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Yield Predictive Worth of Pre-Flowering and Post-Flowering Indicators of Nitrogen Economy in High Yielding Winter Wheat

Witold Szczepaniak, Witold Grzebisz * and Jarosław Potarzycki

Department of Agricultural Chemistry and Environmental Biogeochemistry, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznan, Poland

* Correspondence: witold.grzebisz@up.poznan.pl

Abstract: Indicators of nitrogen economy in winter wheat during vegetative development are a reliable tool for yield prognosis. This hypothesis was verified in a field experiment, carried out in the 2013/2014, 2014/2015, and 2015/2016 seasons. The field experiment, in a two-factor split-plot design, included the following systems of wheat protection (CFP): (i) N + micronutrients, (ii) N + fungicides, (iii) N + micronutrients + fungicides; and N rates: 0, 40, 80, 120, 160, 200, 240 kg N ha^{-1}. The content and accumulation of N in wheat at the beginning of stem elongation and at heading were used for grain density and yield prediction. In the grain-filling phase, the stem N acted as a buffer, stabilizing yield at a high level. The condition for such action was the stem N equilibrium with the ear N at flowering. The N depletion from the leaves during the grain-filling period significantly depended on the grain density. The post-flowering uptake of N by wheat was affected by the grain density, which was affected by the N reserves in the stem. Yield forecast based on pre-flowering indices of nitrogen economy in cereals affects both agronomic decisions aimed at correcting the nutritional status of plants, and farm economics.

Keywords: wheat indicative organs; nitrogen concentration; nitrogen accumulation; grain-filling period; nitrogen remobilization; yield prediction

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1. Introduction

Wheat is one the most sensitive cereals to soil type, place in crop rotation, rate of applied nitrogen fertilizer (N_f), and pesticide protection against diseases [1,2]. The best soil for wheat is chernozem, which in Ukraine provides a yield of 3.5 t ha^{-1} without the use of fertilizers. The actual yield in this country was 3.98 ± 0.21 t ha^{-1} in20142020 [3,4]. In Europe, in this period the highest yields of 8.18 ± 0.45 t ha^{-1} are attainable under good weather conditions, which occur in the United Kingdom and Western Europe. In Poland, the maximum attainable yields are around 10 t ha^{-1}, but the national average does not exceed 5 t ha^{-1} [1,5]. The main reasons for such a deep yield gap between European countries are soil and weather conditions. In Poland, both these factors are less suitable for wheat production than in Western Europe [6]. To achieve high yield, wheat varieties require suitable soil in respect of productivity, physical and chemical characteristics, nitrogen (N) supply, forecrop, soil class, available soil potassium content, crop protection level, i.e., fungicide application, foliar fertilization with macro-, and micronutrients, and available soil phosphorus content [7].

Nitrogen is a key nutritional factor affecting the aggregate yield component of cereals, which is the number of grains per unit area—grain density [8]. Its supply to wheat during wheat's vegetative growth is critical for grain density (GD) and grain yield (GY). However, it should be taken into account that the yield structure formation begins at the onset of the shoot elongation phase [9,10]. In an intensive wheat production system, the main N source is fertilizer nitrogen (N_f). The total dose of the applied N_f and its division into sub-doses requires synchronization with the formation of basic yield components, such as the number of ears, the number of grains per ear, and the grain weight [11]. The second goal of wheat fertilization with N_f is a high protein content in grain [12]. For this reason, the total N_f dose in wheat is as a rule higher than that required for the maximum yield [13]. It is well recognized that wheat plants fertilized with high doses of N_f are sensitive to a broad range of pathogens. Thus, a modern, high-productive wheat system requires the use of very sophisticated methods of crop protection and respective fungicides [14,15]. Data from the Rothamsted Experimental Station unequivocally showed that the inclusion of fungicide protection in wheat production technology significantly increased the yield [16]. In Poland, yield losses caused by pathogens may reach 20–30% or even 50% of the maximum attainable yield [17]. Therefore, fungicide protection of winter wheat against pathogens is an important factor for both the yield and technological quality of grain [16]. Pesticides, apart from having a positive effect on plant health, unfortunately have a negative impact on the environment and human health [15]. The key question is whether organic fungicides will be reduced or even eliminated. An alternative option to protect the wheat canopy during the growing season is the use of micronutrients. It is well known that zinc and copper are used as fungicides. There is some literature data confirming the negative impact of this set of micronutrients on the pressure of pathogens [18,19].

In light of the opinions on wheat yield formation presented above, the question arises concerning the impact of micronutrients applied alone, or with fungicides, on the N economy of winter wheat. The nitrogen economy of seed plants is, in fact, limited to two groups of indicators. The first, which can be called normative or standardized, concerns the content of a nutrient in the entire plant biomass or its indicatory part. The dilemma is that these indicators, mainly the ranges of the nutrient content in the plant, were developed about 50 years ago [20]. From that time until today, new plant varieties have been bred, the yield potential of which is at least twice as high [21]. The second group of indicators focuses on the N balance during the reproductive growth of seed plants, mainly cereals [22]. There is a deep gap; in fact, the lack of indicators of N management in the pre-flowering period would confirm the common opinion about the decisive impact of this period on seeds/grain yield [9,10].

The aim of this study was to develop (i) a set of N economy indicators for the pre-flowering period of winter wheat growth, (ii) to compare the predictive worth of pre- and post-flowering N indicators, and (iii) explain the sources and pathways of N flow to the developing grains after flowering. The basis of the N indices development and evaluation was the field experiment with three systems of wheat canopy foliar protection (micronutrients, fungicide protection, and micronutrients plus fungicide) against the background of progressive N doses (0 to 240 kg ha^{-1}) The obtained N indices were then confronted with the wheat sink capacity expressed as grain density in order to determine both the critical stage of the grain yield forecast and the indicative plant part.

2. Materials and Methods

2.1. Experimental Setup

Basic data on setting up a field experiment were published by Szczepaniak et al. [23].

The study on the content of N, its accumulation, and then remobilization between winter wheat organs during the spring part of the growing season was carried out in the 2013/14, 2014/2015, and 2015/2016 seasons in Smolice (52°42′ N; 17°10′ E), Poland. The field experiment was established on soil formed from loamy sand over sandy loam, classified as Albic Luvisol. The organic carbon (C_{org}) content and pH values were variable, peaking in the 2014/2015 growing season. The content of available nutrients, measured before wheat sowing, i.e., before the application of basic fertilizers (P and K), was in the medium class at least for P, K, Fe, and Zn. This means very favorable conditions for winter wheat growth. Much more variable levels of soil fertility were found for other nutrients, such as Mg, Ca, Cu, and Mn, which in the 2013/2014 growing season were in low or very low classes. The amount of mineral N (N_{min}), measured just before the spring regrowth of winter wheat in a 0.0–0.6 m layer was generally high or very high, as in 2015 (Table 1).

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

Soil, cm	pH	C_{org} %	P	K	Mg	Ca	Cu	Mn	Zn	Fe	N_{min}
			mg kg^{-1}								kg ha^{-1}
2013/2014											
0–30	6.9	1.3	234 VH [5]	231 M	105 VL	988 L	0.4 L	27.2 L	3.6 M	536 H	86.4
30–60	6.7	1.1	234 VH	237 M	103 VL	876 L	0.4 L	25.7 L	3.5 M	541 H	
2014/2015											
0–30	7.1	2.2	185 H	185 M	165 M	2045 M	3.5 M	85.5 M	6.3 H	268 M	129.0
30–60	7.2	2.1	161 H	157 L	155 L	2063 M	3.5 M	93.8 M	5.6 H	269 M	
2015/2016											
0–30	6.6	1.6	202 VH	281 M	165 M	1480 L	2.8 M	61.9 M	6.1 H	347 M	110.0
30–60	6.6	1.4	139 H	222 M	163 L	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 [24]; [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 [25,26].

The local climate of the study area, classified as intermediate between Atlantic and Continental, is seasonal, especially in summer. The meteorological data are presented in Figure 1 and Table 2. The growth conditions of winter wheat assessed on the basis of the Sielianinov hydrothermal indices in the subsequent years of study were very diverse (Table S2). The early vegetation phase of winter wheat in spring 2014 was very good, but 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. The beginning of the 2016 growing season was wet. May was semi-dry, and June was dry. These two months are critical for the development of the number of grains in an ear of winter wheat. Most of the wheat grain-filling period was wet again.

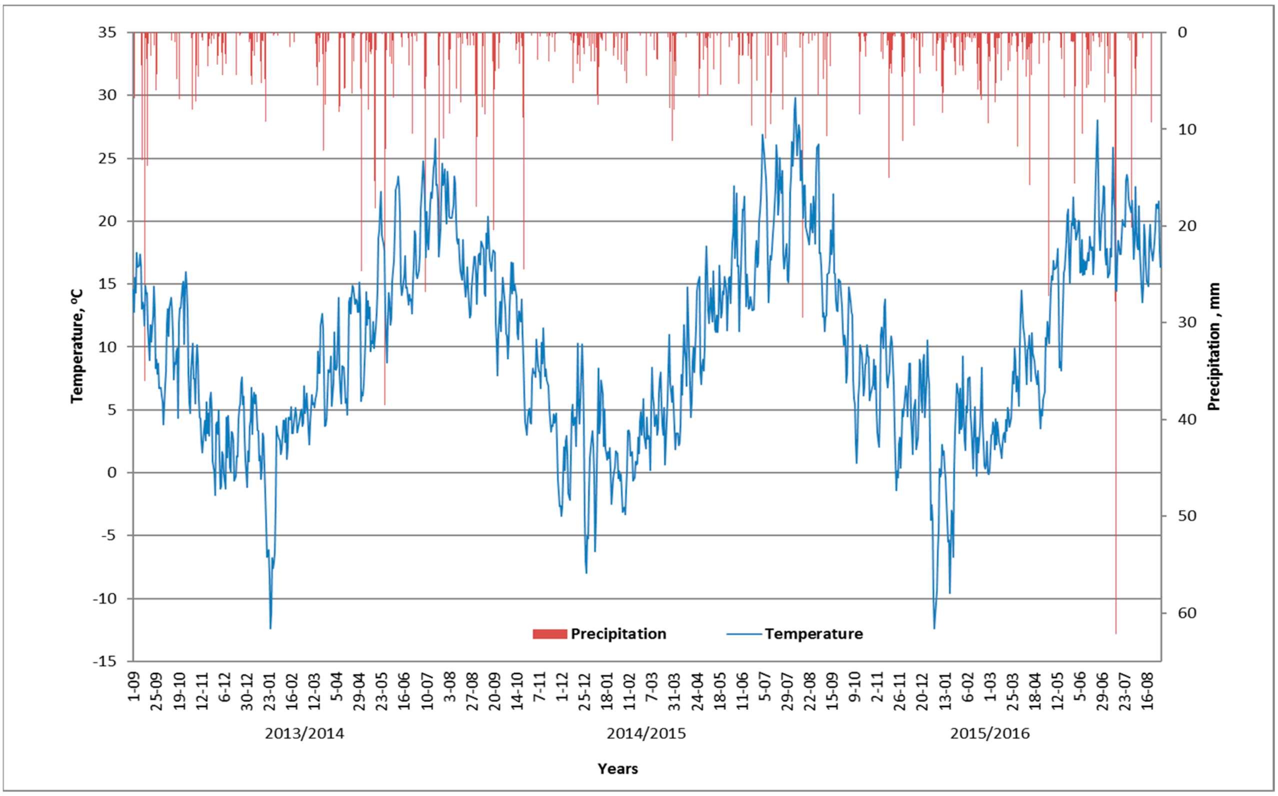

Figure 1. Daily mean air temperature and precipitation during the study, Experimental Station Smolice.

