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Effects of intercropping perennial legumes on intermediate wheatgrass productivity

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ABSTRACT

Context: Intermediate wheatgrass (*Thinopyrum intermedium*; IWG) is a perennial, cool-season grass undergoing domestication as a grain and forage crop. Intercropping perennial legumes with IWG could reduce synthetic N fertilizer inputs, improve forage quality, and provide pollinator resources. However, legumes may also compete with IWG for resources, the extent to which may depend on traits associated with legume species identity and IWG planting density.
Objective: Our objectives were to determine the effects of IWG planting density and legume species identity on IWG grain and vegetative biomass yield in an intercropping system.
Methods: A field trial with IWG planted at two densities, each as monocultures and bicultures with four intercropped legume species, was conducted two sites in Minnesota, USA for two years. Grain and vegetative biomass were measured from the IWG, and vegetative biomass was measured from the legumes. The legume species tested were alfalfa (*Medicago sativa*), Canada milkvetch (*Astralagus canadensis*), Illinois bundleflower (*Desmanthus illinoensis*), and white clover (*Trifolium repens*).
Results: High planting density increased IWG grain and biomass at most site-year combinations. Legume biomass in the intercrop was highly variable among species, sites, and years, yet rarely did it reduce IWG grain and biomass yields below that of the unfertilized IWG monoculture. There was no consistent negative effect of intercropping legumes on total biomass productivity, and when we used the relative yield metric to quantify the land-use efficiency of growing IWG in an intercrop with legumes, we found that for most site-years there was a neutral or positive effect of intercropping legumes. Alfalfa was the most productive legume, with a maximum yield of 5993 kg ha^{-1} at one site-year, and across all treatments higher legume biomass was associated with lower grain yields.
Significance: This study demonstrates that legumes can be intercropped with IWG to generate both grain and forage while possibly limiting synthetic nitrogen inputs. Although vigorously growing legumes may impose competitive effects causing a decline in IWG grain production, competition may not reduce IWG vegetative biomass or overall biomass productivity.

1. Introduction

In natural ecosystems, increases in species richness and functional diversity generally increase productivity and resource-use efficiency (RUE) (Tilman et al., 2012). In contrast, conventional agroecosystems are dominated by monocultures of annual crops whose shallow roots and high fertilizer inputs contribute to low RUE and lead to environmental issues (Isbell et al., 2017). As a primary resource for crop production, synthetic fertilizer accounts for approximately two thirds (~120 Tg N yr^{-1}) of annual anthropogenic nitrogen inputs to terrestrial ecosystems, the majority of which is lost as nitrate (NO_3^-) pollution into waterways or as the potent greenhouse gas nitrous oxide (N_2O) (Fowler et al., 2013). The ‘leakiness’ of nutrients in conventional cropping systems has substantial negative human health and climate impacts. Increasing plant diversity in agriculture by intercropping biologically N fixing (BNF) legumes alongside other crops, a practice referred to as intercropping, can reduce the use of synthetic fertilizers (Li et al., 2023), as well as provide pollinator resources (Boetzl et al., 2023), reduce disease impacts (Zhang et al., 2019), and increase weed suppression (Hauggaard-Nielsen et al., 2001).

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Perennial grain-legume cropping systems may be well-suited for intercropping because their multi-year lifespan allows for opportunities to synchronize nitrogen supply and demand between the legume and non-legume crop (Crews and Peoples, 2005; Duchene et al., 2017). Perennial grains are currently under development in several research and breeding programs around the world because they can reduce erosion and nutrient runoff relative to annual crops. Continuous cover by perennials physically protects soil from wind and water erosion while extensive root systems increase nutrient and water uptake thus limiting leaching and volatilization of unused N (Cox et al., 2002; Glover and Reganold, 2010). Intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host.) Barkw. & D.R. Dewey], a cool-season, sod-forming grass, is one such perennial grain crop, and the first to be approved for sale as a food-grade grain crop in the United States (Bajgain et al., 2020). Although N fertility requirements have been investigated for IWG grain production (Jungers et al., 2017; Dobbratz et al., 2023), few studies have determined the impact of legume intercropping for sustainable N provisioning. There remain major gaps in our understanding of the mechanisms by which N is transferred between the legume and perennial grain crop, or agronomic management strategies that facilitate the uptake of biologically-fixed N by the perennial grain crop.

Much of what is known about the nutrient cycling and plant productivity of legume-grass intercrops has been learned from studying forage systems, and to a lesser extent grain systems (Ofori and Stern, 1987; Thilakarathna et al., 2016). Belowground N transfer from legumes to non-legumes happens primarily through three pathways: the decomposition of roots and nodules (Fustec et al., 2010), the exudation of soluble N-containing compounds from legume roots (Paynel and Cliquet, 2003), and mycorrhizal-mediated transfer of N between plants (He et al. 2009). A meta-analysis of 29 field-scales studies of legume-cereal grain intercrops in temperate agroecosystems found that intercropping increased N_2 fixation by legumes by on average 14 % and as a consequence, N acquisition in cereals by 25 % (Rodriguez et al., 2020). When protein output is a primary goal, legume-cereal intercropping tends to increase yield relative to monocultures (Li et al., 2023).

Intercropping perennial legumes with perennial cereal grain crops can provide many of the benefits observed in annual legume-cereal intercrops, such as reduced N limitation and dependence on synthetic fertilizers (Ryan et al., 2018). Specifically, perennial legume-cereal intercrops maintain deep roots over years which can enhance soil N uptake and soil organic N accumulation (Crews et al., 2022). Additionally, perennial forage legumes like alfalfa have been found to fix more N, or fix more consistently, than annual legumes (Schipanski and Drinkwater, 2012), and the perennial nature of the system may allow for greater synchrony between the N fixation of the legume and the uptake of the accompanying grass crop (Bybee-Finley and Ryan, 2018). The addition of a legume intercrop can also improve overall forage nutritive value, increasing the likelihood that the cropping system can be managed to produce both grain and forage (Law et al., 2022).

In perennial legume-cereal intercrops, the quantity of N transfer from the legume to non-legume depends on numerous factors which are not well-understood. These factors may vary by legume species identity and include traits such as productivity (Reilly et al., 2022), phenology, tissue chemistry, adaptability, and stand persistence (Crews et al., 2022). To develop economically viable perennial grain-legume intercrops, more research is needed on the performance of different legume species and planting and management strategies including plant density. Therefore, the objective of this study was to evaluate the effects of intercropping four legume species, each paired with intermediate wheatgrass (IWG) and grown in two plant density treatments, on biomass, grain yield, and overall aboveground productivity (IWG and legume biomass). Two species that are commonly grown as forages in the region were selected: alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*). Additionally, two species that are native to the upper midwestern United States were selected - Canada milkvetch (*Astralagus canadensis*) and Illinois bundleflower (*Desmanthus illinoensis*) - as native plant species may particularly be important for supporting native pollinator communities. Trials were conducted at two sites over two years using an additive intercrop design, where legumes were seeded into a plot of IWG that had been planted the previous fall. We tested the following hypotheses: 1) the introduction of a legume intercrop increases IWG growth and reproduction, and decreases grain C:N ratio, relative to an unfertilized monoculture control; 2) increased IWG density increases competition within and between the IWG and legume species and causes a reduction in grain yield and overall biomass; 3) greater competitive interactions between IWG and legume species induces stem elongation as part of a shade avoidance strategy and thus increases IWG plant height.

2. Methods

2.1. Site description

The experiment was conducted under non-irrigated conditions at two sites: in Swift, MN (48.874925° N, 95.157180°W), and Roseau, MN (48.877867° N, 95.848230° W). Soils in Roseau are a Zippel very fine sandy loam (coarse-silty, mixed, superactive, calcareous, frigid Typic Endoaquolls) with an average organic matter of 2.8 % and a nitrate N, Olsen P, and soil test K of 67, 4, 95 ppm in the top 15 cm. The soils in Swift are a Percy loam (coarse-loamy, mixed, superactive, frigid Typic Calciaquolls) with an average organic matter of 3.8 % and a nitrate N, Olsen P, and soil test K of 24, 15, and 92 ppm in the top 15 cm. 30-year monthly average low temperature is −15.7C in January and average monthly high temperature is 19.0C in July. 30-year average cumulative precipitation is 649 mm (Table 1).

2.2. Experimental design and establishment

The experiment was a randomized complete split-plot design, with four replicates at each site. Legume intercrop treatment was the whole-plot factor with five levels: no intercrop (monoculture), alfalfa (*Medicago sativa*; ALF), Canada milkvetch (*Astralagus canadensis*; CMV), Illinois bundleflower (*Desmanthus illinoensis*; IBF), and white clover (*Trifolium repens*; WC). Plant density of the intermediate wheatgrass at establishment was applied as either 15 cm row spacing (hereafter referred to as 'high density') or 61 cm row spacing (hereafter referred to as 'low density') as the split-plot factor (Fig. 1). This experimental design was chosen to test the effects both of spacing and intercrop type and their interaction on productivity. Row spacing was selected as the split-plot factor because it was easier to apply in a small area and less likely to be affected by inherent field variability than application of legume type. Each experimental unit (split-plot) was 1.5 m x 6.1 m and separated by a 1.5 m alley between plot rows and no space between plot columns. The seedbed was prepared in mid-August using a disc followed by a field cultivator and then a cultipacker. Intermediate wheatgrass was seeded at 9 kg pure live seed (PLS) ha^{-1} for both plant density treatments on 28 August 2014 using a cone seeder to sow seeds at an approximate depth of 20 mm with 15 cm spacing between rows. Rows were thinned to achieve the approximate 61 cm spacing in the wide-row treatments on 18 September 2014. Intermediate wheatgrass seed was from the fourth breeding cycle for increased grain yield from the Land Institute in Salina, KS (for full description of breeding program see DeHaan et al. (2018)). Legume seed was pre-weighed for each plot to achieve a target seeding rate of alfalfa (16.8 kg PLS ha^{-1}, white clover (6.7 kg PLS ha^{-1}), Illinois bundleflower (11.2 kg PLS ha^{-1}), and Canada milkvetch (13.5 kg PLS ha^{-1}). Alfalfa and white clover were seeded on 15 September 2014 and Illinois bundleflower and Canada milkvetch were frost-seeded by hand into the plots on 1 April 2015. Seed of the native legume species was expected to have more dormancy, thus a frost seeding approach was used to impose cold stratification to improve germination. An additive design was used for two reasons. First, IWG is the primary crop of

Table 1
Monthly and 30-year averages for both temperature and cumulative precipitation in Roseau, MN, USA during the duration of the experiment. Data from the North Dakota Agricultural Weather Network (NDAWN).

