PRODUCTIVE AND ECONOMIC ANALYSIS OF SILAGE MAIZE AND ALFALFA INTERCROPPING

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MASTER OF SCIENCE

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ABSTRACT

Intercropping of maize (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) is not a common practice because alfalfa generally reduces maize grain and biomass yield. The objective of this research was to evaluate the productivity and profitability of maize-alfalfa intercropping. The experiment was conducted in Fargo and Prosper, ND, from 2014 to 2017. The design was a randomized complete block design with four replicates and a split-plot arrangement. Treatments were: 1) maize monoculture, 2) maize intercropped with alfalfa, 3) maize intercropped with alfalfa + prohexadione, and 4) spring-seeded alfalfa (in 2015). Alfalfa established in intercropping with maize had almost double the forage yield in the following year compared with spring-seeded alfalfa, and had higher net returns than silage-maize followed by spring-seeded alfalfa the following year. This system has the potential to get more growers to have alfalfa in the rotation by skipping the typical low yielding alfalfa in establishment year.

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CHAPTER 1: INTRODUCTION

The most common crop sequences in the Corn -Belt region of the US, are continuous maize (*Zea mays* L.) or maize-soybean [*Glycine max* (L.) Merr.]. In the last two decades, forage-based, high diversity, crop rotations have transitioned to less diverse, shorter, and annual crops-based rotations. The increase of farm size, decline on livestock numbers, and the increase of commodity prices has driven the decline on crop diversity (Karlen et al., 2006; Johnston, 2014). The reduced crop diversity in the Corn Belt has resulted in negative economic and environmental impacts, such as loss of soil organic matter, degradation of soil physical characteristics, and increased soil erosion (Sulc and Tracy, 2007; Russelle, 2013). Research has demonstrated that long-term diverse crop rotations produce higher yield in each crop in the rotation compared with monocrops or short rotations, enhancing soil fertility and reducing fertilizer applications to the next crop (Karlen et al., 2006; Sulc and Tracy 2007; Olmstead and Brummer, 2007).

Including perennial crops into a crop rotation, generally results in lower cost of production, reduced soil erosion, and improved soil health (Olmstead and Brummer, 2007). Alfalfa (*Medicago sativa* L.) in rotation with other crops decreases the production cost of the subsequent crop, due to enhanced N_2 fixation, and improved soil health (Olmstead and Brummer, 2007; Zhang et al., 2013). Moreover, when the subsequent crop is maize, adding alfalfa to the crop rotation decreases nitrogen fertilizer costs (Mikic et al., 2015).

Despite of the many benefits alfalfa offers to cropping systems, alfalfa annual forage yield is much lower than that of silage maize, particularly in the establishment year (Grabber, 2016). This has resulted in the reduction of alfalfa production on dairy farms, in favor of continuous silage maize production (Grabber, 2016). With the approval of glyphosate-tolerant alfalfa in 2011, interseeding alfalfa into maize has become a new potential cropping system for forage growers in the Midwest (Hubbard and Hassanein, 2013), since both crops complement each other (Ijoyah, 2012; Sun et al., 2014, 2018). Alfalfa intercropped into maize provides a groundcover after silage maize harvest, in addition to providing forage in the subsequent production years (Grabber, 2016). Interseeding alfalfa into silage maize provides a head start to alfalfa production establishment, doubling the forage yield compared with conventional spring-seeded alfalfa (Grabber, 2016). In addition, intercropped alfalfa during and after maize silage production reduces soil losses of total suspended solids, total nitrogen and phosphorus, and nitrate and dissolved phosphorus (Osterholz et al., 2019).

However, alfalfa interseeding can impact negatively because alfalfa might compete with maize for water and nutrients while the reduced light under the maize's canopy etiolates alfalfa stems, weakening the plants. As a result, plants might die during the winter reducing alfalfa stands (Yost et al., 2015; Grabber, 2016). Additionally, recent research indicates tolerance to shade under the maize canopy varies among alfalfa cultivars (Grabber, 2016). An adequate stand of alfalfa in the first year ranges between 80 to 130 plants m^{-2} (Hall et al., 2004; Grabber, 2016).

In order to improve the survivability of the alfalfa and reduce stem etiolation, growth regulators have been evaluated. These chemicals also work as tools to reduce the competition between alfalfa and maize. Prohexadione-calcium (PHX) [calcium, 1-(4-carboxy-2, 6-dioxocyclohexylidene) propan-1-olate], is a growth regulator that has been utilized in alfalfa to reduce the internode length and improve alfalfa's survivability (Rethwish et al., 2003; Grabber, 2016; Osterholz, et al., 2018). Alfalfa root growth and cold tolerance are also enhanced by growth regulators' application, improving alfalfa stand establishment during the seeding year (Grabber, 2016).

Although the potential of alfalfa-maize interseeding has been studied in Wisconsin since 2010, there are still many questions remaining. Additional research is needed to minimize silage maize yield reduction and increase alfalfa stand establishment. In addition, competition among species, differences in alfalfa cultivars tolerance to shade, maize row spacing, seeding dates, and N management, need to be researched to identify the advantages and disadvantages of this system (Grabber, 2016).

In North Dakota, the growing season is shorter and maize hybrids grown in the state are usually shorter in height and maturity than in Wisconsin (Kucharik, 2008). Thus, the survivability of alfalfa and the competition with maize is likely to be different from that reported in Wisconsin, because light below the maize canopy is probably greater. Additionally, all previous studies have been done only at 76-cm maize row spacing and only for silage maize hybrids (Grabber, 2016).

In North Dakota, alfalfa interseeding in maize has not been studied before and might be a potential system to increase the grower's interest to add alfalfa to the current continuous maize or maize-soybean rotation. Adding alfalfa to the rotation would have many positive economic, as well as, environmental benefits. The maize would serve as a companion crop to alfalfa during establishment and the intercropped alfalfa would serve as a cover crop after maize harvest, preventing soil erosion and enhancing nutrient cycling (Olmstead and Brummer, 2007; Grabber, 2016; Osterholz et al., 2019). In 2019, in North Dakota, grain-maize acreage was 1.3 million and silage maize was 54,946 ha, respectively (NASS, 2019). Alfalfa hay acreage including alfalfa-grass mixtures was 493,716 ha (NASS, 2019). Thus, studying the interseeding alfalfa into maize system in North Dakota could potentially have a positive impact in the state's economy in the near future.

The objectives of this research were: 1) to evaluate the productivity, forage nutritive value, and stand establishment of alfalfa the year after being established in intercropping with maize at two maize row spacing’s compared with spring-seeded alfalfa, 2) to determine if the application of prohexadione-calcium to alfalfa under the maize canopy improves alfalfa establishment and survival, and 3) to calculate the economic benefits of alfalfa-maize intercropping.

CHAPTER 2: LITERATURE REVIEW

2.1. Crop Rotations and Cropping Systems

Agriculture in the U.S. Midwest is dominated by two crops: maize and soybean. In the Corn Belt region, 20% of the maize is produced in monoculture and 80% is in 2-year rotation with soybean (Varvel and Wilhelm, 2003; Karlen et al., 2006; NASS, 2019). Monocultures or short crop rotations systems are negatively affecting soil health, decreasing soil organic matter, and increasing soil erosion (Johnston, 2014). Although not as common, longer crop rotations, including alfalfa or other perennial forages are important in areas with a high concentration of dairy farms (Sulc and Tracy, 2007). In the Corn Belt, alfalfa acreage has only 8% of the total area cultivated but contributes to 80% of the country's alfalfa production (Russelle, 2013).

Maize and soybean acreage has nearly doubled in the last two decades in the Corn Belt and Midwest US states (Ohio, Indiana, Illinois, Iowa, Minnesota, and Wisconsin) (Russelle, 2013; NASS, 2019). Conversely, the total harvested area for hay has decreased in the last two decades mainly due to the reduction of cattle and high commodity prices (Russelle, 2013; NASS, 2019). In addition, Russelle (2013) attributed the decline on alfalfa production to the greater use of maize on ruminant diets.

Long and diverse crop rotations, that include a perennial crop, provide beneficial effects over the ecosystem such as increasing grain crop yield, reducing inputs, and enhancing soil health (Russelle et al., 2007; Sulc and Tracy, 2007; Russelle, 2013). In addition, crop diversity can be enhanced by including a companion crop or a cover crop in the rotation in-between cash crops (Undersander et al., 2011).

The use of companion crops to establish alfalfa is a common practice in the USA to enhance seedling establishment, reduce soil erosion, and increase forage yield in the seeding year

(Hoy et al., 2002; Undersander et al., 2011). Companion crops not properly managed can reduce alfalfa seedling establishment (Simmons et al., 1995; Hoy et al., 2002; Mahli and Foster, 2011). Small grains such as barley (*Hordeum vulgare* L.) and oat *(Avena sativa* L.) are commonly used as companion crops for alfalfa. Intraspecific light competition will depend on companion crop species and cultivar, plant density, plant height (dwarf, semi-dwarf, or conventional), and harvest stage (Simmons et al., 1995; Hoy et al., 2002; Undersander et al., 2011; Mahli and Foster, 2011). The alfalfa underneath the canopy of the companion crop can die by insufficient light or smothering by lodging of the companion crop. At full height, the companion crop reduces the photosynthetically active radiation (PAR) available for alfalfa 59% and 49% under the barley and oat canopy, respectively. Alfalfa seedling mortality ranges from 42 to 50 % under the barley and oat canopy, during the establishment year, but companion crops did not affect alfalfa yield overall. In this study, alfalfa was harvested later in the establishment season and at first flower in the next spring; small grains were harvested at the boot or soft dough stage (Simmons et al., 1995). Oat as a companion crop with alfalfa establishment does not have any negative impact on alfalfa growth. The most important result as a companion crop was the reduction of broadleaves weed density (Hoy et al., 2002). Oat and barley at high seeding rates (124 kg ha^{-1}) decreased forage yield of alfalfa-smooth brome (*Bromus inermis* L.) established in the understory (Mahli and Foster, 2011). Less desirable companion crops for alfalfa are winter wheat (Triticum aestivum L.) and winter rye (Secale cereale L.), due their strong competition with alfalfa seedlings (Undersander et al., 2011).

2.2. Intercropping

Intercropping is a type of multiple cropping in which two or more crops are grown simultaneously in the same field at the same time (Moradi et al., 2014). Plants of different species can be closely arranged to optimize positive plant growth interactions (Belel et al., 2014).

Andrews and Kassam (1976) categorized intercropping into four types:

i) Mixed intercropping: growing two or more crops simultaneously with no different row arrangement. The component crops are intermixed in the available space.

ii) Row intercropping: growing two or more crops simultaneously in alternate rows.

iii) Strip intercropping: growing two or more crops simultaneously in adjacent strips within a field during the same growing season.

iv) Relay intercropping: growing two or more crops with part of the growth cycles overlapping.

Usually, the second crop is sown before the first crop is harvested, then after harvest, the under sown crop resumes growth.

According to Moradi et al. (2014), one of the most important purposes of intercropping is to make more efficient use of the available resources. Additionally, intercropping has shown great potential for increasing biomass yield (Fusuo and Li, 2003). In general, crops in intercropping have greater production stability and higher total seed or biomass yield than their respective monocultures (Smith and Carter, 1998). Growing crops in mixed stands can be more productive than monocultures mainly because of improved temporal solar radiation use efficiency, enhanced weed control, pest suppression, and reduced soil water loss because of rapid development of ground cover (Anil et al., 1998; Belel et al., 2014).

Plants can be closely arranged to optimize and improve positive plant interactions (Smith and Carter, 1998). In intercropping, the highest grain or biomass yields and complementary effects such as availability of nutrients, and availability of water for both crops, occur when crop species' growth periods have their major demands of resources at different times (Li et al., 2006; Reddy and Reddi, 2007). Crops do not compete for the same resources, at the same stage of development; utilizing resources efficiently and likely enhancing nutrient uptake in both crops (Hauggaard-Nielsen et al., 2001; Li et al., 2001; Flores-Sanchez et al., 2012).

2.2.1. Intercropping of annual crops

Intercropping of cereals with legumes in forage production is used commonly to increase forage yield and quality, improve land use efficiency (Li et al., 2006), and profitability per unit land area (Hauggaard-Nielsen et al., 2007). Almost all studies conducted in legume-cereal intercropping (barley-pea (*Pisum sativum* L.), oat-pea, and oat-barley) have shown forage yield advantages compared with the corresponding monoculture (Anil et al., 1998).

Annual cereal and legume intercropping has been the most popular combination used due to the legumes' ability to symbiotically fix nitrogen and reduce soil erosion (Matusso et al., 2012). Legume-cereal intercropping such as pea, faba bean (*Vicia faba* Roth.), and lupin (*Lupinus angustifolius* L.) with barley, offers many advantages such as weed suppression, reduction in diseases, and higher nutrient use efficiency (Anil et al., 1998; Hauggaard-Nielsen et al., 2007). Intercropping of pea, faba bean, and lupin with barley in three consecutive cropping seasons resulted in increased seed yield, especially with pea-barley in sandy and sandy-loam soils. Pea-barley intercropping had a total combined grain yield of 4.6 Mg ha^{-1} in sandy-loam soils, and 4.4 Mg ha^{-1} in sandy soils (Hauggaard-Nielsen et al., 2007). Similarly, faba bean-barley, lupine-barley, and pea-barley produced similar forage dry matter yield than monocultures but with increased crude protein content (Strydhorst et al., 2008). Faba bean-barley, lupin-barley, and pea-barley biomass had 64%, 27%, and 55% higher protein yield, respectively compared with forage barley (79 g kg^{-1}) at the soft dough stage. Faba bean-barley, and lupin-barley had similar forage dry matter yield (12 Mg ha^{-1}). Pea-barley yield was 13.5 Mg ha^{-1} (Strydhorst et al., 2008). The complementary effects of intercropping pea with cereals such as spring wheat (*Triticum aestivum* L.), spring barley, oat, and spring triticale (× *Triticosecale* Witt.) were enhanced when crops phenology and growth period was different. Cereals dominated the mixtures and had a greater contribution to the total forage yield (Sarunaite et al., 2013) while pea increased the crude protein of the forage (Strydhorst et al., 2008). Increased forage yield and nutritive value with intercropping may be related to a change in root development and distribution, likely due to increased nutrient and water use efficiency (Zhang et al., 2013). Pea-barley intercropping induced deeper roots in the cereal and faster lateral root growth in both species compared with the sole crops (Hauggaard-Nielsen et al., 2001).

