Kernza perennial grain and legume dual purpose polycultures in Wisconsin:

effects of row spacing, fertilization, weed management, and legume intercrops

by

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1. Introduction

Kernza as a perennial grain and forage crop

Globally, most of the population's food comes from annual crops (directly as food, or indirectly as animal feed) and 60 to 80% of cropland is dedicated to annual crops (Glover et al., 2007; Pimentel et al., 2012). Increasing land area used for annual row-crop agriculture, that depends on soil disturbance, has resulted in the pollution of marine and freshwater ecosystems, increased greenhouse gas emissions, decreased biodiversity and increased soil erosion (Foley et al., 2011; Pimentel et al., 2012).

One possible solution to these environmental and social problems is to develop perennial agriculture. Perennials such as forages, agroforestry and managed forestry, increase water infiltration rates when compared to intensive row crop production, which in turn provides ecosystem services such as pollution and flood control from runoff (Basche & DeLonge, 2019). In cropping systems with higher degrees of perenniality and diversity, outputs are more stable, they fare better in soil health indicators, and exhibit greater resilience to drought (Sanford et al., 2021). In the case of grain crops, these benefits could be achieved by developing a grain crop that mimics a prairie, including species diversity and perennials (Jackson, 2002).

In the 1980's, intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barksworth & D.R. Dewey) was identified as a candidate for the development of a perennial grain crop, due to its synchronous seed maturity, edible grain, moderate shattering and moderate threshability (Wagoner, 1990; DeHaan et al., 2018). Intermediate wheatgrass (IWG), native to Eurasia, has been widely used in the US and Canada as a perennial forage crop (DeHaan et al., 2018), and its edible seed can be used in baked products or beverages, in combination or replacement of common grains such as wheat, rye, or barley (DeHaan et al., 2018).

Intermediate wheatgrass breeding efforts have been led by The Land Institute, in Salina, Kansas, and the grain is trademarked as Kernza. Over five breeding cycles, average predicted gains from selection were 143, 181 and 60% for seed yield per head, percent naked seed and mass per seed, respectively. So far, there hasn't been any findings that indicate that achieving increased grain yield and high perenniality would be particularly difficult (DeHaan et al., 2018). There have been nine breeding cycles until 2021 (Crain, 2021). Kernza can be grown as a dual purpose perennial grain and forage crop, which reduces the economic risk for the farmer, and provides ecosystem services that are not usually delivered by annual grains. For example, it reduces nitrate leaching (Culman et al., 2013; Jungers et al., 2019).

Although there are potential benefits in ecosystem services and multifunctionality of perennial grains, and Kernza in particular, research is needed to develop agronomical management strategies to increase yields and reduce risks for farmers. Establishment practices, forage nutritive value, maintaining constant yields, weed management and economic assessment are some of the concerns of farmers (Lanker et al., 2019). Among the management strategies that need to be addressed by research are row spacing, intercropping and nitrogen fertilization. Row spacing has an impact in the use of resources such as light, water and nutrients, affecting yield. Out of row spacings of 15, 30, and 62 cm, the wider one increased IWG grain yield (Hunter et al., 2020). In general, there was reported an increase in IWG monoculture grain yields with increasing nitrogen fertilization (Jungers et al., 2017; Zimbric et al., 2020), and after a certain rate was reached, yields declined due to lodging (Jungers et al., 2017). The biomass harvested after grain harvest had similar yield and quality as other forage cool season grass crops, which reinforces the potential of IWG for dual purpose use as grain and forage (Jungers et al., 2017).

Polycultures and intercropping

Intercropping is the growth of two or more species in the same place and time, at least for part of their growing period, and can result in pest control, similar yields with reduced inputs, pollution mitigation and more stable yields per unit area (Brooker et al., 2015). The species involved interact in ways that can be beneficial or disadvantageous, and understanding the underlying physiological traits for this interaction is fundamental to better manage intercropping systems.

Lower grain yields were observed in an IWG and alfalfa bi-culture in sites and years where the conditions were favorable for alfalfa. However, this did not affect IWG nutrient uptake, suggesting a competition for water or light instead (Tautges et al., 2018). In these same sites that yielded less grain in the second year of establishment, the authors observed a possible facilitation of nutrient uptake of IWG by alfalfa in the fourth year of harvest, which may relate to a greater nitrogen availability from fixation over time.

In a study that compared IWG monocrop with an IWG and red clover bi-culture, the bi-culture had better overall forage quality (Favre et al., 2019). Specifically, crude protein in the summer crop residue (after grain harvest) was increased by 69%; in the fall harvest crude protein and relative forage quality were increased by 49% and 11% respectively, while neutral detergent fiber and acid detergent fiber were reduced by 25% and 18% respectively. The authors conclude therefore that Kernza IWG is suitable as a dual-purpose crop, providing apt forage for lactating beef cows, dairy cows and growing heifers in spring and fall.

Legume species for intercropping

Table 1. Traits of legume species used for intercropping.

Legume	Lifespan	Growth habit	Roots	Tolerance to drought	Tolerance to poor drainage	Yield potential	Ease of establish-ment
Alfalfa	Long-lived perennial	Upright	Deep taproot (7 – 9 m)	Excellent	Poor	Very high	Easy
Red clover	Short-lived perennial	Upright	Branching taproot (1.2 – 1.8 m)	Moderate	Fair	High	Very easy
Kura clover	Long-lived perennial	Plastic	Branching taproot (0.6 m)	Moderate	Good	Low	Slow
Berseem clover	Annual	Upright	Shallow taproot (0.1 – 0.15 m)	Poor	Good	High	Easy
Soybean	Annual	Upright	Branching taproot (0.1 – 0.3 m)	Poor	Fair	Low	Easy

Alfalfa (*Medicago sativa* L.) is the leading perennial forage legume in the United States. Plants have a taproot that can penetrate 7 to 9 m (Sheaffer & Gerald, 2007). It grows best in soils that are well drained, neutral in pH, and have high fertility. It is poorly adapted to wet or saline soils and will not tolerate flooding. It has good tolerance to drought. Water use exceeds that of many annual row crops that have a shorter period of vegetative growth. From 5.6 and 8.3 cm ha^{-1} of water are required to produce a metric ton of dry forage, but water use is increased by high air temperatures and low humidity (Sheaffer & Gerald, 2007). Alfalfa intercropped with Kernza can potentially increase forage yields and nutritive value but will also compete for moisture.

Red clover (*Trifolium pratense* L.) is a perennial legume with good tolerance to soil acidity, and poor tolerance to soil alkalinity. It has fair tolerance to wet soils, drought, and cold. The plant has a taproot with secondary branches, but its roots do not penetrate the soil to the extent of alfalfa. It is best adapted where summer temperatures are moderately cool to warm and moisture is sufficient. It has relatively less drought and heat tolerance than alfalfa but is adapted to a wide range of soil types, except those in areas prone to drought or excess moisture. It has lower yields and shorter stand life than alfalfa. Its seedlings are very competitive (Sheaffer & Gerald, 2007). Red clover intercropped with Kernza could provide suitable forage for beef and dairy cattle, especially in grazing systems (Favre et al., 2019).

Kura clover (*Trifolium ambiguum* M. Bieb.) is a perennial with a deep, branching taproot and rhizomes, allowing it to spread vigorously, but seedling vigor is poor. It is adapted to cold and long winters, presents good drought tolerance and tolerance to wet soils. It tolerates frequent defoliation in monoculture or mixture with grass. Under moisture stress and high heat, it becomes dormant (Sheaffer & Gerald, 2007; Zemenchik et al., 2000). Its high persistency, vigor, and good forage nutritive value in mixture with grasses (Kazula et al., 2019) could control weeds and improve suitability of Kernza as a double purpose crop.

Berseem clover (*Trifolium alexandrinum* L.) is an annual legume which lacks drought tolerance because of a short taproot, and can tolerate temporary flooding. It is best adapted to loam and clay soils with good internal drainage with maximum growth at pH of 7-8. Germination is optimum at day/night temperatures of 25/15 ºC (Sheaffer & Gerald, 2007). Berseem clover intercropped with Kernza could improve forage nutritive value (Ross et al., 2004).

Soybean [*Glycine max* (L.) Merr.] is an annual oilseed legume. Traditionally it was used as a forage crop, until it started being processed for oil and protein in the 1930's (Wisconsin Corn Agronomy, 2015). The best soybean yields occur on well-drained, but not sandy, soils having a pH of 6.5 or above. Approximately 80% of the roots are in the top four inches of soil, making soybean susceptible to drought (Purcel, Salmeron & Ashlock, 2014a; Purcel, Salmeron &

Ashlock, 2014b). Due to its high protein content, soybean intercropped with Kernza could increase forage nutritive value.

Knowledge gaps and hypotheses

In order to develop true perennial grain polycultures, several knowledge gaps exist regarding row spacing, fertilization, and management in general. We hypothesize that:

1. Intermediate wheatgrass grain yield and forage biomass will be greater for narrower than wide row spacing, given the higher number of plants per area.
2. Intermediate wheatgrass grain yield and forage biomass will be greater with higher rates of nitrogen fertilization, since N could become a limiting factor in a perennial crop.
3. Intermediate wheatgrass grain yield and forage biomass will be greater by growing it in intercrop with legumes than in unfertilized monoculture, due to the nitrogen fixation process that will supply this element to the crop in the long term.
4. Intermediate wheatgrass grain yield and forage biomass will be higher when removing weeds than in control, due to the reduction in competition for nutrients, light, and water.

