Intermediate wheatgrass nitrogen dynamics: Nitrate leaching prevention and nitrogen supply via legume intercrops

Evelyn Reilly, Jacob M. Jungers[1*], Jessica L. Gutknecht[2], Craig C. Sheaffer[1]

[1] Department of Agronomy and Plant Genetics, University of Minnesota, , St. Paul, MN, USA

[2] Department of Soil Water, and Climate, University of Minnesota, , St. Paul, MN, USA

*** Correspondence:**
Jacob M. Jungers
junge037@umn.edu

Keywords: Intermediate wheatgrass$_1$, nitrate$_2$, groundwater contamination$_3$, nitrate leaching$_4$, perennial grain$_5$, Kernza, drinking water, water quality, (Min.5-Max. 8)

Abstract

Nitrate (NO_3^--N) leaching into groundwater as a result of high rates of nitrogen (N) fertilizer application to annual crops presents human health risks and high costs associated with water treatment. Leaching tends to be worse on certain soil types, such as sandy soils overlying porous bedrock, shallow aquifers, or steep hillslopes. Intermediate wheatgrass ([*Thinopyrum intermedium* (Host.) Barkw. & D.R. Dewey]; IWG) is a perennial grass that produces a novel grain, Kernza® and has the potential to reduce NO_3^--N leaching on sandy soils compared to common annual row crop rotations in the Upper Midwest. We compared grain yields, biomass yields, soil solution NO_3^--N concentration, soil NO_3^--N, soil water content, and root biomass under IWG and a conventionally managed corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] rotation for three years on a Verndale sandy loam in Central Minnesota. Mean soil solution NO_3^--N was 77 to 96% lower under IWG than the annual rotation. Total soil water content did not differ among cropping treatments. Root biomass was 82% lower under soybean than under IWG. IWG grain yields were 854, 434, and 222 kg ha^{-1} for Years 1-3 and vegetative biomass averaged 4.65 Mg ha^{-1} yr^{-1}. Annual crop grain yields were consistent with local averages. These results confirm that IWG effectively reduces soil solution NO_3^--N concentrations even on sandy soils, supporting its potential for broader adoption on vulnerable land.

Introduction

Water quality in the Upper Midwest is threatened by the intensive management practices used in annual cropping systems, including tillage and fertilizer application that leads to nutrient losses and water contamination through leaching and runoff (Randall and Mulla, 2001; Dinnes et al., 2002; Feyereisen et al., 2006; Erisman et al., 2013). While annual commodity crops like corn provide the potential for high economic return, nutrient losses cause eutrophication and hypoxia in surface waters and contamination of groundwater, which poses significant risks to human health (Ward et al., 2018, 2010; Brender et al., 2013). Impacts are often high where shallow aquifers and sandy soils make drinking water sources vulnerable to contamination. This leads to additional water treatment costs of over $5 million for some counties (Keeler et al., 2016). In Southeast Minnesota, for example, conversion of grassland to agriculture is expected to cause a 45% increase in private wells exceeding 10 ppm NO_3^--N, resulting in between $0.7 and 12 million in associated costs over a 20-year period (Keeler and Polasky, 2014). New alternative cropping systems that provide economic returns comparable to those of annual systems and which effectively reduce nutrient losses will be essential for protecting drinking water sources in the future.

Replacing annual crops with perennials has the potential to help reduce NO_3^--N leaching to groundwater and provide other ecosystem services (Asbjornsen et al., 2014; Ferchaud and Mary, 2016). Alternative cropping systems that include perennial grasses for conservation, forage, and biofuel production have lower NO_3^--N leaching loads than corn-soybean [*Glycine max* (L.) Merr.] systems largely because perennial grasses have greater root biomass that extends deeper into the soil, increasing N recovery and reducing leaching (Culman et al., 2013b; Pugesgaard et al., 2015; Ferchaud and Mary, 2016). Deep roots may be particularly important in reducing NO_3^--N leaching since they can expand the total volume of soil from which NO_3^--N can be taken up, and because NO_3^--N is highly mobile and more prone to leaching from deep soil horizons (Maeght et al., 2013). The contrast is striking. NO_3^--N losses in the subsurface drainage water for a corn-soybean system were about 37 times higher than from a Conservation Reserve Program (CRP) planting dominated by perennial grasses; a reduction that was attributed to the greater season-long evapotranspiration (ET) that resulted in less drainage and greater uptake and/or immobilization of N. In that study, average NO_3^--N concentrations in the water during the flow period were 24 mg/L for the corn-soybean rotation and 2 mg/L for the perennial grass CRP (Randall et al., 1997). Although plantings that include perennial grasses are effective at reducing NO_3^--N leaching, a lack of economic return has prevented their large-scale adoption in Midwestern agricultural landscapes.

Intermediate wheatgrass (IWG), [*Thinopyrum intermedium* (Host.) Barkw. & D.R. Dewey] is a perennial cool-season grass being domesticated to produce a grain referred to as Kernza ®, (DeHaan et al., 2018) with the first commercial variety, 'MN-Clearwater', released in 2020 (Bajgain et al., 2020). The crop has potential to provide economic return for producers (Hunter et al., 2020a, 2020b) while reducing NO_3^--N leaching compared to corn (Jungers et al., 2019). IWG initiates growth earlier in the season than warm-season forage and bioenergy grasses and is better able reduce NO_3^--N losses early in the season (Jungers et al., 2019) when they are typically the highest (Randall and Mulla, 2001; Crews and Peoples, 2005). Vegetative regrowth following IWG grain harvest helps reduce post-harvest nitrate losses and erosion.

One potential mechanism by which IWG can reduce NO_3^--N leaching compared to annual crops is related to water demand. Although total growing season ET and drainage was similar between IWG and corn, soil water content was lower under IWG compared to corn and switchgrass at 50 and 100 cm depths (Jungers et al., 2019), suggesting that soil moisture may be stored in other regions of the soil profile. Compared to annual wheat (*Triticum aestivum* L.), IWG had lower soil moisture up to a depth of 70-100 cm, which was associated with NO_3^--N leaching reductions of up to 86% (Culman et al., 2013b). The distribution of IWG root biomass and its effects on soil water content throughout the soil profile are largely unknown.

Reductions in NO_3^--N leaching beneath IWG compared to annual crops can also be related to differences in nitrogen fertilization regimes and associated losses of N in the form of soluble NO_3^--N in the soil water. Soil solution NO_3^--N increased from 0.1 to 0.3 mg L^{-1} when IWG was fertilized with 120 kg N ha^{-1} compared to an unfertilized control, yet this was still lower than the 24.0 mg L^{-1} measured beneath maize fertilized at 160 kg ha^{-1} (Jungers et al., 2019). Integrating legumes such as soybean into annual crop rotations can limit N fertilizing needs, yet the effects of legume crops in rotation on NO_3^--N leaching compared to IWG are unknown.

