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Managing nitrogen fertility and stand density for sustaining Kernza intermediate wheatgrass yields

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Abstract

Kernza intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a promising perennial grain and forage crop, but experiences grain yield decline, potentially due to limited nitrogen (N) and stand overcrowding. We evaluated the effects of N fertilization and stand thinning on grain and forage yield, weed biomass, thousand-kernel weight (TKW), and harvest index (HI). We used a full factorial design with N rates of 0, 75, and 150 kg N ha^{-1} and thinning intensities of 0%, 25%, 38%, or 50% stand density reduction via banded herbicide at two locations in Wisconsin over 2 years. Fertilization and thinning did not interact. Grain yields increased with N fertilization except at Madison in Year 2. At Lancaster, grain yield increased from 293 with no N to 497 and 701 kg ha^{-1} with 75 and 150 kg N ha^{-1}, respectively, across years. At Madison, grain yield increased only in Year 1. Forage mass also increased with N at both sites except Madison in Year 2. At Lancaster, forage mass ranged from 4016 to 6500 kg ha^{-1} across years and N rates. TKW and HI increased with N at both sites, except at Madison in Year 2. Weed biomass was unaffected by treatments. Thinning had no effect on grain yield at Lancaster in Year 1, but in Year 2, grain yield increased from 368 to 505 kg ha^{-1} with 50% thinning. These results suggest that applying 75 kg N ha^{-1} is important for maintaining IWG productivity and that thinning can help sustain grain yield in older stands.

Plain Language Summary

Kernza intermediate wheatgrass is a perennial grass that can be harvested year after year for grain and animal feed. Over time, the grain harvest tends to get smaller. This might happen because the plants are too crowded or because they do not get enough nitrogen. The goal of this study was to see how adding nitrogen fertilizer and thinning (removing some plants) affected grain and forage harvests and weed biomass. Key results are (1) adding nitrogen fertilizer increased both grain and forage

Abbreviations: AONR, agronomically optimum nitrogen rate; HI, harvest index; IWG, intermediate wheatgrass; LA, Lancaster; TKW, thousand-kernel weight; WM, West Madison.

harvests at one location but had no effect at another, (2) thinning the plants did not make a difference early on, but 2 years later, the thinned plots produced 37% more grain at one location and had no impact at the other site, and (3) weed growth was not affected by either treatment. Thus, adding nitrogen fertilizer is important for keeping Kernza productive, and thinning can help maintain grain harvests over time, but its effects may depend on location and timing.

1 | INTRODUCTION

Intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a new perennial grain and forage crop, currently commercialized in the United States as Kernza. The decline in grain yield over time is a significant concern affecting the adoption of IWG for dual-use grain and forage (Dick et al., 2019; Hunter et al., 2020; Jungers et al., 2017, 2018; Lanker et al., 2020; Tautges et al., 2018; Zimbric et al., 2020). Grain yield tends to decline from the first to the third production year. In response to this yield decline, farmers tend to rotate or replant their stand after 3–5 years (Law et al., 2021).

As perennial plants age, vegetative growth and asexual reproduction may be favored under stable conditions rather than grain-producing sexual reproduction (Garnier, 1992). For IWG, fertile tillers have been identified as the primary predictor of grain yield (Fernandez et al., 2020). Additionally, in perennial crops, factors such as kernel number per tiller, proportion of high-yielding tillers, and kernel mass per tiller tend to decrease as the stand ages, contributing to the observed decline in IWG grain yield over time (Canode & Law, 1975; Hunter et al., 2020). Another potential factor contributing to yield decline is above- and belowground intraspecific competition. As the IWG canopy becomes denser post-establishment, light penetration to the crown of the grass is reduced during the fall (Fernandez et al., 2020; Pinto et al., 2021). Given that both the quantity and quality of light are critical for fertile tiller development in grasses (Casal et al., 1985; Deregibus et al., 1985), previous studies have shown that increased canopy density, through greater leaf and tiller biomass, can reduce light penetration to the crown, thereby limiting tillering and ultimately reducing grain yield (Fernandez et al., 2020). Further, perennial grasses allocate substantial carbon to belowground biomass production, leading to increased competition for soil nutrients, another possible reason for grain yield decline over time (DeHaan et al., 2005; Fernandez et al., 2020; Garnier, 1992; Hunter et al., 2020; Sakiroglu et al., 2020; Tautges et al., 2018).

Determining the optimal nitrogen (N) fertilizer rate for a perennial grain is challenging because its larger root systems can access nutrients deeper in the soil profile and store them for years (Ryan et al., 2018; Sprunger et al., 2018). Despite this, IWG is harvested for grain and/or forage, which removes N and other nutrients, making some fertilizer additions necessary. Though the agronomically optimum N rate (AONR) has been reported for the first 3 years of IWG production, little is known about N requirements in older perennial plants to sustain grain production (Jungers et al., 2017; Tautges et al., 2018). Some studies have indicated that fertilizer applied at the AONR had no impact on IWG grain yield in the first year, likely because soil N was not a limiting growth factor (Fernandez et al., 2020; Jungers et al., 2017). However, in subsequent years, grain yields responded to N fertilizer (Fernandez et al., 2020). Similarly, a meta-analysis revealed that IWG grain yield responded more to N fertilizer as the stand ages (Rebesquini et al., 2022). Nitrogen fertilization alone may not be sufficient to protect grain yield potential, but coupling it with an agronomic management practice to increase light penetration to the crown may make sustaining grain yield over time more likely (Pinto et al., 2021).

