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Perennial rye as a grain crop in Alberta, Canada: Prospects and challenges

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Abstract

Perennial crops may present an opportunity to produce grain in a more environmentally and economically friendly manner. We examined principal agronomic traits of perennial cereal rye (*Secale cereale* L. × *S. montanum* Guss 'ACE-1') at two field sites in Alberta, Canada, over two consecutive growing seasons. Treatments included perennial rye, fall rye (*S. cereale* L. 'Hazlett'), spring rye (*S. cereale* L. 'Gazelle'), and perennial forage (meadow brome [*Bromus commutatus* Schrad.] and alfalfa [*Medicago sativa* L.]) with and without N fertilizer addition. Grain yield of the perennial rye in Year 1 averaged 64 and 51% of the fall and spring rye yields at the Breton and Edmonton sites, respectively. Grain yield of the perennial rye in Year 2 at the Edmonton site averaged 42% of the fall and spring rye. Perennial rye at the Breton site in Year 2 was subject to competition with weeds, resulting in minimal grain productivity. Perennial rye at the Edmonton site yielded significantly more aboveground biomass (without grain) than the other rye crops over both years. Likewise, perennial rye at the Breton site produced 1.5 times more aboveground biomass than the perennial forage in Year 1. The experiment was terminated after virtually nonexistent regrowth at both sites in the spring after two growing seasons. Overall, perennial rye may be an option as a dual-purpose forage-grain crop, however, perennial rye cropping beyond 2 yr faces issues of winter survival and weed competition; hence, multi-year perennial rye cropping is not yet a feasible option for cold temperate conditions.

1 | INTRODUCTION

Novel perennial grain crops are of great interest due to their purported ability to rectify several environmental challenges originating from modern agricultural production while continuing to deliver food products (Glover, Reganold, et al., 2010; Ryan et al., 2018). Annual monocrops are often associated with adverse environmental effects such as the loss of soil physical quality, reduced biodiversity, emissions of greenhouse gases and substantial erosion (Jaikumar et al., 2012; Ryan et al., 2018; Zhang et al., 2011). Current efforts to breed perennial grain crops can be divided into two approaches, direct domestication and wide hybridization (T. Cox et al., 2006). Wide hybridization is effectively a shortcut of direct domestication, wherein a wild perennial is crossed with a compatible annual grain and their progeny are selected for

Abbreviations: HI, harvest index; IWG, intermediate wheatgrass; NDVI, normalized difference vegetation index; NHI, N harvest index; NIR, near infrared; NUE, N use efficiency; PE, physiological efficiency; TKW, thousand kernel weight; UE, uptake efficiency.

perenniality (Acharya et al., 2004; Reimann-Philipp, 1995). Higher grain yields from perennial grains developed via hybridization relative to those developed via direct domestication make them more comparable to annual grain crops (Jaikumar et al., 2012; Newell & Hayes, 2018; Ryan et al., 2018). Irrespective of these breeding approaches, current perennial grain crops undergoing development include rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), sorghum [*Sorghum bicolor* (L.) Moench] and intermediate wheatgrass (IWG) (Ryan et al., 2018).

Currently, perennial grain crops do not exist in any considerable commercial sense, as their profitability is a fundamental consideration for producers and is often a driver of management decisions (Hayes et al., 2012). Perennial crops must produce comparable grain yields or offset yield losses by increased aboveground biomass (i.e., vegetative growth that does not include grain, henceforth referred to as just "biomass") for forage and/or by reducing fertilizer input costs. Seed yield and allocation to reproductive structures is typically viewed as being lesser in perennial crops than their annual counterparts, due in part to competing resource sinks within perennial plants and the fact that annual crops have been selected for yield gains for much longer (Bell et al., 2008; Jaikumar et al., 2012; Ploschuk et al., 2005). However, there is potential for considerable yields in perennial crops, and studies have shown that grain yield can be increased while preserving the perenniality of the new cultivars (T. Cox et al., 2006; Moffat, 1996). Additionally, protein contents of grain and biomass, which may differ in a perennial grain relative to an annual counterpart, are necessary considerations relevant to overall quality for human and animal consumption (Newman et al., 2009; Nuttall et al., 2017). Finally, increased fertilizer nitrogen use efficiency (NUE) in perennial crops has the potential to counterbalance high fertilizer costs. An economic assessment by Bell et al. (2008) found that, if used as a dual-purpose grain and forage crop with reduced fertilizer inputs relative to an annual grain crop, perennial wheat could be a profitable option for Australian producers in areas of poor or intermediate soil quality.

Previous research efforts suggest that perennial crops can utilize N more efficiently than annual counterparts potentially due to beneficial relationships with microorganisms in the soil, increased root mass and length, longer growing seasons, internal recycling of N resources, or a combination of the above (Dawson et al., 2008; Glover, Culman, et al., 2010; Lewandowski & Schmidt, 2006). However, a lack of published literature exists to date regarding the NUE of a perennial grain cultivar, as well as how the crop allocates N between vegetative and reproductive structures compared to an annual counterpart based on efficiency metrics such as the N harvest index (NHI), physiological efficiency (PE), and uptake efficiency (UE). Sprunger et al. (2018) found that regardless of N fertilizer application rate, IWG had greater whole plant NUE than annual wheat due to the perennial's greater root mass and enhanced uptake of soil N. However, it is noteworthy that IWG is not a true perennial counterpart of an annual grain, but instead a domesticated forage.

Core Ideas

- Perennial rye grain yield in Year 1 averaged 58% of the annual rye crop yields.
- Grain yield of perennial rye was substantially reduced in Year 2.
- Grain protein productivity of perennial rye can match that of spring rye.
- Abundant tillering of perennial rye may be an opportunity to improve grain yield.
- Winter mortality and weed pressure can undermine multi-year perennial rye cropping.

Finally, further research into how perennial grain crops allocate resources to different plant yield components as well as crop harvest index (HI), a common measure of yield physiology, relative to an annual counterpart can inform future breeding goals as well as the feasibility of incorporating perennial grain crops into long, diversified rotations (Wiebe et al., 2016). Other important considerations for researchers and producers alike include winter survival, spring regrowth, and lodging susceptibility of perennial grain crops. These seasonality aspects and agronomic considerations are crucial to the successful implementation of perennial cropping in temperate regions worldwide where these novel production systems could be affected by harsh winter conditions and early snowfall events (Fowler, 2012; Fowler et al., 1989).

To understand the agronomic potential of a perennial grain cultivar, it must be studied over multiple consecutive years, as production may change with stand age (Jaikumar et al., 2012). Therefore, multi-year field trials were designed and implemented at two sites in central Alberta, Canada (Edmonton and Breton, 2 yr each) to gather essential agronomic information on ACE-1 perennial rye, a model perennial grain cultivar. Perennial rye was selected based on preliminary findings from Lethbridge, AB, summarized by Hayes et al. (2018), who reported on the superior performance of ACE-1, relative to several perennial wheat cultivars. This study is the first of its kind to compare a perennial grain with analogous spring (annual) and fall (biennial) grain, utilizing spring rye (cultivar Gazelle) and fall rye (cultivar Hazlett), respectively. As well, a perennial forage crop typical of the area (meadow brome [*Bromus commutatus* Schrad.] and alfalfa [*Medicago sativa* L.]) was included in the experimental design to compare the potential of perennial rye as a dual-purpose forage-grain crop. The objectives of this study were to

TABLE 1 Baseline soil properties at the Edmonton and Breton field sites from 0-to-30-cm depth

Soil properties	Site	
	Edmonton	Breton
Canadian classification	Black Chernozem	Gray Luvisol
Total C (g C kg^{-1}), 0–30 cm	41.6 ± 7.5	19.2 ± 3.9
Total N (g N kg^{-1}), 0–30 cm	3.6 ± 0.5	1.7 ± 0.3
Available N ($NH_4^+ + NO_3^-$) (mg N kg^{-1}), 0–15 cm[a]	55.5 ± 2.5	48.3 ± 4.5
pH (1:5 H_2O), 0–30 cm	7.3 ± 0.09	6.1 ± 0.08
Bulk density (g cm^{-3}), 5–30 cm	1.0 ± 0.06	1.1 ± 0.06
Soil texture, 0–30 cm	Clay	Loam
Percentage clay, %	48.3	24.8
Percentage silt, %	35.7	41.8
Percentage sand, %	16.0	33.3

[a]Available N samples obtained from the Edmonton and Breton sites on 1 May 2018.

assess yearly biomass and grain yields and compare the 2nd year perennial rye yield components to those of annual and biennial counterparts (spring rye and fall rye, respectively). Additionally, we assessed the protein productivity, HI, NHI, NUE, PE, and UE and the survival, competitiveness and lodging susceptibility of perennial rye compared to spring and fall rye with contrasting growth habits. Overall, the objective of this study was to determine the possibility for perennial rye cropping in central Alberta, where long, cold winters and short growing seasons have limited perennial crop production to highly cold-hardy species (Fowler, 2012).

2 | MATERIALS AND METHODS

2.1 | Sites and experimental design

Field sites were established in Edmonton, AB, Canada (53°29′43.33″, 113°31′59.24″) and Breton, AB, Canada (53°5′16.72″, 114°26′29.35″). Soils at the Edmonton site are a clay texture and are classified as Orthic Black Chernozems. Soils at the Breton site are a loam texture and are classified as Orthic Grey Luvisols, according to the Canadian System of Soil Classification (Table 1). Mean annual air temperature at the Edmonton and Breton sites is 4.2 and 3.4 °C, respectively, with average yearly precipitation of 446 and 479 mm, respectively (Environment Canada, 2020). Hourly temperature and precipitation data was obtained for both sites from permanent weather stations within 1 km of the experimental plots at both sites.