Our objective was to assess the potential of IWG grain production to reduce NO_3^--N leaching compared to an annual soybean-corn-soybean rotation on irrigated sandy soil by measuring soil solution NO_3^--N concentration and soil water content. We hypothesized that soil water NO_3^--N concentrations and soil water content would be lower under IWG, and that this would be related to increased root biomass and rooting depth of IWG compared to corn and soybean. Crop yields and vegetative biomass were measured to assess potential profitability.

Methods

Site Description

Field research was conducted from 2018-2020 at the Central Lakes Community College in Staples, MN, USA (lat. 46.38, long. -94.80). The soil type was a Verndale sandy loam (Typic Argiudoll). The soil contains 1-1.7% organic matter, is excessively well drained, and is considered low fertility potential (USGS Web Soil Survey). Climate data is reported in Table 1. Plots had previously been planted to a corn-soybean rotation followed by barley. From 0-30 cm, mean soil values were 1.7 mg L^{-1} NO_3^--N, 0.34 mg L^{-1} NH_4^+-N, 9.13 ppm P, 72.21 ppm K, and 6.73 kg ha^{-1}.

Table 1. Air temperature and precipitation in Staples during the growth period

Mean monthly air temperature (˚C)					Monthly and season total rainfall (cm)			
Month	2018	2019	2020	30-yr avg.	2018	2019	2020	30-yr avg.
April	1	5	4	7	NA*	5.2	5.1	5.9
May	17	11	12	13	4.2	8.0	2.0	8.4
June	20	18	20	19	12.9	10.9	11.4	11.3
July	21	21	22	21	10	6.2	13.1	9.2
Aug.	20	18	20	20	6.1	7.6	11.2	7.9
Sept.	15	16	13	15	4.6	15.2	3.8	7.3
Oct.	4	5	3	7	9	9.6	3.2	7.3
					46.8*	62.8	49.8	57.3

***Precipitation data not available for April 2018*

Experimental design

Treatments were applied in a randomized block design with two cropping systems replicated once in each of six blocks for a total of twelve plots. Plots were 4.11 by 9.14 m (13.5 by 30 ft.). The annual cropping system was a soybean-corn-soybean rotation. The perennial system was IWG. Soybeans were planted as the first phase of the soybean-corn rotation in May 2018; corn was planted as the second phase in May 2019, and soybean was planted again as the final year of the annual rotation in June 2020. Corn and soybeans were seeded in 75 cm rows at rates of 346,000 and 84,000 seeds ha^{-1}, respectively, with four rows per plot. The corn variety was Organic Viking O.84-95UP Seed Corn and the soybean was Organic MN0810CN.

An improved IWG- Kernza selection designated cycle 4 by Land Institute (Salina, KS) was seeded at a rate of 15 kg ha^{-1}. The IWG was seeded in 15-cm rows with 20 rows per plot on 20 August 2017. Intermediate wheatgrass was fertilized with urea at rates of 80, 100, and 100 kg N ha^{-1} in May 2018, 2019, and 2020 respectively. Urea was split-applied to corn at 140 and 80 kg N/ha in May and June 2019. Soybean was not fertilized. Weed pressure was low and when present, weeds were manually removed in all plots. Plots received 8.9 cm of irrigation between late June and late August in 2018 and 2019 and 7.6 cm between mid-June and mid-August in 2020.

Soil Fertility

Soil was sampled at four depth intervals (0-15, 15-30, 30-45, and 45-60 cm) in June 2019 and October 2020 and analyzed for organic matter, K, P, pH, and available N (NO_3^--N and NH_4^+-N). Samples were taken from eight cores in each plot and aggregated by depth. All soil analyses except NO_3^--N and NH_4^+ were conducted by Agvise Laboratories (Benson, MN; www.agvise.com). The NO_3^--N and NH_4^+ of soil samples were determined by extraction with a 2M KCl solution, where 40 mL solution was added to 10g fresh soil followed by 1 hour shaking (Culman et al., 2013a). Extractions were performed within 48 hours of field collection. NO_3^--N and NH_4^+ analyses of the extractions were performed at the UMN Research Analytical Lab. Method details can be found at http://ral.cfans.umn.edu/tests-analysis/soil-analysis.

Crop Yields

Crop yields were estimated each year from 2018 to 2020. Samples were taken in August of each year when the IWG had reached physiological maturity from two 76 by 76 cm quadrats with a total area of 0.58 m^2. Seed heads were removed from all IWG plants within the quadrat by cutting approximately 2 cm below the basal spikelet. After seed heads were removed, all remaining IWG biomass was harvested to an 8 cm stubble height. The remaining biomass was mechanically harvested and removed from the plots following quadrat sampling.

Biomass and seed heads were dried at 35˚C for 72 hours or until constant mass before being weighed. Grain was removed from spikes using a Wintersteiger LD 350 laboratory thresher (Wintersteiger; www.wintersteiger.com/us/Plant-Breeding-and-Research). Grain was separated from the chaff and other debris by hand-sieving and with a fractionating aspirator (Carter-Day International, Inc.; http://www.carterday.com).

Corn and soybean yield were determined by harvesting a subsection of the middle two rows of each plot. For corn, two 2 m sections of rows were cut from each corn plot. The number of corn stalks cut was recorded for each plot. All ears from the cut stalks were collected, dried (35˚C for 72 hours), shelled, and both cobs and kernels were weighed. Three stalks from each row section were randomly selected, dried, and weighed to estimate stover mass. Soybean yields were determined by harvesting whole plants from two 1 m sections of rows from each plot, followed by drying, threshing, and weighing. Following harvest for yield measurement, the remaining corn and soybean plants were mechanically harvested and removed from the plots.

Root Biomass

Root biomass samples were taken in September 2020 with two 5-cm diameter manual push cores per plot at depths of 0-15, 15-30, 30-45, and 45-60 cm. Roots were separated from soil and debris using a hydropneumatic elutriation system (Smucker et al., 1982), then removed manually from sieves using tweezers. Due to the difficulty of distinguishing live from dead roots, no effort was made to separate them. Roots were dried at 35˚C for 72 hours. Samples were checked after drying for any remaining sand and debris, which was removed before weighing.

Soil solution NO_3^--N concentration

Soil solution NO_3^--N concentrations were determined by collecting soil solution samples with suction lysimeters. Lysimeters consisted of a porous ceramic end cap, a PVC tube, and an airtight rubber stopper (Jungers et al., 2019). Two pairs of 60 and 120 cm lysimeters were installed in each plot. Samples were collected every 7-10 days from April to October each year and analyzed for NO_3^--N concentration by Minnesota Department of Agriculture staff in Staples using a HACH DR 6000 spectrophotometer (Hach, https://www.hach.com).

