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ORIGINAL PAPER

Chemical composition of *Hordeum vulgare* var. *rimpaui* grain under reduced tillage and *Methylobacterium symbioticum* application*

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Abstract

Agronomic practices, including soil tillage and fertilisation, exert a profound influence on the nutritional quality of cereal grains, particularly in organic farming. This study assessed the effect of soil tillage (reduced vs ploughing) and foliar application of the nitrogen-fixing bacterium *Methylobacterium symbioticum* (single and double application) on the chemical composition of black barley (*Hordeum vulgare* var. *rimpaui*) cultivated organically. A field study was conducted in two growing seasons (2023-2024) on an organic farm located in west-central Poland. Grain was analysed for macro- and micronutrients, protein content and amino acid profile, starch, dietary fibre, β-glucan, and total phenols. The crop responses were influenced by the weather conditions. The tillage system had an impact on most of the parameters, with ploughing increasing the content of P, K, Mg, total phenols, and, under less favourable hydrothermal conditions, protein content and amino acid composition. Conversely, reduced tillage led to improved grain density. Application of *M. symbioticum* had a limited effect, primarily enhancing starch content following double application. However, when combined with reduced tillage, the biostimulant enhanced dietary fibre and β-glucan accumulation. Grain yield and size parameters were positively correlated with Mg, Ca, and amino acid contents, whereas micronutrient concentrations were negatively associated with grain size. Overall, the results indicate that reduced tillage combined with microbial fertilisation can improve selected quality traits of black barley grain, supporting its sustainable cultivation in organic systems.

Keywords: reduced tillage, *Methylobacterium symbioticum*, nutritional value, organic farming, black barley

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INTRODUCTION

Cereals constitute a staple human food worldwide; however, increasing attention is being directed toward diversification of cereal raw materials in order to make full use of their nutritional value (Knapowski et al. 2025). This is reflected in the growing interest in ancient grains, which are valued for their high nutritional value and bioactive compounds content (Dragičević et al. 2024). Among these, *Hordeum vulgare* var. *rimpaui* (black barley) is particularly notable for its dark grain colour, attributed to phytomelanin, a pigment known for its strong antioxidant properties (Szczepanek et al. 2023). The primary component of cereal grain is starch (Zhu, 2017), although it is also a rich source of macro- and micronutrients (Laskowski et al. 2019), high-quality protein characterised by essential amino acid composition – EAAI (Biel et al. 2013), and dietary fibre, particularly β-glucan (Mio et al. 2022, Błaszczyk et al. 2025).

Noteworthy, the chemical composition of grain depends on the cereal's genotype as well as environmental factors and agricultural practices (Jaskulska et al. 2018). The weather, including temperature and precipitation, significantly affects cereal yield and quality (Hlisnikovský et al. 2024). In organic farming systems, fertilisation strategies and soil tillage practices are critical determinants of crop performance. Although conventional ploughing facilitates nutrient incorporation (Krauss et al. 2020), reduced tillage is increasingly promoted because of its positive effects on soil structure, organic matter content, microbial activity, erosion control, and lower fuel consumption (Lithourgidis et al. 2005, Khan et al. 2023). However, ensuring adequate nitrogen supply under reduced tillage in organic systems remains challenging (Peigné et al. 2007).

Foliar microbial fertilisers offer a potential solution for sustainable nitrogen management (Rosenblueth et al. 2018). In particular, *Methylobacterium symbioticum*, a nitrogen-fixing bacterium capable of rapid phyllosphere colonisation (Bolla et al. 2025), has been demonstrated to supply 25-50% of the nitrogen demand for various crops, including maize, strawberries, and lettuce, without exerting any adverse effects on soil microbiota, thereby reducing reliance on standard fertilisation practices and maintaining productivity (Torres Vera et al. 2024).

This study aimed to evaluate the effects of reduced tillage and *M. symbioticum* application on the chemical composition of organically grown black barley under varying hydrothermal conditions. It was hypothesised that both tillage systems and bacterial fertilisation influence grain composition, with responses dependent on agrometeorological conditions

MATERIALS AND METHODS

Plant material

The subject of this study was *Hordeum vulgare* L. var. *rimpaui* cv. Kaptur, which is a spring and two-row form of barley. This form is characterised by black-coloured grains and reduction of awns into hooded form. As the Bydgoszcz University of Science and Technology is the breeder of cv. Kaptur, with exclusive rights to it, seeds of this variety of our experiment were obtained through the process of self-multiplication.

