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RESEARCH ARTICLE

Mixed cropping green manure can simultaneously improve the nutrient yield and quality of spring wheat grain under reduced chemical nitrogen supply

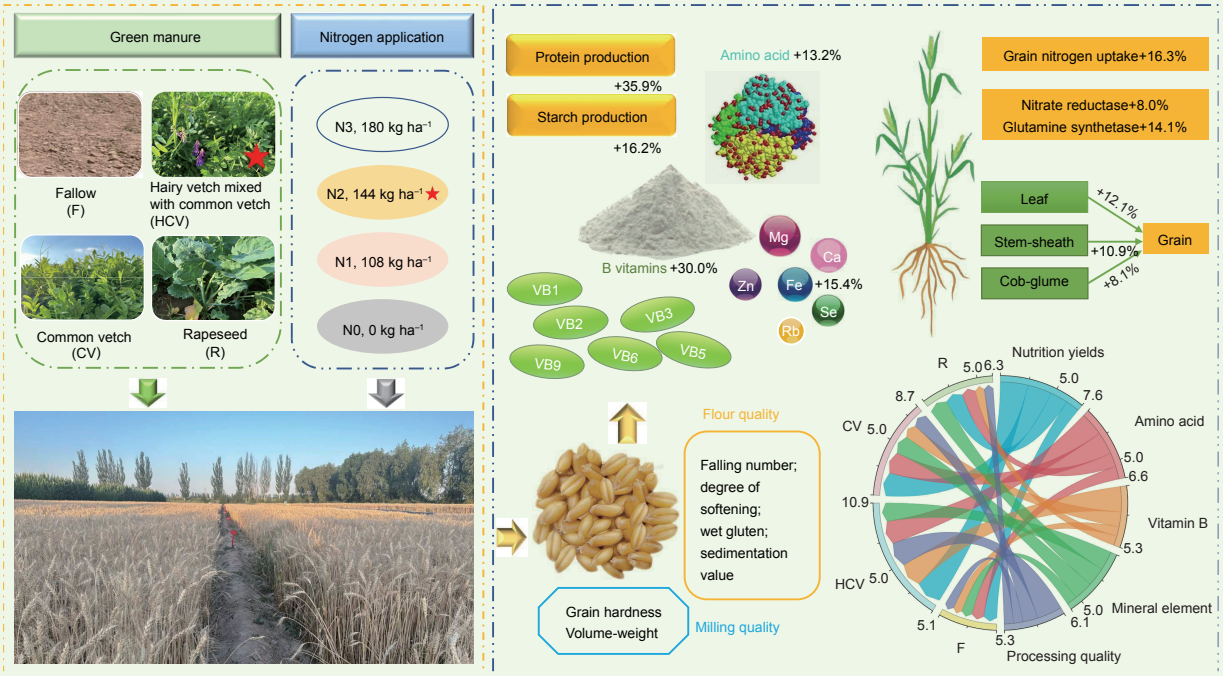
Jingui Wei[1, 2], Fang Yin[1, 2], Yao Guo[3], Zhilong Fan[1, 2], Falong Hu[1, 2], Qiming Wang[1, 2], Shoufa Mao[1], Qiang Chai[1, 2#], Wen Yin[1, 2#]

[1] *State Key Laboratory of Aridland Crop Science, Lanzhou 730070, China*
[2] *College of Agronomy, Gansu Agricultural University, Lanzhou 730070, China*
[3] *College of Life Sciences, Northwest Normal University, Lanzhou 730070, China*

Highlights

- Mixed hairy vetch and common vetch under 20% nitrogen reduction (HCVN2) improved the nutrient yield of wheat grain.
- HCVN2 increased the nutritional and processing qualities.
- HCVN2 enhanced nitrogen accumulation, remobilization, and assimilation.

Graphical abstract



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#Correspondence Qiang Chai, E-mail: chaiq@gsau.edu.cn; Wen Yin, E-mail: yinwen@gsau.edu.cn

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Abstract

Ensuring the provision of adequate and nutritious food for humans through sustainable agricultural development poses a major challenge. Optimizing the nitrogen supply is one of the key factors for improving crop grain yield and quality. Green manure is often used to optimize the nitrogen supply in crop production, but it is unclear whether green manure can maintain nutrient yield and quality of spring wheat under reduced chemical nitrogen input. A split-plot field experiment of several varieties of green manure and mixed cropping with green manure was established in an arid area in 2018. This study aimed to explore the feasibility of using mixed common vetch and hairy vetch, which could simultaneously maintain high nutrient production and grain quality of spring wheat under reduced chemical nitrogen input, and to reveal the mechanism of nitrogen metabolism. The effects of green manure and reduced chemical nitrogen on nutrient yield, amino acid contents, vitamin B contents, mineral contents, and processing quality of spring wheat grain, as well as nitrogen accumulation, remobilization, and assimilation, were examined from 2020 to 2022. Our results showed that a reduced chemical nitrogen input led to lower nutrient production, but green manure could increase the protein and starch yields of wheat grain. HCVN2 (mixed hairy vetch and common vetch under 20% nitrogen reduction) displayed higher protein and starch yields, which increased by 35.9 and 16.2% compared to fallow after wheat harvest with conventional nitrogen application (FN3). Meanwhile, reduced chemical nitrogen reduced the wheat grain quality, but green manure improved wheat grain quality. HCVN2 had higher wheat grain qualities, which improved by 13.2 and 10.0% for essential amino acid and non-essential amino acid contents, by 20.0 and 22.2% for vitamin B and zinc contents, and by 14.0 and 8.6% for falling number and wet gluten compared to FN3, respectively. HCVN2 could simultaneously improve the nutrient production and quality of wheat grain. This was attributed to significantly increasing the nitrogen accumulation and distribution in grain; enhancing the contribution rates of leaf, stem-sheath, and cob-glume nitrogen to grain nitrogen; and promoting the activities of nitrate reductase and glutamine synthetase. Therefore, mixed sown green manure under chemical nitrogen reduced by 20% is promising for improving the nutrient production and quality of spring wheat grain by promoting nitrogen accumulation, remobilization, and assimilation.

Keywords: mixed sown green manure, chemical nitrogen reduction supply, spring wheat, nutrient yield and quality, nitrogen remobilization, enzyme activities of nitrogen assimilation

1. Introduction

Food and nutritional security underpins human survival and constitutes a central pillar of sustainable agricultural development (Lassaletta *et al.* 2016). With global population growth and increasing resource and environmental pressures, improving crop quality is a major global challenge (Lassaletta *et al.* 2016; Ma *et al.* 2021). Although intensive agricultural production has effectively supported the growth of food production over the past few decades, the overuse of nitrogen fertilizers has proven costly and caused negative effects on the ecological environment (Yu *et al.* 2022; Jain 2023). Therefore, it is imperative to maintain the nutrient yield and quality of grain crops while reducing chemical nitrogen input in developing modern agriculture.

Wheat is one of the major grain crops, accounting for between 20% (e.g., United Kingdom) and over 50% (e.g., Central Asia) of the total caloric intake from grain in the human diet (Zörb *et al.* 2018). Wheat grain is rich in nutrient yield compared to other cereal crops (Hermans *et al.* 2021). It includes macronutrients (protein, fat, and carbohydrates) and micronutrients (vitamins and minerals), making it an essential food source for maintaining human health (Hermans *et al.* 2021; Caldelas *et al.* 2023). Meanwhile, the processing quality of wheat grain is largely influenced by the content of storage proteins, which directly affects grain quality and market value (Peng *et al.* 2022). Numerous studies have confirmed that higher wheat quality mainly depends on excellent varieties (Graziano *et al.* 2019) and better agronomic measures, including integrated water and fertilizer management, improved fertilization systems, and optimized cropping patterns (Guzmán *et al.* 2016; Rossmann *et al.* 2019; Wang *et al.* 2022; Caldelas *et al.* 2023). Improving the nitrogen system can promote amino acid synthesis, thereby increasing the protein content and improving wheat quality (The *et al.* 2021). Relevant research has shown that increasing the grain protein content of wheat has an absolute requirement for nitrogen, and higher nitrogen fertilizer levels can enhance the plant's ability to acquire mineral elements, thereby improving baking and processing quality (Zörb *et al.* 2018). However, a reduced supply of chemical nitrogen may limit the genetic potential for maximizing protein accumulation during kernel development (Zhen *et al.* 2020; The *et al.* 2021). Furthermore, a limited nitrogen fertilizer supply can reduce the metabolic activities of amino acids and soluble carbohydrates, leading to lower nutrient production (Savill *et al.* 2018; Ben *et al.* 2020). Therefore, under reduced chemical nitrogen fertilizer input, there is an urgent need to determine how to maintain the nutrient yield and quality of wheat grain.

Crop rotation is an important cultivation measure for improving wheat quality, and leguminous pre-cultivation can effectively increase the grain protein and wet gluten contents of subsequent wheat (Hellemans *et al.* 2018), mainly due to leguminous crops improving soil fertility while replacing part of the chemical nitrogen supply (Zhang *et al.* 2013; Wei *et al.* 2024b). Green manure is incorporated into the main crops to increase crop diversification, especially leguminous green manure which can enrich the soil nitrogen pool, thus

enhancing nitrogen remobilization from vegetative organs to grain and the grain nitrogen distribution, as well as improving the amino acid and protein contents (Sieling and Kage 2021; Peng *et al.* 2022; Lyu *et al.* 2024). In addition, coordinating the nitrogen metabolism of wheat grain by optimizing the nitrogen supply can enhance nitrogen distribution and translocation to increase the amino acid and protein contents, thereby improving nutritional and processing qualities (Savill *et al.* 2018; Ben *et al.* 2020; Yao *et al.* 2023). Higher activities of nitrogen metabolism enzymes can increase nitrate uptake and nitrogen assimilation, thereby facilitating the synthesis of amino acids and proteins in crop grains (Liu *et al.* 2022; Cui *et al.* 2023). Therefore, the impacts of different green manures and mixed cropping green manure on the improvement of the nutrient yield and quality of wheat grains by regulating nitrogen accumulation, remobilization, and assimilation under reduced chemical nitrogen supply remain understudied.

Wheat is one of the main crops in the arid irrigation area of northwestern China, where it is characterized by long-term continuous cropping and higher chemical nitrogen input. Previous studies have indicated that green manure improves the grain yield and resource use efficiency of wheat (Wei *et al.* 2024b). However, whether green manure can improve wheat grain nutrient yield and quality under reduced chemical nitrogen supply remains unclear. We hypothesized that green manure can improve the wheat grain nutrient yield and quality by promoting nitrogen accumulation, remobilization, and assimilation under reduced chemical nitrogen supply. This study aimed to explore the effects of green manure and a reduced chemical nitrogen supply on (i) nutrient yield, (ii) amino acid, vitamin B, and mineral contents, and processing quality, (iii) nitrogen accumulation, remobilization, and assimilation, and (iv) a comprehensive evaluation index of wheat grain quality. The results of this study can provide valuable insights for simultaneously increasing the nutrient yield and quality of the wheat grain to foster the sustainable cultivation of nutritious and healthy food crops for human consumption.

