Organically managed intermediate wheatgrass (*Thinopyrum intermedium*) as a dual-use grain and forage crop

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

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May 2023

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Acknowledgment

This academic achievement is dedicated to the folks that continue to show support for their friends and family in whatever way necessary to get them where they want to go. I learned during Peace Corps that encouraging someone to take the next step can be as easy as saying “I support you”. Throughout this academic journey, I have been shown support from various groups and individuals that have gone far beyond what was required of them and helped me advance to the place I am today. I want to thank my advisors, Dr. Jacob Jungers and Dr. Jessica Gutknecht, for taking a chance and letting me join their labs. Thanks to research techs Katherine Bohn and Jesse Puka-Beals for entertaining my wild ideas and requests, hanging with me, and giving advice in my times of need. Thank you, Dr. Tim Crews and the researchers from The Land Institute, who first introduced me to perennial agriculture and considering place and community. As my community continues to grow, I strive to connect everyone within it because they have so much value to impart on each other and within the world. I can’t stress enough the importance of having a supportive community. Without our community in Gypsum, KS, our family would not have been able to support our farm, which would have jeopardized finishing my degree. I especially want to thank my family for allowing me the breadth to explore my world, inside and out, without shaming me for not staying around our farm. I feel I have a responsibility to the stewardship of that land, and I believe the experiences gained through my explorations will only add more perspective for its stewardship in the future.

I would like to thank the USDA Organic Agriculture Research and Extension Initiative (OREI), The Land Institute, and The Ohio State University for their support of this research project.

Abstract

Intermediate wheatgrass (*Thinopyrum intermedium*), a historically managed forage crop for livestock, is currently being domesticated to produce the cereal grain crop named Kernza®. This study examines using intermediate wheatgrass as both a grain for human consumption and feed for livestock in a dual-use, organically managed system, and consequences for environmental quality. This was done by comparing agronomic and environmental responses to different fertilization strategies (none, commercial mineral fertilizer, or manure) and defoliation to simulate biomass removal for forage. Agronomic measurements included grain, straw, and forage yield as well as environmental effects, specifically nitrogen mineralization, total carbon, total nitrogen, and soil gas emissions. Treatments were carried out at two sites, in south central Minnesota and central Kansas. Results showed that manure increased grain, straw, and forage yields compared to unfertilized treatments in year two in MN and KS. In addition to yields, forage nutritive value increased in manure fertilized treatments compared to unfertilized control treatments in the second year at both sites. Soil extractable nitrogen differed across seasons in MN in years one and two, but KS only differed in year two. There was a difference in nitrogen mineralization among treatments and across seasons in MN in year 2. KS did show an interaction among treatments and season in 2020 and a difference across seasons in both years. Soil gas emissions were higher for CO_2 in manure fertilized plots in the second year in MN, but there were no differences between treatments for CH_4, N_2O, or NH_3. At the end of the experiment, soil carbon was higher in manure fertilized plots in MN. In summary, manure fertilizer improved agronomic variables important to farmers but environmental impacts of this practice should be considered.

Manure application can result in increases in soil gas emissions thus exacerbating human impact on the climate. However, manure showed potential to increase soil organic carbon and potentially offset soil gas emissions associated with manure addition.
KEY WORDS: organic nitrogen fertility, dual-purpose grazing systems, perennial agriculture, greenhouse gas emissions.

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Introduction

Perennial grain crops have been studied as an agricultural strategy to produce food, conserve soil, and reduce nutrient losses from agroecosystems (Cox et al., 2006; Glover, 2022). Kernza® is a perennial cereal grain harvested from improved lines of intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) - a perennial cool-season grass introduced to the United States as a forage grass in the early 20th century (Dehaan et al., 2018; Hitchcock, 1935; Wagoner, 1990). Advanced grain-type lines of IWG have been selected for increased seed size, lodging resistance, threshability, non-shattering, and other domestication traits (Bajgain et al., 2020; Crain et al., 2020; DeHaan et al., 2020). IWG also shows promise in its ability to provide ecosystem services such as soil conservation (Ashworth et al., 2022), mitigating soil greenhouse gas emissions (de Oliveira et al., 2018; Wiesner et al., 2022), and preventing nutrient leaching to drinking water (Jungers et al., 2019; Reilly et al., 2022) compared to annual row crops. Economic advantages of IWG managed as a perennial grain crop include reduced N fertilization compared to some annual grain crops (Jungers et al., 2017), and reduced expenses related to seed and field passes (Law et al., 2022). IWG for grain production is expanding across the United States in organic and conventional acreage to fulfill commercial and consumer demand for Kernza (Lanker et al., 2019; https://kernza.org/the-state-of-kernza/). In Minnesota and Kansas, demand has increased within the value-added sectors for products including breakfast cereals, flour, noodles, and beer (Muckey, 2019). Because IWG was first introduced as a forage grass and is known to have a high forage quality, there is also opportunity to improve the economic viability of IWG by managing it for dual-use grain and forage production (Hunter et al., 2020a; Hunter et al., 2020b).

Intermediate wheatgrass forage can be harvested or grazed during multiple growth stages without interrupting grain production (Hendrickson et al., 2005; Hunter et al., 2020a), and this forage ranges in quality from premium hay with relative feed values (RFV) >160, ideal for lactating dairy cows, to utility quality primarily considered for bedding material (RFV<90) (Newman et al., 2009; Saha et al., 2010). Starting with vegetative growth in the spring, forage can be mechanically harvested or grazed prior to stem elongation, which can generate up to 2 Mg ha^{-1} of forage with relative feed values (RFV) of 150-170 (Favre et al., 2019; Hunter et al., 2020b), which are considered high-quality for cool-season grasses. Weather and field conditions prior to stem elongation can limit dual usage in the spring by affecting access to the field during wet conditions. Digestible dry matter and carbohydrates may also be lost due to increased precipitation during field drying when cut for hay (Fonnesbeck et al., 1986). Following grain harvest at physiological maturity, IWG stems and leaves can be harvested and used as straw for bedding similar to other annual small grain crop residues. Although straw isn't typically thought of as a feed source, IWG post-grain harvest biomass is relatively high in yield and RFV compared to straw from annual small grain crops, and economic analyses have shown that managing this residue for forage can be profitable (Favre et al., 2019; Hunter et al., 2020b). After grain harvest and residue removal, IWG continues to grow throughout the fall only producing vegetative biomass, and at the end of this growing season, yields can reach 4 Mg ha^{-1} with RFV ranging from 100-110, a quality suitable for dry cows and heifers (Newman et al., 2009). Harvesting biomass in both spring and fall can have a positive impact on grain yield by stimulating reproductive tiller growth (Hunter et al., 2020a). Although beneficial across defoliation regimes, spring hay or forage removal has the potential to negatively impact grain yields if harvest occurs after stem elongation through damage to the emerging meristem (Barriball et al., 2022). Fall defoliation post grain harvest, however, has been shown to increase grain yields in the following year compared to non-defoliated plants, therefore forage harvest in the fall presents an opportunity for dual-use as a grain and forage crop with limited risks to subsequent grain yields (Pugliese et al., 2019).

Nitrogen fertilization has been shown to increase IWG grain yield, forage yield, and forage nutritive value (Fagnant et al., 2023; Fernandez et al., 2020; Jungers et al., 2017; Lawrence et al., 1970). Although studies have shown that IWG grain yields were maximized at N fertilizer rates ranging from 61 – 96 kg ha^{-1} (Fernandez et al., 2020; Jungers et al., 2017), few studies have investigated the seasonal dynamics of N use and translocation in this perennial species (Fagnant et al., 2023). At physiological maturity, total N uptake can exceed 150 kg ha^{-1} in the aboveground biomass (Fagnant et al., 2023), and another 30 kg ha^{-1} in root biomass (Dobbratz et al., 2023). Perennial grasses transfer N to different tissues throughout the growing season and can store N in roots and crowns for remobilization during regrowth (Lemus et al., 2008). These complex N dynamics make it challenging to determine the optimal timing of N fertilization for grain and forage yield maximization.

Background levels of soil nitrogen are an important context for N fertilization and IWG N requirements. Soils accumulate inorganic nitrogen over time from decomposed biomass that becomes plant-accessible in the form of ammonium (NH_4+) and nitrate (NO_3-) (Chen et al., 2003). Inorganic forms of nitrogen from commercial fertilizers like urea, sodium nitrate, and ammonium nitrate, may contribute more rapidly to nutrient uptake, while more complex organic sources that require microbial decomposition contribute to the soil inorganic nitrogen pool slowly over time through decomposing biomass, composts, manures, and biologically fixed nitrogen from legume intercrops (Crews et al., 2022; Hendrickson et al., 2005; Lynch et al., 2004). The composition of organic material can affect the rate of mineralization and immobilization. For example, an increase in the C:N ratio above 24 inhibits mineralization and thus favors immobilization by microbes (Magid et al., 2006). Manure is a heterogenous mixture of digested plant material and enteric microbial biomass that varies in C:N composition (Qian & Schoenau, 2002). Where the C:N ratio is higher than N demand from microbes, N is immobilized and unavailable to plants (Chantigny et al., 2002; Musyoka et al., 2019). Therefore, quantifying soil extractable N and net N mineralization are important over the growing season to determine how nitrogen availability differs by nutrient source and treatment during seasonal growth stages. Slow decomposition of manure may be beneficial in a fall grazed dual-use system where additional biomass will be removed (Lemus et al., 2008).

Research on the effects of manure application and defoliation on other perennial grass cropping systems (e.g., pasture, bioenergy grasses) suggests that these management activities could increase the carbon balance of a dual-use perennial cropping system through accumulation of organic matter from above and below ground biomass (Xia et al., 2017, Kim et al., 2022). In addition to below-ground biomass production, a perennial system fertilized with manure may increase soil organic matter (SOM) because of its inherent carbon contribution and slow decomposition (Mori & Hojito, 2015). With its additional carbon and particulate matter, manure eventually stabilizes in forms of sequestered carbon, increasing the carbon resource pool (Cotrufo & Lavallee, 2022; T. Li et al., 2020). However, this additional carbon may have an impact on soil gas emissions, particularly CO_2 emissions due to the release of carbon during decomposition of organic matter by microbes (Chen et al., 2003; Ding et al., 2007). With continually increasing concern over climate change and anthropogenic contributions to the planet, manure application ranks high as a potential contributor of three major greenhouse gasses, CO_2, N_2O, and CH_4 (Bouwman et al., 2002; Calderón et al., 2004; Dalby et al., 2021; Gavrilova et al., 2019; Wyer et al., 2022), so the benefits of manure application must be considered together as well as potential consequences. In addition to climate change concerns with soil greenhouse gas emissions, manure contributes ammonia gas (NH_3) to the atmosphere, a compound affecting human health due to its small particle size (Leytem et al., 2011).