Management strategies that disturb the IWG canopy and potentially stimulate fertile tiller production include wider row spacing (Fernandez et al., 2020; Hunter et al., 2020), defoliation (Bergquist et al., 2022; Culman et al., 2023; Pinto et al., 2021; Pugliese et al., 2019), postharvest burning (Bergquist et al., 2022; Pinto et al., 2021), and thinning established stands (Bergquist et al., 2022; Law et al., 2021; Pinto et al., 2021). Wider row spacing (e.g., 30 cm vs. 15 cm) has been associated with increased grain yield due to enhanced fertile tiller production (Fernandez et al., 2020; Han et al., 2013; Hunter et al., 2020). However, when wider spacing is paired with reduced planting density, the resulting yield increase from tillering may not fully offset the overall yield loss due to fewer plants per unit area (Fernandez et al., 2020). Further, defoliation through summer and/or fall forage harvests has shown positive effects in most studies (Culman et al., 2023; Hunter et al., 2020; Pugliese et al., 2019; Sakiroglu et al., 2020), or no impact in another study (Zimbric et al., 2021). Grain yield stimulation from defoliation was attributed to improved nutrient cycling, reduced intraspecific competition, and increased light penetration (Knapp & Seastedt, 1986; Pugliese et al., 2019). While the timing of defoliation did not affect the proportion of fertile tillers, spring defoliation, more so than fall, has been shown to stimulate tiller production

(Hunter et al., 2020). Burning after harvest has also produced mixed results. One study found no effect on grain yield (Pinto et al., 2021), while another reported increased grain and straw yields (Bergquist et al., 2022). Thinning by killing rows using mechanical (e.g., interrow cultivation or tillage) or chemical (e.g., banded herbicide) methods alters stand density and has been explored as a strategy to improve yield (Bergquist et al., 2022; Law et al., 2021; Pinto et al., 2021).

The timing of thinning plays a crucial role in its effectiveness. When conducted in the fall before IWG entered winter dormancy, strip tillage increased grain yields by 61% compared to no-tillage in the following year, largely due to a higher density of fertile tillers per unit area (Law et al., 2021). In contrast, spring strip tillage reduced competition among fertile tillers but did not result in yield differences compared to no-tillage (Law et al., 2021). Similarly, grain yields following fall interrow cultivation or spring band-applied herbicide showed no differences from control treatments in the second and third production years (Bergquist et al., 2022). Pinto et al. (2021) found that fall-thinning practices, which reduced stand density by 75%, increased light penetration and the proportion of fertile tillers per row but decreased grain yield per area due to the reduced number of rows. This highlights the importance of maintaining adequate row density when thinning to enhance light penetration and fertile tiller production while avoiding yield losses at the field scale. However, thinning practices that open the canopy may also increase weed density and biomass, intensifying competition with IWG. While some studies reported low weed pressure with no discernible effects of thinning (Law et al., 2021), others observed that herbicides applied in the spring or fall for thinning the stand were associated with greater weed pressure compared to no-thinning controls (Bergquist et al., 2022). Given the variability of weed dynamics in cropping systems, it is essential to evaluate the impact of thinning treatments on IWG grain yield, forage mass, and weed pressure to optimize management practices.

The potentially beneficial interaction of N fertilization and stand thinning on IWG grain yield and forage mass to sustain yields over time has not been previously studied. Thus, our objective was to quantify how stand-thinning intensity (0%, 25%, 38%, and 50% of the stand) and N fertilizer rates (0, 75, and 150 kg N ha^{-1}), alone and in combination, influence the yields of IWG. Specifically, we evaluated their effects and interactions on IWG grain and forage yield, weed biomass, IWG thousand-kernel weight (TKW), and harvest index (HI) across two production seasons in mature stands. We hypothesized that supplying N fertilizer would increase grain yield and forage mass relative to the non-fertilized control because N is a major yield-limiting nutrient in perennial cereals; that spring-thinning removing 25% or more of tillers would reduce intraspecific competition and enhance grain yield in the subsequent two harvests without reducing total forage mass; and that the positive yield response to thinning would only occur under non-limiting N conditions, whereas under N-deficient conditions, thinning alone would not overcome nutrient constraints.

Core Ideas

- Intermediate wheatgrass (IWG) forage mass and grain yield mass were greater with 75 kg N ha^{-1} than with no fertilization.
- While greater nitrogen inputs may provide higher overall returns in high-yielding environments like Lancaster, moderate rates still deliver strong economic returns with lower input risk.
- IWG grain yields in thinned stands were up to 37% greater at only one site after 2 years; yields were not affected by thinning in other site-years.

2 | MATERIALS AND METHODS

2.1 | Location characterization

This study was conducted at two sites—the University of Wisconsin-Madison Agricultural Research Stations at Lancaster (42°49′48.5″ N, 90°47′19.1″ W) and West Madison (43°03′42.2″ N, 89°31′54.1″ W). The soil at Lancaster was Fayette silt loam, mildly eroded phase (fine-silty, mixed, superactive, mesic Typic Hapludalfs), and at West Madison was Plano silt loam, gravelly substratum (fine-silty, mixed, superactive, mesic Typic Argiudolls), both with 2%–6% slopes. Prior to the start of the study, six 15-cm deep soil samples were collected on October 14, 2020, from three areas of each field. Samples were analyzed at the UW-Madison Soil and Forage Analysis Laboratory (Table 1).

The experiment was conducted in three old IWG fields: two at Lancaster (LA1 and LA2) and one at West Madison (WM1). The experiment was initiated at LA1 in 2021 and at LA2 and WM1 in 2022 (Table 1). The Lancaster fields, LA1 and LA2, were conducted in different sections of the same large field. The IWG field at Lancaster was planted using a Great Plains 1006 no-till grain drill (Great Plains Manufacturing) with a 38-cm row spacing in the spring of 2018. The field was fertilized with 56 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, and 84 kg K_2O ha^{-1} on April 26, 2018. Broadleaf weeds were managed with 2.4-D amine broadcast applied at 1.07 kg acid equivalent (ae) ha^{-1} on July 6, 2018. From July to October 2018, the field was mowed five times to reduce weed biomass (mainly giant foxtail, *Setaria faberi* L.). On May 5, 2019, IWG was cut to a height of 10 cm for a spring forage harvest. The first grain harvest was in the summer of 2019 from one 0.5 m^2 quadrat per plot where fertile tillers were cut and removed by hand, followed by harvest of the remaining forage to a 10 cm height. Samples were dried at 47°C until constant mass. On

TABLE 1 Description of soil characteristics to 15 cm (including average pH, organic matter [OM%], phosphorus [P], potassium [K], and nitrate–nitrogen [NO_3-N] concentrations in mg kg^{-1}), intermediate wheatgrass (IWG) planting dates, and application dates for nitrogen fertility and thinning treatments at three fields in Wisconsin (Lancaster Field 1, Lancaster Field 2, and West Madison Field 1).

