Intermediate Wheatgrass Grain and Forage Yield Responses to Nitrogen Fertilization

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ABSTRACT

Perennial crops have fewer environmental impacts compared to annual crops, but there are no perennial grains available to replace the annual grains that occupy a majority of U.S. cropland. Here we report grain and biomass yields from an improved breeding population of intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host) Buckworth & Dewey], a perennial grass being domesticated to serve as the first widely grown perennial grain crop. Our objective was to measure grain and biomass yields of this improved grain-type IWG (TLI-C2), a forage variety of IWG (cultivar Rush), and switchgrass (*Panicum virgatum* L.) in response to N fertilization rates ranging from 0 to 200 kg N ha^{-1}. TLI-C2 grain yields responded quadratically to increasing N rates in all but one environment, but yields declined at high N rates due to lodging. TLI-C2 grain yields were highest during the first year of fertilization, yielding 961 and 893 kg ha^{-1} when fertilized at agronomically optimum nitrogen rates (AONRs) of 61 and 96 kg N ha^{-1} for stands seeded in fall of 2011 and spring of 2012, respectively. Grain yields declined with stand age. When fertilized with AONRs for grain, biomass yields of TLI-C2 harvested after grain ranged from 9.2 to 12.3 Mg ha^{-1} and had similar forage and bioenergy quality characteristics compared to Rush, which demonstrates the potential to manage TLI-C2 as a dual-use cropping system for both grain and forage.

Core Ideas

- Intermediate wheatgrass is being bred as a perennial grain crop.
- Optimum N fertilizer rates for intermediate wheatgrass grain yields are reported.
- Intermediate wheatgrass biomass yield and quality is comparable to other biomass crops.
- Intermediate wheatgrass grain yields declined after two years of production.
- Lodging occurred at high N rates, which reduced grain yields.

INCORPORATING PERENNIAL PLANTS into agricultural systems provides a number of environmental benefits compared to annual row crop production (Asbjornsen et al., 2013). Some environmental benefits are related to the deep, dense root systems of perennials. On agricultural soils, biomass allocation to roots is two to four times greater in perennials compared to annuals (Dietzel et al., 2015). Large root systems improve the nutrient use efficiency (Dietzel et al., 2015) and synchrony of plant demands and nutrient supplies (Crews, 2005) compared to annuals, which could reduce synthetic N fertilizer requirements for perennial crops. Water quality could be improved as fertilized perennial crops leach less nitrate than annuals (McIsaac et al., 2010). Reducing N fertilization reduces greenhouse gas emissions related to N fertilizer volatilization (Ruan et al., 2016). Carbon sequestration occurs below perennials as root C is transferred via exudates and turnover to soil organic matter at depths where it is protected from decomposition (Tiemann and Grandy, 2015). Despite the environmental benefits of perennial plants, few species are used as crops in the Midwest as a result of limited markets and low yields.

Perennial plants are found in agriculture as components of pastures, forage crops, and bioenergy crops. Pasture and forage species–such as alfalfa (*Medicago sativa* L.), clovers (*Trifolium* spp.), brome grasses (*Bromus* spp.), etc.–are examples of perennial plants grown on cropland in the United States (Sheaffer et al., 2009), however land area of cropland pasture and forages are decreasing. For example, hay land area declined by 11% from 2002 to 2012, despite a net expansion of total cropland during that time (USDA, 2015; Lark et al., 2015). Perennial crops have also been proposed as feedstock sources for renewable energy (McLaughlin et al., 2002), which has initiated research programs on such crops as switchgrass, mixtures of native prairie plants, miscanthus (*Miscanthus* spp.), and others worldwide. However, perennial-based renewable energy systems have not been economically viable in recent U.S. energy markets, therefore other agriculture markets could be better suited to increase the land area of perennial crops.

Annual cereal grains account for about 50% of human calories and agricultural land area globally, and their extensive production has led to environmental degradation (Tilman, 1999; Monfreda et al., 2008). Replacing annual grains with

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Abbreviations: AONR, agronomically optimum nitrogen rate; CI, confidence interval; IWG, intermediate wheatgrass.

perennial alternatives that provide food directly consumable by humans could increase food and ecosystem security (Glover et al., 2010). Efforts to breed perennial grains are occurring globally (Cox et al., 2010), and recent advancements in genomic technology have increased the pace in wild plant domestication (Sedbrook et al., 2014; DeHaan et al., 2016) and perennial grain development (Kantar et al., 2016).

Intermediate wheatgrass was identified as a perennial species potentially suitable for domestication as a grain crop in 1983 (Wagoner, 1990). The Land Institute in Salina, KS, has made promising progress in breeding IWG for larger seed size and seed yield (DeHaan et al., 2013). Advanced IWG populations had grain yields averaging 1662 kg ha^{-1} during the second year of production and managed with moderate N fertilizer rates (Culman et al., 2013), which is more than four times the yield typically obtained from forage varieties (Weik et al., 2002; Lee et al., 2009). Compared to annual wheat, IWG also reduced N leaching and increased C mineralization in soils (an important driver of C sequestration; Culman et al., 2013). Some advanced IWG genotypes demonstrated suitable qualities for food production, such as high-molecular-weight subunits related to properties of high-quality wheat bread (Zhang et al., 2015). The genetic advancements and environmental benefits of IWG grain production have stimulated commercial interests in this new crop, which is sold under the trade name "Kernza" (The Land Institute, Salina, KS). To meet demands for Kernza, agronomic research on IWG grain production is needed.

