Received: 22 December 2020 | Accepted: 23 July 2021

DOI: 10.1002/agj2.20836

Agronomy Journal

ARTICLE

Soil Fertility and Crop Nutrition

Effects of cultivars and nitrogen management on wheat grain yield and protein

Deepak Ghimire[1] | **Saurav Das**[1] | **Nathan D. Mueller**[2] | **Cody F. Creech**[1] | **Dipak Santra**[1] | **P. Stephen Baenziger**[1] | **Amanda C. Easterly**[1] | **Brian Maust**[1] | **Bijesh Maharjan**[1]

[1] Dep. of Agronomy and Horticulture, Univ. of Nebraska-Lincoln, Lincoln, NE 68583, USA

[2] Nebraska Extension, Univ. of Nebraska-Lincoln, Lincoln, NE 68583, USA

Correspondence
Bijesh Maharjan, Dep. of Agronomy and Horticulture, Univ. of Nebraska-Lincoln, Lincoln, NE 68583, USA.
Email: bmaharjan@unl.edu

Assigned to Associate Editor Natasha Rayne.

Abstract

Low grain protein in hard red winter (HRW) wheat (*Triticum aestivum* L.) is a serious challenge for rainfed wheat growers, particularly in years with elevated grain yield. Proper nitrogen (N) management with adequate N rate and application timing is critical for optimizing grain yield and protein content. This 2-yr experiment evaluated the effects of different N rates and application timings (fall, spring, and split) on grain yield and protein of two HRW wheat cultivars. Field studies were conducted at four different sites across Nebraska under rainfed conditions in 2018/2019 (Year 1) and 2019/2020 (Year 2). A split-plot randomized complete block design with wheat cultivars as the whole plots and factorial combinations of six N rates and three application timings as the subplots was used in four replications. Grain yield was associated positively and grain protein negatively with the water supply to demand ratio (WS/WD) in the season. Freeman cultivar yielded better in a year with higher WS/WD and a newly developed cultivar, Ruth, yielded better in a lower WS/WD year. Nitrogen fertilization significantly increased grain yield in the site-years with moderately higher WS/WD. There was an increase in grain protein with increasing N rates at all site-years. Spring and split-applied N resulted in better grain yield than fall application in the site-year when there was a risk of N loss. This experiment suggested that an effective N management strategy for winter wheat should account for and be adaptable to weather variability to optimize grain yield and protein content.

1 | INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is an important crop in terms of production and market value in the U.S. Great Plains (Weiss et al., 2003). Among six classes of wheat grown in the United States, hard red winter (HRW) wheat accounts for 40% of total wheat production in the United States (Tilley et al., 2012). Hard red winter wheat is commonly used in the preparation of a wide range of flour-based products due to its moderately high protein content (120–140 g kg^{-1}) (Gibson & Newsham, 2018). Grain protein content is an important quality factor of HRW wheat for its end-use functionality in the milling and baking industry (Fuertes-Mendizabal et al., 2013; Fufa et al., 2005; Maghirang et al., 2006; Shewry, 2007). The drop in grain protein below the threshold results

---

Abbreviations: AOR, agronomic optimum rate; GNR, grain nitrogen removal; GS, growth stage; HRW, hard red winter; NRE, nitrogen recovery efficiency; NUE, nitrogen use efficiency; PFP, partial factor productivity.

in reduced revenue for HRW wheat producers as industries set up price adjustments based on the protein content (Woolfolk et al., 2002). Conversely, increasing grain protein content in HRW wheat can benefit wheat producers with additional premiums in grain prices (Dick et al., 2016).

Low grain protein in HRW wheat has been a serious concern among wheat growers and wheat industry, particularly in years with elevated grain yield. Regional quality survey reported a significantly lower average protein content in HRW wheat for 2016 (112 g kg^{-1}) and 2017 (111 g kg^{-1}) compared to the preceding two 5-yr averages (125 and 123 g kg^{-1}) (Plains Grains Inc., 2018).

Grain yield and protein content in wheat are affected by several factors including climate, cultivar, nitrogen (N) management, and soil properties (Peterson et al., 1998, W. Wilson & Gallagher, 1990). Nitrogen is one of the most important management factors affecting grain yield and protein in wheat (Cassman et al., 1992; T. Wilson et al., 2020; Zorb et al., 2018). Determining optimal N rate and application timing is critical for both yield and grain protein. Grain protein content in wheat can be increased by applying N at rates greater than agronomic optimum rate (AOR). As yield reaches its maximum with AOR, the additional N contributes to increasing grain protein (Dick et al., 2016; Goos et al., 1982; Lollato et al., 2019; Macy, 1936; Walsh et al., 2018). However, an increase in grain protein due to additional N may vary by N application timing (Nakano et al., 2008; Romero et al., 2017). Reports suggest that N applied at or before sowing may result in little or no effect on grain protein content (Graham & Stockton, 2019). In contrast, N applied at heading or post-heading stage is found to substantially increase grain protein content (Cooper, 1974; Cruppe et al., 2017; Lollato et al., 2021).

Achieving an adequate protein content and increase in grain yield simultaneously is challenging for wheat producers. Several previous studies reported a negative correlation between grain protein content and grain yield in wheat (Oury & Godin, 2007; Simmonds, 1995; Triboi et al., 2006). The rainfed production system makes it more challenging since it receives annual precipitation lower than annual potential water evapotranspiration (Stewart, 2016). Some studies reported improved grain yield and grain protein content with a considerably high fertilizer N rate in a rainfed environment (Brown et al., 2005; Romero et al., 2017). In Oklahoma under rainfed conditions with an average annual precipitation of 874 mm, Mohammed et al. (2013) reported a linear increase in grain yield along with higher grain protein with an increase in N rates up to 200 kg N ha^{-1}. Application of N rate greater than AOR targeting higher grain yield and grain protein can increase the chance of lodging and thus, negatively affect wheat grain yield and quality (Shah et al., 2019; Zhang et al., 2017). In addition, wheat response to N rate and application timing varies across years and environmental conditions (Mohammed et al., 2013). Therefore, determining the optimum rate and timing of N application that are specific to a region and that account for weather is important from agronomic, economic, and environmental perspectives.

Core Ideas

- Grain yield was associated positively with the water supply to demand ratio in the season.
- There was no yield advantage to spring or split N application over fall except for 1 site-year.
- Application of N increased winter wheat grain protein in both dry and wet years.
- In site-years with low water supply and demand ratio, protein was inversely related to yield.
- Fertilizer N recovery efficiency decreased with an increase in applied N rates.

Grain yield, grain protein content, and their relationships may differ by cultivars besides various other factors including N rates, N application timing, N availability, and growing environment (Gaju et al., 2014; Latshaw et al., 2016; Ortiz-Monasterio et al., 1997; Triboi et al., 2006). Differences in anthesis date, grain-filling duration, kinetics of canopy senescence, N uptake and assimilation, post-anthesis N remobilization efficiency, etc. among cultivars can cause variation in grain yield and grain protein content as well as nitrogen use efficiency (NUE) (Barraclough et al., 2014; Foulkes et al., 2009; Hawkesford, 2017). Currently, ‘Ruth’ and ‘Freeman’, HRW wheat cultivars adapted to dryland environment, are two widely sowed cultivars in Nebraska (Nebraska Wheat Board, 2021). To our knowledge, they are yet to be evaluated together and compared in terms of grain yield, grain protein content, and NUE in different environments and under varying N management.

The objective of this experiment was to evaluate the effects of different N rates and application timing on grain yield and protein content of two cultivars of HRW wheat (Ruth and Freeman) across Nebraska. We hypothesized that higher rates and split application of N would be optimal to maximize winter wheat grain yield along with adequate protein content given an adequate soil moisture.

2 | MATERIALS AND METHODS

2.1 | Experimental sites

In 2018/2019 (Year 1) and 2019/2020 (Year 2), field experiments were conducted under rainfed conditions at four different research stations located across Nebraska: Eastern Nebraska Research and Extension Center near Mead (ENREC, 41.16˚ N, 96.41˚ W, elevation 348 m), Henry J.

Stumpf International Wheat Center near Grant (GRANT, 40.85° N, 101.71, elevation 1,037 m), High Plains Agricultural Lab near Sidney (HPAL, 41.23°N, 103.00°W, elevation 1,310 m), and Panhandle Research and Extension Center near Scottsbluff (PREC, 41.89° N, 103.68° W, elevation 1,197 m). The 30-yr average precipitation (1980–2010) during winter wheat growing season at ENREC, GRANT, HPAL, and PREC are 653, 463, 377, and 360 mm, respectively. The soils at ENREC were Filbert silt loam (fine, smectitic, mesic Vertic Argialboll) in Year 1 and Tomek silt loam (fine, smectitic, mesic Pachic Arguidoll) in Year 2. At GRANT, soil was Mace silt loam (fine-silty, mixed, superactive, mesic Aridic Arguistoll) in Year 1 and Kuma silt loam (fine-silty, mixed, superactive, mesic Pachic Argiustoll) in Year 2. The predominant soil at HPAL was Duroc loam (fine-silty, mixed, superactive, mesic Pachic Haplustoll) in Year 1 and Alliance loam (fine-silty, mixed, superactive, mesic Aridic Argiustoll) in Year 2. At PREC, soil was Tripp very fine sandy loam (coarse-silty, mixed, superactive, mesic Aridic Haplustoll) in both years (USDA-NRCS, 2020). The experimental fields for 2 yr at each research station were located within less than a kilometer apart. Daily precipitation and reference evapotranspiration (ET_o) data for each winter wheat growing season were obtained from weather stations located near the experimental sites (HPRCC, 2021). In-season cumulative precipitation was considered as seasonal water supply (WS) and ET_o as the seasonal water demand (WD).

For each site-year, the experimental layout was split-plot randomized complete block design with four replications. The main plot factor was wheat cultivar. Two HRW wheat cultivars, 'Ruth' (Reg. no. CV-1165, PI 675998; Baenziger et al., 2020) and 'Freeman' (Reg. no. CV-1098, PI 667038; Baenziger et al., 2014) were selected based on their wide use among farmers, broad adaptability, and representation of distinctly different breeding lineages. A factorial combination of three fertilizer N application timings and six N rates were randomly assigned to the split plots. Three N application timings were 100% in fall, 100% in spring, and split (30% in fall and 70% in spring) and six N rates were 0, 25, 50, 75, 100, and 125% of recommended N rate at each site. The 0% N rate is hereafter referred to as the control treatment. The recommended N rate was calculated using Winter Wheat Fertilizer Calculator from the University of Nebraska-Lincoln (UNL) (Hergert & Shaver, 2009) that accounts for nitrate-N from pre-plant soil test (0–120 cm) and fertilizer N and grain prices. The UNL algorithm outputs N rate for yield goal up to 5.04 Mg ha^{-1} and recommends an additional 23 kg N ha^{-1} for yield goal above 5.04 Mg ha^{-1}. The 3-yr average yield (2015–2017) from Nebraska Statewide Winter Wheat Variety Trials for all study locations was <5.04 Mg ha^{-1} (Institute of Agriculture and Natural Resources, 2019). Considering for yield goal <5.04 Mg ha^{-1} and fertilizer N price at US$0.88 per kg, and wheat grain price at $0.165 per kg, recommended N rate was 67 kg N ha^{-1} at GRANT, HPAL, and PREC. At ENREC, the recommended N rate was 90 kg N ha^{-1}, greater than at other three sites considering a greater yield potential (>5.04 Mg ha^{-1}) due to higher seeding rates and better growing conditions. The actual rates of fertilizer N used for different N rate treatments are detailed in Supplemental Table S1.

Ammonium nitrate (34–0–0) was used as the N source and was manually surface broadcast in the plots. The fall portion of N (both in split and fall treatments) was applied approximately 2 wk after sowing. Feekes growth scale (GS), a common scale for staging wheat growth, was used as a guide for subsequent N application (Large, 1954). The spring N application in split and spring treatments was applied near Feekes GS 5 (late tillering) stage of wheat. Fungicide (active ingredients: prothioconazole and tebuconazole at 0.007 and 0.009 g ha^{-1}, respectively) was applied at early flowering stage (Feekes GS 10.5.1) at GRANT in Year 1 and at ENREC in Year 2.

In Year 1, the trials were planted on 4 October, 12 September, 11 September, and 17 September of 2018 at ENREC, GRANT, HPAL, and PREC respectively using plot drills (Almaco, at ENREC, an SRES drill, Seed Research Equipment Solutions, at GRANT and HPAL, and a custom-built drill with a Hege cone at PREC). The sowing dates for Year 2 trials were 26 September, 17 September, 16 September, and 24 September of 2019 at ENREC, GRANT, HPAL, and PREC, respectively. In both years, a seeding rate of 2.44 million seeds ha^{-1} was used at ENREC while a seeding rate of 2.06 million seeds ha^{-1} was used at GRANT, HPAL, and PREC. The dates for fertilizer application and harvest are presented in Figure 1.

2.2 | Field data collection

At each location, pre-sowing soil samples were collected 1 to 7 d before sowing at three to five random spots across the experimental field to a depth of 120 cm at three intervals (0–20, 20–60, and 60–120 cm). Each soil sample was analyzed for nitrate-N (NO_3–N) and other chemical properties (Table 1). Post-harvest soil samples were collected within a week of harvest to a depth of 90 cm from selected plots with N rates (0, 75, 100, and 125%) from all application timing for Ruth cultivar and analyzed for NO_3–N. All soil tests were done using standard procedures (Ward Laboratories Inc.). At PREC in Year 1, 50 kg P ha^{-1} was uniformly applied across the field as soil P test recommended for it (Hergert & Shaver, 2009).

