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Prediction of Grain Yield and Gluten Content in Winter Bread Wheat Based on Nutrient Content in Plant Parts during the Critical Cereal Window

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Abstract: Reliable prediction of winter bread wheat grain yield (GY) and its qualitative parameters (crude protein (CP) and wet gluten (GL) content, wet gluten yield (GLY)) requires evaluation of the plant nutritional status in the Critical Cereal Window (CCW). The reliability of the forecast depends on the dedicated plant characteristics and the correct selection of the diagnostic plant parts. This hypothesis was verified in a one-factor field experiment carried out in the 2013/2014, 2014/2015, and 2015/2016 growing seasons. The field experiment included applying 0, 40, 80, 120, 160, 200, and 240 kg N ha^{-1}. The N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu content in wheat was determined in two growth stages: (i) beginning of booting (BBCH 40) and (ii) full flowering (BBCH 65). The evaluated plant components included the leaves and stem for BBCH 40 and the flag leaf, leaves, stem, and ear of BBCH 65. Grain yields were very high, significantly responding to the increased rates of fertilizer nitrogen (N_f), with a maximum yield of 11.3 t ha^{-1} achieved in 2014 (N rate of 209 kg N ha^{-1}), 13.7 t ha^{-1} in 2015, and 8.6 t ha^{-1} in 2016 (N rate of 240 kg N ha^{-1}). The CP and GL content also increased linearly in accordance with the N_f rates. At the beginning of the booting stage, the GY forecast based on the content of nutrients in the leaves or the stem was 94%. Meanwhile, a slightly higher yield prediction was obtained for leaves during the full flowering stage (95%). The key nutrients comprised K, Ca, and Mn, accounting for 93% of the GY variability. The accuracy of the GL prognosis at BBCH 40, regardless of the plant part, exceeded 99%. Three nutrients, namely, P, Mg, and Zn, explained 98% of the GL variability, and the GLY forecast was high (97%). Both wheat traits depended on Zn, which buffered the action of N and Mg. At the full flowering stage, the highest, yet slightly weaker, predictions of GL and GLY were obtained for leaves (95% and 92%, respectively). At this stage of winter wheat growth, the significant role of Zn and K and the buffering effect of Cu on the action of both nutrients was apparent. The obtained results unequivocally confirm that *the game* for winter wheat grain yield occurs within the Critical Cereal Window. In addition, the end result depends on the plant's N supply during this period and the nutritional status of other nutrients. Application of 40–80 kg N ha^{-1} fertilizer critically impacted the GY and technological quality. Moreover, micronutrients, including Zn and Cu, influence the GY, GL, and GLY considerably. At the beginning of the booting phase (BBCH 40), winter wheat leaves serve as a highly reliable plant component indicator for evaluating nutrient content and quantitative (GY, GLY) and qualitative (GL) characteristics of grain. Moreover, analysis conducted during BBCH 40 allows the farmer to correct the nutritional status of the wheat, taking into account N and other nutrients as necessary.

Keywords: growth stages: booting; full flowering; plant parts: leaves; stem; flag leaf; ear; nutrient content; stepwise regression; path analysis

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1. Introduction

Wheat, next to maize and rice, is the most important food source for humans [1,2]. The key characteristic that sets wheat apart from other food crops is the structure of its crude proteins. Winter wheat is primarily grown to produce flour for the production of bread. The consumption value of cereal flour depends on the protein content and composition [3,4]. Thus, the main goal of wheat production is not only grain yield but also to achieve a sufficiently high content of crude proteins (CP) and wet gluten (GL) [5,6]. Accordingly, the N supply must be high enough to meet both production goals [7]. Indeed, the CP content, and thus GL, in wheat grain is highly associated with the amount of applied nitrogen fertilizer (N_f) [8].

Grain yield results from the degree of expression of two components during the life cycle of the cultivated cereal plant. The first is the number of grains per unit area (grain density, GD), expressed as grains per m^2, while the second is the grain weight (TGW) expressed as 1000 grain weight [9]. GD is an aggregate yield index comprising two primary yield components, namely, ear density (ED; number of ears per m^2) and number of grains per ear (GE) [10]. The process of GD formation by wheat involves several sequential stages of growth. The first component, defining GD (i.e., ED), is formed in the period extending from the beginning of the stem elongation stage to the end of the ear formation stage (heading) [11]. The critical stage of GE, and thus GD, formation begins at the booting stage (BBCH 40–49) and ends at the early milk stage (BBCH 71). Thus, this period of grain crop growth is designated the "Cereals Critical Window" (CCW). Therefore, N management must also consider the optimal distribution during the yield formation period to achieve the production goals.

A factor significantly affecting GE, and consequently GD, is the N supply during the CCW. Its excess results in prolonged stem growth, reducing the growth rate of the ear, which in turn leads to GE reduction [12]. Hence, excessive N_f application during the stem elongation phase may decrease the grain yield (GY) due to an imbalance in the shoot and ear growth dynamics. Meanwhile, N_f application at the end of the booting stage and the early heading is required for two reasons. First, wheat is a cleistogamous plant, meaning that the best-formed flowers are fertilized first. The growth rate and the number of flowers per ear, which determine GE, result from the supply of assimilates, which in turn depends on the plant's N nutritional status [10,11]. Thus, the key goal of late-season N_f application is to maintain the viability of many flowers per ear. The second goal of late-season N_f application is to increase the CP content in the grain [13,14]. These two production goals are consistent with the primary goal of classical farming practice, as their implementation focuses primarily on protein yield. Moreover, these objectives also align with the main economic objective of the farmer, as they boost the economic effect [15].

Using late-season N_f in winter wheat requires determining the N nutrition status of plants immediately before or during the Critical Cereal Window. N content in wheat parts represents the major indicator of winter wheat nutritional status just before flowering [16]. The classic diagnostic procedure in cereals most often concerns the stages of plant growth, terminating at the end of the stem elongation phase. However, these stages are too early to effectively correct the nutritional status of wheat in the CCW [17]. The nitrogen nutrition index (NNI) is an ideal method for assessing the in-season N nutritional status of seed crops; however, its operation is time-consuming [18]. The newest diagnostic technique in agriculture, includes applying spectral methods, providing data on plant N nutrition in real time [19]. Based on the spectral N measurements, Chen et al. [20] developed the NNI indices for winter wheat. However, as is often observed, applying the optimal N concentration did not result in a correspondingly high yield. One reason is the imbalanced status of remaining nutrients responsible for the N economy during the grain-filling period [21].

The yield-forming role of N contained in wheat leaves and stems is well established. However, the assessment of the state of bread wheat N nutrition is used only to predict the GY. To date, no diagnostic studies have predicted protein or gluten content in grain. A CP content of 12.5–13% and GL of 25–28% meet the technological quality of grain for bread wheat [22]. High N_f concentrations are applied during the CCW to increase the CP content in the grain [14,15].

Tissue analysis of winter wheat to determine the plant N status is only used to determine the need for N_f application during the booting or heading stage of cereal plant growth. However, attempts have been made to include other nutrients in the GY prediction for wheat over the past 50 years [23,24]. Indeed, an association has been described between grain leaf and stem N content and GY, and to a lesser extent, protein content. In contrast, the relationships between other nutrients and these wheat traits remain a figurative black box.

Hence, it is necessary to determine the role of nutrients other than N in maintaining N homeostasis during the CCW as the basis for effective N_f use. We postulated that the N balance of winter wheat during this period can be effectively controlled by identifying deficient nutrients. Indeed, recognizing the nutritional status of winter wheat with N, or more broadly with N-limiting nutrients, is the basis for applying N alone or in combination with deficient nutrients.

The minor objective of the study was to predict GY, GL content, and wet gluten yield (GLY) based on the nutrient content in wheat plant parts throughout two stages of its growth (i.e., beginning of booting and full flowering). The main objective of this study is to demonstrate which part of the plant during which stage of development provides the greatest prognostic reliability and practical use for fertilization diagnostics.

2. Materials and Methods

2.1. Experimental Site

The study on the relationship between nutrient content in specific parts of winter wheat and grain yield, GL content, and GLY was carried out in the 2013/2014, 2014/2015, and 2015/2016 seasons in Smolice (52°42′ N; 17°10′ E), Poland. The field experiment was carried out on fertile soil formed from loamy sand over loamy sand, classified as Albic Luvisol. The organic carbon (C_{org}) content and pH values were variable, peaking in the 2014/2015 growing season. The available nutrient content, measured before the application of fertilizers, was medium class or higher for P, K, Mg, Fe, and Zn, indicating highly favorable conditions for wheat growth. Meanwhile, more variable levels of soil fertility were observed in the 2013/2014 season with low class Ca, Cu, and Mn levels. The concentration of mineral N (N_{min}), measured just before the spring regrowth of winter wheat in the 0.0–0.6 m soil layer, was generally high or very high (Table 1).

Table 1. Soil agrochemical characteristics in consecutive growing seasons [1,2,3,4].

Soil, cm	pH	C_{org} %	P	K	Mg	Ca mg kg^{-1}	Cu	Mn	Zn	Fe	N_{min} kg ha^{-1}
						2013/2014					
0–30	6.9	1.3	234 VH [5]	231 H	105 M	988 L	0.4 L	27.2 L	3.6 M	536 H	86.4
30–60	6.7	1.1	234 VH	237 H	103 VM	876 L	0.4 L	25.7 L	3.5 M	541 H	
						2014/2015					
0–30	7.1	2.2	185 VH	185 M	165 MVH	2045 M	3.5 M	85.5 M	6.3 H	268 M	129.0
30–60	7.2	2.1	161 VH	157 M	155 VH	2063 M	3.5 M	93.8 M	5.6 H	269 M	
						2015/2016					
0–30	6.6	1.6	202 VH	281 VH	165 VH	1480 L	2.8 M	61.9 M	6.1 H	347 M	110.0
30–60	6.6	1.4	139 VH	222 VH	163 VH	1504 L	2.5 M	62.0 M	3.7 M	231 M	

[1] 1.0 M KCl soil/solution ratio 1:2.5; m/v; [2] loss-on ignition; [3] Mehlich 3 [25]; [4] 0.01 dm^{-3} $CaCl_2$, soil/solution ratio 1:5; m/v; [5] availability classes: VL, very low; L, low; M, medium; H, high; VH, very high [26–28].

The local climate of the study area, classified as intermediate between Atlantic and Continental, is seasonal, especially in the summer. The early vegetation phase of winter wheat in spring 2014 was very good, however, May was wet. The second part of the season was less favorable as June was very dry and July was dry. The 2015 growing season was generally unfavorable for wheat growth, as indicated by the predominance of dry conditions. Although the beginning of the 2016 growing season was wet, May was semi-dry, and June was dry (Figure 1). May and June are critical for the development of grain number in an ear of winter wheat [10,11]. Subsequently, most of the wheat grain filling period was wet.