Previous research on intermediate wheatgrass has focused on its use as a forage, grazing, and biomass crop (Karn et al., 2006; Liebig et al., 2008; Lee et al., 2009). A number of IWG cultivars are registered with specific trait improvements such as increased forage quality, yield potential, and stand persistence under grazing (Ogle et al., 2011). Biomass yields ranged from 6.7 to 10 Mg ha^{-1} over 4 yr in North Dakota (Wang et al., 2014), and whole plant in vitro dry matter digestibility values have been reported at 649 g kg^{-1} at anthesis (Karn et al., 2006). Likewise, a number of switchgrass cultivars have been evaluated for forage production, and yields were found to be comparable to IWG in the Upper Midwest (Lee et al., 2009; Wang et al., 2014). Like many perennial forage crops, both IWG and switchgrass have also been considered for use as a feedstock source for bioenergy (Sanderson and Adler, 2008). One method to evaluate feedstock sources for bioenergy is to measure the concentration of fermentable carbohydrates in cell walls to predict theoretical ethanol potential (Vogel et al., 2010). Biomass yield and composition of dedicated forage crops such as Rush IWG, and bioenergy crops such as switchgrass, can be used to compare and evaluate the potential for using new crops like grain-type IWG in such markets. Moreover, research on how N fertilization can influence both biomass yield and chemical composition of forage and bioenergy crops is essential (Vogel et al., 2002; Jungers et al., 2015b).

For perennial grain and biomass crops to be profitable and less environmentally harmful, optimum N fertilizer rates need to be determined (Cerrato and Blackmer, 1990; Matson et al., 1998; Randall and Mulla, 2001). For most cereal crops, grain yields increase in response to N fertilizer rates and then plateau at a certain N rate (hereafter referred to as AONR); and beyond that rate, yields can remain stable or decline. Statistical models can be used to determine AONRs and how grain yields

respond to rates in excess of AONRs (Cerrato and Blackmer, 1990; Bullock and Bullock, 1994). Moreover, recent reports have demonstrated tools and techniques for measuring uncertainty around AONRs (Hernandez and Mulla, 2008; Jaynes, 2010) and the importance of that uncertainty (Scharf et al., 2005).

Our objectives were to (i) evaluate the progress in breeding efforts to increase grain yield of IWG by comparing yields of an advanced IWG breeding line (TLI-C2) to those of a forage variety (Rush), (ii) determine the AONR for maximizing grain yields of TLI-C2, and (iii) measure forage yield, quality, and ethanol potential of TLI-C2, Rush, and switchgrass in response to variable N rates and to estimate these characteristics at AONRs.

METHODS

The experiment was conducted at five diverse locations in Minnesota (Table 1). A range of loamy soils was represented that varied in silt and clay content. Locations also varied in the amount and distribution of growing season precipitation. For example, the northern most location at Roseau received less than 500 mm of precipitation during grain filling (June and July), whereas the southern most location at Waseca received more than 1200 mm. A diverse set of growing conditions is necessary for generalizing crop responses to treatments across environments.

All locations were scheduled for seeding in fall of 2011, but because of inclement weather, the Crookston and Roseau locations were seeded in spring 2012. The spring-seeded locations did not produce seed in 2012 because of lack of plant vernalization. To maintain synchrony in treatments through time, N fertilizer treatments for all locations (both spring and fall seeded) were initiated in 2013 and applied for 3 yr. Grain yields from one site (Morris) were lost due to granivory of stored samples by rodents in 2014.

Experimental Design and Sampling

The experimental design was a randomized complete block with a split-plot arrangement and four replications. The main plots were 36 by 3 m and planted to one of three crops; switchgrass (*Panicum virgatum* L. 'Sunburst'; hereafter referred to as "switchgrass"), a forage type IWG (cultivar Rush; hereafter referred to as "Rush"), or an improved grain type IWG (TLI-C2) at rates of 6.7, 18, and 18 kg pure live seed per hectare, respectively. TLI-C2 is a breeding population resulting from two cycles of selection primarily for seed mass per head and mass per seed at The Land Institute in Salina, KS (Zhang et al., 2016). Seeds were planted into 15-cm rows following soybean at Morris, Waseca, and Lamberton and small grains at Roseau and Crookston. Dual Magnum (a.i. S-metolachlor 82.4%) was applied at labeled rates in April of all years to control weeds. Six N fertilization rates were randomly assigned to 6 by 3 m subplots within each main plot. Nitrogen was applied as urea in April of 2013, 2014, and 2015. In 2013, N fertilizer rate treatments were 0, 40, 80, 120, 160, and 200 kg N ha^{-1}. In 2013, N fertilizer rates above 120 kg N ha^{-1} resulted in extreme lodging. There was concern that lodged plants could interfere with neighboring treatments; so N fertilizer rates were adjusted for subsequent years to avoid extreme lodging. In 2014 and 2015, N fertilizer rates were adjusted to 0, 40, 60, 80, 100, and 120 kg N ha^{-1}.

Lodging was scored for all crops at physiological maturity each year. Lodging scores ranged from 0 to 10 and were based

Table I. Description of research sites and average monthly cumulative precipitation and temperature during the growing season from 2012 to 2015.