Objectives

This research has four objectives:

1. Determine the effect of row spacing on IWG grain yield and forage biomass.
2. Determine the effect of nitrogen fertilization on IWG grain yield and forage biomass.
3. Determine the effect of legume species intercropping on IWG grain yield and forage biomass.
4. Determine the effect of weeding on IWG grain yield and forage biomass.

2. Materials and methods

Site Description

An experiment was conducted at the University of Wisconsin-Madison Arlington Agricultural Research Station near Arlington, WI (43°18'6.97" N, 89°21'9.98" W) on a Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudoll; NRCS-USDA, 2020). Seeds were planted in three different seasons: fall of 2016, spring of 2017 and fall of 2017. Data were collected during the 2017, 2018, and 2019 growing seasons. The mean annual temperature is 6.7° C, and the mean annual rainfall is 863 mm (Arguez et al., 2010).

Temperature and precipitation 30 year normals, and daily minimum and maximum temperature data were obtained from the National Oceanic and Atmospheric Administration Climate Data Online Search website (Menne et al., 2012) and monthly precipitation from the Arlington Research Station. Growing degree days (GDD) were calculated using the equation: GDD = (Tmax + Tmin)/2 – Tbase, where Tmax and Tmin are the maximum and minimum daily temperature respectively, and Tbase was 0ºC, as used for cool season grasses (Frank, 1996). Growing degree days were accumulated after 5 consecutive days in which the average temperature was higher than the base temperature (Frank et al., 1985). The accumulation of growing degree days is reported for each year until grain harvest. Monthly precipitation and temperature are shown in Figure 1. Precipitation during the growing season until harvest (April to July) was 428 mm in 2016, 522 mm 2017, 512 mm 2018 and 560 mm 2019. Year 2016 was warmer than a normal year, and years 2018 and 2019 had more precipitation than normal (University of Wisconsin, Division of Extension, 2021).

Soil samples were taken on April 18 2017. In the top 15 cm, NO_3-N averaged 5.1 ppm and NH_4-N averaged 21.1 ppm.

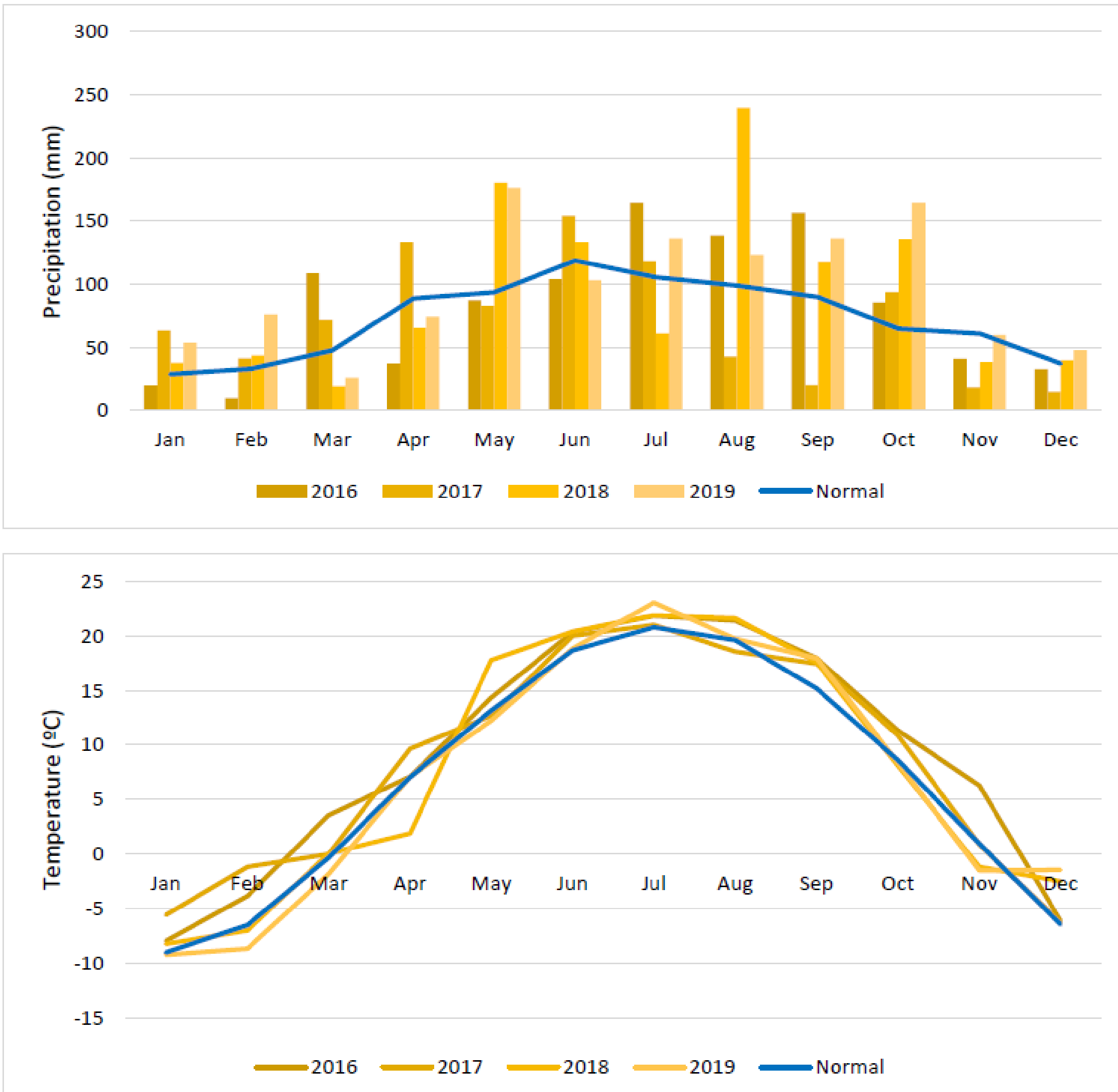

Figure 1. Monthly precipitation (mm) and average temperature (ºC) for years 2016 to 2019 and 30 year monthly normals in Arlington Agricultural Research Station, Wisconsin.

Experimental Design

The experimental design was a randomized complete block in a split-plot arrangement of treatments with five replications (Figure 2). The plot size was 3 m by 4.8 m for fall 2016 and spring 2017, and in fall 2017 the plots were 3 m by 1.5 m. One hundred plots were planted in each planting season, and the total area of the study was one hectare. Row spacing was the main plot factor, which included wide-row (57 cm) and narrow-row (38 cm) spacing treatments. The subplot factor was the cropping system, which was a combination of nitrogen fertilization, intercropping, and weed management, and included ten treatments:

1. IWG monoculture (control)
2. IWG monoculture without weeds (removed by hand)
3. IWG monoculture + 40 kg N ha^{-1}
4. IWG monoculture + 80 kg N ha^{-1}
5. IWG intercropped with alfalfa
6. IWG intercropped with red clover
7. IWG intercropped with Kura clover
8. IWG intercropped with berseem clover
9. IWG intercropped with soybean
10. Annual crop control (corn silage in 2017, oats or corn silage in 2018, oats in 2019)

