Field Crops Research 306 (2024) 109202

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Cultivar-specific phenotypic plasticity of yield and grain protein concentration in response to nitrogen in winter wheat

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ARTICLE INFO

Keywords:
Phenotype
Genotype
Environment
Interaction
Grain protein dilution
Harvest index

ABSTRACT

Context: Owing to the interaction between genotype and environment (G x E), identifying traits to increase wheat yield and grain protein concentration simultaneously or increasing one without affecting the other remains a challenge. Phenotypic plasticity is an insightful perspective to understand G x E.
Objective: To explore the relations of wheat yield and grain protein concentration in response to N input and its physiological basis from the perspective of phenotypic plasticity.
Method: We established a factorial experiment combining 14 winter wheat cultivars and four N fertilization rates (0, 45, 90 and 135 kg ha^{-1}) in eight environments. We analyzed the interaction of cultivar and N combining a phenotypic plasticity framework and a three-phase model of grain yield and protein response to N input. The phases are: phase I, N supply limits both yield and grain protein; phase II, N supply limits grain protein but not yield; and phase III, N supply does not limit yield or grain protein concentration
Results: Grain yield plasticity was positively associated to yield in high-yielding environments without N limitations (phase II) with no cost in low yielding environments, and associated to harvest index. Grain protein plasticity was positively associated to protein in high protein environments without N limitations (phase III). Plasticity of grain protein concentration was negatively associated to grain number m^{-2}, resulting in moderate negative association of protein plasticity and yield. Grain C:N ratio associated weakly and positively with yield plasticity and strongly and negatively for grain protein plasticity.
Conclusion: This work proposes a yield-protein plasticity framework combined with a three-phase model that allows to disclose G × N interactions. Under our experimental conditions, we identified physiological mechanisms associated to yield and protein plasticity.
Implications: Yield and protein plasticity can contribute guiding grower's cultivar selection towards high yield plasticity cultivars when aiming to high yield with acceptable protein levels or high protein plasticity cultivars to ensure high protein at the expense of lower yields. Yield plasticity brings opportunity to breed for high yielding cultivars while maintaining grain protein concentration. Accuracy of N recommendations models and mechanistic crop models can be improved by accounting for G × N interactions through plasticity of yield and grain protein.

1. Introduction

Nitrogen (N) management is crucial to sustainability avoiding both over-fertilization releasing reactive N to the environment and under-fertilization leading to yield gaps and soil mining (Alvarez et al., 2014; Jobbágy and Sala, 2014; Angus et al., 2017; Jaenisch et al., 2021). Understanding and predicting the response of crop yield and grain protein concentration to N fertilization is a long-standing concern in

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https://doi.org/10.1016/j.fcr.2023.109202
Received 10 August 2023; Received in revised form 31 October 2023; Accepted 17 November 2023

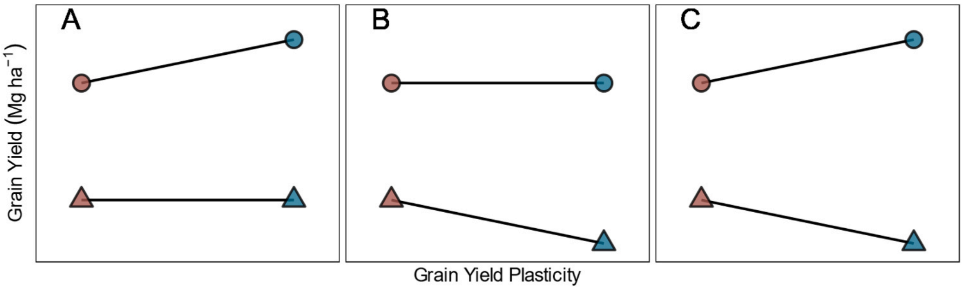

Fig. 1. Hypothetical relations between trait and trait plasticity for a phenotype with high (blue) and low (red) plasticity under conditions that favor high (circles) or low (triangles) traits. The higher plasticity of the blue phenotype arises from (A) responsiveness to favorable conditions, or (B) higher sensitivity to stressful conditions, or (C) high responsiveness to both favorable conditions and stress.

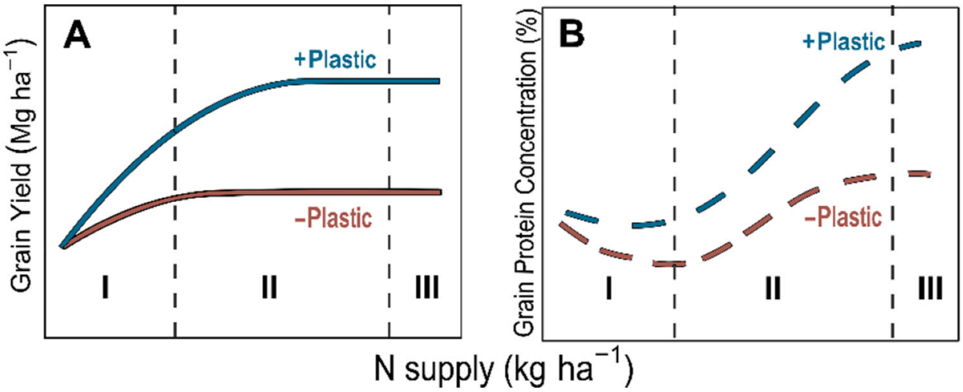

Fig. 2. Conceptual model of grain yield (A; solid lines) and protein (B; dashed lines) in response to N supply for phenotypes with high (blue) and low (red) phenotypic plasticity. Vertical dashed lines are the boundaries of phases I, II, III in the response of yield and protein to N supply. In phase I, N supply limits both yield and grain protein; in phase II, N supply limits grain protein but not yield; and in phase III, N supply does not limit yield or grain protein concentration.

agriculture (Patterson, 1904; Moser, 1932) that remains unsolved due to lagging theoretical frameworks (Cossani and Sadras, 2021; Sadras et al., 2022) to capture the interactions between genotype, environment and management, G x E x M (Van Sanford and MacKown, 1987; Savill et al., 2018; Nehe et al., 2020; Ivić et al., 2021; Lollato et al., 2021).

The association between grain yield and protein concentration is usually negative (Malloch and Newton, 1934; Benzian and Lane, 1981; Triboi et al., 2006; Bogard et al., 2010; Giordano et al., 2023). This is especially important for wheat (*Triticum aestivum* L.), a crop that provides approx. 20% of the human calories (Shewry and Hey, 2015) and the majority of essential amino acids except for lysine, which in the limiting amino acid in all cereals (Shewry, 2007; Shewry and Hey, 2015). The association between yield and grain protein concentration has as genetic component (Monaghan et al., 2001; Oury and Godin, 2007; Nigro et al., 2019; Mosleth et al., 2020), but the interaction with environment (Oury and Godin, 2007) and N supply (Mosleth et al., 2015) can mask genetic effects (*e.g.*, Lollato et al., 2021). The physiological basis of this negative association depends on the degree of source-sink limitation for grain starch and grain N accumulation (Benzian and Lane, 1981; Triboi and Triboi-Blondel, 2002; Triboi et al., 2006; Acreche and Slafer, 2009). Thus, identifying traits to increase yield and protein simultaneously remains a major challenge.

Phenotypic plasticity represents environmentally-induced changes on trait expression of a given genotype (Dewitt and Scheiner, 2004). Phenotypic plasticity depends on the genotype (species, cultivar), the trait (*e.g.*, grain protein concentration, root depth), and the environmental source of variation (*e.g.*, soil moisture, availability of soil N). Its genetic modulation is partially independent of the modulation of the trait *per se* (Bradshaw, 1965; Reymond et al., 2003; Alvarez Prado et al., 2014; Sadras et al., 2016; Kusmec et al., 2018; Laitinen and Nikoloski, 2019; Diouf et al., 2020). The perspective of phenotypic plasticity has offered understanding on G × E × M interactions for agronomically important traits (Sadras et al., 2009; Sadras and Rebetzke, 2013; Alvarez Prado et al., 2014; Sadras et al., 2016, 2019a; Lollato et al., 2020; Diouf et al., 2020; de Felipe and Alvarez Prado, 2021).

In this paper, we explore the relations of wheat yield and grain protein concentration in response to N input and its physiological basis from the perspective of phenotypic plasticity. We advance a conceptual framework that leads to testable hypotheses, and report results from experiments designed to test them.

2. Conceptual framework

2.1. Phenotypic plasticity

Phenotypic plasticity can be quantified as the variance ratio, which is the quotient between the individual variance for a given cultivar VT_{ik} and the variance of the population of cultivars VT_{ip} in a group of environments (Dingemanse et al., 2010; Sadras and Richards, 2014):

$$PP_{ik} = \frac{VT_{ik}}{VT_{ip}} \tag{1}$$

Fig. 1 illustrates trait-plasticity relations using yield as an example. In Fig. 1 (A) plasticity is primarily related to yield responsiveness to favorable conditions, *i.e.* a highly-plastic cultivar outyields its less plastic counterpart under favorable conditions with no difference under low yielding environments. In Fig. 1 (B) plasticity associates with sensitivity to stress with no difference under favorable conditions. In Fig. 1 (C) plasticity associates with both higher yield under favorable conditions and lower yield under stress.

Table 1

Year, location, soil type, geolocation, crop husbandry, and mineral soil N (0–0.6 m) at sowing in eight location-seasons. Fungicide was applied at GS65. Abbreviation: DAS, days after sowing.