Site	Seeded	Coordinates (°N, °W)	Soil type	Apr.	May	June	July	Aug.	Sept.	Oct.
							mm; °C			
Crookston	Spring 2012	47.810,−96.615	Wheatville loam	142.5;4.2	268.2;12.7	411.7;18.7	263.9;21.3	157.2;19.6	130.8;15.5	186.9;7.1
Roseau	Spring 2012	48.878,−95.849	Bearden silty clay loam	115.1;3.3	334.3;11.7	165.6;17.4	263.7;19.9	218.2;18.7	162.1;14.5	155.7;6.3
Lamberton	Fall 2011	44.237,−95.302	Normania clay loam	268.7;6.5	542.3;14.4	482.1;20.4	153.9;22.6	332;20.6	317.8;17.6	159;9.0
Morris	Fall 2011	45.591,−95.876	Tara silt loam	159;5.9	394.5;14	389.1;19.6	273.8;21.7	285.5;20	76.2;16.6	160.3;8.2
Waseca	Fall 2011	44.068,−93.526	Webster clay loam	449.8;6.7	506;14.5	801.6;20.3	408.7;22.1	323.9;20.6	282.2;17.3	194.6;8.9

on both the angle at which the plants were lodging relative to the soil surface, as well as the proportion of the plants that were lodging in the plot. A lodging score of 0 indicated no lodging, whereas a score of 10 indicated 100% of the plants lodging with stems horizontal to the soil surface. Lodging scores were determined by the same observer for all years of this study, therefore ensuring consistency in the relative effect of treatments on lodging.

Plant height was measured by randomly selecting three stems from each plot and measuring them from the ground to the end of the seed head prior to grain harvest. Grain yields were determined from Rush and TLI-C2 by cutting all plants from a 0.5 by 1 m sample area to a 10-cm height in the center of each subplot when grain mass was constant and prior to shattering (mid-August). All remaining biomass was cut and removed from the plot after grain yield measurements.

The plants harvested from the sample area were dried at 60°C for at least 5 d, weighed, and then threshed using a research-scale thresher (Wintersteiger LD350). The lemma and palea were removed (de-hulled) from a subset of dried seed samples from each N rate at all locations mechanically to determine the fraction of grain mass attributable to the lemma and palea. This fraction was averaged and subtracted from all dried grain weights that were not de-hulled.

Biomass yield of Rush and TLI-C2 was determined by subtracting seed weights from total biomass weights measured prior to threshing. Biomass yield was measured from switchgrass after first frost (0°C mid-October) using the same methods as described for IWG. For all crops, biomass was cut to a stubble height of approximately 10 cm. For all biomass samples, a 1-kg subsample was ground and scanned using near-infrared spectroscopy (NIRS) for forage quality metrics including neutral detergent fiber (NDF), acid detergent fiber (ADF), and in vitro total digestible dry matter (IVTD). A subset of ground biomass samples of each crop representing a range of N rate applications was analyzed for determination of forage quality using wet chemistry methods (Goering and Van Soest, 1970) to validate NIRS results annually. The concentration of biomass carbohydrates was estimated using NIRS and validated using wet chemistry on a subset of samples annually. Equation [1] was used to estimate the theoretical ethanol potential based on a 100% conversion efficiency of carbohydrates (Vogel et al., 2010).

$$ETH = [(GLC + GAL + MAN) \times 172.82] \\ + [(XYL + ARA) \times 176.87]] \times 0.00417 \quad [1]$$

where ethanol potential (ETH; $l\,Mg^{-1}$) is estimated by the concentration ($g\,kg^{-1}$) of the following carbohydrates; glucose (GLC), galactose (GAL), mannose (MAN), xylose (XYL), and arabinose (ARA). A subsample of ground biomass was used to determine tissue N content by a gas analyzer. Switchgrass biomass from 2015 was destroyed prior to analyzing for forage and bioenergy characteristics.

Statistical Analysis

Data from the spring and fall seeding dates were analyzed independently to externalize any potential confounding effects related to seeding year. For each seeding date, variation in grain yield by crop type (Rush and TLI-C2), stand age, and N rate was measured using mixed-effects analysis of variance.

Interactions between all main effects were tested. For locations seeded in fall, there was a significant three-way interaction between all main effects, therefore each crop was analyzed independently during each year of the study. For locations seeded in spring, there was a significant interaction between crop type and stand age, crop type and N rate, and N rate and stand age; therefore each crop was analyzed independently during each year of the study. The effect of N fertilizer rate on grain yield was tested for each crop/year combination. A mixed effects model was used to account for random variation from replicates nested within locations. Both a linear model and a quadratic model with N rate as the predictor variable were fit to the grain yield data for each crop/year and compared using Akaike's Information Criterion adjusted for small sample size (AICc; Burnham and Anderson, 2002). The model with the smallest AICc was determined as superior. If the superior model was linear, an AONR could not be estimated. If the superior model included the quadratic term, the equation was reparametarized using Eq. [2] to include an estimate of the X value (N rate) where the response curve peaked; which we refer to as the AONR (Jungers et al., 2015b).

$$Y = \beta_0 + 2\beta_1\beta_2 X + \beta_1 X^2 \qquad [2]$$

Equation [2] was fit using the nlme package in R to 9999 bootstrapped data sets. The mean and 95% confidence intervals (CIs) were calculated and reported as AONRs.

The same methods were used to determine AONRs for biomass yield of switchgrass and Rush. For all crops, IVTD, ADF, NDF, tissue N, ethanol yield, harvest index (except for switchgrass), lodging, and plant height were modeled as a function of N rate for each seeding date and year. A quadratic or linear response was determined using the model selection methods described above for grain yield. If these response variables responded to N rates (either linearly or quadratically), the resulting regression equation was used to predict the value of each response variable at the AONRs for grain (TLI-C2) and biomass (Rush and switchgrass). If there was no effect of N rate on a given response variable, the mean of that response variable across all N rates was reported. Confidence intervals for mean values across N rates were calculated as the product of the standard error and 1.96. The AONRs and predicted values of other response variables when fertilized at AONRs were considered significantly different if 95% CIs did not overlap.