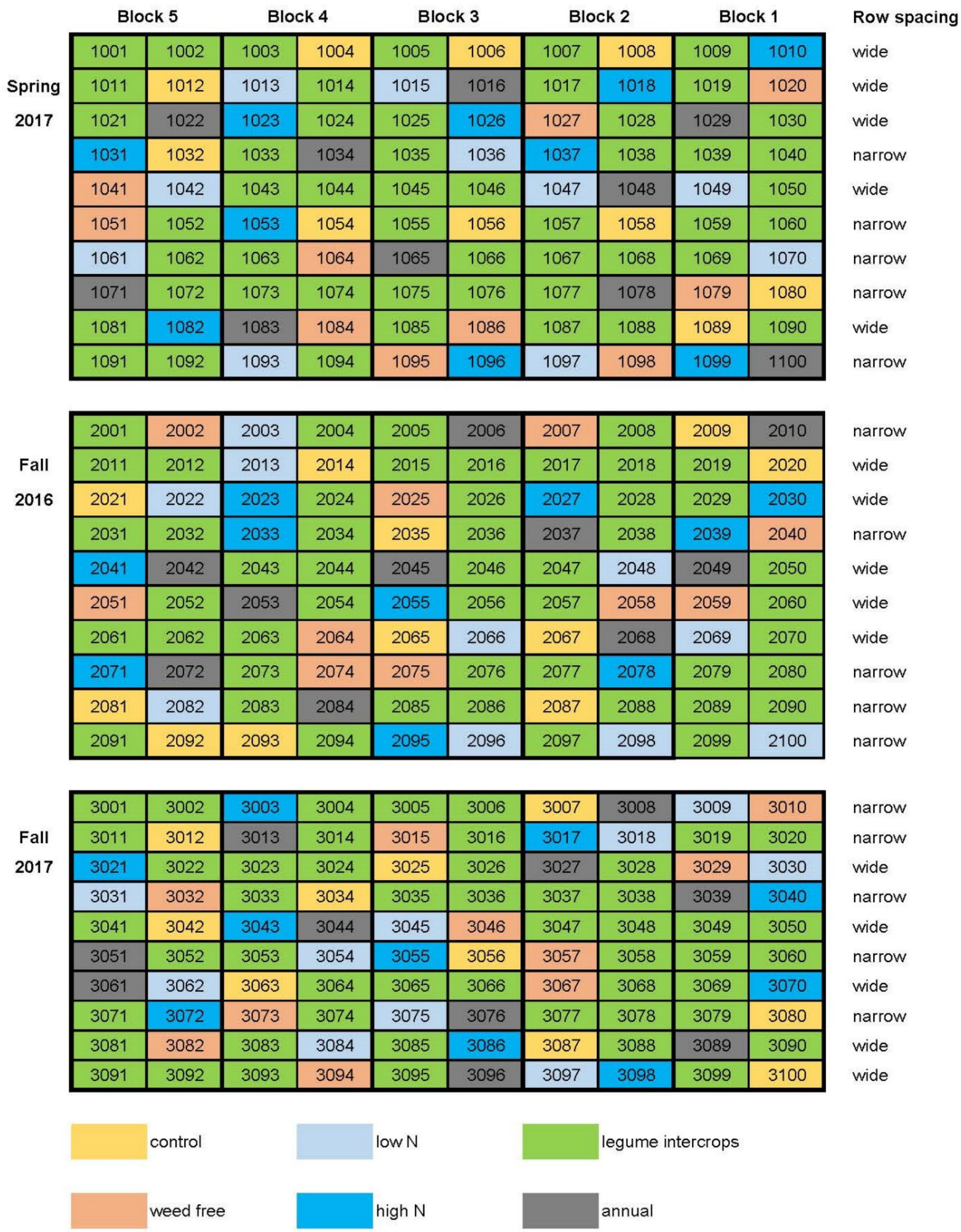

Figure 2. Map of plots and blocks of Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

The previous crop in the experimental area was soybean (both in 2016 and 2017). The IWG germplasm planted was Cycle 4 from The Land Institute, KS. This is the product of four successive breeding cycles for larger grain size among other agronomic traits (DeHaan et. al., 2018).

For the fall 2016 planting, IWG was planted on September 19. The seed was obtained from other IWG fields at the research station. Alfalfa, red clover, Kura clover, and berseem clover intercrops were frost seeded on March 10, 2017. Soybean intercrops were planted on May 15 with a single row seeder, after tilling the soil between IWG rows with a single-row wheel hoe. Planting dates, varieties used and seeding rates are listed in Table 2. In the spring 2017 planting, IWG originated from Iowa was planted on April 12, followed by legume intercrops a week after. Soybean intercrops were planted on May 15, using the same method as for the previous planting. Red clover was replanted on March 22 of 2018, due to potential winter kill. Berseem clover and soybean were replanted on March 22 2018 and May 28 2019. The fall of 2017 planting was established in September, with seeds harvested from the fall of 2016 and spring of 2017 plantings the previous summer. The legume intercrops were scattered by hand on March 22 2018, except soybean, that was planted on May 23 with the same method as before. Soybean and berseem clover were replanted on May 28 and June 4, 2019.

Nitrogen fertilizer was applied as urea in split applications during the spring in 2017 and 2018, and half in the spring and half in the fall in 2019 (Table 3). Weeds were removed by hand, once in spring and once in summer.

Table 2. Planting dates, varieties and seeding rates of intermediate wheatgrass and legume intercrops over three years at Arlington, Wisconsin.

	Planting season		
	Fall 2016	**Spring 2017**	**Fall 2017**
Intermediate wheatgrass	September 19, 2016	April 12, 2017	September 21, 2017
Seeding rate (kg ha^{-1})			
narrow row spacing	13.6	10.8	11.2
wide row spacing	12.6	11.3	11.2
Alfalfa	March 10, 2017	April 19, 2017	March 22, 2018
Variety	Forage First 42-LH (La Crosse Seed; Lacrosse, WI)		
Seeding rate (kg ha^{-1})	19.2	6.7	19.2
Planting	Frost seeded	Drilled	Scattered by hand
Red clover	March 10, 2017	April 19, 2017	March 22, 2018
Variety	Forage First 951 (La Crosse Seed; La Crosse, WI)		
Seeding rate (kg ha^{-1})	17.8	9.0	17.8
Planting	Frost seeded	Drilled	Scattered by hand
Kura clover	March 10, 2017	April 19, 2017	March 22, 2018
Variety	Cossack (University of Wisconsin-Madison; Madison, WI)		
Seeding rate (kg ha^{-1})	20.9	11.2	16.8
Planting	Frost seeded	Drilled	Scattered by hand
Berseem clover	March 10, 2017	April 19, 2017	March 22, 2018
Variety	No variety stated (Albert Lea Seed; Albert Lea, MN)		
Seeding rate (kg ha^{-1})	18.0	11.2	16.8
Planting	Frost seeded	Drilled	Scattered by hand
Soybean	May 15, 2017	May 15, 2017	May 23, 2018
Variety	Viking 2155 (Albert Lea Seed; Albert Lea, MN)		
Seeding rate (seeds ha^{-1})	14,300 to 21,200		
Planting	Single row seeder		

Soybean and berseem clover were planted again on May 23, 2018 for the Fall 2016 and Spring 2017 plantings, and on May 28, 2019, for all plantings. In 2019, berseem clover seed was obtained from Deer Creek Seed Co., Windsor, WI (no variety stated).

Table 3. Dates of fertilization and harvest activities per year and planting season for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

Activity	2017	2018	2019
Nitrogen fertilization	April 18 May 26	May 23 June 6	June 4 September 18
Quadrat grain sampling	August 1	August 7	August 1
Quadrat forage sampling	August 1	August 7	August 1
Grain harvest (combine)	August 1	August 8	August 20
Accumulated GDD from spring to grain harvest (C)	2080*	2111	1901
Forage harvest (whole field), summer	August 8	August 14	September 16
Forage harvest (whole field), fall	October 27	October 24	-

*Spring 2017 accumulated 1833 from planting to harvest in 2017

Data collection

Aboveground biomass and grain were sampled in the summer (Table 3). One 0.25 m^2 quadrat was placed in each plot and IWG forage and legume forage and weeds were cut by hand, separated, and removed. In 2017 and 2018, the biomass was cut at the soil surface; in 2019 it was cut at 10 cm aboveground. The quadrat was placed so that one row of IWG would fit inside the quadrat for the wide row spacing, and two rows for the narrow row spacing. Intermediate wheatgrass grain, IWG forage, and legume forage data were adjusted proportionally to the number of rows within the sampled quadrat, to obtain yields in kilograms per hectare.

Biomass was dried at 60° C for at least 5 days, separated into IWG forage, weeds and legumes, and weighed. Intermediate wheatgrass grain yield was determined by cutting the seed heads from all tillers within the quadrat placed in each plot. Grain was considered to be mature when it reached the Moore 4.5 growth stage (Moore et al., 1991). Grain was dried at 35° C for at least 2 days, threshed with a mechanical seed thresher, and weighed. After grain sampling, the whole plots were harvested with a combine. After the quadrat sampling, all aboveground biomass was removed from the experiment using a mechanical forage harvester (Almaco, FH-88) leaving a stubble height of 10 cm.

Statistical Analysis

The data were tested for normality and homogeneity of variances, and transformed using logarithm or square root to satisfy the assumptions of the analysis. Analysis of variance was performed on IWG grain yield, IWG forage, and legume forage using PROC MIXED procedure in SAS (SAS on Demand, SAS Institute, Cary, North Carolina, USA). Each planting season was analyzed independently, using the following mixed effects model:

$$Y = RS + CS + RS*CS + Block + Year + RS*Year + CS*Year + RS*CS*Year$$

Block, row spacing (RS), cropping system (CS), year of harvest (Year) and all interactions between row spacing, cropping system and year of harvest were treated as fixed effects. Year was not randomized, and was considered a repeated measure. The error term of the main plot was considered a random effect. Means were compared using the Tukey-Kramer honest significant difference test, with an alpha level of 0.05. Contrasts were done to compare differences between IWG control and IWG weed free, with perennial legume intercrop treatments (alfalfa, red clover and Kura clover), nitrogen treatments (40 and 80 kg N ha^{-1}), and soybean intercrop treatment. Annual cropping system treatment wasn't included in the analyses.