	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
	Average Temperature (C)											
2014	NA	NA	NA	NA	NA	NA	NA	18.8	13.8	7.3	−7.5	−8.0
2015	−13.6	−18.0	−1.9	6.0	11.2	17.3	20.3	18.4	16.2	8.1	0.4	−6.3
2016	−13.6	−10.0	−0.3	3.8	13.8	17.7	20.1	19.5	15.0	7.8	4.2	−12.1
30-year Average	−15.7	−13.1	−5.2	3.4	11.2	16.8	19.0	18.0	13.1	5.3	−3.6	−11.6
	Cumulative Precipitation (mm)											
2014	NA	NA	NA	NA	NA	NA	NA	25	54	17	0	0
2015	0	0	0	6	81	63	129	30	39	32	0	0
2016	0	0	0	26	49	127	98	85	108	15	0	0
30-year Average	19	15	23	36	80	107	95	91	73	59	29	23

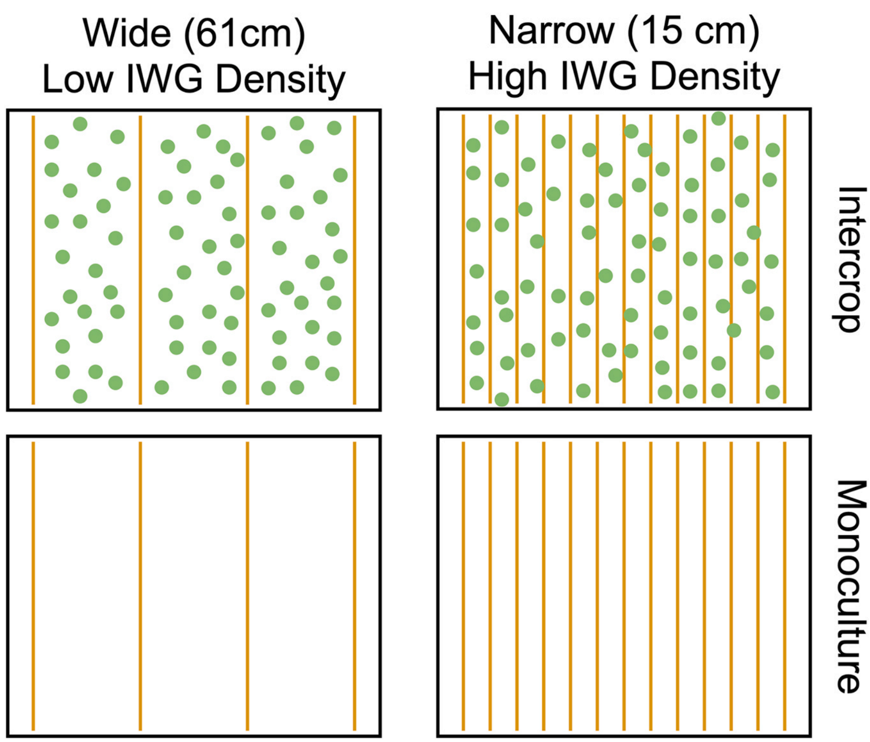

Fig. 1. The intercrop treatment was established with an additive design, where legumes were seeded on top of IWG rows, and the total plant density is thus higher in the intercrop than the monoculture. 15 cm IWG rows were thinned to achieve a low IWG density (shown on left) of 61 cm. Because seeding rate per IWG row was constant, overall IWG plant density is higher in the 15 cm row spacing. The green circles in the diagram represent the intercropped legumes and the yellow lines represent rows of IWG. Items in this figure are not to scale.

interest and legumes alone have low economic value in the study region; thus, an additive design was appropriate for focusing on the effects of intercropping on IWG grain production. Monocultures of the legume species were omitted because of space constraints and the lack of economic viability for these as a stand-alone crop. Second, broadcast seeding legumes over the top of an established IWG stand is more agronomically feasible and a realistic approach to intercropping compared to sowing both species together in alternating rows, which can require specialized seeding equipment. The combination of drilling seeds of the grain species and broadcasting seeds of the legume species is best tested with an additive design.

2.3. Aboveground biomass, grain yield, and plant height

IWG grain and biomass yields were measured annually on 17 August 2015 and 15 August 2016 when the plants reached physiological maturity. At the same dates as harvest in 2016, 5 randomly selected stems with spikes were measured from the soil surface to the top of the spike in each plot. At harvest, a 91 × 46 cm quadrat was placed at three locations within each plot and all grain and biomass was harvested within the quadrat. All spikes were clipped off and weighed. The remaining IWG biomass was cut within the quadrat leaving a stubble of 7.5 cm, bagged, and weighed wet in-field. Legume biomass was cut within each quadrat leaving a 7.5 cm stubble and weighed wet in-field. All seed head samples were dried at 45°C for a minimum of three days before processing. The grain was threshed from the dry IWG seed heads, cleaned of chaff and weighed. A subset of biomass samples was dried at 60°C for a minimum of three days before being weighed to determine moisture content. The average moisture content was determined and subtracted from the biomass wet weights to determine yield on a dry matter basis. Grain carbon (C) and N were determined via combustion analysis at a commercial lab. All remaining biomass left in the plots after hand-sampling for yield was cut to a height of 7.6 cm and raked out of the research plots. No other defoliation events occurred.

2.4. Relative yield/partial land equivalency ratio

In all analyses, the partial land equivalent ratio (PLER), also known as 'relative yield' (RY), was calculated and used as response variable. PLER is the relative yield of an intercropped species compared to its yield when grown in monoculture (Ofori and Stern, 1987). PLER/relative yield (RY) is defined as:

$$RY = \frac{P}{M}$$

where P and M represent yield of the species in intercrop and sole crop respectively. While the land equivalent ratio (LER) is commonly used metric to assess the efficiency of land use of an intercrop, PLER can be interpreted as a measure for the contribution of each species to the efficiency of land use by the cropping system as a whole. Since the experimental design of this study did not include growing the legume in monoculture, we only calculate PLER for IWG biomass and IWG grain yield.

2.5. Statistical analysis

Analysis of variance was conducted using mixed effects models to explain variation in IWG grain yield, IWG vegetative biomass yield, legume biomass yield, harvest index (grain mass/total aboveground dry mass), IWG stem height, and relative yield. Predictor variables were year, site, plant density, and legume intercrop treatment. For all analyses, replicate was included as a random effect along with legume treatment nested within replicate to account for the split-plot design. Three-way and two-way interactions were significant for most response variables. Grain yield was affected by a three-way interaction of intercrop treatment, plant density, and year, as well as plant density, year, and site. Legume yield was affected by a three-way interaction between legume, year, and site (Supplementary Table 1). Therefore, all further analyses were conducted separately for each site and year. Assumptions of the homogeneity of variance were assessed separately for each site-year with Levene's test and visual inspection of residuals plots. These assumptions were violated for legume biomass in all site-years. Therefore, we performed a square root transformation on legume biomass and performed further analyses using the transformed variable, and results were back transformed for presentation. A square root transformation was chosen because visual inspection of the residuals indicated that the variance was proportional to the mean. Other distributions were tested but the square root resulted in a distribution of the residuals that best met the model assumptions. Mixed model analyses and post-hoc pairwise comparison tests were carried out using the statistical software program R (Version 4.1.2) including *nlme* and *emmeans* packages. One-sample t-tests were conducted on relative yields (RY) to test if the estimates were significantly different than 1.

Analysis of variance to explain variation in relative grain yield decline (calculated as the difference in grain yields between the first and second year divided by grain yields in the first year) was also conducted with mixed effect models with plant density and intercrop treatment as fixed effects. The effects of legume intercrop treatment on IWG grain C:N ratio at all sites and years were also tested using a linear mixed effect model with replicate as a random effect, to test whether the legume intercrop influenced the N status of the IWG plant. Analysis of variance was conducted using mixed effect models to explain variation in IWG grain yield and IWG biomass in 2015 with legume biomass and legume species as fixed effects, and replicate as a random effect, and analyses were separated by site. Analysis of variance was conducted using mixed effect models to explain variation in IWG grain yield and IWG biomass in 2016 with legume biomass in 2015 and legume biomass in 2016 as fixed effects, and analyses were separated by site.

3. Results

3.1. Intermediate wheatgrass grain yield

There was substantial variability in grain yield between years and there was a strong effect of both year and site on grain yield (Supplementary Table 1). Both years had comparable monthly average temperatures to the 30-year averages, but 2015 was a particularly dry year, with cumulative precipitation substantially lower than in 2016 and the 30-year average in June, August, September, and October (Table 1).