Winter wheat grains were harvested at maturity from each plot with SPC40 (Almaco) at ENREC, Zurn 150 universal plot combine harvester (Zurn harvesting GmbH & Co.) at GRANT and HPAL, and with a Delta plot combine (Wintersteiger Inc.) at PREC. The average yield per plot and moisture percentage

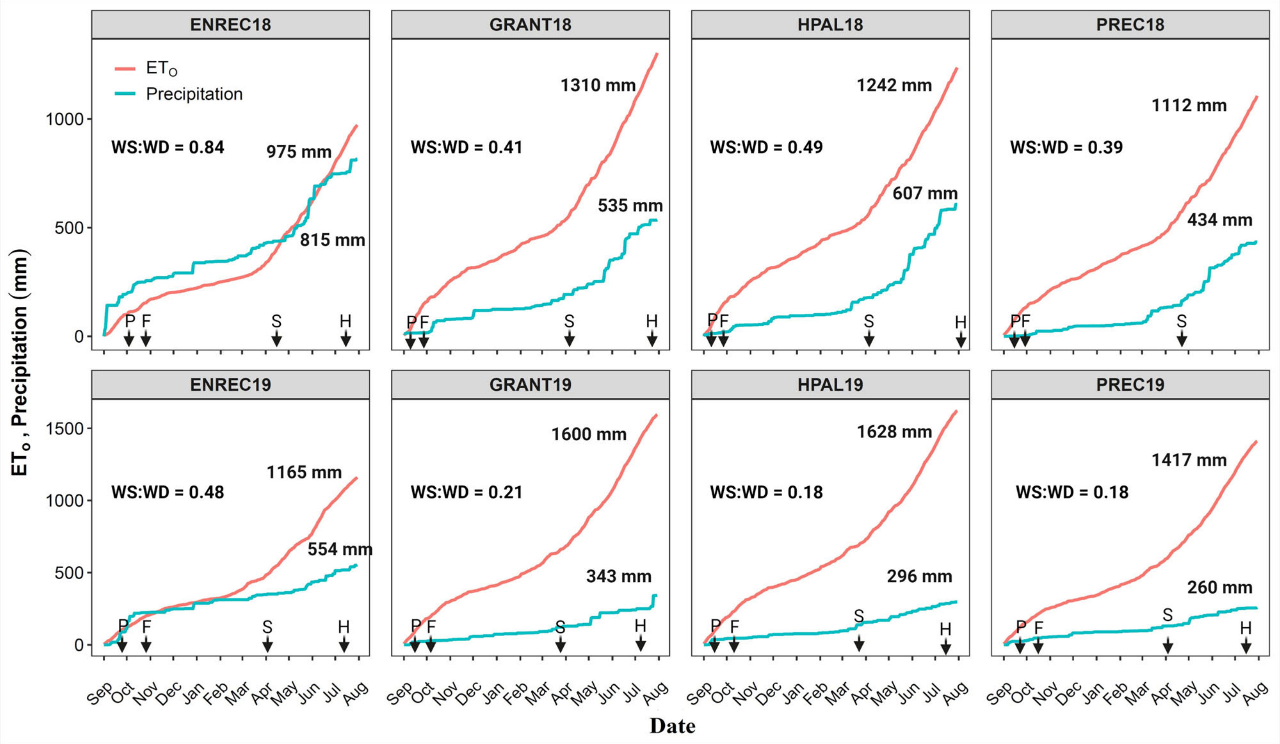

FIGURE 1 Cummulative precipitation and ET$_o$; reference evapotranspiration (mm) at different site-years across Nebraska during winter wheat growing seasons. The arrows shows the dates for sowing (P), fall fertilization (F), spring fertilization (S), and harvest (H). The ratio of water supply and demand during the season (WS/WD) are given for each site-year

TABLE 1 Chemical properties of soil collected pre-sowing at different site-years across Nebraska during winter wheat growing seasons in Year 1 (2018/2019) and Year 2 (2019/2020)

Site-year	Nitrate-N			Olsen P	K	Organic matter	Soil pH
	0–20 cm	20–60 cm	60–120 cm	0–20 cm			
	kg ha^{-1}			mg kg^{-1}			
ENREC18	19.1	28.7	37.2	10.9	268	36	6.7
GRANT18	36.6	16.3	22.4	18.8	427	23	7.2
HPAL18	26.1	23.4	30.5	21.1	647	28	7.8
PREC18	3.1	23.9	35.8	3.6	465	18	8.5
ENREC19	1.2	2.4	2.5	11.9	287	36	7.3
GRANT19	24.8	20.3	23.3	29.4	260	13	6.5
HPAL19	20.5	22.1	23.3	9.7	743	29	7.3
PREC19	42.5	126	52.9	8	545	20	8

of grains were recorded by the weighing system on the plot combine and the reported yield was adjusted to 12% moisture. A subsample of harvested grain from each plot was collected and analyzed for grain protein content using DA 7250 NIR analyzer (Perten Instruments, Inc.). The grain protein content measurement using NIR follows AACC International Method 39-25.01. The NIR analyzer uses novel Diode Array technology and relates reference values and the spectra of samples using multivariate calibrations. Grain protein content was reported on 12% moisture basis, the standard used for grain marketing (Wheat Marketing Center, Inc., 2004).

2.3 | Nitrogen indices

Grain N concentration was calculated from grain protein content using a conversion factor of 5.83 (Merrill & Watt, 1973). Grain nitrogen removal (GNR), nitrogen use efficiency as partial factor productivity (PFP), and nitrogen recovery efficiency (NRE) were calculated using the following equations:

$$\text{GNR}\ \left(\text{kg N ha}^{-1}\right) = \text{Grain yield} \times \text{Grain N concentration} \quad (1)$$

(Lollato et al., 2021)

$$\text{PFP}\ \left(\text{kg kg}^{-1}\right) = \frac{\text{Grain yield}}{\text{N}_{\text{available}}} \quad (2)$$

(Cassman et al., 1998)

$$\text{NRE}\ \left(\text{kg kg}^{-1}\right) = \frac{\text{GNR}_{\text{N1}} - \text{GNR}_{\text{N0}}}{\text{N}_{\text{fertilizer}}} \quad (3)$$

(Varvel & Peterson, 1990)

where N1 and N0 in the subscript represent the values of variables from each fertilized subplot and unfertilized subplot, respectively; $\text{N}_{\text{available}}$ is the total N available in the growing season (presowing soil NO_3–N plus applied fertilizer N); $\text{N}_{\text{fertilizer}}$ is the amount of N applied as inorganic fertilizer in individual subplots.

2.4 | Statistical analysis

Effects of site-year, cultivar, N rate, and N application timing on grain yield and protein were determined using Proc Mixed in SAS 9.4 Software (SAS Institute, 2015) with site nested in year, cultivar, N rate, and N timing as the fixed effects and block and all interactions of block with other terms as random effects. The experimental sites ENREC, GRANT, HPAL, and PREC when referred to as site-years were represented as ENREC18, GRANT18, HPAL18, and PREC18, respectively, for Year 1 and as ENREC19, GRANT19, HPAL19, and PREC19, respectively, for Year 2. Site-year was treated as fixed effects to examine effects of differences in precipitation patterns between the growing seasons and across sites. In addition, effects of cultivar, N rate and application time, and their interactions on response variables (grain yield, grain protein, GNR, NRE, and PFP) were determined separately for individual site-years. The treatment means were calculated using LSMEANS statement and their differences were compared using DIFF option. Differences were considered significant at $P < .05$.

When there was a significant interaction effect of main factors on measured variables, the highest order of interaction effect (3-way > 2-way > main factor effect) was only discussed. A pairwise comparison of means by cultivar in the control treatment at different site-years was carried out in SAS using PROC GLM to compare the cultivars. Environmental index (EI) values were calculated by averaging variables across cultivars as described in Lollato et al. (2021) to explain the cultivar × environment interaction effect on grain yield and grain protein content using the concept of adaptability and stability (Eberhart & Russell, 1966).

Linear regression relationships of total available N with grain yield and grain protein content as well as grain yield with grain protein content and GNR were analyzed for each site-year using PROC REG. The residuals from the linear regression of grain protein content against grain yield (referred as GPD; Grain Protein Deviation hereafter) and GNR against grain yield (GNRD: Grain Nitrogen Recovery Deviation) were used to determine the difference between cultivars, N rate, and N application timings in terms of each relationship (Monaghan et al., 2001).

3 | RESULTS

3.1 | Weather

Total annual precipitation for the wheat growing season in Year 1 (September through July) ranged from 429 mm at PREC18 located in the western region to 815 mm at ENREC18 located in the eastern region of Nebraska (Figure 1). In Year 2, the total annual precipitation ranged from 260 mm at PREC19 to 554 mm at ENREC19. All sites had greater total precipitation in Year 1 and a lower total precipitation in Year 2 compared to the 30-yr normal. In Year 1, the total annual precipitation was 25, 16, 61, and 19% greater than the normal precipitation at ENREC, GRANT, HPAL, and PREC, respectively. In Year 2, ENREC, GRANT, HPAL, and PREC had 15, 26, 21, and 28% lower total annual precipitation than the normal precipitation, respectively. The cumulative growing season ET_o ranged from 975 mm at ENREC18 to 1,628 mm at HPAL19 resulting in WS/WD of 0.18 to 0.84 (Figure 1). Year 2 had WS/WD half of that in Year 1. The trial at PREC in Year 1 was completely lost to a series of hailstorms. At ENREC in Year 1, there was a severe infection of fusarium head blight (FHB) during grain-filling stage.

3.2 | Cultivar differences

Cultivars differed significantly in grain yield at 4 out of 7 site-years (Table 2). Cultivar Ruth had significantly greater grain yield, GPD, GNR, and PFP at ENREC19 and PREC19 (both in Year 2 with lower WS/WD). Cultivar Freeman had greater grain yield and PFP at ENREC18 and HPAL18 and greater GNR at HPAL18 (all in Year 1 with greater WS/WD). Grain protein content was greater with 'Ruth' at ENREC18 and GRANT18 and with 'Freeman' at HPAL19.

Adaptability coefficients for two cultivars were similar in terms of grain yield ($\alpha = 1.01$ for 'Freeman' and 0.989 for

TABLE 2 Pairwise comparison of response variables by cultivars in the control (zero N) treatment at 7 site-years

Site-year	Grain yield F	Grain yield R	Grain protein F	Grain protein R	Grain protein deviation F	Grain protein deviation R	Grain N removal F	Grain N removal R	Partial factor productivity F	Partial factor productivity R
	$\mathrm{Mg\ ha^{-1}}$		$\mathrm{g\ kg^{-1}}$				$\mathrm{kg\ N\ ha^{-1}}$		$\mathrm{kg\ grain\ kg^{-1}\ N}$	
ENREC18	4.47a[a]	3.92b	99.9b	104.3a	–14.3	–15.5	59.15	54.06	45.71a	40.13b
ENREC19	3.33b	4.28a	107.8	111.4	–18.2b	–4.7a	47.89b	63.33a	487.79b	627.73a
GRANT18	5.81	5.87	88.4b	93.9a	–11.7	–5.6	68.29	73.44	67.28	67.96
GRANT19	2.73	2.88	147.1	151.4	14.8	20.6	53.38	57.80	34.86	36.71
HPAL18	2.86a	1.98b	96.7	97.7	–34.2	–42.4	37.03a	25.74b	31.06a	21.54b
HPAL19	2.54	2.64	131.8a	125.8b	–2.5	–7.4	44.29	44.16	33.86	35.21
PREC19	2.70b	3.04a	139.7	142.0	7.1b	12.9a	49.90b	57.14a	10.70b	12.04a

Note. F, Freeman cultivar; R, Ruth cultivar.

[a] Mean values followed by different lowercase letters in each variable column indicate significant difference at $P < .05$ for each site-year.

'Ruth') as well as grain protein ($\alpha = 1.03$ for 'Freeman' and 0.967 for 'Ruth', Figure 2). Grain yield and grain protein content were stable for both cultivars ($r^2 > .94$).

3.3 | Agronomic responses

When responses of grain yield and protein content to N management and cultivar were analyzed with all 7 site-years together, significant interaction effects of site-year with cultivar and N rate were observed (Table 3). Irrespective of cultivar, the site-year GRANT18 had the greatest grain yield and HPAL19 had the lowest among site-years. Grain yield or protein did not vary by N application time. Because of the interaction effects involving site-year as well as the main factor effect of site-year on grain yield and protein content, data analyses were conducted for individual site-years separately and are more elaborately discussed.

