

Article

Compensating Yield Losses Caused by Long-Term Winter Wheat Monoculture Through Crop Protection and Variety Selection

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Abstract

Long-term winter wheat monoculture leads to the deterioration of plant physiological performance and reduced productivity; however, the effectiveness of measures aimed at mitigating these adverse effects has not yet been comprehensively evaluated. The objective of this study was to assess the effects of crop sequence, crop protection intensity, and variety selection on the plant physiological parameters, grain yield, technological grain quality, and mineral composition of winter wheat grain, as well as to determine the extent to which yield losses caused by long-term monoculture can be compensated. The study was conducted during three growing seasons (2022/2023–2024/2025) in a long-term field experiment established in 1967 in Bałcyny, Poland, using a three-factor design with three replications, including two crop sequence systems, three crop protection levels, and two winter wheat varieties. Winter wheat grown in monoculture showed significantly lower leaf greenness index values and grain yield (by 41.4%; $p < 0.05$) compared with crop rotation. In contrast, monoculture significantly increased grain protein content (by 5.8%), gluten content (by 6.6%), Zeleny sedimentation index (by 20.8%), and the concentrations of nitrogen and zinc in grain ($p < 0.05$). Crop protection significantly enhanced grain yield; however, it did not completely eliminate the negative effects of long-term monoculture. The variety Revolver produced significantly higher grain yield and leaf greenness index values than Emil, whereas Emil produced grain with significantly higher protein, gluten, and nitrogen contents. The Monoculture Compensation Efficiency (MCE) index showed that crop protection practices enabled the recovery of 63.8–76.2% of yield losses caused by long-term monoculture. The results indicate that integrated crop protection can only partially mitigate the adverse effects of continuous monoculture and cannot replace the benefits associated with the implementation of a proper crop rotation system.

Keywords: crop rotation; integrated crop protection; grain yield; monoculture compensation



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1. Introduction

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops cultivated worldwide and serves as a major raw material for the milling, baking, and feed industries [1]. Maintaining high and stable grain yields while preserving grain quality remains one of the key challenges of modern agriculture [2]. However, efforts to maximize production often lead to simplified crop rotations and an increased proportion of cereals in crop sequences [3].

In agricultural practice, long-term cultivation of cereals after cereals, including continuous monoculture, is still frequently observed [4,5]. Although such systems may offer organizational and economic advantages, they can also induce a range of unfavorable

biological and agronomic changes [6,7]. As a consequence, reductions in crop productivity, nutrient use efficiency, and physiological activity are commonly reported. The negative effects of simplified crop sequences are most often manifested by lower grain yields, deterioration of technological grain quality, and reduced yield stability [8]. These adverse effects may become particularly severe under unfavorable weather conditions, when plants are additionally exposed to environmental stresses such as drought [9].

Crop rotation is one of the fundamental components of sustainable agriculture and integrated crop production systems [10]. The inclusion of non-cereal crops in crop rotations improves soil structure, suppresses weeds and diseases, and enhances soil fertility [11]. Nevertheless, proper crop rotation is not always practiced, and many farmers adopt simplified cropping sequences. Under such conditions, agronomic measures capable of mitigating the negative effects of long-term wheat monoculture become increasingly important [3].

In simplified crop sequences, both crop protection practices [12] and variety selection [13] may play important roles. More intensive chemical crop protection can reduce weed and pathogen pressure, which are among the major factors limiting yield under long-term monoculture conditions [14–16]. By reducing biotic stress, crop protection can also improve plant physiological performance through the maintenance of leaf assimilatory activity, prolongation of the photosynthetically active period, and enhanced nutrient use efficiency [17]. Similarly, wheat varieties characterized by greater disease resistance, tolerance to environmental stress, and stable yield performance may be less affected by unfavorable crop sequence conditions [18].

Although the adverse effects of long-term cereal monoculture have been widely documented, previous studies have generally focused on individual agronomic practices rather than their combined effects. Most investigations have evaluated crop rotation, cultivar performance, or crop protection separately, with primary emphasis on grain yield and disease incidence [3,5,15]. Consequently, considerably less attention has been paid to the integrated responses of physiological traits, grain technological quality, and mineral composition under long-term monoculture. Furthermore, the interactions among crop sequence, crop protection intensity, and cultivar remain insufficiently understood despite their practical importance for sustainable cereal production. This lack of integrated studies limits our understanding of the mechanisms responsible for maintaining crop productivity under simplified cropping systems.

In addition to grain yield, technological quality traits are of considerable importance due to their direct relevance for the milling and baking industries. Long-term monoculture may alter grain protein content, gluten content, and sedimentation characteristics, thereby affecting grain technological value [19].

Plant responses to monoculture conditions can also be assessed through physiological parameters associated with photosynthetic activity. One of the most widely used indicators is the leaf greenness index (SPAD), which indirectly reflects chlorophyll content and plant nitrogen status [20]. Environmental conditions may additionally influence the uptake and accumulation of mineral nutrients in grain. Particular attention has been paid to nitrogen and zinc, which are involved in protein synthesis and the regulation of enzymatic and antioxidant processes, respectively, thereby affecting both grain yield and quality [21]. Nitrogen and zinc were selected because they are key determinants of grain technological quality and are closely associated with protein synthesis, nitrogen metabolism, and enzymatic activity. Therefore, they were considered representative indicators of the nutritional response of winter wheat to long-term monoculture.

Several agronomic indicators have been proposed to evaluate crop performance under different production constraints, including the Stress Susceptibility Index (SSI) developed by Fischer and Maurer [22], the Stress Tolerance Index (STI) proposed by Fernandez [23],

and more recent integrative approaches that combine and compare multiple stress-related indices for crop evaluation [24]. These indices have been widely applied to assess cultivar performance under drought, nutrient deficiency, and other abiotic stresses. However, none of them directly quantifies the proportion of yield losses caused specifically by long-term monoculture that can be recovered through agronomic management practices. Consequently, they provide limited support for evaluating the effectiveness of crop protection strategies under continuous cereal cultivation.

Despite numerous studies investigating the effects of winter wheat monoculture, there is still a lack of a simple and universal indicator that enables the quantitative assessment of agronomic practices aimed at compensating yield losses caused by long-term continuous cropping. The development of such a tool could provide practical support for farmers by enabling an objective evaluation of the effectiveness of production technologies under simplified crop rotation systems.

Crop sequence, crop protection, and cultivar should not be considered independent management components because they affect different mechanisms regulating crop performance. Crop sequence determines soil fertility, biological activity, and the intensity of weed and pathogen pressure [10,11]. Crop protection modifies these conditions by reducing biotic stress, whereas cultivar characteristics determine the capacity of plants to exploit available resources and tolerate unfavorable environmental conditions [13,18]. Therefore, evaluating the interactions among these factors provides a more realistic assessment of production systems than analyzing each factor separately and contributes to the development of more sustainable crop management strategies.

Therefore, the objective of this study was to evaluate the effects of crop sequence, crop protection intensity, and variety selection on key physiological parameters, grain yield, technological grain quality, and mineral composition of winter wheat grain. In addition, a new indicator, the Monoculture Compensation Efficiency (MCE) index, was proposed and applied to quantify the effectiveness of agronomic practices in compensating yield losses caused by long-term winter wheat monoculture.

2. Materials and Methods

2.1. Experiment Site

Field experiments were carried out in a long-term static field experiment located at the Experimental Production Farm in Bałcyny, northern Poland (53°36' N, 19°51' E), established in 1967 and maintained continuously thereafter (Figure 1). The soil was developed from glacial till and post-glacial sandy-gravel deposits. Before the start of the study (2022), soil pH ranged from 4.7 to 5.3, while the contents of organic carbon, total nitrogen, available phosphorus, available potassium, and available magnesium ranged from 8.8 to 11.6 g kg⁻¹, 0.66 to 1.10 g kg⁻¹, 61.0 to 94.6 mg kg⁻¹, 146.3 to 292.6 mg kg⁻¹, and 32.0 to 56.0 mg kg⁻¹, respectively.

