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Alfalfa Established Successfully in Intercropping with Corn in the Midwest US

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Abstract: Integrating alfalfa (*Medicago sativa* L.) with corn (*Zea mays* L.) for grain will increase biodiversity, reduce the negative environmental impact of corn monoculture and increase farm profitability. The objectives of this research were to evaluate forage productivity and nutritive value, along with stand establishment of alfalfa in a corn grain system in Iowa, Minnesota, and North Dakota. The experimental design was a randomized complete block with four replicates at each site. Treatments included were: sole corn (i.e., check; T1), sole alfalfa (T2), alfalfa intercropped into corn (T3), a prohexadione-treated alfalfa intercropped with corn (T4), and a spring-seeded alfalfa in the year after intercropping (T5), which was planted in plots with T1 the previous year. All sites had below normal rainfall in 2016 and 2017. Corn grain yield was significantly lower when intercropped with alfalfa (T3 and T4) compared with the check corn crop (no alfalfa, T1). Corn grain yield reduction ranged from 14.0% to 18.8% compared with the check (T1). Corn biomass yield was reduced by intercropped alfalfa (T3 and T4) by 15.9% to 25.8%. In the seeding year, alfalfa seasonal forage yield was significantly greater when corn competition was absent in all environments. The intercropped alfalfa from the previous season (T3 and T4) had almost double the forage yield than the alfalfa in the seeding year (spring-seeded alfalfa; T5). In the second production year, there were no meaningful forage yield differences ($p > 0.05$) across all treatments, indicating alfalfa in intercropping systems does not affect forage yield past the first production year. Prohexadione-calcium, a growth regulator, did not affect alfalfa stand density, forage yield and nutritive value. The forage nutritive value was dependent on harvest date not the alfalfa intercropping treatments. Results of our study suggest that establishing alfalfa with corn is feasible and can be a potential alternative for the upper Midwest region. However, when under drought conditions, this system might be less resilient since competition between alfalfa and corn for soil moisture will be intensified under drought or moisture-limited conditions, and this will likely depress corn grain yield. Research targeted to reintroduce perennial crops into the current dominant corn–soybean systems in the US Corn Belt is urgently needed to improve stability and resiliency of production systems.

Keywords: establishment; intercropping; competition; forage yield; stand density; forage nutritive value

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1. Introduction

The removal of perennial crops in the crop rotations by anthropogenic and climatic factors in the Corn Belt region in the USA has resulted in reduced biodiversity, increased soil and nutrients losses, and reduced water quality [1–3]. Less diverse cropping systems are more vulnerable to abiotic and biotic stresses [4–6]. A more diverse, resilient and stable cropping system has the ability to persist over time with minimal variability in productivity over the years, even if subjected to disturbance or adverse conditions such as drought [3]. Under future climate scenarios, it is estimated that more diversified rotations, including alfalfa, can mitigate crop water stress and increase in soil organic carbon [7].

Perennial crops such as alfalfa reduce annual disturbance of soil, which affects many biogeochemical cycles that are key to provide resilience and stability to agroecosystems [3]. Alfalfa provides long-term sustainability contributing to the soil health of cropping systems by affording nitrogen credits to the following crops [8,9]. In addition, alfalfa reduces nitrate leaching (which decreases water pollution), increases biodiversity, critical habitat for wildlife, and soil carbon sequestration [5,10,11].

Even though alfalfa can provide multiple benefits to crop rotations and the environment, Corn Belt farmers have reduced the area planted to alfalfa or replaced alfalfa for more profitable and easy-to-manage annual cropping systems (e.g., silage corn) [12]. Dairy farmers prefer to grow corn for silage instead of alfalfa because alfalfa forage yield in the seeding year is much lower compared with silage corn and about half of the forage yield of corn in a production year [13]. Thus, establishing alfalfa together with another crop, such as corn, in the seeding year provides additional revenue for the farmer to offset the low income in the seeding year [14,15]. Several studies have evaluated alfalfa establishment and forage yield benefits of silage corn–alfalfa intercropping in the US [13–16]. This novel establishment technique has demonstrated potential to increase forage production [15]; however, shading from the corn canopy can hinder alfalfa establishment and seedling survival [13,17]. Nonetheless, when alfalfa establishment is successful, this novel system provides several ecosystem services such as reductions in soil erosion and nutrient loading to water [18,19]. Intercropping corn and alfalfa can increase productivity, profitability [14], and reduce the agricultural carbon footprint particularly in intercropped systems combining C3 and C4 species [7].

In previous alfalfa-silage corn intercropping research [13,14], successful alfalfa establishment required applications of the plant growth regulator, prohexadione-calcium (PHX). However, the alfalfa intercropping research conducted previously was limited to corn silage systems where the corn stover (i.e., residue) was fully removed for forage. Corn grown for grain likely has less photosynthetically active radiation (PAR) available for intercropped alfalfa at the end of the season than earlier-harvested corn planted for ensiling, typically harvested at 65%–70% moisture. For instance, corn harvested for grain leaves more residue (8–12 Mg DM ha^{-1}) on the soil surface [20,21] compared with silage corn in which most of the biomass is removed from the field, resulting in greater continued interference for PAR to recently established alfalfa plants.

There are no previous reports indicating if this novel alfalfa establishment system is adapted to a broader range of environments in corn grain–alfalfa rotations. The objectives of this research were to evaluate the forage productivity and nutritive value, along with stand establishment of alfalfa in a grain corn system in Iowa, Minnesota, and North Dakota.

2. Materials and Methods

2.1. Experimental Sites

This experiment was conducted at two North Dakota State University (NDSU) research sites in Prosper and Forman, ND, and in Rosemount, MN and Ames, IA. Coordinates of each site and soil description are provided in Table 1. Monthly rainfall and minimum, maximum, and average temperature were obtained from nearby weather stations with the North Dakota Agricultural Weather Network [22] and Iowa Environmental Mesonet [23] and Rosemount, MN weather station. All the sites were non-irrigated.

Table 1. Locations and soil description.

Location/State	Latitude	Longitude	Elevation	Soil Type	Soil Characteristics [24]	
			(m.a.s.l.)		Texture	Description
Ames, IA	42°00′46.93″ N	−93°39′50.75″ W	303	Webster-Clarion	Clay loam	Webster: fine-loamy, mixed, super-active, mesic, Typic Endoaquoll Clarion: fine-loamy, mixed, superactive, mesic, Typic Hapludoll
Forman, ND	46°05′03.20″ N	−97°38′06.71″ W	385	Aastad-Forman	Loam	Aastad: fine-loamy, mixed, super-active, frigid, Pachic Argiudoll Forman: fine-loamy, mixed, superactive, frigid, Calcic Argiudoll
Prosper, ND	46°59′57.42″ N	−97°06′57.24″ W	281	Kindred-Bearden	Silty clay loam	Kindred: fine-silty, mixed, super-active, Typic Endoaquoll Bearden: fine-silty, superactive, frigid, Aeric Calciaquoll
Rosemount, MN	44°42′14.93″ N	−93°05′50.92″ W	288	Waukegan	Silt loam	Fine-silty over sandy or sandy-skeletal, mixed, super-active, mesic, Typic Hapludoll

Elevations in meters above sea level (m.a.s.l.).