Both sites were arranged in an identical randomized complete block design consisting of four replicates and eight treatments per block replicate. Treatment structure consisted of two factors, crop (4), and N fertilizer application (2). Crop type consisted of three contrasting grain crop growth habits (perennial rye grain cultivar ACE-1, fall rye grain cultivar Hazlett, spring rye grain cultivar Gazelle) and a perennial forage crop (meadow brome and alfalfa) (Table 2). Within each block replicate, two plots of each cropping treatment were seeded, with one receiving no N fertilizer and one receiving 56 kg N ha^{-1} yr^{-1} in the form of a urea and polymer-coated urea (i.e., environmentally smart nitrogen [ESN]) blend (2:1 ratio) (henceforth referred to as "unfertilized" and "fertilized", respectively). This rate was chosen using preliminary soil test results obtained in May 2018 and the Alberta Farm Fertilizer Information and Recommendation Manager (Government of Alberta, 2021). Each experimental plot measured 8 m in length and 4 m in width, for plots totaling 32 m^2.

2.2 | Plot management

Plot management activities, which varied between crops and sites, are summarized in Supplemental Table S1. The perennial and fall rye were seeded at 90 kg ha^{-1} with a 23-cm row spacing to a depth of 2.5 cm. The spring rye treatment was seeded at 60 kg ha^{-1} with a 23-cm row spacing to a depth of 2.5 cm. For all rye treatments, 15 kg P ha^{-1} in the form of phosphate was placed with the seed. Perennial forage treatments were broadcast seeded at 55 kg ha^{-1} and incorporated. The N fertilizer was broadcasted at the aforementioned rate on the selected plots concurrent with the spring rye seeding every year. Broadleaf weeds were controlled using a combination of Stellar XL herbicide (Corteva Agriscience) applied at 0.9 L ha^{-1} using a backpack sprayer and hand weeding.

2.3 | Field measurements

Grain and biomass yields were measured by hand harvesting 1-m lengths of two adjacent rows at two locations within each replicated plot, at least 1 m from the plot edges. The harvested material was then bagged, threshed, weighed, and oven dried until a constant weight was reached for determination of grain and biomass dry matter. Specific to the Breton site in Year 2, dried plant material from the perennial rye plots was sorted after drying, prior to the final weigh, to differentiate perennial rye biomass from weed growth.

Plant yield components including tiller count and kernels per spike were assessed in Year 2 by counting each component within three 1-m lengths in each grain plot. The purpose of the counts was to characterize the yield components of perennial rye, relative to an annual counterpart. This was done on 30 May 2019, 5 July 2019, 23 July 2019 and 29 Aug. 2019 at the Breton site and 31 May 2019, 11 July 2019, 17 July 2019, 30 July 2019, 13 Aug. 2019, and 28 Aug. 2019 at the Edmonton site.

TABLE 2 Detailed description of crops at Edmonton and Breton field sites (adapted from Kim et al., 2021)

Crop	Description
Perennial rye	Perennial rye crop for grain production (*Secale cereale* L. × *S. montanum* Guss cultivar ACE-1).
Spring rye	Spring rye crop for grain production (*S. cereale* L. cultivar Gazelle). Annual rye or summer rye are alternative designations in the literature.
Fall rye	Fall rye crop for grain production (*S. cereale* L. cultivar Hazlett). Winter rye or biennial rye are alternative designations in the literature.
Perennial forage	Perennial forage crop for hay production. Alfalfa (*Medicago sativa* L.) and bromegrass (*Bromus* spp.) Aboveground biomass is cut and removed two times a year for hay for livestock feeding purposes.

Lodging estimates were completed as per the method described by Caldicott and Nuttall (1979). A square meter quadrat was delineated and marked with flags. A wooden stake was driven into the soil, perpendicular to the soil surface. Proportions of leaning (5–45° from vertical), lodged (45–85° from vertical), and lodged flat (85–90° from vertical) crop were recorded and the lodging index was determined as follows:

$$\begin{aligned}\text{Lodgingindex} &= 1/3\,(\text{percentage area leaning})\\ &\quad + 2/3\,(\text{percentage area lodged})\\ &\quad + (\text{percentage area lodged flat})\end{aligned} \tag{1}$$

Crop stage was assigned based on the Crop Identification and Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBCH) Staging Manual by Lancashire et al. (1991). Because of our focus on perennial cropping, phenology staging was done biweekly during the growing season 2019 in all rye plots by observing leaf counts, tillering, flag leaves, and spikelets.

Normalized difference vegetation index (NDVI) readings were taken early in the growing season (June–July) after fertilizer application to detect differences between the fertilized and unfertilized counterparts for each crop. Measurements of NDVI were taken using a Trimble Greenseeker Handheld Crop Sensor (Vantage Canada) with a plumb bob hung 30 cm from the sensor to ensure measurements were taken consistently from the same height above the growing crop. Three readings were taken from each plot at random to account for spatial differences.

2.4 | Laboratory analyses

Grain and biomass protein were determined using a FOSS DS2500 (Foss Analytics) near infrared (NIR) spectroscope. Samples were scanned from 400 to 2,500 nm as whole grain samples or ground biomass samples using a large product cup with a removable top. To create an NIR calibration curve for each grain and biomass, samples harvested in the 1st year of cropping from each experimental plot were ground and encapsulated in tin capsules (Elemental Microanalysis) and total N was determined using dry combustion in a Flash 2000 Organic Elemental Analyzer (ThermoScientific). Model calibration statistics including R^2, standard error (SE) and standard error of prediction (SEP) for grain, biomass, and perennial forage biomass are as follows: R^2 = .94, SE = .07, SEP = .11; R^2 = .94, SE = .05, SEP = .06 and R^2 = .91, SE = .17, SEP = .24, respectively. Conversion from N concentration into protein concentration was done by multiplying N content by the widely used Jones' Factor of 6.25 (Jones, 1931).

2.5 | Calculations and statistical analyses

Using the dry matter (DM) weight and total N content determination from NIR spectroscopy, HI, grain N partitioning (NHI), fertilizer–NUE in grain production, UE, physiological efficiency (PE), and protein productivity were estimated by comparing each rye treatment with fertilization, to their respective controls (no added N) as follows (Hernandez-Ramirez et al., 2011; Thilakarathna et al., 2020):

$$\text{aboveground HI} = \frac{\text{grain yield}}{\text{grain yield} + \text{aboveground biomass} + \text{residue}} \tag{2}$$

$$\text{NUE of grain} = \frac{\text{grain DM}_{@\text{Nrate}} - \text{grain DM}_{\text{control}}}{\text{N fertilizer rate}} \tag{3}$$

$$\text{UE} = \frac{\text{aboveground N accumulation}_{@\text{Nrate}} - \text{aboveground N accumulation}_{\text{control}}}{\text{N fertilizer rate}} \tag{4}$$

$$\text{PE} = \frac{\text{grain DM}_{@\text{Nrate}} - \text{grain DM}_{\text{control}}}{\text{aboveground N accumulation}_{@\text{Nrate}} - \text{aboveground N accumulation}_{\text{control}}} \tag{5}$$

$$\text{aboveground NHI} = \frac{\text{grain N}}{\text{aboveground biomass N} + \text{residue (stubble) N} + \text{grain N}} \tag{6}$$

$$\text{protein productivity} = \text{protein content} \times \text{biomass DM yield} \; \textit{or} \; \text{grain DM yield} \quad (7)$$

All statistical analyses were performed using version 1.1.383 of R Studio software (R Core Team, 2020). Data normality was tested by the Shapiro–Wilk test, homogeneity of variance was tested by the Bartlett test and plot functions. Non-normality and heteroscedasticity were corrected, when necessary, using a Box–Cox transformation. Significant differences between fertilized and unfertilized NDVI readings were determined using Welch's Two-Sample T Tests. Biomass yield, grain yield, HI, NHI, tiller count, kernel count, thousand kernel weight (TKW), grain protein, biomass protein, grain protein productivity, and biomass protein productivity were analyzed using two-way ANOVA tests with crop and fertilizer as fixed effects. The NUE, PE, and UE were tested using one-way ANOVA with crop as the fixed effect. In some cases, when Box–Cox transformations did not rectify heteroscedasticity, Welch's ANOVA was used in place of ANOVA. All analyses were tested at alpha critical level of .05, and Tukey's Honest Significant Difference from the agricolae (Version 1.3-3) package was used for post-hoc comparisons of means (de Mendiburu, 2019). To assess if grain and biomass yields of the perennial rye were significantly different in Year 2 compared to Year 1, a repeated measures analysis was completed with fertilizer and year as fixed effects, plot ID as the random effect and a first order autoregressive correlation structure to account for temporal autocorrelation.

3 | RESULTS

3.1 | Weather conditions

At the Edmonton site, temperatures were generally similar to the 30-yr average, except for a colder September 2018 and February 2019 (Figure 1a). In September 2017 and 2018, precipitation was greater than the 30-yr average by 39 and 51%, respectively. Conversely, May 2018 and 2019 experienced less rainfall than usual. Conversely, June 2019 was increasingly wet, with a 54% increase in precipitation this month (Figure 1b).

