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Intercropping Alfalfa into Silage Maize Can Be More Profitable Than Maize Silage Followed by Spring-Seeded Alfalfa

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Abstract: Intercropping of silage 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 silage maize–alfalfa intercropping, with a goal to establish alfalfa and increase alfalfa productivity in the first year of production. The experiment was conducted in Fargo and Prosper, ND, USA, in 2014–2017. The design was a randomized complete block with four replicates and a split-plot arrangement. The main plot had two maize row-spacing treatments (RS), 61 and 76 cm, respectively. Treatments in the subplot were: (1) maize monoculture, (2) maize intercropped with alfalfa, (3) maize intercropped with alfalfa + prohexadione-calcium (PHX), and (4) spring-seeded alfalfa in the following year (simulating a maize–spring-seeded alfalfa crop sequence). Both alfalfa and maize were seeded the same day in May of 2014 at both locations. Prohexadione-calcium, a growth regulator to reduce internode length and avoid etiolation of alfalfa seedlings, did not improve alfalfa plant survival. Averaged across locations, RS did not have an effect on silage maize yield and alfalfa forage yield. 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. Considering a two-year system, alfalfa intercropped with maize had higher net returns than a silage-maize followed by a spring-seeded alfalfa sequence. This system has the potential to get more growers to have alfalfa in the rotation skipping the typical low forage yield of alfalfa in the establishment year.

Keywords: silage maize; interseeding; net returns; row spacing; forage yield; forage nutritive value

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1. Introduction

The most common crop sequences in the Corn Belt region of the USA are continuous maize, and maize–soybean (*Glycine max* (L.) Merr.). In the last two to three decades, forage-based, more diverse crop rotations have transitioned to less diverse annual crop-based rotations [1]. The increase in farm sizes, decline in livestock numbers, and increase of commodity prices drove the decline on crop diversity [2,3]. The reduced crop diversity in the Corn Belt has resulted in negative environmental impacts, such as loss of soil organic matter, degradation of soil physical characteristics, and increased soil erosion [4,5]. Research has demonstrated that long-term diverse crop rotations produce higher yield in each crop in the rotation compared with maize monocrop or maize–soybean rotation, enhancing soil fertility, and reducing fertilizer applications to the next crop [2,4,6]. Adding perennial crops, such as alfalfa into an annual crop rotation, generally results in lower cost of production, reduced soil erosion, increases soil organic carbon, and improved soil health [6,7]. Alfalfa in rotation with other crops decreases the production cost of the subsequent crop, due to nitrogen credits and improved soil health [6,8]. Moreover, when the subsequent crop is maize, adding alfalfa to the crop rotation decreases nitrogen fertilizer costs [9,10].

Despite the many economic and environmental benefits that alfalfa provides, annual forage yield of alfalfa is much lower than that of silage maize, particularly in the establishment year [11]. This has resulted in the reduction of alfalfa production and subsequent increase of continuous silage maize production on dairy farms [11]. With the approval of glyphosate-tolerant alfalfa in 2011, interseeding alfalfa into maize to establish alfalfa has become a new potential cropping system for forage growers in the Midwest [12]. Both crops complement each other [13,14]. Alfalfa intercropped into maize provides a ground cover after silage maize harvest, providing forage in the subsequent production years. Intercropping alfalfa and silage maize provides a head start to alfalfa production establishment, doubling the forage yield compared with conventional spring-seeded alfalfa [11]. 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 [15].

Nevertheless, intercropped alfalfa is not always successful, because alfalfa competes with maize for resources, such as water and nutrients, while the reduced available light under the maize canopy etiolates alfalfa stems, weakening the plants. In warm and wet conditions favorable for maize, alfalfa stands decrease. As a result, plants might die during the winter reducing subsequent alfalfa stands [9,11]. Additionally, recent research indicates tolerance to shade under the maize canopy varies among alfalfa cultivars [11].

Grabber [11] evaluated growth regulators applied to interseeded alfalfa and determined that growth regulators improved alfalfa root growth in the year of seeding. The use of prohexadione-calcium (PHX) (calcium, 1-(4-carboxy-2, 6-dioxocyclohexylidene) propan-1-olate) reduced alfalfa internode length, resulting in reduced competition of alfalfa against the maize. Adding adjuvants and ammonium sulfate to PHX decreased alfalfa height by 16% and increased alfalfa stands by 30% compared with the control without PHX [16].

