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Alfalfa intercrop rarely limits productivity of intermediate wheatgrass across diverse edaphic and climate conditions

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Management practices that minimize soil disturbance, reduce inputs, and provide sustainability across a broad range of environmental conditions are needed to ensure productivity into the future. Integrating intermediate wheatgrass (IWG; *Thinopyrum intermedium*), a perennial grain and forage crop, into agroecosystems may help achieve these goals. Additionally, diversification of IWG plantings by intercropping with a perennial legume could further increase other ecosystem services along with increases in nutrient availability. In this large-scale field experiment, IWG performance was assessed over two growing seasons in monoculture, monoculture with nitrogen fertilizer, and in an intercrop with alfalfa (*Medicago sativa*) at seven sites in four states that vary in environmental conditions. At the field scale, IWG grain production was similar in the intercrop and monoculture in both growing seasons at a majority of the sites. Intercropping did not reduce IWG biomass in the second growing season, but IWG biomass was reduced in the intercrop in the first growing season. Nitrogen fertilizer addition did not substantially alter IWG productivity at a majority of the sites. The results reinforce findings that intercropping with alfalfa has minimal impacts on IWG productivity after two growing seasons while providing potential for additional ecosystem services.

KEYWORDS

Kernza®, legume, *Medicago sativa*, perennial agriculture, *Thinopyrum intermedium*

Introduction

As agricultural soils continue to degrade globally and climate variation intensifies, innovative management practices are needed to restore and maintain soil health. Including perennial crops into agroecosystems has shown good potential for rebuilding soils while contributing to beneficial ecosystem services through cessation of tillage and increased contributions below ground through deep root systems (Jungers et al., 2023; Sprunger et al., 2024). Productivity of perennial cropping systems could be limited, however, by lack of temporal rotation with

legume crops to increase nutrients and other crop species to reduce pathogen accumulation (Cox et al., 2005; Rasche et al., 2017). To mitigate these drawbacks, spatial diversification through intercropping may be a viable approach for sustained productivity (Ryan et al., 2018).

Intermediate wheatgrass (IWG; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) shows promise as a novel perennial grain (marketed as Kernza®) and forage crop (Bajgain et al., 2022; DeHaan et al., 2018). Ongoing selective breeding has substantially improved agronomic performance of IWG (Crain et al., 2021, 2020), and research continues to develop best management practices (DeHaan et al., 2025), optimum nutrient inputs (Cassani et al., 2025; Jungers et al., 2017), and planting densities (Fernandez et al., 2020; Hunter et al., 2020) for IWG cropping systems to maximize resource availability for sustained yields over time.

IWG research has also expanded to explore the potential benefits of intercropping IWG with perennial legumes. Performance of IWG could be increased in legume intercrops by simultaneously decreasing competition for nutrient resources, providing facilitative effects, and reducing soil pathogens (Ryan et al., 2018). Beyond increases in IWG productivity, management costs and detrimental environmental impacts of nitrogen fertilizer addition could be reduced, and forage quality and quantity could be increased (Pinto et al., 2022). Recent studies have shown that intercropping with legumes can have positive (Crews et al., 2022) and neutral (Dick et al., 2018; Pinto et al., 2022) effects on IWG performance, and the outcomes of legume-IWG intercrops have varied with the stand age (Crews et al., 2022; Pinto et al., 2024), identity of the legume (Griffin and Jungers, 2025; Pinto et al., 2024), and local climate and edaphic factors (Reilly et al., 2022).

The IWG selection process is still underway for desirable agronomic traits, and continued evaluation of IWG performance and compatibility with potential companion crops is necessary across a broad range of environments. In this study, we built upon previous research by: (1) assessing the performance of a more recent IWG population than used in other studies in fertilized and un-fertilized monocultures and in an intercrop with the perennial legume alfalfa (*Medicago sativa* L.), (2) including an alfalfa monoculture to better understand IWG/alfalfa interactions and determine site-specific conditions affecting alfalfa growth, (3) establishing experiments at seven sites across a broader geographic range than other studies focused on IWG intercrop productivity undertaken thus far to determine if consistent cropping treatment effects emerge regardless of climate and edaphic factor variability. We expected that the nitrogen addition and intercropping treatments would vary among sites in the establishment year due to variation in site characteristics and subsequently increase performance of IWG over time due to increased availability of nutrients.

Materials and methods

Site descriptions

Seven site locations associated with six institutions in the National Science Foundation New Roots for Restoration Biology Integration Institute were established for this study (Table 1; Figure 1). The sites featured soils in either Entisol, Alfisol, or

Mollisol soil orders, which occupy relatively fertile early to mid-stages of soil development. The sites are located along a longitudinal and latitudinal gradient which influences the mean annual precipitation (MAP) and mean air temperature (MAT). A majority ($n = 5$) of the sites were in conventional annual grain agriculture immediately prior to experiment installation, and four of these sites were maintained with tillage. Two sites (UVM and CBG) were fallow fields dominated by a mix of annual and perennial grass species.

Experimental design

The same field experiment was established at each of the seven sites. Experimental plots were arranged in a randomized complete block design with four blocks, and plots within each block were randomly assigned one of four cropping systems: (1) unfertilized IWG monoculture (IWG), (2) fertilized IWG monoculture (IWG + N), (3) unfertilized alfalfa monoculture (Alf), or (4) unfertilized IWG and alfalfa intercrop (IWG + Alf). The IWG seed was TLI 801 (95% germination) derived from the parents of the eighth breeding cycle at The Land Institute in Salina, KS. The alfalfa variety was Hybriforce 3400 (Dairyland Seed, USA; 65.90% pure seed and 80% germination), which was pre-inoculated with rhizobia in a seed coat. Plots (18 rows wide; 0.3078 m on center row spacing; 6.096 m long) were planted (15 lbs. PLS for IWG and alfalfa) at six of the sites in September 2021. The intercrop treatment was a replacement design in which, every other row of IWG was replaced with alfalfa with the same within row seeding rate, reducing the seeding rate and overall crop density per hectare for IWG and alfalfa by 50% relative to the monoculture treatments. Plots were weeded as needed throughout the growing season. Urea (90 kg ha^{-1}) was applied to the fertilized IWG treatment in early spring at all sites except Lawrence (KU), which received Chilean nitrate (90 kg ha^{-1}) to remain under organic cultivation. In 2022, two sites (CBG and UVM) were replanted due to poor establishment, and an additional site (DDPSC) was added in St. Charles, Missouri.