Crimson clover (*Trifolium incarnatum* L.) interseeded into maize 10 to 20 days after maize emergence established successfully, but posed strong competition to maize (Abdin et al., 1998). Interseeded crimson clover competed for soil water with maize, especially at a seeding rate of 22 kg ha^{-1}, decreasing maize grain yield (Parr et al., 2011). However, crimson clover with adequate moisture and rainfall during the growing season, affected less maize grain yield (Abdin et al., 1998). Weed suppression by crimson clover grown during the winter fallow period in continuous maize may lead to a reduction in herbicide use. At maize planting, crimson clover reduced weed biomass 22 to 46% (Baeberi and Mazzoncini, 2001).

Intercropping maize grain with bambara groundnut [*Vigna subterranean* (L.) Verdc.], and peanut (*Arachis hypogeae* L.) had a significant effect on plant height, total fresh yield, and crude protein content, and also increased the use efficiency of solar radiation, available water, and nutrients compared with monocultures (Ali and Mohammad, 2012; Belel et al., 2014). Additionally, maize-cowpea *(Vigna unguiculata* L.*)* intercropping increased N, P, and K uptake compared with the maize alone (Belel et al., 2014). This was also observed in a maize-wheat-soybean intercropping where N, P, and K nutrient uptake was greater in intercropping than each sole crop (Li et al., 2001).

Intercropping silage maize and sunflower (*Helianthus annuus* L.) increased forage quality (fiber, fat, and protein content) compared with the sole crops. Silage maize produced high dry matter yield and sunflower silage was higher in fat and crude protein in comparison with silage maize. Also, lactating cow intake of intercropping silage maize and sunflower, were similar to the silage maize alone but increased the milk production (Anil et al., 1998).

Intercropping of two different cereals also has shown increased yield and N use efficiency. Total grain yield of wheat and maize increased from 40 to 70% when both crops were intercropped. Nitrogen uptake in wheat monoculture was 188 kg N ha^{-1} while in wheat intercropped with maize was 270 kg N ha^{-1} (Li et al., 2001).

2.2.2. Intercropping of perennial legumes and cereals

Intercropping perennial legumes with annual cereals is less common in high input agriculture but is gaining interest to increase biodiversity in cropping systems. Red clover (*Trifolium pratense* L.), kura clover (*Trifolium ambiguum* Bieb.), and alfalfa are the most common perennial legumes grown in intercropping with winter wheat or maize. When intercropping perennial legumes and annual crops, differences in physiological ecology (physiological responses of both crops growing together to the environment), root spatial distribution, and nutrient requirements need to be considered (Zhang et al., 2011).

Red clover, intercropped with winter wheat, not only can supply forage, but also supplies N to the crop (Blaser et al., 2007). To maximize red clover forage yield and quality once the wheat or triticale has been harvested, it is recommended to frost seed in early March, and seed the clover at a rate of 900-1200 seed m^{-2}. Frost seeding involves broadcasting seeds just after snowmelt in late winter or early spring. As the ground freezes and thaws, the seeds are incorporated into the soil, and germination occurs only when the soil water and temperature are optimal (Sarrantonio and Gallandt, 2003). Legumes are favored by frost-seeding compared with grasses, but it is likely that wheat will out-compete the red clover in the spring of the year of establishment (Blaser et al., 2007).

Red clover intercropped with silage maize, provided soil erosion protection and did not significantly reduced silage maize yields. The reduction of soil loss was 48 to 76% less compared with silage maize alone. Moreover, intercropped red clover increased silage maize biomass yields compared with sole maize, without nitrogen fertilizer application (Wall et al., 1991). Red clover intercropped with maize, and seeded 10 to 20 days after maize emergence at a rate of 10 kg ha^{-1}, did not decreased maize grain yield, especially with adequate seasonal rainfall (Abdin et al., 1998).

Intercropping maize grain and kura clover, allowed the clover to become a living mulch in maize, especially under no-tillage conditions. Kura clover did not affect maize grain yield, and continued growing after harvesting maize, until a hard fall frost. Kura clover seeded in early spring, affected maize grain yield due to competition between the two species, and also cool-weather favored kura clover growth instead of maize. An excellent winter ground cover was provided by kura clover, covering approximately 60% of the soil surface (Zemenchik et al., 2000). Loss of maize plant population and slow maize development were reported due to kura clover competition and wet-cool springtime soils, when kura clover was intercropped with maize grain (Sawyer et al, 2010).

Other perennial or biennial clovers that are utilized in intercropping with winter wheat, to suppress weed growth are alsike clover (*Trifolium hybridum* L.), white clover (*Trifolium repens* L.), and yellow sweetclover (*Melilotus officinalis* L.). Usually, they are sown on frozen ground in early spring and provide good organic matter production and N_2-fixation before the next spring crop planting the season after winter wheat harvest (Ross et al., 2001).

Alfalfa in intercropping, improves the productivity of the intercrop, or subsequent crop due to increased nutrient availability and organic matter, among others (Belel et al., 2014). Alfalfa intercropped with forage sorghum (*Sorghum bicolor* L. Moench) had greater forage yield than forage sorghum alone. Alfalfa-forage sorghum biomass yield ranged between 15.4 and 17.0 Mg ha^{-1} and forage sorghum between 14.6 and 16.7 Mg ha^{-1} (Hallam et al., 2001).

Grain-maize and alfalfa intercropping, in which alfalfa was seeded two months before maize, enhanced maize and alfalfa biomass yield compared with the monocultures. Also, Zhang et al., (2011) suggested that in intercropping, alfalfa utilized resources better than maize and produced greater yield. Alfalfa was a superior competitor, and its productivity dominated the total average biomass yield, during the three years of the experiment (Zhang et al., 2011). In China, intercropping of alfalfa and maize increased maize grain yield, alfalfa forage biomass, land use efficiency, and economic income per unit area compared with the monocultures (Zhang et al., 2011; Sun et al., 2014, 2018).

Land equivalent ratio (LER) is defined as “an index of intercropping advantage and a reflection of the degree of interspecific competition or facilitation in an intercropping system”

(Zhang et al., 2011). Another definition of LER is the “relative land area required as a sole crop to produce the same yields as intercropping” (Mead and Willey, 1980).

Land equivalent ratio measures the levels of intercrop interference on the cropping system. Then, a LER higher than 1.0 indicates an intercropping advantage or positive inter-specific interference that exist in the mixture, and LER less than 1.0 reflects mutual antagonism or competition in the intercropping system (Zhang et al., 2011). A LER value for alfalfa intercropped into maize grain was always more than 1.0, since the year following the establishment, the intercropping system had superior yield compared with maize or alfalfa alone (Zhang et al., 2011). Alfalfa and maize grain intercropping resulted in higher productivity and a temporal and spatial complementarity, which optimized resource utilization and promoted intercropping advantages (Zhang et al., 2013). As expected, alfalfa in monoculture during the seeding year had lower biomass yield than maize in monoculture (Zhang et al., 2011).

In another study, maize was relay-cropped into alfalfa established two months earlier. Maize was still in the seedling V3 (third leaf) stage and shorter than alfalfa by the time the latter reached bloom stage (Sheaffer et al., 1988; Ransom and Endres, 2014). Alfalfa and maize used complementary niches for enhanced light interception (Sun et al., 2014). Interseeding alfalfa into maize at the same seeding date as maize, favored alfalfa establishment (Pendleton et al., 1957). Usually, early in the spring availability of soil water and cool temperatures favor alfalfa’s growth. In an alfalfa-maize intercropping study, alfalfa seeded at the same time as maize or later in the season did not decrease maize grain yield and provided cover in the fall and in the spring of the year following seeding (Exner and Cruse, 1993).

2.2.3. Ecosystem services provided by intercropping

An ecosystem service is defined as "a concept that distinguishes the long-term role that healthy ecosystems play in the sustainable provision of human wellbeing, economic development, and poverty alleviation across the globe" (Turner and Daily, 2008). Daily and Matson (2008) described this concept as a bridge between the environment and human well-being. To integrate an ecosystem service as a decision-making tool it must be credible, replicable, scalable, and sustainable. The dynamic of the diversity in an ecosystem, is measured not only by the amount of different species, but also by the relationship between space and time. To implement this new concept, an economic policy that favors the diversification in land uses and the diversity among land users is required (Swift et al., 2004).

According to Kragt and Robertson (2014), there are four classes of ecosystem services:

1. Provisioning services: the products directly obtained from the ecosystems.
2. Supporting services: services that are necessary for the production of all other ecosystem services.
3. Regulating services: the benefits obtained from the regulation of ecosystem processes.
4. Cultural services: the nonmaterial benefits people obtain from ecosystems.

Alfalfa provides several supporting and regulating ecosystem services, among them, soil health improvement, N_2 fixing, N credits for the next crop, pollinators and wildlife habitat, water retention in the soil, and mitigation of nitrate leaching and P run-off (Carter and Scheaffer, 1983; Power, 2010; Undersander et al., 2011; Dell et al., 2018). Alfalfa has shown great potential to control erosion and enhance soil structure, due to its deep root system and increase in soil organic carbon (Dell et al., 2018; Niu et al., 2020). In addition, soil stability was enhanced and surface sediment run-off was decreased when alfalfa was included in the cropping system (Wu et al., 2011).

Nitrogen is the main limiting nutrient for crop growth. Fortunately, legumes have the ability to fix atmospheric N_2 in symbiotic association with rhizobia, which converts it to plant-available forms. Rhizobia fixes atmospheric N_2 into N-containing compounds which are used for amino acids synthesis in the plant (Russelle, 2004; Undersander et al., 2011). Nitrogen fixation rate depends directly on the supply of N from other available sources. Legumes uptake N from fertilizers, from the mineralization of plant residues, and indirectly, from the recycled-N in the manure from animals fed with alfalfa (Peterson and Russelle, 1991). Russelle (2004) reported that N_2 fixation by alfalfa ranged from 45 to 477 kg N ha^{-1} annually in the Mississippi River Basin. Alfalfa only fixes the additional nitrogen needed to grow, because it is an adaptive process, and alfalfa will uptake and deplete N available from all other sources first (Russelle, 2004). In an intercropping system composed by forages legumes and grasses, N_2 fixation by the legume will provide the grass with enough N, reducing the need of N fertilizers (Aponte et al., 2019). When alfalfa is grown together with grasses, 80% of the N taken up by the grass comes from the N_2 fixation of the legume (Carlsson and Huss-Danell, 2003).

Nitrogen transfer from legumes to non-legumes may occur by N-release from decaying nodules when crops are growing together, or after the legume residue roots have been incorporated into the soil (Anil et al., 1998; Zhang et al., 2013). Other pathways to transfer nitrogen from legume to grasses are excretion of nitrogen from the legume root and nodules, and grasses and legumes root interconnection by mycorrhizal fungi (Haystead et al., 1988; Russelle et al., 1994). Mycorrhiza hypha allows the extension of the root system and enhances the plant's access to nutrients. In white clover and perennial ryegrass (*Lolium perenne* L.), and due the presence of mycorrhizae the transfer of N from the legume to the grass increased dry matter yield in both crops (Haystead et al., 1988). Mycorrhiza-colonized roots increased plant ability to release N into the soil. In N-limited pastures, it is common to find mycorrhiza, and the exchange of N is polarized from legume to non-legume (Haystead et al., 1988).

Sun et al. (2014), reported that in alfalfa-maize intercropping, N was transferred from alfalfa by N released from decaying alfalfa nodules and roots, enhancing soil N availability, improving soil physical and chemical properties, and maize growth. It is believed that in intercropping the cereal roots deplete the legume root rhizosphere of N, obligating the legume to increase N_2 fixation, increasing the N content in both crops (Hauggaard-Nielsen et al., 2001).

Additionally, alfalfa-maize based cropping systems improve the N economy of the cropping system increasing productivity per unit time and space, and net returns in comparison with monoculture (Thayamini and Brintha, 2010). In a study conducted in Minnesota, alfalfa added 47 to 72 kg ha^{-1} of N credit to the silage maize crop, increasing silage maize yield in 1.8 Mg ha^{-1}. The average net return to N was \$44 ha^{-1} (Coulter et al., 2012).

Nitrogen applications based on crop requirements, play an important role on farmer's budget. Excess N fertilizer increases the risk of leaching into groundwater. In maize following alfalfa, the risk of NO_3-N leaching increases when applications exceeded 45 kg N ha^{-1}. Nitrogen credits from 3- to 7-yr old alfalfa stands - to maize, during the first year can reach 168 kg ha^{-1}. Maize silage following a 3- to 7-yr old alfalfa application of 40 kg N ha^{-1} was beneficial to obtain economically optimum maize silage yield (Yost et al., 2012).

Maize seeded after a 3-yr old alfalfa stand, did not required N fertilizer, mainly due to the net N deposition in the alfalfa residue. Maize seeded after a 1- or 2-yr old alfalfa stand required N fertilizer, at 97 and 64 kg N ha^{-1}, respectively, for optimum maize grain yield (Yost et al., 2015). The N credit from maize to alfalfa also was reported by Niu et al. (2020) who indicated that the N credit was due to an increase in soil microbial biomass and mineralization rate in a 9-year continuous alfalfa stand compared with maize following alfalfa after 3-years of alfalfa.

In North Central states, alfalfa intercropped with maize can continue to grow about one month after the maize has been harvested, providing enough time to the alfalfa plants to store carbohydrates in the roots to avoid winter injury (Undersander et al., 2011). Then, the total N acquisition (N soil + N_2 fixed) in alfalfa-maize intercropping system is greater compared with the sole crop, especially following a maize monoculture (Zhang et al., 2013).

Intercropping changes the N distribution in different organs of the maize plant. In maize intercropped with alfalfa, N content of maize stalks was higher than in stalks of sole maize (Zhang et al., 2013). The maize stalk N content was higher under alfalfa intercropping compared with the maize sole crop. Little attention has been paid to root distribution in combined perennial legume-annual cereal crops with intercropping. Enhanced biomass yield of both crops in an intercropping system is most likely due to a complementary root spatial distribution and compatible root development that allows both crops to improve nutrient uptake (Zhang et al., 2013). If roots cover a larger soil volume, which is the case with intercropping, the uptake of P will be increased (Anil et al., 1998). Legumes increase the P uptake in association with some cereals, because root exudate compounds into the rhizosphere that enhances the mobilization of insoluble phosphate (Li et al., 2001).