We tested for associations between grain yield, lodging, and plant height. We calculated the Spearman correlation coefficient between grain yield and lodging score using data from all locations during the first year of fertilizer application (2013; when a wider range of N rates were applied). We also modeled lodging score as a function of N fertilizer rate using mixed effects models for each seeding date/year combination using the methods described above. We determined if the relationship was linear or quadratic using the model selection methods described above, which was deemed the null model for the subsequent selection steps. The null model for lodging at each region/stand age combination was compared to an alternative model that included plant height as a covariate. The models were compared using the maximum likelihood ratio test to determine if plant height was a useful predictor to explain variation in lodging.

RESULTS AND DISCUSSION

Averaged across N rates and years, grain yields of TLI-C2 were 100 and 108% higher than Rush, the forage variety of IWG, at the fall ($F_{1, 335} = 334.7$, $P < 0.001$) and spring seeded ($F_{1, 235} = 265.7$, $P < 0.001$) locations, respectively. This is convincing evidence that breeding efforts to increase grain yields of IWG have been successful. TLI-C2 grain yields at the spring seeded locations were highest during the first production year and peaked at an average of 971 kg ha^{-1} when fertilized at 80 kg N ha^{-1}. Compared to a study in Michigan that tested an improved IWG population from the first cycle of breeding, first year grain yields were substantially lower than our results, however the authors attributed low first year yields to a late fall seeding date (Culman et al., 2013). Second year grain yields were higher in the Michigan study (Culman et al., 2013) and peaked at 1662 kg ha^{-1}. In our study, second year grain yields at the fall-seeded locations peaked at 996 kg ha^{-1} at the 80 kg N ha^{-1} fertilizer rate. Despite being lower than the IWG yields reported by Culman et al. (2013), TLI-C2 yields are substantially larger than seed yields of IWG forage varieties published in previous studies. For example, seed yields of IWG forage varieties peaked at 242 kg ha^{-1} across an aspect gradient in South Dakota and 442 kg ha^{-1} across a fertilization gradient in Saskatchewan (Loeppky et al., 1999; Lee et al., 2009). These yield estimates included the lemma and palea fractions of the seed, therefore inflating grain yields compared to estimates used in our study.

Agronomic Optimum Nitrogen Rates

Grain yields of TLI-C2 responded quadratically in response to increasing N rates for five of six seeding date/year combinations (Fig. 1). Agronomically optimum N rates for maximizing TLI-C2 grain yields ranged from 61.0 to 96.4 kg N ha^{-1} (Table 2). These AONRs are less than those identified for maximizing yields of other annual crops including wheat (Chen et al., 2004; Liang et al., 2008), corn (*Zea mays* L.) (Sindelar et al., 2012), and rice (*Oryza sativa* L.) (Wang et al., 2004), all of which usually yield more grain than IWG and require at least 100 kg N ha^{-1} for maximized grain yields. These studies found that annual crop yield response functions other than the quadratic (i.e., functions that did not model a decrease in yields with higher N rates) best fit their data. Many functions have been tested to identify AONRs for various crops including linear plateau models, quadratic plateau models, and logistic curve models (Cerrato and Blackmer, 1990). It is important to note that yield decreases in our study as a response to high N rates were not artifacts of the quadratic model, but instead occurred as a result of other physiological changes that are discussed below. Since yield declines occurred for some environments at high N rates, we chose to confine our analysis to linear and quadratic model testing.

Reporting uncertainty in AONR estimates is important for developing nutrient management guidelines that maximize crop yields and farmer profits while minimizing environmental damage (Jaynes, 2010). Similar to previous studies, we used 95% confidence intervals (CIs) as a metric for measuring uncertainty in our estimates of AONRs (Hernandez and Mulla, 2008; Jaynes, 2010). Although the AONR was highest for TLI-C2 grain production during the last year of study

Table 2. Agronomically optimum nitrogen rates (AONRs) and 95% confidence intervals for maximizing biomass yield (switchgrass and Rush) or grain yield (TLI-C2). Grain and biomass yield predictions, and 95% confidence intervals, at AONR for each crop, seeding date and year combination.

Crop	Seeding date	Year	AONR	2.5%	97.5%	Grain yield	2.5%	97.5%	Biomass yield	2.5%	97.5%
			——— kg N ha⁻¹ ———			———————— kg ha⁻¹ ————————					
Switchgrass											
	Fall (2011)	2013	149.7	135.3	177.5	na†	na	na	12,180	11,885	12,423
		2014	99.6	66.4	230.2	na	na	na	11,580	10,537	13,052
		2015	81.1	73.6	272.4	na	na	na	15,857	15,483	18,989
	Spring (2012)	2013	127.8	110.1	143.9	na	na	na	11,760	11,248	12,357
		2014	Linear‡	na	na	na	na	na	15,340	12,020	18,660
		2015	Linear‡	na	na	na	na	na	15,440	13,648	17,233
Rush											
	Fall (2011)	2013	0§	na	na	456	408	511	10,900	10,534	11,266
		2014	108.1	71.8	163.3	73	59	87	10,166	9,414	10,624
		2015	120.5	76.1	213.3	38	31	46	8,336	7,651	9,281
	Spring (2012)	2013	116.7	88.1	155.5	443	402	483	10,667	10,181	11,214
		2014	81.1	43.6	136.0	150	123	179	10,216	9,400	11,048
		2015	116.6	83.4	225.3	103	89	117	8,355	7,695	10,756
TLI-C2											
	Fall (2011)	2013	61	35.7	75.9	961	916	1005	11,983	11,584	12,365
		2014	ns¶#	na	na	237	174	300	9,687	8,867	10,507
		2015	94.7	66.2	132.8	153	132	179	9,269	8,698	9,868
	Spring (2012)	2013	96.4	79.3	109.9	893	841	947	11,880	11,408	12,345
		2014	86.6	70.2	118.9	386	344	432	12,267	11,520	12,977
		2015	77.3	74.1	368.8	265	252	573	9,353	8,500	10,278

† na, not applicable.