Soil Water Content

Soil water content was measured in 2019 and 2020 at 10, 20, 30, 40, 60, and 100 cm using a Delta-T Devices PR2/6 Probe (Delta-T Devices 2021). One soil water content probe access tube was installed in each plot in May 2019. Readings were taken five times throughout the growing season in 2019 and six times during the growing season in 2020. Total water content was calculated for each plot and date using trapezoidal integration (Hupet et al., 2004).

Statistical Analysis

Analysis of variance (ANOVA) was conducted using mixed effects models to explain variation in soil water NO_3^--N concentration, soil water, soil NO_3^--N, root biomass, and crop yields. Predictor variables for the ANOVA were cropping system, depth (for soil variables), and their interaction. Years were analyzed separately because the annual crop varied. Cropping system was treated as a categorical variable; depth was treated as a categorical variable for root biomass and soil NO_3^--N. NO_3^--N concentrations from the 60 and 120 cm depths were not statistically different and thus were averaged for the analysis. Plot position was included in the model as a covariate to account for possible lateral movement of N from the adjacent plot treatment. Data were analyzed with replicate as a random effect. For the lysimeter data, which included two pairs of lysimeters per plot, plot was nested within replicate in the random effects structure. Mean comparisons using Tukey's adjusted P value were used to generate estimated means for effects. Statistical analysis was carried out using statistical software program R (Version 3.5.2 GUI 1.70) including *emmeans* and *nlme* packages (R Core Team, 2018; Length, 2019; Pinheiro et al., 2019).

Results

Soil Solution NO_3^--N Concentration

Soil solution NO_3^--N concentration differed by cropping system treatment in all three years (Table 2, Figure 1), but did not vary by sampling depth or show an interaction effect in any year (p-values > 0.1). The average soil water NO_3^--N concentration was 77%, 96%, and 96% lower in the perennial system than the annual system in Years 1-3, respectively. The IWG system averaged 4.3, 0.8, and 0.3 mg L^{-1} in years 1-3, while the annual rotation averaged 19.0, 22.1, and 7.8 3 mg L^{-1} in years 1-3. The IWG system means show a downward trend over time, while corn had a higher mean than either year of soybean. The second year of soybean, in the third year of the rotation, was lower than the first year, possibly due to drought conditions.

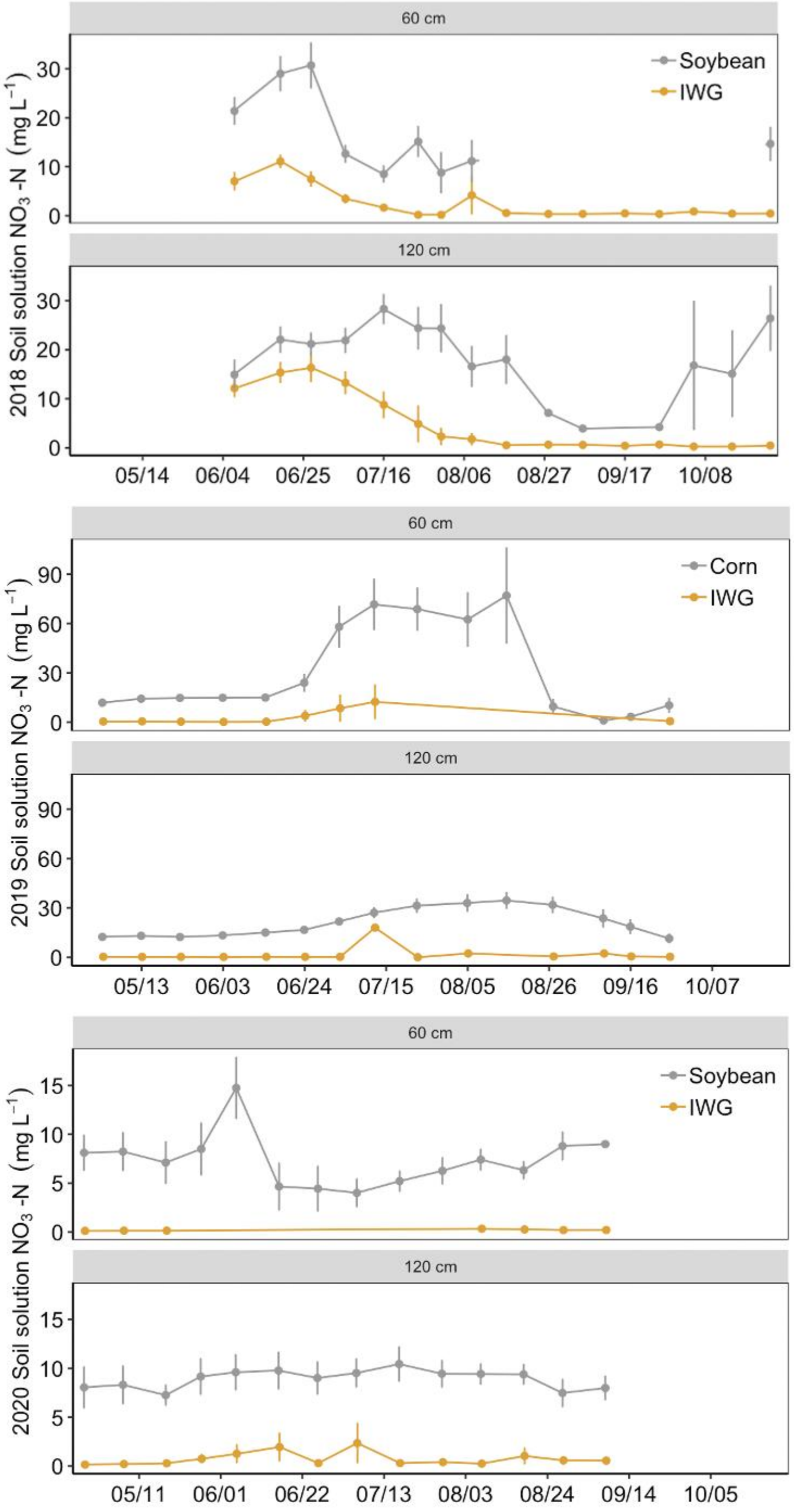
Throughout the seasons, both intra- and inter-annual variation was observed. NO_3^--N concentrations under IWG initially had mean values between 10 and 20 mg L^{-1} in Year 1 but declined to nearly zero by the end of July 2018 and remained at those levels for all three years except for occasional deviations. Corn and soybean showed distinct patterns. In 2018, NO_3^--N concentrations under soybean were initially high, declining to near zero in mid-September, but increasing to previous levels after harvest. In 2019, NO_3^--N concentrations under corn were between 10 and 20 mg L^{-1} but spiked to levels over twice that between late June and late August. Concentrations slowly declined over the remainder of the year. In 2020, mean NO_3^--N concentrations under soybean were consistently around 10 mg L^{-1} at the 120 cm depth.

