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Optimizing Crop Productivity and Fertility Practices in Intermediate Wheatgrass

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OPTIMIZING CROP PRODUCTIVITY AND FERTILITY PRACTICES IN INTERMEDIATE WHEATGRASS

by

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OPTIMIZING CROP PRODUCTIVITY AND FERTILITY PRACTICES IN INTERMEDIATE WHEATGRASS

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The intensive management associated with many annual crops often includes recurring tillage, fertilization, and pesticide applications, which contribute to environmental concerns such as water pollution and soil erosion. Intermediate wheatgrass (*Thinopyrum intermedium*), recognized under the trade name Kernza®, is a perennial grass that can be managed to produce grain and biomass while providing desired environmental benefits such as soil conservation and nutrient cycling. There has been limited research on best management practices and crop productivity for this alternative dual-use crop in Nebraska. A field experiment was conducted beginning in 2021 to assess nitrogen (N), phosphorus (P), and potassium (K) management practices for intermediate wheatgrass (IWG) grain and forage production and how it changes over the years of the stand. Additionally, a meta-analysis of 16 independent studies was conducted to evaluate the impact of nitrogen rates across sites and years on intermediate wheatgrass productivity. The meta-analysis found limited effects on grain yield in year 1 but optimal rates ranging from 51-150 kg N ha^{-1} in later years. In our field experiment for years 1 and 2, N, P, and K rates did not impact yields, while second year yields were much lower than the first year due to dry spring conditions during anthesis. Our findings highlight the importance of strategizing the fertilization practices across the intermediate wheatgrass

stand years in order to maximize grain and forage production. Moreover, IWG demonstrated its potential as a low-input, alternative, and dual use cash crop for Nebraska producers. Its adaptability makes it an appealing option for various industries, including food, restaurants, and bakeries. The grain holds promise for crafting bread, pasta, and could also serve as key ingredient in the production of alcoholic beverages like beer.

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Table of contents

List of Tables

List of Figures

List of Equations

CHAPTER 1: GENERAL INTRODUCTION

The state of Nebraska, located in the Midwestern region of the United States, has significant agricultural production based on annual crops and beef cattle. According to the USDA National Agricultural Statistics Service, the state is in the top 5 for soybean and corn production and is in the top 15 for wheat production in the U.S. (USDA NASS 2023). According to the agricultural census, it is estimated that around 50% of Nebraska's cropland uses no-till management, which is 85% greater than 20 years ago. However, the use of cover crops on annual cropland remains limited, accounting for only about 4% of the total acres, per the 2017 census data. In terms of beef cattle production, this practice offers opportunity for forage crops, and the state still has a sizable acreage of hay production, for example (Hiller et al., 2009). However, increasing corn and soybean production has taken over the acreage of previously cultivated perennial or cool-season crops. Furthermore, annual cropping systems are typically more susceptible to climate variability's adverse effects than sustainable agricultural approaches (Knutson et al., 2011; Sumberg and Giller, 2022).

With a growing global population and the important aspect of climate change, the agricultural cropping systems are facing a challenge: how to simultaneously increase food production and reduce environmental impacts when using finite resources (Pereira, 2017). In recent years, the interest in perennial crops as a more sustainable alternative to traditional annual crops has been growing. These crops, characterized by their deep and extensive root systems, offer a range of ecosystem services, helping to improve and maintain soil health and fertility, and soil water conservation (Asbjornsen et al., 2014; Basche and Edelson, 2017).

Perennial crops as a resilient alternative to mitigate drought impacts in agriculture

Severe weather events, such as drought, create a challenge to agriculture by threatening food security and food production across the globe. In the face of this threat, the adoption of perennial crops emerges as a promising strategy to enhance agricultural resilience (Picasso et al., 2022). Perennial crops can develop deep root systems that enable them to access water resources more efficiently, reducing their susceptibility to drought stress. Moreover, perennial crops often exhibit greater tolerance to extreme weather conditions such as prolonged periods of water scarcity through strategies such as dehydration tolerance, greater soil resource acquisition capabilities, or reduced growth potential, for example (Volaire et al., 2009; Keep et al., 2021). As a result, integrating perennial crops into agricultural systems can play an important role in mitigating the adverse effects of drought and safeguarding global food production (DeHaan et al., 2023).

Intermediate Wheatgrass

Intermediate Wheatgrass (IWG) (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey), also known as Kernza®, is a cool season perennial grass native to Eurasia. IWG stands as a multifunctional crop, and can serve as both forage and grain production, offering year-round protection and a deep belowground root system. Its aboveground biomass provides valuable forage for livestock, while the grain it produces holds promise as a novel ingredient for food products (Favre et al., 2019; Bajgain et al., 2022). Beyond its agronomic uses, Kernza offers several ecosystem services, including soil health and water quality improvement, carbon sequestration, and enhanced nutrient cycling (Pugliese, 2019; Ashworth et al., 2023; Rakkar et al., 2023). These characteristics make Kernza a promising candidate in sustainable agriculture, addressing the dual challenges of food production and environmental conservation in an era of climate change uncertainties.

The knowledge gap concerning Kernza fertilization across its established years highlights a critical area of research in sustainable agriculture. Kernza, as a perennial crop, presents unique challenges and opportunities, particularly in terms of maintaining grain yields as the stand ages. One key challenge is the decline in grain yields observed as Kernza stands mature (Jungers et al., 2017a; Altendorf et al., 2021). Unlike annual crops that are replanted every year, Kernza establishes itself over multiple years, where grain yields tend to decrease. This phenomenon is not yet fully understood, and the factors contributing to this decline in fertility need further investigation. Maintaining consistent grain yields across stand years is vital for the economic viability of Kernza farming. Farmers need to anticipate yield trends and develop management practices that can help decrease the decline in productivity as the stand ages. This may involve adjusting fertilization strategies, optimizing planting density, or selecting Kernza varieties that exhibit greater yield stability.

Thesis organization

Considering the identified gaps, the overall objective of this research was to advance the understanding of what are the best management practices in terms of fertility to maximize grain and biomass yield across the stand years of intermediate wheatgrass.

Chapter two investigated the response of Kernza to different agronomic practices, with the objective of optimizing nitrogen, phosphorus, and potassium management for Kernza grain and forage production over the stand years of the crop. Chapter three is a meta-analysis conducted to understand the nutrient needs from season-to-season based on nitrogen rate studies with intermediate wheatgrass, and to determine the rate effects on grain yield and biomass production across different years of the crop's development.

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CHAPTER 2: ASSESSING FERTILITY NEEDS AND CROP YIELDS IN THE FIRST TWO YEARS OF INTERMEDIATE WHEATGRASS IN EASTERN NEBRASKA

Abstract

The intensive management associated with many annual crops including corn and soybean includes annual tillage as well as frequent fertilization, and pesticide applications, which contribute to environmental degradation concerns such as water pollution and soil erosion. Intermediate wheatgrass (*Thinopyrum intermedium*), recognized under the trade name Kernza® by The Land Institute, is a cool-season low-input perennial grass that can be managed to produce grain and biomass while providing desired environmental benefits such as soil conservation and nutrient cycling. We conducted an experiment located in Eastern Nebraska through the multi-institution Kernza CAP, focusing on analyzing Kernza yields at different nitrogen (N) rates of 0, 45, 90, 135, and 180 kg ha^{-1}, different N application timings (spring, fall, and split-application), and N different sources (synthetic and organic fertilizer) in years 1 and 2 (fall 2021 through summer 2023). Also, treatments with phosphorus (P) at a rate of 56 kg ha^{-1} and potassium (K) at a rate of 168 kg ha^{-1} were applied. Plots were designated to either receive or not receive the P and K application. This trial evaluates different fertilizer management practices for Kernza compared with annual cropping systems (corn and soybeans), where a previous crop (oat) was implemented before planting Kernza. Grain yield, summer and fall biomass production, plant heights as well as lodging, and weed assessments were evaluated in the first two years of the project. We detected few differences between agronomic qualities (plant height, lodging, weeds) in year one although there were less lodging and fewer weeds in the fall-applied N treatment. In year two, limited differences again were observed between treatments, with no lodging occurrence, and weed incidence remained low. The significant differences observed in three out of twelve treatments sampled for fall forage production suggest that N rates around 90 kg ha^{-1} perform better than higher rates, considering factors such as grain yield, lodging, and fall biomass production. There was a large grain yield decline in the second stand year, likely a result of not only stand age, but also the extreme dry conditions faced by the crop during the 2023 growing season. Our findings suggest that rainfed Kernza in especially dry years occurring later in the crop's stand may not be optimal for grain production, however it can still provide strong above-ground biomass production. This study demonstrates the crop's potential as an alternative dual use crop that can endure periods of water stress while requiring lower fertility and herbicide needs compared to annual crops.

Keywords: perennial crops; resilient cropping systems; intermediate wheatgrass; Kernza; fertility management; nitrogen.

Abbreviations: IWG, intermediate wheatgrass.

Introduction

To meet the challenges of the growing population demand and climate change, future cropping systems must simultaneously increase food production and reduce environmental impacts while using the same unit area of land or input resources (Smith, 2013; Franco et al., 2021). This concept of ecological intensification can be achieved through a range of management practices within cropping systems, but it is essential to prioritize the enhancement of environmental quality, profitability, and productivity of agriculture, and also consider the social dimension of improving the quality of life for agricultural producers (Spiegal et al., 2018; Sanford et al., 2021). In recent years, there has been an increased interest in perennial grain crops as potential alternatives to annual cash crops. There is a growing body of literature that recognizes the ecosystem services provided by perennial crops, mainly associated with improving soil health and fertility (Rakkar et al., 2023; DeHaan et al., 2023). These improvements are facilitated by the presence of massive and deep root systems, which have a significant impact on soil physical and biological health indicators, carbon storage, nutrients and water availability, erosion control, among others (Jungers et al., 2017a; Duchene et al., 2020).

Since it was reported in 1983, Intermediate wheatgrass (IWG) (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey) has attracted considerable interest (Wagoner, 1990). Intermediate wheatgrass, a cool-season perennial grass native to Eurasia, has been undergoing domestication by The Land Institute (Salina, KS), and is marketed under the trade name Kernza® (DeHaan et al., 2018). Similar to other perennial plants, Kernza demonstrates significant potential as a multi-functional crop. It can be used as a dual-purpose crop while providing several soil-related benefits through year-round protection of above- and below-ground areas, facilitated by its deep root system and high aboveground biomass production (Picasso et al., 2022; Culman et al., 2023).

As the impacts of drought events continue to rise in the U.S. and are expected to grow in frequency and severity in the future, addressing drought-tolerant solutions for agriculture has become increasingly urgent (Kuwayama et al., 2019). Resilience, as described by Sanford et al. (2021), emerges as the capacity to respond to perturbations or extreme weather events. Within an agricultural context, one aspect of this resilience translates to the ability of plants to rebound and maintain productivity under unfavorable conditions. In comparison to annual crops, perennial cropping systems such as Kernza may exhibit enhanced resilience and stability during periods of extreme climate, as a result of documented improvements in water infiltration rates, soil structure, nutrient supply, and other soil parameters (Basche and DeLonge, 2017; Sanford et al., 2021) , as well as through exhibiting perennial crop stress strategies such as resource conservation to ensure multi-season survival which are not utilized by annual crops (Volaire et al., 2009; Keep et al., 2021).

To date, several studies have investigated the benefits of Kernza to the cropping systems, and other studies have explored how to manage this crop best to be a potential cash crop for producers. This study aims to contribute to this growing area of research by exploring critical factors related to the fertility management of Kernza and providing new insights into the environmental benefits of a perennial cropping system compared to 'business-as-usual' annual cropping systems such as corn and soybeans. Our objectives were: (1) evaluate the response of Kernza to different agronomic practices; and (2) optimize nitrogen (N), phosphorus (P), and potassium (K) management for Kernza grain and forage production over the stand years of the crop.

Materials and Methods

The present study is part of a five-year, multi-institution project funded by the USDA-NIFA Sustainable Agricultural Systems program. The project activities began in 2020 and will continue until 2025. The experiment was established in spring 2021 at the Eastern Nebraska Research, Extension and Education Center (ENREC) (9'24.68"N, 96°25'30.41"W 41°). The soils are predominantly classified as Filmore silt loam (50.8% of the area) and Yutan silty clay loam (43.9% of the area) (USDA-NRCS Web Soil Survey), with 26.8% clay, 13.4% sand, and 59.9% silt in the 0-20 cm soil layer.