‡ Where crop yields responded linearly to N rates, response variables were estimated at the highest N rate applied for the given year. AONR was not calculated.

§ AONR estimate was negative, thus response variables were averaged at the 0 kg N ha⁻¹ fertilizer rate.

¶ Where biomass or grain yields did not respond to N rates, response variables were averaged across all N rates. Confidence intervals are estimated as the product of the standard error of the mean and 1.96.

ns = not significant. Grain or biomass yields did not respond to N rates, therefore an AONR was not calculated.
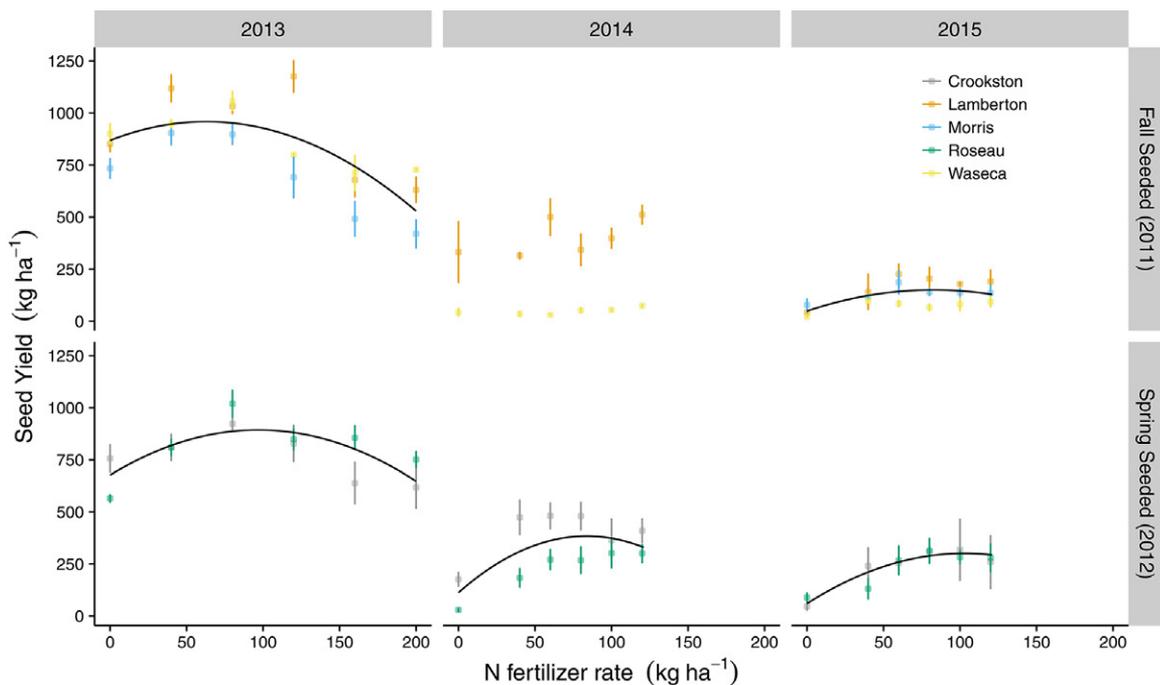

Fig. 1. Grain yields of TLI-C2 intermediate wheatgrass in response to N fertilizer rates. Data points represent mean grain yields (± SE) at each N fertilization rate by site and separated by seeding date and year. Regression lines represent fitted grain yields by N rate averaged across sites within each seeding date and year (site treated as random effect). Grain yields in 2014 at sites seeded in fall were not significantly different across N rates.

(Table 2), 95% CIs of the AONRs overlapped across years; which suggests that AONRs may not be significantly different across stand ages for this crop. When comparing AONRs among crops in 2013, a lack of overlap of the 95% CIs of the AONRs suggests that switchgrass required more N fertilizer to reach maximum biomass yield compared to TLI-C2 for grain yield at spring seeded locations. Increasing levels of N fertilization of switchgrass resulted in an exponential increase in N_2O emissions, which can negate the climate change mitigation benefits of this bioenergy crop (Ruan et al., 2016). Additionally, reduced N fertilizer requirements for TLI-C2 offers economic incentives for adoption of this perennial crop.

Lodging

Grain yield declines at high N rates were likely a result of lodging (Supplemental Fig. S1). During the first year of N fertilization (2013), we observed substantial lodging at the 160 and 200 kg N ha^{-1} fertilization rates. As expected, grain yields were lower in plots with higher lodging scores at N fertilization rates of 80 kg N ha^{-1} and above (spearman correlation = −0.75; $P < 0.001$). Lodging generally occurred between boot stage and heading, which could have interfered with grain development. As observed in cereal grains, lodging can decrease grain yields by

disrupting pollination and/or reducing photosynthesis as a result of shading from lodged plants (Mulder, 1954). The possible physiological changes associated with lodging at high N rates include (i) increased length of culm internodes–which reduces the plant's ability to support developing seed heads under environmental forces such as wind or rain, and/or (ii) increased plant canopy development–which leads to shading and etiolation of basal culms (Mulder, 1954; Pinthus, 1973). After accounting for effects of N fertilizer, we found that plant height did not explain additional variation in lodging for most seeding date/year combinations (Supplemental Table S1). Surprisingly, plant height was negatively correlated with lodging score (b = −0.08; $P < 0.001$) at the spring-seeded locations in 2013, which contradicts the experimental results from Mulder (1954). This suggests that IWG plant height may not be the major physiological change in response to N fertilization that contributes to lodging, and that selecting for shorter plants to breed for lodging resistance may not be successful. Instead, stem diameter or stem composition may also be an important cause of lodging.