Table 2. Mean soil solution NO_3^--N was higher in annual than perennial treatments in all years. Different lower-case letters denote statistical significance at $P < 0.05$.

Year	Treatment	NO_3^--N (mg L^{-1})	SE		p-value
2018	Perennial	4.3	1.3	a	0.0003
2018	Annual	19.0	1.3	b	
2019	Perennial	0.8	3.2	a	0.0036

2019	Annual	22.1	2.5	b	
2020	Perennial	0.3	1.0	a	0.0025
2020	Annual	7.8	1.0	b	

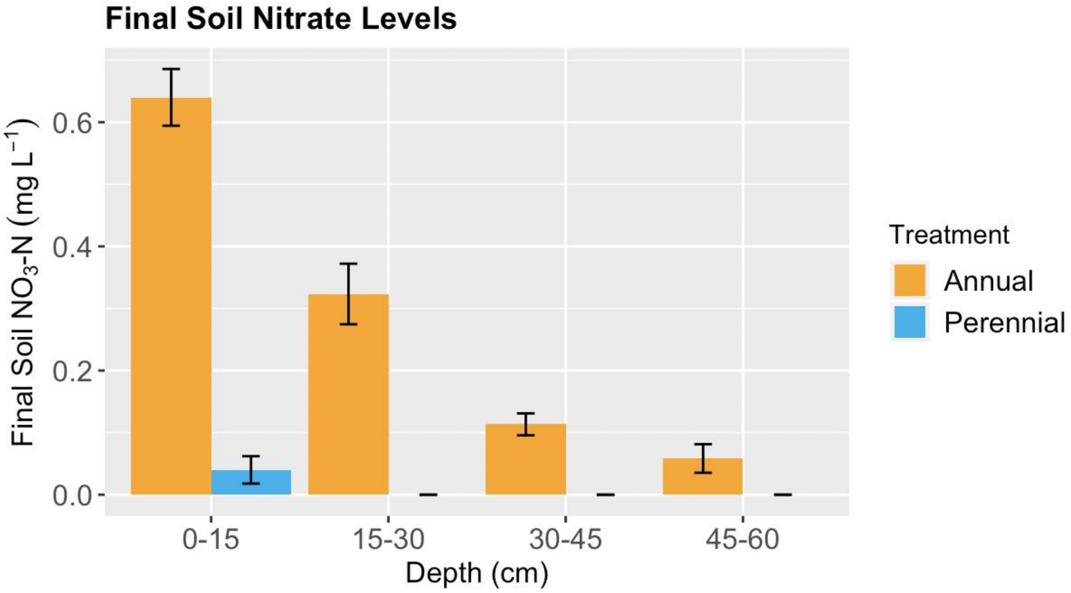
Figure 1. Soil solution NO_3^--N under annual and perennial treatments showed different patterns due to crop, depth, and year, with IWG showing consistently low concentrations after full establishment


60 cm
120 cm
2018 Soil solution NO3 -N (mg L−1)
Soybean
IWG
0
10
20
30
05/14
06/04
06/25
07/16
08/06
08/27
09/17
10/08
60 cm
120 cm
2019 Soil solution NO3 -N (mg L−1)
Corn
IWG
0
30
60
90
05/13
06/03
06/24
07/15
08/05
08/26
09/16
10/07
60 cm
120 cm
2020 Soil solution NO3 -N (mg L−1)
Soybean
IWG
0
5
10
15
05/11
06/01
06/22
07/13
08/03
08/24
09/14
10/05

Soil N

There was an effect of cropping system treatment, depth, and a depth by interaction (p-values <0.0001) on soil NO$_3^-$-N measured at the end of the study in 2020 (Figure 2). Perennial system values were 0.04, 0, 0, and 0 mg L^{-1} (±0.027) at 0-15, 15-30, 30-45, and 45-60 cm respectively and annual system values were 0.64, 0.32, 0.11, and 0.06 mg L^{-1} (±0.027). Soil NO$_3^-$-N was greater in the annual cropping system compared to IWG at 0-15, 15-30, and 30-45 cm depths, and the difference was greatest at the 0-15 cm depth.

Figure 2. Final soil NO$_3^-$-N was higher under the annual treatment than the IWG at the first three depths



Root Biomass

Root biomass collected at the end of the study in 2020 was affected by treatment, depth, and a treatment by depth interaction (p-values <0.001), so root biomass was compared separately at each depth. Root biomass was greater under IWG compared to the annual cropping system at all depths (Table 3). The magnitude of the difference was greatest at 0-15 cm, and this decreased with depth. Soybean root biomass was 80%, 85%, 81%, and 83% lower than IWG root biomass at 0-15, 15-30, 30-45, and 45-60 cm respectively. Summed over all the depths, total IWG root biomass was 13.73 Mg ha^{-1} while soybean root biomass was 2.54 Mg ha^{-1}, 82% lower ($p < 0.001$).

Table 3. Root biomass was higher under the perennial than the annual system at all measured depth intervals. Different lower-case letters denote statistical significance at $P < 0.05$.

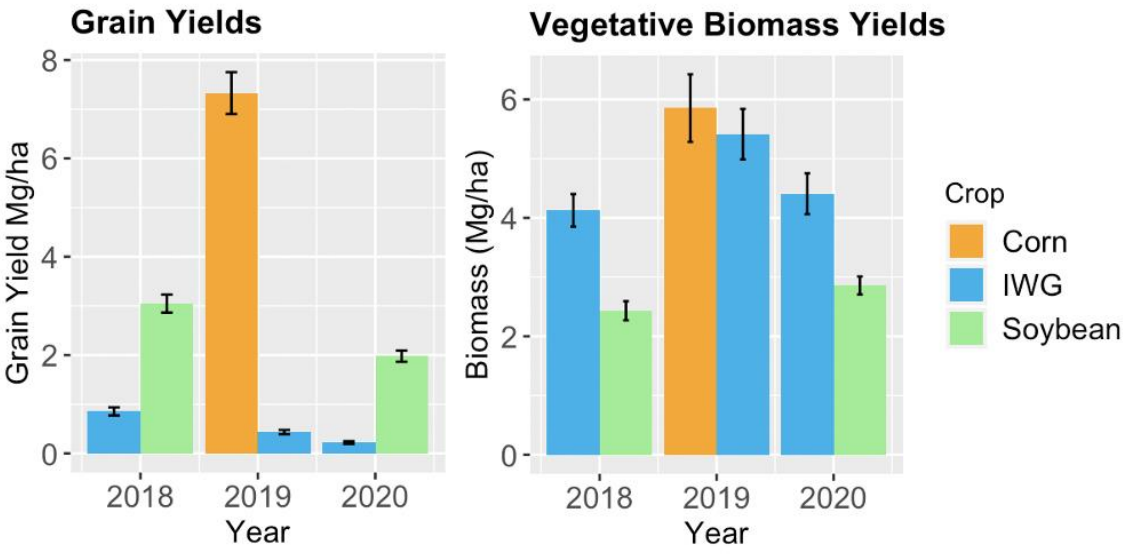
Crop	**Depth**	**Root Mass (Mg ha^{-1})**	**SE**		p-value
IWG	0-15cm	8.57	1.06	a	0.002
Soybean	0-15cm	1.69	1.06	b	
IWG	15-30cm	2.82	0.26	a	0.0013
Soybean	15-30cm	0.42	0.26	b	
IWG	30-45cm	1.30	0.16	a	0.0058
Soybean	30-45cm	0.25	0.16	b	
IWG	45-60cm	1.03	0.13	a	0.0039
Soybean	45-60cm	0.17	0.13	b	