	Lancaster		West Madison
Factor	**Field 1**	**Field 2**	**Field 1**
pH	6.4		6.6
OM (%)	2.4		3.2
P (mg kg^{-1})	18		27
K (mg kg^{-1})	127		145
NO_3-N (mg kg^{-1})	5.1		6.6
Planting date	April 23, 2018		September 20, 2019
Planting density	11 kg PLS ha^{-1}		11 kg PLS ha^{-1}
IWG germplasm	TLI-C4		TLI-C5
Previous crop	Corn silage (*Zea mays* L.)		Alfalfa (*Medicago sativa* L.)
Stand age at treatment implementation (years)	3	4	3
Pretreatment N fertilization date	March 22, 2021	March 22, 2021	May 4, 2021
N fertilization Treatment date	March 29, 2021 April 11, 2022	April 11, 2022 April 10, 2023	April 4, 2022 April 10, 2023
Thinning treatment date	April 5, 2021	April 27, 2022	April 27, 2022
Harvest date	August 3, 2021 (Year 1) July 29, 2022 (Year 2)	July 28, 2022 (Year 1) July 31, 2023 (Year 2)	July 27, 2022 (Year 1) August 1, 2023 (Year 2)

March 22, 2021, 22 kg N ha^{-1}, 106 kg P_2O_5 ha^{-1}, and 213 kg K_2O ha^{-1} were applied.

In early September 2019, the WM1 seedbed was prepared using a moldboard plow, vertical tiller, and a cultipacker. IWG seed was planted with a 3-m-wide International Harvester grain drill (International Harvester of Canada Limited) with a 36-cm row spacing. On May 12, 2020, 2–4 D was applied at 1.07 kg ae ha^{-1} to manage broadleaf weeds. Grain was harvested using a ZÜRN 150 combine (ZÜRN Harvesting GmbH & Co. KG), and the remaining straw was cut to 10 cm and baled in summer 2020. In spring 2021, 90 kg N ha^{-1} was applied as urea, and grain was harvested by combine in summer 2021.

Climate data were obtained from the National Oceanic and Atmospheric Administration (NOAA, 2024). The 30-year average monthly precipitation was 72 mm at Lancaster and 75 mm at West Madison (Figure S1). During the study period, precipitation was slightly higher at West Madison but overall similar between sites (Figure S1). Temperatures were also consistent across both sites and years (Figure S2). Fields were not irrigated.

2.2 | Experimental design

The experimental design in each of the three fields was a randomized complete block with four replications. The treatment design constituted a full factorial of two factors: spring N fertilization and spring stand thinning. The N fertilization factor comprised three levels: 0, 75, and 150 kg N ha^{-1}. At LA1, 22 kg N ha^{-1} was also applied across the entire field in early spring of the treatment year (Table 1). Nitrogen fertilization treatments will be subsequently referred to as low, moderate, and high N, respectively, across fields. The stand thinning factor consisted of four levels: 0%, 25%, 38%, and 50% stand thinned by band-applied herbicide (described below) to reduce the number of IWG rows per plot. Thinning resulted in new average row spacing of 38, 51, 61, and 76 cm, respectively, at Lancaster, and 36, 48, 58, and 72 cm, respectively, at West Madison. The control treatment was neither fertilized nor thinned.

Plot size was based on the row spacing of the IWG stand at each location and was configured to include eight rows per plot. Consequently, plot size was 6.1 m long × 3.0 m wide at Lancaster and 4.6 m long × 2.8 m wide at West Madison. The experiment totaled 144 plots across treatment levels and fields (three N rate levels × four thinning density levels × four replications × three fields). Plots were separated by 1-m-wide alleys, which were maintained by mowing.

Nitrogen was applied in the form of 44-0-0 polymer-coated urea. A 3-m-wide Gandy spreader was utilized at Lancaster, while a 0.9-m-wide Gandy push spreader (The Gandy Company) was used at West Madison to apply N fertilizer at spring green-up (Table 1). At 15 cm of spring growth,

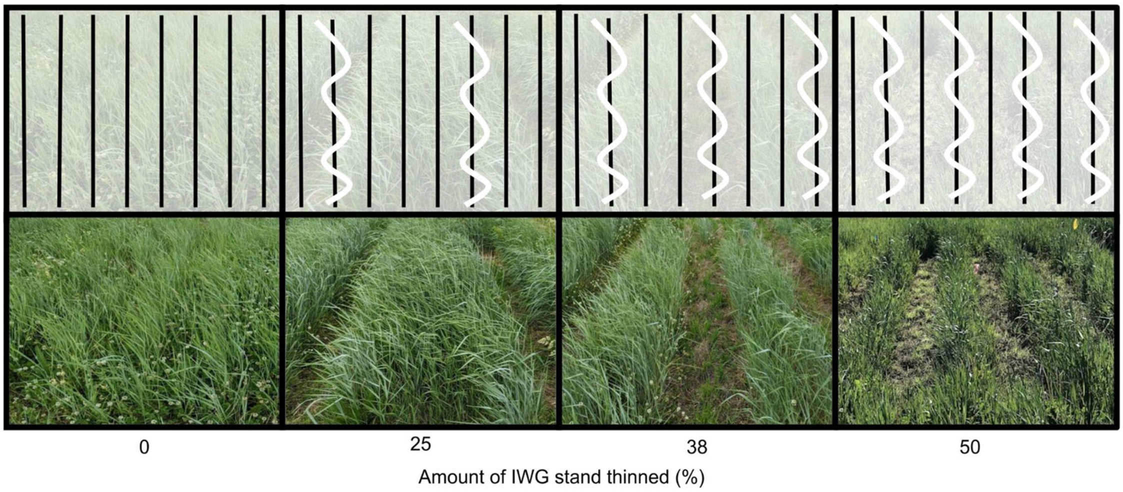

FIGURE 1 Illustration of the four thinning levels implemented in this study. White areas represent the rows removed through banded herbicide applications, while black lines depict the remaining rows. Photos show an example of each treatment at the West Madison Agricultural Research Station, Wisconsin, on June 7, 2021. IWG, intermediate wheatgrass.

thinning treatments were implemented by applying glyphosate (Roundup PowerMAX) at a rate of 0.74 kg ae ha^{-1} using a CO_2-pressurized backpack sprayer and a 3-m-wide boom with TeeJet AIXR 110015 nozzles (Spraying Systems Co.) on zero, two, three, or four of eight total rows of IWG per plot to reduce stand density by 0%, 25%, 38%, and 50%, respectively (Figure 1). After 2 weeks, IWG mortality was visually assessed to be 100%.