2. Materials and methods

2.1. Experimental site description

This study was implemented at the Hexi Oasis Agricultural Research Experimental Station of Gansu Agricultural University (37.95°N, 102.63°E), located in Wuwei City in northwestern China. This region experiences an annual mean temperature of 7.2°C, annual rainfall of approximately 200 mm, accumulated temperature of 3,513°C (above 0°C), and a frost-free period lasting 155 d. The soil organic matter, total N, available P, and available K at the 0–30 cm depth were respectively 15.5 g kg^{-1}, 0.83 g kg^{-1}, 23.7 mg kg^{-1}, and 157.1 mg kg^{-1}.

2.2. Experimental design and field management

This study established a split-plot field experiment in 2018, with data collection from 2020 to 2022. Sixteen treatments consisting of four cropping patterns and four nitrogen application rates were tested with three replicates. The experimental main plot had four cropping patterns of wheat multi-cropped green manure, including mixed hairy vetch and common vetch (HCV), common vetch (CV), rapeseed (R), and fallow (F) after the wheat harvest; and four nitrogen application rates of the local conventional nitrogen rate (N3, 180 kg ha^{-1}), nitrogen reduced by 20% (N2, 144 kg ha^{-1}) and 40% (N1, 108 kg ha^{-1}), and a zero-nitrogen application rate (N0, 0) formed the split-plots. The area of each plot was 40 m^2.

Three types of green manures, common vetch (cv. Lanjian 2), hairy vetch (cv. Turkmen Vicia villosa Roth), and rapeseed (cv. Qingza 5) were sown after the wheat harvest and incorporated into the field *via* crushing in late October of the same year. The hairy vetch mixed with common vetch treatment included 187.5 and 37.5 kg ha^{-1} of common vetch and hairy vetch, respectively. The planting densities of common vetch and rapeseed were 225 and 7.5 kg ha^{-1}, respectively. Spring wheat (Yongliang 4) was sown on 18 March at a planting density of 450 kg ha^{-1} and harvested on 20 July. All nitrogen (urea, 46.6% N) and phosphate fertilizers (superphosphate, 18% P_2O_5) were used as base fertilizers. Wheat received 80% of the chemical nitrogen fertilizer and 100% of the phosphate fertilizer (90 kg ha^{-1}), while the green manure only received 20% of the nitrogen fertilizer. No K fertilizer was needed, mainly because the K content was high enough in this area to meet the needs of crop growth. The irrigation quota was administered at amounts of 750, 900, and 750 m^3 ha^{-1} during the seedling, booting, and grain-filling stages of the wheat growing season, respectively, with a drip irrigation system. Moreover, 700 and 900 m^3 ha^{-1} of irrigation were applied at the seedling and budding stages of the green manure, respectively.

2.3. Measurements and calculations

Nutrient yield and processing quality A seed moisture meter (PM-8188, KETT Co., Japan) was used to measure the moisture content of wheat grain, and the grain yield of wheat was calculated based on the grain yield of 4 m^2 per plot with 13% water content at full maturity. Wheat grain protein, starch, falling number, grain hardness, degree of softening, wet gluten, and sedimentation value were determined with a near-infrared grain analyzer (FOSS, NIRS-DS2500, Denmark). Protein and starch yields (kg ha^{-1}) were calculated by multiplying the protein and starch contents (%) in each grain organ by the corresponding wheat grain yield.

Determination of free amino acid contents The free amino acid contents in wheat grain were determined using a high-performance liquid chromatography-inductively coupled plasma mass spectrometer (HPLC-ICP-MS, Agilent Technologies, USA, Agilent 1290–6460) (Tsochatzis *et al.* 2019). After removing impurities, each sample was washed three times with deionized water and dried in an oven at 35°C. To begin the extraction process, 100 mg of wheat flour was transferred to a 2 mL microcentrifuge tube, and 1 mL of 0.5 mol L^{-1} aqueous hydrochloric acid was added. The sample was shaken at 8,000 r min^{-1} for 20 min, followed by sonication in a 25°C water bath for an additional 20 min. Subsequently, the sample was centrifuged at 20,000×g for 20 min. Finally, 250 μL of the extraction supernatant was

transferred to an Agilent chromatographic sample vial and diluted to 1 mL using 80% acetonitrile in water. The elution conditions are shown in Appendix A.

Determination of vitamin B contents The contents were determined using ultra-high pressure liquid chromatography (UHPLC, Agilent Technologies, USA, Agilent 1260II) (Tsochatzis *et al.* 2019). To initiate the experiment, 500 mg of wheat flour was transferred into a 2 mL brown centrifuge tube. Next, 800 μL of ultra-pure water and 40 μL of 0.1 mol L^{-1} sodium hydroxide were added, followed by 800 μL of 1 mol L^{-1} phosphate buffer (pH=5.5). The sample was thoroughly mixed using a Vortex Genie 2 and subsequently subjected to ultrasonication for 15 min. After 24 h, the mixture was centrifuged at 4°C and 14,000×g for 10 min. The resulting supernatant was passed through a 0.22 μm microporous filter membrane, and the filtrate was stored at 4°C in a chromatographic sample bottle from Agilent. The elution conditions are shown in Appendix B.

Determination of mineral contents An inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7800, USA) was used for sample analysis (Zhang and Rui 2012). A total of 100 mg of wheat flour was transferred to a digestion tube and mixed with 5 mL of concentrated nitric acid until the flour was dissolved completely. The mixture was allowed to settle before being transferred to a microwave digestion furnace, where it was heated for 2–3 h. After cooling to room temperature, the digestion tube lid was cautiously opened, and the acid was poured out slowly to ensure the clarity of the liquid. The remaining solution was then transferred to a 25 mL volumetric bottle using a measuring tube.

Nitrogen transportation and distribution Twenty representative wheat plants were collected at the flowering and maturity stages in the field. Scissors and tweezers were used to separate each organ, and their fresh weights were obtained. The plant organs were dried using the oven-drying method at 105°C for 30 min, followed by 80°C until a constant weight of the sample material was achieved. After oven drying, all the sample materials were weighed and pulverized using a micro plant sample shredder (Shanghai, China). The nitrogen content was determined using an Elementar vario MACRO cube (Elementar, Hanau, Hessen, Germany) (Wei *et al.* 2024a). Nitrogen uptake in each plant organ was calculated by multiplying the product of nitrogen content and the corresponding dry matter accumulation. The proportion of nitrogen accumulated in each plant organ was defined as nitrogen distribution (ND_i). The nitrogen distribution and transportation quantity (NTQ_i) and transportation contribution rate (NTC_i) in the *i*th plant organ were obtained using the following eqs.:

$$ND_i\ (\%)=\frac{NA_i}{NA_t}\times 100 \tag{1}$$

$$NTQ_i=NA_{flowering}-NAL_{maturity} \tag{2}$$

$$NTC_i\ (\%)=\frac{NTQ_i}{NA_{grain}}\times 100 \tag{3}$$

where NA_i is nitrogen accumulation in the *i*th plant organ, while NA_t and NA_{grain} are the aboveground and grain nitrogen accumulation in wheat plants, respectively. The different wheat organs included leaf, stem-sheath, cob-glume, and grain. $NA_{flowering}$ and $NA_{maturity}$ are the largest and final amounts of nitrogen accumulation in the *i*th plant organ.

Activities of enzymes involved in nitrogen assimilation Fresh samples of wheat grain were obtained at 7, 14, 21, 28, and 35 d after flowering. They were quickly wrapped in aluminum foil, labeled, frozen in liquid nitrogen, brought back to the laboratory, and stored in a –80°C ultra-low temperature freezer. The nitrate reductase and glutamine synthase activities were analyzed using a detection kit by following the manufacturer's instructions (Beijing Solarbio Science and Technology Co., Ltd., China). Each sample was weighed at 0.1 g, mixed with the reagent, and fully reacted according to the steps in the instructions. The measurement method involved pipetting 200 μL of supernatant into a 96-well plate, using a multi-mode reader (SynergyHTX, Bio Tek, America) to measure the absorbance at 340 and 540 nm and calculating the enzymatic activities.

Entropy-TOPSIS for multiple functional assessment of wheat quality A comprehensive evaluation index for nutrient yield and quality was established by comprehensively considering the protein yield, starch yield, amino acid contents, vitamin B contents, mineral contents, and processing quality of the wheat grain (Yang *et al.* 2024). Index weighting was used to calculate the comprehensive evaluation index of each treatment through Entropy-TOPSIS with the following steps:

Normalize the indicators:

$$b_{ij}=\frac{X_{ij}-X_{j\min}}{X_{j\max}-X_{j\min}}\ (\text{positive indicator}) \tag{4}$$

$$b_{ij}=\frac{X_{j\max}-X_{ij}}{X_{j\max}-X_{j\min}}\ (\text{negative indicator}) \tag{5}$$

where b_{ij} represents the normalized value of the *j*th index in the *i*th treatment, and X_{ij} represents the average value of the *j*th index in the *i*th treatment. In addition, $X_{j\max}$ and $X_{j\min}$ are the maximum and minimum values of each scoring component, respectively.

Calculate the entropy value (H_{ij}) of each evaluation index:

$$H_{ij}=\frac{-1}{\ln m}\sum_{j=1}^{m} f_{ij}\ln f_{ij} \tag{6}$$

$$f_{ij}=\frac{b_{ij}}{\sum_{j=1}^{m} b_{ij}} \tag{7}$$

where i=1, 2, …, n, and j=1, 2, …, m. When b_{ij}=0, $\ln f_{ij}$ is modified as follows:

$$f_{ij}=\frac{(1+b_{ij})}{\sum_{j=1}^{m}(1+b_{ij})} \tag{8}$$

Calculate the entropy weight (W_j) of the evaluation index:

$$W_j=\frac{1-H_j}{n-\sum_{i=1}^{n} H_i} \tag{9}$$

Construct a weighted normalized decision matrix (Z):

$$Z=(W_j\times f_{ij})_{n\times m}=\begin{pmatrix} Z_{11} & Z_{12} & \cdots & Z_{1m} \\ Z_{21} & Z_{22} & \cdots & Z_{2m} \\ \cdots & \cdots & \cdots & Z_{3m} \\ Z_{n1} & Z_{n2} & \cdots & Z_{nm} \end{pmatrix} \tag{10}$$

Use the positive ideal solution (Z_j^+) and the negative ideal solution (Z_j^-) to determine the positive ideal solution vector Z^+

and the negative ideal solution vector Z^-:

$$Z_j^-=\min(Z_{1j}, Z_{2j}, \cdots, Z_{nj}) \quad (11)$$

$$Z_j^+=\max(Z_{1j}, Z_{2j}, \cdots, Z_{nj}) \quad (12)$$

Calculate the Euclidean distances between each index and the positive and negative ideal solutions:

$$D_i^-=\sqrt{\sum_{j=1}^{m}(Z_j^- - Z_{ij})^2} \quad (13)$$

$$D_i^+=\sqrt{\sum_{j=1}^{m}(Z_j^+ - Z_{ij})^2} \quad (14)$$

Calculate the comprehensive evaluation index CEI_i of each crop rotation, in which the proximity between the evaluation object and the optimal scheme was calculated as follows:

$$CEI_i=\frac{D_i^-}{D_i^+ + D_i^-} \ (0\leq CEI_i\leq 1) \quad (15)$$

Nutritional yields (CEI_{NY}), amino acid contents (CEI_{AA}), vitamin B contents (CEI_{VB}), mineral contents (CEI_{MC}), and processing quality (CEI_{PQ}) were considered to be equally important in wheat quality. The comprehensive evaluation index (CEI) of wheat quality was calculated using the following formula:

$$CEI=\frac{CEI_{NY}+CEI_{AA}+CEI_{VB}+CEI_{MC}+CEI_{PQ}}{5} \quad (16)$$

2.4. Data statistics and analysis

A one-way analysis of variance (ANOVA) was used to compare the differences in all indicators in response to green manure and nitrogen levels for each treatment. Differences were identified as significant at the $P<0.05$ level. Furthermore, for the effects of green manure and nitrogen reduction levels on each treatment, a two-way ANOVA was performed to analyze each indicator. In this study, all the data were tested for normality (Q-Q plot) and homogeneity of variance ($P>0.1$). They were found to be approximately normal and met the assumptions of ANOVA for the homogeneity of variances. Graphs were constructed using Origin 2023b software (OriginLab Corporation, USA). Data analysis was performed using SPSS 26.0 software (IBM, Chicago, USA) through ANOVA. Mean values were compared using Duncan's multiple range test at a significance level of $P\leq 0.05$. The Mantel test analysis among wheat quality, nitrogen transportation, and nitrogen distribution used R software, with four R packages ("linkET", "ggplot2", "dplyr", and "vegan").