Year	Location	Env.	Soil	Latitude	Longitude	Sowing	N fertilization (DAS)	Fungicide (DAS)	Harvest (DAS)	Initial mineral soil N (kg ha-$^{-1}$)	
										NO3-N	NH4-N
2020	Ashland Bottoms	AM20	Belvue silt loam	39°08′31″N	96°37′52″W	10/25/2019	133	207	245	32.9	22.3
	Great Bend	GB20	Taver loam	38°21′46″N	98°52′10″W	10/16/2019	146	211	253	135.2	11.9
	Hutchinson	HU20	Ost loam	37°55′43″N	98°01′50″W	10/10/2019	153	209	263	222.9	51.9
	Viola	VI20	Nalim loam	37°18′36″N	98°40′12″W	10/17/2019	147	202	242	31.8	20.4
2021	Ashland Bottoms	AM21	Belvue silt loam	39°08′31″N	96°37′52″W	10/14/2020	146	208	250	54.3	39.8
	Ashland Bottoms	AB21	Bismark grove silt loam	39°07′57″N	96°37′11″W	10/13/2020	147	211	251	50.2	59.4
	Hutchinson	HU21	Ost loam	37°55′43″N	98°01′50″W	10/8/2020	151	209	251	72.3	53.2
	Manhattan	MA21	Kahola silt loam	38°24′00″N	98°54′00″W	10/7/2020	153	218	243	74.6	92.3

2.2. *Evaluation of yield and grain protein response to N for cultivars with contrasting plasticity*

The responses of grain yield and grain protein concentration to N supply are usually explored with empirical functions (Gastal et al., 2015) despite their limitations (Sadras et al., 2019b). The relationship of yield and grain protein concentration to N supply can be divided in three phases (Stone and Savin, 1999), as depicted in Fig. 2.

In phase I, N supply limits both yield and grain protein concentration. Here, increase in N supply increases grain yield at a diminishing return until a point in which N becomes non-limiting. In this phase, increasing yield associates with reduced grain protein concentration. The large response of yield to N supply in phase I associates with enhanced tillering, leaf expansion, increasing capture of radiation and canopy photosynthesis (Gastal et al., 2015). The reduction in grain protein concentration is due to a dilution effect, as starch accumulates faster than protein (Triboi et al., 2006).

In phase II, grain yield plateaus but N supply still limits grain protein concentration. In this phase, N suffices for maintaining both high crop photosynthetic capacity and rate of carbon deposition in grain. Grain protein accumulation relies primarily on mobilization of vegetative N (Van Sanford and MacKown, 1987; Martre et al., 2003) complemented with N uptake during grain fill (Barneix, 2007; Bogard et al., 2010), when there is a tradeoff between maintenance of shoot N required to sustain radiation use efficiency and allocation of N to grain protein (Sinclair and de Wit, 1976).

In phase III, N supply ensures maximum grain yield and grain protein concentration and increases in N supply are neutral for both. In some cases, excessive N supply can reduce grain yield, *e.g.,* in crops relying on stored soil water, by increasing water use early in the season at the expense of water use during reproduction, and reducing carbohydrate reserves (van Herwaarden et al., 1998; Lollato et al., 2019; Sadras et al., 2019b).

We hypothesize that (i) phenotypic plasticity of yield and grain protein concentration are negatively related; (ii) yield plasticity will be associated to yield in high yield environments without trade-offs under low yielding environments; (iii) protein plasticity is associated to protein in high protein environments without trade-offs under low protein environments (Fig. 1A). Nitrogen supply contributes to environmental variation defining a range of yield and grain protein expression environments conceptualized in Fig. 2. Therefore, we also hypothesize that differences among phenotypes with contrasting plasticity are more likely under non-N limiting conditions (phase II for yield and phase III for grain protein).

This paper focuses on N management to simultaneously improve grain yield and protein concentration (or improving one without negatively affecting the other) of winter wheat cultivars with the objective to answer the following questions:

i. Are yield and grain protein plasticity associated to responsiveness to favorable conditions, stress tolerance, or a combination of both (Fig. 1)?
ii. How does N supply modulate the phenotype (yield and grain protein) of cultivars with contrasting plasticity? At which phases of trait response to N supply do differences among cultivars arise?
iii. Which are the physiological mechanisms associated to phenotypic plasticity under varying N supply?

3. Method

3.1. *Crop husbandry, plant material, and experimental design*

Rainfed field experiments were conducted in eight environments resulting from the combination of seasons and locations in Kansas, US (Table 1). A factorial experiment was established in each environment that combined four N rates (0, 45, 90 and 135 kg N ha^{-1}) and 14 high-

Table 2

Characteristics of the 14 winter wheat commercial cultivars included in the study: year of release, average protein deviation, maturity, plant height, drought tolerance and standability. Phenotypic plasticity (unitless), calculated as variance ratio (Eq. 1), is shown for yield, its components, grain protein concentration, and crop N traits. Abbreviations: year of release (YOR), grain protein deviation from the grain yield ~ grain protein regression line (GPD), maturity (MAT, 1 = early; 5 = medium; 9 = late), plant height (PH), drought tolerance (DT, 1 = excellent; 5 = good; 9 = poor straw strength: 1 = excellent; 5 = good; 9 = poor), standability (Sta), harvest index (HI), N uptake (Nup), N harvest index (NHI), N utilization efficiency (NUtE).

Cultivar	YOR	GPD	MAT*	PH	DT*	Sta*	Phenotypic Plasticity (dimensionless)												
		(%)		(cm)			Yield	Protein	Biomass	HI	Grains m^{-2}	Grain weight	Heads m^{-1}	Grains $head^{-1}$	Biomass Nup	Above-ground Nup	Stover N concentra-tion	NHI	NUtE
Everest	2009	-0.14 ± 0.01	2	69.7 ± 6.5	7	2	0.99	0.87	0.68	0.74	0.8	0.49	0.75	0.68	0.97	0.94	1.05	0.81	0.88
WB Grainfield	2012	-0.49 ± 0.05	5	78.3 ± 7.3	5	4	1	0.97	1.08	1.01	1.1	1	0.85	0.8	0.99	0.94	0.72	0.91	1.18
WB4458	2012	0.57 ± 0.05	4	73.8 ± 6.9	6	2	1.11	1.07	0.79	0.53	0.66	0.56	0.57	0.78	0.96	1.04	1.01	0.86	0.79
DoubleStop	2013	0.92 ± 0.09	6	75.6 ± 7.0	5	4	0.92	0.98	1.47	0.58	0.82	0.94	1.09	0.47	1.19	1.24	0.9	0.84	0.64
SY Monument	2014	-0.25 ± 0.02	6	75.6 ± 7.0	6	5	0.9	0.85	1.19	0.74	0.99	0.73	0.92	0.62	1.08	1.08	0.93	0.8	0.99
Bentley	2015	-0.12 ± 0.01	4	79.6 ± 7.4	4	7	1.13	0.92	0.98	0.93	1.07	0.89	0.88	0.92	0.95	1.13	0.83	0.82	0.98
WB4269	2015	-0.16 ± 0.01	4	68.4 ± 6.3	5	3	1.12	0.74	0.67	0.71	1.24	0.56	1.29	0.61	0.96	0.9	0.92	0.74	0.63
WB4303	2015	0.12 ± 0.01	3	72.5 ± 6.7	6	1	0.98	1	0.82	1.17	1.03	0.9	0.49	1.87	1.09	1	1.29	1.18	0.86
Larry	2016	-0.26 ± 0.02	5	75.0 ± 7.0	6	3	0.89	0.93	0.97	1.09	0.83	0.73	0.65	0.77	0.88	0.9	0.85	0.99	1.05
LCS Chrome	2016	0.43 ± 0.04	8	80.4 ± 7.5	4	3	0.71	1.07	1.12	1.21	0.79	0.99	0.77	0.59	0.97	0.86	1.27	1.58	0.97
Tatanka	2016	-1.18 ± 0.11	6	73.2 ± 6.8	6	7	1.04	0.89	0.77	0.61	1.33	0.63	1.31	0.55	1.04	0.93	0.89	0.83	1.3
Zenda	2016	-0.28 ± 0.03	4	76.6 ± 7.1	7	3	0.9	0.8	0.95	0.57	0.87	0.53	0.77	0.61	1.07	0.87	0.98	1.04	0.85
Bob Dole	2017	0.11 ± 0.01	4	83.5 ± 7.8	6	5	0.96	1.07	1.25	0.79	0.74	0.97	0.7	0.83	1.01	0.89	0.74	0.98	1.15
Green Hammer	2018	0.89 ± 0.08	4	78.2 ± 7.3	4	1	0.97	1.18	0.96	0.63	0.78	0.82	0.56	0.96	0.86	1.32	1.5	1.28	0.82

[*] sources: https://bookstore.ksre.ksu.edu/pubs/mf991.pdf; http://www.ramwheatdb.com/database.php

Table 3
Analytical solutions implemented for identifying the starting point of the phases described in Fig. 2 and fitted in Fig. 5. In phase III, only those cultivars that reached maximum protein were compared. Abbreviations: N supply (N_s).