Experimental design and management

The experiment was designed as a randomized complete block design with four replications. Kernza intermediate wheatgrass, MN-Clearwater variety (MN1504) (Bajgain et al., 2020), was planted following spring oats, into 38-cm rows, with a seeding rate of 12 kg ha^{-1}. Prior to planting, the soil was tilled to prepare the seedbed and remove weeds. Twelve different treatments were randomly assigned to 15 by 6 m plots, where ten treatments were planted with Kernza on September 8, 2021, and two of the treatments were assigned as the “business-as-usual” conventional cropping system (corn-soybean rotation) and established Spring 2022 (Table 2.1). Within the Kernza plots, five different

N fertilization rates were applied: 0, 45, 90, 135, and 180 kg N ha^{-1}. Synthetic nitrogen, applied as urea, was used in nine of the treatments at various times, including Fall (late October), Spring (late April), and Split (Fall and Spring) applications. Additionally, one organic N treatment was applied in the Fall using poultry manure at a rate of 180 kg N ha^{-1}. In the synthetic nitrogen treatments, the experiment also investigated phosphorus (56 kg N ha^{-1}) and potassium (168 kg N ha^{-1}) omission, where some plots received both P and K applications during the Fall, while others received only one of these nutrients. Fertilizer was hand-applied to each plot using hand crank spreaders.

Table 2.1. Description of treatments separated by nutrient, application time, and rate (kg ha^{-1}).

		Spring	**Fall**			
Treatment number	**Treatment name**	**N**	**N**	**P_2O_5**	**K_2O**	**SM***
			kg ha^{-1}			
1	BAU 1 (Corn)	180	0	112	168	Y
2	BAU 2 (Soybeans)	0	0	0	0	N
3	0-56-168	0	0	56	168	Y
4	45(spring)-56-168	45	0	56	168	N
5	90(spring)-56-168	90	0	56	168	Y
6	135(spring)-56-168	135	0	56	168	N
7	180(spring)-56-168	180	0	56	168	Y
8	90(fall)-56-168	0	90	56	168	Y
9	90(split)-56-168	45	45	56	168	N
10	Poultry manure	x	180	x	x	Y
11	90(spring)-56-0	90	0	56	0	N
12	90(spring)-0-168	90	0	0	168	N

*SM – Soil moisture monitoring treatments, if included – yes (Y) or no (N).

Weed surveys were conducted simultaneously (during stem elongation and anthesis stages) to evaluate the relative abundance of broadleaf and grass weeds in the Kernza treatments. This assessment focused on the inter-row space and canopy cover, where 0 indicated 0% weed coverage in the inter-row or canopy, 1 indicated 1-20%, 2 indicated 21-50%, 3 indicated 51-80%, and 4 indicated 81-100% weed coverage in the inter-row or canopy cover. Furthermore, lodging assessments were conducted for all treatments during anthesis and physiological maturity each year. The lodging scores varied between 0 and 9 and were determined based on two factors: the angle of the lodged plants relative to the soil surface and the percentage of plants in the plot that exhibited lodging. A score of 0 denoted no lodging, while a score of 9 represented complete lodging, with all plant stems horizontal to the soil surface. To ensure consistency in evaluating the treatments' relative effects, the same observer conducted all the assessments throughout the study period.

Before harvesting, plant height was measured by randomly selecting five stems from each plot and measuring them from the ground to the end of the seed head. When grain mass was constant and seedheads were not shattering yet (late-July), grain yields and biomass were determined by hand-cutting all plants using a 114.3 cm x 114.3 cm quadrat placed on the ground and centered over the correct number of rows, first cutting all the seed heads on stems of plants rooted inside the quadrat, and then collecting the remaining biomass at a height of 7-10 cm above the soil surface. The seedheads and biomass harvested from the sample area were dried and weighed, and lastly, the seedheads were threshed to obtain the grain weight of each sample. The remaining grain in the plots was harvested using a combine, and the straw was baled and removed from the field. During the Fall season, the forage from three selected treatments (3, 5, and 7, as listed in Table 2.1) was collected using the same quadrat area as that used for grain harvest. Subsequently, the dry weight of each treatment was recorded.

Soil Monitoring

Soils were monitored in selected treatments (Table 2.1) in order to compare key fertility treatments to annual crop controls. Before establishing the experiment in 2021, a baseline soil sampling was conducted at three different intervals (0-20 cm, 20-50 cm, and 50-100 cm), in four different points of each plot. All four sub-samples were aggregated and mixed well to result in one sample per plot per depth, kept refrigerated until analyzed or processed for shipping. These samples were analyzed for organic matter, pH, and micro and macronutrients. In subsequent years (2022 and 2023), composite soil samples were collected from the same plots. These samples were taken at a depth of 0-20 cm and analyzed for chemical composition.

Additionally, soil moisture tubes were placed in the same plots, and moisture measurements were collected using a Delta-T Devices PR2/6 Probe (Delta-T Devices, 2021), which features six sensors distributed across the probe and provides readings of volumetric soil water content (%) and millivolts (mV) to indicate moisture levels at different depths of the soil profile. Samples were collected every week from April until harvest each year.

Statistical Analysis

Statistical analyses were performed using *R* Studio (R Core Team, 2021). A two-way analysis of variance (ANOVA) with mixed effects was performed using the *lme4* package to evaluate the impact of different treatments on the response variables of grain yields, summer biomass, plant height, and soil chemical components. In this analysis, the explanatory variables of treatments and years, and their interaction, were treated as fixed terms, meaning we considered them to have consistent effects on the response variable, while block was considered a random factor. We used the same statistical model for variables with data available for only one year, such as soil moisture and fall forage yields, but with treatments as the only fixed effect and block as random.

For lodging, we analyzed the results for each year separately, since in 2023 no lodging occurred in any treatments. For NDVI results, we conducted separate analyses for each date to assess the impact of nitrogen application on the plants. Treatment means were compared using Tukey's HSD test at $P < 0.05$. The relationship between plant height and lodging was analyzed through Spearman correlation analysis at a significance level of $P < 0.05$.

Results

Variable weather conditions over the two years of the experiment

In 2021 (establishment year), total precipitation exceeded historical averages, with significant rainfall occurring in August (before planting) and October (after planting). In 2022, despite lower total precipitation, the field received well-distributed rainfall in May and June. However, in 2023, severe drought conditions throughout May and early June impacted the growth and development of plants (Table 2.2). Specifically, May 2023 rainfall was just over 4 mm compared to the long-term average of 122 mm. June 2023 rainfall was 98 mm compared to a long-term average of 124 mm ("PRISM Climate Group," 2021).

Table 2.2. Monthly mean air temperature and precipitation during 2021, 2022, 2023, and historical data for the experimental field. (Data extracted from PRISM Climate Group, Oregon State University).

	2021		**2022**		**2023**		**1991-2020**	
Month	**Precip (mm)**	**T (°C)**	**Precip (mm)**	**T (°C)**	**Precip (mm)**	**T (°C)**	**Precip (mm)**	**T (°C)**
Jan	31.2	-2.5	8.1	-6.1	29.1	-2.8	17.6	-4.9
Feb	21.4	-9.9	3.21	-3.4	39.9	-2.1	22.7	-2.5
Mar	126.7	7.2	51.1	3.8	20.6	1.7	41.1	4
Apr	55.4	9.9	28.6	8.3	49.3	10.4	76.9	10.3
May	94.7	16	105.2	16.5	4.1	18.2	121.6	16.5
Jun	82.5	23.9	75.8	23	98.1	23	123.9	22.3
Jul	67.9	23.6	21.3	24.9	196.1	23	85.3	24.5
Aug	203.6	23.8	62.0	23.7			97.7	23.2
Sep	34.6	19.9	30.9	19.7			77.4	18.8
Oct	130.3	13.1	18.9	11.3			58.9	11.7
Nov	11.3	5.9	15.1	2.8			34.7	3.9
Dec	8.2	1.6	27.2	-5.2			30.1	-2.4
	Total 868.3	**Mean 11.0**	**Total 447.7**	**Mean 9.9**	**Total (Jan-July) 437.5**		**Total 788.5**	**Mean 10.4**

Soil moisture measurements taken throughout the 2023 growing season within the monitored treatments (Figure 2.1) indicate a decrease in soil moisture percentages from April to May, particularly in shallower depths. However, as the season progressed and July brought higher precipitation levels, there was a subsequent increase in soil moisture across most treatments and depths. At a depth of 1000 mm, there was a noticeable increase in moisture content, particularly in the corn-soybean treatment, which suggests that roots of these plants may not be effectively absorbing moisture from these deeper levels of soil profile. It is also apparent from the figure that higher nitrogen rates (180 kg ha^{-1}) applied in spring or fall usually accounted for the lowest soil moisture content averages across all depths when compared to lower N rates of 90 kg ha^{-1}.

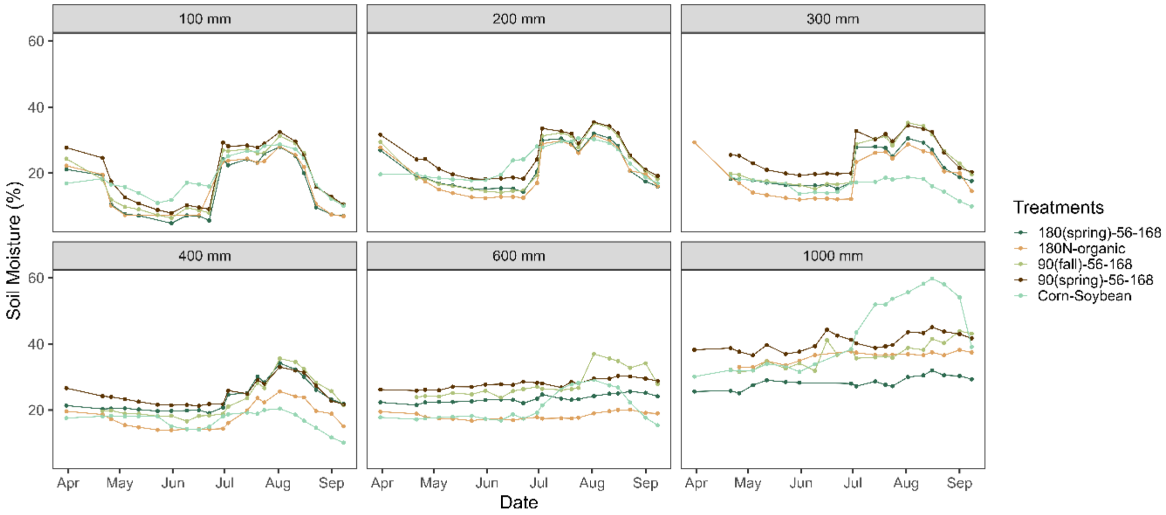

Figure 2.1. Average volumetric soil water content (%) at different depths of the soil profile throughout the 2023 growing season in the monitored treatments. Measurements were acquired using the Delta-T Devices PR2/6 Probe.

Grain yields

In the first stand year, there were no statistically significant differences in Kernza grain yields among treatments (Figure 2.2). However, the organic treatment exhibited the highest yield, with a mean estimate of 1340 ± 58 kg ha^{-1}, while the 90(spring)-56-168 treatment had the lowest yield, with a mean estimate of 1062 ± 148 kg ha^{-1}. The treatment without any nitrogen application (0-56-168; Table 2.1) outperformed treatments with the higher nitrogen rates such as 135 and 180 kg ha^{-1} of N (treatments 6 and 7, Table 2.1). Similar to the first year, in 2023 (stand year 2), no statistical differences were observed between treatments. However, a highly significant difference emerged when comparing results across the two years (Figure 2.2). The yield demonstrated a marked decrease from one year to the next, with the highest yield of 298.3 ± 113 kg ha^{-1} with the 90(spring)-0-168 treatment. The lowest yield across treatments observed in stand year two was in the organic treatment (poultry manure), averaging 176.8 ± 57 kg ha^{-1}. This contrasts with the first year when the organic treatment had the highest grain yield among all treatments. The limited rain during critical stages of the crop's development likely contributed to the significant decrease in grain yield from year one.

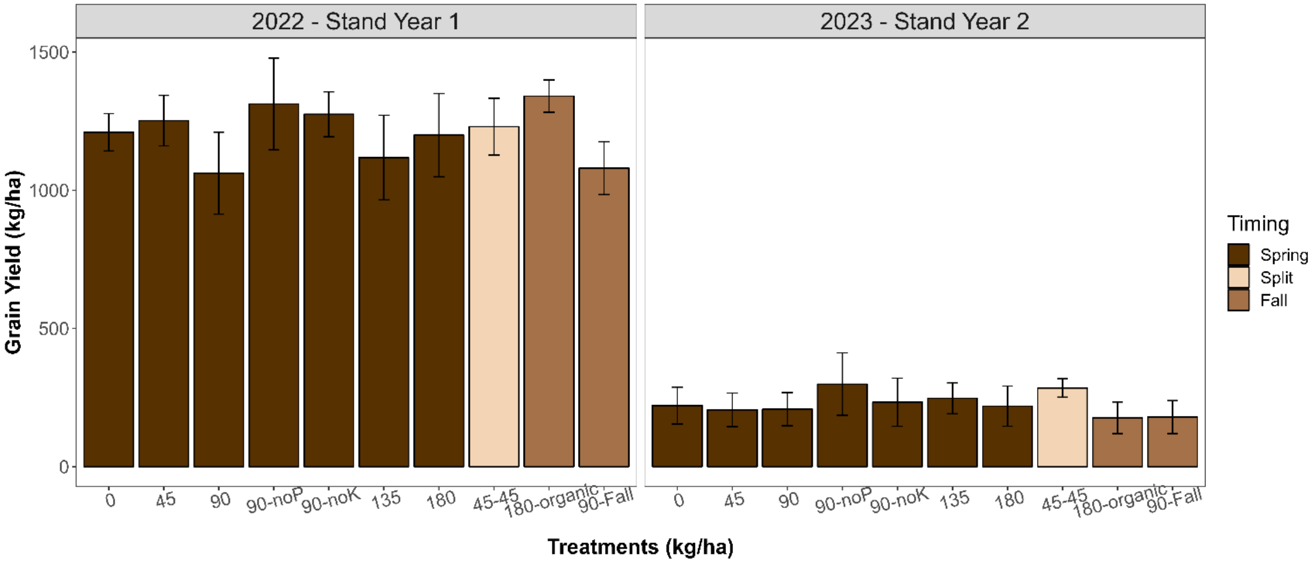

Figure 2.2. Grain yield of intermediate wheatgrass in years 1 and 2 of the crop's stand in response to nitrogen, phosphorus, and potassium (NPK) application rates at different application times. Significant differences at $p < 0.05$ were analyzed according to Tukey's HSD test.