Data collection

Preliminary soil samples (0–25 cm depth) were collected from each plot location at each site (16 plots $\times$ 7 sites = 112 samples) in the establishment year to assess baseline differences in soil abiotic factors. Soil samples were sent to the Kansas State University Soils Lab (Manhattan, KS, USA) to assess Sikora pH, pH, cation exchange capacity, potassium, calcium, sodium, organic matter (loss on ignition), phosphorus (Bray extraction), total organic carbon, total nitrogen, and total phosphorus.

Biomass was collected by harvesting crops (10 cm above the soil surface) for 3-m-long sections of three representative rows (3 rows $\times$ 3 m long = 9 linear meters) for each plot. The row width was considered to be halfway into the inter-row space (0.15 m) on either side of the focal row (0.30 m total harvest width per row).

Alfalfa was harvested when flowering reached between 10 and 15% across an entire experimental site (typically two to three harvests per growing season). The harvested alfalfa was weighed in the field, and a subsample was collected. The subsample was weighed at field moisture, oven dried (48 h at 60°C) and reweighed. The moisture content was used to calculate the biomass of the entire harvested sample. The remaining rows of alfalfa

TABLE 1 Location, soil classification, site conditions, and climate descriptions of the seven site locations used for the field experiment.

Institution	Closest population center	Site Abbr.	Latitude, longitude	Soil classification	MAP (mm yr ⁻¹)	MAT (°C)	Land-use before planting	Field preparation for planting	Planting year
The Land Institute	Salina, KS	TLI	38.767846, -97.571326	Coarse-silty, mixed, superactive, mesic Fluventic Haplustolls	685–864	12.2–13.9	Annual wheat/soy/sorghum crop rotation with tillage	Disc tillage and soil packer	2021
University of Kansas	Lawrence, KS	KU	39.020694, -95.262861	Coarse and fine-silty, mixed, superactive, mesic Fluventic Hapludolls	813–1,067	12.2–13.9	Organic annual wheat and soybean with tillage	Rotary tiller and cultipacker	2021
University of Missouri	Novelty, MO	MU	39.950250, -92.065098	Fine, smectitic, mesic Vertic Epiaqualfs	940–1,041	11.1–12.2	No-till corn/soy rotation with a silver maple alley crop	Tree removal, disc tillage, and cultivator	2021
Donald Danforth Plant Science Center (Planthaven Farm)	O'Fallon, MO	PHF	38.849349, -90.685239	Fine, smectitic, mesic Aeris Chromic Vertic Epiaqualfs	940–1,195	11.1–13.9	Corn/soy rotation with tillage	Re-graded, tilled, and cultipacker	2021
Donald Danforth Plant Science Center (DDPSC field research site)	St. Charles, MO	DDPSC	38.848133, -90.457331	Coarse-silty, mixed, superactive, mesic Cumulic Hapludolls	889–1,194	10.0–13.9	Corn/soy rotation with tillage	Rotary tiller	2022
Chicago Botanic Garden	Mettawa, IL	CBG	42.234232, -87.918064	Fine, illitic, mesic Aeris Epiaqualfs	711–1,016	7.2–12.2	Mowed fallow grass field	Glyphosate burn down and tilled	2022
University of Vermont	Burlington, VT	UVM	44.428964, -73.205408	Sandy, isotic, frigid Typic Haplorthods and mixed, mesic Typic Udipsamments	762–1,270	2.8–11.1	Mowed fallow grass field	Rotary tiller	2022

after sampling were cut and left as mulch in the plot. The alfalfa from the rows sampled and the subsample were also returned to the plot after data was recorded. The repeated harvest methodology was used to maximize the potential nitrogen availability for IWG in the intercrop. Cutting of alfalfa can stimulate nitrogen release into the soil through increased root exudation and the decomposition of alfalfa aboveground biomass as well as roots can contribute to the nitrogen pool (Crews et al., 2022, 2016).

IWG was harvested once per growing season at grain maturity (typically between the end of July and end of August). Entire seed heads were harvested from 3 m sections along three different representative rows (rows that were not influenced by edge effects) in each treatment plot. Then the biomass (without seed heads) was harvested from those rows as described for alfalfa above. The seed heads and biomass were kept separate. The seed heads were dried (48 h at 60 °C), shipped to TLI for threshing, and the clean seed (grain) was dried (48 h at 60 °C) and weighed.

Statistical analyses

Non-metric multidimensional scaling (MetaMDS; *vegan* package in R statistical software; Oksanen et al., 2019) was used to visually inspect differences among sites in soil properties. Intrinsic analysis (*envfit*; *vegan* package in R) was used to assess specific soil properties contributing to the separation of sites. A PERMANOVA (adonis2: *vegan* package in R) followed by pairwise PERMANOVAs (pairwise.perm.manova; RVAideMemoire package in R; Hervé, 2018) were used to statistically test for differences among the sites.