The regressions of grain yield and grain protein content against WS/WD ratio suggested that grain yield tends to increase linearly (slope = 2.27) as the water supply and demand ratio increases while grain protein content decreased

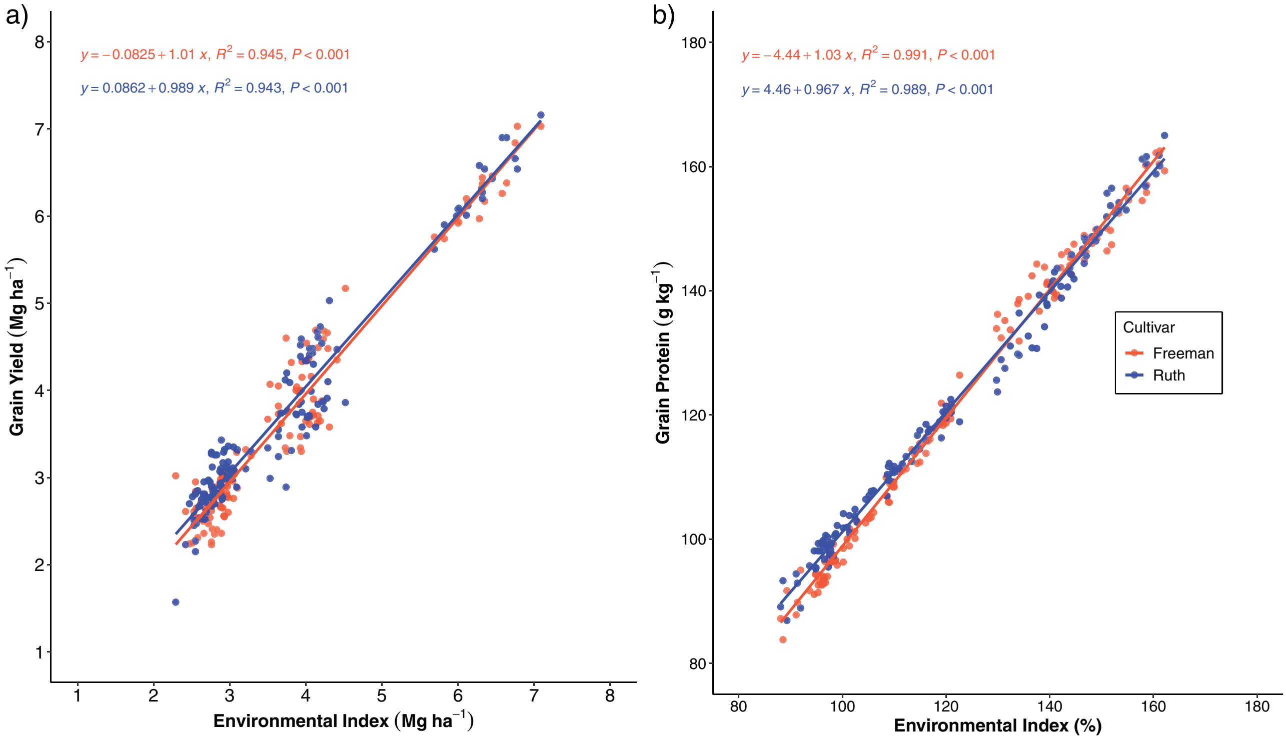

FIGURE 2 Linear regression of (a) grain yield and (b) grain protein content for different cultivars against environmental index

TABLE 3 Mean grain yield and grain protein of winter wheat affected by site-year, cultivar, time of N application and N rate during winter wheat growing seasons in Year 1 (2018/2019) and Year 2 (2019/2020) across Nebraska

Source of variation	Yield	Protein
	Mg ha^{-1}	g kg^{-1}
Site-year (SY)		
ENREC18	4.0 b[a]	109.4 e
GRANT18	6.3 a	95.1 g
HPAL18	3.3 c	97.7 f
ENREC19	4.0 b	116.5 d
GRANT19	2.9 d	155.2 a
HPAL19	2.7 d	134.9 c
PREC19	2.9 d	145.2 b
P value	**<.0001**[b]	**<.0001**
Cultivar (C)		
Ruth	3.9	119.9
Freeman	3.8	118.8
P value	.0670	.1193
Time of N application (T)		
Split	3.8	119.5
Fall	3.8	119.2
Spring	3.8	119.3
P value	0.5874	0.8157
N rate (R)[c]		
0	3.6d	114.5f
25	3.7c	116.1e
50	3.8bc	118.2d
75	3.9b	120.0c
100	3.9ab	122.7b
125	4.0a	124.5a
P value	**<.0001**	**<.0001**
	P value	
Interactions		
SY × C	**<.0001**[c]	**<.0001**
SY × T	.2521	.0707
SY × R	**<.0001**	**.0109**
C × T	.1527	.4413
C × R	.6763	.5507
T × R	.8830	.9560
SY × C × T	.8831	.9978
SY × C × R	.5893	.6402
SY × T × R	.7898	.7089
C × T × R	.7605	.7865
SY × C × T × R	.7869	.8907

[a] Mean values followed by different letter in each column and section indicate significant difference at a given *P* value.

[b] *P* value lower than .05 is in bold fonts.

[c] Unit of N rate is percentage of recommended N rate.

quadratically with an increase in WS/WD and plateaued at WS/WD of 0.43 (Figure 3). All three sites in Year 2 had a lower WS/WD ratio and resulted in lower grain yield and higher grain protein content as compared to the sites in Year 1 with comparatively higher WS/WS ratio.

3.4 | Grain yield at individual site-years

Regression analysis showed a significant linear increase in grain yield with available N at GRANT18, HPAL18, ENREC19, and HPAL19 (Figure 4). The slope of regression was 0.021 Mg per kilogram available N at HPAL18 compared to 0.011 at GRANT18, 0.004 at ENREC19, and 0.003 at HPAL19. Grain yield at ENREC18 was negatively correlated to available N with a slope of −0.003. In Year 2, grain yield response to available N was not significant at GRANT and PREC.

There was a significant interaction effect of cultivar and N rate on grain yield at ENREC19 and PREC19 (Table 4). At ENREC19, grain yield ranged from 3.32 Mg ha^{-1} with 'Freeman' (the control treatment) to 4.71 Mg ha^{-1} with 'Ruth' (the 125% N treatment) (Figure 5a). At ENREC19, grain yield with 'Ruth' was always greater than 'Freeman' at each of the applied N rates including the control. Within each cultivar, grain yield response to N rates was different. With 'Ruth', the 125% N rate treatment had significantly greater grain yield than the rest of N rates whereas, with 'Freeman', the high N rates (50, 75, 100, and 125% N rates) had greater yield than the control treatment and 25% N rate.

At PREC19, grain yield with 'Ruth' was always greater than 'Freeman' at each of the applied N rates except the control (Figure 5b). With 'Freeman', grain yield with the 25% N rate was significantly greater than with higher N rates except for the 125% N rate. With 'Ruth', grain yield did not vary by N rate except that the 50% N rate had greater grain yield than the control.

There was a significant cultivar × N application timing interaction effect on grain yield at ENREC19 (Figure 5c). For 'Ruth', there were greater yields with split and spring N applied treatments compared to the fall treatment. In contrast, no yield difference by N application timing was observed with 'Freeman'.

Cultivar had a significant effect on grain yield at 5 out of 7 site-years (Table 4). Grain yield was significantly greater for 'Ruth' compared to 'Freeman' at GRANT19, ENREC19, and PREC19, all in Year 2, while 'Freeman' yielded significantly greater than 'Ruth' at ENREC18 and HPAL18 both in Year 1 (Table 4).

No significant effect of N application timing on grain yield was observed at site-years except at GRANT18, where the

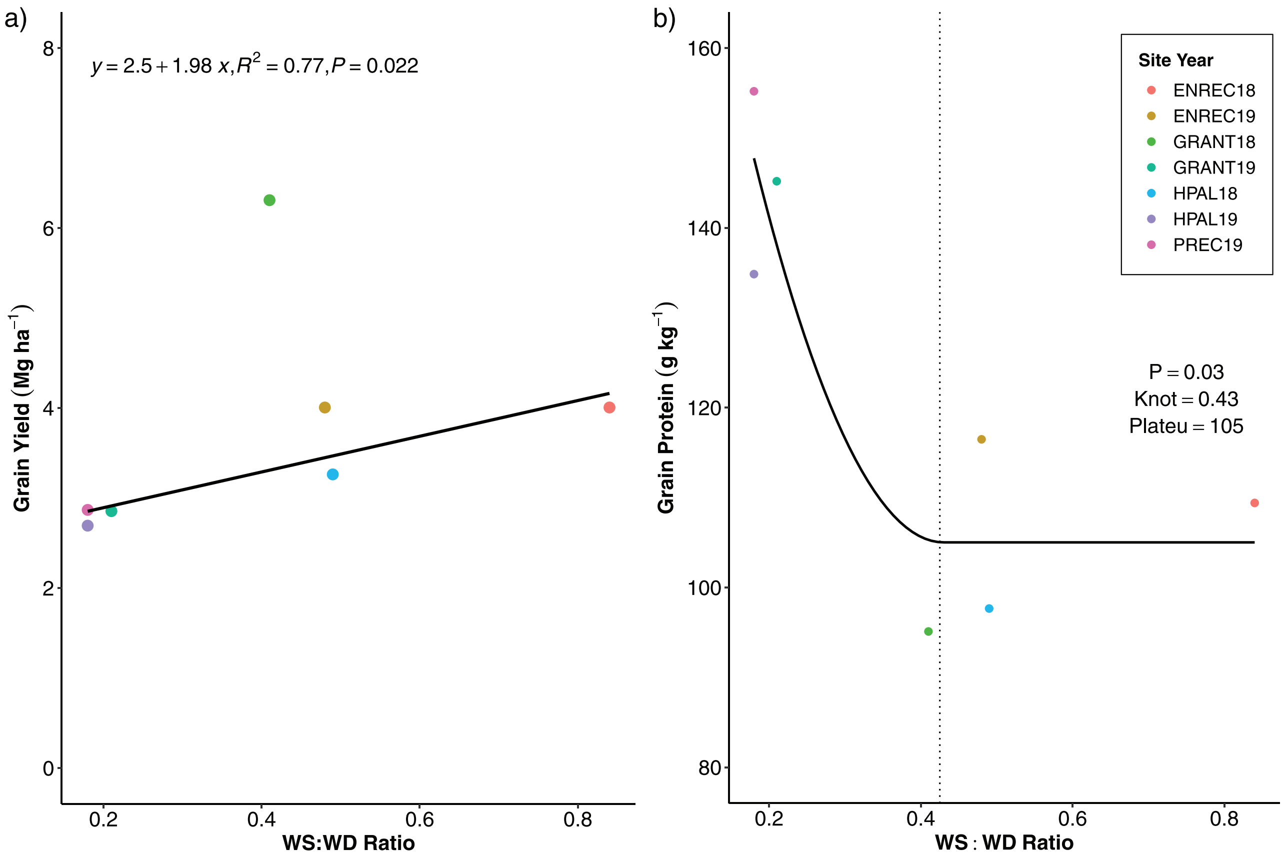

FIGURE 3 Regression of winter wheat (a) grain yield and (b) grain protein content for different site-years against water supply and water demand ratio (WS/WD). Grain yield data from GRANT18 was excluded from regression considering it as an outlier in Figure 3a

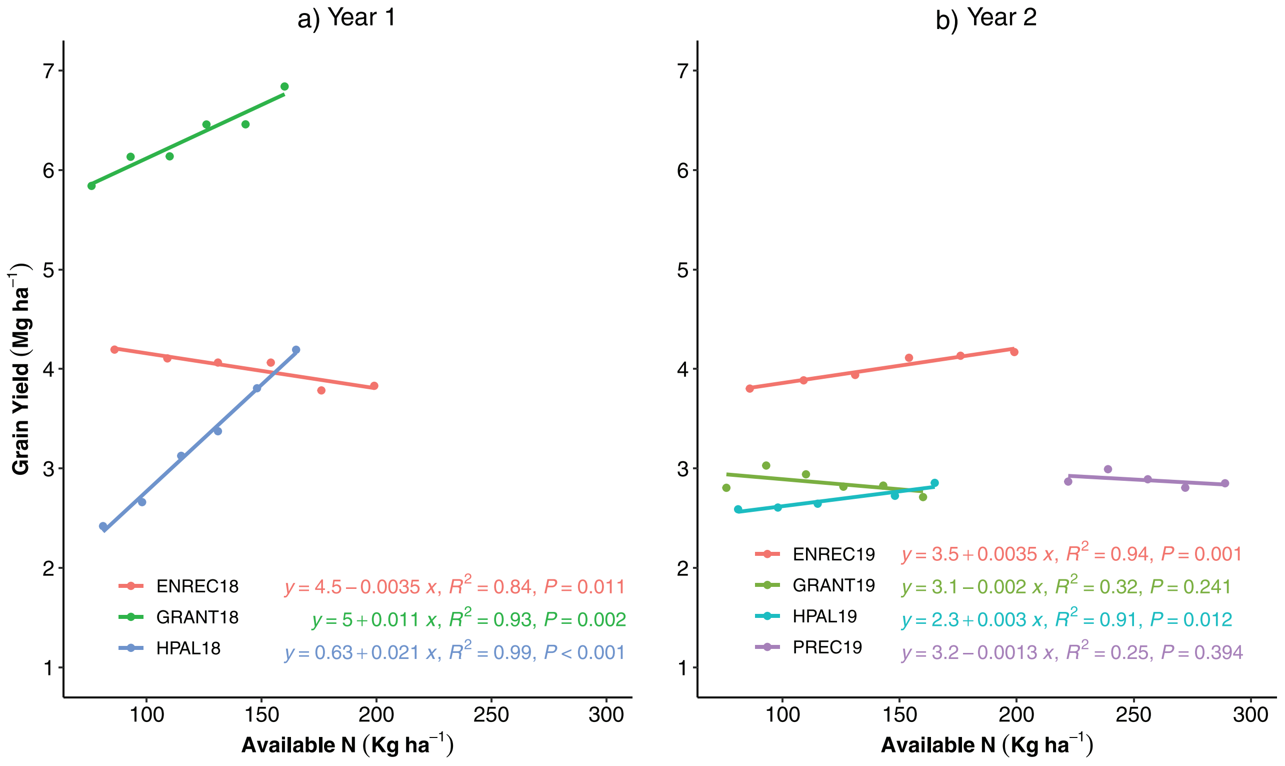

FIGURE 4 Linear regression (with regression coeffient and *P* value) of winter wheat grain yield against available N (kg ha^{-1}) at different experimental sites across Nebraska during winter wheat growing season in (a) Year 1 (2018/2019) and (b) Year 2 (2019/2020)

TABLE 4 Mean grain yield of winter wheat for each site-year as affected by cultivar, N application time, and N application rate

Source of variation	Grain yield ENREC18	 GRANT18	 HPAL18	 ENREC19	 GRANT19	 HPAL19	 PREC1
	Mg ha^{-1}						
Cultivar (C)							
Ruth	3.64b[a]	6.35	3.07b	4.43a	2.94a	2.71	3.18a
Freeman	4.37a	6.27	3.46a	3.59b	2.77b	2.68	2.56b
P value	**<.0001**[b]	.76	**.032**	**.010**	**.009**	.660	**.009**
Time of N application (T)							
Split	4.06	6.38a	3.33	4.04	2.86	2.69	2.81
Fall	4.04	6.16b	3.31	3.93	2.85	2.73	2.94
Spring	3.93	6.39a	3.15	4.06	2.86	2.67	2.86
P value	.558	**.019**	.268	.056	.992	.713	.156
N rate (R)[c]							
0	4.19	5.84d	2.42d	3.80b	2.81	2.59	2.87
25	4.11	6.13c	2.66d	3.86b	3.03	2.61	2.99
50	4.06	6.14c	3.13c	3.94b	2.94	2.65	2.89
75	4.06	6.46b	3.37c	4.11a	2.82	2.74	2.81
100	3.83	6.46b	3.81b	4.14a	2.83	2.73	2.85
125	3.78	6.84a	4.19a	4.19a	2.71	2.86	2.81
P value	.2048	**<.0001**	**<.0001**	**<.0001**	.093	.097	.391
	P value						
Interactions							
C × T	.5112	.714	.795	**.008**	.954	.122	.186
C × R	.868	.409	.211	**.004**	.914	.635	**.044**
T × R	.561	.557	.647	.108	.325	.193	.409
C × T × R	.350	.396	.765	.273	.497	.262	.409

[a] Mean values followed by different letter in each column and section indicate significant difference at a given *P* value.