3. Results

Table 4.P-values for the analysis of variance for grain, IWG forage, and legume forage yields for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

	IWG grain			IWG forage			Legume forage		
	Fall 2016	**Spring 2017**	**Fall 2017**	**Fall 2016**	**Spring 2017**	**Fall 2017**	**Fall 2016**	**Spring 2017**	**Fall 2017**
Block	0.17	0.17	0.06	0.06	0.16	0.92	0.23	0.93	0.10
Row spacing	**0.03**	0.08	0.14	**0.01**	**0.01**	0.47	0.08	0.24	0.33
Cropping system	**0.02**	**<0.01**	**0.02**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Row spacing * cropping system	0.92	**<0.01**	0.9	0.28	0.38	0.42	0.27	0.34	0.76
Year	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Row spacing * year	0.25	**0.02**	0.95	**0.05**	0.16	0.07	0.41	0.74	0.44
Cropping system * year	**<0.01**	**<0.01**	**0.02**	**<0.01**	**<0.01**	**0.01**	**<0.01**	**<0.01**	**<0.01**
Row spacing * cropping system * year	0.31	0.22	0.68	0.39	0.16	0.91	0.37	0.18	0.90

No three-way interaction was detected for any variable (Table 4). An interaction between the cropping system and year of harvest was observed for all variables and all planting seasons. Therefore, results are presented by year. In general, no row spacing by year or cropping system interaction was detected, with a few exceptions: row spacing by year for grain yield in the Spring 2017 and for IWG forage in the Fall 2016; row spacing by cropping system for grain yield in the Spring 2017. Differences between years and between cropping systems were detected for all variables and planting seasons. Differences between row spacing were found in few cases (Table 4).

Grain yields declined each year for all the planting dates (Table 5, Figure 3). From the first to the second year of harvest, the Spring 2017 declined 74% and the Fall 2017 planting declined 84%. Fall 2016 planting declined 43% from the first to the second year of harvest, and 93% from the first to the third year. Intermediate wheatgrass forage yields declined each year for the two fall planting seasons, 24% and 54% from first to second and third year in the Fall 2016 planting, and 28% from the first to the second harvest in the Fall 2017 planting. The Spring planting presented a different behavior: the first forage harvest yielded less, it was done just four months after establishment. From the second to the third harvest, yields were similar. Legume forage yields tended to be greater in the later harvests. For the Fall 2016 planting, the first two years yielded the same, and yields increased over 60% in the third year. For the Spring 2017 harvest, the third year yielded almost twice as much as the first, while the harvest in the second year wasn't significantly different from either. The Fall 2017 planting yields increased over 10 times from the first to the second year.

Table 5. Intermediate wheatgrass grain, IWG forage, and legume forage yield by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

	Planting season							
	Fall 2016			**Spring 2017**			**Fall 2017**	
	2017	**2018**	**2019**	**2017**	**2018**	**2019**	**2018**	**2019**
	kg ha^{-1}							
IWG grain	597 a	342 b	43 c	-	286 a	74 b	669 a	104 b
IWG forage	4264 a	3238 b	1978 c	500 b	2564 a	2641 a	3872 a	2802 b
Legume forage	744 b	539 b	1213 a	525 b	666 ab	1047 a	70 b	1032 a

Means for the same variable within a planting date followed by the same letter are not different by the Tukey's honest significant test at 5%.

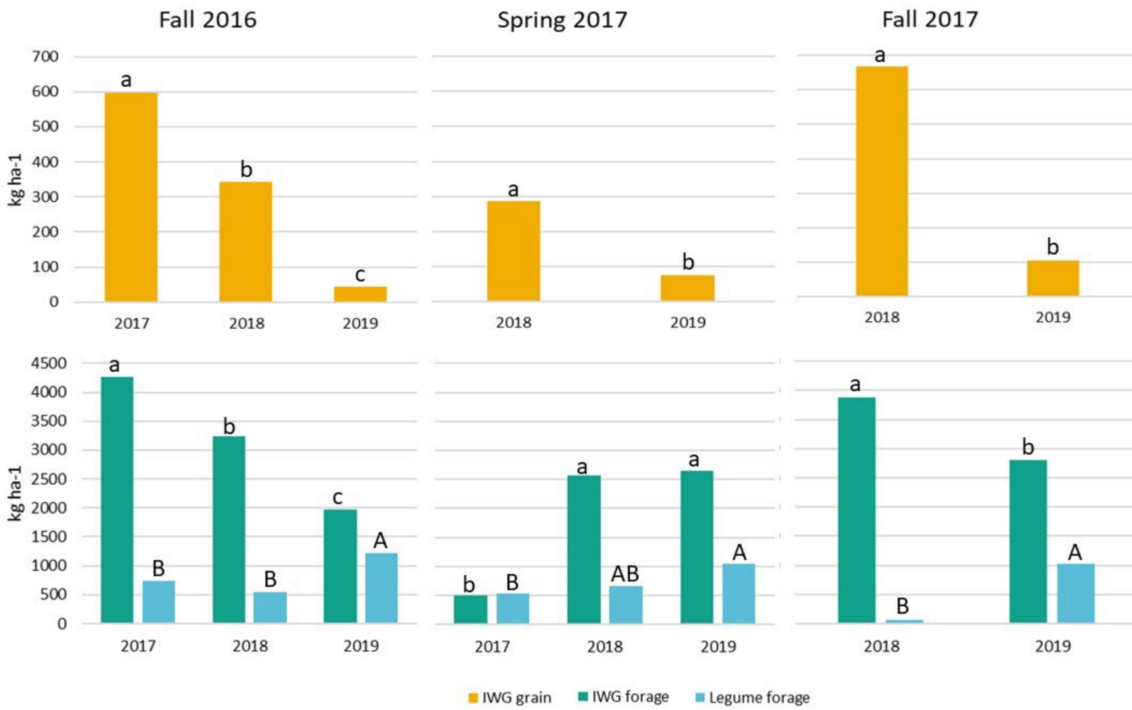

Figure 3. Intermediate wheatgrass grain, IWG forage, and legume forage yield by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

Row spacing

Row spacing did not affect grain yield in 5 out of 7 combinations of years and planting seasons (Table 6). The wider row spacing yielded higher than the narrow row spacing in two cases: the first year of harvest (2017) of the Fall 2016 planting and in the second year of harvest (2019) of the Spring 2017 planting.

Intermediate wheatgrass forage in the Fall 2016 planting was higher for the wide row spacing in 2017, followed by the narrow row spacing the same year of harvest. The narrow row spacing in 2019 had the lowest yields for that planting season, followed by the wide row spacing that year. In the Spring 2017 planting, the highest yields were obtained in the wide row spacing in year 2019, and the lowest in both row spacings in year 2017. In the Fall 2017 planting season, the tendency is similar to that observed in the Fall 2016 planting, where forage yields decline over time, but there aren't any differences between row spacings in the same year.

For legume forage, the tendency is similar to the other variables, in which when there were differences, the wide row spacing yielded more. In the Fall 2016 planting, the highest yield was obtained in 2019 with wide row spacing. The lowest yield was in the narrow row spacing in 2018. In the Spring 2017 planting, the highest yield was obtained with wide row spacing in 2019, and the lowest was with the narrow row spacing in 2017. In the Fall 2017 planting, there are no differences between row spacings in the same year.

Table 6. Intermediate wheatgrass grain, IWG forage and legume forage by row spacing, planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

		Planting season															
	Row spacing	Fall 2016						Spring 2017						Fall 2017			
		2017		2018		2019		2017		2018		2019		2018		2019	
		kg ha^{-1}															
IWG grain	Narrow	527	b	300	c	37	d	-		289	a	49	c	763	a	120	b
	Wide	672	a	389	c	50	d	-		280	a	109	b	580	a	90	b
IWG forage	Narrow	3362	b	2903	bc	1742	d	322	c	2231	b	1895	b	3714	a	3163	ab
	Wide	5273	a	3590	c	2229	cd	718	c	2921	ab	3510	c	4033	a	2464	b
Legume forage	Narrow	532	cd	439	d	1148	ab	491	b	517	ab	959	ab	64	b	884	ab
	Wide	994	abc	649	bcd	1279	ab	561	ab	833	ab	1139	ab	77	b	1191	ab

Narrow row spacing: 38 cm. Wide row spacing: 57 cm.

Means for the same variable within a planting date followed by the same letter are not different by the Tukey's honest significant test at 5%.