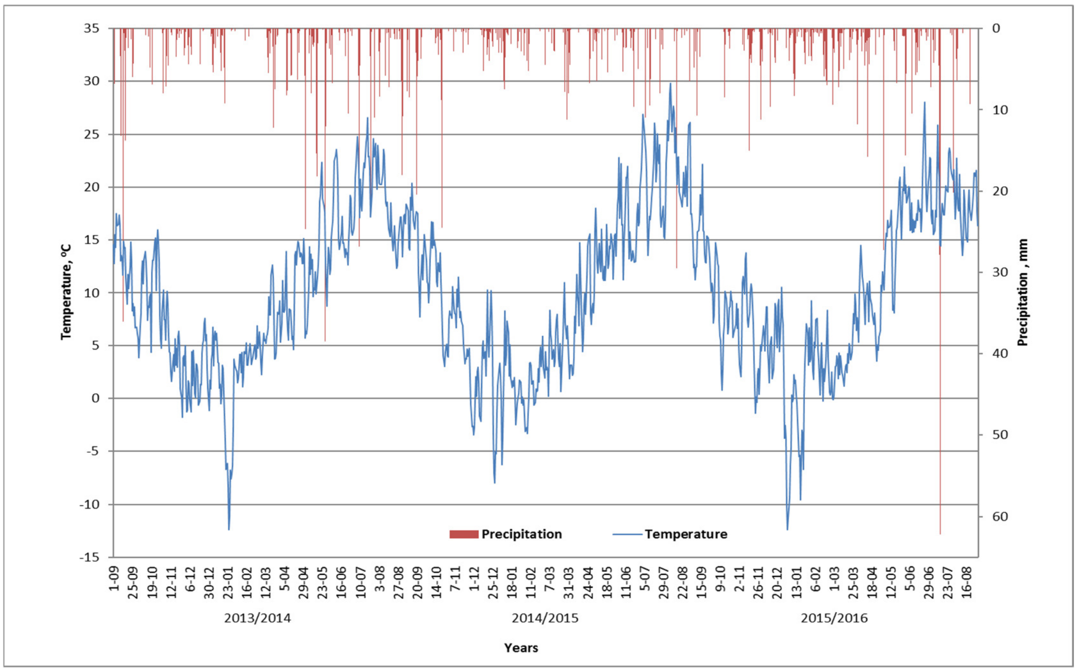

Figure 1. Daily mean air temperature and precipitation at the Experimental Station Smolice during the study.

2.2. Experimental Design

The data used in this study was based on a one-factor field experiment with four time replicates comprising control N plot and six plots with increasing concentrations of fertilizer N: 40, 80, 120, 160, 200, 240 kg ha^{-1}.

The total area of one plot was 22.5 m^2 (1.5 × 15 m). The winter wheat cv. Wydma was sown annually in the fourth week of September at 300 grain m^{-2}. The forecrop was winter oilseed rape. The wheat was harvested the following year at the end of July from an area of 19.5 m^{-2}. Nitrogen was applied in the form of ammonia nitrate (34:0:0) in accordance with the experimental schedule:

(1) Application of 40 and 80 kg N ha^{-1} at the end of winter, before beginning winter wheat vegetation in spring.
(2) Supplement to 160 kg N ha^{-1} at the end of tillering/beginning of shoot elongation (BBCH 29/30).
(3) Supplement to 240 kg N ha^{-1} when the flag leaf became visible (BBCH 39).

At each subsequent date, nitrogen fertilizer was applied to the top dressing using a fertilizer spreader. Phosphorus was applied at a rate of 17.2 kg P ha^{-1} in the form of triple superphosphate (46% P_2O_5). Potassium was applied at a rate of 100 kg K ha^{-1} as Korn-Kali (K-MgO-Na_2O-SO_3 → 40-6-3-12.5). Both fertilizers were applied to the soil two weeks before wheat sowing. Plant protection was conducted in accordance with the codex of good practice.

2.3. Plant Sampling

The plant material for the determination of dry matter and nutrient content in the indicatory parts of winter wheat was collected at the beginning of the booting stage (BBCH 40) and at the full flowering stage (BBCH 65). A single sample was partitioned based on the plant growth stage into leaves, stems, flag leaf, and ears. The N content was determined in plant material using the standard macro Kjeldahl method [29]. For mineral nutrients, the collected plant sample was dried at 65 °C and then mineralized at 550 °C. The obtained ash was dissolved in 33% HNO_3. P concentration was measured by the vanadium-molybdenum method using a Specord 2XX/40 (Analytik Jena, Jena, Germany) at a wavelength of 436 nm. The concentration of K, Mg, Ca, Fe, Mn, Zn, and Cu was determined using flame type atomic absorption spectrometry. The results were expressed on a dry matter basis.

2.4. Statistical Analysis

The effects of the individual experimental factor (year, N concentrations) and their interactions with grain yield, GL content, and GLY were assessed by means of a two-way ANOVA (Y and N concentrations). Means were separated by honest significant difference (HSD) using Tukey's method when the F-test indicated significant factorial effects at the level of $p < 0.05$. Trends in the responses of wheat traits, namely, GY, GL content, and GLY, to increasing N_f concentrations, were determined using a linear and quadratic regression model. The relationships between the traits were analyzed using Pearson correlation and linear regression. STATISTICA 12 software was used for all statistical analyses (StatSoft Inc., Tulsa, OK, USA, 2013). In the second step of the diagnostic procedure, stepwise regression was applied to define an optimal set of nutrients based on GY, GL content, and GLY. In the computational procedure, a consecutive variable was removed from the multiple linear regressions in a step-by-step manner. The best regression model was chosen based on the highest F-value. Path analysis was conducted based on Konys and Wiśniewski [30]. The values of direct and indirect path coefficients were ordered in four classes: negligible (0.00–0.09), low (0.10–0.19), moderate (0.20–0.29), and high (0.30–0.99) [31]. In the developed path diagrams, the coefficients in the negligible class were omitted.

3. Results

3.1. Winter Wheat Biomass at Critical Stages of Grain Yield Formation

Yields of winter wheat grain were characterized by high year-to-year variability (Table 2). On average, the highest GY was obtained in 2015, and the lowest in 2016. The GY achieved in 2016 was one-third lower than that in 2015. Yields under the N_f control (no N_f applied) increased in the following order: 2016 (74.9%) < 2014 (90.6%) < 2015 (100%). The increase in GY in response to increasing concentrations of N_f was consistent with the quadratic regression model in the first season (2013/2014) and linear in the remaining two (Figures 2 and S1).

Table 2. Grain yield, protein and gluten content, and protein and gluten yield.

Factor	Factor	GY	CP	GL	STA	CPY	GLY	STAY
	Level	**t ha^{-1}**		**%**			**t ha^{-1} DW**	
Years (Y)	2014	9.84 b	11.2 c	21.6 c	63.6 a	1.18 b	2.16 b	6.25 b
	2015	11.27 a	11.9 b	23.8 b	63.3 b	1.37 a	2.76 a	7.11 a
	2016	7.22 c	14.0 a	29.0 a	61.8 c	1.02 c	2.13 b	4.45 c
Fc, p		285.7 ***	142.0 ***	145.3 ***	97.0 ***	83.1 ***	61.8 ***	325.8 ***
Nitrogen concentration, kg ha^{-1} (N)	0	6.42 e	10.5 e	20.1 d	64.4 a	0.67 d	1.28 d	4.14 d
	40	8.24 d	11.2 de	21.d	63.8 ab	0.90 c	1.74 c	5.28 c
	80	9.03 d	11.4 d	22.1 d	63.6 b	1.01 c	1.96 c	5.76 c
	120	10.00 c	12.3 c	24.5 c	62.9 c	1.21 b	2.41 b	6.30 b
	160	10.27 bc	13.1 b	26.7 b	62.4 cd	1.33 b	2.70 b	6.41 ab
	200	10.96 ab	13.90 a	28.63 ab	61.78 de	1.51 a	3.09 a	6.78 ab
	240	11.18 a	14.17 a	29.56 a	61.51 e	1.57 a	3.28 a	6.88 a
Fc, p		82.7 ***	58.6 **	56.7 ***	52.0 ***	117.0.0 **	113.1 ***	72.3 ***
Source of variation in the studied interactions								
Y × N		**	ns	ns	ns	***	***	**

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and ns, non-significant. Legend: CP, total protein content; GL, total gluten content; STA, total starch content; CPY, total protein yield; GLY, total gluten yield; STAY, total starch yield; GY, grain yield.

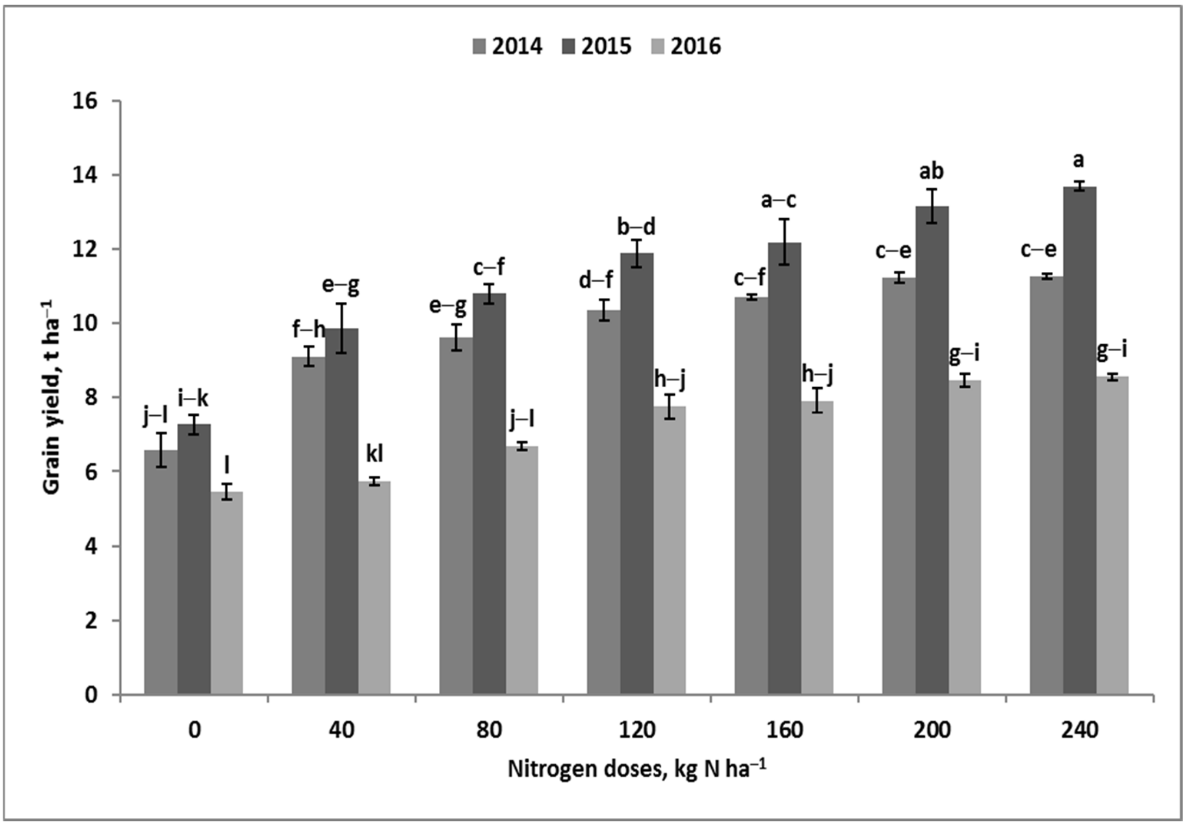

Figure 2. Effect of increasing nitrogen concentrations in subsequent years of study on grain yield of winter wheat. Similar letters indicate a lack of significant differences using Tukey's test. The vertical bar in the column refers to the standard error of the mean.