Site and experiment treatments

Field experiments were set up on an organic farm (52°09′17″N, 16°29′54″E) in the village of Wolkowo, in the Wielkopolskie Voivodeship, Poland. The experiments were conducted in two consecutive years (2023-2024). The soil at the experimental sites was classified as a Luvisol (IUSS WRB) and characterised as sandy loam soil. The chemical analysis of soil samples was conducted by an accredited laboratory of the National Chemical and Agricultural Station in accordance with the major Polish standards (PN-ISO 10390:1997, PN-R-04023:1996, PN-R-04022:1996, PN-R-04020:1994, PN-R-04017:1992, PN-R-04016:1992, PN-R-04019:1993, PN-R-04021:1994). Soil pH determined in KCl was 6.5. According to the Polish classification criteria (IUNG-PIB), the soil had a very high content of plant available phosphorus (37.1 mg P 100 g^{-1}) and magnesium (10.1 mg Mg 100 g^{-1}), and a high content of potassium (18.4 mg K100 g^{-1}). The content of Cu, Zn, Mn and Fe were as follows: 3.20, 18.45, 108.0 and 866.5 mg kg^{-1} of soil. The content of organic carbon in the soil was found to be 1.16%. The preceding crop for barley was potatoes.

The experiment was established in a split-plot design with four replications. Two tillage methods, ploughing and reduced tillage, and single or dual application of plant-stimulating nitrogen nutrition with BlueN (*Methylobacterium symbioticum)*, along with a control, were tested.

Weather conditions

The weather conditions during the black barley growing seasons differed between the study years. Temperatures were lower from the first ten-day period of March to 10th April, then throughout May, as well as in late June 2023 compared with the same periods in 2024, while the remaining periods were similar (Table 1). In 2023, the lowest mean daily air temperature was recorded between the 1st and 10th of April (4.36°C), whereas in 2024 it occurred in mid-March (6.65°C). The period with the highest temperatures lasted between 11th and 20th July in both years, with an average temp. of 20.76°C and 21.09°C, respectively.

Table 1

The meteorological conditions at the experimental location

Year	March		April			May			June			July		Total/ Avg.
	11-20	21-31	1-10	11-20	21-30	1-10	11-20	21-31	1-10	11-20	21-30	1-10	11-20	
Precipitation (mm)														
2023	12.80	10.70	11.30	22.00	5.90	15.90	12.20	0.00	0.00	15.10	21.80	7.20	15.30	150.20
2024	3.00	13.60	14.90	16.30	0.40	2.70	0.30	30.40	8.40	30.20	29.50	33.30	31.80	214.80
Temperature (C°)														
2023	5.37	7.26	4.36	8.53	9.15	10.20	11.49	13.18	17.41	16.93	19.63	19.42	20.76	12.59
2024	6.65	9.43	13.77	8.65	8.59	14.44	15.26	18.05	16.86	16.15	21.20	19.22	21.09	14.57
	emergence		leaf devel.	tillering		stem elongation		booting	heading/ flowering		grain development and filling			

Data were obtained from the IUNG Station in Wielichowo.

The total precipitation during the black barley growth (mid-March to mid-July) was 150.20 mm in 2023 and 214.80 mm in 2024 (Table 1). The distribution of precipitation differed between the years: from mid-March through the following two months, which is the main period of plant development and biomass accumulation, there was nearly 1.8-fold less rainfall in 2024 than in 2023. In contrast, during the stages of grain development and filling (June-July), there was more rainfall in 2024.

Agrotechnical methods

The main plot factor, soil tillage, encompassed two practices: ploughing and reduced tillage. In the autumn preceding the barley season, the following soil tillage practices were carried out: 1) conventional moulboard ploughing to a depth of 25 cm, or 2) reduced tillage, employing a chisel plough with narrow tines to the same depth (25 cm). In both years, the spring soil preparation involved two passes with a disc harrow–string roller unit. Prior to sowing, plants were fertilised with powdered phosphate rock (40 kg ha^{-1} P_2O_5), and kainite (90 kg ha^{-1} K_2O). Weeding harrowing was performed diagonally to the rows at the 3-4 leaf stage.