For crop specific responses, biomass and grain of IWG and alfalfa biomass (total biomass collected in all harvests within a growing season) were analyzed on a per row basis (g m⁻²). For field-scale analysis, IWG biomass and grain were converted to kilograms per hectare accounting for the 50% reduction in IWG density in the mixture treatment. A split plot in time mixed model approach (proc

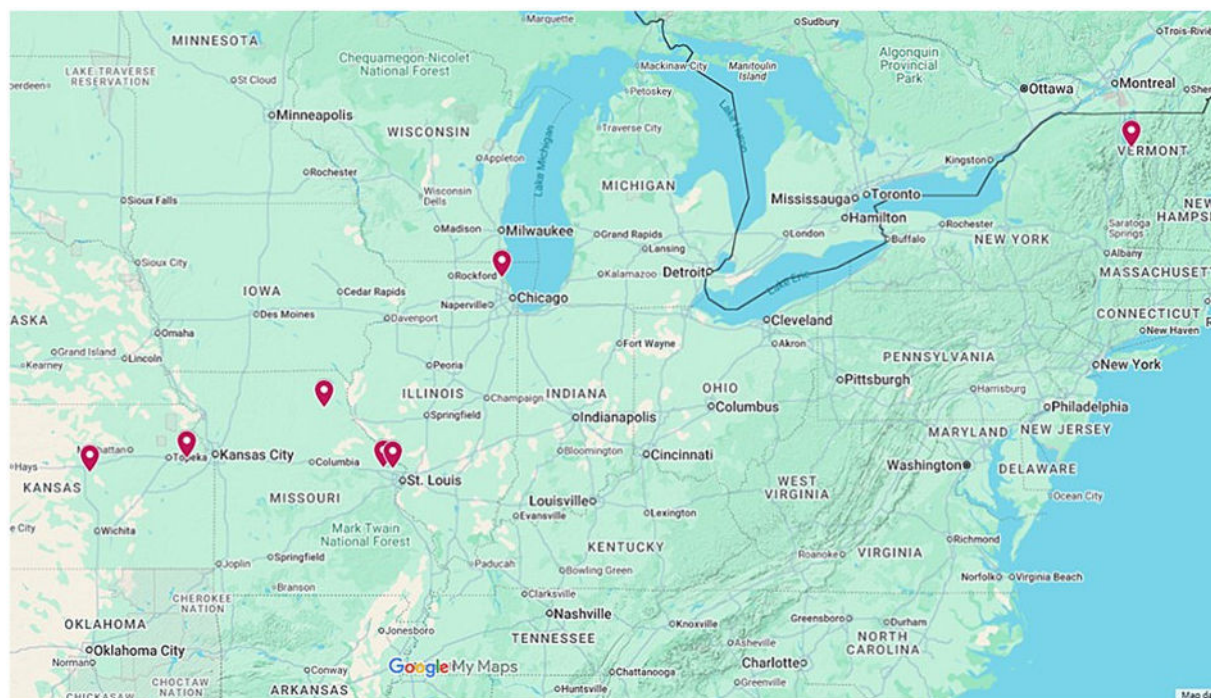


FIGURE 1

Map showing the location of seven sites where the long-term field experiments were established (From Google Maps by Google).

mixed; SAS Inc. Cary, NC, USA) was used with stand age, site, and cropping treatment, as well as their interactions, as fixed effects. Stand age represents time since planting rather than the calendar year a site was established (as in Culman et al., 2023). A block by site interaction was included as a random effect to account for the nesting of block at each site, and the three-way interaction of block, site, and treatment was included as a random effect so the appropriate error term would be used in the split plot in time analysis. Denominator degrees of freedom were calculated using Satterthwaite approximation. Tukey's HSD multiple comparisons were used to determine differences within groups following a significant result ($\alpha < 0.05$). To explore significant interactions, the Slice argument (Littell et al., 2006) was used to do partitioned analyses of the main effects (F tests) within the levels of the other factors. To understand variability in cropping treatment effects, cropping treatment effects were assessed at each stand age within each site (significant Stand age $\times$ Site $\times$ Cropping Treatment interaction). To understand how responses varied within a cropping treatment over time at each site, the effect of stand age was assessed within each cropping treatment at each site (significant Stand age $\times$ Site $\times$ Cropping Treatment interaction). To understand how responses varied within cropping treatment across stand age, the effect of each cropping treatment between stand ages were assessed at each site (significant Site $\times$ Treatment interaction). If an F test was significant, Tukey's HSD multiple comparisons were used to determine differences among groups.

For each model, the normality (Q-Q plot and histogram) and variance (residuals vs. predicted) were assessed to verify statistical assumptions. In the analysis of crop-specific (g m^{-2}) and field-scale (kg ha^{-1}) responses, IWG biomass was natural log transformed, IWG grain was square-root transformed, and alfalfa biomass was natural log transformed to meet statistical assumptions. Least square means

and 95% confidence limits were back-transformed for graphical presentation.

Results

Soil properties

Soil properties varied across the experimental sites (Pseudo $F_{6,111} = 446.55$, $p = 0.001$, $R^2 = 0.96$; Figure 2), and all the sites were significantly different from each other (Bonferroni adjusted $p = 0.02$ for all pairwise site comparisons).

The separation of sites was driven by high cation exchange capacity, calcium, potassium, and pH at TLI, high sodium, total organic carbon, magnesium, organic matter, and total nitrogen at CBG, and high total and extractable phosphorus at UVM (Figure 2).

Crop per row responses (g m^{-2})

IWG biomass (g m^{-2} of row)

Across sites and cropping treatments, IWG biomass production per row increased from the first to second growing season (first growing season: LS mean = 267 g m^{-2} of row; 95% lower confidence limit = 248, 95% upper confidence limit = 287; second growing season: 497 g m^{-2} of row; 462, 534; significant main effect of Stand Age; Table 2). Across sites and stand age, IWG biomass per row was greater in the intercrop (364 g m^{-2} of row; 326, 407) and greater in fertilized monoculture (418 g m^{-2} of row; 374, 468) than the unfertilized monoculture (317 g m^{-2} of row; 284, 355; significant main effect of Cropping Treatment; Table 2).