3. Results

3.1. Effect of green manure on the nutrient yield of wheat grain under reduced nitrogen supply

The protein and starch yields were significantly reduced with nitrogen reduction and improved with green manure (Fig. 1). Compared with the conventional nitrogen application rate (N3), nitrogen reduced by 20% (N2) lowered the protein yield by 6%, nitrogen reduced by 40% (N1) lowered the protein and starch yields by 31.6 and 11.4%, and the no nitrogen application rate (N0) led to 58.5 and 26.9% reductions in the protein and starch yields, respectively. Protein yield increased by 46.4, 35.0, and 15.5% in mixed HCV, CV, and R compared to the F treatment, respectively. HCV and CV enhanced the starch yield by 16.3 and 13.8% in comparison to F. Protein and starch yields increased by 27.5 and 10.8% with HCVN3 and by 35.9 and 16.2% with HCVN2 compared to FN3, respectively. The protein yield of HCVN2 was enhanced by 10.7 and 40.8% in comparison to CVN2 and RN2, while the starch yield of HCVN2 was improved by 5.4 and 19.2% compared to CVN2 and RN2, respectively. Therefore, mixed hairy vetch and common vetch gave higher protein and starch yields to improve the nutrient yield while reducing chemical nitrogen usage by 20%.

3.2. Nutritional quality of wheat grain in response to green manure under reduced nitrogen supply

Amino acid contents Nitrogen reduction significantly reduced the amino acid contents, but green manure increased them (Fig. 2). Nitrogen supply reduction resulted in lower essential and non-essential amino acid contents, with reductions of 7.0 and 5.9% in N2, 14.0 and 13.8% in N1, and 23.1 and 23.5% in N0 compared to N3, respectively. The essential amino acid contents were improved by 18.8, 15.2, and 7.6% with HCV, CV, and R compared to F, respectively. HCV and CV enhanced non-essential amino acid contents by 12.0 and 7.6% compared to F. For the essential and non-essential amino acid contents, HCVN2 increased them by 13.2 and 10.0% over FN3, compared to 5.1 and 5.2% over CVN2, and 5.3 and 12.9% over RN2, respectively. In terms of amino acid composition, HCVN2 enhanced the lysine, threonine, isoleucine, leucine, and valine contents by 20.7, 18.8, 23.1, 32.3, and 13.2% compared with FN3, respectively. HCVN2 increased the glutamic acid, proline, aspartic acid, serine, glycine, alanine, cystine, tyrosine, and histidine contents by 12.1, 11.1, 6.4, 5.6, 6.8, 6.9, 7.0, 6.5, and 9.0% compared to FN3, respectively. Therefore, mixed hairy vetch and common vetch under 20% reduced nitrogen had higher amino acid contents, which can lay the foundation for high nutritional quality in wheat grain.

Vitamin B contents Nitrogen reduction significantly lowered the vitamin B contents, but green manure enhanced them (Fig. 3). The vitamin B contents of N1 and N0 were reduced by 12.5 and 32.4% compared with N3; and they were enhanced by 26.6, 19.3, and 9.5% with HCV, CV, and R compared to F, respectively. However, it is notable that HCVN2 enhanced the vitamin B contents by 20.0%, including increases of 22.6 and 42.8% in vitamin B1 and B2, 20.6 and 35.8% in vitamin B3 and B5, and 25.6 and 28.4% in vitamin B6 and B9 compared with FN3, respectively. Compared to CVN2 and RN2, HCVN2 enhanced the vitamin B contents by 8.6 and 23.1%. These results suggest that mixed hairy vetch and common vetch under a 20% nitrogen reduction increased the vitamin B contents, thus further improving the micronutrient contents of wheat grain.

Mineral contents As shown in Table 1, nitrogen reduction had a detrimental impact on the mineral contents, but green manure had a positive effect. Compared with N3, the mineral contents were reduced by 11.4 and 21.3% with N1 and N0, and they were improved by 8.2 and 7.8% with HCV and CV compared with F, respectively. Importantly, HCVN2 raised

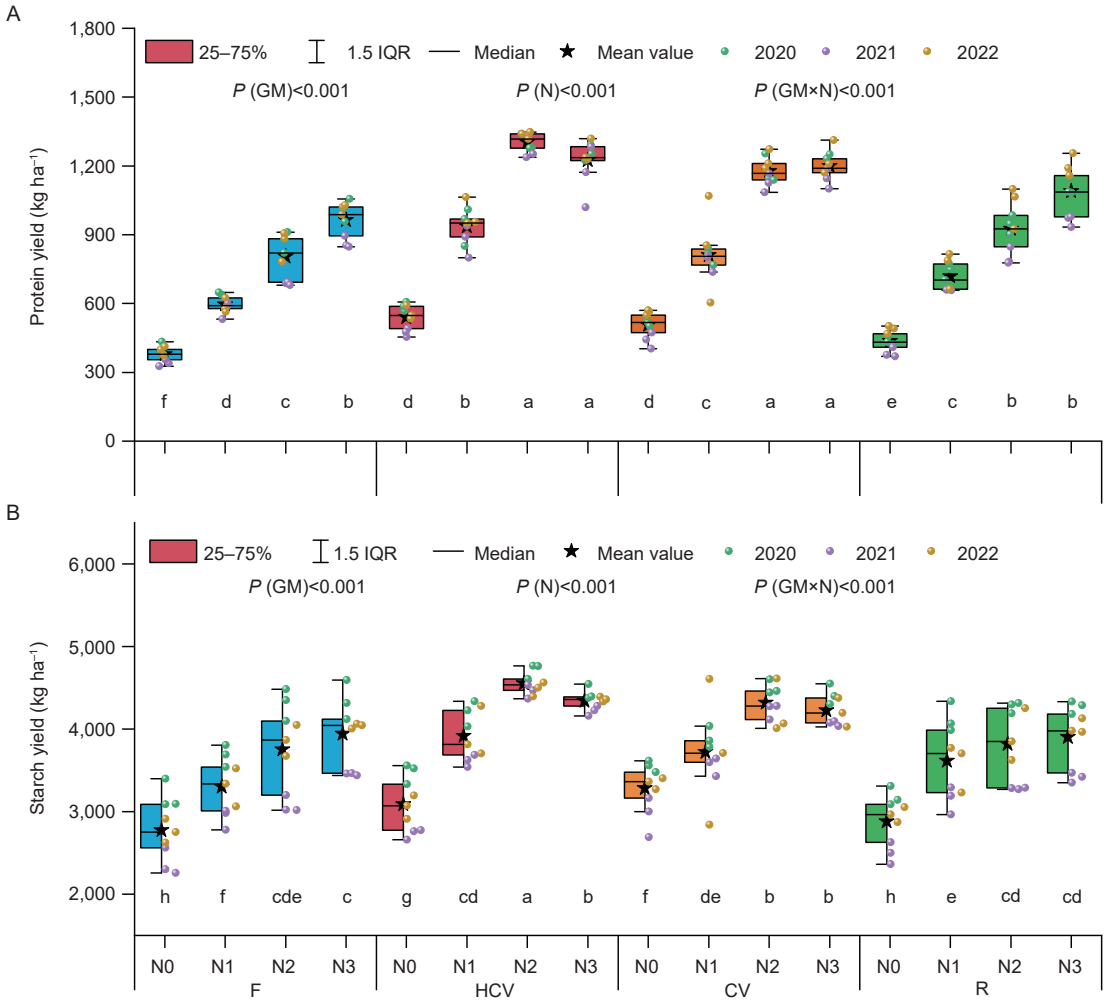


Fig. 1 Protein (A) and starch (B) yields of wheat grain with green manure (GM) under reduced nitrogen (N) supply. F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate. Error bars indicate the SE of the means (n=9, 2020–2022). Different lowercase letters above bars indicate significant differences between treatments at P<0.05.

the mineral contents by 8.6 and 23.1% compared to CVN2 and RN2. HCVN2 exhibited higher contents of individual minerals, and magnesium (10.1%), calcium (6.0%), iron (26.0%), zinc (22.2%), and selenium (12.9%) were higher than in FN3. Overall, the above results show that mixed hairy vetch and common vetch under a 20% nitrogen reduction improved the mineral contents, thus enhancing the micronutrient contents of wheat grain.

3.3. Effect of green manure and reduced nitrogen supply on the processing quality of wheat grain

Milling quality The grain hardness and volume-weight were affected by nitrogen supply reduction, green manure, and their interactions (Table 2). N1 increased the grain hardness by 8.4% compared with N3. HCV and CV reduced grain hardness by 6.9 and 3.6%, but they increased the volume-weight by 5.4 and 4.2% compared to F, respectively. HCVN2 increased the volume-weight by 4.2% compared to FN3. There were no significant differences in grain hardness or volume-weight among HCVN2, CVN2, and RN2. These results indicate that mixed hairy vetch and common vetch under a 20% nitrogen reduction improved milling quality, thus further increasing processing quality.

Flour quality The data showed that a reduced nitrogen input had a negative effect on flour quality, but green manure had a positive effect (Table 2). N2 reduced the sedimentation value by 6.0%, and N1 lowered the falling number, wet gluten, and sedimentation value by 12.3, 10.8, and 16.1%, respectively, but it increased the degree of softening by 16.8% compared with N3. However, HCV and CV had higher flour quality characteristics, which improved by 13.2 and 10.1% for falling number, 8.6 and 6.7% for wet gluten, and 13.1 and 9.0% for sedimentation compared with F, respectively. HCVN2 was higher than FN3 for falling number, wet gluten, and sedimentation by 14.0, 7.8, and 7.2%, respectively. Interestingly, HCVN2 increased the falling number by 4.9 and 13.9% compared to CVN2 and RN2. HCVN2 enhanced the wet gluten and sedimentation values by 13.0 and 13.7% compared to RN2, respectively. Overall, mixed hairy vetch and common vetch under a 20% nitrogen reduction can improve flour quality to enhance the processing quality of wheat grain.