Our goal was to understand the potential agronomic and environmental impacts of managing IWG as a dual-use grain forage crop in response to different N fertilization sources. Specifically, our objectives were to 1) evaluate grain, forage yield, and forage nutritive value of IWG when managed with and without a fall forage harvest event, and 2) to evaluate the effect of two organic N fertilizer sources (cattle manure and sodium nitrate) on grain and forage yield, soil nutrient status, soil nitrogen mineralization potential, and gas emissions (CO_2, N_2O, CH_4, and NH_3). The study was conducted at two locations in the central US over three years, and we predicted that manure fertilizer would have a positive impact on yield and other agronomic measurements, while soil nitrogen mineralization rates, as well as soil gas emissions, would vary across fertilizer and defoliation treatments.

Methods

Experimental Sites

This study was conducted at the University of Minnesota Rosemount Research and Outreach Center, located in Rosemount, Minnesota (44°41'18.0"N 93°04'28.1"W) and in Salina, Kansas at The Land Institute (38° 46' 32.4012"N, 97° 35' 41.9136"W) from August 2019 through November 2022. 30-year historical weather data were available from weather stations present within 2 miles of each site (https://www.ncei.noaa.gov). The Minnesota daily climate and precipitation values were obtained from the Minnesota Department of Natural Resources online database (MN-DNR, 2023). The Minnesota site was previously in alfalfa, which was terminated with 2, 4-d at recommended rates followed by tillage with a chisel plow and a tandem disc prior to planting IWG. IWG cv ‘MN-Clearwater’ was then planted at a rate of 13 kg pure live seed ha^{-1} on 38-cm rows in September of 2019. The experiment was conducted on a Port Byron (Fine-silty, mixed, superactive, mesic Typic Hapludolls) with a soil pH of 6.5, 3.6 percent organic matter, 0.1 mmhos/cm soluble salts (Web Soil Survey), 8 ppm Bray-1 phosphorus (P) , and 111 ppm potassium (K).

The Kansas site was previously in wheat and was prepared with a conventional disk harrow prior to planting IWG cv ‘MN-Clearwater’ at a rate of 13 kg pure live seed ha^{-1} on 30 cm rows in September 2019. The soil was a Detroit silty clay loam (Fine, smectitic, mesic Pachic Argiustolls) with a soil pH of 7.2 (1:1water/soil, Web Soil Survey) with 3.0

percent organic matter [Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online. Accessed on 01/01/2023].

At both sites, baseline soil characteristics were measured. Baseline analysis in MN included N (ppm), P (ppm), and K(ppm). P and K were determined by collecting four soil cores per block, each 0.86 cm in diameter from a depth of 0-15 cm deep with a hand probe. Samples were aggregated by block and sent to a commercial laboratory for analysis (University of Minnesota Soil Testing Lab). The Bray-1 method and Ammonium Acetate Extraction method were used to determine phosphorus and potassium concentrations respectively. Soil inorganic nitrogen was calculated from initial nitrogen mineralization samples in Minnesota by analyzing colorimeter data and summing the available nitrate and ammonium at UMN in the Gutknecht lab. Baseline analysis in KS included only soil % C and % N. Kansas samples were tested at the Kansas State Stable Isotope Mass Spec Lab (SIMSL) by mass spectrometry for total CN analysis at the Kansas State University Soil Testing Lab for general fertility analysis.

Experimental Design and Treatment Implementation

The experimental design was a split-plot randomized complete block with four replications. Fertilizer treatments were applied at the main plot level and defoliation treatments were applied at the split plot level. Whole plots measured 3x4.6 m in MN and 7.3x22.9 m in KS. Experimental units (sub-plots) were 3 x 2.3 m and 11.4 x 3.6 m respectively. There were three main-plot fertilizer treatments which included a control (no fertilizer), Allganic® Nitrogen Plus 15-0-2 (sodium nitrate, $NaNO_3$, applied at the MN site only), and livestock manure applied annually in the spring (Table 1). In

Minnesota, the livestock manure was sourced from dairy cattle housed on bedded pack (manure and straw bedding mixture), whereas manure was sourced from a beef cattle feedlot in KS. Multiple samples were taken from each source and either sampled individually, tested, and averaged, or homogenized and tested. Samples from MN were analyzed as received at Stearns DHIA Laboratories for % moisture, % dry matter, % Total Kjeldahl N (TKN), % organic N, % inorganic (ammonium and nitrate) N, % P_2O_5, and % K_2O. Samples from KS were analyzed as received at Servi-tech Laboratories for % moisture, % dry matter, % Total Kjeldahl N (TKN), % organic N, % inorganic (ammonium and nitrate) N, and % P_2O_5. The lab report included estimated first year available nutrients for solid surface applied as reported from the laboratory and converted to kg Mg^{-1} (Table 1). Phosphorus was tested with the Bray-1 method and potassium with acid digestion.

Grain, Straw, and Forage Yields and Nutritive Value

Grain yield was determined by harvesting seed heads at physiological maturity on 8/3/2020, 7/26/2021, and 7/20/2022 in MN; and 7/18/2020, 7/19/2021, and 7/18/2022 in KS. All live plants were cut within two separate 76 cm by 76 cm quadrats randomly placed within each subplot in MN and from two separate 50 cm by 50 cm quadrats randomly placed within each plot in KS. Seed heads were removed from the stems just below the lowest spikelet (see Heineck et al. 2022 for description of seed head structure), dried at 35°C for five days or until samples maintained constant mass. Once samples were dried, seed heads were counted and threshed using a Wintersteiger LD 350 with a 2x6 mm concave screen and cleaned using the Carterday fractionating aspirator. After removing seed heads, all residual biomass (hereafter referred to as straw) within the sample quadrats was cut leaving a 10 cm stubble height, dried at 35 °C, and weighed for dry matter yield. In defoliation subplots, forage biomass was sampled using the same methods described for straw biomass at physiological maturity. There were two defoliation treatments, none or fall defoliation. The defoliation treatment was performed after the first harvest on 10/19/2020 and 10/22/2021 in MN and 11/11/2020, 11/19/2021, and 11/19/2022 in KS. All remaining biomass outside the quadrat sampling area was cut and removed from the defoliation treatment plots.

Nutritive value was determined on all vegetative biomass samples (straw and forage). Samples were ground to 6 mm using a Wiley Laboratory Mill model 4, and then to 1 mm using the Foss Cyclotec General Purpose Sample Mill**.** Ground samples were analyzed using near infrared reflectance (NIR) spectroscopy (Perton DA 7250) following methods described in Puka-Beals et al., 2022. Forage nutritive value including crude protein (CP), neutral detergent fiber (NDF), NDF digestibility (NDFD), and acid detergent fiber (ADF) and used to calculate the relative feed value (RFV) based on equations from Moore and Undersander (2002).

RFV = (DMI, % of BW) * (DDM, % of DM) / 1.29

Soil Net N mineralization and Extractable N

Net N mineralization was measured using an *in-situ* incubation method (Dobbratz et al., 2023; Crews et al., 2022). Briefly, 2 PVC tubes of 23 cm depth and 5 cm diameter are inserted into each plot at each timepoint. One of the two tubes is immediately pulled from the plot and the soil in the tube is analyzed for extractable N as the time zero sample. The second PVC tube is left to incubate in place for approximately 28 days, after which soil from the tube is then extracted and analyzed for extractable N. After soil was removed from the PVC tube it was immediately sieved to 2 mm. 10 g were then weighed into 50 ml conical centrifuge tubes and extracted with a 1 M KCl solution. Soil sample N extraction was performed according to protocols outlined in Culman et al. 2013. Preparations for analysis were made using colorimetric analyses for nitrate (Doane et al., 2003) and ammonium (Sinsabaugh et al., 2000) modified for 96 well plate-based analysis. In this procedure, reagents were used to react with nitrogen in the extracted solutions to produce a color detectable by a colorimeter at a specific wavelength. The extractions were analyzed using the Bio-Tek Synergy ™ HT Multi-Detection Microplate reader for analyzing ammonium and nitrate concentrations. The concentrations (ppm) of ammonium and nitrate were then determined by comparing absorbances of extracted samples to a standard curve of known concentrations of ammonium and nitrate. Once analyzed, the time zero and incubated samples were adjusted for dry soil weight in order to determine extractable nitrate and ammonium as mg N kg^{-1} soil. The net nitrogen mineralization rate in the spring, summer, and fall was calculated as mg N kg^{-1} soil day^{-1} by first summing the concentrations of ammonium and nitrate for each extraction and then taking the difference between the incubated and time zero samples and dividing it by the number of days incubated *in-situ*. Kansas also extracted samples according to Culman et al. 2013. Samples were then sent to the Kansas State Soil Testing lab where they were analyzed for ammonium and nitrate concentrations. The ammonium and nitrate present in time zero extractions were summed and considered the extractable mineral nitrogen at each time point.

Final Soil C and N.

Final soil samples were collected in MN from each plot to characterize the effects of fertilizer application on soil % C and % N at the termination of the experiment. 3 probe samples were taken from each plot at 23 cm depth and aggregated. Samples were then sent to Brookside Laboratories, Inc. New Bremen, OH for analysis on a combustion analyzer.

Soil Gas Emissions

Soil gas emissions were measured using a Gasmet® DX4040 portable FTIR gas analyzer. Gas samples were taken in a chamber measuring 16.2 x 52.7 x 10.2 cm (8708 cm^3) made from 18-gauge stainless steel. Measurements were made by placing the chamber onto a sampling anchor made of the same stainless-steel material, with the bottom removed, and inserted into the soil. The anchors were inserted into the soil so that edges were nearly flush with the soil surface, at least 24 hr prior to the first sampling event, and left *in-situ* for the duration of the season when possible. If field activities required for anchors to be removed, they were reinstalled after the activities were complete and not sampled again until they had been in place for at least 24 hr (Bergquist 2019). Sampling events were performed on roughly a biweekly basis. The instrument was calibrated by Gasmet Technologies to identify concentrations (ppm) of carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3). Gas measurements were taken in commercial fertilized (MN only), manure fertilized, and control plots at 21 second intervals, over a time span of 6 min to determine the concentration of gas emissions over time. The gas emissions rates were then determined using linear regression as a function of gas emissions increase over time. The slope of the predicted linear regression equation was used as the gas emission rate for each sampling event. Cumulative seasonal emissions were estimated using trapezoidal integration of all emissions rates over time (Levy et al., 2017). This was done by calculating the area of the trapezoid formed between the emissions rates of two given dates and then summing the areas of all the trapezoids over the course of the growing season to calculate total cumulative amounts of each gas emitted (Levy et al., 2017) as kg ha^{-1} yr^{-1}. Measurements were taken biweekly from spring after IWG emerged from dormancy (Rosemount: 5-22-2020, 4-05-2021; Salina: 3-31-2020, 3-31-2021) until fall when it began to go dormant again (Rosemount: 11-19-2020, 10-25-2021; Salina: 11-27-2020, 11-20-2021). Growing season cumulative GHG fluxes were compared among the fertilizer treatments at both sites in 2020 and 2021, and in all fertilizer×defoliation treatment combinations in MN in 2021. GHGs were not measured in the defoliation treatments in KS and were not measured in any plots in 2022.