2.3 | Data collection

IWG grain, forage, and weed biomass were collected in both study years. Year 1 refers to 3 months after thinning, and Year 2 refers to 15 months after thinning. Thinning treatments were applied only once, in the spring of Year 1, while N fertilization treatments were applied in the spring of Year 1 and Year 2. Grain and forage were harvested at physiological maturity (dates provided in Table 1). Although we refer to the product as forage in this study, IWG harvested simultaneously with the grain is of very low quality and resembles straw. Due to differences in row spacing, grain and forage were hand-harvested from a 0.3-m × 1.5-m quadrat at Lancaster and a 0.3-m × 1.4-m quadrat at West Madison. Each quadrat included four original rows to capture the thinning treatment effect. For the 38% thinning treatment, samples were harvested from two quadrats per plot and averaged. All fertile tillers within the quadrat were cut, dried at 47˚C for 10 days, and threshed using a mechanical thresher to estimate grain yield. Forage and weed biomass were clipped to a 10 cm height, separated, dried at 47˚C for 10 days, and weighed to determine dry biomass. Dry matter yields per hectare were extrapolated from quadrat data. The TKW was calculated by weighing 100 dry, dehulled kernels and multiplying by 10. HI was calculated as the ratio of dry grain mass to the total mass of dry grain and forage. The number of fertile tillers was measured by counting all fertile tillers within each quadrat at the time of harvest, which was collected only at WM1 and LA2 in 2023, both in Year 2.

2.4 | Statistical analysis

Grain yield data from Lancaster and West Madison for Years 1 and 2 were pooled for initial analysis, which showed two-way interactions between N fertilizer and location (p-value $<$ 0.0001) and between year and location (p-value < 0.0001), and a three-way interaction among N fertilization, location, and year (p-value < 0.01). Thus, data were analyzed by location.

For the Lancaster model (Model 1), treatments and field were treated as fixed effects, block was included as a random effect nested within field, and year was modeled as a repeated measure:

$$\begin{aligned} Y_{ijklm} = {} & \mu + T_i + N_j + F_k + Y_l + \beta_m + T_i \times N_j + T_i \times F_k \\ & + T_i \times Y_l + N_j \times F_k + N_j \times Y_l + F_k \times Y_l + T_i \\ & \times N_j \times F_k + T_i \times N_j \times Y_l + T_i \times F_k \times Y_l + N_j \\ & \times F_k \times Y_l + T_i \times N_j \times F_k \times Y_l + \varepsilon_{ijklm} \end{aligned} \quad (1)$$

where Y_{ijklm} = response variable (grain yield, forage mass, weed biomass, TKW, or HI); μ = overall mean; T_i = effect of thinning; N_j = effect of N fertilizer; F_k = effect of field (LA1 or LA2); Y_l = effect of year; β_m = block; ε_{ijklm} = random residual. Residuals were assumed to be independent and normally distributed with constant variance. These assumptions were assessed using residual plots in RStudio, version 2022.07.1 (RStudio, PBC). Year was included as a fixed effect to account for repeated measures across time, and no specific residual covariance structure was modeled. Interactions were treated as fixed effects and evaluated separately if significant at $\alpha = 0.05$. The model was analyzed using the lmer function from the lme4 package in R. Simple linear regressions were also conducted for each response variable, with N fertilizer rate and thinning intensity evaluated as separate predictors. Analyses were performed by location and year when significant interactions were detected. The lm function in R was used to extract regression equations, p-values, and R^2 values. Regression lines were only included in figures when relationships were statistically significant ($\alpha = 0.05$).

For the West Madison model (Model 2), treatments were treated as fixed effects, block was included as a random effect, and year was modeled as a repeated measure:

$$Y_{ijkl} = \mu + T_i + N_j + Y_k + \beta_l + T_i \times N_j + T_i \times Y_k + N_j \times Y_k + T_i \times N_j \times Y_k + \varepsilon_{ijkl} \quad (2)$$

where Y_{ijkl} = response variable (grain yield, forage mass, weed biomass, TKW, or HI); μ = overall mean; T_i = effect of thinning; N_j = effect of N fertilizer; Y_k = effect of year; β_l = block; ε_{ijkl} = random residual. Residuals were assumed to be independent and normally distributed, and these assumptions were checked using residual plots. Grain yield data were square-root transformed to meet model assumptions and back-transformed for presentation. Interactions were considered fixed effects and evaluated separately when significant at $\alpha = 0.05$. Simple linear regressions were conducted as described for the Lancaster model.

3 | RESULTS

3.1 | Effects of N fertilization

No interactions occurred between N fertilization and IWG thinning treatments for measured variables (Table 2), so N and thinning effects were analyzed separately. Additionally, the N fertilization by field interaction at Lancaster was not significant for measured variables (Table 2), so data were pooled across fields for analysis. Grain yields across all fields responded positively to N fertilization, except at West Madison Year 2, which showed no response (Table 3). Pooled grain yields for Lancaster fields had a slope of 2.72 kg grain per kg of N. Using this regression equation (p-value < 0.01), grain yields were 293 kg ha^{-1} with no N fertilizer, 497 kg ha^{-1} with moderate N, and 701 kg ha^{-1} with high N (Figure 2a). At West Madison, the response to N fertilization was significant in only Year 1, with a slope of 1.22 kg grain per kg of N (p-value < 0.01); grain yields were 128 kg ha^{-1} with no N fertilizer, 221 kg ha^{-1} with moderate N, and 313 kg ha^{-1} with high N (Figure 2a). Grain yield was not affected by N fertilization at West Madison in Year 2 (p = 0.18; Table 3). The number of fertile tillers m^{-2} was not measured in Year 1. In Year 2, the regression was not significant at West Madison (p = 0.07), indicating no effect of N rate on fertile tiller number. In contrast, at Lancaster, the number of fertile tillers increased linearly with N fertilization ($p < 0.001$; fertile tillers m^{-2} = 112 + 1.7 × N rate). This corresponds to 95% and 210% more fertile tillers with moderate and high N rates, respectively, compared to the non-fertilized control in Lancaster Year 2.

Forage mass showed a positive linear response to N fertilization, except at West Madison Year 2, where no response was observed (Table 3). Using the regression equations for Lancaster Years 1 and 2 (both p-values < 0.01), forage mass at moderate and high N rates was 26% and 52% greater for Year 1, and 55% and 109% greater for Year 2, compared to the non-fertilized control. Forage mass at West Madison Year 1 (p-value 0.02) with moderate and high N rates resulted in 29% and 58% greater yields than the control (Figure 2b).