Plots planted with corn and soybean as part of the soil moisture monitoring treatments were harvested to assess the grain yields. Table 2.3 compares the experiment yields with the county 10-year averages and with the yields from 2012, considered the last major drought year for the Saunders Co. Overall, the grain yields for corn were approximately 16.45% lower than the 10-year county averages, while the soybean yields were 74% below the 10-year county averages (USDA NASS National Agriculture Statistics Service Quickstats Database).

Table 2.3. Corn and soybean averages from the experiment (2022) compared to the county 10-year average and to the 2012 county average yields (USDA NASS 2023).

	2022 Experiment Yields		2022 Saunders Co. Average		Saunders Co. 10-year Average Yield		Saunders Co. 2012 Yields (last major drought year)	
	kg ha^{-1}	bu ac^{-1}	kg ha^{-1}	bu ac^{-1}	kg ha^{-1}	bu ac^{-1}	kg ha^{-1}	bu ac^{-1}
Corn	9.5	152	10.6	170	11.1	177	7.4	118
Soybean	2.1	31	3.1	47	3.6	54	2.4	36

Summer and Fall biomass yields

There were no statistically significant differences in summer forage yields across the treatments for year 1 (Figure 2.3). The 90(spring)-0-168 treatment achieved the highest yield of 8220 ± 590 kg ha^{-1}, while the 90(fall)-56-168 treatment had the lowest yield, estimated at an average of 6925 ± 421 kg ha^{-1}. Similar to the grain yield results, for the summer forage biomass in year one, the 0N treatment had better yields when compared to treatments that received other nitrogen application rates. In the second year of the intermediate wheatgrass stand, as in the first year, there were no statistically significant differences in yield across treatments. The highest yield was observed with the 90(spring)-56-0 treatment, totaling 7416.3 ± 805, while the lowest yield was obtained with the 90(spring)-56-168 treatment, totaling 5138.5 ± 619.5.

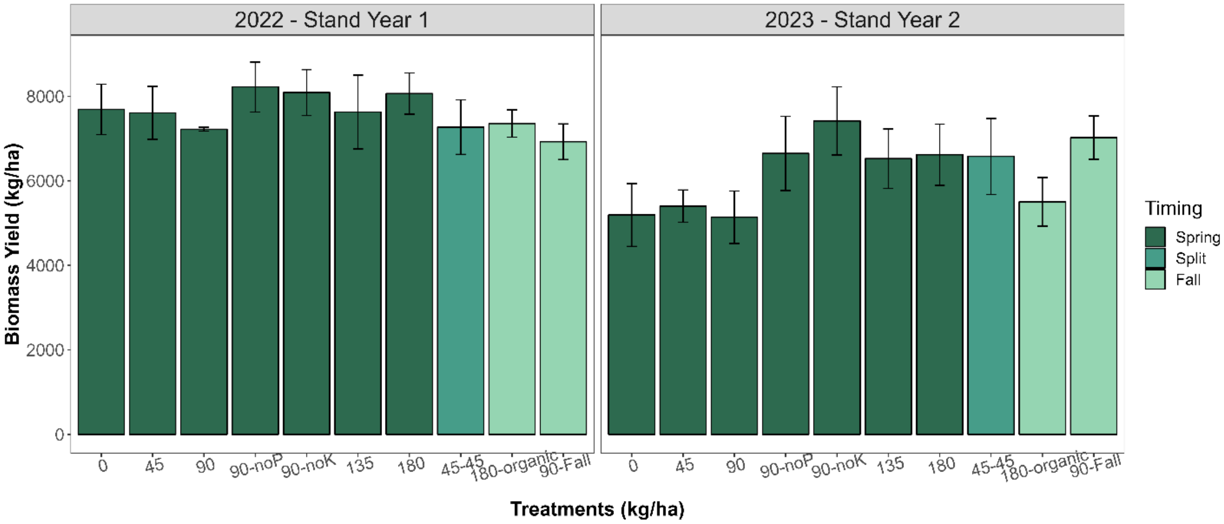

Figure 2.3. Summer forage yield of intermediate wheatgrass in years 1 and 2 of the crop's stand in response to nitrogen, phosphorus, and potassium (NPK) application rates at different application times. Significant differences at $p < 0.05$ were analyzed according to Tukey's HSD test.

We found significant differences ($p = 0.05$) when comparing the fall forage yields of both the 180(spring)-56-168 and 90(spring)-56-168 treatments with the 0N treatment (Figure 2.4) for the first Kernza stand year. The treatments that received 180 and 90 kg ha^{-1} N yielded 4447 ± 419 and 4381 ± 530 kg ha^{-1} of forage, while the 0N control treatment produced 1857 ± 492 kg ha^{-1} of forage during the Fall. Only three treatments were sampled.

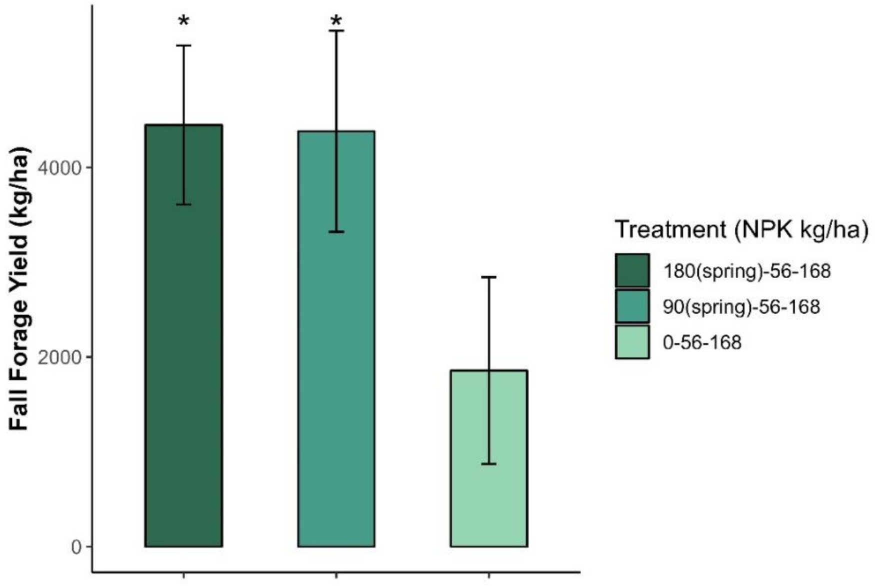

Figure 2.4. Fall forage yield of intermediate wheatgrass in year 1 in response to nitrogen, phosphorus, and potassium (NPK) application rates in three different treatments. Data are means ± SE. Significant differences at $p < 0.05$ according to Tukey's HSD test are indicated with asterisks above the error bars.

Plant height, lodging and NDVI

We found a significant difference in plant height between years ($p < 0.05$), representing a large decrease in plant height from one year to another. However, we did not find differences between treatments in either year (Fig 2.5). These findings suggest that the lower average height observed in 2023 also may be attributed to the drought conditions experienced during the growing season.

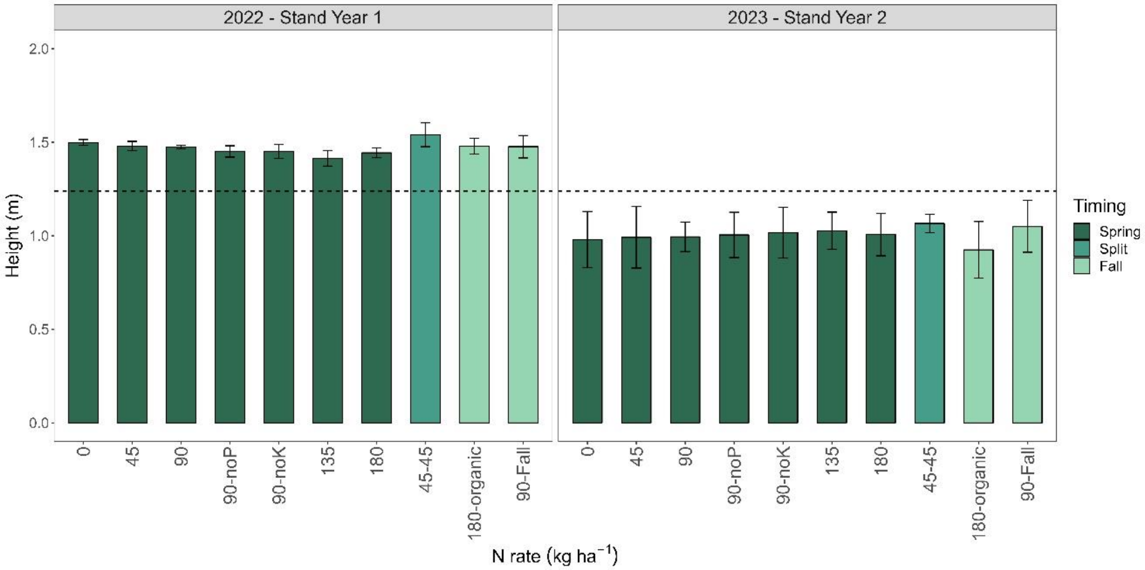

Figure 2.5. Average plant height (m) by the different treatments in years 1 and 2 of the crop's stand. Measurements were taken one day before harvest. Significant differences at $p < 0.05$ were analyzed according to Tukey's HSD test. Dashed line represents the average height across the two years.

During the initial year of the experiment, significant lodging was observed at higher nitrogen (N) rates, specifically at 135 and 180 kg ha^{-1} (Figure 2.6). As anticipated, grain yields were negatively affected in the plots with higher lodging scores. The lodging predominantly occurred during the period between anthesis and harvest, potentially disrupting grain development. We examined the relationship between plant height and lodging by conducting the Spearman correlation analysis. The obtained correlation was negative (-0.42; $P < 0.05$), suggesting that plant height may not be the primary factor influencing lodging. There was no lodging observed in any of the treatments during the second year of the intermediate wheatgrass stand, either during anthesis or before harvest when the measurements were taken.

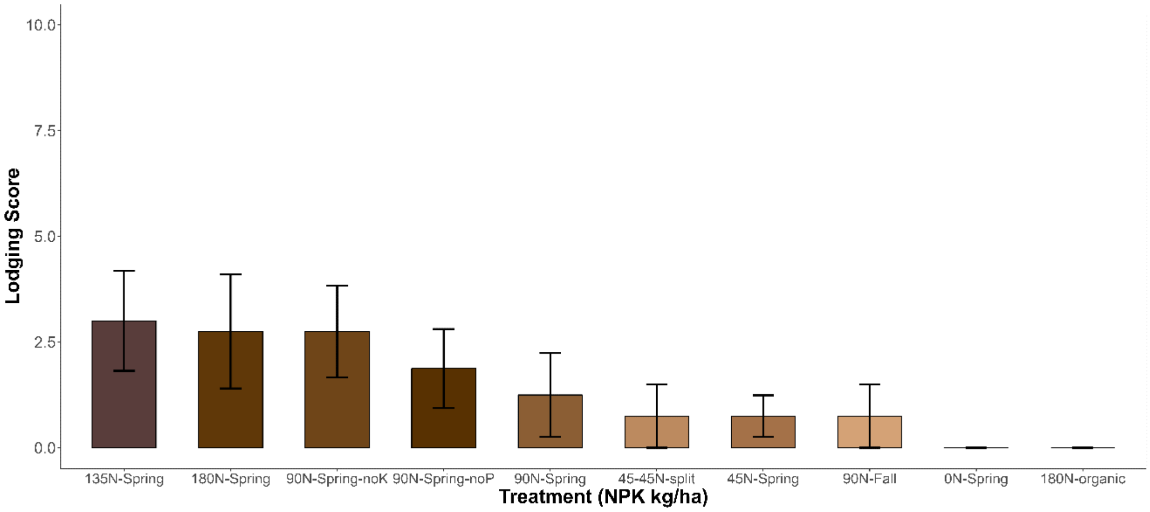

Figure 2.6. Estimated lodging score by different treatments before harvest. Error bars depict the standard errors of the mean. Significant differences at $p < 0.05$ were analyzed according to Tukey's HSD test.

