



Adapted and yet evolving: Long-term agronomic, nutritional and genetic assessment of the buckwheat variety Čebelica (*Fagopyrum esculentum* Moench) under Central European conditions

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ABSTRACT

Common buckwheat is valued for its adaptability, nutritional quality and potential to enhance sustainable cropping systems. However, yield instability and limited data on long-term genetic and nutritional stability remain key challenges. To address this, we conducted a nine-year field study (2012–2020) of the Slovenian variety Čebelica under Central European conditions, integrating agronomic, environmental, nutritional and genetic analyses. Grain yields varied widely (498.8–1418.0 kg ha⁻¹), primarily due to climatic differences, with the precipitation as the dominant factor ($F = 4.324$, $P = 0.027$). Crop rotation explained 39.9 % of yield variation, with highest productivity following oats, maize or red clover. In contrast, protein (10.1–12.5 %) and fat (2.6–3.2 %) contents were highly stable ($CV < 6$ %), indicating robust physiological regulation. Micro-nutrient trends showed increasing iron and zinc, and decreasing sodium. Redundancy analysis revealed that precipitation, soil phosphorus and solar radiation explained over 75 % of trait variation, highlighting complex environmental control. Solar radiation influenced a trade-off between protein/fat and total phenolic content, suggesting metabolic shifts under varying light. Genetic analysis using nine SSR loci showed high diversity ($H_o = 0.863$) and a significant genetic shift between 2014 and 2015. Principal coordinate analysis and Mantel test ($r = 0.556$, $P = 0.005$) confirmed temporal genetic structuring, indicating ongoing adaptation despite the use of a single variety. These findings demonstrate that Čebelica is a nutritionally stable yet genetically dynamic variety, well suited for climate-resilient agriculture. This work offers rare, long-term insights into the ecological, agronomic and genetic dynamics of an underutilised but valuable crop.

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) has gained renewed interest in recent years due to its agronomic versatility, nutrient richness and suitability for low-input and climate-resilient cropping systems. Although classified as a pseudocereal, buckwheat offers several advantages over conventional cereals, including a short growth cycle (70–90 days), adaptability to marginal soils, minimal fertiliser requirements and natural weed suppression through allelopathic effects (Vieites-Álvarez et al., 2024). These characteristics make it an attractive component in diversified crop rotations, especially under the increasing pressure of biodiversity loss, soil degradation and climatic instability (Small, 2017; Verza et al., 2025).

The nutritional profile of buckwheat is particularly remarkable. It provides high-quality proteins rich in lysine and arginine, amino acids

that are usually limited in real grains, as well as significant amounts of bioactive compounds such as rutin, quercetin and chlorogenic acid (Rao and Poonia, 2023; Sofi et al., 2023; Sonawane et al., 2024). These compounds contribute to its antioxidant, anti-inflammatory and anti-diabetic properties (Joshi et al., 2025). In addition, buckwheat fortifies essential micronutrients such as iron, zinc, selenium and magnesium, elements that are increasingly being considered in bio-fortification strategies to combat hidden hunger in cereal-based diets (Sofi et al., 2023). As a result, buckwheat is becoming increasingly popular as a functional food and as an ingredient in gluten-free products, nutraceuticals and plant-based protein alternatives (Pirzadah et al., 2020; Jin et al., 2022).

Despite these strengths, the widespread adoption of buckwheat is limited by its notoriously unstable yields, which are often attributed to indeterminate flowering, self-incompatibility and high sensitivity to

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abiotic stress during flowering and grain filling (Chrungoo and Chettry, 2021). Conventional breeding efforts have produced relatively few varieties with reliable performance in different agroecological zones, exacerbating the limited commercial expansion of the crop (Chettry and Chungoo, 2021). This unpredictability of yields is particularly problematic in regions already facing climate-related agricultural risks.

A largely overlooked aspect of buckwheat cultivation is its interaction with previous crops in crop rotation systems (Vieites-Álvarez et al., 2024). While buckwheat is often used as a green manure or cover crop due to its rapid biomass accumulation and ability to suppress weeds (McKenzie-Gopsill, 2025), little is known about how different previous crops influence its yield and grain composition. Understanding these interactions is critical to optimising crop rotation and ensuring the sustainability of cropping systems that include buckwheat.

Another unexplored dimension is the genetic stability of buckwheat under repeated cultivation. As an obligate outcrossing species with high genetic variability, buckwheat is susceptible to temporal genetic shifts due to selection pressure, environmental variability or changes in pollinator dynamics (Singh et al., 2020; Miyashita et al., 2023). While population-level genetic diversity has been documented in landraces and wild varieties (Pipan et al., 2023; Balázová et al., 2024), few longitudinal studies have investigated how continuous cultivation over time affects the genetic structure of modern cultivars. Given the potential effects of genetic drift and environmental filtering, long-term studies are crucial to assess whether buckwheat maintains its phenotypic and genomic integrity across seasons.

To fill these knowledge gaps, we conducted a nine-year field study (2012–2020) with the Slovenian buckwheat variety Čebelica under Central European conditions. Our objectives were: (1) to assess the interannual stability of agronomic and nutritional traits; (2) to evaluate the effects of previous crops on yield and quality parameters; and (3) to monitor temporal genetic changes using microsatellite markers (SSR). By integrating agronomic, compositional, molecular and environmental analyses, this study provides unique long-term insights into the adaptability and resilience of buckwheat. These results are of direct relevance for climate-smart agriculture, crop rotation planning and variety improvement in temperate climates.

2. Materials and methods

2.1. Experimental site, plant material, and crop rotation

A long-term field trial was conducted over nine consecutive growing seasons (2012–2020) with the Slovenian buckwheat variety (*Fagopyrum esculentum* Moench) Čebelica at the experimental field station of the Agricultural Institute of Slovenia in Jablje (46°08'33.78" N, 14°33'22.72" E; 302 m a.s.l.). The site is characterised by Umbrian Planosol soils with a silty loam texture and a bulk density of 1.61 g cm⁻³. Buckwheat was

grown each year as the main summer crop, sown in May–June and harvested between September and October. The field design included annual variations of previous crops, including cereals (wheat, oat, barley, maize), grasses (annual ryegrass) and legumes (red clover), which allowed evaluation of the effects of crop rotation on agronomic and nutritional performance (Table 1). Seeds from previous seasons were used for sowing each year, as the Agricultural Institute of Slovenia performs maintenance breeding for the Čebelica variety. Germination tests were carried out before sowing to ensure the viability of seeds and to optimise the sowing density (400 seeds m⁻²). The field trials were set up in triplicate with plot size of 100 m² under standardised agronomic practise without fertiliser treatment. After harvest, the seeds were air-dried in a ventilated wooden drying chamber at room temperature until the moisture content dropped below 11 %. Soil properties were monitored annually in the top 25 cm, including pH, organic matter and plant-available phosphorus (P₂O₅) and potassium (K₂O) to account for nutrient status and interannual variability. In addition, meteorological data, including cumulative monthly rainfall, mean daily air temperature, relative humidity and solar radiation, were obtained from the Slovenian Environment Agency (ARSO) via the Jablje meteorological station, which is located in the immediate vicinity of the experimental plots (Supplementary Table S1).