Although the potential of alfalfa–maize silage intercropping system has been studied and reported in Wisconsin in the USA [11,15–17], it is unknown whether this system is adapted to a broader range of environments and maize row spacing. The objectives of this research were: (1) to determine if establishment of alfalfa in intercropping with maize affects forage yield and alfalfa plant density, (2) to evaluate if maize row spacing and the application of PHX influences alfalfa establishment in intercropping and maize yield, and (3) to determine if the returns over two growing seasons are higher for alfalfa established through intercropping with silage maize or silage-maize followed by spring-seeded alfalfa the following year.

2. Materials and Methods

2.1. Experimental Sites

The experiment was conducted at two North Dakota State University (NDSU) research sites, in Fargo (46°52′ N, 96°48′ W, elevation 274 m) and Prosper (46°58′ N, 97°3′ W, elevation 280 m), ND, USA. The soil type in Fargo was mapped as Fargo–Ryan clay soil (Fargo: fine, smectitic, frigid Typic Epiaquerts; Ryan: fine, smectitic, frigid Typic Natraquerts) while the soil type at Prosper was mapped as Kindred–Bearden silty clay loam (Kindred: fine-silty, mixed, superactive Typic Endoaquoll; Bearden: fine-silty, mixed, superactive, frigid Aeric Calciaquoll) [18]. Monthly rainfall and minimum, maximum, and average temperature were obtained from nearby weather stations with the North Dakota Agricultural Weather Network [19]. Only 2014 and 2015 weather data were considered since in 2016 and 2017, only alfalfa yield was evaluated.

During the two-year experimental period, at both Prosper and Fargo, the growing season minimum and maximum temperatures were similar to the normal long-term temperature, with slightly warmer November 2014 through January 2015, likely enhancing alfalfa stand survival (data not shown). In 2014, after sufficient rainfall in early season, plants experienced drier conditions through summer until final harvest in October at both locations. In 2015, at both locations, the month of May was exceptionally wet, but towards the end of the season, the rainfall was below normal.

2.2. Experimental Design and Management

The experimental design was a randomized complete block with four replicates and a split-plot arrangement. The main plots had two row spacings (61 cm and 76 cm) of maize and the subplots were four treatments: (i) sole maize, (ii) alfalfa intercropped with maize, (iii) alfalfa intercropped into maize with one application of prohexadione-calcium (PHX), and (iv) spring-seeded alfalfa in 2015. Both crops were established in 2014. Sole maize treatment was done for a single year (2014) only. In 2015, the plots that had alfalfa intercropped with maize the previous year (with and without PHX) were evaluated for forage yield.

Previous crop at both locations was hard red spring wheat (*Triticum aestivum* L.). In 2014, conservation tillage, consisting of two passes of chisel plowing and one pass of disking, was used to prepare the seedbed for planting alfalfa. No-tillage was used before alfalfa was seeded in the spring of 2015. A glyphosate-tolerant alfalfa cultivar, Presteez RR (purity: 65.9%; germination: 73%; hard seed: 15%, fall dormancy rating 3, and winter survival rating (1) at a seeding rate of 15 kg pure live seed (PLS) ha^{-1} was used. The silage maize hybrid used was 2MD96 RR from Peterson Farms, Prosper, ND, USA (96 relative maturity (RM), with the Roundup Ready® trait). Maize was seeded with a two-row maize drill at 76 cm (Planter John Deere, 7100 MaxEmerge, Moline, IL, USA), and a different cone plot planter was used to plant maize at 61 cm (Wintersteiger, Plotseed XL, Salt Lake City, UT, USA). The targeted maize plant density was 87,932 plants ha^{-1} for both row spacings.

Alfalfa was seeded immediately after seeding the maize plots for treatments (2) and (3). The alfalfa was seeded with the same plot planter as for maize at 61 cm, but planting eight rows at the time, at 15-cm row spacing. Each experimental unit was 6-m in length and had either four rows of maize or four rows of maize and 16 rows of alfalfa seeded on the same seeding date (Tables 1 and 2).