Meteorological conditions differed among the growing seasons in terms of air temperature and precipitation distribution. Deviations of monthly mean air temperature and monthly precipitation totals from the long-term averages are presented in Figures 2 and 3.

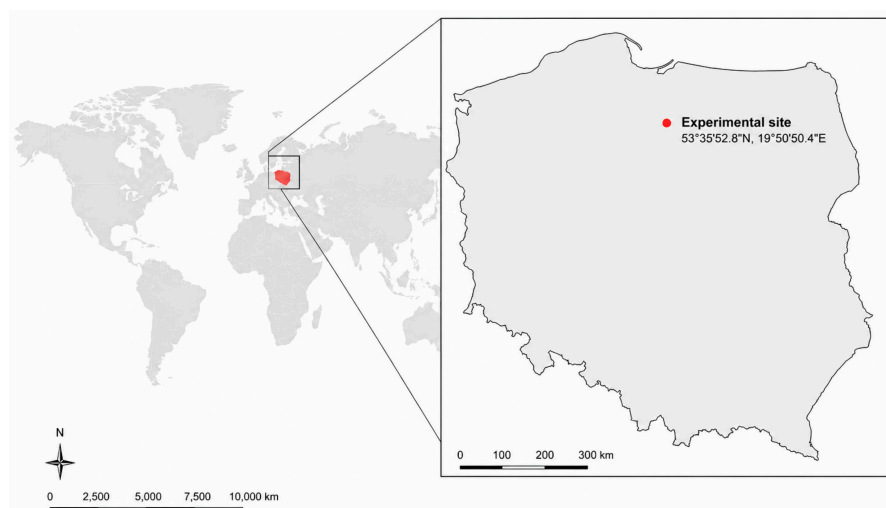


Figure 1. Location of the experimental site in Poland (53°35'52.8'' N, 19°50'50.4'' E).

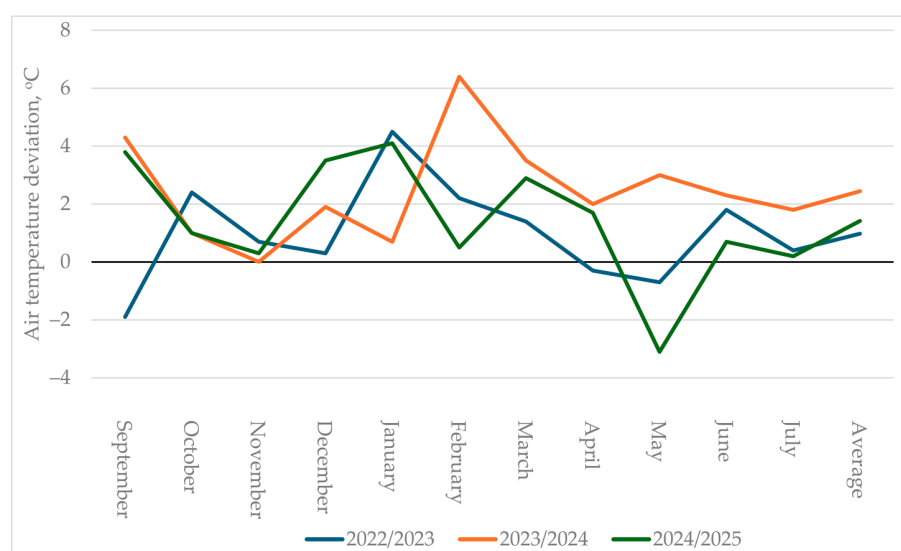


Figure 2. Deviations of monthly mean air temperature from the long-term average (1951–2015) during the winter wheat growing seasons of 2022/2023, 2023/2024, and 2024/2025.

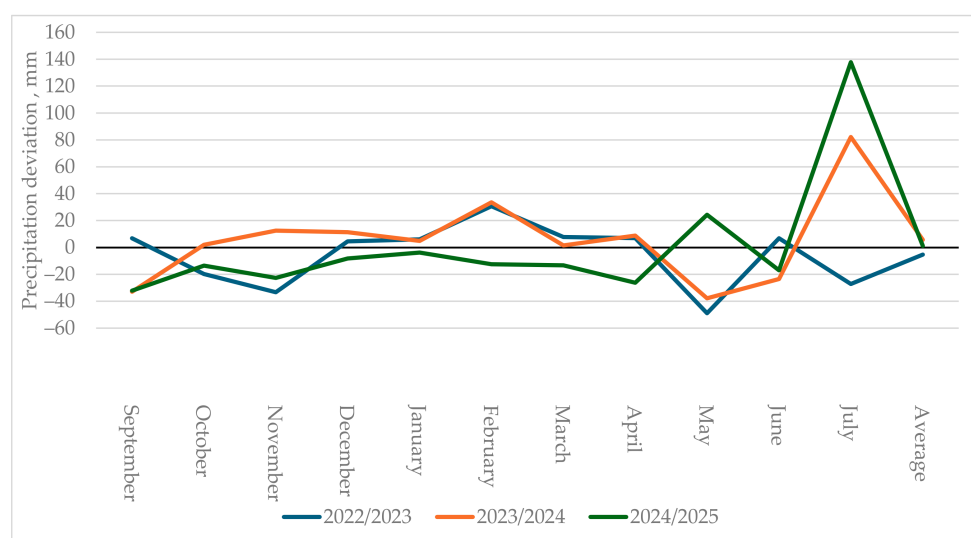


Figure 3. Deviations of monthly precipitation totals from the long-term average (1951–2015) during the winter wheat growing seasons of 2022/2023, 2023/2024, and 2024/2025.

2.2. Experimental Design

The results presented in this study were obtained from winter wheat (*Triticum aestivum* L.) grown during the 2022–2025 growing seasons, corresponding to the harvest years 2023, 2024, and 2025. The experiment was conducted in a long-term static field trial established in 1967 and maintained in a split-block arrangement with three replications (Figure S1). The total experimental area was 10,022 m². The sowing plot area was 27.0 m², while the harvest area was 21.0 m².

The experiment was arranged as a three-factor design including two crop sequence systems, three crop protection levels, and two winter wheat varieties. The experimental factors were as follows:

- I. Crop sequence system (Cs):
 - A) six-course crop rotation: sugar beet (*Beta vulgaris* L.)—maize (*Zea mays* L.)—spring barley (*Hordeum vulgare* L.)—spring pea (*Pisum sativum* L.)—winter oilseed rape (*Brassica napus* L.)—winter wheat (*Triticum aestivum* L.);
 - B) continuous winter wheat monoculture maintained since 1967.
- II. Crop protection level (Cp):
 - A) without protection (untreated control);
 - B) herbicide treatment;
 - C) herbicide + fungicide treatment.
- III. Variety (V):
 - A) Emil;
 - B) Revolver.

Detailed information on all herbicide and fungicide applications, including commercial products, active ingredients, application rates, is presented in Supplementary Tables S1 and S2, whereas cultivar characteristics are presented in Table S3.

The experiment consisted of a factorial arrangement with 12 treatment combinations (2 crop sequence systems × 3 crop protection levels × 2 winter wheat varieties), each replicated three times, giving a total of 36 experimental plots in each growing season.

The selected crop protection treatments represent three levels of management intensity commonly encountered in commercial winter wheat production. The untreated control was included to assess the full impact of long-term monoculture without chemical intervention. The herbicide treatment represents the minimum chemical protection commonly applied to control weeds, whereas the combined herbicide and fungicide treatment reflects a more intensive crop protection strategy intended to reduce both weed competition and disease pressure. This experimental arrangement enabled the assessment of the extent to which increasing crop protection intensity can compensate for the negative effects of continuous monoculture. The cultivars Emil and Revolver were selected because they are registered winter wheat cultivars adapted to Polish environmental conditions and differ in agronomic performance and yield potential. Their inclusion enabled the evaluation of whether cultivar characteristics influence the capacity of winter wheat to tolerate long-term monoculture and to respond to increasing crop protection intensity.