Monthly air temperature at the Breton site deviated from the 30-yr monthly average (1980–2010) in May 2018, which was slightly warmer and September 2018, February 2018, and February 2019, which were all colder than average (Figure 2a). Average monthly precipitation greatly differed from the 30-yr average for the 2018–2019 (Year 2) growing season, as conditions were substantially wetter in the months of June and July (Figure 2b).

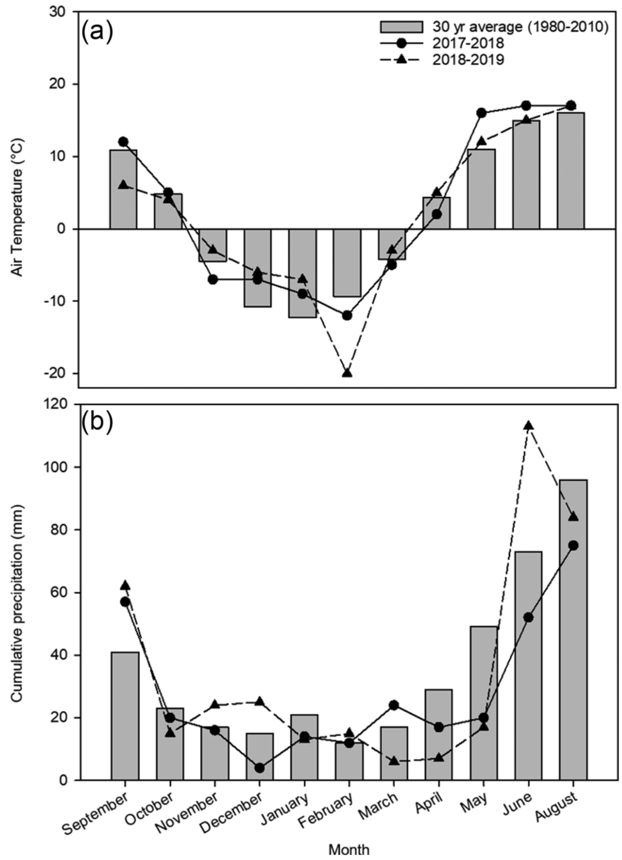

FIGURE 1 (a) Temperature and (b) precipitation obtained from Alberta Information Service (ACIS, 2020) for the Edmonton site for Year 1 (2017–2018) and Year 2 (2018–2019) of the field experiment

3.2 | Yield and yield components

3.2.1 | Year 1

At the Edmonton site, crop was the only factor affecting grain yield in Year 1 ($p < .001$), whereas fertilizer and the interaction of fertilizer and crop were insignificant (Table 3). Perennial rye had lower grain yield than both fall and spring rye, yielding 46 and 56% of fall and spring rye, respectively. In terms of biomass, the perennial rye crop had greater biomass ($p < .001$) than the other rye crops but did not differ from the perennial forage. Harvest index was affected by crop alone ($p < .001$). The HI ranked from lowest to highest was as follows: perennial rye < spring rye < fall rye (Table 3). Likewise, for TKW at the Edmonton site, the only significant factor was crop ($p < .001$). On average, TKW was lowest in perennial rye (31.8 ± 0.5 g) and greater in fall (33.0 ± 0.4 g) and spring rye (35.9 ± 0.5 g) (Table 4).

Crop type was the only significant factor determining grain yield at the Breton site in Year 1, similar to the Edmonton site (Table 3). Perennial rye yields were the lowest; both

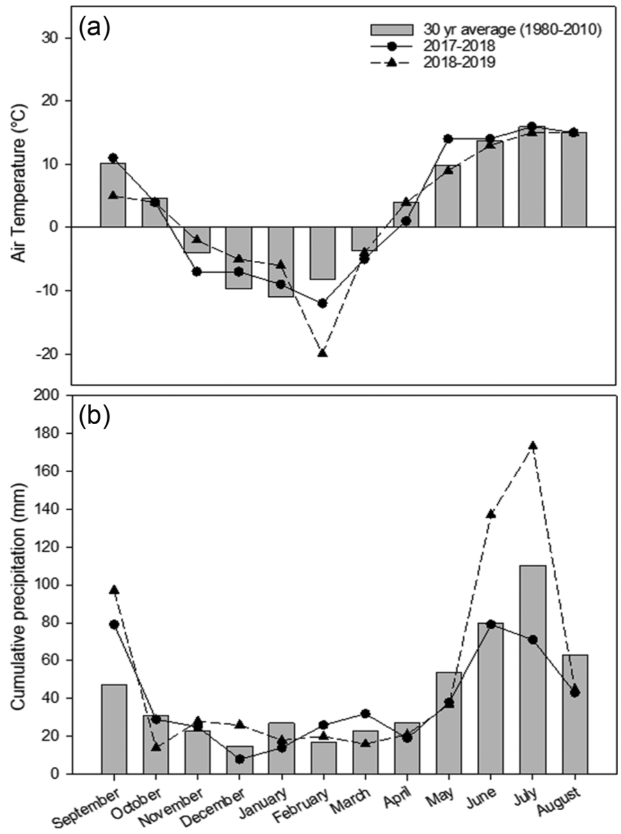

FIGURE 2 (a) Temperature and (b) precipitation obtained from Alberta Information Service (ACIS, 2020) for the Breton site for Year 1 (2017–2018) and Year 2 (2018–2019) of the field experiment

spring and fall rye had greater grain yields ($p < .001$). There was no effect of fertilizer on grain yield for any treatment, nor an interaction between crop and fertilizer. On average, perennial rye yield was 52 and 64% of fall and spring rye yield, respectively. Similarly, crop type was the only factor determining biomass at the Breton site in Year 1 ($p < .001$). Neither fertilizer, nor the interaction of crop and fertilizer was significant. Notably, the greatest biomass productivity was from the perennial rye crop and the lowest from the perennial forage crop. The perennial rye crop produced more than 1.5 times more biomass than the perennial forage plots. Analysis of HI indicated an effect of crop ($p < .05$), but not fertilizer nor the interaction of fertilizer and crop. No difference in HI between perennial rye and fall rye was found, but perennial rye HI was significantly lower than that of spring rye (Table 3).

Breton TKW was affected by the interaction between crop and fertilizer ($p < .05$), due to the increase in TKW in fall rye when no N was applied. Crop type was also significant ($p < .001$), but fertilizer was not. Consequently, fall rye without N addition had the highest TKW (39.1 ± 0.6 g), whereas perennial rye TKW (31.8 ± 0.5 g, on average) did not differ from any of the other treatments (Table 4).

3.2.2 | Year 2

Grain yield at the Edmonton site was affected by crop ($p < .01$) (Table 3). Perennial rye produced, on average, 38 and 46% of the grain yields of fall and spring rye, which were not different from one another. Conversely, perennial rye at the Edmonton site in Year 2 produced more biomass than all the other crops ($p < .001$). Specifically, the perennial rye produced 68% more biomass than the perennial forage crop, on average. Neither fertilization, nor the interaction of crop and fertilizer were significant. Differences in HI were found for all crops ($p < .001$), with perennial rye having the lowest HI values and fall rye the highest (Table 3).

Notably, perennial rye had more total tillers per plant than the other crops, but less kernels per spike ($p < .001$ and $p < .001$), which had a lower TKW than both fall and spring rye crops ($p < .001$). Each yield component had no effect of fertilizer, nor was an interactive effect detected. Estimated productive tillers as a percentage of total tillers was substantially reduced in the perennial rye crop treatments relative to the spring and fall rye. Notably, the proportion of productive tillers in the perennial rye crop was on average less than half of that of the spring rye crop (Table 4).

Grain yield quantification was challenging at the Breton site in Year 2 for the perennial rye treatments, due to strong competition from weeds. Only certain areas of the plot with perennial rye dominance were sampled when possible, thus grain yield measurements from Breton in Year 2 are not an accurate mean estimate and are included solely to demonstrate that grain production is possible for consecutive seasons (Table 3). For fall rye and spring rye, both crop and fertilization affected grain yield ($p < .05$ and $p < .05$), but their interaction did not. Spring rye had greater grain yield than fall rye, and unfertilized plots yielded less grain than their fertilized counterparts did. Biomass yield was affected by crop alone ($p < .001$). No differences were discerned between contrasting rye growth habits (i.e., perennial, fall, and spring), however, all three rye crops produced more biomass than the perennial forage.

Perennial rye had a greater number of total tillers, relative to the other two rye crops, similar to the Edmonton site ($p < .001$). However, perennial rye had a lower number of kernels per spike ($p < .001$). Notably, none of the aforementioned yield components were affected by fertilization nor the interaction between fertilization and crop. Representative TKW data was not possible for perennial rye at the Breton site in Year 2, due to lack of sample collection in all the field replicates. No difference in TKW was found between the fall rye or spring rye crops. Again, estimated productive tillers were much lower for the perennial rye crop relative to the fall and spring rye crops, with the average proportion of productive tillers relative to the total being only 43% of the spring rye crop (Table 4).