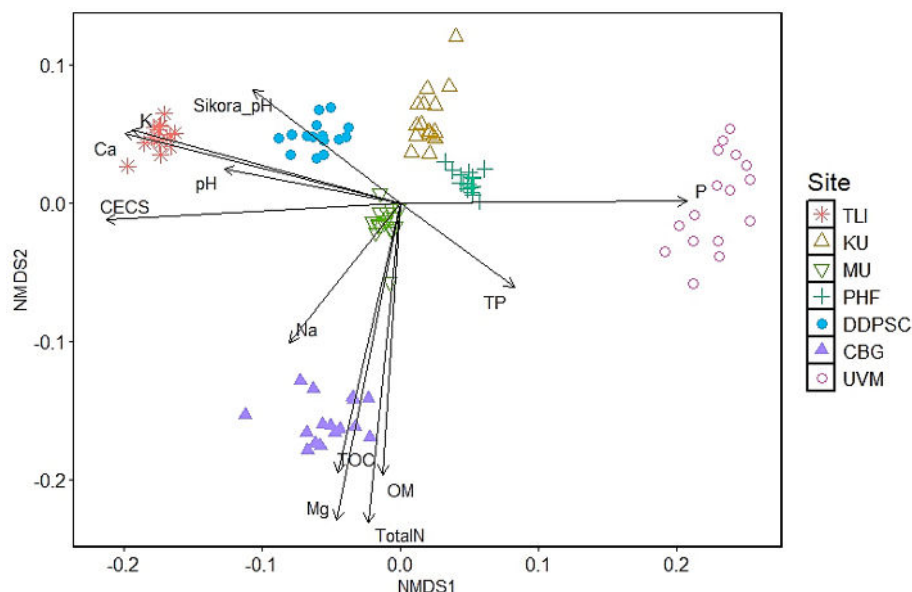


FIGURE 2

Nonmetric multidimensional scaling ordination of soil properties at all seven sites. Vectors of soil properties were calculated using intrinsic analysis.

However, the effects of stand age and cropping treatment were not consistent across sites (significant Stand Age $\times$ Site $\times$ Cropping Treatment interaction; Table 2). One year after establishment, cropping treatment only had a significant effect at UVM (Supplementary Table S1), where IWG biomass in the nitrogen addition treatment was greater than in the intercrop and greater than in the unfertilized monoculture (Figure 3A). After 2 years of growth, cropping treatment had a significant effect at three sites (KU, MU, and CBG; Supplementary Table S1). At KU and MU, IWG biomass was greater in the intercrop than the unfertilized monoculture, and at CBG the intercrop had the least amount of IWG biomass (Figure 3B).

Within cropping treatments, IWG biomass production per row decreased significantly in the unfertilized monoculture from year one to year two at TLI and marginally at KU and MU (Supplementary Table S2; Supplementary Figure S1). Biomass was similar in both years in the intercrop at three sites (TLI, KU, and CBG) and increased at the other four sites (MU, PHF, DDPSC, and UVM). Fertilizer effects over time were more variable among sites, as fertilized monocultures increased at four sites (PHF, DDPSC, CBG, and UVM), decreased at one site (TLI), and was similar in both years at one site (KU).

IWG grain (g m^{-2} of row)

IWG grain production per row was similar in the first and second year of the experiment across sites and cropping treatments (non-significant main effect of Stand Age; Table 2), and grain production per row was greater in the intercrop (56 g m^{-2} of row; 50, 62) and greater in the fertilized monoculture (51 g m^{-2} of row; 45, 56) than the unfertilized monoculture (40 g m^{-2} of row; 36, 46) across sites and stand ages (significant main effect of Cropping Treatment; Table 2).

Similar to IWG biomass, the effects of stand age and cropping treatment also varied with site (significant Stand Age $\times$ Site $\times$ Cropping Treatment interaction; Table 2). After 1

year of growth, cropping treatment had an impact on IWG grain production at three sites (TLI, PHF, and DDPSC; Supplementary Table S1), and there was not a consistent pattern of cropping treatment effects among these sites. The unfertilized and fertilized monocultures had greater grain production than the intercrop at TLI. The nitrogen addition treatment had greater grain production than the unfertilized monoculture and marginally greater grain production than the intercrop ($p = 0.08$ in pairwise comparison) at PHF. The intercrop had more grain than the unfertilized and fertilized monocultures at DDPSC (Figure 3C).

After 2 years of growth, IWG grain production significantly varied with cropping treatment at five sites (TLI, KU, MU, DDPSC, and CBG; Supplementary Table S1; Figure 3D) and marginally varied at one site (PHF) with a more consistent cropping treatment effect among these sites than in the establishment year. With the exception of CBG, the intercrop produced more grain than unfertilized monoculture at these sites. In addition, the intercrop had greater grain production per row than the fertilized monoculture at TLI and DDPSC. At KU the fertilized monoculture had more grain than the unfertilized monoculture. At CBG, the nitrogen addition treatment had greater grain production than the intercrop.

IWG grain production per row remained stable in the intercrop treatment at two (TLI and MU) of the three sites with stable biomass production over time and marginally decreased at the other site (KU; Supplementary Table S2; Supplementary Figure S2). In contrast, IWG grain decreased in the unfertilized monocultures at all three of these sites in the fertilized monocultures at two (TLI, MU and marginally at KU). Three of the four sites with increased biomass production over time (PHF, CBG, and UVM) had similar increases in IWG grain production with the exception of similar grain yields both years in the fertilized monocultures at one site (PHF). The highly productive DDPSC site, where biomass increased over time in all cropping treatments, had reduced IWG grain in all cropping treatments in the second growing season (Supplementary Figure S2).

TABLE 2 Results from mixed model analysis of the effects of stand age, site, and cropping treatment on per row (g m^{-2}) IWG biomass, IWG grain yield, and alfalfa biomass for the seven sites with 2 years of growth.