Yields decreased between year 1 and year 2, on average, by 87 % in Roseau and 77 % in Swift (Tables 2–5). There was no effect of intercrop treatment in Roseau in 2015 (Table 2), but there was in Roseau in 2016, where the IWG grain yield in the alfalfa biculture had 67 % lower yield than in the monoculture (Table 3). In Swift 2015, the IWG in the alfalfa intercrop had a 64 % lower grain yield than the monoculture and was significantly lower than all other treatments (Table 4), but there was no effect in Swift 2016 (Table 5). IWG plant density also did not have a significant effect on grain yield at most site years, except at Roseau in 2015, where the high plant density had a significantly higher grain yield (Table 2). There were no interactions between intercrop treatment and plant density in any site-year. At both Roseau ($F_{(4,26)} = 2.86$, $p = 0.04$) and Swift ($F_{(4,26)} = 2.86$, $p = 0.03$), the presence of a legume intercrop had little effect on relative grain yield decline (calculated as the difference in grain yields between the first and second year divided by grain yields in the first year) when compared to the monoculture control. When compared pairwise to the monoculture control, there were no significant differences (Sidak post-hoc tests, $p < 0.05$) in either Roseau or Swift.

3.2. Intermediate wheatgrass biomass

The effect of intercrop treatment on IWG biomass varied between the two sites. There was no effect of intercrop treatment at Roseau in both years. There was a strong effect of intercrop treatment on IWG biomass at the Swift site in both years, although the magnitude and direction of this effect varied between years. In 2015, IWG biomass in the alfalfa treatment was 48 % lower than the IWG biomass in the monoculture, and there was no significant difference between the monoculture and all other intercrop treatments. In contrast, in 2016, the IWG biomass in the alfalfa treatment was 77 % higher than in the monoculture, and the other three intercrop treatments had significantly higher IWG biomass than the monoculture, as well (Tables 3, 4).

At both Roseau and Swift, there was no effect of plant density in 2015 but there was a strong effect of plant density on IWG biomass in the second year of both sites, with 3.6 times higher IWG biomass in the high plant density compared to the wide spacing at Swift and 3.1 times higher IWG biomass in the high plant density at Roseau. There was no interaction between plant density and intercrop treatment in the Roseau site, but in the Swift site, there was an interaction between intercrop treatment and plant density in 2016, where the increase in IWG biomass in the intercrops compared to the monoculture was greater in the high plant density than the low plant density (Fig. 2). In the high plant density, IWG biomass was greater when intercropped with alfalfa compared to Canada milkvetch.

3.3. Legume biomass

Across both sites and years, there was significant variation in legume biomass among legume species in the intercrop (Tables 1–4). The alfalfa intercrop was the most productive, with on average nine-times greater biomass than the other legume species in 2015 and two-times greater biomass than the other legume species in 2016 (Fig. 3). There was no effect of plant density on legume biomass across intercrop treatments.

Table 2
ANOVA analyses for Roseau in 2015 with predictor variables of legume intercrop and plant density treatments. One-sample t-tests were conducted on relative yields (RY) to test if the estimates were significantly different than 1. RY < 1 indicates intercrop competition while RY < 1 indicates facilitation. Pairwise Tukey means comparisons tests were conducted for all other variables, and treatments that share a letter are not significantly different from one another. * indicates $p < .05$, ** indicates $p < .005$, *ns* is non-significant $p > 0.05$.

	Grain Yield (kg ha^{-1})	IWG Veg. (kg ha^{-1})	Legume Veg. (kg ha^{-1})	Harvest Index	NPP (kg ha^{-1})	RY Grain	RY Biomass
ANOVA							
Legume (L)	ns	ns	*	ns	ns	ns	ns
IWG density (D)	**	ns	ns	**	ns	ns	ns
L×D	ns	ns	ns	ns	ns	ns	ns
Legume[a]							
NONE	754 a	14360 a	NA	0.049 a	15115 a	NA	NA
ALF	619 a	12358 a	2327 a	0.045 a	15305 a	0.901 ns	0.900 ns
CMV	915 a	16284 a	355 ab	0.054 a	17554 a	1.396 ns	1.252 ns
IBF	872 a	16785 a	262 b	0.050 a	17919 a	1.287 ns	1.266 ns
WC	832 a	13953 a	86 b	0.056 a	14872 a	1.166 ns	1.067 ns
IWG Density							
High	968	14985	495	0.060	16449	1.132 ns	1.148 ns
Low	614	14418	736	0.040	15768	1.155 ns	1.035 ns

[a] None = IWG monocultures, no legume intercrops; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*)

Table 3
ANOVA analyses for Roseau in 2016 with predictor variables of legume intercrop and plant density treatments. One-sample t-tests were conducted on relative yields (RY) to test if the estimates were significantly different than 1. RY < 1 indicates intercrop competition while RY < 1 indicates facilitation. Pairwise Tukey means comparisons tests were conducted for all other variables, and treatments that share a letter are not significantly different from one another. * indicates $p < .05$, ** indicates $p < .005$, *ns* is non-significant $p > 0.05$.

	Grain Yield (kg ha^{-1})	IWG Veg. (kg ha^{-1})	Legume Veg. (kg ha^{-1})	Harvest Index	NPP (kg ha^{-1})	IWG Height (cm)	RY Grain	RY Biomass
ANOVA								
Legume (L)	*	ns	*	*	ns	*	*	ns
IWG density (D)	ns	**	ns	**	**	*	ns	ns
L×D	ns	ns	ns	ns	ns	ns	ns	ns
Legume[a]								
NONE	130 a	5271 a	NA	0.031 ab	5401 a	90.3 ab	NA	NA
ALF	43 b	4093 a	3147 a	0.016 b	7283 a	97.7 b	0.371 **	0.780 **
CMV	64 ab	4414 a	2345 a	0.023 ab	6823 a	82.9 a	0.636 **	0.826 **
IBF	117 ab	4295 a	1128 a	0.034 a	5540 a	84.2 a	1.165 ns	0.836 ns
WC	109 ab	4579 a	1339 a	0.032 ab	6026 a	91.7 ab	1.232 ns	0.956 ns
IWG Density								
High	87	6919	1513	0.012	8520	82.3	0.879 ns	0.922 *
Low	98	2142	1670	0.042	3909	96.4	0.879 ns	0.839 ns

[a] None = IWG monocultures, no legume intercrops; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*)

Table 4
ANOVA analyses for Swift in 2015 with predictor variables of legume intercrop and plant density treatments. One-sample t-tests were conducted on relative yields (RY) to test if the estimates were significantly different than 1. RY < 1 indicates intercrop competition while RY < 1 indicates facilitation. Pairwise Tukey means comparisons tests were conducted for all other variables, and treatments that share a letter are not significantly different from one another. * indicates $p < .05$, ** indicates $p < .005$, *ns* is non-significant $p > 0.05$.

	Grain Yield (kg ha^{-1})	IWG Veg. (kg ha^{-1})	Legume Veg. (kg ha^{-1})	Harvest Index	NPP (kg ha^{-1})	RY Grain	RY Biomass
ANOVA							
Legume (L)	**	*	**	*	ns	**	*
IWG density (D)	ns	ns	ns	ns	ns	ns	*
L×D	ns	ns	ns	ns	ns	ns	ns
Legume[a]							
NONE	638 a	10843 a	NA	0.058 ab	11676 a	NA	NA
ALF	232 b	5734 b	5993 b	0.039 b	11959 a	0.389 **	0.567 **
CMV	678 a	11030 a	696 a	0.058 ab	12405 a	1.229 ns	1.207 ns
IBF	685 a	9966 a	584 a	0.065 a	11235 a	1.177 ns	1.050 ns
WC	668 a	9524 ab	467 a	0.069 a	10659 a	1.100 ns	0.961 ns
IWG Density							
High	624	10080	1615	0.058	12356 *	0.908 ns	0.870 ns
Low	536	8759	1481	0.057	10817 *	1.049 ns	1.044 ns

[a] None = IWG monocultures, no legume intercrops; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*)

Table 5
ANOVA analyses for Swift in 2016 with predictor variables of legume intercrop and plant density treatments. One-sample t-tests were conducted on relative yields (RY) to test if the estimates were significantly different than 1. RY < 1 indicates intercrop competition while RY < 1 indicates facilitation. Pairwise Tukey means comparisons tests were conducted for all other variables, and treatments that share a letter are not significantly different from one another. * indicates $p < .05$, ** indicates $p < .005$, *ns* is non-significant $p > 0.05$.

	Grain Yield (kg ha^{-1})	IWG Veg. (kg ha^{-1})	Legume Veg. (kg ha^{-1})	Harvest Index	NPP (kg ha^{-1})	IWG Height (cm)	RY Grain	RY Biomass
ANOVA								
Legume (L)	ns	**	*	*	**	**	ns	*
IWG density (D)	ns	**	ns	**	**	**	**	ns
L×D	ns	**	ns	ns	0.065	ns	ns	ns
Legume[a]								
NONE	31	2675 a	NA	0.017 ab	2706 a	79 a	NA	NA
ALF	111	4709 b	3196 a	0.029 a	8015 c	91 b	5.327 ns	1.726 **
CMV	75	3763 b	1831 ab	0.018 ab	5669 b	83 ab	3.890 ns	1.433 **
IBF	23	3821 b	1305 b	0.008 b	5148 b	76 a	1.239 ns	1.355 **
WC	46	3860 b	1285 b	0.015 ab	5190 b	83 ab	3.124 ns	1.423 **
IWG Density								
High	71	5905	1400	0.011	7376	77.5	4.570 **	1.475 **
Low	43	1626	1646	0.024	3315	87.8	1.262 ns	1.299 **

[a] None = IWG monocultures, no legume intercrops; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*)

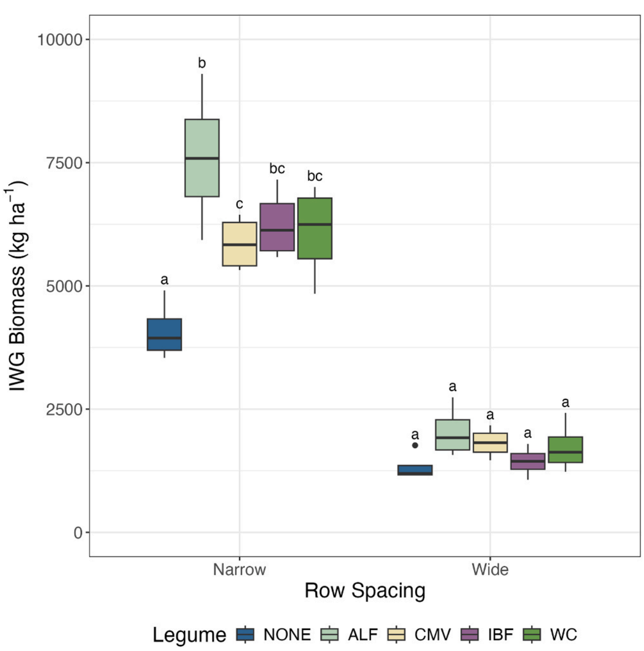

Fig. 2. At the Swift site in 2016 only, there was an interaction between plant density and intercrop treatment for IWG biomass, where the difference between the monoculture and all other intercrop treatments was greater in the high plant density than the low ($p < .005$). Shared letters indicate no significant difference between treatments within a given site-year (to the level of $p > 0.05$). Intercrop treatment abbreviations: NONE = IWG monocultures, no legume intercrop; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*).