In the second or third ten-day period of March in 2023 and 2024, respectively, *H.* v. *rimpaui* was sown at a depth of 3-4 cm, with a row spacing of 12.5 cm. The sowing density was established at either 350 or 450 seeds (no per m^2); however, subsequent data analysis was performed on averages from both sowing densities.

The biostimulation of black barley with nitrogen-fixing bacteria encompassed single and double application, along with the control. In the objects with a single treatment as well as the first dose in the object with double application, the preparation was applied at full tillering (BBCH 25). The second dose in objects with two applications of the biofertiliser was applied at the stem elongation stage (BBCH 32/33). A dose of 0.33 kg ha^{-1} of the biofertiliser consisting of *Methylobacterium symbioticum* (BlueN), together with 0.2 l ha^{-1} Cropvit FeMo, was used in both treatments. As the farm is conducting certified organic production, no chemical fungicides or insecticides were used during the experiment. Black barley was harvested at the full grain maturity stage, in the middle of July in both years. A plot harvester was used for this purpose.

Assessment of grain parameters

H. v. *rimpaui* grain samples for chemical analysis were collected from each plot during harvest. Prior to the evaluation of macroelements, the grains were subjected to wet mineralisation in concentrated sulphuric acid. The macronutrient composition of the grain samples was determined by the vanadium–molybdenum method for phosphorus, flame photometry for potassium and calcium, and Atomic Absorption Spectrometry (AAS) method for magnesium. The analysis of Mn, Fe, Zn and Cu commenced with mineralisation in a mixture of concentrated hydrochloric and nitric acids at a 1:3 ratio. Subsequently, the content of the elements was determined by the AAS method on a Varian AA240FS spectrometer (Kozera et al. 2023).

The total protein content was calculated on the basis of the nitrogen content in grains determined by the Kjeldahl method. The analysis of amino acids in the black barley grain commenced with the acid hydrolysis of the grain using 6 M hydrochloric acid at 110°C. The composition of the amino acids was then determined using an AAA-400 amino acid analyser (Kozera et al. 2023). The total amino acid content (AA_{total}), along with the sums of EAA (essential amino acids) and NEAA (non-essential amino acids), was calculated from the determined amino acid profiles. Moreover, the integrated index of exogenous amino acids (EAAI) was calculated from the formula (FAO, 2013):

$$EAAI = (c_1/c_{01} \text{ x } c_2/c_{02} \text{ x } \text{....x } c_n/c_{0n})^{1/n},$$

where: $c_1, c_2, ..., c_n$ – the content of successive exogenous amino acids in the examined protein,

$c_{01}, c_{02}, ..., c_{0n}$ – the content of successive exogenous amino acids in the reference protein (chicken egg protein).

The total polyphenol content of the grain was evaluated using the Folin-C Colorimetric AOAC SMPR method 2015.009 (AOAC, 2015). The total fibre content in black barley grain was determined using an enzymatic method, following the procedure in accordance with the AOAC 991.43 reference method (AOAC, 2023). The AOAC 995.16 method was used to analyse the β-glucan content (AOAC, 2023) and the AOAC 996.11 method (AOAC, 2023) was employed to determine the starch content.

Statistical analysis

The fundamental statistical descriptor (mean) was calculated from the data obtained. The Shapiro-Wilk test was employed to ascertain the normality of the data distribution. A MANOVA was conducted to determine the influence of several factors (soil tillage, biofertilisation and year of study) on the analysed grain parameters. In the event of significant differences, a Tukey *post hoc* test was employed (at level α = 0.05). The statistical significances (*p*-values) are presented in the Supplementary Materials (Tables S1 and S2). The Pearson's correlation coefficient served to ascertain the relationships between chemical grain composition and grain size parameters. The statistics were computed using the Statistica 13.0 PL package (Statsoft, Poland). The graph illustrating the content of Cu, Mn, Zn, and Fe in the *H. vulgare* var. *rimpaui* grain was prepared using Grapher 21 (Golden Software, Golden, CO, the USA).

RESULTS

The effect of soil tillage

The contents of phosphorus (P), potassium (K), and magnesium (Mg) in *Hordeum vulgare* var. *rimpaui* grain were significantly influenced by the soil tillage system (Supplementary Materials, Tables S1, S2 and Table 2).