2.2. Experimental Design and Management

The experimental design was a randomized complete block with four replicates at each site. The treatments were: sole corn (T1), sole alfalfa (T2), alfalfa intercropped into corn (T3), a PHX-treated alfalfa intercropped with corn (T4), and a spring-seeded alfalfa in the year after intercropping (T5), which was planted in plots with T1 the previous year. Prohexadione rate was 0.5 kg a.i. ha^{-1}. Citric acid (0.935 kg ha^{-1}), ammonium sulfate (1.12 kg ha^{-1}) and crop oil concentrate (2.34 L ha^{-1}) were added to the PHX solution and applied in a volume of 187 L ha^{-1}. Prohexadione was applied only to alfalfa plants when alfalfa was about 20 cm in height and corn was at about an 8-leaf stage (V8).

A glyphosate-tolerant alfalfa cultivar, RR Presteez (Croplan, fall dormancy 3 and winter survival 1) was used at Forman, Prosper, and Rosemount locations. In Ames, the Pioneer 54QR04 alfalfa (fall dormancy 4 and winter survival 2) was used in both years. Corn hybrid planted at Forman and Prosper were Winfield 3337VT2P/RIB (93 RM) in 2016 and Peterson 2MD02 (102 RM) in 2017. At Ames, DeKalb DKC57-75RIB (107 RM) was used both years while at Rosemount DeKalb DKC45-65RIB (95 RM) was used. Seeding dates of corn and alfalfa for each treatment and at each location and year are provided in Table 2.

Table 2. Seeding dates and prohexadione-calcium application dates at all locations and years.

Location	Corn Seeding	Alfalfa Seeding	Prohexadione-Calcium	Spring Alfalfa Seeding
Ames, IA	17 May 2016	17 May 2016	24 June 2016	16 May 2017
Ames, IA	16 May 2017	16 May 2017	5 July 2017	
Forman, ND	3 May 2016	4 May 2016	17 June 2016	2 May 2017
Prosper, ND	5 May 2016	5 May 2016	16 June 2016	2 May 2017
Prosper, ND	12 May 2017	12 May 2017	26 June 2017	
Rosemount	5 May 2017	5 May 2017	11 July 2017	

Corn was planted in 76 cm row spacings at all locations. A 4-cone planter at Prosper (Almaco, Nevada, IA, USA), an 8-row planter equipped with fertilizer banders at Forman (John Deere, JD 7200 Moline, IL, USA), a 4-row planter at Ames (Kinze, Williamsburg, IA, USA), and a 2-row corn drill at Rosemount (John Deere, 7100 MaxEmerge, Moline, IL, USA) were used. Alfalfa was seeded at 15 cm between rows at Prosper, Forman, and Rosemount with a plot drill (Wintersteiger, Austria) and with a grain drill at Ames (Almaco, Nevada, IA, USA). Each experimental unit was 6 m in length and had either four rows of maize (check plots) or four rows of maize and 16 rows of alfalfa seeded on the same seeding date. Data was collected in the two-center rows of corn in each experimental unit. The alfalfa alone plots had 8 rows of alfalfa and the forage yield was collected in the 6-center rows. Borders were sufficient to limit light into intercropped treatments.

Alfalfa was harvested using a flail forage harvester (Carter MFG CO., Inc., Brookston, IN, USA) at Prosper and Forman, and hand-harvested at all other locations with additional mowing performed at Ames. Two-plot center rows of corn were harvested using a plot combine at Prosper and Forman in 2016 (Zürn 150, Waldenburg, Germany), and Ames (John Deere 4 row combine, Moline, IL, USA) and hand harvested in Rosemount. Alfalfa and corn harvest dates are presented in Table 3. Corn stover was not removed in the fall of the seeding year. Corn stover was left in the fall in order to serve as a windbreaker and to increase snow capture during the winter, thus will protect interseeded alfalfa from winter kill; however, corn stover could potentially smother alfalfa seedlings. Following the year of alfalfa establishment, residual corn stover was rotary mowed early in the spring before alfalfa started to grow at all locations, except Ames.

Table 3. Harvest dates of alfalfa and corn at all locations and years.

	Alfalfa				**Spring-Seeded Alfalfa**		**Corn**
Location/Year	**Harvest 1**	**Harvest 2**	**Harvest 3**	**Harvest 4**	**Harvest 1** †	**Harvest 2** †	
Ames, IA							
2016 (seeding year)			10 Nov.				13 Nov.
2017	31 May	20 July	13 Sept.				
2018	1 June	12 July	22 Aug.	26 Oct.			
Ames, IA							
2017 (seeding year)	.		23 Nov.				30 Nov.
2018	1 June	12 July	22 Aug.	26 Oct.	12 July	8 Sept.	
2019	4 June	10 July	8 Sept.	3 Nov.			
Forman, ND							
2016 (seeding year)	20 July	22 Aug.	10 Oct.				14 Oct.
2017	31 May	5 July	1 Aug.	11 Oct.	1 Aug.	11 Oct.	
Prosper, ND							
2016 (seeding year)	19 July	23 Aug.	10 Oct.				14 Oct.
2017	31 May	29 June	1 Aug.	4 Oct.	14 July	4 Oct.	
2018	29 May	28 June	1 Aug.	5 Sept.			
Prosper, ND							
2017 (seeding year)	20 July		4 Oct.				2 Nov.
2018	29 May	28 June	1 Aug.	5 Sept.	9 July	5 Sept.	
2019	3 June	15 July	19 Aug.				
Rosemount							
2017 (seeding year)	11 July	27 July	2 Oct.	-			2 Oct.
2018	13 June	7 Aug.	31 Oct.	-	31 Oct.		
2019	14 June	8 Aug.	23 Sept.	-			

† Harvest dates (cut 1 and 2) of spring-seeded alfalfa treatment following corn. In the seeding years in Iowa, Minnesota and Prosper, ND environments the first harvest were obtained at the time of the third cut of other treatments, thus the date is indicated in H3.

Weed control was conducted with glyphosate (isopropylamine salt of N-(phosphono methyl) glycine) at 0.84–0.91 kg a.i. ha^{-1} at all locations and years. In Ames, additional to the glyphosate application, S-ethyldipropylthiocarbamate (EPTC) at 6.35 kg a.i ha^{-1} was applied before planting the experiment.

Alfalfa was sprayed to control potato leafhopper (*Empoasca fabae* Harris) at most locations. At the North Dakota locations, malathion (0,0-dimethyl phophorodithioate of diethyl mercaptosuccinate) at 171 mL a.i. ha^{-1} was sprayed in early August in 2016, 2017, and 2018. In Minnesota locations, lambda-cyhalothrin ([1α(S*), 3α(Z)]- (±)-cyano-(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate) at 140 mL ha^{-1} and permethrin (3-phenoxyphenyl)methyl (±)cis-trans 3-(2,2 dichloroethenyl)-2,2,dimethylcyclopropanecarboxylate) at 197 mL a.i. ha^{-1} were applied for potato leafhopper control. In Ames, dimethoathe (O,O dimethyl phosphorodithioate S-ester with 2-mercapto-N-methylacetamide) at 585 mL a.i. ha^{-1} was applied twice in 2016 and three times in 2017 to control potato leafhoppers.