Lodging trends in response to N rates were similar among the Rush and TLI-C2 IWG crops (Supplemental Fig. S1), however lodging scores were often higher in Rush compared to TLI-C2 when fertilized at AONRs (Fig. 2A). Switchgrass
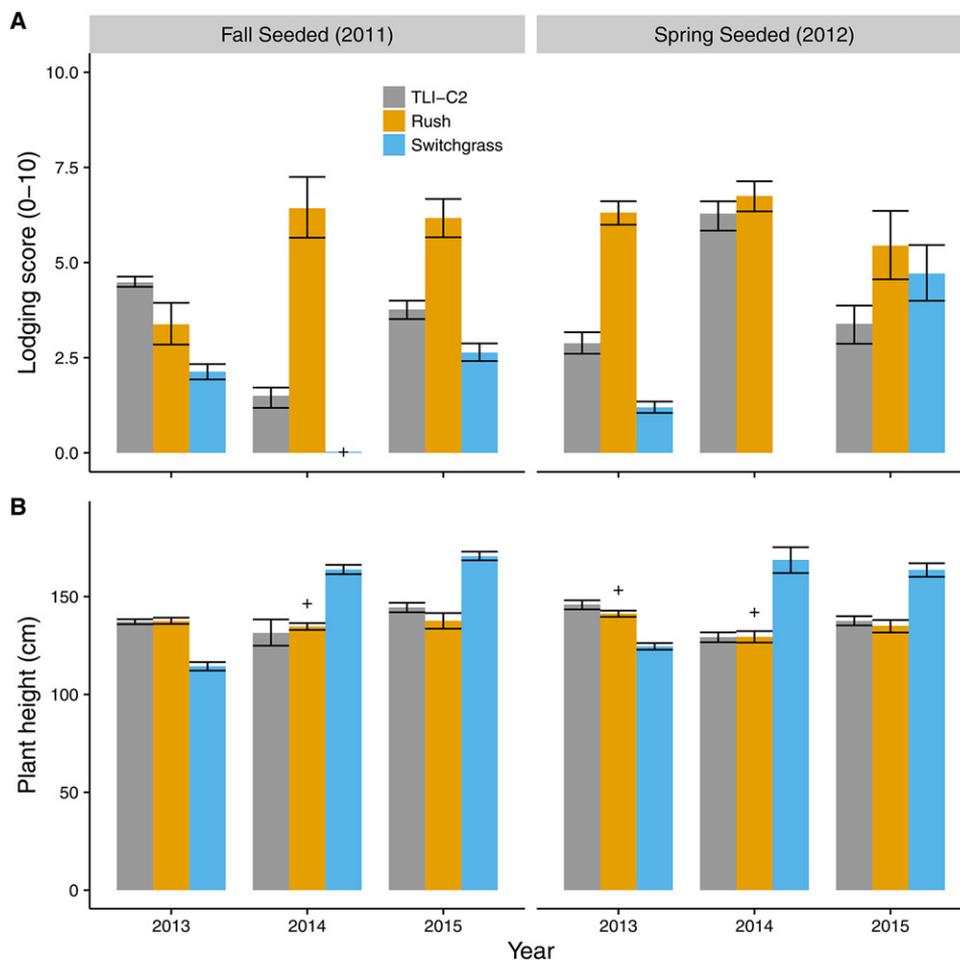

Fig. 2. (A) Estimated lodging score and (B) plant height from TLI-C2 intermediate wheatgrass when fertilized with agronomically optimum nitrogen rates (AONR) for grain production, and Rush and switchgrass when fertilized with AONR for biomass production. When response variables were not affected by N fertilizer, the mean value across all N fertilizer rates is presented and indicated by +. For environments where AONR was not calculated (see Table 2), the mean response variable was averaged for the 0 (no yield response to N fertilizer) or highest (linear yield response to N fertilizer) N fertilizer rate.

had low lodging scores compared to IWG during the first 2 yr of the study when fertilized at AONRs (Fig. 2A). By 2015, the effect of N fertilization on switchgrass lodging increased (Supplemental Fig. S1). Unlike IWG, lodging was minimal for switchgrass at N fertilizer rates exceeding AONRs (Supplemental Fig. S1), even though N requirements for switchgrass maximum biomass yield were met in 2013 and 2014 (as indicated by a quadratic response to N fertilizer; Table 2). The mechanisms driving lodging at high N fertilizer rates in IWG does not appear to be acting on switchgrass during the first 2 yr of production.

Changes in Grain Yield with Stand Age

Grain yields of TLI-C2 declined substantially with stand age (Table 2). At the fall-seeded locations, unfertilized stands were harvested for grain in 2012, whereas spring-seeded stands grew vegetatively and did not produce grain. Unfertilized grain yields from the fall-seeded locations in 2012 averaged 848 kg ha^{-1} (SE = 35.9). All locations produced high grain yields in 2013, and then experienced grain yield declines in 2014 and 2015 (Table 2). Since the fall-seeded locations produced an additional year of high grain yields before the yield decline, we recommend that IWG grown for grain in the Upper Midwest be planted in fall.