2.2. Grain yield and compositional analysis of buckwheat grains

2.2.1. Measurement of grain yield and sample preparation

Grain yield (GY) was recorded at physiological maturity for each plot in all years from 2012 to 2020. After harvest, grains were cleaned and weighed, and yield was calculated in kilogrammes per hectare (kg ha⁻¹) based on dry weight. All yields were adjusted for moisture content to a standardised level of 11 %. After harvest, representative grain samples (~2 kg per replicate plot) were collected for compositional analysis. Subsamples were homogenised and ground to a fine powder using a Retsch MM 400 ball mill (30 Hz, 60 s) prior to all chemical analyses. All tests were performed in triplicate.

2.2.2. Protein and fat analysis

The crude protein content was determined according to the Kjeldahl method (ISO 5983–2) (Goyal et al., 2022), whereby the nitrogen content was converted into protein using a factor of 6.25. The procedure included digestion with concentrated sulphuric acid, distillation with sodium hydroxide and titration with standard hydrochloric acid in the presence of a mixed indicator. The crude fat content was determined by Soxhlet extraction with petroleum ether as solvent (Thiex et al., 2003). 5.0 g of the ground sample was extracted for six hours. The solvent was removed by evaporation and the remaining fat residue was dried and weighed. The results were expressed as a percentage of dry weight (% w/w).

Table 1
Crop rotation patterns and environmental parameters base on an average 6-month growing period from May to October (2012–2020) at the Jablje field site in Slovenia.

	2012	2013	2014	2015	2016	2017	2018	2019	2020
Previous crop	Wheat/annual ryegrass	Corn/barley	Red clover/wheat	Annual ryegrass/barley	Annual ryegrass/barley	Wheat	Oat/red clover	Wheat/annual ryegrass	Wheat/barley
Rainfall (mm)	132.93	96.73	137.03	105.30	119.87	109.67	141.62	133.90	151.88
Temperature (°C)	17.08	16.94	16.50	17.21	16.54	17.05	17.65	17.38	16.73
Relative humidity (%) RH)	81.89	81.05	83.87	83.33	84.94	81.50	84.98	81.26	82.41
Solar radiation (W m ⁻²)	219.97	292.55	274.65	273.21	274.32	294.94	285.35	281.12	180.05
pH	7.28	7.08	7.33	7.35	7.41	7.24	7.11	7.28	7.30
Organic matter (%)	3.35	3.43	3.67	3.38	3.30	3.52	3.34	3.35	3.37
Available P (mg P ₂ O ₅ 100 ⁻¹ g ⁻¹)	19.50	19.67	19.00	18.67	18.89	15.56	13.33	19.50	16.87
Available K (mg K ₂ O 100 ⁻¹ g ⁻¹)	15.17	18.67	19.00	17.50	15.33	17.78	14.78	15.17	16.00

P, phosphorus; K, potassium.

2.2.3. Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) colourimetric method (Singleton et al., 1999). Phenols were extracted from the homogenised samples with 70 % ethanol and 10 % methanol (v/v), followed by vortexing and ultrasonic extraction (three cycles). The extracts were centrifuged ($15,000 \times g$, 5 min) and filtered through $0.45 \mu\text{m}$ PTFE syringe filters. A reaction mixture consisting of 30 μL extract, 0.5 mL 10 % FC reagent and 0.5 mL 10 % Na_2CO_3 was incubated and the absorbance was measured spectrophotometrically. TPC was expressed as mg gallic acid equivalents (GAE) per gram (mg GAE g^{-1}) dry weight.

2.2.4. Elemental analysis (macro- and micronutrients)

The elemental composition of the grains was analysed using inductively coupled plasma mass spectrometry (ICP-MS). A total of 14 elements were measured, six of which were macro-nutrients: magnesium (Mg), phosphorus (P), sulphur (S), potassium (K), calcium (Ca) and sodium (Na), and eight micro-nutrients: vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn) and molybdenum (Mo). For each sample, 250 mg of homogenised grain was digested in a mixture of 6.0 mL nitric acid (65 %, Suprapur) and 2.0 mL hydrogen peroxide (30 %, Suprapur) using an Ethos UP microwave digestion system (Milestone, Italy). After digestion, the volume was made up to 50 mL with high purity deionised water. Elemental concentrations were measured using an Agilent ICP-MS 7900 instrument (Tokyo, Japan) equipped with an octopole reaction system. Helium was used as collision gas (5.0 mL min^{-1} in He mode, 10.0 mL min^{-1} in HEHe mode). Calibration was performed using IV-STOCK-50 multi-element standards (Inorganic Ventures, USA), with the P and S standards added separately. The accuracy was validated with two certified reference materials: NIST SRM 1573a (tomato leaves) and NIST SRM 1547 (peach leaves). Results were expressed on a dry weight basis as g kg^{-1} (macro-nutrients) or mg kg^{-1} (micro-nutrients).

2.2.5. DNA extraction and genetic analysis

Genomic DNA was extracted in each year (2012–2020) from ten individual plants from which a young, healthy leaf tissue was taken. The DNeasy Plant Pro Kit (Qiagen) was used without the addition of PS buffer in the homogenisation step, as described in Pipan et al. (2018). Extracted DNA was checked for quality and quantity using a fluorimeter (Qubit 3.0; ThermoFisher Scientific, Waltham, MA, USA) and diluted to a final concentration of $12.6 \text{ ng } \mu\text{L}^{-1}$. A set of nine genus-specific SSR markers was used to analyse the genetic/allelic diversity of the genotypes studied between years. The markers were selected based on their specificity, high polymorphism and representative distribution in the genome and therefore come from two denomination groups: *Fem* (Iwata et al., 2005) (five markers: *Fem1303*, *Fem1322*, *Fem1407*, *Fem1582* and *Fem1840*) and *GB-FE* (Ma et al., 2009) (four markers: *GB-FE025*, *GB-FE035*, *GB-FE054* and *GB-FE121*). Depending on the primer pair, PCR reactions were performed under touch-down conditions as described in Pipan and Meglič (2019) (Supplementary Table S2). Fragment analysis was performed with a genetic analyser (3500; Applied Biosystems, Waltham, MA, USA) and allele sizes were determined by comparison with an internal size standard (GeneScan-500 ROX; Applied Biosystems) using GeneMapper 6.0 software (Applied Biosystems) as described in Pipan et al. (2023).

2.3. Data analysis

2.3.1. Agronomic trait analysis and environmental stability

All statistical analyses were performed in R (version 4.2.2; R Core Team, 2022). First, we built linear mixed-effects models using the lme4 package (Bates et al., 2025) to assess the effects of year, previous crop and their interaction on each measured trait. In this framework, 'Year' was treated as a random effect because it represents a random sample of environmental conditions from a larger population, allowing for

generalization beyond the specific years studied and providing robust estimates of variance components for stability assessment (Piepho et al., 2003). The model structure included random intercepts for these factors to account for variability between cropping seasons, crop rotations and their interactions: $\text{Response} \sim 1 + (1 | \text{year}) + (1 | \text{previous crop}) + (1 | \text{year:previous crop})$. Variance components were estimated using Restricted Maximum Likelihood (REML) and the proportion of total variance explained by each component was calculated to assess the relative contributions of each source to variability. To test the significance of the random effects, likelihood ratio tests (LRTs) were performed by comparing the full model with nested models, each of which excluded a random effect. These comparisons were based on maximum likelihood (ML) estimation, and significance was determined at $P < 0.05$. To investigate multivariate patterns in the relationships between years, crop rotations and traits, we performed principal component analysis (PCA) using the packages 'FactoMineR' (Lê et al., 2008) and 'factoextra' (Kassambara and Mundt, 2017).