The CP, GL, and starch (STA) contents exhibited significant seasonal variability, however, no interaction between Y $\times$ N_f was found. The highest CP and GL content was recorded in 2016, concomitant with the lowest STA (Table 2). The GL content significantly depended on CP (r = 0.99 ***). Meanwhile, both these grain traits showed a negative association with STA (r = -0.98 *** and r = -0.99 ***). The effect of increasing N_f concentrations on these characteristics was progressive, stabilizing their values at the maximum level on plots fertilized with 200–240 kg N ha^{-1}. However, the opposite trend was observed for STA, the content of which was the highest under the N_f control. None of these qualitative grain traits exhibited significant relationships with GY (Table S1).

Unlike GY, CPY and GLY followed a linear pattern in response to increasing N_f concentrations (Figures 3, S2 and S3). The CPY and GLY trends partially aligned with those of GY, with large differences between their highest values in 2015 and significantly lower yields in the remaining years. The yield gap for both characteristics between 2015 and 2016 widened with increasing N_f concentrations, reaching ~30% on the plot with 240 kg N ha^{-1}. The pattern of STA yield (STAY) was consistent with the linear model in 2016, whereas in the remaining years, it best fit the quadratic regression model (Figure S4). In 2014, the maximum STAY of 7.06 t ha^{-1} was recorded for the 198 kg N ha^{-1} N_f plot. In 2015, these two basic characteristics were respectively 8.28 t ha^{-1} and 236 kg N ha^{-1}. GY was the main driver of yields of all examined grain quality characteristics. A perfect relationship was observed between GY and STAY (Table S1; r = 1.0).

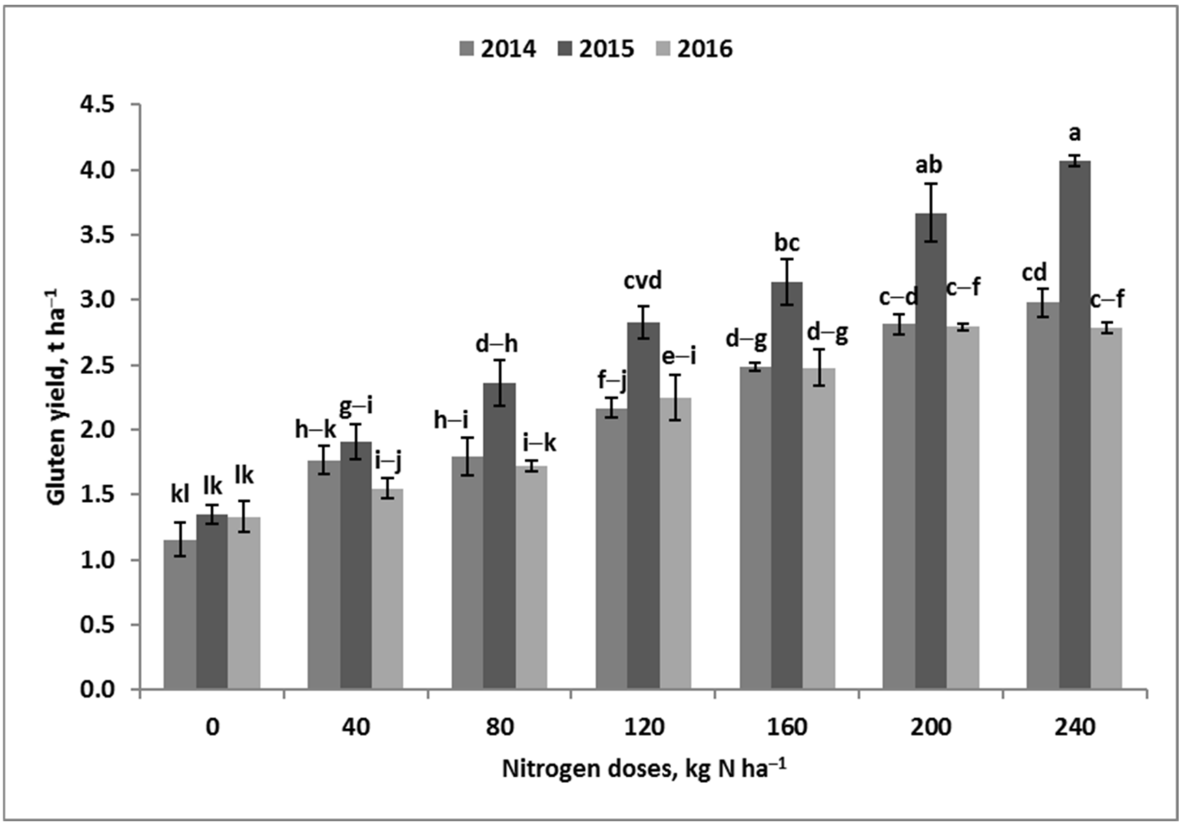

Figure 3. Effect of increasing nitrogen concentrations in subsequent years of study on wet gluten yield of winter wheat. Similar letters indicate no significant differences using Tukey's test. The vertical bar in the column refers to the standard error of the mean.

The biomass of leaves (LE) and stems (ST) at the beginning of winter wheat booting (BBCH 40) was variable in the subsequent years (Appendix A, Table A1). Both components in the first two seasons were at a similar level, while significantly higher values were recorded in the 3rd season. LE40 responded significantly to the years and N_f concentrations, but not to the interaction of both. Meanwhile, ST40 was sensitive to the Y × N interaction, primarily due to the extremely high biomass on the N control plot and that fertilized with 40 kg N ha^{-1} (Figure S5). Differences between the seasons were the greatest in these two plots. However, while no dependence was observed for GY and STAY on these wheat traits, both these canopy characteristics were positively and significantly associated with CP and GL and negatively with STA. Additionally, their impact on CPY and GLY was positive but low (Table S2).

All components of winter wheat biomass at full flowering (BBCH 65) responded significantly to both years and N_f concentrations (Table A1). Compared to the booting stage, the biomass of leaves (LE65) slightly decreased in 2014 and 2016 but increased in 2015. In the case of stem biomass (ST65), its net increase during this period was the highest in 2015 (+48%). The biomass of ears (EA65) responded to N_f in the same manner as stems and leaves at BBCH 40. At full wheat flowering, only ST65 was sensitive to the Y × N interaction. Its trends in response to N_f concentrations followed the quadratic regression model (Figure S6). All wheat biomass components and total wheat biomass at BBCH 65 showed no significant association with GY (Table S1). Meanwhile, LE65, EA65, and TB65 exhibited positive and significant associations with CP and GL and negative associations with STA (Table S2). LE65 was also identified as a very good predictor of CPY and, in particular, GLY, but not STAY.

3.2. Nutritional Composition of Winter Wheat Parts at Booting—BBCH 40

3.2.1. Leaves

Nutrient content in winter wheat leaves at the BBCH 40 stage showed a significant year-to-year variability and sensitivity to increasing N_f concentrations. However, no response to the Y × N interaction was observed (Table 3). Regarding the year, significantly lower P, K, Mg, and Ca contents were recorded in 2015 than in the other years. The opposite trend was observed for Cu. The effect of increasing N_f concentrations was clear, as a progressive increase in the nutrient contents was observed. The most important difference in the effect of N_f concentrations concerned a stabilizing trend in nutrient content. This was most evident for P, the content of which was stable across all N_f concentrations. Meanwhile, Mn content stabilized at 40 kg N ha^{-1}, and Mg, Ca, and Cu from 80 kg N ha^{-1}. In the case of N, K, Fe, and Zn, stabilization began from 120 kg N ha^{-1}.

Table 3. Nutrient content in winter wheat leaves at the beginning of the booting stage (BBCH 40).

Factor	Factor	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
	Level			% DM				mg kg^{-1} DM		
Years	2014	2.53 b	0.42	3.58 a	0.20 b	0.17 b	75.5 a	58.3 a	10.7 b	3.7 b
(Y)	2015	3.08 a	0.31	2.90 c	0.12 c	0.14 c	68.6 b	32.9 b	11.7 ab	6.0 a
	2016	3.24 a	0.36	3.37 b	0.23 a	0.23 a	50.9 c	36.7 b	13.2 a	5.5 a
Fc, p		21.7 ***	26.7 ***	25.0 ***	189.4 ***	70.0 ***	42.0 ***	18.0 ***	3.6 *	27.3 ***
Nitrogen	0	2.40 d	0.31 a	2.93 c	0.16 b	0.15 c	47.0 c	30.0 b	7.8 c	3.7 a
rates, kg ha^{-1}	40	2.67 cd	0.35 ab	2.97 c	0.17 bc	0.16 bc	55.0 c	36.5 ab	10.5 c	4.4 bc
(N)	80	2.90 bc	0.38 a	3.26 bc	0.19 ab	0.19 ab	69.2 b	42.6 ab	11.1 bc	5.0 ab
	120	3.19 ab	0.40 a	3.57 ab	0.20 a	0.20 a	74.5 ab	52.1 a	14.5 ab	6.1 a
	160	3.58 a	0.39 a	3.69 a	0.20 a	0.21 a	79.2 a	51.9 a	15.5 a	6.0 a
Fc, p		19.6 ***	7.1 ***	14.4 ***	11.1 ***	12.8 ***	29.1 ***	5.4 **	12.7 ***	11.1 ***
				Source of variation of the studied interactions						
Y × N		ns	ns	ns	ns	ns	ns	ns	ns	ns

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$; ns, non-significant.

Among the nine examined nutrients, a significant relationship was only observed between Fe content and GY (Table S3). The prediction of GY based on the nutrient content in leaves, according to the coefficient of determination for the path analysis (PA-R^2), was high (94%; Table S4). However, according to the stepwise regression analysis, the best set of nutrient predictors comprised N, Ca, and Mn (Table A2). Additionally, N content showed a moderate association with Ca and very strong relationships with Zn and Cu. In contrast, N, K, Zn, and especially Mg were significantly associated with Ca. The content of Mn was moderately associated with Fe and strongly with P and K. The path analysis indicated a strong correlation between Fe and GY, while the direct value of the path coefficient for Fe was low (Table S4). Its high value resulted from the indirect but moderate positive effect of P and Mn. Mg exerted the greatest but negative direct effect on GY; however, an indirect effect elicited by P and Ca partially ameliorates this effect.