Response	Factor	DF(Num, Den)	F	P
IWG biomass	Stand age (SA)	1,63	323.65	<0.0001
	Site (S)	6,63	82.24	<0.0001
	Cropping treatment (CT)	2,63	6.19	0.0035
	SA $\times$ S	6,63	105.97	<0.0001
	SA $\times$ CT	2,63	6.58	0.0025
	S $\times$ CT	12,63	1.97	0.0427
	SA $\times$ S $\times$ CT	12,63	3.90	0.0002
IWG grain	Stand age (SA)	1,104	1.68	0.1981
	Site (S)	6,21.1	28.24	<0.0001
	Cropping treatment (CT)	2,104	10.33	<0.0001
	SA $\times$ S	6,104	33.6	<0.0001
	SA $\times$ CT	2,104	5.71	0.0045
	S $\times$ CT	12,104	4.07	<0.0001
	SA $\times$ S $\times$ CT	12,104	2.70	0.0033
Alfalfa biomass	Stand age (SA)	1,41.2	9.77	0.0033
	Site (S)	6,41.2	53.46	<0.0001
	Cropping treatment (CT)	1,41.3	5.72	0.0214
	SA $\times$ S	6,41.1	61.19	<0.0001
	SA $\times$ CT	1,41.2	5.62	0.0226
	S $\times$ CT	6,41.2	6.34	<0.0001
	SA $\times$ S $\times$ CT	6,41.1	3.43	0.0077

Significant *p* values (< 0.05) are in bold.

Alfalfa biomass (g m^{-2} of row)

Alfalfa biomass varied with stand age, site, and cropping treatment (significant three-way interaction; Table 2). After 1 year of growth, alfalfa biomass was greater in the monoculture than the intercrop at PHF, and biomass was similar in both cropping treatments at the other six sites (Figure 4A). After 2 years of growth, alfalfa biomass was greater in monoculture at DDPSC and UVM, and similar in both cropping treatments at the five other sites (Figure 4B).

Field scale IWG biomass and grain (kg ha^{-1})

IWG biomass (kg ha^{-1})

IWG biomass at the field scale increased from the first (2,118 kg ha^{-1} ; 1,970, 2,277) to second (3,945 kg ha^{-1} ; 3,670, 4,241) growing season across sites and cropping treatments (significant main effect of Stand age; Table 3). There was substantial variation in IWG biomass production among sites across cropping treatments, and biomass values ranged from 221 kg ha^{-1} at UVM to 5,319 kg ha^{-1} at KU in the first growing season and from 1,679 kg ha^{-1} at CBG to

8,307 kg ha^{-1} at DDPSC in the second growing season (Supplementary Table S3).

At the field scale across stand age and sites, intercropping (1,819 kg ha^{-1} ; 1,628, 2033) resulted in a reduction in IWG biomass production relative to the unfertilized monoculture (3,172 kg ha^{-1} ; 2,838, 3,546) and the fertilized monoculture (4,185 kg ha^{-1} ; 3,744, 4,667; significant main effect of Cropping treatment; Table 3). However, cropping treatment effects varied with site (significant Site $\times$ Treatment interaction; Table 3), and three sites (KU, MU, and DDPSC) had similar IWG biomass in the intercrop and the unfertilized monoculture across stand age (Supplementary Table S4; Supplementary Figure S3A).

Cropping treatment and site effects also varied with stand age (significant Stand age $\times$ Site $\times$ Cropping Treatment interaction; Table 3). Reduced IWG biomass in the intercrop was most evident in the first growing season as five of the seven sites (TLI, MU, PHF, CBG, and UVM) had significantly less biomass than either monoculture (Figure 5A; Supplementary Table S5). At KU IWG biomass production in the intercrop was marginally less ($p = 0.08$) than in the unfertilized monoculture, and the fertilized monoculture produced more biomass than the intercrop. At DDPSC all cropping treatments resulted in similar IWG biomass productivity.

In contrast to the first growing season, the impacts of reduced IWG planting density in the intercrop were minimal in the second growing season. Five of the seven sites (TLI, KU, MU, DDPSC, and UVM) had similar IWG biomass production in the intercrop and unfertilized monoculture (Figure 5B; Supplementary Table S5). At CBG and PHF the unfertilized monoculture had more biomass than the intercrop, but the effect was only marginal at PHF ($p = 0.09$ in pairwise comparison). At three sites (KU, PHF, and CBG), the fertilized monoculture produced more IWG biomass than the intercrop.

IWG grain (kg ha^{-1})

IWG grain production at the field scale marginally decreased from the first (413 kg ha^{-1} ; 375, 452) to second (371 kg ha^{-1} ; 336, 410) growing season across sites and cropping treatments (marginally significant main effect of Stand Age; Table 3). There was also substantial variation in IWG grain production among sites across cropping treatments, and biomass values ranged from 54 kg ha^{-1} at CBG to 826 kg ha^{-1} at KU in the first growing season and from 222 kg ha^{-1} at TLI to 749 kg ha^{-1} at PHF in the second growing season (Supplementary Table S3).

IWG grain production at the field scale was reduced in the intercrop relative to either monoculture across stand age and sites, as intercropping (280 kg ha^{-1} ; 244, 318) reduced grain production relative to the unfertilized monoculture (407 kg ha^{-1} ; 364, 453) and to the fertilized monoculture (507 kg ha^{-1} ; 458, 558; main effect of Cropping Treatment; Table 3). As with biomass, cropping treatment effects varied with site (significant Site $\times$ Treatment interaction; Table 3). The reduced grain production in the intercrop relative to the unfertilized monoculture was driven by significantly lower yields across stand age at two sites (TLI and CBG) and marginally lower yields at another two sites (PHF and UVM). The other three sites (KU, MU, and DDPSC) had similar grain production in the unfertilized monoculture and the intercrop (Supplementary Table S4; Supplementary Figure S3B).

