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RESEARCH ARTICLE

Interseeding alfalfa into corn silage increases corn N fertilizer demand and increases system yield

William R. Osterholz[1] · **Matthew D. Ruark**[2] · **Mark J. Renz**[2] · **John H. Grabber**[3]

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Abstract

Interseeding alfalfa (*Medicago sativa* L.) into corn (*Zea mays* L.) harvested as silage can increase rotation productivity and reduce negative environmental impacts, but the importance of N fertilizer management for successful implementation of the interseeding system remains unexplored. Nitrogen fertilizer enhanced leaf chlorophyll content, corn silage yield, and total N content of corn with interseeded alfalfa and conventional solo-seeded corn at two locations in southern Wisconsin. However, greater N rates (additional 83 kg N ha^{-1} at one location; could not be estimated at second location) were needed to maximize corn silage yield when alfalfa was interseeded, suggesting that alfalfa effectively competed with corn for N. Maximum corn silage yields were depressed by 7–16% when alfalfa was interseeded, but interseeded alfalfa yields in the subsequent year were 40–160% greater than spring-seeded alfalfa, resulting in greater total forage yield over the 2-year study period. Alfalfa plant density after corn silage harvest was greatest at low N rates, but all N rates resulted in acceptable stands with good yields in the second year of the studies. This is the first demonstration that application of additional N fertilizer can ensure high interseeded corn silage yields without causing major issues with alfalfa establishment. Additionally, split N application where half of the N rate was broadcast at planting and the balance banded along the corn row as a side-dressing did not influence most corn or alfalfa N responses compared to a single broadcast application at planting. Results of this work and previous studies suggest near maximal yields of corn silage can be obtained in this interseeding system if N is applied at 224 kg ha^{-1}, but further refinement of fertilizer and other crop management practices is, however, needed to maximize the forage production and environmental stewardship potential of this system.

Keywords Interseeding · Corn · Alfalfa · Nitrogen fertilizer management · Crop nitrogen uptake

1 Introduction

Intercropping of legumes and cereal crops can potentially increase the efficiency of resource exploitation and overall plant production (Duchene et al. 2017). Dairy forage production could utilize intercropping through interseeding of alfalfa into corn silage, where alfalfa and corn are concurrently planted in the same field in the same growing season. In this system, corn is harvested for silage while alfalfa serves as a cover crop and then is utilized for forage production in subsequent growing seasons (Fig. 1; Grabber 2016). Previous work indicates interseeding corn with alfalfa could provide improvements to the typical corn silage-alfalfa crop rotations that produce a large portion of forage grown in temperate regions by increasing alfalfa production and rotation profitability compared to conventional corn-alfalfa rotations (Grabber 2016; Osterholz et al. 2018a; Osterholz et al. 2020). In addition, interseeded alfalfa can provide significant ground cover during corn growth and following corn silage harvest, mitigating soil erosion and nutrient losses associated with corn silage production (Osterholz et al. 2019). Therefore, the interseeded corn-alfalfa cropping system warrants further development.

One important factor for optimization of crop yields and mitigation of environmental impacts in this interseeding system is N fertilizer management. Asynchrony between N supply and crop N demand can reduce crop yields and also increase the risk of N losses (Hong et al. 2007; Eagle et al. 2017). Studies conducted over the past several decades have

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✉ William R. Osterholz
will.osterholz@usda.gov

[1] USDA-ARS Soil Drainage Research Unit, Columbus, OH 43210, USA

[2] University of Wisconsin-Madison, Madison, WI 53706, USA

[3] USDA-ARS Dairy Forage Research Center, Madison, WI 53706, USA

Fig. 1 Interseeding alfalfa with corn at time of planting (left) allows establishment of alfalfa during corn production. The interseeded alfalfa continues to grow following corn silage harvest (right), thus jumpstarting alfalfa production the following year while providing soil and water conservation benefits.

shown legumes intercropped with corn can act as competitors for N and reduce corn productivity, particularly in environments with limited N availability (Kurtz et al. 1952; Smeltekop et al. 2002; Sawyer et al. 2010). Evidence from interseeded corn-legume systems suggests that increased rates of N fertilizer could reduce N competition between the crops and thus help to mitigate the corn yield penalty associated with interseeding (Jellum and Kuo 1997). An initial study in Wisconsin showed that interseeded alfalfa reduced dry matter yields of silage corn by an average of 15% at a fertilization rate of 180 kg N ha^{-1}, but in subsequent studies where fertilization was increased to 224 kg N ha^{-1} the corn yield reductions associated with interseeding averaged 5% (Grabber 2016, Osterholz et al. 2018a, Grabber et al. unpublished data).

In addition to the rate of N fertilizer applied, timing and placement of fertilizer can influence crop N uptake dynamics, competition, and risk of environmental losses (Kovács et al. 1995, Crews and Peoples 2005, Nkebiwe et al. 2016). Split application of N fertilizer may better synchronize N supply with corn N demand and thereby reduce risk of loss (Crews and Peoples 2005). In addition, placement of a side-dressed fertilizer application as a band along the corn row could concentrate the available N near corn roots and thus favor uptake by corn rather than the interseeded alfalfa.

In addition to the potential effects on corn productivity, N management is likely to influence interseeded alfalfa establishment, which is an important component of success of the interseeded system (Osterholz et al. 2020). Previous research has shown alfalfa establishment in the interseeded system can vary substantially with management factors such as corn seeding rate, presumably due to competitive interactions with the corn crop (Osterholz et al. 2018a). Although N fertilization would not be expected to directly impact plant density of alfalfa during establishment (Hannaway and Shuler 1993), it could indirectly influence seedling survival of interseeded alfalfa due to effects on corn productivity and competitiveness. For example, if higher N fertilizer rates enhance corn productivity, stress on alfalfa seedlings could increase thereby reducing alfalfa establishment success. As N fertilizer management potentially influences both corn yield and alfalfa establishment, improved understanding of these effects will be vital to successful implementation of this interseeding system.

The overall goals of this study were (1) to explore the effects of N fertilizer rate and application approach on corn silage production and alfalfa establishment and production in the interseeded corn-alfalfa system and (2) to compare the performance of the interseeded cropping system to a conventional solo-seeded corn-alfalfa rotation. Based on previous studies, we expected to observe N competition between corn and interseeded alfalfa and tested several specific hypotheses:

1.) Greater N rates will be required to maximize corn yield in the interseeded system.
2.) Yield of corn interseeded with alfalfa will be reduced compared to solo-seeded corn at low N fertilizer rates.
3.) A N fertilizer application approach designed to favor corn N uptake will enhance the ability of corn to compete with alfalfa for available N, particularly at lower N rates.
4.) Increased N rates will reduce interseeded alfalfa establishment and subsequent yield.