[b] *P* value lower than .05 is in bold fonts.

[c] Unit of N rate is % of recommended N rate.

split and spring N-applied plots had significantly greater yield compared to fall-applied treatments (Table 4).

Grain yield varied by N rate at GRANT18, HPAL18, and ENREC19 (Table 4), 3 out of 4 site-years where yield had a positive linear correlation with available N rates. Grain yields were in the order 125% > 100% = 75% > 50% = 25% > 0% at GRANT18 and 125% > 100% > 75% = 50% > 25% = 0% at HPAL18. Grain yield at N rates of 125, 100, and 75% were greater than the yield at 50, 25, and 0% at ENREC19. Nitrogen rate did not have any significant effect on grain yield at ENREC18, GRANT19, HPAL19, and PREC19.

3.5 | Grain protein

Linear regression of grain protein content against available N was significantly positive at all site-years at $P < .05$ and at GRANT18 at $P < .10$ (Figure 6). The slopes of regression were between 0.0384 and 0.163 g kg^{-1} protein per kilogram available N. Slopes were greater in Year 2 than in Year 1 for HPAL and GRANT and the reverse was true for ENREC.

There was a significant interaction effect of cultivar by N rate on grain protein content at HPAL18 (Table 5), where 'Ruth' had significantly greater protein content than 'Freeman' at N rates other than the control and 125% N (Figure 7). Grain protein content varied by different N rates with 'Freeman'. The 125% N rate treatment had greater grain protein content than the rest of the N rates. The control and 100% N treatment had greater grain protein content than the 25% treatment. Grain protein content did not vary by N rate with 'Ruth'.

There was a significant N application timing × N rate interaction effect on grain protein content at HPAL19 (Table 5). Among fall-applied treatments, the 125% N rate had significantly greater grain protein content than any other N rates but 100% N (Figure 8). The 100% N rate had greater grain protein content than the control and the 25% N rate. Among split-applied treatments, the higher N rates (125, 100, and 75% N) had greater grain protein content than the control and the 50% N rate. Among spring-applied treatments, the 125 and 100% N rates had greater grain protein content than in the control

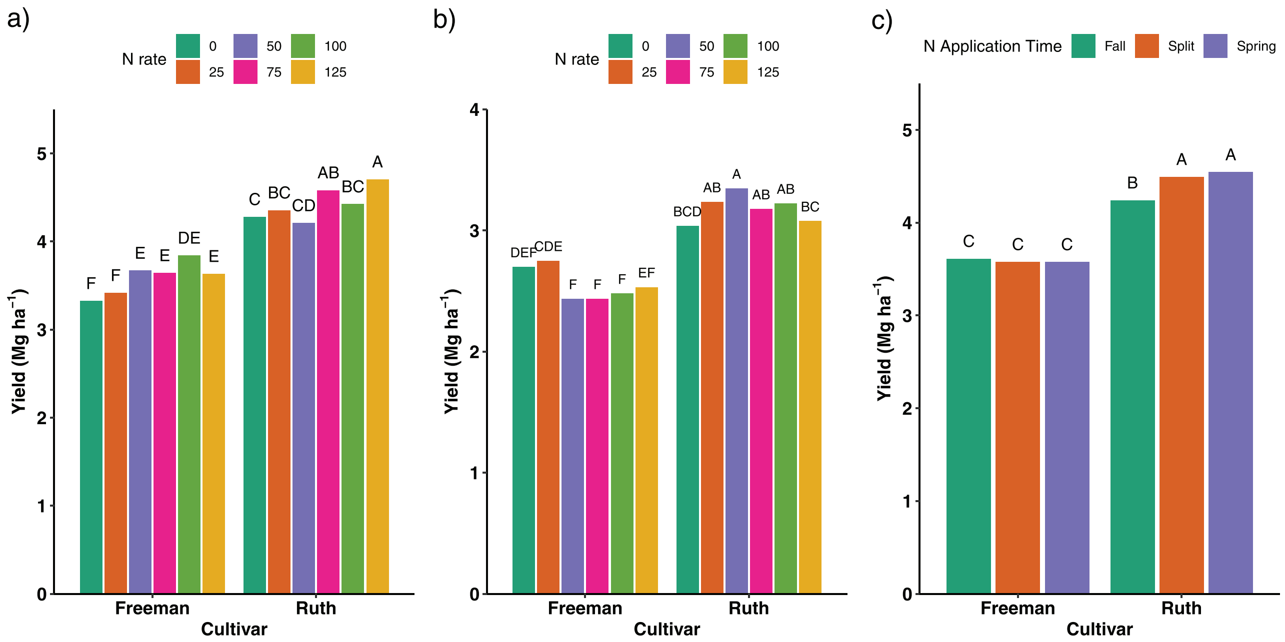

FIGURE 5 Winter wheat grain yield as affected by (a) Interaction of cultivar and N rate at ENREC19 (2019/2020); (b) Interaction of cultivar and N rate at PREC19 (2019/2020); and (c) Interaction of cultivar and N application time at ENREC19 (2019/2020). Bars with different uppercase letters indicate significant difference in mean grain yield at $P < .05$

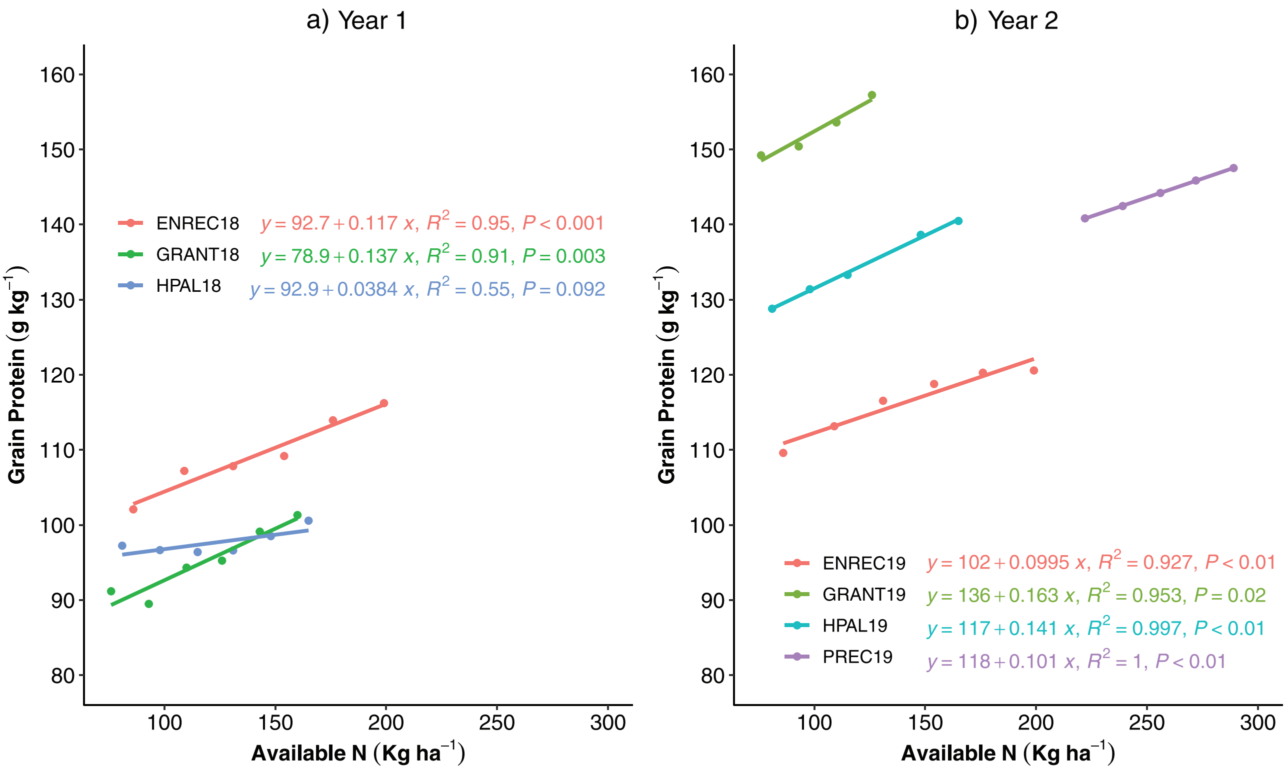

FIGURE 6 Linear regression (with regression coeffient and P value) of winter wheat grain protein against available N (kg ha^{-1}) across different experimental sites across Nebraska during winter wheat growing seasons in (a) Year 1 (2018/2019) and (b) Year 2 (2019/2020)

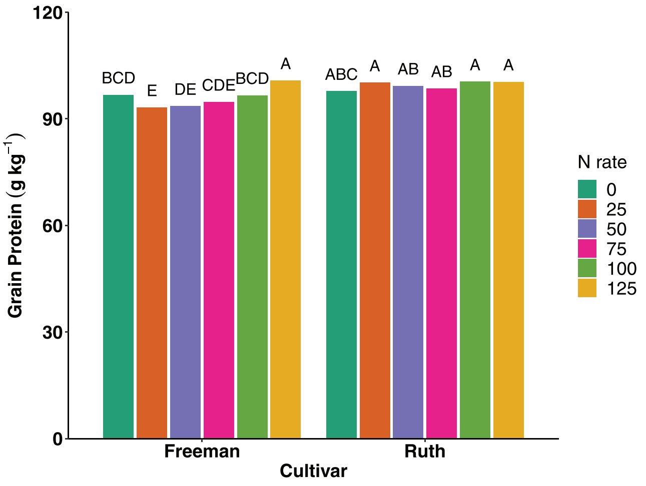

FIGURE 7 Grain protein of winter wheat as affected by the interaction of cultivar and N rate at HPAL18 (2018/2019). Bars with different uppercase letters indicate significant difference in mean grain protein at $P < .05$

and the 25% N rate. Grain protein content at any N rate did not vary by N application time.

The main factor effect of cultivar on grain protein content was significant at HPAL in both Years 1 and 2 (Table 5). In Year 1 when 'Freeman' had greater grain yield than 'Ruth', the effect of cultivar on grain protein content was reversed ('Freeman' < 'Ruth') that year. In Year 2, where yield did not vary by cultivar, grain protein content was significantly greater with 'Freeman' than 'Ruth' Nitrogen application timing did not have any significant effect on grain protein content at any of the site-years.

A significant effect of N rate on grain protein content was observed at all site-years (Table 5). The plots applied with a higher N rate had greater protein compared to low or no N-applied plots. The highest protein content was always below 102 g kg^{-1} at GRANT and HPAL in Year 1. In contrast, greater grain protein content (>102 g kg^{-1}) were achieved at all N-applied plots at ENREC in Year 1 with the highest

TABLE 5 Mean grain protein content of winter wheat for each site-year affected by cultivar, N application time, and N application rate

	Grain protein						
Source of variation	**ENREC18**	**GRANT18**	**HPAL18**	**ENREC19**	**GRANT19**	**HPAL19**	**PREC19**
	g kg^{-1}						
Cultivar (C)							
Ruth	110.7	96.6	99.4a[a]	117.0	155.9	132.0b	145.3
Freeman	108.1	93.6	95.9b	115.9	154.5	137.7a	145.1
P value	.21	.20	**.02**	.82	.40	**.01**	.91
Time of N application (T)							
Split	109.2	95.1	98.3	116.7	153.9	135.5	146.4
Fall	108.1	94.6	97.5	116.1	156.9	135.8	144.9
Spring	110.9	95.6	97.2	116.7	154.8	133.2	144.3
P value	.07	.69	.36	.53	.15	.08	.11
N rate (R)[b]							
0	102.1c	91.2 cd	97.2b	109.6e	149.2d	128.8e	140.8e
25	107.2b	89.5d	96.7b	113.0d	150.4 cd	131.4de	142.5de
50	107.8b	94.3bc	96.4b	116.5c	153.6bc	133.3 cd	144.2 cd
75	109.2b	95.2b	96.6b	118.8b	157.3ab	136.6bc	145.9bc
100	113.9a	99.1a	98.5ab	120.3ab	160.1a	138.6ab	147.5ab
125	116.2a	101.3a	100.6a	120.7a	160.6a	140.5a	150.3a
P value	**<.0001**[c]	**<.0001**	**.0039**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
	P value						
Interactions							
C × T	.9915	.6937	.4057	.6558	.9233	.4802	.4672
C × R	.9565	.5510	**.0236**	.0771	.3909	.2482	.2972
T × R	.4554	.4055	.3827	.4626	.8872	**.0380**	.6026
C × T × R	.7533	.2554	.8098	.1566	.7591	.1677	.4572

[a] Mean values followed by different letter in each column and factor indicate significant difference at a given *P* value using least-squares means.

[b] Unit of N rate is % of recommended N rate.

[c] *P* value lower than 0.05 is in bold fonts.