Grain yield persistence has been a major challenge for the development of perennial crops, and of the limited data available, most current perennial crops (including other studies testing IWG) show yield declines with stand age (Vico et al., 2016). Other studies also have reported IWG grain yield declines after the first 2 yr of production (Weik et al., 2002; Lee et al., 2009). It is unclear why grain yields of TLI-C2 declined with stand age. Grain yield declines for other perennial crops have been attributed to mortality (Larkin et al., 2014; Vico et al., 2016). Although we did not measure plant population or ground cover in this study, our observations suggest that population density did not decrease, an observation supported by the relatively stable biomass yields with stand age (Table 2, Supplemental Fig. S2). Instead, we observed fewer seed heads per plant, which resulted in a decrease in the harvest index with stand age in the spring (F = 172.4; P < 0.001) and fall seeded locations (F = 462.5; P < 0.001; Supplemental Fig. S3). Based on anecdotal observations, we hypothesize that grain yields declined as a function of increasing plant density. As stands aged, the inter-row space became occupied with new individuals from either shattered seed or rhizome recruitment. If stands are increasing in plant density, individual plants could be expressing the shade avoidance syndrome in response to a decrease in red/far red light ratio. A decrease in red/far red ratio with plant density has been shown to reduce grain yield and harvest index in spring rapeseed (Rondanini et al., 2014), maize (Maddonni and Otegui, 2006), and wheat (Ugarte et al., 2010). Prolonging grain yields might also be possible by reducing plant density via establishment in wider rows. Lawrence (1980) found that seed yields of the perennial grass *Elyus angustus* (Trin) were higher in wider rows, and that the difference in grain yields between wide and narrow rows increased with stand age. Research on the effects of plant density and light quality on grain yield are needed to discern potential mechanisms driving yield declines with stand age.

Biomass Yield and Quality

Biomass yields of TLI-C2 were comparable to crop varieties bred for forage (Rush) and bioenergy (switchgrass) markets (Table 2). Prior to 2015, biomass yields of TLI-C2 were similar to yields of switchgrass when managed at AONR for grain yield, and similar or greater than biomass yields of Rush (Table 2). This suggests that breeding efforts for increased grain yield have not changed other physiological traits related to biomass yield. Biomass from IWG can be sold into forage or bioenergy markets as an additional revenue stream for producers interested in growing TLI-C2 for grain. It is expected that price premiums from the sustainability attributes of TLI-C2 grain will improve the economic viability of this new crop; however revenue from harvested residue is likely to be essential to the profitability of producing improved IWG for grain. Although biomass quantity is a major driver of revenues for forage and bioenergy sales, biomass quality is also of concern. The IVTD values were significantly higher for IWG compared to switchgrass (Fig. 3C). The predicted relative feed value (RFV) of TLI-C2 biomass at AONR ranged from 68 to 80, which was comparable to Rush (range at AONR: 70–78). Hay with RFV of less than 130 is categorized as "utility", which receives the lowest price at auction (National Agricultural Statistics Service, 2016).

Biomass yields of Rush responded quadratically to increasing N rates during all years (Table 2). The AONRs for Rush biomass ranged from 81.1 to 120.5 kg N ha^{-1} and were highest (and most variable based on 95% CIs) during the last year of the study. The predicted AONR in 2013 for the fall-seeded locations was negative and thus nonsensical. The quadratic biomass yield response of Rush to N was driven by the strong decline in yield with high N rates (Supplemental Fig. S2).

Switchgrass biomass yields responded to N rates quadratically during the first year of fertilization when N rates up to 200 kg N ha^{-1} were applied (Table 2). The AONRs for switchgrass during the first year of fertilization were 127.8 and 149.7 kg N ha^{-1} for the spring- and fall-seeded locations, respectively; and CIs were relatively small for these estimates (Table 2). In subsequent years, when the N fertilizer range was decreased (0–160 kg N ha^{-1}), switchgrass yields plateaued only at the fall-seeded locations, and the AONR was lower during these years compared to the first year of fertilization. The CIs for AONR estimates were largest in 2014 and 2015, indicating that the altered N fertilizer range (0 to 160 kg N ha^{-1}) was not wide enough to capture a reliable estimate of AONR for switchgrass biomass production. Other studies have estimated AONRs for switchgrass ranging from 49 to 168 kg N ha^{-1} in Illinois (Anderson et al., 2013) and 140 kg N ha^{-1} in Iowa (Heggenstaller et al., 2009), which is in the range of our AONR estimates during the first year of N fertilization.