Table 1. Seeding dates and prohexadione-calcium application dates for 2014 and 2015 at Fargo and Prosper, ND, USA.

Location	Maize Seeding	Alfalfa Seeding	Prohexadione-Calcium	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

Table 2. Harvest dates (HV) of alfalfa and maize at Fargo and Prosper, ND, USA, for 2014, 2015, and 2016.

Location/Year	Alfalfa						Maize
	HV1	HV2	HV3	HV4	HV1 Trt 4 †	HV2 Trt 4 †	HV1
Fargo 2014	8 October	-	-	-	-	-	26 September
Prosper 2014	8 October	-	-	-	-	-	26 September
Fargo 2015	19 June	14 July	11 August	1 October	5 August	1 October	-
Prosper 2015	19 June	10 July	5 August	1 October	5 August	1 October	-
Fargo 2016	2 June	28 June	1 August	25 August	-	-	-
Prosper 2016	2 June	28 June	1 August	25 August	-	-	-
Fargo 2017	31 May	29 June	1 August	4 October	-	-	-
Prosper 2017	31 May	29 June	1 August	4 October	-	-	-

† Harvest dates of spring-seeded alfalfa in 2015 (Trt 4).

Prohexadione-calcium (PHX), at 0.5 kg a.i. ha^{-1}, was applied to alfalfa foliage when growth attained 20 cm in height and maize was at V8 stage [20] to obtain increased alfalfa leaf/stem ratio, and improved survivability of alfalfa under the maize canopy. Application was made using a one-nozzle manual sprayer (Roundup® Model 190259, 1-Gallon Premium Sprayer, The Fountain Head Group, New York Mills, NY, USA). The product was applied over the alfalfa, but under the maize canopy.

In 2014, when maize was at V4 stage of growth, 120 kg N ha^{-1} as urea fertilizer (CH_4N_2O) were applied to all plots When maize was at V5 stage all plots were fertilized with gypsum (170 g kg^{-1} of SO_4) at a rate of 30 kg ha^{-1}. Thereafter, 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 each year following recommendations from Franzen and Berti [21]. Weeds were controlled with glyphosate (isopropylamine salt of N-(phosphonomethyl) glycine) at 0.84–0.91 kg a.e. ha^{-1} as required.

2.3. Sampling and Analysis

Soils were sampled before the crops were planted at all locations each year. Samples were analyzed for pH, organic matter, and available P and K at the 0- to 15-cm depth. Additionally, NO_3-N concentration was determined at the 0 to 15-cm and 15 to 60-cm depths. The NO_3-N concentration was determined by the transnitration of salicylic acid method [22]. The Olsen method and the ammonium acetate tests were used for available P and K determination, respectively [23]. Baseline soil test results are shown in Table 3. All soil sample analyses were conducted by the North Dakota State Soil testing lab.

Table 3. Soil chemical analysis baseline for the experimental sites at Fargo and Prosper, ND, USA in 2014 and 2015.

Location/Year	N-NO_3	P	K	OM	pH †
	kg ha^{-1}	mg kg^{-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.

Crops were harvested at the recommended plant height and growth stage to maximize both forage yield and quality (Table 2) [24]. The number of alfalfa plants and stems per plant were determined in a 1-m^2 before each harvest. Alfalfa plant height was measured to the nearest 1-cm from at least three stems on different plants in each plot prior to every harvest. In the seeding year, alfalfa biomass yield was calculated from a 1-m^2 area subplot plot before each harvest, in alfalfa–maize plots. Thereafter (2015–2017), alfalfa plots were harvested using a plot forage harvester (Carter MFG CO., Inc., Brookston, IN, USA), taking the six-center rows from each plot. Harvested biomass was weighed in the field, and a sample of fresh forage of about 2 kg was taken. Samples were air dried at 55 °C in a forced-air oven until dry. Samples were then weighed to calculate percent moisture at harvest and determine dry matter forage yield.