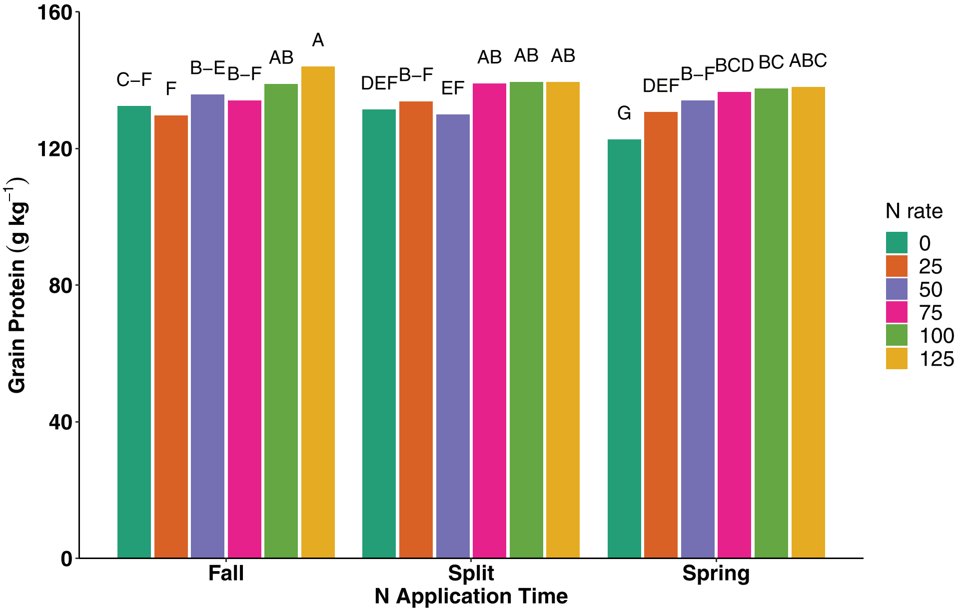

FIGURE 8 Grain yield of winter wheat as affected by the interaction of N application time and N rate at HPAL19 (2019/2020). Bars with different uppercase letters indicate significant difference in mean grain protein at $P < .05$

(116 g kg^{-1}) in plots applied with 125% N rate. In Year 2, all sites had grain protein content >102 g kg^{-1} irrespective of N rate and as high as 161 g kg^{-1} at GRANT19.

3.6 | Other agronomic responses (GNR, NRE, and PFP)

There was a significant interaction effect of cultivar and N rate on GNR at ENREC19 and PREC19 (Table 6). Cultivar Ruth tended to have greater GNR (57.1–75.3 kg N ha^{-1}) than ‘Freeman’ (46.8–61.3 kg N ha^{-1}) across different N rates. A significant interaction effect of cultivar and N application timing was observed at ENREC19. Cultivar Ruth had greater GNR compared to ‘Freeman’ for all N application timings (65.7–71 vs. 55–55.7 kg N ha^{-1}) and GNR differed significantly by N timings for only ‘Ruth’ with greater GNR for spring and Split N timings compared to fall. The main factor effect of cultivar on GNR was significant at ENREC18 and GRANT19 where ‘Ruth’ had greater GNR (62.4 and 60.8 kg N ha^{-1}, respectively) than ‘Freeman’ (53.2 and 56.8 kg N ha^{-1}, respectively). The main factor effect of N rate on GNR was significant at GRANT18, HPAL18, and HPAL19. The GNR ranged from 70.9 to 92.0, 31.4 to 56.1, and 44.2 to 53.3 kg N ha^{-1} at GRANT18, HPAL18, and HPAL19, respectively, with the lowest GNR at zero N rate and the highest GNR at the highest N rate. At all 3 site-years, there was a linear increase in GNR with N rate.

A significant three-way effect of cultivar, N application timing, and N rate on NRE was observed at ENREC19 and HPAL19 (Table 6). Cultivar Freeman with 25% N rate applied in fall resulted in the greatest NRE at HPAL19 while ‘Ruth’ with 25% N rate applied in Split had the greatest NRE at ENREC19. There were no obvious trends for differences in NRE by cultivar, N application timing, or N rate. There was a significant interaction effect of cultivar and N rate on NRE at PREC19. Cultivar Ruth had significantly greater NRE (0.74–3.64 kg N kg^{-1} fertilizer N) than ‘Freeman’ (0.59–3.09 kg N kg^{-1} fertilizer N) for each of the N rates except for the highest N rate (125%). There was an increase in NRE for each cultivar usually with a decrease in N rates. At ENREC18, ‘Freeman’ had greater NRE compared to ‘Ruth’ (0.12 > –0.07 kg N kg^{-1} fertilizer N). Nitrogen rate had a significant main factor effect on NRE at GRANT19 where NRE for 25% N rate was significantly greater than other N rates and the increase in NRE was observed with a decrease in N rates.

Significant interaction effects of cultivar and N rate on PFP was observed at ENREC19 and HPAL18 (Table 6). Cultivar Ruth had a higher PFP than ‘Freeman’ at the lower N rates (0 and 25%) at ENREC19 and vice-versa for the control N treatment at HPAL18. There was no significant difference in PFP by cultivars at other N rates with one exception at HPAL18 where ‘Freeman’ with 25% N rate had a significantly higher PFP than ‘Ruth’ with 75% N rate.

There was a significant main factor effect of cultivar at 2 site-years. Cultivar Ruth had a greater PFP than ‘Freeman’ at PREC19 and the reverse was true at ENREC18. The main factor effect of N application timing on PFP was significant at GRANT18 where Split and Spring N application had greater PFP compared to fall N application. There were significant main factor effects of N rate on PFP at 5 site-years which include both wet and dry site-years (ENREC18, GRANT18,

TABLE 6 Three-way ANOVA *F*-test probabilities for grain nitrogen removal (GNR), nitrogen recovery efficiency (NRE), and partial factor productivity (PFP) as affected by cultivar (C), N rate, N application timing (Timing), and their interaction at 7 site-years in Nebraska

Variables and site-year	C	Timing	Nrate	C × Timing	C × N rate	Timing × N rate	C × Timing × N rate
GNR							
ENREC18	**<.0001**	.72	.91	.37	.54	.75	.39
ENREC19	**.02**	**.04**	**<.0001**	**.01**	**.02**	.13	.47
GRANT18	.27	**.01**	**<.0001**	.52	.73	.26	.55
GRANT19	**.0029**	.88	.30	.89	.99	.22	.46
HPAL18	.10	.30	**<.0001**	.70	.22	.85	.80
HPAL19	.14	.28	**<.0001**	.12	.93	.21	.13
PREC19	**.004**	.11	.40	.24	**.03**	.18	.33
NRE							
ENREC18	**.02**	.29	.95	.29	.11	.26	.63
ENREC19	**.03**	**.009**	.94	**.007**	**<.0001**	.11	**.02**
GRANT18	.46	.07	.13	.96	.74	.05	.56
GRANT19	.15	.93	**.006**	.47	.91	.95	.26
HPAL18	.47	.39	.58	.66	.86	.94	.98
HPAL19	.26	.32	.94	**.01**	.99	.32	**.02**
PREC19	**<.0001**	.07	**<.0001**	.70	**.004**	.80	.94
PFP							
ENREC18	**<.0001**	.53	**<.0001**	.80	.80	.36	.49
ENREC19	**.010**	.40	**<.0001**	.33	**<.0001**	.84	.91
GRANT18	.80	**.03**	**<.0001**	.81	.55	.66	.68
GRANT19	.09	.93	**<.0001**	.93	.95	.32	.49
HPAL18	**.049**	.51	**.049**	.67	**.02**	.78	.65
HPAL19	.50	.80	**<.0001**	.13	.82	.44	.46
PREC19	**.01**	.24	**<.0001**	.23	.08	.32	.53

Note. Significance level of effects <.05 are given in bold numbers.

GRANT19, HPAL19, and PREC19). At all of these site-years, zero N rate had the greatest PFP (11.37–67.62 kg grain yield kg^{-1} total available N) and PFP decreased with the increase in N rates.

3.7 | Relationship among agronomic variables

A significant linear relationship was observed between grain protein content and grain yield at 5 site-years out of 7 with slope coefficient ranging from −5.93 to 3.24 (g kg^{-1} grain protein content per Mg ha^{-1} grain yield) (Figure 9a). However, the fit of regression was low (R^2 = .03–.35). Among the significant regressions, 2 site-years each had negative (GRANT19 and PREC19) and positive (HPAL18 and ENREC19) slopes. The regression at each site-year by N rate showed an inconsistent trend of slope coefficients across N rates (Supplemental Table S2). Analysis of variance on residuals of the above linear regression showed a significant main factor effect of N rate on GPD which suggested that grain protein content increased with increasing N rates when accounted for grain yield.

The GNR increased significantly with the increase in grain yield at all site-years with slope coefficients ranging from 11.6 to 19.3 kg N Mg^{-1} grain yield (Figure 9e). Analysis of variance on residuals for the regression showed a significant main factor effect of cultivars and N rate on GNRD. Cultivar Ruth had significantly higher GNR than 'Freeman' (Figure 9f) and GNR increased with an increase in N rates (Figure 9g).

3.8 | Postharvest residual mineral soil N

Residual total mineral N were in the order 125% N = 100% N > 75% N = 0% N at ENREC19 (Table 7). Residual mineral N was significantly greater with the 125% N than the control and 75% N at HPAL18. The N rate treatments at 125 and 100% had greater residual mineral N than the control at HPAL19. Residual mineral N did not vary by N rates at ENREC18, GRANT18, GRANT19, and PREC19. Overall, all

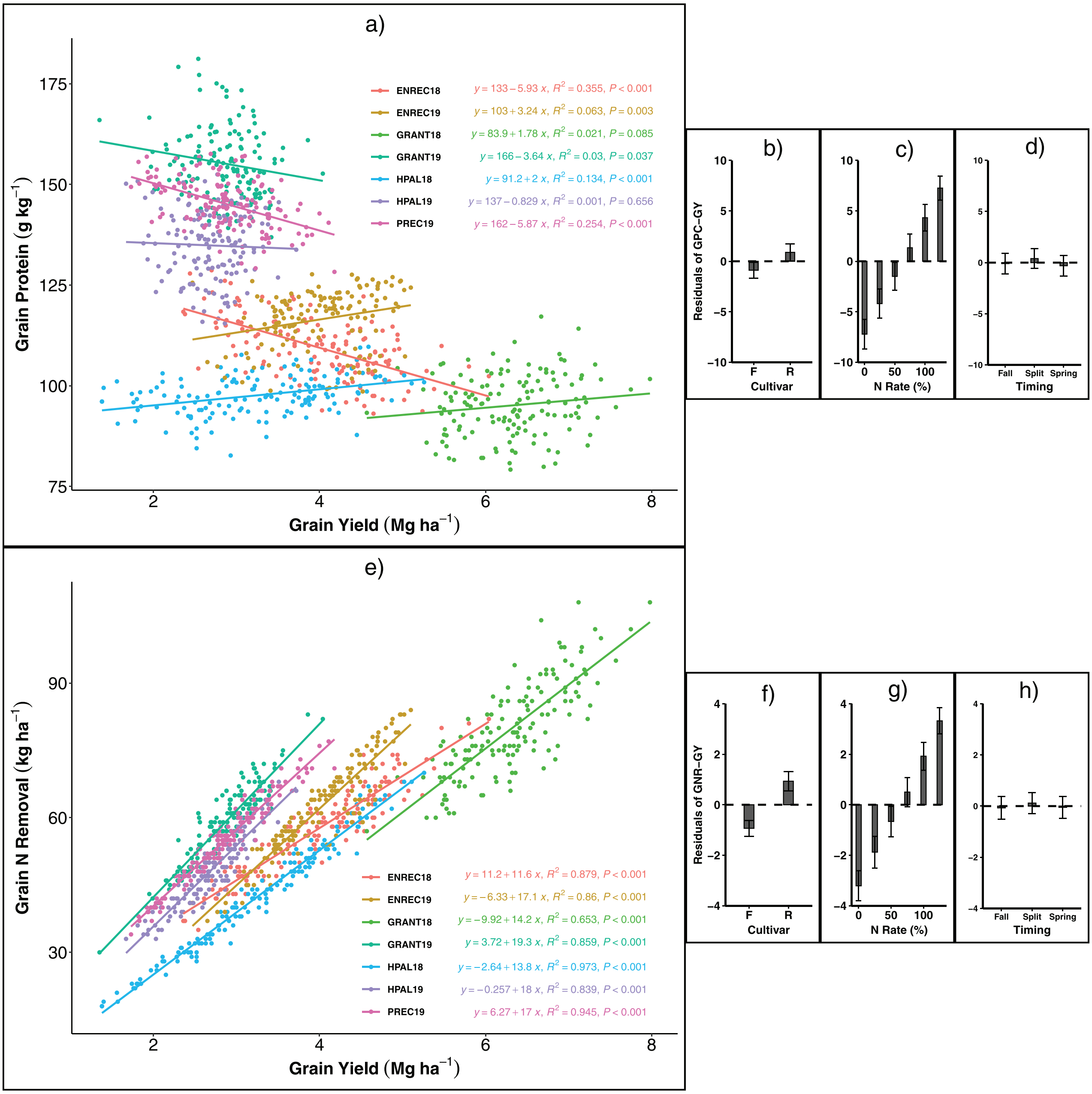

FIGURE 9 Relationship (a) between grain yield and grain protein content, and (e) between grain yield and grain N removal. Analysis of variance for the residuals of the regressions in (a) and (e) are shown for (b, f) N rate, (c, g) cultivar, and (d, h) N application timing. Error bars represent standard error

sites in Year 2 tended to have greater residual mineral N than in Year 1.

4 | DISCUSSION

4.1 | Interannual differences in grain yield and grain protein

The experimental sites in this study cover a varied precipitation gradient and represent overall agroclimatic and production zones of winter wheat growing areas in Nebraska (Peterson, 1992). Greater grain yield in Year 1 than in Year 2 can be attributed to greater monthly and cumulative water supply compared to water demand during the growing season. Among all site-years with no disease pressure and hail damage, GRANT18 had a moderate cumulative WS/WD ratio (0.41) and distinctively higher ratio in March through June (0.53–0.70) making ample water available during critical growth stages and thereby, had the highest of all grain yields (Bian et al., 2016). Mohammed et al. (2013) and Deng et al. (2006) also reported similar results in a winter wheat

TABLE 7 Residual total mineral (nitrate + ammonium) N in postharvest soil samples (0–90 cm) under different N rates at all site-years across Nebraska during winter wheat growing seasons in Year 1 (2018/2019) and Year 2 (2019/2020)

N rate[a]	ENREC18	ENREC19	GRANT18	GRANT19	HPAL18	HPAL19	PREC19
	kg N ha^{-1}						
0	28.0	53.1b[b]	45.8	131.1	19.2b	43.9b	219.8
75	34.9	75.4b	49.8	155.0	17.3b	51.1ab	230.0
100	33.5	110.0a	51.5	100.2	20.1ab	67.7a	261.0
125	32.2	101.7a	53.9	112.8	26.5a	63.6a	294.7
P value	.51	**<.0001**	.69	.14	**.042**	**.028**	.52

Note. Significance level of effects <.05 are given in bold numbers.