Caveats and methodological limitations.

Differences in plot sizes between the two locations may have an impact on the homogeneity of the plots because of soil heterogeneity. The different previous crops at each site could have had legacy effects on fertilizer uptake and nitrogen pool longevity in relation to the stand. In KS, no defoliated treatments were analyzed for soil gas emissions. Gasmet equipment and labor capacity of the program limited the maximum number of measurements that could be taken at that site. The frequency of measurements was also higher in Kansas (every week) for the first year. For the same reason, MN moved to a biweekly measurement to balance labor capacity with workload. N mineralization at the Kansas site was only measured in the control defoliated treatments, whereas in MN both defoliated and control plots were measured, so the effect of defoliation on N mineralization could not be determined at the Kansas site. In 2022 at KS, fertilizer was switched to a composted beef manure rather than feedlot manure from the two previous years. For this source, the appropriate nitrogen application rate was calculated based on total available nitrogen communicated by the vendor but did not undergo laboratory analysis to determine 1st year available N. Finally, Kansas did not have a commercial fertilizer treatment which will leave a knowledge gap of how this type of fertilizer compares to organic manure fertilizer.

Statistical analysis

Statistical analyses were conducted using R version 4.0.2 (06-22-2020). Linear mixed effects models were constructed using the ‘nlme′ package (Pinheiro et al., 2022) to determine the fixed effects of fertilizer type, defoliation, and their interaction on response variables. Models were created to account for the split-plot design by including main plot treatment nested within blocks as random effects. Data were analyzed separately for each site-year combination to account for large differences in growing seasons across locations and differences in agronomic management (N application rates and timing, defoliation, etc.). In 2020, models only included fixed effects of fertilizer type as defoliation treatments were not yet imposed. Agronomic response variables were IWG grain yield, IWG straw yield, IWG forage yield, and IWG straw and forage RFV. Agronomic response variables were tested for normality and homogeneity of variances. In 2021 and 2022, models that included fertilizer type, defoliation, and their interaction were tested to determine effects on all agronomic variables except for fall forage yield from MN in 2022. Environmental response variables measured were net N mineralization and extractable N over the course of spring, summer, and fall; soil gas emissions across each yearly growing season in 2020 and 2021; and total C and N in the soil at the end of the experiment. Environmental response variables were also tested for normality and homogeneity of variances. In 2021 and 2022, models were created following the agronomic model examples including fertilizer type, defoliation, season, and their interaction. These predictor variables were tested to determine effects on all environmental response variables except for defoliation treatments, and final C and N in KS in any year. For N mineralization and extractable nitrogen, which were measured multiple times within a growing season, a mixed effects model was run first that included the full combination of season, defoliation, and fertilization treatments, and all interactions as fixed effects in site years with a defoliation treatment present. Block was included as a random effect. Defoliation was never significant for extractable N or N mineralization (Table S1) so this treatment was removed from the model statistics presented in this manuscript. The full model was therefore season, fertilization treatment, and their interactions. Means were calculated with the ‘emmeans’ package at $\alpha = 0.05$. Post-hoc comparisons of means were conducted using Tukey’s HSD as implemented in the ‘emmeans’ package (Lenth, 2022).

Results

Precipitation

Cumulative yearly precipitation was on average 25 % lower over the three years of the study compared to the 30-yr average in Minnesota. The last two years averaged 29 %

lower compared to the 30-yr average, and the 2021 growing season was considered a historic drought. The Kansas cumulative precipitation was 10 % lower than the 30-yr average in all years and had exceptionally low precipitation between November 2021 and February 2022 with only 16 % of its normal rainfall (Table 2a). Temperature was similar to the 30-yr averages at both sites for the duration of the study (Table 2b).

Grain, Straw, Forage Yield, and Nutritive Value

Grain yield was not affected by fall defoliation in any site-year combination in this study. Grain yield was not affected by fertilizer treatment in 2020 at either location but did vary by fertilizer type at both locations in 2021 and at MN in 2022 (Table 3). In 2021 at MN, commercial fertilizer increased grain yield by 109 % compared to the control, while manure fertilizer resulted in yields that were similar to both treatments (Figure 1). In KS, grain yields were 92 % greater in the manure treatment compared to the control in 2021. In 2022 at MN, grain yields from commercial and manure treatments were similar and both were greater than the control treatment. The same was true for Kansas where grain yields in the manure treatment were greater than the control (Figure 1).

Effects of treatments on straw yields were similar to those on grain yields with no significant effects in the first year at either location (Table 3, Figure 1). However, there was an interaction between the fertilizer and defoliation treatments in 2021 at MN (Table 3, Figure 2). Fall defoliation in 2020 resulted in an increase in 2021 straw yield compared to the non-defoliated treatment when fertilized with commercial fertilizer. There was a main effect of fertilizer on straw yield in 2022 at MN, whereby both fertilized treatments were greater in yield compared to the control. At KS in 2021, manure fertilizer increased straw yield by 41 % compared to the control (Figure 1). In 2022, KS straw yields were higher in the fall defoliation treatments than uncut treatments (Figure 2).

Straw RFV was not affected by fertilizer treatment in 2020 at either location (Table 3, Figure 1, Table S2). In 2021 at MN, RFV was greater in the manure fertilizer treatment compared to the control with the commercial fertilizer similar to both (Figure 1). In 2022, straw RFV was greater in the manure treatment compared to both the commercial fertilizer and control treatments in MN. At Kansas, RFV was similar between the manure and the control treatments in both 2020 and 2021 (Figure 1).

Forage yield, the biomass that was harvested during the fall defoliation event, was not affected by fertilizer treatment in 2020 at either site or at KS in 2021. It was, however, greater when fertilized with manure (1413 kg ha^{-1}) than in the control (844 kg ha^{-1}) at MN in 2021, while commercial fertilizer yields (1263 kg ha^{-1}) were not statistically different from either the manure or control treatments (Table 4). There was no effect of fertilizer on forage yield in Kansas. Forage RFV was 17 % higher in MN and 15 % higher in KS in the manure fertilized plots than in the unfertilized control treatments in 2021 (Table 4, Figure 3). Higher RFV was also true for manure compared to the commercial fertilizer in MN in 2021. Forage protein (%) was also not affected by fertilizer treatment in 2020 at either site (Table 4) but in 2021 varied by fertilizer treatment in both MN and KS and was 34 % and 30 % higher in protein, respectively, in the manure fertilized plots in comparison to the control treatments (Table 4, Figure 4).

Soil Gas Flux

Fertilization and defoliation did not affect soil CO_2, CH_4, N_2O or NH_3 flux in any site-year combination except for MN in 2021 (Table 5), where the cumulative CO_2 flux was greater in the manure fertilizer treatment by 61 % and 116 % compared to the commercial fertilizer and control treatments, respectively (Table 6). The average CO_2 flux was more than 2 times greater in the manure treatment compared to the control at KS in 2020, but this difference was only marginally significant (Tables 5 and 6).

N Mineralization

In Minnesota in 2020, there were no significant fertilization treatment effects or significant changes over the three N mineralization sampling events (spring, summer, and fall (Table 7). N mineralization means varied from -0.18 mg N kg^{-1} soil day^{-1} in summer 2020 to 0.1375 mg N kg^{-1} soil day^{-1} in the spring 2020, with summer exhibiting significantly negative N mineralization rates in both treatments ($P<0.05$) indicating immobilization (Figure 5). In 2020 in Kansas, sampling season along with a fertilizer by sampling season interaction were significant main treatment effects (Table 7). N mineralization was significantly higher in the summer than in the fall with spring similar to both. A fertilizer by season interaction showed the summer manure treatment had significantly higher N mineralization than the control (Figure 5) and was the only treatment or time combination with N mineralization rates statistically different from zero ($P>0.05$). Minnesota did have an effect of fertilization and sampling season in 2021. The manure treatment showed the highest N mineralization rate in spring, compared to the summer and fall control treatments, with spring control, summer and fall manure treatments similar to both. Rates were significantly positive across all sampling seasons with means ranging from the lowest at 0.142 in the summer to the highest at 0.378 mg N kg^{-1} soil day^{-1} in the spring, (P>0.05) demonstrating mineralization. Kansas showed significant effects of sampling season in 2021 (Table 7) but there were no significant differences based on post-hoc means comparisons, and mineralization rates in Kansas in 2021 were only marginally significantly different than zero (P = 0.0728 for control plots and 0.0663 for manure plots).

Extractable Nitrogen

In Minnesota, sampling season exhibited significant treatment effects on soil extractable N in 2020 where extractable N was highest in summer and spring, and approximately 80 % lower in fall than in the summer (Figure 6). In 2021, Minnesota showed significant differences in extractable nitrogen across fertilizer treatments and sampling season (Table 7). There was also an interactive effect between fertilizer treatment and sampling season such that the manure treatment was higher than the control only in summer of 2021 (Table 7, Figure 6). This increase in the manure treatment was related to an overall higher extractable N in summer 2021 in Minnesota (Table S4). Kansas did not show a significant effect from fertilizer in 2021 but did show an effect of sampling season. The spring sampling had the highest extractable N followed by the summer with similar amounts to both spring and fall, fall having the lowest extractable N of the season (Table 7, Figure 6)

Final soil C and N

Total soil % N at MN from 0- to 23-cm was affected by fertilizer treatment in 2022 (P = 0.003) but not by defoliation treatment (P = 0.948) or their interaction (P = 0.289). Total soil % N in the manure fertilizer treatment was higher (0.28 %) compared to commercial fertilizer (0.24 %) and control (0.25 %) treatments. Total soil % C from 0- to 23- cm was also affected by fertilizer treatment at MN in 2022 (P = <0.001) but not by defoliation treatment (P = 0.404) or their interaction (P = 0.741). Total soil % C in the manure fertilizer treatment was higher (2.25 %) compared to the commercial fertilizer (1.90 %) and control (1.92 %) treatments.