Crop yield

Grain yield was higher for the annual rotation crops than for IWG in all years (p-values < 0.001). IWG grain yields were 0.85, 0.43, and 0.22 Mg ha^{-1} for Years 1, 2, and 3 respectively. Annual grain yields in Mg ha^{-1} were 3.05 (soybean), 7.33 (corn), and 1.98 (soybean) for Years 1, 2, and 3 respectively (Figure 3).

Intermediate wheatgrass vegetative biomass yields (Figure 3) were 4.12, 5.41, and 4.41 Mg ha^{-1} for Years 1, 2, and 3 respectively, and annual biomass yields were 2.43 (soybean), 5.85 (corn), and 2.86 (soybean) Mg ha^{-1}. Intermediate wheatgrass had higher biomass than soybean in both years (p-values = 0.001 and 0.009) but biomass yield similar to corn (p = 0.322). Intermediate wheatgrass grain yields declined over time while vegetative biomass yields increased somewhat.

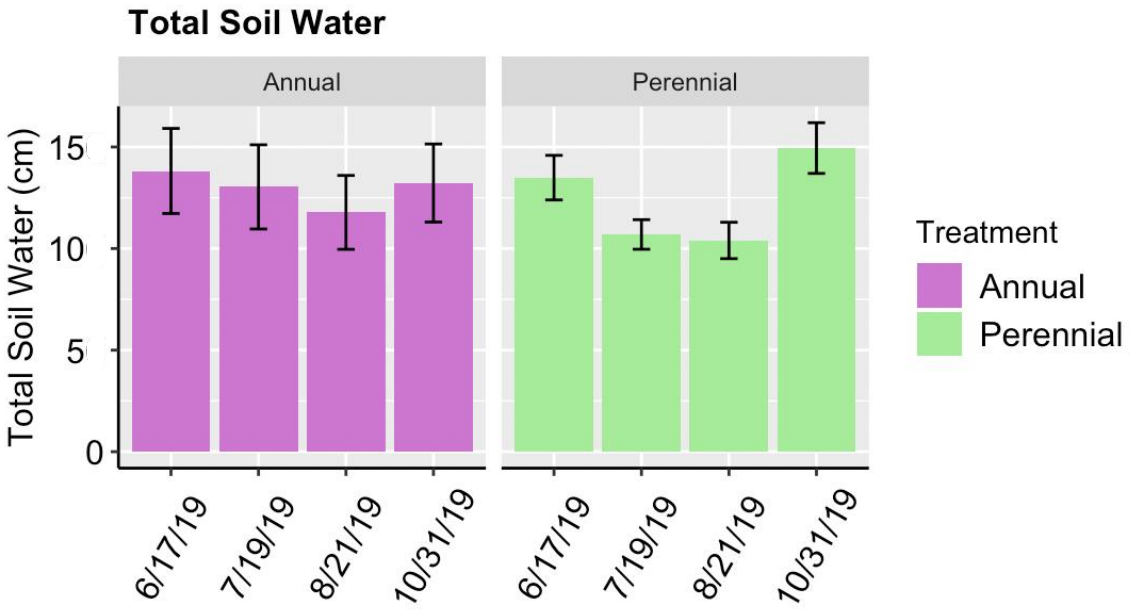
Figure 3. Grain yields were higher for both corn and soybean than IWG, while IWG biomass yields were similar to corn and higher than soybean



Soil Water

In 2019, cropping system treatment did not affect total soil water content (p-value = 0.796), but there was an effect of sampling date and an interaction (p-values < 0.001). Soil water content did not differ between treatments on any sampling date, but it did differ between dates within treatments (Figure 4). Average soil water content values were 13.7, 11.9, 11.1, and 14.1 cm for June 17th, July 19th, August 21st, and October 31st, respectively, and the July and August values were lower than the June and October values ($p < 0.001$). In 2020, neither cropping system treatment ($p = 0.753$) nor sampling date (p = 0.965) were significant, and there was no interaction effect (p = 0.206). Mean soil water content for both treatments and all dates was 12.9 cm.

Figure 4. In 2019, soil moisture differed by date, but not by treatment



Discussion

Soil Water NO_3^--N Concentration

Consistent with previous findings, we observed drastically lower concentrations of soil solution NO_3^--N beneath IWG compared to an annual cropping system. Concentrations under IWG were initially between 10 and 20 mg L^{-1} during June and July of 2018, but by August approached zero and remained very low for the duration of the experiment. This was likely a result of the time needed for IWG establishment, during which its ability to take up nitrate would have been more limited due to a smaller root system.

This is the first study to compare IWG soil water NO_3^--N levels on sandy soils in Minnesota where NO_3^--N leaching is of greatest concern. Here, we show that the potential for IWG to mitigate NO_3^--N leaching is similar in this vulnerable landscape compared to regions of Minnesota with silt and silt-loam soils that have higher water holding capacity. It is meaningful to confirm other observations of lower NO_3^--N concentration under IWG on a sandy soil where the aquifer is vulnerable to NO_3^--N leaching.

We found three-year average NO_3^--N concentrations of 1.82 mg L^{-1} for IWG and 16.3 mg L^{-1} for the corn-soybean rotation, values consistent with those observed in other work, and for IWG, values comparable to mixtures of perennial grasses and forbs found in Conservation Reserve Program (CRP) plantings that averaged 2 mg L^{-1} (Randall et al., 1997). Comparison plots of corn fertilized at 80 and 160 kg ha^{-1} had average nitrate concentrations of 8.38 and 23.97 mg L^{-1}, comparable to our results.