2 Methods

2.1 Experiment description

A nitrogen fertilizer rate experiment was conducted during the 2017–2018 growing season at two locations: the Arlington Agricultural Research Station (AARS; 43° 18′ N, 89° 20′ W) on a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls) and the USDA-ARS Dairy Forage Research farm near Prairie du Sac (PDS, 43° 21′ N, 89° 24′ W) on a Richwood silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). Prior crops at AARS were corn silage fertilized with 171 kg N ha^{-1} in 2016 and soybean (*Glycine max* (L.) Merr.) in 2015. Prior crops at PDS were second-year corn silage fertilized with 112 kg N ha^{-1} in 2016 and first-year corn silage after alfalfa that was grown without fertilizer N in 2015. No manure was applied at either site in the preceding 2 years before the study.

The experimental design was a three-factor randomized complete block design, replicated in three blocks at each location. The factors were cropping system (corn with interseeded alfalfa vs. solo-seeded corn followed the next year with spring-seeded alfalfa), N fertilizer rate (0, 56, 112, 168, 224, and 280 kg N ha^{-1}) applied to corn, and N fertilizer application approach (100% broadcast applied prior to planting (PP) vs. 50% broadcast at planting and 50% banded along the row as a side-dress (BSD)). Individual plots were 3 m (4 corn rows) wide by 6 m long, with all measurements taken from the middle two corn rows. A border area 6 m wide surrounding the experimental area on all sides was planted to corn to minimize the penetration of light from neighboring grassed alleyways.

2.2 Management details

Crop varieties were a dual-purpose corn hybrid (A6267, 102 d maturity, Agrigold, St. Francisville IL) and a leafhopper-resistant alfalfa variety (55H94, Pioneer, Johnston IA) previously identified in an alfalfa variety trial as performing well in the interseeding system (Grabber unpublished data). Corn was no-till planted on 76 cm rows at a population of 79,000 plants ha^{-1} with rows oriented north-south. Interseeded alfalfa was no-till planted at a rate of 18 kg (pure live seed) ha^{-1} in 16.5 cm rows in the corn inter-row area, but with every 5th row skipped to avoid planting directly over the corn row. Solo-seeded corn and corn interseeded with alfalfa were planted concurrently on May 15, 2017, at AARS and May 5, 2017, at PDS. Spring-seeded alfalfa was no-till planted in the 0, 112, and 224 kg N ha^{-1} treatments only, as previous year N rate was not expected to have a large effect on spring-seeded alfalfa yield. Spring-seeded alfalfa was the same variety (Pioneer 55H94) used for interseeding and was planted at the same rate, with equivalent row spacing except without the row skips used in the interseeded system. Spring-seeded alfalfa was planted on April 26, 2018, and April 24, 2018, at AARS and PDS, respectively.

Weed control during 2017 was achieved using broadcast pre-emergence applications of 1 kg a.i. ha^{-1} glyphosate (N-(phosphonomethyl)glycine) (Roundup PowerMax, Bayer, Leverkusen Germany) and 1.26 kg a.i. ha^{-1} encapsulated acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide) (Warrant, Bayer, Leverkusen Germany). A post-emergence application of 0.28 kg a.i. ha^{-1} bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) (Buctril, Bayer, Leverkusen Germany) was applied when interseeded alfalfa reached ~20 cm in height to further reduce weed populations, and additional hand weeding ensured limited weed pressure. In 2018 at PDS, spring-seeded alfalfa was treated with 1.7 kg a.i. ha^{-1} 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid, dimethylamine salt) (Butyrac, Albaugh, Ankeny IA) in mid-June followed by 0.28 kg a.i. ha^{-1} clethodim ((E)-2-(1-((3-chloro-2-propenyl)oxy)imino)propyl)-5-(2-(ethylthio)propyl)-3-hydroxy-2-cyclohexen-1-one) (Select 2EC, Winfield Solutions, St. Paul MN) in mid-July. No weed control was employed in 2010 for spring-seeded alfalfa at AARS.

An application of 0.45 kg a.i. ha^{-1} prohexadione calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate) (Kudos, Fine Americas Inc., Walnut Creek CA) mixed with crop oil concentrate (12.5 mL L^{-1}), citric acid (5 g L^{-1}), and ammonium sulfate (10 g L^{-1}) in a carrier volume of 190 L ha^{-1} was applied to interseeded alfalfa using drop nozzles when it reached ~30 cm in height. Previous research has shown that similar applications of this plant growth regulator with the same mix of adjuvants can significantly improve interseeded alfalfa establishment (Grabber 2016; Osterholz et al. 2018a, 2018b).

The six rates of N were hand applied as urea which was coated with N-(n-Butyl) thiophosphoric triamide (Agrotain, Koch Agronomic Services, Wichita KS) to minimize the risk of NH_3 volatilization. The PP application was made at corn planting. The BSD application was applied at planting and when corn reached V5 growth stage on June 16 and June 9 at AARS and PDS, respectively. No-till production systems were implemented at both sites; therefore, nitrogen was not incorporated following application.

Broadcast fertilizer applications of phosphorus (71 and 56 kg P ha^{-1} at AARS and PDS, respectively) as well as potassium, sulfur, and boron (251 kg K, 28 kg S, and 2.2 kg B ha^{-1} at both locations) were made during the fall of 2015 preceding the initiation of the experiment. An additional broadcast fertilizer application was made during the fall of 2017 prior to alfalfa production in 2018; rates applied were 39 and 17 kg P ha^{-1} at AARS and PDS, respectively, 372 and 167 kg K ha^{-1} at AARS and PDS, respectively, and 28 kg S and 2.2 kg B ha^{-1} at both locations. Fertilizer rates were determined by conducting soil tests and consulting university recommendations for corn silage and alfalfa production (Laboski et al. 2012).

2.3 Measurements

A SPAD-502DL meter (Konica Minolta, Osaka Japan) was utilized to measure corn leaf chlorophyll levels at two corn growth stages: V10 (10 collared leaves) and R2 (kernel blister) corresponding to July 13 and 7 and August 23 and 22 at AARS and PDS, respectively. Chlorophyll meters have been widely used to indicate the N status of corn (Blackmer and Schepers 1995; Hawkins et al. 2007). In each plot, 10 corn plants growing in the middle two rows were randomly selected, and readings were taken from the middle third of the uppermost collared leaf at V10 and the ear leaf at R2, visually affirming that each measured leaf appeared healthy. The 10 measurements were averaged to a single value for each plot. Relative chlorophyll meter (RCM) values were then calculated with the max value set as the average of the 280 kg N ha^{-1} solo-seeded corn plots (Hawkins et al. 2007).