Table 2. Values of the Sielianinov hydrothermal index (*k*) for winter wheat (March–July).

Growing Season	March	April	May	June	July
2013/2014	2.0 qw	1.3 qd	3.4 ew	0.6 vd	1.1 qd
2014/2015	2.8 vw	0.8 d	0.6 vd	0.7 vd	0.7 vd
2015/2016	4.3 ew	1.7 qw	1.2 qd	0.7 vd	2.2 w

Legend: classes of weather conditions: very dry, vd: 0.41 < k < 0.7; dry, d: 0.71 < k < 1.0; quite dry, qd: 1.01 < k < 1.50; quite wet, qw: 1.51 < k < 2.0; wet, w: 2.01 < k < 2.5; very wet, vw: 2.51 < k < 3.0; extremely wet, ew: k > 3.01 [27].

2.2. Experimental Design

The field experiment, arranged in a two-factor split-plot design, was replicated four times and included:

1. Three systems of wheat canopy foliar protection in the spring part of the growing season (CFP):
    - 1.1. N + foliar fertilization with micronutrients (N + Mi, i.e. fungicide control—FC);
    - 1.2. N + fungicide protection (N + P, FP);
    - 1.3. N + micronutrients + fungicide protection (N + Mi + FP, MiFP).
2. Rates of applied fertilizer N: 0, 40, 80, 120, 160, 200, and 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 the rate of 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 following experimental schedule:

(1) 80 kg N ha^{-1}: the late winter, before the beginning of winter wheat vegetation in spring;
(2) 160 kg N ha^{-1}: at the end of tillering/beginning of the shoot elongation (BBCH 29/30);
(3) 240 kg N ha^{-1}: at the stage of a flag leaf visible (BBCH 39).

Phosphorus was applied at the rate of 17.2 kg P ha^{-1} in the form of triple superphosphate (46% P_2O_5). Potassium was applied at the 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 two weeks before wheat sowing. Foliar application of micronutrients and fungicides was carried out in accordance with the experimental schedule, as shown in Table 3.

Table 3. Time schedule and composition of micronutrients and fungicides applied to wheat.

Stage of Wheat Growth	Microelements	Fungicide
BBCH 30/31	Cu + Mn → 60 + 140 g ha^{-1}	Capalo 337.5 SE → 1.5 dm^3 ha^{-1}
BBCH 39	Cu + Mn + Zn → 15 + 60 + 100 g ha^{-1}	Adexar Plus → 2 dm^3 ha^{-1}
BBCH 65	-	Osiris 65 EC → 2 dm^3 ha^{-1}

2.3. Plant Sampling

The plant material for the determination of the dry matter and N content was collected at four stages of winter wheat growth: (i) the beginning of stem elongation (BBCH 31), (ii) the beginning of heading (BBCH 50), (iii) the full flowering (BBCH 65), and (iv) maturity (BBCH 90) [28]. A single plant sample, depending on the stage of wheat growth, was partitioned into leaves, stems, ears, chaffs, and grain. The N content was determined in the plant parts using the standard macro-Kjeldahl procedure [29]. The content of N was expressed on a dry matter basis. The chlorophyll meter SPAD-502Plus was used to measure the absorbance of the flag leaf. This index expresses the relative amount of chlorophyll in a plant leaf [30].

2.4. Calculated Parameters

Based on the primary data about the plant biomass and the content of N in particular parts of the plant, the following set of N indicators was calculated:

Nitrogen accumulation (N_a) in wheat parts in subsequent stages of wheat growth:

$$N_a = WB \times N, \text{ kg N ha}^{-1} \tag{1}$$

Total nitrogen accumulation in respective stages, TN, kg N ha^{-1}:

$$TN50 = N_aL + N_aS \tag{2}$$

$$TN65 = N_aL + N_aS + N_aE \tag{3}$$

$$TN90 = N_aL + N_aS + N_aCh + N_aG \tag{4}$$

$$TNVe = N_aL + N_aS + N_aCh \tag{5}$$

Nitrogen total balance (ΔTN, ΔTN50, ΔTN65):

$$\Delta TN50 = TN50 - TN31, \text{ kg N ha}^{-1} \tag{6}$$

$$\Delta TN65 = TN65 - TN50, \text{ kg N ha}^{-1} \tag{7}$$

Nitrogen remobilization quota (NRQ):

$$NRQ = TN65 - TNVe90, \text{ kg N ha}^{-1} \tag{8}$$

Nitrogen remobilization efficiency (E-NRQ):

$$\text{E-NRQ} = (NRQ/TN65) \times 100\% \tag{9}$$

Contribution of remobilized nitrogen into the grain (C-NRQg):

$$\text{C-NRQg} = (NRQ/N_aG) \times 100\% \tag{10}$$

Nitrogen post-flowering uptake (NPFU):

$$NPFU = TN90 - TN65, \text{ kg N ha}^{-1} \tag{11}$$

Efficiency of post-flowering N uptake (E-NPFU):

$$\text{E-NPFU} = (NPFU/TN90) \times 100\% \tag{12}$$

Contribution of post-flowering N uptake to the grain yield (C-NPFUg):

$$\text{C-NPFUg} = (NPFU/NG90) \times 100\% \tag{13}$$

Nitrogen partial remobilization (NRL, NRS, NRCh):

$$NRL = N_aL65 - N_aL90, \text{ kg N ha}^{-1} \tag{14}$$

$$NRS = N_aS65 - N_aS90, \text{ kg N ha}^{-1} \tag{15}$$

$$NRCh = N_aE65 - N_ach90, \text{ kg N ha}^{-1} \tag{16}$$

Efficiency of N partial remobilization (E-NRL, E-NRS, E-NRCh):

$$\text{E-NRL} = [(NRL/N_aL65) \times 100]\% \tag{17}$$

$$\text{E-NRS} = [(NRS/N_aS65) \times 100]\% \tag{18}$$

$$\text{E-NRCh} = [(\text{NRL}/\text{N}_a\text{E65}) \times 100]\% \tag{19}$$

where: N—nitrogen content in wheat parts, % DW; WB—the dry weight of plant parts, kg N ha^{-1}; BBCH: 31, 50, 65, 90—stages of wheat growth [28]; L, S, E, Ch, G—wheat parts: leaves, stems, ears, chaffs, and grain, respectively; T: 31, 50, 65, 90—total N accumulation by wheat (leaves + stem + ear, + grain) in respective stages, kg N ha^{-1}.

2.5. Statistical Analysis

The effects of individual research factors (year, foliar protection, N doses) and their interactions on the grain yield and indices of nitrogen use efficiency were assessed by means of a two-way ANOVA. Means were separated by honest significant difference (HSD) using Tukey's method when the F-test indicated significant factorial effects at the level of $p < 0.05$. The relationships between the traits were analyzed using Pearson correlation and linear regression. The stepwise regression was applied to define the optimal set of variables for a given plant's characteristics. The best regression model was selected based on the highest F-value for the entire model. STATISTICA 12 software was used for all statistical analyses (StatSoft Inc., 2013, Tulsa, OK, USA).

3. Results

3.1. Patterns of Nitrogen Concentration in Winter Wheat

The content of N in all the organs of winter wheat in the critical stages of its growth was significantly and positively correlated with grain density per unit area (GD), grain yield (GY), and the SPAD index (Table A1). Detailed data and discussion on GD and GY can be found in the paper by Szczepaniak et al. [23]. The highest values of correlation coefficients (r) were obtained for wheat organs (leaves and stems) at the BBCH 50 stage. Stepwise regression analysis performed up to BBCH 65 of wheat growth showed a much wider set of independent variables for the main discussed traits, i.e., GD and GY:

3. Yield (GY):

$$\text{GY} = -5.98 + 1.83\text{N31} + 6.01\text{N50S}\ for\ n = 21,\ R^2 = 0.90,\ and\ p\ \leq 0.01 \tag{20}$$

4. Grain density (GD):

$$\text{GD} = -16.73 + 3.27\text{N31} + 6.64\text{N50S} + 6.1\text{N65E}\ for\ n = 21,\ R^2 = 0.98,\ and\ p\ \leq 0.01 \tag{21}$$

Both equations show that in the vegetative period of wheat growth in spring, the key variable for a significant GY prognosis was the content of N in wheat at BBCH 31 (N31) and in the stems at BBCH 50. The significant GD forecast compared to GY was additionally dependent on the N content in wheat ears at BBCH 65. The first two wheat N traits responded differently to the weather in the subsequent years of the study and to experimental factors (Table 4). Only N31 showed a significant response to the method of wheat canopy foliar protection (CFP). Moreover, it was significantly dependent on the interaction of Y × CFP and Y × N, but not on the interaction of Y × CFP × N. The first interaction revealed the significant impact of the years (2014 < 2015 < 2016). This significance resulted from a higher N content in wheat grown in the FP and MiFP main plots compared to Mi, i.e., the fungicide control (FC) main plot in 2015 (Figure S1). The second interaction indicated a particularly high N content in wheat in the N control plot in 2015 and 2016 (Figure 2). Consequently, the significant impact of the applied N_f was much more emphasized in 2014 than in the other two years, in which no response was found to the use of 80 kg N ha^{-1}.

Table 4. The content of nitrogen in winter wheat parts in critical stages of growth, % DW.