TABLE 3 Dry matter (DM) of aboveground biomass yield (without grain), grain yield, and harvest index (HI) for perennial rye, fall rye, spring rye crops, and perennial forage at the Edmonton and Breton sites for 2018 and 2019

Crop + fertilization	Grain yield Edmonton	Grain yield Breton	Biomass yield (aboveground biomass without grain) Edmonton	Biomass yield (aboveground biomass without grain) Breton	HI Edmonton	HI Breton
	kg DM ha^{-1}				kg grain DM kg^{-1} grain and biomass DM	
	Year 1					
Perennial grain + N	2,170 ± 130 Aa	2,810 ± 190 Aa	2,980 ± 190 Aa	8,370 ± 470 Aa	0.43 ± 0.02 Aa	0.19 ± 0.05 Aa
Perennial grain + 0 N	2,450 ± 160 Aa	3,190 ± 220 Aa	3,550 ± 190 Aa	9,880 ± 190 Aa	0.41 ± 0.008 Aa	0.17 ± 0.06 Aa
Fall grain + N	4,890 ± 240 Ba	5,640 ± 280 Ba	2,050 ± 110 Ca	6,450 ± 310 ABa	0.71 ± 0.007 Ba	0.31 ± 0.1 ABa
Fall grain + 0 N	5,120 ± 320 Ba	5,840 ± 180 Ba	2,100 ± 150 Ca	6,570 ± 420 ABa	0.71 ± 0.005 Ba	0.30 ± 0.1 ABa
Spring grain + N	4,030 ± 190 Ba	3,520 ± 60 Ca	2,480 ± 100 BCa	5,960 ± 190 Ba	0.62 ± 0.005 Ca	0.36 ± 0.004 Ba
Spring grain + 0 N	4,140 ± 270 Ba	3,610 ± 180 Ca	2,320 ± 140 BCa	6,180 ± 300 Ba	0.64 ± 0.008 Ca	0.36 ± 0.006 Ba
Perennial forage+ N	na	na	2,500 ± 190 ABa	3,470 ± 280 Ca	na	na
Perennial forage + 0 N	na	na	3,020 ± 380 ABa	3,750 ± 320 Ca	na	na
	Year 2					
Perennial grain + N	860 ± 40 Aa	860[a]	6,500 ± 370 Aa	4,050 ± 720 Aa	0.12 ± 0.01 Aa	[a]
Perennial grain + 0 N	1,040 ± 170 Aa	500[a]	9,000 ± 1250 Aa	4,000 ± 630 Aa	0.11 ± 0.02 Aa	[a]
Fall grain + N	2,430 ± 400 Ba	2,220 ± 270 Aa	3,320 ± 430 Ba	4,940 ± 380 Aa	0.39 ± 0.02 Ba	0.31 ± 0.03 Aa
Fall grain + 0 N	2,600 ± 150 Ba	1,820 ± 170 Ab	3,650 ± 140 Ba	4,350 ± 410 Aa	0.42 ± 0.01 Ba	0.30 ± 0.02 Aa
Spring grain + N	2,070 ± 240 Ba	2,870 ± 180 Ba	4,400 ± 360 Ca	5,870 ± 90 Aa	0.33 ± 0.01 Ca	0.33 ± 0.01 Aa
Spring grain + 0 N	2,060 ± 210 Ba	2,130 ± 73 Bb	4,990 ± 380 Ca	4,770 ± 250 Aa	0.29 ± 0.01 Ca	0.31 ± 0.01 Aa
Perennial forage+ N	na	na	2,340 ± 190 Da	2,620 ± 220 Ba	na	na
Perennial forage + 0 N	na	na	2,600 ± 159.0 Da	2,640 ± 260 Ba	na	na

Note. na, not applicable.Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column ($\alpha = .05$).

[a]Breton: Year 2 perennial rye values are not representative due to sampling bias but are included to demonstrate grain production possibility in the absence of significant weed pressure. Only one replication of grain yield for each fertilizer treatment was possible, thus no standard errors are presented and perennial rye was not included in statistical analyses.

TABLE 4 Yield components for perennial rye, fall rye and spring rye crops for Year 2 at the Edmonton and Breton sites

Crop + fertilization	Tiller count	Kernel count	Thousand kernel weight	Estimated productive tillers[a]
	no. per plant	no. per spike	g	% of total
	Edmonton			
Perennial rye + N	17.4 ± 1.2 Aa	28.3 ± 2.8 Aa	27.4 ± 0.3 Aa	12.5
Perennial rye + 0 N	17.3 ± 1.7 Aa	25.8 ± 1.3 Aa	27.7 ± 0.6 Aa	15.2
Fall rye + N	6.4 ± 0.1 Ba	34.0 ± 1.4 Ba	28.5 ± 0.4 Ba	28.4
Fall rye + 0 N	6.4 ± 0.2 Ba	34.7 ± 1.3 Ba	29.6 ± 1.0 Ba	26.4
Spring rye + N	5.6 ± 0.3 Ca	37.0 ± 1.1 Ba	31.3 ± 0.2 Ca	33.9
Spring rye + 0 N	6.1 ± 0.2 Ca	38.0 ± 1.0 Ba	31.9 ± 0.1 Ca	30.5
	Breton			
Perennial rye + N	8.6 ± 0.5 Aa	28.4 ± 1.9 Aa	31.0[b]	25.3
Perennial rye + 0 N	7.8 ± 0.6 Aa	28.4 ± 1.9 Aa	30.9[b]	17.4
Fall rye + N	5.0 ± 0.1 Ba	33.0 ± 1.1 Ba	30.6 ± 1.9 Aa	50.3
Fall rye + 0 N	6.1 ± 0.9 Ba	33.2 ± 1.0 Ba	34.1 ± 0.6 Aa	35.8
Spring rye + N	3.8 ± 0.1 Ca	36.0 ± 1.1 Ba	30.6 ± 0.7 Aa	61.4
Spring rye + 0 N	3.8 ± 0.1 Ca	35.2 ± 0.9 Ba	30.4 ± 1.3 Aa	47.4

Note. Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column ($\alpha = .05$).
[a]Estimated productive tillers per plant was calculated as grain productivity (g m^{-2})/[kernel weight (g) × kernel count (no. per spike) × plant count (plants m^{-2})].
[b]Breton: Year 2 had minimal grain productivity. Perennial rye values are not included in the statistical analysis as only one replication for each fertilizer treatment was possible, thus no standard errors are presented.

3.2.3 | Changes in perennial rye grain and biomass yields from year 1 to year 2

Year was found to be a significant factor when assessing the capability of a 2-yr-old perennial rye crop to maintain grain and biomass yields (Supplemental Table S2). Year was a factor for grain yield at the Edmonton site from Year 1 to Year 2. Grain yield showed a decline in the second season of perennial rye growth ($p < .001$). There was no effect of fertilizer, nor was there an interactive effect of year and fertilizer. Biomass yield was affected by year at both Edmonton and Breton sites ($p < .001$ and .01, respectively). The Edmonton site showed increases in biomass from Year 1 to Year 2, whereas the Breton site showed a decline from Year 1 to Year 2.

3.3 | Grain and biomass protein and protein productivity

At the Edmonton site, both grain protein and biomass protein concentrations were solely dependent on crop in both Year 1 ($p < .001$ and $p < .001$) and Year 2 ($p < .001$ and $p < .001$) (Table 5). Grain protein of the perennial rye was higher than the other rye growth habits (fall and spring rye) for both years. Predictably, biomass protein productivity was highest for the perennial forage plots for both years. In Year 1, perennial rye had the second greatest biomass protein productivity, which was greater than spring rye. In Year 2, no discernable differences were detected across the three rye crops in terms of biomass protein (Table 5).

Grain protein productivity was lower in the perennial rye plots for both Years 1 and 2 at the Edmonton site, based on crop type alone, and no differences were detected between the fall and spring rye crops ($p < .001$ and .01). Similar to the Breton site, biomass protein productivity for perennial rye in both years was greater ($p < .001$ and .01) than the other rye crops, but lower than the perennial forage plots. In Year 2 this trend held for fall and spring rye, however, no difference between perennial rye and perennial forage was established in terms of biomass protein productivity. No effect of fertilizer, nor the interaction of crop and fertilizer, was found for the Edmonton site for any of the protein concentration or protein productivity calculations in either year (Table 5).

At the Breton site, both grain protein and biomass protein concentrations were solely dependent on crop in Year 1 ($p < .001$ and $p < .001$) and Year 2 ($p < .05$ and .001, respectively). Grain protein content, from highest to lowest in Year 1: perennial rye > fall rye > spring rye. Fall rye was higher in protein than spring rye. As expected, biomass protein for both Years 1 and 2 was highest for the perennial forage plots. Notably, in Year 1, perennial rye biomass had the second greatest biomass protein after the perennial forage crop; but switched to having the lowest biomass protein in Year 2 (Table 5).