Corn silage was harvested on September 12 and September 6, 2017, at AARS and PDS, respectively. A plot harvester with a built-in weighing scale was used to chop and weigh the middle two corn rows in each plot. A well-homogenized subsample of chopped material was taken from the plot combine for silage N concentration analysis. The subsample was dried at 60 °C for at least 2 weeks and weighed to estimate silage dry matter content, ground to pass a 2-mm sieve, and analyzed in duplicate for N concentration on a Leco Tru-Mac elemental analyzer (Leco Corp., St. Joseph MI). Corn N uptake was calculated by multiplying N concentration by dry matter yield. Corn silage yields are reported on a dry matter (DM) basis.

Approximately 1 month following corn silage harvest, alfalfa stand density was assessed in a representative 0.186 m^2 area in the middle inter-row of each interseeded plot. Soil was excavated ~5 cm deep along the alfalfa rows to distinguish and count the individual alfalfa crowns. The following year, interseeded alfalfa was harvested 4 times (early June, late June/early July, late July, and late August) and spring-seeded alfalfa was harvested 3 times (late June/early July, late July, and late August), with yield recorded from a 1.5-m-wide swath in the middle of each plot using a plot harvester with built-in weighing scale. To calculate DM content, a homogenous subsample of harvested material was dried at 60 °C for at least 2 weeks. Weeds harvested along with alfalfa were included in the plot yields, but two spring-seeded plots at AARS had extremely high weed cover and were considered outliers, and therefore were excluded from the alfalfa yield and combined 2-year yield analyses. Yields from the individual harvests were summed to calculate the total second-year alfalfa yield. In addition, first-year corn silage yields and second-year alfalfa yields were summed to calculate combined the 2-year yields

2.4 Statistics

Statistical analyses were conducted using the NLIN, REG, and GLIMMIX procedures in SAS v.9.4 (SAS Institute, Cary NC). Residuals were visually checked to ensure acceptable levels of normality and equal variances. Locations were analyzed individually, as initial analyses of variance (ANOVA) revealed significant location by treatment interactions for most response variables. Fixed effects of cropping system, N fertilizer rate, and N fertilizer application approach treatments were analyzed by a nested ANOVA, where factorial combinations of these effects were nested beneath a factor testing the 0N control against all treatments with N applied (Piepho et al. 2006). Block was included as a random effect. Mean separations were conducted using the LINES feature of proc GLIMMIX in SAS. Nitrogen rate responses were analyzed by linear or nonlinear regression and in the latter case quadratic plus plateau regression models were used to estimate the value of the plateau and the N join point (the N rate where maximum value was achieved). Initial exploratory analyses showed that model fits were comparable between linear plateau and quadratic plateau models, as the root mean square errors (RMSE) and coefficient of determination (R^2) were similar (data not shown).

3 Results and discussion

3.1 Weather conditions

Weather conditions were generally favorable for corn and alfalfa production throughout the 2-year study period at both locations. Temperatures were near the 30-year average with no extreme hot or cold spells (Table S1). Growing season precipitation was variable but was sufficient for crop growth. In the first year, when corn was grown with or without interseeded alfalfa, excessive precipitation was received in July at PDS followed by relatively dry conditions at both locations in August and September. Furthermore, in the second year during alfalfa production, both locations had excessive precipitation in May and August, and a drier than normal July.

3.2 Corn response to N

At low N rates, corn RCM values were significantly greater in solo-seeded corn than corn with interseeded alfalfa at both locations at both the V10 and R2 corn growth stages (Tables 1, 2). Nitrogen application approach generally did not affect RCM values at either location or measurement time, although the V10 measurement at PDS showed a significant three-way interaction between N rate, cropping system, and N application approach, which was driven by the lower RCM values for the PP application at low N rates (56N and 112N) but not at higher N rates in the interseeded system (data not shown). Corn RCM values showed a distinct quadratic plateau response to N rate when measured at both V10 and R2 growth stages. Corn with interseeded alfalfa had a greater N join point (N rate at which the plateau was reached) than solo-seeded corn at both sites and both growth stages (Table 3). At the V10 measurement, the join point for RCM values was 177 and

Table 1 Analysis of variance F-statistics and *P*-values for effects of cropping system (interseeded corn/alfalfa vs solo-seeded corn), N fertilizer rate, and N fertilizer application approach on relative chlorophyll meter (RCM) readings at V10 and R2 corn growth stages, corn silage yield, and corn silage N content at harvest at two locations. *P*-values are in parentheses. NS indicates not significant at the $P < 0.05$ level.

	RCM (V10)	RCM (R2)	Silage yield	Silage N content
Arlington Agriculture Research Station				
0N control vs. N treatments	66.5 (<0.0001)	136.9 (<0.0001)	58.0 (<0.0001)	96.2 (<0.0001)
N rate (R)	8.0 (<0.0001)	19.1 (<0.0001)	7.4 (0.0001)	30.6 (<0.0001)
Cropping System (CS)	16.1 (<0.0001)	8.3 (0.0009)	22.1 (<0.0001)	22.7 (<0.0001)
R x CS	3.6 (0.01)	4.4 (0.005)	2.9 (0.03)	1.5 (NS)
N application approach (A)	3.4 (NS)	0.1 (NS)	0.0 (NS)	0.2 (NS)
R x A	0.3 (NS)	0.4 (NS)	0.1 (NS)	0.3 (NS)
CS x A	0.0 (NS)	0.0 (NS)	0.0 (NS)	0.1 (NS)
R x CS x A	0.4 (NS)	0.2 (NS)	0.5 (NS)	0.3 (NS)
Prairie du Sac				
0N control vs. N treatments	277.7 (<0.0001)	77.6 (<0.0001)	57.0 (<0.0001)	104.3 (<0.0001)
R	85.3 (<0.0001)	37.1 (<0.0001)	20.7 (<0.0001)	72.9 (<0.0001)
CS	89.0 (<0.0001)	6.7 (0.003)	77.5 (<0.0001)	67.2 (<0.0001)
R x CS	29.8 (<0.0001)	5.3 (0.002)	3.2 (0.02)	0.4 (NS)
A	3.4 (NS)	0.01 (NS)	0.1 (NS)	0.2 (NS)
R x A	4.2 (0.006)	1.3 (NS)	1.3 (NS)	0.3 (NS)
CS x A	4.0 (0.05)	0.2 (NS)	0.1 (NS)	0.2 (NS)
R x CS x A	4.1 (0.007)	0.3 (NS)	1.9 (NS)	1.4 (NS)

87 kg N ha^{-1} greater in corn with interseeded alfalfa at AARS and PDS, respectively. Estimation of the differences in join points at the R2 measurement was not possible as RCM values of the interseeded corn did not reach a plateau within the range of N rates used in this experiment. Although the join points at R2 for the interseeded corn could not be estimated, they were greater than 280 kg N ha^{-1} and so occurred at a higher N rate than that observed at the V10 measurement (Table 3). This indicates that N limitation in the interseeded corn was more pronounced at the later R2 measurement timing compared to the earlier V10 measurement timing.