3.4. Harvest index

Harvest index is the ratio of grain mass to total aboveground dry mass. At all site-years except Roseau 2015, there was an effect of intercrop treatment on the harvest index of IWG, where the alfalfa treatment had a significantly lower harvest index than the Illinois bundleflower and white clover treatments (Tables 1–4). At both Roseau and Swift, there was a strong effect of plant density in only the second year, in which the low plant density had a significantly higher harvest index.

3.5. Net primary productivity

Net primary productivity (NPP) is the sum of the aboveground biomass of both the legume intercrop and IWG. In both years at Roseau, there was not an effect of intercrop treatment on NPP. At Swift, there was not an effect of intercrop treatment on NPP in 2015 (Table 4), but there was in 2016, where the alfalfa-IWG biculture had three times greater NPP than the monoculture, and significantly higher NPP than all other legume intercrops (Table 5). At Roseau in 2015, there was no effect of plant density on NPP (Table 2), but at Roseau in 2016, the high plant density had 2.2 times the NPP of the low plant density (Table 3). A similar effect of plant density on NPP was observed at Swift, where there was no effect in 2015 (Table 4) but in 2016 the high plant density had on average 2.2 times the NPP of the low plant density (Table 5).

3.6. Relative yield (RY)/partial land equivalency ratio

The effects of plant density and intercrop treatment on RY varied between sites. In Roseau in 2015, there was no effect of plant density or intercrop treatment on the relative yield of IWG grain or biomass, and none of the treatments differed significantly from 1, meaning that there was no evidence that the intercrop represented a positive or negative change in the land use efficiency of IWG production (Fig. 4 Table 2). In Roseau in 2016, there was no effect of plant density on any measure of RY. There was no effect of intercrop treatment on the RY of vegetative biomass, but there was an effect on RY grain, in which the alfalfa intercrop has a significantly lower relative yield than the white clover or Illinois bundleflower treatments. Both the alfalfa and Canada milkvetch treatments had relative yields of biomass and grain that were significantly less than 1 (Table 3).

In Swift, the effect of plant density on RY of grain varied between years. In 2015, the low-density treatment had a higher relative yield of biomass than the high-density treatment (Fig. 4Table 4). In 2016, the high plant density had a higher RY of grain than the low plant density (Table 5). The relative yield of both grain and biomass in the alfalfa treatment increased dramatically between 2015 and 2016. In 2015, alfalfa had on average the lowest relative yield in terms of both grain and biomass, significantly lower than the Illinois bundleflower and white clover treatments in terms of grain and Illinois bundleflower and Canada milkvetch in terms of biomass production (Table 4). In 2016, in contrast, the alfalfa treatment had on average the highest relative yields of all the legume treatments. In 2016, all intercrop treatments had relative yields of biomass significantly higher than 1 (Table 5).

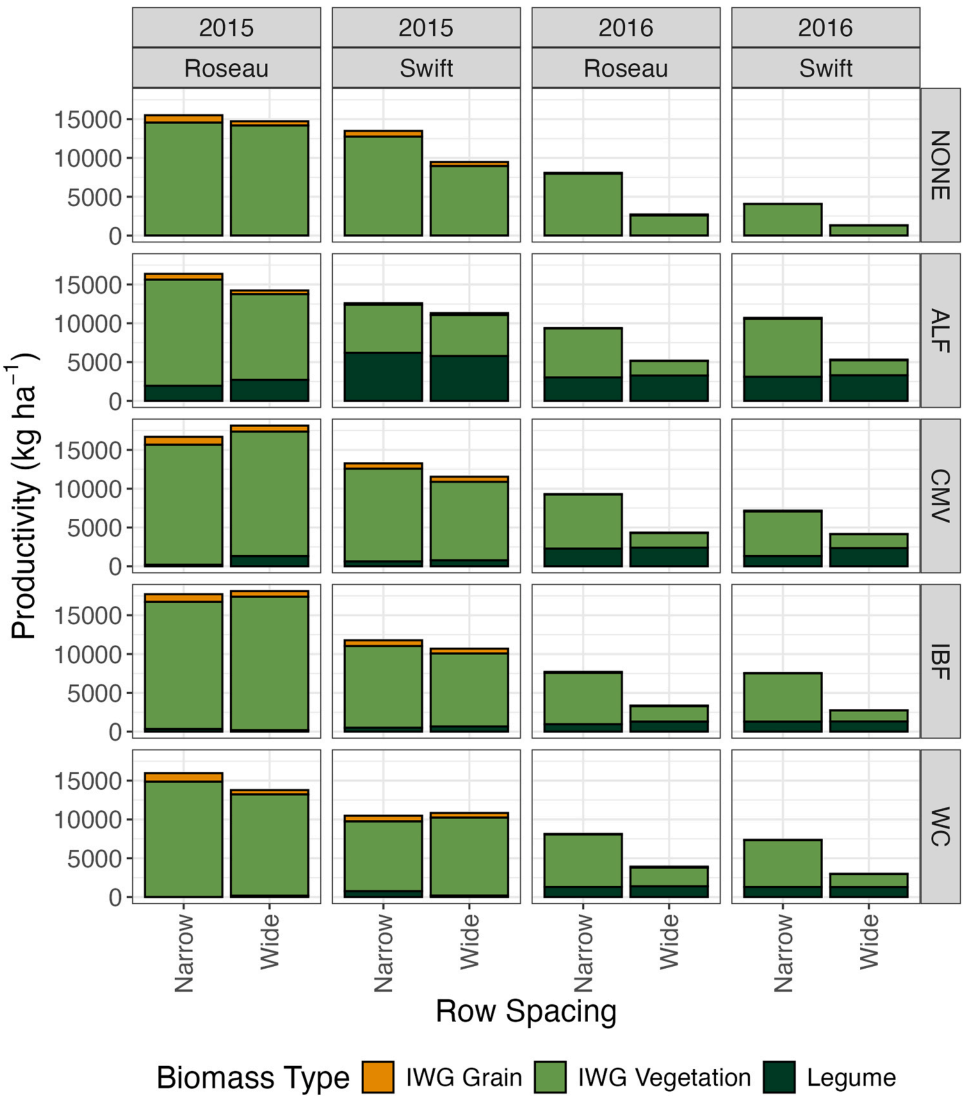

Fig. 3. The alfalfa intercrop produced the greatest overall biomass of all the legume species. Total biomass production varied between both years and sites. Weights are averaged across replicates and separated by plant density, site, and year. Intercrop treatment abbreviations: NONE = IWG monocultures, no legume intercrop; ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*).

3.7. *Traits associated with grain-legume interactions: IWG height, grain C:N ratio, and legume biomass*

i. *Intermediate wheatgrass stem height*

Intermediate wheatgrass stem height was measured at both sites in 2016 only. At both sites, plant height differed between intercrop treatments, and the IWG in the alfalfa intercrop was on average the tallest intercrop and significantly taller than the IWG in the Illinois bundleflower treatment in both Swift and Roseau (Tables 2, 4). The presence of an intercrop did not affect IWG height compared to the monoculture control, except for the IWG in the alfalfa treatment in Roseau, which was on average taller by 12 cm than the monoculture control (Table 3). In both Roseau and Swift, the IWG plants in the low-density treatment were significantly taller, by 10 and 14 cm, respectively, than in the high-density treatment.

ii. *Grain C:N ratio*

To determine if the presence of a legume intercrop would influence the nitrogen status of IWG, we assessed the C:N ratio of the IWG grain in the wide spacing at both sites in 2015 and 2016. The IWG monoculture was not included. We found that the C:N ratio did not differ significantly between intercrop treatments ($F_{3,\,44} = 0.586$, $p = 0.646$).

iii. *Legume biomass*

In addition to analyzing legume biomass as a response variable (see Section 3.3 above), which may be affected by both plant density and legume species, here we analyzed legume biomass as an explanatory variable to test the hypothesis that increased legume biomass causes heightened competition with IWG and thus a decline in IWG grain yield and biomass. In 2015, there was a negative effect of legume biomass on both IWG grain yield and biomass at both sites (Fig. 5 Table 6). The lack of significant interaction between legume species and biomass in this model indicates that this relationship was consistent for all four legume species.

In 2016, fewer relationships were observed between legume biomass and IWG grain and biomass compared to 2015. There was no effect of legume biomass on IWG vegetative biomass in either site. At Swift, there was also no effect of legume biomass on grain yield, but at Roseau, higher legume biomass in both the prior year and current year was inversely correlated with IWG grain yield (Fig. 5 Table 7). This inverse linear relationship was significant even when two points with high leverage, where the legume yield was zero, were removed from the analysis ($F_{1,13} = 18.899$, $p < 0.005$).