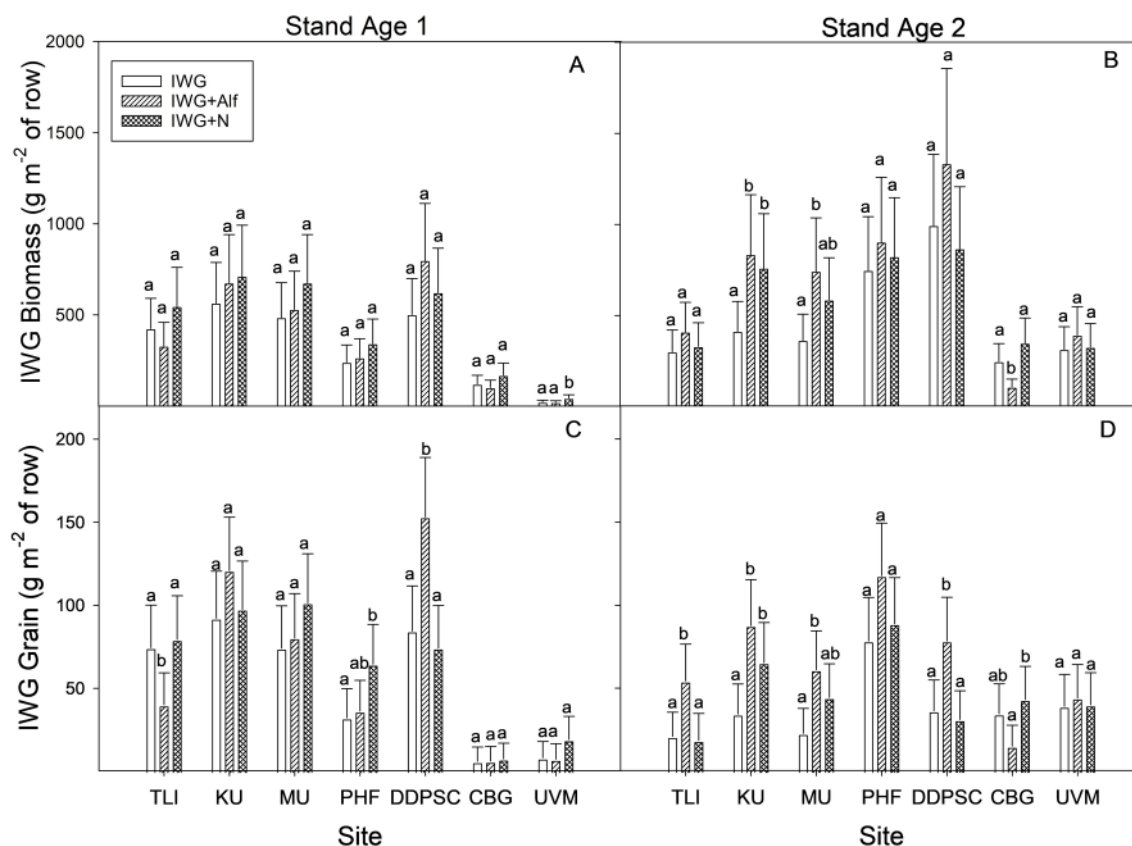


FIGURE 3

Average per row (ls mean g m^{-2} of row + 95% confidence limit) IWG biomass (A,B), and IWG grain yields (C,D) at each of the seven sites at each stand age. Bars with different letters within a site and stand age were significantly different ($p < 0.05$; Tukey's HSD).

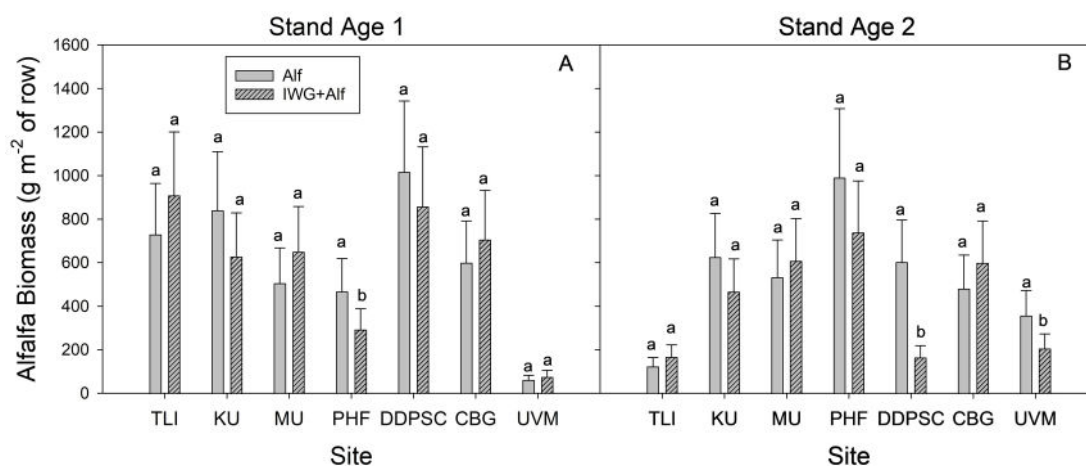


FIGURE 4

Average per row (ls mean g m^{-2} of row + 95% confidence limit) alfalfa biomass at each of the seven sites at after 1 (A) and 2 (B) years of growth. Bars with different letters within a site and stand age were significantly different ($p < 0.05$; Tukey's HSD).

Cropping treatment and site effects on grain production at the field scale varied with stand age (significant Stand age $\times$ Site $\times$ Cropping Treatment interaction; Table 3). In the first growing season, the impacts of reduced IWG planting density were less pronounced than biomass results, as the intercrop and unfertilized monoculture had similar IWG grain production at a majority