Maize plant height was taken measuring five random plants from the center two-rows. Maize was harvested by hand, in two 4.6-m long rows (total area harvested was 2.8 m^2 in 61 cm-row spacing, and 3.5 m^2 at 76-cm row spacing) leaving a 5-cm stubble height, to calculate the biomass yield at 65% moisture. Plants were weighed in the field (fresh weight), and then, a sample of two complete plants was dried to calculate water content. Once maize biomass was harvested, all maize plants were cut off and removed from the field with a maize silage chopper (New Holland FP 240, Racine, WI, USA) in Fargo, and by hand in Prosper.

Dried samples of alfalfa and maize were ground in Wiley Mill to pass through a 1-mm sieve. Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and neutral detergent fiber digestibility (NDFD) were determined with a XDS near-infrared reflectance (NIR) rapid content analyzer (Foss, Denmark), following the methods described by Abrams et al. [25]. Selected samples were sent to the Animal Sciences Laboratory at North Dakota State University to correct the calibration curve.

2.4. Statistical Analysis

Analysis of variance and mean comparisons were conducted using the MIXED procedure of SAS 9.4 [26]. Location was considered a random effect and years fixed in the statistical analysis. The row spacing and the treatments were considered fixed effects. Each harvest was analyzed individually. Mean separations were performed with f-protected least significant difference (LSD) comparisons at the $p \leq 0.05$ probability level. Error mean squares were compared for homogeneity among environments according to the folded f-test and if homogeneous; then, a combined analysis was performed across environments. Data are shown by location if the interaction between treatment and location was significant. Otherwise, data were averaged across locations.

2.5. 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. We used yield data from the experimental study for the three sequences in the economic analysis. However, since maize was not planted in Year 2, the simulation assumes maize silage yield in Year 1 and Year 2 as the same. Many farmers grow maize in monoculture without a reduction in silage yield.

Constructed budgets were developed using input costs, and financial information from Haugen [27] and Swenson and Haugen [28]. The budget used was developed for dry land, eastern North Dakota. All budgets consisted of two consecutive years. Inputs from "cradle" (crop planting) to farm gate (harvesting) are included for this analysis, thus costs of production included input expenses for land preparation, seeding, fertilizer, and pest management, and harvesting (Table 4).

Table 4. 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
Harvest:			
Silage harvester	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 [29].

Maize seed cost 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 cost of alfalfa seed was $12.75 kg^{-1}, and included the cost of inoculation and seed treatment [30]. Land preparation, sowing, spraying, and harvesting equipment most commonly used in the region were used in the analysis (Table 5). Machinery costs included labor, repairs, fuel and oil, depreciation, and machinery overhead and were based on values of dollars per hectare obtained from Lazarus [29] and Haugen [27].

Table 5. Alfalfa seasonal forage yield at two locations from 2014–2017 averaged across two row spacings (61 and 76 cm) in Fargo and Prosper, ND, USA.

Treatment	Fargo				Prosper			
	2014	2015	2016	2017	2014	2015	2016	2017
	Mg DM 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, %	14.90	7.09	10.91	6.73	34.5	7.37	10.32	6.64

Prohexadione-calcium (PHX), rate of 0.5 kg a.i. ha^{-1}; least significant difference (LSD); coefficient of variation (CV). Not significant (NS).

Herbicide cost, in the maize and alfalfa-seeding year were fixed at $48.11 and $44.18 ha^{-1}, respectively, according to Aakre [31]. In the intercropping system (alfalfa + maize), the herbicide was applied twice during the growing season over both crops at the same time. 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 glyphosate tolerance. The seed cost included insecticide seed treatment for corn wireworm (*Melanotus communis* Gyllenhal), Western corn rootworm, white grub (*Holotrichia serrata*), and cutworm (Order: Lepidoptera) [28].