Phase	Analytical solution	Description
I	$N_s(kgNha^{-1}) = \min(N_s)$	Grain yield and protein comparisons at minimum N supply.
II	$\frac{dYield}{dN_s} = 0, \quad where \frac{dYield}{dN_s} = \lim_{w\to 0}\frac{Yield(N_s + w) - Yield(N_s)}{w}$	Grain yield and protein comparisons at the agronomic optimum N supply (AON). Where AON is the starting point of phase II and represents the N supply that maximizes yield which is represented by the first derivative equal to zero and $w = \Delta N_s$ value approaching zero.
III	$\frac{dProtein}{dN_s} = 0, \quad where \frac{dProtein}{dN_s} = \lim_{w\to 0}\frac{Protein(N_s + w) - Protein(N_s)}{w}, \quad forN_s > \frac{dYield}{dN_s} = 0$	Comparisons of grain protein at N supply that maximizes protein (NMP). Where NMP is the starting point of phase III and represents the N supply that maximizes protein which is represented by the first derivative equal to zero and $w = \Delta N_s$ value approaching zero.

yielding, agronomically adapted winter wheat cultivars with contrasting grain protein concentration (Table 2). The combination of locations and seasons with N rates originated 32 N-environments in which cultivars were evaluated. Treatments were arranged in a split-plot design with three or four replicates depending on the location, with N rates assigned to main plot and varieties to subplots. Fertilizer N was applied as granulated urea (46–0–0) during spring tillering following winter dormancy at GS25 (Zadoks et al., 1974). All plots received 56 kg ha^{-1} of diammonium phosphate at sowing (10 kg of N ha^{-1} and 11 kg of P ha^{-1}) and 22 kg ha^{-1} of sulfur as gypsum (0–0–0–23 S) together with N application, to avoid sulfur limitations (Salvagiotti et al., 2009; Jaenisch et al., 2020).

Trials at Viola, Ashland Bottoms, Manhattan, and Great Bend were sown under no-tillage after soybean (*Glycine max L. Merrill*), and the trials in Hutchinson were established under conventional tillage after winter canola (*Brassica napus L.*) in 2020 and no-till after soybean in 2021. Crop husbandry was similar across locations and seasons (Table 1). Imidacloprid insecticide and tebuconazole fungicide were applied to the seed. Each variety was sown at a rate of 260 seed m^{-2} (adjusting for variety-specific seed size) with a Great Plains 506 no-till drill. Plots were 9.1 m long (trimmed to 7.2 m prior to harvest) and 7 rows wide with a row spacing of 0.19 m. Weeds and insects were controlled as needed using commercial pesticides during the season. To avoid confounding effects of differential disease resistance among varieties (Cruppe et al., 2021), crops were treated with foliar fungicides at anthesis with 89 ml ha^{-1} of picoxystrobin [(Methyl (∝E)-∝-(methoxymethylene)− 2-[[[6-(trifluoromethyl)− 2 pyridinyl]oxy] methyl]benzeneacetate] plus 36 ml ha^{-1} cyproconazole [∝-(4-chlorophenyl)-∝-(1-cyclopropylethyl)− 1 H-1,2, 4-triazole-1-ethanol].

3.2. Measurements

Initial mineral soil N and texture were characterized before sowing in a composite sample of 15 individual soil cores at each 0–15 and 15–60 cm soil depth (Table 2, Supplementary Table 1). Initial mineral soil N content was determined by N-KCl extraction. Daily weather data were retrieved from Kansas Mesonet stations (Patrignani et al., 2020) located near the field experiments. Weather data was summarized for (i) the critical period for yield determination between − 300°Cd and 100°Cd centered on anthesis using a base temperature of 4.5 °C (Fischer, 1985; Cossani and Sadras, 2021) and (ii) grain fill, from 100 °Cd to 600

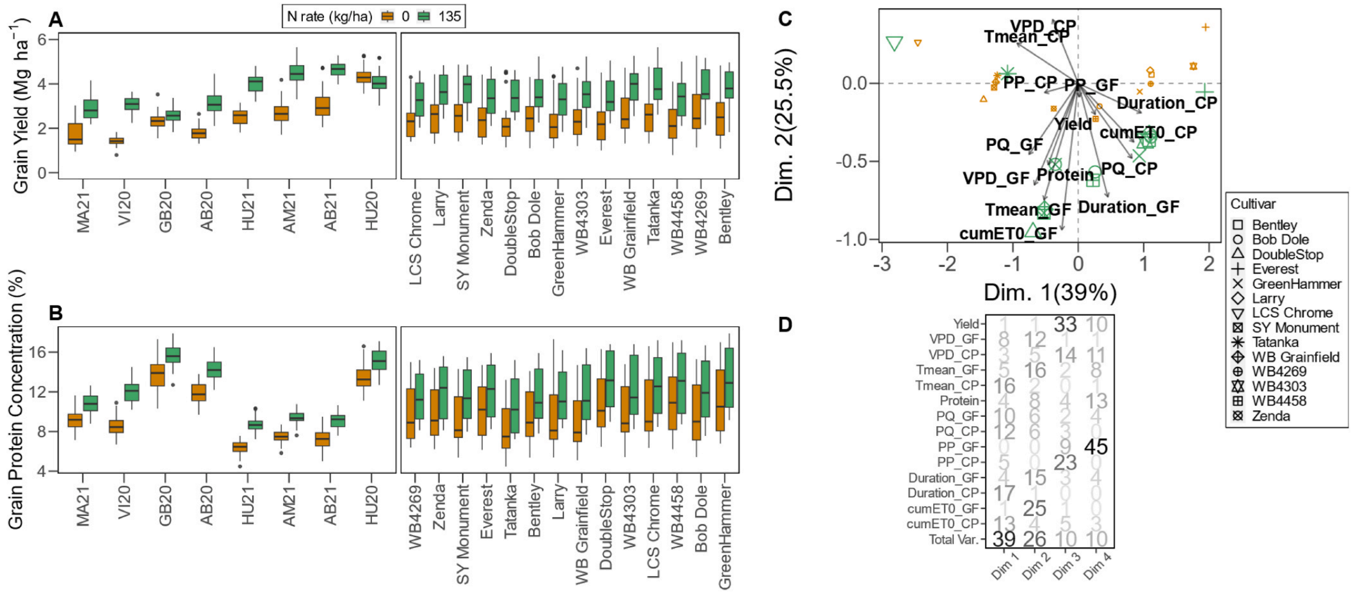

Fig. 3. Frequency distribution of grain yield (A) and grain protein concentration (B) pooled for cultivars and location-season on the most contrasting N rates of 0 and 135 kg N ha^{-1}. The box represents the interquartile range and the line inside the box the median. Whiskers represent 1.5x the interquartile range. Cultivars are sorted in increasing order according to their phenotypic plasticity of yield (A) and protein (B); and location-seasons are sorted from left to right according to the mean grain yield in increasing order. Principal component analysis for grain yield, grain protein concentration and environmental variables: biplot (C) and variance contribution within dimension (D) in experiments carried out in Kansas in 2020 and 2021. In C and D, the color of the points represents the extreme N rates (0 kg N ha^{-1} orange, 135 kg N ha^{-1} green), while shapes represent cultivars. Abbreviations: Ashland Bottoms 2A2 field (AB), Ashland Bottoms M3 field (AM), Great Bend (GB), Hutchinson (HU), Manhattan (MA), Viola (VI), season 2019–2020 (20), season 2020–2021 (21), mean temperature (Tmean), vapor pressure deficit (VPD), photothermal quotient, (PQ), cumulative reference evapotranspiration (cumET0), critical period (CP) and grain filling period (GF).

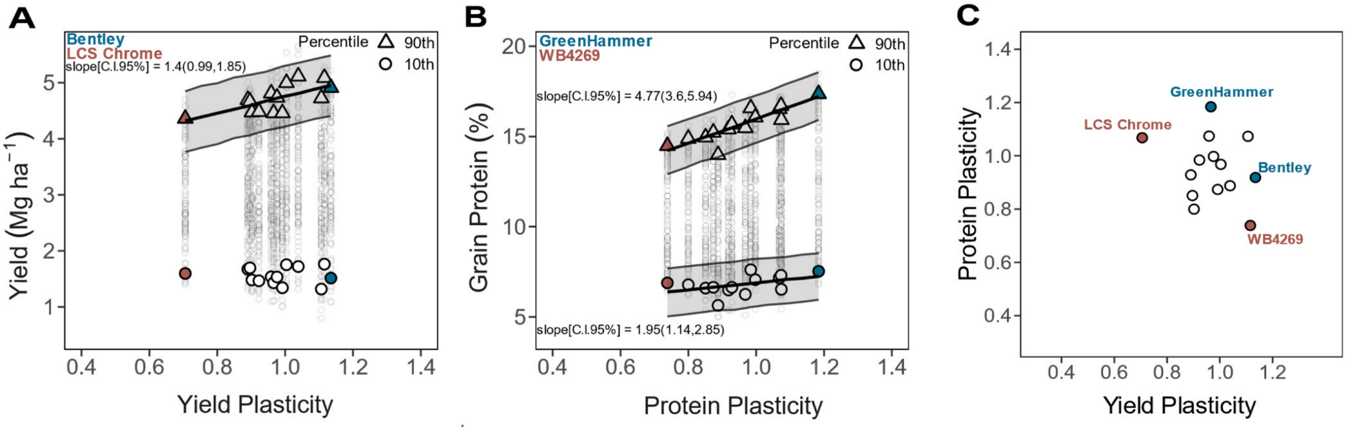

Fig. 4. Trait-plasticity relationships for grain yield (A) and protein (B), and relationship between plasticity of grain protein and yield (C). Colors highlight the least (red) and the most plastic (blue) cultivars for each trait. Solid lines (posterior median) and ribbons (posterior 95% credible interval) are shown when the 95% credible interval of the slope of the relation is not overlapping zero. Inset text shows the slope posterior median and 95% credible interval.

