



OPEN ACCESS

EDITED BY

Mohamed Ait-El-Mokhtar,
University of Hassan II Casablanca, Morocco

REVIEWED BY

Mushtaq Ahmad Khan,
Hazara University, Pakistan
Nitin Gudadhe,
Navsari Agricultural University, India

*CORRESPONDENCE

Jun Zhang
✉ 627085986@qq.com

RECEIVED 20 October 2024

ACCEPTED 30 December 2024

PUBLISHED 14 January 2025

CITATION

Zhang J, He W, Wei Z, Chen Y and Gao W (2025) Integrating green manure and fertilizer reduction strategies to enhance soil carbon sequestration and crop yield: evidence from a two-season pot experiment. *Front. Sustain. Food Syst.* 8:1514409. doi: 10.3389/fsufs.2024.1514409

COPYRIGHT

© 2025 Zhang, He, Wei, Chen and Gao. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Integrating green manure and fertilizer reduction strategies to enhance soil carbon sequestration and crop yield: evidence from a two-season pot experiment

Jun Zhang^{1,2,3*}, Wei He^{1,2,4}, Zheng Wei^{1,2}, Yifei Chen^{1,2} and Weichun Gao^{1,2}

¹Shaanxi Dijian Guantian Investment and Construction Co., Ltd., Baoji, China, ²Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an, China, ³Zhongshan High Standard Farmland Construction Group Baoji Co., Ltd., Baoji, China, ⁴School of Water Resources and Hydropower, Xi'an University of Technology, Xi'an, China

The excessive use of chemical fertilizers in agricultural production has led to diminishing returns, necessitating alternative methods to enhance soil fertility and reduce fertilizer dependency. One promising approach is the integration of leguminous green manure, which improves soil structure, enhances nutrient cycling, and supports sustainable farming practices. However, the application of green manure in systems with continuous fertilizer reduction remains underexplored. This study addresses this gap by investigating the effects of reducing nitrogen and phosphorus fertilizers (N-P) by up to 24% in conjunction with multiple cropping of soybean green manure on soil fertility, organic carbon fractions, and wheat yield. The research employed a pot experiment conducted over two wheat-growing seasons (March 2021 to July 2022) at an experimental station in Baoji, China. Treatments included CK (control, no fertilizer), CF (conventional fertilizer), and reduced N-P fertilizer applications by 6% (RF6), 12% (RF12), 18% (RF18), and 24% (RF24). Key findings revealed that RF12 had no significant impact on wheat grain yield compared to CF. The incorporation of soybean green manure significantly improved soil alkaline nitrogen by 22.3% and available phosphorus by 30.7%, while high-labile organic carbon (H-LOC) and microbial biomass carbon (MBC) increased by 34.5 and 29.6%, respectively. Additionally, a notable increase of 12.4% in soil organic carbon content was observed, suggesting enhanced carbon sequestration potential. This study provides valuable insights into sustainable agricultural practices by demonstrating that incorporating leguminous green manure alongside moderate fertilizer reduction can maintain crop yield, improve soil nutrient availability, and increase organic carbon content, thus supporting reduced reliance on chemical fertilizers and promoting long-term soil fertility and carbon sequestration.

KEYWORDS

green manure, fertilizer reduction, soil carbon sequestration, crop yield, sustainable agriculture

1 Introduction

Soil carbon sequestration plays a pivotal role in promoting sustainable agriculture and mitigating climate change, as it underpins soil fertility and ecosystem functionality. Chemical fertilizers, while integral to enhancing crop productivity, have increasingly been associated with environmental concerns such as nutrient leaching, greenhouse gas emissions, and soil acidification, alongside diminishing returns due to excessive use (Liu et al., 2022; Hou et al., 2023). This has necessitated the exploration of alternative strategies, such as the use of green manure, which offers potential benefits for soil organic carbon (SOC) dynamics and sustainable farming systems. Leguminous green manure, in particular, is distinguished by its capacity for biological nitrogen fixation and nutrient release, which not only improve soil structure and fertility but also modulate SOC dynamics (Mandal et al., 2003; Carter et al., 2014). These dynamics are governed by distinct SOC fractions, including highly labile organic carbon (H-LOC), low-labile organic carbon (L-LOC), and recalcitrant organic carbon (ROC), each contributing uniquely to SOC storage and turnover (Rahmati et al., 2020; Rossi et al., 2020). However, the specific effects of green manure incorporation on these SOC fractions remain inadequately understood.

The integration of green manure into soil introduces both organic carbon and nitrogen, which may significantly influence SOC fractions and their cycling. Green manure, as a high-quality residue with a low carbon-to-nitrogen (C/N) ratio, is prone to rapid decomposition, facilitating the release of nutrients and contributing to SOC pools (Xu et al., 2021; Lyu et al., 2024). This contrasts with the effects of synthetic fertilizers, which primarily provide inorganic nutrients and indirectly affect SOC dynamics by altering microbial activity (Li et al., 2018; He et al., 2020). The interplay between green manure and synthetic fertilizers is further complicated by phenomena such as the priming effect, where the addition of fresh organic matter accelerates the decomposition of existing SOC (Liu et al., 2015; Zheng et al., 2022). Such trade-offs raise critical questions about the balance between adding new organic carbon (e.g., cellulose and lignin from green manure) and triggering the mineralization of stable carbon stocks. Understanding these mechanisms is essential for optimizing carbon sequestration strategies while mitigating potential SOC losses due to accelerated turnover.