To directly address the relationship between trait variation and environmental parameters, and to complement the variance partitioning approach, we performed a redundancy analysis (RDA) with the package vegan (Oksanen et al., 2007). The trait data were standardised (z-scores) and the response and explanation matrices were structured accordingly. A forward selection procedure was used to identify the most significant environmental predictors. This was done using the 'ordiR2step()' function, whereby the R^2 was adjusted to prevent overfitting (Blanchet et al., 2014). The statistical significance of the full model and the selected variables was assessed at 999 permutations.

The stability of traits in different environments (defined by combinations of year and previous crop) was assessed using three univariate stability indices: coefficient of variation (CV), environmental variance (EV) and mean absolute deviation (MAD). The CV, calculated as $\text{CV} = (\sigma / \mu) \times 100$, where σ is the standard deviation and μ is the mean, represents the relative variability. However, as the CV can be influenced by changes in the mean, we tested its reliability using Taylor's Power Law (TPL). A significant positive correlation between $\log_{10}(\text{variance})$ and $\log_{10}(\text{mean})$ would indicate that the CV is dependent on the mean, which could affect its usefulness as a stability measure (De Notaris et al., 2023). The EV, calculated as $\text{EV} = \sum (Y_i - \bar{Y})^2 / (n - 1)$, where Y_i is the trait value in the i th environment and \bar{Y} is the overall mean, quantifies the dispersion independent of the mean. The MAD, defined as $\text{MAD} = \sum |Y_i - \bar{Y}| / n$ offers a robust alternative to variance-based measures. To assess the response of traits to environmental variation, we applied Finlay-Wilkinson regression (Finlay and Wilkinson, 1963). The mean values of the traits for each environment were regressed against the overall mean value of the environment: $Y_{ij} = b_0 + b_1 \bar{Y}_j + e_{ij}$. The regression slope (b_1) reflects adaptability, with values close to 1 indicating average sensitivity, while $b_1 < 1$ indicates greater stability (low sensitivity) and $b_1 > 1$ indicates increased sensitivity to environmental fluctuations (Becker and Leon, 1988).

2.3.2. Microsatellite-based genetic diversity and temporal structure analysis

To analyse the genetic diversity, the microsatellite data were processed with the package 'poppr' (Kamvar et al., 2014). Basic genetic diversity parameters, including number of alleles per locus (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon information index (I) and inbreeding coefficient (F_{is}) were calculated using the package 'popgenreport' (Adamack et al., 2014). Tests for deviations from Hardy-Weinberg equilibrium were performed for each locus using the package 'pegas' (Paradis, 2010), with P -values adjusted for multiple comparisons using the Benjamini-Hochberg method.

To investigate temporal genetic dynamics over the nine-year study period (2012–2020), we used three complementary analytical approaches. First, a genetic similarity network with 3D effect was created using the 'igraph' package (Csardi, 2013) to visualise the genetic relationships between sample years. Second, a principal coordinate

analysis (PCoA) based on Nei genetic distances was performed using the 'ape' package (Paradis et al., 2019) to quantify genetic differentiation between temporal samples and calculate the percentage of variation explained by each coordinate to assess the strength of temporal structuring. Thirdly, the analysis of ancestry proportions was performed using the package 'adegenet' (Jombart, 2008), where the optimal number of genetic clusters was determined by the Bayesian information criterion. To quantify the relationship between genetic differentiation and temporal distance, we implemented an isolation-by-time analysis using a Mantel test in the package 'vegan' (Oksanen et al., 2007), correlating genetic distances with temporal distances (measured in years) and using 9999 permutations to assess statistical significance.

3. Results

3.1. Soil and climate conditions during the study period

The experimental period (2012–2020) showed significant variability in climatic and edaphic conditions. Annual rainfall (as an average 6-month growing period from May to October) ranged from 96.73 mm (2013) to 151.88 mm (2020), with 2018 and 2020 being particularly wet years (141.62 mm and 151.88 mm, respectively). Relative humidity fluctuated between 81.05 % (2013) and 84.98 % (2018), while mean temperatures remained stable (16.50–17.65 °C). Solar radiation showed contrasting extremes, peaking in 2013 (292.55 W m⁻²) and dropping sharply in 2020 (180.05 W m⁻²), indicating varying light availability over the seasons. In terms of soil parameters, pH and organic matter were moderately stable, with a pH close to neutral (7.08–7.41) and organic matter consistently ≥3.30 %. However, plant-available phosphorus decreased significantly in 2017 (15.56 mg P₂O₅ 100⁻¹ g⁻¹) and 2018 (13.33 mg P₂O₅ 100⁻¹ g⁻¹), while plant-available potassium fluctuated between 14.78 mg K₂O 100⁻¹ g⁻¹ (2018) and 19.00 mg K₂O 100⁻¹ g⁻¹ (2013, 2014), indicating possible nutrient limitations in certain years. These variations in rainfall, temperature and soil fertility likely contributed to the observed year-to-year differences in crop performance, particularly in yields and nutrient uptake dynamics.

3.2. Trend analysis and sources of variation

Between 2012 and 2020, grain yield (GY) and quality traits as well as the nutrient profile showed considerable variability due to both differences between years and previous crop effects, as shown by linear mixed models (LMM). The year effect was strongest for yield, explaining

46.7 % of the variance ($\chi^2 = 4.28$, $P = 0.038$). The highest yields were achieved in 2013 (1267.00 ± 71.15 kg ha⁻¹) and 2018 (1418.00 ± 71.15 kg ha⁻¹). While 2018 was characterised by above-average precipitation, 2013 had the lowest recorded rainfall during the growing season. This suggests that other environmental or agronomic factors such as high solar radiation or favourable crop rotation may have contributed to the unexpectedly high yield in 2013. In contrast, the sharp yield declines in 2016 (498.83 ± 71.15 kg ha⁻¹) and 2019 (644.83 ± 71.15 kg ha⁻¹) corresponded to drier or less favourable seasonal weather conditions. The influence of the previous crop was marginally significant and explained 39.9 % ($\chi^2 = 3.56$, $P = 0.059$) of the yield variance, with yields ranging from 657.08 ± 63.34 kg ha⁻¹ after barley to 1451.00 ± 126.69 kg ha⁻¹ after oats. Crop rotations with maize (1444.00 ± 126.69 kg ha⁻¹) and red clover (1328.00 ± 89.58 kg ha⁻¹) also resulted in significantly higher yields, which were more than twice as high as those after barley (Table 2; Fig. 1; Supplementary Tables S3 & S4).

For the quality parameters, protein content varied between 10.13 ± 0.04 % (2016) and 12.53 ± 0.06 % (2017) and was almost entirely determined by year, which explained 97.3 % of the variation ($\chi^2 = 24.68$, $P < 0.001$). Total phenolic content (TPC) followed a similar trend, peaking in 2020 (10.26 ± 0.03 mg GAE g⁻¹), with the year explaining 99.6 % of the variation ($\chi^2 = 40.27$, $P < 0.001$). In contrast, fat content, although relatively stable over time, was significantly affected by the interaction between year and crop rotation (38.4 % of the variance, $\chi^2 = 51.7$, $P < 0.001$), with no significant main effect of crop rotation or year alone. For the nutrient profiles, K, Ca, Na and S showed strong year effects, with year explaining more than 97 % of their variation, with K concentrations, for example, increasing 1.7-fold from 2012 to 2020 ($\chi^2 = 42.01$, $P < 0.001$). The Mg and P demonstrated dual regulation by year and rotation with year explaining 72.7 % and 76.7 % and year × previous crop interaction explaining 25.5 % and 23.1 % of their variation, respectively. As for micronutrients, they were all strongly influenced by year, which explained >70 % of their variation ($P < 0.001$). They were not significantly affected by the previous crop alone, but most showed small but significant year × previous crop interaction effects. In particular, Mo (24.2 % variance, $\chi^2 = 69.46$, $P < 0.001$) and V (18.6 % variance, $\chi^2 = 37.58$, $P < 0.001$) showed relatively high interaction effects, with Mo content tripling in wheat rotations compared to maize, while V content decreased significantly from 2012 to 2020. An exception was Co, which was only significantly influenced by the interaction effect (51.4 % of the variance, $\chi^2 = 8.49$, $P < 0.001$) (Table 2; Fig. 1).