In a study conducted in Wisconsin, Osterholz et al. (2019) reported that compared with maize silage grown without interseeded alfalfa and followed by spring-seeded alfalfa, interseeded maize/alfalfa reduced losses of total suspended solids by 49% to 87%, total N by 37% to 74%, and total P by 37% to 81%, respectively. In this same study, total runoff volume and losses of dissolved solids, dissolved P, and NO_3-N were reduced in the alfalfa-maize interseeding system compared with the conventional maize silage followed by alfalfa rotation.

In a maize-alfalfa based system, the alfalfa root system can significantly improve soil fertility and physico-chemical properties. In maize-alfalfa intercropping, alfalfa roots reach down to more than 90-cm in depth and the maize roots only to 40-cm in depth (Sun et al., 2014). In the North Central states, alfalfa roots typically can grow down to 1.2-to 2.4-m in depth (Russelle, 2003). The alfalfa hay crop can extract significant amounts of NO_3-N to soil depths of 90, 180, 210, and 270 cm from Year 1 to Year 4 (Entz et al., 2001).

One important consideration in intercropping with alfalfa is water use. Alfalfa is a high water user and has a high water use efficiency (quantity of water necessary to produce a unit of crop field), and this is due mainly to the high biomass yield, its long-life cycle, and a deep root system (Anil et al., 1998). Alfalfa water use is about 10 cm of water for Mg of dry matter yield under rainfed conditions (Carter and Sheaffer, 1983; Lindenmayer et al., 2011). Alfalfa's high water use can dry up the soil profile to 2-m deep causing water stress to the intercropped or following crop (Undersander et al., 2011). Alfalfa water use efficiency (WUE) values fluctuate between 0.12 to 0.17 Mg of biomass ha^{-1} cm^{-1} of water (Bauder et al., 1978; Carter and Shaeffer, 1983; Lindermayer et al., 2011), while maize WUE fluctuates between 0.11 to 0.23 Mg of biomass ha^{-1} cm^{-1} (Olson, 1971; Noorwood, 2000). In maize, water availability is a critical factor for biomass and grain yield production, because the roots are shallow and mostly distributed above 40-cm in depth. In alfalfa, roots can penetrate more than 10-m in depth, but most roots are located in the first 60-cm of soil. Both crops compete for water and nutrients in the shallow soil layers (Sun et al., 2014). The absence of tillage during the alfalfa's life cycle decreases the breakdown of the soil structure, in comparison with an annual crop (Wu et al., 2011). Alfalfa covered-plots had 1.77 times higher infiltration rate than bare-soil, reducing surface run-off and soil sediment loss (Wu et al., 2011).

Maize, under a conventional system, once harvested, leaves the soil uncovered during the winter and early spring, increasing soil wind and water erosion and nutrients losses by run-off (Landis et al., 2008). When a variety of crops are present in the field, in rotation or in intercropping, insect populations and diversity increases, providing valuable ecosystem services such as crop pollination and pest control (Landis et al., 2008; Eberle et al., 2015).

2.2.4. Intercropping of alfalfa with maize to replace conventional spring seeding of alfalfa

Intercropping of maize and alfalfa can also be used to establish alfalfa one year ahead, increasing alfalfa forage yield on farms in high need of forage, such as dairy farms (Sun et al., 2014; Grabber, 2016). Alfalfa seasonal forage yield is much lower than silage maize, thus many dairy farmers have moved to continuous silage maize production (Sulc and Tracy, 2007; NASS, 2019). One-way to increase alfalfa yield in the typically low-yield seeding year, is interseeding alfalfa into maize (Zhang et al., 2011; Grabber, 2016).

In alfalfa-maize intercropping, light competition is an important factor to consider. Maize light interception is about 80 to 90% at full plant height, thus only 20 to 10% of PAR light is available for the alfalfa under the leaf canopy (Matusso et al., 2012). In intercropping, when alfalfa and maize are seeded at the same time, usually alfalfa grows slower than maize. As maize gets taller, reduced PAR light cause alfalfa’s stem internodes to elongate, weakening the plant and increasing the risk of winterkill (Matusso et al., 2012; Grabber 2016).

To prevent stems from etiolating and plants from weakening, growth regulators can be used to control stem elongation (Grabber, 2016). Prohexadione-calcium (PHX) (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate) is a gibberellic acid inhibitor. Currently sold under a variety of trade names by multiple companies (Apogee® by BASF, Kudos® 27.5 WDG by Fine Americas, and Anuew™ by Nufarm US), PHX is used commercially in the USA to limit shoot growth in fruit trees, peanuts (*Arachis hypogea* L.), nursery crops, turf, vegetable crops, and grass grown for seed production. In all cases, PHX reduces vegetative growth, balancing canopy development and fruit or seed production (Rethwish et al., 2003).

Gibberellins (GAs) are plant hormones with an active role in plant growth and cell elongation; and are found in flowers, roots, fruits, seeds, and leaf primordia in meristems. Gibberellic acid regulates stem elongation and the mobilization of endosperm reserves during early stages of seed germination. The synthesis of GAs occurs in developing seeds and fruits, young leaves of developing apical buds and elongation shoots, and the apical regions of roots (Hopkins and Hüner, 2008). Some growth retardants block the synthesis of GAs. Prohexadione-calcium alters the biosynthesis of growth-active GAs, reducing longitudinal shoot growth. Prohexadione-calcium inhibits the enzyme flavanone 3-hydroxylase by hydroxylation of the 3β bond. This enzyme is necessary for flavonoids biosynthesis (Evans et al., 1999; Costa et al., 2001). The biological functions of flavonoids in plants are related with the defense against UV-B radiation, pathogen infection, nodulation, and pollen fertility (Hopkins and Hüner, 2008).

Usually, PHX is applied as a foliar spray or soil drench, to reduce stem elongation, obtaining shorter and compact plants, with dark green foliage (Hopkins and Hüner, 2008). The growth suppresser effect of PHX typically lasts for 2-5 weeks. It is absorbed by the foliage and the uptake is generally complete within 8 hours following application. Then, it is translocated acropetally (Costa et al., 2001). When tricarballylic acid is formed from PHX, is incorporated into the plant matrix (Evans et al., 1999). In soil, PHX decomposes, mostly to carbon dioxide, with a half-life less than 7 days and does not persist in the plant or affect directly vegetative growth the following season (Evans et al., 1999). Prohexadione-calcium reduces internode elongation, resulting in increased alfalfa leaf/stem ratio and improved survivability of alfalfa under the maize canopy (Rethwish et al., 2003; Grabber, 2016).

Prohexadione-calcium applied under the maize canopy, when alfalfa reached 18-cm in height, resulted in a first-year alfalfa forage yield increase of 1.5 Mg ha^{-1} (Grabber, 2016). The growth regulator was able to reduce alfalfa biomass and seedling mortality. Alfalfa interseeded into maize with application of PHX had a forage yield of 10.9 Mg ha^{-1} in the first year of production while silage maize had a forage yield of 22.1 Mg ha^{-1}. Alfalfa-maize interseeding reduced maize plant height and decreased maize dry matter biomass yield in 10%, when maize was planted.

The first application of PHX was done four to six weeks after planting (Grabber, 2016). Early application of PHX also has a positive effect over the alfalfa stand density, increasing the number of plants per m^{-2}. Stand densities went from 157 plant m^{-2} without PHX, to 236 plants m^{-2} with rates of PHX in between 0.6 to 2.4 kg a.e. ha^{-1}. The rates of PHX were not statistically different over the stand density. Rates of 0.60 to 2.40 kg ha^{-1} were used over 10-28 cm height seedlings, and the result was the reduction of alfalfa biomass by 0.41- 0.76 Mg ha^{-1}. Moreover, reduction of seedling mortality was observed in all treatments with PHX, compared with spring-seeded alfalfa (Grabber, 2016). In addition, a variety of adjuvants and ammonium sulfate added to PHX were effective in reducing alfalfa height by an average of 16% within 4 weeks after treatment and increasing alfalfa stand counts after maize harvest by 30% compared with the non-PHX treated control (Osterholz et al., 2018).

2.3. Economics: Alfalfa, Maize, and Alfalfa-Maize Intercropping

Some benefits of intercropping are flexibility, maximization of profit, minimization of risk, soil conservation, ecosystem services, and soil fertility improvements. Nevertheless, intercropping may reduce the yield of one or both crops (Belel et al., 2014). Intercropping uses land more efficiently and typically increases LER and net returns (Smith and Carter, 1998; Brintha and Seran, 2009; Ijoyah and Dzer, 2012). Sun et al. (2014), reported that the main economic benefits of the maize-alfalfa intercropping are reduced inputs and N credits to maize.

A cost reduction between 8 and 14% was obtained when forage sorghum was interseeded into alfalfa (Hallam et al., 2001). The cost of interseeded sorghum-alfalfa was \$54 Mg^{-1} and the cost of alfalfa in monocrop with three harvests in a season was \$ 63 Mg^{-1} (Hallam et al., 2001). Sorghum grain yield as a monocrop was higher than in intercropping with alfalfa, but the risk of soil erosion was greater. Impact in the environment should be taken into consideration when deciding the crops to intercrop. The focus must not be only on profitability, but land sustainability (Hallam et al., 2001).

Farmers who add alfalfa to maize-soybean based rotations can obtain significant economic gains. Average production costs for a 2-yr maize-soybean rotation were higher than the cost for a 4-yr rotation (maize-soybean-oat/alfalfa-alfalfa) in Minnesota, although net return was higher for the 2- yr maize-soybean rotation (Delbridge et al., 2011). This was expected since the study was done when maize and soybean price were high. The high production cost of the 2-yr maize-soybean rotation, was \$68 ha^{-1} greater than the 4-yr rotation, mainly due to the higher fertilizer and pesticide applications (Delbrige et al., 2011). Conversely, a simulated 5-yr rotation in Iowa, including maize-soybean-oat/alfalfa-alfalfa-alfalfa resulted in a 24% net income increase compared with a maize-soybean-maize-soybean-maize, including government farm support payments for the row crops (Olmstead and Brummer, 2007). The average production cost of a 2-yr maize-soybean rotation, was \$487 $ha^{-1}yr^{-1}$ and of a 4-yr maize-soybean-oat/alfalfa-alfalfa was \$405 ha^{-1} yr^{-1} (Delbridge et al., 2011).

Typical fall operations for glyphosate-tolerant alfalfa fall establishment in dryland systems include fertilizer application disking, harrowing, rolling, planting, and spraying. While maize spring field operations include disking, anhydrous ammonia application, planting, spraying (at least twice), combining, grain transportation, drying, and chopping stalks (Klein et al., 2015). Inputs for both crops include seed, fertilizers, herbicides, and insecticides, but maize needs additional services such as scouting and crop insurance (Klein et al., 2015).

In Nebraska, the cost for establishment including the overhead was \$551 ha^{-1} for alfalfa and \$1,245 ha^{-1} for maize in dryland (Klein et al., 2015). In North Dakota, the estimated cost to produce maize and alfalfa is \$1,261 ha^{-1} and \$ 601 $ha^{-1,}$ respectively (NDSU Extension, 2018). In Iowa, the cost of alfalfa establishment, including the overhead was between \$430 and \$543 ha^{-1}, assuming average Iowa equipment use and a land surface of 65 ha (Hallam et al., 2001).

In Wisconsin, alfalfa-maize intercropping rotations had annual net returns of \$303 to \$367 ha^{-1}, with a 3-yr maize and 3-yr alfalfa sequence identified as the most profitable (Osterholz et al., 2020). Conventional rotations provided lower annual net returns of \$260 to \$320 ha^{-1} and the most profitable rotation was a 4-yr maize and 4-yr alfalfa sequence. Sensitivity analyses demonstrated that intercropped alfalfa had a robust economic benefit, with increased net returns obtained when the maize yield penalty was limited to less than 20% or the success rate of alfalfa establishment exceeded 49% of attempts for the most profitable intercropped rotation sequence (Osterholz et al., 2020).

CHAPTER 3: MATERIALS AND METHODS

3.1. Experimental Sites

The experiment was conducted at two North Dakota State University (NDSU) research sites at Fargo (46°52′N, 96°48’W, elevation 274 m) and Prosper (46°58’N, 97°3’W, elevation 280 m). The soil type at Prosper is a Kindred-Bearden silty clay loam (Perella: fine-silty, mixed, superactive Typic Endoaquoll; Bearden: fine-silty, mixed, superactive, frigid Aeric Calciaquoll) while the soil type at Fargo is Fargo-Ryan clay soil (fine, montmorillonitic, frigid, Vertic Haplaquoll with a leached and degraded nitric horizon) (Soil Survey 2014, 2016). Monthly rainfall and minimum, maximum, and average temperature was obtained from the North Dakota Agricultural Weather Network (NDAWN, 2017).

3.2. Experimental Design and Management

The experimental design at both locations was a randomized complete block (RCB) with four replicates and a split-plot arrangement. The main plots were two maize row spacings (61 cm and 76 cm) and the subplots were the alfalfa intercropping treatments: i) sole maize, ii) alfalfa intercropped with maize, iii) alfalfa intercropped with maize with one application of PHX, and iv) spring-seeded alfalfa in 2015. The experiment was started in 2014. In 2015, the plots that had intercropped alfalfa the previous year were left to grow and were evaluated for forage yield.

Previous crop at both locations was hard red spring wheat with conservation tillage consisted of two passes of chisel plowing and one pass of disking to prepare the seedbed for planting alfalfa in 2014. No-tillage was used before alfalfa was seeded in the spring of 2015.

A glyphosate-resistant alfalfa cultivar, Presteez RR (alfalfa label information: pure live seed: 65.9%; germination: 73%; hard seed: 15%, fall dormancy rating: 3, Winter survival rating:1) and the silage maize hybrid 2 MD 96 RR (96 d relative maturity, Roundup Ready™) were used for this study.

In 2014, maize plots were seeded on 29 May with a two-row maize drill at 76-cm (7100 MaxEmerge, Moline, IL), and a different-cone plot planter (Wintersteiger, Plotseed XL, Salt Lake City, UT) was used to plant maize at 61-cm. Alfalfa was seeded right after seeding maize with the two alfalfa rows spaced 7.5-cm apart. The alfalfa was seeded with the same plot planter, but with 8 rows spaced at 15-cm row spacing. Each experimental unit of 6-mt long, had either four rows of maize or four rows of maize intercropped with 16 rows of alfalfa seeded on the same seeding date (Table 3.1). The targeted maize plant density was 87,932 plants ha^{-1} for both row spacings. The seeding rate for alfalfa was 15 kg ha^{-1} of pure live seed (PLS) (only correcting seeding rate by seed germination (80%).