Factor	Factor Level	N31	N50L	N50S	N65L	N65S	N65E	N65FL	N90L	N90S	N90Ch	N90G	SPAD65
Year (Y)	2014	3.2 ^{c}	2.7 ^{b}	1.3 ^{b}	1.5 ^{b}	0.8 ^{b}	1.9 ^{c}	1.9 ^{c}	1.2 ^{a}	0.3 ^{b}	0.5 ^{c}	2.0 ^{c}	552.3
	2015	3.8 ^{b}	3.3 ^{a}	1.4 ^{a}	1.4 ^{b}	0.9 ^{a}	3.0 ^{a}	2.5 ^{b}	0.9 ^{b}	0.3 ^{b}	1.0 ^{a}	2.1 ^{b}	553.1
	2016	4.0 ^{a}	3.4 ^{a}	1.4 ^{a}	2.3 ^{a}	0.8 ^{b}	2.1 ^{b}	3.2 ^{a}	1.2 ^{a}	0.5 ^{a}	0.8 ^{b}	2.4 ^{a}	552.4
p		***	***	***	***	***	***	***	***	***	***	***	ns
Foliar canopy protection (CFP)	N + Mi	3.5 ^{b}	3.1	1.3	1.8	0.8 ^{b}	2.3	2.5	1.3 ^{a}	0.4	0.8 ^{a}	2.2	536.7 ^{c}
	N + P	3.7 ^{a}	3.2	1.4	1.8	0.9 ^{a}	2.4	2.5	1.0 ^{b}	0.4	0.7 ^{b}	2.1	555.6 ^{b}
	N + Mi + P	3.7 ^{a}	3.1	1.4	1.7	0.8 ^{a}	2.3	2.6	1.0 ^{b}	0.4	0.7 ^{b}	2.1	565.6 ^{a}
p		***	ns	ns	ns	***	ns	ns	***	ns	**	ns	***
Nitrogen rates (kg N ha^{-1})	0	2.9 ^{b}	2.4 ^{c}	1.1 ^{a}	1.2 ^{d}	0.6 ^{d}	2.1 ^{c}	2.0 ^{d}	0.8 ^{d}	0.3 ^{d}	0.7 ^{d}	1.8 ^{f}	455.2 ^{g}
	40	3.8 ^{a}	2.8 ^{b}	1.2 ^{ab}	1.4 ^{cd}	0.7 ^{cd}	2.2 ^{c}	2.1 ^{d}	0.9 ^{c}	0.3 ^{cd}	0.7 ^{cd}	1.9 ^{e}	502.9 ^{f}
	80	3.8 ^{a}	3.0 ^{b}	1.3 ^{b}	1.5 ^{c}	0.7 ^{cd}	2.3 ^{bc}	2.2 ^{d}	1.0 ^{bc}	0.4 ^{cd}	0.7 ^{cd}	2.0 ^{f}	526.8 ^{e}
	120	3.8 ^{‡,a}	3.3 ^{a}	1.4 ^{a}	1.7 ^{b}	0.8 ^{c}	2.4 ^{ab}	2.6 ^{c}	1.1 ^{b}	0.4 ^{bc}	0.8 ^{bc}	2.1 ^{d}	552.4 ^{d}
	160	3.8 ^{‡,a}	3.5 ^{†,a}	1.5 ^{†,a}	2.1 ^{a}	0.9 ^{b}	2.4 ^{ab}	2.8 ^{bc}	1.3 ^{a}	0.4 ^{ab}	0.9 ^{ab}	2.3 ^{c}	594.5 ^{c}
	200	3.8 ^{‡,a}	3.5 ^{†,a}	1.5 ^{†,a}	2.1 ^{a}	1.0 ^{a}	2.5 ^{a}	3.0 ^{ab}	1.3 ^{a}	0.4 ^{a}	0.8 ^{a}	2.4 ^{b}	611.6 ^{b}
	240	3.8 ‡	3.5 ^{†,a}	1.5 ^{†,a}	2.2 ^{a}	1.1 ^{a}	2.5 ^{a}	3.2 ^{a}	1.4 ^{a}	0.5 ^{a}	0.9 ^{a}	2.5 ^{a}	625.1 ^{a}
p		***	***	***	***	***	***	***	***	***	***	***	***
Source of variation for interactions													
Y × CFP		***	ns	ns	ns	ns	ns	*	**	ns	*	ns	ns
Y × N		***	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns
CFP × N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***
Y × CFP × N		ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns

Similar letters means a lack of significant differences using Tukey's test; ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. Legend: N—nitrogen content, % DW; 31, 60, 65, 90—growth stages of winter wheat; L, S, E, FL, Ch, G—leaves, stem, ear, flag leaf, chaffs, grain, wheat parts (organs); ‡ the first N_f dose of 80 kg N ha^{-1} was only applied; † the N_f dose was supplemented to 160 kg N ha^{-1}.

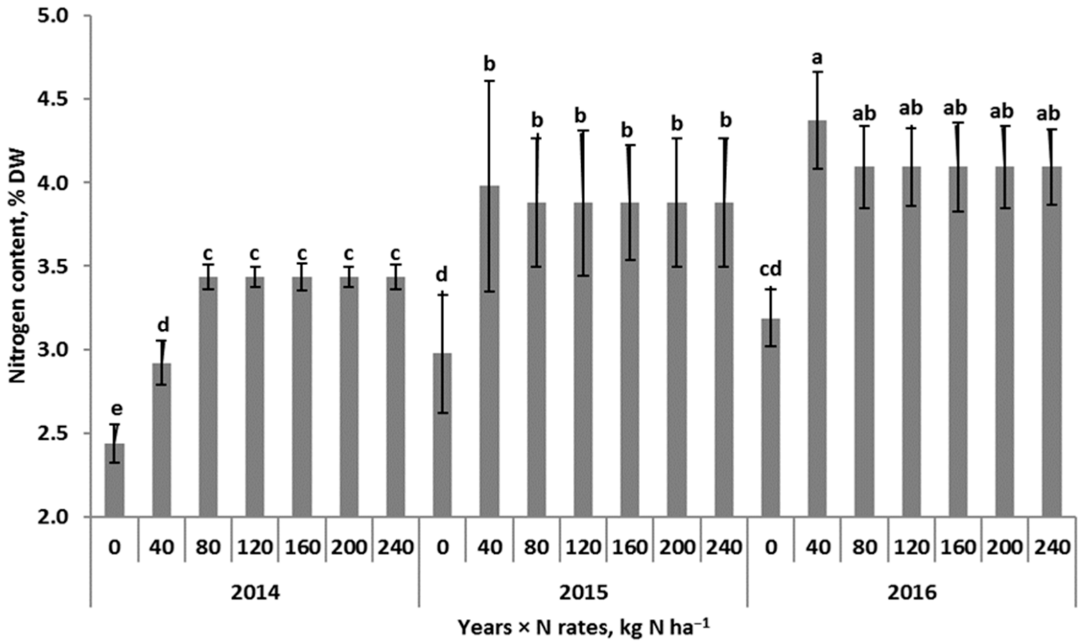

Figure 2. Effect of increasing nitrogen rates on the content of nitrogen in winter wheat at the beginning of the stem elongation phase in subsequent years of study. Similar letters means a lack of significant differences using Tukey's test. The vertical bar in the column refers to the standard deviation value.

The N content in wheat stems at BBCH 50 (N50S) was the second major predictor of GD and GY. This N trait responded significantly to both years and N doses, but no interaction was found between them (Table 1). N50 S was significantly lower in 2014 than in the remaining years of the study. The effect of increasing N doses on N50 S progressed up to 120 kg ha^{-1}, then stabilized. The highest increase was observed between plots of 80 and 120 kg N ha^{-1}. The obtained regression model showed that the maximum N content of 1.5% was reached at the N_f dose of 236.8 kg ha^{-1}:

$$\text{N50 S} = -0.0000058\text{N}_f^2 + 0.0036\text{N}_f + 1.09 \text{ for n} = 7, \text{R}^2 = 0.97, p \leq 0.01 \quad (22)$$

The third component in the second equation, i.e., the content of N in wheat ears at BBCH 65 (N65E) followed the same pattern as N50S. However, the effect of the years was more pronounced. The highest N content was found in 2015, a year with the highest GD and GY. The N content in the stem increased in accordance with increasing N_f doses, being consistent with the linear regression model:

$$\text{N65E} = 0.018\text{N}_f + 21.2 \text{ for n} = 7, \text{R}^2 = 0.94 \text{ and } p \leq 0.01 \quad (23)$$

However, the difference between the content of N on the N control plot and the variant with 240 kg N ha^{-1} was low, reaching only 0.4%.

Considering the entire growing season of winter wheat in spring, the set of variables, predicting GY changed partly, and is as follows:

$$\text{GY} = -7.87 + 1.82\text{N31} + 3.6\text{N50S} - 2.09\text{N90L} + 3.51\text{N90G} \; for \; \text{n} = 21, \; \text{R}^2 = 0.98, \; and \; p \; \leq 0.01 \quad (24)$$

As shown in this equation, GY, in addition to N31 and N50S, also depended on two other variables, including the N content in leaves and grain at BBCH 90 (N90L; N90G). N90L, similarly to N31, showed a significant response to the interaction of Y × CFP and Y × N. Equation (24) indicates that the increased content of N in wheat leaves at BBCH 90 negatively affected the yield. The main reason for this trend was the much higher N content in wheat leaves, which was recorded, regardless of the year, on the FC object. In contrast, the lowest values of N90L were recorded in 2015 (Figure 3). This relationship was fully corroborated by the Y × N interaction. As shown in Figure A1, in 2014 and 2016, the N content in leaves at BBCH 90 increased almost linearly with the applied N doses. A completely different pattern was observed in 2015. In this particular year, the N90L followed the quadratic regression model. Its increase was recorded, up to 160 kg N ha^{-1}. The N content in wheat grain (N90G) showed a significant response to the years and N_f doses, but no interaction between these factors was found (Table 4). The N90G, averaged over experimental factors, increased in the following order: 2014 < 2015 < 2016. The effect of the applied N_f was consistent with the increased N rates.

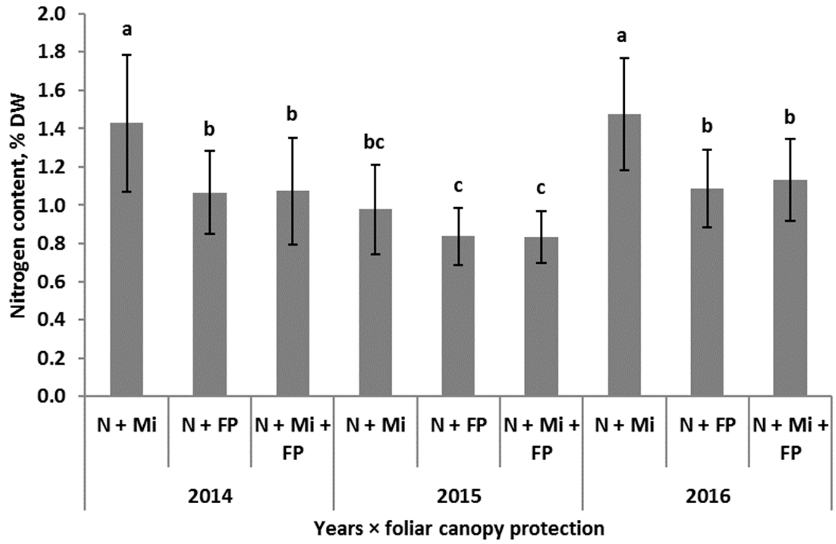

Figure 3. The content of nitrogen in winter wheat leaves at maturity in response to the method of foliar canopy protection in subsequent years of study. Similar letters means a lack of significant differences using Tukey's test. Legend: N + Mi—nitrogen plus foliar applied micronutrients; N + FP—nitrogen + foliar applied fungicides; N + Mi + FP—nitrogen plus foliar applied micronutrients and fungicides. The vertical bar in the column refers to the standard deviation value.

The SPAD index, determined at the BBCH 65 stage, using the flag leaf as the indicative plant part, turned out to be the best GY predictor (Table A1). It showed a significant response to the CFP × N interaction (Figure A2). Regardless of the experimental object, the trend of this index followed the same linear course in response to increasing N_f doses. As shown in equation no. 25, this index was significantly dependent on two wheat N traits, i.e., N31 and the content of N in the flag leaf (N65 FL). The obtained equation is as follows:

$$\mathrm{SPAD} = 105.1 + 50.3\mathrm{N31} + 103.9\mathrm{N65FL}\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.94,\ and\ p\ \leq 0.01 \quad (25)$$

Despite similar yearly patterns of N31 and N65 FL, the correlation coefficient between these two variables was weak (r = 0.54*). The content of N in the flag leaf of wheat (N65 FL) was significantly affected by the Y × CFP interaction (Figure S2). In 2014, an increase in N65 FL on objects protected with fungicides compared to FC was observed. In 2015, N65 FL values were significantly higher compared to 2014, but no difference was found between the main plots. An even stronger trend was observed in 2016, when N65 FL was the highest within the study period. Moreover, the highest N content was recorded in the FC main plot. The fungicidal protection of the wheat canopy caused a slight decrease in this flag leaf characteristic.