Table 2

The contents of P, K, Mg, and Ca in grain and grain density of the *H. vulgare* var. *rimpaui* in 2023 and 2024

Year	Soil tillage	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Grain density ($kg\ hl^{-1}$)
2023	ploughing	3.96 *a*	6.98 *a*	0.929 *a*	0.635 *a*	54.53 *a*
	reduced	3.54 *b*	6.54 *a*	0.918 *a*	0.640 *a*	55.15 *a*
2024	ploughing	4.09 *a*	6.49 *a*	0.860 *a*	0.091 *a*	50.67 c
	reduced	3.98 *a*	6.21 *a*	0.827 *a*	0.086 *a*	51.74 *b*
Mean	ploughing	4.02 *A*	6.73 *A*	0.894 *A*	0.363 *A*	52.60 *B*
	reduced	3.76 *B*	6.38 *B*	0.872 *B*	0.363 *A*	53.45 *A*

a, *b*, *A*, *B* – values with different letters significantly differ at $p<0.05$

In both study years and on average across years, grain from ploughed plots was characterized by higher contents of P, K, and Mg than from plots with reduced tillage. In contrast, calcium (Ca) content remained unaffected by the tillage method, indicating relatively stable accumulation of this element irrespective of soil management practices.

Grain density was significantly affected by both the year of study and soil tillage. Higher grain density was observed in 2023 than in 2024. Reduced tillage positively influenced grain density in 2024 and with respect the mean values for the two-year period, whereas no significant differences between the tillage methods were observed in 2023.

The analysis of micronutrient contents in *H., vulgare* var. *rimpaui* grain revealed a significant effect of the year of study (Supplementary Materials, Tables S1, S2 and Figure 1). In 2024, the grains contained more copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) compared with those harvested in 2023. Moreover, in 2024, soil tillage significantly affected the concentrations of Cu, Mn, and Fe. Higher contents of Cu and Fe were observed in grains obtained under ploughing, whereas reduced tillage promoted the accumulation of Mn. In contrast, zinc content was not significantly influenced by the soil tillage methods, indicating a relatively stable response of this element to soil management practices.

The soil tillage methods were found to affect the content of total phenols in black barley grains (Supplementary Materials, Tables S1, S2 and Table 3).

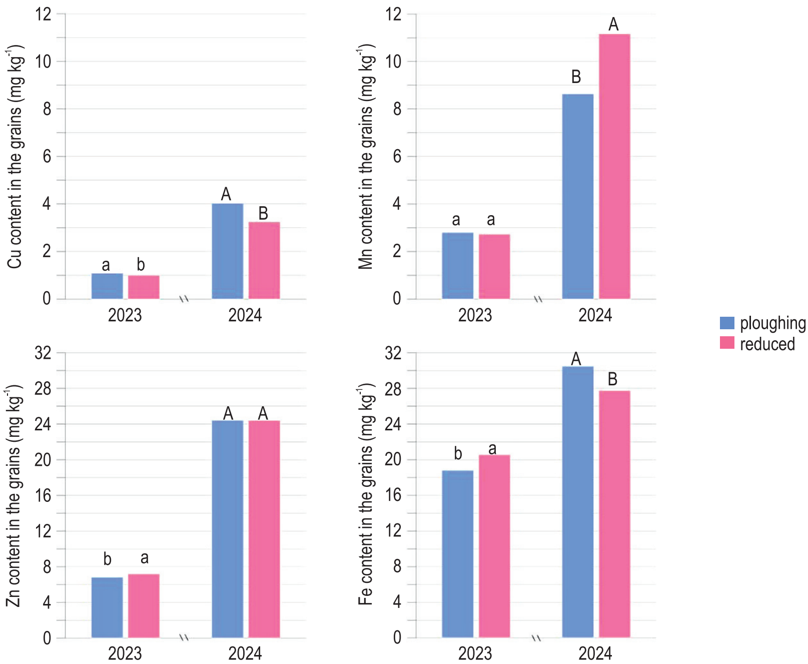

Fig. 1. The content of Cu, Mn, Zn, and Fe in the *H. vulgare* var. *rimpaui* grain in 2023 and 2024: *a*, *b*, *A*, *B* – values with different letters significantly differ at p<0.05

Table 3

The total phenols content (g GAE kg^{-1}) in 2023 and 2024

Soil tillage	Years		
	2023	2024	mean
Ploughing	2.266 *a*	2.268 *a*	2.267 *a*
Reduced	2.224 *b*	2.216 *b*	2.220 *b*

a – *b* – values with different letters significantly differ at p<0.05.