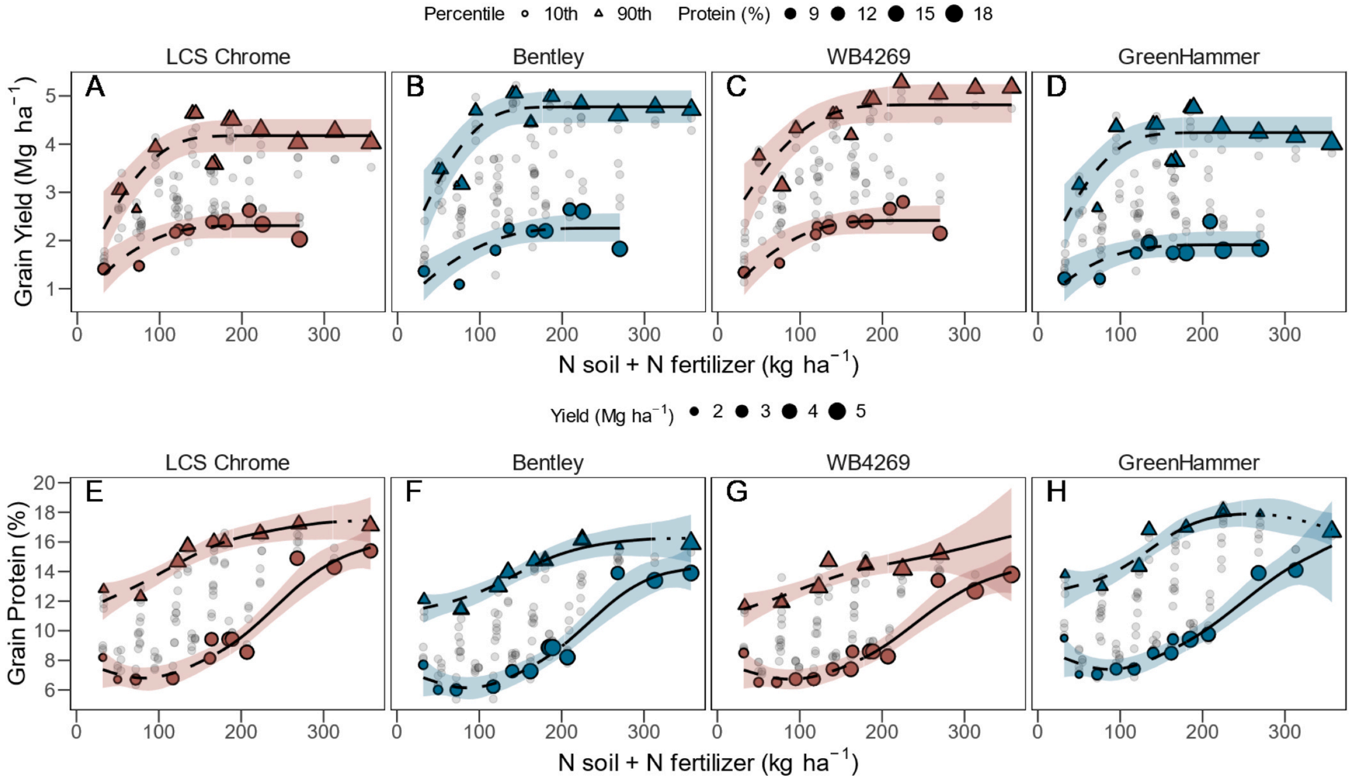

Fig. 5. Wheat grain yield (A-D) and protein (E-F) response to N supply under conditions conducive to high (90th percentile, triangles) and low (10th percentile, circles) trait expression for cultivars with contrasting phenotypic plasticity. Red symbols are for cultivars with low plasticity for yield (LCS Chrome) and protein (WB4269), and blue is for cultivars with high plasticity for yield (Bentley) and protein concentration (Green Hammer). Linetype indicates the phases conceptualized in Fig. 2: dashed (phase I), solid (phase II), dotted (phase III). Lines represent the posterior medians and ribbons the 95% estimation credible interval for model predictions. The size of the symbol shows protein concentration in the yield panels (A-D) and yield in the protein panels (E-H).

°Cd after anthesis using a base temperature of 0 °C (DuPont et al., 2006).

At physiological maturity (GS 93), shoot biomass was collected from an area of 0.19 m^{-2} in a middle row of each plot and immediately dried at 60 °C for 72 h. Following shoot sampling, the entire plots were harvested with a Massey Ferguson 8XP small plot combine. Grain weight and moisture were measured on-site during harvest with Harvest Master monitor (Juniper Systems, Inc., Logan UT, USA) incorporated to the combine, and yield adjusted to dry matter accounting for the plot area plus borders. Dry shoot samples were partitioned to determine yield components: heads m^{-2}, grains head^{-1}, grains m^{-2}, thousand kernel weight, shoot biomass, and harvest index. We determined grain N and stover N concentration with LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI, 2005.) and calculated (i) N harvest index (the ratio between N in the grain and N in shoot); (ii) N utilization efficiency, as the amount of grain produced per unit shoot N uptake; and (iii) stover and shoot N uptake.

3.3. *Phenotypic plasticity*

Phenotypic plasticity was quantified with Eq. 1, for each cultivar and

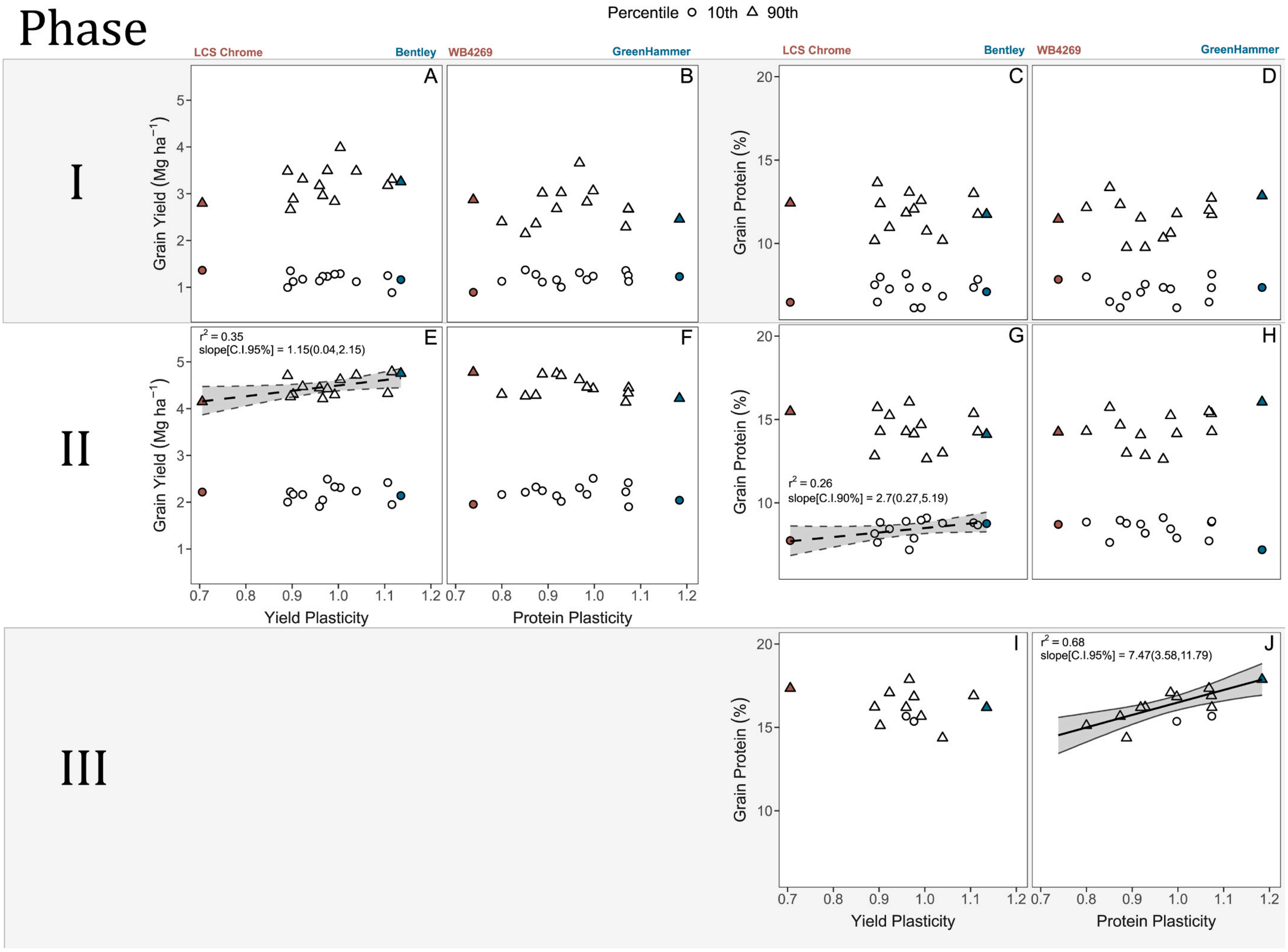

Fig. 6. Association of yield and protein plasticity with grain yield (A, B, E, F) and protein (C, D, G, H, I, J) at the starting point of phases described in Fig. 2 and Table 3 (phase I - N limits yield and protein; phase II, N limits for protein but not for yield; phase III – non-N limitation). In yield panels shapes represent high (triangles) and low (circles) yielding environments; in protein panels shapes represent high (triangles) and low (circles) protein environments. Lines shown only when the slope's credible interval not overlaps zero at different confidence levels (95% - solid lines; 90% - dashed lines).

trait of interest, across all combinations of four N rates and eight location-seasons (32 N-environments). To establish whether plasticity was primarily related to responsiveness to favorable conditions, stress tolerance, or a combination of both (Fig. 1) we plotted trait-plasticity relations (Sadras and Richards, 2014; Sadras et al., 2016) and fitted bayesian hierarchical linear-regression models, described below, using the data for the 10th and 90th percentile. The 10th percentile corresponds to environments with low trait expression (*e.g.*, low grain yield, low grain protein concentration) and the 90th percentile corresponds to environments of high trait expression (*e.g.*, high yield, high protein concentration). Thus, whether the percentiles corresponded to stressful or favorable environments depended on the trait. Percentiles in our dataset seek to return an objective measure of upper and lower limits of traits, excluding undue influence of extremes (Sadras et al., 2017).