Table 2

Effects of year, previous crop and their interaction on the agronomic quality and nutrient traits of the buckwheat variety Čebelica (2012–2020).

Traits		Year		Previous crop		Interaction	
		χ^2	P-value (Significance)	χ^2	P-value (Significance)	χ^2	P-value (Significance)
Yield	GY	4.28	0.038*	3.56	0.059	94.59	< 0.001***
Quality traits	Protein	24.68	< 0.001***	2.52	0.112	10.53	0.001**
	Fat	2.59	0.107	0	1	51.7	< 0.001***
	TPC	40.27	< 0.001***	0	1	13.82	< 0.001***
Macro-nutrients	Mg	5.86	0.016*	0.02	0.877	96.71	< 0.001***
	P	6.36	0.012*	0	1	76.45	< 0.001***
	S	36.13	< 0.001***	3.89	0.049*	1.62	0.203
	K	42.01	< 0.001***	0	1	44.44	< 0.001***
	Ca	29.55	< 0.001***	1.52	0.217	1.58	0.209
	Na	61.3	< 0.001***	6.83	0.009**	0	1
	V	7.99	0.005**	0	1	37.58	< 0.001***
	Cr	11.95	< 0.001***	0	1	56.15	< 0.001***
Micro-nutrients	Mn	35.43	< 0.001***	0.65	0.419	161.14	< 0.001***
	Fe	52.33	< 0.001***	0	1	178.42	< 0.001***
	Co	0	1	0	1	8.49	0.004**
	Cu	30.45	< 0.001***	0	1	43.45	< 0.001***
	Zn	45.3	< 0.001***	0.05	0.821	5.26	0.022*
	Mo	6.19	0.013*	0	1	69.46	< 0.001***

GY, grain yield; TPC, total phenolic content.

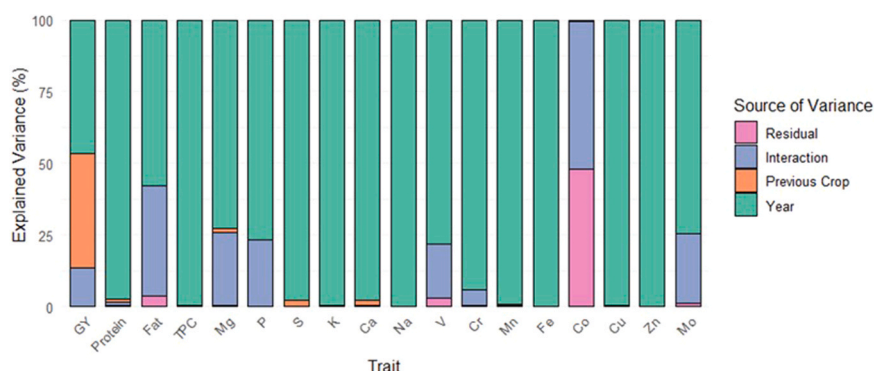


Fig. 1. Contribution of the previous crop, the year, their interactions and the residuals to the total explained variance for the analysed traits.

3.3. Multivariate patterns and principal component analysis

The PCA biplot provided substantial insight into the complex relationships between crop rotations, years and the analysed parameters and confirmed the primary sources of variation identified by LMM. The first two principal components explained 64.7 % of the total variance, with PC1 and PC2 accounting for 40 % and 24.7 %, respectively (Fig. 2). The biplot showed three clear temporal clusters spanning the nine-year study period. The earliest years (2012–2013) are positioned predominantly on the negative side of PC1 and were characterised by low nutrient concentrations, although they were strongly associated with Na and V. The year 2014 occupied a central position along PC 1 but exhibited a strong positive loading on PC2. By contrast, barley-annual ryegrass rotations during the transition years (2015–2016) clustered in the lower left quadrant and showed strong negative associations with GY, protein content and most nutrients, while showing a positive correlation with TPC and Mo. The most recent years (2017–2020) were concentrated on the positive side of PC1 and showed strong positive associations with most micro- and macro-nutrients. Despite the dominant year effect, crop rotation patterns remained relatively consistent, with wheat-red clover rotations generally associated with higher GY, protein levels and micronutrients such as Fe and Zn. Red clover showed a particularly strong association with protein and Fe in 2014 and with

micronutrient enrichment in 2018. In contrast, the barley-annual ryegrass rotations in the earliest years (2012–2013) formed discrete clusters associated with increased TPC and Mo (Fig. 2). PCA underpinned the ANOVA-derived three-class model for nutrient regulation, with climate-dominated traits (e.g. K, S, Na, TPC) showing vectors aligned with year clusters, while dual-control nutrients (Mg, P) were intermediate and traits responding to interactions (e.g. Mo, fat, V) showed distinct alignments consistent with the year \times previous crop effect.

3.4. Association between agronomic traits and environmental parameters

To directly address the multivariate relationship between trait variation and continuous environmental parameters, we performed a Redundancy analysis (RDA). This analysis revealed that variation in agronomic and nutritional traits was mainly explained by rainfall ($F = 4.324$, $P = 0.027$), soil phosphorus (Soil_P) ($F = 3.577$, $P = 0.033$) and solar radiation ($F = 2.987$, $P = 0.041$), which accounted for 31.76 %, 24.88 % and 19.57 % of the total constrained variance, respectively. In contrast, other environmental parameters such as organic matter, soil pH, relative humidity and temperature did not contribute significantly to the explained variance ($P > 0.05$), suggesting that their influence on trait variation was minimal during the study period (Table 3). The RDA