Fertilizer rates for corn were 120 kg N ha^{-1} in Prosper and Forman, 168 kg N ha^{-1} in Ames, both years, and 100 kg N ha^{-1} in Rosemount. Nitrogen fertilizer at all locations was urea. Fertilization with phosphorus and potassium in Ames was at 112 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1}, respectively. In Rosemount, 50 and 246 kg ha^{-1} of P_2O_5 and K_2O were applied, respectively. In North Dakota sites, all experiments were fertilized in the seeding and first production year with 67 and 112 kg ha^{-1} of P_2O_5 and K_2O, respectively.

2.3. Sampling and Analysis

Soils were sampled to a depth of 15 cm before the experiment was planted at each location, and samples were analyzed for pH, organic matter, and available P and K. Additionally, NO_3-N concentration was determined for the 0 to 15 cm and 15 to 60 cm depths. The N concentration was determined by the transnitration of salicylic acid method [25]. The Olsen method and the ammonium acetate tests were used for available P and K determination, respectively [26] (Table 4).

Table 4. Soil chemical analysis baseline for each location and year.

Location/Year	N-NO_3	P	K	OM	pH †
	kg ha^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	
Ames 2016	76	9	80	43	6.6
Ames 2017	64	2	80	45	6.5
Forman 2016	60	28	382	57	6.3
Prosper 2016	95	33	358	42	7.3
Rosemount 2017	17	17	112	42	5.9

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

Aboveground corn biomass yield was calculated from a linear meter from the two-center rows of each plot. All plants were cut off by hand, weighed in the field (fresh weight), and then a sample of two complete plants was dried to calculate water concentration. Corn grain yield was determined by the combine harvester and corrected to 150 g kg^{-1} water concentration. Average corn plant height was determined measuring five random plants from the center two rows. At Ames, alfalfa biomass was hand-harvested from two 0.5 m^2 quadrats per plot. Samples were put in cloth bags and placed inside a forced-air oven at 49 to 55 °C, depending on site, until dry, and then weighed.

Alfalfa plants were counted from the same two 0.5 m^2 area in each plot from which biomass samples were collected in North Dakota and Minnesota locations and only stems were counted in Ames, IA. Alfalfa plants were counted without digging them out. However, the authors were careful when taking plant and stem counts. Each plant was separated/identified by its own crown (at the ground level) to determine if it was a single plant or a cluster. Stems arising from each plant was counted immediately after identifying individual plants. Stems that were 5 cm or longer were counted as stems. Plant height was measured to the nearest 1-cm from at least three stems on randomly selected plants in each plot prior to every harvest.

Photosynthetically active radiation (PAR) readings under and above the canopy were collected in 2016 and 2017 at all North Dakota environments by placing a ceptometer in between the two center rows. Three readings were taken from each experimental unit and the ceptometer provided the average readings. Intercepted PAR light percentage was calculated using the following formula:

$$\text{Intercepted PAR light}(\%) = \frac{\text{PAR above the canopy} - \text{PAR below the canopy}}{\text{PAR above the canopy} \times 100} \quad (1)$$

Dried samples of alfalfa were grinded in a Model 4 cutting mill (Eberbach Corporation, Ann Arbor, MI, USA) to pass through a 1-mm sieve. Samples from all locations were analyzed at North Dakota State University, Forages Lab. Concentrations of crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), and neutral detergent fiber digestibility (NDFD) were determined with an NIRS, XDS analyzer (Foss, Denmark), following the methods described by Abrams et al. [27].

2.4. Statistical Analysis

Analysis of variance and mean comparisons were conducted using the mixed procedure of SAS [28]. Analysis of variance was conducted separately for each state. Environments (defined as a combination of location and year) were combined within a state for corn yield and plant height. For alfalfa evaluations, environments were combined within a same state and production year. Even though the experimental design and treatments were the same in all locations in each state, differences in management and number of harvests per season in each state made it impossible to perform a combined analysis over the six environments. For alfalfa yield and stand in North Dakota, three environments were combined for the seeding year and first production year: Prosper and Forman seeded in 2016 and Prosper seeded in 2017. Only two environments were combined for the second production year: Prosper in 2016 and 2017. In Iowa, the experiment was started in 2016 and 2017 so both environments were combined for the analysis of the seeding, first, and second production years. In Minnesota, only one environment was considered in the analysis for each year. Environments (location/year) within a state were considered a random effect. Treatments were considered fixed factors in the statistical analysis. Harvests were considered as a fixed factor in the analysis for nutritive value analysis only; for all other alfalfa variables harvests were analyzed separately. For plant and stem density, the evaluations were only done in fall and spring, so the harvest was not a factor in the analysis. Least square means pair comparisons were conducted with the pdiff function of the mixed procedure [28]. The standard error and degrees of freedom of each pair comparison were used to calculate the protected Fisher's least square differences (LSD) at the $p \leq 0.05$ probability level.

3. Results

3.1. Corn Grain and Biomass Yield and Plant Height

Corn grain yield was significantly lower ($p \leq 0.05$) when intercropped with alfalfa (T3 and T4) in Iowa and North Dakota compared with the check corn crop (no alfalfa check; T1) (Table 5). Grain yield reduction ranged from 14.0% to 18.8% from the check. Biomass yield of corn was also affected by intercropped alfalfa (T3 and T4), causing a reduction in yield of 15.5% to 25.8%. Corn plants intercropped with alfalfa (T3 and T4) were 6.7% to 9.0% shorter than the check corn crop (T1) in Iowa and North Dakota environments (Table 5); however, this reduction was not significant. The application of PHX to the intercropped alfalfa (T4) did not affect corn grain or biomass yield.

Table 5. Corn grain yield, above-ground biomass yield, and plant height means for each treatment and significance of effects (source of variation, SOV) in the model averaged across locations within a state.

Treatment	Iowa			Minnesota			North Dakota ‡		
	Grain	**Biomass**	**Height**	**Grain**	**Biomass**	**Height**	**Grain**	**Biomass**	**Height**
	Mg ha^{-1}		(m)	Mg ha^{-1}		(m)	Mg ha^{-1}		(m)
Corn alone (check, T1)	14.2	33.3	2.25	12.2	23.9	2.69	14.9	29.4	2.39
Alfalfa + corn (T3)	11.8	27.1	2.10	9.7	20.9	2.60	12.1	22.3	2.23
Alfalfa + corn + PHX † (T4)	11.9	28.0	2.09	9.7	20.2	2.59	12.8	23.3	2.17
LSD (0.05)	2.0	3.8	NS	NS	2.0	NS	1.2	5.4	NS
	% reduction from check								
Alfalfa + corn	17.0	18.6	6.7	20.5	12.5	3.3	18.8	25.8	6.7
Alfalfa + corn + PHX	16.0	15.9	7.0	20.5	15.5	3.7	14.0	22.0	9.0
SOV	Significance ($p < F$)								
Env	NS	NS	NS	-	-	-	NS	NS	NS
Trt	**	*	NS	NS	**	NS	*	*	NS
Trt × Env	NS	NS	NS	-	-	-	**	NS	NS

*, **, Significant at ≤0.05, and 0.01 probability level, respectively; NS, non-significant ($p > 0.05$). † PHX: prohexadione calcium, rate of 0.5 kg a.i. ha^{-1}. ‡ North Dakota results are averaged across three environments (Env); Prosper includes 2016 and 2017 and Forman 2016. Iowa results are averaged across two environments: Ames, 2016 and 2017. Least significant difference (LSD) values are compared between treatments within a same state and variable.