Further, Normalized Difference Vegetation Index (NDVI) measurements were collected before harvest in 2022, and at three different timings in 2023: before Spring fertilization (2023-04-26), 4 weeks after Spring fertilization (2023-05-23), and before harvest (2023-07-24) (Figure 2.7). In 2022, a significant difference ($p <0.05$) was observed when comparing the treatment with 180 kg N ha^{-1} applied in the Spring to the control treatment (0N). In 2023, significant differences were detected before Spring fertilization for treatments with 180 kg N ha^{-1} and 90 kg N ha^{-1} applied in the Fall when compared to the control and 90N-Spring treatments. After the Spring fertilization, the treatment receiving 180 kg N ha^{-1} was significantly higher when compared to the 0 kg N control treatment.

NDVI measurements taken before harvest in 2023 presented higher values compared to those in 2022. The 0 kg N control treatment displayed the lowest NDVI value, while treatments receiving nitrogen application showed higher values.

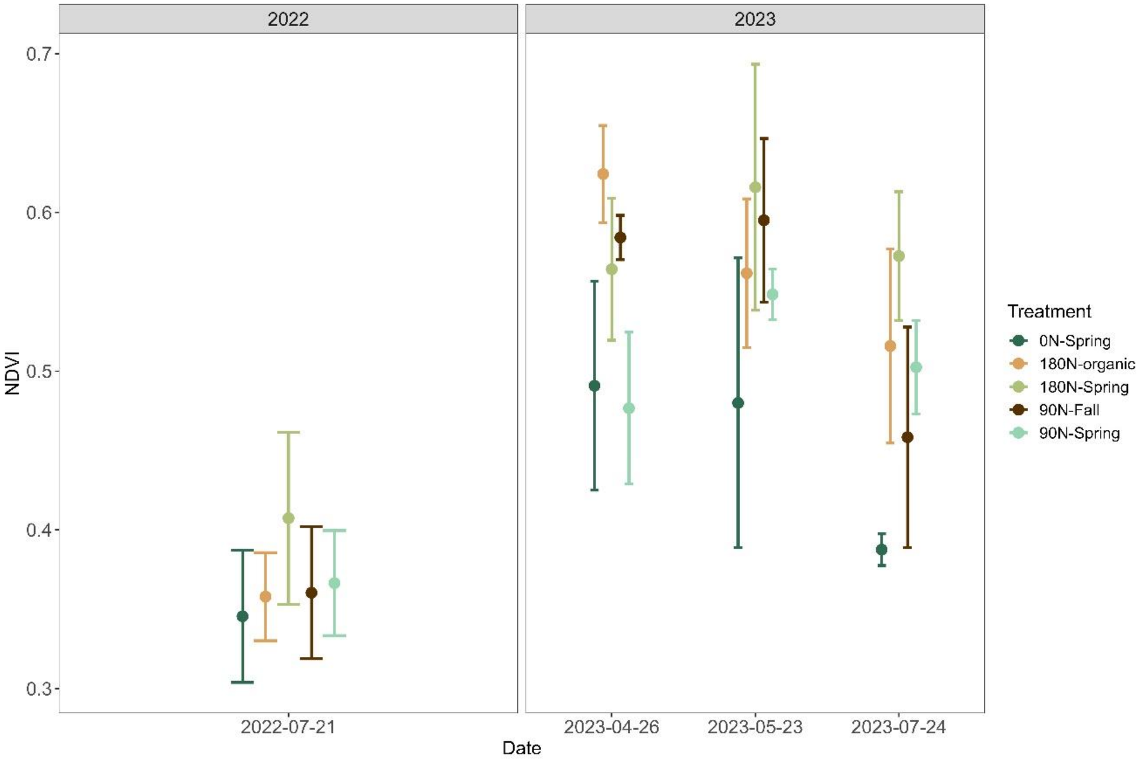

Figure 2.7. Mean NDVI values and standard deviation at five different treatments. Measurements were taken before harvest in 2022, and in 2023, before N application, after N application, and before harvest.

Weed Assessments

Results from the weed assessment indicate that across both years 1 and 2 of the Kernza stand, there was a low incidence of weeds. This can be attributed to the adequate establishment of the crop during its first year of production, where no herbicides were applied during the initial two years of the crop's stand. In contrast, when comparing

Kernza to annual crops (corn and soybean), while their average weed scores were also low, herbicide applications were required to manage weed growth in their respective plots (Table 2.4).

In addition to assessing weed presence, we also evaluated the occurrence of volunteer Kernza in the inter-row using the same scoring system. In year 1, the assessment was scored as zero for all treatments since, as would be expected, there was no occurrence of volunteer Kernza during the first production year. However, during the second year of the Kernza stand, Kernza plants emerged in the inter-rows, and the average scores ranged from 0.125 to 0.875 across different treatments, representing greater than zero but less than 20% weed cover. This evaluation is of significance due to the natural shattering that may occur from *Kernza* plants before harvest, particularly when the grains are reaching physiological maturity. Thus, volunteer IWG cover is expected to increase as the stands ages.

Table 2.4. Average weed scores for each treatment in years 1 and 2 of the crop's stand. Scores represent an average of visual assessment ratings for the inter-row and canopy. The scoring system ranges from 0 to 4, where: 0 indicates 0% of the inter-row space or canopy occupied by weeds; 1 indicates 1-20% of the inter-row space or canopy occupied by weeds; 2 indicates 21-50% of the inter-row space or canopy occupied by weeds; 3 indicates 51-80% of the inter-row space or canopy occupied by weeds; and 4 indicates 81-100% of the inter-row space or canopy occupied by weeds. Average volunteer IWG in year 2 is presented following the same score criteria.

	Average Weed Score		Average inter-row volunteer IWG
	Year 1	Year 2	Year 2
BAU 1 (Corn)	0.25	0	0
BAU 2 (Soybeans)	0.06	1.14	0
0-56-168	0.31	0	0.75
45(spring)-56-168	0.28	0	0.875
90(spring)-56-168	0.22	0	0.75
135(spring)-56-168	0.28	0	0.5
180(spring)-56-168	0.22	0	0.125
90(fall)-56-168	0.13	0	0.5
90(split)-56-168	0.25	0	0.75
Chicken manure	0.38	0	0.875
90(spring)-56-0	0.28	0	0.75
90(spring)-0-168	0.34	0	0.5

Soil nutrients

The soil test reports conducted at three times – before planting in 2021, during the first Kernza stand year in 2022, and again during the second Kernza stand year in 2023 – reveal consistently elevated levels of P, ranging from 57-91 ppm, and K, ranging from 325-432 ppm (high to very high). Magnesium (Mg) exhibited medium to high values across all treatments throughout these years. Calcium (Ca), on the other hand, initially displayed low levels in most treatments in 2021, with some treatments evolving to medium in 2022 and 2023 (Table 5). The percentage of soil organic matter exhibited a consistent decline across the years for all the treatments, with significant differences observed in most of the treatment-year combinations.

Table 2.5. Average of chemical properties across six different treatments in the 0-20 cm soil depth across three years: 2021 – baseline soil sampling, 2022 – first Kernza stand year, and 2023 – second Kernza stand year. Different letters denote statistically different values across treatment-years combinations within each respective variable.

Treatment	**Year**	**Organic Matter (%)**	**Soil pH**	**Phosphorus (ppm)**	**Potassium (ppm)**	**Magnesium (ppm)**	**Calcium (Ca)**	**CEC**
0-56-168	2021	4.1 a	5.8 a	57.5 a	431.7 a	238 a	2102.7 a	17.5 a
	2022	3.8 a	5.8 a	66.5 a	381.5 a	239 a	2031.5 a	17 a
	2023	3 b	6 a	70.2 a	403.5 a	241.7 a	2222.2 a	17.4 a
180N-organic	2021	4 a	5.9 a	72 a	379.5 a	271.2 a	2184.2 a	17.5 a
	2022	3.9 a	6 a	85.2 a	344.2 a	271.7 a	2266 a	17.4 a
	2023	3 b	6.3 a	91.7 a	401.7 a	296.2 a	2507 a	18.4 a
180(spring)-56-168	2021	3.8 a	5.8 a	59 a	398.5 a	241.2 a	2019 a	16.7 a
	2022	3.8 a	5.8 a	73.5 a	325.2 a	246.7 a	1998.5 a	16.8 a
	2023	2.9 b	5.7 a	82.5 a	428.5 a	244 a	2093 a	17.8 a
90(fall)-56-168	2021	3.8 a	5.7 a	69 a	386 a	260 a	2103.7 a	17.9 a
	2022	3.9 a	6 a	70.5 a	333.7 a	261 a	2208.5 a	17 a
	2023	3 a	6.1 a	78.5 a	395 a	258.7 a	2528.7 a	18.8 a
90(spring)-56-168	2021	4.1 a	5.7 a	60.2 a	420.2 a	249 a	1932.2 a	17.1 a
	2022	3.8 a	5.9 a	69 a	354.2 a	250.7 a	2039.7 a	16.7 a
	2023	3 b	5.8 a	73.5 a	377.5 a	246.7 a	2132 a	17.6 a
Corn-Soybean	2021	3.9 a	5.7 a	64 a	404.7 a	232 a	1936.2 a	17.2 a
	2022	3.8 a	5.7 a	80.7 a	392.7 a	245.5 a	1994.2 a	17.2 a
	2023	2.9 b	5.7 a	90.2 a	385.7 a	241 a	2084.7 a	17.3 a
				F-statistic and significance				
	Treatment (T)	0.5	1.2	0.8	0.8	2.4*	1.8	0.6
	Year (Y)	10.5***	1.9	1	0.5	0.3	1.8	0.3
	T * Y	0.2	1.3	0.3	0.6	0.8	1.3	0.4

*Asterisks indicate statistical significance at the respective probability levels: *p = 0.05, **p = 0.01, ***p = 0.001.

Discussion

Impact of weather on overall experiment

Although weather is always an important factor in agriculture experiments, the trends in precipitation, especially the lack of spring precipitation in the second year, were particularly notable at our location. The year of 2021 had higher total precipitation in comparison to the historical data, and during the months of August (before planting) and October (after planting), the experimental field received a considerable amount of rain that helped Kernza establish in the field ("PRISM Climate Group," 2021). The scarcity of rainfall during 2022 did not impact grain and biomass yields, given the adequate distribution of rain during critical periods of the crop (April-June), where the plants are going through stem elongation and heading growth stages. Also, according to the U.S. Drought Monitor, more than 90% of the Saunders County area was under a "moderate drought" for the months of April and May 2022.

While precipitation levels from January through July of 2023 (437.5 mm) closely align with historical averages for the same period (489.6 mm) and almost match the total precipitation of 2022 for the entire year (447.8 mm), the drought conditions of specific periods likely had a negative impact on the second Kernza production year. During the whole month of May and the beginning of June, when the demand for water and nutrients is higher, precipitation levels were far below the historical averages, impacting the growth and development of the plants. According to the U.S. Drought Monitor, at the beginning of May, 46% of the Saunders County area was experiencing extreme drought, with 26% in severe drought, 16% in moderate drought, and 12% in exceptional drought.

However, by the end of the month, the situation had worsened, with 46% of the area facing exceptional drought and the remaining 54% under extreme drought conditions. The impact of such dry conditions was particularly evident in the height of the plants. As mentioned in a global synthesis by Daryanto et al. (2016), when cereal grain crops like corn and wheat experience drought conditions during the reproductive stage, yield losses are greater when drought conditions occur during the vegetative stage. Moreover, plant height of wheat can be reduced by 35% at stem elongation and 23% at booting stage when experiencing dry conditions (Rijal et al., 2020), which is similar to what we observed in such stages for intermediate wheatgrass.

In terms of yield components, a decrease in the number of heads and kernels per spike can be caused by drought stress before the anthesis stage for cereal crops like wheat. Further, water deficit during the early grain development stage can decrease the grain size since the grain filling duration is reduced (Sarto et al., 2017; Dhakal, 2021). The dry conditions experienced by Kernza in 2023 had similar effects, with the critical stage of heading facing an extreme drought. These findings suggest that, in terms of grain production, harvesting Kernza for grain may not be optimal in dry years. However, it has demonstrated a strong capacity for forage production even when experiencing extreme drought. In wheat and perhaps other cereal crops, the agronomically important traits of biomass and grain yield are not always connected. For example, a study conducted by Paul et al. (2016) compared a drought-sensitive wheat cultivar with a drought-tolerant cultivar. They observed that the sensitive cultivar had a higher biomass production, but lower grain stability compared to the tolerant cultivar under severe drought conditions.

For perennial grasses, this increase in biomass production can be associated with improved water-use efficiency, since productivity and persistence of perennial grasses during dry periods are determined by the volume of soil exploited by the roots (Sheaffer et al., 1992). Therefore, in a dual-purpose and rainfed system, Kernza may be seen as a resilient alternative crop with strong above- and below-ground biomass production, providing year-round soil cover and offering ecosystem services benefits through its deep root system.