[a] Unit of N rate is percentage of recommended N rate.

[b] Means followed by same letter in each column are not significantly different at $p < .05$ using least-squares means.

experiment in Oklahoma, where grain yield was higher in years that received adequate precipitation during spring. These results support those by Partignani et al. (2014) in which as little as 250 mm growing season precipitation was enough to maximize winter wheat grain yield in Oklahoma, as long as precipitation distribution was favorable.

Severe infection of FHB caused yield loss at ENREC in Year 1 and therefore, there was no greater yield at ENREC in Year 1 than in Year 2 as was the case with other sites. For the same reason, ENREC did not yield the highest among the sites in Year 1 although it had a higher yield potential as it received more precipitation and higher recommended N and seeding rates than other sites.

The decline in yield across site-years with a lower WS/WD ratio (0.18–0.21) compared to site-years with a greater WS/WD ratio (0.41–0.84) led to a higher grain protein content in drier site-years over wet site-years. Increased grain yield favored by adequate precipitation might have left minimal residual N in soil for crop uptake at the flowering stage and hence, lower grain protein content level in site-years with greater WS/WD ratio. Grain yield is reported to negatively correlate with grain protein content under low soil N condition and higher grain yield (Fowler, 2003; Kibite & Evans, 1984). This inverse grain yield–protein relation is attributed to the dilution of grain N resulted from a higher accumulation of carbohydrates in the kernel (Grant et al., 1985; Terman, 1979). Higher plant biomass from increased N uptake during early growth stages and low remobilization of N to the grains might also be a potential reason for inverse grain yield–protein relation (Gaju et al., 2014). However, the positive relationship between grain yield and protein observed at some site-years suggests that the negative relationship is not universal (Lollato et al., 2021; Torrion et al., 2019). Grain protein content is sometimes identified as an indicator of N sufficiency (Goos et al., 1982). The lower grain protein content (<12 g kg^{-1}) in site-years with greater WS/WD ratio (0.41–0.84) observed even with the high N rates suggest that additional N input could achieve greater yield and protein content in high-yielding environments.

4.2 | Cultivar differences

The linear regression of grain yield and grain protein content against environmental index suggested that both cultivars had broad adaptability for grain yield and grain protein content. Depending on annual precipitation, 'Ruth' yielded higher in a dry year (three out of four sites) and 'Freeman' yielded higher in a wet year (two out of three sites). Differences in yield between cultivars can be attributed to the cultivar characters as reported by Baenziger et al. (2020). Freeman is a cultivar developed for broad adaptation given temperature, elevation, and precipitation gradients present across the state and it yielded greater than 'Ruth' under wet environment. Ruth as a semi-dwarf cultivar was developed more recently considering the moisture-limited environment and it yielded better than 'Freeman' under dry environment. Greater PFP was observed with 'Ruth' in drier site-years and with 'Freeman' in wet site-years also attest to similar advantage each cultivar has depending on growing environments. Otherwise, a statewide dryland wheat variety trial reported that grain yields averaged across years (2015–2017) were similar for these two cultivars in all the three wheat-growing regions in the Northeast (Southeast-includes ENREC, west central-includes GRANT, and West-includes HPAL and PREC) (Institute of Agriculture and Natural Resources, 2019). A late-maturing cultivar will have a warmer grain-filling condition and might accumulate more protein (Castro et al., 2007). Although 'Ruth' has moderately late maturity compared to 'Freeman', there were no consistent cultivar differences in grain protein. In a given site-year where one cultivar had greater yield also had lower protein which rather suggested an inverse yield–protein relationship.

4.3 | Nitrogen application time

Most of the N uptake by wheat occurs during stem elongation (Feekes GS 6–10) and N application prior to this stage has greater N loss potential (Zebarth et al., 2007). There was a substantial precipitation event (total of 48 mm) within 10 d after the fall application of fertilizer at GRANT18 (Figure 1). Precipitation is one of the important drivers of N loss from the root zone via nitrate leaching (Maharjan et al., 2014; Singh et al., 1995). Denitrification can also be a significant pathway for N loss in medium/heavy texture soils with water-logged conditions (Kaur et al., 2020). The possible loss of applied N might have resulted in a lower yield for fall N-applied plots compared to split or spring treatments at GRANT18. At ENREC19, a similar increase in yield with spring or split N application was observed over fall application for 'Ruth'. There was only around 2 mm precipitation within 1 wk of fall N application at ENREC19. Therefore, this difference in yield by N application timing for 'Ruth' suggests that this cultivar can potentially benefit from split or spring N application over fall application under rainfed condition even in a dry year given no or minimal disease pressure. Given the high variability in weather and grain yield response to applied N, split or spring application of N provides additional advantage allowing to make economy-based decision on whether to fertilize or terminate winter wheat as a cover crop in spring and plant other crops such as maize in spring.

Improvement in grain protein content of winter wheat with split-applied N was reported in previous studies (Dhillon et al., 2020; Garrido-Lestache et al., 2004; Lollato et al., 2021). Our result aligned with the finding of Graham and Stockton (2019) who observed no significant increase in grain protein content by split N application over the at-sowing N application. Other studies reported an increase in grain protein content with fertilizer N top-dressed and foliar-applied in late spring (Mohammed et al., 2013). Dick et al. (2016), Weber et al. (2008), and Zebarth et al. (2007) found that split N applied late-season during flag leaf stage (Feekes GS 9) and the post-flowering stage (Feekes GS 10.5.4) increased grain protein content in winter wheat in Oklahoma. In our experiment, the spring application of N was made at or around Feekes GS 5. Thus, this earlier application of spring dose of N fertilizer might have contributed to vegetative growth but not to increasing grain protein content in spring-applied N treatments.

Available N for crop uptake beyond vegetative stages or remobilization of plant accumulated N to grains would contribute to increasing grain protein content (Doyle & Shapland, 1991; Ottman et al., 2000; Vaughan et al., 1990). Therefore, it would require applying N rates greater than those considered in this study (particularly in spring of years with enough moisture) or applying additional N after the booting stage to observe an increase in grain protein content (Wang et al., 2008). Foliar application of N may be an effective strategy for in-season N management, when feasible, especially in dry conditions where N uptake from soil may be severely impacted (Dick et al., 2016; Woolfolk et al., 2002; Wyatt et al., 2018). However, one should account for a potential risk of leaf burn associated with late-season foliar N application, particularly at higher N rates and thereby, impacting grain yield (Cruppe et al., 2017). Urea with urease and nitrification inhibitor was reported to enhance winter wheat yield and protein in the U.S. southern Plains (Adams et al., 2018). Advanced fertilizer technology such as polymer coating and chemical inhibitors slow N transformation in soil and thereby, may improve wheat yield and protein with a sustained supply of N during the season. In-season N management for greater protein should account for associated costs and returns (Corassa et al., 2018).

4.4 | Nitrogen rate

In a dryland environment such as Nebraska, grain yield and protein content can be co-limited by water and N availability (Cossani & Sadras, 2018; Sadras et al., 2016). Modeled indices of colimitation capturing water and N interaction affected wheat yield and nutrient use efficiency (Sadras, 2004). The site-years with a moderate WS/WD ratio (0.41–0.49) showed a significant increase in grain yield with an increase in N rates. These results aligned with the findings of Walsh et al. (2018) who reported an improved yield with increased fertilizer N application at experimental sites that had good soil moisture resulted from timely precipitation in a semi-arid environment. Availability of water during critical growth stages enhances NUE in wheat (Ayad et al., 2010; Ma et al., 2019) and thereby, increases crop yield as N rate increases. When moisture is not limiting, an increase in available N to plants enhances dry matter accumulation by affecting leaf area, radiation interception, and photosynthetic efficiency (Duan et al., 2019; Srivastava et al., 2018). The positive linear response of grain yield with no plateau to N rates (including 125% of recommended N rate) observed at some site-years suggested that there is a potential to further increase grain yield by applying additional N when there is adequate soil moisture and low disease pressure.

The current Nebraska winter wheat recommendation suggests for ≤112 kg N ha^{-1} for a maximum grain yield of 5 Mg ha^{-1} with ≥120 g kg^{-1} grain protein content under rainfed conditions (Hergert & Shaver, 2009). It does not directly account for the yield potential but rather, economics (cost of fertilizer and grain) of the production. As observed in the experiment, potential grain yield for a given site varied by year based on the weather conditions and the grain yield response to applied N also varied as a function of WS/WD. Therefore, a more robust fertilizer N recommendation can be developed with a consideration of yield potential that is co-limited by water and fertilizer. This can further open the opportunity for in-season N management using crop sensors that can determine crop N stress and need (Mullen et al., 2003; Raun et al.,

2005) and crop water stress and needs (Sun et al., 2019). In addition to crop canopy reflectance data, a series of variables such as weather and soil moisture should be accounted for while developing a decision framework accompanied by machine-learning techniques to improve the accuracy and reliability of in-season N rate recommendation (Colaço et al., 2021).

The decrease in yield with increasing N rate treatments at ENREC18 might be because of the effect of FHB on yield across all treatments. A similar result was reported by Varga et al. (2005) where no benefits in winter wheat grain yield was observed by increasing N rate under high disease severity and no fungicide application. Severity of FHB could be higher in wheat fertilized with higher N rates (Lemmens et al., 2004) and hence, a negative response of yield to N rate as was observed at ENREC18.

A drier soil environment in Year 2 due to lower precipitation during the growing season masked potential benefits of adding N at all sites (GRANT19, HPAL19, and PREC19) except for ENREC19 as evidenced by higher residual mineral N particularly under high N rate treatments in Year 2 than in Year 1. Greater yield with increasing rates of N was observed at ENREC19 because yield potential was considerably higher there in Year 2 compared to other sites. Although Year 2 was a drier year than normal, ENREC19 was not too dry nor as drastically dry as the other three sites. At PREC19, there was also a high pre-plant residual nitrate-N (220 kg ha^{-1}) at 0-to-120-cm depth and such high residual soil N reduces fertilizer NUE (Duan et al., 2019; Matson et al., 1998) and makes N application less effective to increase yield, particularly in a drier environment.

Increase in grain protein content with N rate observed across site-years in this experiment align with the findings of Mohammed et al. (2013) in an experiment conducted in Oklahoma, where they reported an increase of grain protein content with increasing fertilizer N in winter wheat while the increment in protein content was variable across years. There are other studies that reported similar results (Bhatta et al., 2017; Lollato et al., 2019; Zhang et al., 2017). However, Abedi et al. (2011) reported that over-application of fertilizer N (360 kg N ha^{-1}) can result in a drop in protein content in wheat grains due to dilution effect from increased grain yield.

4.5 | Other agronomic responses (GNR, NRE, and PFP)

The average NRE observed across site-years (0.02–0.25 kg N kg^{-1} fertilizer N, excluding PREC19) was lower compared to the estimated global NUE of 0.35 kg N kg^{-1} for cereal crops (Omara et al., 2019) and those reported for the U.S. Great Plains region (Lollato et al., 2021). The exceptionally higher NRE observed at PREC19 (1.52 kg N kg^{-1} fertilizer N) could be attributed to the higher pre-plant residual NO_3–N (Yan et al., 2014), which increased available N for uptake while it was not accounted for in NRE calculation. A decrease in PFP with the increase in N rates, as observed in the study, has been widely reported (Lollato et al., 2021; Yang et al., 2019) and can be attributed to inefficient crop N uptake or loss of N via different pathways.

5 | CONCLUSION

Nitrogen fertilization increased grain yield only in wet environments while it increased grain protein content in both dry and wet environments. Spring and split-applied N can be effective over fall application in those years when there is a greater risk of applied N loss via leaching and denitrification. Ruth, as a semi-dwarf most recently developed cultivar for greater adaptation in the Nebraska and neighboring states demonstrated potential to benefit from spring or split N application given no disease pressure. An inverse relationship between grain yield and protein was prominent in dry environments while there was a positive relationship in wet and disease-free environment. Available soil N for crop uptake beyond the vegetative stage is critical to increasing grain protein content. Further research on applying fertilizer N beyond Feekes GS 10 or the use of available fertilizer technologies such as controlled- or slow-release N to achieve prolonged and sustained soil N through the flowering stage is warranted. An effective N management strategy for winter wheat should account for and be adaptable to weather variability, particularly available moisture during spring to optimize grain yield and protein content. This may be achieved using in-season N management tools like crop canopy sensors, the potential of which needs to be further explored in dryland winter wheat. Variability in weather and its impact on yield potential observed in this experiment indicated that directly accounting for yield potential into the N recommendation algorithm, which is missing in the current algorithm, can be useful for successful N management. Future studies on the economics of the cost associated with differential N fertilization and the returns from yield gains and protein premiums can aid winter wheat growers in making appropriate decisions. With the premium being paid for higher proteins, it might be worth an effort to incorporate grain protein component directly into the N recommendation as attempts are made to revise and improve current algorithms.

ACKNOWLEDGMENTS

We acknowledge Nebraska Wheat Board and University of Nebraska-Lincoln Institute of Agriculture and Natural Resourses for funding support.