2.3. Agronomic Practices

All remaining agronomic practices were carried out according to standard recommendations for winter wheat cultivation in north-eastern Poland (Table 1). Pest control measures were applied uniformly across all experimental plots when economic thresholds for pest occurrence were exceeded.

Table 1. Agronomic management practices applied in the long-term winter wheat field experiment.

Agronomic Practice	Description
Tillage	Conventional tillage system: pre-sowing plowing followed by seedbed preparation immediately before sowing using a cultivator and tooth harrow.
Nitrogen (N) fertilization	The total nitrogen rate was 120 kg N ha ^{−1} , applied in three splits: (1) 30 kg N ha ^{−1} before sowing as urea (46% N); (2) 60 kg N ha ^{−1} at BBCH * 26–27 as ammonium nitrate (34% N); and (3) 30 kg N ha ^{−1} at BBCH 30–39, also as ammonium nitrate (34% N).
Phosphorus (P) fertilization	Phosphorus was applied before sowing at 34.9 kg P ha ^{−1} as granular superphosphate (40% P).
Potassium (K) fertilization	Potassium was applied before sowing at 83 kg K ha ^{−1} as potassium chloride (60% K).
Sowing	Plot seed drill SPZ 1.5 (P.W. PROMAR Sp. z o.o., Poznań, Poland).
Sowing dates	13 September 2022, 20 September 2023, and 13 September 2024.
Harvest dates	4 August 2023, 10 July 2024, and 4 August 2025.

* BBCH—Biologische Bundesanstalt, Bundessortenamt and CHemical industry scale.

Insect pest control was applied uniformly across all experimental treatments whenever economic threshold levels were exceeded (Table S4). Although fungal diseases were observed during the experiment, particularly in untreated plots, disease severity was not quantitatively assessed because this was beyond the scope of the study. Consequently, the effects of fungicide application should be interpreted as the overall reduction in biotic stress rather than the control of individual pathogens.

2.4. Sampling and Analysis

2.4.1. Leaf Greenness Index (SPAD) and Photosynthetic Rate

During the growing season of winter wheat, leaf greenness index (SPAD) and net photosynthetic rate were determined at two growth stages: BBCH 31–35 (stem elongation) and BBCH 51–55 (heading).

The leaf greenness index was measured using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Osaka, Japan). SPAD measurements were performed on the youngest fully expanded leaves of ten randomly selected plants in each plot.

Net photosynthetic rate was determined using an LCI Portable Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, UK). Measurements were conducted on five randomly selected plants per plot. For each leaf, ten consecutive readings were recorded at 5 s intervals and averaged for statistical analysis. All measurements were performed on cloudless days between 09:00 and 11:00 a.m. under natural light conditions. The results were expressed as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

2.4.2. Grain Yield Determination

Winter wheat was harvested at full technological maturity using a plot combine harvester (Wintersteiger Classic 1540, Wintersteiger AG, Ried im Innkreis, Austria). Grain was collected separately from each experimental plot, and grain weight was recorded. Grain yield was adjusted to a standard moisture content of 14% and expressed as t ha^{-1} .

2.4.3. Determination of Nitrogen and Zinc Concentrations in Grain

Grain samples were collected from each experimental plot after harvest. The samples were cleaned of mechanical impurities, dried to constant weight, and ground to obtain a homogeneous analytical material.

Total nitrogen (N) concentration was determined using the Kjeldahl method after wet digestion in concentrated H_2SO_4 with a catalyst. Analyses were performed using

a Kjeltect™ steam distillation system (FOSS, Hillerød, Denmark). The results were expressed as g kg^{−1} dry matter (DM).

Zinc (Zn) concentration was determined after wet digestion of plant material in a mixture of concentrated HNO₃ and HClO₄ acids. Zinc concentrations were measured by atomic absorption spectrometry (AAS) using an AA-7000 spectrometer (Shimadzu Corporation, Kyoto, Japan). The results were expressed as mg kg^{−1} dry matter (DM) of grain. Grain samples from each experimental plot (three field replications per treatment) were analyzed.

2.4.4. Grain Quality Parameters

Grain quality parameters, including crude protein content, wet gluten content, and the Zeleny sedimentation index, were determined using an Infratec™ 1241 Near-Infrared Analyzer (FOSS, Hillerød, Denmark). Analyses were performed using near-infrared reflectance spectroscopy (NIRS) based on the manufacturer's calibration models. Protein and wet gluten contents were expressed as percentages (%), whereas the Zeleny sedimentation index was expressed in milliliters (mL). Grain quality analyses were performed on samples collected from each experimental plot ($n = 3$ field replications).

2.4.5. Monoculture Compensation Efficiency (MCE)

To quantitatively assess the effectiveness of crop protection treatments in mitigating the adverse effects of long-term winter wheat monoculture, the Monoculture Compensation Efficiency (MCE) index was calculated. This index expresses the percentage of yield losses caused by monoculture that were compensated for by the application of chemical crop protection.

For the herbicide treatment (H), MCE was calculated according to the following equation:

$$MCE_H = \frac{X_{MCH} - X_{MC0}}{X_{CRH} - X_{MC0}} \times 100$$

For the herbicide–fungicide treatment (HF), the Monoculture Compensation Efficiency (MCE) index was calculated using the following equation:

$$MCE_{HF} = \frac{X_{MCHF} - X_{MC0}}{X_{CRHF} - X_{MC0}} \times 100$$

where

X_{MCH} —is the value of the analyzed trait in monoculture following herbicide application,

X_{MC0} —is the value of the analyzed trait in monoculture without protection,

X_{CRH} —is the value of the analyzed trait in crop rotation following herbicide application,

X_{MCHF} —is the value of the analyzed trait in monoculture following herbicide and fungicide application,

X_{CRHF} —is the value of the analyzed trait in crop rotation following herbicide and fungicide application.

An MCE value of 0% indicates no compensation for the adverse effects of monoculture, whereas a value of 100% indicates complete compensation, with the trait reaching the level obtained under crop rotation. Values exceeding 100% indicate an additional positive effect of the applied crop protection treatment beyond full compensation of monoculture-related losses. The MCE index was developed as a descriptive agronomic indicator intended to quantify the relative recovery of monoculture-induced yield losses following crop protection treatments. Its broader applicability should be verified in future studies conducted under different environmental and agronomic conditions.

2.5. Statistical Analysis

Statistical analyses were performed using mixed-effects models in TIBCO Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA).

Linear mixed-effects models were fitted with year (Y), crop sequence (Cs), crop protection (Cp), and variety (V) as fixed effects and replication as a random effect. In addition to the main effects, selected two-way interactions ($Y \times Cs$, $Cs \times Cp$, and $Cs \times V$) were included in the final models. The crop protection $\times$ variety ($Cp \times V$) interaction was not included. Higher-order interactions ($Cs \times Cp \times V$ and interactions involving year with two or more experimental factors) were not included to avoid overparameterization and reduced statistical power, given the experimental structure and three field replications per treatment combination. Statistical significance was assessed at $p \leq 0.05$.

Prior to the statistical analyses, the assumptions of the linear mixed-effects models were verified. Normality of residuals was assessed using the Shapiro–Wilk test. Homogeneity of variance was evaluated using Levene’s test and residual-versus-fitted value plots. The diagnostic analyses confirmed that the assumptions of the models were satisfactorily met, and therefore no data transformation was required. Mean comparisons were performed using Tukey’s honestly significant difference (HSD) test at a significance level of $\alpha = 0.05$. Relationships among the analyzed traits were evaluated using Pearson’s correlation coefficients, and the correlation structure was visualized using heatmaps. The multivariate relationships among the studied variables were further explored using principal component analysis (PCA). Prior to PCA, all variables were standardized to a mean of 0 and a standard deviation of 1. Prior to principal component analysis (PCA), the suitability of the dataset was evaluated using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett’s test of sphericity. The obtained KMO value (0.697) and the significant Bartlett’s test ($\chi^2 = 2811.60$, $df = 55$, $p < 0.001$) confirmed that the correlation structure of the data was appropriate for PCA. Before analysis, all variables were standardized to a mean of zero and a standard deviation of one.