Ploughing contributed to a higher concentration of total phenols, with the effect being observed in both study years.

In 2024, soil tillage significantly affected both the protein content and protein quality of *Hordeum vulgare* var. *rimpaui* grain (Supplementary Materials, Tables S1, S2 and Table 4). Grain from mouldboard ploughing was characterised by a higher protein content compared with reduced tillage. Similar trends were observed for the content of essential amino acids (EAA), non-essential amino acids (NEAA), total amino acids (AA_{total}), as well as for the essential amino acid index (EAAI).

Table 4

The protein content in grain of *H. vulgare* var. *rimpaui* and protein quality in 2024

Soil tillage	Protein content (% DM)	EAA ($g\ kg^{-1}$ DM)	NEAA ($g\ kg^{-1}$ DM)	AA_{total} ($g\ kg^{-1}$ DM)	EAAI (% DM)
Ploughing	9.56 *a*	245.8 *a*	437.9 *a*	683.8 *a*	49.57 *a*
Reduced	9.18 *b*	239.3 *b*	418.6 *b*	657.8 *b*	47.81 *b*

EAA – content of essential amino acids, NEAA – content of non-essential amino acids, AA_{total} – content of amino acids, EAAI – essential amino acids index, *a* – *b* – values with different letters significantly differ at $p<0.05$

The effect of *Methylobacterium symbioticum* biostimulant

Biostimulation with *Methylobacterium symbioticum* significantly affected the starch content in *Hordeum vulgare* var. *rimpaui* grain in both years of the study (Supplementary Materials, Tables S1, S2 and Table 5). In 2023, the application of the biostimulant resulted in pronounced differences among treatments, with the double application leading to the highest starch content compared with the control and single application. In 2024, the effect of biostimulation on starch content was less pronounced, although grains from plants treated with *M. symbioticum* tended to exhibit higher starch levels than the control.

Table 5

The content of starch in grain of the *H. v. rimpaui* in study years

Year	Biostimulation	Starch content (% DM)
2023	BlueN 1x	57.18 *d*
	BlueN 2x	60.48 *a*
	control	58.01 *cd*
2024	BlueN 1x	59.03 *abc*
	BlueN 2x	59.93 *ab*
	control	58.61 *bcd*

a – *d* – values with different letters significantly differ at $p<0.05$

The effect of interaction between soil tillage and biostimulant

The interaction between soil tillage and biostimulation significantly affected protein content and protein quality parameters in *Hordeum vulgare* var. *rimpaui* grain in 2023 (Supplementary Materials, Tables S1, S2 and Table 6). Under reduced tillage, the application of *Methylobacterium symbioticum* significantly increased the content of protein and the contents of essential amino acids (EAA), non-essential amino acids (NEAA), total amino acids (AA_{total}) as well as the essential amino acid index (EAAI) compared

Table 6

The protein content in grain and protein quality of *H. vulgare* var. *rimpaui* in 2023

Soil tillage	Biostimulation	Protein content (% DM)	EAA (g kg^{-1} DM)	NEAA (g kg^{-1} DM)	AA $_{total}$ (g kg^{-1} DM)	EAAI (% DM)
Ploughing	BlueN 1x	9.57 *ab*	274.4 *ab*	491.1 *ab*	765.4 *ab*	55.21 *ab*
	BlueN 2x	9.81 *ab*	282.9 *ab*	495.9 *ab*	778.8 *ab*	59.75 *ab*
	control	9.88 *ab*	284.2 *ab*	499.4 *ab*	783.6 *ab*	56.96 *ab*
Reduced	BlueN 1x	10.36 *a*	301.0 *a*	522.6 *a*	823.5 *a*	59.77 *a*
	BlueN 2x	9.52 *ab*	275.5 *ab*	499.8 *ab*	775.3 *ab*	55.19 *ab*
	control	9.05 *b*	261.1 *b*	482.3 *b*	743.4 *b*	53.13 *b*

EAA – content of essential amino acids, NEAA – content of non-essential amino acids, AA_{total} – content of amino acids, EAAI – essential amino acids index, *a* – *b* – values with different letters significantly differ at p<0.05.

with the control. The most pronounced effects were observed following a single application of the biostimulant under reduced tillage conditions. In contrast, under ploughing, biostimulation did not result in significant differences in any of the analysed protein-related parameters.