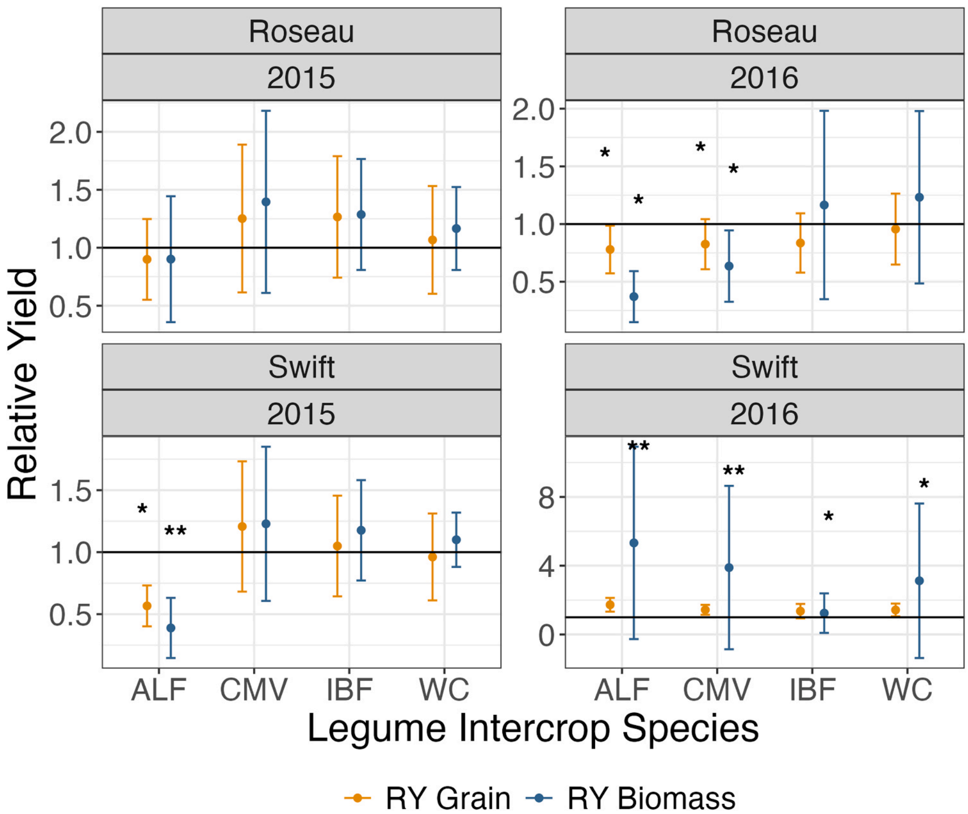

Fig. 4. Relative yield of IWG grain and vegetative biomass in Swift and Roseau in 2015 and 2016. Points indicate means over replicates and plant density and error bars indicate standard error of the mean. A one-sample *t*-test was performed on each group to test whether it differed significantly from 1, which is marked with the horizontal black line on all plots. Significant divergence from 1 indicates that the IWG grown in an intercrop contributed to greater efficiency of land use (greater than 1) or less efficient land use (less than 1). ** indicates $p < 0.005$ and * indicates $p < 0.05$. Intercrop species abbreviations: ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Atragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*).

4. Discussion

4.1. *Effect of Legume Intercrop on IWG*

For most of the legume species, intercropping did not impose competitive effects on IWG in ways that decreased IWG biomass and/or grain yield compared to monocultures. However, the results suggest that these relationships can be highly variable among legume species, sites, and years. We found that two of the four intercrop species - Illinois bundleflower and white clover - can be intercropped with IWG without a negative effect on IWG biomass. For these two intercropped treatments, the IWG biomass did not differ significantly from the monoculture in any site-year combination. This was largely true for grain yield, as well, except for Roseau in 2016, where the presence of the intercrop was linked to lower grain yields for all intercrop treatments. These results support the findings of Reilly et al. (2022) and Dick et al. (2018) who found a similar lack of change in IWG grain yield when clover was intercropped.

In contrast to Illinois bundleflower and white clover, the alfalfa intercrop more often had negative impacts on both IWG grain and biomass, and it was also the legume with the greatest biomass. Alfalfa was more productive than the other legume species in this study, with nine times greater biomass than other legume species in the first year and two times greater biomass in the second year (Fig. 3). Productive growth, especially in the first year, may have allowed the alfalfa to compete for light, soil water, and/or soil nutrients more efficiently than IWG, thus leading to net competitive effects on IWG. At Swift, the dominance of alfalfa relative to IWG decreased in the second year, which resulted in IWG biomass yields being greater than those in the monoculture. This competitive dominance of alfalfa in an IWG intercrop has been observed by Dick et al. (2018) in the third year of an intercrop study compared to white clover, although they did not observe any negative effects on IWG grain and biomass yield compared to the monoculture.

In this study, other legume intercrops likely imposed some competitive effects, but to a lesser extent than alfalfa. We found that across all legume intercropping treatments, legume biomass was negatively correlated with IWG grain yield and biomass in the first year (Fig. 5). Legumes with greater biomass in the first year may have competed for light or other soil resources without providing facilitative benefits. The legumes may also have allocated more resource to rapid growth than to N fixation, which can be costly. However, the ability to model the relationship between legume biomass and IWG grain in this study is limited. In the first year, there was poor legume establishment in several plots, which may have increased variation within treatments. In the second year, IWG grain yield across all treatments was greatly reduced, which may have masked other treatment effects. Reilly et al. (2022) also found an inverse relationship between IWG grain yield and legume intercrop biomass. Additionally, in their study, legume biomass in the first year was positively correlated with grain yield and N transfer in the third year, which they speculate may have been because plots with high legume biomass in the first year allowed for greater root growth and tissue inputs following senescence, facilitating N transfer and higher grain yields in subsequent years. This finding suggests that had the present study continued into a third or fourth year, we may have seen evidence of facilitation in treatments with high first-year legume biomass. Delayed facilitative effects of alfalfa on IWG have been observed in other studies (Crews et al., 2022).

The land use efficiency of growing IWG in an intercrop with legumes was quantified using the relative yield (RY) metric, also known as partial

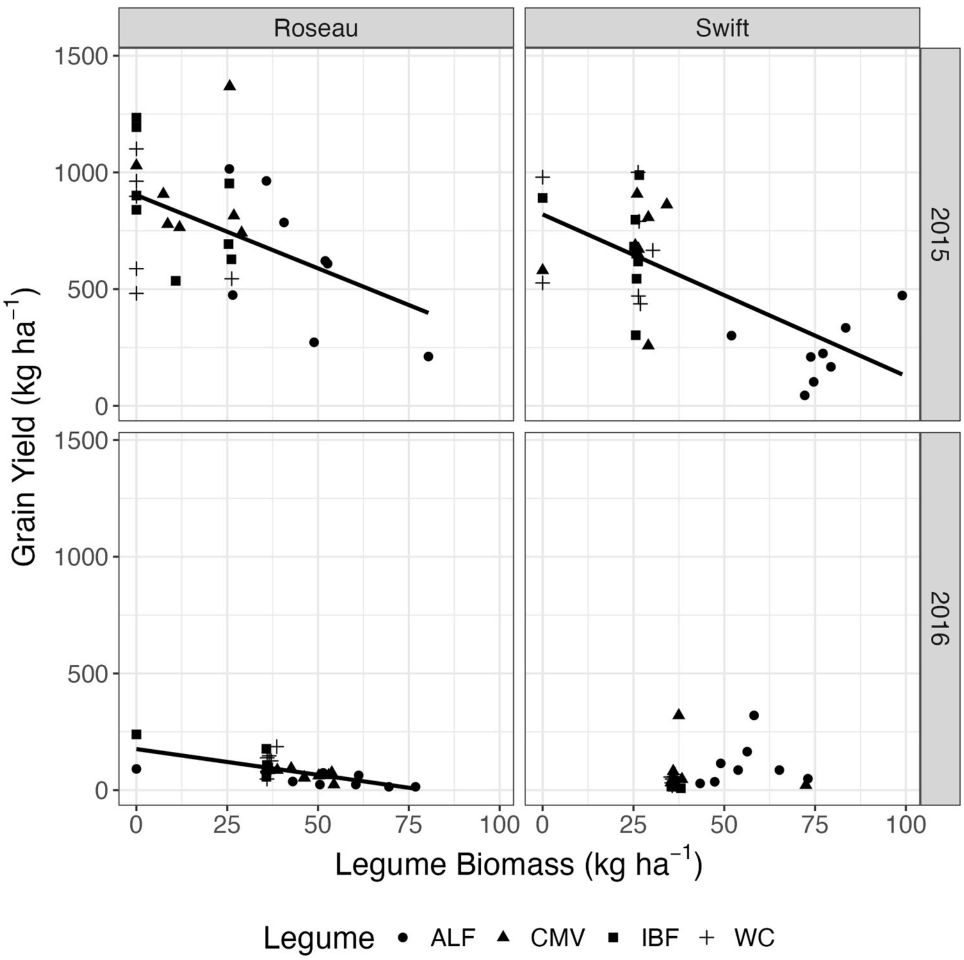

Fig. 5. The effect of legume biomass on IWG grain yield at Roseau 2015 ($F_{1,14} = 8.5925$, $p < 0.05$), Swift 2015 ($F_{1,11} = 30.57$, $p < 0.005$), Roseau 2016 ($F_{1,12} = 40.46$, $p < 0.005$), and Swift 2016 (non-significant). Legume biomass was square-root transformed. Points are plot-level observations, and the solid line is the regression line plotted on top of the field observations. There was no significant relationship between legume yield and grain yield in Swift in 2016. Intercrop species abbreviations: ALF = alfalfa (*Medicago sativa*); CMV = Canada milkvetch (*Astragalus canadensis*); IBF = Illinois bundleflower (*Desmanthus illinoensis*); WC = white clover (*Trifolium repens*).