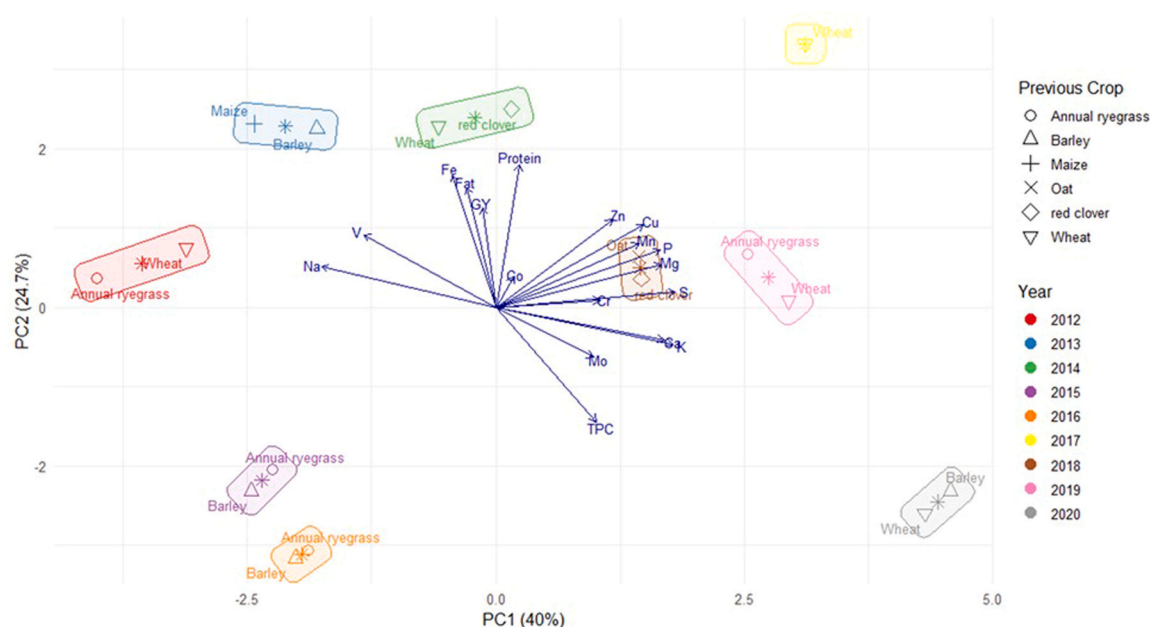


Fig. 2. Principal component analysis (PCA) biplot of agronomic, nutritional and elemental traits across years and crop rotations. GY, grain yield; TPC, total phenolic content.

Table 3

Redundancy analysis (RDA) of environmental variables explaining trait variation in the buckwheat variety Čebelica over nine years.

Variable	F-value	Variance explained	Proportion constrained (%)	P-value
Rainfall	4.324	2.63	31.76	0.027
Soil_P	3.577	2.06	24.88	0.033
Solar radiation	2.987	1.62	19.57	0.041
Organic matter	0.943	0.73	8.82	0.61
pH	0.721	0.61	7.37	0.721
Relative humidity	0.554	0.45	5.43	0.78
Temperature	0.42	0.18	2.17	0.85

Soil_P, plant-available phosphorus in soil. Bold font indicates statistical significance.

biplot showed a clear temporal structuring along the first two canonical axes RDA1 and RDA2, which explained 19.87 % and 10.68 % of the total variance, respectively. The most recent years (2017–2020) are clustered on the positive side of RDA1 and are closely related to increased precipitation and increased values of GY, TPC, protein and various macro- and micro-nutrients (e.g. K, Ca, Fe, Zn, Mn, Mo). The year 2020 in particular is the most pronounced and indicates an environment characterised by high rainfall and strong trait expression. In contrast, the years 2012–2016 were grouped on the negative side of RDA1, with 2012 and 2015 clearly separated from the others. These years are consistent with higher plant-available phosphorus and higher solar radiation, and are associated with elevated Na and V levels, as well as modest protein and fat contents. The year 2014 appears to be isolated along RDA2 and reflects a unique combination of moderate rainfall and high SR with a distinct trait signature, including elevated Na, fat and protein levels (Fig. 3).

3.5. Stability patterns

Stability analysis over nine growing seasons revealed conspicuous patterns of environmental susceptibility and trait-specific stability. GY showed the highest instability, with a CV of 36.5 %, a MAD of 394.4, extreme environmental variance (EV = 118.085) and a steep negative regression slope (bi = -18.1). In contrast, protein and fat showed remarkable stability with a low CV (4.7–6.0 %), minimal MAD (0.13–0.63) and negligible environmental variance (EV ≤ 0.47). However, TPC showed moderate variability (CV = 23.71 %) but a clear positive temporal trend (bi = 0.20), possibly reflecting either plant

response to cumulative environmental stresses or developmental changes in the perennial crop. Among the macronutrients, K showed stable accumulation (CV = 15.69 %, bi = 0.12), while Na showed extreme interannual fluctuations (CV = 51.07 %) and a dramatic downward trend (bi = -2.27), possibly indicating altered soil salinity patterns or changes in uptake regulation. P and Mg showed intermediate stability (CVs of 13.50 % and 16.26 %, respectively), with both showing a slight but consistent annual increase (bi = 0.05 and 0.04) (Table 4).

The micro-nutrients showed complex and divergent stability patterns. Mn exhibited both considerable environmental variance (EV = 20.86) and strong positive temporal accumulation (bi = 0.38), associated with a near perfect Taylor's power law correlation ($r = 0.94$, $P = 0.005$), suggesting that its variability is inextricably linked to mean concentration. Fe had a similarly high environmental variance (EV = 198.68), but without TPL confounding ($P = 0.69$), suggesting that its variability is due to external factors rather than mean-dependent scaling. Zn is an interesting case of apparent stability (CV = 11.55 %) despite moderate environmental variance (EV = 6.41), with a consistent upward trend (bi = 0.17), possibly reflecting gradual enrichment in soil or improved plant uptake efficiency. Of note, several micro-nutrients (V, Cr, Mo) had high CVs (39.91–59.96 %) but relatively low absolute environmental variance, indicating relatively large but numerically small variations in their concentrations (Table 4).

3.6. Genetic diversity analysis

Genotyping using nine polymorphic SSR loci revealed considerable genetic variation in the buckwheat variety Čebelica, with marked differences in diversity patterns between loci. The number of alleles per locus (Na) varied from 2.44 (Fem1322) to 8.89 (Fem1407), with a mean of 6.2 ± 2.3 alleles per locus. All loci showed high observed heterozygosity ($H_o = 0.667$ – 1.000), with seven of nine loci showing a significant excess of heterozygosity (Fis = -0.732 to -0.128) over expected values ($H_e = 0.502$ – 0.819). The effective number of alleles ($N_e = 2.014$ – 5.865) and the Shannon information index ($I = 0.752$ – 1.938) confirmed the high discriminatory power of the markers, with the loci Fem1407 ($N_a = 8.89$, $I = 1.918$) and GBFE121 ($N_a = 8.78$, $I = 1.938$) showing particularly exceptional diversity. A single locus (Fem1407) exhibited heterozygote deficiency (Fis = 0.103), which contrasts with the general pattern of heterozygote excess observed throughout the marker set (Table 5).

Genetic diversity parameters analysed over nine consecutive years (2012–2020) showed considerable variation in allele numbers, with the highest values for Na and Ne occurring in 2018 ($N_a = 6.89$ and $N_e =$

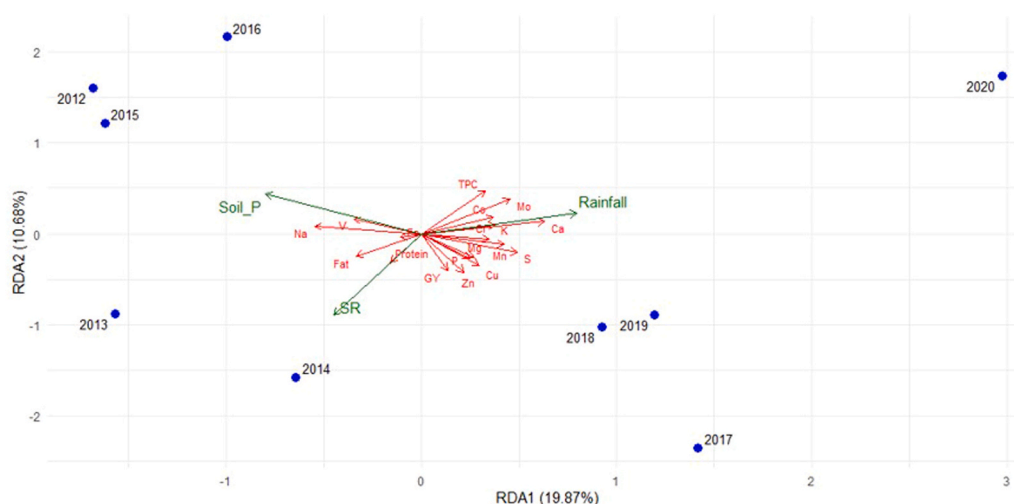


Fig. 3. Redundancy analysis (RDA) biplot of agronomic traits and environmental drivers in the buckwheat variety Čebelica over time (2012–2020). GY, grain yield; TPC, total phenolic content; Soil_P, plant-available phosphorus in soil; SR, solar radiation.