Corn silage yield and N content was significantly greater in solo-seeded corn than corn with interseeded alfalfa at both locations (Tables 1, 2). Nitrogen application method did not significantly impact corn yield or N content at either location and this factor was omitted from the subsequent regression analyses of N response. Quadratic plateau models generally fit both corn yield and corn N content data at AARS; however, at PDS, a yield plateau was not reached so a quadratic model was sufficient to model the N response (Fig. 2, Table 3). At AARS, the regression join point for corn silage yield was 83 kg N ha^{-1} greater in the interseeded treatment compared to solo-seeded corn (Fig. 3). The solo-seeded corn maximum yield at AARS was 1.4 Mg DM ha^{-1} greater than interseeded corn, while at PDS solo-seeded corn yielded 3.5 Mg DM ha^{-1} more than interseeded corn at the maximum N rate. At both sites, differences between interseeded and solo-seeded corn silage yields were most pronounced at lower N rates (Fig. 2, Table 3). For example, at the 0N rate, corn with interseeded alfalfa yielded 5.0–5.2 Mg ha^{-1} less DM than solo-seeded corn.

Silage N content of corn interseeded with alfalfa was lower than solo-seeded corn across a range of N rates (Table 1). The maximum corn silage yield was attained at a lower N rate than the maximum corn silage N content in both interseeded and solo-seeded corn at AARS, indicating that the additional corn N uptake at higher N rates was not effective at increasing yields but did increase N content. Such excess or "luxury" N uptake has been previously observed in corn silage (Lawrence et al. 2008). In terms of practical N management, the results of this and previous studies (e.g., Grabber 2016; Osterholz et al. 2018a) indicate near maximal corn silage yields in the interseeding system can be obtained if N is applied at 224 kg N ha^{-1} on high yield potential silt loam soils in southern Wisconsin. For comparison, state extension recommendations indicate 151–219 kg N ha^{-1} should be applied to corn on these soils depending on the nitrogen fertilizer:corn grain price ratio (Laboski et al. 2012). Additional N response experiments conducted in diverse growth environments with higher maximum N rates are, however, needed to provide further confirmation and refinement of recommended N fertilizer rates for corn interseeded with alfalfa.

Table 2 Corn relative chlorophyll meter (RCM) readings, corn silage yield, corn silage N uptake, alfalfa fall stand density, and second-year alfalfa yield at two locations for two cropping systems (interseeded corn/alfalfa vs. corn-alfalfa rotation) at 6 N rates from 0 to 280 kg N ha^{-1}. Treatment means and least significant differences were calculated by nested analyses of variance. Means are averaged across N application approaches. "+A" indicates corn with interseeded alfalfa, "-A" indicates solo-seeded corn. Different lowercase letters within rows indicate treatment means were different. chlorophyll meter values.

N rate	0		56		112		168		224		280	
Cropping system	+A	-A	+A	-A	+A	-A	+A	-A	+A	-A	+A	-A
Arlington Agriculture Research Station												
RCM 1	0.80e	0.88d	0.86d	0.98ab	0.94c	0.99ab	0.96bc	1.01a	0.98abc	1.01a	1.00ab	1.00ab
RCM 2	0.68f	0.71ef	0.78e	0.92cd	0.87d	0.95abc	0.94bc	0.97abc	1.00ab	0.99ab	0.99ab	1.00a
Silage yield (Mg DM ha^{-1})	11.2f	16.4de	14.4e	19.6abc	18.5cd	21.2a	19.0bc	21.0ab	19.9abc	20.7ab	19.1bc	20.7ab
Silage N content (kg N ha^{-1})	82g	121f	109fg	167de	157e	194bc	188cd	215ab	208abc	229a	201bc	229a
Alfalfa fall stand density (plants m^{-2})	243a	-	226ab	-	205abc	-	211abc	-	187bc	-	173c	-
Interseeded alfalfa yield (Mg DM ha^{-1})	10.6ab	-	11.1a	-	9.9b	-	10.6ab	-	10.0b	-	10.9a	-
Spring-seeded alfalfa yield (Mg DM ha^{-1})	-	5.1b	-	-	-	6.0b	-	-	-	7.1a	-	-
Prairie du Sac												
RCM 1	0.72g	0.87e	0.78f	0.94c	0.90d	0.99ab	0.97bc	1.00a	0.99ab	0.99a	1.00a	1.00a
RCM 2	0.75c	0.74c	0.75c	0.87b	0.84b	0.96a	0.97a	1.00a	1.02a	1.00a	1.01a	1.00a
Silage yield (Mg DM ha^{-1})	9.6g	14.6ef	11.0g	16.9cd	13.7f	17.8bc	15.7de	17.8bc	15.8de	19.3ab	16.4cd	19.9a
Silage N content (kg N ha^{-1})	72h	100fg	81gh	125e	108f	151d	139de	182bc	150bc	197ab	173c	207a
Alfalfa fall stand density (plants m^{-2})	247a	-	226a	-	196ab	-	184b	-	175b	-	132c	-
Interseeded alfalfa yield (Mg DM ha^{-1})	10.8a	-	10.7a	-	10.1ab	-	10.0abc	-	9.9bc	-	9.4c	-
Spring-seeded alfalfa yield (Mg DM ha^{-1})	-	4.1a	-	-	-	4.4a	-	-	-	5.0a	-	-

Table 3 Quadratic plateau regression parameter estimates for N rate response of corn leaf relative chlorophyll meter (RCM) values at two dates, corn silage yield, and N content at two locations. The regression model was quadratic where the estimated join point was greater than the maximum N rate of 280 kg ha^{-1} (values in italics). Separate models were fit for the two cropping systems studied: "+A" indicates corn with interseeded alfalfa, "-A" indicates solo-seeded corn. *For quadratic plateau models the value is the plateau, for quadratic models the value is the maximum at the highest N rate of 280 kg N ha^{-1}.