The interaction between soil tillage and biostimulation significantly affected the dietary fibre content in *Hordeum vulgare* var. *rimpaui* grain (Supplementary Materials, Tables S1, S2 and Table 7). In general, higher

Table 7

The dietary fibre content of *H. vulgare* var. *rimpaui* grain, mean for 2023-2024

Soil tillage	Biostimulation	Dietary fibre (% DM)
Ploughing	BlueN 1x	33.32 *ab*
	BlueN 2x	31.75 *c*
	control	32.64 *bc*
Reduced	BlueN 1x	33.71 *ab*
	BlueN 2x	34.10 *a*
	control	32.77 *bc*

a – *c* – values with different letters significantly differ at p<0.05

fibre content was recorded under reduced tillage than under ploughing. Within this tillage system, biostimulation with *Methylobacterium symbioticum* resulted in an increase in dietary fibre content, with the double application resulting in the highest values. Under ploughing, the effect of biostimulation on dietary fibre content was less pronounced, and the values obtained for the biostimulant treatments did not differ substantially from those of the control.

The interaction between soil tillage and biostimulation significantly affected the *β*-glucan content in *Hordeum vulgare* var. *rimpaui* grain in 2023 (Supplementary Materials, Tables S1, S2 and Table 8). Under reduced tillage, the application of *Methylobacterium symbioticum* resulted in higher *β*-glucan content compared with the control. Both single and double applications of the biostimulant promoted *β*-glucan accumulation, with the highest values observed following the single application. In contrast, under ploughing, biostimulation did result in significant differences in *β*-glucan content compared with the control.

Table 8

The *β*-glucan content in grain of *H. vulgare* var. *rimpaui* in 2023

Soil tillage	Biostimulation	*β*-glucan content (% DM)
Ploughing	BlueN 1x	5.16 *bc*
	BlueN 2x	5.19 *bc*
	control	5.34 *abc*
Reduced	BlueN 1x	5.54 *a*
	BlueN 2x	5.43 *ab*
	control	5.07 *c*

a – *c* – values with different letters significantly differ at $p<0.05$

The correlation between chemical composition of grain, grain yield and grain parameters

The statistical analysis revealed a statistically significant correlation between the content of P, K, Mg, Ca, Cu, Zn, Fe, Mn, protein (TP), EAA, NEAA, AA, EAAI, and *β*-glucan in *H.* v. *rimpaui* grain and its grain yield, thousand grain weight (TGW), and grain density (Table 9). The present study found no significant correlations between starch, dietary fibre, total phenol content, and grain yield, TGW, or grain density of black barley (data

Table 9

Pearson's correlation coefficients between chemical composition of grain, grain yield, thousand grain weight and grain density

Specification	P	K	Mg	Ca	Cu	Zn	Fe	Mn	TP	EAA	NEAA	AA_{total}	EAAI	*β*-glucan
Grain yield[+]	-0.21[#]	0.42	0.75	0.93	-0.89	-0.93	-0.88	-0.91	0.36	0.80	0.87	0.86	0.84	-0.24
TGW[+]	-0.44	ns	0.61	0.90	-0.89	-0.90	-0.86	-0.85	ns	0.65	0.77	0.73	0.69	-0.26
Grain density	-0.39	ns	0.56	0.87	-0.88	-0.87	-0.86	-0.80	ns	0.60	0.70	0.67	0.64	-0.28

TGW – thousand grain weight, TP – total protein content, EAA – content of essential amino acids, NEAA – content of non-essential amino acids, AA_{total} – content of amino acids, EAAI – essential amino acids index. [#] Presented correlation coefficients are significant at $p<0.05$, ns – correlation coefficient insignificant. [+] Presented in the article Błaszczyk et al. 2026.

not presented). As can be observed, grain yield, TGW and grain density exhibited very strong (r>0.8) or strong (r>0.6) positive correlation with the content of Mg, Ca, EAA, NEAA, AA_{total}. A weak but positive correlation was observed with K and total protein (TP) content.