As indicated previously, our results demonstrated that in 2022, the corn and soybean yields were below long-term county averages. Compared to the last major drought year in 2012, even with presumed genetic gains over the prior decade, soybean yields were 14% lower in 2022 (31 bushels in 2022 versus 36 bushels in 2012) with corn yields 28% higher (152 bushels in 2022 versus 118 bushels in 2012). Given the increasing frequency of extreme weather events due to climate change, exploring alternative agricultural practices within existing cropping systems, such as the corn-soybean rotation, is essential. Conventional annual cropping systems that rely on rainfed practices may be more susceptible to extreme weather conditions and less resilient to climate-related challenges than perennial cropping systems. Additionally, it is important to consider the profitability of such annual crop systems, especially given the fluctuating prices of commodities and inputs from year to year (Eeswaran et al., 2021; Volsi et al., 2022). A dual use crop offers another source of income in more extreme years, since the production tends to decrease, and the prices of forage tends to increase as drought becomes more severe (Good, 2023).

Relative performance of Kernza in Eastern Nebraska

We found limited differences between the many fertility treatments in grain and summer biomass production during the first two years of the intermediate wheatgrass stand. Additionally, we found that grain yields at our location in the first year match and even outperform those at other studies. A recent study conducted by Culman et al. (2023) found that grain yields in the first year of a multi-site trial ranged from 494 to 1075 kg ha^{-1} (for experiments from 2014/2015 to 2017/2018 in several US states and Canada). Pinto et al. (2022) reported a grain yield of 945 kg ha^{-1} for the first year of Kernza monoculture. In our study, grain yields during the first year ranged from 1062 to 1340 kg ha^{-1}. Another study conducted in Minnesota found that when fertilized at a rate of 80 kg ha^{-1}, Kernza peaked at an average grain yield of 971 kg ha^{-1} in the first year, while in our study, a similar rate (90 kg ha^{-1}) resulted in an average grain yield of 1312 kg ha^{-1}. As reported in other studies, year two was expected to have a grain yield decline due to stand aging (Jungers et al., 2017a; Hunter et al., 2020; Fernandez et al., 2020). Our site also experienced a yield decline due to stand age, however the decline was more severe due to the dry spring season in 2023, with a mean yield of 227 ± 3 kg ha^{-1} across treatments.

A significant aspect of Kernza in the first production year was the significant amount of summer forage production, ranging from 6924 to 8219 kg ha^{-1}. This outperformed yields from a study conducted in Wisconsin, which reported a forage yield mean of 6141 kg ha^{-1} from the first year of Kernza grown in monoculture (Favre et al., 2019). Moreover, it surpassed the average of 6000 kg ha^{-1} across all sites in the multi-site trial reported by Culman et al. (2023). However, for the second stand year, intermediate wheatgrass experienced a decline in biomass yield. Although this yield decline suggests that, as the crop's stand matures, there is a growing demand for higher nitrogen inputs to optimize biomass production, this study did not find significant differences between N rate treatments. Previous studies have also reported biomass yield decline from the second year of intermediate wheatgrass stand (Jungers et al., 2017a; Tautges et al., 2018; Culman et al., 2023). As already mentioned, the experiment encountered severe drought conditions during the 2023 growing season. It is possible, therefore, that the reduction in biomass yield is a result of not only stand age, but also water deficit and heat stress.

A notable difference with respect to fertility is that the fall forage production varied between the three treatments sampled (180(spring)-56-168; 90(spring)-56-168; and 0-56-168), with the highest yield of 4447 kg ha^{-1} in the 180(spring)-56-168 treatment. This is the first series of results in the experiment where we observed significant differences between fertility treatments. Favre et al. (2019) reported the fall forage yield of 1394 kg ha^{-1} for the first stand year, while Pinto (2022) reported a yield of under 2500 kg ha^{-1} for Kernza monoculture – lower than the 0 kg N control treatment yield observed in our study (1857 kg ha^{-1}). These findings indicate that while nitrogen application may not have a significant impact on grain and summer forage yields in the first production year, it was important later in the growing season for the fall forage production. The nitrogen applied in the previous spring appears to play an important role in achieving better forage yields during this period. Interestingly, the fall forage yield from the higher nitrogen rate was similar to that of the lower nitrogen rate. However, only three treatments were sampled for the fall forage production, and more investigation is needed on how other treatments perform.

Thus, considering grain, summer forage, and fall forage yields as depicted in figures Figure 2.2, Figure *2.3*, and Figure *2.4*, our results show that Kernza demonstrated a good performance for year one when 90 kg ha^{-1} was applied in the spring. However, it is difficult to identify the optimal N rate since no statistically significant differences were found for grain and summer forage yields. Jungers et al. (2017) found that the agronomically optimal nitrogen rates (AONR) for an intermediate wheatgrass forage variety ranged between 81.1 to 120.5 kg ha^{-1}, and for an improved grain-type Kernza, the AONR ranged from 61.0 to 96.4 kg ha^{-1}. For year two, the limited differences between treatments in grain and forage production also restricted our ability to detect the optimal nitrogen rate. This lack of response can likely be attributed to the extremely dry weather conditions, which appeared to influence the limited response to various NPK treatments. Another important aspect to consider is the soil composition in the experimental area. From 2021 (baseline soil sampling) through 2023, the 0-20 cm soil layer consistently exhibited high or very high levels of P and K, which makes it hard to detect the best management practices in terms of P and K applications, as it appears that additional nutrient applications were not compensating for the soil's existing nutrient abundance.

The Normalized Difference Vegetation Index (NDVI) measures surface vegetation growth, and finds application in predicting grain yields, biomass, chlorophyll level, or nitrogen variability, for example (Hassan et al., 2019). A higher NDVI value corresponds to better growth and increased in vegetation coverage (Xu et al., 2021). In

2022, prior to harvesting, the NDVI values were similar between treatments, with significant differences observed 180 kg N ha^{-1} and the control treatment. In 2023, differences emerged across the season, and measurements taken before harvest were higher than those of 2022. As mentioned in the literature, temperature is an important factor in NDVI changes (Wang et al., 2021). Moreover, NDVI can serve as a response variable to identify and quantify drought disturbances, with lower values reflecting vegetation stress (Tucker, 1979). Nevertheless, our results are somewhat unclear since the 2023 values were higher, despite the presence of drought disturbances throughout the season. This could be an indicator of strong biomass growth at the times of measurement.

Other agronomic aspects reflecting Kernza performance

We observed higher lodging rates with N rates ranging from 90 to 180 kg ha^{-1} in the first year of the experiment, with a more pronounced lodging occurring at the 135 and 180 kg N ha^{-1} rates applied in the spring. This lodging is likely related to the lower grain yields observed in both the 180(spring)-56-168 and 135(spring)-56-168 treatments, although there were no statistical differences between treatments. Several reports have shown that lodging can result in economic and yield losses, as well as difficulties during harvesting in cereals (Frahm et al., 2018; Khobra et al., 2019; Niu et al., 2022). Khobra et al. (2019) points out that lodging can be associated with various plant traits, including plant height, culm traits, stem anatomical traits, among others. Our analysis, consistent with the findings of Frahm et al. (2018) and Jungers et al. (2017) for Kernza, found no correlation between plant height and lodging. This suggests that other plant traits may have influenced the occurrence of lodging. Surprisingly, during the second year of the Kernza stand, no lodging was observed either during anthesis or prior to harvest.

As indicated previously, Kernza demonstrated effective weed suppression through successful stand establishment during the initial two years of the trial, with no need for herbicide application in both years, proving to be a low-input crop due to its low management required after planting. In contrast, in the conventional plots where corn and soybeans were planted, herbicides were applied in both years to control weeds. Perennial crops such as Kernza can control weed development with ground cover throughout the year, making them more competitive against weed competition (Zhang et al., 2011; Duchene et al., 2023).

Conclusion

In this study we found limited responses of NPK treatments during the initial two years of the Kernza stand, where N rates of 0, 45, 90, 135, and 180 kg ha^{-1} were applied. Several factors might have contributed to this. Firstly, the crop established well in the first year, benefiting from favorable precipitation levels during crucial growth stages, along with high organic matter as well as P and K content in the soil. Next, the second year of the experiment encountered a severe drought, which likely accounts for the limited responses between treatments. However, we observed significant differences in fall forage production with three of the twelve treatments sampled. Specifically, N rates of 90 kg ha^{-1} and 180 kg ha^{-1} had very similar yields and exhibited significant differences compared to the 0N treatment. Despite limited fertility differences, our findings highlight the importance of adopting a strategic approach to fertilization practices across the Kernza stand years to optimize grain and forage production. Moreover, Kernza demonstrated its potential as a low-input, alternative, and dual-use cash crop for Nebraska producers, given its ability to produce high yields in its first year and high biomass across two years, without herbicides and with lower fertility needs compared to annual crops.

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CHAPTER 3: THE IMPACT OF NITROGEN RATES ACROSS SITES AND YEARS ON INTERMEDIATE WHEATGRASS PRODUCTIVITY: A META-ANALYSIS

Abstract

Intermediate wheatgrass (IWG) (*Thinopyrum intermedium*), marketed under the trade name Kernza® by The Land Institute, is a cool-season low-input perennial grass that can be managed to produce grain and biomass. This crop has shown a great potential to be used as a cash crop while providing many desired benefits to soil conservation, nutrient cycling, and better nitrogen use efficiency. Although many studies with intermediate wheatgrass have answered some questions about its use and benefits, little is known about the fertilizer requirements that best fit the needs of this crop. Research has found different impacts of fertility responses in different years of IWG development, making the determination of nutrient needs in different years a challenge. Understanding how soil-year effects of nitrogen rates affect IWG growth and development is critical. A meta-analysis was conducted to evaluate the impact of nitrogen rates across sites and years on intermediate wheatgrass productivity. Our search returned 16 individual published studies plus one multi-site trial with unpublished data shared by collaborators. We selected studies based on two main criteria: 1) studies needed to include an experimental design that assessed the effects of at least two nitrogen (N) rates of intermediate wheatgrass, and 2) studies needed to report grain yield or biomass components of Intermediate Wheatgrass. Through a generalized boosted regression tree (BRT) model, we found that the most important factors influencing grain yield were stand year followed by N rate. There were limited effects on grain yield in year 1 but optimal N rates ranging from 51-150 kg ha^{-1} in later years. Additionally, we found that seeding rate was an important factor influencing biomass production across years, followed by N rate. We also conducted a sensitivity analysis, which confirmed the robustness of our results, and we did not identify any evidence of publication bias. Thus, considering the dual objectives of maximizing grain yield and biomass production over the years of the stand, a broad approach in terms of management practices for IWG is needed.

Keywords: perennial crops; intermediate wheatgrass; nitrogen rate; grain yield; biomass production.

Introduction

Nitrogen (N) plays an essential role in crop physiology, growth, and development, and its use has increased over the years due to the increase in food production around the world (Javed et al., 2022). One of the keys to advancing a more sustainable agricultural system is to manage N inputs that optimize crop yields without contributing to air or water N pollution (Lu et al., 2019; Yan et al., 2020). More than 70% of global food comes from annual cereal crops, legumes, and oilseeds such as wheat, soybeans, corn, and rice, which often require high rates of N fertilizer inputs (Pimentel et al., 2012). Additionally, the intensive management associated with many annual cropping systems can cause negative impacts on ecosystems including soil erosion, water pollution, soil compaction, greenhouse gas emissions, and detrimental impacts on species biodiversity (Gomiero et al., 2011; Pimentel et al., 2012).

Several conservation practices continue to gain traction with producers, including no-till, cover crops, and crop rotation, which are broadly understood to reduce some negative environmental impacts and to increase profitability (Hobbs, 2007; Palm et al., 2014). Another alternative approach to increasing ecosystem security is introducing perennial crops into cropping systems. There are several benefits to utilizing perennial crops compared to annual crops, especially regarding soil conservation and N cycling efficiency (Culman et al., 2013). Perennials exhibit a greater root mass and higher levels of plant root carbon compared to annual cropping systems, resulting in increased soil organic carbon (SOC) and the ability to maintain stable levels of carbon and nitrogen in the soil over time (Glover et al., 2010). In addition, perennial crops can contribute to food, fiber, fuel, and feed co-products, therefore diversifying production (Asbjornsen et al., 2014).

Intermediate wheatgrass (IWG) (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey) is a cool-season perennial grass that has been marketed under the trade name Kernza®, and it is used as a forage and grain production crop. This crop has been under breeding programs led by researchers at The Land Institute (Salina, KS) and other institutions across the U.S. and globally. As compared to perennial crops utilized for bioenergy or livestock, a perennial grain such as Kernza® offers great potential as a dual-purpose (forage and grain) cash crop that provides many desired benefits to soil conservation and nutrient cycling (Culman et al., 2013; Law et al., 2022). Intermediate wheatgrass has also demonstrated a capacity for carbon sequestration and nitrogen retention due to its large amount of root biomass (Pugliese et al., 2019; Law et al., 2022). A recent study by Rakkar et al. (2023) demonstrated that perennial cropping systems involving IWG intercropped with alfalfa improved the mean weight diameter of water-stable aggregates, indicating improved soil physical health when compared to annual crops. The intercropping system also had reduced soil nitrate levels, similar to what Reilly et al. (2022) found when comparing intermediate wheatgrass to an annual crop. With its noticeable agronomic and environmental benefits, IWG has drawn wide attention as a crop with enormous potential as an alternative to annual crops.