		Intercept	Linear coefficient	Quadratic coefficient	Join point (kg N ha^{-1})	Plateau or max*
Arlington Ag Research Station						
RCM 1	+A	0.80	0.00142	−0.000003	272	0.99
	-A	0.88	0.00265	−0.000014	95	1.00
RCM 2	+A	0.67	0.00228	−0.000004	*>280*	*1.00*
	-A	0.71	0.00510	−0.000024	106	0.98
Corn silage yield (Mg DM ha^{-1})	+A	10.8	0.0869	−0.00022	199	19.5
	-A	16.4	0.0776	−0.00034	116	20.9
Corn silage N content (kg N ha^{-1})	+A	69	0.984	−0.00177	278	206
	-A	123	0.828	−0.00161	256	229
Prairie du Sac						
RCM 1	+A	0.69	0.00232	−0.00000431	269	1.00
	-A	0.87	0.00159	−0.00000485	182	1.00
RCM 2	+A	0.69	0.00187	−0.00000225	*>280*	*1.08*
	-A	0.73	0.00285	−0.00000758	188	1.00
Corn silage yield (Mg DM ha^{-1})	+A	8.9	0.0537	−0.00096	279	16.4
	-A	15.0	0.0264	−0.00003	*>280*	*20.1*
Corn silage N content (kg N ha^{-1})	+A	63.2	0.427	−0.00011	*>280*	*173*
	-A	96.0	0.612	−0.00075	*>280*	*208*

The greater RCM value and yield responses to increasing N rates for corn with interseeded alfalfa suggests that competition for N between the interseeded corn and alfalfa dominated N interactions in the interseeded system. Similar N competition dynamics were previously observed in interseeded corn and annual snail medic (*Medicago scutellata* Mill), where presence of medic reduced N concentrations and N uptake in corn (Smeltekop et al. 2002). While the legume species and management details will likely influence the degree of competition with interseeded corn, our findings and the work by Smeltekop et al. (2002) suggest interseeded legume seedlings typically compete with corn for available N, rather than provide additional N for corn growth, rather than provision significant N to corn through biological N fixation. While productive alfalfa stands can fix N in excess of 300 kg ha^{-1} year^{-1} and newly seeded stands typically fix N on the order of 150 kg ha^{-1} in the seeding year (Heichel et al. 1981; Peterson and Russelle 1991), newly fixed N would be largely unavailable to a competing crop prior to termination of the alfalfa crop. Additionally, N fixation by the interseeded alfalfa was likely lower than previous observations from solo-seeded stands. Alfalfa N fixation is inhibited when large quantities of available soil N are present as was the case at higher N fertilization rates in this study (Cherney and Duxbury 1994). Furthermore, competition from the corn canopy for light reduced alfalfa growth in the higher N rate treatments in late summer, with growth effectively ceasing in August (Grabber and Osterholz, unpublished data). As N fixation is dependent on the carbohydrates produced from active photosynthesis, N fixation was likely prevented during this period of suppression. Additional studies utilizing ^{15}N isotope methods could confirm the absence of significant N provisioning from alfalfa fixation to corn in the interseeded system.

An important observation of this work was that even at the highest N rates there was a persistent corn silage yield reduction of 7–16% with interseeded alfalfa. While application of N fertilizer at rates greater than 112 kg N ha^{-1} appeared to reduce the magnitude of this yield reduction, N fertilizer did not fully overcome the negative effect of interseeded alfalfa in this study. Previous studies conducted at multiple sites from 2014 to 2019 with a single N rate of 224 kg N ha^{-1} have shown on average a 5% reduction in corn silage yield when alfalfa is interseeded (Osterholz

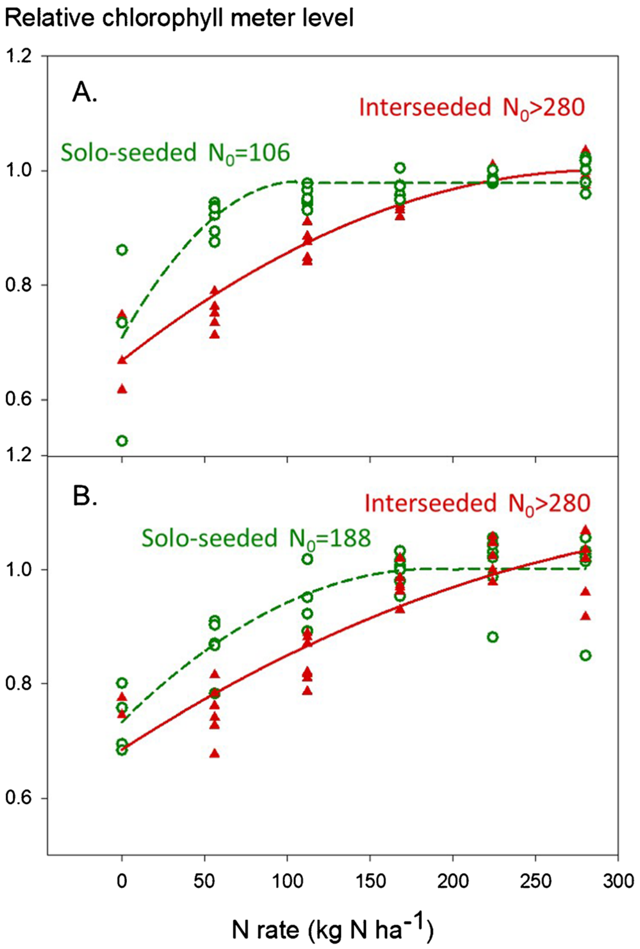

Fig. 2 Nitrogen rate response of relative chlorophyll meter readings of corn ear leaf at the R2 growth stage at AARS (A) and at PDS (B) locations. Separate models were fit to the two cropping systems: corn with interseeded alfalfa is represented by red solid line and triangles, solo-seeded corn by green hollow circles and dashed line. Lines represent the modeled quadratic plateau, data points represent values for individual plots, and N_0 is the join point N rate in kg N ha^{-1}.