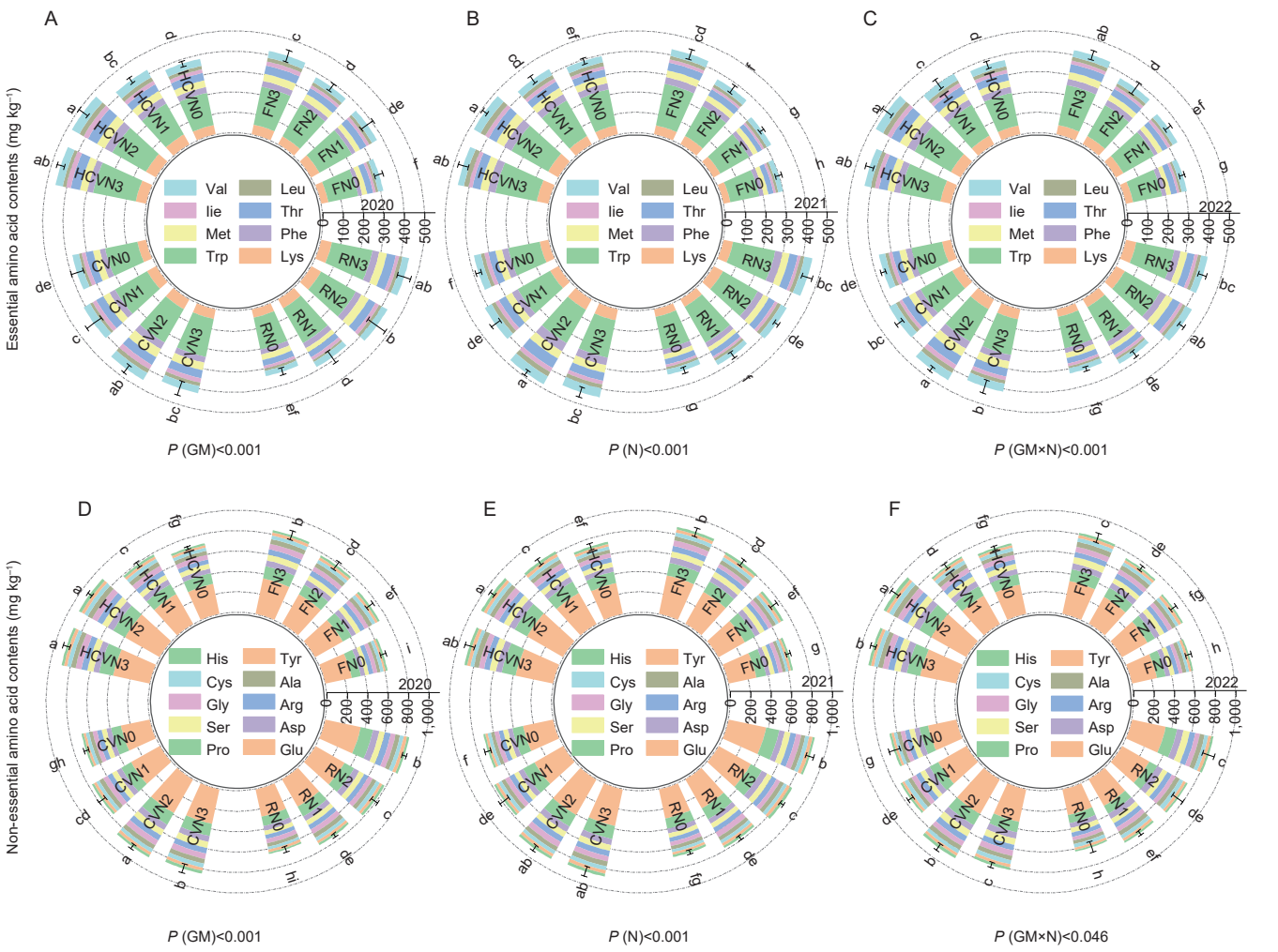


Fig. 2 Essential amino acid (A–C) and non-essential amino acid (D–F) contents of wheat grain as affected by green manure (GM) and reduced nitrogen (N) supply. F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate. Error bars indicate the SE of the means (n=3). Different lowercase letters above bars indicate significant differences between treatments at P<0.05.

3.4. Nitrogen metabolism mechanism for improved nutrient yield and quality in wheat grain with green manure and nitrogen reduction

Nitrogen accumulation and distribution A nitrogen supply reduction led to lower nitrogen accumulation, but green manure could increase nitrogen accumulation in wheat (Fig. 4). Grain nitrogen accumulation was lower by 5.0 and 22.6% with N2 and N1, and grain nitrogen distribution was lower by 8.4% with N1 compared to N3. HCV and CV increased grain nitrogen accumulation by 25.2 and 18.8%, and they enhanced nitrogen distribution in the grain by 9.3 and 7.6% compared to F, respectively. The nitrogen accumulation and distribution of grain in HCVN2 were 16.3 and 9.0% higher than in FN3, and 6.0 and 15.3% higher than in RN2. Therefore, mixed hairy vetch and common vetch under a 20% nitrogen reduction increased grain nitrogen accumulation in wheat.

Nitrogen remobilization A reduction in the nitrogen supply decreased the amount of nitrogen transported from vegetative organs to grain, but green manure improved the quantity and contribution rate of nitrogen transport from vegetative organs to grain in wheat (Appendix C). The amounts of nitrogen transported from leaf, stem-sheath, and cob-glume to grain were reduced by 25.1, 24.3, and 24.8%, respectively, in N1 compared with N3. The amounts of nitrogen transported from leaf, stem-sheath, and cob-glume to grain were enhanced by 25.5 and 22.2%, 33.7 and 31.7%, and 29.1 and 26.6% in HCV and CV compared with F, respectively. The contribution rates from leaf and stem-sheath to grain of HCV were enhanced by 5.2 and 6.1% compared to F. The amounts of nitrogen transported from leaf, stem-sheath, and cob-glume to grain were improved by 33.3, 28.8, and 18.9% in HCVN2 compared to FN3. The contribution rates from leaf, stem-sheath, and cob-glume to grain were increased by 12.1, 11.9, and 11.1% with HCVN2 compared to FN3, respectively. Therefore, mixed hairy vetch and common vetch under a 20% nitrogen reduction can improve the nitrogen transportation amounts and contribution rates from vegetative organs to grain in wheat.

Nitrogen assimilation Nitrate reductase and glutamine synthetase activities were significantly decreased with nitrogen input reduction and improved with green manure

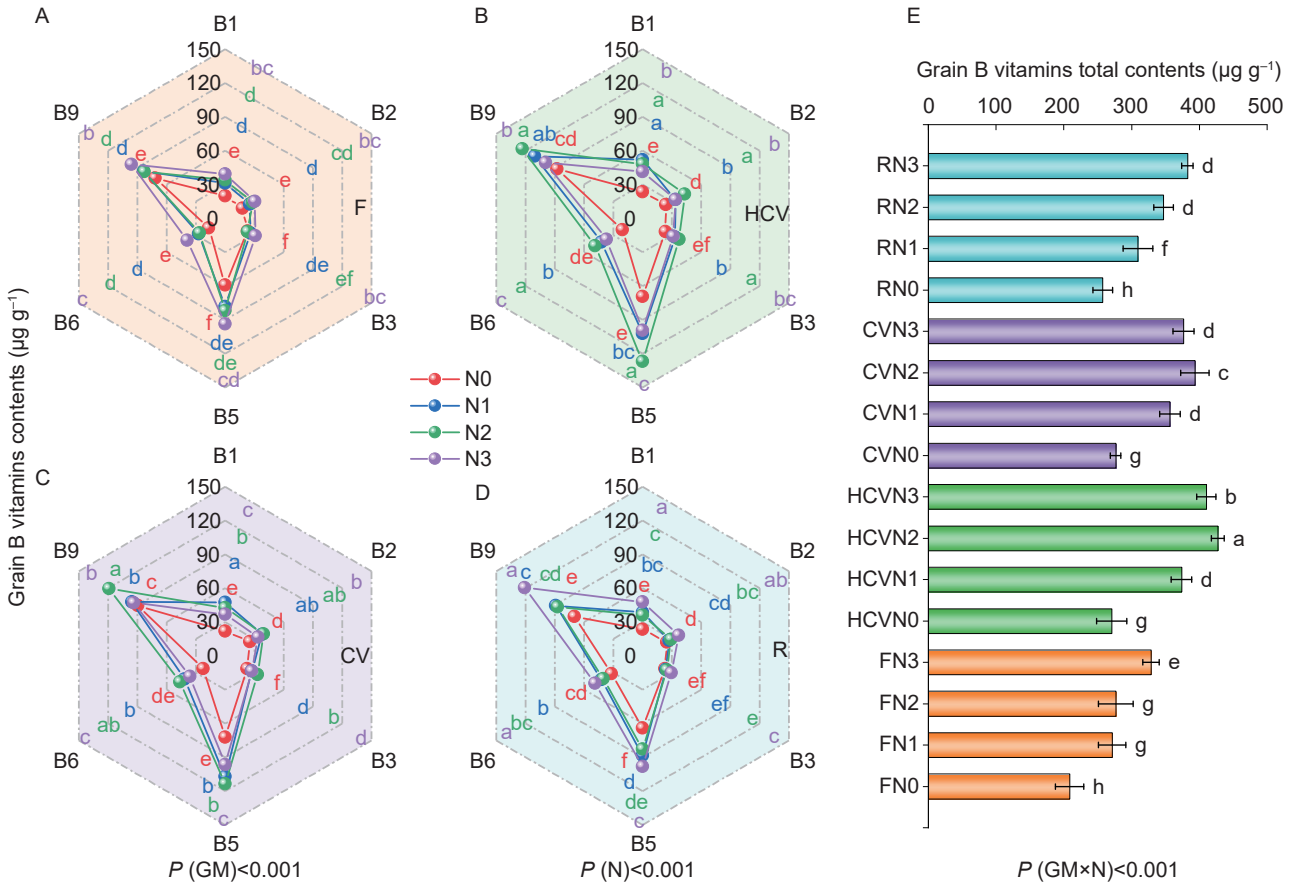


Fig. 3 Vitamin B contents of wheat grain with green manure (GM) under reduced nitrogen (N) supply. F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate. Error bars indicate the SE of the means (n=9, 2020–2022). Different lowercase letters above bars indicate significant differences between treatments at P<0.05.