Table 6
Statistical significance (*= $p < 0.05$, ** = $p < 0.005$, *ns* is non-significant) of legume biomass as influenced by legume species on IWG grain and biomass yield at Swift and Roseau in 2015.

	Fixed Effects	Grain Yield	IWG Biomass
Swift 2015	legume biomass	*	**
	legume species	ns	ns
	legume species: biomass	ns	ns
Roseau 2015	legume biomass	**	**
	legume species	ns	ns
	legume species: biomass	ns	ns

land use equivalency, in which 1 represents no improvement in land use equivalency in the intercrop compared to the monocrop. Relative yield did not vary among the monocrop and intercrop treatments in the first year at Roseau, which may be due in part to the relatively slow establishment of all legume species compared to the IWG (Fig. 3). In contrast, in the first year at Swift, there was evidence that competition with alfalfa caused a decline in both IWG grain and biomass in the intercrop. The change in relative yield of IWG grain production between years was largest for the alfalfa intercrop in Swift, which increased an average of 0.389–5.327 between the first and second years (Fig. 4). These findings support the hypothesis that benefits of intercropping legumes into grains may take multiple years to come to fruition. Crews et al. (2022) observed RY increase from 0.8 to 3.4 in IWG-alfalfa intercrop over 5 years. They found no significant difference in the influence of above- and below-ground alfalfa inputs to IWG productivity but speculate that delays in facilitative effects may be due to the fact that legume N-fixation and root turnover into bioavailable forms of N often takes multiple years.

Table 7
Statistical significance (*= $p < 0.05$, ** = $p < 0.005$, *ns* is non-significant) of legume biomass in 2015 and 2016 on IWG grain and biomass yield at Swift and Roseau in 2016.

	Fixed Effects	Grain Yield	IWG Biomass
Swift 2016	2015 legume biomass	ns	ns
	2016 legume biomass	ns	ns
	2015 legume biomass: 2016 legume biomass	*	ns
Roseau 2016	2015 legume biomass	**	ns
	2016 legume biomass	**	ns
	2015 legume biomass: 2016 legume biomass	0.065	ns

In the second year at Swift, where all the intercropped treatments had higher NPP than the monoculture treatment, it is likely that the legume was still imposing some competition for light, water, or nutrients. For example, we found that IWG plant height was greater when intercropped with alfalfa compared to the monoculture, which could have been a response to light limitation in the presence of a productive legume. However, that may have been offset by an increase in soil available N made possible by the biological N fixation of the legume, leading to an increase in total productivity. A limitation of this study is that we did not measure traits such as biological N fixation or N transfer between the legumes and grass, and thus we cannot identify mechanisms to explain why in Swift in the second year the presence of a legume increased overall productivity. However, Li et al. (2021), Reilly et al. (2022), and Crews et al. (2022) have detected transfers of N between legumes and IWG using ^{15}N natural abundance methods.

4.2. Effect of plant density on the IWG intercrop

Few studies have investigated the effect of plant density on IWG grain yield managed as a monoculture or intercrop. Averaged over intercropping treatments, plant density effects on IWG grain and biomass productivity were variable. It is important to note that the within-row plant density was the same for both treatments, so the high plant density had four times the number of plants per hectare than the low plant density (Fig. 1). However, we did not find four times the grain or biomass yield in the high plant density at all site-years as might be expected. As a tillering grass species, IWG has a propensity to spread and occupy open space, which likely occurred in this study. The high plant density treatment had greater biomass in the second year at both sites – 3.2 times higher in Roseau and 3.6 times higher in Swift.

There was an interaction between plant density and intercrop treatment at Swift on IWG biomass, where the difference between the monoculture and all other intercrop treatments was greater in the high plant density than the low plant density (Fig. 2). The addition of alfalfa into narrow rows increased IWG biomass relative to the IWG monoculture, but alfalfa intercropping did not affect IWG when managed with the wider rows (Fig. 2). It is possible that the high plant density decreased the distance between the species' root systems, facilitating greater transfer of N from the legume to the grass. Legume-grass N transfer has been shown to be greatest when roots are in closer proximity or in direct contact (Xiao et al., 2004; Meng et al., 2015), as close proximity reduces the distance rhizodeposits and other N compounds must travel through soil via mass flow. There was no interaction between plant density and legume intercrop for IWG grain, however. Taken together, these results suggest that optimal planting densities may be applicable to different intercropped legume species, and that higher density plantings may increase overall biomass for alfalfa intercrops, without a negative impact on grain yield.

4.3. Effect of intercrop on IWG plant height

We also hypothesized that higher plant density, taller plants, and more productive legume intercrops would increase competition for light and induce the IWG plant to grow taller to better compete for light. Stem elongation is a typical shade-avoidance response to crowding in dense stands that has been characterized in other plant species (Smith, 1982; Maliakal et al., 1999; Postma et al., 2021). Averaged across plant density treatments, alfalfa, which produced the greatest biomass, coexisted with IWG plants that were taller than in some of the other treatments (Tables 2, 4). High variability in legume abundance between sites and treatments prevented us from drawing the conclusion that competition with neighboring plants induced IWG to grow taller, but these findings suggest more perennial grass-legume intercrop studies should explore the effects of legume intercrop on stem height and leaf length.

We hypothesized that denser planting would be associated with increased plant height, as grasses are known to adopt a shade-avoidance strategy in which stems elongate to reach for light. Surprisingly, we found the opposite relationship between plant density and IWG plant height of what we expected. The low plant density treatment had taller IWG plants, by 10 cm and 14 cm at Swift and Roseau, respectively. These taller shoots may be a result of competition among tillers within a plant instead of between individual plants. Studies in other grasses have found that higher within-row tiller density can increase shoot and leaf length as a result of lower light levels in the canopy (Simic et al., 2009; Casal et al., 1985). Although seeding rate per row was the same in both the low and high plant density, it is possible that plants in the low-density treatment produced more aboveground biomass because of reduced intra-specific crowding from neighboring IWG rows. However, we did not measure within-row tiller density so we cannot test this hypothesis here. Although the effects of planting density on biomass allocation are highly variable between taxa and poorly understood (Poorter et al., 2012), reduced crowding may also reduce root:shoot ratio and encourage greater aboveground biomass production (Rehling et al. 2021). If this is true for IWG, then the low plant density treatment may have produced more tillers such that by the middle of the second growing season the stems were induced to grow taller to compete for light. Further studies could assess tiller production as well as total biomass to better characterize the plant's allocation response to density.

4.4. Agronomic performance

Overall, variability in legume biomass, IWG biomass, and IWG grain yield was high among all years and sites, which could have been related to management challenges associated with intercropping. Previous research has quantified the effect of delaying the date of seeding in the fall on IWG establishment and yield in northern Minnesota (Jungers et al., 2022), but little is known about the effect of fall seeding date on legume establishment. The seeding date of 15 September was considered late for forage legumes in northern Minnesota (Sheaffer et al., 2023), but the alfalfa established well based on first year biomass yield. The fall-seeded white clover yielded substantially less biomass than the alfalfa in the first year possibly because of poor establishment from late seeding. It is recommended that white clover be seeded about 40 days prior to a killing frost (Sheaffer et al., 2023; SARE), but this occurred only 25 days after seeding at the research sites used for this study. Native legumes like the Illinois bundleflower and Canada milkvetch used in this study exhibit seed dormancy and it is possible that temperature and moisture conditions may not have been suitable for breaking this dormancy.

Intercropping monocot and dicot species as we did in this study substantially limits the ability to rely on chemical herbicides for weed control as many herbicides have modes of action that target one of these groups of plants. We observed substantial weed pressure the second year, in which we also observed steep decline in grain yield across all sites and treatment. Future research should quantify the weed suppression capabilities of certain legume species when intercropped with IWG. Other agronomic practices such as early spring mowing and fall burning could suppress weeds and should be tested in IWG/legume intercropping systems.

All the treatments had substantially lower yields in the second year, and low overall yields in the second year may have masked treatment effects. Interannual yield decline in both intercropped and monoculture IWG is a well-documented issue (Zhen et al., 2023), which may be due to both N limitation (Fernandez et al., 2020) and competition between tillers in dense stands (Law et al., 2021), as well as other unknown factors. If competition for resources or light quality in the canopy was a driving factor in yield decline in this study, of which mixed evidence has been found in IWG and other cool season grasses (Pinto et al., 2021; Chastain et al., 1997), we might expect to see a strong effect of plant density on grain yield. The fact that decreased plant density was not correlated with a decline in grain yield suggests that nitrogen limitation may be the driving factor. Tautges et al. (2018) found that intercropping IWG with alfalfa reduced yield decline relative to the monoculture. It's possible that legumes did not prevent a yield decline in this study because N limitation was greater in these northern MN sites compared with locations in the Tautges et al. (2018) study.

Our findings that intercropping legumes, especially alfalfa, had an either positive or neutral impact on IWG biomass supports the viability of growing IWG with legumes for harvest as a dual-use grain and forage crop. Other studies have found additional benefits of intercropping clover and alfalfa with IWG for forage, including higher crude protein content and greater fiber digestibility (Favre et al., 2019; Pinto et al., 2021). The ability to harvest for forage could provide farmers with an additional source of revenue, especially on years that grain yields are low (Culman et al., 2023).

4.5. Future research directions

Longer-term studies that also measure N fixation and transfer, using ^{15}N natural abundance methods, are needed to better characterize interannual changes in facilitation between perennial grain intercrops and uncover their underlying mechanisms. The relative performance of the intercrops in this study was highly variable between the first and second years, and if the study had continued for 3–5 years, we would have likely observed further interannual variability. Similar studies of IWG-perennial legumes intercrop saw facilitation effects only on or after the third year (Tautges et al., 2018; Crews et al., 2022). This delay may be because N transfer to IWG happens in part through root turnover and mineralization, and it likely takes more than two years for the legumes to build a substantial root system that can lead to N inputs into the soil (Burity et al., 1989; Dubach and Russelle, 1994).