Cropping system

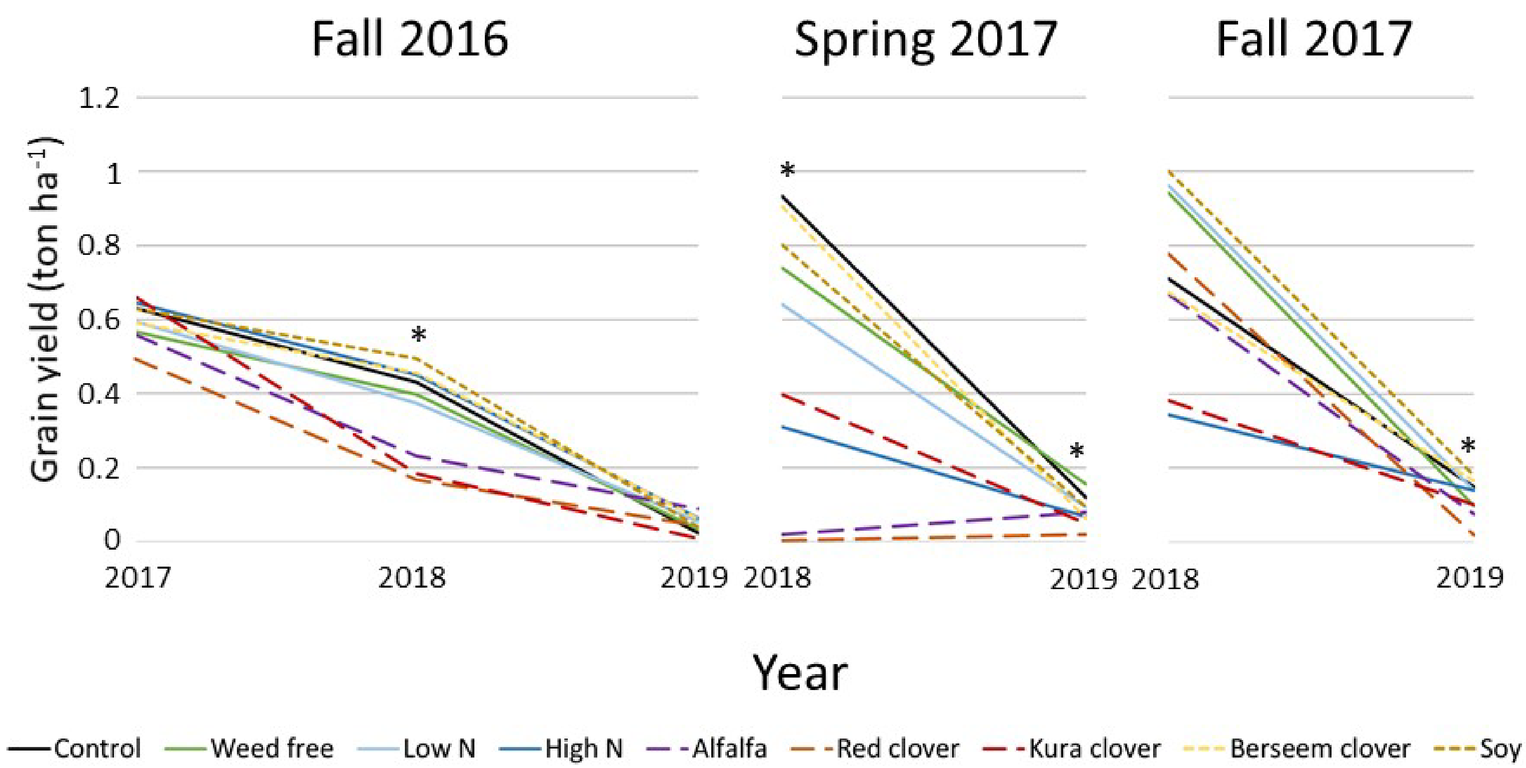

Figure 4. IWG grain yields by cropping system in each planting season and year (means across row spacings) for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.
Asterisks show years where differences between cropping systems were found.

In the first and the third harvests of the Fall 2016 planting (years 2017 and 2019), there weren't any differences between cropping systems (Table A 1 in the Appendix shows estimates and their significant differences). In the second harvest (2018) there were some differences: the highest yielding cropping system was soybean intercrop, which was different from the lowest ones, Kura clover and red clover (Figure 4). Red clover was the lowest yielding cropping system in that year, and control, high N, berseem clover and soybeans were significantly higher in comparison. All cropping systems show numerical decrease in yield, but most of them don't show differences between the first and second harvest, except the three perennial legumes (alfalfa, red clover, Kura clover) which showed a steeper decline in yield. From the second to the third year of harvest, all the cropping systems decline, except alfalfa and red clover (the decline in these intercrops was a numerical trend, but not significant).

In the Spring 2017 cropping systems there are some differences in both years of harvest (Figure 4). In 2018, the first year of harvest, the red clover and alfalfa intercrops yielded less than all the other cropping systems. In the second year of harvest, the weed free treatment yielded more than the red clover intercrop. All other cropping systems yielded the same. Some of the cropping systems show a decline in yield: control, low N, Kura clover, berseem clover, and soybean. The other cropping three systems (weed free, high N, alfalfa, and red clover) don't present any changes.

In the Fall 2017 planting, there aren't any differences in the first year of harvest. In the second year, all cropping systems were similar, except for red clover, which was the lowest and similar only to weed free, alfalfa and Kura clover (Figure 4). Some cropping systems show a significant decline in yield between the first and second year of harvest, like weed free, low N, alfalfa, red clover and soybean. The others (control, high N, Kura clover and berseem clover) don't show a decline.

In the second year of harvest of the Fall 2016 and Fall 2017 plantings, threshed grain yields were higher in the control and weed free cropping systems than in the perennial legume intercrops (Table 7). In the Spring 2017 planting, the control and weed free yielded more grain than the perennial legume intercrops in both years of harvest.

Nitrogen

The nitrogen treatments yielded more grain than the control and weed free treatments in the third year of harvest of the Fall 2016 planting (Table 7). The soybean intercrop did not show any differences with the control and weed free treatments for any of the planting dates and harvest years.

Intermediate wheatgrass forage yields were higher in control and weed free treatment than in the perennial legumes cropping systems, in the second year of the Fall 2016 planting and the second and third year of the Spring of 2017 planting (Table 8). Forage yields were higher for the nitrogen treatments than for the control and weed free treatment in the third year of the Fall 2016 planting and the second year of the Fall 2017 planting. The soybean intercrop yielded higher IWG forage yields than the control and weed free treatments in the second year of the Fall 2017 planting.

Table 7. Intermediate wheatgrass grain contrast estimates (Est, kg ha^{-1}) and P-values (P) by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

	Planting season													
	Fall 2016						**Spring 2017**				**Fall 2017**			
	2017		**2018**		**2019**		**2018**		**2019**		**2018**		**2019**	
	Est	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**
Perennial legumes vs Control + Weed free	0	n.s.	**-40**	**<0.01**	1	n.s.	**-10**	**<0.01**	**-10**	**<0.01**	-9	n.s.	**-10**	**<0.01**
Nitrogen (High, Low) vs Control + Weed free	0	n.s.	0	n.s.	**6**	**0.02**	-9	n.s.	-9	n.s.	-9	n.s.	-9	n.s.
Soybean vs Control + Weed free	0	n.s.	5	n.s.	1	n.s.	-9	n.s.	-9	n.s.	-9	n.s.	-9	n.s.

(Negative estimates indicate that control+weed free had higher values.)

Table 8. Intermediate wheatgrass forage contrast estimates (Est, kg ha^{-1}) and P-values (P) by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

	Planting date															
	Fall 2016						**Spring 2017**						**Fall 2017**			
	2017		**2018**		**2019**		**2017**		**2018**		**2019**		**2018**		**2019**	
	Est	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**	**Est**	**P**
Perennial legumes vs Control + Weed free	-7	n.s.	**-578**	**<0.01**	-15	n.s.	-25	n.s.	**-1158**	**<0.01**	**-150**	**<0.01**	-3	n.s.	-8	n.s.
Nitrogen (High, Low) vs Control + Weed free	36	n.s.	25	n.s.	**186**	**<0.01**	-6	n.s.	-14	n.s.	60	n.s.	35	n.s.	**12**	**0.01**
Soybean vs Control + Weed free	4	n.s.	63	n.s.	7	n.s.	-5	n.s.	-1	n.s.	0	n.s.	-1	n.s.	**12**	**0.03**