Table 4

Stability indices for agronomic quality and nutrient traits in of the buckwheat variety Čebelica across nine growing seasons.

Trait	CV	EV	bi	MAD	TPL_Correlation	TPL_P-value
GY	36.547	118085.16	−18.089	394.372	0.47	0.202
Protein	6.015	0.465	−0.031	0.628	0.26	0.469
Fat	4.71	0.013	−0.01	0.133	−0.184	0.611
TPC	23.71	2.456	0.201	0.925	−0.445	0.198
Mg	16.26	0.12	0.044	0.378	0.038	0.916
P	13.503	0.21	0.052	0.568	0.231	0.549
S	82.192	0.709	0.136	0.267	0.356	0.489
K	15.689	0.51	0.12	0.46	0.562	0.189
Ca	44.082	0.088	0.046	0.249	0.214	0.684
Na	51.065	148.786	−2.273	8.395	0.11	0.814
V	59.958	0.002	−0.007	0.041	0.609	0.2
Cr	41.901	0.026	0.012	0.135	0.632	0.178
Mn	21.996	20.864	0.381	5.527	0.943	0.005
Fe	31.943	198.682	−1.616	16.358	0.313	0.687
Co	32.425	0	0	0.011	−0.08	0.92
Cu	12.001	0.532	0.066	0.934	0.023	0.965
Zn	11.547	6.409	0.167	2.15	−0.279	0.65
Mo	39.911	0.028	0.016	0.163	0.639	0.245

GY, grain yield; TPC, total phenolic content; CV, coefficients of variation; EV, environmental variance; bi, regression slopes; MAD, mean absolute deviation; TPL, Taylor's Power Law results.

Table 5

Genetic diversity metrics of the buckwheat variety Čebelica based on nine SSR loci across nine consecutive years (2012–2020).

	Locus	Na	Ne	I	Ho	He	Fis
Loci	Fem1303	6	2.992	1.372	0.933	0.659	−0.415
	Fem1322	2.444	2.014	0.752	0.789	0.502	−0.57
	Fem1407	8.889	5.865	1.918	0.733	0.815	0.103
	Fem1582	6.111	3.568	1.457	0.889	0.704	−0.273
	Fem1840	3.778	2.265	0.994	0.667	0.55	−0.2
	GBFE025	3	2.4	0.941	1	0.58	−0.732
	GBFE035	7.333	5.054	1.758	0.9	0.796	−0.134
	GBFE054	8	5.396	1.828	0.933	0.798	−0.171
	GBFE121	8.778	5.807	1.938	0.922	0.819	−0.128
	Mean	6.037 ± 0.288	3.929 ± 0.204	1.440 ± 0.052	0.863 ± 0.016	0.691 ± 0.014	−0.280 ± 0.031
Years	2012	6.222	3.862	0.59	0.889	0.726	−0.224
	2013	5.444	3.504	0.56	0.844	0.69	−0.224
	2014	5.778	3.783	0.619	0.933	0.727	−0.285
	2015	6.667	4.49	0.553	0.833	0.754	−0.106
	2016	6	3.646	0.538	0.811	0.718	−0.13
	2017	5.333	3.528	0.56	0.844	0.712	−0.186
	2018	6.889	4.768	0.59	0.889	0.748	−0.188
	2019	6.333	4.106	0.597	0.9	0.713	−0.262
	2020	5.667	3.676	0.546	0.822	0.696	−0.181
	Mean	6.037 ± 0.288	3.929 ± 0.204	1.440 ± 0.052	0.863 ± 0.016	0.691 ± 0.014	−0.198 ± 0.021

Na, number of alleles per locus; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; I, Shannon information index; Fis, inbreeding coefficient.

4.76) and the lowest values in 2017 (Na = 5.33 and Ne = 3.504), with Na and Ne mean values of 6.037 ± 0.288 and 3.929 ± 0.204 . The Shannon information index (I) remained high (mean I = 1.440 ± 0.052), reflecting both high allelic richness and high evenness. Genetic diversity was also high in all years, with mean observed heterozygosity (Ho) and expected heterozygosity (He) of 0.863 ± 0.016 and 0.691 ± 0.014 , respectively. In every year, Ho was higher than He, indicating a persistent excess of heterozygosity throughout the study period. This pattern was further supported by a strongly negative inbreeding coefficient (Fis), which ranged from −0.106 to −0.285 (mean Fis = -0.198 ± 0.021) (Table 5).

To elucidate the temporal genetic dynamics of the studied variety during the nine-year study period (2012–2020), we performed a comprehensive analysis of the genetic structure using several complementary analytical approaches. At a threshold distance of 0.1147, the 3D-Effect Genetic Similarity Network (Fig. 4A) showed a clear temporal structuring, with the earlier years (2012–2014) forming a distinct cluster compared to the later years (2015–2020), with 2015 appearing as a transition node associated with both temporal groups. Strong connectivity can be seen between chronologically adjacent years,

particularly between 2017–2019, suggesting a gradual genetic shift. Remarkably, 2020 shows a moderate genetic distance to the other years and is mainly connected to 2018. This network structure suggests a chronological progression of genetic composition, with a clear shift between 2014 and 2015, followed by an internal reorganisation within the more recent group (2016–2020). Principal coordinate analysis (PCoA) of genetic distances provided quantitative confirmation of this pattern (Fig. 4B). The first two principal coordinates, which explained 77.7 % of the total genetic variation (51.9 % and 25.8 %, respectively), distinguished two primary genetic clusters corresponding to different time periods: an earlier cluster covering the years 2012–2014 and a later cluster containing the years 2015–2020. The temporal progression, represented by the connecting red line, showed a straightforward pattern of genetic changes with a clear shift between 2014 and 2015, right at the origin of the coordinates. This transition marked the boundary between the two genetic clusters, with a movement from negative to positive values along the first main coordinate. These results were also confirmed supported by analysing the ancestry proportions (Fig. 4C), which identified two distinct genetic clusters over the study period, with cluster 1 (blue) dominating in the early populations