3.2. Patterns of Nitrogen Accumulation in Winter Wheat

Key traits of winter wheat, i.e., GY and GD, were significantly correlated with the amount of N accumulated in plant organs (N_a) during the spring part of the growing season

(Table A2). The stepwise regression analysis carried out for N_a during the pre-flowering stages of wheat growth clearly emphasized the dominant role of two of the eight studied traits. The increase in GY was consistent with the increased N_a in stems (N_aS50). On the other hand, GD significantly depended on both N_aS50 and the amount of N_a in the ears at the BBCH 65 stage (N_aE65). The relevant regression models are shown below:

$$\mathrm{GY} = 0.95 + 0.08\mathrm{N_aS50}\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.88,\ p\ \leq 0.01 \tag{26}$$

$$\mathrm{GD} = 0.94 + 0.08\mathrm{N_aS50} + 0.1\mathrm{N_aE65}\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.94,\ p\ \leq 0.01 \tag{27}$$

Both these N_a traits showed a significant response to the years and experimental factors. At the same time, no significant interaction was found between them and the years (Table 5). These two characteristics, averaged over the years, reached their highest values in 2016 and were almost twice the lowest in 2014. The effect of foliar protection was very similar for both traits. Significantly higher N_a in these two wheat organs was recorded for the fungicide-protected plants. Both N_a traits showed nearly the same patterns in response to the progressively increasing N_f doses. A significant increase in N_a was recorded in response to the increased N_f dose up to 160 kg ha^{-1}, after which it stabilized. The N_a pattern for these two wheat organs best fits the quadratic regression model:

$$\mathrm{N_aS50} = -0.001\mathrm{N_f^2} + 0.43\mathrm{N_f} + 70\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.98,\ p\ \leq 0.01 \tag{28}$$

$$\mathrm{N_aE65} = -0.001\mathrm{N_f^2} + 0.4\mathrm{N_f} + 66.7\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.96,\ p\ \leq 0.01 \tag{29}$$

The maximum N_aS50 and N_aE65 of 116.2 and 104.1 kg ha^{-1}, respectively, was achieved for the respective N_f optima of 216.9 and 181.8 kg ha^{-1}.

The set of prognostic traits for GD and GY, considered throughout the winter wheat growing season, was the same. The differences in their predictive power, apart from N_aL90, were noted only for appropriate coefficients of the obtained equations. The obtained models are presented below:

$$\mathrm{GD} = 4.24 + 0.09\mathrm{N_a31} - 0.11\mathrm{N_aL90} + 0.05\mathrm{N_aG90}\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.98\ and\ p\ \leq 0.01 \tag{30}$$

$$\mathrm{GY} = 1.75 + 0.04\mathrm{N_a31} - 0.11\mathrm{N_aL90} + 0.03\mathrm{N_aG90}\ for\ \mathrm{n} = 21,\ \mathrm{R}^2 = 0.98,\ p\ \leq 0.01 \tag{31}$$

What is most important is the fact that the N_aL90 exerted a negative impact on GD, and consequently on GY. This wheat N trait responded to the studied factors, but not to their interaction (Table 6). Its highest value in subsequent years of study was recorded in 2016, the year with the lowest GY. Leaves of wheat treated only with micronutrients accumulated 23% more N at BBCH 90 N compared to those protected with fungicides. The effect of increasing the N_f dose increased N_aL90 up to 160 kg ha^{-1}. The amount of N in wheat at the beginning of the stem elongation phase (N_a31) was dependent on the Y × N interaction (Table 5). Significant differences between the years were noted for the first three N treatments (Figure S3).

The N_aG90, as shown in equations 30 and 31, achieved, among the studied wheat N traits, the highest values of the correlation coefficient with GD and GY (Table A2). This N characteristic of winter wheat responded significantly to the years and experimental factors. Nevertheless, the CFP × N interaction was the most important for this N trait, which, however, showed no interaction with the years. The effect of increasing N_f doses on N_aG90 was, in fact, very similar, regardless of the method of wheat protection (Figure 4). However, the N accumulation gap, i.e., the difference in N_a between the fungicide-treated objects and fungicide control, increased in accordance with the N_f dose. It ranged from 13 and 15 kg N ha^{-1} for the N control plot, and increased up to 37 and 48 kg N ha^{-1} on the plot fertilized with 240 kg N ha^{-1} for the FP and MiFP objects, respectively.

Table 5. Nitrogen accumulation in winter wheat during vegetative stages, kg N ha^{-1}.

Factor	Factor Level	N_aT31	N_aL50	N_aS50	N_aT50	N_aL65	N_aS65	N_aE65	N_aT65
Year (Y)	2014	59.1 ^{c}	56.7 ^{c}	79.4 ^{c}	136.1 ^{c}	23.1 ^{c}	51.4 ^{c}	62.7 ^{c}	137.1 ^{c}
	2015	78.7 ^{b}	75.4 ^{b}	102.9 ^{b}	178.3 ^{b}	34.4 ^{b}	94.8 ^{b}	92.6 ^{b}	221.8 ^{b}
	2016	106.7 ^{a}	92.3 ^{a}	122.2 ^{a}	214.5 ^{a}	58.6 ^{a}	74.4 ^{a}	119.2 ^{a}	252.3 ^{a}
p		***	***	***	***	***	***	***	***
Foliar protection (CFP)	N + Mi	81.5	73.2	96.4 ^{b}	169.6	37.3	68.1 ^{b}	86.9 ^{b}	192.4 ^{b}
	N + P	81.5	74.1	103.2 ^{ab}	177.3	41.2	79.4 ^{a}	94.9 ^{a}	215.4 ^{a}
	N + Mi + P	81.5	77.1	104.9 ^{a}	182.0	37.6	73.1 ^{b}	92.7 ^{ab}	203.4 ^{ab}
p		ns	ns	*	ns	ns	***	*	**
Nitrogen rates (kg N ha^{-1})	0	49.9 ^{c}	43.8 ^{d}	68.8 ^{d}	112.6 ^{d}	18.0 ^{e}	46.2 ^{e}	64.7 ^{c}	128.8 ^{e}
	40	77.6 ^{b}	61.7 ^{c}	89.9 ^{c}	151.6 ^{c}	25.6 ^{de}	57.5 ^{de}	82.4 ^{b}	165.5 ^{d}
	80	88.6 ^{‡,a}	69.7 ^{bc}	94.5 ^{bc}	164.3 ^{bc}	32.4 ^{cd}	66.9 ^{cd}	94.6 ^{ab}	194.0 ^{cd}
	120	88.6 ^{‡,a}	78.5 ^{b}	106.7 ^{ab}	185.1 ^{ab}	40.5 ^{bc}	75.3 ^{bc}	100.0 ^{a}	215.8 ^{bc}
	160	88.6 ^{‡,a}	90.0 ^{†,a}	116.9 ^{†,a}	206.8 ^{†,a}	47.5 ^{ab}	84.2 ^{ab}	96.7 ^{a}	228.4 ^{ab}
	200	88.6 ^{‡,a}	90.0 ^{†,a}	116.9 ^{†,a}	206.8 ^{†,a}	53.8 ^{a}	93.4 ^{a}	102.1 ^{a}	249.3 ^{ab}
	240	88.6 ^{‡,a}	90.0 ^{†,a}	116.9 ^{†,a}	206.8 ^{†,a}	53.2 ^{a}	91.1 ^{a}	100.0 ^{a}	244.3 ^{a}
p		***	***	***	***	***	***	***	***
Source of variation for interactions									
Y × CFP		ns	ns	ns	ns	ns	ns	ns	ns
Y × N		**	ns	ns	ns	**	ns	ns	ns
CFP × N		ns	ns	ns	ns	ns	ns	ns	ns
Y × CFP × N		ns	ns	ns	ns	ns	ns	ns	ns

Similar letters means a lack of significant differences using Tukey's test; ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; ns—non-significant; ‡—constant N rate of 80 kg ha^{-1}; †—constant N rate of 160 kg ha^{-1}. Legend: N_a—nitrogen accumulation; 31, 50, 65—stages of wheat growth according to BBCH scale; T, L, S, E—total, leaves, stem, ear, respectively; ‡ the first N_f dose of 80 kg N ha^{-1} was only applied; † the N_f dose was supplemented to 160 kg N ha^{-1}.

Table 6. Nitrogen accumulation in winter wheat parts at harvest, kg N ha^{-1}.

Factor	Factor Level	N_aL90	N_aS90	N_aCH90	N_aVe90	N_aG90	N_aT90
Year (Y)	2014	14.8 ^{b}	20.3 ^{c}	10.1 ^{c}	45.1 ^{b}	179.5 ^{c}	224.6 ^{b}
	2015	15.1 ^{b}	27.6 ^{b}	24.9 ^{b}	67.5 ^{a}	231.0 ^{b}	298.6 ^{a}
	2016	19.6 ^{a}	32.5 ^{a}	15.5 ^{a}	67.6 ^{a}	164.4 ^{a}	232.0 ^{b}
p		***	***	***	***	***	***
Foliar protection (CFP)	N + Mi	18.8 ^{a}	26.6	17.2	62.6 ^{a}	174.4 ^{c}	237.0 ^{b}
	N + P	15.3 ^{b}	27.9	17.2	60.4 ^{ab}	196.9 ^{b}	257.3 ^{a}
	N + Mi + P	15.3 ^{b}	25.9	16.0	57.2 ^{b}	203.6 ^{a}	260.9 ^{a}
p		***	ns	ns	*	***	***
Nitrogen rates (kg N ha^{-1})	0	8.6 ^{e}	17.7 ^{d}	10.8 ^{c}	37.1 ^{e}	111.1 ^{g}	148.2 ^{g}
	40	12.8 ^{d}	22.2 ^{cd}	14.5 ^{b}	49.5 ^{d}	147.7 ^{f}	197.2 ^{f}
	80	15.3 ^{cd}	25.0 ^{bc}	16.2 ^{b}	56.6 ^{cd}	169.4 ^{e}	226.0 ^{e}
	120	16.7 ^{ab}	26.5 ^{bc}	17.3 ^{ab}	60.5 ^{bc}	199.6 ^{d}	260.1 ^{d}
	160	20.9 ^{a}	29.6 ^{ab}	19.3 ^{a}	69.8 ^{ab}	219.5 ^{c}	289.4 ^{c}
	200	19.7 ^{a}	32.2 ^{a}	19.4 ^{a}	71.4 ^{a}	239.8 ^{b}	311.2 ^{b}
	240	21.3 ^{a}	34.5 ^{a}	20.1 ^{a}	75.9 ^{a}	254.3 ^{a}	330.1 ^{a}
p		***	***	***	***	***	***
Source of variation for interactions							
Y × CFP		ns	ns	ns	ns	**	ns
Y × N		ns	ns	***	ns	***	**
CFP × N		ns	ns	ns	ns	**	ns
Y × CFP × N		ns	ns	ns	ns	ns	ns

Similar letters means a lack of significant differences using Tukey's test; ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; ns—non-significant; constant N rate of 80 kg ha^{-1}; Legend: N—nitrogen accumulation; 90—stage of wheat growth according to BBCH scale; L, S, Ch, Ve—leaves, stem, chaffs, total sum of vegetative parts, respectively.