Further, N fertilization had no effect on weed biomass at either location but did influence both TKW and HI (Table 2). At Lancaster, TKW increased by 5% and 10%, and HI increased by 17% and 34%, at moderate and high N rates, respectively, compared to the non-fertilized control (Table 3, Figure 2c,d). At West Madison, TKW and HI responded positively to N fertilization in Year 1, with TKW increasing by 6% and 11% and HI increasing by 27% and 54% at moderate and high N rates, respectively, compared to the control. No significant response in TKW or HI was observed at West Madison in Year 2.

3.2 | Effects of IWG stand thinning

The effects of stand thinning varied across fields. At Lancaster, thinning effects did not differ between fields, so data were pooled for analysis (Table 2). In Year 1, grain yields at Lancaster were unaffected by thinning (Table 4). However, in Year 2, grain yield increased linearly with thinning intensity, with a slope of 2.73 kg grain per percent thinned (p = 0.04; Figure 3a). Based on the regression equation, grain yield at 50% thinning was 505 kg ha^{-1}, a 37% increase over the non-thinned control yield of 368 kg ha^{-1}. Despite this yield response, the number of fertile tillers at LA2 in Year 2 did not differ with levels of thinning (p-value 0.73; data not shown). In contrast, grain yields at West Madison in both Years 1 and 2 were not influenced by thinning (Table 4). As above, the number of fertile tillers at West Madison in Year 2 did not differ with levels of thinning (data not shown).

TABLE 2 *F*-values and significance levels from the analysis of variance evaluating the main effects of thinning, nitrogen (N) fertilization, field, and year, as well as their interactions, on grain yield, forage mass, weed biomass, thousand-kernel weight (TKW), and harvest index.

	Grain	Forage	Weeds	TKW	Harvest index
	Lancaster				
Thinning (*T*)	0.26	1.79	2.06	2.31	4.69**
N fertilization (*N*)	56.91***	39.94***	0.86	20.24***	25.69***
Field (*F*)	31.29***	6.03*	0.02	67.99***	21.99**
Year (*Y*)	30.16***	0.59	0.71	54.30***	16.02***
$T \times N$	0.32	0.36	0.94	0.42	0.52
$T \times F$	0.30	1.38	1.18	1.39	1.50
$N \times F$	0.68	0.13	0.15	1.61	2.36
$T \times Y$	7.41**	2.32	0.53	2.04	2.51
$N \times Y$	0.45	8.85***	2.48	0.11	1.93
$F \times Y$	0.06	0.05	3.49	70.29***	0.70
$T \times N \times F$	0.69	0.43	0.95	0.65	0.67
$T \times N \times Y$	1.53	0.69	0.32	1.31	0.62
$T \times F \times Y$	1.74	1.91	1.13	1.34	0.13
$N \times F \times Y$	1.23	2.19	0.76	0.14	2.03
$T \times N \times F \times Y$	1.07	0.63	0.82	1.24	0.69
	West Madison				
T	2.27	4.12**	0.014**	0.49	0.6325
N	12.79***	4.50**	0.78	3.52*	6.791**
Y	157.20***	20.80***	0.47	29.23***	56.88***
$T \times N$	0.82	1.04	0.92	1.36	1.925
$T \times Y$	0.77	1.01	0.76	1.55	0.8648
$N \times Y$	6.52**	0.075	0.54	6.39***	4.992**
$T \times N \times Y$	0.31	2.23*	0.14	1.04	1.92

Note: The dataset includes measurements from two fields near Lancaster, WI, and one field near West Madison, WI, collected from 2021 to 2023.
*,**, and *** denote significance levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

Lancaster forage mass decreased linearly with thinning intensity, with a slope of −15.90 kg forage per percent thinned ($p < 0.01$; Figure 3b). Using this regression equation, forage mass at 50% thinning was 4664 kg ha^{-1}, which is 15% lower than the non-thinned control yield of 5459 kg ha^{-1}. Thinning had no effect on forage mass in West Madison. Thinning also did not affect weed biomass (data not shown) or TKW in any site (Table 4). However, it did influence HI at Lancaster, where it increased linearly with thinning intensity (Figure 3c).

4 | DISCUSSION

The differences in yield between Lancaster and West Madison highlight the impact of environmental conditions, management history, and stand age on IWG performance. Poor initial establishment at West Madison, likely due to location-specific factors such as lower soil fertility and higher weed pressure, substantially limited productivity. Weed competition further affected IWG productivity at West Madison. Although the total weed biomass was similar across locations, the ratio of forage to weed biomass was much lower at West Madison, nearing 1:1, compared to a 3.4:1 ratio at Lancaster (data not shown). This suggests greater weed competition with IWG at West Madison compared to Lancaster, affecting both grain and forage mass. A strong field effect was observed across most response variables at Lancaster, except for weed biomass (Table 2). This field effect likely reflects differences in stand age between the fields. At the start of the study, the stand age at LA1 was 3 years, while at LA2, it was 4 years. Consequently, data were collected over stand ages of 3–4 years at LA1 and 4–5 years at LA2. Thus, the field effect is confounded with the stand age effect. Declining grain yield with increasing stand age is a well-documented characteristic of IWG (Hunter et al., 2020; Jungers et al., 2017; Zimbric et al., 2020). As LA2 was 4 years old at the study's start, 1 year older than LA1, it likely had lower initial grain yields due to its more advanced stand age.

TABLE 3 Regression slope, intercept, *p*-value, and R^2 values for the effects of nitrogen fertilizer rate on intermediate wheatgrass (IWG) grain yield, forage mass, thousand-kernel weight (TKW), and harvest index (HI) in Years 1 and 2 at Lancaster and West Madison, WI.

	Slope	Intercept	*p*-value	R^2
	Lancaster			
Grain yield, Years 1 and 2	2.72	293.26	<0.01	0.39
Forage mass, Year 1	14.05	4016.02	<0.01	0.24
Forage mass, Year 2	23.35	3199.68	<0.01	0.43
TKW, Years 1 and 2	4.6e-3	6.88	<0.01	0.08
HI, Years 1 and 2	1.7e-4	0.076	<0.01	0.11
	West Madison			
Grain yield, Year 1	1.22	128.16	<0.01	0.35
Grain yield, Year 2	NS	48.74	0.18	NS
Forage mass, Year 1	8.53	2212.29	0.02	0.11
Forage mass, Year 2	NS	1461.42	0.22	NS
TKW, Year 1	4.6e-3	6.07	<0.01	0.21
TKW, Year 2	NS	6.95	0.75	NS
HI, Year 1	2.1e-4	0.058	<0.01	0.17
HI, Year 2	NS	0.038	0.42	NS

Note: Data from both years are combined when there was no significant interaction between year and nitrogen rate ($\alpha = 0.05$); otherwise, regressions are shown separately by year.
Abbreviation: NS, not significant.