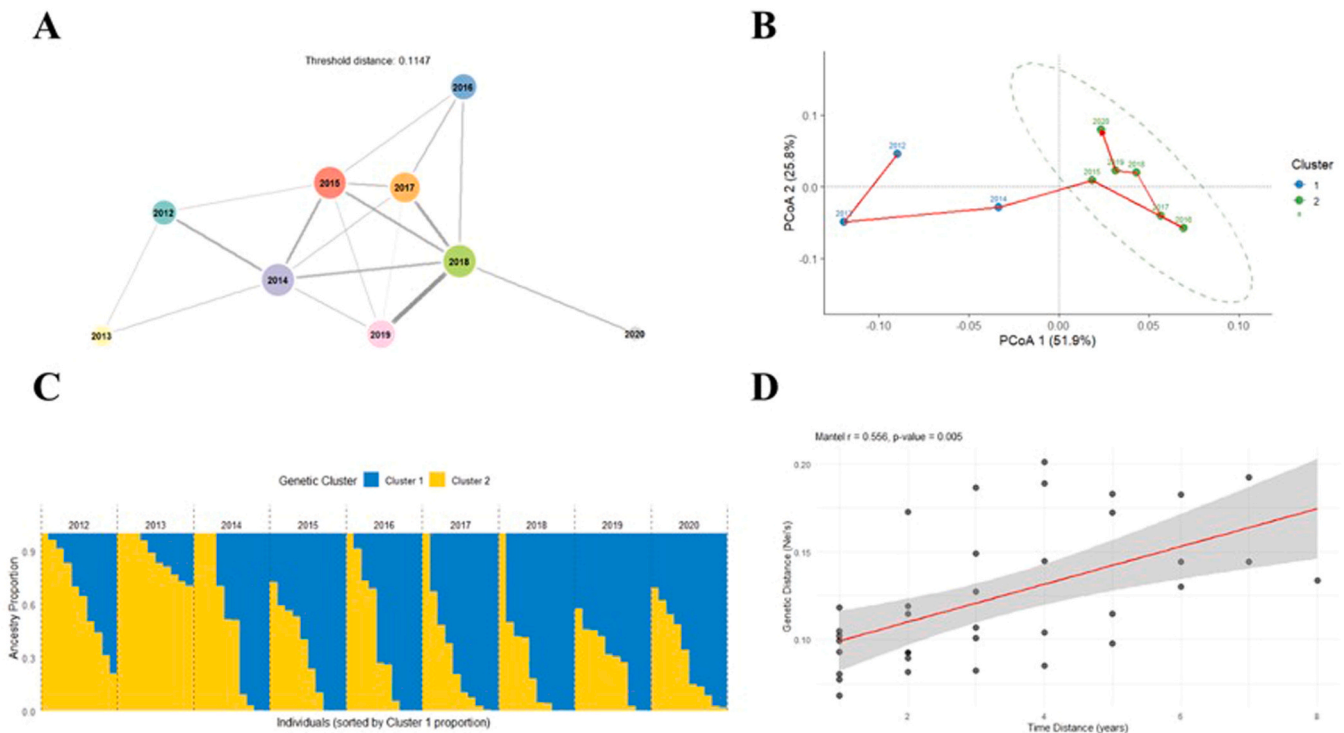


Fig. 4. Temporal dynamics of genetic structure in the buckwheat variety Čebelica (2012–2020). (A) 3D network analysis of microsatellite data showing annual genetic clustering; edge thickness reflects genetic similarity. (B) Principal Coordinate Analysis (PCoA) illustrating temporal genetic differentiation. (C) Ancestry proportion analysis indicating shifts in genetic clusters over time. (D) Isolation-by-time analysis showing a correlation between genetic and temporal distances (Mantel test).

(2012–2014) but gradually decreasing thereafter, while cluster 2 (yellow) became increasingly dominant from 2015 onwards. The most dramatic transition in cluster proportions occurred between 2014 and 2015, which corresponds exactly to the split point observed in both the network and PCoA analyses. Statistical validation by isolation-by-time analysis (Fig. 4D) confirmed a significant positive correlation between genetic and temporal distance ($r = 0.556$, $P\text{-value} = 0.005$), showing that genetic differentiation increases proportionally to temporal separation.

4. Discussion

This long-term study provides comprehensive insights into the agronomic performance, nutrient stability and genetic dynamics of the buckwheat variety Čebelica under Central European conditions. By integrating trait variation with environmental, rotational and genetic analyses, we uncover the complex interplay between climate variability, management practises and genotype stability and provide rare, longitudinal evidence of how outcrossing crops respond to real-world agro-ecological stresses.

4.1. Yield variability due to climate and crop rotation

The nine-year cultivation of the Čebelica variety achieved an average yield that was well above the documented values for European varieties under comparable conditions (0.72 t ha^{-1}) and slightly above the global average (0.94 t ha^{-1}) (Domingos and Bilsborrow, 2021). However, grain yield showed high interannual variability, ranging from 498.8 to $1418.0 \text{ kg ha}^{-1}$, largely due to rainfall variability and previous crop effects. The highest yields were recorded in 2013 and 2018, both years with above-average rainfall, while the lowest yields corresponded to drier or less favourable conditions, particularly in 2016 and 2019. In these low-yielding years, dry periods occurred during flowering and grain filling, which are known to be the most sensitive development

phases of buckwheat. Drought stress during these stages likely contributed to reduced productivity, which is confirmed by previous studies highlighting the plant's susceptibility to water stress and photoperiod effects during reproductive development (Rangappa et al., 2023b; Ali and Hussain, 2024). These results demonstrate the high productivity potential of the Čebelica variety under optimal climate conditions, but also emphasise its sensitivity to seasonal climatic fluctuations. Furthermore, the marginally significant effect of previous crop on yield ($P = 0.059$) supports the hypothesis that crop rotation can serve as a strategic lever to optimise buckwheat production. Crop rotations with oats, maize and red clover increased yields by more than 100 % compared to barley, suggesting that nitrogen-fixing or deep-rooting previous crops can improve nutrient availability in the soil. This interpretation is supported by our soil analyses, which showed higher levels of plant-available phosphorus and potassium in years following red clover and maize rotations. While nitrogen was not directly measured, the enhanced nutrient status aligns with the expected benefits of these crops (Luo et al., 2025; González-García et al., 2025).

4.2. Temporal stability of grain composition and nutrient trends

In contrast to the pronounced variability observed in grain yield, the compositional characteristics of buckwheat grains, especially crude protein and fat content, showed remarkable temporal stability over the nine-year period. The low coefficients of variation ($CV < 6 \%$) and minimal environmental variance emphasise the ability of the variety to maintain important nutritional traits under fluctuating climatic and edaphic conditions. These results are consistent with previous studies that have reported robust physiological regulation of macronutrient accumulation in buckwheat (Colak, 2025; Tian et al., 2025; Ullah et al., 2025). However, TPC showed high variability ($CV \approx 24 \%$) and a steady increase over time, likely reflecting cumulative response to environmental stressors or shifts in secondary metabolism associated with changes in crop rotation and nutrient status. Phenolic compounds play a

crucial role in plant defence and oxidative stress regulation, and their increasing levels over time may indicate increased antioxidant investment under field stress (Naikoo et al., 2019). In addition, the element profile showed a clear temporal dynamic in the nutrients. The macro-nutrients K, Ca, S and Na were strongly influenced by interannual climate variability, due to their mobility and dependence on soil solution dynamics, especially rainfall and plant-available phosphorus (Rangappa et al., 2023a). A significant decrease in Na content indicates reduced soil salinity or down-regulation of Na⁺ transport, which could benefit buckwheat quality by improving flavour and osmotic regulation. In contrast, Mg and P were regulated by both year and previous crop, indicating more complex environmental and biotic interactions. Their availability is strongly influenced by microbial activity in the rhizosphere and root architecture, both of which depend on crop rotation and soil inheritance (Ahmed et al., 2023; Sardans et al., 2023). These dual control patterns emphasise the importance of incorporating microbiome and nutrient cycling considerations into crop rotation design.