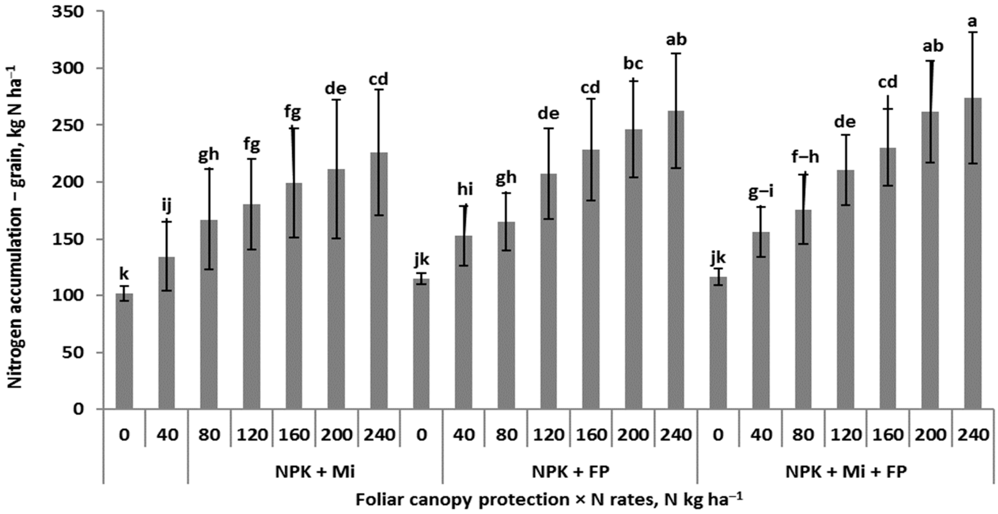

Figure 4. Nitrogen accumulation patterns in winter wheat grain as a result of interaction of canopy foliar protection and applied fertilizer doses. Similar letters means a lack of significant differences using Tukey's test. Legend: N + Mi—nitrogen plus foliar applied micronutrients; N + FP—nitrogen + foliar applied fungicides; N + Mi + FP—nitrogen plus foliar applied micronutrients and fungicides. The vertical bar in the column refers to the standard deviation value.

The total N_a in wheat and its particular organs at the BBCH 65 stage was significantly sensitive to the years and experimental factors, but no interaction between them was found (Table 5). At this stage of wheat growth, regardless of the year, ears accumulated the highest, and the leaves the lowest amount of N. The pattern of the total N_a response to weather conditions in the subsequent years of the study was the same as recorded for the leaves and ears. The N_a, regardless of the plant part, increased in the order of 2014 < 2015 < 2016 (Figure 5). A more stable, and at the same different N_a trend (lower coefficient of variation), was recorded for the stems. This wheat organ, in contrast to leaves and ears, accumulated the largest amount of N in 2015, the year with the highest GD and GY. The effect of CFP was significant for the stems and ears, clearly indicating the dominance of fungicide protection over other wheat canopy protection treatments. It should be emphasized that the lowest N_a was recorded on the FC main plot. The effect of the increasing N_f rates on N_a was organ-specific. The tendency for stems and leaves, despite stabilization on the plot with 200 kg N ha^{-1}, follows the linear regression model ($R^2 \geq 0.9$ and $p \leq 0.01$). In contrast, the trend for ears is best described by the quadratic regression model, reaching a maximum of 103.1 kg ha^{-1} for N_f of 181.8 ha^{-1}.

At wheat maturity, the yearly pattern of TN was driven by the Y × N interaction. It should be underlined that TN was almost entirely dependent on N_a90 (Table A2; Figure 4). Overall, N_a in all vegetative parts of wheat was significantly influenced by the years, and the lowest values were recorded in 2014. The total N_a in the vegetative wheat biomass, despite the year-to-year and/or organ variabilities, was the same in 2015 and 2016. Two facts summarize the main differences between the years. First, the most stable trait of N_a was recorded for the stem, whose share in the total N ranged from 41% in 2015 to 48% in 2016 (Table 6; Figure 5). Secondly, the highest share of ears in the total N_a, as noted in 2015, corresponded to its lowest share in leaves. The effect of wheat canopy protection appeared only for leaves, and N_a was significantly lower for plants treated with fungicides. The total N_a in vegetative wheat parts responded significantly to the progressive increase in N_f doses, but the interaction with years was recorded only in the case of chaffs (Figure S4). The effect of weather in the subsequent growing season on N_a in chaffs was visible in all years. In 2014 and 2016, it was marked by an increase in N_a on plots fertilized with N_f compared to the N_f control. In 2015, it increased consistently with the increased N_f doses up to 160 kg N ha^{-1}, then it stabilized.

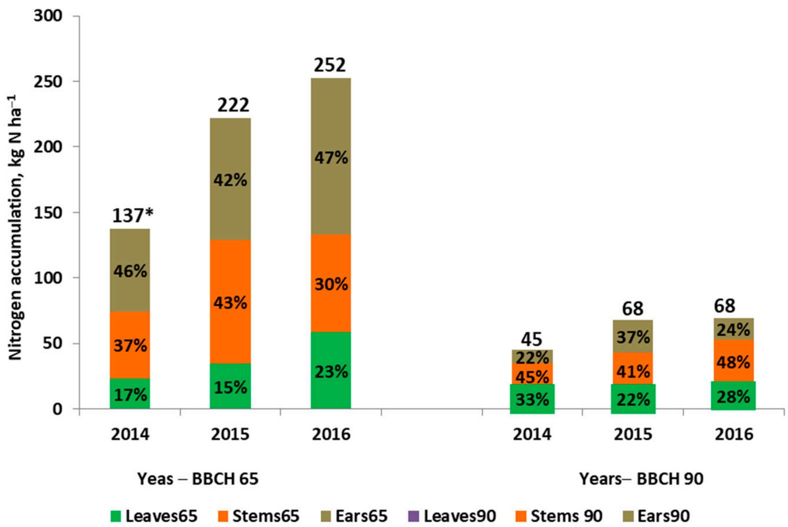

Figure 5. The amount and structure of nitrogen accumulation by winter wheat at full flowering and at maturity. * total amount of N_a in the plant.

3.3. Indicators of Nitrogen Management by Winter Wheat

All 14 traits associated with N remobilization and translocation between wheat organs during the spring part of the growing season showed a marked impact of the years (Table 4). A significant effect of canopy foliar protection of winter wheat was found only for five traits: nitrogen remobilization quota NRQ, the efficiency of NRQ (E-NRQ), and nitrogen remobilization from leaves, stems, and ears (NRL90, NRS90, NRCh90). A significant influence of N_f was noted for six N indicators, including the last three from the above list. In addition to this set, a significant response to N_f doses was noted for N balance between wheat plants at BBCH 31 to BBCH 50 (ΔTN50), NRQ, and nitrogen post-flowering uptake (NPFU).

The N_aRQ index turned out to be the best predictor of both GD and GY (r = 0.94). It responded to the years and experimental factors, but not to interactions between them (Table 7). NRQ in 2015, averaged over experimental factors, was higher by 67.7%, and in 2016 it was twice as high, compared to 2014. Its value on the main FC plot was significantly lower compared to the main plots protected with fungicides. This decrease was 16.4% and 11.3% for FP and MiFP plots, respectively. The N_aRQ response to the increased N_f doses, averaged over other factors, best fits the quadratic regression model:

$$\mathrm{NRQ} = -0.0016\mathrm{N}_f^2 + 0.71\mathrm{N}_f + 91 \; for \; \mathrm{n} = 7, \;\; \mathrm{R}^2 = 0.98, \;\; p \; \leq 0.01 \tag{32}$$

This equation clearly shows that the maximum N_aRQ of 169.8 kg N ha^{-1} was achieved at a N_f optimum of 221.9 kg N ha^{-1}. Two derivatives of N_aRQ indices, i.e., the efficiency of the remobilized N (E-N_aRQ) and its contribution to the remobilized N to the grain N pool (C-N_aRQg) showed a quite different response to the studied factors. The first one, E-N_aRQ, ranged from 59% to 74% and the observed changes were significantly dependent on the CFP × N interaction (Figure S5). The effect of increasing N_f doses on E-N_aRQ was significantly dependent on CFP. Its tendency on the FC plot first decreased to a N_f of 160 kg ha^{-1}, and then increased again. The opposite tendency was observed in the objects with fungicides, where the E-N_aRQ indices increased up to a N_f of 120 kg ha^{-1}, then stabilized over two N_f doses, and finally decreased at the maximum N_f dose. However, this index was poorly correlated with GD and GY (Table A3). The C-N_aRQg index responded significantly only to the years, increasing in the following order: 2014 < 2015 < 2016. What is most important is that its value in 2016 exceeded 100%. In addition, this index was negatively correlated with GD and GY.

Table 7. Indices of nitrogen management by winter wheat.