Leguminous green manure, with its high nitrogen content and low C/N ratio, is hypothesized to stimulate SOC turnover by enhancing microbial activity (Zhou et al., 2021; Fan et al., 2022). Simultaneously, the reduction of synthetic nitrogen (N) and phosphorus (P) fertilizers may moderate these dynamics, potentially limiting microbial decomposition rates and influencing SOC stabilization (Han et al., 2021; Liang et al., 2022). This study seeks to investigate the combined effects of green manure incorporation and reduced synthetic fertilizer inputs on SOC fractions (H-LOC, L-LOC, and ROC) and their turnover. The central hypothesis posits that specific SOC fractions will exhibit differential responses, with some fractions being stimulated while others are stabilized, depending on the balance between nutrient inputs and microbial activity. Additionally, the study aims to elucidate how these practices influence the overall carbon sequestration potential of soils, thereby advancing the understanding of sustainable fertilizer management in monoculture systems.

This investigation addresses critical knowledge gaps through a field experiment examining the impacts of green manure

incorporation and fertilizer reductions on SOC fractions, soil nutrient dynamics, and crop productivity. The findings aim to inform sustainable agricultural practices, offering a scientific basis for strategies that enhance SOC sequestration while maintaining agricultural yields.

2 Materials and methods

2.1 Experimental site

The outdoor pot experiment was conducted from March 2021 to July 2022 at the experimental station of the College of Agriculture, Baoji College of Arts and Sciences (107°1' E, 34°4' N). The site is located in the midsection of the northern slope of the Taibai Mountains, characterized by a temperate continental climate, with an average annual temperature of 7.5–8.2°C, annual precipitation ranging from 180 to 270 mm, and annual evaporation of 1,500–2000 mm. The test soil was the topsoil (0–20 cm) from a wheat field where fertilizer reduction had been implemented for three consecutive years. The soil type was irrigated gray desert soil. Before the experiment started (in 2018), the basic physical and chemical properties of the soil were: pH 7.79, organic matter content 19.5 g/kg, alkaline nitrogen 62.13 mg/kg, available phosphorus 29.85 mg/kg, and available potassium 175.95 mg/kg.

2.2 Experimental design

The pot experiment of multiple cropping green manure after wheat harvest included six treatments: no fertilizer (CK), conventional fertilizer (CF) (applying nitrogen and phosphorus fertilizers only), and nitrogen and phosphorus fertilizer reductions of 6, 12, 18, and 24% (RF6, RF12, RF18, and RF24, respectively). Each treatment was replicated three times. Fertilizers were applied during the wheat season, while no fertilizer was applied during the green manure season. Before the experiment, soil samples from each treatment were taken to measure the basic soil properties.

The pots used in the experiment were high-density polyethylene rectangular containers, 46.5 cm long, 35.0 cm wide, and 22.0 cm high, with each pot filled with 40.0 kg of air-dried soil. After aging, the soil layer was about 20 cm deep. The pots were buried flush with the ground to simulate field soil temperatures. During both the wheat and soybean growing seasons, soil moisture content was controlled to maintain 40–60% of the field water-holding capacity using the weighing method. Wheat was sown on April 1, 2021 (variety: Xin Chun 38), with two rows per pot, row spacing of 15 cm, and each row 10 cm away from the pot edge. After sowing, 80 plants per pot were retained. After wheat was harvested on July 6, soil and plant samples were collected to measure wheat yield and soil fertility indicators. Soybean plants were sown on July for green manuring purpose, following the same sowing method as wheat. After emergence, 10 plants per row were retained, and the green manure was plowed under during the full flowering stage, with plant and soil samples collected. At the time of plowing, the aboveground biomass and nutrient contents of the green manure for each treatment are shown in Table 1. The same wheat growing operations were repeated in 2022, with soil

TABLE 1 Aboveground biomass, dry matter and nutrient contents of soybean in each treatment.