Table 3.1. Seeding dates and proxehadione (PHX) application dates for maize and alfalfa at Fargo and Prosper, ND in 2014 and 2015.

Location	Maize	Intercropped alfalfa	Intercropped alfalfa + PHX	Spring-seeded alfalfa
Fargo	29 May 2014	29 May 2014	2 July 2014	2 June 2015
Prosper	23 May 2014	23 May 2014	2 July 2014	1 June 2015

In 2014, proxehadione (PHX) was applied to alfalfa when maize was at V8 stage and alfalfa was 18-20cm in height (Table 3.1), using a rate of 0.5 kg a.i. ha^{-1}. The equipment used was a premium manual sprayer, with 3.78 L capacity, made of reinforced PVC, and with one nozzle (Roundup 1-Gallon Premium Sprayer, USA). The product was sprayed over the alfalfa, but under the maize canopy.

Weed control was done with glyphosate (isopropylamine salt of N-(phosphonomethyl) glycine), at a rate of 0.84 kg a.i. ha^{-1} depending on the weed pressure. In 2014, the product was applied twice during the growing season over both crops at the same time, using a three-nozzle CO_2 backpack sprayer. From 2015 to 2017, glyphosate was sprayed once every year.

In 2014, 120 kg N ha^{-1} as urea fertilizer were applied to all plots. Alfalfa was fertilized with 30 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1}, as mono ammonium phosphate (11:52:0) and potassium chloride (0:0:60), in the fall of both years following recommendations by (Franzen and Berti, 2017). After maize showed symptoms of sulfur deficiency (V5 stage), in Fargo in 2014, all plots were fertilized with gypsum (17% of SO_4) with a rate of 30 kg ha^{-1} in Fargo.

Maize was harvested by hand in the two-center plot rows with a 2.8 m^2 harvested area for the 61 cm-row spacing and 3.5 m^2 harvested area for the 76-cm row spacing. Maize stubble height after forage harvest was 5-cm. A forage moisture of 65% was used to calculate forage biomass yield. Once maize biomass was harvested all remaining maize plants in the plot were cut off and removed from the field with a maize silage chopper (New Holland FP 240, Racine, WI), in Fargo and by hand in Prosper. Alfalfa was harvested using a flail forage harvester (Carter MFG CO., Inc., Brookston, IN) in 2015 (Table 3.2).

Table 3.2. Harvest dates (HV) of alfalfa and maize at Fargo and Prosper, ND, USA, for 2014 to 2017.

	----------------------------------Alfalfa----------------------------------						Maize
Location/year	HV1	HV2	HV3	HV4	HV1 Yr2†	HV 2 Yr2†	
Fargo 2014‡	8 Oct.						26 Sept.
Prosper 2014	8 Oct.						26 Sept.
Fargo 2015	19 June	14 July	11 Aug.	1 Oct.	5 Aug.	1 Oct.	
Prosper 2015	19 June	10 July	5 Aug.	1 Oct.	5 Aug.	1 Oct.	
Fargo 2016	2 June	28 June	1 Aug.	25 Aug.	18 July		
Prosper 2016	2 June	28 June	1 Aug.	25 Aug.	18 July		
Fargo 2017	31 May	29 June	1 Aug.	4 Oct.			
Prosper 2017	31 May	29 June	1 Aug.	4 Oct.			

† Harvest dates of spring-seeded alfalfa
‡ Years indicate year of harvesting

3.3. Sampling and Analysis

Soil samples were taken at both locations and both years at a 0- to 15-cm depth and tested for pH, organic matter, P, and K. The N-NO_3 analysis was done from the soil samples taken at the 0- to 15-cm and 15- to 60-cm depths. Samples were sent to the North Dakota State University Soil testing lab and N-NO_3 was determined with the transnitration of salicylic acid method (Cataldo et al., 1975). The Olsen method and the ammonium acetate tests were used for available P and K determination, respectively (Franzen, 2010) (Table 3.3).

Table 3.3. Soil test results from the experimental sites at Fargo and Prosper, ND, in 2014 and 2015.

Location/year	N-NO_3	P	K	OM	pH†
	kg ha^{-1}	------mg kg^{-1}------		g kg^{-1}	
Fargo 2014	234	15	420	59	7.8
Prosper 2014	184	33	308	38	6.5
Fargo 2015	115	19	399	66	7.8
Prosper 2015	79	38	300	40	6.3

†pH, organic matter (OM), P-Olsen and K at 0-15 cm depth, N-NO_3 at 0-60 cm depth.

In 2014, biomass yield of alfalfa was calculated from the total biomass harvested from 1-m^2 area in each plot. The number of alfalfa plants was counted in the same 1-m^2 before harvesting. Plant height was taken measuring three plants per plot.

In 2015, the six-center alfalfa rows of the plots, 6-m long were harvested with a plot forage harvester (Carter MFG CO., Inc., Brookston, IN), weighed in the field, and a sample of fresh forage of about 2 kg was taken and dried to calculate moisture and determine dry matter forage yield (Table 3.2). Forage yield in each harvest and total seasonal yield of alfalfa were calculated. Harvests were conducted as close as possible to the plant height and growth stage required for prime hay quality, Relative Feed Value (RFV) greater than 151. (Table 3.4).

Table 3.4. Target maturity stage and plant height for each harvest to obtain prime alfalfa hay[†], in 2015 at Fargo and Prosper, ND.

Harvest	Plant height (cm)	Maturity stage
First	80	Late-vegetative -Early bud
Second	60	Late-bud -Early flower
Third	50	Early-flower - Late flower
Fourth	40	Late-flower

[†]Sheaffer et al., 1988.

Maize biomass samples were placed in burlap bags and weighed. The samples were dried at 70°C for four days, and then weighed to determine dry weight.

Dried samples of alfalfa and maize, were grounded to 1-mm mesh and sent to the University of Wisconsin, Madison, forage quality laboratory for forage quality analysis with a Near Infrared Spectroscopy (NIRS) apparatus (Foss-Sweden Model 6500, Minneapolis, MN). Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), in vitro dry matter digestibility (IVDMD), and neutral detergent fiber digestibility (NDFD) was determined, following the method described by Abrams et al. (1987).

Plant N uptake of alfalfa and maize were calculated by multiplying the dry matter biomass yield by the total N content (CP/6.25) utilizing the Kjeldahl method (Speirs and Mitchell, 2013).

3.4. Statistical Analysis

Statistical analysis was conducted using standard procedures for a randomized complete-block design with a split-plot arrangement. Each year was analyzed separately. Each location was considered a random effect in the statistical analysis. The different intercropping treatments and row spacing were considered fixed effects. Analysis of variance and mean comparisons were conducted using the Mixed Procedure of SAS (SAS Institute Inc., 2014). Trait error mean squares were compared for homogeneity among locations according to the folded *F*-test and if homogeneous, then a combined ANOVA was performed across locations. Treatment means separation was determined by *F*-protected LSD comparisons at the $P \leq 0.05$ probability level.

3.5. Cost and Economic Analysis

Economic analysis was done on three 2-year sequences: i) maize Year 1- maize Year 2; ii) maize + alfalfa Year 1 - alfalfa Year 2; and iii) maize Year 1 - spring-seeded alfalfa Year 2. The maize + alfalfa was the intercropping treatment without application of PHX, since this growth regulator did not show any effect on this study.

Constructed budgets were developed using rates and financial information from Haugen (2017) and Swenson and Haugen (2014). The budget used was developed for eastern North Dakota, and all costs related with irrigation for maize were taken out of the original budget since the research was conducted on dryland (NDSU, 2018). All budgets consist of two consecutive years. Costs of production considered included input expenses for land preparation, seeding, fertilizer, and pest management (Table 3.5).

Seed price for maize was calculated using the price per thousand kernels (TK) ($\$3.5$ TK^{-1}) and multiplied for a target plant density of 87,932 ha^{-1}. The price of alfalfa seed was $12.75 kg^{-1}, included the cost of inoculation and seed treatment (NDSU, 2018). Land preparation, sowing, spraying, and harvesting equipment, most commonly used in the region were in the analysis (Table 3.5). Machinery costs included labor, repairs, fuel and oil, depreciation, and machinery overhead were based on values of dollars per hectare obtained from Lazarus (2014) and Haugen (2017).

Herbicide cost, in the maize and alfalfa seeding year were fixed at $48.11 and $44.18 ha-^{1}, respectively according to Aakre (2014). In the intercropping system, the herbicide was applied twice during the growing season over both crops at the same time, using a surface (Boom sprayer, self-propelled, 20.4 m). No insecticide application was necessary since the maize seed contained traits for the European corn borer (*Ostrinia nubilalis*) and Western corn rootworm (*Diabrotica virgifera virgifera*) control in addition to being tolerant to glyphosate. The seed cost includes insecticide seed treatment for corn wireworm (*Melanotus communis* Gyllenhal), Western corn rootworm, white grub (*Holotrichia serrata*), and cutworm (Order: Lepidoptera) (Swenson and Haugen, 2014).

Harvesting equipment for this analysis included forage silage harvester for maize, and square baler, mower, hay rake, and a hay swather-conditioner for alfalfa. Drying and transport costs were not considered in the analysis. For each system, crop insurances cost, machinery repair cost, operating interest, miscellaneous costs, and fixed costs calculated based on Swenson and Haugen (2014) were included as “other costs”.

Economic output was calculated based on maize silage and alfalfa hay value at harvest with current prices multiplied by the yield. Maize silage dry matter yield obtained in this study was used for the economic analysis. Silage yield used was of 13.8 Mg ha^{-1} dry matter yield, and 39.4 Mg ha^{-1} maize silage at 65% moisture for all treatments. Since this study did not show reduction in maize silage yield in treatments with intercropped alfalfa, the same maize silage yield value was used for all treatments that had maize. Silage value was calculated according to LaPorte (2019), assuming a medium maize grain yield of 8.4 Mg ha^{-1} and, a maize grain price of \$177 Mg^{-1} (\$4.5 bu^{-1}). A conversion factor was calculated to transform maize grain price (\$4.5 bu^{-1}) to silage maize value at 65% moisture (LaPorte, 2019), resulting in a value of \$41.1 $Mg^{-1}$of maize silage at 65% moisture. For alfalfa, the average yield obtained at 76-cm row spacing were used for the economic analysis; forage dry matter yield for I intercropped alfalfa in Year 2 was 10.2 Mg ha^{-1} and for spring-seeded alfalfa was 5.5 Mg ha^{-1}.

The net revenue from a two-year system was estimated as the difference between the total revenue and the total production cost for a consecutive two-year period. A sensitivity analysis was performed to validate the results obtained. This analysis considered several potential maize grain prices (between \$32.0 and \$50.3 Mg^{-1}), and alfalfa hay prices (\$125 to \$181 Mg^{-1}), and calculated profit fluctuations for each of those scenarios.

Table 3.5. Summary of inputs, rates used, and description used for cost calculations in alfalfa and maize.

Inputs	Rate	Price per unit	Description
Seeds	kg ha^{-1}	\$ kg^{-1}	
Maize	21.00	14.65	MD 96RR
Alfalfa	10.00	12.75	Presteez RR
Fertilizers			
N	150.00	0.881	Urea, applied only to maize
P_2O_5	30.00	0.947	Mono ammonium phosphate
K_2O	50.00	0.881	Potash (KCl)
Herbicide	0.84 + 0.21	9.34 + 247.1	Glyphosate (2 applications) + pyroxasulfone (Zidua)
Machinery	Units ha^{-1}	\$ ha^{-1}	
Soil preparation:			
Chisel plow	1	28.2	11.3 m, Tractor 310 HP
Field cultivator	1	12.8	9 m, Tractor 360 HP
Planting:			
Small grain drill	1	31.1	4.6 m, Tractor 130HP
Row crop drill with cart	1	41.8	15.8 m, Tractor 260 HP
Chemicals:			
Chemical sprayer	1	31.4	24.4 m, Self-prop
Spreading fertilizer	1	15.2	24.4 m, Tractor 130 HP
Harvesting:			
Silage harvesting	1	83.4	2 row, 1.5 m, Tractor 105 HP
Large square baler	1	24.3	6.1 m, Tractor 130 HP
Mower	1	26.7	2.7 m , Tractor 40 HP
Hay rake	1	12.7	2.7 m, Tractor 40 HP
Hay swather-conditioner	1	21.5	4.3 m, Tractor 60 HP

†All machinery and fuel values necessary for each operation were extracted from Lazarus (2014).

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Climatic Characteristics

During the 2-year experimental period, at both Prosper and Fargo, the growing season minimum and maximum temperatures were similar to the 30-year average with slightly warmer temperatures November 2014 through January 2015 that probably enhanced alfalfa stand survival (Table 4.1). In 2014, after sufficient rainfall early in the season, plants experienced drier conditions compared with the 30-year average rainfall in the summer until final harvest in October at both locations. The rainfall deficit from May to October in 2014 was -106.3 mm and -150.6 mm in Fargo and Prosper, respectively, compared with the long-term average (Table 4.1). In 2015, at both locations, May was exceptionally wet and towards the end of the season, the rainfall conditions were below normal. The rainfall deficit compared with the long-term average from May to October in 2015 was -35.7-mm and -23.7-mm in Fargo and Prosper, respectively (Table 4.1).

4.2. Alfalfa Forage Yield

The analysis of variance indicated a significant effect for the interaction between location and intercropping treatment for total forage yield of alfalfa in the seeding year (2014), first (2015), second (2016), and third (2017) production years (Table 4.2). Row spacing did not influence alfalfa yield in any of the locations and years. The alfalfa forage yield of intercropping treatments were different only in 2015 (Table 4.2).

Table 4.1. Monthly average growing-season rainfall and maximum, minimum, and average temperatures in Prosper and Fargo, in 2014 and 2015.