Harvesting equipment included a forage silage harvester for maize, and a 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 [28], 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, followed by a sensitivity analysis to assess the validity of the findings under different assumptions and prices. 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. This maize yield was the average biomass yield obtained in the study at 76-cm row spacing, which is the most common row spacing used in the Corn Belt region. The dry matter yield of 13.8 Mg ha^{-1} was converted to silage yield at 65% moisture, which is equal to 39.4 Mg ha^{-1} for all treatments. Maize silage yields in treatments with, and without intercropped alfalfa were not different. Thus, the same maize silage yield value was used for all treatments that had maize. Silage yield losses between treatments were not observed in this study. However, similar research has shown that intercropping maize with alfalfa usually has a yield penalty of up to 30% [17]. Thus, we included a sensitivity analysis of net returns with a price of $41.1 Mg^{-1} silage maize at 65% moisture, and $166 Mg^{-1} for 0 to 30% silage yield losses due to intercropping.

Silage value was calculated according to LaPorte [32], assuming a medium maize grain yield of 8.4 Mg ha^{-1} and a maize grain price of $177 Mg^{-1} ($4.5 bushel^{-1} of maize grain). A conversion factor was calculated to transform maize grain price ($4.5 bushel^{-1}) to silage maize value at 65% moisture [32], resulting in a value of $41.1 Mg^{-1} of maize silage at 65% moisture. For alfalfa, the average yield obtained in the experiment at 76-cm row spacing were used for the economic analysis; forage dry matter yield for 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.

3. Results and Discussion

3.1. Alfalfa Forage Yield and Plant Density

Results for alfalfa forage yield differed by locations and are discussed separately. Row spacing did not influence alfalfa forage yield, thus forage yield is presented averaged across row spacings. In 2014, alfalfa biomass yield was similar in alfalfa with or without prohexadione-calcium (PHX) application (Table 5), indicating that PHX did not improve alfalfa biomass yield. In contrast, Grabber [11] tested several rates of PHX indicating rates between 0.6 and 1.2 kg a.i. ha^{-1} increased alfalfa stand survival, and biomass yield in October compared with both the check and the 2.4 kg a.i. ha^{-1} rate. Maize plants in North Dakota grow shorter (varies with hybrids and season) than in Wisconsin, letting light get through the canopy. This might explain why a response to PHX was not observed in this study.

The alfalfa seasonal forage yield that was intercropped in 2014 was about twice that of the spring-seeded alfalfa in 2015 (Figure 1). Grabber [11] reported 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 only about 5.0 Mg ha^{-1}. Establishing alfalfa during the maize year overcomes the low forage yield in the seeding year, likely providing an economic advantage. Alfalfa seasonal biomass yield in the second and third production years (2016–2017)'s yield was not significantly different among treatments (Table 5).