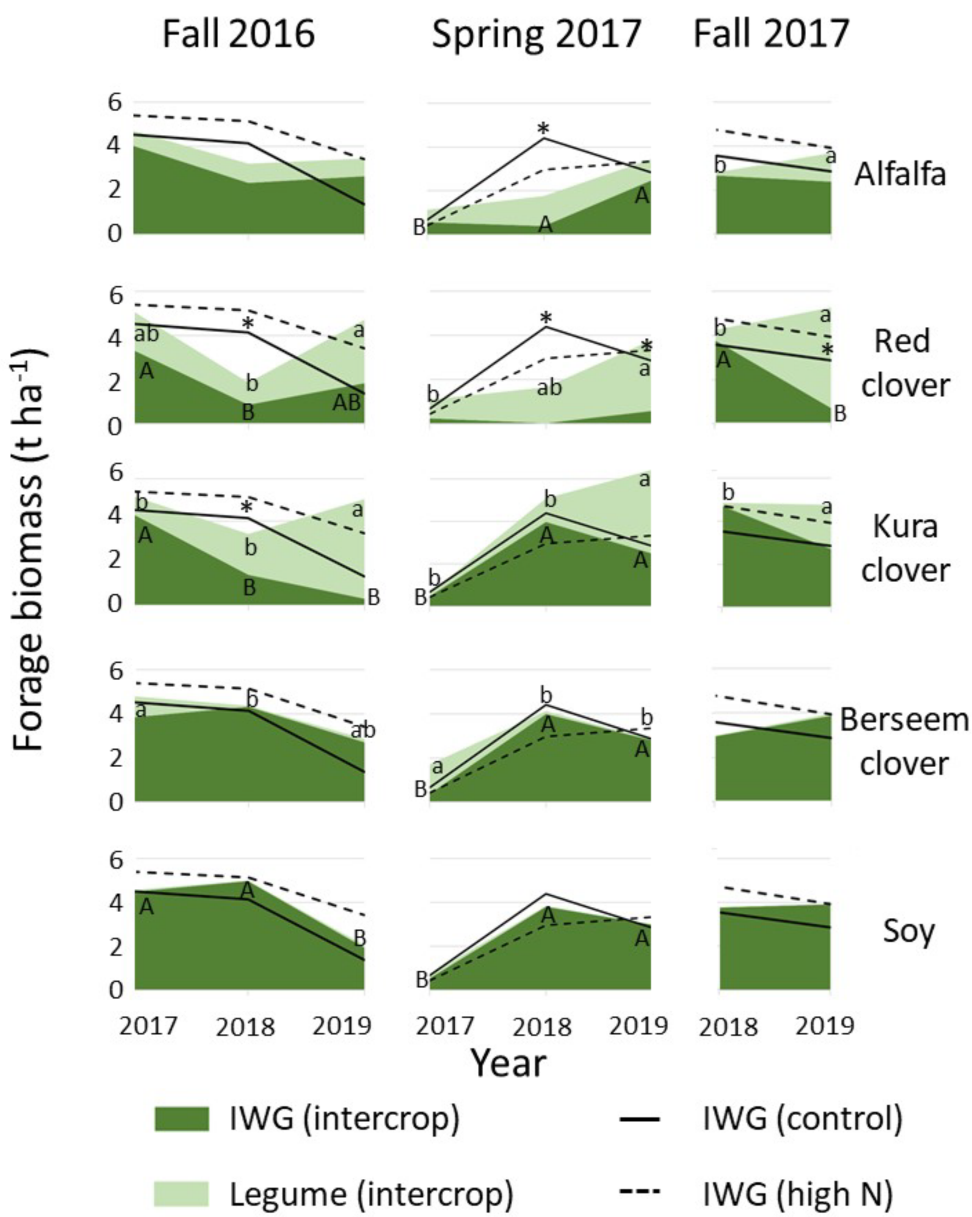

Figure 5. Intermediate wheatgrass and legume forage (in metric tons per hectare) by planting season and legume intercrop cropping system for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

The stacked area graphs show IWG forage in dark green, bottom area, and the legume forage in light green, above the IWG area. The IWG forage yields of the control and the high nitrogen treatments in each planting date are represented by lines for reference. Within each graph, asterisks denote a significant difference between IWG forage in that cropping system versus the control. Differences between legume forage yields among harvest years of the same planting season and cropping system are denoted by lower case letters, and differences between IWG forage yields for each legume intercrop are denoted by upper case letters. Means followed by the same letter are not different by Tukey's honest significant difference test at the 5%.

In the Fall 2016 planting, the IWG + alfalfa cropping system yielded the same IWG forage as the control (Figure 5). Alfalfa forage did not present any differences among the three years of harvest. In the intercrop with red clover, IWG forage was lower than the control in 2018, but it didn't show differences in the other years. Red clover forage was lowest in the second year and highest in the third year, while the second year was similar to both. Kura clover intercrop presented a similar behavior: IWG forage was lower than the control in the second year of harvest, and Kura clover forage increased with time. The two first years had similar yields, but in the third year it increased. In the intercrop with berseem clover, IWG forage didn't show any differences with the control, and berseem forage decreased in the second year, but the third yielded similar to both previous harvests. Intermediate wheatgrass forage yields in the soybean intercrop were similar to the control, and there aren't any differences in soybean forage between the three years of harvest. For all years in this planting, the high nitrogen treatment did not differ from the control. In 2018, this nitrogen treatment yielded more IWG forage than the three perennial legumes. In 2019, it was only higher than the Kura clover intercrop.

In the Spring 2017 planting in the IWG and alfalfa cropping system, the IWG forage obtained was less than the control in the second year of harvest, 2018. It didn't differ from the control in the other years. Alfalfa forage yields didn't differ between the three years of harvest. In the red clover cropping system, IWG yields were lower than the control in 2018 and 2019. The highest red clover forage yield was obtained in 2019, and the lowest in 2017.The year 2018 wasn't different from either. In the intercrop with Kura clover, there weren't any differences in IWG forage when compared to the control. The third year of harvest produced the highest Kura clover forage yields, with the two previous years yielding the same, and less than the third. Berseem clover intercrop did not have an effect on IWG forage yields when compared to the control. Berseem clover forage was higher in the first year of harvest, and the two later years yielded the same. The IWG forage obtained in the soybean intercrop was the same as in the control for all years, and soybean forage didn't show any differences in the three harvests. The high nitrogen treatment yielded the same IWG forage as the control in all years. In 2018, it yielded more forage than the alfalfa and red clover intercrops, and in 2019 it yielded more than only the red clover intercrop.

In the Fall 2017 planting, alfalfa intercrop produced the same amount of IWG forage as the control. Alfalfa forage was higher in 2019, the second year of harvest. In the intercrop with red clover, IWG forage was lower than the control in 2019, and red clover forage was higher that same year. The Kura clover intercrop produced statistically the same amount of IWG forage than the control, and Kura clover forage was higher on the second year of harvest. Berseem clover and soybean intercrops didn't show any differences on IWG forage with the control, or in amount of legume forage through the two years of harvest. The high nitrogen treatment didn't show any statistical difference with any of the intercrops during the two years.

4. Discussion

Yield decline with age

As observed in other studies, IWG grain yield declined as the stand aged from years one to three (Hunter et al., 2020; Fernandez et al., 2020; Tautges et al., 2018). Hunter et al. (2020) found that yield decline was driven by a reduction in grain number per high yielding spike and proportion of high yielding spikes with age. Finding ways to reduce intraspecific competition and increase the allocation of resources to sexual reproduction of IWG will be fundamental in stabilizing yields. Tilling between the rows in the fall reduced grain yield decline, probably due to a decrease in intraspecific competition (Law et al., 2020). A study by Pinto et al. (2021) showed that mechanical or chemical thinning increased light penetration, which might help reduce grain yield decline, but results are not conclusive. Intermediate wheatgrass forage also presented a decline with age, which is contrary to what has been found in most literature (Hunter et al., 2020; Fernandez et al., 2020; Tautges et al., 2018).

Comparing with annual winter wheat trials performed at the same agricultural research station as this experiment, the grain yield averages of years 2017, 2018 and 2019 was 6,906 .kg ha^{-1} (Conley et al., 2017; Conley et al., 2018; Conley et al., 2019). Yields per year were higher than the three year average in 2017 (7,896 kg ha^{-1}), and lower in 2018 and 2019, with this last year producing the lowest yields (6,543 kg ha^{-1} and 6,277 kg ha^{-1} respectively). Assuming these yields were affected by weather in similar ways as IWG, we can have an approximation of how age and weather affected IWG grain yields separately. The year 2019 was a very low year for the three planting seasons: yields were between 104 and 43 kg ha^{-1}, even if it was the second or third year of harvest. To compare, in 2018, the fall 2016 second year of harvest yielded 342 kg ha^{-1}, much lower than the second year of harvest of the fall of 2017 planting. It is possible that in the year 2019, yields were affected more by poor weather conditions than by the age of the stand. The first years of harvest for the fall 2016 and fall 2017 plantings yielded somewhat similar. Intermediate wheatgrass might have higher yield potential in its first year, and weather could have a minor effect than in subsequent years.

Regarding IWG forage yields, oat and barley trials performed at the same agricultural research station as this experiment, forage averages for the years 2017, 2018 and 2019 were 5,535 kg ha^{-1} (Gutierrez et al., 2017; Gutierrez et al., 2018; Gutierrez et al., 2019). Yields per year were higher than the three year average in 2017 and 2018 (6,350 and 6,919 kg ha^{-1}, respectively), and lower in 2019 (3,336 kg ha^{-1}). Intermediate wheatgrass forage seems not to have been affected in the same way as barley and oat forage, since the second year of harvest for the fall 2016 planting (year 2018) seems similar to the second year of harvest for the fall 2017 planting (year 2019), instead of the harvest of 2019 being much lower.

Row spacing

Wide row spacing increased grain and IWG forage, though this effect wasn't seen in all plantings and harvest years. The hypothesis that IWG grain yield and forage biomass would be higher for narrower row spacings was rejected. Grain yield was higher in the wider row spacing in Fall 2016 planting in 2017 (first year) and in the Spring 2017 planting in 2019 (second year), with differences of 145 kg ha^{-1} and 60 kg ha^{-1} respectively. These results are similar to what Hunter et al. (2020) found, where out of row spacings of 15, 30 and 61 cm, the wider row spacings tended to increase grain yield in some of the years. The authors suggest the reduction in lodging and an increase in tiller ha^{-1} resulting from the wider row spacing may be responsible for increasing yields. Considerable lodging was observed in our experiment, possibly explaining the differences among row spacings.

Wider row spacing reduces competition for water, nutrients and light, which may produce more grain per row (Craine & Dybzinski, 2013). For this reason, post-harvest mechanical thinning is recommended to increase harvest index (Law, 2020). However, the lower number of rows per hectare in wider row spacings may counteract this increase. While grain yield per row was not evaluated, it must have been higher enough at wider row spacing in the two planting season - harvest year combinations to compensate for the fewer rows per ha. Furthermore, IWG seeding rate per hectare was almost the same for the two row spacings, which means that for the wide row spacing there were more seeds per row. Higher grain yields for wider row spacings is consistent with the findings of Pinto et al. (2021), who found that increasing light penetration to the soil surface had a positive impact on Kernza yield components per row, although not enough to compensate for a reduction in rows per area.