Although many studies with IWG have answered some questions about its use and benefits, a knowledge gap remains about the optimal N requirements of the crop. Research has found different impacts of fertility responses in different years of IWG development (Jungers et al., 2017b). This is similar to what is known about other perennial crops such as miscanthus and switchgrass, with both crops responding positively to the observed applied N rates ranging from 0 to 202 kg ha^{-1}, with the effect being more noticeable in older stands of miscanthus and middle-aged stands of switchgrass (Sharma et al., 2022). Several studies investigating IWG and its fertilizer needs are conducted on short time scales under specific management and climate conditions, which may make it difficult to detect differences and make broader recommendations.

Quantitative systematic reviews, referred as meta-analyses, comprise statistically analyzed data obtained from the published literature, to help determine what is known and what is unknown in a certain topic (Philibert et al. (2012). Moreover, meta-analyses provide us with the capability to investigate the effect of different variables on the outcomes of individual experiments, with the focus in this case being on grain and biomass yield responses. The objective of this study is to understand the nutrient needs from season-to-season through a meta-analysis of N rate studies with intermediate wheatgrass and to determine rate effects on grain yield and biomass production across different years of the crop's development.

Materials and methods

Database construction

We searched and selected studies based on two main criteria: 1) studies needed to include an experimental design that assessed the effects of at least two nitrogen rates of intermediate wheatgrass, and 2) studies needed to report grain yield or biomass components of Intermediate Wheatgrass. The literature search was performed in the Web of Science and in Scopus databases in January 2023, using the following keywords:

"kernza" OR "intermediate wheatgrass" OR "*Thinopyrum intermedium*" AND

"nitrogen" OR "nitrogen rate*" OR "nitrogen application*"

The search returned 50 papers from Scopus and 70 papers from Web of Science. Combining both databases, a total of 81 full-text records were reviewed based on their abstracts, ultimately returning 16 papers that fit our criteria (Figure 3.1). Articles were excluded because they either did not include at least two nitrogen rates in the experiment design or did not report grain yield or biomass components. Additionally, unpublished data from a multi-site trial that met all criteria was added to the database to compare the results to those obtained from the published literature, and to make broader N recommendations. Including the unpublished datasets served as a check on publication bias as the results were derived from collaborators within the relatively small community of researchers investigating intermediate wheatgrass fertility management (McLeod and Weisz, 2004). When data were not reported in tables, we retrieved them from figures using 'Web Plot Digitizer' (https://apps.automeris.io/wpd/). The majority of the studies were conducted in North America (14), with fewer in Canada (2) and Europe (1).

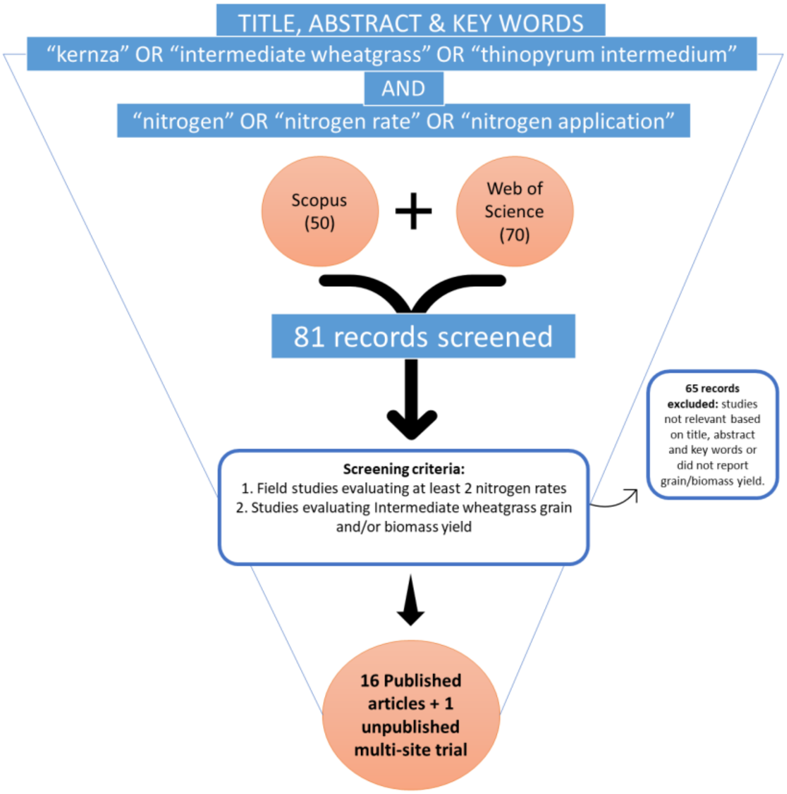

Figure 3.1. Approach used to screen articles from Scopus and Web of Science.

Data analysis

All data manipulation, analysis and graphics were completed with *R studio* (version 2023.3.1.446) (R Core Team, 2021). A response ratio was developed to evaluate the effects of different N rates on IWG yield and forage biomass. The response ratio is the ratio of the response variable of higher to standard (control) N rate:

(3.1)

$$Response\ ratio = \ln \frac{High\ N\ Rate}{Standard\ N\ rate}$$

Values above zero represent an increased effect of high N rate, while values below zero represent a decreased effect of high N rate. Additionally, a weighting factor (*Wi*) was included in the statistical model based on the experimental and control replications (*Reps*) of each study (Philibert et al., 2012):

(3.2)

$$Wi = \frac{Experimental\ Reps\ x\ Control\ Reps}{Experimental\ Reps + Control\ Reps}$$

Table 3.1. Study reference, lowest nitrogen rate (kg/ha), nitrogen treatment rates (kg/ha), number of response rations, grain and biomass yield components (if reported – yes or no), location of the study, and other experimental factors reported in each study (RS – row spacing; FT – fertilizer type; AT – application time; SY – stand year; SR – seeding rate).

Study no.	Reference	Lowest N rate	Treatment or higher N rates	No of response ratios	Grain yield Y/N	Biomass Y/N	Experiment location	Other experimental factors
1	Loeppky et al., 1999	0	50, 100, 150	3	Y	N	Canada	RS, FT, AT, SR
2	Lee et al., 2009	0	150	2	Y	Y	South Dakota	RS, FT, AT, SY, SR
3	Jungers et al., 2017	0	40, 60, 80, 100, 120, 160, 200	70	Y	N	Minnesota	RS, FT, AT, SY, SR
4	Sakiroglu et al., 2020	90	134	2	Y	Y	Wisconsin & Minnesota	RS, FT, AT, SY, SR
5	Zimbric et al., 2021	90	135	2	Y	Y	Wisconsin	RS, FT, AT, SY, SR
6	Taugtes et al., 2018	0	80, 60	15	Y	Y	Minnesota	RS, FT, AT, SY, SR
7	Jungers et al., 2019	0	40, 120, 160	6	Y	Y	Minnesota	RS, FT, AT, SY, SR
8	Culman et al., 2013	90	135	2	Y	Y	Michigan	RS, FT, AT, SY, SR
9	Fernandez et al., 2020	0	20, 40, 60, 80	48	Y	Y	Minnesota	RS, FT, AT, SY, SR
10	Frahm et al., 2018	0	40, 80	10	Y	Y	Minnesota	RS, FT, AT, SY, SR
11	Reilly et al., 2022	0	45, 90	10	Y	Y	Minnesota	RS, FT, AT, SR
12	Fagnant et al., 2023	0	50, 100, 150	16	N	Y	Belgium	RS, FT, AT, SY, SR
13	Crews et al., 2022	0	75, 150	10	Y	Y	Kansas	RS, FT, AT, SY, SR
14	Sprunger et al., 2018	90	135	6	N	Y	Michigan	RS, FT, AT, SY, SR
15	MacKown & Northup, 2010	45	90, 135	8	N	Y	Oklahoma	FT, AT, SY, SR
16	Lawrence & Ashford, 1968	0	75, 150, 225, 300, 375	25	N	Y	Canada	RS, FT, AT, SY
				Total = 235	Total = 178	Total = 158		

Table 3.2 Data from multi-site trial (which includes several sites published in Culman et al., 2023 and Pugliese, 2019 although this publication does not include results explicitly from the N rate treatments): lowest nitrogen rate (control (kg ha^{-1}), higher nitrogen rate treatment, number of response ratios, and yield components (yes or no).

Site	Planting year	Lowest N rate	Treatment or Higher N rates	No of response ratios	Grain yield (Y/N)	Biomass (Y/N)
Colorado	2014	80	120	3	Y	Y
Kansas	2014	75.8	107	3	Y	Y
New York	2014	80	120	3	Y	Y
Ohio	2014	80	120	3	Y	Y
Wisconsin	2015	80	120	2	Y	Y
Michigan	2010	90	120	2	Y	N
				Total = 16		

From the 16 published studies, 235 individual response rations were created to comprise the dataset (Table 2.1). Of the 235 paired observations, 215 represent 0 N rate controls, and 20 represent higher to lower N rate comparisons. From the unpublished data, 16 response ratios were added to the database – all higher to lower N rate comparisons – (Table 2.2), totaling 251 comparisons by site-experiment year. Each paired observation was generated by calculating the ratio of higher to lower N rates for an individual experimental year (equation 1). Furthermore, when experiments included designs with N rates plus other factors such as seeding rate, application time, row spacing, and more, individual response ratios were generated to solely focus on the effect of a higher and lower N rate within a single site-experiment year, while keeping all other experimental factors constant. We categorized experiments into two groups: those that included a zero-control nitrogen rate compared to higher N rates, and those that compared lower to higher nitrogen rates (Table 2.1) and analyzed them separately.

To identify the factors with the strongest effects on grain and biomass yield, we fit a generalized boosted regression tree (BRT) model (Elith et al., 2008). This model utilized six distinct predictors using the R package gbm (Greenwell et al., 2022). Our model configuration involved a shrinkage rate of 0.01, 1 node depth, and 300 trees following a Gaussian distribution. Variable importance was quantified using the relative influence metric. This model was only applied for the zero-control nitrogen group, as the lower to higher nitrogen group had insufficient additional treatment data to proceed with this model. To assess the impact of predictors identified as important by the BRT, we applied mixed-effect models using the lme4 package in R, using intermediate wheatgrass stand year and each of the predictors as the fixed terms. We included a random term for each experiment because, in general, there are significant discrepancies between studies and from a statistical perspective, studies are blocks and their effects must be viewed as random (St-Pierre, 2001). We calculated mean effect sizes and their corresponding 95% confidence intervals using the emmeans package for both grain and biomass yield across various years of the crop’s development.

We examined publication bias by analyzing histograms to identify potential variations in the frequency of published studies in relation to their effect sizes (Basche and DeLonge, 2017; Andrews and Kasy, 2019). Such variations might indicate a bias against publishing studies that do not show significant positive or negative effects. Moreover, we conducted a sensitivity analysis using a Jackknife technique, removing individual studies, and recalculating the overall effect size of nitrogen rates on grain and biomass yield using the same statistical model (Philibert et al., 2012).

Results

The BRT analysis revealed that, in the context of grain yield, two key moderators emerged as the primary drivers of its variation: (a) stand year, and (b) nitrogen rate (Figure 3.2). The remaining three moderators had relatively minor influence, with a combined contribution of less than 18%. On the other hand, when considering biomass production over the years, the analysis demonstrated a different pattern, with seeding rate having the highest relative influence (35.51%), and nitrogen rate and stand year with similar importance (26.51% and 24.89% respectively). The remaining three moderators demonstrated limited significance, together contributing less than 14%. It is worth noting that the predictor related to application season lacked sufficient data for inclusion in the grain yield model, as the majority of the studies used the same application season – spring – for fertilizer application.