With respect to grain protein productivity at the Breton site, a crop effect ($p < .001$) was detected at the Breton

TABLE 5 Grain and aboveground biomass protein for perennial rye, fall rye, spring rye crops, and perennial forage

Crop + fertilization	Grain protein		Biomass protein		Grain protein productivity		Biomass protein productivity	
	Edmonton	Breton	Edmonton	Breton	Edmonton	Breton	Edmonton	Breton
	%				kg protein ha^{-1}			
	Year 1							
Perennial rye + N	18.4 ± 0.1 Aa	16.7 ± 0.2 Aa	7.5 ± 0.2 Aa	5.1 ± 0.4 Aa	400 ± 20 Aa	470 ± 30 Aa	220 ± 20 Aa	430 ± 50 Aa
Perennial rye + 0 N	18.4 ± 0.2 Aa	16.7 ± 0.3 Aa	7.5 ± 0.3 Aa	5.0 ± 0.4 Aa	450 ± 30 Aa	530 ± 30 Aa	260 ± 20 Aa	490 ± 40 Aa
Fall rye + N	13.9 ± 0.2 Ba	12.6 ± 0.3 Ba	6.3 ± 0.3 ABa	4.3 ± 0.4 Ca	680 ± 30 Ba	710 ± 40 Ba	130 ± 10 Ba	270 ± 20 Ba
Fall rye + 0 N	13.9 ± 0.2 Ba	12.5 ± 0.2 Ba	6.4 ± 0.3 ABa	4.1 ± 0.2 Ca	710 ± 50 Ba	730 ± 20 Ba	130 ± 10 Ba	270 ± 14 Ba
Spring rye + N	15.1 ± 0.3 Ca	14.5 ± 0.3 Ca	5.5 ± 0.2 Ba	4.6 ± 0.2 ACa	610 ± 30 Ba	510 ± 10 Aa	140 ± 10 Ba	280 ± 10 Ba
Spring rye + 0 N	15.2 ± 0.6 Ca	14.6 ± 0.5 Ca	5.5 ± 0.2 Ba	4.7 ± 0.2 ACa	630 ± 60 Ba	530 ± 30 Aa	130 ± 10 Ba	290 ± 30 Ba
Perennial forage+ N	na	na	14.6 ± 1.4 Ca	11.7 ± 0.9 Ba	na	na	370 ± 40 Ca	400 ± 40 Aa
Perennial forage + 0 N	na	na	15.5 ± 0.6 Ca	10.2 ± 0.6 Ba	na	na	470 ± 50 Ca	490 ± 40 Aa
	Year 2							
Perennial rye + N	14.1 ± 0.2 Aa	16.0[a]	5.4 ± 0.2 Aa	5.2 ± 1.3 Aa	130 ± 10 Aa	140[b]	350 ± 20 Aa	230 ± 40 Aa
Perennial rye + 0 N	13.7 ± 0.6 Aa	17.2[a]	4.9 ± 0.3 Aa	3.9 ± 0.04 Aa	150 ± 30 Aa	90.0[b]	440 ± 60 Aa	160 ± 30 Ab
Fall rye + N	13.2 ± 0.7 Ba	13.8 ± 0.5 Aa	5.5 ± 0.3 Aa	6.5 ± 0.4 Aa	280 ± 40 Ba	300 ± 30 Aa	180 ± 30 Ba	320 ± 10 Ba
Fall rye + 0 N	13.1± 0.5 Ba	13.7 ± 0.4 Aa	5.7 ± 0.6 Aa	6.6 ± 0.4 Aa	330 ± 20 Ba	250 ± 20 Aa	220 ± 10 Ba	280 ± 10 Bb
Spring rye + N	12.1 ± 0.3 Ba	13.4 ± 0.1 Ba	4.1 ± 0.3 Aa	5.3 ± 0.3 Aa	250 ± 20 Ba	370 ± 20 Aa	230 ± 20 BCa	380 ± 10 Ba
Spring rye + 0 N	12.3 ± 0.4 Ba	12.8 ± 0.1 Ba	5.5 ± 0.3 Aa	6.0 ± 0.6 Aa	250 ± 20 Ba	270 ± 10 Aa	280 ± 30 BCa	250 ± 30 Bb
Perennial forage+ N	na	na	13.1 ± 0.8 Ba	12.2 ± 0.9 Ba	na	na	310 ± 20 ACa	320 ± 20 Ba
Perennial forage + 0 N	na	na	12.8 ± 0.3 Ba	11.8 ± 1.4 Ba	na	na	330 ± 30 ACa	320 ± 30 Bb

Note. na, not applicable. Uppercase letters denote significant differences between crops based upon post hoc analysis after ANOVA, lowercase letters indicate significant differences between fertilizer application levels. The same letters indicate no significant differences within column; different letters indicate significant differences within column ($\alpha = .05$).

[a]Breton: Year 2 had minimal grain productivity. Perennial rye values are not included in the statistical analysis as only one replication for each fertilizer treatment was possible, thus no standard errors are presented.

site in Year 1. Perennial rye showed no difference in protein productivity from spring rye, but both perennial and spring rye were lower than fall rye. In Year 2, there was a clear reduction in perennial rye protein productivity as a result of severely reduced grain yield. No differences between the spring and fall rye were discerned. Conversely, when considering biomass protein productivity, perennial rye had greater protein productivity than the other rye crops but did not differ from the perennial forage ($p < .001$). In Year 2, biomass protein productivity was affected by crop ($p < .001$) as well as fertilizer ($p < .01$). Perennial rye plots had lower biomass protein productivity than all other crops (when encompassing grain and forage), and fertilized treatments had greater biomass protein productivity than those without fertilizer addition (Table 5).

3.4 | Nitrogen use efficiency metrics: NUE, UE, PE, and NHI

Neither site showed differences in NUE between grain crops in Year 1. Correspondingly, no numerical trends in NUE were discernible due to high variability in the dataset for both Edmonton and Breton. Notably, only the Edmonton site in Year 2 showed significance ($p < .05$), wherein perennial rye had greater NUE than fall rye but was not different from spring rye (Supplemental Table S3).

Similarly, no differences in UE were discernible between rye crops at both sites in Year 1, and all treatments showed a low or even negative uptake efficiency. For both years at the Edmonton site, perennial rye showed no difference from either the fall or spring treatments, despite the UE being numerically higher (Supplemental Table S3).

Consistently, no differences in PE in Year 1 at either site could be discerned. However, despite statistical insignificance, perennial rye at the Breton site showed an apparent reduction in PE relative to spring rye in Year 1. Perennial rye at the Edmonton site in Year 2 had lower PE than fall rye ($p < .01$), but was not different than spring rye, despite being markedly diminished (Supplemental Table S3).

Perennial rye consistently had the lowest numerical NHI across years and sites, but this reduction was not statistically significant at the Breton site in Year 1. Conversely, at the Edmonton site in Year 1, NHI was lower for the perennial rye than the fall and spring rye ($p < .001$). This trend held consistent at the Edmonton site in Year 2, where the perennial rye had lower NHI than the other two rye crops ($p < .001$) (Supplemental Table S4). Overall, differences in NHI were dependent on crop type alone, meaning fertilizer application did not affect NHI for any crop type in any year.

3.5 | Canopy greenness: NDVI

Few differences were discerned between fertilized and unfertilized counterparts of the same crop over both growing seasons (Figures 3 and 4). At the Edmonton site, fertilized fall rye had significantly higher NDVI than in the corresponding unfertilized fields on two of the sampling dates over both Years 1 and 2 of the study ($p < .05$ and $p < .05$). However, in Year 2 the difference between fertilized and unfertilized fall rye became insignificant later in the season. In Year 2 at the Edmonton site, fertilized spring rye exhibited higher NDVI than unfertilized spring rye ($p < .05$). At the Breton site, only perennial forage in Year 2 showed higher NDVI due to the fertilizer addition and on only one sampling date.

Phenological differences between the spring rye crop and the perennial and fall rye crops can be seen in the NDVI measurements for Year 1 (Figure 3). A trend of declining NDVI values for the perennial and fall rye crops are indicative of declining leaf area index (LAI), whereas the spring rye crop shows a trend of increasing LAI over the measurement period for each site.

3.6 | Staging

Detailed staging was completed for both sites in Year 2 (Figure 5). No differences were discerned between fertilized and unfertilized plots of the same crop, thus only crop is shown as a factor. At both sites, the perennial rye matured faster than the spring rye, maintaining a significant lead in maturity over most of the growing season, until all rye crops reached similar maturity in late August 2019.

3.7 | Susceptibility to lodging

Lodging measurements were done when visual evidence of lodging was apparent at either site. This corresponded to two dates at the Breton site, 19 Sept. 2018 and 22 July 2019. Substantial lodging in the perennial rye and spring rye plots was observed in 2018 after an early autumn snowfall prior to harvest of the aforementioned plots. The lodging indices for the perennial rye + N and the spring rye + N were 68.5 ± 2.7 and 97.9 ± 0.8%, respectively. Conversely, when crop standability measurements were done on 22 July 2019, lodging was only recorded in the perennial rye + N plots with no evidence of lodging in the other rye crops. The average lodging index of the perennial rye + N was 65.2 ± 6.1%.