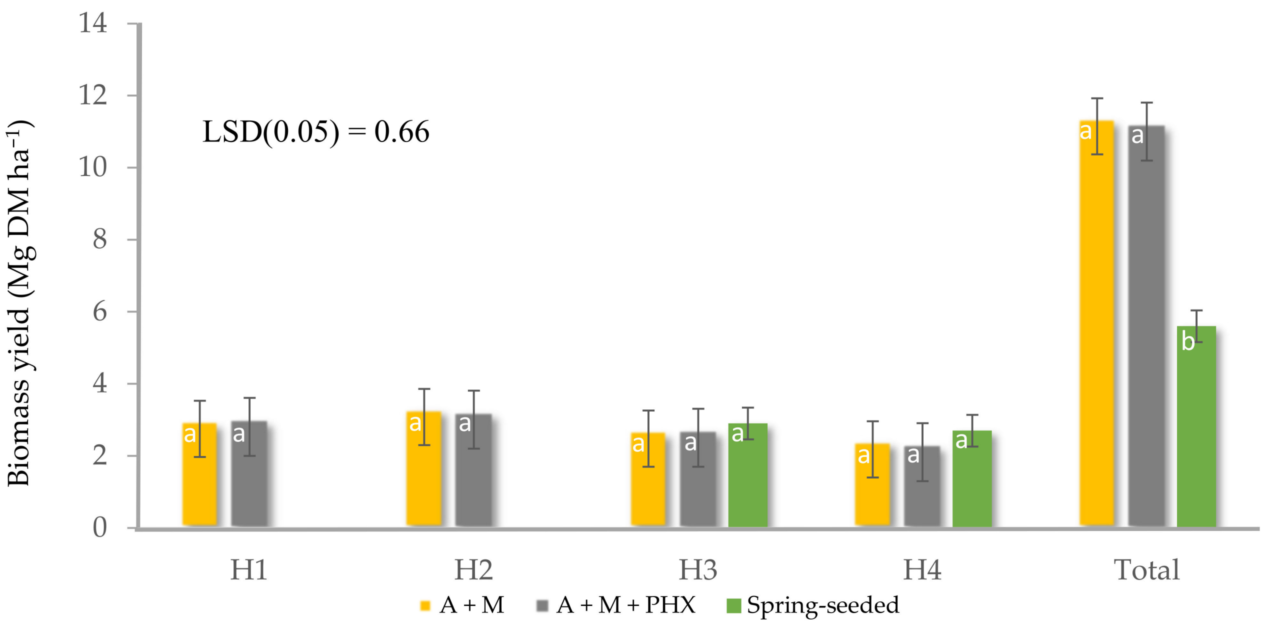

Figure 1. Alfalfa biomass yield (dry matter) of four harvests (H) in 2015; for spring-seeded alfalfa (A spring), maize and alfalfa (M + A) intercropping without prohexadione-calcium (PHX) application and with PHX application (M + A + PHX) averaged across locations, Fargo and Prosper, ND, USA in 2015. Least significant differences (LSD) value (lowercase letters) is to compare between harvests and treatments ($p \leq 0.05$).

Only the interaction between treatment and row spacing was significant; thus, the results are presented averaged across environments. In 2014, the alfalfa plant density was lower at the 61-cm row spacing for the PHX-treated alfalfa compared with the non-treated alfalfa, but similar in both treatments at 76-cm row spacing (Table 6). Intercropped alfalfa stands had a least 113 plants m^{-2} in the fall of 2014, which is within the range of 80–130 plants m^{-2} considered as adequate stand for the seeding year [11,33,34]. Grabber [11] reported just the opposite as the PHX treatment increased stand survival compared with the non-treated check. This might have been due to maize hybrids in our experiment, that were earlier maturing and shorter than in Wisconsin, allowing more light within the canopy.

Table 6. Alfalfa plant density in the fall of 2014, spring of 2015, and fall of 2015 in two maize row spacings averaged across two locations, Fargo and Prosper, ND, USA.

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

Least significant differences (LSD_1) to compare between means for the same treatment with different row spacing. LSD_2 to compare between means for the same row spacing with different treatments, PHX: prohexadione-calcium, rate of 0.5 kg a.i. ha^{-1}.

In the spring of 2015, alfalfa plant density was similar in plots that had different maize row spacings in 2014. Between the fall 2014 and the spring 2015, plant density decreased across all treatments and row spacings (Table 6). However, a reduction of 50 to 60% of plant density in the first winter of alfalfa is common in North Dakota, regardless of harvest management or winter temperatures [35]. Although Grabber [11] reported that the initial stand establishment was three times greater than in this study, the reduction in stand from July to October of the same season was of about 40–50% for both the treated with PHX and control treatments. Alfalfa self-thinning of stands by intraspecific competition in the seeding year has been previously reported [36]. Alfalfa plant stands declined between the spring and fall of 2015. Alfalfa seeded in 2015 had similar plant density in the fall (seeding year) to that of the alfalfa seeded in 2014 (seeding year) at both row spacings. Spring-seeded alfalfa in 2015 had about three-fold greater plant density than alfalfa established in 2014 in Fargo and twice the plant density at Prosper (Table 6). Plant density was not taken in 2016 and 2017, since there were no differences in forage yield. It is unlikely that alfalfa plant density was different among treatments.

3.2. Maize Biomass (Silage) Yield

There was a significant interaction between treatment, row spacing, and location on total maize biomass yield ($p \leq 0.05$) (Data not shown). In Prosper, monoculture maize produced higher maize biomass yield than maize from alfalfa-intercropping systems at 61-cm row spacing (Table 7). This was not observed at 76-cm row spacing averaged across both locations, 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 intercropped with 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 [11] reported that 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 7. Maize biomass yield and plant height for two row spacings (61 and 76 cm) averaged across locations, Fargo and Prosper, ND, USA in 2014.