Management of crop residues may have influenced N dynamics and agronomic outcomes in this study. We removed all biomass from the plots after grain harvest as recommended for IWG monoculture grain production. The removal of aboveground biomass may have reduced the amount of N transfer from the legumes in this study. Because IWG-legume intercrops can be mulched and left in place for weed control and nitrogen addition, more research that examines the effect of cutting or mulching timing on IWG productivity would be useful. Herbage removal can affect nodule senescence and root turnover in legumes. For example, Butler et al. (1959) and Wilson (1942) found in greenhouse studies that shading and defoliation is linked to higher root and nodule turnover in white clover, red clover, and lotus. In this study, cutting and greater shading in the high plant density may have stimulated root turnover in the second year and contributed to N transfer between the legume and IWG.

Of the four legume species grown in this study, white clover and alfalfa are the most commonly grown as forages in Upper Midwest cropping systems. Thus, these species may be more accessible for farmers to grow in an intercrop with IWG, as Canada milkvetch and Illinois bundleflower seeds are substantially more expensive. Although prices for native seeds are highly variable, as of 2023 Minnesota seed companies were selling Canada milkvetch for approximately US $133 kg^{-1} (Prairie Moon Nursery, 2025) while white clover seed was sold for around US$10 kg^{-1} (Albert Lea Seed Company, 2025). However, Canada milkvetch and Illinois bundleflower are both native to the Great Plains and Great Lakes regions of North America, and thus may provide particular benefits for native pollinators and other wildlife. Integrating native perennial species into row-crop systems has been found to increase native pollinator and bird abundance (Asbjornsen et al., 2014; Schulte et al., 2016; 2017). This research suggests that it may be possible to integrate native legumes through intercropping, as well, with neutral to positive impacts on grass seed and biomass production.

5. Conclusions

High IWG density had few negative effects on productivity and would be recommended for ensuring ground cover and the probability of maximizing grain and biomass yields. We also found that although the performance of the intercrop was highly variable between sites, species, and years, rarely did the presence of an intercrop reduce grain and biomass yields below that of the unfertilized IWG monoculture. The fact that there was no consistent negative effect of intercropping legumes on net primary productivity is a benefit for dual-use grain and forage IWG producers, as legumes have higher nutritive value and digestibility than IWG biomass. Higher legume biomass was linked to lower grain yields across treatments, and alfalfa was the most productive intercrop, suggesting that vigorously growing legume intercrops can exert negative competitive effects on IWG, leading to reduced grain yield but not necessarily reduced biomass productivity. An economic assessment based on the current prices of forage and IWG grain (which vary by region and season) can be conducted to evaluate the tradeoffs of legume biomass and grain yield and inform producer decision making. At one site, relative yield increased from year 1 to 2, a shift which was largest for the alfalfa intercrop. This finding indicates that facilitation by legumes may require more than one year of growth to realize potential N benefits to IWG production, as mechanisms of nitrogen transfer such as root production, N fixation, and turnover may not be realized until at least the second year.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2025.109954.

Data availability

Data will be made available on request.

References

Albert Lea Seed Company. 2025. Ladino White Clover Seed for Sale. Albert Lea Seed. ⟨https://alseed.com/product/ladino-white-clover/⟩ (accessed 1 April 2025).

Asbjornsen, H., Hernandez-Santana, V., Liebman, M., Bayala, J., Chen, J., Helmers, M., Ong, C.K., Schulte, L.A., 2014. Targeting perennial vegetation in agricultural landscapes for enhancing ecosystem services. Renew. Agric. Food Syst. 29 (2), 101–125. https://doi.org/10.1017/S1742170512000385.

Bajgain, Prabin, Zhang, Xiaofei, Jungers, Jacob M., DeHaan, Lee R., Heim, Brett, Sheaffer, Craig C., Wyse, Donald L., Anderson, James A., 2020. MN-clearwater', the first food-grade intermediate wheatgrass (Kernza Perennial Grain) cultivar. J. Plant Regist. 14 (3), 288–297. https://doi.org/10.1002/plr2.20042.

Boetzl, Fabian A., Douhan Sundahl, Anna, Friberg, Hanna, Viketoft, Maria, Bergkvist, Göran, Lundin, Ola, 2023. Undersowing oats with clovers supports pollinators and suppresses arable weeds without reducing yields. J. Appl. Ecol. 60 (4), 614–623. https://doi.org/10.1111/1365-2664.14361.

Burity, H.A., Ta, T.C., Faris, M.A., Coulman, B.E., 1989. Estimation of nitrogen fixation and transfer from alfalfa to associated grasses in mixed swards under field conditions. Plant Soil 114 (2), 249–255. https://doi.org/10.1007/BF02220805.

Butler, G.W., Greenwood, R.M., Soper, Kathleen, 1959. Effects of shading and defoliation on the turnover of root and nodule tissue of plants of Trifolium repens, Trifolium pratense, and Lotus uliginosus. N. Z. J. Agric. Res. 2 (3), 415–426. https://doi.org/10.1080/00288233.1959.10418027.

Bybee-Finley, K.Ann, Ryan, Matthew R., 2018. Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture 8 (6), 80. ⟨https://doi.org/10.3390/agriculture8060080⟩.

Casal, J.J., Deregibus, V.A., Sánchez, R.A., 1985. Variations in tiller dynamics and morphology in Lolium multiflorum Lam. vegetative and reproductive plants as affected by differences in red/far-red irradiation. Ann. Bot. 56 (4), 553–559. https://doi.org/10.1093/oxfordjournals.aob.a087040.

Chastain, T.G., Kiemnec, G.L., Cook, G.H., Garbacik, C.J., Quebbeman, B.M., Crowe, F.J., 1997. Residue management strategies for kentucky bluegrass seed production. Crop Sci. 37 (6), cropsci1997.0011183X003700060029x. https://doi.org/10.2135/cropsci1997.0011183X003700060029x.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H., Jackson, W., 2002. Breeding perennial grain crops. Crit. Rev. Plant Sci. 21 (2), 59–91. https://doi.org/10.1080/0735-260291044188.

Crews, Timothy E., Peoples, Mark B., 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutr. Cycl. Agroecosystems 72 (2), 101–120. https://doi.org/10.1007/s10705-004-6480-1.

Crews, Timothy E., Kemp, Laura, Bowden, James H., Murrell, Ebony G., 2022. How the nitrogen economy of a perennial cereal-legume intercrop affects productivity: can synchrony be achieved? Front. Sustain. Food Syst. 6. ⟨https://www.frontiersin.org/articles/10.3389/fsufs.2022.755548⟩.

Culman, Steve, Pinto, Priscila, Pugliese, Jennie, Crews, Timothy, DeHaan, Lee, Jungers, Jacob M., Larsen, Jamie, et al., 2023. Forage harvest management impacts "Kernza" intermediate wheatgrass productivity across North America. Agron. J. 115 (5), 2424–2438.

DeHaan, Lee, Christians, Marty, Crain, Jared, Poland, Jesse, 2018. Development and evolution of an intermediate wheatgrass domestication program. Sustainability 10 (5), 1499.

Dick, C., Cattani, D., Entz, M.H., 2018. Kernza intermediate wheatgrass (Thinopyrum intermedium) grain production as influenced by legume intercropping and residue management. Can. J. Plant Sci. 98 (6), 1376–1379. https://doi.org/10.1139/cjps-2018-0146.

Dobbratz, Michelle, Jungers, Jacob M., Gutknecht, Jessica L.M., 2023. Seasonal plant nitrogen use and soil N pools in intermediate wheatgrass (*Thinopyrum intermedium*). Agriculture 13 (2), 468. https://doi.org/10.3390/agriculture13020468.

Dubach, Markus, Russelle, Michael P., 1994. Forage legume roots and nodules and their role in nitrogen transfer. Agron. J. 86 (2), 259–266. https://doi.org/10.2134/agronj1994.00021962008600020010x.

Duchene, Olivier, Vian, Jean-François, Celette, Florian, 2017. Intercropping with legume for agroecological cropping systems: complementarity and facilitation processes and the importance of soil microorganisms. A review. Agric., Ecosyst. Environ. 240 (March), 148–161. https://doi.org/10.1016/j.agee.2017.02.019.

Favre, Jeremie R., Castiblanco, Tatiana Munoz, Combs, David K., Wattiaux, Michel A., Picasso, Valentin D., 2019. Forage nutritive value and predicted fiber digestibility of kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. Anim. Feed Sci. Technol. 258 (December), 114298. https://doi.org/10.1016/j.anifeedsci.2019.114298.

Fernandez, Christopher W., Ehlke, Nancy, Sheaffer, Craig C., Jungers, Jacob M., 2020. Effects of nitrogen fertilization and planting density on intermediate wheatgrass yield. Agron. J. 112 (5), 4159–4170. https://doi.org/10.1002/agj2.20351.

Fowler, David, Coyle, Mhairi, Skiba, Ute, Sutton, Mark A., Cape, J.Neil, Reis, Stefan, Sheppard, Lucy J., et al., 2013. The global nitrogen cycle in the twenty-first century. Philos. Trans. R. Soc. B: Biol. Sci. 368 (1621), 20130164. https://doi.org/10.1098/rstb.2013.0164.

Fustec, Joëlle, Lesuffleur, Fabien, Mahieu, Stéphanie, Cliquet, Jean-Bernard, 2010. Nitrogen rhizodeposition of legumes. a review. Agron. Sustain. Dev. 30 (1), 57–66. https://doi.org/10.1051/agro/2009003.

Glover, Jerry D., Reganold, John P., 2010. Perennial grains: food security for the future. Issues Sci. Technol. 26 (2), 41–47.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Interspecific competition, N use and interference with weeds in pea–barley intercropping. Field Crops Res. 70 (2), 101–109. https://doi.org/10.1016/S0378-4290(01)00126-5.