A previous study in Minnesota found that nitrate concentrations beneath IWG averaged 0.09 to 0.3 mg L^{-1} when fertilized with 80 kg N ha^{-1}. Despite only receiving 20 kg N ha^{-1} more fertilizer annually in this study, nitrate concentrations were more than ten times greater. This is likely related to the low organic matter and coarse structured soil found in our study (Jungers et al., 2019). In contrast, a study on sandy soil in Michigan found lysimeter NO_3^--N concentrations varying from 10-20 mg L^{-1} under IWG during the first year but virtually undetectable amounts in the second year, possibly due to the soil texture as well as slow stand establishment (Culman et al., 2013b). All three of these studies modeled total NO_3^--N leaching, which largely reflected trends in NO_3^--N concentrations. For example, models estimated total leaching of 21.7 kg N ha^{-1} under corn compared to 0.2 kg N ha^{-1} under IWG (Jungers et al., 2019). This suggests that total leaching would be much lower under IWG relative to an annual rotation in Central Minnesota as well, given the large difference in average soil water nitrate concentrations.

This is also among the first studies to compare soil water NO_3^--N levels to an unfertilized legume crop, and these results are even more striking given that only the corn phase of the corn-soybean rotation was fertilized with N while the IWG was fertilized at a relatively high rate. Despite applying up to 100 kg N ha^{-1} of urea annually to the IWG, lower NO_3^--N levels were observed in the IWG compared to the unfertilized soybean. Biologically fixed N may have been mineralized after exudation or sloughing of soybean roots, which may have contributed to higher soil water NO_3^--N levels compared to IWG. Significant N demand by IWG may have also contributed to the large difference in soil water NO_3^--N. N removal from a perennial IWG stand can be up to 140 kg N ha^{-1} in the second year and 100 kg N ha^{-1} in the third (Tautges et al., 2018), indicating that it can take up at least that much annually. Though it can use that much N, fertilizing at those rates is not typically recommended due to the tendency to lodge at high N rates (Jungers et al., 2017).

Soil NO_3^--N

In addition to lower soil water NO_3^--N concentration, we also found less NO_3^--N in the soil after three years of IWG production compared to the annual rotation system. This suggests that the IWG assimilated NO_3^--N more thoroughly from the soil than the annual rotation system, especially because the Year 3 crop was unfertilized soybean. NO_3^--N remaining in the soil is a major factor determining the concentration of dissolved NO_3^--N in soil water, which in turn determines total leaching loads (Randall and Mulla, 2001; Culman et al., 2013a; Jungers et al., 2019). The low levels of NO_3^--N under IWG also suggest that the plants may have been N-limited, despite being fertilized at the high end of optimal rates (Jungers et al., 2017).

Root biomass

Root biomass was similar to other reported values for the third year of an IWG stand and for soybean plots. We found 13.7 Mg ha^{-1} root biomass under IWG and 2.5 Mg ha^{-1} under soybean, including mass sampled from 0 – 60 cm. Previous work has found IWG belowground biomass to be 3.28 Mg ha^{-1} in the first 10 cm, on average, in plots fertilized at 90 and 134 kg ha^{-1} in Minnesota and Wisconsin (Sakiroglu et al., 2020). In treatments harvested once annually and fertilized at 72-81 kg N ha^{-1}, observed total root biomass from 0-20 cm peaked between 3.5 and 4 Mg ha^{-1} in June and July, declining to 1 Mg ha^{-1} at the end of the growing season (Pugliese et al., 2019). IWG fertilized at 80 kg N ha^{-1} had root biomass of 4.10, 7.32, and 9.51 Mg ha^{-1} (0-60 cm depth) in Years 1-3 of a three-year study, while a soybean-corn-soybean rotation had root biomass of 2.22, 2.93, and 2.30 Mg ha^{-1} in Years 1-3 (Bergquist, 2019). This indicates that while IWG root biomass accumulates over time, annual root biomass does not. Our findings also indicate that IWG increases root biomass effectively on this soil type, enabling its efficient N uptake even on sandy soil.

Soil Water

While we did not observe a difference in average total soil moisture between treatments, others have. In a similar comparison of perennial and annual systems, soil under *Miscanthus* and switchgrass had less soil water than a corn-soybean system earlier in the season, but later in the season switchgrass had higher soil water and *Miscanthus* had less (McIsaac et al., 2010). Other studies have shown decreases in soil moisture under IWG compared to annual wheat (Culman et al., 2013b) and corn (Jungers et al., 2019). It has also been observed that soil water content tended to be higher under annuals than semi-perennials, and that there was less drainage from semi-perennials and perennials than annuals (Ferchaud and Mary, 2016). Lower soil water content suggests higher plant water use and translates to reduced drainage, one of the factors impacting nitrate leaching. Since soil water did not differ in this experiment, reduced nitrate concentration rather than drainage volume may be of particular importance on sandy soils.

It is also established that increased root biomass increases water and nutrient uptake and could lead to lower soil water, supporting previous findings (Ehdaie et al., 2010; Matsunami et al., 2012; Carvalho et al., 2014). In our study, the similar soil moisture contents observed in the perennial and annual treatments may have been a function of the low water holding capacity of the sandy soil, which may have promoted drainage regardless of root biomass.

Grain and Biomass Yields

Intermediate wheatgrass grain yields at our sandy site were comparable to previous reports from sites with higher soil fertility levels. Under similar fertilizer treatments, reported first year values range from 763 kg ha^{-1} (Zimbric et al., 2020) to 1089 kg ha^{-1} at sites in Wisconsin (Favre et al., 2019) and from 893 kg ha^{-1} (Jungers et al., 2017) to 1150 kg ha^{-1} (Fernandez et al., 2020) in Minnesota. Second and third-year yields tend to be much lower, typically ranging from 150 kg ha^{-1} (Fernandez et al., 2020) to 630 kg ha^{-1} (Sakiroglu et al., 2020) in Year 2 and from 153 kg ha^{-1} (Jungers et al., 2017) to 371 kg ha^{-1} (Zimbric et al., 2020) in Year 3. Our yields suggest that this soil type and climate is appropriate for IWG grain and biomass production with irrigation.

Forage production is important for profitable IWG systems, since a major challenge of IWG grain production is the substantial yield declines in years 2 and 3 (Jungers et al., 2017; Pugliese et al., 2019; Hunter et al., 2020a). Intermediate wheatgrass biomass yields in this study are similar to those of other reports in Minnesota, though they are at the lower end of the range. Reported summer aboveground biomass values include 5130 kg ha^{-1} in the second year and 5850 kg ha^{-1} in the third year for IWG fertilized at 90 and 134 kg ha^{-1} in Wisconsin and 10600 kg ha^{-1} for third year stands in Minnesota (Sakiroglu et al., 2020). Similarly, summer yields of approximately 6200 kg ha^{-1} were reported for first year monocultures fertilized at 100 and 68 kg N ha^{-1} as urea (Favre et al., 2019). Biomass yields averaged 13400 to 14320 kg ha^{-1} for control treatments in a management study fertilized at 56 kg ha^{-1} the previous year (Pinto et al., 2021). First and second year biomass yields of 8000 to 9000 kg ha^{-1} have also been reported for IWG fertilized at 80 kg N ha^{-1}. Third-year yields were lower, closer to 7000 kg ha^{-1} (Fernandez et al., 2020).