3.4. Bayesian process models and statistical analysis

Bayesian statistics are attracting interest in ecology and agriculture (Dorazio, 2016), including in the analysis of grain yield response to N fertilization in maize (*Zea mays L.*)(Correndo et al., 2021) and wheat (Ouedraogo and Brorsen, 2018). In contrast to frequentist approaches in which model parameters are estimated as fixed over true quantities (Dennis, 1996; Ellison, 2004), bayesian models (i) use the sample data to update prior knowledge (beliefs and hypotheses); (ii) grant inference over model parameters, as they are considered random; (iii) base inference on posterior distributions and credible intervals rather than strict p-values and confidence intervals; and (iv) allow for a direct measurement of the probability of an event to be true (Hooten and Hefley, 2019).

To quantify the association between phenotypic plasticity and N with grain yield and protein concentration we fitted bayesian hierarchical models using the *brms* package (Bürkner, 2017) in R (R Core Team, 2022). The models were fitted with a Markov Chain Monte Carlo algorithm, combining three chains with 10,000 iterations each, discarding the first 5000 as burn in interval. Thinning rate was set to 5 and an adapt delta to 0.99. Samples were used for estimating median and 95% credible intervals of posterior distributions. We used uninformative priors derived from previous published literature (de Oliveira Silva et al., 2020a) and unpublished data from N response studies conducted in Kansas (five studies conducted in 27 site-years and adding to 1492 observations) to describe yield and protein concentration response process to phenotypic plasticity, N supply, and crop N traits. Weather data was used to explore associations between grain yield, protein concentration using principal component analysis (PCA).

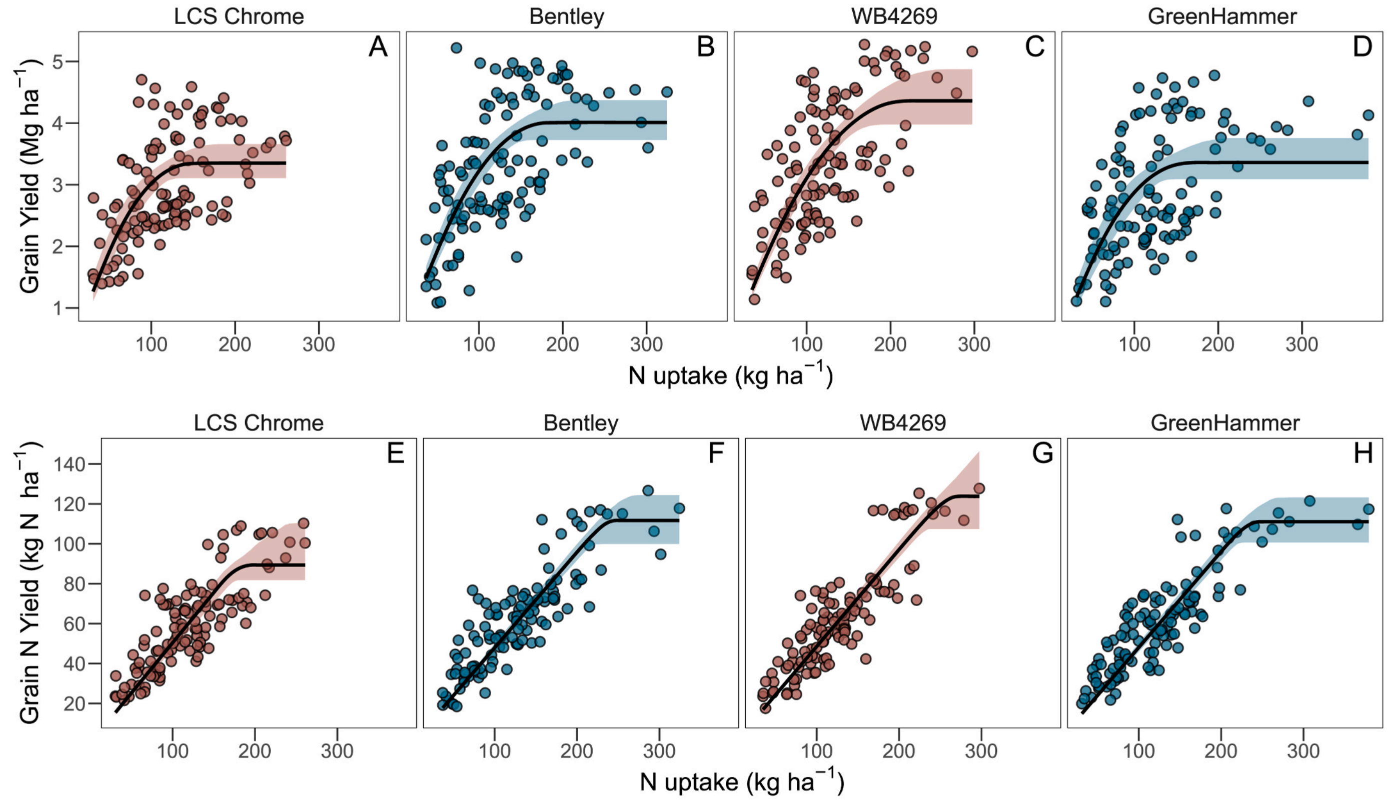

Fig. 7. Wheat grain yield (A-D) and N yield (E-H) response to N uptake across environments for cultivars with contrasting plasticity for yield (LCS Chrome, Bentley) and protein (WB4269, Green Hammer). Solid lines represent the posterior medians and ribbons the 95% estimation credible interval for model predictions.

3.4.1. Phenotypic plasticity response models

Based on previous studies (Sadras et al., 2016, 2019a) yield and grain protein concentration association with phenotypic plasticity was defined with the following model:

$$y_i \sim N(\mu_i, \sigma_i^2) \tag{2}$$

$$\mu_i = \beta_0 + \beta_1 x_i \tag{3}$$

Where β_0 is the intercept and β_1 the slope of the association between plasticity of a given trait and the trait itself under high (90th percentile) and low (10th percentile) trait expression environments. Percentiles defining trait expression environment were obtained by splitting the predictor variable into equally spaced intervals and fitting the corresponding model using the 10th and 90th percentile across all data contained within each interval (Sadras et al., 2016, 2019a). We used uniform priors for the intercept β_0 that allow the model to cover all possible variability (Supplementary Table 4). For β_1 we defined an uninformative and normally distributed sample prior with an expected value of zero and standard deviation of 50. To explore associations of grain yield and protein plasticity and yield components and N traits, we also implemented bayesian hierarchical linear models described above with their respective priors provided in Supplementary Table 4.

3.4.2. Grain yield and protein concentration response to N supply models

For cultivars with contrasting phenotypic plasticity for yield, we described their differential yield response to N supply (mineral soil N at sowing + N fertilizer) with quadratic-plateau models for high (90th percentile) and low (10th percentile) yielding environments:

$$yield_i(tn \quad ha^{-1}) \sim N(\mu_i, \sigma_i^2) \tag{4}$$

$$\mu_i = \begin{cases} \beta_0 + \beta_1 x_i + \beta_2 x_i^2 & x_i < AON \\ \beta_0 + \beta_1 AON + \beta_2 AON^2 & \text{otherwise.} \end{cases} \tag{5}$$

$$\begin{aligned} &\beta_0 \sim N(1.0, 1.7), \\ &\beta_1 \sim N(0.02, 0.06), \\ &AON \sim N(130, 50), \\ &\sigma_i^2 \sim half - student - t(3, 0, 2.5). \end{aligned} \tag{6}$$

Where β_0 is the yield when N supply equals zero and β_1 the linear component and β_2 was decomposed as the term $\frac{-\beta_1}{2 \times AON}$ where AON is the agronomic optimum N supply, which is equivalent to the first derivative equal to zero (Fig. 2). This model assumes that total N supply comes from two sources: mineral soil N at sowing, fertilizer N; thus, mineralization of N from organic matter and atmospheric N depositions are part of the error of the model. Thus, β_0 represents the expected grain yield when N supply from soil and fertilizer is zero.