TABLE 4 Regression slope, intercept, *p*-value, and R^2 values for the effects of thinning rate on intermediate wheatgrass grain yield, forage mass, thousand-kernel weight (TKW), and harvest index (HI) in Years 1 and 2 at Lancaster and West Madison, WI.

	Slope	Intercept	*p*-value	R^2
	Lancaster			
Grain yield, Year 1	NS	600.36	0.23	NS
Grain yield, Year 2	2.73	368.37	0.05	0.04
Forage mass, Years 1 and 2	−15.90	5459.29	0.04	0.02
TKW, Years 1 and 2	NS	7.05	0.10	NS
HI, Years 1 and 2	1.9e-4	0.073	<0.001	0.16
	West Madison			
Grain yield, Years 1 and 2	NS	159.00	0.42	NS
Forage mass, Years 1 and 2	NS	2373.38	0.16	NS
TKW, Years 1 and 2	NS	6.61	0.61	NS
HI, Years 1 and 2	NS	0.051	0.21	NS

Note: Year 1 represents measurements taken 3 months after thinning, and Year 2 represents measurements taken 15 months after thinning. Data from both years are pooled when there was no interaction between year and thinning rate ($\alpha = 0.05$); otherwise, regressions are shown separately by year.
Abbreviation: NS, not significant.

4.1 | Effects of nitrogen fertilization

Grain yield consistently increased with N fertilization compared to non-fertilized stands, though the response varied slightly across locations. Despite low yields at West Madison, all fields in the study demonstrated similar responses to N fertilization. As grain and forage are harvested and removed over the years, fertilization becomes necessary to replenish soil N levels and sustain grain yields (Fernandez et al., 2020; Pugliese et al., 2019). Pre-fertilization nitrate-N concentrations in our fields were low in the upper 15 cm, 6.6 mg kg^{-1} at West Madison and 5.1 mg kg^{-1} at Lancaster (Table 1), falling below the level that is known to be sufficient for row crops at 20 mg kg^{-1} nitrate-N in the surface soil (USDA, 2014). Even though IWG does not require as much N as row crops, the levels at West Madison and Lancaster

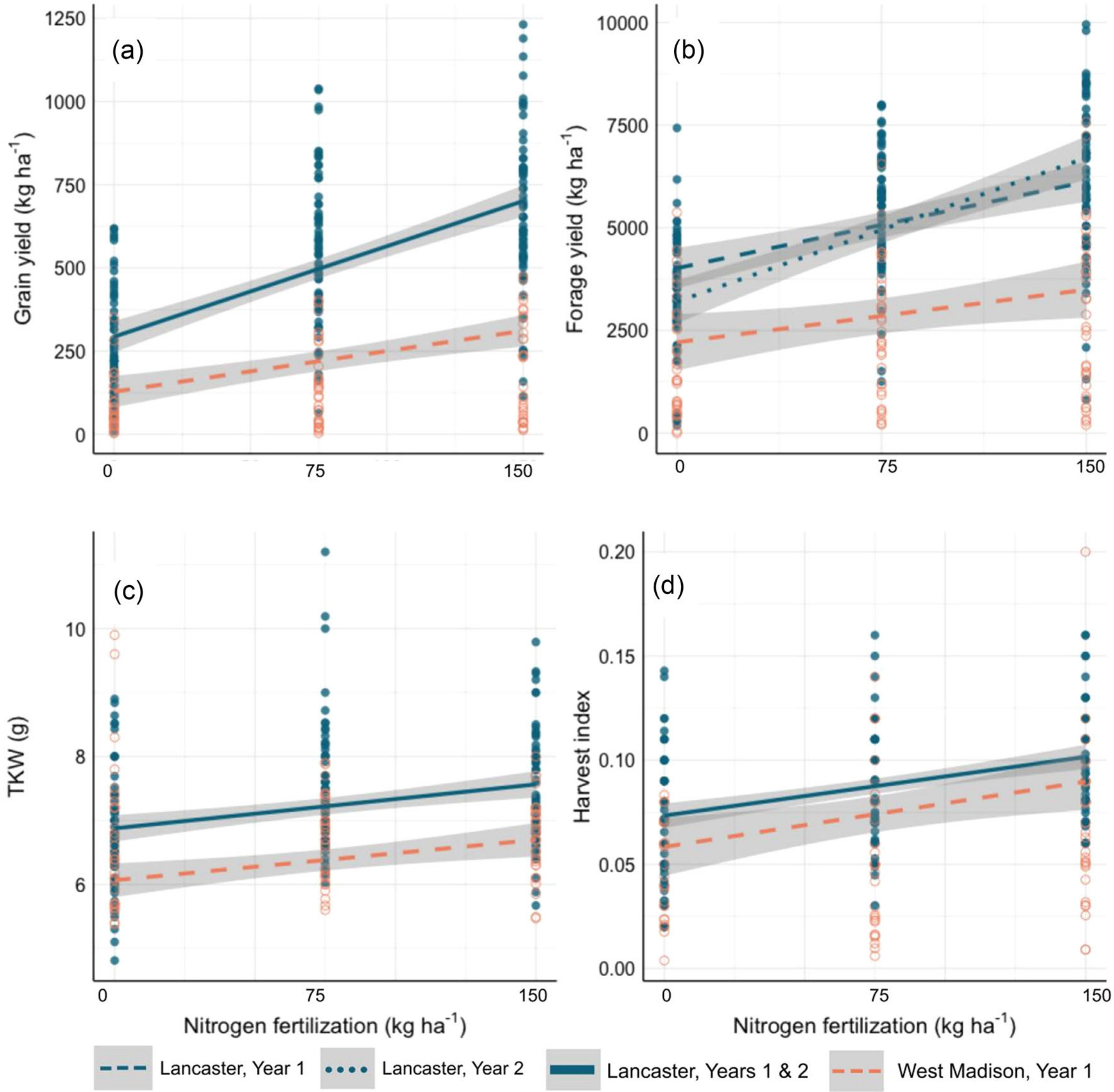

FIGURE 2 (a) Intermediate wheatgrass grain yield, (b) forage mass, (c) thousand-kernel weight (TKW), and (d) harvest index in Years 1 and 2 at Lancaster and West Madison, WI, across nitrogen fertilizer rates. Regression lines are shown only for relationships significant at the $\alpha = 0.05$ level. Years were combined when there was no significant interaction with year. All regression equations and associated statistics are provided in Table 3.

are still substantially lower than adequate (USDA, 2014). Given these conditions, we anticipated yield responses to N fertilization.