	Fargo	Prosper	Fargo			Prosper		
Month			Min	Max	Aver.	Min.	Max	Aver.
	Rainfall (mm)		Temperature ( )					
					2015			
Jan.	0.0	0.0	-21.4	-9.3	-15.6	-22.3	- 8.7	-15.6
Feb.	0.0	0.0	-20.7	-9.9	-15.0	-21.5	- 9.5	-15.6
Mar.	0.0	0.0	-10.8	0.0	- 5.6	-10.6	0.4	-5.0
April	78.6	79.9	- 0.9	10.2	4.4	- 0.8	10.4	5.0
May	49.8	52.1	8.1	19.7	13.9	7.2	20.1	13.9
June	140.3	107.2	14.4	25.0	19.4	13.7	25.4	19.4
July	34.1	33.3	14.7	26.6	20.6	13.7	26.9	20.6
Aug.	37.1	60.5	15.6	26.1	21.1	14.4	26.8	20.6
Sept.	51.3	46.7	9.8	22.0	16.1	8.0	22.6	15.0
Oct.	7.7	9.1	3.0	14.7	8.9	1.3	15.1	8.3
Nov.	0.0	0.0	-9.7	-1.1	-5.6	-10.6	-1.1	-6.1
Dec.	0.0	0.0	-10.2	-2.7	-6.7	-11.1	-2.8	-7.2
Total	398.8	388.7						
Total May-Oct.	320.3	308.8						
Normal May-Oct.	426.2	459.5						
Dev. May-Oct.	-106.3	-150.6						
30-year avg.	574.3	594.4						
					2014			
Jan.	0.0	0.0	-13.8	-4.5	-8.9	-14.7	-4.5	-9.4
Feb.	0.0	0.0	-19.2	-8.6	-13.9	-20.4	-8.6	-14.4
Mar.	0.0	0.0	-5.4	7.1	0.6	-7.0	7.0	0.0
April	15.9	20.1	0.6	15.7	8.3	-0.9	16.1	7.8
May	199.7	148.7	6.7	18.6	12.8	5.5	18.7	12.2
June	63.8	109.8	13.8	25.4	19.4	13.0	25.9	19.4
July	71.0	88.4	16.6	27.9	22.2	15.1	27.6	21.1
Aug.	54.3	36.3	14.1	26.6	20.6	12.2	26.5	19.4
Sept.	41.0	21.8	11.8	24.8	18.3	9.9	25.0	17.2
Oct.	31.8	30.8	4.4	16.1	10.6	2.5	16.3	9.4
Nov.	0.0	0.0	-2.8	7.1	2.2	-3.8	7.1	1.7
Dec.	0.0	0.0	-8.5	-2.0	-5.0	-10.3	-2.8	-6.7
Total	477.5	455.9						
Total May-Oct.	461.9	435.8						
Normal May-Oct.	426.2	459.5						
Dev. May-Oct.	-35.7	-23.7						
30-year avg.	574.3	594.4						

†NDAWN (2017).

Table 4.2. Analysis of variance and error mean squares for total seasonal alfalfa forage yield from 2014 to 2017 in response to maize row spacing (RS) and intercropped alfalfa with and without prohexadione application.

SOV	df	2014	df	2015	2016	2017
Loc	1	0.604*	1	31.12*	0.21	2.83
Rep (loc)	6	0.137*	6	0.54	2.64	6.67
RS	1	0.001	1	2.49	0.13	1.61
RS x loc	1	0.004	1	0.37	3.53	0.66
Loc x RS x rep	6	0.063	6	1.12	2.21	0.60
Trt	2	2.109	4	168.80*	6.32	6.11
Trt x loc	2	0.343*	3	5.56*	1.48	2.90*
Trt x RS	2	0.005	4	0.67	5.03	1.54
Trt x RS x loc	2	0.029	3	0.10	2.94	1.12
Error	24	0.004	34	0.46	3.20	0.75
CV,%		24.180		7.28	10.63	6.74

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment
*Significant at 0.05 probability level

In 2014, alfalfa biomass yield was similar for alfalfa with or without PHX application, indicating PHX did not improve alfalfa biomass yield (Table 4.3). Conversely, Grabber (2016) tested several rates of PHX indicating rates between 0.6 and 1.2 kg a.i. ha^{-1} increased alfalfa biomass yield in October compared with both the check and the 2.4 kg a.i. ha^{-1} rate. Maize plants in North Dakota grew much shorter than in Wisconsin letting light get through the leaf canopy to the soil surface. This might explain why a response to PHX was not observed in this study.

In the following year, 2015, the spring-seeded alfalfa was harvested twice in the season. While the alfalfa established in 2014 (alfalfa –maize with or without PHX) was harvested four times in 2015 (Fig. 4.1). Alfalfa seasonal biomass yield was about twice the yield of the spring-seeded alfalfa (Figure 4.1) at both locations (Table 4.3).

Table 4.3. Alfalfa seasonal forage yield at two locations from 2014-2017 averaged across two row spacings (61 and 76 cm) in Fargo and Prosper.

	Fargo				Prosper			
Treatment	2014	2015	2016	2017	2014	2015	2016	2017
	------------------------------------Mg ha^{-1}------------------------------------							
Spring-seeded alfalfa	-	5.51	16.68	11.12	-	5.75	17.05	15.80
Alfalfa + maize	0.59	10.19	17.57	10.80	0.61	12.38	17.43	14.93
Alfalfa + maize + PHX	0.65	10.03	16.19	11.34	0.50	12.41	16.94	14.83
LSD (0.05)	NS	0.66	NS	NS	NS	0.82	NS	NS
CV, %	14.9	7.09	10.91	6.73	34.50	7.37	10.32	6.64

†PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1}

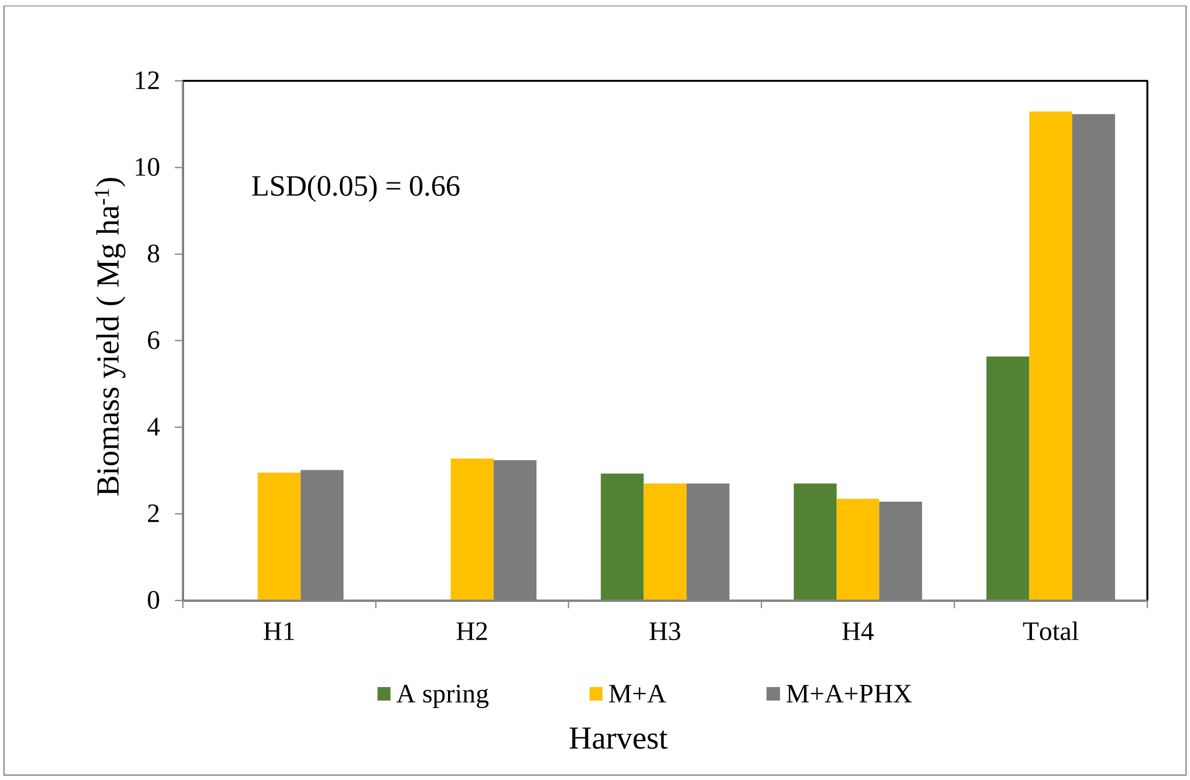

Fig. 4.1. Alfalfa biomass yield (dry matter) of four harvests (H) in 2015; for spring-seeded alfalfa (A spring), maize and alfalfa (M+A) intercropping without prohexadione (PHX) application and with PHX application (M+A+PHX) averaged across locations, Fargo and Prosper in 2015.

Grabber (2016) reported a similar response of doubling alfalfa forage yield in the first production year when comparing silage maize-alfalfa system versus spring-seeded alfalfa in Wisconsin. This is a notable difference since alfalfa forage production in the seeding year is low. Establishing alfalfa during the maize production year skips the low forage yield in the seeding year that could likely provide an economic advantage. In this experiment, the growth regulator (PHX) did not affect forage yield of alfalfa across locations. Alfalfa seasonal biomass yield in the second and third production years (2016-2017) yield was not significantly different among treatments (Table 4.3). This indicates that establishing alfalfa with maize does not influence forage yield past the first production year.

4.3. Alfalfa Plant Density

Alfalfa plant density was significant ($P \leq 0.05$) for the treatments in the fall of 2014, and for the interaction between row spacing and treatment and treatment by location in the fall of 2015 (Table 4.4). No differences in plant density were observed in the spring of 2015 for any factor in the analysis (Table 4.4). In 2014, plant density was lower at the 61-cm row spacing for the PHX-treated alfalfa compared with the non-treated alfalfa at 61-cm and both treatments at the 76-cm row spacing (Table 4.5). Intercropped alfalfa stands had at least 113 plants m^{-2} in the fall of 2014, which is within the range 80-130 plants m^{-2} considered as an adequate stand for the seeding year (Hall et al., 2004, Grabber 2016, Berti and Samarappuli, 2018). Oppositely, Grabber (2016) reported, the PHX treatment increased stand survival compared with the alfalfa non-treated check. This might have been due to the maize hybrids in our experiments were earlier maturing and shorter than in Wisconsin, likely allowing greater light penetration through the leaf canopy to the soil surface.

Table 4.4. Analysis of variance and mean squares for alfalfa plant density in the fall of 2014, spring of 2015, and fall of 2015 at two locations Fargo and Prosper, ND.

SOV	df	2014	2015 spring	2015 fall
Loc	1	1156*	435	2451*
Rep (loc)	6	1586	681	483
RS	1	123	592	2
RS x loc	1	35	512	8
RS x loc x rep	6	862	749	638
Trt	1	4381*	1104	30146
Trt x loc	1	81	50	4778*
Trt x RS	1	1579	1225	790*
Trt x RS x loc	1	168	21	46
Error	31	1232	374	373
CV%		24	27	25

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment
*Significant at 0.05 probability level

Table 4.5. Alfalfa plant density in the fall of 2014, spring of 2015, and fall of 2015 at two row spacings averaged across two locations, Fargo and Prosper, ND.

	2014		2015 spring		2015 fall	
	Row spacing (cm)					
Treatment	61	76	61	76	61	76
	---------------------no. plants m^{-2}-----------------------					
Alfalfa + maize 2014	154	139	81	76	55	53
Alfalfa + maize + PHX 2014	113	138	57	76	42	53
Spring-seeded alfalfa	.	.	.	.	125	
LSD_1 (0.05)	16					
LSD_2 (0.05)	46					

LSD_1 to compare among means within a same treatment but different row spacing.
LSD_2 to compare means with row spacing and same or equal treatment
†PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1}

Table 4.6. Alfalfa plant density in the fall of 2014, spring of 2015, and fall of 2015 at two locations, Fargo and Prosper, ND averaged across two row spacings, 61 and 76-cm, but only for 2014 seeded alfalfa.

	2014		2015 spring		2015 fall	
Treatment	Fargo	Prosper	Fargo	Prosper	Fargo	Prosper
	-----------------------no. plants m^{-2}-------------------------					
Alfalfa + maize 2014	162	131	73	83	50	58
Alfalfa + maize + PHX 2014	143	107	64	68	45	49
Spring-seeded alfalfa	-	-	-	-	152	98
LSD (0.05)	NS		NS		11	

†PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1}

In the spring of 2015, alfalfa plant density was similar between row spacings and treatments (Table 4.4). Plant density decreased to less than half the density in the previous fall regardless of treatment and row spacing (Table 4.5). A plant density reduction of 50 to 60% in the first overwintering of alfalfa is common in North Dakota, regardless of management or winter temperatures (Berti et al., 2012). Although Grabber's (2016) initial stand establishment was three times greater than in this study, the reduction in stand from July to October of the same season was about 40-50% for both treated and untreated treatments. Alfalfa self-thinning of stands by intraspecific competition in the seeding year has been previously reported by Mattera et al. (2013). Alfalfa plant stands decreased between spring and fall of 2015. This was probably due to self-thinning during the season. Spring-seeded alfalfa had similar plant density in the fall of 2015 (seeding year) to that of the alfalfa in the fall of 2014 (seeding year) at both row spacings. Spring-seeded alfalfa in 2015 had about three times greater plant density than alfalfa established in 2014 in Fargo and twice the plant density at Prosper (Table 4.6). This result is expected since 2015 spring-planted alfalfa had not been exposed to a winter yet. Unfortunately, plant density was not taken in 2016 and 2017, but since there were no differences in forage yield it could be suggested that plant density was not different among treatments.

4.4. Maize Biomass Yield and Plant Height

The analysis of variance indicated a significant interaction between location row spacing, and treatment. In Prosper, monoculture maize produced significantly higher maize biomass yield than maize from alfalfa-intercropping systems at 61-cm row spacing. This response was not observed at 76-cm row spacing or in Fargo at both row spacings. This is an indication that at a narrower row spacing intraspecific competition between maize and alfalfa can reduce biomass yield. Alfalfa interseeded in maize without PHX caused a significant reduction in maize plant height at 76- cm row spacing, averaged across locations, but this did not affect the biomass yield. In contrast to our results, Grabber (2016) reported alfalfa without PHX treatment, at any rate, reduced maize height by 0.27 m and maize biomass yield by 3.5 Mg ha^{-1}.

Table 4.7. Analysis of variance and mean squares for maize biomass yield, maize plant height, and plant density in the fall of 2014 at two locations, Fargo and Prosper, ND.

SOV	df	Biomass yield	df	Plant height	No. plants
Loc	1	191.10*	1	0.088*	0.45
Rep (loc)	6	6.61*	6	0.026	7.01
RS	1	1.03	1	0.114*	246.86*
RS x loc	1	0.03	1	0.001	0.19
RS x loc x rep	6	7.03	6	0.021	9.08
Trt	2	28.80	2	0.124	8.10
Trt x loc	2	7.52*	2	0.026	0.69
Trt x RS	2	5.18	2	0.027*	8.10
Trt x RS x loc	2	15.31**	2	0.002	4.64
Error	48	2.29	56	0.019	4.13
CV%		11.40		5.116	16.98

†SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment
*, **Significant at 0.05, and 0.01probability levels, respectively.