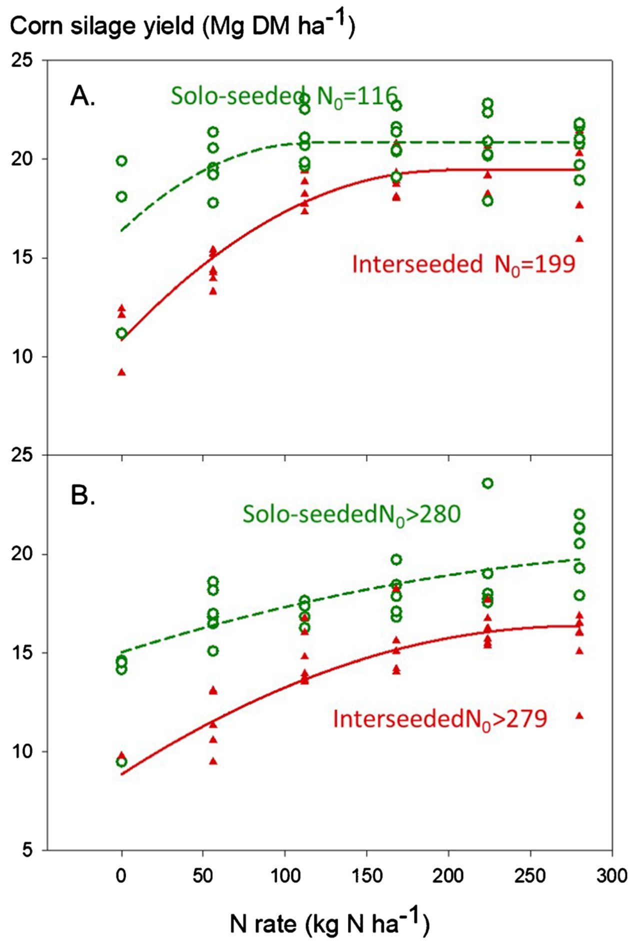

Fig. 3 Corn silage yield response to N fertilizer rates at AARS (A) and PDS (B) locations. Separate models were fit to the two cropping systems: corn with interseeded alfalfa is represented by red solid line and triangles, solo-seeded corn by green hollow circles and dashed line. Lines represent the modeled quadratic plateau regression, data points represent values for individual plots, and N_0 is the join point N rate in kg N ha^{-1}.

et al. 2018a; Grabber et al. unpublished data). Competition for water or nutrients other than N (such as P or K) could potentially explain the persistent yield reduction, but we consider this unlikely in the current study due to applications of sufficient P and K fertilizer to satisfy crop requirements as well as sufficient precipitation during the interseeding growing season (Table S1). Another possibility is that the corn yield decline was determined early in the growing season when corn seedlings detected the presence of interseeded alfalfa. Corn seedlings have been shown to modify their growth in response to the altered light quality cause by the presence of other vegetation and these effects can extend through the growing season to ultimately reduce biomass accumulation and yield (Page et al. 2009). Further research is required to identify the mechanisms behind frequent corn yield reduction in the interseeding system and to develop management practices for minimizing impacts on corn yield.

Altering the N application approach did not influence N uptake efficiency or mitigate N competition between the interseeded crops. Splitting the N application to better coincide with corn N demand combined with placing the N fertilizer in a narrow band over the corn row did not increase the ability of corn to compete for N. At PDS, there was a small but significant improvement in N status of interseeded corn at the V10 growth stage when the two lowest N rates were applied using the BSD approach, but this effect did not extend to later in the growing season. The placement of the side-dressed N was likely ineffective as it was applied in early June during a period of rapid crop growth, so roots of both crops are likely to have fully explored the inter-row area soon after fertilizer

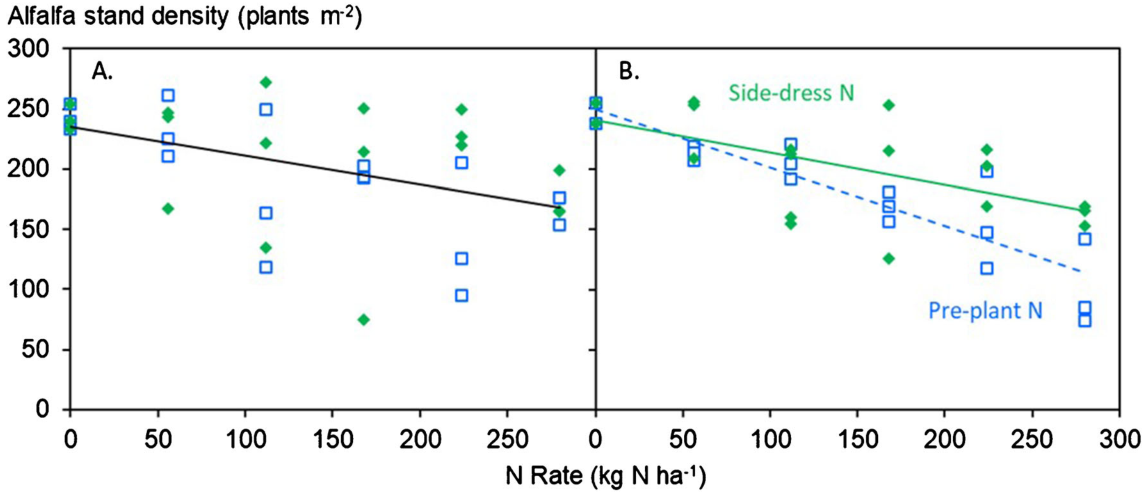

Fig. 4 Nitrogen application rate effects on fall interseeded alfalfa stand density at two locations, AARS (A) and PDS (B). Regressions for nitrogen application approaches are presented separately for PDS, with blue hollow squares and dashed line representing pre-plant N application and solid green diamonds and line representing split plus banded side-dress N application and AARS regression equation $y = -024x + 235$; PDS pre-plant N regression equation $y = -0.48x + 249$; PDS side-dress N regression equation $y = -0.26x + 240$.

application and thus accessed any available soil N. Furthermore, nitrate is highly mobile under wet soil conditions so adequate soil moisture in June also likely encouraged rapid diffusion of N throughout the rooting zone. The N fertilizer response of corn can differ between pre-plant and in-season side-dress applications, but typically this occurs only under extreme weather conditions (Kovács et al. 2015). While heavy precipitation occurred in July in this study, the moderate precipitation during the early part of the interseeding year likely limited the importance of N application timing. As there was no advantage to the BSD application approach, it appears that manipulating N application in this manner was not an effective approach for reducing the intercrop N competition. By comparison, the PP broadcast appears to be an adequate and less laborious N application approach for the interseeded system. However, a side-dress approach would offer additional flexibility for producers to alter N application rates in response to extreme weather events or if N deficiency was detected early in the growing season.

The differences in the corn yield response to N rate between the two sites could be explained in part by differences in precipitation, as N losses are known to vary dramatically under different precipitation regimes (Eagle et al. 2017). Wet conditions at PDS, particularly in July, could have resulted in loss of N via leaching or denitrification, thus increasing the N fertilizer required to maximize yield at this site. Additionally, management at PDS may have enhanced the N fertilizer requirement at this site, as corn silage was previously grown with a low N rate, thus likely minimizing soil N availability prior to experiment initiation. However, despite these differences, similar effects of interseeded alfalfa on corn yield and the response to N fertilizer were observed at both locations.