3. Results

3.1. Leaf Greenness Index (SPAD) and Photosynthetic Rate

Analysis of variance revealed significant effects of year, crop sequence, and chemical crop protection on SPAD values measured at both BBCH 31–32 and BBCH 51–55 growth stages (Table 2). A significant variety effect was detected only at BBCH 31–32. Significant interactions were also observed between year and crop sequence for both measurement dates, as well as between crop sequence and crop protection at BBCH 51–55.

Table 2. Analysis of variance (F- and p -values) for the leaf greenness index and photosynthetic rate in winter wheat. Significant effects are indicated at $p < 0.05$.

Treatments	Leaf Greenness Index				Photosynthetic Rate			
	BBCH _{31–32}		BBCH _{51–55}		BBCH _{31–32}		BBCH _{51–55}	
	F	p	F	p	F	p	F	p
Year (Y)	49.284	<0.001	48.423	<0.001	31.47	<0.001	71.374	<0.001
Crop sequence (Cs)	107.322	<0.001	93.264	<0.001	1.982	0.160	2.575	0.113
Crop protection (Cp)	162.227	<0.001	122.141	<0.001	1.983	0.144	2.068	0.134
Variety (V)	14.320	<0.001	1.053	0.308	2.719	0.103	0.656	0.421
$Y \times Cs$	16.951	<0.001	22.735	<0.001	1.088	0.517	0.675	0.512
$Cs \times Cp$	2.538	0.086	22.235	<0.001	0.400	0.672	0.923	0.402
$Cs \times V$	0.196	0.659	4.113	0.046	0.000	0.990	5.690	0.020

SPAD values were significantly higher under crop rotation than under continuous monoculture (Figure 4). Crop protection treatments increased SPAD values at both growth stages, with the highest values recorded following the combined application of herbicides and fungicides. The variety Revolver exhibited significantly higher SPAD values than Emil at BBCH 31–32. The highest SPAD values were observed in the crop rotation system receiving both herbicide and fungicide treatments, whereas the lowest values occurred in monoculture without chemical protection. The differences between these treatments amounted to 8.59 and 9.12 SPAD units at BBCH 31–32 and BBCH 51–55, respectively.

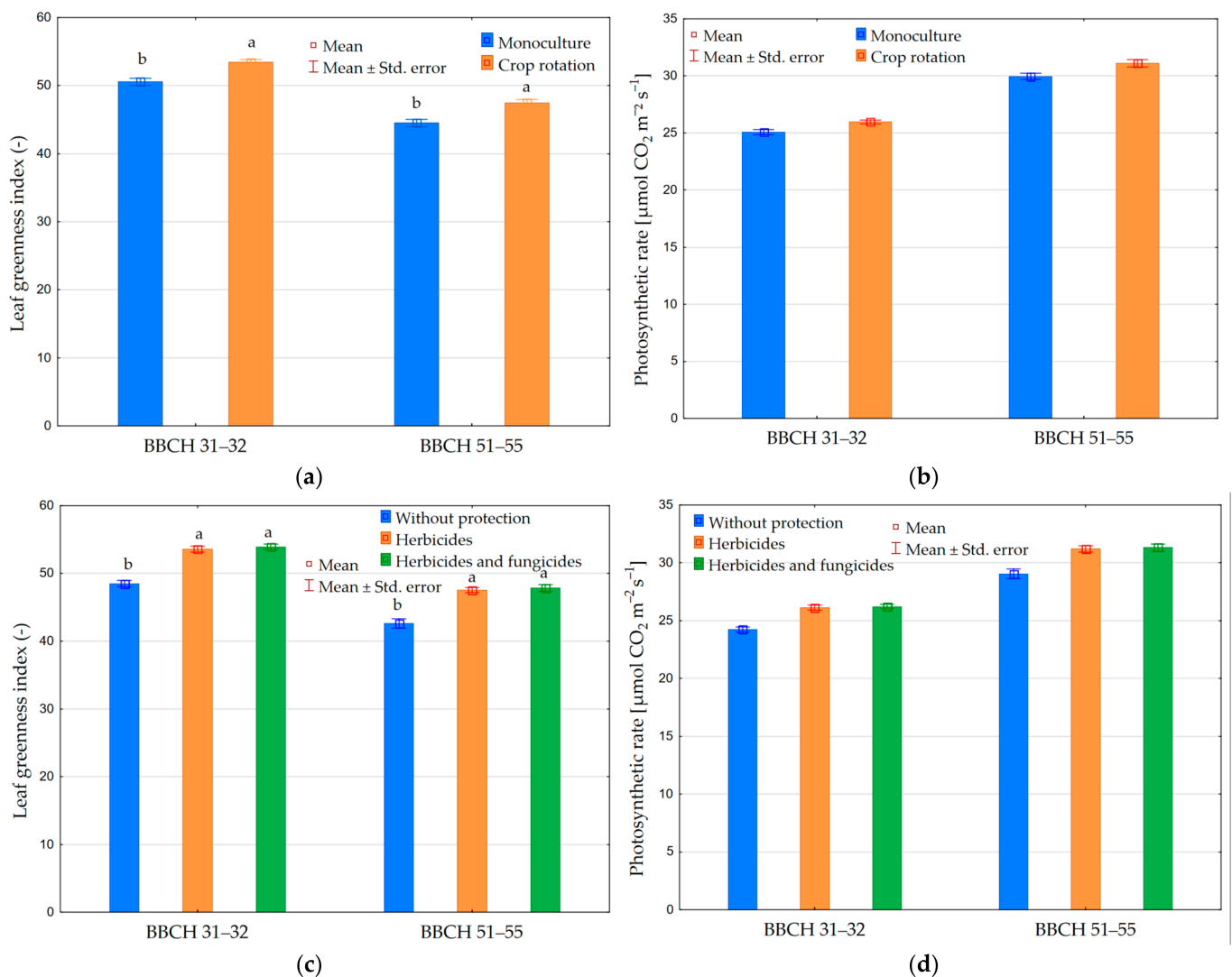


Figure 4. Effects of crop sequence on the leaf greenness index (a) and photosynthetic rate (b), and effects of crop protection on the leaf greenness index (c) and photosynthetic rate (d). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Error bars indicate the standard error (SE).

In contrast, no significant main effects of crop sequence, crop protection, or variety were found for net photosynthetic rate (Table 2, Figure 4). However, significant interactions were detected for crop sequence $\times$ crop protection and crop sequence $\times$ variety. Net photosynthetic rate was higher at BBCH 51–55 than at BBCH 31–32, ranging from 29.06 to 31.32 and from 24.24 to 26.22 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. Detailed mean values for all treatment combinations are provided in Supplementary Table S5.

3.2. Grain Yield and Technological Grain Quality

Grain yield was significantly affected by year, crop sequence, crop protection treatment, and variety (Table 3). The mean grain yield obtained under crop rotation was 41.4% higher than that recorded under continuous monoculture (Figure 5). Crop protection significantly increased grain yield. Compared with the untreated control, herbicide applications increased yield by 75.0%, whereas the combined application of herbicides and fungicides increased yield by 81.5%. The mean grain yield of the variety Revolver was 17.7% higher than that of Emil.