Black and Reitz (1969) found that IWG seed yields in the northern Great Plains were higher in wider row spacings (152 cm) in dry years and in narrow row spacings (76 cm) in wet years, with little overall effect across 5 yr. The two row spacings used in our experiment (57 and 38 cm) were narrower than what used by these authors. Out of the two years in which higher yields for wide row spacings were observed, 2017 was similar to the normal, and 2019 had higher precipitation. Our findings are not consistent with the findings by Black and Reitz.

The effect of row spacing on IWG forage was similar as for grain: the wider row spacing yielded higher than the narrow row spacing in two cases: the first year of harvest (2017) of the Fall 2016 planting and in the third year of harvest (2019) of the Spring 2017 planting, with differences of 1911 and 1615 kg ha^{-1}, respectively. These results differ from Hunter et al. (2020b) findings, where narrower row spacings (of 15 and 30 cm) produced more summer forage (straw) than the widest row spacing (61 cm). This difference in results could be explained by the seeding rates in our experiment, which in kg ha^{-1} were almost the same for both row spacings (resulting in higher seeding rate per row in the wider row spacing). In contrast, Hunter et al. (2020) used constant seeding rate per row in their different row spacings, resulting in lower seeding rates per ha for the wider row spacings. In the experiment by Pinto et al. (2021), the seeding rate per row was also constant, and their results show that vegetative biomass per row was higher when light penetration increased due to a combination of residue removal and chemical or mechanical thinning was used, resulting in the termination of 3 out of every 4 rows, which is similar to planting in wider row spacing. Legume forage didn't vary with row spacing in any given planting date and harvest year.

In the experiment by Hunter et al. (2020b), straw (summer forage) yield and potential value was similar to the cost of producing straw and grain, reducing the risk of grain production for the farmers. This reinforces the use of IWG as a dual purpose grain and forage crop. Even in years when Kernza yields are low (due to weather events, or due to the yield decline that is usually observed), harvesting for straw will make the crop profitable. Farmers could harvest grain in the first one or two years, and keep the crop for forage and straw harvests in subsequent years.

Legume intercropping

The hypothesis that IWG grain and forage biomass would be higher by legume intercropping was rejected. In cases where there was an effect, it was negative. In the Fall 2016 planting, differences in grain yield among cropping systems were observed in the second year of harvest. Soybean intercrop yielded more grain than Kura and red clover intercrops. Red clover intercrop yielded the least amount of grain, and was lower than the control, high N, and berseem clover and soybean intercrops. Red clover and alfalfa intercrops yielded less grain than other cropping systems in the Spring 2017 planting in the first year of harvest (2018). In the second year (2019), red clover yielded less than the weed free treatment. In the Fall of 2017 planting, in the first year of harvest, all cropping systems yielded the same amount of grain. In the second year (2019), red clover was the lowest yielding, and less than the control, low and high N, and berseem clover and soybean intercrops. The soybean intercrop was the highest yielding cropping system, and yielded more than red clover.

Soil was tilled before planting soybean, and soybean forage yields were low (Figure 5), which suggests the effect of this intercrop may have been due to the soil tillage and not the soybean crop. Pinto et al. (2021) found an increase in grain yield per row resulting from the mechanical thinning of a Kernza stand done with a rototiller. Law et al. (2020) found an increase in grain production after fall strip-tillage of Kernza, but no effect of spring tillage.

No studies have been made that evaluate grain yields of intercropping red clover, Kura clover, berseem clover or soybeans with IWG, but other species have been studied, especially alfalfa. Dick et al. (2018) found that interseeding IWG with alfalfa, sweet clover or white clover did not affect IWG grain yields. Tautges et al. (2018) found that in an intercrop with alfalfa, IWG grain yields were reduced in sites where alfalfa growth is favored, and usually the intercrop yielded less than the fertilized and unfertilized monocultures. This changes by the fourth year of harvest, where IWG grain yield of the alfalfa biculture is the same or higher than the fertilized monoculture. They found a positive correlation between alfalfa biomass production in year 3 and grain yield, harvest index and nutrient uptake in year 4, suggesting the effect of alfalfa on IWG may be cumulative.

In the Fall 2016 planting, the three perennial legume intercrops showed a decline in grain yield from the first to the second harvest. From the second to the third harvest, on the other hand, all cropping systems showed a decline, except alfalfa and red clover. In the Spring 2017 planting, the control, low N, and Kura clover, berseem clover, and soybean intercrops showed a grain yield decline from the first to the second harvest. In the Fall of 2017 planting, the cropping systems that showed a grain yield decline were weed free, low N, alfalfa, red clover and soybean.

Weik et al. (2002) found that white clover under-sown in IWG stands could improve grain yield persistence, probably due to greater availability of nitrogen from N_2 fixation. Tautges et al. (2018) found that in an intercrop with alfalfa, the biculture presented less grain yield decline than the monoculture and fertilized monoculture over four years of harvest.

Intermediate wheatgrass forage was reduced by legume intercrops in the Fall 2016 planting in the second year of harvest (2018) in the red clover and Kura clover intercrops. In the Spring 2017 planting, the alfalfa intercrop IWG forage yield was less than the control in the second year of harvest (2018). In the same planting date, intercropping with red clover resulted in lower IWG yields in 2018 and 2019. In the Fall 2017 planting, the only intercrop that had an effect over IWG forage was red clover in the second year of harvest (2019), where a reduction in yield was observed.

Over all the planting dates and harvest years of the experiment, the legume intercrops that had an effect over IWG forage were red clover, Kura clover and alfalfa. All these intercrops reduced IWG forage yield, but the effects were not consistent through all the planting dates or years.

Legume forage yields that showed differences through different harvests within a planting date were: red clover and Kura clover, that increased with time in all planting dates; berseem, that tended to decrease its yields with time in two of the planting dates; and alfalfa, that increased its yields only in one planting date and year.

Dick et al. (2018) found that interseeding IWG with alfalfa did not affect IWG seed or biomass yields, and alfalfa forage yield didn’t vary in the two harvests of the experiment. This differs from our findings of alfalfa intercrop reducing IWG forage yields in one planting date - year, and alfalfa forage increasing in another planting date - year. The experiment by Dick et al. was planted in the fall, with the alfalfa interseeded in the next spring, and the effect of alfalfa over IWG forage in our experiment occurred in the spring planting, when both species were planted a week apart. Planting IWG in the fall will give it time to establish and grow before adding another species in the intercrop.

Tautges et al. (2018) found that in the second year of harvest, a fertilized IWG monoculture yielded more IWG forage than an alfalfa - IWG biculture and an unfertilized IWG monoculture, but by the fourth year, the biculture yielded the same as the fertilized monoculture and more than the unfertilized monoculture. In an experiment with IWG and legume mixtures (alfalfa, birdsfoot trefoil and kura clover), the mixture with alfalfa produced the highest total forage yield, and alfalfa had the lowest yield decline. Birdsfoot trefoil and kura clover were less vigorous than alfalfa in mixture with IWG (Sleugh et al., 2000).

For all the legume species that were used, there are a several cases where adding the legume forage to the IWG forage makes the intercrop as a whole yield more than the control (Figure 5). This indicates that legume intercrops have the potential to increase total biomass yields in a Kernza dual purpose system, what presents a benefit for farmers with dual purpose systems.

In general, there was competition by the legumes in the intercrops. This could have been caused by high seeding rates used (Table 2), which were closer to the recommended rates for pure stands than for mixes (Duiker & Curran, 2007; Hackney et al., 2000; Min, 2011; NRCS-USDA, n.d.; Undersander, n.d.). Therefore, lower legume seeding rates should be used in next experiments. Any species considered as an intercrop should preferably be a less competitive perennial, or an annual with fast establishment and initial growth.

Legumes were not mowed or harvested in the spring, which could have been a factor in the competition between legumes and IWG. Future IWG intercrop research should include mowing or harvesting of forage during the spring, to determine whether this practice can increase grain or forage yields. This is compatible with the dual purpose use of Kernza, that includes forage harvests in the spring and fall.

Considering that strip tillage in the fall could improve yields (Law et al., 2020), and the observed competition by legumes, one practice that should be considered in future trials is the strip tillage of the stand in the fall after harvest, and at the same time the planting of an annual legume that would grow in the fall and senesce in winter, liberating the nitrogen captured by root nodules (Walley et al., 1996). This would provide fixation of nitrogen and other benefits associated to diversity, while at the same time minimizing competition between species. In the case of using alfalfa or other perennial crop, these could be tested in a planting after the first year of harvest to reduce competition with IWG, but the potential benefit of nitrogen liberation from root nodules would be reduced (Walley et al., 1996).

Nitrogen

The hypothesis that IWG grain and forage biomass would be higher at higher rates of nitrogen fertilization was not rejected. Higher nitrogen fertilization rates had a positive effect on yields; however, this was not observed in all planting seasons-years. High and low nitrogen fertilization treatments did not show yield differences among each other, or when compared to the control. In some of the planting season - harvest year combinations, these treatments yielded more than the intercrop with red clover, and in one harvest year they yielded more than the alfalfa intercrop. The contrast analysis showed that for the Fall 2016 planting season in the 2019 harvest, both yielded more than the two unfertilized monocultures (control and without weeds).