Discussion

Across all sites, grain and straw yields did not significantly differ across treatments in the first year suggesting that the fields may have had sufficient native nitrogen available to support first year growth. This observation has been made in other first year IWG stands as well (Dobbratz et al., 2023, Sprunger et al., 2018). Soil extractable N was between 6 and 10 mg kg^{-1} soil in the top 15 cm in spring of the first growing season, which may be adequate (Vogel et al. 2002) but also may be considered low for grass crop production. Residual N from previous crops may be assimilated prior to soil sampling as IWG growth begins relatively early in the growing season (Jungers et al., 2018), adding support to the hypothesis that native soil N was sufficient in year 1 of our study. IWG at Minnesota had the benefit of following an alfalfa crop that can supply nitrogen credits of up to 168 kg ha^{-1} in the first year, and 84 kg ha^{-1} N two years after termination, likely contributing to its sustained yields (Mohr et al., 1999, Yost et al., 2014). Perennial systems have been observed to increase root biomass and take up nitrogen from below 150 cm in low nitrogen scenarios (Claassen & Marler, 1998; Entz et al., 2001), so soil available N below the sampling depth may have also been taken up by IWG in the first spring. In Minnesota, grain yields were similar among fertilizer treatments, but lower when unfertilized. In year 1, native soil N may have met IWG's nitrogen requirement, as discussed, but this is somewhat surprising for years 2 and 3 since the fertilizer rate of the manure addition level was determined based on an estimated mineralization rate of the organic N in the manure and IWG's annual N fertilization best practices. The actual outcome of organic N mineralization and total available N throughout the growing season can vary substantially (Eghball et al., 2002). Under relatively dry years like those experienced in this study, one could expect to see lower actual first-year available N than estimated, so it could be that the N level added was less than planned. MN showed a decline in yields over the course of the study with a 12 % drop in grain yield after the first year, and showing a greater decline of 85 % between 2021 and 2022. Grain yields did respond positively to both manure and commercial fertilizer addition during certain site-year combinations. In 2022, the positive effect of N fertilizer on grain yield became even more pronounced with commercial fertilized plots being 235 % higher yielding than the control. Kansas exhibited negative overall trends in grain yields as well, falling 69 % in 2021 and experiencing yield declines sooner than Minnesota. Additionally, a 48 % yield decrease from control treatment yields compared to fertilized treatments was observed. Declining yields over time is a trait in IWG that is consistent across all growing regions and is exacerbated by nitrogen deficiency (Jungers et al., 2017, Fernandez et al., 2020; Hunter et al., 2020; Lanker et al., 2020; Law et al., 2022). In Minnesota, soil extractable N was lower in all seasons in 2021 compared to 2020. This suggests that nitrogen limitation through time may be contributing to yield decline, and this is further supported by the greater reduction in grain and straw yields in the control compared to the fertilized treatments in this study. Sprunger et al. (2018) suggested there was a greater N requirement for IWG in subsequent years after seeding to supply total plant biomass, including a larger root system. Greater N uptake in root tissue could explain the lower yields over time compared to the first year (Sainju et al., 2017). Precipitation preceding grain harvest may have also contributed to differences in grain yield across years. During 2021, both locations experienced precipitation deficits of 31 % in the two months prior to grain harvest. These months are critical for grain development and a lack of moisture likely affected grain yield.

Straw yields followed similar trends to grain yields in response to fertilizer treatments where no effects were seen in 2020 and positive effects of fertilizer compared to the control were seen in 2021 and 2022. This suggests that IWG roughly allocates N fertilizer to reproductive and vegetative tissues similarly, which is akin to tissue allocation patterns observed in Dobbratz et al. (2023). Although the fall defoliation treatment did not affect grain, it did affect straw yields. The effects of defoliation differed by fertilization treatment in MN in 2021 where straw yields increased with defoliation when fertilized with commercial fertilizer, but defoliation had no effect in the control and manure treatments (Figure 2). A possible explanation of higher straw yields from commercially fertilized treatments could be that a greater proportion of the commercial fertilizer is available early in the growing season compared to the manure, thus more available N may have been taken up by the IWG while the plants were allocating N to stem and leaf growth, as opposed to N from manure made available through mineralization, thus becoming available later in the growing season when vegetative growth is largely complete (Crews & Peoples, 2004; Crews & Peoples, 2005, Fagnant et al., 2023).The removal of biomass may have also decreased the C:N ratio in the treatment, which would have reduced competition for N from microbes and allowed IWG to better take up commercial N (McSwiney et al., 2010).

Another explanation for the interaction between defoliation and commercial N addition in 2021 and increased yield in defoliated treatments in 2022 is the increase of light penetration. Removal of detritus may have increased infrared light penetration below the canopy of the plant (Deregibus 1983). The ability of infrared light to be less obstructed by foliage and reach the crown in the defoliated plots in combination with nitrogen availability may have stimulated more biomass through tillering (Pinto et al., 2021). Increased rubisco activity as well as water conservation from diminished foliage could also be contributing to the higher yields (Harrison et al., 2010).

Forage yields are determined from vegetative regrowth following grain harvest and have been proposed as an important source of secondary economic revenue for Kernza producers (Hunter et al., 2020b). In the first year in both MN and KS, forage yields were statistically the same across fertilizer treatments. Differing from trends in the other response variables, however, the manure treatment yielded more fall forage than the commercial fertilizer treatment in 2021. The difference between the two treatments may be due to a gradual accrual of extractable nitrogen over the season from manure decomposition rather than the immediately available, and short-lived accessibility of nitrogen provided by the commercial fertilizer (Diekmann et al., 1993; Esteller et al., 2009). Although our data on soil N dynamics do not consistently support this across locations, we did see an almost two-fold greater N mineralization rate in the manure treatment compared to the commercial treatment at MN in 2021 (Table 7, Figure 5).

Grain, straw, and fall forage yields were in the range of previously reported values in MN and KS and support the idea that IWG can be managed as an economically viable dual-use crop. Typical grain yields from MN can range between 500 and 1000 kg ha^{-1}, with MN Clearwater averaging 696 kg ha^{-1} in a multi-site variety trial (Bajgain et al., 2020). In this study, MN first- and second-year fertilized yields were above average compared to previous studies (Favre et al., 2019). Kansas yields are typically lower than locations in the upper Midwest (Tautges et al., 2018; Crews et al., 2022), but have been shown to achieve yields in the 400-500 kg ha^{-1} range during the 1st growing season. KS grain yields were relatively high in the first year but lower in years two and three. Straw and forage yields were lower than reported in previous studies (Hunter et al., 2020b) but did show positive responses to nitrogen. Unlike grain yield, straw and forage yields were stable and did not decline through time. This could lend itself to transitioning primarily to a forage crop after peak grain harvests have been achieved (Puka-Beals et al., 2022). Considering a crop as dual use in time is a potential way to reap the highest production value from each system without impacting the yield of the other. Forage and straw RFV and CP were consistent with previous research (Hunter et al., 2020b; Favre et al., 2019), which supports the dual-use potential.

Protein content of forage is an important component of feed for livestock. Forage with protein content below 7% is considered low quality feed, but as protein content goes up, so does digestibility of dry matter by cattle (Mathis et al., 2007). Higher protein feeds are important to the rumen of growing beef and lactating dairy cattle (Poppi & McLennan, 1995; Satter & Roffler, 1975). Protein content in the manure-fertilized fall forage was greater than the control and commercial fertilizer treatments at both sites in 2021, and averaged 9.5 and 11.2 % at MN and KS, respectively. Fall forage protein content can be positively correlated with soil N availability in perennial forage grasses (Johnson et al., 2001). We found that soil extractable N was lower during the summer and into the fall in 2021, suggesting a possible lack of nutrients during fall vegetative growth (Figure 6). Although not statistically significant, at MN the mean extractable N and N mineralization was higher in the manure compared to the control in all seasons and years. High variability around the means may have limited statistical power to make definitive conclusions about N availability and cycling when comparing treatments, but perhaps this slight difference could have influenced the protein content of fall forage. We also saw a slight increase in straw biomass protein in the manure fertilized plots, although grain harvest indicated there was sufficient N to yield similar harvests from both manure and commercial fertilized treatments. Differences in yield and protein contents among fertilizer types may have been related to their chemical composition. While $NaNO_3$ only contains the NO_3^- ion to contribute to plant growth, manure contains both NO_3^- and NH_4^+. NH_4^+ is more energetically favorable for plant uptake and is converted primarily to amino acids i.e. protein (Engels & Marschner, 1995; Liu et al., 2016; Wang & Macko, 2011). This could explain the higher protein content of the forage and straw from the manure treatments. Manure may also contain additional micro- and macronutrients that are released and incorporated that were not available from the commercial fertilizer like phosphorus, potassium, and sulfur (Eghball et al., 2002; Saviozzi et al., 2006).

Relative feed value (RFV) is a composite metric of nutritive value that incorporates digestibility. It is calculated using acid detergent Fiber (ADF) and neutral detergent fiber (NDF), which is composed of several plant structural component measurements gathered from cellulose, lignin, hemicellulose, and carbohydrates (Undersander et al., 2002). We found that RFV was greater in the manure treatments compared to the control and fertilizer treatments. Since crude protein is not considered in the RFV calculation, there is an indication that the manure treatment affected forage tissue chemistry in other ways not directly related to protein. Hemicellulose, a main component of NDF determination, is used to calculate % dry matter intact (DDI), which is then used to calculate RFV. As hemicellulose concentration goes down, i.e. lower NDF, RFV increases. N availability to the plant has been shown to negatively correlate with the amount of hemicellulose produced by the plant (Liu et al., 2016), so as the plant has N available through manure, hemicellulose decreases resulting in a lower NDF which is reflective of a higher quality feed and increased digestibility.

We saw similar effects of increased forage yield and nutritive value after IWG fertilization as have been seen with fertilized winter wheat, another dual-use crop (Sij et al., 2011). As a dual use crop, IWG is different from winter wheat because it can provide forage over multiple seasons without replanting, increasing the amount of feed available to livestock while decreasing costs of production through reduced soil preparation and replanting costs, and higher net return from the combination of hay and grain than winter wheat (Law et al., 2022). In addition to costs of planting winter wheat, there are other environmental costs of annual production. The potential environmental costs of wheat's low nitrogen use efficiency compared to IWG (Sprunger et al., 2018) are associated with annual grains losing nitrogen to leaching, leading to inefficient N use and potential water contamination (Reilly et al., 2022). Unlike wheat, IWG will continue taking up available nitrogen in the soil with its extensive root system, reducing the "leakiness" of fertilizer application and improving nitrogen use efficiency. Livestock producers will easily be able to incorporate this system into their operation as there is little difference in grazing strategy compared with dual-use wheat. This perennial system will be an asset to farmers across many aspects of the farm, both environmental and economic.

