 | 85 |
| Residual | 84 | 3.32 | 0.26 | 0.01 | 0.13 | 3.00 | <0.001 | 1.24 | 2.5 | 3.0 | 143 |
| CV, % | | 6.05 | 5.24 | 5.29 | 5.44 | 2.21 | 5.21 | 4.51 | 3.7 | 2.6 | 7.7 |

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

† Acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), and relative feed quality (RFQ)

Table A4. Analysis of variance and mean square values for corn grain nutritive value for five treatments (Trt) and four environments (Env), Prosper and Hickson, ND, in 2020 and 2021

| | df | Ash | Fat | CP | Fiber |
|---|---|---|---|---|---|
| Env | 3 | 4.28 | 0.0224 | 2.307 | 0.109 |
| trt | 3 | 0.39 | 0.0002 | 0.506 | 0.001 |
| Env x trt | 9 | 0.25 | 0.0002 | 0.357 | 0.001 |
| Env(rep) | 12 | 0.16 | 0.0004 | 0.251 | 0.001 |
| Residual | 36 | 0.14 | 0.0001 | 0.092 | <0.001 |
| CV, % | | 1.68 | 0.33 | 4.06 | 0.91 |

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

† Crude protein (CP)

Table A5. Analysis of variance and mean square values for corn biomass nutritive value for five treatments (Trt) and four environments (Env), Prosper and Hickson, ND, in 2020 and 2021.

| SOV | df | ADF† | Starch | NDF | ADL | CP | P | Ash |
|---|---|---|---|---|---|---|---|---|
| Env | 3 | 49.01* | 64.47 | 19.68 | 7.47* | 229.89 | 0.0006 | 4.28 |
| trt | 3 | 0.70 | 14.32 | 3.65 | 0.03 | 95.90 | 0.0006 | 0.39 |
| Env x trt | 9 | 0.87 | 5.45 | 7.17 | 0.14 | 9.92 | 0.0005 | 0.25 |
| Env(rep) | 12 | 3.87* | 21.44* | 4.95 | 0.48* | 49.05 | 0.0004* | 0.16 |
| Residual | 36 | 1.37 | 7.06 | 2.62 | 0.17 | 16.47 | 0.0002 | 0.14 |
| CV, % |  | 3.90 | 8.76 | 2.72 | 6.94 | 16.12 | 13.66 | 4.65 |

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

† Acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and crude protein (CP),