3.2. *Alfalfa Forage Yield*

Alfalfa seeding year seasonal forage yield was significantly greater ($p \leq 0.05$) when alfalfa did not have to compete with corn (Table 6). In Iowa and Minnesota experiments, only one alfalfa harvest was performed in the seeding year, while in North Dakota the experiments three cuts were performed at all environments, except at Prosper, ND, in 2017, where only two harvests were taken. Alfalfa biomass yield under the corn canopy at the end of the season (H3) were similar between PHX-treated (T4) and untreated alfalfa (T3), indicating that application of PHX did not reduce alfalfa biomass production in the seeding year (Table 6).

Table 6. Alfalfa mean forage/biomass yield in the seeding year averaged across environments within a same state and significance of effects (source of variation, SOV) in the model.

Treatment	Iowa	Minnesota	North Dakota ‡			
	H1	**H1**	**H1**	**H2**	**H3**	**Total**
	Forage/biomass yield (Mg ha^{-1})					
Alfalfa alone (T2)	1.14	1.41	3.13	3.58	1.07	7.79
Alfalfa + corn (T3)	0.57	0.46	-	-	0.55	0.55
Alfalfa + corn + PHX † (T4)	0.40	0.39	-	-	0.39	0.39
LSD ¶ (0.05)	0.34	0.55	-	-	0.12	0.73
SOV	Significance ($p < F$)					
Env	NS	-	-	-	***	***
Trt	***	***	-	-	***	***
Trt × env	***	-	-	-	***	***

*** Significant at ≤ 0.001 probability level, respectively; NS, non-significant ($p > 0.05$). † PHX: prohexadione calcium at 0.5 kg a.i. ha^{-1}. ‡ North Dakota results are averaged across three environments except in Prosper in 2017. Prosper 2016 and 2017 and Forman 2016 were combined. In Iowa two environments were combined, Ames 2016 and 2017 and Minnesota results are from one environment, Rosemount 2017. Only one harvest (H1) was conducted in the seeding year in all treatments at the end of the season in Iowa and Minnesota environments. In North Dakota environments, only the alfalfa alone was harvested three times (H1, H2, and H3). All other treatments were harvested once at the time of the third harvest (H3). Alfalfa average yield for treatments T3 and T4 was taken at the end of the season. However, yield data is presented on H3, but it corresponds to the first harvest of treatments T4 and T3. ¶ Least significant difference (LSD) values are compared between treatments within a same state and harvest date.

In the first production year, alfalfa seeded without corn in the previous year (T2) had a significantly higher seasonal forage yield than alfalfa treatments established under the corn canopy (T3 and T4) at all environments (Table 7). The main difference in forage yield was observed in the first harvest of the first production year at all environments. The reduction in seasonal forage yield from alfalfa established alone (T2) compared with alfalfa treatments (T3 and T4) ranged between 27% and 33.5% in Iowa environments, 34.7% to 47.1% in Minnesota, and 13.8% to 19.1% in North Dakota.

Table 7. Alfalfa forage yield in the first production year for four harvests (H1, H2, H3, and H4), and total seasonal averaged across environments (Env) within a same state † and significance of effects (source of variation, SOV) in the model.

Treatment	H1	H2	H3	H4	Total
	Forage yield (Mg ha^{-1})				
	Iowa				
Alfalfa alone (T2)	5.77	2.55	2.12	-	10.46
Alfalfa + corn (T3)	3.47	2.24	1.92	-	7.63
Alfalfa + corn + PHX ‡ (T4)	2.95	2.03	1.94	-	6.95
Spring-seeded alfalfa ⁋ (T5)	-	0.70	1.06	-	1.41
LSD (0.05)	1.22	0.46	0.48	-	1.32
SOV	Significance ($p < F$)				
Env	**	NS	NS	-	***
Trt	***	**	***	-	***
Trt × env	NS	NS	NS	-	NS
	Minnesota				
Alfalfa alone (T2)	4.40	2.42	-	-	6.85
Alfalfa + corn (T3)	2.45	2.07	-	-	4.47
Alfalfa + corn + PHX (T4)	1.97	1.63	-	-	3.62
Spring-seeded alfalfa (T5)	-	1.15	-	-	1.15
LSD (0.05)	0.88	0.66	-	-	1.24
SOV	Significance ($p < F$)				
Trt	***	**			***
	North Dakota				
Alfalfa alone	4.54	3.74	3.21	3.90	14.47
Alfalfa + corn	3.13	3.64	3.06	3.77	12.60
Alfalfa + corn + PHX (T4)	2.87	3.51	2.87	3.67	11.70
Spring-seeded alfalfa (T5)	-	-	2.98	2.59	5.32
LSD †† (0.05)	0.60	NS	NS	NS	0.96
SOV	Significance ($p < F$)				
Env	NS	***	NS	***	NS
Trt	*	NS	NS	NS	***
Trt × env	NS	NS	*	***	NS

*, **, *** Significant at ≤0.05, 0.01, and 0.001 probability level, respectively; NS, non-significant ($p > 0.05$). † North Dakota results are averaged across three environments. Prosper 2017 and 2018 and Forman 2017 were combined. In Iowa, two environments were combined: Ames 2017 and 2018, and Minnesota results are from one environment: Rosemount 2018. ‡ PHX: prohexadione calcium at 0.5 kg a.i. ha^{-1}. ⁋ Spring-seeded alfalfa is in the seeding year while all other treatments are in the first production year. †† Least significant difference (LSD) values are compared between treatments within a same state and harvest date.

The seasonal forage yield of alfalfa, established when intercropped with corn the previous season (T3 and T4), was significantly higher than the alfalfa treatment seeded in the spring of the first production year (T5) (Table 7). The PHX application did not increase alfalfa forage yield compared with alfalfa without PHX application (Table 7). Spring-seeded alfalfa (T5) seasonal yield was from two harvests in North Dakota and only one harvest in Iowa and Minnesota. Spring-seeded alfalfa (T5) had between 51% and 57% less seasonal forage yield than the alfalfa established in intercropping with corn the previous season (T3 and T4) in North Dakota. The forage yield of the spring-seeded alfalfa (T5) in Minnesota and Iowa was between 74% and 83% lower than the alfalfa established in intercropping the previous year (T3 and T4), but this was due to having only one harvest in the spring-seeded alfalfa (T5) while in North Dakota the seasonal forage yield was the result of two harvests, which is typical for the area.

In the second production year, alfalfa seasonal forage yield for all treatments was statistically the same at all environments ($p > 0.05$) (Table 8). The differences among treatments in the first production year from alfalfa alone (T2) compared with alfalfa established in intercropping with corn (T3 and T4) were no longer presented. The spring-seeded alfalfa (T5) seasonal forage yield was the same as all other alfalfa treatments even if it was in the first production year while all other alfalfa treatments (T2, T3, and T4) were in the second production year. However, a few significant differences in forage yield between treatments were observed when analyzed by each harvest in both Minnesota and North Dakota environments. In Minnesota, the alfalfa alone (T2) had a significantly lower forage yield than the other treatments in the second and third harvest (Table 8). In North Dakota, the spring-seeded alfalfa (T5) had a greater forage yield than all other treatments in both the first and second harvest, although in the second harvest spring-seeded alfalfa (T5) was significantly different only from T4 ($p \leq 0.05$).