Conversely, a very strong negative correlation was identified between grain yield, TGW, grain density and the contents of Cu, Zn, Fe, and Mn (r<-0.8). For P and β-glucan content, this relationship was weakly negative.

DISCUSSION

Soil tillage and biofertilisation influenced the content of macro- and microelements, protein content and its quality, as well as starch and functional ingredients analysed in the study. Nonetheless, the influence of variable meteorological conditions throughout the growing seasons proved pivotal.

The study observed a tendency for higher contents of Mg and Ca in barley grains in 2023 than in 2024. This may be attributable to more favourable weather conditions for grain formation (Tables 1, 2). In 2023, there was a higher thousand grain weight than in 2024 – 36.6 g and 29.1 g, respectively (Błaszczyk et al. 2026) as well as greater grain density (Table 2); there were significant positive correlations between those grain traits and contents of Mg and Ca (Table 9), and significant positive correlations between those grain traits and contents of Mg and Ca were proved.

Ca exhibited particularly high variability and strong correlations, which is consistent with its xylem-dependent transport and sensitivity to plant water status (Kabir et al. 2025). Reduced soil moisture likely constrained Ca movement to spikes, explaining the higher grain Ca concentrations observed under lower drought stress in the first year (Tables 1, 2). Mg demonstrated reduced responsiveness to water availability (Tables 1, 2), indicating its mobility within the phloem and its capacity for redistribution within the plant. Nevertheless, drought can still reduce grain Mg content through limitations in root uptake and remobilization (Mengutay et al. 2013). The study revealed that potassium and phosphorus concentrations exhibited minimal sensitivity to variations in weather conditions (Tables 1, 2, 9). The weak negative relationship between phosphorus and grain size supports the view that grain P accumulation is largely controlled by remobilisation from vegetative tissues, a process potentially enhanced by drought-induced senescence (El Mazlouzi et al. 2022).

The micronutrient composition of black barley grain was found to be significantly affected by the study year. Concentrations of Cu, Mn, Zn, and Fe were found to be significantly elevated in the second year in comparison with 2023 (Figure 1). This observation is concomitant with the presence of a strong negative correlation between micronutrient levels and grain size parameters (Table 9). This finding suggests the occurrence of dilution effects in larger, more developed grains, which is consistent with evidence that micronutrients in *H.* v. *rimpaui* are primarily localised in the outer grain layers, which represent a greater proportion of less developed grains (Błaszczyk et al. 2025). Furthermore, grain density was found to be influenced by weather conditions (Table 2), with higher values observed in 2023 than in 2024. These findings are consistent with earlier reports which indicated that limitations caused by water stress on the processes of grain development and filling result in a reduction in barley grain yield, and an increase in grain size (Hoyle et al. 2020).

Grain density was found to be significantly higher under reduced tillage, a finding that is consistent with previous observations (Adil et al. 2024) and likely reflects improved soil moisture retention and water availability, which in turn favours grain development. The larger grains produced under this system were associated with lower concentrations of P, K, and Mg (Table 2), which supports the hypothesis of a nutrient dilution effect (Calderini et al. 2003). Tillage-related differences in micronutrient composition, particularly in the second year, were comparable to earlier findings showing higher Cu and Fe under conventional tillage and higher Mn under reduced tillage (Woźniak et al. 2014*a*). These patterns have been hypothesised to reflect discrepancies in mineral uptake, which are driven by soil moisture conditions, organic matter mineralisation rates, and nitrogen availability. Collectively, these factors influence micronutrient accessibility to plants.

The findings of the present study demonstrated that ploughing resulted in an increase in the total phenol concentrations present within the grains of black barley (Table 3). As Ma et al. (2021) asserted, the agronomic management has the capacity to affect the total phenol content. Ploughing has been shown to reduce of water available in the soil (Adil et al. 2024). This, in turn, can induce water stress in plants, which has been identified as one of the primary triggers to stimulate plant species to produce phenols, which have been shown to help overcome stressful conditions (Nowak et al. 2023).