Grain protein concentration was modeled using a generalized additive model (GAMs) that allows flexibility on the shape of the relationships between the predictor and explanatory variables with smoothing functions or splines (Pedersen et al., 2019). We defined GAM models for high (90th percentile) and low (10th percentile) protein environments:

$$protein_i(\%) \sim N(\mu_i, \sigma_i^2) \tag{7}$$

$$\mu_i = \beta_0 + \beta_1 + f(Nsupply) \tag{8}$$

$$\begin{aligned} &\beta_0 \sim half - student - t(3, 14.4, 2.5), \\ &X\beta_{1:3} \sim Cauchy(0, 1), \\ &\sigma_i^2 \sim half - student - t(3, 0, 2.5). \end{aligned} \tag{9}$$

Where μ_i is the predicted grain protein concentration in the i^{th} percentile, β_0 is the intercept and β_1 is the effect of the cultivar and

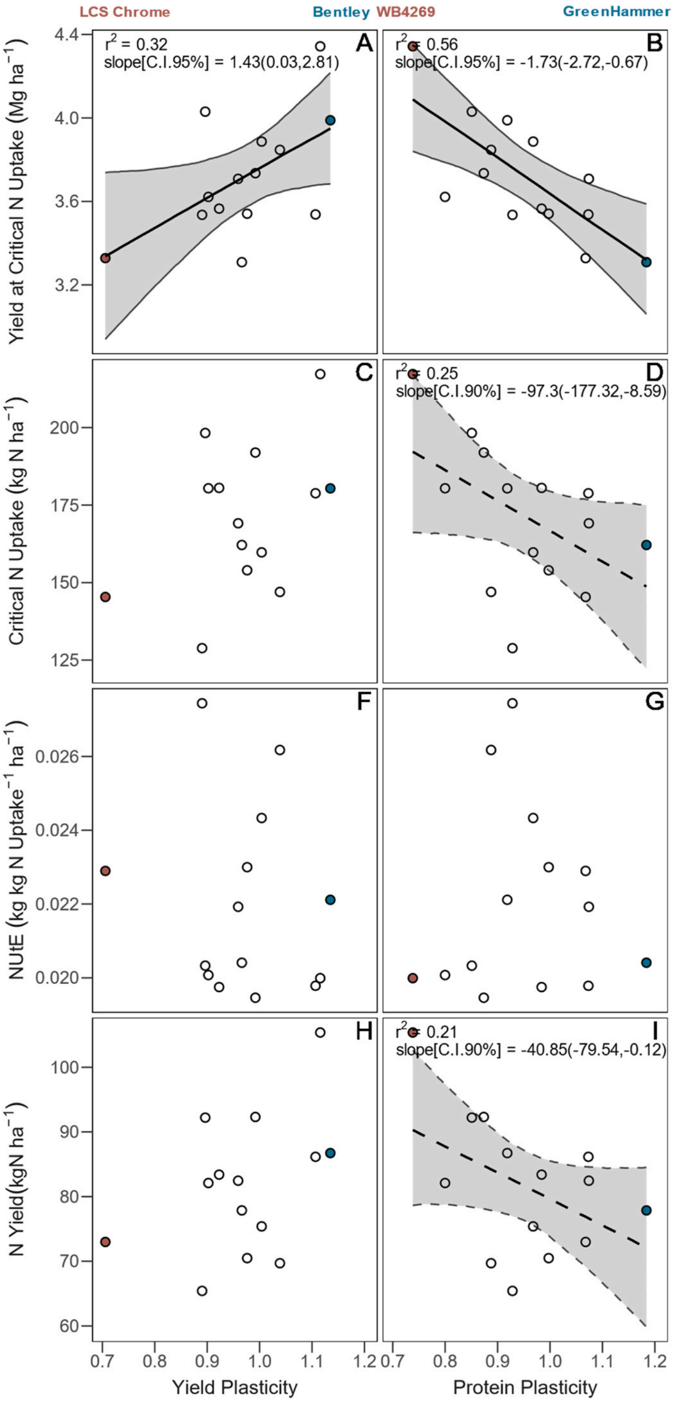

Fig. 8. Association between yield and protein plasticity and yield at the critical N uptake, critical N uptake, N utilization efficiency (NUtE) and N Yield for contrasting yield and protein plasticity cultivars pooled across environments. Lines shown only when the slope's credible interval does not overlap zero at 95% (solid line) and 90% (dashed lines). Inset slopes represent the median and credible interval.

$f(Nsupply)$ represent the smoothing effects of N supply on grain protein concentration. We implemented uninformative priors provided by the default option in *brms* package in R (Eq. 9).

To analyze yield and protein response to N against our three-phase model described in Fig. 2, we compared cultivars at the starting point of the phases by including traits derived from yield (Eq. 8) and protein (Eq. 9) response to N models (Fig. 5) as described in Table 3.

3.4.3. *Relationships between phenotypic plasticity, yield components and crop N traits*

Linear bayesian regression models were used to quantify the associations between yield components and N traits (Section 2.2) with yield and grain protein plasticity as follows:

$$crop\_trait_{ij} \sim N\left(\mu_{ij}, \sigma_{ij}^2\right) \tag{10}$$

$$\mu_{ij} = \beta_0 + \beta_1 x_{ij} \tag{11}$$

Were μ_{ij} is the expected value of the *jth* trait of the *ith* cultivar with plasticity equal to x_{ij}, β_0 is the intercept and β_1 is the slope indicating the change in a given crop trait per unit yield or protein plasticity.

The relationship between shoot N uptake and grain yield was described with the following process model:

$$yield_i(tn \quad ha^{-1}) \sim N(\mu_i, \sigma_i^2) \tag{12}$$

$$\mu_i = \begin{cases} \beta_0 + \beta_1 x_i + \beta_2 x_i^2 & x_i < NC \\ \beta_0 + \beta_1 NC + \beta_2 NC^2 & \text{otherwise} \end{cases} \tag{13}$$

$$\begin{aligned} &\beta_0 \sim \delta(0), \\ &\beta_1 \sim N(0.0347, 2), \\ &NC \sim U(0, 400), \\ &\sigma_i^2 \sim half - student - t(3, 0, 2.5). \end{aligned} \tag{14}$$

Where β_0 was set to zero by implementing a point mass distribution prior centered to zero, x_i represents the aboveground N uptake (kg N ha^{-1}); β_1 and β_2 are the linear and quadratic terms respectively, and NC is the critical N uptake that represents the N uptake that maximizes grain yield. Nitrogen utilization efficiency at NC (*i.e.:* NUtE; kg grain/kg N uptake) was derived from the quotient between the posterior distribution of grain yield at the NC and NC.

Similarly, the relationship between grain N yield and shoot N uptake was modeled as:

$$Nyield_i \quad (kgN \quad ha^{-1}) \sim N(\mu_i, \sigma_i^2) \tag{15}$$

$$\mu_i = \begin{cases} \beta_0 + \beta_1 x_i + \beta_2 x_i^2 & x_i < NC \\ \beta_0 + \beta_1 NC + \beta_2 NC^2 & \text{otherwise.} \end{cases} \tag{16}$$

$$\begin{aligned} &\beta_0 \sim \delta(0), \\ &\beta_1 \sim N(0.76, 10), \\ &AON \sim U(0, 400), \\ &\sigma_i^2 \sim half - student - t(3, 0, 2.5). \end{aligned} \tag{17}$$

Where β_0 was set to zero by implementing a point mass distribution prior centered to zero, x_i is shoot N uptake (kg N ha^{-1}), β_1 is the linear term and NC is the critical N uptake that maximizes grain N yield.

We used a quadratic model to describe the relationship between NHI and HI as follows:

$$Nyield_i \sim N(\mu_i, \sigma_i^2) \tag{18}$$

$$\mu_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 \tag{19}$$

$$\begin{aligned} &\beta_0 \sim \delta(0), \\ &\beta_1 \sim N(1, 2), \\ &MaxNHI \sim U(0, 1), \\ &\sigma_i^2 \sim half - student - t(3, 0, 2.5). \end{aligned} \tag{20}$$

Where β_0 was set to zero by implementing a point mass distribution prior centered to zero, β_1 is the linear term and β_2 was expressed as the first derivative equal to zero ($MaxNHI = \frac{-\beta_1}{2\times\beta_2}$).

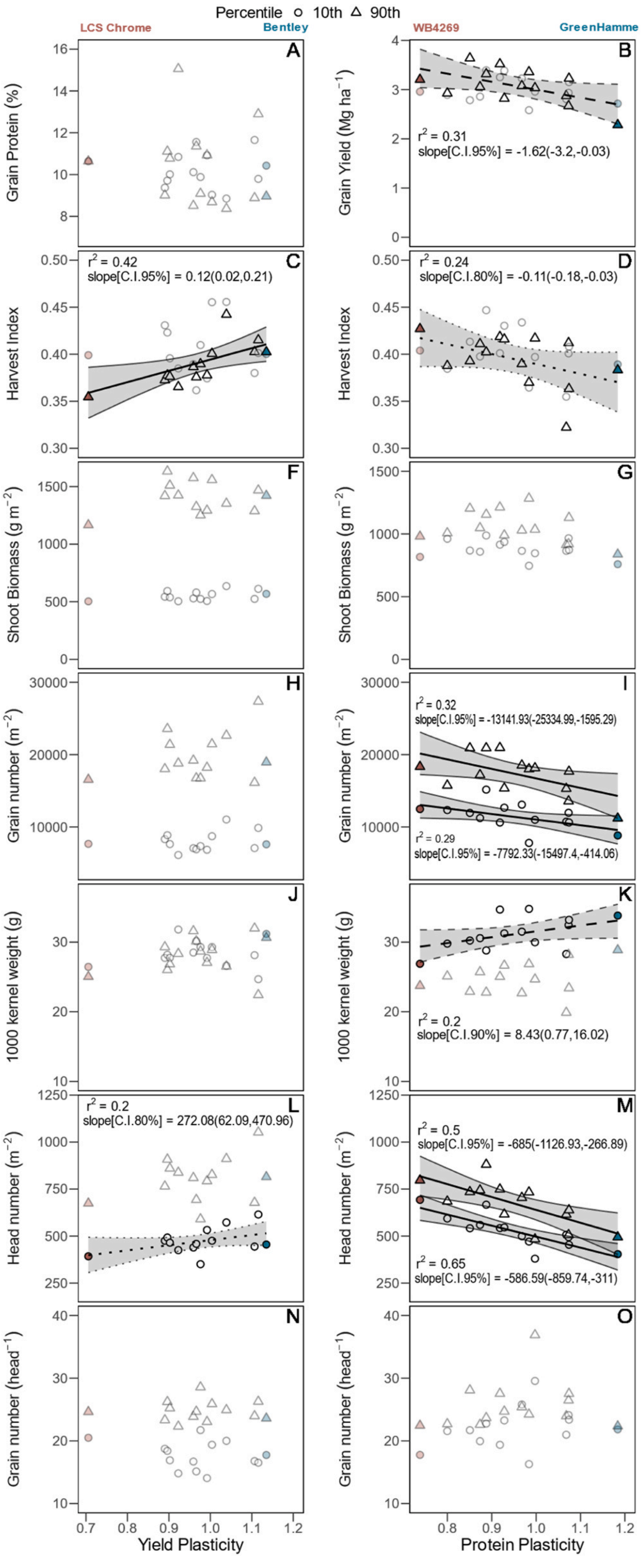

Fig. 9. Relations between grain yield phenotypic plasticity (A, C, F, H, J, L, N) and protein (B, D, G, I, K, M, O) phenotypic plasticity and crop traits (grain protein, grain yield, harvest index, shoot biomass, grain number m^{-2}, thousand kernel weight, head number m^{-2}, and grain number head $^{-1}$) for crops grown in low (10th percentile, circles) and high (90th percentile, triangles) yield and protein environments. Points and lines shown only when the slope's credible interval does not overlap zero at 95% (solid lines), 90% (dashed lines) and; 80% (dotted lines). Inset slopes represent the median and credible interval.