The relatively low biomass yields at this site relative to some other sites suggests that strategies to increase biomass without reducing grain production would be beneficial, especially since grain yield declined over time as is generally the case. This might entail fertilizing at higher rates to increase biomass production or intercropping with a legume to increase forage quality.

Conclusion

We found that soil solution NO_3^--N concentrations were 77-96% lower under IWG than the annual corn-soybean rotation, but soil water content was the same. This indicates that the IWG used more N, which is also demonstrated by very low residual soil N levels at the end of the experiment relative to the annual. The lower N concentrations in soil solution would be expected to translate to reductions in total leaching load of a similar magnitude. The increased uptake of N by IWG was likely facilitated by its greater root biomass, which was 5.4 times higher than that under the annual system. Despite the challenges associated with production of IWG on low-fertility sandy soils, grain yields were comparable to other locations and the system would likely be profitable in the first year for grain alone. Biomass yields would support additional revenue streams in subsequent years to improve economic viability.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

The Author Contributions section is mandatory for all articles, including articles by sole authors. If an appropriate statement is not provided on submission, a standard one will be inserted during the production process. The Author Contributions statement must describe the contributions of individual authors referred to by their initials and, in doing so, all authors agree to be accountable for the content of the work. Please see here for full authorship criteria.

Funding

This project was funded by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources.

Acknowledgments

The authors would like to thank Lindsay Wilson, Katherine Bohn, and the rest of the Sustainable Cropping Systems Lab staff for their dedication and assistance with essential research activities. We would also like to thank Ryan Perish and Hannah Barrett for their invaluable contributions to data collection and site maintenance. We thank Matthew Leung and Manbir Rakkar for assisting with soil nitrate extractions.

References

Asbjornsen, H., Hernandez-Santana, V., Liebman, M., Bayala, J., Chen, J., Helmers, M., et al. (2014). Targeting perennial vegetation in agricultural landscapes for enhancing ecosystem services. *Renew. Agric. Food Syst.* 29, 101–125. doi:10.1017/S1742170512000385.

Bajgain, P., Zhang, X., Jungers, J. M., DeHaan, L. R., Heim, B., Sheaffer, C. C., et al. (2020). 'MN-Clearwater', the first food-grade intermediate wheatgrass (Kernza perennial grain) cultivar. *J. Plant Regist.* 14, 288–297. doi:10.1002/plr2.20042.

Bergquist, G. (2019). Biomass Yield and Soil Microbial Response to Management of Perennial Intermediate Wheatgrass (Thinopyrum Intermedium) as Grain Crop and Carbon Sink.

Brender, J. D., Weyer, P. J., Romitti, P. A., Mohanty, B. P., Shinde, M. U., Vuong, A. M., et al. (2013). Prenatal Nitrate Intake from Drinking Water and Selected Birth Defects in Offspring of Participants in the National Birth Defects Prevention Study. *Environ. Health Perspect.* 121, 1083–1089. doi:10.1289/ehp.1206249.

Carvalho, P., Azam-Ali, S., and Foulkes, M. J. (2014). Quantifying relationships between rooting traits and water uptake under drought in Mediterranean barley and durum wheat: Root traits and water uptake. *J. Integr. Plant Biol.* 56, 455–469. doi:10.1111/jipb.12109.

Crews, T. E., and Peoples, M. B. (2005). Can the Synchrony of Nitrogen Supply and Crop Demand be Improved in Legume and Fertilizer-based Agroecosystems? A Review. *Nutr. Cycl. Agroecosystems* 72, 101–120. doi:10.1007/s10705-004-6480-1.

Culman, S. W., Snapp, S. S., Green, J. M., and Gentry, L. E. (2013a). Short- and Long-Term Labile Soil Carbon and Nitrogen Dynamics Reflect Management and Predict Corn Agronomic Performance. *Agron. J.* 105, 493–502. doi:10.2134/agronj2012.0382.

Culman, S. W., Snapp, S. S., Ollenburger, M., Basso, B., and DeHaan, L. R. (2013b). Soil and Water Quality Rapidly Responds to the Perennial Grain Kernza Wheatgrass. *Agron. J.* 105, 735–744. doi:10.2134/agronj2012.0273.

DeHaan, L., Christians, M., Crain, J., and Poland, J. (2018). Development and Evolution of an Intermediate Wheatgrass Domestication Program. *Sustainability* 10, 1499. doi:10.3390/su10051499.

Dinnes, D. L., Karlen, D. L., Jaynes, D. B., Kaspar, T. C., Hatfield, J. L., Colvin, T. S., et al. (2002). REVIEW AND INTERPRETATION. *Agron. J.* 94, 19.

Ehdaie, B., Merhaut, D. J., Ahmadian, S., Hoops, A. C., Khuong, T., Layne, A. P., et al. (2010). Root System Size Influences Water-Nutrient Uptake and Nitrate Leaching Potential in Wheat: Root System and Nutrient Uptake in Wheat. *J. Agron. Crop Sci.* 196, 455–466. doi:10.1111/j.1439-037X.2010.00433.x.

Erisman, J. W., Galloway, J. N., Seitzinger, S., Bleeker, A., Dise, N. B., Petrescu, A. M. R., et al. (2013). Consequences of human modification of the global nitrogen cycle. *Philos. Trans. R. Soc. B Biol. Sci.* 368, 20130116. doi:10.1098/rstb.2013.0116.

Favre, J. R., Castiblanco, T. M., Combs, D. K., Wattiaux, M. A., and Picasso, V. D. (2019). Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. *Anim. Feed Sci. Technol.* 258, 114298. doi:10.1016/j.anifeedsci.2019.114298.

Ferchaud, F., and Mary, B. (2016). Drainage and Nitrate Leaching Assessed During 7 Years Under Perennial and Annual Bioenergy Crops. *BioEnergy Res.* 9, 656–670. doi:10.1007/s12155-015-9710-2.

Fernandez, C. W., Ehlke, N., Sheaffer, C. C., and Jungers, J. M. (2020). Effects of nitrogen fertilization and planting density on intermediate wheatgrass yield. *Agron. J.* 112, 4159–4170. doi:10.1002/agj2.20351.

Feyereisen, G. W., Wilson, B. N., Sands, G. R., Strock, J. S., and Porter, P. M. (2006). Potential for a Rye Cover Crop to Reduce Nitrate Loss in Southwestern Minnesota. *Agron. J.* 98, 1416–1426. doi:10.2134/agronj2005.0134.

Hunter, M. C., Sheaffer, C. C., Culman, S. W., and Jungers, J. M. (2020a). Effects of defoliation and row spacing on intermediate wheatgrass I: Grain production. *Agron. J.* 112, 1748–1763. doi:10.1002/agj2.20128.