The trends in micro-nutrients were both element-specific and of agricultural importance. Micro-nutrients Fe and Zn, important targets for biofortification, increased steadily over time, probably due to soil enrichment, microbial promotion or selection of nutrient-efficient genotypes. This is critical given the global burden of micronutrient deficiency and the role of agronomic biofortification as a sustainable solution (Dhaliwal et al., 2022). In contrast, Mo, V and Co showed strong year \times crop interactions and high variability likely due to shifts in redox status, organic inputs and root exudate-mediated complex formation (Ansari et al., 2023). The strong increase of Mo in wheat rotations indicates a strong role of residue quality and microbial turnover (Wu et al., 2021). These dynamics emphasise the need for micronutrient monitoring tailored to the crop rotation and system type, especially in low-input or organic farming systems.

4.3. Trait-environment relationships

The multivariate PCA and RDA analyses reinforced the dominant influence of climate and previous crop on trait expression. PCA showed a clear temporal structuring of agronomic and compositional traits over the nine-year period and identified three distinct phases: early (2012–2014), transitional (2015–2016) and recent (2017–2020). These phases correspond closely with interannual variations in rainfall and solar radiation and illustrate the sensitivity of trait profiles to environmental changes. Such clustering indicates not only the plasticity of traits in response to external influences, but also a possible course of adaptation or physiological acclimatisation under field conditions. The RDA further illustrated the hierarchical control of trait variation by environmental variables, with rainfall, plant-available phosphorus (Soil P) and solar radiation emerging as the most important determinants of trait variation. This result is consistent with ecological theory, which states that the water and nutrient availability act as primary environmental filters that influence plant responses to functional traits under variable conditions (Brunet et al., 2025). In particular, rainfall had the strongest influence, emphasising its role in regulating both grain yield and nutrient uptake in shallow-rooted, fast-growing crops such as buckwheat (Vieites-Álvarez et al., 2024).

The availability of phosphorus also proved to be an important environmental factor, especially in the earlier years of the study. As a nutrient with low mobility in the soil, phosphorus is essential for root development, flowering and seed set in buckwheat, a crop known for its relatively shallow root system and sensitivity to nutrient imbalances (Kolarić et al., 2021). Higher soil P levels in the early years were associated with higher fat and sodium content, but not with higher yield or overall nutrient enrichment, suggesting possible nutrient interactions or sub-optimal uptake under drier conditions. These results emphasise the importance of balanced phosphorus availability, especially in crop rotations, as buckwheat performance is highly dependent on both nutrient accessibility and moisture availability.

The strong influence of solar radiation emphasises its role in regulating both primary metabolism and the synthesis of secondary metabolites such as phenols. It is known that higher irradiance increases the production of flavonoids and shifts resource allocation to antioxidant protection (Ferreyra et al., 2021; Qin et al., 2025). In our study, early years with higher solar radiation were associated with an increase in fat, protein and sodium, while wetter years with lower solar radiation favoured greater accumulation of macro- and micronutrients. This suggests a trade-off between photoprotection and nutrient uptake determined by the interaction of light intensity and water availability. The lack of significant effects of temperature and relative humidity could be due to the relative stability of these variables during the growth periods (16–17 °C and 81–83 % relative humidity, respectively).

4.4. Temporal genetic differentiation reflects gradual drift and adaptive structuring

A remarkable result of this long-term study is the clear temporal genetic differentiation observed in a single buckwheat variety (Čebelica) grown in the field. Although the variety was maintained uniform, the SSR data showed a consistently high level of heterozygosity ($H_o = 0.863$), exceeding expectations at random mating ($H_e = 0.691$), and a strongly negative inbreeding coefficient ($F_{is} = -0.198$), suggesting that Čebelica exhibits strong outcrossing behaviour and may be subject to balancing selection. High heterozygosity is expected in obligate outcrossers such as buckwheat, but the observed excess may indicate selection favouring heterotic genotypes, especially under fluctuating environmental conditions (Abdul-Rahman et al., 2021; Johnson et al., 2023). This supports the findings from buckwheat (Barcaccia et al., 2016; Joshi et al., 2019) and other outcrossing species (Khoury et al., 2022) where environmental heterogeneity maintains allelic diversity over time.

Multivariate genetic analyses revealed a sharp genetic transition between 2014 and 2015, which was accompanied by significant changes in environmental parameters (e.g. lower phosphorus availability, changes in rainfall) and crop rotations (e.g. introduction of red clover and oats). These changes probably led to new selection pressures that altered the genetic structure of the population. This pattern is consistent with ecological filtering, where genotypes that are better adapted to the new conditions prevail over time (Joshi et al., 2019; Li et al., 2021). As buckwheat is dependent on insect pollination, changes in pollinator density or pollination behaviour, whether due to climate variability, plant phenology or landscape changes, could alter gene flow patterns and mating dynamics (Liu et al., 2020). Variability in pollination success is known to cause temporal and spatial genetic structures in other insect-pollinated crops and wild plants (Castro et al., 2021; Feigs et al., 2022).

4.5. Implications for climate-resilient cropping and genetic conservation

This study positions the buckwheat variety Čebelica as a promising crop for climate-resilient systems, combining stable nutritional quality with genetic adaptability. The variety's ability to maintain protein and fat content despite environmental fluctuations, alongside gradual genetic restructuring, indicates physiological robustness and evolutionary responsiveness - traits essential for adapting to a changing climate. This dual resilience supports a shift towards integrated crop assessment, where multi-year agronomic, environmental and genetic monitoring can reveal hidden adaptive dynamics. This approach is particularly important for outcrossing species, where uncontrolled temporal changes can affect both performance and conservation value. These results also challenge the assumption of genetic uniformity in seed systems and emphasise the need for regular monitoring in both formal and farmer-managed contexts. Embracing genetic change, rather than merely controlling it, can increase resilience and support the overall goals of sustainable, biodiverse agriculture.

5. Conclusion

This long-term field study provides unique insights into the ecological, nutritional and genetic performance of the buckwheat variety Čebelica (*Fagopyrum esculentum* Moench) over nine growing seasons. Under fluctuating environmental conditions, the variety showed remarkable stability in composition, especially in protein and fat content, confirming its nutritional reliability. In parallel, directional changes in traits such as total phenolic content and selected micro-nutrients indicate adaptive metabolic responses to cumulative environmental stressors. Importantly, temporal genetic analyses revealed significant structuring over time, demonstrating that evolutionary forces such as selection and gene flow actively reshape genetic composition even within a named variety. This plasticity, observed without targeted breeding, emphasises the dynamic nature of outcrossing crops and their potential for in situ adaptation. By integrating agronomic, environmental and genomic data over a period of nearly a decade, this study goes beyond conventional short-term assessments and presents a modelling framework for evaluating crop resilience under real-world conditions. The results highlight the potential of underutilised species such as buckwheat to make an important contribution to climate-resilient and food-secure agriculture. Furthermore, they emphasise the need to exploit genetic and phenotypic variability rather than restrict it – a crucial consideration for conservation, breeding and agricultural sustainability in a time of rapid environmental change.

CRedit authorship contribution statement

Vladimir Meglič: Writing – review & editing, Funding acquisition.
Barbara Pipan: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.
Mohamed Neji: Writing – original draft, Visualization, Software, Data curation.
Lovro Sinković: Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors mentioned above declare that they have no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eja.2025.127895](https://doi.org/10.1016/j.eja.2025.127895).

Data availability

Data will be made available on request.

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