The prediction of GL content based on nutrient contents in wheat leaves, expressed as PA-R^2, was 99.5%. (Table S5). Five of the nine examined nutrients were significantly associated with the quality parameters of grain (Table S3). The strongest and, at the same time, positive relationships with CP and GL were found for N, Ca, and Zn. Slightly lower dependence strengths were observed for Cu and Mg. Meanwhile, this set of nutrients showed strong, negative correlations with STA. Stepwise regression analysis identified P, Mg, and Zn as key predictors of GL content (Table A2). These three nutrients explain 98% of the obtained GL variability. P was significantly correlated with Mg and K, Mn, and Fe. However, Mg was correlated with P and K and strongly with Ca. Zinc, next to K and Ca, was strongly associated with N and Cu (Table S3). Path analysis showed that Zn exerted the strongest and most stable impact on GL (Table S5; Figure 4).

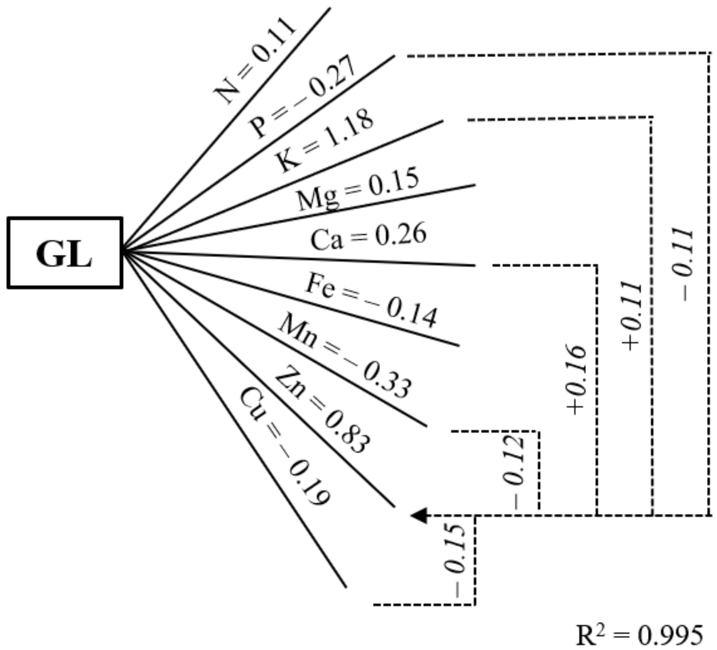

Figure 4. Path diagram of the direct and indirect effects of nutrients in winter wheat leaves at the booting stage on wet gluten content (GL). Negligible values of the indirect path coefficients (<0.10) are not included in the figure.

The GLY prediction based on leaf nutrient content, expressed as PA-R^2, was 96.9%. Excluding Ca, GLY was significantly correlated with the same nutrients that determined its content (Table S3). However, according to stepwise regression analysis, the best set of nutrients comprised N, Mg, and Mn (Table A2). Path analysis confirmed that Mg elicited the greatest effect, with the highest yet negative direct path coefficient value (Table S6). The negative value of this coefficient was partially ameliorated by other nutrients, with Ca, having the greatest impact. The highest correlation coefficient was found for Zn. However, the path coefficient for Zn was moderate. The increase in this value resulted primarily from the positive but indirect effects of N and Ca (Figure 5).

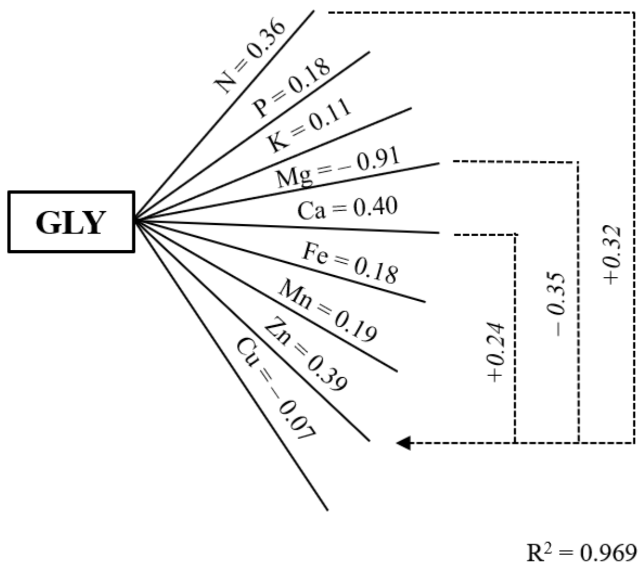

Figure 5. Path diagram of direct and indirect effects of nutrients in winter wheat leaves at the booting stage on the wet gluten yield (GLY). Negligible values for the indirect path coefficients (<0.10) are not included in the figure.

3.2.2. Stems

The content of the examined nutrients in the winter wheat stem was much lower than in the leaves (Table 4). All showed significant year-to-year variability. The lowest content in 2015, the year with the highest yield, was found for P, K, and Mg. While the reverse trend was observed only for Cu. Excluding Mn, the trend in nutrient content aligned with the N_f rates. The difference between the response patterns concerned only the concentration of N_f at which the content of a given nutrient stabilized. The most labile was Zn, its content increased progressively with N_f concentration. The content of P, Fe, and Cu stabilized at 80 kg N ha^{-1}, while others at 80 kg N ha^{-1}. The content of five of the nine examined nutrients (P, Mg, Ca, Fe, and Zn) was dependent on the Y × N interaction. Based on the stepwise regression analysis, only the variability of P, Mg, and Zn was sufficient to meet the conditions to reliably predict GY, GL, and GLY (Table A2). Mg exhibited consistently negative relationships in all equations. The key factor affecting the Mg content was the year (Figure S7) with the highest Mg content recorded in 2016, the year with the lowest yield. In addition, Mg content responded poorly to increased N_f rates. A similar trend was observed for Mg content in 2015, the year with the highest yield, however, the Mg content was twice as low as in 2016. The correlation analysis clearly confirmed a strong association between Mg and P, Mg and Zn, and P and Zn.

Table 4. Nutrient content in winter wheat stems at the beginning of the booting stage (BBCH 40).

Factor	Factor Level	N	P	K % DM	Mg	Ca	Fe	Mn	Zn mg kg^{-1} DM	Cu
Years (Y)	2014	1.21 b	0.23 b	2.71 a	0.12 b	0.04 a	33.9 a	30.8 a	11.9 b	2.0 c
	2015	1.37 a	0.21 c	2.16 b	0.09 c	0.03 b	31.5 b	18.2 b	13.0 b	3.1 a
	2016	1.36 a	0.29 a	2.51 a	0.21 a	0.03 b	24.9 b	21.5 b	15.2 a	2.8 b
Fc, p		5.7 **	83.7 ***	24.1 ***	761.4 ***	153.5 ***	19.7 ***	30.8 ***	11.5 ***	33.0 ***
Nitrogen rates, kg ha^{-1} (N)	0	1.08 c	0.21 c	1.96 c	0.11 d	0.03 c	24.9 b	20.5	9.5 c	2.0 b
	40	1.13 bc	0.23 b	2.25 bc	0.12 cd	0.03 b	25.3 b	22.2	11.1 c	2.3 b
	80	1.26 b	0.24 ab	2.40 b	0.13 bc	0.03 b	30.8 a	25.0	13.4 b	2.7 a
	120	1.53 a	0.26 a	2.83 a	0.14 ab	0.04 a	34.4 a	25.7	15.6 ab	3.1 a
	160	1.56 a	0.27 a	2.87 a	0.14 ab	0.04 a	35.1 a	24.2	17.3 a	3.0 a
Fc, p		20.6 ***	18.4 ***	23.4 ***	15.8 ***	38.4 ***	15.2 ***	1.8 ns	28.0 ***	13.8 ***
Source of variation of the studied interactions										
Y × N		ns	***	ns	**	***	***	ns	*	ns

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$, ns, non-significant.

The GY prediction based on the nutrient content in winter wheat stems, expressed as PA-R^2, was 94.3%, slightly higher than for the leaves (Table S8). The Mg imbalance was clearly confirmed by the respective path coefficient, which was strongly negative. Its value was mitigated to the greatest extent by K, for which the corresponding path coefficient was very high and positive. The buffering function of K was also recorded for Fe, which showed the highest positive correlation coefficient and negative path coefficient with GY. The overall level of GL prediction based on the nutrient content in the winter wheat stem reached 99.7% (Table S9). Mg showed high stability in the path and correlation coefficients (Figure 6; Table S9). The negative impact of P was counterbalanced by the positive impact of Zn and, to a much lesser extent, by K. Thus, Zn had the highest path coefficient value, ameliorating the negative direct effects for N and P while stabilizing the effect of K and Mg. The prediction of GLY, as shown by PA-R^2, was 94.8% (Table S10). Among the two predictive nutrients (i.e., Mg and Zn), Zn was characterized by the most stable path coefficient value. It was stabilized by K and simultaneously destabilized by N, P, and Mg.

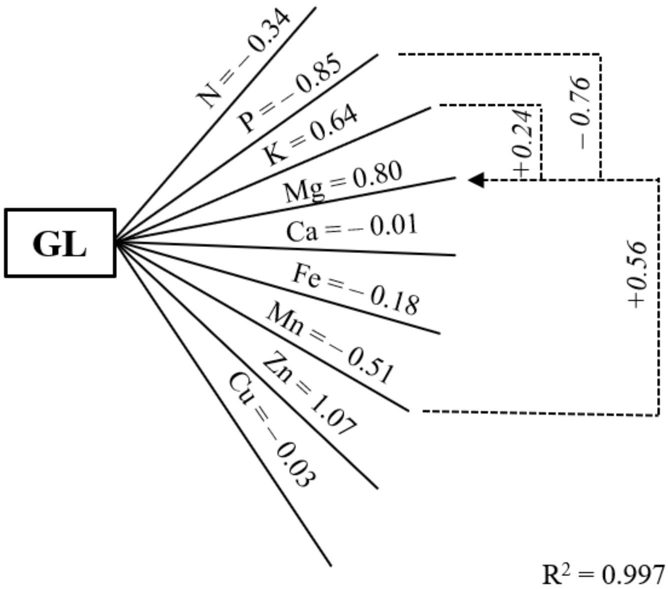

Figure 6. Path diagram of the direct and indirect effects of nutrients in the winter wheat stem at the full flowering stage on the wet gluten yield (GLY). Negligible values of the indirect path coefficients (<0.10) are not included in the figure.

3.3. Nutritional Composition of Winter Wheat Parts at Full Flowering—BBCH 65

3.3.1. Flag Leaf

The nutrient contents in the winter wheat flag leaf at the BBCH 65 stage was significantly driven by the weather in subsequent study years (Table A3). It should be emphasized that there was no interaction effect between N_f concentrations and the years, except for Zn. The lowest Mg and Fe contents were measured in 2015, the year with the highest GY; meanwhile, the K, Ca, Zn, and Cu contents peaked. SPAD index values were high with no variability across the years. Increased rates of applied N_f significantly affected the content of the examined nutrients, except Mg. The nutrient contents stabilized at different N_f concentrations. The Ca and Mn content stabilized at 40 kg N ha^{-1}. Additionally, P, K, Fe, and Cu stabilized at 40 kg N ha^{-1}, while N and Zn stabilized at 160 kg N ha^{-1}.