Switchgrass biomass yields ranged from 11.6 to 15.9 Mg ha^{-1} at AONRs ranging from 81.1 to 149.7 kg N ha^{-1} (Table 2). Switchgrass yields were slightly larger than a national average of 10.9 Mg ha^{-1} estimated from a review of 324 published reports (Wang et al., 2010), and greater than switchgrass yields in Minnesota when fertilized with optimum N rates (Jungers et al., 2015b) and long-term yields when fertilized with 67 kg N ha^{-1} (Jungers et al., 2015a).
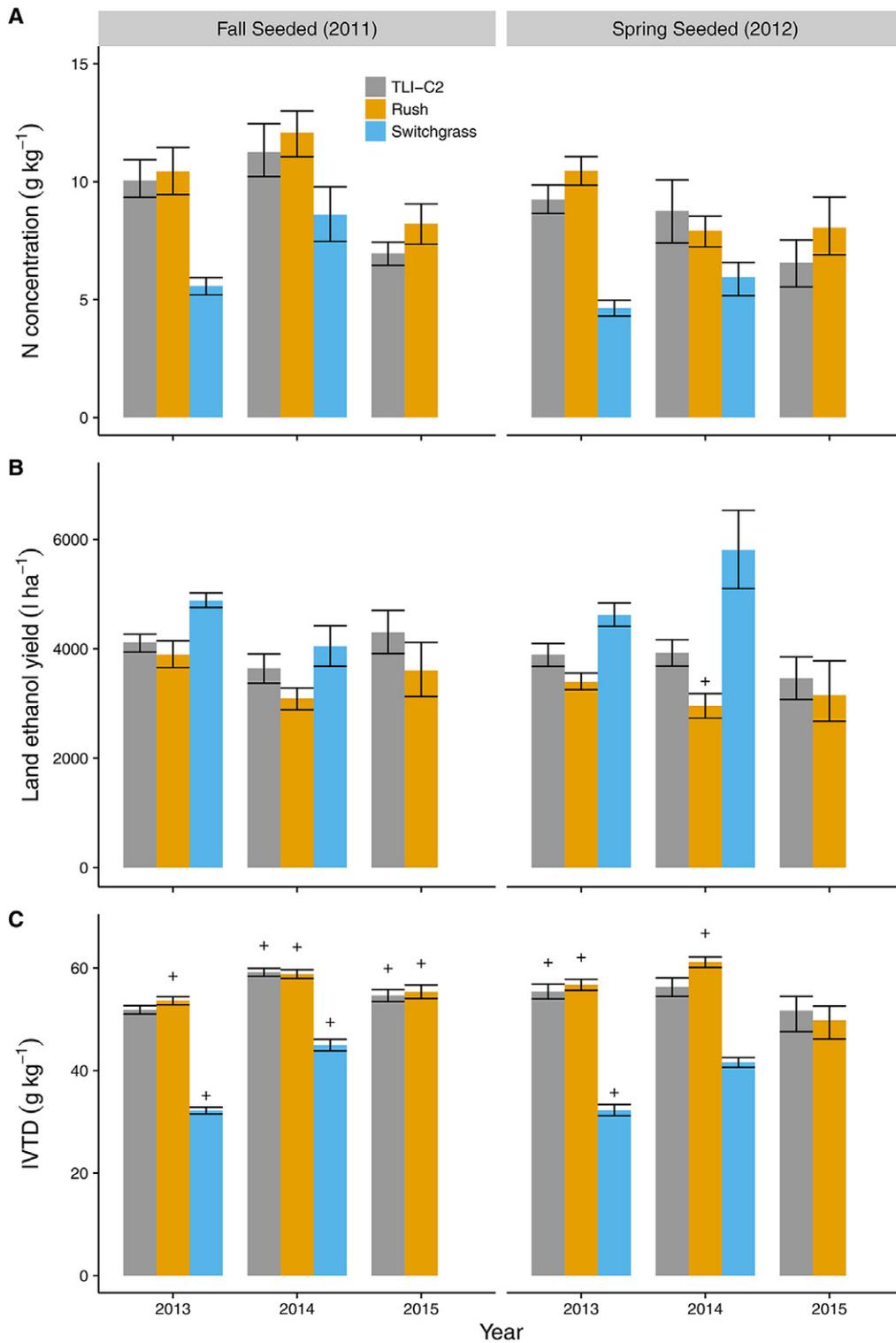
Fig. 3. (A) Nitrogen concentration, (B) land ethanol yield, and (C) in vitro total digestibility (IVTD) of biomass harvested from TLI-C2 intermediate wheatgrass when fertilized with agronomically optimum nitrogen rates (AONR) for grain production, and Rush and Switchgrass when fertilized with AONR for biomass production. When response variables were not affected by N fertilizer, the mean value across all N fertilizer rates is presented and indicated by +. For environments where AONR was not calculated (see Table 2), the mean response variable was averaged for the 0 (no yield response to N fertilizer) or highest (linear yield response to N fertilizer) N fertilizer rate.

Theoretical ethanol potential of switchgrass when managed at AONR for biomass yield ranged from an estimated 362 to 401 L Mg^{-1}, which is less than other reported values (Schmer et al., 2012; Jarchow et al., 2012; Jungers et al., 2015b). When measured on an area basis, ethanol yields were greater for switchgrass compared to TLI-C2 and Rush (Fig. 3B). The higher ethanol potential of switchgrass was not surprising since C4 grasses often have higher concentrations of hemicellulose compared to C3 grasses like IWG (Jarchow et al., 2012). Time of harvest may also have contributed to differences in ethanol potential between IWG and switchgrass. Rush and TLI-C2 biomass was harvested immediately after grain harvest, while switchgrass was harvested multiple weeks after seeds had shattered and plants senesced. Harvesting switchgrass when it was more mature compared to IWG likely resulted in greater concentrations of lignocelluloses, which in turn increased the ethanol potential of the switchgrass crop (Waramit et al., 2011). The difference in harvest timing may have also resulted in the difference in forage quality between the IWG and switchgrass crops. Switchgrass biomass harvested after seed set had lower ADF and higher IVTD values compared to biomass harvested after a frost (Guretzky et al., 2010).

CONCLUSION

Perennial crops provide a suite of environmental benefits compared to annuals, but markets are limited for most of the perennial crops currently available. Harvesting grain for direct human consumption from a perennial crop could increase its economic value, thus leading to increased land area and subsequent environmental improvements. We found that grain yields of a new population of intermediate wheatgrass bred for grain production–TLI-C2–were substantially larger than yields of the forage intermediate wheatgrass variety Rush; suggesting that breeding efforts to increase grain yields of this perennial crop have succeeded. To maximize grain yields in the Upper Midwest, TLI-C2 should be seeded in fall and fertilized with between 60 and 100 kg N ha^{-1} annually after the first year. Two agronomic challenges were confirmed and require further investigation to determine mechanisms and solutions: (i) lodging occurred at moderate to high N rates, which was associated with lower grain yields and (ii) grain yields declined with stand age. We show that residual biomass removed after grain harvest is consistently high yielding and has potential for forage compared to the dedicated forage and bioenergy crops Rush and switchgrass; therefore potentially reducing the economic risks for adopting farmers if sold as a secondary source of revenue.

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