Nitrogen mineralization

Nitrogen mineralization by microbes in the soil increases the available soil N pool, potentially for plant utilization (Schimel & Bennett, 2004). The ability of microbes to mineralize nitrogen can depend on climate, moisture, plant matter type, and stage of decomposition affecting plant growth and uptake (Habibur Rahman et al., 2013; Du et al., 2020). Spring mineralization and extractable nitrogen across treatments at both sites during the spring of 2020 were minimal with little difference between treatments (Figure 5, Figure 6). Microbial activity is heavily influenced by moisture availability and temperature, which in moisture's absence or lower seasonal temperatures, can slow microbial activity (Gomez et al., 2020). Minnesota had no precipitation in April, and nearly half of the normal precipitation in May. Weather variation likely contributed to low mineralization in the subsequent sampling. Because of decreased rainfall, mineralization remained statistically zero during much of the season in MN and exhibited immobilization in the summer. Kansas also exhibited low nitrogen mineralization throughout the season, only showing significant mineralization during the summer of 2020 in fertilized treatments (Figure 5). A potential influence on the mineralization measurement could be the loss of available N from denitrification which can be a problem during *in-situ* incubation methods. Microbial breakdown of NO_3 from disturbed soils leading to N_2 release can result in an underestimation of nitrogen mineralization (Hatch et al., 1998; Kastl et al., 2015). By monitoring the extractable N across the season, we saw that extractable nitrogen pools increased from spring into summer before decreasing into the fall (Figure 6). With Minnesota demonstrating higher yields, protein, and forage nutritive value in the manure fertilized forage harvest compared to the unfertilized control treatments, the lack of net mineralization rates suggests that there may have been methodological factors influencing the accounting of N. Differences between mineralization and extractable N results could justify the assumption that denitrification within the sampling tubes was occurring, leading to an under accounting of nitrogen mineralization.

In the spring of 2021, an increase was observed in mineralization in Minnesota, but not in Kansas. Du et al. (2020) showed that the stage of biomass decomposition had an increased effect on nitrogen release, and that alfalfa had a delayed release of nitrogen starting 6-12 months after primary decomposition is initiated. This may explain the increase in mineralization in MN and not in KS. Abundant moisture from spring rains in both sights ensured moisture was available for microbial decomposition, benefitting their ability to release nutrients from organic matter in the soil (Sierra, 1997). This led to mineralization during the summer, but little difference between treatments. In the fall, mineralization fell to zero statistically in both locations. Fall is generally a time of relatively low precipitation in Kansas but was also considerably drier than average in MN because of drought. As a result, extractable N was low at both sites in the fall of the second year.

Soil gas emissions

Soil gas emissions from IWG and its accompanying fertilizers are of particular interest when considering perennial agriculture's potential ability to mitigate global climate change. IWG has been shown to be a net carbon sink, but it has also demonstrated losses of carbon through respiration (de Oliveira et al., 2018). Additionally, manure respiration can result in twice the soil respiratory CO_2 compared to unfertilized control treatments (Rochette & Gregorich, 1998). In line with this previous research, annual CO_2 emissions were nearly doubled in the manure treatment compared with the control in MN in 2021. With the addition of C available from the decomposing biomass present in the manure, it may have provided nutrients necessary for increased microbial activity, thus CO_2 respiration (Li et al., 2019, Shakoor et al., 2021, Ding et al., 2007b). Kansas trended toward an increase in CO_2 flux in the manured treatments compared to the control treatments, although the P-value was not significant ($p = 0.054$). With an increase in soil CO_2 emissions from manure, this finding must be considered in terms of its contribution to global CO_2 concentration in the atmosphere. Manure application can result in both positive and negative impacts. For example, application of manure has been shown to build soil organic carbon and provide phosphorus and potassium in addition to nitrogen, an objectively positive impact (Das et al., 2023; Gerzabek et al., 1997; Dao & Cavigelli, 2003).

There were some caveats to our measurements that may have influenced the accounting of gas emissions. Manure is highly variable both in spatial distribution and composition which could lead to variation in gas flux between plots and biased accounting of soil gas emissions (Aguirre-Villegas et al., 2018). There is research to suggest that with the addition of nitrogen, root respiration will increase CO_2 respiration of the plant roots (Zhang et al., 2014). Contrary to this research, sodium nitrate supplied in MN did not significantly increase respiration compared to the control, even though there was increased plant productivity as demonstrated by the grain and straw yields. In this case, labile carbon and nutrients found in the manure may have stimulated microbial growth while the nitrogen provided by the inorganic fertilizer source did not (Curiel Yuste et al., 2007).

Precipitation events also created a unique methodological challenge for this study because of moisture's increased effect on microbial respiration in combination with the timing of flux measurements. Measurements of CO_2 before rain events could have resulted in an under estimation of emissions due to suppressed microbial activities in drier environmental conditions. In contrast, taking measurements after rainfall events may have biased the accuracy toward an over estimation of CO_2 flux due to ideal moisture conditions for microbial activity (Xu et al., 2004). In many cases, measurements were taken after precipitation events to ensure the capture of microbial respiration, possibly skewing measurements toward over estimation of CO_2. Although post precipitation measurements were favored, Minnesota experienced two years of drought based on the 30-year precipitation average, which likely reduced respiration rates comparatively. Kansas also experienced lower precipitation rates in the fall of 2021, which may have reduced the microbial activity during the latter part of that year. Gas measured in this way cannot fully describe gas emissions during a season because the atmosphere requires constant measurement to capture the flux of a dynamic system (Levy et al., 2017). Therefore, gas flux may have been affected by collection methods resulting in biased estimates toward an over or underestimation of the actual gas flux. In future research, bridging the measurement gaps will be important for giving a more accurate portrayal of soil gas emissions.

Seasonal emissions of NH_3, N_2O, and CH_4 were not significantly different from the control. However, some measurement considerations should be considered in the future. Two-thirds of NH_3 has been shown to volatilize within the first 10 hours after manure fertilizer application (Gordon et al., 2001). Measurements within this timeframe were not achieved within this study. Additionally, application of partially composted manure, which has had time to release volatile compounds and break down into more stable forms, may have contributed to the lower measurements of NH_3 (S. Zmora-Nahum, 2018). To calculate NH_3 emissions accurately, gas measurements should be taken directly after manure application and frequently during the first few days after application to account for volatilization. Sporadic or reduced precipitation may have contributed to varying N_2O fluxes, especially in MN. N_2O accounting should take into consideration soil moisture, since N_2O is highly influenced by moisture exhibiting higher emissions when soil has greater than 70% water filled pore space (Ruser et al., 2006). With sufficient moisture from rainfall and warmer temperatures, large pulses of N_2O can be emitted into the atmosphere (Zheng et al., 2000). In either site, variation in gas fluxes cannot be fully accounted for because measurements were not continuously taken (Levy et al., 2017). Finally, CH_4 is typically produced in anaerobic environments including in livestock rumen through enteric fermentation (Moss et al., 2000). In aerobic environments, methanogens are less likely to produce CH_4 because of rapid aeration of manure applied on the soil surface (Phan et al., 2012). This may be an explanation for the similarities between the control and fertilized treatments for methane.

Soil Carbon

The balance between C accumulation and losses are important when considering the impact of agriculture. In MN, soil C was higher in the manure treatment compared with the control or commercial fertilization treatments in 2022, the third and final treatment year. The carbon content of manure was not directly measured in this study but can range from 155 to 251 g kg^{-1} for air dried dairy and beef cattle manure (Hartz et al., 2000). As the manure decomposes, up to 52 % of the C in applied manure can be lost through respiration, however some is still converted into soil organic matter (Hao et al., 2004). For example, manure application has increased SOC as much as 35 % in conventional tillage cropping systems when fertilized with livestock manure in long term studies (Gross and Glaser, 2021). Observations of increased SOC in response to annual manure fertilization have been shown to increase linearly over time, and eventually reach a stabilized state where C content remains relatively constant (Das et al., 2023; Gerzabek et al., 1997). In our study we saw a 29 % respiratory loss of C from soil CO_2 in manure treatments. Additionally, we saw an increase in total soil carbon (2.25 %) in manure compared to the control (1.92 %) and commercial (1.90 %) fertilizer treatments. This is important when considering carbon as a part of soil aggregation, a main driver of nutrient retention (Jiao et al., 2006). The amount of carbon entering or leaving the system as well as the degree of recalcitrance of the organic matter containing the C (i.e. particle size and amount of lignin in the OM) can affect the quantity of C that stays in the soil as soil organic matter, straw being a readily decomposable C source. In MN, manure was applied at a rate of 7.2 Mg ha^{-1} dry matter to supply our desired rate of application of 88 kg N ha^{-1}, which was based on the expected amount of first year available N in dairy cattle manure. After 29 % of the manure carbon was respired, the other 71 % of remaining carbon, or 0.79 Mg C $ha^{-1}yr^{-1}$, was available annually to build the soil organic carbon pool. Over three years, estimated remaining C from manure was 2.38 Mg C ha^{-1}, which is very close to the estimated amount of carbon gain we observed from soil tests, 2.77 Mg C ha^{-1}. Carbon accumulation takes years to see appreciable increases and can vary depending on the amount applied, variations in manure carbon content between species and within species i.e., dairy versus beef cattle, moisture content of the manure, as well as method of application. Nevertheless, we observed soil C accumulation with manure application.

Farm Scale

All results must be put into the context of how implementation would occur at the farm scale. When considering fertilization of manure at this scale, many more aspects of the farm are taken into consideration including economics, resource availability, and the environment to name a few. Considering the nitrogen availability in manure required to fertilize an entire hectare of IWG at the rate recommended to achieve similar grain and biomass yields, ~56.2 Mg ha^{-1} (25.1 tons $acre^{-1}$) would have to be applied to achieve the nitrogen quantity sufficient to raise equivalent yields. Finding access to large quantities of manure could be difficult depending on the location of the farm and could result in unintended consequences like over-application of phosphorus (Kleinman & Sharpley, 2003). It is best to consider the resources, feasibility of application, equipment, and the capacity of the environment to cycle the applied nutrients, to match the potential outcomes for the farmer when considering manure application as a fertilizer source.

Areas for future research

One important aspect to address when considering future research is moving from a simulated grazing system to a live grazing approach. Several studies have measured simulated grazing impact on agronomic variables, but few incorporate live grazing. This would be an improvement in the accuracy of a grazing study because it would introduce simultaneous fertilization and animal impact, both key components of stand management in a dual-use system.