The Ca content in the flag leaf, as indicated by the stepwise regression analysis, was the key nutrient associated with GY, GL, and GLY (Tables A2 and S11). The overall effect of flag leaf nutrient content on GY was 93.1%, as indicated by PA-R^2 (Table S12). The path coefficient for Ca was the highest among the examined nutrients and was highly stable, as evidenced by the constant correlation coefficient. The negative indirect impact of Zn on Ca, despite the significant and positive correlation between both nutrients, was counterbalanced by the positive impact of K on Ca. Moreover, Zn was significantly correlated with GY and Ca.

The nutrient content in the flag leaf explained 97.4% of the GL content in grain (Table S13). The nutrients predicting GL included N, Ca, Fe, and Cu (Table A2). Only Ca showed a negative relationship with GL. Among this set of nutrients, the highest path coefficient values were found for N and Cu. However, the correlation coefficient for N was markedly higher and was identified as a key predictor of GL. In addition, this coefficient was significantly and positively affected by Cu and non-significantly by Fe. According to the stepwise regression analysis, GLY was significantly predicted by Ca, Fe, and Cu (Table A2). Among this set of nutrients, the highest path coefficient was found for Ca (Table S12), which was enhanced, however moderately, by K and Cu. K was the nutrient with the highest correlation coefficient and was moderately associated with Ca.

3.3.2. Leaves

The nutrient contents in winter wheat leaves at the BBCH 65 stage were significantly affected by year and increasing N_f concentrations. Responses to the interaction of Y × N were noted for N, Fe, Zn, and Cu (Table 5). Compared to the BBCH 40 stage, the content of N, P, Fe, Zn, and Cu decreased, however, this effect was year-specific. In 2015 compared to other years, the N and P content had the largest decrease, and Zn and Cu the smallest. The opposite trend was noted for Mg, Ca, and Mn. The most considerable changes were obtained for Ca. Its content in 2015 increased 4.5-fold, doubled in 2014, and was stable in 2016. An increase in Mg content occurred in all years, however, the highest was recorded in 2015 (>2-fold). In general, the lowest N, P, and Fe content was recorded in 2015, a year with the highest yield. The opposite tendency was observed for Ca, Zn, and Cu. The effect of progressively increasing N_f concentrations was nutrient-specific. The lowest stabilization, that is, high response to increasing N concentrations, was noted for N, K, Fe, Zn, and Cu. The content of this set of nutrients stabilized beginning with 160 kg N ha^{-1}. The content of P and Ca stabilized at 80 kg N ha^{-1}. Meanwhile, the Mg and Mn content stabilized at 40 kg N ha^{-1}.

Table 5. Nutrient content in winter wheat leaves at full flowering (BBCH 65).

Factor	Factor	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
	Level			**% DM**				**mg kg^{-1} DM**		
Years (Y)	2014	1.47 b	0.28 b	2.92 b	0.28 a	0.37 b	55.1 b	89.5 a	5.6 c	2.50 c
	2015	1.35 b	0.22 c	3.21 a	0.27 a	0.63 a	51.9 b	46.4 b	11.0 a	5.43 a
	2016	2.31 a	0.31 b	3.41 a	0.16 b	0.24 c	66.1 a	41.2 b	8.3 b	3.88 b
Fc, p		65.4 ***	52.8 ***	17.2 ***	90.1 ***	381.0 ***	30.8 ***	26.5 ***	45.6 ***	113.8 ***
Nitrogen rates, kg ha^{-1} (N)	0	1.08 d	0.24 c	2.55 d	0.21 b	0.33 c	47.0 e	37.9 b	5.3 d	3.13 c
	40	1.45 cd	0.25 bc	2.93 cd	0.22 ab	0.37 bc	53.0 de	50.5 ab	6.5 cd	3.52 bc
	80	1.39 cd	0.27 ac	2.97 c	0.23 ab	0.40 ac	54.2 ce	53.8 ab	6.0 cd	3.29 c
	120	1.77 bc	0.27 ac	3.27 bc	0.23 ab	0.45 a	57.4 bd	65.2 ab	8.0 bc	3.80 ab
	160	2.00 ab	0.28 ab	3.41 ab	0.24 ab	0.45 a	63.2 ab	65.3 ab	9.5 ab	4.37 bc
	200	2.06 ab	0.28 ac	3.67 a	0.26 a	0.45 a	62.1 ac	62.6 ab	10.8 a	4.72 a
	240	2.21 a	0.30 ab	3.46 ab	0.26 a	0.43 ab	67.1 a	77.8 a	12.1 a	4.76 a
Fc, p		17.7 ***	5.0 ***	18.4 ***	3.2 **	9.4 ***	11.3 ***	2.7 *	17.4 ***	10.4 ***
Source of variation of the studied interactions										
Y × N		*	ns	ns	ns	ns	*	ns	*	*

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$, respectively; ns, non-significant.

According to the stepwise regression analyses, K content was the key nutrient in winter wheat leaves affecting GY, GL, and GLY (Tables A2 and S15). The GY prediction based on leaf nutrient content, as indicated by PA-R^2, was 95.4% (Table S16). A detailed analysis showed that GY was best predicted based on K, Ca, and Mn contents (Table A2). Meanwhile, according to the path analysis, Ca had the greatest direct effect on GY (Figure 7; Table S16). Its effect was moderately enhanced by Cu and Mg and negatively by Mn. As a result, the correlation coefficient for Ca reached the highest value among the examined nutrients. The prognostic set of nutrients for GL, besides K and Ca, included Cu. The GL prediction, as indicated by PA-R^2, was 97.8% (Table S15). The direct effect of K on GL was significantly lower than for Ca (negative) and Cu (positive). However, it showed the highest correlation coefficient value among the examined nutrients. Moreover, the path coefficient for K was strongly enhanced by Cu and moderately by N. The GLY prediction, as indicated by PA-R^2, was 95.9% (Table S18). The key set of nutrients comprised K, Mn, and Cu (Table A2). Among these, Cu exerted the highest direct effect; the correlation coefficient was moderately enhanced only by K.

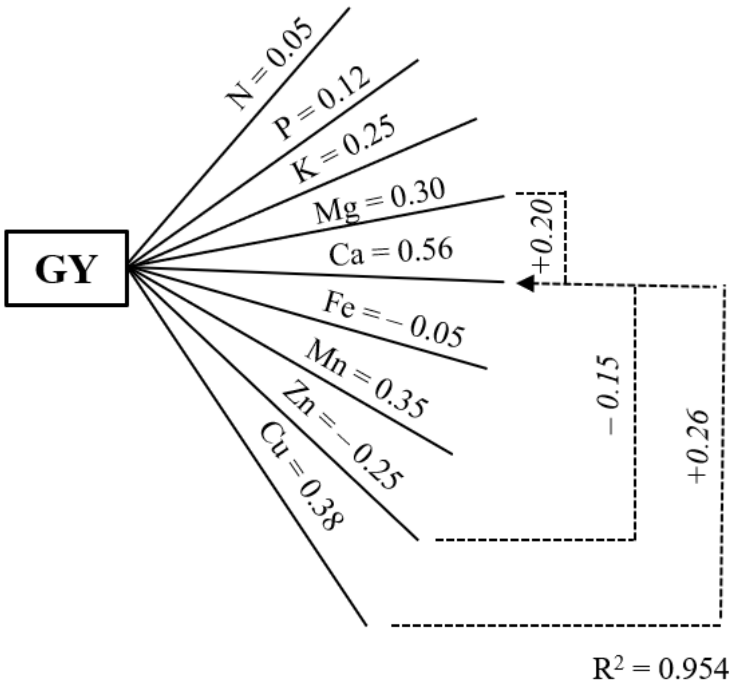

Figure 7. Path diagram of direct and indirect effects of nutrients in the winter wheat stem at the full flowering stage on the grain yield (GY). Negligible values of the indirect path coefficients (<0.10) are not included in the figure.

3.3.3. Stems

The key factor affecting the nutrient contents in the winter wheat stem was the year (Table A4). The highest content of N, Ca, and Fe compared to other years was recorded in 2015. The opposite tendency was found for P, K, Mg, and Mn. The effect of increasing N_f concentrations was nutrient-specific. No response to N_f concentrations was noted for Ca or Mn. Meanwhile, Fe was stable from 40 kg N ha^{-1}, Cu from 120 kg N ha^{-1}, and the remaining nutrients from 160 kg N ha^{-1}. Only the N and Ca content responded significantly to the Y × N interaction. The dominance of N in 2015 over the remaining years was observed in the N control plots and those fertilized with up to 120 kg N ha^{-1} (Figure S8). The N content exhibited significant relationships with GY, GL, and GLY (Table S19).

GY was significantly associated with six of the nine examined nutrients, with the highest value for Cu followed by Fe (Table S19). The GY prediction based on the content of nutrients in the stem, as indicated by PA-R^2, was 90.3% (Table S20). According to the stepwise regression analysis, the predictive set of nutrients comprised Ca and Zn (Table A2). Ca, considered separately, was moderately related to GY, poorly and negatively correlated with Mg and Mn, and positively correlated with Fe and Cu. Meanwhile, Zn exhibited strong positive relationships with P, K, and Mn. In addition, Zn had the highest path coefficient value (Table S20), however, it was significantly reduced by the indirect effects of P and Mn. In contrast, Ca had a low path coefficient value, which increased due to the positive but indirect effect of Mn. The GL prediction based on stem nutrient content, as indicated by PA-R^2, was 98.5% (Table S21). Based on the stepwise regression analysis, the best set of variables comprised Mg, Mn, and Zn (Table A2). Mg was weakly but positively correlated with P, and negatively with Ca (Table S19). However, Mg showed the highest path coefficient value, approaching 1.00, which Ca moderately reduced. The GLY prediction based on stem nutrient content, as indicated by PA-R^2, was 93.4% (Table S22). The key effect on this wheat trait, based on stepwise regression analysis, was elicited by Mg and Mn (Table A2). However, the direct effect of both nutrients on GLY was only moderate. The highest path coefficient value was recorded for Zn, which Mn strongly reduced.

3.3.4. Ears

The most pronounced differences in the nutrient contents in winter wheat ears were the highest for N and Ca and the lowest for Mg and Mn in 2015 compared to the other years (Table A5). All differences were large. The Ca content was twice as high and N 40 (50%) higher than in the remaining years. The same range of differences was noted for Mg and Mn, respectively. The effect of increasing N_f concentrations on the nutrient content was specific, except for P and Cu. The Mn content stabilized on the plot fertilized with 40 kg N ha^{-1}, the N and Ca stabilized at 80 kg N ha^{-1}, K and Zn at 120 kg N ha^{-1}, and Fe at 200 kg N ha^{-1}. The Y $\times$ N interaction was noted only for Cu, however, the year was the dominant factor. GY showed significant associations with five of the nine studied nutrients, with the highest correlation coefficient for Mg (Table S23).