3.3 Alfalfa response to N

Fall alfalfa stand density was linearly related to N rate at both locations ($p = 0.02$ and $p < 0.0001$ at AARS and PDS, respectively), as alfalfa stand density decreased as N rate increased (Fig. 4). The decrease in fall alfalfa stand density with greater N rates suggests that alfalfa seedling mortality was enhanced at high N rates. The greater corn productivity at high N rates likely altered the microclimate beneath the corn canopy, with reduced light availability and increased humidity, in turn increasing stress and potentially enhancing disease pressure on the interseeded alfalfa seedlings. However, despite the reduced alfalfa stand density at high N rates, all alfalfa stands were deemed sufficient for forage production as previous research has shown first production yields do not substantially increase above a stand density of ~140 plants m^{-2} (Tesar and Marble 1988). Interseeded alfalfa establishment could be favored by applying lower rates of N fertilizer, but this would risk a trade-off of large reductions in corn silage yield without a meaningful increase in alfalfa yield, which is not likely to be acceptable to producers.

At AARS, the N application approach was not associated with differences in alfalfa stand density ($p = 0.08$). However, at PDS, the BSD approach resulted in slightly greater stand density compared to the PP approach ($p = 0.02$, Fig. 4). Additionally, interactions between N rate and application approach were not significant at either location ($p > 0.1$). The cause of the N application approach effect at PDS is unclear. Stress on the interseeded alfalfa from competition with corn was not likely dissimilar in the BSD and PP application approaches, as corn productivity was not different in these treatments. Further research may elucidate the mechanism by which N application placement and timing impacts alfalfa establishment.

Second-year interseeded alfalfa yield at PDS was significantly influenced by N rate ($p = 0.009$) and N application approach ($p = 0.001$) as well as their interaction ($p = 0.009$). Interseeded alfalfa yield was reduced by 15 to 20% at the greatest N application rates of 224 and 280 kg N ha^{-1} when the PP but not the BSD application approach was used (Fig. 5). As noted above, at PDS, the interseeded alfalfa stand density at high N rates was reduced when N was applied PP which may be responsible for the yield difference. At AARS, interseeded alfalfa yield was influenced by N rate ($p = 0.02$) but not by N application approach or the interaction of N rate and application approach ($p > 0.1$). In contrast to PDS, the N rate effect at AARS did not indicate a consistent pattern of change with N rates (Fig. 5). Overall, N rate and application method did not have pronounced and consistent effects on interseeded alfalfa yield, as the interseeded alfalfa stands produced around 10.5 Mg DM ha^{-1} in the subsequent year across all N rates. Interseeded alfalfa yielded more than spring seeded at both locations ($p < 0.0001$) yield was at least 41% greater than spring-seeded alfalfa yield for the individual N rates, and when averaged across the N rate treatments was 68% and 128% greater at AARS and PDS, respectively (Table 2). Previous studies also found first-year yields of interseeded alfalfa were 60 to 130% greater than conventional spring-seeded alfalfa (Grabber 2016; Osterholz et al. 2018a).

Spring-seeded alfalfa yield was influenced by the previous year N rate at AARS, where the yield of 7.1 Mg ha^{-1} for the 224N rate was greater than the yield of 5.1 and 6.0 Mg ha^{-1} for the 0N and 112N rates, respectively ($p = 0.04$; Fig. 5). However, at PDS, the spring-seeded alfalfa yields were not affected by N rates. Additionally, the N application approach did not affect spring-seeded alfalfa yield at either location ($p > 0.1$). The N rate effect at AARS was likely due to residual soil N promoting early-season alfalfa and weed growth. Newly seeded alfalfa yield has been shown to respond positively to greater N availability early in the growing season (Hannaway

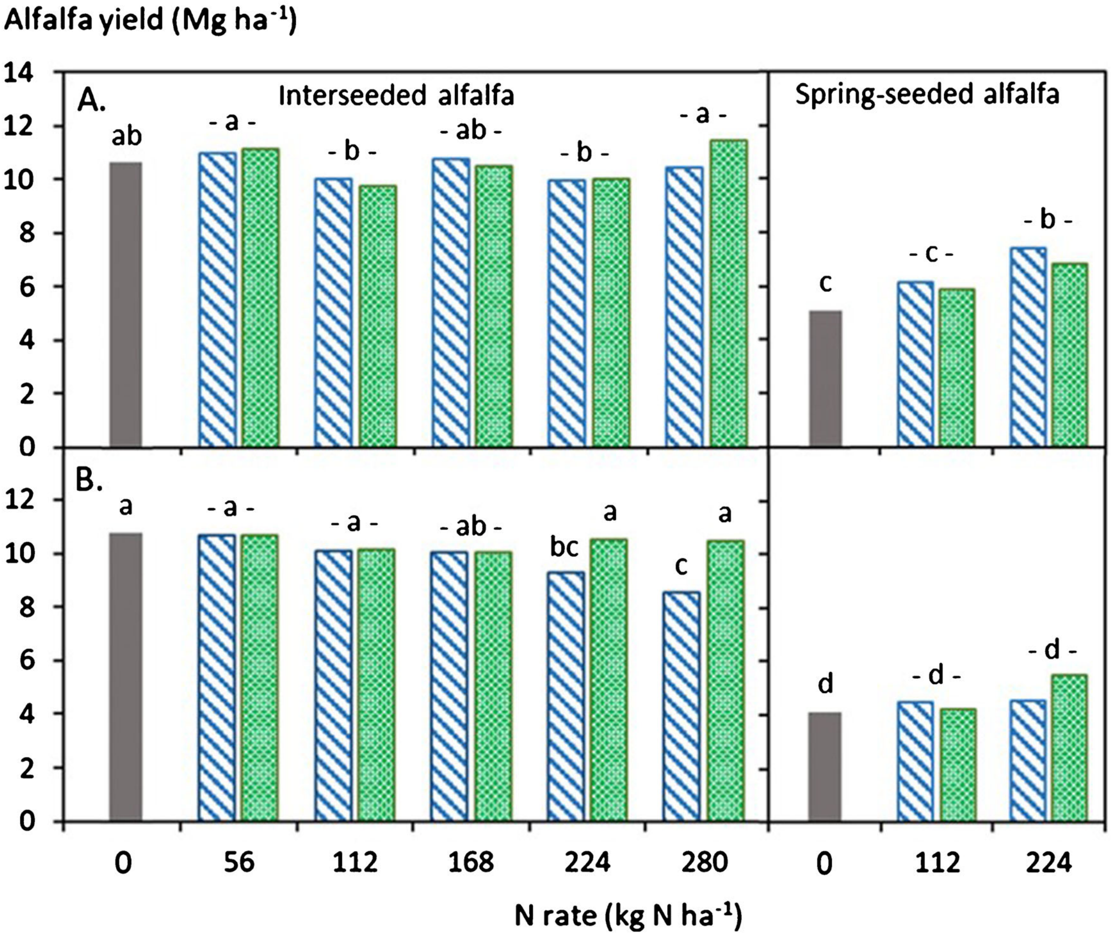

Fig. 5 Alfalfa yield the year following corn silage production as influenced by cropping system (interseeded vs spring-seeded), N rate, and N application approach at two locations, (A) AARS and (B) PDS. Solid gray bars represent no N application, diagonal blue bars represent pre-plant N application, and checked green bars represent split banded side-dress N application. Bars with different lower case letters within a location were significantly different (LSD, $p < 0.05$). Separate analyses of variance were used to test differences between N rate and application approach treatments within the cropping systems and between treatments shared across the cropping systems.