Another finding from this study was that fertilizer type was shown to impact plant productivity in many of the agronomic variables. One variable that stood out was the difference between forage quality of the manure fertilizer treatment compared to the control in MN. Future research should pursue the study of other nitrogen fertilizer sources including but not limited to commercial inorganic, multispecies manure, biological nitrogen fixation, and enhanced efficiency fertilizers (EEF) that protect nitrogen from rapid release (Ransom et al., 2020). Slow-release nitrogen over time may lead to improved fertilization of IWG being utilized for both grain and animal feed by contributing to forage quality later in the season. It was hypothesized that the gradual breakdown of manure and release of nitrogen over time may align better with the synchrony of the plant's physiological requirements; synchrony and physiological requirements referring to the nutrient availability to the plant during key production stages i.e., grain and leaf biomass growth (Jungers et al., 2018). Fertilizers all have their unique composition and solubilities that may lend themselves to immediate use by the plants, or gradual breakdown and release overtime. Knowing which fertilizers best fit in a dual-use system and improve nitrogen use efficiency will be beneficial for future management.

Conclusion

Commercial inorganic and manure fertilizer treatments resulted in similar grain and biomass yields when applied at similar available nitrogen rates. Manure provided an additional benefit of higher RFV and protein content, which could be a result of the higher nitrogen mineralization and soil extractable N levels measured over the growing seasons. Microbial activity associated with additional nitrogen mineralization doubled CO_2 emissions in manured fertilized treatments and are important to consider when deciding to apply manure or a commercial inorganic fertilizer. However, soil C increases from manure relative to commercially fertilized and unfertilized IWG may offset the increase in emissions. Producers should evaluate the impact of these fertilizer applications at field scale to determine whether they are appropriate for their operations based on resource availability and impact to their specific environments, as well as their impact globally.

Table 1. Fertilizer characterization. Fertilizers [a] were characterized before application for total Kjedahl Nitrogen (%), Organic N (kg ton^{-1}), Inorganic N (kg ton^{-1}), 1st year available (kg ton^{-1}), and rate of application (kg N ha^{-1}).

Fertilizer Type	Location	Year	Date applied	TKN	Organic N	Inorganic N	1st yr Available N	Rate applied
				%		(kg Mg^{-1})		(kg N ha^{-1})
Manure (Dairy cattle)	MN	2020	22-May	0.79	6.43	0.09	1.23	60
		2021	06-Apr	0.55	4.15	0.37	0.91	88
		2022	19-Apr	0.62	4.61	0.48	0.82	88
Commercial	MN	2020	19-May	0.75	NA	6.17	6.17	70
		2021	22-Apr	0.75	NA	6.17	6.17	70
		2022	19-Apr	0.75	NA	6.17	6.17	70
Manure (Beef cattle)	KS	2020	07-Apr	0.63	3.95	1.15	2.39	70
		2021	22-Apr	0.78	2.47	0.62	3.09	112
Composted Manure		2022[a]	16-Mar	NA	8.23	NA	NA	112

a. Kansas manure in 2022 came from a different source than previous years and was composted. Manure was applied based on the advertised organic N content and applied according to the rates previously established in the treatments.

Table 2a. Average monthly precipitation (inches). Precipitation by month and year from MN and KS in 2020, 2021, and 2022 referenced to their respective 30 years averages (https://www.ncei.noaa.gov).

Location	Year	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
MN	30 yr avg	34.12	0.96	0.90	1.80	3.10	4.32	4.88	4.51	4.43	3.44	2.86	1.71	1.21
	2020	27.27	2.30	0.60	1.36	0.00	2.25	8.97	2.27	3.98	1.10	3.01	0.95	0.48
	2021	23.85	0.82	0.33	2.37	2.04	2.73	1.57	1.39	4.58	3.00	2.33	1.02	1.67
	2022	24.85	0.59	0.48	2.65	3.16	4.22	0.88	2.32	7.17	0.43	0.17	1.32	1.46
KS	30 yr avg	29.69	0.71	0.87	1.82	2.72	5.04	3.75	3.92	3.71	2.65	2.16	1.22	1.12
	2020	26.77	0.98	1.39	1.12	1.33	4.73	3.62	7.62	0.68	1.81	0.12	2.51	0.86
	2021	26.11	0.99	0.18	2.94	2.61	6.62	1.01	1.44	4.07	4.07	2.10	0.00	0.08
	2022	27.28	0.37	0.17	1.79	0.64	8.72	3.74	4.25	1.55	3.49	0.47	1.16	0.93

Table 2b. Monthly maximum and minimum temperature (F°). Temperatures (C°) by month and year from MN and KS in 2020, 2021, and 2022 referenced to their respective 30 years averages (https://www.ncei.noaa.gov).

Location		Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
MN	T max	30 yr avg	25.9	28.3	40.4	64.5	66.0	81.3	83.9	81.0	69.2	50.2	46.9	32.1
		2020	26.6	17.1	48.1	54.7	67.9	85.8	84.0	82.6	75.5	64.0	43.8	30.8
		2021	18.1	22.2	37.6	47.1	69.1	81.3	83.5	79.4	74.1	61.0	42.1	21.2
		2022	22.2	27.0	39.9	55.8	67.8	77.3	81.3	79.0	72.2	58.6	41.5	27.7
	T min	30 yr avg	10.5	5.2	25.1	40.6	47.1	58.8	63.2	59.5	48.1	31.5	27.2	14.4
		2020	12.4	0.2	28.7	35.0	47.0	61.5	60.8	59.6	49.6	43.1	25.5	12.7
		2021	-4.9	-0.3	21.1	30.2	48.3	59.3	62.7	59.0	50.9	36.8	25.4	8.4
		2022	3.3	6.9	20.4	33.7	46.6	57.1	60.9	58.4	50.1	36.9	23.4	10.8
KS	T max	30 yr avg	43.3	47.4	59.3	68.2	72.9	92.2	90.2	89.9	81.1	66.5	62.6	49.4
		2020	45.7	36.8	62.9	66.3	72.5	91.2	92.3	93.1	86.8	70.2	60.2	55.6
		2021	44.9	48.1	58.4	69.7	77.8	89.0	93.6	95.1	87.4	74.0	54.1	42.0
		2022	41.4	46.3	57.3	66.9	76.8	88.1	92.8	90.1	81.9	69.1	55.1	43.2
	T min	30 yr avg	23.6	23.9	37.3	39.1	72.9	67.1	70.3	65.0	54.3	4.0	34.3	23.9
		2020	23.9	14.1	36.5	39.1	53.5	66.2	67.5	69.4	61.3	41.0	34.3	25.1
		2021	16.5	16.8	30.1	41.2	56.5	66.1	69.5	67.7	59.0	43.0	30.0	19.6
		2022	20.1	23.6	33.2	42.3	53.4	64.2	69.1	67.1	58.2	44.9	32.1	22.6

Table 3. Statistical results of IWG agronomic yield measurements. P-values were generated from mixed effects models based on a split-plot complete randomized block design with defoliation as the split plot treatment. Bold numbers with an asterisk indicate P values less than 0.05.

		Grain			Straw			Straw RFV		
Site	Treatment	2020	2021	2022	2020	2021	2022	2020	2021	2022
					kg ha^{-1}				unitless	
MN	Fertilizer	0.411	**0.011***	**<0.001***	0.188	**0.012***	**<0.001***	0.392	**0.003***	**<0.001***
	Defoliation	NA	0.231	0.454	NA	0.335	0.692	NA	**<0.001***	0.394
	Fert×Def	NA	0.203	0.473	NA	**0.040***	0.384	NA	0.871	0.205
KS	Fertilizer	0.716	**0.023***	**<0.001***	0.754	**0.043***	0.607	0.562	0.294	NA
	Defoliation	NA	NA	0.408	NA	NA	**0.037***	NA	NA	NA
	Fert×Def	NA	NA	0.693	NA	NA	0.207	NA	NA	NA

Table 4. Statistical results of treatments on forage yield, forage RFV, and forage % protein. P-values were generated from mixed effects models based on a randomized block design. Bold numbers with an asterisk indicate P values less than 0.05.

		Forage Yield		RFV		Protein	
Site	Treatment	2020	2021	2020	2021	2020	2021
MN	Fertilizer	0.465	**0.022***	0.633	0.002	0.563	**<0.001***
KS	Fertilizer	0.535	0.489	0.826	0.043	0.638	**0.020***

Table 5. Statistical results of treatments on soil gas emissions. P-values were generated from mixed effects models based on a randomized block design with split plots for defoliation treatments. Bold numbers with an asterisk indicate P values less than 0.05.

		CO_2		CH_4		N_2O		NH_3	
	Treatment	2020	2021	2020	2021	2020	2021	2020	2021
MN	Fertilizer	0.848	**0.002***	0.457	0.391	0.609	0.274	0.611	0.213
	Defoliation	NA	0.685	NA	0.120	NA	0.093	NA	0.498
	Fert×Def	NA	0.221	NA	0.230	NA	0.460	NA	0.329
KS	Fertilizer	**0.054***	0.480	0.245	0.521	0.602	0.144	0.195	0.254
	Defoliation	NA	NA	NA	NA	NA	NA	NA	NA
	Fert×Def	NA	NA	NA	NA	NA	NA	NA	NA

Table 6. Mean CO_2 flux. Presented are the means of control, manure, and commercial fertilizer treatments for CO_2 (kg ha^{-1} yr^{-1}) at both Minnesota and Kansas sites (2020-2021). Means were averaged across defoliation treatments in 2021 when defoliation occurred. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

	Units: kg ha^{-1} yr^{-1}	CO_2	
	Fertilizer Treatment	2020	2021
MN	Commercial	361±308	1381±194b
	Control	471±308	1029±194b
	Manure	611±308	2218±194a
KS	Control	1179±325	1082±159
	Manure	2345±325	1260±159

Table 7. Statistical results of treatments on nitrogen (N) mineralization and extractable N measurements. P-values were generated from mixed effects models based on a randomized block design. Bold numbers with an asterisk indicate P values less than 0.05.