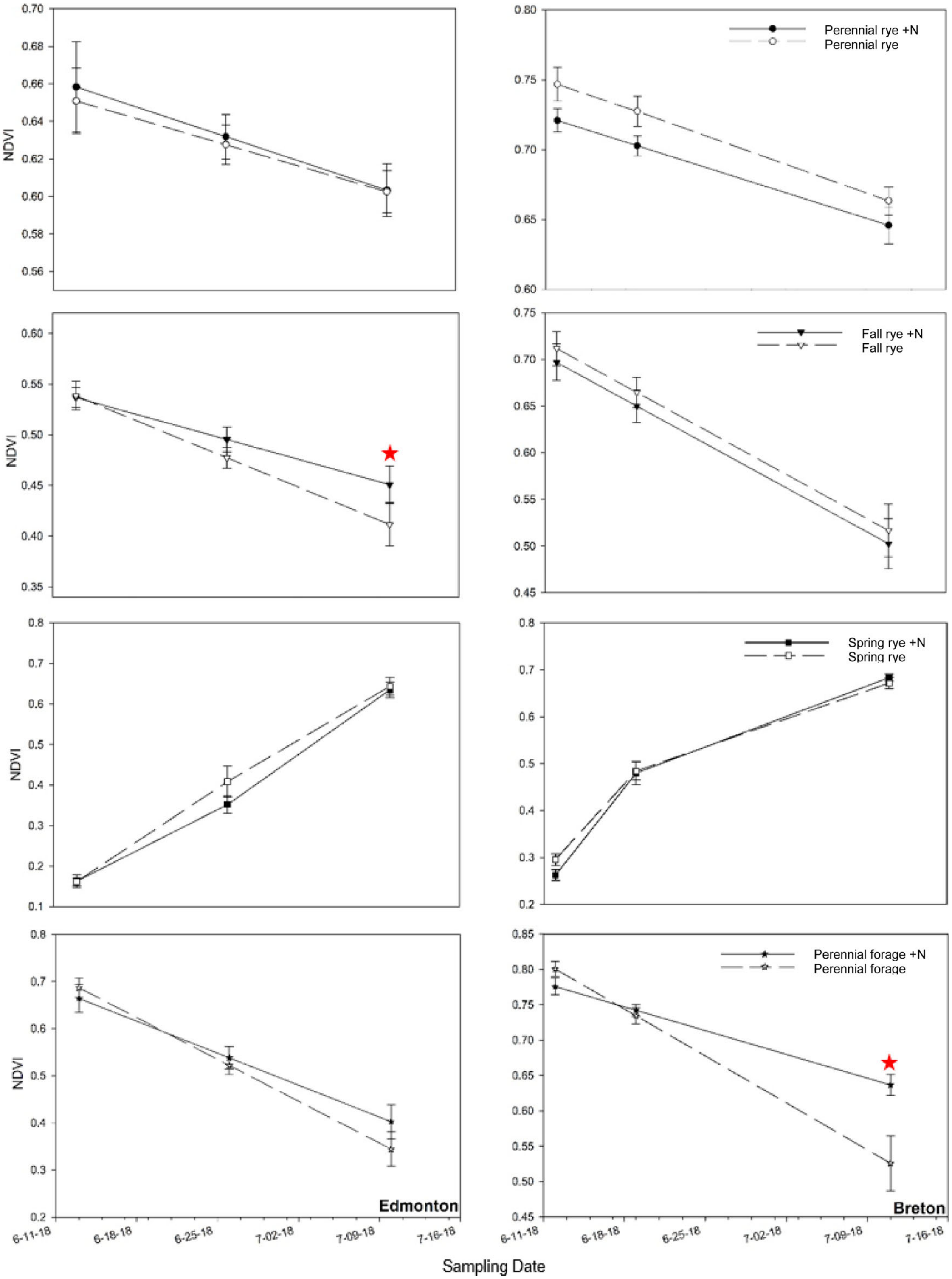

FIGURE 3 Normalized difference vegetation index (NDVI) measurements of (circles) perennial rye, (triangles) fall rye, (squares) spring rye, and (stars) perennial forage at the (left) Edmonton and (right) Breton sites for both (filled symbols) fertilized and (unfilled symbols) unfertilized treatments in 2018. Red stars indicate significant differences between the fertilized and unfertilized counterparts of each crop type based on a Welch's two sample *t* test (α = .05). Note the different *y* axis scales across panels

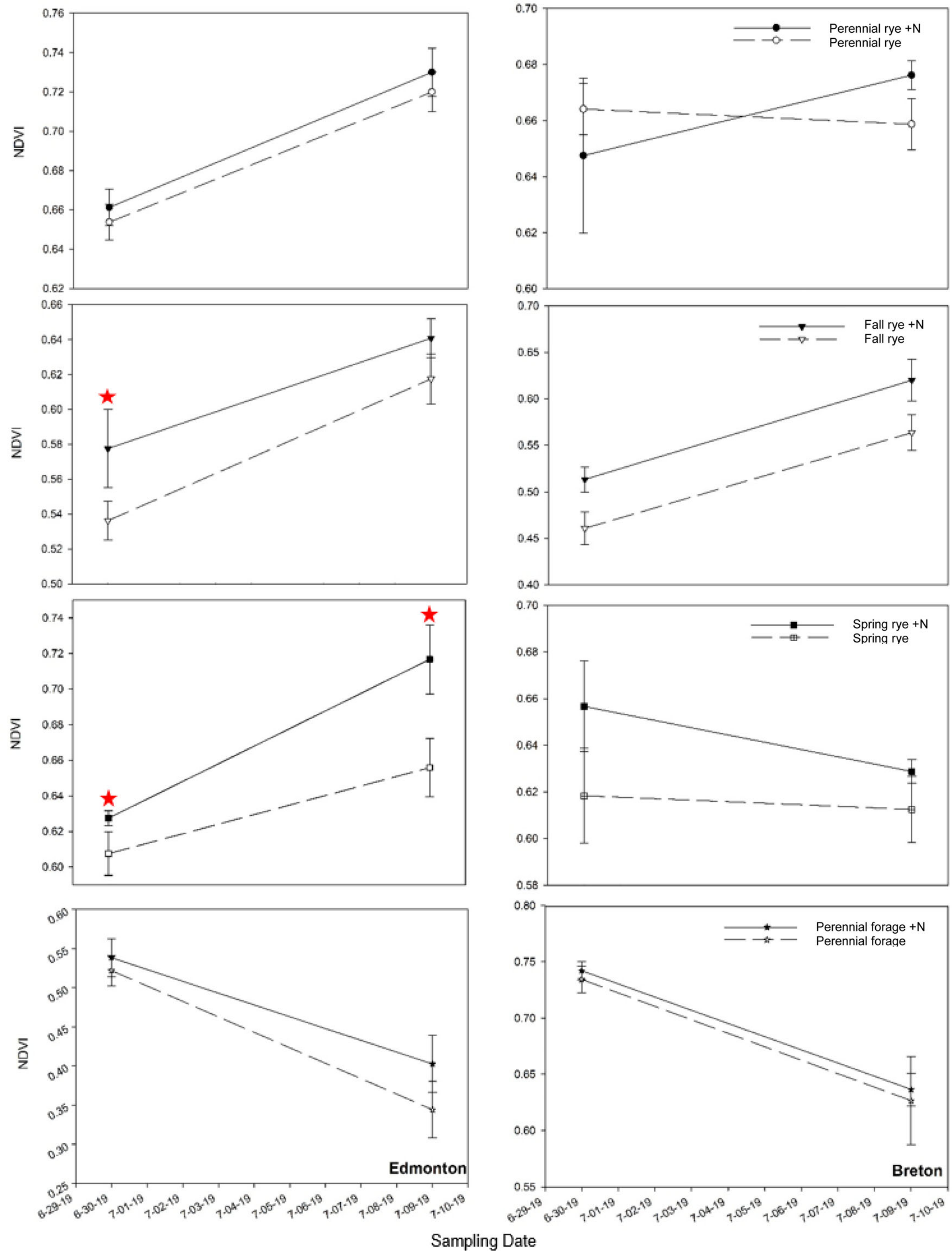

FIGURE 4 Normalized difference vegetation index (NDVI) measurements of (circles) perennial rye, (triangles) fall rye, (squares) spring rye, and (stars) perennial forage at the (left) Edmonton and (right) Breton sites for both fertilized (filled symbols) fertilized and (unfilled symbols) unfertilized treatments in 2019. Red stars indicate significant differences between the fertilized and unfertilized counterparts of each crop type based on a Welch's two sample t test ($\alpha = .05$)

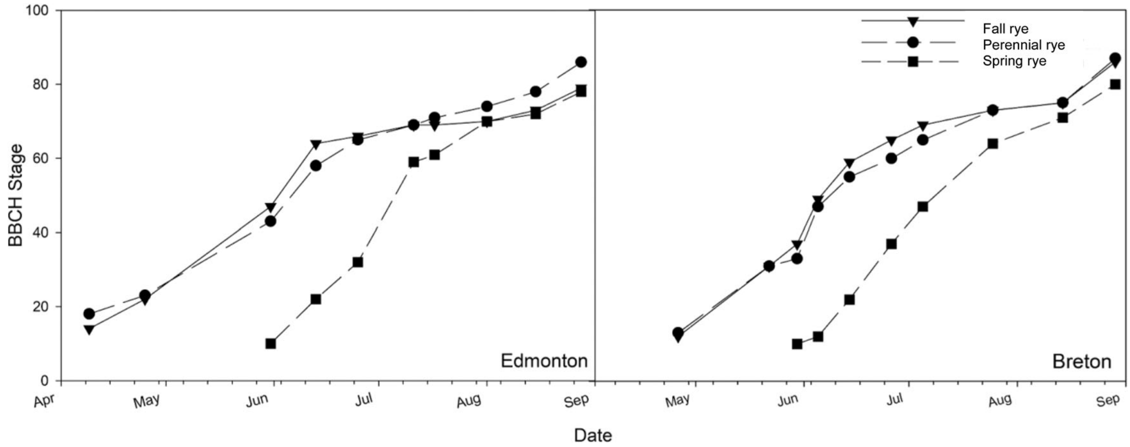

FIGURE 5 Perennial rye, fall rye and spring rye staging based on the BBCH staging manual for Year 2 (2019) for the (left) Edmonton and (right) Breton sites

3.8 | Competitiveness of perennial rye crops

Weed pressure from the soil seed bank in the perennial rye plots at the Breton site in Year 2 resulted in significantly reduced plant density and yield of the perennial rye. Specifically, only 40 ± 5% of perennial rye plots were composed of perennial rye, on average. At the Breton site, the remaining ∼60% of the plant matter in perennial rye plots was comprised of timothy (*Phleum pratense* L.) (10 ± 3%), white clover (*Trifolium repens* L.) (12 ± 3%), ryegrass (*Lolium perenne* L.) (2 ± 10%) and various unidentified perennial grasses (36 ± 4%). Notably, the prevalence of perennial grasses hindered attempts to control them with herbicides due to the risk of damaging the perennial rye crop itself.