The GY prediction based on the nutrient content in the ear, as indicated by PA-R^2, was 92.1% (Table S24). However, as demonstrated by the stepwise regression analysis, the key predictive nutrients were N and K (Table A2). N content was strongly and positively correlated with Ca and negatively correlated with Mg and Mn (Table S23). K was negatively associated with P and Mg and positively with Zn. The path analysis confirmed the dominant effect of N and K on GY. The path coefficient for K was very high and stable, however, was significantly reduced by Mg and increased by N. Meanwhile, the path coefficient for N was reduced by Mg and Mn (Table S24). The predictive value of the nutrient content for GL prediction was, as indicated by PA-R^2, 92.3% (Table S25). N and Mg were the key predictive nutrients, as demonstrated by stepwise regression analysis (Table 5). The key direct effect of both nutrients was confirmed (Table S25), however, the interactions between them were contradictory. That is, the indirect effect of N on Mg was strongly negative and only partially restored by Mn. Meanwhile, Mg and Mn strongly reduced the path coefficient value for N. The GLY prediction based on ear nutrient content, as indicated by PA-R^2, was 88.5% (Table S26). The best set of variables, resulting from stepwise regression analysis, consisted of N, K, and Mg (Table 5). The greatest direct effect on GLY was exerted by N, while Mg and Mn strongly reduced it. Nevertheless, the correlation coefficient value for N was the highest among the examined nutrients. The direct effect of Mg was reduced by N and K; the negative impact of Mg on the K path coefficient was confirmed.

4. Discussion

4.1. Vegetative Indicators of Grain Yield and Qualitative Characteristics of Grain

Winter wheat yields achieved on the N control plots were exceptionally high, with 6.6, 7.3, and 5.4, respectively, in 2014, 2015, and 2016. The environmental yield Gap (EYG) based on this dataset was 1.8 t ha^{-1}. Such high yields resulted from (i) high soil fertility, (ii) high N_{min} content at the beginning of winter wheat regrowth in the spring, and (iii) winter oilseed rape forecrop. The importance of winter oilseed rape as a forecrop for winter wheat is well-documented [32,33]. These three factors formed the basis for the operation of the applied N_f. The maximum GY in 2014 amounted to 11.3 t ha ha^{-1} and was obtained at an N_f rate of 209 kg N ha^{-1}. In the remaining years, GY increased linearly with N_f concentrations, reaching a maximum of 13.7 t ha^{-1} in 2015 and 8.6 t ha^{-1} in 2016. Hence, the agronomic-environmental yield gap (A-EYG) almost tripled, reaching 5.1 t ha^{-1}. Such a large difference indicates the key impact of N_f concentrations on the GY, regardless of the weather patterns in a given year. The observed level of variability in winter wheat yields is typical under the production conditions in Poland [34]. Grain yields in 2015 and 2016 increased according to a linear regression model in response to increasing N_f rates, thus pointing to N supply as a key limiting factor. However, in 2015, the maximum GY was 60% higher than in 2016. This discrepancy indicates a disturbance in the N supply. The main cause of the decreased GY in 2016 was the excessive mass of vegetative wheat parts during the CCW.

Despite having great importance in the wheat variety, qualitative characteristics of grain, such as CP and GL content, are limited by the N supply, which is largely related to the N_f concentration [35,36]. The GL content was significantly correlated with the CP content, therefore the prediction procedure in this study was focused on GL and GLY. Stable, high CP and GL content, meeting the required quality standards, was achieved on plots fertilized with 200 and 240 kg N ha^{-1}. The increase in CP typically caused a decrease in the STA content, but not the STAY, which was perfectly correlated with GY. The trend in CPY and GLY differed from GY and was consistent with the linear model in response to increasing N_f.

The wheat growth period before flowering is considered critical for the number of grains per unit area (grain density, GD) [37]. Therefore, a strong association between the total wheat canopy biomass or between the biomass of particular wheat organs with GY is expected. Such a dependence was not observed in the booting or full flowering phases of wheat growth. Moreover, in the booting phase, the ratio of the stem weight to leaf weight (ST/LE) was relatively constant (3.36 ± 0.37) regardless of the experimental factors (CV = 11%). More complicated patterns were observed at BBCH 65, when the relationship between ear weight to leaf weight (EA/LE) and ear weight to stem weight (EA/ST) significantly affected grain yield. Both ratios were inversely correlated with GY:

1. EA/LE: GY = −3.39 EA/LE + 16.5 for n = 14, R^2 = 0.58 and $p \leq 0.001$
2. EA/ST: GY = −10.0 EA/ST + 14.2 for n = 14, R^2 = 0.34 and $p \leq 0.05$

These two equations clearly indicate that any extension of the particular ratio leads to a decrease in GY. The narrowest values of both indices, as found in 2015, clearly indicate the potential of the vegetative parts of the winter wheat canopy to maintain ear productivity. Indeed, it is well-documented that the GD of wheat, as a cleistogamous plant, depends on the number of fertile flowers [11].

A very clear relationship was observed between the leaf biomass in both consecutive stages of winter wheat growth and the GL content. The observed, linear dependence was stronger at full flowering, when the leaf biomass exhibited a quadratic course in response to increasing N_f rates. The highest biomass was obtained at an optimal N_f of 195 kg ha^{-1}. For stems, no significant association was observed with CP and GL at this stage. In the ears, the biomass reached saturation at an N_f of 161 kg ha^{-1}. Meanwhile, the CP and GL contents increased linearly with the increase in N_f rates. The advantage of 2015 over other years was due to the net increase in leaf biomass and stem biomass during the CCW. This type of dependence clearly points to leaves and ears as the key sources of N for the growing grains. This type of dependence is observed only for high-yielding winter wheat [16].

4.2. Prognosis of Grain Yield and Quality Characteristics of Grain

The period of wheat growth from the beginning of the booting phase to the beginning of flowering phase is considered crucial for GD as the key yield component [37]. The results of this study clearly showed that in the CCW period, the N content in the vegetative parts of winter wheat was significantly reduced. Moreover, in both stages, the N content in the indicative parts of the wheat did not exhibit a significant relationship with GY, however, linearly and positively affected CP and GL content. Hence, a significant association was confirmed between the N content in the leaves and the stem at BBCH 40 with CP and GL contents. However these relationships were much stronger for leaves. Even stronger relationships were observed between these two traits for the flag leaf and all leaves at the full flowering stage. For stems, this relationship was significant but much weaker, and for ears it was insignificant. This type of relationship indicates that N content is the limiting factor for these two traits of winter wheat. This dependency was much stronger during flowering.

It should be noted that the N content in both wheat parts at BBCH 40, regardless of the weather in the consecutive seasons, increased progressively with increasing N_f concentrations. Thus, the non-saturation N status of wheat explain why the CP, and especially GL, content was below the threshold norms [22]. The saturation of leaves with N, despite slight variability in subsequent years, was observed only during the full flowering stage, covering fertilization variants from 160 to 240 kg N ha^{-1} (maximum CV < 13%). Thus, the N_f application during the booting stage significantly increased the CP content, and consequently, GL content. These results confirm the important role of late-season nitrogen application to bread wheat on the quality characteristics of grain. However, this fertilization treatment does not always lead to increased yield [14].

4.2.1. Booting Stage

The content of the examined nutrients in winter wheat at the onset of booting was identified as a good predictor of GY, GL, and GLY. Their leaf and stem content explained 94% of the GY variability.

The N content in leaves at the beginning of the booting stage (BBCH 40), regardless of the year, was associated with the highest yields, assumed above 10 t ha^{-1}, ranging from 2.7% to 3.8%. There is no standard range for N content in winter wheat leaves for this phase. However, the obtained range is much narrower than that set by Bergman [17] for wheat just before the booting stage (2–5%; end of the shooting phase, BBCH 37). Regardless of the year, the optimal range for N content in the wheat stem was narrow, oscillating around 1.5%. According to the stepwise regression analysis, the set of nutrients for GY prediction differed depending on the plant part:

1. Leaves: $GY = 2.89 + 3.45N - 46.5Ca + 0.1Mn$ for $n = 10, R^2 = 0.81, p \leq 0.001$
2. Stems: $GY = 2.88 + 2.77K - 34.7Mg + 1.49Cu$ for $n = 10, R^2 = 0.91, p \leq 0.001$

The first piece of information derived from these equations points to the stem as the plant part with a greater prediction level. The second concerns N, with the linear GY dependent on the N content only in the year with the lowest yield. Such a relationship indicates the presence of a non-nutritional factor limiting the yield. Additionally, both equations clearly show that the excess Ca in leaves and Mg in stems led to decreased yield. Excessive accumulation of Ca in leaves at the BBCH 40 stage was observed in 2016, the year with the lowest yield. It should, therefore, be treated as a disturbance of the yield formation processes. Such a phenomenon was also observed in spelt wheat [38]. The negative path coefficient and correlation coefficient for Mg in leaves, especially in stems, indicate that its supply to wheat was excessive in relation to the needs, leading to its low efficiency. Based on the path analysis results, Ca and Mg interacted antagonistically. Hence, a simple and practical method to control the Ca content is to apply Mg to wheat foliage at the beginning of the booting phase [39]. The predictive role of Fe, demonstrated for both parts of the plant (correlation analysis), as well as Mn (leaves) and Cu (stems), indicates the important role of micronutrients for the high-yielding winter wheat.

The prediction of GL content based on the nutrient content in wheat leaves and stems at the beginning of booting was 99.5% and 99.7%, respectively. Thus, the accuracy of the prognosis was very high, exceeding 99%. Based on the path analysis, the direct effect of N was negative. Regardless of the plant part, the GL prognosis was based on three nutrients: P, Mg, and Zn. Among these, the key N controlling function was assigned to Zn. Zn exhibited the highest path coefficient value and the highest indirect impact on N, particularly considering stems. Meanwhile, Mg was the key nutrient determining the GL content. Its stable effect on GL was strongly supported by the indirect effect of Zn and, in part, by K. However, the effect of Zn requires further investigation as its higher content in the stem buffered the action of other nutrients. The mitigating effect was strongest for N and P, which had negative path coefficients. The importance of Zn in winter wheat production was fully confirmed for GLY prognosis. As in the case of GL, GLY exhibited the highest direct dependence on Zn content and the highest impact on N; a much stronger effect was observed for the stem. To date, the importance of Zn in wheat has been reported primarily in the semi-dry areas of wheat cultivation and recently in China [40,41]. These studies found that Zn is an essential nutrient for stabilizing the action of N in high-yielding wheat at the onset of the booting phase, which is crucial for yield formation while also being decisive for CP and GL content.

4.2.2. Full Flowering Stage

The assessment for the predictive worth of the winter wheat parts in the full flowering stage was carried out based on determination coefficients for the nutrients. The resulting series are as follows (based on 2-percentage difference):

(1) GY: LE65 > FLE65 $\geq$ EA65 > ST65;
(2) GL: ST65 $\geq$ LE65 $\geq$ FLE65 > EA65;
(3) GLY: LE65 > ST65 = FLE65 > EA65.