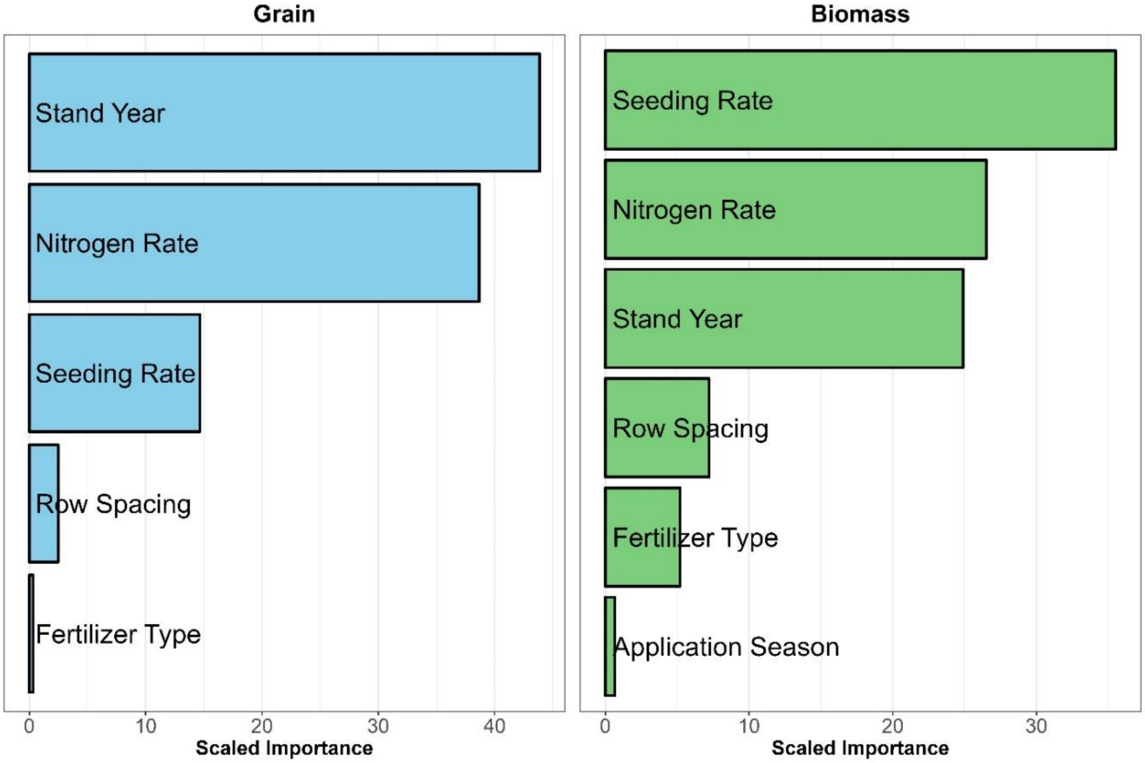

Figure 3.2. Scaled importance of five predictors for grain yield and six predictors for biomass. Variable importance derived from a generalized boosted regression tree.

Grain yield

In studies that included zero nitrogen rate as the control rate, there was no significant difference from zero during the first year of intermediate wheatgrass development. However, in the second, third, and fourth years, the crop showed a positive response to nitrogen application, with more pronounced effects on the latter years of the crop's stand (Figure 3.3A). Response ratios based on experiments with higher to lower nitrogen rate comparisons had a different pattern, with no positive effects from nitrogen application in all three years of the crop's stand (Figure 3.3B).

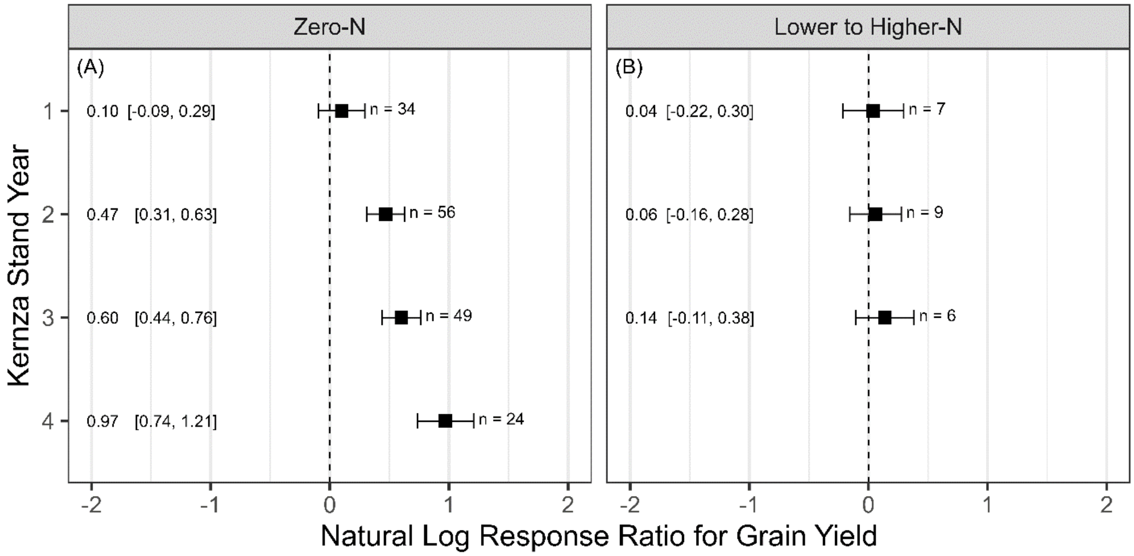

Figure 3.3. Natural log response ratio for grain yield separated by year of IWG stand. The figure includes papers that had a zero-control nitrogen rate (A) and papers that had higher to lower N comparisons (B) (mean effect and ± 95% confidence interval, n = number of paired comparisons in each Kernza stand year).

As the BRT model revealed, stand year and nitrogen rate have a high influence on IWG grain yield. In order to gain a more comprehensive understanding of how grain yield responds to different nitrogen rates in different years of the crop's stand, we categorized the N rates into five distinct groups: <50 kg ha^{-1}, 51-79 kg ha^{-1}, 80-99 kg ha-1, 100-149 kg ha^{-1}, and rates equal or greater than 150 kg ha^{-1}. Subsequently, we conducted an analysis to assess the impact of each nitrogen rate group across different stand years (Figure 3.4).

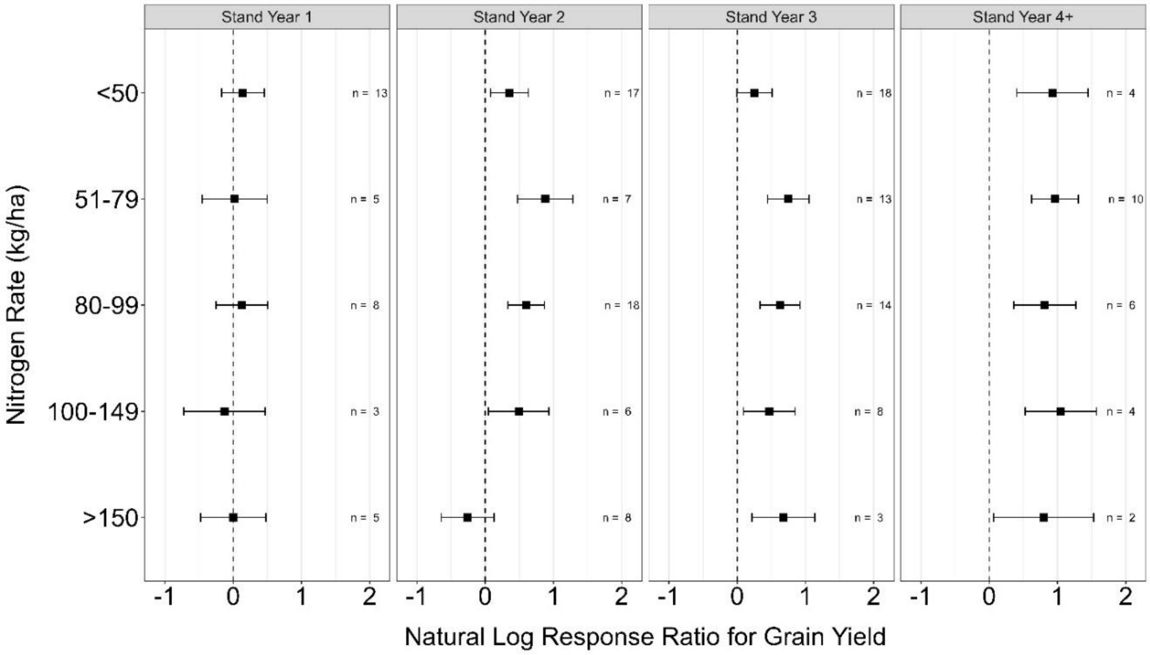

Figure 3.4. Natural log response ratio for grain yield to nitrogen rate groupings by IWG stand year. The figure includes only papers that had a zero-control nitrogen rate (mean effect and ± 95% confidence interval, n = number of paired comparisons in each Kernza stand year).

In line with the findings for year one (Figure 3.3), our observations did not reveal any significant difference from 0 for any of the N rates during the first year (Figure 3.4). However, for the second stand year, we observed a significant response in grain yield when nitrogen rates ranging from 51 and 150 kg ha^{-1} were applied, while rates equal to or greater than 150 kg ha^{-1} had a negative response to grain yield. For stand years 3 and 4, it became evident that nearly all the N rates had a positive effect on grain yield. These results indicate the critical role of nitrogen application, particularly for older stands of IWG, in maximizing grain yield. It is important to note that, for some of the N rate groups and stand years, the number of paired comparisons is relatively limited. Therefore, more evidence and data are needed to make broader conclusions.

Biomass

A positive response ratio of intermediate wheatgrass to nitrogen application was found for stand years two, three, and four of aboveground total biomass, with no effect of nitrogen application in year one, but positive effects in subsequent years (Figure *3.5*A). In experiments with higher to lower N comparisons (Figure 3.5B), despite the limited number of paired comparisons for each year, we also observed similar trends where older stands showed a significant response to nitrogen application in the fourth year.

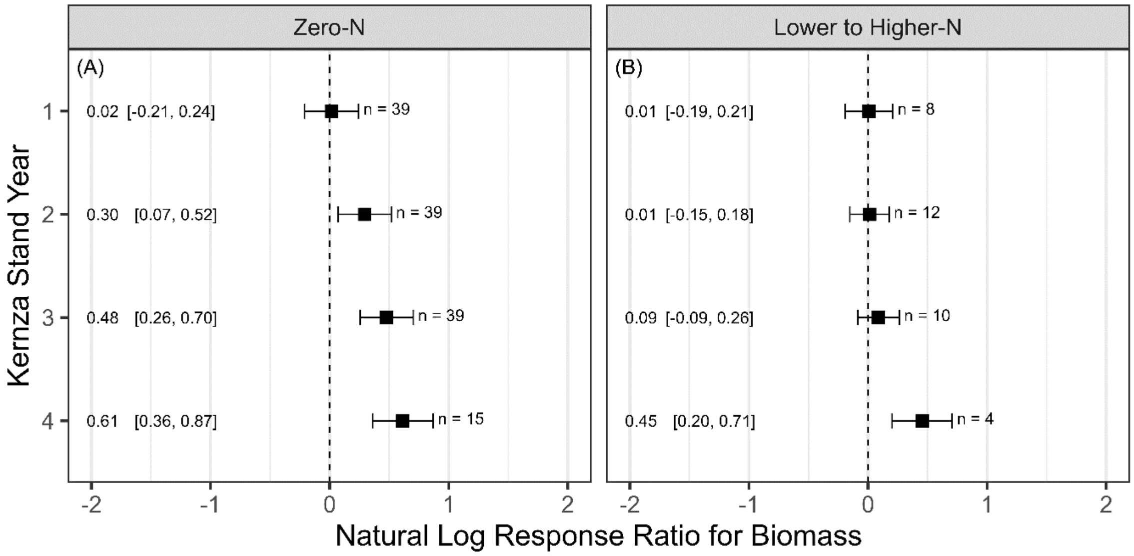

Figure 3.5. Natural log response ratio for biomass separated by year of intermediate wheatgrass stand. The figure includes papers that had a zero-control nitrogen rate (A) and papers that had higher to lower N comparisons (B) (mean effect and ± 95% confidence interval, n = number of paired comparisons in each Kernza stand year).

Sensitivity analysis and publication bias assessment

The results from the publication bias analysis using histograms indicated that there was no bias against publishing studies with an effect size around zero (Figure 3.6). Also, removing any individual study in the sensitivity analysis did not change the statistical significance of the mean effect size for grain yield (Figure *3.7*A) or biomass (Figure *3.7*B) by stand year, as the mean and error bars had similar response ratios compared to the original overall mean and 95% confidence intervals.

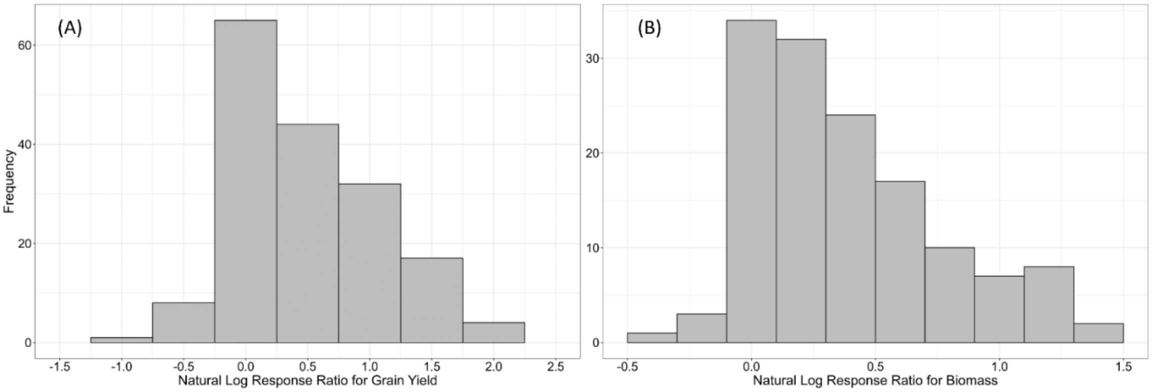

Figure 3.6. Histograms for the frequency of observations of (A) grain yield response ratios and (B) biomass response ratios to evaluate publication bias.