and Schuler 1993; Bélanger and Richards 2000). Significant residual soil N was likely present following corn silage harvest at AARS at high N rates, as the join point N rate for the solo-seeded corn was only 116 kg N ha^{-1}. Soil N mineralization after corn silage harvest may have further contributed to build up of plant available soil N. Additionally, weed biomass was not separated from the harvested alfalfa and weed growth in the spring-seeded alfalfa at AARS was observed to be particularly vigorous prior to the initial harvest in early July. Thus, enhanced weed growth in response to residual N also likely contributed to the N management effects on spring-seeded alfalfa yield.

3.4 Total 2-year yield

Combined yield (corn silage + alfalfa) over the 2 years of the experiment was not significantly different between the interseeding and conventional systems at AARS ($p > 0.1$), but 2-year yield was significantly greater in the interseeded system compared to the conventional system at PDS ($p = 0.005$). Therefore, corn silage yield reduction in the interseeding system was compensated for by an increase in alfalfa yield, and surpassed the conventional system yield in one location. Additionally, N rate significantly influenced the 2-year yield at both AARS and PDS ($p < 0.001$). Two-year yields at the 224N rate were 25.7 and 24.3 Mg DM ha^{-1} for the interseeded and conventional systems at PDS and averaged 29.2 Mg DM ha^{-1} for both systems at AARS, which was 6% and 7–11% greater than the yields at the 112N rate at AARS and PDS, respectively. Nitrogen application approach and interactions with crop system and N rate were not significant factors determining 2-year yield at either location ($p > 0.1$).

3.5 Potential environmental and economic implications

Competition for available N between corn and interseeded alfalfa and continued growth of alfalfa following corn harvest is expected to convey an environmental benefit of lower risk of N leaching losses. Interseeded alfalfa likely depletes the highly leachable soil nitrate pool during the growing season and results in a smaller residual soil nitrate pool after corn harvest, as observed in a previous study of interseeded corn-legume systems (Grabber et al. 2014). Further research quantifying soil N pools at different N application rates is needed to confirm and quantify this benefit in the interseeded corn-alfalfa system. Previous work has shown that interseeded alfalfa provides groundcover before and after corn silage harvest and the subsequent spring, which leads to large reductions in soil erosion and nutrient losses in runoff (Osterholz et al. 2019). However, it should be noted that the greater corn N requirement due to interseeded alfalfa could be considered an environmental trade-off, as N fertilizer production generates significant greenhouse gas impact (Snyder et al. 2009). Additionally, on commercial dairy farms, manure typically contributes a significant portion of the N required by corn silage (Powell et al. 2007) and thus, manure could be used to help meet the greater N requirements of corn interseeded with alfalfa. The agronomic and environmental impact of utilizing manure as an N source in the interseeding system requires further study.

The additional N fertilizer required to maximize corn yield in the interseeded system will incur additional economic costs. However, the value of the alfalfa yield increase provided by interseeding was likely more than sufficient to overcome the additional N fertilizer requirements. A recent economic analysis of interseeded corn and alfalfa assumed an additional 45 kg N ha^{-1} was required for corn silage production when alfalfa was interseeded, yet forage rotations utilizing interseeding still provided a robust increase in net returns compared to conventional corn silage-alfalfa rotations (Osterholz et al. 2020). This study assumed manure was available from a linked dairy enterprise, and results may have differed if the entire N requirement was purchased as fertilizer. Furthermore, adoption of the interseeding system would likely change the ratio of cropland dedicated to corn silage and alfalfa. For example, the improvement in alfalfa yield may encourage more frequent planting of alfalfa, but the length of time alfalfa stands are maintained may be shorter as producers seek to maintain high levels of corn silage production. Thus, the potential implications of the interseeding system at both the farm and landscape scales are deserving of further attention.

4 Conclusions

Corn interseeded with alfalfa improved the total 2-year yield of corn followed by alfalfa by combining corn silage production with alfalfa establishment. Here we show for the first time the importance of additional N fertilizer applied to corn silage interseeded with alfalfa for ensuring high corn silage yields. The response of corn to N fertilizer additions was significantly stronger when alfalfa was interseeded, particularly at low N rates. Interseeding alfalfa resulted in a 52–62% reduction in corn silage yield when N rate was 0 kg N ha^{-1}, but only a 7–16% reduction at N rates that maximized corn yield. Corn yields in the interseeded system at one location were maximized at a N rate of 199 kg N ha^{-1}, which was 83 kg N ha^{-1} greater than the conventional corn-alfalfa rotation, while maximum yield was not reached by either treatment in the second location. Results of this and previous work, however, suggest an N application rate of 224 kg N ha^{-1} could be used to ensure high yields in the interseeding system on high yield potential soils in southern Wisconsin. Interseeded alfalfa establishment was negatively impacted by increases in N application rate, but the differences in stand density did not consistently translate into alfalfa yield declines as interseeded alfalfa yield did not show a strong relationship with N rate. Despite efforts to design a N fertilizer application approach favoring corn N uptake using split timing and banded placement, N application approach was largely not important in determining the N response of corn productivity and corn-alfalfa competition dynamics, although at one location alfalfa establishment and second-year alfalfa yield was slightly enhanced when high N rates were split applied and banded. The interseeded corn-alfalfa systems produced significantly more total forage over 2 years compared to the conventional corn-alfalfa rotation at one of two locations, where the total 2-year forage yields at the highest yielding N rate of 224 kg N ha^{-1} were 7% greater in the interseeded system compared to the conventional corn-alfalfa rotation. While maximum interseeded corn yield was 1.4–3.3 Mg ha^{-1} lower than that of a conventional solo-seeded corn, second-year alfalfa yields were 2.8–4.9 Mg ha^{-1} greater in interseeded alfalfa compared to spring-seeded alfalfa. The interseeded corn-alfalfa system has potential to improve the productivity and environmental impact of corn and alfalfa-based cropping systems, and insights developed in this study provide a basis for further development of optimal N management practices for this system.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13593-021-00711-1.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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