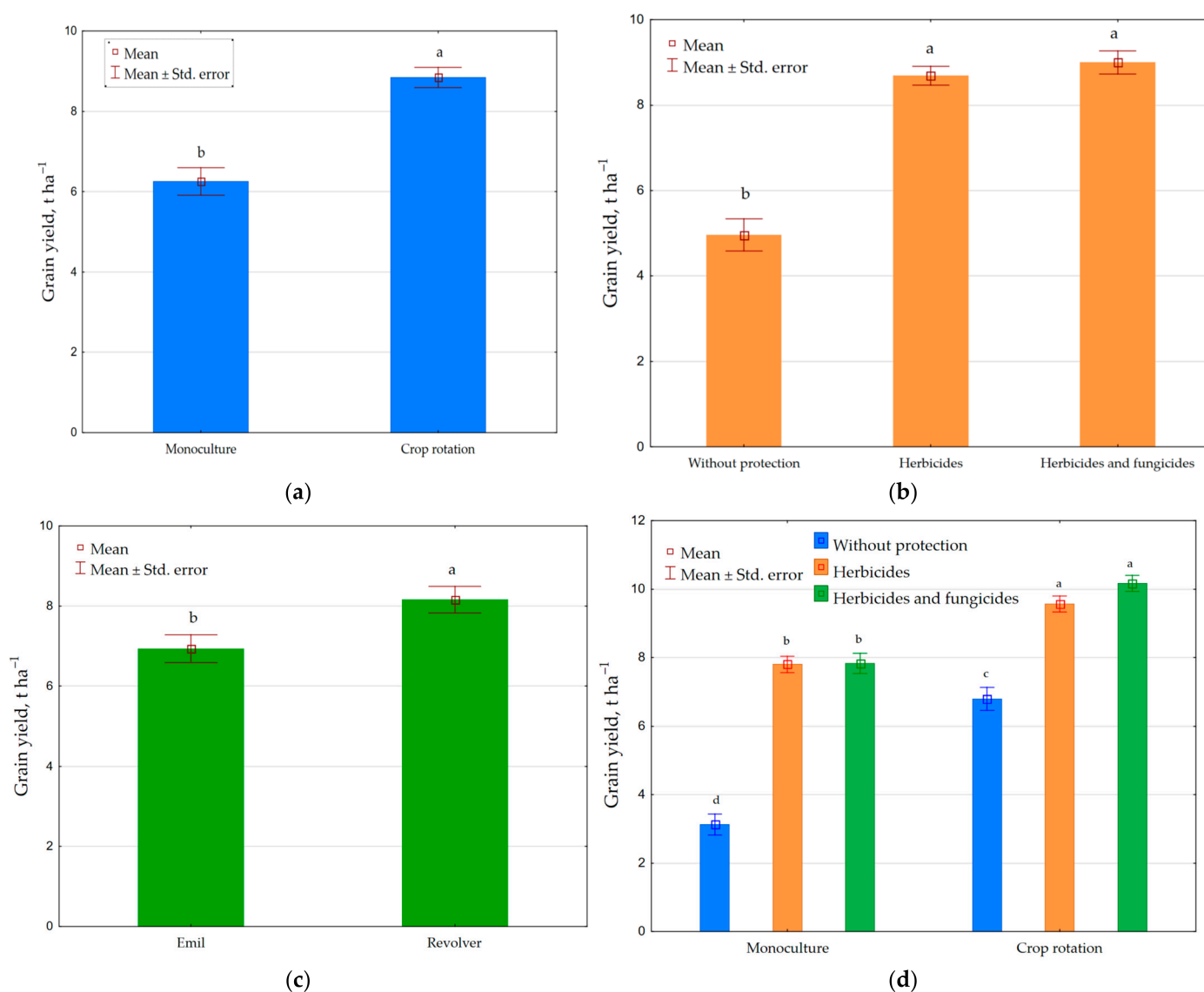


Figure 5. Winter wheat grain yield as affected by crop sequence (a), crop protection (b), variety (c), the crop sequence × crop protection interaction (d). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Error bars indicate the standard error (SE).

For the crop sequence × crop protection interaction, the highest grain yield was obtained under crop rotation with combined herbicide and fungicide application (10.17 t ha⁻¹), whereas the lowest yield was recorded in the monoculture without chemical protection (3.13 t ha⁻¹). The crop sequence × variety interaction was not significant ($p = 0.051$). Detailed mean values for all treatment combinations (crop sequence × crop protection × variety) in individual growing seasons (2023–2025) are presented in Supplementary Table S5.

Table 3. Analysis of variance (F- and *p*-values) for grain yield, protein content, gluten content, and Zeleny sedimentation index of winter wheat. Significant effects are indicated at *p* < 0.05.

Treatments	Grain Yield		Protein Content		Gluten Content		Zeleny Index	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Year (Y)	30.379	<0.001	58.887	<0.001	35.708	<0.001	186.277	<0.001
Crop sequence (Cs)	453.571	<0.001	58.073	<0.001	54.821	<0.001	142.385	<0.001
Crop protection (Cp)	454.575	<0.001	30.672	<0.001	21.079	<0.001	24.950	<0.001
Variety (V)	101.937	<0.001	71.649	<0.001	102.908	<0.001	52.402	<0.001
Y × Cs	2.097	0.130	24.716	<0.001	57.658	<0.001	35.931	<0.001
Cs × Cp	21.579	<0.001	2.026	<0.139	1.292	0.281	10.418	<0.001
Cs × V	3.944	0.051	85.755	<0.001	102.908	<0.001	98.206	<0.001

The analysis of technological grain quality revealed significant effects of all experimental factors on protein content, wet gluten content, and the Zeleny sedimentation index (Table 3). Grain harvested from the monoculture system was characterized by higher protein and wet gluten contents, as well as a higher Zeleny index, compared with grain produced under crop rotation (Table 4). In contrast, bulk density was higher under crop rotation than under monoculture.

Table 4. Effects of crop sequence, crop protection, variety and year on protein content, gluten content and Zeleny sedimentation index. Data are presented as means ± standard error (SE). Within each factor, means followed by different letters in the same column differ significantly according to Tukey's HSD test (*p* < 0.05).

Treatments	Protein Content, %	Gluten Content, %	Zeleny Index, mL
Monoculture Crop rotation	10.64 ± 0.16 a 10.06 ± 0.11 b	Crop sequence 20.57 ± 0.40 a 19.29 ± 0.27 b	28.12 ± 0.95 a 23.27 ± 0.82 b
		Crop protection 20.73 ± 0.61 a 19.58 ± 0.29 b 19.50 ± 0.27 b	27.71 ± 1.69 a 24.89 ± 0.79 b 24.49 ± 0.71 b
Without protection Herbicide Herbicides and fungicides	10.78 ± 0.25 a 10.17 ± 0.10 b 10.11 ± 0.12 b	Variety 20.81 ± 0.39 a 19.06 ± 0.27 b	27.17 ± 1.12 a 24.23 ± 0.69 b
Emil Revolver	10.68 ± 0.16 a 10.03 ± 0.10 b	Year 18.90 ± 0.47 b 20.46 ± 0.14 a 20.43 ± 0.53 a	22.86 ± 0.73 b 23.00 ± 1.09 b 31.23 ± 1.03 a

3.3. Mineral Composition of Grain (N and Zn)

Analysis of variance revealed significant effects of year, crop sequence, crop protection treatment, and variety on grain nitrogen concentration (Table 5). In the case of zinc concentration, all experimental factors except variety had a significant effect. Significant interactions were also observed for year × crop sequence, crop sequence × crop protection, and crop sequence × variety.

Grain harvested from winter wheat grown in continuous monoculture contained higher concentrations of both nitrogen and zinc than grain obtained under crop rotation (Table 6). The highest concentrations of both nutrients were recorded in the untreated plots. Through the study years, the highest nitrogen concentrations were observed in 2024 and 2025, whereas the highest zinc concentration was recorded in 2024.

Table 5. Analysis of variance (F- and *p*-values) for nitrogen (N) and zinc (Zn) contents in winter wheat grain. Significant effects are indicated at *p* < 0.05.

Treatments	N		Zn	
	F	<i>p</i>	F	<i>p</i>
Year (Y)	263.226	<0.001	709.025	<0.001
Crop sequence (Cs)	83.347	<0.001	267.899	<0.001
Crop protection (Cp)	12.635	<0.001	219.021	<0.001
Variety (V)	35.947	<0.001	0.003	0.954
Y × Cs	99.137	<0.001	509.297	<0.001
Cs × Cp	8.091	0.001	75.217	<0.001
Cs × V	50.396	<0.001	40.400	<0.001

Table 6. Nitrogen (N) and zinc (Zn) contents in winter wheat grain as affected by crop sequence, crop protection, variety and year. Data are presented as means ± standard error (SE). Within each factor, means followed by different letters in the same column differ significantly according to Tukey's HSD test (*p* < 0.05).