Table 8. Alfalfa forage yield for four harvests (H1, H2, H3, and H4) and total in the second production year averaged across environments (Env) within a same state [†].

Treatment (Trt)	H1	H2	H3	H4	Total
			Forage yield (Mg ha^{-1})		
			Iowa		
Alfalfa alone (T2)	3.87	1.71	1.22	0.85	7.66
Alfalfa + corn (T3)	3.55	1.74	1.37	0.86	7.52
Alfalfa + corn + PHX [‡] (T4)	3.46	1.87	1.39	0.98	7.40
Spring-seeded alfalfa [⁋] (T5)	3.16	1.90	1.13	0.96	7.46
LSD [††] (0.05)	NS	NS	NS	NS	NS
SOV			Significance ($p < F$)		
Env	***	NS	***	NS	***
Trt	NS	NS	NS	NS	NS
Trt × env	NS	NS	NS	NS	NS
			Minnesota		
Alfalfa alone (T2)	4.52	2.55	1.16	-	8.23
Alfalfa + corn (T3)	3.71	3.22	1.44	-	8.07
Alfalfa + corn + PHX [‡] (T4)	4.04	3.59	1.66	-	9.29
Spring-seeded alfalfa [⁋] (T5)	4.46	3.28	1.69	-	9.44
LSD (0.05)	NS	0.69	0.39	-	NS
SOV			Significance ($p < F$)		
Trt	NS	NS	-	-	NS
			North Dakota		
Alfalfa alone (T2)	3.55	3.77	3.25	2.62	11.88
Alfalfa + corn (T3)	3.51	3.49	3.24	2.87	11.67
Alfalfa + corn + PHX [‡] (T4)	3.52	3.31	3.18	2.64	11.33
Spring-seeded alfalfa [⁋] (T5)	4.02	3.92	3.25	2.76	12.58
LSD (0.05)	0.40	0.47	NS	NS	NS
SOV			Significance ($p < F$)		
Env	NS	NS	NS	NS	NS
Trt	*	*	NS	NS	NS
Trt × env	NS	NS	NS	NS	NS

*, *** Significant at ≤ 0.05, and 0.001 probability level, respectively; NS, non-significant ($p > 0.05$); SOV, source of variation. [†] North Dakota results are averaged across three environments; Prosper 2018 and 2019 were combined. In Iowa, two environments were combined: Ames 2018 and 2019. Minnesota results are from one environment, Rosemount 2018. [‡] PHX: prohexadione calcium at 0.5 kg a.i. ha^{-1}. [⁋] Spring-seeded alfalfa is in the first production year while all other alfalfa treatments are in the second production year. [††] Least significant difference (LSD) values are compared between treatment means within a same state and harvest date.

3.3. Alfalfa Stem and Plant Density and PAR

Stem density in the fall of the seeding year was greater for alfalfa established alone (T2) in Iowa environments (Table 9). As expected, in the fall of the first production year the spring-seeded alfalfa treatment (T5) had the greatest stem density in Iowa. However, in both Minnesota and North Dakota environments plant density was not different among treatments ($p > 0.05$) (Table 9). Stem density from the fall in the seeding year to the next spring decreased in Iowa by 21% for the alfalfa alone (T2). In Minnesota, plant density reduction ranged between 36% and 61% depending on the treatment. The application of PHX did not improve alfalfa stand survival and shading by corn only reduced alfalfa stands in Iowa.

Table 9. Plant density or stem density of alfalfa for each treatment in the fall of the seeding year (Fall SY), the spring of the first production year (Spring Y1) and in the fall of the first production year (Fall Y1).

Treatment	Iowa			Minnesota			North Dakota [†]		
	Fall SY	**Spring Y1**	**Fall Y1**	**Fall SY**	**Spring Y1**	**Fall Y1**	**Fall SY**	**Spring Y1**	**Fall Y1**
	Stems m^{-2}			Plants m^{-2}			Plants m^{-2}		
Alfalfa alone (T2)	441	346	87	42	27	21	49	58	41
Alfalfa + corn (T3)	194	230	107	62	24	19	48	57	40
Alfalfa + corn + PHX [‡] (T4)	182	225	108	34	20	18	43	60	41
Spring-seeded alfalfa [¶] (T5)		363	90			12		67	44
LSD (0.05) [††]	140	78	NS	NS	NS		NS	NS	NS

NS, non-significant ($p > 0.05$). [†] North Dakota results are averaged across three environments. Prosper 2016 and 2017 and Forman 2016 were combined for the Fall SY. For the Spring Y1 and Fall Y1 Prosper 2017 and 2018 and Forman 2017 were combined. In Iowa, two environments were combined: Ames 2016 and 2017 for the Fall SY, and Ames 2017 and 2018 were combined for the Spring Y1 and Fall Y1. Minnesota results are from one environment: Rosemount 2017 for the Fall SY, and Rosemount 2018 for the Spring Y1 and Fall Y. [‡] PHX: prohexadione calcium, at 0.5 kg a.i. ha^{-1}. [¶] Spring-seeded alfalfa is in the first production year while all other alfalfa treatments are in the second production year. [††] Least significant difference (LSD) values are compared between treatments within a same state and year.

The analysis of variance for PAR indicated significant effects ($p \leq 0.05$) for environment, date, treatment by date, date by environment, and treatment by date by environment for North Dakota environments (data not shown). The intercepted radiation was measured at all locations and treatments in 2016, but only the average intercepted photosynthetically active radiation (PAR) for alfalfa alone (T1) and corn alone (T2) at two North Dakota environments in 2016 are presented (Figure 1). The PAR interception at other locations was similar to North Dakota environments, which is why it is not shown.

The PAR intercepted by corn without intercropped alfalfa (T2) increased to 80%–85% by canopy closure around mid-July (Figure 1). Thereafter, minimal changes in PAR ($p \leq 0.05$) were observed until the end of summer, with a slight decline in September (Figure 1). Even after corn was mature, the intercepted PAR by corn remained above 80%, indicating that radiation available for alfalfa intercropped into corn is less than 20% after mid-July. Intercepted PAR by corn intercropped with alfalfa was no different than corn alone ($p > 0.05$), thus data is not shown.

The alfalfa alone treatment (T2) interception of PAR varied with location (Figure 1). The PAR interception reached 49% and 84% before the first harvest in Forman and Prosper, respectively. At Forman, the PAR interception was measured 12 days before the first harvest while at Prosper it was measured on the same day of harvest. After the first cut, alfalfa intercepted as much light as corn before the second harvest.