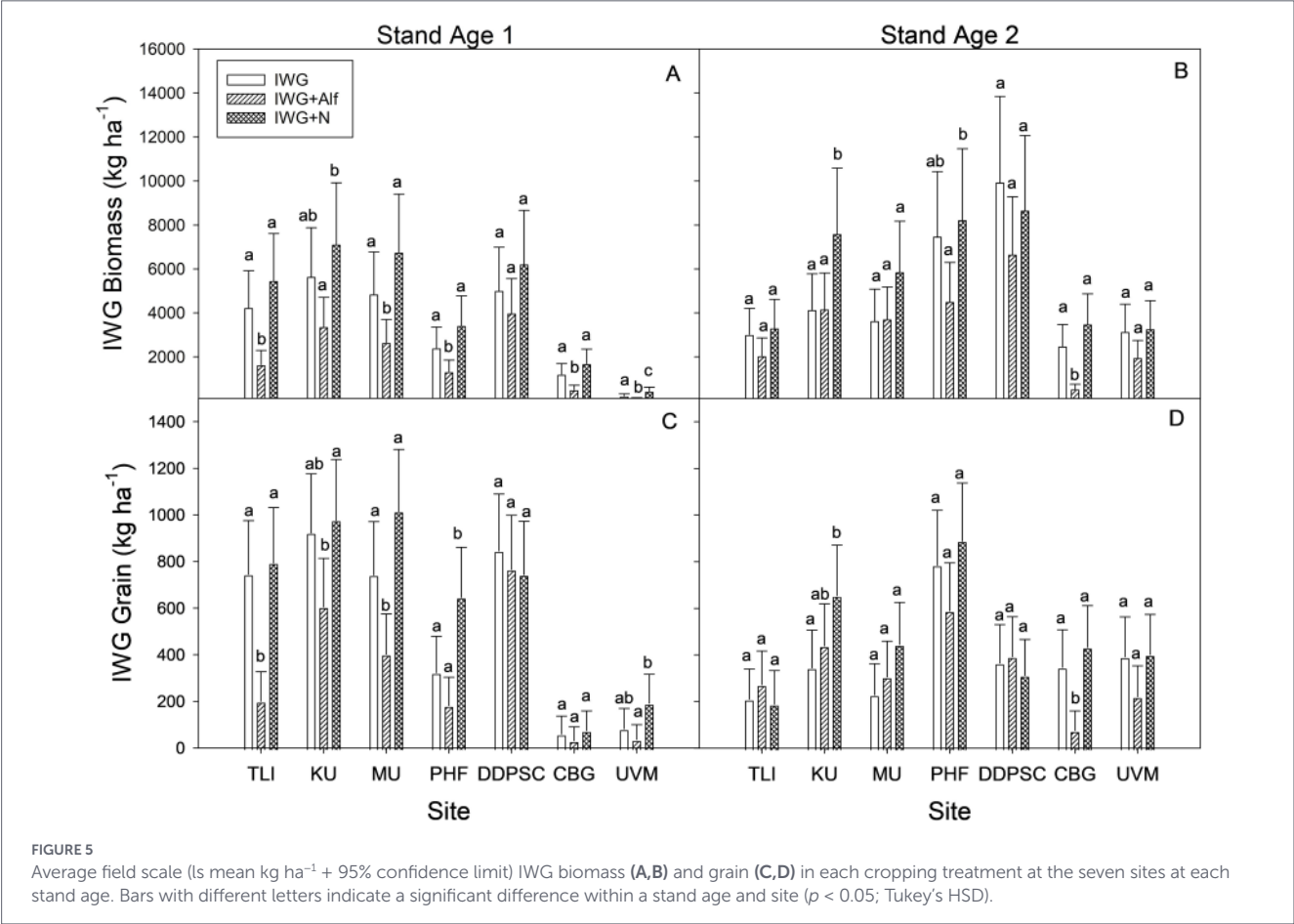
of the sites (marginally so at KU, PHF, DDPSC, CBG, UVM; Supplementary Table S5; Figure 5C). However, fertilized monocultures had more grain than the intercrop at a majority of sites (TLI, MU, PHF, UVM, and at KU).

After 2 years of growth, cropping treatment effects were minimal as grain production was similar among all cropping treatments at five

TABLE 3 Results from mixed model analysis of the effects of sampling year, site, and cropping treatment on IWG biomass and grain yield at the field-scale (kg ha⁻¹).

Response	Factor	DF(Num,Den)	F	P
IWG biomass	Stand age (SA)	1,63	323.65	<0.0001
	Site (S)	6,63	82.24	<0.0001
	Cropping treatment (CT)	2,63	58.02	<0.0001
	SA × S	6,63	105.97	<0.0001
	SA × CT	2,63	6.58	0.0025
	S × CT	12,63	1.97	0.0427
	SA × S × CT	12,63	3.90	0.0002
IWG grain	Stand age (SA)	1,104	3.21	0.0762[†]
	Site (S)	6,21.2	26.9	<0.0001
	Cropping treatment (CT)	2,104	33.12	<0.0001
	SA × S	6,104	34.07	<0.0001
	SA × CT	2,104	5.06	0.0008
	S × CT	12,104	2.03	0.0284
	SA × S × CT	12,104	3.18	0.0007

Significant *p* values (< 0.05) are in bold and [†]indicates marginal significance (*p* < 0.1).



sites (TLI, marginally at MU, PHF, DDPSC, and marginally at UVM; [Supplementary Table S5](#); [Figure 5D](#)). Of the two sites where cropping treatment was significant (KU, CBG), the intercrop had similar grain production as the unfertilized and fertilized monocultures at one site (KU), and the intercrop had lower grain production than the fertilized and unfertilized monoculture at the other (CBG; [Figure 5D](#)).

Discussion

Intercropping IWG with a legume in perennial agriculture systems could potentially increase many ecosystem services ([Akchaya et al., 2025](#); [Ryan et al., 2018](#)), including sustained productivity with decreased inputs over time ([Crews et al., 2022](#)). In this study

we assessed a recent selection cycle of IWG in monoculture with and without nitrogen fertilizer and intercropped with alfalfa at multiple sites with variable soil conditions across a broad geographic range. Contrary to expectations, intercropping with alfalfa did not significantly affect IWG grain production at the field scale (kg ha^{-1}) in the first or second growing season nor affect IWG biomass in the second growing season at a majority of the sites, though it did tend to result in lower IWG biomass in the establishment year. Similar field-scale (kg ha^{-1}) IWG biomass and grain yields have been reported in IWG/alfalfa intercrop studies in the first growing season (Crews et al., 2022; Dick et al., 2018; Pinto et al., 2024). The contrasting results presented here could in part be due to the broad geographic range of the site locations, as site-specific variability across North America has been shown to alter IWG biomass more than grain production (Culman et al., 2023). Overall, our results align with previous studies that show intercropping with alfalfa has minimal impact on IWG performance within the first two growing seasons (Dick et al., 2018; Pinto et al., 2022), and there is potential to increase ecosystem services through increased crop diversity across a broad range of environments without substantial impacts on IWG performance.