AUTHOR CONTRIBUTION STATEMENT

Deepak Ghimire: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Writing-original draft, Writing-review & editing. Saurav Das: Data curation, Formal analysis, Writing-original draft, Writing-review & editing. Cody Creech: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing-original draft, Writing-review & editing. Dipak Santra: Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing-original draft, Writing-review & editing. Nathan Mueller: Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing-review & editing. Brian Maust: Investigation, Methodology, Project administration, Resources, Writing-review & editing. Amanda Easterly: Data curation, Investigation, Methodology, Project administration, Resources, Writing-review & editing. P. Stephen Baenziger: Conceptualization, Funding acquisition, Investigation, Methodology, Writing-review & editing. Bijesh Maharjan: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing-original draft, Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Deepak Ghimire https://orcid.org/0000-0001-6718-2302
Cody F. Creech https://orcid.org/0000-0002-5334-4814
Dipak Santra https://orcid.org/0000-0001-7484-6672
Bijesh Maharjan https://orcid.org/0000-0002-4728-7956

REFERENCES

Abedi, T., Alemzadeh, A., & Kazemeini, S. A. (2011). Wheat yield and grain protein response to nitrogen amount and timing. *Australian Journal of Crop Science*, *5*(3), 330–336.

Adams, C. B., Thapa, S. B., Fan, Y., & Park, S. (2018). Agronomic and economic effects of two enhanced-efficiency urea fertilizer technologies on southern Great Plains winter wheat. *Agronomy Journal*,*110* (3), 1097–1102. https://doi.org/10.2134/agronj2017.08.0485

Ayad, J. Y., Al-Abdalla, A. M., & Saoub, H. M. (2010). Variation in root water and nitrogen uptake and their interactive effects on growth and yield of spring wheat and barley genotypes. *International Journal of Botany*, *6*(4), 404–413. https://doi.org/10.3923/ijb.2010.404.413

Baenziger, P. S., Graybosch, R. A., Regassa, T., Klein, R. N., Kruger, G. R., Santra, D. K., Xu, L., Rose, D. J., Wegulo, S. N., Jin, Y., Kolmer, J., Hein, G. L., Chen, M.-S., Bai, G., Bowden, R. L., & Poland, J. (2014). Registration of 'NE06545' (Husker Genetics Brand Freeman) hard red winter wheat. *Journal of Plant Registrations*, *8*(3), 279–284. https://doi.org/10.3198/jpr2014.02.0009crc

Baenziger, P. S., Graybosch, R. A., Rose, D. J., Xu, L., Guttieri, M. J., Regassa, T., Klein, R. N., Kruger, G. R., Santra, D. K., Hergert, G. W., Wegulo, S. N., Jin, Y., Kolmer, J., Hein, G. L., Bradshaw, J., Chen, M.-S., Bai, G., Bowden, R. L., El-Basyoni, I., & Lorenz, A. (2020). Registration of 'NE10589' (Husker Genetics Brand Ruth) hard red winter wheat. *Journal of Plant Registrations*, *14*(3), 388–397. https://doi.org/10.1002/plr2.20068

Barraclough, P. B., Lopez-Bellido, R., & Hawkesford, M. J. (2014). Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. *Field Crops Research*, *156*, 242–248. https://doi.org/10.1016/j.fcr.2013.10.004

Bhatta, M., Regassa, T., Rose, D. J., Baenziger, P. S., Eskridge, K. M., Santra, D. K., & Poudel, R. (2017). Genotype, environment, seeding rate, and top-dressed nitrogen effects on end-use quality of modern Nebraska winter wheat. *Journal of the Science of Food and Agriculture*, *97*(15), 5311–5318. https://doi.org/10.1002/jsfa.8417

Bian, C., Ma, C., Liu, X., Gao, C., Liu, Q., Yan, Z., Ren, Y., & Li, Q. (2016). Responses of winter wheat yield and water use efficiency to irrigation frequency and planting pattern. *PLOS ONE*, *11*(5), e0154673. https://doi.org/10.1371/journal.pone.0154673

Bijay-Singh, Yadvinder-Singh, & Sekhon, G. S. (1995). Fertilizer-N use efficiency and nitrate pollution of groundwater in developing countries. *Journal of Contaminant Hydrology*, *20*(3), 167–184. https://doi.org/10.1016/0169-7722(95)00067-4

Brown, B., Westcott, M., Christensen, N., Pan, B., & Stark, J. (2005). *Nitrogen management for hard wheat protein enhancement (PNW 578)*. Pacific Northwest Extension. https://www.extension.uidaho.edu/publishing/pdf/PNW/PNW0578.pdf

Cassman, K. G., Bryant, D. C., Fulton, A. E., & Jackson, L. F. (1992). Nitrogen supply effects on partitioning of dry matter and nitrogen to grain of irrigated wheat. *Crop Science*, 32 (5). https://doi.org/10.2135/cropsci1992.0011183X003200050038x

Cassman, K. G., Peng, S., Olk, D. C., Ladha, J. K., Reichardt, W., Dobermann, A., & Singh, U. (1998). Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Research*, *56*(1), 7–39. https://doi.org/10.1016/S0378-4290(97)00140-8

Castro, M., Peterson, C. J., Rizza, M. D., Dellavalle, P. D., Vázquez, D., IbáÑez, V., & Ross, A. (2007). Influence of heat stress on wheat grain characteristics and protein molecular weight distribution. In H. T. Buck, J. E. Nisi, & N. Salomón (Eds.), *Wheat production in stressed environments* (pp. 365–371). : Springer. https://doi.org/10.1007/1-4020-5497-1_45

Colaço, A. F., Richetti, J., Bramley, R. G. V., & Lawes, R. A. (2021). How will the next-generation of sensor-based decision systems look in the context of intelligent agriculture? A case-study. *Field Crops Research*, *270*, 108205. https://doi.org/10.1016/j.fcr.2021.108205

Cooper, J. E. (1974). Effects of post-planting applications of nitrogenous fertilizers on grain yield, grain protein content, and mottling of wheat. *Queensland Journal of Agriculture and Animal Science*, *31*, 33-42.

Corassa, G. M., Hansel, F. D., Lollato, R., Pires, J. L. F., Schwalbert, R., Amado, T. J. C., Guarienti, E. M., Gaviraghi, R., Bisognin, M. B., Reimche, G. B., Santi, A. L., & Ciampitti, I. A. (2018). Nitrogen management strategies to improve yield and dough properties in hard red spring wheat. *Agronomy Journal*, *110*(6), 2417–2429. https://doi.org/10.2134/agronj2018.02.0075

Cossani, C. M., & Sadras, V. O. (2018). Water–nitrogen colimitation in grain crops. *Advances in Agronomy*, 150, 231–274. https://doi.org/10.1016/bs.agron.2018.02.004

Cruppe, G., Edwards, J. T., & Lollato, R. P. (2017). In-season canopy reflectance can aid fungicide and late-season nitrogen decisions on winter wheat. *Agronomy Journal*, *109*(5), 2072–2086. https://doi.org/10.2134/agronj2016.12.0720

Deng, X.-P., Shan, L., Zhang, H., & Turner, N. C. (2006). Improving agricultural water use efficiency in arid and semiarid areas of China. *Agricultural Water Management*, *80*(1), 23–40. https://doi.org/10.1016/j.agwat.2005.07.021

Dhillon, J., Eickhoff, E., Aula, L., Omara, P., Weymeyer, G., Nambi, E., Oyebiyi, F., Carpenter, T., & Raun, W. (2020). Nitrogen management impact on winter wheat grain yield and estimated plant nitrogen loss. *Agronomy Journal*, *112*(1), 564–577. https://doi.org/10.1002/agj2.20107

Dick, C. D., Thompson, N. M., Epplin, F. M., & Arnall, D. B. (2016). Managing late-season foliar nitrogen fertilization to increase grain protein for winter wheat. *Agronomy Journal*, *108*(6), 2329–2338. https://doi.org/10.2134/agronj2016.02.0106

Doyle, A.d, & Shapland, R.a (1991). Effect of split nitrogen applications on the yield and protein content of dryland wheat in northern New South Wales. *Australian Journal of Experimental Agriculture*, *31*(1), 85–92. https://doi.org/10.1071/EA9910085

Duan, J., Shao, Y., He, L., Li, X., Hou, G., Li, S., Feng, W., Zhu, Y., Wang, Y., & Xie, Y. (2019). Optimizing nitrogen management to achieve high yield, high nitrogen efficiency and low nitrogen emission in winter wheat. *Science of the Total Environment*, *697*, 134088. https://doi.org/10.1016/j.scitotenv.2019.134088

Eberhart, S. A., & Russell, W. A. (1966). Stability parameters for comparing varieties. *Crop Science*, *6*(1), 36–40. https://doi.org/10.2135/cropsci1966.0011183X000600010011x

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S., & Shewry, P. R. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research*, *114*(3), 329–342. https://doi.org/10.1016/j.fcr.2009.09.005

Fowler, D. B. (2003). Crop nitrogen demand and grain protein concentration of spring and winter wheat. *Agronomy Journal*, *95*(2), 260–265. https://doi.org/10.2134/agronj2003.2600

Fuertes-Mendizábal, T., González-Torralba, J., Arregui, L. M., González-Murua, C., González-Moro, M. B., & Estavillo, J. M. (2013). Ammonium as sole N source improves grain quality in wheat. *Journal of the Science of Food and Agriculture*, *93*(9), 2162–2171. https://doi.org/10.1002/jsfa.6022

Fufa, H., Baenziger, P. S., Beecher, B. S., Graybosch, R. A., Eskridge, K. M., & Nelson, L. A. (2005). Genetic improvement trends in agronomic performances and end-use quality characteristics among hard red winter wheat cultivars in Nebraska. *Euphytica*, *144*(1), 187–198. https://doi.org/10.1007/s10681-005-5811-x

Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S., & Foulkes, M. J. (2014). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Research*, *155*, 213–223. https://doi.org/10.1016/j.fcr.2013.09.003

Garrido-Lestache, E., López-Bellido, R. J., & López-Bellido, L. (2004). Effect of N rate, timing and splitting and N type on bread-making quality in hard red spring wheat under rainfed Mediterranean conditions. *Field Crops Research*, *2–3*(85), 213–236. https://doi.org/10.1016/S0378-4290(03)00167-9

Gibson, M., & Newsham, P. (2018). Bread. In Gibson, M. & Newsham, P., *Food science and the culinary arts* (pp. 121–131). Elsevier. https://www.sciencedirect.com/science/article/pii/B9780128118160000105

Goos, R. J., Westfall, D. G., Ludwick, A. E., & Goris, J. E. (1982). Grain protein content as an indicator of N sufficiency for winter wheat. *Agronomy Journal*, *74*(1), 130–133. https://doi.org/10.2134/agronj1982.00021962007400010033x

Graham, C. J., & Stockton, M. (2019). Winter wheat response to fertilizer type and timing in western South Dakota. *Agronomy Journal*, *111*(3), 1433–1440. https://doi.org/10.2134/agronj2018.06.0391

Grant, C. A., Stobbe, E. H., & Racz, G. J. (1985). The effect of fall-applied N and P fertilizer and timing of N application on yield and protein content of winter wheat grown on zero-tilled land in MANITOBA. *Canadian Journal of Soil Science*, *65*(4). https://doi.org/10.4141/cjss85-068

Hawkesford, M. J. (2017). Genetic variation in traits for nitrogen use efficiency in wheat. *Journal of Experimental Botany*, *68*(10), 2627–2632. https://doi.org/10.1093/jxb/erx079

Hergert, G. W., & Shaver, T. M. (2009). *Fertilizing winter wheat (EC 143)*. University of Nebraska-Lincoln Extension, https://extensionpublications.unl.edu/assets/pdf/ec143.pdf

High Plains Regional Climate Center (HPRCC). (2021). *High Plains Regional Climate Center homepage*. https://hprcc.unl.edu

Institute of Agriculture and Natural Resources (2019). Nebraska winter wheat variety test results. University of Nebraska-Lincoln. https://cropwatch.unl.edu/winter-wheat-variety-test-results

Kaur, G., Singh, G., Motavalli, P. P., Nelson, K. A., Orlowski, J. M., & Golden, B. R. (2020). Impacts and management strategies for crop production in waterlogged or flooded soils: A review. *Agronomy Journal*, *112*(3), 1475–1501. https://doi.org/10.1002/agj2.20093

Kibite, S., & Evans, L. E. (1984). Causes of negative correlations between grain yield and grain protein concentration in common wheat. *Euphytica*, *33*(3), 801–810. https://doi.org/10.1007/BF00021906

Large, E. C. (1954). Growth stages in cereals-illustration of the Feekes scale. *Plant Pathology*, *3*, 128–129. https://doi.org/10.1111/j.1365-3059.1954.tb00716.x

Latshaw, S. P., Vigil, M. F., & Haley, S. D. (2016). Genotypic differences for nitrogen use efficiency and grain protein deviation in hard winter wheat. *Agronomy Journal*, *108*(6), 2201–2213. https://doi.org/10.2134/agronj2016.02.0070

Lemmens, M., Haim, K., Lew, H., & Ruckenbauer, P. (2004). The effect of nitrogen fertilization on *Fusarium* head blight development and deoxynivalenol contamination in wheat: Impact of nitrogen fertilization. *Journal of Phytopathology*, *152*(1), 1–8. https://doi.org/10.1046/j.1439-0434.2003.00791.x

Lollato, R. P., Figueiredo, B. M., Dhillon, J. S., Arnall, D. B., & Raun, W. R. (2019). Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: A synthesis of long-term experiments. *Field Crops Research*, *236*, 42–57. https://doi.org/10.1016/j.fcr.2019.03.005

Lollato, R. P., Jaenisch, B. R., & Silva, S. R. (2021). Genotype-specific nitrogen uptake dynamics and fertilizer management explain contrasting wheat protein concentration. *Crop Science*, *61*(3), 2048–2066. https://doi.org/10.1002/csc2.20442

Ma, G., Liu, W., Li, S., Zhang, P., Wang, C., Lu, H., Wang, L., Xie, Y., Ma, D., & Kang, G. (2019). Determining the optimal N input to improve grain yield and quality in winter wheat with reduced apparent N loss in the North China Plain. *Frontiers in Plant Science*, *10*, 181. https://doi.org/10.3389/fpls.2019.00181

Macy, P. (1936). The quantitative mineral nutrient requirements of plants. *Plant Physiology*, *11*(4), 749–764. https://doi.org/10.1104/pp.11.4.749