Site	Treatment	N-Min		Extractable N	
		2020	2021	2020	2021
MN	Fertilizer Treatment	0.393	**0.015***	0.1290	**0.031***
	Season	0.053	**0.009***	**<0.001***	**<0.001***
	Fert×Season	0.726	0.686	0.708	**0.006***
KS	Fertilizer Treatment	0.115	0.720	0.834	0.548
	Season	**0.011***	**0.035***	0.571	**0.002***
	Fert×Season	**0.025***	0.554	0.669	0.092

Figure 1. Mean grain yield (kg ha^{-1}), straw yield (kg ha^{-1}), and straw relative feed value (RFV) by fertilizer treatment at Minnesota and Kansas sites in 2020-2022. Means were averaged across defoliation treatments in 2021 and 2022 when defoliation occurred. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of P <0.05, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

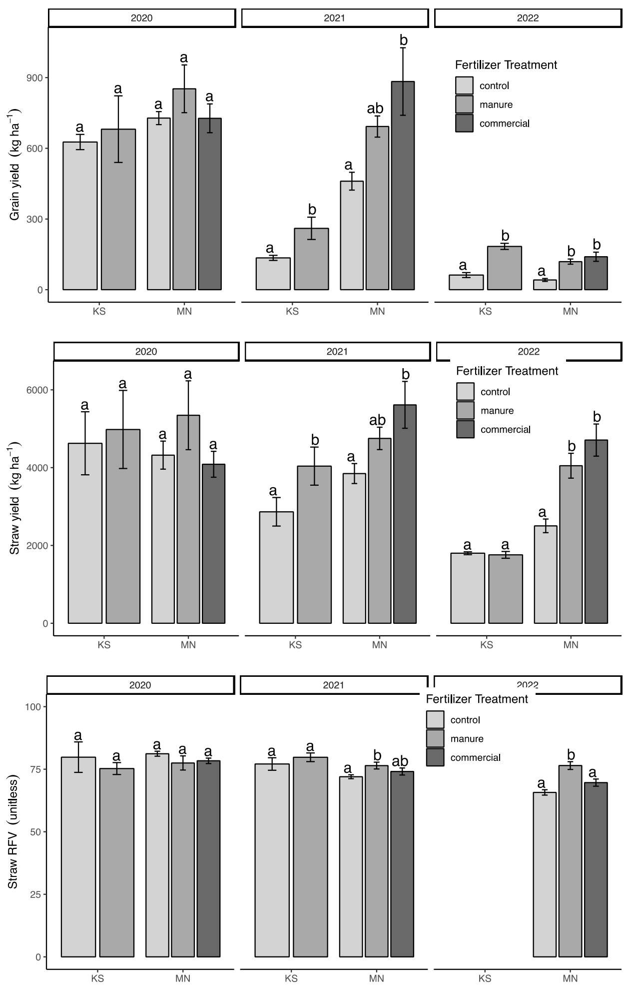


Figure 2. Mean straw yield (kg ha^{-1}) by fertilizer and defoliation treatments at Minnesota and Kansas. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

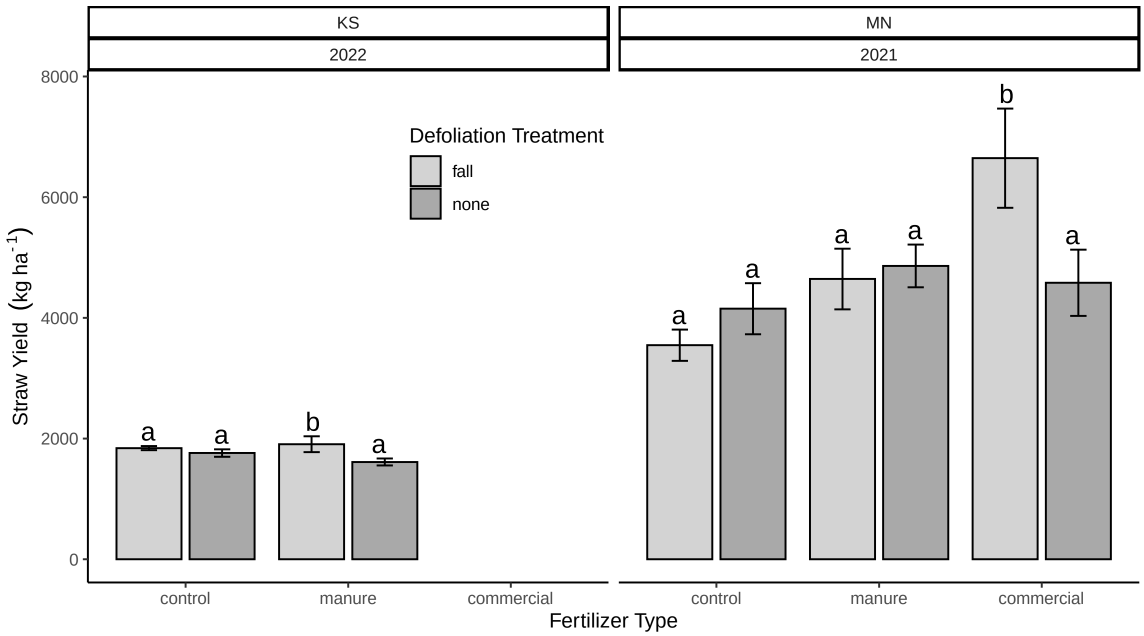


Figure 3. Mean forage relative feed value (RFV) by fertilizer type at Minnesota and Kansas in 2020 and 2021. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of P <0.05, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

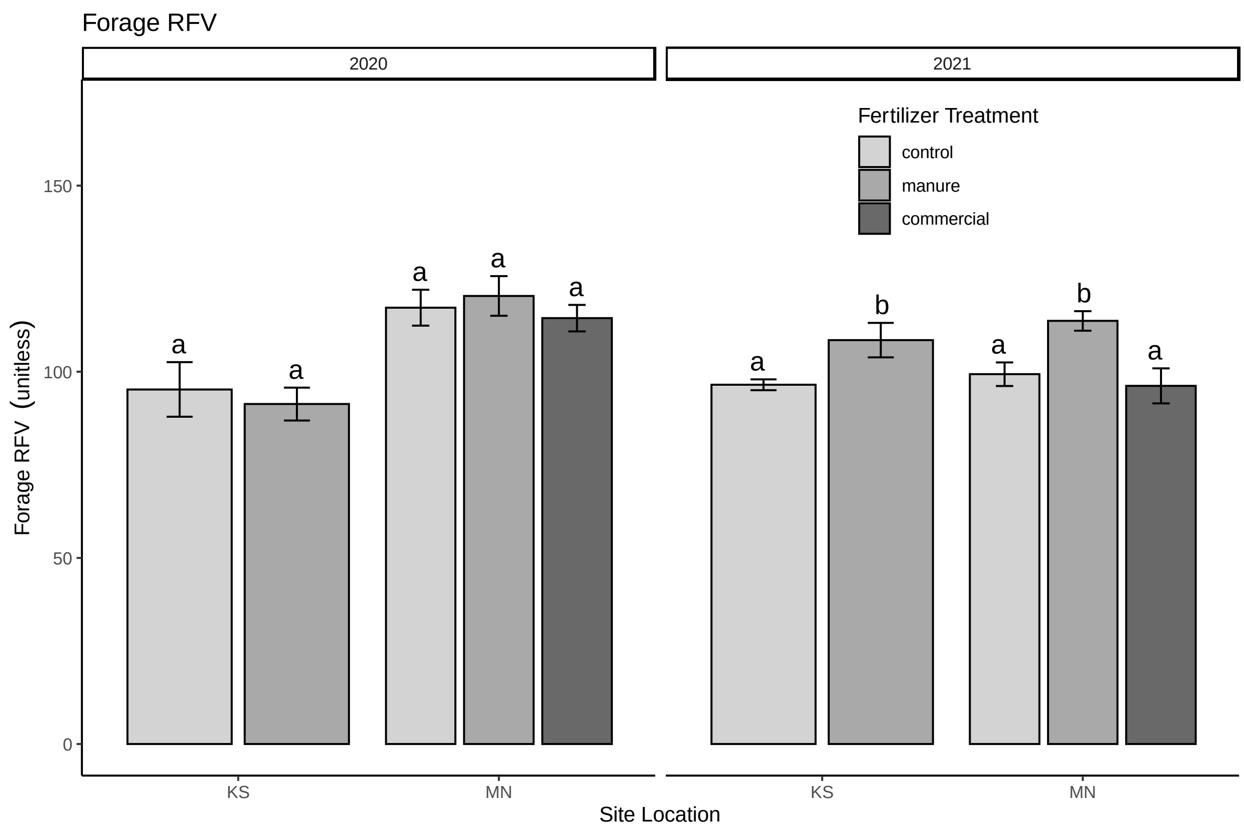


Figure 4 Mean forage protein (%) by fertilizer type at Minnesota and Kansas in 2020 and 2021. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of P <0.05, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

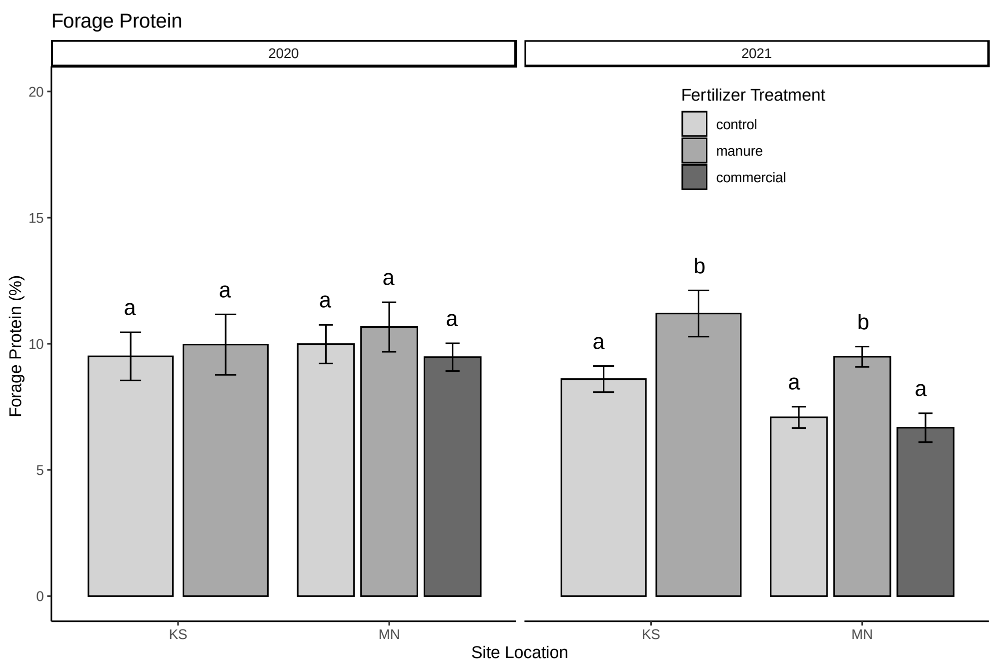


Figure 5. Mean nitrogen mineralization by fertilizer type and season at Minnesota and Kansas in 2020 an 2021. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