Table 1 Mineral contents of wheat grain with green manure under reduced nitrogen input in 2021

Green manure[1)]	Nitrogen level[2)]	Magnesium (μg g^{-1})	Calcium (μg g^{-1})	Iron (μg g^{-1})	Zinc (μg g^{-1})	Selenium (μg g^{-1})
F	N0	848.3 f	286.4 f	11.28 e	21.23 g	0.056 h
	N1	987.3 d	335.5 de	13.05 de	26.31 d	0.086 e
	N2	1,030.3 c	332.9 de	13.33 de	28.26 b	0.094 cd
	N3	1,085.9 b	383.2 b	14.88 bcd	27.11 c	0.104 b
HCV	N0	9,18.0 e	297.4 ef	12.81 de	24.98 e	0.067 g
	N1	9,86.6 d	358.1 bcd	13.99 cde	27.43 c	0.087 de
	N2	1,195.3 a	406.3 a	18.75 a	33.13 a	0.117 a
	N3	1,159.3 a	393.9 ab	14.12 cde	30.52 a	0.114 a
CV	N0	912.8 e	299.1 ef	12.59 de	22.92 fg	0.074 f
	N1	1,082.5 b	340.2 d	11.76 de	27.46 c	0.089 de
	N2	1,142.6 ab	411.2 a	17.57 ab	29.92 ab	0.101 b
	N3	1,131.9 ab	382.3 b	16.74 abc	27.78 c	0.085 e
R	N0	882.3 ef	310.3 e	11.42 e	21.90 g	0.096 bc
	N1	903.5 ef	358.1 bcd	11.76 de	24.42 ef	0.069 fg
	N2	1,101.1 b	373.1 b	16.47 abc	26.92 d	0.094 cd
	N3	1,091.3 b	410.6 a	19.42 a	29.56 ab	0.106 ab
Significance test (P-value)						
Green manure level (GM)		0.004	0.039	0.049	0.000	0.000
Nitrogen level (N)		0.000	0.000	0.000	0.000	0.000
GM×N interaction		0.015	0.021	0.042	0.000	0.000

[1)] F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed.
[2)] N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate.
Values followed by different letters in the same column are significantly different at P<0.05.

Table 2 Grain processing quality with green manure under reduced nitrogen supply

Year	Green manure[1)]	Nitrogen level[2)]	Grain hardness (%)	Volume-weight (g L^{-1})	Falling number (s)	Degree of softening (F.U.)	Wet gluten (%)	Sedimentation value (mL)
2020	F	N0	55.74 a	752.77 f	241.39 h	120.97 a	28.98 ef	22.94 ef
		N1	56.41 a	760.00 e	265.27 f	112.33 c	30.36 de	26.33 d
		N2	50.38 fgh	764.71 e	281.21 e	105.86 d	31.71 cd	27.12 d
		N3	51.57 def	778.69 de	300.30 d	97.94 f	32.41 bc	30.21 b
	HCV	N0	53.14 cd	793.91 bc	256.38 g	115.60 b	32.31 bc	26.67 d
		N1	50.92 efg	797.61 bc	296.52 d	103.13 e	31.31 cd	31.54 ab
		N2	49.45 gh	817.07 a	341. 52 a	94.43 g	35.03 a	32.14 ab
		N3	47.17 i	810.10 a	323.87 bc	88.50 h	34.48 a	34.46 a
	CV	N0	54.83 ab	777.83 de	253. 85 g	117.51 b	30.64 d	24.02 e
		N1	53.59 bc	801.54 b	277.68 e	106.76 d	30.32 de	27.35 cd
		N2	50.79 efg	804.52 ab	327.46 b	95.51 fg	34.28 a	32.51 ab
		N3	48.93 h	785.48 cd	316.39 c	89.82 h	33.44 ab	33.03 a
	R	N0	55.45 a	764.55 e	252.79 g	118.31 b	28.21 f	21.91 f
		N1	52.20 cde	789.83 cd	264.68 f	111.14 c	30.24 de	24.10 e
		N2	51.03 efg	785.72 cd	292.90 d	101.47 e	31.59 cd	28.98 bc
		N3	51.73 def	804.47 ab	317.46 c	94.75 g	33.95 a	30.98 b
2021	F	N0	56.33 a	753.37 h	230.79 h	124.72 a	27.85 f	24.20 ef
		N1	55.80 ab	757.69 h	257.62 fg	110.34 c	29.97 de	26.24 d
		N2	51.86 e	765.13 g	269.00 ef	107.60 d	31.24 cd	29.58 c
		N3	50.10 fg	778.55 f	294.69 c	96.77 f	32.90 b	32.23 b
	HCV	N0	53.16 cd	796.36 d	258.26 fg	116.56 b	30.17 de	24.58 e
		N1	50.37 f	798.48 d	286.84 cd	102.20 e	31.80 bc	30.78 c
		N2	49.98 fg	820.39 a	332.07 a	96.09 f	35.27 a	33.78 a
		N3	46.62 h	811.57 b	319.12 ab	91.01 gh	34.92 a	35.24 a
	CV	N0	53.49 c	779.81 f	289.58 c	116.54 b	28.84 ef	24.21 ef
		N1	52.37 de	799.31 d	274.69 de	104.55 e	30.42 cd	29.45 c
		N2	52.56 de	809.31 bc	319.75 ab	95.82 f	35.17 a	34.01 a
		N3	47.13 h	801.13 d	317.72 b	88.63 h	34.26 a	34.52 a
	R	N0	55.78 ab	768.19 g	249.45 g	115.60 b	28.16 f	22.72 f
		N1	55.05 b	787.83 e	270.22 ef	111.57 c	28.86 ef	26.62 d
		N2	48.66 gh	784.32 ef	300.37 c	103.40 e	31.24 cd	29.68 c
		N3	50.84 ef	803.32 cd	314.42 b	92.86 g	34.45 a	33.85 a
2022	F	N0	59.43 a	752.70 h	238.37 h	119.47 a	28.75 g	23.20 gh
		N1	58.18 b	759.12 gh	270.33 f	113.30 b	29.70 fg	27.20 e
		N2	56.08 bc	764.69 g	280.47 e	106.11 c	30.09 fg	29.90 c
		N3	52.63 de	777.92 f	305.99 d	99.18 d	32.38 de	29.77 c
	HCV	N0	55.49 cd	794.51 cd	249.64 g	115.32 b	32.52 d	25.78 f
		N1	52.12 ef	786.78 e	307.00 d	103.33 cd	30.88 ef	29.86 c
		N2	51.32 ef	822.94 a	353.27 a	94.50 de	35.01 a	32.93 ab
		N3	49.40 f	812.25 b	337.92 b	89.09 f	34.46 b	34.20 a
	CV	N0	56.82 b	780.10 f	241.63 gh	111.93 bc	33.44 c	26.67 ef
		N1	56.86 b	796.77 c	288.23 e	104.03 c	30.12 fg	27.18 e
		N2	49.36 f	808.79 b	331.29 bc	96.09 de	35.81 a	31.76 b
		N3	46.51 g	807.28 b	323.22 c	90.90 ef	34.41 bc	33.90 a
	R	N0	58.58 ab	766.46 g	248.27 gh	117.30 a	29.13 g	21.82 h
		N1	58.42 b	790.10 d	268.83 f	108.39 c	30.35 fg	24.12 g
		N2	52.71 de	784.04 ef	307.89 d	102.49 cd	30.37 fg	28.27 d
		N3	55.43 cd	806.01 b	324.67 c	92.17 e	34.23 bc	32.06 ab
Significance test (*P*-value)								
Green manure level (GM)			0.000	0.010	0.000	0.000	0.000	0.000
Nitrogen level (N)			0.000	0.037	0.008	0.000	0.000	0.001
GM×N			0.000	0.028	0.002	0.000	0.000	0.006

[1)] F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed.
[2)] N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate.
Values followed by different letters in the same column are significantly different at $P<0.05$.

(Fig. 5). The nitrate reductase and glutamine synthetase activities were reduced by 13.6 and 4.6% with N2 and by 22.9 and 44.4% with N1 compared to N3, respectively. HCV and CV increased the nitrate reductase activity by 32.4 and 18.5% and enhanced glutamine synthetase activity by 22.2 and 13.6% compared to F, respectively. Specifically, HCVN2 increased the nitrate reductase activity by 8.0, 9.8, and 49.3% after 7–21 d of flowering when compared with FN3, CVN2, and RN2, respectively. Glutamine synthetase activity after 7–21 d of flowering was enhanced by 14.1 and 24.9%

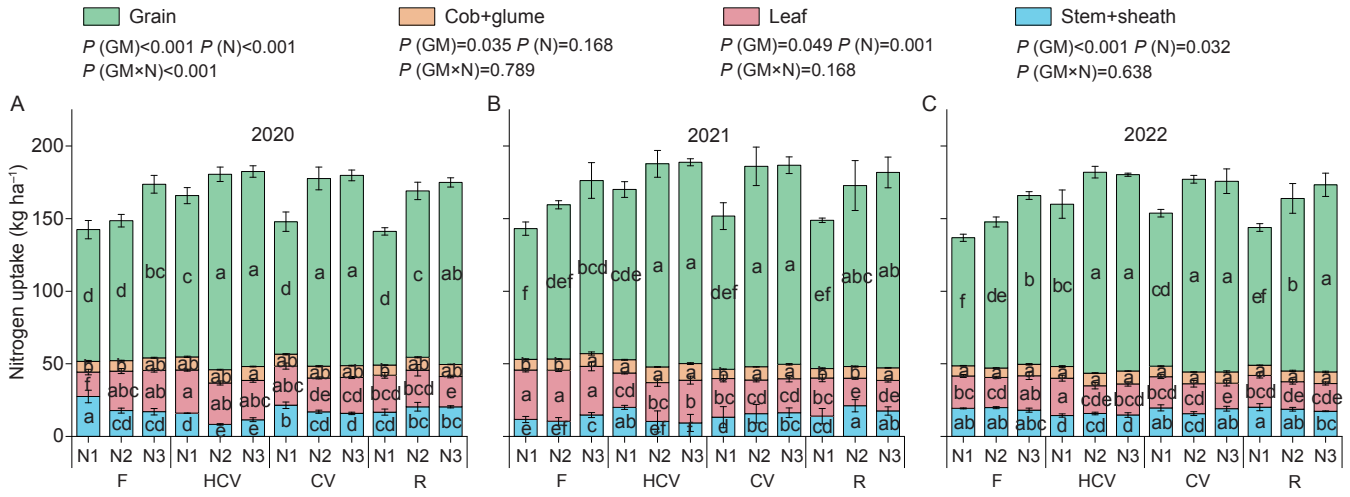


Fig. 4 Nitrogen (N) uptake as affected by green manure (GM) under reduced nitrogen supply. F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate. Error bars indicate the SE of the means (n=3). Different lowercase letters above bars indicate significant differences between treatments at P<0.05.