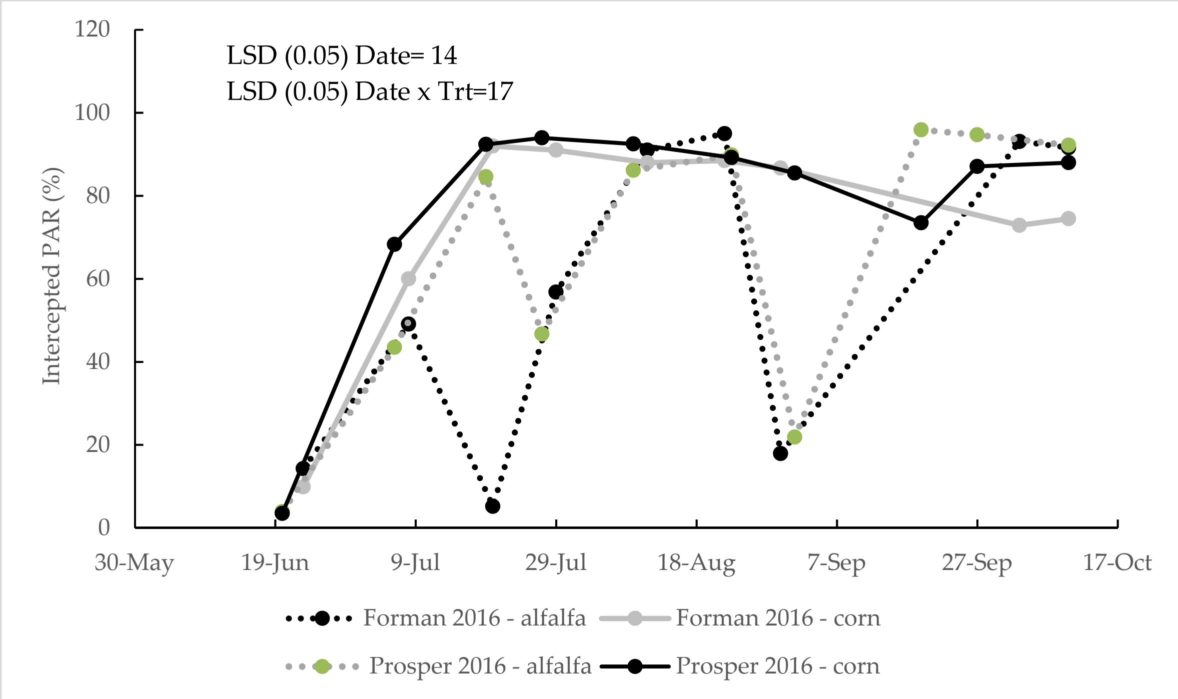

Figure 1. Photosynthetically active radiation (PAR) intercepted by corn (T1) and alfalfa (T2) in monoculture at two locations (Prosper and Forman, ND) in 2016.

3.4. Alfalfa Nutritive Value

Forage nutritive value varied mainly by harvest date in all experiments and years, with no differences due to the treatments within a same harvest ($p > 0.05$) (Table 10). In the first production year, there was a significant harvest by environment and treatment by harvest by environment for CP, NDFD, and NDF ($p \leq 0.05$) in North Dakota. In Iowa, harvest by treatment was significant for CP ($p \leq 0.05$) and harvest by environment was significant for both CP and NDF (Table 10). The CP was lower in the first harvest compared with all other harvests in Iowa regardless of the treatment (Table 11). The lowest CP concentration in North Dakota environments was in the fourth harvest in the first production year. Fiber digestibility was higher in the first harvest both in Iowa and North Dakota environments. The NDF ranged from 371 to 413 g kg^{-1} among all harvest and locations.

Treatments averaged across harvest dates were significant for NDFD and NDF in the Minnesota environment in the first production year; however, the interaction between treatment and harvest date was not significant in this state (Table 11). In Minnesota, in the first production year, the alfalfa coming from intercropping (T3 and T4) had significantly higher NDFD (353–358 g kg^{-1}) and lower NDF (465–483 g kg^{-1}) than the alfalfa without corn (T2) (345 and 529 g kg^{-1}, respectively). However, all alfalfa established the previous year had much higher NDFD and less NDF than the spring-seeded alfalfa (T5) (243 and 582 g kg^{-1}, respectively). In the second production year, in Minnesota, alfalfa alone (T2) and alfalfa coming from intercropping (T3 and T4) had an average NDFD across all harvest dates of 435 g kg^{-1} while spring-seeded alfalfa (T5) was 410 g kg^{-1} (data not shown).

Table 10. Sources of variation and significance of effects for crude protein (CP), neutral detergent fiber digestibility (NDFD), and neutral detergent fiber (NDF) of alfalfa in the first and second production year.

	Iowa			Minnesota			North Dakota		
SOV	**CP**	**NDFD**	**NDF**	**CP**	**NDFD**	**NDF**	**CP**	**NDFD**	**NDF**
				First Production Year					
Trt	NS	NS	NS	NS	*	***	NS	NS	NS
Trt × Env †	NS	NS	NS	-	-		NS	NS	NS
H	NS	NS	NS	***	***	***	*	NS	NS
H × Env	***	NS	**	-	-	-	*	***	***
Trt × H ‡	**	NS	NS	NS	NS	NS	NS	NS	NS
Trt × H × Env	NS	NS	NS	-	-	-	***	***	***
				Second Production Year					
Trt	NS	NS	NS	NS	**	NS	NS	NS	NS
Trt × Env	NS	NS	NS	-	-	-	NS	NS	NS
H	***	NS	NS	***	***	***	NS	NS	NS
H × Env	***	***	***	-	-	-	***	***	**
Trt × H	NS	NS	NS	NS	**	NS	NS	NS	NS
Trt × H × Env	NS	NS	NS	-	-		NS	NS	NS

*, **, *** Significant at ≤0.05, 0.01, and 0.001 probability level, respectively; NS, non-significant ($p > 0.05$). † Environment= (Env). North Dakota results are averaged across three environments. Prosper 2017 and 2018 and Forman 2017 were combined for the first production year and Prosper 2018 and 2019 for the second production year. In Iowa, two environments were combined, Ames 2016 and 2017 for the first production year and Ames 2017 and 2018 for the second production year. Minnesota results are from one environment: Rosemount 2017 in the first production year and Rosemount 2018 for the second production year. ‡ Treatments (Trt), Harvest (H).

Table 11. Forage nutritive value analysis, crude protein (CP) and neutral detergent fiber digestibility (NDFD) for each harvest and treatment in the first production year †.