Factor	Factor	ΔTN50	ΔTN65	NRQ	E-NRQ	C-NRQg	NPFU	E-NPFU	C-NPFUg	NRL	NRS	NRCh	E-NRL	E-NRS	E-NRCh
	Level	**kg N ha^{-1}**			**%**		**kg N ha^{-1}**	**%**		**kg N ha^{-1}**			**%**		
Year (Y)	2014	77.0 ^{b}	1.1 ^{b}	92.0 ^{c}	66.3 ^{b}	51.8 ^{c}	87.5 ^{a}	73.8 ^{a}	48.2 ^{a}	8.3 ^{a}	31.1 ^{a}	52.6 ^{a}	37.8 ^{c}	58.1 ^{b}	83.1 ^{b}
	2015	99.5 ^{a}	43.5 ^{a}	154.2 ^{b}	68.5 ^{b}	69.1 ^{b}	76.8 ^{a}	37.4 ^{b}	30.9 ^{b}	19.4 ^{b}	67.1 ^{c}	68.0 ^{b}	49.1 ^{b}	69.5 ^{a}	72.2 ^{c}
	2016	107.9 ^{a}	37.7 ^{a}	184.7 ^{a}	72.3 ^{a}	115.4 ^{a}	−20.3 ^{b}	−5.8 ^{c}	−15.4 ^{c}	39.0 ^{c}	41.0 ^{b}	103.8 ^{c}	61.9 ^{a}	54.2 ^{c}	86.6 ^{a}
p		***	***	***	***	***	***	***	***	***	***	***	***	***	***
Foliar protection (CFP)	N+Mi	88.1	22.7	129.7 ^{b}	66.3 ^{b}	79.4	44.7	32.4	20.6	18.5 ^{a}	41.5 ^{a}	67.7 ^{a}	39.3	58.8	79.5
	N+P	95.8	38.2	155.0 ^{a}	70.3 ^{a}	82.1	41.9	31.3	17.9	25.9 ^{b}	51.5 ^{b}	77.6 ^{b}	50.6	61.3	80.8
	N+Mi+P	100.5	21.4	146.2 ^{a}	70.4 ^{a}	74.6	57.5	41.6	25.4	22.4 ^{ab}	47.1 ^{ab}	76.7 ^{b}	48.9	61.7	81.7
p		ns	ns	***	**	ns	ns	ns	ns	***	***	***	**	ns	ns
Nitrogen rates (kg N ha^{-1})	0	62.7 ^{c}	16.1	91.7 ^{d}	69.8	83.5	19.4 ^{c}	28.3	16.5	9.4 ^{a}	28.4 ^{a}	53.9 ^{a}	35.4 ^{d}	58.9	82.1
	40	74.0 ^{bc}	13.9	116.0 ^{cd}	68.1	82.9	31.7 ^{bc}	37.8	17.1	12.8 ^{a}	35.3 ^{ab}	67.8 ^{b}	39.1 ^{cd}	58.2	80.6
	80	75.7 ^{bc}	29.7	137.4 ^{bc}	69.7	84.6	32.0 ^{bc}	29.1	15.4	17.1 ^{ab}	41.9 ^{bc}	78.4 ^{bc}	40.5 ^{c}	60.2	82.1
	120	96.6 ^{ab}	30.7	155.3 ^{ab}	70.8	80.9	44.2 ^{bc}	31.5	19.1	23.8 ^{bc}	41.8 ^{cd}	82.7 ^{bc}	50.8 ^{ab}	62.9	82.0
	160	118.2 ^{‡,a}	21.6	158.5 ^{ab}	67.6	74.3	61.0 ^{ab}	38.5	25.7	26.5 $^{b–d}$	54.6 ^{de}	77.4 ^{bc}	51.0 ^{ab}	61.5	78.7
	200	118.2 ^{‡,a}	42.5	178.0 ^{a}	69.9	76.5	61.9 ^{ab}	35.9	23.5	34.0 ^{cd}	61.2 ^{de}	82.7 ^{c}	55.7 ^{a}	63.3	80.1
	240	118.2 ^{‡,a}	37.5	168.4 ^{a}	67.3	68.5	85.8 ^{a}	44.8	31.5	31.9 ^{d}	79.9 ^{e}	79.9 ^{c}	51.3 ^{b}	59.1	79.0
p		***	ns	***	ns	ns	***	ns	ns	***	***	***	***	ns	ns
Source of variation for interactions															
Y × CFP		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × N		ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
CFP × N		ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × CFP × N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Similar letters means a lack of significant differences using Tukey's test; ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; ns—non-significant; ‡—constant N rate of 80 kg ha^{-1}. Legend: 50, 65—stages of wheat growth according to BBCH scale; L, S, E, Ch—leaves, stem, ear, chaffs, respectively; NRQ—N remobilization quota; E-NRQ—N remobilization efficiency; C-NRQg—contribution of remobilized N into grain; NPFU—N post-flowering N uptake; E-NPFU—efficiency of post-flowering N uptake; C-NPFUg—contribution of the post-flowering N uptake into grain N yield; NRL, NRS, NRCh—N partial remobilization; E-NRL, E-NRS, E-NRCh—efficiency of N partial remobilization; ‡ the first N_f dose of 80 kg N ha^{-1} was only applied; the N_f dose was supplemented to 160 kg N ha^{-1}.

The NRQ indices correlated strongly with the amount of N remobilized from the wheat organs (leaves, stems, and ears) during the grain-filling period (Table A3). Moreover, all these organ-specific indices were significantly and positively correlated with GD and GY. The highest correlation coefficient was noted for ΔNRS:

$$GD = 0.21\Delta NRS + 8.73 \; for \; n = 21, \; R^2 = 0.81, \; p \; \leq 0.01 \quad (33)$$

$$GY = 0.11\Delta NRS + 3.78 \; for \; n = 21, \; R^2 = 0.82, \; p \; \leq 0.01 \quad (34)$$

The steepness of the direction coefficient for the obtained equations indicates that GD was more sensitive than GY to the amount of N remobilized from the stems during the grain-filling period (GFP). Despite the highest R^2 for ΔNRS, only ΔNRL showed a significant response to the Y × N interaction (Figure 6). The impact of the year on the N remobilized from leaves at BBCH 90 was affected by the season. The most striking differences between the years are presented for the N control plot and the 200 kg N ha^{-1} plot. The amount of N remobilized from the leaves at BBCH 90 compared to BBCH 65 was, in 2015 and 2016, 2.6-, and 10.6-fold higher than in 2014. This tendency on the 200 kg N ha^{-1} plot was 2.2-, and 5.1-fold higher. The efficiency of N remobilized from leaves, unlike the other two wheat organs at BBCH 90, was significantly and positively correlated with both GD and GY (Table A3).

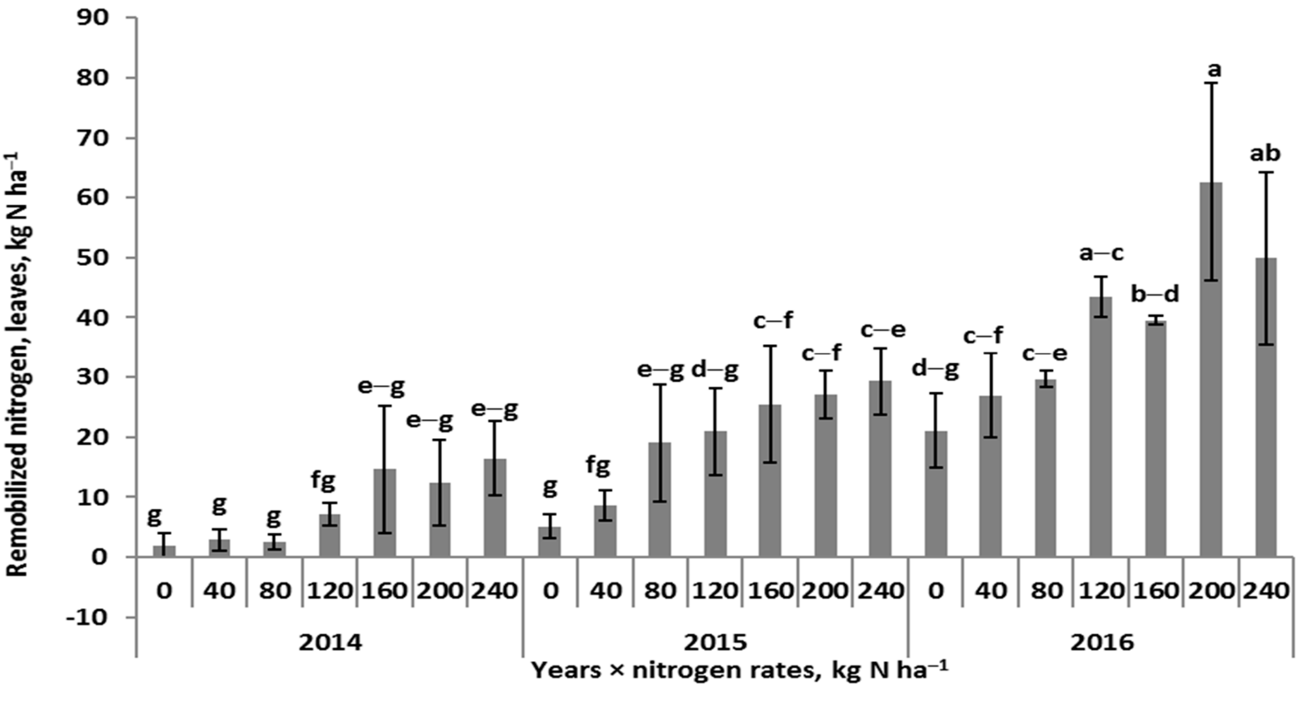

Figure 6. Effect of increasing nitrogen rates on the amount of remobilized N from leaves of winter wheat during the grain-filling period. Similar letters means a lack of significant differences using Tukey's test. The vertical bar in the column refers to the standard deviation value.

The post-flowering N uptake (NPFU) by winter wheat from the soil was significantly affected by the years and N doses (Table 4). There was no interaction between the factors studied. In terms of years, the net soil N uptake was recorded in 2014 and 2015, but not in 2016. The year-average effect of N_f was, in general, consistent with its increasing doses. A realistic assessment of N_f impact on NPFU, based only on 2014 and 2015, showed much higher values, especially in the case of treatments with a low N_f dose (Figure S6). Plants grown in the N control plot took up more—almost 40 kg N ha^{-1}. The value of the index was over three times higher in the 240 kg N ha^{-1} plot, reaching 125 ha^{-1}. The efficiency of post-flowering N uptake was almost 75% and was twice as high as in 2015. Its share in total N in grain was also higher in 2014, approaching 50%. In 2015, it was high, but 17% lower.

4. Discussion

The essence of the study was to identify the main N determinants of the grain yield of winter wheat in an intensive production system. This system is based on progressively increasing N rates and canopy protection against pathogens. For the farming economy, it is important to identify the key characteristics of N management in the wheat canopy during both vegetative and reproductive growth, as well as throughout the growing season. It was assumed that N economy indicators, validated on the basis of grain yield and grain density, allow determining not only the critical phase of winter wheat growth, but also the diagnostic plant part.

4.1. Nitrogen Content in Wheat Organs as Yield Predictor

Reliable predictions of GY and/or its components, such as GD and TGW, are the basis for effective N management in seed-producing crops. This also applies to cereals [31,32]. The N content in indicative plant parts is often used as a classic indicator, and more precisely as a yield predictor during the growing season [33,34]. The conducted study showed that only four out of 11 variables tested for the content of N in wheat parts in the critical stages of its growth were useful in predicting both GD and consequently GY. The thousand-grain weight (TGW) was only significantly correlated with the N content in wheat at BBCH 31. The importance of this stage was due to the fact that it clearly reflected the response of wheat to the first dose of N_f. It was applied just before the beginning of winter wheat vegetation (Figure S2). The importance of this stage for the formation of grain yield by wheat is consistent with some previous studies [9,10]. In agricultural practice, this stage is used to determine the N_f dose at the beginning of the stem elongation phase [35]. The basic yield traits, i.e., GD and GY decreased in the following order: 2015 > 2014 > 2016 [23]. The low GY in 2014 can be explained by the lower N_f, because the N_f dose of 80 kg N ha^{-1} was still insufficient to raise the N content in wheat at BBCH 31 to 4% DW, as reached in 2015. In contrast, the low yield in 2016 was due to a too-high N_f dose applied before the BBCH 31 stage. As emphasized by Mizuta et al. [36], an excessive N_f dose before the stem elongation phase causes strong wheat tillering, resulting in an excessive number of shoots and their strong competition for light. The highest yield in 2015 can be partially explained by the action of applied fungicides, which increased the N content in plants (Figure S1).

In the case of an excessive supply of N, the balance between the growth rate of the stem and the growth rate of the ear is disturbed. It has been documented that prolonged or sustained stem growth reduces the supply of assimilates to the growing ear and thus reduces the number of viable, fertile flowers [37]. Our study clearly showed that the N content in the ears should be treated as a significant variable of the GY prediction. The highest N content in the ears was recorded in 2015 when the GD was picked [31].