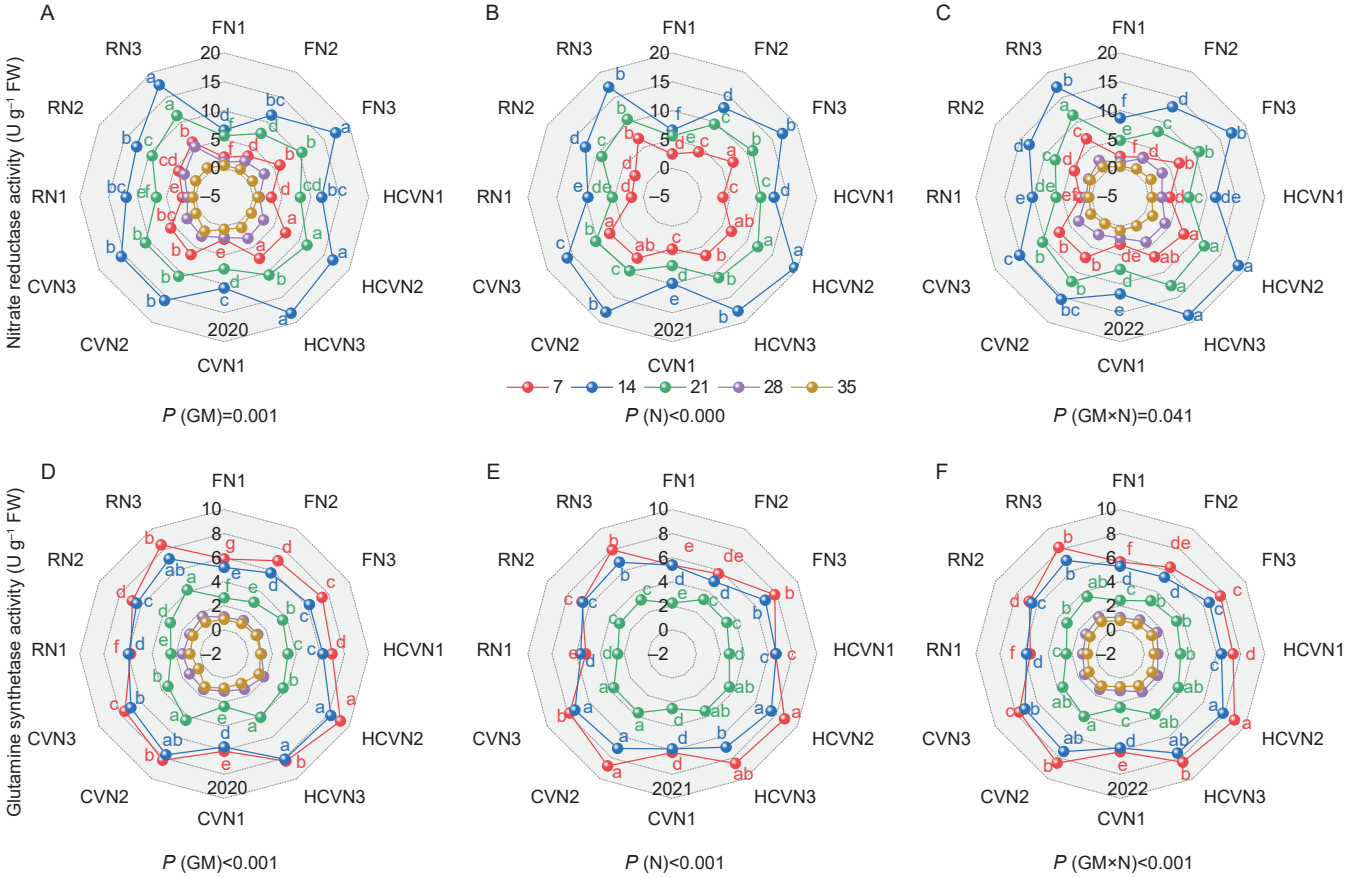


Fig. 5 Activities of nitrate reductase (A–C) and glutamine synthetase (D–F) with green manure (GM) under reduced nitrogen (N) supply in wheat grain. F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate.

with HCVN2 compared to FN3 and RN2, respectively. Thus, mixed hairy vetch and common vetch with a 20% reduction in nitrogen can improve nitrate reductase activity and glutamine synthetase activity to improve grain nitrogen assimilation.

3.5. Comprehensive evaluation index of wheat grain quality, and correlation analysis and principal component analysis among wheat grain quality, nitrogen accumulation, nitrogen remobilization, and nitrogen assimilation

Comprehensive evaluation index of wheat grain quality Nutrient production, amino acid contents, vitamin B contents, mineral contents, and processing quality were used to determine the comprehensive evaluation index of wheat grain quality by entropy-TOPSIS (Fig. 6). The contributions of each component to the overall comprehensive evaluation index are shown in Fig. 6-A. Nutrient production accounted for the largest share of the comprehensive evaluation index of wheat grain quality, followed by the amino acid, mineral, and vitamin B contents, and then processing quality. Nitrogen input reduction resulted in lower nutrient production and quality of wheat grain (Fig. 6-B and C). HCV had a higher contribution than CV, R, or F in nutrient production, amino acid, vitamin B, and mineral contents, processing quality, and the comprehensive evaluation index (Fig. 6-A and B). HCVN2 showed the highest comprehensive evaluation index of wheat grain quality, which was 73.8, 22.3, and 134.0% higher than FN3, CVN2, and RN2, respectively (Fig. 6-C). Therefore, mixed hairy vetch and common vetch improved the nutrient production and quality of wheat grain under a 20% reduction in chemical nitrogen.

Mantel test analysis and principal component analysis among wheat grain quality, nitrogen accumulation, nitrogen remobilization, and nitrogen assimilation The results showed that essential amino acids, non-essential amino acids, wet gluten, and protein yield had significant positive correlations, indicating that the wet gluten and protein yield of wheat grain were improved with an increase in amino acids (Appendix D-a). The Mantel test analysis showed that grain nitrogen accumulation, nitrogen remobilization from vegetative organs to grain, nutrient production, and quality of wheat grain had significant positive correlations (Appendix D-a), implying that greater grain nitrogen accumulation and remobilization can improve the nutrient production and quality of wheat grain. The principal component analysis indicated that nitrate reductase activity, glutamine synthetase activity, grain nitrogen accumulation, and nitrogen remobilization from leaf to grain had significant positive correlations (Appendix D-b, PC1), suggesting that improved nitrogen assimilation is beneficial for increasing nitrogen remobilization from leaf to grain and grain nitrogen accumulation, thereby improving the nutritional quality of wheat grain. Therefore, mixed hairy vetch and common vetch under a 20% nitrogen reduction proved to be an effective strategy for improving the nutrient yield and quality of wheat grain by increasing nitrogen accumulation, remobilization, and assimilation.

4. Discussion

4.1. Effect of green manure on nutrient production in wheat grain under reduced nitrogen supply

In this study, green manure increased the production of nutrients in wheat grain. This occurred mainly because returning green manure to the field improves the soil physical and chemical properties, enriches nutrient pools, increases microbial diversity, and optimizes ecological functions, thereby promoting the absorption of nutrients by crops (Xie *et al.* 2016; Chen *et al.* 2020; Liu *et al.* 2022). Leguminous green manure generally accelerates the rate of mineralization more than crucifers due to the lower C/N ratio, which leads to higher soil mineral nitrogen content (Ma *et al.* 2021; Gao *et al.* 2023), ultimately increasing nutrient production. However, planting green manure also leads to higher soil water consumption, which reduces subsequent crop yields (He *et al.* 2016; Alvarez *et al.* 2017). On the other hand, in the arid irrigation area of northwestern China, a nitrogen reduction of 20% and green manure retention was reported to reduce soil evaporation and promote transpiration, thus

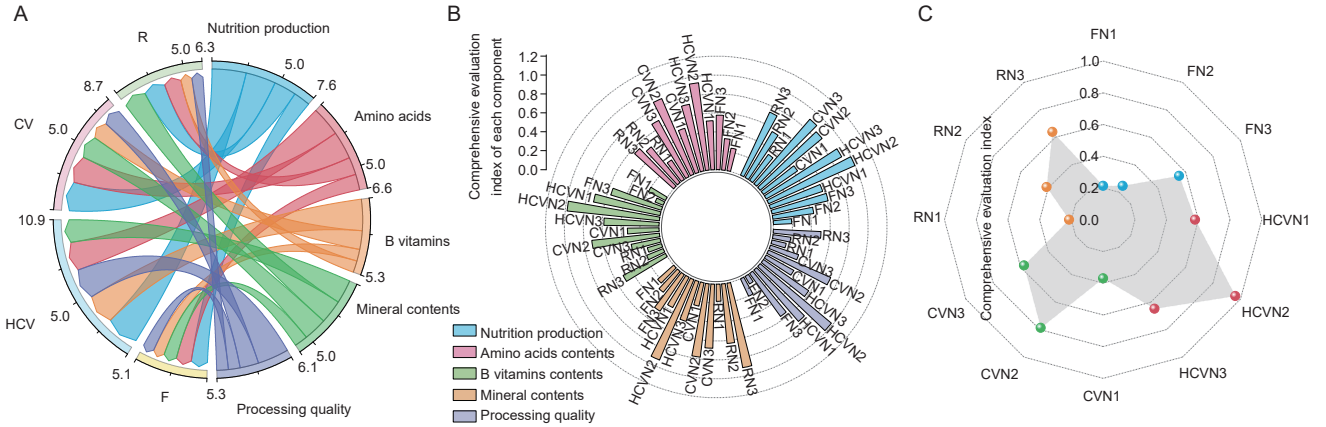


Fig. 6 Contributions of different green manures to wheat grain quality (A) and the comprehensive evaluation index of nutrient yield and quality of spring wheat grain with green manure and reduced nitrogen input (B and C). F, fallow; HCV, mixed hairy vetch and common vetch; CV, common vetch; R, rapeseed. N0, zero-nitrogen application; N1, nitrogen reduced by 40%; N2, nitrogen reduced by 20%; N3, conventional nitrogen application rate.

increasing crop yields and maintaining economic benefits (Wang *et al.* 2024). Notably, legume crops require minimal water during their early growth stages, and their roots primarily extract water from the upper soil layers (Ma *et al.* 2021).

In the present study, mixed hairy vetch and common vetch led to higher nutrient production in wheat grain under nitrogen reduced by 20%. The main reasons may be as follows: Green manure promotes nitrogen uptake in vegetative organs and transportation to synthesize amino acids and proteins (Savill *et al.* 2018; Ben *et al.* 2020; Lyu *et al.* 2024); and plants absorb part of the soil mineral nitrogen after flowering, which increases the storage protein contents in grains (Peng *et al.* 2022). Leguminous green manure can fix nitrogen from the atmosphere by its nodules to increase the soil total nitrogen content, thus promoting the soil organic cycle and enhancing crop nutrient uptake (Zhang *et al.* 2021; Yu *et al.* 2024); and green manure promotes carbon and nitrogen cycling, which can increase crop yields (Snyder *et al.* 2016). Furthermore, mixed green manure supplies a larger amount of organic material due to the complementary effects of their canopy structures, which enhances light capture and energy efficiency (Li *et al.* 2023; Wei *et al.* 2024b). In addition, mixed cropping systems create spatial advantages in the rhizosphere for water and nutrient utilization, which facilitates mutual uptake between plants (Li *et al.* 2023). Furthermore, relying solely on green manure as a nutrient source generally results in a lower nutrient supply capacity (Gao *et al.* 2023), and this can be attributed to the lower immediate availability of mineral nutrients in organic sources compared with inorganic fertilizers, as well as a temporary decline in soil fertility (Ma *et al.* 2021). The nutrient requirements of crops vary at different growth stages, so combined chemical nitrogen and green manure can better meet the needs of the crops (Gao *et al.* 2023).