3.4.4. *Model selection*

For representing the most convenient relationship between grain yield and protein concentration in response to N supply, we compared two models for each trait: (i) a model in which the parameters were estimated across cultivars and (ii) a model with parameters estimated for each cultivar. We compared linear-plateau and quadratic-plateau models and selected the model with the best fit. Comparisons were performed using *model_performance* function from the *performance* package in R (Lüdecke et al., 2021). Models with the lowest widely applicable information criterion were retained.

4. Results

4.1. *Environments, yield, and grain protein*

Growing season rainfall ranged from 358 to 511 mm, and accumulated reference evapotranspiration ranged from 552 to 714 mm (Supplementary Figure 1). Across environments, mean seasonal T_{min} ranged from 1.2 to 2.7 °C; mean seasonal T_{max} ranged from 13.2 to 16.1 °C; the photothermal quotient ranged from 1.37 to 2.19 MJ m^{-2} d^{-1} °C^{-1} in the critical period and from 0.90 to 1.15 m^{-2} d^{-1} °C^{-1} during grain fill (Supplementary Figure 1).

Across all sources of variation, grain yield averaged 3.16 Mg ha^{-1} and ranged from 0.79 to 5.65 Mg ha^{-1} (Table 2). Average grain yield increased from 2.50 Mg ha^{-1} ($Q_{2.5\%}$ =1.14 Mg ha^{-1}; $Q_{97.5\%}$ =4.60 Mg ha^{-1}) in the unfertilized control to 3.60 Mg ha^{-1} ($Q_{2.5\%}$ = 2.24; $Q_{97.5\%}$ = 5.15 Mg ha-^{1}) with the highest N rate (Fig. 3A). Average grain protein concentration was 10.9% and increased from 9.9% ($Q_{2.5\%}$ = 5.7%; $Q_{97.5\%}$ = 15.9%) in the unfertilized control to 12.1% ($Q_{2.5\%}$ = 7.8%; $Q_{97.5\%}$ = 16.8%) with the highest N rate (Fig. 3B). The variation in yield and grain protein in unfertilized crops can be partially explained by contrasting initial mineral soil N and organic N mineralization across environments (Table 1, Supplementary Table 1), while the range in the highly fertilized crops suggests that N supply did not limit grain yield (Goos et al., 1982).

Yield associated positively with photothermal quotient during the critical period, the duration of the grain filling period, and to a lesser extent, to the duration of the critical period (Fig. 3C). Yield correlated negatively with time to anthesis and with mean temperature during the critical period. Grain protein concentration increased with photothermal quotient and mean temperature during grain fill (Fig. 3C).

4.2. *Phenotypic plasticity of grain yield and grain protein concentration*

Grain yield plasticity varied 1.6-fold, from 0.70 for LCS Chrome to 1.13 for Bentley (Table 2). Grain yield plasticity associated with responsiveness to high yielding environments, whereas all cultivars returned similar yield in low yield environments (Fig. 4A). A comparison of the same analysis using a frequentist approach return similar results and is in Supplementary Figure 3.

Plasticity of grain protein concentration varied 1.6-fold, from 0.73 for WB4269 to 1.18 for Green Hammer (Table 2). Grain protein plasticity associated with divergent trait-plasticity relations for the 90th and 10th percentiles, *i.e.,* highly plastic phenotypes increased protein concentration more markedly in high than in low protein environments (Fig. 4B, inset Fig. 4B). A frequentist approach of this same analysis returned similar results and is in Supplementary Figure 3.

4.3. *Grain yield and protein concentration response to N for cultivars with contrasting plasticity for yield and grain protein concentration*

Under high yielding conditions, plasticity was unrelated to yield on phase I (*i.e.:* minimal N supply) and associated positively to yield in phase II (Fig. 6E). For instance, Bentley, the cultivar with highest phenotypic plasticity for yield, outyielded LCS Chrome by 0.59 Mg ha^{-1} ($C.I._{95\%}$ = 0.17 – 1.01 Mg ha^{-1}). Under low yielding conditions, there

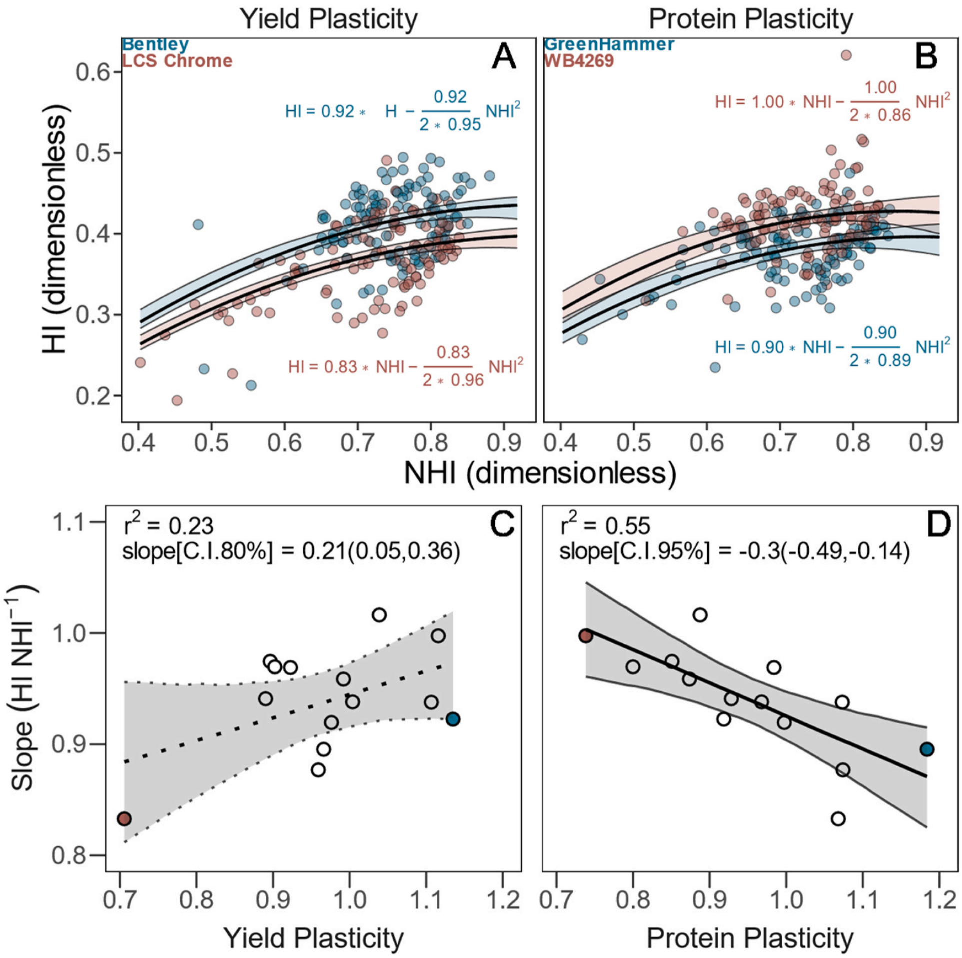

Fig. 10. Allometric relation between N harvest index (NHI) and harvest index (HI) for contrasting yield (A) and protein (B) plasticity cultivars; $R^2 = 0.34$. Association between yield (C) and protein (D) plasticity and the linear term of the quadratic models presented above. Lines shown only when the slope's credible interval not overlaps zero at different confidence levels (95% - solid lines; 90% - dashed lines; 80% - dotted lines). Inset slopes represent the median and credible interval.

were no yield differences among cultivars (Fig. 5A-D, Supplementary Figure 4, Supplementary Table 3).

Cultivar-specific grain protein response to N supply was only observed under high grain protein environments (Fig. 5E-H, Supplementary Figure 4), which is denoted by a better performance (lower WAIC; Supplementary Table 3) of the model when parameters were estimated for each of the cultivars. Under low protein environments, the pooled model performed better (Supplementary Table 3), indicating similar grain protein response to N supply among cultivars. In high protein environments, plasticity of grain protein concentration associated with higher protein in phase III (Fig. 6J).