In Minnesota, Jungers et al. (2017) identified an agronomic optimum nitrogen rate (AONR) of 61–96 kg N ha^{-1} for the TLI-C2 IWG variety. While higher N rates can increase grain production, the ideal rate depends on balancing yield gains, input costs, and environmental risk. Based on 2024 prices, \$4.41 kg^{-1} for clean, dehulled Kernza grain (Fancher, personal communication, 2024) and \$0.71 kg^{-1} for polymer-coated urea (Jay-Mar, Inc., personal communication, 2024), we estimate a return of \$11.29 per kg N at Lancaster and \$4.67 per kg N at West Madison, using regression-based yield responses. Grain prices fluctuate over time and can vary substantially between conventional, organic, and Regenerative Organic Certified markets; therefore, return calculations must be tailored to local grain prices at the time of interest. Because our models assume a linear yield response, return per unit N remains constant across N rates, though total returns increase with higher N applications. While greater N inputs may provide higher overall returns in high-yielding environments like Lancaster, moderate rates still deliver strong economic returns with lower input risk. High N rates also increase the risk of lodging, which can reduce yield (Fernandez et al., 2020; Jungers et al., 2017; Koeritz et al., 2015) and pose environmental risks such as ground and surface water contamination. Moreover, excessive N application contributes to broader environmental issues, including leaching, volatilization, runoff, and greenhouse gas emissions (Erisman et al., 2011; Fowler et al., 2013). Considering these trade-offs and the consistent economic and yield benefits of moderate fertilization across locations, we recommend moderate N rates

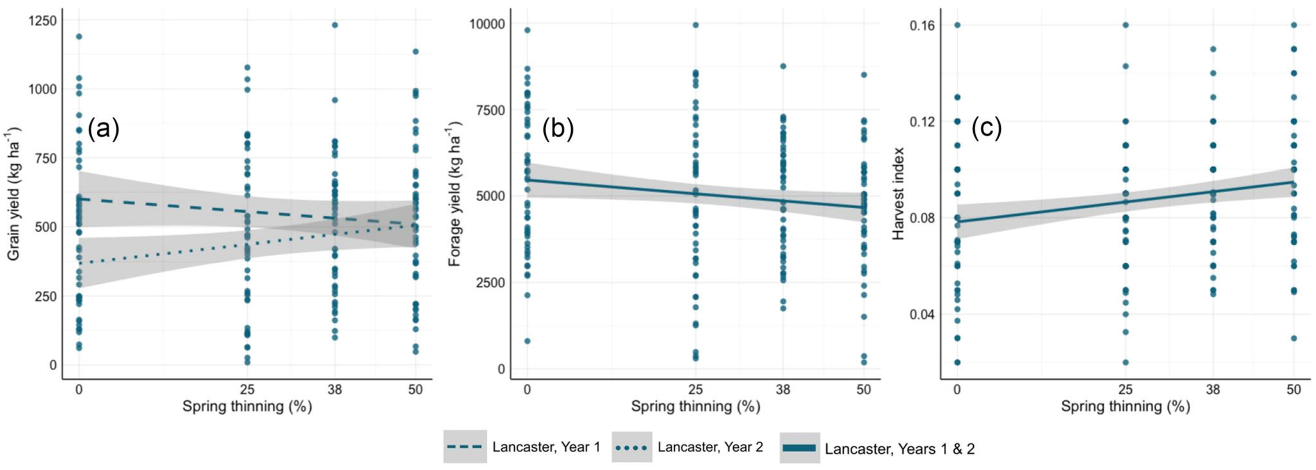

FIGURE 3 (a) Intermediate wheatgrass grain yield in Year 2 (15 months after thinning), (b) forage mass in Year 1 (3 months after thinning), and (c) harvest index in Year 2 at Lancaster, WI, across thinning rates. Regression lines are shown only for relationships significant at the $\alpha = 0.05$ level. All regression equations and associated statistics are provided in Table 4.

for IWG grain production to optimize both profitability and environmental sustainability.

Our summer forage mass was consistent with those reported in recent studies. For instance, Culman et al. (2023) observed summer forage mass ranging from 1600 to 8000 kg ha^{-1} in the third production year under moderate N fertilization rates (80–90 kg N ha^{-1}) across nine distinct locations. Similarly, our forage mass fell within this range, with Lancaster yields toward the upper range and West Madison yields toward the lower range. Nitrogen fertilization has been shown to increase forage mass compared to non-fertilized controls (Pugliese et al., 2019). Consistent with this, Zimbric et al. (2020) reported a 14% increase in summer aboveground biomass with 135 kg N ha^{-1} compared to 90 kg N ha^{-1}. Our findings align, showing summer aboveground biomass increases of 17% and 19% in Lancaster and West Madison, respectively, when high N rates were applied compared to moderate rates.

Fernandez et al. (2020) identified the proportion of fertile tillers as the most reliable indicator of grain yield, noting it was unaffected by N fertilization. Although we did not measure the proportion of fertile tillers in our study, we observed that the number of fertile tillers increased by 1.66 m^{-2} for every kg of N applied in Lancaster Year 2. This trend aligns with our finding that IWG grain yield increased with higher N fertilization rates. Fernandez et al. (2020) also found a positive correlation between TKW and grain yield, with TKW influenced by both N fertilization and planting density. However, while low planting density increased kernel mass, the specific effect of N fertilization on TKW was not reported.