Treatment	N, %	Zn, mg kg ^{−1}
Crop sequence		
	Monoculture	1.57 ± 0.03 a
Crop rotation		
	Crop rotation	1.46 ± 0.03 b
Crop protection		
	Without protection	1.56 ± 0.05 a
	Herbicide	1.48 ± 0.03 b
Herbicides and Fungicides		
	Herbicides and Fungicides	1.52 ± 0.04 b
Variety		
	Emil	1.55 ± 0.03 a
	Revolver	1.48 ± 0.03 b
Year		
	2023	1.32 ± 0.03 b
	2024	1.62 ± 0.03 a
	2025	1.62 ± 0.03 a

For the year × crop sequence interaction, the highest nitrogen and zinc concentrations were found in the monoculture system in 2024, reaching 1.75% and 44.96 mg kg^{−1}, respectively. Detailed treatment means for individual years are presented in Supplementary Table S5.

3.4. Correlation Analysis Between Physiological Traits, Grain Yield, and Grain Quality Parameters of Winter Wheat

The strongest positive correlations were observed between the leaf greenness index (SPAD) and net photosynthetic rate (Figure 6). Grain yield was strongly and positively correlated with both SPAD values (*r* = 0.878 for BBCH 31–32 and *r* = 0.824 for BBCH 51–55) and net photosynthetic rate (*r* = 0.733 and *r* = 0.626 at BBCH 31–32 and BBCH 51–55, respectively).

Positive relationships were also found between grain nitrogen and zinc concentrations and grain quality parameters. Nitrogen concentration was positively correlated with protein content (*r* = 0.657), wet gluten content (*r* = 0.590), and the Zeleny sedimentation index (*r* = 0.576). Similar relationships were observed for zinc concentration, which was positively correlated with protein content (*r* = 0.578), wet gluten content (*r* = 0.436), and the Zeleny sedimentation index (*r* = 0.570).

In contrast, grain yield was negatively correlated with protein content (*r* = −0.405), wet gluten content (*r* = −0.329), and the Zeleny sedimentation index (*r* = −0.299).

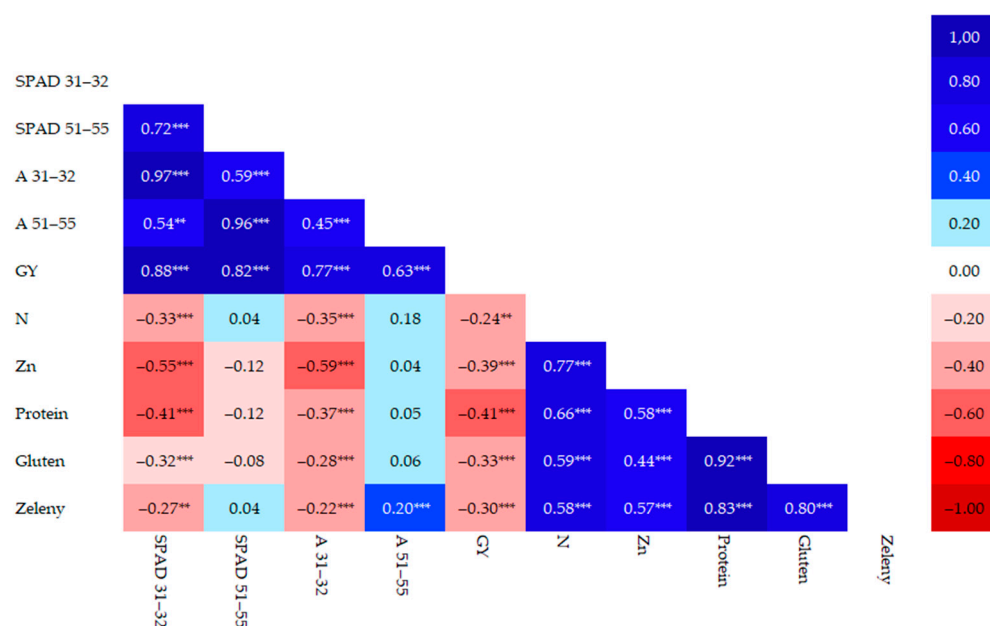


Figure 6. Heatmap of Pearson correlation coefficients among physiological parameters and grain yield, nutrient contents, and grain quality traits in winter wheat. Positive correlations are shown in blue and negative correlations in red. Asterisks indicate significant correlations (** $p < 0.01$, *** $p < 0.001$); no asterisk indicates a non-significant correlation ($p \geq 0.05$). SPAD 31–32—leaf greenness index; SPAD 51–55—leaf greenness index; A 31–32—photosynthetic rate, BBCH 31–32; A 51–55—photosynthetic rate, BBCH 51–55; GY—grain yield, N—nitrogen, Zn—zinc.

Principal component analysis (PCA) revealed that the first two principal components explained 49.59% and 29.97% of the total variance, respectively (Figure 7a). Together, the first two principal components explained 79.56% of the total variance, indicating that the two-dimensional PCA solution provided an adequate representation of the multivariate relationships among the analyzed variables. Therefore, interpretation of the PCA biplots was considered reliable. The PCA biplot showed a clear separation between grain quality traits and physiological and yield-related parameters. Protein content, wet gluten content, the Zeleny sedimentation index, and grain nitrogen and zinc concentrations were grouped on the negative side of PC1. In contrast, grain yield, SPAD values, and net photosynthetic rate measured at BBCH 31–32 and BBCH 51–55 were located on the positive side of PC1. The PCA score plot indicated a distinct separation of the monoculture treatments without crop protection (M–Wp–E and M–Wp–R) from the remaining experimental treatments (Figure 7b). Most combinations involving crop rotation and the application of crop protection treatments were clustered in the central and positive regions of PC1, reflecting their association with improved physiological performance and higher grain yield. Based on the PCA score plot, the treatment combinations can be ranked according to their agronomic performance. The most favorable combinations were Cr–HF–R and Cr–HF–E, followed by Cr–H–R and Cr–H–E. The least favorable treatments were M–Wp–E and M–Wp–R, which were clearly separated from the remaining combinations and associated with lower physiological activity and grain yield. Overall, the crop rotation combined with herbicide and fungicide treatment, particularly for the cultivar Revolver, represented the most favorable treatment combination, whereas continuous monoculture without crop protection was associated with the least favorable agronomic performance.