The SPAD index is often used to determine the N status of crop plants and consequently to predict the yield [30]. Our study fully confirmed this opinion, but the predictive worth of this index was also influenced by two other variables, i.e., N content in BBCH 31 and in the flag leaf at BBCH 65. The observed stable continuity of the N content from the beginning of the stem elongation phase up to the full flowering of winter wheat indicates the occurrence of a phenomenon that can be called "Crop Plant Nitrogen Memory"(CPNM). This term means that the N status encoded by the crop plant in the early stages of its growth cannot be overcome by the high rates of N_f applied in the later stages. The N status of wheat, established in the early stages of its development, had a significant impact on the formation of the yield components. This phenomenon was revealed both in 2014 and 2016. In both years, despite the contrastive content of N, the number of ears per unit area was reduced.

The physiological N sink of the cereal has two components. The first is GD, which determines the demand of the growing grain for N. The second is the capacity of a single grain to accumulate N, which is expressed as the content of N [38]. However, the key issue of wheat production is not the seasonal variability of the N content, but its stabilization against the background of increasing N_f doses, and thus an increase in the wheat yield. The N content in grain is naturally inconsistent with the TGW. An increase in TGW results in a decrease in N content and vice versa [39]. As shown in Table 4 and Figure S7, grain saturation with N was revealed only for the plants grown on the control N and in the plots fertilized with the low N_f dose. This figure clearly documents that wheat well fed with N did not limit the grain yield. This simply means that the N source during GFP was not a limiting factor of grain yield.

4.2. Nitrogen Management by Wheat during the Growing Season

Nitrogen accumulation by cereals is considered a good predictor of grain yield, or its key yield elements, such as GD in seed plants [11]. The obtained results clearly showed that during the pre-flowering period of winter wheat growth, all the studied traits of the N economy were significantly correlated with both GD and GY (Table A2). The performed stepwise analysis showed that an early GY prediction can be based on the N accumulated in the stem at BBCH 50 (Equation (26)). However, assuming a strong dependence of GY on GD, the N amount in the ears at BBCH 65 should also be included in the regression model (Equation (27)). The unbalanced stem growth due to an over-supply of N during the booting phase of wheat growth can suppress ear development [37]. This phenomenon was fully revealed in 2016 and, as a consequence, it was manifested in a reduction in the number of grains per ear [23]. In 2016, the amount of N in the wheat stem at BBCH 50 was 19% higher compared to 2015, but GE was 28% lower. As a result, the grain yield in 2015 was 11.04 t ha^{-1}, and in 2016 only 6.67 t ha^{-1} [23]. The imbalance in the N economy during the vegetative period of wheat growth was also confirmed by the quadratic regression models obtained for N_aS50 and N_aE65. The N_f maxima of both models were 216.9 and 181.8 kg ha^{-1}, clearly indicating its surplus, which was more striking for ears.

The grain-yield prognosis (GD and GY), taking into account the entire spring growing season of winter wheat, was based on the actual state of three N traits: N_a31, N_aL90, and N_aG90 (equations (30) and (31)). First, these two models, as well as those shown in equations 21 and 25, support the importance of N plant status at BBCH 31 as the key wheat N trait for determining GY and its main component, i.e., GD. This relationship clearly confirms the opinion that the critical phase of yield formation by winter wheat commences at the beginning of the stem elongation phase [10]. Moreover, the significant impact of N_a31 on GD and GY directly corroborates the concept of Crop Plant Nitrogen Memory, as presented in the previous section. This concept should be extended to the formation of yield components. It is well documented that too low a N_f dose results in a reduction in the number of ears, which could be compensated in the later stages of wheat growth by a greater number of grains per ear [40]. This study clearly showed that the insufficient N_f dose at the beginning of winter wheat vegetation resulted in a reduction in both the number of ears and the number of grains per ear. These two characteristics are primary components of the GD.

4.3. Indicators of N Management in Winter Wheat during the Spring Vegetation

The in-depth explanation of the impact of the N economy in winter wheat on grain yield was based on the indicators of its balance in subsequent phases of growth during the spring growing season. The study clearly highlights the crucial importance of two periods. The first concerns the vegetative period of wheat development. This was associated with an increase in the total N accumulation in the wheat canopy in the early phase of the stem growth, i.e., ΔTN50 (BBCH31 to BBCH 50). The second and finalizing phase of yield formation by winter wheat concerns the reproductive stages of wheat development. This issue actually concerns the sink/source relationship, represented on the one hand by the demand of the growing grains for N, and its sources on the other hand [41]. The main source of N for grain is the vegetative wheat parts, from which it is first remobilized and then translocated to the growing grains. The second source of N is its direct uptake from the soil during the post-flowering period of wheat growth [42]. The variability in N_aRQ explained 88% of GD and GY variability. The predictive worth of N_aPFU was significantly lower, reaching only 56% (Table A3). Therefore, the vegetative wheat parts were the main source of N to the growing grains. The key reason for the observed phenomenon was too-low GD [31]. The obtained results confirm the opinion that in conditions of high N accumulation in wheat before flowering, its uptake from the soil is not important [43]. The excess of N resources in vegetative wheat parts was also confirmed by the significantly higher N content in the grain as observed in 2016 (Table 1). In 2014 and 2015, the main source of N in grain was also derived from the vegetative wheat parts. At the same time, there was a significant contribution of N uptake from the soil in total grain N. It reached 48% in 2014 and 31% in 2015. In both years, the N reserves of N accumulated by wheat during the pre-flowering growth were too low to meet the requirements of the growing grains. Thus, under the greater GD, a plant is forced to take up N from the soil N [44]. The greatest increase in GD was recorded in the object where fungicides and micronutrients were applied simultaneously.

The significant impact of NRQ on both GD and GY was inherent in the N remobilization from particular parts of wheat during the post-flowering period of its growth (Table A3). All these plant parts contributed significantly to the accumulation of N in wheat grain, as indicated by the organ-specific efficiency. This index, averaged for all examined factors, was the highest for the ears, and then for the stems (Table 7, Figure 5). The highest relationships between N remobilized from particular wheat organs, as indicated by the R^2 coefficient, were noted for the stems. The value of this dependence confirms the importance of this plant part for the formation of yield components, GD, and as a consequence GY [45]. However, the best indicators of wheat N economy response to variable weather conditions were leaves. This is consistent with some literature reports [46]. However, our study clarified some dependencies. The obtained negative relationship between the amount of N in wheat leaves at BBCH 90 and GD, and GY clearly highlights three facts:

(1) Leaves are an important, but not dominant, source of N for the growing grains;
(2) Too-low GD, as the physiological sink of winter wheat, had no potential to exploit N resources in leaves during the grain-filling period. This was the case observed in 2016, despite the high remobilized efficiency.
(3) A strong decrease in the content and amount of N was revealed in leaves, but provided for the high demand of the growing grains. This was the case observed in 2014 and 2015.

5. Conclusions

Three sets of indicators were used to explain the N economy of high-yielding wheat. The wheat canopy was protected against pathogens by foliar application of micronutrients, fungicides, or both. All the examined indicators were related to grain density and grain yield. The six conclusions of this study need to be emphasized. The key one is the N content in the wheat at the beginning of the stem elongation phase. The primary N status of wheat plants, which can be called the Crop Plant Nitrogen Memory, significantly affected the formation of the yield component. The SPAD index determined on the flag leaf can be used as an in-season tool to make a reliable grain-yield prognosis. Its prognostic credibility increases when the N content in wheat at BBCH 31 is also included in the model. The prognostic usability of the nitrogen remobilization quota can be considered a diagnostic tool for grain yield. Its use, however, requires a good diagnosis of the N flow from the particular vegetative wheat organs during the grain-filling period of the grain. Their individual contribution to the grain N pool depends not only on their size at the beginning of wheat flowering, but also on the degree of depletion during GFP. The stem requires special attention during this period. This part of wheat proved to be the main predictor of GD, and consequently GY, during both the vegetative wheat growth and the GFP. The stem N is the decisive factor for yield, provided that it has the same proportion in the total N with the ears at the full flowering stage. The sensitivity of winter wheat to weather during the growing season is best reflected by N flow in the leaves during GFP. Despite the lowest N pool at flowering, its remobilization significantly depends on GD. The higher the GD, the deeper N depletion from leaves is to be expected.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13010122/s1: Figure S1. Effect of the foliar method of winter wheat canopy protection on the content of nitrogen at the beginning of the stem elongation phase in consecutive years of study. Figure S2. Effect of the foliar method of winter wheat canopy protection on the content of nitrogen at the beginning of the stem elongation phase in consecutive years of study. Figure S3. Effect of the foliar method of winter wheat canopy protection on the content of nitrogen at the beginning of the stem elongation phase in consecutive years of study. Figure S4. Effect of increasing nitrogen rates on the amount of accumulated nitrogen in chaffs of winter wheat at maturity in subsequent years of study. Figure S5. Effect of interaction of foliar protection of winter wheat canopy and nitrogen rates on the efficiency of the remobilized nitrogen during the grain filling period. Figure S6. The effect of nitrogen rates on the post-flowering N uptake from the soil; averaged for 2014 and 2015 growing seasons. Figure S7. The relationship between the grain yield of winter wheat and the content of nitrogen in grain.

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Appendix A

Table A1. Correlation matrix of basic yield components and the content of nitrogen in winter wheat parts, n = 21.

Traits	GD	TGW	N31	N50L	N50S	N65L	N65S	N65E	N65FL	N90L	N90S	N90Ch	N90G	SPAD
GY	0.99 ***	0.59 **	0.84 ***	0.90 ***	0.91 ***	0.84 ***	0.80 ***	0.87 ***	0.84 ***	0.46 *	0.74 ***	0.60 **	0.81 ***	0.93 ***
GD	1.00	0.47 *	0.84 ***	0.93 ***	0.92 ***	0.86 ***	0.80 ***	0.89 ***	0.85 ***	0.55 ***	0.76 ***	0.68 ***	0.84 ***	0.94 ***
TGW		1.00	0.44 *	0.29	0.39 *	0.26	0.41 *	0.32	0.34	−0.31	0.23	−0.13	0.25	0.42
N31			1.00	0.73 ***	0.67 **	0.58 **	0.53 *	0.61 **	0.54 *	0.35	0.50 *	0.45 *	0.55 *	0.72 ***
N50L				1.00	0.94 ***	0.90 ***	0.81 ***	0.83 ***	0.91 ***	0.71 ***	0.87 ***	0.82 ***	0.89 ***	0.92 ***
N50S					1.00	0.89 ***	0.79 ***	0.85 ***	0.87 ***	0.59 ***	0.79 ***	0.72 ***	0.85 ***	0.90 ***
N65L						1.00	0.92 ***	0.86 ***	0.94 ***	0.75 ***	0.91 ***	0.86 ***	0.96 ***	0.93 ***
N65S							1.00	0.81 ***	0.89 ***	0.60 ***	0.91 ***	0.72 ***	0.93 ***	0.91 ***
N65E								1.00	0.86 ***	0.61 ***	0.74 ***	0.70 ***	0.85 ***	0.90 ***
N65FL									1.00	0.73 ***	0.91 ***	0.81 ***	0.97 ***	0.94 ***
N90L										1.00	0.76 ***	0.89 ***	0.79 ***	0.68 ***
N90S											1.00	0.83 ***	0.95 ***	0.87 ***
N90Ch												1.00	0.85 ***	0.78 ***
N90G													1.00	0.95 ***

***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. Legend: GY—grain yield; GD—grain density; TGW—thousand-grain weight; N—nitrogen content, % DW; 31, 60, 65, 90—growth stages of winter wheat; L, S, E, FL, Ch, G—leaves, stem, ear, chaffs, grain, wheat parts (organs).