He, Xinhua, Xu, Minggang, Qiu, Guo Yu, Zhou, Jianbin, 2009. Use of 15N stable isotope to quantify nitrogen transfer between mycorrhizal plants. J. Plant Ecol. 2 (3), 107–118. ⟨https://doi.org/10.1093/jpe/rtp015⟩.

Isbell, Forest, Adler, Paul R., Eisenhauer, Nico, Fornara, Dario, Kimmel, Kaitlin, Kremen, Claire, Letourneau, Deborah K., et al., 2017. Benefits of increasing plant diversity in sustainable agroecosystems. J. Ecol. 105 (4), 871–879. https://doi.org/10.1111/1365-2745.12789.

Jungers, J.M., Schiffner, S., Sheaffer, C., Ehlke, N.J., DeHaan, L., Torrion, J., Noland, R., Franco, J.G., 2022. Effects of seeding date on grain and biomass yield of intermediate wheatgrass. Agron. J. 114 (4), 2342–2351. https://doi.org/10.1002/agj2.21083.

Jungers, Jacob M., DeHaan, Lee R., Betts, Kevin J., Sheaffer, Craig C., Wyse, Donald L., 2017. Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. Agron. J. 109 (2), 462–472. https://doi.org/10.2134/agronj2016.07.0438.

Law, Eugene P., Pelzer, Christopher J., Wayman, Sandra, DiTommaso, Antonio, Ryan, Matthew R., 2021. Strip-tillage renovation of intermediate wheatgrass (Thinopyrum intermedium) for maintaining grain yield in mature stands. Renew. Agric. Food Syst. 36 (4), 321–327. https://doi.org/10.1017/S1742170520000368.

Law, Eugene P., Wayman, Sandra, Pelzer, Christopher J., Culman, Steven W., Gómez, Miguel I., DiTommaso, Antonio, Ryan, Matthew R., 2022. Multi-criteria assessment of the economic and environmental sustainability characteristics of intermediate wheatgrass grown as a dual-purpose grain and forage crop. Sustainability 14 (6), 3548. https://doi.org/10.3390/su14063548.

Li, Chunjie, Stomph, Tjeerd-Jan, Makowski, David, Li, Haigang, Zhang, Chaochun, Zhang, Fusuo, van der Werf, Wopke, 2023. The productive performance of intercropping. Proc. Natl. Acad. Sci. 120 (2), e2201886120. https://doi.org/10.1073/pnas.2201886120.

Li, Shoujiao, Jensen, Erik Steen, Liu, Nan, Zhang, Yingjun, Dimitrova Mårtensson, Linda-Maria, 2021. Species interactions and nitrogen use during early intercropping of intermediate wheatgrass with a white clover service crop. Agronomy 11 (2), 388. https://doi.org/10.3390/agronomy11020388.

Maliakal, Satya K., McDonnell, Kristin, Dudley, Susan A., Schmitt, Johanna, 1999. Effects of red to far-red ratio and plant density on biomass allocation and gas exchange in impatiens capensis. Int. J. Plant Sci. 160 (4), 723–733. https://doi.org/10.1086/314157.

Meng, Lingbo, Zhang, Aiyuan, Wang, Fei, Han, Xiaoguang, Wang, Dejiang, Li, Shumin, 2015. Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front. Plant Sci. 6. ⟨https://www.frontiersin.org/articles/10.3389/fpls.2015.00339⟩.

Ofori, Francis, Stern, W.R., 1987. Cereal–Legume Intercropping Systems. In: Brady, N.C. (Ed.), Advances in Agronomy, 41. Academic Press, pp. 41–90. https://doi.org/10.1016/S0065-2113(08)60802-0.

Paynel, Florence, Cliquet, Jean, 2003. N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds. Agronomie 23 (5–6), 503–510. https://doi.org/10.1051/agro:2003022.

Pinto, Priscila, De Haan, Lee, Picasso, Valentin, 2021. Post-harvest management practices impact on light penetration and kernza intermediate wheatgrass yield components. Agronomy 11 (3), 442. https://doi.org/10.3390/agronomy11030442.

Poorter, Hendrik, Niklas, Karl J., Reich, Peter B., Oleksyn, Jacek, Poot, Pieter, Mommer, Liesje, 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193 (1), 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.

Postma, Johannes A., Hecht, Vera L., Hikosaka, Kouki, Nord, Eric A., Pons, Thijs L., Poorter, Hendrik, 2021. Dividing the pie: a quantitative review on plant density responses. Plant, Cell Environ. 44 (4), 1072–1094. https://doi.org/10.1111/pce.13968.

Prairie Moon Nursery. 2025. Astragalus canadensis - Canada Milk Vetch. ⟨https://www.prairiemoon.com/astragalus-canadensis-canada-milk-vetch⟩ (Accessed 1 April 2025).

Rehling, Finn, Sandner, Tobias M., Matthies, Diethart, 2021. Biomass partitioning in response to intraspecific competition depends on nutrients and species characteristics: a study of 43 plant species. J. Ecol. 109 (5), 2219–2233. https://doi.org/10.1111/1365-2745.13635.

Reilly, Evelyn C., Gutknecht, Jessica L., Tautges, Nicole E., Sheaffer, Craig C., Jungers, Jacob M., 2022. Nitrogen transfer and yield effects of legumes intercropped with the perennial grain crop intermediate wheatgrass. Field Crops Res. 286 (October), 108627. https://doi.org/10.1016/j.fcr.2022.108627.

Rodriguez, Carolina, Carlsson, Georg, Englund, Jan-Eric, Flöhr, Adam, Pelzer, Elise, Jeuffroy, Marie-Hélène, Makowski, David, Jensen, Erik Steen, 2020. Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. Eur. J. Agron. 118 (August), 126077. https://doi.org/10.1016/j.eja.2020.126077.

Ryan, Matthew R., Crews, Timothy E., Culman, Steven W., DeHaan, Lee R., Hayes, Richard C., Jungers, Jacob M., Bakker, Matthew G., 2018. Managing for multifunctionality in perennial grain crops. BioScience 68 (4), 294–304. https://doi.org/10.1093/biosci/biy014.

Schipanski, Meagan E., Drinkwater, Laurie E., 2012. Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant Soil 357 (1), 147–159. https://doi.org/10.1007/s11104-012-1137-3.

Schulte, Lisa A., MacDonald, Anna L., Niemi, Jarad B., Helmers, Matthew J., 2016. Prairie strips as a mechanism to promote land sharing by birds in industrial agricultural landscapes. Agric. Ecosyst. Environ. 220 (March), 55–63. https://doi.org/10.1016/j.agee.2016.01.007.

Schulte, Lisa A., Niemi, Jarad, Helmers, Matthew J., Liebman, Matt, Gordon Arbuckle, J., James, David E., Kolka, Randall K., et al., 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. Proc. Natl. Acad. Sci. 114 (42), 11247–11252. https://doi.org/10.1073/pnas.1620229114.

Sheaffer, Craig C., Ehlke, Nancy J., Albrecht, Kenneth A., Peterson, Paul R., 2023. Forage Legumes. Clovers, Birdsfoot Trefoil, Cicer Milkvetch, Crownvetch and Alfalfa. In: Minnesota Agricultural Experiment Station Bulletin, 608, 2018.

Simic, Aleksandar, Vuckovic, Savo, Maletic, Radojka, Sokolovic, Dejan, Nenad Djordevic, 2009. The impact of seeding rate and inter-row spacing on Italian Ryegrass for seed in the first harvest year. Turk. J. Agric. For. 33 (5), 425–433. https://doi.org/10.3906/tar-0812-12.

Smith, H., 1982. Light quality, photoperception, and plant strategy. Annu. Rev. Plant Physiol. 33 (1), 481–518. https://doi.org/10.1146/annurev.pp.33.060182.002405.

Sustainable Agriculture Research and Education, 2023."White Clover: Trifolium repens."⟨https://www.sare.org/publications/managing-cover-crops-profitably/legume-cover-crops/white-clover/⟩. Accessed 1 April 2025.

Tautges, Nicole E., Jungers, Jacob M., DeHaan, Lee R., Wyse, Donald L., Sheaffer, Craig C., 2018. Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. Bi-culture with Alfalfa in the Upper Midwestern USA. J. Agric. Sci. 156 (6), 758–773. https://doi.org/10.1017/S0021859618000680.

Thilakarathna, Malinda S., McElroy, Michel S., Chapagain, Tejendra, Papadopoulos, Yousef A., Raizada, Manish N., 2016. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. Agron. Sustain. Dev. 36 (4), 58. https://doi.org/10.1007/s13593-016-0396-4.

Tilman, David, Reich, Peter B., Isbell, Forest, 2012. Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. Proc. Natl. Acad. Sci. 109 (26), 10394–10397. https://doi.org/10.1073/pnas.1208240109.

Wilson, J.K., 1942. The loss of nodules from legume roots and its significance. Agron. J. 34 (5), 460–471. https://doi.org/10.2134/agronj1942.00021962003400050006x.
Xiao, Yanbo, Li, Long, Zhang, Fusuo, 2004. Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15N techniques. Plant Soil 262 (1), 45–54. https://doi.org/10.1023/B:PLSO.0000037019.34719.0d.
Zhang, Chaochun, Dong, Yan, Tang, Li, Zheng, Yi, Makowski, David, Yu, Yang, Zhang, Fusuo, van der Werf, Wopke, 2019. Intercropping cereals with faba bean reduces plant disease incidence regardless of fertilizer input; a meta-analysis. Eur. J. Plant Pathol. 154 (4), 931–942. https://doi.org/10.1007/s10658-019-01711-4.
Zhen, Xiaoxing, Michelle Dobbratz, Jungers, Jacob M., Walid Sadok, 2023. Is interannual grain yield decline of intermediate wheatgrass influenced by management and climate in the Upper Midwest? Agric. Ecosyst. Environ. 362 (2024), 108856.