3.9 | Winter survival

Winter survival of the perennial rye crop was 50 and 48% in the fertilized and unfertilized plots in Year 1 at the Edmonton site, and 58 and 52% for the fertilized and unfertilized plots in Year 1 at the Breton site, respectively. After the second winter, survival of the perennial rye crop at the Edmonton site in Year 2 was highly diminished, with only 31 and 24% of the plants remaining in the fertilized and unfertilized plots. Winter survival at the Breton site in Year 2 showed an increase from Year 1, with 61% of the original plant count for both fertilized and unfertilized plots. However, at the time of this plant count early in the growing season, we were unable to differentiate between the various grass species detailed in the section entitled Staging and therefore there is a high probability that the winter survival of the perennial rye in Year 2 at the Breton site was much lower. In Year 3, the experiment was concluded because perennial rye crop exhibited negligible survival at the Breton site and no survival at the Edmonton site.

4 | DISCUSSION

4.1 | Yield potential of a perennial rye crop

The perennial rye crop at both the Edmonton and Breton sites showed decreased grain yield relative to fall and spring rye, which is consistent with earlier reports (Table 3) (Cattani, 2019; DeHaan & Van Tassel, 2014; Hayes et al., 2018). Yield reduction in the perennial rye crop in this instance can be attributed to reduced kernels per spike and TKW for both sites in the 1st and 2nd years of cropping (Table 4). Reduced grain yield in perennials is the result of natural selection, as energy is allocated to structures such as roots and stems that increase competitiveness and longevity over seeds, thus seed size in perennial plants is generally smaller than in annuals (S. Cox et al., 2018; DeHaan et al., 2005; Wagoner & Schaeffer, 1990). Notably, Huang et al. (2018) reported no declines in yield in a perennial rice cultivar, PR23, when grown at several sites in China and Laos, indicating that the trade-off between perenniality and yield is not definite, and thus continued research can improve perennial grain prospects (T. Cox et al., 2006). Preliminary research on the model perennial chosen for this study, ACE-1 perennial rye, also suggested issues with floret fertility and chromosome pairing during meiosis may reduce yield in tetraploid varieties of perennial rye, such as ACE-1 (Acharya et al., 2004; Hayes et al., 2018). However, diploid varieties of perennial rye, such as Reimann–Phillip, have shown improved spike fertility, suggesting that informed breeding efforts can reduce sterility (Hayes et al., 2018. Overall, annual crops have the advantage of intensive, long-term breeding efforts whereas perennial grains are a relatively new breeding endeavor, with much of the current research being led by The Land Institute in Kansas since the early 1990s (T. Cox et al., 2002; Jackson & Jackson, 1999).

Although this study did not directly measure productive vs. unproductive tillers in the field, we conducted post hoc estimations of the proportion of productive tillers (Table 4). These back-calculations indicated that the proportion of productive tillers in perennial rye was substantially reduced relative to the spring and fall rye crops. This illustrates why the increased total tiller count of the perennial rye crop was not conducive to increased rye yield in this crop. The majority of perennial rye tillers did not bear grain, which is a common attribute in perennial rye crops (Cattani, 2019; Wagoner & Schaeffer, 1990). These findings present an opportunity for the development of breeding goals and should inform future research into perennial rye improvement, whereby perennial rye crop yield can be optimized by selecting offspring that confer increased yields by trading off with reduced unproductive tillering.

A major concern with perennial grain crops is a decline in grain yield with increasing stand age (Jungers et al., 2018; Murphy et al., 2010; Pimentel et al., 2012; Ploschuk et al., 2005). This is consistent with our results, as the grain yield of the perennial rye crop declined in Year 2 relative to Year 1 at the Edmonton site (Supplemental Table S2). This is in contrast to a study by Jaikumar et al. (2012) that found no reductions in grain yield between 1-yr-old and 2-yr-old perennial rye and wheat. We hypothesize that this may be the result of different perennial rye cultivars used (Rival rye vs. ACE-1 rye), or the result of environmental conditions that favored vegetative growth over grain production in our experiment, as evidenced by grain yield reductions in both spring and fall rye crops in Year 2 as well. Notably, both sites experienced overall decreases in grain yield across all grain treatments, potentially a result of the abnormally cold and wet growing season at both sites in Year 2 (Figures 1 and 2) (ACIS, 2020). Colder temperatures affect seed filling, ultimately reducing grain yield and increased precipitation can diminish yields, specifically in rye crops (Mantri et al., 2012; Peltonen-Sainio, Hakala, et al., 2011). Thus, Year 2 reductions in grain yield may have been the result of the specific environmental conditions of this growing season, instead of perennial rye genetic shortfalls.

An earlier economic analysis by Bell et al. (2008) ascertained that a perennial grain crop could be profitable if it produced 40% of the grain yield of an analogous annual crop and the harvested biomass was used as forage. According to Bell et al. (2008), both the Breton site and the Edmonton site in Year 1 may be profitable despite averaging only 55% grain yield of the other grain crops. In Year 2, the Edmonton site barely achieved this threshold, averaging 42% of the other crops' grain yield. While grain yield in Year 2 was minimal at the Breton site, we hypothesize that had the Breton perennial rye plots not experienced such substantial reductions in yield as a result of poor competition with weed growth, reductions in grain yield could still have materialized, due to colder-than-average fall and winter conditions that may have resulted in winter damage and consequently reduced grain yield (Peltonen-Sainio, Hakala, et al., 2011). Notably, the economic analysis by Bell et al. (2008) accounted for reduced fertilizer and seeding costs of a perennial grain as well as the reduced market price, but neglected the potential benefits of reduced erosion, increases in soil organic matter, and other ecosystem services that are postulated with the adoption of perennial grains (Ryan et al., 2018). Further, the analysis by Bell et al. (2008) estimated a set price for perennial grain, which in reality would be subject to change depending on markets and grain quality. Thus, a more detailed economic analysis is still required to fully capture these several externalities and uncertainties to conclude if a perennial grain crop is profitable in the long term.

Aboveground biomass yields of perennial rye at the Edmonton and Breton sites in Year 1 were the greatest of all treatments including the perennial forage, regardless of fertilization (Table 3). This is partially the result of greater tillering in perennial rye relative to the other rye crops (Table 4). Several studies have emphasized that prolific biomass production is a consistent trait of many perennial grasses (Acharya et al., 2004; Fedenko et al., 2013; Shinners et al., 2010). Notably, this can present the breeding opportunity for reallocation of this assimilated C away from unproductive tillers to grain via breeding as mentioned above (T. Cox et al., 2006; Jaikumar et al., 2012). Interestingly, in Year 2 at the Edmonton site, biomass production of perennial rye increased relative to Year 1, whereas this trend was not evident at the Breton site. Based on our observations, this was less the result of reduced biomass from individual plants of perennial rye and instead the result of weed growth competition in the perennial rye plots at the Breton site. Indeed, by the end of Year 2, less than half of each perennial rye plot was occupied by perennial rye.

The relationship of grain to total aboveground biomass of a crop, referred to as HI, is a measure of efficiency for plants producing grain. Harvest index of the perennial rye was universally reduced in this study relative to the spring and fall rye, due to less proportion of assimilated C being allocated to grain over biomass (Table 3). Reduced HI of perennial rye relative to annual counterparts is consistent with literature, as the evolutionary advantage of a wild perennial is highly dependent on the survival of vegetative structures and thus more photosynthate is allocated to nonsexual growth (T. Cox et al., 2006; Culman et al., 2013; DeHaan et al., 2005; Jaikumar et al., 2012). However, this metric places the importance of grain production over total plant productivity (DeHaan & Van Tassel, 2014). Total primary productivity includes vegetative biomass, which is an important characteristic of perennial grains that may serve as dual-purpose forage and grain crops (Ryan et al., 2018; Snapp et al., 2019).

Interestingly, no effect of fertilization was found for grain yield, biomass yield, or HI for any treatment in Year 1, or at the Edmonton site in Year 2 (Table 3). The Edmonton site is characterized by Black Chernozemic soil, which is highly fertile. Highly fertile soils may not show a yield response to fertilizer addition (Tausz et al., 2017; Thilakarathna et al., 2020). Specifically, Campbell et al. (2005) studied the effects of fertilization on grain and biomass yields in a Chernozemic soil and found that when compared to an unfertilized control, fertilization negligibly altered yields in the 1st years of the experiment and obvious yield increases took several growing seasons to materialize. As well, while the Breton site is underlain by a generally less fertile Gray Luvisolic soil, its land use history was that of a mixed perennial grass stand grown for forage harvest for at least 60 yr prior to this experiment. The soil was tilled for the first time in June 2017 prior to experiment establishment. Thus, there was ample N from mineralizing roots and grass residues and increased soil organic matter decomposition from the tillage disturbance, a legacy effect that can last up to 3 yr after conversion of a perennial grass stand (Mukumbuta & Hatano, 2020; Thilakarathna & Hernandez-Ramirez, 2021). Thilakarathna and Hernandez-Ramirez (2021) documented how growing perennial forage in Breton raises soil organic matter and N concentrations, which is then available for subsequent crop uptake upon simulated tillage and cropping. This is supported by the lack of differences found between fertilized and unfertilized NDVI readings in the crop canopies for the majority of sampling dates, indicating that crops in the unfertilized plots did not experience reductions in N availability or uptake that may have translated into lower NDVI readings, relative to their fertilized counterparts. Indeed, only 12% of average NDVI readings showed differences between fertilized and unfertilized crops. Overall, several more growing seasons and successive grain harvests may be required to detect consistent differences in fertilized vs. unfertilized plots for both the Edmonton and Breton sites.