Both IWG biomass and grain production varied greatly among sites, as reported in previous studies assessing IWG performance across North America (Cassani et al., 2025; Culman et al., 2023). The two sites with the lowest yields across treatments after one year of growth (CBG and UVM) were the only two sites not in agricultural production leading up to experiment installation (Table 1; Supplementary Table S3). Lack of agricultural management, such as consistent nutrient inputs, weed control, and drainage maintenance for years prior to planting of the IWG stands, may have contributed to decreased IWG productivity in the establishment year. However, in the second growing season, these two sites increased in grain yield and biomass production, produced more grain than one site (TLI), and had comparable grain yields to two other sites (MU and DDPSC). While declines in IWG grain yield often occur over time, as occurred at four of the sites (TLI, KU, MU, and DDPSC), when IWG establishment density is low, grain yield can remain stable or increase from the first to second growing season (Dehaan et al., 2025). The grain yields at PHF also followed this trajectory, as it only produced more grain than CBG and UVM in the first year, then had the highest grain yield of all the sites in the second year. In contrast to CBG and UVM, PHF was in agricultural production prior to experiment installation, which suggests grain yield trends over time were not solely determined by land-use history.

While previous studies have suggested early competition between alfalfa and IWG in intercropping plantings (Crews et al., 2022; Griffin and Jungers, 2025; Reilly et al., 2022; Tautges et al., 2018), we found no evidence of alfalfa limiting IWG performance, as per row (g m^{-2}) IWG performance in the intercrop was similar or greater than in the unfertilized monoculture at all the sites in the first year. In the second growing season, intercropping benefits to per row (g m^{-2}) IWG performance were more pronounced with stronger impacts on grain than biomass. The repeated harvesting of alfalfa is not standard practice in IWG/alfalfa experiments (but see Crews et al., 2022). This management likely decreased competition for resources, especially above ground, throughout the

growing season. One site (CBG) had less per row (g m^{-2}) IWG biomass in the intercrop relative to the monocultures. As previously discussed, CBG was one of two sites that was not in agricultural production prior to experiment installation, which may have contributed to the different outcome. Similar to IWG, alfalfa tended to perform similarly in monoculture and the intercrop at most sites in the first growing season, and this trend continued into the second growing season. Alfalfa biomass was reduced in the intercrop at one site in the first growing season and two sites in the second growing season. This suggests that IWG could possibly limit alfalfa growth under certain conditions. While a specific mechanism was not identified, the inclusion of the alfalfa monoculture in the experimental design allowed for insights into whether alfalfa performance in the intercrop was due to interactions with IWG rather than just site conditions limiting performance (similar performance in the intercrop and monoculture).

Although nitrogen amendments can have positive effects on IWG performance (Jungers et al., 2017) and the application rate in this study was within the recommended range (Zhen et al., 2024), nitrogen addition had a minimal impact on IWG biomass and grain production at a majority of the sites. This suggests that IWG performance in monoculture was not greatly limited by nitrogen availability in the first two years of growth. A majority of the sites were in agricultural production prior to experiment installation, which can make nutrients readily available and decrease nitrogen addition impacts. The minimal nitrogen response also suggests that the increased per row (g m^{-2}) performance of IWG in the intercrop was not due to the facilitative effects of alfalfa, but rather reduced density of IWG. For example, increased row spacing in IWG monocultures can decrease intra-specific competition and increase access to above and below-ground resources, which can lead to an increase in the production of fertile tillers (Fernandez et al., 2020; Hunter et al., 2020). Without an additional IWG monoculture cropping treatment planted at double the row spacing (as in Crews et al., 2022; Pinto et al., 2022, 2024), uncoupling alfalfa presence from decreased IWG density was limited. Regardless, reducing IWG density through substitutive planting in an intercrop would be a better option than increased row spacing in monoculture due to similar IWG productivity and the potential for the other ecosystem benefits from increased crop diversity, such as reduced weed pressure and increased pollinator habitat (Ryan et al., 2018). Also, as stand age increases and nutrients become more limited, the beneficial facilitative effects of alfalfa could potentially become more prominent (Crews et al., 2022).

The IWG germplasm used in this study was from a more recent population with greater selection for desirable agronomic traits than IWG used in other experiments, which may have influenced responses to site conditions and cropping treatments (Zhen et al., 2024). While breeding of the IWG germplasm used in this experiment was not specifically for planting in intercrops, trait selection and the breeding environment could change nutrient use requirements and efficiency of IWG, as well as have farther reaching effects, like altering interactions with soil biota (McKenna et al., 2024). For these reasons, the continued assessment of IWG performance in different geographic, edaphic, and cropping treatment contexts will be necessary as breeding efforts continue.

Conclusion

This study shows little harm to IWG performance when intercropping with alfalfa beyond reduced IWG biomass at the field-scale in the establishment year, and this drawback could be potentially offset by simultaneous forage harvest of both crops at IWG biomass harvest to boost total biomass quantity and quality (Pinto et al., 2022). Yet the responses in this study were collected over a relatively short time frame, so further monitoring and sampling of responses over longer periods is necessary to fully understand intercropping impacts in perennial agroecosystems. As resources become more limited in monoculture, additional benefits of intercropping IWG with alfalfa may become more apparent (Crews et al., 2022). Also, though increased access to resources can be assumed, explicit testing of nutrient availability and as well as nitrogen transfer will be needed for confirmation of facilitative alfalfa effects (Reilly et al., 2022). Beyond resource quantification and transfer, root architecture (Yang et al., 2022) and soil microbiomes (Wang et al., 2017) can also contribute to increased crop performance in intercrops. Knowing the relative contributions of all of these factors to IWG performance would provide a more holistic understanding of intercropping systems and could provide insight into management options that could further increase productivity and sustainability of IWG plantings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LP: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. MD: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. RU: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. KO: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. NZ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. LV: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. EW: Conceptualization, Data curation,

Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. KH: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. AM: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. TC: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2026.1816743/full#supplementary-material>

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