Yield at critical N uptake was positively associated to yield plasticity (Fig. 8A). To exemplify, the plastic cultivar for yield Bentley outyielded the stable LCS Chrome by 0.57 Mg ha^{-1}(C.I.$_{95\%}$ = 0.28 – 0.87 Mg ha^{-1}) (Figs. 7A, 8B; Fig. 8A). Yield plasticity was unrelated to critical N uptake, N utilization efficiency and N yield (Figs. 8C, 9F, 9H). Protein plasticity was negatively related to grain yield at critical N uptake (Fig. 8B). For instance, grain yield at critical N uptake was 0.81 Mg ha^{-1}(C.I.$_{95\%}$ = 0.43 – 1.25 Mg ha^{-1}) higher in WB4269, stable for protein, compared to Green Hammer, with high plasticity for grain protein concentration (Figs. 7C, 8D). Critical N uptake negatively associated with protein plasticity (Fig. 8D), which is reflected in the greater N uptake of WB4269 with low plasticity for grain protein concentration compared to the more plastic Green Hammer (Q$_{50\%}$ = 32.6 kgN; C.I.$_{95\%}$ = 4.51 – 58.4 kgN) (Figs. 7C, 7D; Fig. 8D). Protein plasticity was unrelated to N utilization efficiency (Fig. 8G) and negatively related to N yield (Fig. 8I).

4.4. *Associations between phenotypic plasticity and crop traits*

Grain yield plasticity was unrelated to protein in high and low yielding environments (Fig. 9A). Under high yielding conditions, yield plasticity was positively associated to harvest index (Fig. 9C), while under low yield scenarios yield plasticity associated positively to heads m^{-2} (Fig. 9L). Yield was unrelated to other physiological traits in high yielding environments (Fig. 9).

Grain protein plasticity was unrelated to yield in low protein environments and negatively associated to yield in high protein environments (Fig. 9B). At low protein conditions, protein plasticity associated negatively to harvest index, grains m^{-2} and heads m^{-2} (Figs. 9D, 9I, 9M) and positively to kernel weight (Fig. 9K). In high protein environments, protein plasticity was negatively associated with grain yield, partially due to lower grain m^{-2} and heads m^{-2} (Figs. 9B, 9I, 9M).

Fig. 10A and 10B shows the allometric relationship between NHI and HI for cultivars with contrasting phenotypic plasticity. Grain yield plasticity was weakly positively related to HI per unit NHI (Fig. 10C). In contrast, protein plasticity was negatively associated with HI per unit NHI (Fig. 10D).

5. Discussion

We combined a three-phase model of yield and grain protein concentration in response to N supply with a perspective of phenotypic plasticity to analyze the interaction between cultivar and N fertilization under contrasting environmental conditions in winter wheat. Our findings are bounded by the combination of eight environments and 14 modern semi-dwarf cultivars with a narrow range in plant height (Wang et al., 2023). Location-season and N fertilization contributed to the variation in environmental conditions (Fig. 3), in addition to cultivar × environment interactions typical of agronomic settings (Supplementary Figure 1).

In our experiments initial N supply was sufficient to support tillering potential (Table 1). Reflecting common practice in the region (Jaenisch et al., 2021) crops were fertilized during spring after winter dormancy (GS 25). Timing of N application can contribute to synchronizing crop N supply and N demand (Hawkesford, 2012; Giordano et al., 2023). For instance, N applications at or after jointing will most likely increase grain protein concentration rather than yield (Lollato et al., 2021; Souza et al., 2022; Giordano et al., 2023). Understanding the interaction between yield and protein plasticity and late-season N application over grain yield and protein remains to be investigated.

Yield plasticity was agronomically adaptive as it associated with greater yield in high yielding environments, in line with previous studies in the study region (Grogan et al., 2016; Lollato et al., 2020). For instance, on a trial conducted with 299 winter wheat cultivars across 11 environments in the study region, Grogan et al. (2016) found that plasticity of yield was related to maximum yields (slope = 2.737 Mg $ha^{-1}plasticity^{-1}$) and unrelated to yield across low yielding environments, supporting our findings in Fig. 4A. At the expense of lower yield, and particularly in low grain protein environments (Fig. 4B), plasticity of grain protein concentration associated with high grain protein regardless of environmental variation. Nitrogen was a key factor related to the expression of phenotypic plasticity as the associations between trait and plasticity for yield and protein were only apparent under non-N limiting conditions, phase II for yield and phase III for grain protein concentration. Grain yield plasticity related positively to greater yield under favorable yielding conditions and was unrelated to grain protein content (Fig. 9A), highlighting the possibility of breeding for high yield plasticity while maintaining protein concentration levels.

Protein plasticity associated negatively to critical N uptake (Fig. 8D), highlighting that drivers of protein plasticity can be associated to the ability of the crop to capture N. Empirical evidence supports the co-regulation of crop N uptake by N supply and crop biomass accumulation (Gastal and Lemaire, 2002; Gastal et al., 2015). Crop biomass accumulation, in turn, drives plant N demand (Devienne-Barret et al., 2000), which drives N uptake under high N supply (Gastal and Lemaire, 2002) through physiological mechanisms including: (i) density of N transporters per unit root length; (ii) intrinsic activity of N transporters; (iii) activity of N transporters modulated by photosynthesis signaling; and (iv) shoot N satiety signals (Gojon et al., 2009; Nacry et al., 2013).

The association between yield plasticity and yield in favorable environments was partially mediated by the higher harvest index of cultivars with higher yield plasticity (Fig. 9C). In contrast, yield often associates more strongly with biomass (Slafer et al., 2014; de Oliveira Silva et al., 2020b; Jaenisch et al., 2022). This reinforces the notion that a trait and its plasticity are partially independent traits with partially independent genetics (Bradshaw, 1965; Reymond et al., 2003; Alvarez Prado et al., 2014; Diouf et al., 2020; Happ et al., 2021). Thus, selection for yield potential may favor low grain protein whereas selection for yield plasticity could favor high yield in non-N limiting environments while maintaining acceptable grain protein concentration.

There were environment-dependent associations between plasticity of grain protein concentration and grain yield per se, whereby cultivars with higher plasticity for grain protein concentration had lower grain number and yield in low protein environments (Fig. 9I). Higher protein plasticity associated with higher kernel weight (Fig. 9K), which is linked to the number of endosperm cells in the grain, in turn associated with grain N sink capacity (Fradgley et al., 2021). High kernel weight and grains per head in cultivars with high protein plasticity did not compensate the lower grain number; this is consistent with the high plasticity of grain number and the stability of grain weight (Bradshaw, 1965; Sadras, 2007, 2021; Sadras and Slafer, 2012; Slafer et al., 2014, 2022). Thus, cultivars with higher plasticity of grain protein concentration largely expressed the trade-off between grain number and grain weight, partially related to the reduction in survival of distal florets and lower proportion of constitutively small grains (Slafer et al., 2022).

Simultaneous improvement of yield and protein must consider the target trait and its interaction with management and the environmental source of variation. Grain protein deviation has taken countless research efforts (Monaghan et al., 2001; Bogard et al., 2010; Mosleth et al., 2015) as a target trait to break the negative association of yield and protein. Nonetheless, a study mapping two populations of 159 and 189 locally adapted genotypes on six environments showed that selection for grain protein deviation was positively associated to protein but still negatively affecting grain yield (Rapp et al., 2018). In our research, yield plasticity was positively associated to yield in high yield, non-N limiting environments and was unrelated to protein, opening paths for increasing yield without affecting grain protein content. The positive association between NHI and HI is widespread in crops (Ciampitti and Vyn, 2011; Tamagno et al., 2017) including wheat (Desai and Bhatia, 1978); it reflects a tight link between the allocation of carbon and N to grain. However, the strength of the association varies with grain protein concentration (Lemaire and Ciampitti, 2020). The negative association between the slope of the allometric relation between NHI and HI and protein plasticity partially explains the negative associations between protein plasticity and grain yield, as HI became less sensitive to variations in NHI with increasing phenotypic plasticity for grain protein concentration.

6. Conclusion

This article proposes a phenotypic plasticity framework to analyse yield and protein response to N, disclosing G × N interactions and identifying higher yielding cultivars that exploit non-N limiting conditions. Our findings have agronomic, breeding and modelling implications: (i) releasing grain yield and protein plasticity information on commercial wheat cultivars data sheet can guide growers towards high yield plasticity cultivars when aiming to high yield with acceptable protein levels or high protein plasticity cultivars to ensure high protein at the expense of lower yields; (ii) dissecting the genetic basis of wheat yield phenotypic plasticity remains a challenge. Yield plasticity has partially its own genetic regulation and was unrelated to grain protein concentration and protein plasticity in this dataset. Breeding programs may benefit as yield plasticity can be subject to direct selection in order to breed for high yielding cultivars while maintaining grain protein concentration levels; (iii) accuracy of N recommendations and mechanistic crop simulation models can be improved by accounting for G × N interactions through plasticity of yield and grain protein. This study provides a basis for wheat growth models to account for yield and protein plasticity through G × E dependent physiological processes such as grain carbon and N allocation.

Funding

This research was partially sponsored by the Kansas Wheat Commission, by the Kansas Agricultural Experiment Station, and by the Kansas Cooperative Extension Service. The Wheat Initiative provided partial funds for the publication of this paper as an open access.

CRediT authorship contribution statement

NG: Investigation, Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. VS: Conceptualization, Writing – review & editing. AC: Methodology, Writing – review & editing. RL: Resources, Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Romulo P. Lollato reports financial support was provided by Kansas Wheat Commission. Romulo P. Lollato reports financial support was provided by Kansas Agricultural Experiment Station. Romulo P. Lollato reports financial support was provided by Kansas Cooperative Extension Service.

Data Availability

Data will be made available on request.

Acknowledgements

The research question explored in this paper initiated during discussions within the Expert Working Group in Agronomy of the Wheat Initiative. The authors acknowledge the support received from the visiting scholars and graduate students (Luiz O. Pradella, Luke P. Ryan and Jorge A. Romero Soler) within the Wheat and Forage Production Program in the Department of Agronomy, Kansas State University, in helping to collect data used in this manuscript. This is research contribution no. 24-101-J from the Kansas Agricultural Experiment Station.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2023.109202.

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