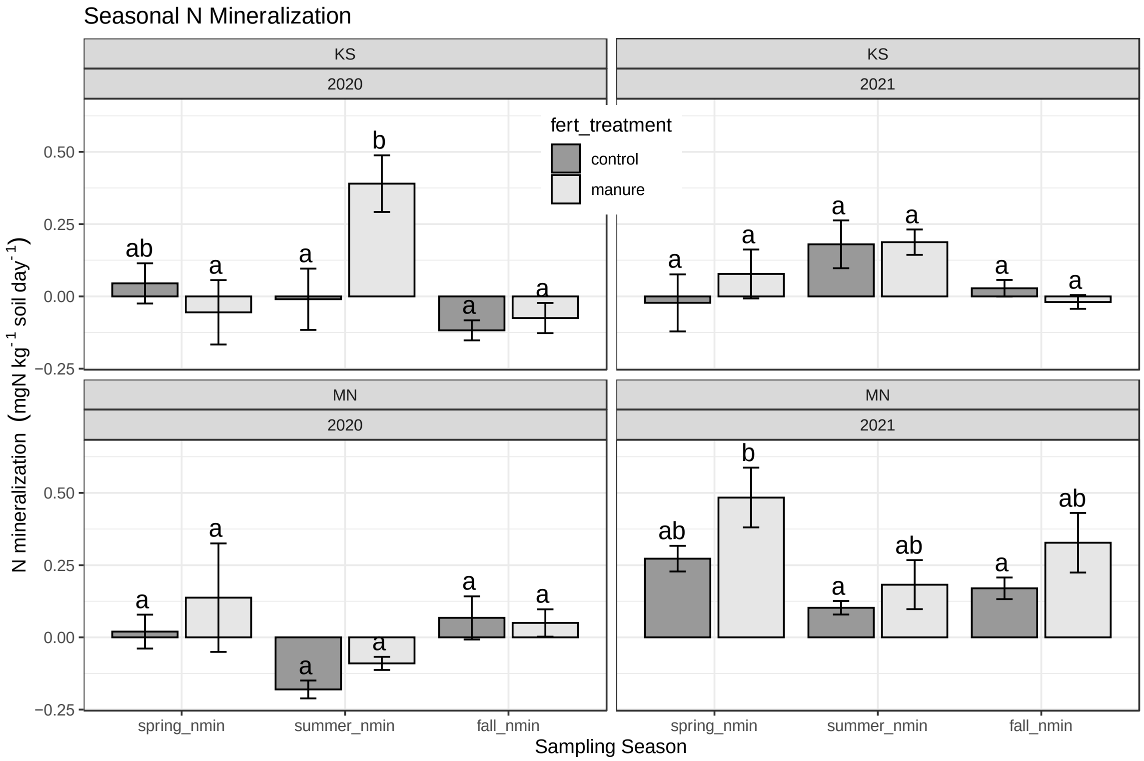
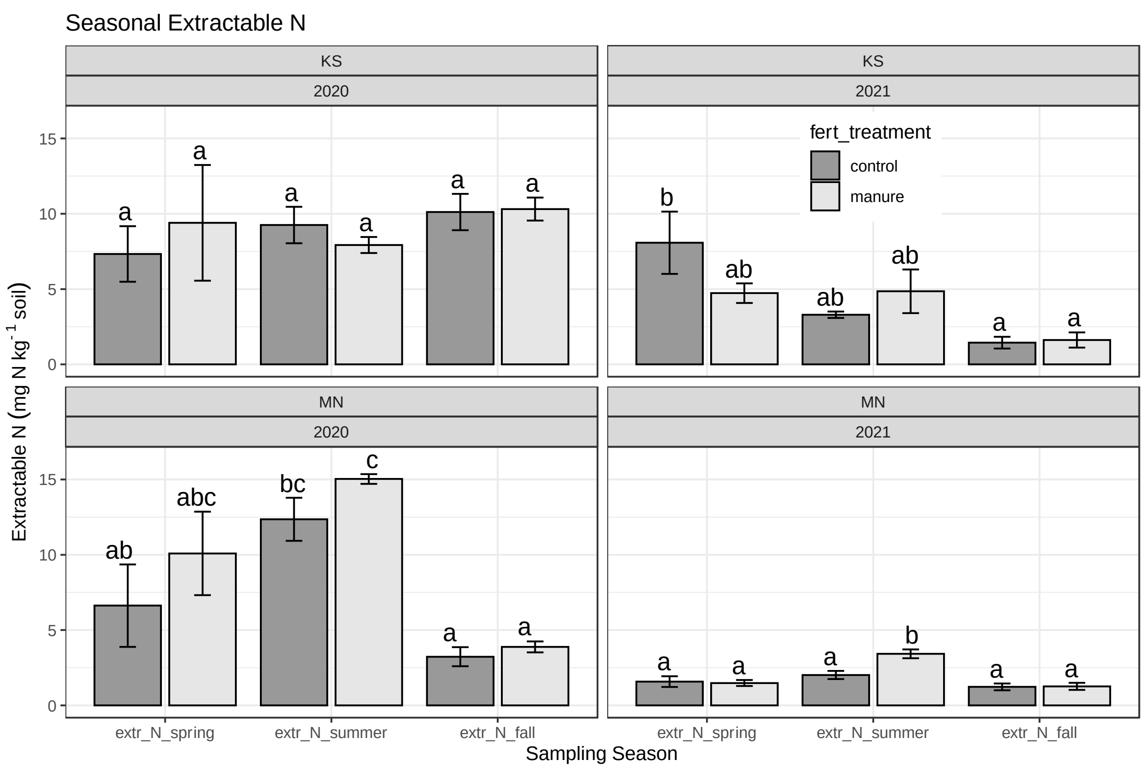


Figure 6 Mean soil extractable nitrogen by fertilizer type and season at Minnesota and Kansas in 2020 and 2021. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.



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SUPPLEMENTAL

Website Resources:

Weather

https://www.dnr.state.mn.us/climate/historical/select_data.html?sid=217107&sname=ROSEMOUNT%20RESEARCH%20AND%20OUTREACH%20CENTER&sdate=por&edate=por&temperature=true

Table S1 Mean comparison of agronomic yields.

Site	Treatment	Grain kgha^{-1}			Straw unitless			Straw RFV		
	Fertilizer	2020	2021	2022	2020	2021	2022	2020	2021	2022
MN	commercial	726 ±74.5	883±85.3a	140±14.9a	4081±494	5605±369a	4702±323a	81.2±1.84	74.1±1.08ab	58.5±1.34b
	control	727 ±74.5	461±85.3b	41±14.9b	4315±494	3843±369b	2500±323b	78.3±1.84	72.0±1.08b	53.6±1.34c
	manure	851 ±74.5	692±85.3ab	119±14.9a	5338±494	4744±369ab	4043±323a	77.5±1.84	76.4±1.08a	64.4±1.34a
KS	control	627±102	135±34.9b	104±13.2b	4626±911	2865±354b	1800±57	73.8±5.38	77.1±1.74	NA
	manure	681±102	260±34.9a	226±13.2a	4981±911	4039±354a	1758±57	75.2±5.38	79.9±1.74	NA
	Defoliation									
MN	none	NA	617±69.7	95±13.0	NA	4524±305	3824±264	NA	76.7±0.97a	71.3±1.09
	fall	NA	740±69.7	106±13.0	NA	4938±305	3673±264	NA	71.7±0.97b	69.9±1.09
KS	control	NA	NA	172±13.2	NA	NA	1685±57b	NA	NA	NA
	fall	NA	NA	158±13.2	NA	NA	1873±57a	NA	NA	NA

Presented are the means of control, manure, and commercial fertilizer treatments agronomic yields (kg ha^{-1}) at both Minnesota and Kansas sites (2020-2022). Means of defoliation treatments are also included (kg ha^{-1}). Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

Table S2 Mean comparison of forage RFV and protein

Site	Treatment	Mean Forage RFV and Protein			
		RFV		Protein	
		unitless		%	
	Fertilizer	2020	2021	2020	2021
MN	commercial	114±4.6	96±3.6b	9.47±0.9	6.67±0.7b
	control	117±4.6	99±3.6b	9.98±0.9	7.08±0.7b
	manure	120±4.6	113±3.6a	10.66±0.9	9.49±0.7a
KS	control	95±6.8	97±3.4b	9.50±0.8	8.60±0.5b
	manure	93±6.8	109±3.4a	10.30±0.8	11.20±0.5a

Presented are the means of control, manure, and commercial fertilizer treatments for forage RFV (unitless) and forage protein (%**)** at both Minnesota and Kansas sites (2020-2021). Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.

Table S3 Sampling dates for nitrogen (N) mineralization and extractable nitrogen (N)

	MN	Spring	Summer	Fall
2020	Initial	6/10/2020	7/21/2020	9/15/2020
	Final	7/8/2020	8/19/2020	10/16/2020
2021	Initial	5/24/2021	6/30/2021	8/18/2021
	Final	6/16/2021	8/4/2021	9/15/2021
	KS			
2020	Initial	5/10/2020	7/6/2020	8/28/2020
	Final	6/10/2020	8/4/2020	9/28/2020
2021	Initial	5/13/2021	7/6/2021	9/27/2021
	Final	6/9/2021	8/3/2021	10/19/2021

Initial and Final dates when 23 cm samples were removed from all treatments for nitrogen (N) mineralization and extractable nitrogen testing (N) Table S4. Mean nitrogen mineralization (mg N kg $^{-1}$ soil day^{-1}) and Extractable Nitrogen (mg N kg $^{-1}$ soil) across spring, summer, and fall sampling.

Table S4 Mean nitrogen mineralization and extractable nitrogen.

Year	2020			
Treatment	MN		KS	
Fertilizer	N Min	Extr N	N Min	Extr N
control	-0.031	7.40	-0.027	8.90
manure	0.032	9.67	0.087	9.21
Season				
spring	0.078	8.36b	0.184ab	8.36
summer	-0.135	13.69c	0.379b	8.59
fall	0.059	3.56a	0.093a	10.21

Year	2021			
Treatment	MN		KS	
Fertilizer	N Min	Extr N	N Min	Extr N
control	0.182a	1.61a	0.062	3.73
manure	0.331b	2.06b	0.082	4.27
Season				
spring	0.378b	1.53a	0.028ab	6.40c
summer	0.142a	2.72b	0.184b	4.08b
fall	0.249ab	1.25a	0.004a	1.53a

Presented are the means of control, manure, and commercial fertilizer treatments for N mineralization (mg N kg^{-1}soil day^{-1}) and extractable N (mg N kg^{-1} soil) at both Minnesota and Kansas sites (2020-2021). Means were averaged across fertilizer treatments in 2020-2021. Treatment means sharing the same letter within each site × year combination are not statistically different at a threshold of $P < 0.05$, based on mixed effects models analysis (see methods). Error bars represent 1 standard error from the mean.