Table 4.8. Maize biomass yield and plant height for two row spacings (61 and 76 cm) averaged across locations, Fargo and Prosper, ND, in 2014.

	Fargo		Prosper		Average	
Treatment	61	76	61	76	61	76
	--------------Mg ha^{-1}---------------				---------------cm-----------	
Maize	11.07	12.20	17.27	15.38	276	283
Alfalfa + maize	10.67	10.45	12.05	15.15	265	266
Alfalfa + maize + PHX	11.02	11.05	14.15	13.60	265	282
LSD (0.05)			2.72		12	

†PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1}

4.5. Alfalfa Forage Nutritive Value

Row spacing, treatment, treatment by row spacing, and all interactions with location were not significant for most nutritive components, except for crude protein and ash content. Crude protein was significant for the treatment effect and treatment by location interaction in the third harvest. Ash content was significant for treatment effect in the first and third harvest and significant for the interaction between location by row spacing in the second and fourth harvest (Tables 4.9-4.12).

Crude protein concentration was lower in the spring-seeded alfalfa (Table 4.13). The third harvest for the intercropped alfalfa planted in 2014 was actually done about the same time as the first harvest for the spring-seeded alfalfa. First cut of the seeding year could have had lower crude protein since it was harvested in the summer and likely had higher stem to leaf ratio. Stems usually have much less protein than leaves (Pecetti et al., 2017). The interaction treatment by location for crude protein in the third cut was probably due to differences in ranking between treatments from alfalfa that was intercropped in 2014.

The non-treated alfalfa had higher ash content (101 g kg^{-1}) than alfalfa treated with PHX (95.3 g kg^{-1}) ($P \leq 0.05$) and both had higher ash content than the spring-seeded alfalfa first cut (80.8 g kg^{-1}) (Table 4.14). It is possible, but unlikely that PHX-treated alfalfa had shorter internodes and hence higher leaf to stem ratio, which might explain the lower ash content. The PHX inhibit the biosynthesis of gibberellins, shortening the internodes (Evans et al., 1999; Costa et al., 2001). But we have to consider that the PHX was applied in 2014 and these results are from 2015 harvested alfalfa, almost one year after PHX application.

Spring-seeded alfalfa first cut was about the same time as the third cut of the alfalfa established in 2014. Spring-seeded alfalfa was likely shorter (not measured) at first cut, with higher leaf to stem ratio than alfalfa established in 2014. Alfalfa stems usually have a higher ash content than leaves.

In the second harvest, the alfalfa ash content was higher ($P \leq 0.05$) in the alfalfa coming from the 61-cm row spacing in Prosper in 2014, but not in Fargo. In the fourth harvest, the highest ash content was in the alfalfa coming from the 76-cm row spacing in 2014 in Fargo (Table 4.15). This response could be due to soil contaminating some of the samples. The row spacing should not have any effect in the year where only alfalfa was present.

Table 4.9. Analysis of variance and mean squares of alfalfa for the first harvest for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), in-vitro dry matter digestibility (IVDMD), ash, lignin, total digestible nutrients (TDN), neutral detergent fiber digestibility (NDFD), and relative forage quality (RFQ) at two locations, Fargo and Prosper in 2015.

SOV	df	CP	ADF	NDF	IVDMD	Ash	Lignin	TDN	NDFD	RFQ
Loc	1									
Rep(loc)	6									
RS	1	47.5	120	55	3.4	0.03	22.80	140.0	830.0	28
Loc x RS	1	94.5	3	3	17.5	38.30	7.03	3.8	5.4	6
RS x rep x loc	6	47.0	200	319	163.0	7.11	11.00	232.0	88.0	197
Trt	1	16.5	2	21	0.1	258.80*	0.03	3.8	9.0	25
Loc x trt	1	34.0	2364	666	280.0	1.53	7.03	318.0	1116.0	365
Trt x RS	1	109.0	253	378	154.0	101.55	7.03	282.0	0.3	288
Loc x trt x RS	1	215.0	190	300	92.0	34.03	7.03	247.0	63.3	220
Error	12	45.2	263	55	214.0	64.40	7.03	289.0	532.0	230
CV%		2.8	5	5	2.0	8.17	4.81	2.5	4.9	8

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment
*Significant at 0.05 probability level

Table 4.10. Analysis of variance and mean squares of alfalfa second harvest for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), in-vitro dry matter digestibility (IVDMD), ash, lignin, total digestible nutrients (TDN), neutral detergent fiber digestibility (NDFD), and relative forage quality (RFQ) at two locations, Fargo and Prosper in 2015.

SOV	df	CP	ADF	NDF	IVDMD	Ash	Lignin	TDN	NDFD	RFQ
Loc	1									
Rep(loc)	6									
RS	1	34.0	420.5	751	267.0	15.0	8.0000	480.5	205.0	480.5
Loc x RS	1	23.0	84.5	124	2.6	72.0*	8.0000	84.5	108.8	162.0
Loc x rep x RS	6	43.0	145.6	270	213.0	8.7	10.2000	164.0	292.0	110.0
Trt	1	3.8	18.0	23	3.4	1.1	0.0001	21.1	132.0	40.5
Loc x trt	1	0.3	72.0	58	2.3	4.5	0.5000	91.1	5.3	60.5
Trt x RS	1	34.0	0.5	30	8.7	21.1	2.0000	0.5	26.3	2.0
Loc x trt x RS	1	9.0	162.0	116	65.9	12.5	12.5000	180.5	306.3	50.0
Error	12	36.6	210.0	293	141.5	15.5	5.0800	233.6	125.4	179.8
CV%		2.5	4.9	5	1.4	4.4	4.4700	2.3	2.1	7.2

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment
*Significant at 0.05 probability level

Table 4.11. Analysis of variance and mean squares of alfalfa third harvest for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), in-vitro dry matter digestibility (IVDMD), ash, lignin, total digestible nutrients (TDN), neutral detergent fiber digestibility (NDFD), and relative forage quality (RFQ) at two locations Fargo and Prosper in 2015.

SOV	Df	CP	ADF	NDF	IVDMD	Ash	Lignin	TDN	NDFD	RFQ
Loc	1									
rep(loc)	6									
RS	1	11.0	3.5	0.0001	5.3	3.5	1.7	8.3	150.5	0.1
Loc x RS	1	25.5	54.2	44.1000	62.6	7.5	9.2	60.8	391.0	60.8
Loc x rep x RS	6	45.7	27.2	41.4000	44.9	20.8	6.0	32.3	154.4	51.6
Trt	1	3333.6***	650.9	166.4000	202.9	765.2**	22.1	730.3	4883.1	422.1
Loc x trt	1	171.1*	99.4	212.0000	74.3	8.8	45.0	127.0	294.1	262.1
Trt x RS	1	53.1	269.1	388.6000	64.5	9.8	6.4	326.1	26.0	504.1
Loc x trt x RS	1	69.1	51.9	107.3000	150.4	16.0	3.5	60.3	244.8	127.3
Error	12	45.6	72.0	118.4000	77.0	17.7	5.1	83.3	186.4	135.8
CV, %		2.7	3.3	3.6000	1.0	4.7	5.0	1.3	2.8	5.0

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 4.12. Forage nutritive value of alfalfa for the fourth harvest in two locations Fargo and Prosper in 2015**.**

SOV	df	CP	ADF	NDF	IVDMD	Ash	Lignin	TDN	NDFD	RFQ
Loc	1									
rep(loc)	6									
RS	1	0.2	46.0	108.0	0.1	123.5	8.3	50.0	252.1	150.5
Loc x RS	1	38.5	6.0	16.3	3.7	266.0 *	14.1	6.0	133.3	35.0
Loc x rep x RS	6	22.1	498.8	573.8	228.6	44.5	23.0	568.6	190.9	440.5
Trt	1	108.1	34.1	65.3	11.0	16.5	36.8	34.9	695.8	41.3
Loc x trt	1	28.9	27.3	22.8	12.3	46.3	7.6	31.3	255.8	2.9
Trt x RS	1	54.6	144.1	147.3	41.9	18.9	4.1	165.9	407.9	183.3
Loc x trt x RS	1	108.4	498.1	743.6	306.3	30.8	43.6	562.1	315.0	618.9
Error	12	50.3	253.0	298.0	130.0	70.0	21.9	293.0	414.0	320.6
CV, %		3.0	5.5	5.1	1.4	9.1	8.2	2.5	4.3	8.9

SOV=Source of variation, Loc=location; RS=row spacing; Trt= treatment

*Significant at 0.05 probability level

Table 4.13. Crude protein concentration interaction between treatments and location for the third cut of the 2015 season for alfalfa that was intercropped with maize in 2014 averaged across two row spacings (61 and 76 cm).

Treatment	Fargo	Prosper	Mean
	--------------g kg^{-1}------------		
Spring-seeded alfalfa	233.0	231.5	232.3
Alfalfa + maize	257.8	257.1	257.4
Alfalfa + maize + PHX	254.6	259.7	257.1
LSD (0.05)	19.9		

†The third harvest corresponds to the first harvest of the spring-seeded alfalfa in 2015.

Table 4.14. Ash content of alfalfa for the first and third harvest averaged across two locations, Fargo and Prosper, ND in 2015.

	Harvest 1	Harvest 3
Treatment	mean	Mean
	-----------------------g kg^{-1}--------------------------	
Spring-seeded alfalfa	-	80.8
Alfalfa + maize	101.0	93.1
Alfalfa + maize + PHX	95.3	92.5
LSD (0.05)	5.5	4.5

†PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1},
‡Ash content in 2015, averaged across two locations, Fargo and Prosper.
The third and fourth harvests corresponds to the first and second harvest of the spring-seeded alfalfa in 2015.

Table 4.15. Ash content of alfalfa for the second and fourth for the interaction between two row spacings and two locations, Fargo and Prosper, ND in 2015 and averaged across three treatments.

	Harvest 2		Harvest 4	
Row spacing	Fargo	Prosper	Fargo	Prosper
61	91.6	90.1	92.0	87.2
76	93.3	85.7	100.0	85.7
LSD (0.05)	3.6		6.6	

4.6. Economic Analysis

When comparing net return after two years of silage maize with silage maize intercropped with alfalfa, the latter showed a higher positive net return (Table 4.16). Extra seed cost, and planting cost associated with the sowing of alfalfa increased the production cost in the first year, compared with the maize monoculture. However, lower production cost and higher revenue generated from alfalfa hay compared with silage maize in the second year contributed to the positive net return after the two-year period. When comparing the two systems that had alfalfa, the sequence silage maize followed by spring-seeded alfalfa had a r lower net return, compared with a positive net return when alfalfa was intercropped with silage maize in in the first year of the sequence, even when the latter had a higher production cost. This is mainly due to the lower forage yield in the spring-seeded alfalfa system.

Osterholz et al. (2020) compared several rotations of corn and alfalfa with and without intercropping and the annual net return ranged between \$303 to \$367 ha^{-1}. All annual returns in this study were positive as where the biennial sequences estimated in our study. Osterholz et al., (2020) net returns were calculated with a higher silage maize yield of, 20.3 Mg DM ha^{-1}, while in our study we used only 13.8 Mg DM ha^{-1}, which correspond to the average biomass yield across locations obtained in the experiment. However, the study in Wisconsin was done at lower maize grain prices than our study calculated with a grain price of \$177 Mg^{-1}. Thus, the net return of silage maize rotations were similar to those calculated for a 2 year sequence of silage maize (\$379 Mg^{-1}). The alfalfa forage yield used by Osterholz et al. (2020) in the economic analysis were very similar to those used in our analysis. Osterholz et al. (2020) used alfalfa forage yields of 11.4 Mg ha^{-1} and 5.8 Mg ha^{-1} for alfalfa coming from intercropping with maize the year before and spring-seeded alfalfa, respectively. In our analysis, we used 10.2 Mg ha^{-1} and 5.5 Mg ha^{-1} alfalfa yield coming from intercropping and for spring-seeded alfalfa, respectively. In conditions of much lower maize silage yield potential such of those obtained in North Dakota, establishing alfalfa while growing maize allows for positive net returns.

The sensitivity analysis was conducted varying the maize and alfalfa prices (Table 4.17). The results of the sensitivity analysis using a fixed yield value for silage maize of 13.8 Mg ha^{-1} indicated that, having a \$125 Mg^{-1} alfalfa hay price with a \$36.6.0 Mg^{-1} maize silage price would be sufficient to having positive net revenue from the sequence with intercropped alfalfa with maize followed by alfalfa in Year 2.(Table 4.17). Only at a price of maize silage greater than \$50.3 Mg^{-1} and an alfalfa price greater than \$166 Mg^{-1}, the maize-maize sequence is more profitable than the maize + alfalfa intercropped- alfalfa sequence. Maize-alfalfa intercropping 2-year system was always more profitable that the usual practice of silage maize in Year 1 followed by spring-seeded alfalfa

Osterholz et al. (2020) sensitivity analysis was calculated by varying maize silage yield penalty and alfalfa establishment success rates. Maize yield penalty had a greater impact on net returns than alfalfa stands. In our experiment, we did not observe maize silage yield reduction at 76-cm row spacing in maize with intercropped alfalfa compared with maize in monoculture. We did not have an alfalfa sole crop to determine alfalfa stand reduction in this study. In a scenario of silage maize yield greater than 13.8 Mg ha^{-1}, which is very achievable in areas of the Midwest with more rain during the summer, the alfalfa-maize intercropping system can have positive net returns even at low maize and alfalfa prices.

In addition, intercropping systems offer several ecosystem services that could be valued or at least taken into consideration as a path towards sustainable production of alfalfa and maize-based feed production. Gaba et al. (2015) demonstrated that exists evidence that intensive cropping systems have led to a decline in biodiversity and also, threatening the environment. This caused damage to an important number of ecosystem services such as nutrient cycling, regulation of climate and water quality, and soil erosion just for mention some of them.

Syswerda and Robertson (2014) demonstrated that grain yield were positively correlated with nitrate leaching and negatively correlated with plant diversity and Belel et al. (2014) reported soil fertility improvements.

Promoting intercropping systems as maize and alfalfa that have multiple positive effects on the environment (ground coverage, weed control, pollinators, less N applications) can benefit agricultural ecosystems; however valuing ecosystem services in annual budgets at every farm management planning guide will be very challenging (Shulz et al, 2020).