In our study, TKW increased with N fertilization in all fields except West Madison Year 2, with no difference between moderate and high N rates. This suggests that moderate N fertilization is sufficient to enhance kernel mass in IWG. On average, kernel mass increased by 11% across all three fields with moderate N rates, contributing to the overall increase in grain yield. While this response is not well-documented for IWG, similar findings have been observed in winter wheat, where N fertilization increased TKW compared to non-fertilized controls (Hussain et al., 2006; Litke et al., 2017). However, in winter wheat, a common tradeoff exists in which increases in kernel number m^{-2} often come at the expense of kernel size (Vicentin et al., 2024). To better understand IWG yield components, we estimated kernel number m^{-2} by dividing grain yield (g m^{-2}) by TKW (g 1000 kernels^{-1}). Kernel number m^{-2} was strongly correlated with grain yield ($R^2 = 0.95$; Figure S3), while the relationship between kernel number m^{-2} and kernel mass was weak ($R^2 = 0.05$; Figure S4). This suggests that kernel mass remained relatively stable even as kernel number increased. This pattern, modest increases in both kernel number m^{-2} and kernel mass, contrasts with the typical tradeoff observed in annual wheat and may reflect differences in IWG's reproductive development. These results suggest that N fertilization plays a key role in improving both kernel mass and kernel number and thus enhancing IWG grain yield.

The HI was 31% and 40% greater with moderate and high N fertilization, respectively, compared to the control. As a measure of reproductive efficiency, HI reflects the proportion of total aboveground crop biomass allocated to grain yield. In IWG, a decline in HI over time typically indicates that resources are not fully constrained across the stand, as total biomass often remains stable or increases with age (Law et al., 2021). In our study, despite higher forage mass associated with N fertilization, HI increased, highlighting the role of N in maintaining grain yield efficiency over time. This suggests that N fertilization not only supports forage production but also mitigates the decline in grain yield and HI commonly observed in aging stands, ensuring sustained productivity in perennial grain systems. Also, it should be noted that IWG grain yields are generally low compared to annual small grains, but sustained breeding efforts are underway to increase grain yields and kernel mass while maintaining the crop's deep, fibrous root system and its associated environmental benefits.

4.2 | Effects of IWG stand thinning

Thinning in the Lancaster fields affected grain yield, forage mass, and harvest index; the effects of spring-thinning on grain yield in the Lancaster fields varied depending on the time after thinning (Table 2). Grain yield and HI were unaffected by thinning at Year 1 but increased with greater thinning intensity at Year 2 (Table 4). These results align with findings from Law et al. (2021), where spring-thinning reduced competition among reproductive tillers without affecting overall yield in the thinning year. Similarly, Bergquist et al. (2022) found no impact on grain yield during the thinning year when using spring-thinning with band-applied herbicides compared to controls. In contrast, Pinto et al. (2021) reported a decrease in grain yield following 75% fall-thinning of an IWG stand, likely due to the aggressive thinning intensity used. In our study, we deliberately applied less aggressive thinning rates (0%, 25%, 38%, and 50%) to identify an optimal rate for reducing intraspecific competition while maintaining stand productivity. Despite these more moderate thinning intensities, our results suggest that spring-thinning does not improve grain yields in Year 1. The field at West Madison showed no response to thinning, likely due to poor stand vigor and the high proportion of weeds relative to forage. This lack of response was evident across grain and forage mass, TKW, and HI (Table 4).

In contrast, we found that grain yield and HI at Year 2 were highest with the greatest thinning intensity for both Lancaster fields (Figure 3a,c), indicating that this treatment increased grain yield as well as the ratio of grain to total biomass. This suggests that reduced stand density in the fall may have a greater effect on IWG grain yields than spring stand density. IWG is a highly competitive grass that becomes denser over time, limiting light penetration to its crown during the fall (Fernandez et al., 2020; Pinto et al., 2021). Light quantity and quality are critical for fertile tiller production in grasses (Casal et al., 1985; Deregibus et al., 1985). As the canopy thickens, reduced light reaching the crown may inhibit tillering in IWG, leading to lower grain yields (Fernandez et al., 2020). Law et al. (2021) provide further evidence for this mechanism. They found that applying strip tillage to an aging IWG stand in the fall increased grain yields by 61% the following year compared to a non-tilled control, whereas spring tillage had no effect on yields. Combined with our findings, this strongly indicates that lower stand density in the fall is key to boosting grain yields in the following year. So, for farmers considering spring treatments to reduce stand density, such as banded herbicide applications or strip tillage, it is important to note that these treatments are unlikely to affect yields in the year they are applied. Instead, the impact becomes apparent in the subsequent year, after the reduced stand density in the fall.

Although the interaction between thinning and year for forage mass at Lancaster was not significant at the 0.05 level, it was significant at <0.10 (Table 2). This, combined with the significant regression, suggests that thinning reduced summer forage mass at Lancaster Year 1. This pattern aligns with findings from Law et al. (2021), who reported lower forage mass following spring-thinning compared to controls, and Pinto et al. (2021), who observed diminished forage mass after fall-thinning with an aggressive intensity of 75%. In contrast, Bergquist et al. (2022), using a less severe thinning intensity of 40% applied with band herbicides, found no difference in summer forage mass between spring-thinned and non-thinned treatments. In our study, thinning intensity reached up to 50%, potentially contributing to the observed forage mass decline, as it was more extreme than the thinning approach used by Bergquist et al. (2022). Also, Bergquist et al. (2022) observed greater weed biomass in all banded herbicide thinning treatments compared to the control, but we did not find that thinning affected weed biomass.

5 | CONCLUSIONS

This study highlights the importance of environment and management in IWG productivity. These analyses showed that moderate N fertilization increased grain yields, forage mass, TKW, and HI compared to no fertilization. While IWG yields were greater for higher than moderate N fertilization, the moderate rate was more cost-effective and posed less environmental risk. Thinning effects varied by site. At Lancaster, spring-thinning improved grain yields and HI in Year 2 compared to the non-thinned control, likely by reducing fall canopy density and improving light availability. However, spring-thinning treatments did not affect yields in Year 1 at Lancaster or at West Madison where poor stand vigor limited response. While thinning at 50% reduced forage mass compared to the non-thinned control at Lancaster Year 1, weed biomass was not affected. These results suggest that 50% thinning, timed to optimize fall light conditions, can enhance grain production the following year. By combining 50% thinning with moderate N fertilization, growers can effectively manage aging stands of IWG to maintain productivity.

AUTHOR CONTRIBUTIONS

Erica D. Shoenberger: Data curation; formal analysis; investigation; writing—original draft; writing—review and editing. **David E. Stoltenberg**: Conceptualization; methodology; supervision; writing—review and editing. **Valentin D. Picasso**: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data will be available publicly in the Dryad.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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