Treatment	**Iowa**		**Minnesota**		**North Dakota**	
	CP	**NDFD**	**CP**	**NDFD**	**CP**	**NDFD**
			CP and NDFD concentration (g kg^{-1})			
			First harvest			
Alfalfa alone (T2)	173	459	158	460	240	471
Alfalfa + corn (T3)	180	453	173	486	233	472
Alfalfa + corn + PHX ‡ (T4)	179	499	173	500	229	476
			Second harvest			
Alfalfa alone (T2)	198	396	199	345	238	458
Alfalfa + corn (T3)	209	404	199	353	254	460
Alfalfa + corn + PHX ‡ (T4)	207	402	200	358	250	461
Spring-seeded alfalfa ¶ (T5)	-	-	149	243	-	NS
			Third harvest			
Alfalfa alone (T2)	247	421	-	-	220	422
Alfalfa + corn (T3)	250	429	-	-	219	438
Alfalfa + corn + PHX ‡ (T4)	253	424	-	-	249	441
Spring-seeded alfalfa ¶ (T5)	198	380	-	-	243	407
			Fourth harvest			
Alfalfa alone (T2)	197	394	-	-	174	412
Alfalfa + corn (T3)	226	399	-	-	183	424
Alfalfa + corn + PHX ‡ (T4)	218	388	-	-	189	427
Spring-seeded alfalfa ¶ (T5)	254	455	-	-	189	374
LSD_1 (0.05) Trt x Harvest	*3*	NS	NS	NS	NS	NS
LSD_2 (0.05) Trt	NS	NS	NS	2	NS	NS

† North Dakota results are averaged across three environments. Prosper 2017 and 2018 and Forman 2017 were combined. In Iowa two environments were combined: Ames 2017 and 2018, and Minnesota results are from one environment, Rosemount 2018. ‡ PHX: prohexadione calcium, at 0.5 kg a.i. ha^{-1}. ¶ Spring-seeded alfalfa is in the seeding year while all other treatments are in the first production year. *LSD_1*, Least Significant Differences at a $p \leq 0.05$ for the comparison of treatment means for a same harvest within a same column. *LSD_2* compares treatment means across all harvests within a same column. *NS*, non-significant ($p > 0.05$).

4. Discussion

4.1. Corn Grain and Biomass Yield and Plant Height

Alfalfa intercropped into corn decreased both grain and biomass yield. The yield penalty, however, depended on rainfall in critical months for corn growth. Intercropped alfalfa may compete for water and reduce both grain and biomass yield. The rainfall amount observed in 2016 was 62% and 60% of normal, respectively, in June and August, in the North Dakota locations (Table 12). The rainfall deficit in June at both locations likely gave a head start to the intercropped alfalfa (T3 and T4). In June, based on PAR readings, alfalfa had enough light available (>80%) to compete aggressively for water with corn (Figure 1). In Iowa, a deficit in rainfall was observed in June in 2016 and in June through September in 2017. Even though above average rainfall was observed in May 2017, the experiment was not planted until May 16, so the rain was enough to allow both crops to emerge. However, there was not enough moisture available the remainder of the season. Maximum and minimum temperatures at each location were similar to the 30-year average temperatures (data not shown).

Table 12. Accumulated rainfall and deviation from normal rainfall in 2016 and 2017 from May to September at Fargo, Prosper, ND, Ames, IA, and Rosemount MN.

	2016		2017	
	Rainfall (mm)	**Dev. Normal † (mm)**	**Rainfall (mm)**	**Dev. Normal (mm)**
Month	Ames, IA			
May	109	−10	189	71
June	24	−103	48	−79
July	149	34	37	−77
August	209	87	93	−29
September	200	119	46	−35
Total	691	128	563	−150
	Forman, ND			
May	66	−3	43	−2
June	46	−57	20	−49
July	146	62	83	−19
August	51	−3	19	−65
September	23	−35	171	117
Total	332	−36	440	27
	Prosper, ND			
May	82	5	17	−61
June	38	−63	88	−12
July	88	0	50	−38
August	26	−40	53	−14
September	61	−5	152	86
Total	295	−103	359	−39
	Rosemount, MN			
May	-	-	182	70
June	-	-	91	−40
July	-	-	139	25
August	-	-	129	17
September	-	-	42	−41
Total	-	-	584	32

† Normal rainfall is from 30-year average at each location.

In previous studies without intercropping, they reported alfalfa maximum rooting depth averaged 177 cm with 50% of the root mass between 0–20 cm depth, while in corn maximum rooting depth was 118 cm with 50% of the root mass between 0–11 cm deep in the seeding year [29,30]. Alfalfa estimated water use in Minnesota is about 600 mm in a season with a water use efficiency of 0.69 kg m^{-3} [31]. Nevertheless, in our study, the biomass accumulated of alfalfa in intercropping at the end of the season ranged between 393 and 576 kg ha^{-1} which would explain only a small portion of the water used by alfalfa. In addition, it has been reported that alfalfa and corn in intercropping favor root development instead of above ground biomass accumulation [32].

Conversely to our results, Grabber [13] and Berti et al. [15] did not observe a significant reduction in silage corn biomass yield treatments where it was intercropped with alfalfa in normal rainfall conditions. It is possible that in average-rainfall situations, the environment within the canopy of silage corn does not favor alfalfa growth and thus decreases competition with corn early in the season. Corn silage is usually planted at a higher plant density than corn for grain, which might aid to outcompete the alfalfa under the canopy. There are no reports of alfalfa-corn intercropping under dry conditions.

Another factor to consider in alfalfa-corn intercropping is nitrogen fertilization. In our study we fertilized with 168, 100, and 120 kg N ha^{-1} for Iowa, North Dakota, and Minnesota locations, respectively. We could speculate that if the N rates were higher, the corn grain and biomass yield would have been greater and would not have been affected as much by alfalfa competition, especially in environments with above normal rainfall (Rosemount 2017). In below normal rainfall conditions, it could be argued that additional N would have not affected corn yield due to decreased N uptake.

The competitive ability and grain yield of corn in intercropping with alfalfa likely varies according to the nitrogen rate applied. Jellum et al., [33] calculated that 83 kg ha^{-1} more N was required for intercropped silage corn with alfalfa to reach the same critical biomass yield as the corn in monoculture. However, this study was conducted over 30 years ago when yield potential of corn hybrids was significantly less and no glyphosate tolerant crops were available. In addition, in this study corn was interseeded into established alfalfa not at the same time as in our study. Nevertheless, understanding the interaction of N rates with intercropping treatments would be key to optimize both corn and alfalfa yield.

4.2. Alfalfa Forage Yield

The alfalfa alone treatment (T2) had a much greater yield than the intercropped alfalfa (T3 and T4) in the seeding and first production year. This indicates that the competition for light and perhaps nutrients in the seeding year does reduce alfalfa yield potential in the first production year. Alternatively, it is likely that late harvest of corn as grain gave alfalfa little time to accumulate alfalfa biomass before winter, and this could have delayed or reduced spring growth of alfalfa the following year. Alfalfa biomass yield under the corn canopy at the end of the season were similar between PHX-treated and untreated alfalfa, indicating application of PHX did not reduce alfalfa biomass production in the seeding year. Prohexadione is a growth retardant. It reduces the internode length in alfalfa, so it was not expected to reduce biomass.

Mattera et al. [34] determined that radiation interception by alfalfa increases as plants grow and the canopy closes. However, alfalfa under reduced incident radiation grows much slower. In our study, based on PAR readings, alfalfa growing under a corn canopy received less than 20% of the incident radiation once the corn canopy closed, at about mid-July. Similarly, other researchers have reported corn canopy intercepts 80% to 90% of PAR [35,36]. In addition, alfalfa cultivars have different tolerance to shade [16].

Even though sole alfalfa (T2) had a higher forage yield in seeding and first production years, the intercropped system might have a more positive impact in the long term (second production year). In Iowa and North Dakota environments, there was an observed reduction of the total forage yield from the first to the second production year for sole alfalfa (T2), while it was similar for the intercropped systems (T3 and T4). In Minnesota, the forage yield increased from the first to the second production year in all treatments, but much more for the intercropped systems (T3 and T4). In this study, the third production year was not evaluated, but literature indicates that alfalfa forage yield potential decreases as the plant ages after the third or fourth year [37,38].