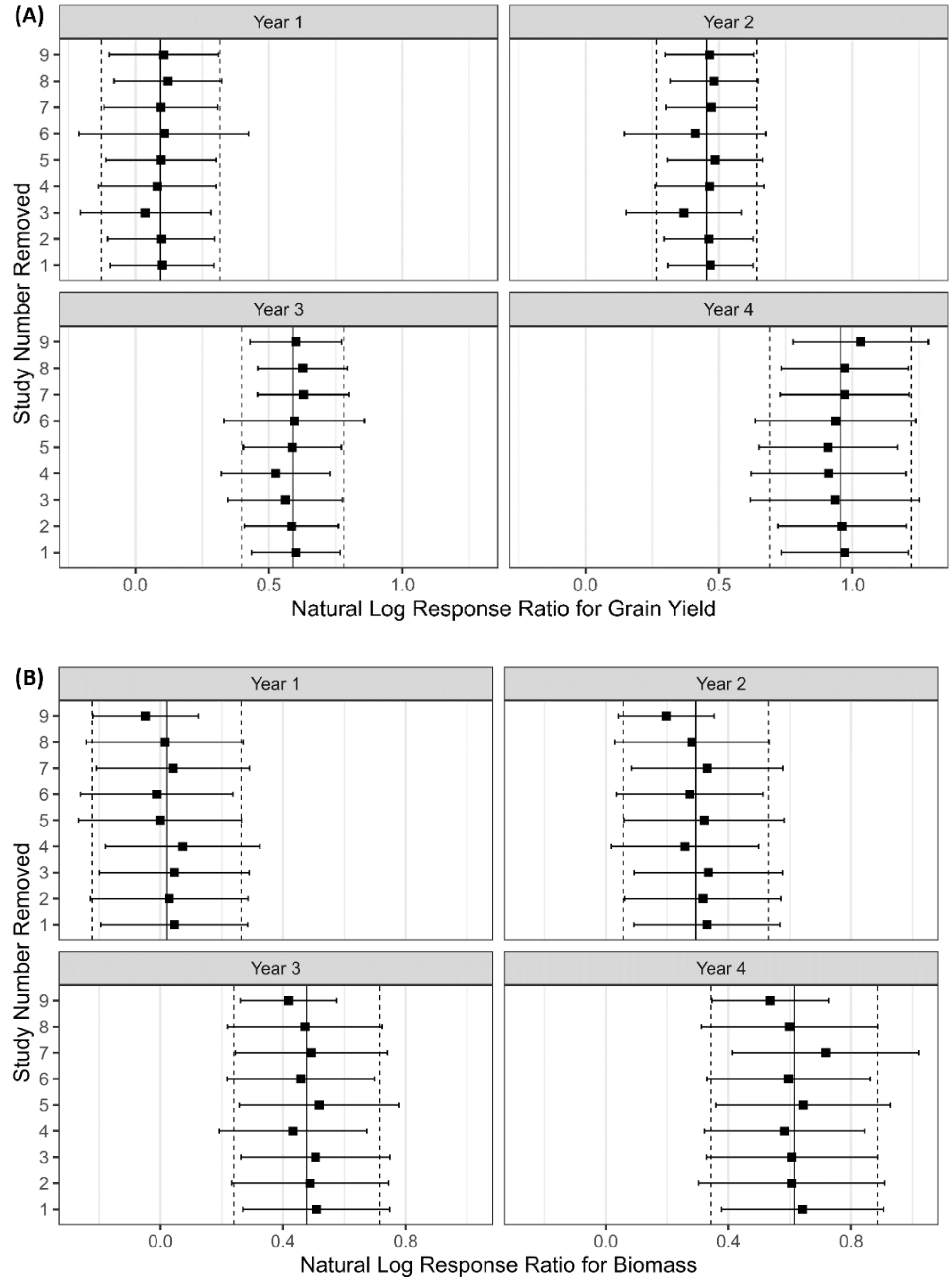

Figure 3.7. Results of Jackknife analysis to assess sensitivity of the analysis to study inclusion for (A) grain and (B) biomass. The x axis notes the change in mean effect size and revised 95% confidence intervals as individual studies were removed (y axis). The original overall mean effect is represented by the solid line and the original 95% confidence intervals are represented by the dotted lines.

Discussion

Our analysis found that older stands of intermediate wheatgrass exhibit a more pronounced response to nitrogen with respect to grain yield and aboveground biomass production. The reasons behind the limited differences observed in year one for grain and biomass remain somewhat unclear but are in alignment with other published intermediate wheatgrass experiments. Several reports have shown that IWG typically achieves higher grain yields in its initial year of development, followed by a subsequent decline in yield for the later years (Jungers et al., 2017b; Frahm et al., 2018; Tautges et al., 2018; Fernandez et al., 2020; Crews et al., 2022). These findings underscore the importance of nitrogen management strategies, particularly as the crop matures.

The observed pattern of yield decline over the years in IWG aligns with findings from previous studies evaluating other perennial grasses. Loeppky et al. (1999) reported a similar trend with smooth bromegrass, where seed yield consistently decreased over the years, regardless of the nitrogen rate, with the first year having the highest yield compared to the second and third years. Nazli et al. (2020) reported a substantial reduction in yield ranging from 19% to 78% for cool-season grasses, including reed canary grass, tall fescue, and perennial ryegrass during the second and third year of their study. Other studies conducted with the perennial grass miscanthus also reported a yield decline after several years of growth (Clifton-Brown et al., 2007; Angelini et al., 2009; Larsen et al., 2014).

We found that stand year had the highest relative importance in terms of grain yield between the predictors analyzed in this study, followed by nitrogen rate. Thus, it is fundamental to understand the site-year effect of nitrogen rates on IWG performance for making broader recommendations regarding nutrient management for IWG over time. Even though there were limited differences in year one among all N rates, the N application may play a crucial role in facilitating a good establishment on IWG plants during the initial stand year as well as impacting total biomass production (possibly of importance in a dual use system). This, in turn, can lead to enhanced growth and development in subsequent years.

Moreover, we found that during the second stand year, the optimal nitrogen rates range from 51 to 149 kg ha^{-1}, with rates below 51 and exceeding 150 kg ha^{-1} exhibiting a negative impact on grain yield. As noted by Jungers et al. (2017b), agronomically optimum nitrogen rates (AONR) for IWG grain yields varied between 61.0 to 96.4 kg N ha^{-1}, and their findings recommended to fertilize the plants annually after the first year in order to maximize grain yields. Furthermore, their study also reported that N rates exceeding 150 kg ha^{-1} resulted in a decline in grain yield, likely due to lodging.

In the context of the higher to lower N rate comparisons, we observed limited differences across years for grain yield, and a positive response in biomass production to higher N rates for older stands, which aligns with the patterns observed with the zero N experiments. However, it is important to note that the range of nitrogen included in the comparisons – the lowest control was 45 kg ha^{-1} while highest treatment was 135 kg ha^{-1} – may ultimately fall within a range near to agronomically optimal in later years of the crop’s stand. Thus, this included range of N rates tested in the higher to lower comparisons may have limited our ability to detect significant differences from this portion of the dataset. However, including the higher to lower comparisons as well as the unpublished studies adds confidence to our analysis of the 0 N controls and the finding of Jungers et al. (2017) for agronomically optimal rates as the crop matures. This limitation underscores the value of using zero control nitrogen rates in experimental designs.

In terms of aboveground biomass production, the BRT analysis highlights that stand year is not the primary influential factor. Instead, it identified seeding rate as the primary factor, followed by nitrogen rate. Similar to grain yield, biomass showed more positive responses to nitrogen application in older stands. However, as the BRT model suggests, biomass production can be sustained over the years through effective management of other variables. Culman et al. (2023) found that biomass was more affected by management (harvest frequency) than stand year. In another study, Frahm et al. (2018) reported that biomass yield was significantly affected by N rate in only one of five site-years. Additionally, another study reported that grain yield decline over time might also be related to tiller competition in dense stands (Law et al., 2021), as also hypothesized by Jungers et al. (2017b), whom noted the emergence of IWG in the inter-row spaces over the years in consequence of shattering or rhizome recruitment. These findings suggest that, while certain factors like forage harvest frequency, or higher seeding rates might not necessarily benefit grain yield, they can have a positive impact in biomass yield. Small cereal grain crops such as winter wheat are understood to be influenced by plant density, where the number of plants is a function of different yield environments (i.e., limited environments in terms of resources and weather, require a higher number of plants per m^2 to attain the same grain yield as high yield environments) (Bastos et al., 2020).

Moreover, we recognize the limitations of this analysis, with limited research that fit our criteria given the nature of this alternative perennial crop. Additionally, it is important to mention that meta-analyses inherently have an unbalanced design since they rely on existing studies. While it is always possible that results are influenced by studies with greater response ratios, our sensitivity analysis did not show different trends when individual studies were removed. Therefore, future investigations should aim to broaden our understanding of the relationships between grain and biomass yield with other experimental factors like sowing date, precipitation accumulation, fertilizer application timing, seeding rate, and soil fertility for example. These factors are likely to have important impacts on the response of IWG yield across different years.

Conclusion

The overall trend observed by this analysis is that intermediate wheatgrass grain yield showed no differences between higher and lower nitrogen rates in its first stand year, while for subsequent years the optimal N rate ranged from 51 to 149 kg ha^{-1}. These findings underscore the importance of considering the multi-year effects of nitrogen management strategies in perennial grain cropping systems. While nitrogen rate played a crucial role in maximizing grain yield, the trend was different for biomass production. We found that seeding rate was an important factor influencing biomass production across years. Additionally, our results suggest that higher N rates are required as the stand ages. However, it is important to consider other agronomic plant traits, as higher rates can lead to problems such as lodging.

Overall, this synthesis improves our understanding of the site-year effects of nitrogen rates on the intermediate wheatgrass performance. Our results suggest the need for a broad approach in terms of management practices to optimize N rates in intermediate wheatgrass, particularly considering the dual objectives of maximizing grain yield and biomass production over many years of crop growth. Future work could delve deeper into the mechanisms driving these trends, such as the effects of seeding rate and other factors that were not able to be comprehensively included in this study (such as sowing date, environment, soil fertility, soil type, etc.).

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CHAPTER 4: CONCLUSION

The objective of my thesis was to determine the most effective management practices for optimizing crop productivity and fertility in intermediate wheatgrass grain and biomass production. I addressed this question through two distinct approaches: firstly, a field experiment conducted in Nebraska aimed to assess how intermediate wheatgrass responded to different fertility treatments. Secondly, a meta-analysis of 16 individual studies was conducted to evaluate the impact of different nitrogen rates on intermediate wheatgrass.

In chapter 2, our field experiment involved 12 different treatments focusing on nitrogen (N), phosphorus (P), and potassium (K) management to evaluate intermediate wheatgrass grain and forage production. We also compared a perennial cropping system to business-as-usual corn and soybean, considering factors like soil moisture, and soil chemical composition. Additionally, other agronomic aspects of intermediate wheatgrass were evaluated, including plant height, lodging and weed assessments. We found that for years 1 and 2, N, P, and K rates did not impact yields, while second year yields were much lower than the first year likely due to dry spring conditions during anthesis. Despite this, Kernza demonstrated a great capacity for forage production even when experiencing an extreme drought, although grain production was highly affected by the weather in the second year 2. Additionally, Kernza proved to be a low-input crop during the initial two years of the experiment, requiring no herbicide application and minimum tillage requirements other than planting.

In chapter 3, I assessed the impact of nitrogen rates across sites and years on intermediate wheatgrass grain and biomass yields by conducting a meta-analysis of N rate studies. We selected the studies based on two main criteria: 1) studies needed to include an experimental design that assessed the effects of at least two nitrogen rates of intermediate wheatgrass, and 2) studies needed to report grain yield or biomass components of intermediate wheatgrass. Our search found 16 published studies that fitted our criteria, plus one multi-site trial with unpublished data shared by collaborators. We found limited effects on grain yield in year 1, but optimal N rates ranging from 51-150 kg ha^{-1} in later years of the crop stand. A generalized boosted regression tree model found that, in terms of grain yield, stand year and N rate were the factors with strongest effect. On the other hand, seeding rate had the strongest effect on biomass production, followed by N rate. Thus, it is important to consider other agronomic aspects such as sowing date, environment, soil fertility, etc., when growing intermediate wheatgrass in a dual-purpose system.

Together, Chapter 2 and Chapter 3 provide a more comprehensive understanding of fertility management practices on intermediate wheatgrass grain and biomass production. Our findings highlight the importance of strategizing the fertilization practices across the intermediate wheatgrass stand years in order to maximize grain and forage production. Moreover, intermediate wheatgrass demonstrated its potential as a low-input alternative, and dual-use crop for Nebraska producers.