Meteorological conditions modulated the effects of soil tillage and biostimulation on protein accumulation in black barley grain (Table 4, 6). Under adverse weather conditions, higher protein content and quality were observed in ploughing tillage, likely associated with lower grain density (Table 2) and greater nitrogen accumulation relative to denser grains where dilution effects occur (Magliano et al. 2014). In the first year, under more favourable weather conditions, the impact was exerted by the interplay of soil tillage and biostimulation. Ploughing likely enhanced nitrogen availability through accelerated decomposition and mineralisation of organic N, supporting a stable nutrient supply (Sandhu et al. 2022). In reduced tillage, the enhanced efficacy of *M. symbioticum* in N-fixation and its provision to plants is likely associated with its augmented capacity for fixation under conditions of diminished N availability to plants, which might occur in this tillage system (Valente et al. 2024).

Starch content in *H.* v. *rimpaui* grain tended to increase in both years following double bacterial application (Table 5). This response may be linked to phytohormone production by *Methylobacterium* spp., particularly cytokinins (Palberg et al. 2022), which can regulate starch biosynthesis and accumulation (Zhang et al. 2022). The elevated starch levels observed may therefore reflect enhanced bacterial activity. However, contrasting reports indicate no significant effect of *M. symbioticum* on grain starch (Bolla et al. 2025), suggesting that metabolic responses are strongly modulated by the genotype, environmental conditions, and agronomic management (Sanjenbam et al. 2022).

The content of dietary fibre and β-glucan in black barley grain (Tables 7, 8) was found to be influenced by the interaction between soil tillage and biostimulation; however, the effect on β-glucan was only evident in 2023. Under the conditions of this study, both compounds tended to be higher under reduced tillage, a finding that aligns with the reports of an increased fibre accumulation in cereals from the reduced tillage (Woźniak et al. 2014*b*). Currently, there is a paucity of data on the impact of *Methylobacterium symbioticum* on dietary fibre, including β-glucan levels in grains. A long-term study on the content of β-glucan in barley demonstrated that its content is primarily influenced by weather conditions and genotype, with N fertilization exerting either no or a negligible effect (Khaleghdoust et al. 2024). The synergistic effect of tillage and fertilisation on β-glucan was found in research conducted by Bogale et al. (2025), in which the application of bio-algae fertiliser to barley grown in reduced tillage increased its content. The authors ascribed this effect to the synergistic influence of the higher nutrient and water availability, better soil structure, and enhanced plant metabolism. Given the inconsistent effects of sole factors and the paucity of data on the influence of *M. symbioticum* on dietary fibre and β-glucan, further research is needed to elucidate that point.

CONCLUSIONS

The study revealed that soil tillage affected the content of macro- and microelements, protein and protein quality, and total phenols in *H.* v. *rimpaui* grain. A higher content of P, K, Mg, as well as total phenols was determined in grains from ploughing tillage. In contrast, significantly higher grain density was observed in grain from reduced tillage. In less favourable weather conditions for barley growth, ploughing also stimulated the Cu and Fe content, as well as protein content, EAA, NEAA, AA_{total}, and EAAI. The effect of the *M. symbioticum*-based biostimulant on grain quality was relatively weaker. It was only shown that double application promoted the accumulation of starch in the barley grain.

The interaction between soil tillage and biostimulant significantly influenced protein content and quality, as well as dietary fibre and *β*-glucan content. Under favourable weather conditions of the growing season, a single application of *M. symbioticum* in reduced tillage stimulated the protein content, and EAA, NEAA, AA_{total}, EAAI. Only in reduced tillage did the application of biostimulant have a beneficial effect on *β*-glucan content in grain. Mg and Ca content, as well as EAA, NEAA, AA_{total}, and EAAI, exhibited a positive correlation with grain size (TGW and grain density). Conversely, micronutrients showed a negative correlation.

It can be concluded that reduced tillage can enhance grain quality parameters, especially in combination with *Methylobacterium symbioticum* application. This suggests the possibility of implementing these elements of technology in organic cultivation of *H.* v. *rimpaui*.

Author contributions

Conceptualization – K.B. and M.S., methodology – K.B. and M.S., software – K.B., validation – K.B. and M.S., formal analysis – K.B. and M.S., investigation – K.B., and M.S., resources – K.B. and M.S, data curation – K.B. and M.S., writing – original draft, K.B., writing – review & editing, K.B. and M.S., visualization – K.B., supervision – K.B. and M.S. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Supplementary material

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