4.3. Alfalfa Stem and Plant Density

Plant density in North Dakota and Minnesota environments was lower than expected for a seeding year stand and spring of the year after planting [16,39], however it is not unusual to have extensive winter-kill and plant density reduction during the first winter [38]. Our plant count approach could have underestimated stand density relative to previous research where crowns were dug out. The differences detected in alfalfa plant density among treatments varied across environments and were insufficient to explain the alfalfa forage yield differences observed in the first production year.

Prohexadione-calcium (PHX) application did not improve alfalfa stand survival at any location. In the seeding year, establishment of alfalfa under the corn was poor in Rosemount 2017 for both treated and untreated alfalfa. This is in contrast to observations of Grabber [13] and Grabber et al. [16] who reported a significant increase of alfalfa stem density when PHX was applied to intercropped alfalfa in Wisconsin. However, as in this study, Grabber et al. [16] indicated that there was no significant response to PHX on alfalfa stem density in the studies conducted in Michigan and Pennsylvania. Grabber et al. [16] also reported differences in shade tolerance among alfalfa cultivars, but the cultivars used in this study were not included in the Grabber et al. [16] study.

Adequate plant density of intercropped alfalfa is desired to ensure long-term forage production. However, due to a later start of the season and the early-maturing corn grain hybrids chosen in our study in North Dakota and Minnesota, the corn likely closed the canopy later than the corn would in the studies conducted in Wisconsin [13,16] and may have allowed improved survival of alfalfa seedlings. Corn silage grows typically taller than corn for grain, which reduces available light within the canopy for alfalfa to grow [36].

4.4. Alfalfa Forage Nutritive Value

Crude protein of alfalfa was mostly influenced by harvest date in the first and second production years at all environments. Alfalfa crude protein concentration largely depends on the moisture and temperature conditions as it develops. Typically, alfalfa is much taller in the first harvest than subsequent harvests, since it develops in cooler conditions with plenty of soil moisture, so development to the reproductive stage is delayed. However, a taller plant generally has a greater stem-to-leaf ratio decreasing the fiber digestibility, a response observed in Minnesota in this study. In dry and warmer-than-average springs, the plant will switch to the reproductive stage faster and bloom when still very short (30–40 cm tall). The 2018 spring was dry and it warmed up very fast, which made the plants flower with less stem elongation. A shorter plant has a greater leaf to stem ratio, hence higher protein concentration [40–42]. This explains the strong interaction between harvest and environment observed in this study. Additionally, taller, denser canopies of alfalfa also have a lot more foliar disease pressure, which often results in loss of lower leaves prior to harvest [43].

The variation observed in neutral detergent fiber digestibility (NDFD) in this study was likely due to weather conditions at each environment in each harvest. A strong harvest by environment interaction was significant for North Dakota in the first production year and in all environments in the second production year. The spring-seeded alfalfa had much lower NDFD than the other alfalfa treatments in the first production year only in Minnesota, which is likely due to the fact that only one harvest was conducted that year and was let to fully bloom before harvest, while the other alfalfa treatments were all harvested twice at early bloom stage. We did evaluate lignin content (data not shown) in this study, but chose not to present it because lignin content had a significant negative correlation ($p \leq 0.0001$) with NDFD, thus NDFD represents lignin differences. Correlation coefficients between lignin and NDFD ranged between -0.456 and -0.965, depending on environment and production year.

Neutral detergent fiber digestibility indicates the ability of rumen microbes to convert plant fiber into smaller molecules and energy. Neutral detergent fiber (NDF) is composed primarily of the structural carbohydrates cellulose and hemicellulose, and a complex structural polyphenol, lignin [41]. As the plant matures, lignin deposition increases, reducing fiber digestibility (NDFD) [40]. Additionally, hot dry weather increases lignin deposition. Lignin is not digested by the microorganisms in the rumen and can also obstruct the conversion of cellulose and hemicellulose to sugar molecules [40,41]. Lignin also accumulates in warm and dry weather when the plant is forced to change to reproductive stage [40]. Thus, this supports that the NDFD values obtained in this study (409–476 g kg^{-1}), were lower than alfalfa NDFD values typically reported in the literature in alfalfa grown under normal rainfall and temperature conditions [44].

Vigorous plants likely have thicker and stronger stems, which would explain lower digestibility and higher lignin content. Likewise, Fonseca et al. [45] found a positive correlation between plant vigor and NDF concentration, with taller stems more fibrous than shorter ones.

The variation in both NDFD and crude protein might be explained by differences in spring-seeded alfalfa harvest dates, which were 14 July in Prosper and 1 August in Forman. Lower than normal rainfall in North Dakota locations delayed the first harvest of the spring-seeded alfalfa in 2017. Although it is generally believed shorter plants have better forage nutritive value, the stress probably affected the expression of the multifoliolate trait. The alfalfa cultivar in this study was RR Presteez, which has the multifoliolate trait. Research done in Italy concluded that alfalfa cultivars with the multifoliolate trait showed low expression of the trait under stress, reducing NDFD and crude protein. Alfalfa in drought stress environments had 12.3% lower plant NDF and 9.7% lower leaf protein [46]. Additionally, drought stress can have a direct negative effect on symbiotic N_2 fixation, which can reduce crude protein in alfalfa stems [47].

Although there was not many differences in alfalfa forage nutritive value, in a large-scale, this system may reduce the nutritive value of the alfalfa. Corn harvested for grain will leave very low-quality residue (stover) that likely will end up in the bales of the first cut of alfalfa in the following season. The system was originally designed for silage corn, which leaves almost no residue [13], but our intent with this research was to determine if this system can also work for corn harvested for grain. Managing or removing the corn residue will have a cost that would need to be considered in the system's profitability. In addition, the effect of corn stover on alfalfa seedlings survival will need to be researched.

In summary, establishing alfalfa in intercropping with corn for grain is feasible and has multiple benefits to soil health and the environment in comparison with corn monoculture, as demonstrated by several pieces of research [14,15,19,30,32,37,48]. It has been demonstrated that alfalfa-silage corn intercropping systems have a higher total net revenue than corn monocultures, despite the yield penalty to corn in the seeding year [14,15,30]. All these studies have been conducted in silage corn and not corn for grain.

5. Conclusions

Intercropping alfalfa with corn resulted in a decrease in corn grain and biomass yield in most environments. However, the yield penalty was compensated for by increased alfalfa forage yield in the first production year in comparison with spring-seeded alfalfa.

Variations in alfalfa plant density did not explain alfalfa forage yield differences among treatments. The growth regulator applied to improve stand survival of intercropped alfalfa did not have an influence on plant density or forage yield. Establishing alfalfa in intercropping did not influence forage nutritive value. The forage nutritive value was strongly dependent on the date of harvest, not the treatments.

According to the results, establishing alfalfa with corn is feasible and can be an alternative for farmers to diversify their cropping system in the upper Midwest region, even under soil moisture limited conditions. Research targeted to reintroduce perennial crops into the current dominant corn–soybean systems in the US Corn Belt is urgently needed to improve stability and resiliency of production systems.

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