Table 4.16. Economic analysis of three different systems for a two-year (yr) period containing silage maize, silage maize with intercropped alfalfa, and silage maize followed by spring-seeded alfalfa.

Variable	Maize -Maize		Maize + Alfalfa - Alfalfa		Maize - Spring-seeded alfalfa	
	Yr-1	Yr-2	Yr-1	Yr-2	Yr-1	Yr-2
Inputs	--$ ha^{-1}--					
Land preparation						
Chisel plow	28.2	28.2	28.2	0.0	28.2	28.2
Field cultivator	12.8	12.8	12.8	0.0	12.8	12.8
Seeding				0.0		
Row crop planter	41.8	41.8	41.8	0.0	41.8	0.0
Small grain drill	0.0	0.0	31.1	0.0	0.0	31.1
Seeds				0.0		
Maize seed	307.8	307.8	307.8	0.0	307.8	0.0
Alfalfa seed	0.0	0.0	127.5	0.0	0.0	127.5
Fertilization						
Application-broadcast	15.2	15.2	15.2	15.2	15.2	15.2
N	148.0	148.0	148.0	0.0	148.0	0.0
P	28.4	28.4	28.4	28.4	28.4	28.4
K	44.1	44.1	44.1	44.1	44.1	44.1
Chemicals						
Sprayer	31.4	31.4	15.7	15.7	31.4	15.7
Herbicide						
Pre-emergent (pyraxofluzole)	51.9	51.9	0.0	0.0	51.9	0.0
Glyphosate	15.7	15.7	15.7	15.7	15.7	15.7
Harvesting						
Silage harvesting	83.4	83.4	83.4	0.0	83.4	0.0
Mower	0.0	0.0	0.0	106.7	0.0	53.3
Hay rake	0.0	0.0	0.0	50.7	0.0	25.4
Hay swather-conditioner	0.0	0.0	0.0	86.0	0.0	43.0
Large square baler	0.0	0.0	0.0	97.2	0.0	48.6
Other costs	622.1	622.1	622.1	590.9	622.1	524.6
Production cost	1430.7	1430.7	1521.7	1050.6	1430.7	1013.5
Total production cost		**2861.4**		**2572.2**		**2444.2**
Outputs						
Silage	1620.5	1620.5	1620.5	0.0	1620.5	0.0
Hay	0.0	0.0	0.0	1693.2	0.0	913.0
Total revenue	1620.5	1620.5	1620.5	1693.2	1620.5	913.0
Revenue of two-year system		**3241.0**		**3313.7**		**2533.5**
Net return two-year system		**379.6**		**741.5**		**89.1**

†Data used for outputs were maize yield 13.8 Mg ha^{-1} for all treatments and alfalfa hay yield 10.8 Mg ha^{-1} for full production year alfalfa and 5.5 Mg ha^{-1} for spring-seeded alfalfa.

Table 4.17. Sensitivity analysis for total net return after two years, produced from maize and alfalfa for three different systems containing maize, maize intercropped with alfalfa, and maize followed by spring-seeded alfalfa.

Price of alfalfa (\$ Mg^{-1})	Price of maize silage (\$ Mg^{-1})				
	32.0	**36.6**	**41.1**	**45.7**	**50.3**
	Maize - Maize (\$ ha^{-1})				
125	-338.6	24.37	379.2	742.0	1104.7
143	-338.6	24.37	379.2	742.0	1104.7
166	-338.6	24.37	379.2	742.0	1104.7
181	-338.6	24.37	379.2	742.0	1104.7
	Maize with intercropped alfalfa (\$ ha^{-1})				
125	-35.9	145.5	322.9	504.3	685.7
143	147.7	329.1	506.5	687.9	869.3
166	382.1	563.5	740.5	922.3	1103.7
181	535.3	716.7	894.1	1075.5	1256.9
	Maize followed by spring-seeded alfalfa (\$ ha^{-1})				
125	-495.5	-313.8	-136.4	45.0	226.4
143	-396.2	-214.8	-37.4	144.0	325.4
166	-269.2	-88.3	89.1	270.5	451.9
181	-187.2	-5.8	171.6	353.0	534.4

CHAPTER 5: CONCLUSIONS

Alfalfa established in intercropping with maize had almost double the forage yield in the following year compared with spring-seeded alfalfa following a crop of silage maize. The application of prohexadione-calcium to alfalfa under the maize canopy did not improve alfalfa establishment and survival when intercropped with silage maize indicating that alfalfa can be established in intercropping with silage maize in the northwestern US Corn Belt region without significant stand reduction and maize silage yield at 76-cm row spacing. Silage maize biomass yield was the lowest at the narrowest row spacing of 61-cm at only one location, but this was not observed at the 76-cm row spacing, which is the most common row spacing used by growers in the Corn Belt. Intercropping maize and alfalfa did not affect forage nutritive value in alfalfa harvested the following year compared with spring-seeded alfalfa, except for crude protein and ash, which were significant for some of the effects. Alfalfa intercropped with maize has higher net returns than a silage-maize followed by a spring-seeded alfalfa the following year. This system may help to get more growers to include alfalfa in their rotation by skipping the low seeding year yield.

REFERENCES

Aakre, D. 2013. Custom farm work rates. EC499. North Dakota State Univ. Ext. Serv., Fargo, ND.

Abdin, O., Bruce E. Coulman, D. Cloutier, M.A. Faris, X. Zhou and D.L. Smith. 1998. Yield and yield components of maize interseeded with cover crops. Agron. J. 90:63-68.

Abrams, S.M., J. Shenk, and F.E. Westerhaus. 1987. Determination of forage quality by near infrared reflectance spectroscopy: Efficacy of broad-based calibration equations. J. Dairy Sci. 70:806-813. doi:10.3168/jds.S0022-0302(87)80077-2

Ali, S., and H.S. Mohammad. 2012. Forage yield and quality in intercropping of forage maize with different cultivars of berseem clover in different levels of nitrogen fertilizer. J. Food Agric. and Envir. 10(1):602-604.

Andrews D.J., and A.H. Kassam. 1976. The importance of multiple cropping in increasing world food supplies. American Soc. Agron. 27:1-10.

Anil, L., J. Park, R.H. Phipps, and F.A. Miller. 1998. Temperature intercropping of cereal for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass and Forage Sci. 53:301-317.

Aponte, A. D. Samarappuli, and M.T. Berti. 2019. Alfalfa grass-mixtures in comparison to grass and alfalfa monocultures. Agron. J. 111 (2):628-638 doi: 10.2134/agronj2017.12.0753

Bauder, J.W., A. Bauer, J.M. Ramirez, and D.K. Cassel. 1978. Alfalfa water use and production in dryland and irrigated sandy loam. Agron. J. 70:95-99.

Baeberi, P., and M. Mazzoncini. 2001. Changes in weed community composition as influenced by cover crop and management system in continuous maize. Weed Science 49:491-499.

Belel, M.D., R.A. Halim, M.Y. Rafii, and H.M. Saud. 2014. Intercropping of maize with some selected legumes for improved forage production: a review. J. Agric. Sci. 6 (3):48-62.

Berti, M.T., R. Nudell., and D.W. Meyer. 2012. Fall harvesting of alfalfa in North Dakota impacts plant density, yield, and nutritive value. Forage and Grazinglands, doi: 10.1094

Berti, M.T. and D. Samarappuli, 2018. How does sowing rate affect plant and stem density, forage yield, and nutritive value in glyphosate-tolerant alfalfa? Agronomy 8: 169 doi:10.3390/agronomy8090169

Blaser B.C., J.W. Singer, and L.R. Gibson. 2007. Winter cereal, seeding rate, and intercrop seeding rate effect on red clover yield and quality. Agron. J. 99:723-729.

Brintha, I., and T.H. Seran. 2009. Effect of paired row planting of radish (*Raphanus sativus* L.) intercropped with vegetable amaranths (*Amaranthus tricolor* L.) on yield components in sandy Regosol. J. Agric. Sci. 4:19-28.

Carlsson, G., and K. Huss-Danell. 2003. Nitrogen fixation in perennial forage legumes in the field. Plant and Soil 253:353-372.

Cataldo, B.A., M. Haroon, L.E. Schrader, and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6:71-80.

Carter P.R., and C.C. Sheaffer. 1983. Alfalfa response to soil water deficits. Growth, forage quality, yield, water use, and water-use efficiency. Crop Sci. 23(4):669-675.

Costa, G., C. Andreotti, F. Bucchi, E. Sabatini, C. Bazzi, S. Malaguti, and W. Rademacher. 2001. Prohexadione-Ca (Apogee®): growth regulation and reduced fire blight incidence in pear. HortSci. 36(5):931-933.

Coulter, J., M. Russelle, M. Yost, C. Sheaffer, and D. Kaiser. 2012. Maximizing on-farm nitrogen and carbon credits from alfalfa to maize. Final technical report. Bull. S-10. Minnesota Maize Research and Promotion Council and Minnesota Maize Growers Assoc. St. Paul, MN.

Daily, G.C., and P.A. Matson. 2008. Ecosystem services: from theory to implementation. PNAS 105(28):9455-9456.

Delbridge, T.A., J.A. Coulter, R.P. King, C.C. Sheaffer, and D.L. Wyse. 2011. Economic performance of long-term organic and conventional cropping systems in Minnesota. Agron. J. 103:1372-1382.

Dell, C.J., H.T. Gollany, P.R. Adler, R.H. Skinner, and R.W. Polumsky. 2018. Implications of observed and simulated soil carbon sequestration for management options in maize-based rotations. J. Environ. Qual. 47(4): 617-624. doi: 10.2134/jeq2017.07.0298.

Eberle, C.A., M.D. Thom, K.T. Nemec, F. Forcella, J.G. Lundgren, et al. 2015. Using pennycress, camelina, and canola cash cover crops to provision pollinators. Ind. Crops Prod. 75. doi: 10.1016/j.indcrop.2015.06.026.

Entz, M.H., W.J. Bullied, D.A. Forster, R. Gulden, and J.K. Vessey. 2001. Extraction of subsoil nitrogen by alfalfa, alfalfa-wheat, and perennial grass systems. Agron. J. 93:495-503.

Evans, J.R., R.R. Evans, C.L. Regusci, and W. Rademacher. 1999. Mode of action, metabolism, and uptake of BAS 125W, Prohexadione-calcium. HortSci. 34(7):1200-1201.

Exner D.N., and R.M. Cruse. 1993. Interseeded forage legume potential as winter ground cover, nitrogen source and competitor. J. Prod. Agric. 6 (2):226-231.

Flores- Sanchez D., A. Pastor, B.H. Janssen, E.A. Latinga, W.A. Rossing, and M.J. Kropff. 2012. Exploring maize-legume intercropping systems in Southwest Mexico. Agroecol. Sust. Food J. 37(7):739-761.

Franzen, D.W. 2010. North Dakota fertilizer recommendation tables and equations: based on soil test and yield goals. Bull. SF-882 (Revised). North Dakota State Univ. Ext. Serv., Fargo, ND.

Franzen, D.W. and M.T. Berti. 2017. Alfalfa soil fertility requirements in North Dakota soils. Bull. SF1863. North Dakota State University Extension Service.

Fusuo, Z., and L. Li. 2003. Using competitive and facultative interaction in intercropping systems enhances crop productivity and nutrients use efficiency. Plant and Soil 248:305-312.

Gaba, S., F. Lescourret, , S. Boudsocq, J. Enjalbert, P. Hinsinger, E.P Journet, M.L Navas, J. Wery, G. Louarn, E. Malézieux, E. Pelzer, M. Prudent and H. Ozier-Lafontaine.2015. Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. Agron. Sustain. Dev. 35:607–623 DOI 10.1007/s13593-014-0272-z (Verifies 21 January 2021)

Grabber J.H. 2016. Prohexadione-calcium improves stand density and yield of alfalfa interseeded into silage maize. Agron. J. 108:1-10.

Hall, M.H., C.J. Nelson, J.H. Coutts, and R.C. Stout. 2004. Effect of seeding rate on alfalfa stands longevity. Agron. J. 96:717-722.

Hallam, A., I.C. Anderson, and D.R. Buxton. 2001. Comparative economic analysis of perennial, annual, and intercrops for biomass production. Biomass and Energy 21:407-424.

Haugen, R. 2017 Custom Farm Work Rates on North Dakota Farms, 2016. Bull EC499 February 2017. North Dakota State University Extension.

Hauggaard-Nielsen H., B. Jornsgaard, J. Kinane, and E.S. Jensen. 2007. Grain legume-cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agr. Food Syst. 23(1): 3-12.

Hauggaard-Nielsen H., P. Ambus, and E.S. Jensen. 2001. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutr. Cycling in Agro-Ecosyst. 65:289-300.

Haystead, A., N. Malajczuk, and T.S. Grove. 1988. Underground transfer of nitrogen between pasture plants infected with vesicular-arbuscular mycorrhizal fungi. New Phytol. 108:417-423.

Hopkins, W.G., and N.P.A. Hüner. 2008. Introduction to plant physiology. 4th Ed. John Wiley & Sons, Inc, Hoboken, NJ.

Hoy M.D., K.J. Moore, J.R. George, and E.C. Brummer. 2002. Alfalfa yield and quality as influenced by establishment method. Agron. J. 94:65-71.

Hubbard, K., and N. Hassanein. 2013. Confronting coexistence in the United States: organic agriculture, genetic engineering, and the case of Roundup Ready alfalfa. Agric Hum Values 30:325-335.

Ijoyah, M.O. 2012. Review of intercropping research: studies on cereal-vegetable based cropping system. Scientific J. of Crop Sci. 1(3):55-62.

Ijoyah, M.O., and D.M. Dzer. 2012. Yield performance of okra (*Abelmoschus esculentus* L. Moench), and maize (*Zea mays* L.) as affected by time of planting of maize in Makurdi, Nigeria. Int. Sch. Res. Net. doi:10.5402/2012/485810.

Johnston, C.A. 2014. Agricultural expansion: land use shell game in the U.S. Northern Plains. Landscape Ecol. 29:81-95.

Karlen, D.L., E.G. Hurley, S.S. Andrews, C.A. Cambardella, D.W. Meek, M.D. Duffy, and A.P. Mallarino. 2006. Crop rotation effects on soil quality at three Northern maize/soybeans Belt locations. Agron. J. 98:484-495.