Generally, among the four examined plant parts, the highest prognostic value was found for LE65 and the lowest for EA65. In the case of GY, the prognostic usefulness of leaves was higher, while for GL and GLY, it was much weaker than that obtained at the beginning of the booting stage.

Predictions of GY based on the nutrient content in the flag leaf, as well as for all leaves, clearly showed that N was not the limiting nutrient. It should also be emphasized that the N content in both wheat parts responded weakly to the indirect effects of other nutrients. However, the dependence of GY on the content of N in stems and ears was noted. Meanwhile, the path coefficient for N in stems was low. Moreover, the coefficient of determination for these parts of wheat was much lower than for leaves. The key nutrient directly limiting GY was Ca:

1. Flag leaf: $\mathrm{GY} = 2.3 + 13.1\mathrm{Ca} + 0.04\mathrm{Mn}$ for $n = 14, \mathrm{R}^2 = 0.85, p \leq 0.001$
2. Leaves: $\mathrm{GY} = -2.23 + 1.53\mathrm{K} + 10.8\mathrm{Ca} + 0.04\mathrm{Mn}$ for $n = 14, \mathrm{R}^2 = 0.93, p \leq 0.001$
3. Stems: $\mathrm{GY} = -2.8 + 232.2\mathrm{Ca} + 0.35\mathrm{Zn}$ for $n = 14, \mathrm{R}^2 = 0.73, p \leq 0.001$

The dependence of GY on the nutrient contents in leaves differed entirely from that recorded at the beginning of the booting phase. It should be emphasized that the linear relationship for the Ca $\times$ GY, indicating deficiency of Ca content in the flag leaf, was observed only in years with lower yield than in 2015. In this particular yield, the relationship adhered to the quadratic regression model. The optimum Ca content of 0.64% DM resulted in a yield of 13.3 t ha^{-1}. In the remaining years, the maximum Ca content in the flag leaf for the maximum GY was 0.31%, amounting to 11.3 and 8.6 t ha^{-1} in 2014 and 2016, respectively. The action of Ca resulted not only from the increase in its content in the leaves in 2015 during the CCW period but also from the simultaneous increase in the Zn and Cu contents. The stem nutrient set for GY prediction comprised Ca and Zn. This suggests that Zn is a critical nutritional factor influencing GY.

Although stem nutrient content was a slightly better predictor of GL content than leaves, the latter was more suitable for predicting GLY:

A. Wet gluten (GL):
   1. Leaves: $\mathrm{GL} = 1.0 + 7.6\mathrm{K} - 15.8\mathrm{Ca} + 1.5\mathrm{Cu}$ for $n = 14, \mathrm{R}^2 = 0.94, p \leq 0.001$
   2. Stems: $\mathrm{L} = 7.87 + 90.1\mathrm{Mg} - 0.32\mathrm{Mn} + 1.06\mathrm{Zn}$ for $n = 14, \mathrm{R}^2 = 0.90, p \leq 0.001$
B. Wet gluten yield (GLY):
   1. Leaves: $\mathrm{GLY} = -2.1 + 0.6\mathrm{K} + 0.02\mathrm{Mn} + 0.4\mathrm{Cu}$ for $n = 14, \mathrm{R}^2 = 0.92, p \leq 0.001$
   2. Stems: $\mathrm{GLY} = 0.83 - 0.06\mathrm{Mn} + 28\mathrm{Zn}$ for $n = 14, \mathrm{R}^2 = 0.79, p \leq 0.001$

The K content in leaves was determined to be the leading nutrient affecting the GL content, ultimately GLY. Based on the path analysis, K showed a high indirect response to Cu and N. Moreover, the negative direct effect of Ca was mitigated by Cu. Although GLY depended on K, the yield driver was Cu. However, the positive impact of Cu on Zn content cannot be overlooked. The optimal set of nutrients for GL prediction comprised Mg, Mn, and Zn. The highest value of the path coefficient was for Mg, which Ca reduced. GLY was driven by Zn, the direct effect of which was reduced primarily by Mn. Regarding the leaves, a strong interaction between Zn x Cu was observed.

In light of these results, a key question arises regarding the rationality of N_f application to bread wheat at the beginning of the booting stage. A significant increase in the CP content, especially GL and GY, after applying at least 40 kg N ha^{-1} confirms the rationality of this treatment. Notably, the N, K, Fe, Zn, and Cu content in leaves stabilized at an N_f concentration of 160 kg N ha^{-1}. In this set, K, Cu, and Zn were crucial in predicting GY, GL, and GLY. At this point in the discussion, the question should be asked whether the late-season application of solid N fertilizer can be replaced by foliar application of N and/or foliar application of N with micronutrients. Foliar fertilization of winter wheat with N is a technological option as it can increase the PC content; however, a yield increase is possible only with a low basic N concentration [42,43]. This strategy has been assessed for foliar fertilization of wheat, mainly with urea [44]. A maximum Cu, Fe, and Mn uptake rate occurs immediately before flowering [45]. Thus, simultaneous foliar application of N and micronutrients can increase the photosynthetic activity of leaves. Indeed, Cu foliar application can increase the flag leaf area and chlorophyll content, both of which positively affect the GY of wheat [46].

5. Conclusions

The results unequivocally confirmed that the game of winter wheat GY occurs in the CCW yield formation period. In addition, the game's end result depends not only on the plant's N supply during this period but also on the nutritional status of other nutrients. Micronutrients, including Zn and Cu, were critical factors in forming GY, GL content, and GLY.

The non-saturation of winter wheat leaves with N at the beginning of the booting phase indirectly indicates the need for the so-called late-season N fertilizer application. In the case of high-yielding winter wheat, applying 40 to 80 kg N ha^{-1} immediately before the CCW period ensured high yield and high technological quality of grain. The positive reaction of winter wheat traits to late applied N_f logically points to N as a limiting factor. It can, therefore, be concluded that in high-yielding winter wheat, even a good nutritional state with N at booting does not guarantee the required fluor quality.

However, the yield-forming effect of N, as indicated by its content in indicatory wheat parts during the CCW period, cannot be limited to the N content alone. The degree of prediction of the tested winter wheat traits confirmed this assumption. It should be emphasized that only GY prediction was more reliable at the full flowering stage than the booting stage. The significant and positive effect of Ca content in the leaves, flag leaf, and stem during full flowering on GY was likely due to the buffering effect of Zn and Cu. The accuracy of the GL prognosis at BBCH 40, regardless of the plant part, exceeded 99%. Three nutrients, namely, P, Mg, and Zn, explained 98% of the variability in GL, while the GLY forecast was high (97%). Both wheat traits depended on Zn, which buffered the action of N and Mg. During the full flowering stage, the highest predictions of GL and GLY, however weaker compared with the booting stage, were obtained for leaves (95% and 92%, respectively). At this particular stage of winter wheat growth, the significant role of Zn and K and the buffering effect of Cu on the action of both were revealed. The obtained results indicate the beginning of the booting phase as the key to correcting the N nutritional status of the plant. The leaves meet the requirements of the basic indicator of winter wheat at this stage. In farming practice, the shortage of Mg, Zn, and Cu during the CCW can be controlled by foliar application of fertilizers alone or with N.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13102649/s1, Figure S1: Regression models of winter wheat grain yield response to concentrations of applied nitrogen; Figure S2. Regression models of winter wheat crude protein yield response to concentrations of applied nitrogen; Figure S3. Regression models of winter wheat wet gluten yield response to concentrations of applied nitrogen; Figure S4. Regression models of winter wheat starch yield response to concentrations of applied nitrogen; Figure S5. Effect of increasing nitrogen concentrations in subsequent years on winter wheat biomass at full flowering; Figure S6. Regression models of winter wheat biomass at full flowering response to concentrations of applied nitrogen; Figure S7. Effect of increasing nitrogen concentrations in subsequent years on magnesium content in winter wheat leaves at full flowering; Figure S8. Effect of increasing nitrogen concentrations in subsequent years on nitrogen content in stems of winter wheat at full flowering. Table S1. Correlation matrix between winter wheat traits at the beginning of the full flowering (BBCH 65) and grain yield, *n* = 14; Table S2. Correlation matrix between winter wheat traits at the beginning of the booting stage (BBCH 40) and grain yield, *n* = 10; Table S3. Correlation matrix between leaf nutrient contents and quantitative and qualitative traits of winter wheat at the beginning of booting (BBCH 40), *n* = 10; Table S4. Components of path analysis for the grain yield response to the nutrient content in winter wheat leaves at the onset of booting (BBCH 40), *n* = 10; Table S5. Components of path analysis for the gluten content response to the nutrient content in winter wheat leaves at the onset of booting (BBCH 40), *n* = 10; Table S6. Components of path analysis for the gluten yield response to the nutrient content in winter wheat leaves at the onset of booting (BBCH 40), *n* = 10; Table S7. Correlation matrix between nutrient contents in stems and quantitative and qualitative traits of winter wheat at the beginning of booting (BBCH 40), *n* = 10; Table S8. Components of path analysis for the grain yield response to the nutrient content in stems of winter wheat at the onset of booting (BBCH 40), *n* = 10; Table S9. Components of path analysis for the gluten content response to the nutrient content in stems of winter wheat at the onset of booting (BBCH 40), *n* = 10; Table S10. Components of path analysis for the gluten yield response to the nutrient content in stems of winter wheat at the onset of booting (BBCH 40), *n* = 10; Table S11. Correlation matrix between nutrient contents in the flag leaf and quantitative and qualitative traits of winter wheat at full bloom (BBCH 65), *n* = 14; Table S12. Components of path analysis for the grain yield response to the nutrient content in the flag leaf of winter wheat at full bloom (BBCH 65), *n* = 14; Table S13. Components of path analysis for the gluten content response to the nutrient content in the flag leaf of winter wheat at full bloom (BBCH 65), *n* = 14; Table S14. Components of path analysis for the gluten content response to the nutrient content in the winter wheat flag leaf at full bloom (BBCH 65), *n* = 14; Table S15. Correlation matrix between nutrient contents in leaves and quantitative and qualitative traits of winter wheat at full bloom (BBCH 65), *n* = 14; Table S16. Components of path analysis for the grain yield response to the nutrient content in winter wheat leaves at full bloom (BBCH 65), *n* = 14; Table S17. Components of path analysis for the gluten content response to the nutrient content in winter wheat leaves at full bloom (BBCH 65), *n* = 14; Table S18. Components of path analysis for the gluten yield response to the nutrient content in winter wheat leaves at full bloom (BBCH 65), *n* = 14; Table S19. Correlation matrix between nutrient contents in the stem and quantitative and qualitative traits of winter wheat at full bloom (BBCH 65), *n* = 14; Table S20. Components of path analysis for the grain yield response to the nutrient content in the winter wheat stem at full bloom (BBCH 65), *n* = 14; Table S21. Components of path analysis for the gluten content response to the nutrient content in the winter wheat stem at full bloom (BBCH 65), *n* = 14; Table S22. Components of path analysis for the gluten yield response to the nutrient content in the winter wheat stem at full bloom (BBCH 65), *n* = 14; Table S23. Correlation matrix between nutrient contents in the ear and quantitative and qualitative traits of winter wheat at full bloom (BBCH 65), *n* = 14; Table S24. Components of path analysis for the grain yield response to the nutrient content in the winter wheat ear at full bloom (BBCH 65), *n* = 14; Table S25. Components of path analysis for the gluten content response to the nutrient content in the winter wheat ear at full bloom (BBCH 65), *n* = 14; Table S26. Components of path analysis for the gluten yield response to the nutrient content in the winter wheat ear at full bloom (BBCH 65), *n* = 14.