Treatment	Fargo		Prosper		Average	
	Row Spacing (cm)					
	61	76	61	76	61	76
	Mg DM ha^{-1}				Plant Height (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	

Prohexadione-calcium (PHX), rate of 0.5 kg a.i. ha^{-1}; least significant differences (LSD).

3.3. Alfalfa Forage Nutritive Value

Row spacing, treatment, and treatment by row spacing were not significant for most nutritive components analyzed (NDF, ADF, ADL, and NDFD) (results not shown), except for crude protein and ash content. Crude protein was significant for the treatment effect only in the third harvest, and ash content was significant for treatment in the first and third harvest.

Crude protein concentration was lower in the spring-seeded alfalfa, compared with alfalfa that was established in intercropping in 2014 in the third harvest (Table 8). Crude protein of first, second, and fourth harvest were not different among treatments or row spacings, thus results are not presented. The third harvest date for the intercropped alfalfa planted in 2014 was harvested at about the same time as the first harvest for the spring-seeded alfalfa. The first harvest (third for 2014 alfalfa) in 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 [37]. This probably was the reason for the differences in crude protein concentration.

Table 8. Crude protein concentration interaction between treatments and location for the third harvest of the 2015 season for alfalfa that was intercropped with maize in 2014 averaged across two row spacings (61 and 76 cm), at Fargo and Prosper, ND, USA.

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, PHX: prohexadione-calcium; least significant differences (LSD).

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. The non-treated alfalfa had higher ash content (101 g kg^{-1}) than PHX-treated alfalfa (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 9). 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. [38,39].

Table 9. Ash content of alfalfa in four harvests averaged across two locations, Fargo and Prosper, ND, USA in 2015.

Treatment	Harvest 1	Harvest 2		Harvest 3	Harvest 4	
	Mean	61	76	Mean	61	76
			g kg^{-1}			
Spring-seeded alfalfa	-	-	-	80.8	87.9	92.8
Alfalfa + maize	101.0	90.2	90.5	93.1	90.4	91.2
Alfalfa + maize + PHX	95.3	94.6	86.5	92.5	90.3	94.3
LSD (0.05)	5.5	NS		4.5	NS	

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, PHX: prohexadione-calcium; least significant differences (LSD). Not significant (NS).

The first harvest of the 2015 spring-seeded alfalfa was about the same time of year as the third harvest of the alfalfa established in 2014. At the time of harvest, spring-seeded alfalfa plots may have had a higher plant density, thus a better ground coverage compared with that of alfalfa plots seeded in 2014. During harvest using the forage harvester, 2014-seeded alfalfa samples may have had a higher soil contamination resulting in a higher ash content. This would explain a lower ash content for spring-seeded alfalfa established in 2015.

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 10). 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 10. Ash content of alfalfa for the second and fourth harvest for the interaction between two row spacings and two locations, Fargo and Prosper, ND, USA in 2015 and averaged across treatments.

Row Spacing	Harvest 2		Harvest 4	
	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	

3.4. Economic Analysis

Silage maize intercropped with alfalfa had a higher net return than mono-cropped silage maize (Table 11). Although we did not have field data for the mono-cropped maize (two-year maize sequence), we assumed that both years would have the same silage yield and costs. 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 lower net return, compared with alfalfa intercropped with silage maize in in the first year of the sequence, even though the latter had a higher production cost. This was mainly due to the lower forage yield from the spring-seeded alfalfa system.

Table 11. 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	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	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
Production cost (2-year)		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 2-year system		3241.0		3313.7		2533.5
Net return 2-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.

The sensitivity analysis was conducted by varying the maize and alfalfa prices (Table 12). The maize–alfalfa intercropping two-year system was always more profitable than the usual practice of silage maize followed by spring-seeded alfalfa. It is only at a price of maize silage greater than $50.3 Mg^{-1} and an alfalfa price greater than $166 Mg^{-1} that the two-year maize silage sequence was more profitable than the maize + alfalfa intercropped–alfalfa sequence.

Table 12. Sensitivity analysis for total net return after two years for three 2-year sequences: maize followed by maize, alfalfa intercropped with maize, and maize followed by spring-seeded alfalfa.