Table A2. Correlation matrix of basic yield components and the amount of nitrogen accumulated in winter wheat parts, n = 21.

Traits	TN31	N_aL50	N_aS50	TN50	N_aL65	N_aS65	N_aE65	TN65	N_aL90	N_aS90	N_aCh90	N_aVe90	N_aG90	TN90
GD	0.88 ***	0.93 **	0.95 ***	0.95 ***	0.92 ***	0.92 ***	0.94 ***	0.96 ***	0.71 ***	0.83 ***	0.85 ***	0.82 ***	0.96 ***	0.96 ***
GY	0.83 ***	0.91 ***	0.94 ***	0.94 ***	0.89 ***	0.91 ***	0.92 ***	0.94 ***	0.64 ***	0.80 ***	0.79 ***	0.77 ***	0.96 ***	0.95 ***
TN31	1.00	0.84 ***	0.81 ***	0.84 ***	0.76 ***	0.75 ***	0.87 ***	0.82 ***	0.77 ***	0.76 ***	0.85 ***	0.81 ***	0.77 ***	0.80 ***
N_aL50		1.00	0.95 ***	0.99 ***	0.92 ***	0.90 ***	0.85 ***	0.92 ***	0.82 ***	0.88 ***	0.88 ***	0.89 ***	0.93 ***	0.95 ***
N_aS50			1.00	0.99 ***	0.92 ***	0.92 ***	0.88 ***	0.94 ***	0.72 ***	0.83 ***	0.82 ***	0.82 ***	0.93 ***	0.93 ***
TN50				1.00	0.94 ***	0.92 ***	0.88 ***	0.94 ***	0.78 ***	0.87 ***	0.86 ***	0.87 ***	0.94 ***	0.95 ***
N_aL65					1.00	0.97 ***	0.85 ***	0.97 ***	0.80 ***	0.91 ***	0.89 ***	0.90 ***	0.96 ***	0.97 ***
N_aS65						1.00	0.86 ***	0.98 ***	0.72 ***	0.91 ***	0.86 ***	0.86 ***	0.95 ***	0.95 ***
N_aE65							1.00	0.93 ***	0.61 **	0.73 ***	0.78 ***	0.73 ***	0.86 ***	0.85 ***
TN65								1.00	0.74 ***	0.89 ***	0.87 ***	0.86 ***	0.96 ***	0.96 ***
N_aL90									1.00	0.86 ***	0.90 ***	0.96 ***	0.73 ***	0.80 ***
N_aS90										1.00	0.92 ***	0.97 ***	0.88 ***	0.92 ***
N_aCH90											1.00	0.97 ***	0.84 ***	0.89 ***
N_aVe90												1.00	0.85 ***	0.95 ***
N_aG90													1.00	0.99 ***

***, ** indicate significant differences at $p < 0.001$, $p < 0.01$, respectively. Legend: GY—grain yield; GD—grain density; N_a—amount of accumulated N; 31, 60, 65, 90—growth stages of winter wheat; L, S, E, FL, Ch, G—leaves, stem, ear, chaffs, grain, wheat parts (organs).

Table A3. Correlation matrix of basic yield components and the indices of nitrogen economy in winter wheat, n = 21.

Traits	ΔTN50	ΔTN65	NRQ	E-NRQ	C-NRQg	NPFU	E-NPFU	C-NPFUg	NRL	NRS	NRCh	E-NRL	E-NRS	E-NRCh
GD	0.86 ***	0.53 *	0.94 ***	0.14	−0.45 *	0.75 ***	0.33	0.45 *	0.89 ***	0.90 ***	0.89 ***	0.76 ***	0.41	−0.15
GY	0.87 ***	0.52 *	0.94 ***	0.21	−0.46 *	0.76 ***	0.34	0.46 *	0.89 ***	0.90 ***	0.88 ***	0.80 ***	0.45 *	−0.08
ΔTN50	1.00	0.31	0.86 ***	0.13	−0.48 *	0.76 ***	0.23	0.48 *	0.89 ***	0.87 ***	0.70 ***	0.78 ***	0.41	−0.19
ΔTN65		1.00	0.69 ***	0.31	0.06	0.19	−0.13	−0.06	0.67 **	−0.69 **	0.62 **	0.55 *	0.40	0.07
NRQ			1.00	0.36	−0.23	0.59 **	0.07	0.23	0.97 ***	0.97 ***	0.92 ***	0.88 ***	0.60 **	0.01
E-NRQ				1.00	0.50 *	−0.29	−0.47 *	−0.50 *	0.29	0.28	0.45 *	0.62 **	0.86 ***	0.85 **
C-NRQg					1.00	−0.89 **	−0.83 ***	−1.00 **	−0.31	−0.28	0.07	−0.10	0.32	0.56 **
NPFU						1.00	0.72 ***	0.89 ***	0.65 **	0.61 **	0.43	0.42	−0.02	−0.46 *
E-NPFU							1.00	0.83 ***	0.12	0.11	0.01	−0.06	−0.35	−0.48 *
C-NPFUg								1.00	0.31	0.28	0.07	0.10	−0.32	−0.56 **
NRL									1.00	0.97 ***	0.80 ***	0.88 ***	0.53 ***	−0.09
NRS										1.00	0.82 ***	0.86 ***	0.56 **	−0.11
NRCh											1.00	0.77 ***	0.61 **	0.24
E-NRL												1.00	0.75 ***	0.22
E-NRS													1.00	0.63 **

***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; ns—non-significant; Legend: GY—grain yield; GD—grain density; 50, 65—stages of wheat growth according to BBCH scale; L, S, E, Ch—leaves, stems, ear, chaffs, respectively; NRQ—N remobilization quota; E-NRQ—N remobilization efficiency; C-NRQg—contribution of remobilized N into grain; NPFU—N post-flowering uptake; E-NPFU—efficiency of post-flowering N uptake; C-NPFUg—contribution of the post-flowering N uptake in the grain N yield; NRL, NRS, NRCh—N partial remobilization; E-NRL, E-NRS, E-NRCh—efficiency of N partial remobilization.

Appendix B

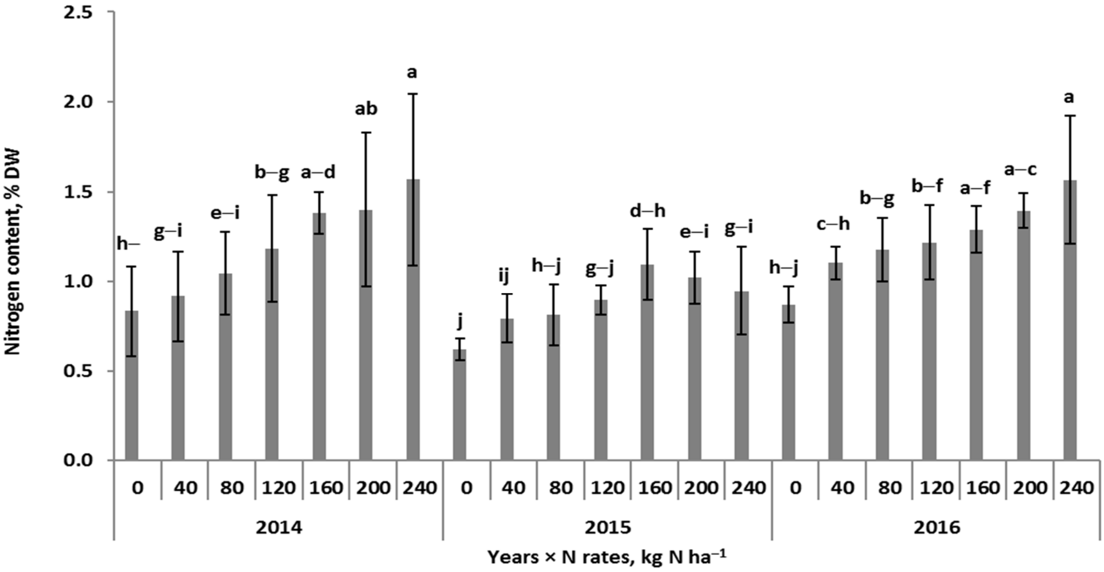

Figure A1. Effect of nitrogen rates on the content of nitrogen in winter wheat leaves at maturity. Similar letters means a lack of significant differences using Tukey's test. The vertical bar in the column refers to the standard deviation value.

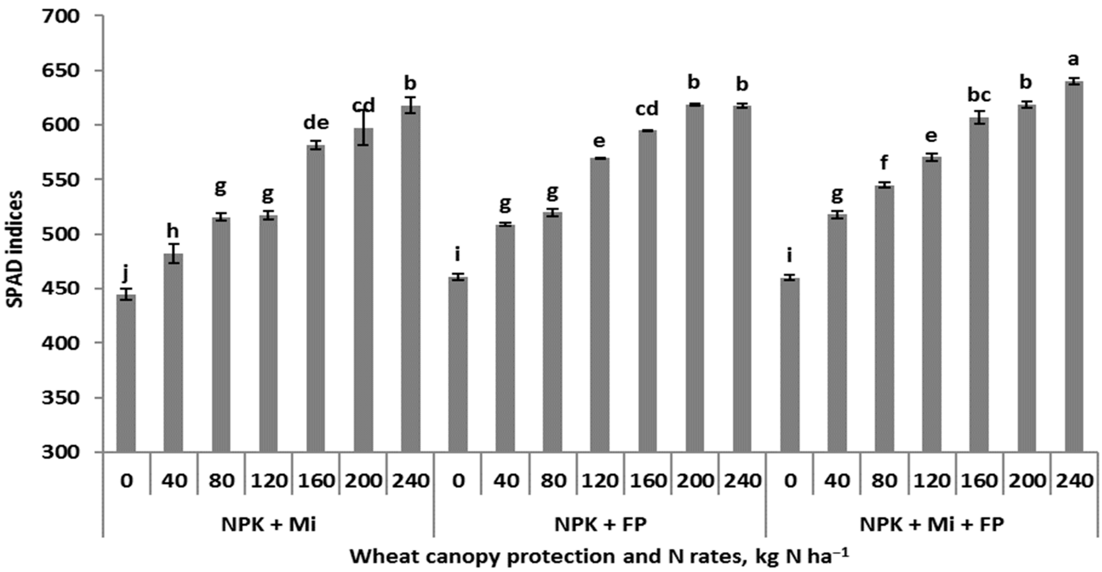

Figure A2. Response of SPAD indices to interaction of winter wheat canopy foliar protection and nitrogen rates. Similar letters means a lack of significant differences using Tukey's test. The vertical bar in the column refers to the standard deviation value. Legend: N + Mi—nitrogen plus foliar applied micronutrients; N + FP—nitrogen + foliar applied fungicides; N + Mi + FP—nitrogen plus foliar applied micronutrients and fungicides. The vertical bar in the column refers to the standard deviation value.

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