4.2. Nutritional quality of wheat grain with green manure under reduced nitrogen input

The results suggested that a reduced nitrogen input will reduce the amino acid contents, but mixed common vetch and hairy vetch improved the amino acid contents under a 20% nitrogen reduction. The main reasons are as follows: (i) the application of a lower nitrogen fertilizer input reduces the soil mineral nitrogen contents, thereby lowering the concentration of substrates for amino acid synthesis (Zhang *et al.* 2021; Wei *et al.* 2024a); (ii) mixed sown green manure return can improve the soil organic carbon and mineral nitrogen contents, thus enhancing nitrate absorption and promoting the metabolic processes of carbon and nitrogen (Zörb *et al.* 2018; Wei *et al.* 2024b); (iii) higher nitrogen absorption supports strong metabolic activities and abundant storage in nutritive organs, which can improve the activities of nitrogen metabolic enzymes and promote amino acid synthesis (The *et al.* 2021); and (iv) mixed sown green manure return promotes crop nitrogen remobilization and reassimilation at pre-anthesis (Wei *et al.* 2024b), ultimately promoting amino acid synthesis (Huang *et al.* 2020). Previous studies have shown that amino acids are affected mostly by nitrogen uptake during grain filling, and green manure decomposition can provide some of the nutrients for grain growth (Soltani *et al.* 2014; Zhang *et al.* 2021).

In this study, green manure increased the vitamin B contents, with leguminous green manure showing a greater effect than rapeseed green manure. This could be due to increases in multiple nutrients with leguminous green manure residue returning to the soil, thus indirectly enhancing the accumulation of microelements in the plants (Ma *et al.* 2021; Zhang *et al.* 2021). Furthermore, rapeseed green manure enhances the mobilization of fixed phosphorus in the soil through its well-developed cluster roots (Yu *et al.* 2024). Notably, this study is the first to demonstrate an increase in the vitamin B contents of wheat grain through a field experiment.

Previous studies have demonstrated that the zinc content of wheat grain increases under a higher nitrogen application rate (Hermans *et al.* 2021; Hui *et al.* 2022; Wang *et al.* 2022), which also explains why nitrogen reduction resulted in a lower zinc content of the wheat grain. Besides, rapeseed green manure significantly increased the iron, zinc, and selenium contents in wheat grain. It especially affected the selenium content, which may be due to rapeseed's strong ability to enrich selenium and the secretion of organic acids from its roots (Lyu *et al.* 2022). The zinc content of wheat grain was significantly increased with mixed common vetch and hairy vetch, which may be related to the promotion of beneficial root exudates and enhancement of multiple soil elements by different crops, thus increasing the absorption of mineral elements (Soltani *et al.* 2014; Costerousse *et al.* 2021). In addition, the combination of nutrients provided by green manure, such as calcium, magnesium, zinc, and iron, has the potential to enhance the nutritional quality of crops (Soltani *et al.* 2014; Grüter *et al.* 2018).

4.3. Effect of green manure and reduced nitrogen input on the processing quality of wheat grain

The findings showed that a reduced nitrogen application rate negatively affected grain processing quality. Lower nitrogen fertilizer input reduces the grain protein content, wet gluten content, and processing quality (Zheng *et al.* 2020), mainly because nitrogen remobilization from pre-anthesis nutrient organs is reduced, and the synthesis of grain storage proteins also depends on nitrogen assimilation during the post-anthesis period (The *et al.* 2021; Wang *et al.* 2023a). Furthermore, the lower chemical nitrogen supply reduces grain nitrogen accumulation (Wei *et al.* 2024b), thus lowering the grain protein content and processing quality. This study also found that mixed hairy vetch and common vetch under a 20% nitrogen reduction had a strong advantage in improving grain processing quality. This may be attributed to the increases in soil active carbon and nitrogen fractions and associated enzymatic activities with mixed sowing green manure (Yu *et al.* 2024), thereby indirectly improving the wet gluten content, sedimentation value, and falling number (Yao *et al.* 2023). Mixed sowing green manure can promote crop growth and nitrogen utilization to improve the concentrations of protein and wet gluten, thus improving the processing quality

of crops (Tarui *et al.* 2013; Zörb *et al.* 2018; Gao *et al.* 2023).

4.4. Green manure and nitrogen reduction increased the nutrient value and quality of wheat grain by improving nitrogen accumulation, remobilization, and assimilation

It is noteworthy that mixed hairy vetch and common vetch increased grain nitrogen accumulation, nitrogen remobilization from vegetative organs to grain, and the activities of nitrogen metabolism enzymes, thus increasing the amino acid, protein, and wet gluten contents under a 20% reduction in chemical nitrogen. This effect can be attributed to the fact that mixed sown green manure enhances the soil mineral nitrogen content and promotes crop N utilization (Wei *et al.* 2025), which improves grain nitrogen accumulation from the environment at post-anthesis for the synthesis of more amino acids and proteins (Zhang *et al.* 2021; Liu *et al.* 2022). In addition, mixed sown green manure can increase the activities of nitrogen metabolizing enzymes in plants to promote amino acid synthesis (Wei *et al.* 2024b), and ultimately improve grain nutrient yield and processing quality (Peng *et al.* 2022; Lyu *et al.* 2024). Besides, the green manure returns increased nitrogen accumulation, remobilization, and assimilation to improve the production of nutrients and quality of wheat grain. On the one hand, green manure return can increase the soil organic carbon content to provide more energy materials and carbon skeletons for nitrogen metabolic processes, further synergistically promoting nitrogen metabolism at post-anthesis to increase grain protein and starch yields (Savill *et al.* 2018; Huang *et al.* 2020; Cui *et al.* 2023). On the other hand, optimizing photosynthetic performance and the activities of nitrogen metabolism enzymes can enhance nitrogen remobilization from leaf to grain at post-anthesis and grain nitrogen accumulation, ultimately increasing the carbohydrate, amino acid, protein, and wet gluten contents (Ben *et al.* 2020; Sieling and Kage 2021; The *et al.* 2021). In addition, leguminous crops improve soil health and enhance the chlorophyll and antioxidant enzymes of the main crops, which can promote the transfer of nitrogen to the grain and thus improve grain quality (Wang *et al.* 2023a, b).

4.5. Implications and perspectives

One novelty of this study is the use of mixed sown common vetch and hairy vetch, which can simultaneously maintain high nutrient production and grain quality of spring wheat while reducing the chemical nitrogen input. Compared with common vetch and rapeseed, mixed hairy vetch and common vetch significantly improved the nutrient yield and grain quality of wheat grain. This occurred mainly because mixed sown green manure has spatial advantages in the different canopy structures and root distributions, thus increasing the amount of green manure returned to the field (Wei *et al.* 2024b). In addition, leguminous pre-crops enhanced soil health and microelement contents, which increased nitrogen accumulation, remobilization, and assimilation. Therefore, mixed sown hairy vetch and common vetch can improve the nutrient yield and quality of wheat grain under a 20% nitrogen reduction by promoting nitrogen accumulation, remobilization, and assimilation.

The scientific evidence in this study provides valuable insights for advancing current policies and accelerating the implementation of various scientific research projects, which can accelerate the green transition of cropping systems in China. Specifically, planting green manure can strengthen the function of farmland ecological services, as it is critical for achieving long-term grain production resilience, improving soil health, and ensuring environmental sustainability. However, one limitation of this study is that it only used local wheat cultivars. Next, we should carry out field studies on wheat grain quality with multiple sites and varieties, mainly focusing on the mechanisms of chemical structures and the molecular mechanisms of carbon and nitrogen metabolism. The insights gained from this study will help guide future agronomic practices in the region and other areas with similar climatic and soil characteristics, ensuring the nutrient yield and quality of wheat grain for local communities, so as to provide new ideas for the green development of agriculture.

5. Conclusion

A lower chemical nitrogen input reduced the nutrient yield of wheat grain, but green manure improved it. Mixed cropping green manure under a nitrogen supply reduced by 20% showed high protein and starch levels. Meanwhile, the reduced chemical nitrogen reduced the grain quality of wheat, but green manure improved it. Mixed cropping green manure under chemical nitrogen reduced by 20% enhanced the amino acid, vitamin B and mineral contents, as well as processing quality. It also simultaneously improved the nutrient yield and quality of wheat grain, which was attributed to increasing grain nitrogen accumulation, nitrogen transportation from vegetative organs to grain, and the activities of nitrogen metabolism enzymes. Therefore, mixed cropping green manure can simultaneously improve the nutrient production and quality of wheat grain by promoting nitrogen accumulation, remobilization, and assimilation.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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References

Alvarez R, Steinbach H S, De Paepe J L. 2017. Cover crop effects on soils and subsequent crops in the pampas: A meta-analysis. *Soil & Tillage Research*, **170**, 53–65.

Ben S, González-Torralba J, Collar C, Aranjuelo I, Morales F. 2020. Durum wheat grain yield and quality under low and high nitrogen conditions: Insights into natural variation in low- and high-yielding genotypes. *Plants*, **9**, 1636.

Caldelas C, Rezzouk F Z, Aparicio N, Diez-Fraile M C, Diez-Fraile M C. 2023. Interaction of genotype, water availability, and nitrogen fertilization on the mineral content of wheat grain. *Food Chemistry*, **404**, 134565.

Chen J R, Qin W J, Chen X F, Cao W D, Qian G M, Liu J, Xu C X. 2020. Application of Chinese milk vetch affects rice yield and soil productivity in a subtropical double-rice cropping system. *Journal of Integrative Agriculture*, **19**, 2116–2126.

Costerousse B, Quattrini J, Gruter R, Frossard E, Thonar C. 2021. Green manure effect on the ability of native and inoculated soil bacteria to mobilize zinc for wheat uptake (*Triticum aestivum* L.). *Plant and Soil*, **467**, 287–309.

Cui H X, Luo Y L, Li C H, Chang Y L, Jin M, Li Y, Wang Z L. 2023. Effects of nitrogen forms on nitrogen utilization, yield, and quality of two wheat varieties with different gluten characteristics. *European Journal of Agronomy*, **149**, 126919.

Gao S J, Zhou G P, Chang D N, Liang H, Nie J, Liao Y L, Lu Y H, Xu C X, Liu J, Wu J, Han S, Wang H, Liu C Z, Lv Y H, Huang Y B, He C M, Geng M J, Wang J H, He T G, Li Z Y. 2023. Southern China can produce more high-quality rice with less N by green manuring. *Resources*, *Conservation and Recycling*, **196**, 107025.

Graziano S, Marando S, Prandi B, Boukid F, Marmiroli N, Francia E, Pecchioni N, Sforza S, Visioli G, Gullì M. 2019. Technological quality and nutritional value of two durum wheat varieties depend on both genetic and environmental factors. *Journal of Agricultural and Food Chemistry*, **67**, 2384–2395.

Grüter R, Meister A, Schulin R, Tandy S. 2018. Green manure effects on zinc and cadmium accumulation in wheat grains (*Triticum aestivum* L.) on high and low zinc soils. *Plant and Soil*, **422**, 437–453.

Guzmán C, Autrique J E, Mondal S, Singh R P, Govindan V, Morales-Dorantes A, Posadas-Romano G, Crossa J, Ammar K, Peña R J. 2016. Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. *Field Crops Research*, **186**, 157–165.