4.2 | Substantial protein productivity of a perennial rye crop

Total grain protein is one component that determines the profitability of a grain crop (Asseng et al., 2002). In accordance with the present study, ample literature has documented increased protein in perennial grain crops relative to annual grain crops (Pimentel et al., 2012; Marti et al., 2016; Ryan et al., 2018). For both the Edmonton and Breton sites, the perennial rye had increased grain protein relative to the fall and spring rye, whose protein contents were within the expected range (Table 5). Protein content of annual rye grain in Alberta is generally 12% but can reach as high as 14.5% depending on cultivar (Alberta Agriculture & Forestry, 2016; Arendt & Zannini, 2013) Protein productivity is the product of grain protein concentration and grain yield, thus it encompasses both metrics into a single density parameter and represents the overall ability of a crop to produce grain protein (Asseng et al., 2002). As a result of greater grain protein, the grain protein productivity of the perennial rye at the Breton site in Year 1 was comparable to that of spring rye. Interestingly, increased protein concentration in the perennial rye was able to overcome lower yields and deliver the same grain protein productivity in the perennial crop as an annual crop. Conversely, in both years at the Edmonton site, the protein productivity was lower than the fall and spring rye despite having greater grain protein concentration, due to insufficient grain yields. Thus, the grain protein productivity of a perennial rye crop may be comparable to an annual rye crop in specific circumstances, but more research is needed as accurate yield measurements were precluded at the Breton site in Year 2.

Similarly, the perennial rye biomass protein was greater in Year 1 at both sites compared to the biomass protein of the other rye treatments, but was lower than the perennial forage biomass, likely due to the presence of alfalfa in the perennial forage mix, a legume with a higher crude protein content than most grasses (Table 5) (Deng et al., 2020). Notably, increased biomass yield in the perennial rye resulted in comparable protein productivity between the perennial rye and perennial forage at the Breton site in Year 1 and the Edmonton site in Year 2. Forages are the main source of food for ruminants and those with a legume are highly valued as animal feed because they are an inexpensive source of protein (Radovic et al., 2009; Wilkins & Humphreys, 2003). Thus, comparable protein productivity from perennial rye biomass highlights its practicality as a dual-purpose forage-grain crop.

4.3 | Nitrogen use efficiency of a perennial rye crop

Common measures of NUE include NUE, PE, UE, and NHI and optimizing these metrics is a significant challenge for world agriculture, particularly in grain crops (Jamil, 2020). Both NUE (a measure of how grain yield increases with fertilizer application) and UE (a measure of whole-aboveground plant N increase with fertilizer application) were small for all rye crops across both sites and years (Supplemental Table S3). Our NUE values were lower when compared to previous research on grain crops in Black Chernozemic soils comparable to the Edmonton site (Thilakarathna et al., 2020) and Gray Luvisolic soils like the Breton site (Malhi et al., 2011). We hypothesize that this may be attributed to a combination of two things: primarily, the fertilizer application method in our study. To reduce damage to the perennial rye plots, fertilizer was broadcast onto the plots and left unincorporated; a method that can suffer significant losses due to NH_3

volatilization (Alberta Agriculture & Forestry, 2016; Romero et al., 2017). Secondly, baseline soil fertility conditions at both Edmonton and Breton sites were relatively high, as discussed in the section entitled Yield potential of a perennial rye crop. Thus, a combination of these two factors may have resulted in low NUE and UE and masked the effects of fertilizer application.

Further, perennial rye showed no improvement in NUE or UE relative to annual. We postulate that this null result may be because we did not measure root contributions to overall plant NUE in our study, as a previous study by Sprunger et al. (2018) found increases in the whole plant NUE (when accounting for the roots) of the perennial IWG compared to annual wheat. Notably, Sprunger et al. (2018) calculated NUE for aboveground and belowground components separately as well as for the whole plant and found that the aboveground NUE was not different between the perennial and annual, which is congruent with the findings of the present study. The increase in whole plant NUE reported by Sprunger et al. (2018) was the result of increased root biomass and root N content in the perennial crop. This finding was further confirmed in a related work by Kim et al. (2021), who found twofold the root mass in perennial rye in the 15-to-30-cm subsurface soil layer and greater root N density than a spring rye crop. Additionally, the abovementioned lodging of the perennial rye crop observed at the Breton site in Year 2 is likely indicative of an over application of fertilizer, which would mask any NUE effects.

The NHI is the ability of a plant to partition N into grain over other vegetative sinks, which is an important metric for the economy of grain quality and allocation efficiency (Dobermann, 2007; Jamil, 2020). Lower NHI for the perennial rye is predictable, based on the overall perennial life strategy for longevity, which prioritizes allocation to vegetative structures over grain (DeHaan & Van Tassel, 2014; DeHaan et al., 2007; Snapp et al., 2019). Notably, T. Cox et al. (2002) postulated that the trade-off between vegetative structures and grain in perennial rye crops would only be required in the 1st year. Results for the Edmonton site contradict this hypothesis, as the NHI of the perennial rye was reduced relative to fall and spring rye in Year 1 and again in Year 2 (Supplmental Table S4). While no differences in NHI were determined for the Breton site in Year 1 between any of the rye crops, the perennial rye had reduced NHI on average. This indicates transfer of N to grain in the perennial rye crop was reduced relative to fall and spring rye crops, despite perennial rye having the highest grain protein content (Lopez-Bellido & Lopez-Bellido, 2001). Similarly, reduced PE in the perennial rye at the Edmonton site in Year 2 supports the diminished ability for perennial rye to translate increased whole plant N content into increased grain yield.

4.4 | Challenges with growth, survival, and competitiveness of perennial rye

Perennial rye initially matured faster than its fall and spring counterparts but slowed considerably as the season progressed, eliminating the ability for an earlier grain harvest, a prospect that would have reduced the risk of an early season snowfall damaging yields, inducing lodging, and often even impeding harvest in western Canada. However, the rapid vegetative growth may allow for a forage harvest early in the season and not impede the perennial rye crops ability to regrow and produce a considerable grain harvest (Ates et al., 2017; Pugliese et al., 2019). Testing this hypothesis should be included in a future study, as a second biomass harvest for forage may improve the profitability of the perennial rye, but a mismatch in the timing of biomass harvest could unintentionally deplete root carbohydrate reserves and compromise successful regrowth of perennial rye (Ferraro & Oesterheld, 2002).

Unfortunately, the current climate of central Alberta does not lend itself to the over winter success of existing grain crops, let alone novel perennial grains (Cattani et al., 2019; Salmon et al., 2015). In addition to reduced yields, high winter mortality also precludes the ability to study the long-term benefits on soil health that are purported in the literature with perennial grain cropping (Crews & Cattani, 2018; Ryan et al., 2018). Furthermore, the competitiveness of the perennial rye against weed pressure at the Breton site was insufficient to support a considerable grain yield past 1 yr, despite worries that perennial grains could become invasive (Schlautmann et al., 2018).

5 | CONCLUSIONS

The perennial grain, ACE-1 perennial rye, may be a viable option relative to spring and fall rye in cold temperate environments if harvested as a dual-purpose forage and grain crop; however, grain yields may be reduced after the 1st year, reducing its feasibility as a cash crop. Biomass yields and unproductive tillering of the perennial rye were considerably elevated, also indicating the significant potential for perennial rye to fix atmospheric C, lending to the strategic possibility for breeding efforts to physiologically redistribute resource allocation from vegetative structures to grain. The perennial rye crop produced ample grain protein productivity because of increased grain protein concentration, but only when grain yields were sufficient to sustain a considerable harvest in the 1st year of growth. Generally, NUE parameters did not differ between growth habits (perennial vs. fall vs. spring) for aboveground biomass, indicating that gains in NUE in perennial rye crops likely come from increased belowground allocation or at lower fertilization rates. Challenges associated with increasing weed pressure and winter mortality of perennial rye crops prevented more than two production cycles, as well as their monitoring beyond 2 yr at either study site. Overall, perennial rye requires further development prior to consideration as a suitable option as a grain crop in agroecosystems that experience conditions comparable to those in central Alberta, Canada.

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AUTHOR CONTRIBUTIONS

Erin Jane Daly: Data curation; Formal analysis; Investigation; Software; Validation; Visualization; Writing – original draft; Writing – review & editing. Guillermo Hernandez-Ramirez: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing. Keunbae Kim: Data curation; Investigation. Dick Puurveen: Investigation; Project administration. Chloe Ducholke: Data curation. Lori Oatway: Formal analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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