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Appendix A

Table A1. Characteristics of winter wheat canopy at critical stages of yield formation, t ha^{-1} DW.

Factor	Factor Level	Booting, BBCH 40 LE40	ST40	TB40	Flowering, BBCH 65 LE65	ST65	EA65	TB65
Years (Y)	2014	1.89 b	6.55 b	8.44 b	1.5 b	7.4 c	3.5 b	12.4 c
	2015	2.15 b	6.89 b	9.04 b	2.3 a	10.2 a	3.1 b	15.6 b
	2016	2.63 a	8.64 a	11.26 a	2.4 a	9.1 b	5.7 a	17.2 a
Fc, p		18.2 ***	40.4 ***	38.4 ***	32.1 ***	26.3 ***	114.7 ***	31.1 ***
Nitrogen rates, kg ha^{-1} (N)	0	1.59 c	5.71 c	7.30 c	1.3 c	7.2 c	3.1 c	11.6 c
	40	2.06 b	7.26 b	9.32 b	1.6 bc	7.8 ba	3.5 bc	12.9 bc
	80	2.30 ab	7.96 ab	10.27 ab	2.0 ab	9.3 ac	4.1 ab	15.4 ab
	120	2.44 ab	7.67 ab	10.12 ab	2.3 a	10.3 a	4.6 a	17.2 a
	160	2.73 a	8.18 a	10.91 a	2.4 a	10.1 a	4.7 a	17.3 a
	200	-	-	-	2.4 a	9.1 ab	4.4 ab	15.9 a
	240	-	-	-	2.3 a	8.6 ab	4.3 ab	15.2 ab
Fc, p		14.7 ***	18.7 ***	20.4 ***	12.4 ***	7.3 ***	8.8 ***	10.1 ***
Source of variation of the studied interactions								
Y × N		ns	**	*	*	*	ns	ns

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$; ns, non-significant. Legend: LE, leaf biomass; ST, stem biomass; EA, ear biomass; TB, total biomass; 40, 65 stages of winter wheat growth.

Table A2. Summary of statistical evaluation of indices obtained for stepwise regression and path analyses.

Wheat Traits	Statistical Indices	LE40	ST40	FL65	LE65	ST65	EA65
Grain yield—GY	Coefficient of determination —path analysis	0.938	0.943	0.930	0.954	0.903	0.921
	Coefficient of determination —stepwise regression	0.814	0.887	0.846	0.931	0.734	0.798
	Indicative nutrients —stepwise regression	N, Ca, Mn	K, Mg, Cu	Ca, Mn	K, Ca, Mn	Ca, Zn	N, K
Wet gluten content—GL	Coefficient of determination —path analysis	0.995	0.997	0.974	0.978	0.985	0.923
	Coefficient of determination —stepwise regression	0.978	0.929	0.942	0.943	0.897	0.778
	Indicative nutrients —stepwise regression	P, Mg, Zn	P, Mg, Zn	N, Ca, Fe, Cu	K, Ca, Cu	Mg, Mn, Zn	N, Mg
Wet gluten yield—GLY	Coefficient of determination —path analysis	0.969	0.948	0.930	0.959	0.934	0.885
	Coefficient of determination —stepwise regression	0.947	0.849	0.896	0.924	0.787	0.770
	Indicative nutrients —stepwise regression	N, Ca, Mn	Mg, Zn	Ca, Fe, Cu	K, Mn, Cu	Mn, Zn	N, K, Mg

Legend: LE, leaf biomass; ST, stem biomass; EA, ear biomass; TB, total biomass; 40, 65 stages of winter wheat growth.

Table A3. Nutrient content and SPAD index in the winter wheat flag leaf at full flowering (BBCH 65).

Factor	Factor Level	N	P	K % DM	Mg	Ca	Fe	Mn mg kg^{-1} DM	Zn	Cu	SPAD
Years (Y)	2014	2.08 c	0.30 a	2.30 b	0.34 a	0.30 b	68.3 a	83.3 a	3.8 c	2.8 b	564.6
	2015	2.52 b	0.25 b	2.59 a	0.26 c	0.56 a	49.0 b	35.8 b	11.0 a	4.6 a	563.9
	2016	3.13 a	0.29 b	2.33 b	0.30 b	0.25 b	62.6 a	33.4 b	5.9 b	4.1 a	568.4
Fc, p		29.2 ***	23.5 ***	12.7 ***	30.3 ***	61.8 ***	32.1 ***	71.1 ***	43.7 ***	31.7 ***	1.8 ns
Nitrogen rates, kg ha^{-1} (N)	0	2.18 c	0.25 c	1.99 d	0.29	0.29 b	45.0 c	35.7 a	3.9 c	2.8 b	460.2 f
	40	2.03 c	0.26 bc	2.29 c	0.30	0.31 ab	53.3 bc	45.7 ab	4.9 bc	3.3 b	517.8 e
	80	2.13 c	0.27 ac	2.31 bc	0.30	0.35 ab	52.2 bc	47.2 ab	5.2 bc	3.4 b	544.8 d
	120	2.49 bc	0.28 ab	2.51 ac	0.29	0.38 ab	63.6 ab	50.8 ab	6.7 bc	3.7 ab	570.5 c
	160	2.90 ab	0.30 a	2.59 ac	0.29	0.43 ab	66.5 a	53.0 ab	8.3 ab	4.5 a	607.0 b
	200	3.01 ab	0.29 ab	2.64 a	0.31	0.44 a	67.7 a	57.7 ab	11.0 a	4.6 a	618.8 b
	240	3.29 a	0.29 ab	2.52 ab	0.31	0.42 ab	71.4 a	65.6 a	8.2 ab	4.6 a	640.2 a
Fc, p		10.9 ***	5.9 ***	11.8 ***	0.4 ns	3.4 **	13.4 ***	3.4 *	8.1 ***	7.6 ***	535.8 ***
Source of variation of the studied interactions											
Y × N		ns	ns	ns	ns	ns	ns	ns	*	ns	ns

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$; ns, non-significant.

Table A4. Nutrient content in winter wheat stems at full flowering (BBCH 65).

Factors	Factor Level	N	P	K % DM	Mg	Ca	Fe	Mn	Zn mg kg^{-1} DM	Cu
Years (Y)	2014	0.77 b	0.17 a	2.44 a	0.15 b	0.032 b	24.1 b	47.0 a	14.9	2.74 a
	2015	0.93 a	0.14 c	1.85 b	0.11 c	0.046 a	33.9 a	9.2 b	8.5	2.76 a
	2016	0.79 b	0.16 b	1.97 b	0.20 a	0.033 b	22.3 b	15.6 b	8.0	2.33 b
Fc, p		9.8 ***	17.0 ***	38.1 ***	516.1 ***	31.8 ***	11.7 ***	94.0 ***	68.8 ***	6.4 **
Nitrogen rates, kg N ha^{-1} (N)	0	0.57 d	0.13 c	1.56 d	0.14 c	0.033	20.2 a	21.9	7.7 d	2.11 c
	40	0.70 bc	0.14 c	1.83 cd	0.14 c	0.034	22.1 ab	27.8	9.5 cd	2.38 c
	80	0.70 cd	0.14 c	1.77 cd	0.14 c	0.036	26.5 ab	23.3	8.4 bd	2.19 bc
	120	0.79 bc	0.15 bc	2.04 bc	0.15 bc	0.038	23.2 ab	23.0	9.8 bd	2.55 ac
	160	0.91 ab	0.17 ab	2.33 ab	0.16 ab	0.039	32.7 ab	22.6	11.3 ac	2.82 ab
	200	1.01 a	0.18 a	2.55 a	0.16 a	0.040	30.1 a	21.8	12.5 ab	3.15 a
	240	1.04 a	0.18 a	2.51 a	0.16 a	0.040	32.7 a	27.2	14.0 a	3.07 a
Fc, p		15.8 ***	9.8 ***	25.3 ***	12.4 ***	1.9 ns	3.4 **	0.6 ns	10.2 ***	8.1 ***
Source of variation of the studied interactions										
Y × N		*	ns	ns	ns	*	ns	ns	ns	ns

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$; ns, non-significant.

Table A5. Nutrient content in winter wheat ears at full flowering (BBCH 65).

Factors	Factor	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
	Level			% DM					mg kg^{-1} DM	
Years (Y)	2014	1.95 b	0.33 b	1.51 a	0.16 b	0.015 c	37.3 ab	44.8 a	37.3 a	3.65 b
	2015	2.96 a	0.32 b	1.38 b	0.11 c	0.030 a	36.5 a	19.4 c	28.0 b	4.09 ab
	2016	2.11 b	0.38 a	0.94 c	0.22 a	0.019 b	40.1 a	30.8 b	25.8 b	3.94 a
Fc, p		113.8 ***	22.3 ***	326.5 ***	428.2 ***	419.6 ***	5.2 **	115.9 ***	71.0 ***	5.7 **
Nitrogen rates, kg N ha^{-1} (N)	0	2.10 c	0.33	1.16 c	0.16 ab	0.020 c	35.3 c	28.2 b	26.3 c	3.63
	40	2.15 bc	0.34	1.21 bc	0.17 ab	0.021 bc	36.1 bc	31.2 ab	27.8 bc	4.13
	80	2.34 ac	0.32	1.21 bc	0.15 b	0.020 ac	34.6 c	30.3 ab	28.7 bc	3.85

Table A5. *Cont.*

Factors	Factor	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
	Level			% DM				mg kg^{-1} DM		
	120	2.35 ac	0.35	1.31 ab	0.16 ab	0.021 ac	37.6 bc	30.2 ab	31.3 ab	3.78
	160	2.43 ac	0.34	1.32 ab	0.16 ab	0.021 ac	37.6 bc	31.0 ab	31.2 ab	4.08
	200	2.47 ab	0.36	1.38 a	0.17 a	0.022 ab	43.2 a	33.0 ab	32.2 ab	3.94
	240	2.53 a	0.36	1.34 a	0.17 a	0.023 a	41.2 ab	37.8 a	35.0 a	3.84
Fc, p		4.3 **	1.7 ns	10.8 ***	2.3 *	4.2 **	6.3 ***	2.9 *	7.1 ***	1.4 ns
Source of variation of the studied interactions										
Y × N		ns	ns	ns	ns	ns	ns	ns	ns	*

Similar letters in the column indicate no significant differences between experimental treatments using Tukey's test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$; ns, non-significant.

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