Alfalfa Price ($ 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	24.4	379.2	742.0	1104.7
143	−338.6	24.4	379.2	742.0	1104.7
166	−338.6	24.4	379.2	742.0	1104.7
181	−338.6	24.4	379.2	742.0	1104.7
			Maize + alfalfa–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	741.5	922.3	1103.7
181	535.3	716.7	894.1	1075.5	1256.9
			Maize–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

Osterholz et al. [17] compared several rotations of maize 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 were the biennial sequences estimated in our study. Osterholz et al. [17] 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 corresponds to the average biomass yield across locations obtained in the experiment. However, the study in Wisconsin was done at lower maize grain prices than in our study, which was calculated with a grain price of $177 Mg^{-1}. Thus, the net return of silage maize rotations were similar to those calculated for a two-year sequence of silage maize ($379 Mg^{-1}) The alfalfa forage yield used by Osterholz et al. [17] in the economic analysis were very similar to those used in our analysis. Osterholz et al. [17] used alfalfa forage yields of 11.4 Mg ha^{-1} and 5.8 Mg ha^{-1} for alfalfa coming from intercropping with maize in the previous year and spring-seeded alfalfa, respectively.

Even though, in our study, maize biomass yield loss, due to competition with alfalfa in intercropping, were not significant ($p \leq 0.05$), other researchers have reported silage yield penalties of up to 30% [17], and thus a sensitivity analysis simulating a yield penalty to maize silage was conducted. At an alfalfa price of $166 Mg^{-1} and a maize silage price lower than $36.6 Mg^{-1}, the maize–alfalfa intercropping system had greater net returns than the two-year maize sequence (maize monoculture) even if the yield penalty was 30% (Table 13). At a price of $41.1 Mg^{-1} and 45.7 Mg^{-1}, the maize–alfalfa intercropping system had greater returns than the two-year maize silage sequence, but only if the maize biomass yield penalty was less than 25% and 10%, respectively. At greater maize prices than $45.7 Mg^{-1}, net returns were greater for the two-year maize silage system. The business-as-usual system with maize followed by spring-seeded alfalfa had lower net returns than alfalfa established in intercropping, even with a silage-maize yield penalty of 30%, regardless of maize price.

Table 13. Sensitivity analysis for total net return after two years, for silage maize intercropped with alfalfa system assuming silage-maize yield losses from 0–30% due to intercropping.

	Price of Silage Maize (\$ Mg^{-1})				
Silage Maize Yield Penalty	**32.0**	**36.6**	**41.1**	**45.7**	**50.3**
	Maize + alfalfa–alfalfa (\$ ha^{-1})				
No loss	383	564	742	923	1105
10%	257	420	580	743	906
15%	194	348	499	653	807
20%	131	276	418	563	708
25%	68	204	337	473	609
30%	4	131	256	383	510

Intercropping systems offer several ecosystem services that could be valued or at least taken into consideration as a path towards the sustainable production of alfalfa and maize-based feed production. Gaba et al. [40] demonstrated that intensive cropping systems have led to a decline in biodiversity. This caused damage to an important number of ecosystem services such as nutrient cycling, regulation of climate and water quality, and soil erosion, just to mention a few. Syswerda and Robertson [41] demonstrated that maize grain yield were positively correlated with nitrate leaching and negatively correlated with plant diversity and Belel et al. [42] reported soil fertility improvements if intercropping was used. 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 [43].

4. Conclusions

Alfalfa established in intercropping with maize had almost double the forage yield in the following year versus 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. Silage maize biomass yield was the lowest at the narrowest row spacing of 61 cm at only one location. Biomass yield was similar at the 76-cm row spacing regardless of location. Alfalfa intercropped with maize had greater net returns than a silage-maize followed by a spring-seeded alfalfa the following year, which is the typical crop sequence in the region for growers who grow silage maize and alfalfa. Even if maize silage yield is reduced due to intercropping, establishing alfalfa with maize had greater returns than maize followed by spring-seeded alfalfa. The results of this study indicate that intercropping alfalfa with maize, in order to establish alfalfa a year ahead, is promising and might get more growers to consider including alfalfa in rotation with silage maize. However, more research is needed to evaluate this system in a large scale.

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