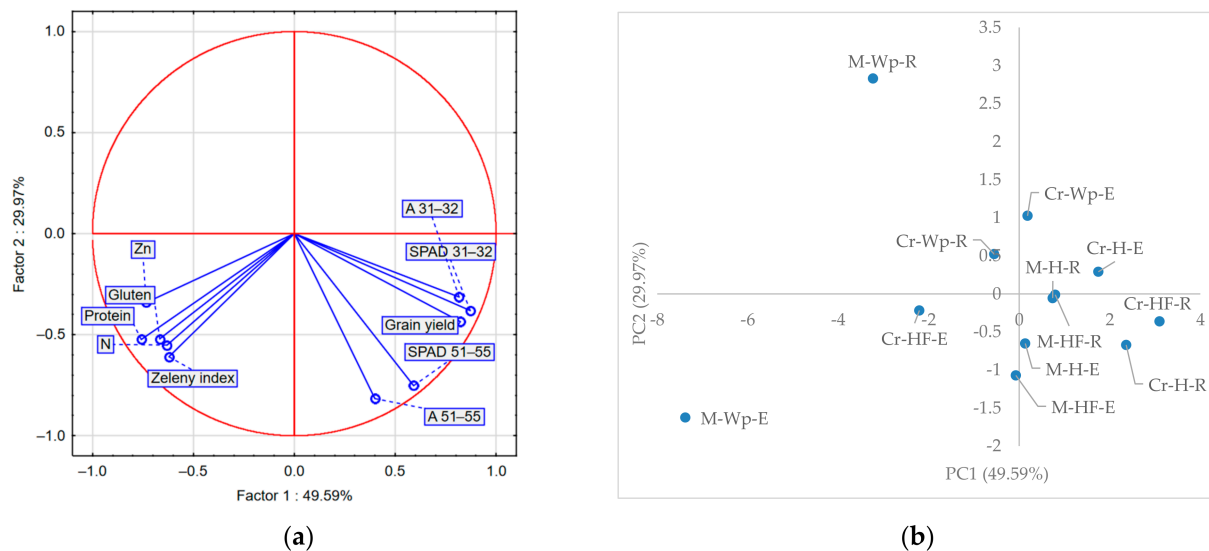


Figure 7. Principal component analysis (PCA) showing the relationships among physiological traits (leaf greenness index and photosynthetic rate), grain yield, nitrogen (N) and zinc (Zn) concentrations, and grain quality traits (protein content, gluten content, and Zeleny sedimentation index) in winter wheat. (a) PCA correlation circle of the measured variables. (b) PCA score plot of the experimental treatments. SPAD 31–32, leaf greenness index at BBCH 31–32; SPAD 51–55, leaf greenness index at BBCH 51–55; A 31–32, photosynthetic rate at BBCH 31–32; A 51–55, photosynthetic rate at BBCH 51–55; Grain yield, N, nitrogen, Zn, zinc, M—monoculture, Cr—crop rotation, Wp—without protection, H—herbicide, HF—herbicide + fungicide, E—Emil, R—Revolver.

3.5. Compensation Efficiency of Yield Losses Caused by Monoculture (MCE)

The values of the Monoculture Compensation Efficiency (MCE) index indicated that chemical crop protection compensated for approximately 64% to 76% of the grain yield losses caused by long-term winter wheat monoculture (Figure 8). Slightly higher MCE values were recorded for the variety Emil than for Revolver. Moreover, compensation efficiency was marginally greater following herbicide application alone than after the combined application of herbicide and fungicide treatments.

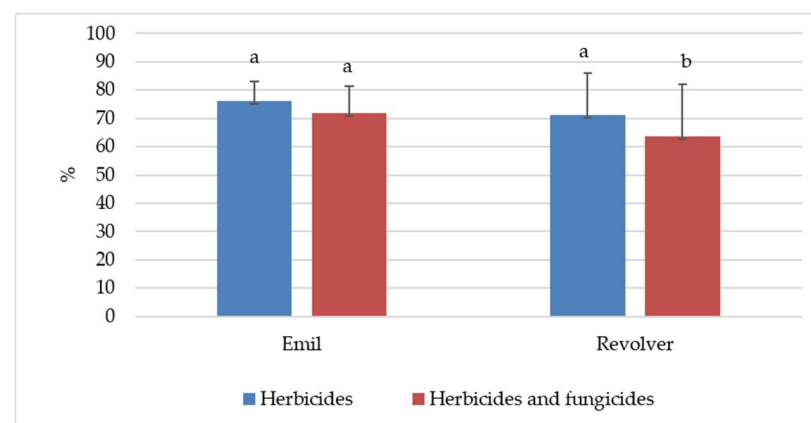


Figure 8. Compensation efficiency (MCE, %) of winter wheat grain yield losses caused by long-term monoculture as affected by crop protection $\times$ variety. Data are presented as means for 2023–2025. Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$). Error bars indicate the standard error (SE).

Overall, the results demonstrate that chemical crop protection substantially mitigated the negative effects of continuous monoculture on grain yield; however, it was insufficient to fully compensate for the yield advantages associated with crop rotation.

4. Discussion

4.1. Leaf Greenness Index (SPAD) and Photosynthetic Rate

Long-term cultivation of winter wheat under continuous monoculture resulted in lower SPAD values at both measurement dates. The SPAD is widely recognized as a reliable indicator of chlorophyll content, plant nitrogen status, and overall physiological condition [20,25,26]. Mehrabi et al. [26] demonstrated a close relationship between SPAD values, nitrogen use efficiency, and cereal productivity. The reduction in SPAD values observed under long-term monoculture may result from the combined effects of several mechanisms. Continuous cultivation of the same crop species gradually deteriorates the biological properties of soil, reduces microbial diversity, and promotes the accumulation of soil-borne pathogens [27,28]. Restricted root development decreases the efficiency of water and nutrient uptake, particularly nitrogen, which is a fundamental component of chlorophyll. Consequently, leaves undergo earlier senescence, the activity of the photosynthetic apparatus is reduced, and the intensity of processes responsible for biomass accumulation declines [29]. The magnitude of the SPAD reduction observed in the present study appears greater than that reported in many shorter-term experiments. This difference is likely associated with the exceptional duration of the experiment, in which continuous winter wheat monoculture has been maintained since 1967. Such prolonged management probably intensified cumulative changes in soil biological properties and pathogen pressure, thereby amplifying the physiological response of winter wheat.

The results of the present study indicate that crop protection treatments increased SPAD values. This effect can be primarily attributed to reduced competition from weeds for water, light, and nutrients, as well as to decreased pressure from foliar diseases [30]. Schierenbeck et al. [31] reported that effective fungicide protection prolongs leaf functionality, delays chlorophyll degradation, and improves the efficiency of photosynthetically active radiation utilization. Khatami et al. [32] pointed out that herbicides, in addition to controlling weeds, may also affect crop plants by causing temporary disturbances in physiological processes and symptoms of phytotoxicity, particularly under stressful environmental conditions or in highly sensitive varieties. Since disease severity was not quantitatively evaluated, the beneficial effects of fungicide application should be interpreted as the combined alleviation of biotic stress rather than as evidence of control of specific fungal diseases.

Net photosynthesis is highly dependent on instantaneous environmental conditions; therefore, single measurements of gas exchange are often characterized by greater variability [33] than SPAD measurements [20]. As a result, differences among treatments may be more difficult to detect statistically, even when long-term stress factors affect the functioning of the photosynthetic apparatus. Correlation analysis and PCA revealed strong positive relationships among SPAD values, net photosynthetic rate, and grain yield. The strong positive correlation between SPAD and net photosynthetic rate observed in the present study therefore indicates that leaves with higher chlorophyll content generally exhibited higher photosynthetic activity, although the greater temporal variability of gas-exchange measurements limited the detection of significant treatment effects in the analysis of variance.

These findings indicate that treatments characterized by higher chlorophyll content and better physiological status of the photosynthetic apparatus also achieved higher grain yields.

These results are consistent with the observations of Thomas and Ougham [34], who demonstrated that maintaining leaf greenness prolongs the period of active photosynthesis, promotes more efficient nitrogen remobilization, and enables a longer supply of assimilates

to developing grains. Consequently, this trait may contribute to sustaining high cereal productivity, particularly under stress conditions [35].

Although SPAD values responded significantly to crop sequence and crop protection, net photosynthetic rate did not show corresponding significant main effects. This apparent discrepancy may result from the different physiological nature of these parameters. The SPAD reflects leaf chlorophyll content and nitrogen status, which are relatively stable indicators of plant physiological condition [20]. In contrast, net photosynthetic rate represents an instantaneous physiological process that is strongly influenced by short-term environmental conditions during measurement, including irradiance, leaf temperature, vapor pressure deficit, and stomatal conductance. Consequently, photosynthetic measurements generally exhibit greater within-treatment variability than SPAD values, which may reduce the statistical significance of treatment effects despite the existence of strong positive correlations between both parameters. This interpretation is supported by the strong positive correlation observed between SPAD values and net photosynthetic rate in the present study.