He G, Wang Z H, Li F C, Dai J, Ma X L, Li Q, Xue C, Cao H B, Wang S, Liu H, Luo L C, Huang M, Malhi S S. 2016. Soil nitrate-N residue, loss and accumulation affected by soil surface management and precipitation in a winter wheat-summer fallow system on dryland. *Nutrient Cycling in Agroecosystems*, **106**, 31–46.

Hellemans T, Landschoot S, Dewitte K, Van Bockstaele F, Vermeir P, Eeckhout M, Haesaert G. 2018. Impact of crop husbandry practices and environmental conditions on wheat composition and quality: A review. *Journal of Agricultural and Food Chemistry*, **66**, 2491–2509.

Hermans W, Mutlu S, Michalski A, Langenaeken N A, Courtin C M. 2021. The contribution of sub-aleurone cells to wheat endosperm protein content and gradient is dependent on cultivar and N-fertilization level. *Journal of Agricultural and Food Chemistry*, **69**, 6444–6454.

Huang X, Wang C Y, Hou J F, Du C Y, Liu S J, Kang J, Lu H F, Xie Y X, Guo T C, Ma D Y. 2020. Coordination of carbon and nitrogen accumulation and translocation of winter wheat plant to improve grain yield and processing quality. *Scientific Reports*, **10**, 10340.

Hui X L, Wang X S, Luo L C, Wang S, Guo Z K, Shi M, Wang R Z, Lyons G, Chen Y L, Cakmak I, Wang Z H. 2022. Wheat grain zinc concentration as affected by soil nitrogen and phosphorus availability and root mycorrhizal colonization. *European Journal of Agronomy*, **134**, 126469.

Jain A K. 2023. Greenhouse gas emissions from nitrogen fertilizers. *Nature Food*, **4**, 139–140.

Lassaletta L, Billen G, Garnier J, Bouwman L, Velazquez E, Mueller N D, Gerber J S. 2016. Nitrogen use in the global food system: Past trends and future trajectories of agronomic performance, pollution, trade, and dietary demand. *Environmental Research Letters*, **11**, 095007.

Li C, Li W Q, Luo Y L, Jin M, Chang Y L, Cui H X, Sun S F, Li Y, Wang Z L. 2023. Mixed cropping increases grain yield and lodging resistance by improving the canopy light environment of wheat populations. *European Journal of Agronomy*, **147**, 126849.

Liu R, Zhou G P, Chang D N, Gao S J, Han M, Zhang J D, Sun X F, Cao W D. 2022. Transfer characteristics of nitrogen fixed by leguminous green manure crops when intercropped with maize in northwestern China. *Journal of Integrative Agriculture*, **21**, 1177–1187.

Lyu C H, Li L, Liu X W, Zhao Z Q. 2022. Rape straw application facilitates Se and Cd mobilization in Cd-contaminated seleniferous soils by enhancing microbial iron reduction. *Environmental Pollution*, **310**, 119818.

Lyu H Q, Li Y, Wang Y L, Wang P F, Shang Y P, Yang X H, Wang F, Yu A Z. 2024. Drive soil nitrogen transformation and improve crop nitrogen absorption and utilization — A review of green manure applications. *Frontiers in Plant Science*, **14**, 1305600.

Liu L, Weng Y, Fang J, Zhao Z, Du S. 2022. Understanding the effect of GO on nitrogen assimilation in wheat through transcriptomics and metabolic process analysis. *Chemosphere*, **296**, 134000.

Ma D K, Yin L N, Ju W L, Li X K, Liu X X, Deng X P, Wang S W. 2021. Meta-analysis of green manure effects on soil properties and crop yield in Northern China. *Field Crops Research*, **266**, 108146.

Peng Y C, Zhao Y, Yu Z T, Zeng J B, Xu D G, Dong J, Ma W J. 2022. Wheat quality formation and its regulatory mechanism. *Frontiers in Plant Science*, **13**, 834654.

Rossmann A, Buchner P, Savill G P, Hawkesford M J, Scherf K A, Mühling K H. 2019. Foliar N application at anthesis alters grain protein composition and enhances baking quality in winter wheat only under a low N fertiliser regimen. *European Journal of Agronomy*, **109**, 125909.

Savill G P, Michalski A, Powers S J, Wan Y, Tosi P, Buchner P, Hawkesford M J. 2018. Temperature and nitrogen supply interact to determine protein distribution gradients in the wheat grain endosperm. *Journal of Experimental Botany*, **69**, 3117–3126.

Sieling K, Kage H. 2021. Apparent fertilizer N recovery and the relationship between grain yield and grain protein concentration of different winter wheat varieties in a long-term field trial. *European Journal of Agronomy*, **124**, 126246.

Snyder E M, Karsten H D, Curran W S, Malcolm G M, Hyde J A. 2016. Green manure comparison between winter wheat and corn: Weeds, yields, and economics. *Agronomy Journal*, **108**, 2015–2025.

Soltani S, Khoshgoftarmanesh A H, Afyuni M, Shrivani M, Schulin R. 2014. The effect of preceding crop on wheat grain zinc concentration and its relationship to total amino acids and dissolved organic carbon in rhizosphere soil solution. *Biology and Fertility of Soils*, **50**, 239–247.

Tarui A, Matsumura A, Asakura S, Asakura S, Yamawaki K, Hattori R, Daimon H. 2013. Evaluation of mixed cropping of oat and hairy vetch as green manure for succeeding corn production. *Plant Production Science*, **16**, 383–392.

The S V, Snyder R, Tegeder M. 2021. Targeting nitrogen metabolism and transport processes to improve plant nitrogen use efficiency. *Frontiers in Plant Science*, **11**, 628366.

Tsochatzis E, Papageorgiou M, Kalogiannis S. 2019. Validation of a HILIC UHPLC-MS/MS method for amino acid profiling in triticum species wheat flours. *Foods*, **8**, 514.

Wei J G, Chai Q, Yin W, Fan H, Guo Y, Hu F L, Fan Z L, Wang Q M. 2024a. Grain yield and N uptake of maize in response to increased plant density under reduced water and nitrogen supply conditions. *Journal of Integrative Agriculture*, **23**, 122–140.

Wei J G, Fan Z L, Hu F L, Mao S F, Yin F, Wang Q M, Chai Q, Yin W. 2024b. Legume green manure can intensify the function of chemical nitrogen fertilizer substitution via increasing nitrogen supply and uptake of wheat. *The Crop Journal*, **12**, 1222–1232.

Wei J G, Yin W, Chai Q, Fan Z L, Hu F L, Zhao L H. 2025. How does the long-term return of mix-sowing green manures increase nitrogen

utilization and decrease ecological costs of wheatland under reduced chemical nitrogen input? *Resources, Environment and Sustainability*, **21**, 100220.
Wang P F, Yu A Z, Wang F, Wang Y L, Lyu H Q, Shang Y P, Yang X H, Liu Y L, Yin B, Zhang D L, Chai Q, 2024. Nitrogen reduction by 20% with green manure retention reduces soil evaporation, promotes maize transpiration and improves water productivity in arid areas. *Field Crops Research*, **315**, 109488.
Wang W, Li M Y, Zhu R, Mo F, Khan A, Batool A, Zhang W, Lu J S, Zhu Y, Wang B Z, Yang Y M, Wang J, Tao X P, Xiong Y C. 2023a. Leaf senescence, nitrogen remobilization, and productivity of maize in two semiarid intercropping systems. *European Journal of Agronomy*, **150**, 126943.
Wang W, Li M Y, Zhu S G, Khan A, Tao X P, Huang G F, Liu Y H, Zhang W, Tao H Y, Gong D S, Song C, Xiong Y C. 2023b. Plant facilitation improves carbon production efficiency while reducing nitrogen input in semiarid agroecosystem. *Catena*, **230**, 107247.
Wang Y J, Tao Z Q, Wang D M, Wang Y J, Yang Y S, Zhao G C, Shi S B, Chang X H. 2022. An economic and viable approach to improve wheat quality in Qinghai-Tibetan Plateau, China. *Journal of Integrative Agriculture*, **21**, 2227–2240.
Xie Z J, Tu S X, Shah F, Xu C X, Chen J R, Han D, Liu G R, Li H L, Muhammad I, Cao W D. 2016. Substitution of fertilizer-N by green manure improves the sustainability of yield in double-rice cropping system in south China. *Field Crops Research*, **188**, 142–149.
Yang X L, Xiong J R, Du T S, Ju X T, Gan Y T, Li S, Xia L L, Shen Y J, Pacenka S, Steenhuis T S, Siddique K H M, Kang S, Butterbach-Bahl K. 2024. Diversifying crop rotation increases food production, reduces net greenhouse gas emissions and improves soil health. *Nature Communications*, **15**, 198.
Yao C S, Ren J, Li H R, Zhang Z, Wang Z M, Sun Z C, Zhang Y H. 2023. Can wheat yield, N use efficiency and processing quality be improved simultaneously? *Agricultural Water Management*, **275**, 108006.
Yu R, Zhang H Y, Chang F D, Song J S, Wang J, Wang X Q, Kan Z R, Zhao N, Li X H, Ma J, Li Y Y. 2024. Mixed sowing of feedrape and *Vicia villosa* can substitute nitrogen fertilizer to improve soil multifunctionality in the Hetao Irrigation District. *Catena*, **235**, 107617.
Yu X, Keitel C, Zhang Y, Wangeci A N, Dijkstra F A. 2022. Global meta-analysis of nitrogen fertilizer use efficiency in rice, wheat and maize. *Agriculture*, *Ecosystems and Environment*, **338**, 108089.
Zhang D B, Zhang C, Ren H L, Xu Q, Yao Z Y, Yuan Y, Yao P W, Zhao N, Li Y Y, Zhang S Q, Zhai B N, Wang Z H, Huang D L, Cao W D, Gao Y J. 2021. Trade-offs between winter wheat production and soil water consumption *via* leguminous green manures in the Loess Plateau of China. *Field Crops Research*, **272**, 108278.
Zhang H, Rui Y K. 2012. Determining mineral elements in four kinds of grains from Beijing market by ICP-MS simultaneously. *Journal of Saudi Chemical Society*, **16**, 31–33.
Zhang X X, Gao J S, Cao Y H, Ma X T, He J Z. 2013. Long-term rice and green manure rotation alters the endophytic bacterial communities of the rice root. *Microbial Ecology*, **66**, 917–926.
Zhen S M, Deng X, Xu X, Liu N N, Zhu D, Wang Z M, Yan Y M. 2020. Effect of high-nitrogen fertilizer on gliadin and glutenin subproteomes during kernel development in wheat (*Triticum aestivum* L.). *Crop Journal*, **8**, 38–52.
Zheng B Q, Fang Q, Zhang C X, Mahmood H, Zhou Q, Li W Y, Li X N, Cai J, Wang X, Zhong Y X, Huang M, Cao W D, Dai T B, Jiang D. 2020. Reducing nitrogen rate and increasing plant density benefit processing quality by modifying the spatial distribution of protein bodies and gluten proteins in endosperm of a soft wheat cultivar. *Field Crops Research*, **253**, 107831.
Zörb C, Ludewig U, Hawkesford M J. 2018. Perspective on wheat yield and quality with reduced nitrogen supply. *Trends in Plant Science*, **23**, 1029–1037.

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