Hunter, M. C., Sheaffer, C. C., Culman, S. W., Lazarus, W. F., and Jungers, J. M. (2020b). Effects of defoliation and row spacing on intermediate wheatgrass II: Forage yield and economics. *Agron. J.* 112, 1862–1880. doi:10.1002/agj2.20124.

Hupet, F., Bogaert, P., and Vanclooster, M. (2004). Quantifying the local-scale uncertainty of estimated actual evapotranspiration. *Hydrol. Process.* 18, 3415–3434. doi:10.1002/hyp.1504.

Jungers, J. M., DeHaan, L. H., Mulla, D. J., Sheaffer, C. C., and Wyse, D. L. (2019). Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agric. Ecosyst. Environ.* 272, 63–73. doi:10.1016/j.agee.2018.11.007.

Jungers, J. M., DeHaan, L. R., Betts, K. J., Sheaffer, C. C., and Wyse, D. L. (2017). Intermediate Wheatgrass Grain and Forage Yield Responses to Nitrogen Fertilization. *Agron. J.* 109, 462–472. doi:10.2134/agronj2016.07.0438.

Keeler, B. L., Gourevitch, J. D., Polasky, S., Isbell, F., Tessum, C. W., Hill, J. D., et al. (2016). The social costs of nitrogen. *Sci. Adv.* 2, e1600219. doi:10.1126/sciadv.1600219.

Keeler, B. L., and Polasky, S. (2014). Land-use change and costs to rural households: a case study in groundwater nitrate contamination. *Environ. Res. Lett.* 9, 074002. doi:10.1088/1748-9326/9/7/074002.

Length, R. (2019). *emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.3.* Available at: https://CRAN.R-project.org/package=emmeans.

Maeght, J.-L., Rewald, B., and Pierret, A. (2013). How to study deep roots—and why it matters. *Front. Plant Sci.* 4. doi:10.3389/fpls.2013.00299.

Matsunami, M., Matsunami, T., Ogawa, A., Toyofuku, K., Kodama, I., and Kokubun, M. (2012). Genotypic Variation in Biomass Production at the Early Vegetative Stage among Rice Cultivars Subjected to Deficient Soil Moisture Regimes and Its Association with Water Uptake Capacity. *Plant Prod. Sci.* 15, 82–91. doi:10.1626/pps.15.82.

McIsaac, G. F., David, M. B., and Mitchell, C. A. (2010). *Miscanthus* and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. *J. Environ. Qual.* 39, 1790–1799. doi:10.2134/jeq2009.0497.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2019). *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-139,*. Available at: https://CRAN.R-project.org/package=nlme.

Pinto, P., De Haan, L., and Picasso, V. (2021). Post-Harvest Management Practices Impact on Light Penetration and Kernza Intermediate Wheatgrass Yield Components. *Agronomy* 11, 442. doi:10.3390/agronomy11030442.

Pugesgaard, S., Schelde, K., Larsen, S. U., Laerke, P. E., and Jørgensen, U. (2015). Comparing annual and perennial crops for bioenergy production - influence on nitrate leaching and energy balance. *GCB Bioenergy* 7, 1136–1149. doi:10.1111/gcbb.12215.

Pugliese, J. Y., Culman, S. W., and Sprunger, C. D. (2019). Harvesting forage of the perennial grain crop kernza (Thinopyrum intermedium) increases root biomass and soil nitrogen cycling. *Plant Soil* 437, 241–254. doi:10.1007/s11104-019-03974-6.

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing Available at: https://www.R-project.org/.

Randall, G. W., Huggins, D. R., Russelle, M. P., Fuchs, D. J., Nelson, W. W., and Anderson, J. L. (1997). Nitrate Losses through Subsurface Tile Drainage in Conservation Reserve Program, Alfalfa, and Row Crop Systems. *J. Environ. Qual.* 26, 1240–1247. doi:10.2134/jeq1997.00472425002600050007x.

Randall, G. W., and Mulla, D. J. (2001). Nitrate Nitrogen in Surface Waters as Influenced by Climatic Conditions and Agricultural Practices. *J. Environ. Qual.* 30, 337–344. doi:10.2134/jeq2001.302337x.

Sakiroglu, M., Dong, C., Hall, M. B., Jungers, J., and Picasso, V. (2020). How does nitrogen and forage harvest affect belowground biomass and nonstructural carbohydrates in dual-use Kernza intermediate wheatgrass? *Crop Sci.* 60, 2562–2573. doi:10.1002/csc2.20239.

Smucker, A. J. M., McBurney, S. L., and Srivastava, A. K. (1982). Quantitative Separation of Roots from Compacted Soil Profiles by the Hydropneumatic Elutriation System [1]. *Agron. J.* 74, 500–503. doi:10.2134/agronj1982.00021962007400030023x.

Tautges, N. E., Jungers, J. M., DeHaan, L. R., Wyse, D. L., and Sheaffer, C. C. (2018). Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture *v.* bi-culture with alfalfa in the Upper Midwestern USA. *J. Agric. Sci.* 156, 758–773. doi:10.1017/S0021859618000680.

Ward, M. H., Kilfoy, B. A., Weyer, P. J., Anderson, K. E., Folsom, A. R., and Cerhan, J. R. (2010). Nitrate Intake and the Risk of Thyroid Cancer and Thyroid Disease. *Epidemiology* 21, 389–395. doi:10.1097/EDE.0b013e3181d6201d.

Ward, M., Jones, R., Brender, J., de Kok, T., Weyer, P., Nolan, B., et al. (2018). Drinking Water Nitrate and Human Health: An Updated Review. *Int. J. Environ. Res. Public. Health* 15, 1557. doi:10.3390/ijerph15071557.

Zimbric, J. W., Stoltenberg, D. E., and Picasso, V. D. (2020). Effective weed suppression in dual-use intermediate wheatgrass systems. *Agron. J.* 112, 2164–2175. doi:10.1002/agj2.20194.

Delta-T Devices (2021). PR2 Profile Probe - analogue version. Cambridge, UK. www.delta-t.co.uk/product/pr2/

MPCA (2014). Minnesota Nutrient Reduction Strategy. Minnesota Pollution Control Agency. https://www.pca.state.mn.us/sites/default/files/wq-s1-80.pdf

USDA – NRCS (2021). Web Soil Survey. USDA – Natural Resources Conservation Service. https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

Supplementary Material

Supplementary Material should be uploaded separately on submission, if there are Supplementary Figures, please include the caption in the same file as the figure. Supplementary Material templates can be found in the Frontiers Word Templates file.

Please see the Supplementary Material section of the Author guidelines for details on the different file types accepted.

Data Availability Statement

The datasets [GENERATED/ANALYZED] for this study can be found in the [NAME OF REPOSITORY] [LINK]. Please see the Data Availability section of the Author guidelines for more details.