4.2. Grain Yield, Technological Grain Quality, and Grain Mineral Composition (N and Zn)

Increasing crop rotation diversity improves nitrogen use efficiency, supports the maintenance of high soil fertility, and enables the achievement of higher and more stable cereal yields [3,36]. This effect is associated with more efficient utilization of soil resources, beneficial changes in microbial activity, and enhanced nitrogen availability for subsequent crops [3,36]. The results obtained in the present study confirm this mechanism. The reduction in grain yield observed under monoculture was accompanied by deterioration in plant physiological traits, indicating a close relationship between the condition of the photosynthetic apparatus and the productivity of winter wheat. Lower SPAD values recorded under monoculture may reflect an earlier decline in photosynthetic activity and accelerated leaf senescence. These processes are considered among the earliest symptoms of increasing plant stress and may lead to a shorter period of active biomass accumulation and reduced grain yield [29].

The 41.4% reduction in grain yield observed in the present study exceeded that reported in many previous studies of simplified cereal production systems. This discrepancy most likely reflects the exceptional duration of the experiment and the cumulative biological changes that developed over more than five decades of continuous winter wheat cultivation.

Appropriately managed crop protection practices can mitigate yield losses caused by monoculture [8,37]. In the present study, both herbicide application alone and the combined application of herbicides and fungicides improved crop productivity. This effect can be attributed to reduced weed competition, which improves the utilization of light, water, and nutrients by crop plants [38], as well as to reduced disease pressure [39].

In our previous study [38], long-term monoculture was shown to promote shifts in weed species composition and increase weed competitiveness against winter wheat, whereas effective herbicide protection significantly reduced weed infestation. The results presented here extend these findings by demonstrating that improved productivity was directly associated with the maintenance of a better physiological status of the plants.

Despite the substantial reduction in grain yield, winter wheat grown under monoculture simultaneously exhibited higher protein and wet gluten contents, as well as a higher Zeleny sedimentation index. This phenomenon is commonly associated with the dilution effect, whereby the concentration of nitrogenous compounds and mineral nutrients decreases as grain yield increases. Therefore, the higher protein concentration observed

under monoculture should be interpreted primarily as a concentration effect resulting from lower grain yield rather than as evidence of improved crop performance.

This mechanism has been well documented in studies on wheat grain quality [40]. Nitrogen and zinc were selected because of their fundamental roles in grain protein synthesis, technological quality, and plant physiological performance, making them suitable indicators of the nutritional consequences of long-term monoculture. The results concerning grain mineral composition support this interpretation. In the present experiment, grain produced under monoculture was characterized by higher nitrogen and zinc concentrations than grain obtained under crop rotation.

Nitrogen is the principal constituent of grain storage proteins; therefore, its concentration is closely associated with protein and gluten contents [41]. Zinc plays an important role in nitrogen metabolism and protein synthesis [21]. Consequently, zinc concentration in grain is frequently positively correlated with protein concentration and selected technological quality parameters of wheat [42,43]. Under the conditions of the present study, the higher zinc concentration observed in monoculture was most likely a consequence of reduced grain yield rather than increased uptake of this element.

The higher grain yield of the variety Revolver indicates its greater capacity to adapt to long-term monoculture conditions. Wheat varieties differ in nitrogen use efficiency, root system architecture, and tolerance to biotic and abiotic stresses, all of which influence their ability to maintain high productivity under limited environmental resources [44,45]. These differences may become particularly evident under long-term monoculture, where plants are exposed to increased stress pressure [19].

4.3. Compensation Efficiency of Yield Losses Caused by Monoculture (MCE)

The obtained MCE values indicate that the applied crop protection treatments enabled the recovery of 64–76% of the grain yield losses caused by long-term continuous winter wheat cultivation. This finding suggests that appropriately managed chemical crop protection can substantially mitigate the adverse effects of simplified crop sequences; however, it cannot fully replace the benefits associated with a properly designed crop rotation [46,47].

Unlike previously proposed productivity or stress tolerance indices, the MCE index should not be regarded as a universal indicator of crop resilience. At the present stage, the index should be regarded as a descriptive agronomic indicator rather than a universally validated assessment tool. Instead, it should be considered a complementary management-oriented metric designed specifically to evaluate the effectiveness of agronomic practices in recovering yield losses caused by long-term monoculture. This result is consistent with numerous long-term studies demonstrating that the positive effects of crop rotation arise from its simultaneous influence on the physical, chemical, and biological properties of soil, the suppression of weeds and pathogens, and the improvement of nutrient cycling [48]. Therefore, no single agronomic practice can completely substitute for the benefits derived from species diversity within crop rotations.

The present findings are also in agreement with our previous studies conducted within the same long-term experimental site involving winter oilseed rape cultivation [16]. Those studies demonstrated that continuous winter oilseed rape monoculture increased weed infestation and intensified competition for water and nutrients, thereby deteriorating the growing conditions for subsequent winter wheat crops. Similarly, Stępień [38] reported that effective herbicide protection significantly reduced weed infestation in both crop rotation and monoculture systems.

The incomplete compensation of yield losses observed in the present study confirms that the reduction in productivity under monoculture is a multifactorial phenomenon.

Consequently, even effective weed control and disease suppression cannot eliminate all factors responsible for yield decline under long-term continuous cropping [49].

The higher MCE values recorded for the variety Emil indicate that the effectiveness of compensatory measures depends not only on the applied crop management strategy but also on the genetic characteristics of the variety. This finding highlights the growing importance of selecting varieties with high yield stability and superior adaptation to stress conditions as a key component of sustainable cereal production systems [39].

The proposed MCE index should be regarded as a proof-of-concept requiring further validation under different environmental conditions, soil types, crop species, and management systems. Future studies should evaluate its robustness across independent long-term experiments and compare its performance with other productivity- and resilience-based indicators. Such validation will determine the broader applicability of the MCE index as a decision-support tool in sustainable crop production. The present study was conducted at a single long-term experimental site and included only two winter wheat cultivars. Furthermore, disease severity and soil microbial communities were not assessed directly. Therefore, further studies under different environmental conditions are needed to validate the broader applicability of the proposed MCE index.

5. Conclusions

The present study demonstrated that long-term winter wheat monoculture significantly reduced leaf greenness (SPAD) and grain yield, while increasing grain protein, wet gluten, Zeleny sedimentation index, and grain concentrations of nitrogen and zinc as a consequence of the dilution effect associated with lower productivity. Crop protection substantially improved physiological performance and grain yield; however, even the combined application of herbicides and fungicides compensated for only 64–76% of the yield losses caused by continuous monoculture, confirming that chemical protection cannot fully replace the agronomic and ecological benefits of diversified crop rotations.

The contrasting responses of the two cultivars indicate that high yield potential and the capacity to compensate for monoculture-induced yield losses are not necessarily associated. While Revolver generally achieved higher grain yield, Emil exhibited higher MCE values, suggesting greater responsiveness to agronomic interventions. Therefore, cultivar selection should be considered together with crop protection and crop rotation when developing sustainable production strategies.

From a practical perspective, diversified crop rotation should remain the primary strategy for maintaining high and stable winter wheat productivity. Where continuous wheat cultivation cannot be avoided, integrated crop management combining effective weed and disease control with the selection of well-adapted cultivars offers the greatest potential for reducing, although not eliminating, productivity losses. Therefore, chemical crop protection should be regarded as a complementary practice rather than a substitute for crop rotation.

The proposed Monoculture Compensation Efficiency (MCE) index provides an additional tool for evaluating the effectiveness of agronomic practices aimed at mitigating yield losses under long-term monoculture. Future studies should validate its applicability across different environments, soil types, crop species, and management systems to determine its broader usefulness in sustainable crop production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture16161692/s1>, Figure S1: Layout of experimental plots in the field experiment. 2023, 2024, and 2025 indicate the years in which winter wheat was grown in the crop rotation system. CR—crop rotation; CC—monoculture (continuous cropping). Plots marked with the year of cultivation indicate the location of winter wheat within the crop rotation system;

Table S1: Herbicides used on sites: “Herbicide” and “Herbicide + Fungicide”; Table S2: Fungicides used on site: “Herbicide + fungicide”; Table S3: Characteristics of the winter wheat varieties used in the study; Table S4: Insecticides used on site: “Without protection”, “Herbicide” and “Herbicide + fungicide”; Table S5: Mean values of all 12 treatment combinations in individual growing seasons (2023–2025).

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