Soil analyses done at the beginning of the experiment showed that there isn't a nitrogen deficit in the planted site. April 18 2017 soil samples averaged 5.1 ppm of NO_3-N and 21.1 ppm of

NH_4-N in the top 15 cm, which could explain the lack of an effect of nitrogen on grain yields. The differences with red clover are better explained by the fast and competitive growth observed in this species (Figure 5): it was the only species that presented a reduction in IWG grain yields in all planting seasons. High soil fertility at the beginning of the experiment, paired with incidence of lodging (observed in the field) and high variability of the obtained yields, could explain the lack of an effect of nitrogen fertilization on grain yields in this experiment. This lack of nitrogen limitation might also explain why IWG was not benefited by the nitrogen fixation provided by legumes.

Tautges et al. (2018) observed in general greater yields in a fertilized monoculture than in an unfertilized monoculture. In a study by Fernandez et al. (2020), nitrogen applications of 0 to 80 kg N ha^{-1} had no effect on grain yield in the first year, but increased yields in years 2 and 3. Frahm et al. (2018) observed that nitrogen fertilizer application of 80 kg N ha^{-1} decreased grain yields in one of five site-year combinations when compared to 40 kg N ha^{-1}, but both were similar to the unfertilized treatment. They suggest high lodging rates at this site-year as a possible explanation for this decrease.

There were no differences in IWG forage yield between the two different nitrogen fertilization rates or the control for any of the planting seasons and harvest years. The contrast analysis showed that forage yields were higher for the nitrogen treatments than for the control and weed free treatments in the third year of the Fall 2016 planting and the second year of the Fall 2017 planting (both in 2019). Forage yields didn’t show differences as the stand ages, except in Spring 2017, where the first year presented lower yields in both fertilization rates, and increased in the second, maintaining yields in the third. This pattern is consistent with the control and other treatments, and yields were low due to the short time for plant growth between establishment in spring and harvest in summer. The two fertilization rates used yielded more IWG forage than the red clover, alfalfa and Kura clover intercrops in some of the planting season-year combinations.

Fernandez et al. (2020) observed a positive response of IWG biomass yield to increasing rates of nitrogen (from 0 to 20, 40, 60 and 80 kg N ha^{-1}). Tautges et al. (2018) observed an increase in IWG straw in most of their sites during the four years of experiment, with fertilization rates of 60 kg N ha^{-1} in the second year and 80 kg N ha^{-1} in the third and fourth. Frahm et al. (2018) observed an increase in IWG biomass yields with nitrogen fertilization of 80 kg N ha^{-1} in only one of the studied site-years, and no effect with a rate of 40 kg N ha^{-1}. These studies show that there is a tendency of nitrogen application to increase IWG forage yields, but there are some exceptions. Our results don't show this tendency so clearly. As with the pattern observed for grain yield, nitrogen was probably not a limiting nutrient at the beginning of the experiment, so its application didn't show an effect, except in the contrast analysis with the unfertilized monocultures.

Weed management

The hypothesis that IWG grain and forage biomass would be higher by removing weeds was rejected. There wasn't an effect of removing weeds on yield. Grain yield of plots where weeds were removed did not show any differences from the control in any of the planting seasons and years that were harvested. In some planting season-year combinations, these plots yielded more than alfalfa, red clover or Kura clover. Grain yield decline showed an inconsistent response to this treatment. The contrast analysis showed that the weed free and control yielded higher than the perennial legume intercrops in four planting season-years, and they yielded less than the nitrogen treatments in one case. Zimbric et al. (2020) found no effect of biweekly weed removal from IWG plots on grain yields in second or third year harvests, suggesting weed competition is not a problem for this crop. Our findings are consistent with this study; however, most of the weeds present at the beginning of the experiment were annuals, so further research should be done that includes IWG weed management in stands with a high presence of perennial weeds.

The cropping system without weeds did not show any differences in IWG forage yield with the control, and yields higher than in the red clover, Kura clover or alfalfa intercrops in some cases in the Fall 2016 and Spring 2018 plantings. There wasn't a decline of yields as the stand aged; the control declined in the third year of the Fall 2016 planting. The contrast analysis showed that the control and weed free treatments forage yields were higher than the perennial legume cropping systems in three planting season-year combinations; lower than the nitrogen treatments in two combinations; and lower than the soybean intercrop in one case. Zimbric et al. (2020), similar to their findings on grain yield, found no effect of biweekly weed removal from IWG plots on IWG forage yields in second or third year harvests, and no effect on yield decline. Our results are similar to this study; however, the removal of weeds appears to have prevented forage yield decline in one planting season-year.

5. Conclusions

The effect of row spacing was inconsistent, but in some environments the wider row spacing had higher grain and IWG forage yields. Intercropping with legumes decreased grain yields in some environments, although total biomass might be higher in the intercrops. The effect of nitrogen fertilization increased yields in some of the planting seasons and years. There wasn't an effect of weed removal on yields.

Based on these results, more research is needed to determine optimal row spacings and other factors that have an interaction with this variable, such as lodging, light interception, or below ground competition. Regarding legume intercrops, due to the important potential benefits of incorporating nitrogen fixing crops into perennial grain crop production, it is crucial to find species and intercrop management techniques that improve grain yields, or contribute to stabilizing yields over time. Nitrogen fertilization is recommended only in soils with low levels of this nutrient, in order to reduce negative environmental impacts from nitrate leaching and reduce unnecessary costs. Finally, weed removal in stands with high presence of annual weeds is not necessary for this crop, since it is highly competitive against weeds.

Considering all the potential benefits of this dual purpose perennial grain crop system, research should continue in order to find the best management for this crop.

6. Literature

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7. Appendix

Table A 1. IWG grain for each cropping system by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

Cropping system	Planting season: Fall 2016 2017	Fall 2016 2018	Fall 2016 2019	Spring 2017 2018	Spring 2017 2019	Fall 201 2018	Fall 201 2019
	kg ha^{-1}						
Control	632 a	432 abc	23 g	934 a	118 cdef	711 abcd	149 de
Weed free	569 a	398 abcd	35 fg	740 ab	156 bcde	943 a	98 ef
Low N	596 a	374 abcd	55 fg	642 abc	94 def	963 ab	138 de
High N	647 a	451 abc	63 efg	311 abcde	67 def	344 abcde	138 de
Alfalfa	561 a	233 bcde	90 efg	20 fg	80 def	669 abcd	75 ef
Red clover	494 ab	167 def	43 fg	3 g	20 fg	778 abc	19 f
Kura clover	663 a	184 cdef	9 g	397 abcd	50 efg	382 abcde	100 ef
Berseem clover	592 a	456 abc	62 efg	906 a	62 ef	675 abcd	164 cde
Soybean	630 a	495 ab	40 fg	802 ab	94 def	1002 a	179 bcde

Means followed by a common letter are not significantly different by Tukey’s honest significant difference test at the 5% level of significance.

Table A 2. IWG forage for each cropping system by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

Cropping system	Planting season: Fall 2016 2017		Fall 2016 2018		Fall 2016 2019		Spring 2017 2017		Spring 2017 2018		Spring 2017 2019		Fall 2017 2018		Fall 2017 2019	
	kg ha^{-1}															
Control	4517	abc	4140	abcd	1368	fgh	671	bcd	4398	a	2702	a	3577	a	2853	a
Weed free	3893	abcd	3711	abcde	2090	cdefg	635	bcd	3563	a	3141	a	4229	a	2222	ab
Low N	4671	abc	4025	abcd	2644	abcdefg	654	bcd	4064	a	4278	a	4620	a	3788	a
High N	5395	a	5143	a	3418	abcdefg	427	d	2963	ab	3376	a	4763	a	3925	a
Alfalfa	4044	abcd	2329	bcdefg	2642	abcdefg	571	cd	386	cd	2661	a	2671	ab	2392	ab
Red clover	3355	abcdef	850	gh	1828	defg	238	d	14	d	638	bcd	3767	a	677	b
Kura clover	4333	abcd	1446	efgh	311	h	467	d	3989	a	2349	abc	4842	a	2700	a
Berseem clover	3841	abcd	4317	abcd	2702	abcdefg	386	d	3998	a	2680	a	2939	ab	3876	a
Soybean	4487	abc	4974	ab	1928	defg	547	cd	3816	a	2905	a	3764	a	3913	a

Means followed by the same letter are not significantly different by Tukey's honest significant difference test at the 5% level of significance.

Table A 3. Legume forage for each intercropping system by planting season and year of harvest for Kernza intermediate wheatgrass and legume intercropping experiment at Arlington, WI.

Cropping system	Planting season: Fall 2016 2017		Fall 2016 2018		Fall 2016 2019		Spring 2017 2017		Spring 2017 2018		Spring 2017 2019		Fall 2017 2018		Fall 2017 2019	
	kg ha^{-1}															
Alfalfa	661	cdef	872	cd	801	cde	567	cdef	1391	bcd	936	cde	125	de	1313	bc
Red clover	1771	bc	1004	c	2882	ab	843	cde	1644	abc	3360	ab	467	cd	4577	a
Kura clover	857	cde	1936	bc	4734	a	157	def	1096	cde	4234	a	36	de	2061	b
Berseem clover	964	cd	36	g	163	defg	1311	c	137	def	27	f	1	e	107	de
Soybean	69	fg	24	g	112	efg	169	def	40	ef	9	f	16	de	1	e

Means followed by the same letter are not significantly different by Tukey's honest significant difference test at the 5% level of significance.