Maghirang, E. B., Lookhart, G. L., Bean, S. R., Pierce, R. O., Xie, F., Caley, M. S., Wilson, J. D., Seabourn, B. W., Ram, M. S., Park, S. H., Chung, O. K., & Dowell, F. E. (2006). Comparison of quality characteristics and breadmaking functionality of hard red winter and hard red spring wheat. *Cereal Chemistry*, *83*(5), 520–528. https://doi.org/10.1094/CC-83-0520

Maharjan, B., Venterea, R. T., & Rosen, C. (2014). Fertilizer and irrigation management effects on nitrous oxide emissions and nitrate leaching. *Agronomy Journal*, *106*(2), 703–714. https://doi.org/10.2134/agronj2013.0179

Matson, P. A. (1998). Integration of environmental, agronomic, and economic aspects of fertilizer management. *Science*, *280*(5360), 112–115. https://doi.org/10.1126/science.280.5360.112

Merrill, A. L., & Watt, B. K. (1973). Energy value of foods: Basis and derivation *(Agriculture Handbook 74)*. USDA-ARS. https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Classics/ah74.pdf

Mohammed, Y. A., Kelly, J., Chim, B. K., Rutto, E., Waldschmidt, K., Mullock, J., Torres, G., Desta, K. G., & Raun, W. (2013). Nitrogen fertilizer management for improved grain quality and yield in winter wheat in Oklahoma. *Journal of Plant Nutrition*, *36*(5), 749–761. https://doi.org/10.1080/01904167.2012.754039

Monaghan, J. M., Snape, J. W., Chojecki, A. J. S., & Kettlewell, P. S. (2001). The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. *Euphytica*, *122*(2), 309–317. https://doi.org/10.1023/A:1012961703208

Mullen, R. W., Freeman, K. W., Raun, W. R., Johnson, G. V., Stone, M. L., & Solie, J. B. (2003). Identifying an in-season response index and the potential to increase wheat yield with nitrogen. *Agronomy Journal*, *95*(2), 347–351. https://doi.org/10.2134/agronj2003.3470

Nakano, H., Morita, S., & Kusuda, O. (2008). Effect of Nitrogen application rate and timing on grain yield and protein content of the bread wheat cultivar 'Minaminokaori' in southwestern Japan. *Plant Production Science*, *11*(1), 151–157. https://doi.org/10.1626/pps.11.151

Nebraska Wheat Board (2021). *Nebraska wheat top planted varieties*. https://nebraskawheat.com/wp-content/uploads/2021/04/2021-Variety-Survey.pdf

Omara, P., Aula, L., Oyebiyi, F., & Raun, W. R. (2019). World cereal nitrogen use efficiency trends: Review and current knowledge. *Agrosystems, Geosciences & Environment*, *2*(1), 180045. https://doi.org/10.2134/age2018.10.0045

Ortiz-Monasterior, J. I., Sayre, K. D., Rajaram, S., & Mcmahon, M. (1997). Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Science*, *37*(3), 898–904. https://doi.org/10.2135/cropsci1997.0011183X003700030033x

Ottman, M. J., Doerge, T. A., & Martin, E. C. (2000). Durum grain quality as affected by nitrogen fertilization near anthesis and irrigation during grain fill. *Agronomy Journal*, *92*(5), 1035–1041. https://doi.org/10.2134/agronj2000.9251035x

Oury, F.-X., & Godin, C. (2007). Yield and grain protein concentration in bread wheat: How to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica*, *157*(1), 45–57. https://doi.org/10.1007/s10681-007-9395-5

Patrignani, A., Lollato, R. P., Ochsner, T. E., Godsey, C. B., & Edwards, J. T. (2014). Yield gap and production gap of rainfed winter wheat in the southern Great Plains. *Agronomy Journal*, *106*(4), 1329–1339. https://doi.org/10.2134/agronj14.0011

Peterson, C. J. (1992). Similarities among test sites based on cultivar performance in the hard red winter wheat region. *Crop Science*, *32*(4), 907–912. https://doi.org/10.2135/cropsci1992.0011183X003200040014x

Peterson, C. J., Graybosch, R. A., Shelton, D. R., & Baenziger, P. S. (1998). Baking quality of hard winter wheat: Response of cultivars to environment in the Great Plains. *Euphytica*, *100*(1), 157–162. https://doi.org/10.1023/A:1018361502435]

Plains Grains Inc. (2018). *Hard red winter wheat – 2017 Regional quality survey*.Plains Grains Inc., https://www.plainsgrains.org/wp-content/uploads/2018/10/2017-HRWW-Report__HiRes-FINAL.pdf

Raun, W. R., Solie, J. B., Stone, M. L., Martin, K. L., Freeman, K. W., Mullen, R. W., Zhang, H., Schepers, J. S., & Johnson, G. V. (2005). Optical sensor-based algorithm for crop nitrogen fertilization. *Communications in Soil Science and Plant Analysis*, *36*(19–20), 2759–2781. https://doi.org/10.1080/00103620500303988

Romero, C. M., Engel, R. E., Chen, C., Wallander, R., & Jones, C. A. (2017). Late-fall, winter, and spring broadcast applications of urea to no-till winter wheat. II. Fertilizer N recovery, yield, and protein as affected by NBPT. *Soil Science Society of America Journal*, *81*(2), 331–340. https://doi.org/10.2136/sssaj2016.10.0333

Sadras, V. O. (2004). Yield and water-use efficiency of water- and nitrogen-stressed wheat crops increase with degree of co-limitation. *European Journal of Agronomy*, *21*(4), 455–464. https://doi.org/10.1016/j.eja.2004.07.007

Sadras, V. O., Hayman, P. T., Rodriguez, D., Monjardino, M., Bielich, M., Unkovich, M., Mudge, B., & Wang, E. (2016). Interactions between water and nitrogen in Australian cropping systems: Physiological, agronomic, economic, breeding and modelling perspectives. *Crop and Pasture Science*, *67*(10), 1019–1053. https://doi.org/10.1071/CP16027

SAS Institute. (2015). *SAS 9.4 in-database products: User's guide* (5th ed.). SAS Institute.

Shah, L., Yahya, M., Shah, S. M. A., Nadeem, M., Ali, A., Ali, A., Wang, J., Riaz, M. W., Rehman, S., Wu, W., Khan, R. M., Abbas, A., Riaz, A., Anis, G. B., Si, H., Jiang, H., & Ma, C. (2019). Improving lodging resistance: Using wheat and rice as classical examples. *International Journal of Molecular Sciences*, *20*(17). 4211. https://doi.org/10.3390/ijms20174211

Shewry, P. R. (2007). Improving the protein content and composition of cereal grain. *Journal of Cereal Science*, *46*(3), 239–250. https://doi.org/10.1016/j.jcs.2007.06.006

Simmonds, N. W. (1995). The relation between yield and protein in cereal grain. *Journal of the Science of Food and Agriculture*, *67*(3), 309–315. https://doi.org/10.1002/jsfa.2740670306

Srivastava, R. K., Panda, R. K., Chakraborty, A., & Halder, D. (2018). Enhancing grain yield, biomass and nitrogen use efficiency of maize by varying sowing dates and nitrogen rate under rainfed and irrigated conditions. *Field Crops Research*, *221*, 339–349. https://doi.org/10.1016/j.fcr.2017.06.019

Stewart, B. A. (2016). Dryland farming. In Smithers, G., Trinetta, V. & Knoerzer, K., *Reference module in food science*. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.02937-1

Sun, H., Feng, M., Xiao, L., Yang, W., Wang, C., Jia, X., Zhao, Y.u, Zhao, C., Muhammad, S. K., & Li, D. (2019). Assessment of plant water status in winter wheat (*Triticum aestivum* L.) based on canopy spectral indices. *PLOS ONE*, *14*(6), e0216890. https://doi.org/10.1371/journal.pone.0216890

Terman, G. L. (1979). Yields and protein content of wheat grain as affected by cultivar, N, and environmental growth factors. *Agronomy Journal*, *71*(3), 437–440. https://doi.org/10.2134/agronj1979.00021962007100030014x

Tilley, M., Chen, Y. R., & Miller, R. A. (2012). Wheat breeding and quality evaluation in the US. In S. P. Cauvain (Ed.), *Breadmaking* (2nd ed., pp. 216–236). Woodhead Publishing. https://doi.org/10.1533/9780857095695.1.216

Torrion, J. A., Walsh, O. S., Liang, X., Bicego, B., & Sapkota, A., (2019). Managing 'egan' wheat with a gene for high grain protein. Agrosystems. *Geosciences & Environment*, *2*(1), 1–8. https://doi.org/10.2134/age2019.03.0019

Triboi, E., Martre, P., Girousse, C., Ravel, C., & Triboi-Blondel, A.-M. (2006). Unravelling environmental and genetic relationships between grain yield and nitrogen concentration for wheat. *European Journal of Agronomy*, *25*(2), 108–118. https://doi.org/10.1016/j.eja.2006.04.004

USDA–NRCS. (2020). *Web soil survey*. : USDA–NRCS. http://websoilsurvey.sc.egov.usda.gov/app/websoilsurvey.aspx

Varga, B., Svecnjak, Z., Macesic, D., & Uher, D. (2005). Winter wheat cultivar responses to fungicide application are affected by nitrogen fertilization rate. *Journal of Agronomy and Crop Science*, *191*(2), 130–137. https://doi.org/10.1111/j.1439-037X.2004.00133.x

Varvel, G. E., & Peterson, T. A. (1990). Nitrogen fertilizer recovery by corn in monoculture and rotation systems. *Agronomy Journal*, *82*(5), 935–938. https://doi.org/10.2134/agronj1990.00021962008200050019x

Vaughan, B., Westfall, D. G., & Barbarick, K. A. (1990). Nitrogen rate and timing effects on winter wheat grain yield, grain protein, and economics. *Journal of Production Agriculture*, *3*(3), 324–328. https://doi.org/10.2134/jpa1990.0324

Walsh, O., Shafian, S., & Christiaens, R. (2018). Nitrogen fertilizer management in dryland wheat cropping systems. *Plants (Basel, Switzerland)*, *7*(1), 9. https://doi.org/10.3390/plants7010009

Wang, Z.-H., Li, S.-X., & Malhi, S. (2008). Effects of fertilization and other agronomic measures on nutritional quality of crops. *Journal of the Science of Food and Agriculture*, *88*(1), 7–23. https://doi.org/10.1002/jsfa.3084

Weber, E. A., Graeff, S., Koller, W. - D., Hermann, W., Merkt, N., & Claupein, W. (2008). Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat (*Triticum aestivum* L.). *Field Crops Research*, *106*(1), 44–52. https://doi.org/10.1016/j.fcr.2007.10.011

Weiss, A. (2003). Assessing winter wheat responses to climate change scenarios: A simulation study in the U.S. Great Plains. *Climatic Change*, *58*(1), 119–147. https://doi.org/10.1023/A:1023499612729

Wheat Marketing Center, Inc. (2004). *Wheat flour testing methods. Wheat Marketing Center, Inc.* https://nebraskawheat.com/wp-content/uploads/2014/01/WheatFlourTestingMethods.pdf

Wilson, T. L., Guttieri, M. J., Nelson, N. O., Fritz, A., & Tilley, M. (2020). Nitrogen and sulfur effects on hard winter wheat quality and asparagine concentration. *Journal of Cereal Science*, *93*, 102969. https://doi.org/10.1016/j.jcs.2020.102969

Wilson, W. W., & Gallagher, P. (1990). Quality differences and price responsiveness of wheat class demands. *Western Journal of Agricultural Economics*, *15*(2), 254–264. https://www.jstor.org/stable/40988089

Woolfolk, C. W., Raun, W. R., Johnson, G. V., Thomason, W. E., Mullen, R. W., Wynn, K. J., & Freeman, K. W. (2002). Influence of late-season foliar nitrogen applications on yield and grain nitrogen in winter wheat. *Agronomy Journal*, *94*(3), 429–434. https://doi.org/10.2134/agronj2002.4290

Wyatt, E. C., Bushong, J. T., Macnack, N. E., Mullock, J. L., Taylor, R., & Raun, W. R. (2018). Influence of droplet size of foliar-applied nitrogen on grain protein content of hard red winter wheat. *Crops & Soils*, *51*(3), 48–58. https://doi.org/10.2134/cs2018.51.0301

Yan, X., Ti, C., Vitousek, P., Chen, D., Leip, A., Cai, Z., & Zhu, Z. (2014). Fertilizer nitrogen recovery efficiencies in crop production systems of China with and without consideration of the residual effect of nitrogen. *Environmental Research Letters*, *9*(9), 095002. https://doi.org/10.1088/1748-9326/9/9/095002

Yang, D., Cai, T., Luo, Y., & Wang, Z. (2019). Optimizing plant density and nitrogen application to manipulate tiller growth and increase grain yield and nitrogen-use efficiency in winter wheat. *PeerJ*, *7*, e6484. https://doi.org/10.7717/peerj.6484

Zebarth, B. J., Botha, E. J., & Rees, H. (2007). Rate and time of fertilizer nitrogen application on yield, protein, and apparent efficiency of fertilizer nitrogen use of spring wheat. *Canadian Journal of Plant Science*, *87*(4), 709–718. https://doi.org/10.4141/CJPS06001

Zhang, M., Wang, H., Yi, Y., Ding, J., Zhu, M., Li, C., Guo, W., Feng, C., & Zhu, X. (2017). Effect of nitrogen levels and nitrogen ratios on lodging resistance and yield potential of winter wheat (*Triticum aestivum* L.). *PLOS ONE*, *12*(11), e0187543. https://doi.org/10.1371/journal.pone.0187543

Zörb, C., Ludewig, U., & Hawkesford, M. J. (2018). Perspective on wheat yield and quality with reduced nitrogen supply. *Trends in Plant Science*, *23*(11), 1029–1037. https://doi.org/10.1016/j.tplants.2018.08.012

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Ghimire D, Das S, Mueller ND, Creech CF, Santra D, Baenziger PS, Easterly AC, Maust B, Maharjan B. Effects of cultivars and nitrogen management on wheat grain yield and protein. *Agronomy Journal* 2021;1–21. https://doi.org/10.1002/agj2.20836