Klein, R.N., R.K. Wilson, J.T. Groskopf, and J.A. Jansen. 2015. 2016 Nebraska crop budgets: alfalfa. Institute of Agriculture and Natural Resources. Univ. of Nebraska-Lincoln Ext. EC872. Available at: http://cropwatch.unl.edu/image/2016Budgets/Maize%20Dryland%20Budgets%2015-23.pdf (verified 16 February 2020).

Kragt, M.E., and M.J. Robertson. 2014. Quantifying ecosystem services trade-offs from agricultural practices. Ecological Economics 102:147-157.

Kucharik C.J. 2008. Contribution of planting date trends to increase maize yields in the Central United States. Agron. J. 100:328-336

Landis, D.A., M.M. Gardiner, W. van der Werf, and S.M. Swinton. 2008. Increasing maize for biofuel production reduces biocontrol services in agricultural landscapes. PNAS 105(51):20552-20557.

Laporte, J. 2019. Farm Management- Price standing corn silage, Michigan State University Extension 19 August 2019. Available at: https://extension.msu.edu/ (verified 27 January 2021).

Lazarus, W.F., 2014. Machinery Cost Estimates. University of Minnesota Extension, St. Paul, MN.

Li, L., J. Sun, F. Zhang, X. Li, S. Yang, and Z. Rengel. 2001. Wheat/maize or wheat/soybean strip intercropping: I. Yield advantage and interspecific interactions on nutrients. Field Crops Res. 71:123-137.

Li, L., J. Sun, F. Zhang, T. Guo, F.A. Smith, and S.E. Smith. 2006., Root distribution and interactions between intercropped species. Oecologia 147:280-290.

Lindenmayer R.B., N.C. Hansen, J. Brummer, and J.G. Pritchett. 2011. Deficit irrigation of alfalfa for after-savings in the Great Plains and Intermountain West: A review and analysis of the literature. Agron. J. 103(1):45-50.

Mahli, S., and A. Foster. 2011. Cover crop seeding rate effects on forage yields of oat and barley and under seeded bromegrass–alfalfa mixture. Commun. Soil Sci. Plant Anal. 42:2344-2350.

Mattera, J., L.A. Romeroa, A.L. Cuatrin, P.S. Maizeaglia, and A.A. Grimoldi. 2013. Yield components, light interception and radiation use efficiency of lucerne (*Medicago sativa* L.) in response to row spacing. Eur. J. Agron. 45: 87-95.

Matusso, J.M., J.N. Mugwe, and M. Mucheru-Muna. 2012. Potential role of cereal-legume intercropping systems in integrated soil fertility management in small holder farming systems of sub-Saharan Africa. Third Ruforum Biennial Meeting 24-28 September 2012, Entebbe, Uganda.

Mead, R. and Willey, R.W. 1980. The concept of a land equivalent ratio and advantages. Experimental Agric. 16: 217-226.

Mikic A., B. Cupina, D. Rubiales, V. Mihailovic, L. Sarunaite, J. Fustec, S. Antanasovic, S. Krstic, L. Bedoussac, L. Zoric, L. Dordevic, and V. Peric. 2015. Models, developments, and perspectives of mutual legume intercropping. p. 337-419 *In* Sparks, D.L. (ed.). Advances in Agronomy. Vol. 130.

Moradi, H., M. Noori, A. Sobhkhizi, M. Fahramand, and K. Rigi. 2014. Effect of intercropping in agronomy. J. Novel Applied Sci. 3 (3):315-320.

NASS, National Agricultural Statistics Service. 2019. Crop production 2019: Summary. US Dep. of Agriculture (USDA). ISSN: 1057-7823. Available at: http://www.usda.gov/nass/PUBS/ (verified 3 March 2020).

NDAWN, North Dakota Agricultural Weather Network. 2017. NDAWN Center. North Dakota State Univ., Fargo, ND. Available at: http://ndawn.ndsu.nodak.edu (verified 14 January 2021).

Niu, Y., Z. Luo, L. Cai, J.A. Coulter, Y. Zhang, and M. Berti. 2020. Continuous monoculture of alfalfa and annual crops influence soil organic matter and microbial communities based on the substrate utilization pattern analysis in rainfed Loess Plateau of China. Agronomy 10:1054, doi:10.3390/agronomy10071054

North Dakota State University (NDSU). 2018. Projected budgets for crop and livestock. North Dakota State University Extension. Available at: https://www.ag.ndsu.edu/farmmanagement/crop-budget-archive (verified January 21, 2021).

Noorwood, C.A. 2000. Water use and yield of limited-irrigated and dryland maize. Soil Sci. Soc. America J. 64 (1):365-370.

Olmstead, J., and E.C. Brummer. 2007. Benefits and barriers to perennial forage crops in Iowa maize and soybean rotations. Renewable Agric. and Food Syst. 23(2):97-107

Olson, T.C. 1971. Yield and water use by different populations of dryland maize, grain sorghum, and forage sorghum in the Western Corn belt. Agron. J. 63(1):104-106.

Osterholz, W.R., J.H. Grabber, and M.J. Renz. 2018. Adjuvants for prohexadione-calcium applied to alfalfa interseeded into maize. Agron. J. 110(6): 2687–2690. doi: 10.2134/agronj2017.09.0564.

Osterholz, W.R., M.J. Renz, W.E. Jokela, and J.H. Grabber. 2019. Interseeded alfalfa reduces soil and nutrient runoff losses during and after maize silage production. J. Soil Water Conserv. 74(1): 85-90. doi: 10.2489/jswc.74.1.85.

Osterholz, W.R., M.J. Renz, W.E. Jokela, and J.H. Grabber. 2020. Alfalfa establishment by interseeding with silage corn projected to increase profitability of corn silage-alfalfa rotations. Agron. J. 112:4120-4132 doi: 10.1002/agj2.20312

Parr M., J. M. Grossman, S. C. Reberg-Horton, C. Brinton, and C. Crozier. 2011. Nitrogen delivery from legume cover crops in no-till organic maize production. Agron. J. 103:1578-1590.

Pecetti, L., P. Annicchiarico, C. Scotti, M. Paolini, V. Nanni, and A. Palmonari. 2017. Effects of plant architecture and drought stress level on lucerne forage quality. Grass Forage Sci. 72(4):714-722.

Pendleton, J.W., J.A. Jackobs, F.W. Slife, and H.P. Bateman. 1957. Establishing legumes in maize. Agron. J. 49(1):44-48.

Peterson, T.A, and M. Russelle. 1991. Alfalfa and the nitrogen cycle in the Corn Belt. J. Soil Water Conserv. 46(3):229-235.

Power, A.G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Available at: http://rstb.royalsocietypublishing.org/content/365/1554/2959.short (verified 19 January 2021)

Ramson, J., and G.J. Endres. 2014. Maize: growth and management. North Dakota State Univ., Ext. Serv., Fargo, ND. A1173.

Reddy, T.Y., and G.H.S. Reddi. 2007. Principles of Agronomy. 3rd ed. Kalyam Publ., India.

Rethwisch, M.D., R. Perez, B.J. Griffin, A. Bradley, and M. Reay. 2003. Alfalfa yield and quality responses to applications of three types of plant growth regulators. Forage and grain report. Univ. of Arizona. Available at https://repository.arizona.edu/handle/10150/205397 (verified 20 January 2021).

Ross S.M., J.R. King, R.C. Izaurralde, and J.T. O'Donovan. 2001. Weed suppression by seven clover species. Agron. J. 93:820-827.

Russelle, M.P. 2003. The role of perennial forages in prevention and remediation of nitrate impacts. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.544.7381&rep=rep1&type=pdf (Verified 20 January 2021).

Russelle, M.P. 2004. The environmental impacts of N_2 fixation by alfalfa. p. 57-62 *In* Proc. 2004 National Alfalfa Symposium and 34th California Alfalfa Symposium. 13-15 Dec. 2004, San Diego, CA. Univ. Coop. Ext. and Alfalfa Workgroup.

Russelle, M.P. 2013. The alfalfa yield gap: a review of the evidence. Forage and Grazinglands. doi 10.1094/FG-2013-0002-RV.

Russelle, M.P., D.L. Allan, and C.J.P. Gourley. 1994. Direct assessment of symbiotically fixed nitrogen in the rhizosphere of alfalfa. Plant and Soil 159:233-243.

Russelle, M.P., M.H. Entz, and A.J. Franzluebbers. 2007. Reconsidering integrated crop-livestock systems in North America. Agron. J. 99:325-334.

Sarrantonio M. and E. Gallandt. 2003. The role of cover crops in North American cropping systems. J. of Crop Prod. 8 (1): 53-74.

Sarunaite L., I. Deveikyte1, A. Arlauskiene, Z. Kadziuliene, and S. Maiksteniene. 2013. Pea and spring cereal intercropping systems: Advantages and suppression of broad-leaved weeds. Pol. J. Environ. Stud. 22(2):541-551.

SAS Institute, 2014. SAS User's Guide: Statistics; SAS Inst.: Cary, NC, USA, 2014.

Sawyer, J.E., P. Pedersen, D.W. Barker, D.A. Ruiz Diaz, and K. Albrecht. 2010. Intercropping maize and kura clover: Response to nitrogen fertilization. Agron. J. 102:568-574.

Schulz, V.S., C. Schumann, S. Weisenburger, M. Müller-Lindenlauf, K. Stolzenburg, K. Möller.2020. Row-Intercropping Maize (*Zea mays* L.) with Biodiversity-Enhancing

Flowering-Partners-Effect on Plant Growth, Silage Yield, and Composition of Harvest Material. Agriculture 10 (11):524.

Sheaffer, C.C., G.D. Lacefield, and V.L. Marble. 1988. Cutting schedules and stands. p. 411-437 *In*: Alfalfa and Alfalfa improvement. Agronomy Monograph 29. ASA, CSSA, SSSA. Madison, WI. doi: 10.2134/agronmonogr29 (Verified 20 January 2021).

Simmons S.R., C.C. Scheafer, D.C. Rasmusson, D.D. Stuthman, and S.E. Nickel. 1995. Alfalfa establishment with barley and oat companion crop differing in stature. Agron. J. 87:268-272.

Smith, M.A., and P.R. Carter. 1998. Strip intercropping maize and alfalfa. J. Prod. Agric. 11(3):345-353.

Soil Survey. 2014. Official Soil Series Description (OSD)-Bearden USDA-NRCS. https://soilseries.sc.egov.usda.gov/OSD_Docs/B/BEARDEN.html (verified 6 January 2021).

Soil Survey 2016. Official Soil Series Description (OSD)-Fargo USDA-NRCS. https://soilseries.sc.egov.usda.gov/OSD_Docs/F/FARGO.html (verified 6 January 2021).

Speirs, J., and W.J. Mitchell. 2013. Estimation of nitrogen by Kjeldahl`s method note in the ammonia distillation. J. Inst. Brewing. 42:247-250.

Strydhorst S., J.R. King, K.J. Lopetinsky, and K.N. Harker. 2008. Forage potential of intercropping barley with faba bean, lupin, or field pea. Agron. J. 100(1):182-190.

Sulc, R.M., and B.F. Tracy. 2007. Integrated crop-livestock in the U.S. Corn belt. Agron. J. 99:335-345.

Sun, T., Z. Li, Q. Wu, T. Sheng, and M. Du. 2018. Effects of alfalfa intercropping on crop yield, water use efficiency and overall economic benefit in the Corn belt of Northeast China. Field Crops Res. 216: 109-119.

Sun, B., Y. Peng, H. Yang, Z. Li, Y. Gao, C. Wang, Y. Yan, and Y. Lui. 2014. Alfalfa (*Medicago sativa* L.)/maize (*Zea mays* L.). Intercropping provides a feasible way to improve yield and economics incomes in farming and pastoral areas of Northeast China. PLOS One. 9(10):e110556.

Swenson, A. and R. Haugen 2014. Projected 2015 Crop budgets North Central North Dakota. Bull. EC1654. Farm Management Planning guide December 2014.

Swift, M.J., A.M.N. Izac, and M. van Noordwijk. 2004. Biodiversity and ecosystem services in agricultural landscapes-Are we asking the right questions? Agric. Ecosyst. Environ. 104:113-134.

Syswerda, S.P. and G.P. Robertson. 2014. Ecosystem services along a management gradient in Michigan (USA) cropping systems. Agriculture, Ecosystems & Environment 189: 28-35.

Thayamini, H.S., and I. Brintha. 2010. Review on maize based intercropping. J. Agronomy. 9(3):135-145.

Turner R.K., and G.C. Daily. 2008. The ecosystem services framework and natural capital conservation. Environ. Resource Econ. 39:25-35.

Undersander D., D. Cosgrove, E. Cullen, C. Grau, M.E. Rice, M. Renz, C. Scheaffer, G. Shewmaker, and M. Sulc. 2011. Alfalfa Management Guide. American Soc. of Agronomy, Crop Sci. Soc. of America and Soil Sci. Soc. of America. Madison, WI.

Varvel G.E., and W.W. Wilhelm. 2003. Soybean nitrogen contribution to maize and sorghum in Western Corn Belt rotations. Agron. J. 95:1220-1225.

Wall, G.J, E.A. Pringle, and R.W. Sheard. 1991. Intercropping red clover with silage maize for soil erosion control. Can. J. Soil Sci. 71: 137-145.

Wu, S., P. Wu, H. Feng, and G.P. Merkley. 2011. Effects of alfalfa coverage on runoff, erosion and hydraulic characteristics of overland flow on loess slope plots. Front. Environ. Sci. Engin. 5(1):76-83.

Yost, M.A., Coulter, J.A., M.P. Russelle, C.C. Sheaffer, and D.E. Kaiser. 2012. Alfalfa nitrogen credit to first-year maize: potassium, regrowth, and tillage timing effects. Agron. J. 104:953-962.

Yost, M.A., M.P. Russelle, J.A. Coulter, M.A. Schmitt, C.C. Sheaffer, and G.W. Randall. 2015. Stand age affects fertilizer nitrogen response in first year maize following alfalfa. Agron. J. 107(2):486-494.

Zemenchik R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Maize production with kura clover as a living mulch. Agron. J. 92:698-705.

Zhang, G., Z. Yang, and S. Dong. 2011. Interspecific competitiveness affects the total biomass yield in alfalfa and maize intercropping system. Field Crops Res. doi: 10.1016/j.fcr.2011.06.006 (verified 27 July 2020)

Zhang, G., Ch. Zhang, Z. Yang, and S. Dong. 2013. Root distribution and N acquisition in an alfalfa and maize intercropping system. J. Agriculture Sci. 5(9):128-141.