Treatment	Biomass (g pot ⁻¹)	Dry matter (g pot ⁻¹)	Organic carbon (%)	Total nitrogen (%)	Total phosphorus (%)	Total potassium (%)
CK	358.46 ± 6.06 c	100.99 ± 1.33 c	40.01 ± 0.29 b	3.31 ± 0.06 b	0.62 ± 0.01 a	1.54 ± 0.03 a
CF	537.98 ± 7.39 a	166.16 ± 1.96 a	41.68 ± 0.35 a	3.56 ± 0.07 a	0.62 ± 0.02 a	1.53 ± 0.02 a
RF6	538.78 ± 6.87 a	165.24 ± 1.79 a	42.30 ± 0.40 a	3.31 ± 0.06 b	0.53 ± 0.01 c	1.50 ± 0.03 b
RF12	563.94 ± 5.95 a	174.32 ± 1.56 a	42.43 ± 0.29 a	3.38 ± 0.05 a	0.57 ± 0.01 b	1.52 ± 0.03 a
RF18	485.10 ± 5.60 b	154.66 ± 1.67 b	41.76 ± 0.23 a	3.12 ± 0.05 c	0.63 ± 0.01 a	1.53 ± 0.03 a
RF24	478.56 ± 6.47 b	163.14 ± 1.85 a	42.04 ± 0.35 a	3.15 ± 0.06 c	0.57 ± 0.01 b	1.51 ± 0.03 a
Two-factor ANOVA (Significance)						
Year (Y)	ns	ns	ns	ns	**	**
Treatment (T)	ns	ns	**	*	**	**
Interaction (Y × T)	ns	ns	ns	ns	**	**

CK, CF, RF6, RF12, RF18, and RF24 represent the treatments of no fertilizer, conventional nitrogen and phosphorus fertilizer, and nitrogen and phosphorus fertilizer reductions of 6, 12, 18, and 24%, respectively. Different letters in the same column represent significant differences at the 5% significance level following the LSD test. ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; “ns” indicates no significance ($p > 0.05$).

TABLE 2 Fertilizer application dose and fertilization strategy of each treatment.

Treatment	Base fertilizer (kg hm ⁻²)		Toppdressing (kg hm ⁻²)		Total nutrient content (kg hm ⁻²)	
	N	P ₂ O ₅	N	P ₂ O ₅	N	P ₂ O ₅
CK	–	–	–	–	–	–
CF	72.00	32.00	228.00	101.35	300.00	133.35
RF6	67.68	30.08	214.32	95.27	282.00	125.35
RF12	63.36	28.16	200.64	89.18	264.00	117.34
RF18	59.04	26.24	186.96	83.10	246.00	109.34
RF24	54.72	24.32	173.28	77.02	228.00	101.34

CK, CF, RF6, RF12, RF18, and RF24 represent the treatments of no fertilizer, conventional nitrogen and phosphorus fertilizer, and nitrogen and phosphorus fertilizer reductions of 6, 12, 18, and 24%, respectively. The values in the table represent the fertilizer application rates for each treatment.

samples taken before sowing (March 28) and after harvest (July 1) to determine nutrient contents.

The fertilization strategy for the wheat season was as follows: base fertilizer accounted for 24% of the total nutrients, while topdressing accounted for 76% of the total nutrients (with 10% applied during the seedling stage, 30% during the jointing stage, 20% during the booting stage, 10% during the flowering stage, and 6% during the ripening stage). The fertilization amounts for each treatment are shown in Table 2. Urea (N ≥ 46.4%) and superphosphate (P₂O₅ ≥ 46%) were used as the nitrogen and phosphorus sources for the base fertilizer. During topdressing, ammonium phosphate (N ≥ 12%, P₂O₅ ≥ 61%) was used as the phosphorus source, and urea was used to supply the remaining required nitrogen.

2.3 Measured items and methods

2.3.1 Plant sample collection and measurement

Wheat and green manure samples were collected at the time of harvest and plowing, respectively. After being killed at 105°C for 30 min, the samples were dried at 75°C to a constant weight to measure dry matter. The wheat plants were divided into grain, husk, and straw (stem and leaves), while the soybean plants were divided into stems, leaves, and pods. Each plant part was ground and passed

through a 0.5 mm sieve for determining the contents of carbon (C), nitrogen (N), phosphorus (P), and potassium (K). The carbon content of the plants was measured using the potassium dichromate heating method, while nitrogen, phosphorus, and potassium contents were measured using the digestate after H₂SO₄-H₂O₂ digestion. Nitrogen content was determined using the Nessler's reagent colorimetric method, phosphorus using the vanadium molybdenum yellow colorimetric method, and potassium using a flame photometer (Cong et al., 2023).

2.3.2 Soil sample collection and measurement

Soil samples were collected five times: during wheat sowing in 2021 (S1), after wheat harvest (S2), after the plowing of green manure (S3), during wheat sowing in 2022 (S4), and after wheat harvest in 2022 (S5). For each pot, six evenly distributed sampling points were selected, and soil was taken from a depth of 20 cm using a stainless steel soil auger with an inner diameter of 3 cm. The soil samples were then air-dried, ground, and sieved through 1.00 mm (18 mesh) and 0.15 mm (100 mesh) sieves. Soil samples passing through the 1.00 mm sieve were used to measure soil pH, alkaline nitrogen (AN), available phosphorus (AP), and available potassium (AK), while samples passing through the 0.15 mm sieve were used to measure soil organic carbon (SOC) and total nitrogen (TN). Soil pH was measured using a potentiometer with a water-to-soil ratio of 2.5:1. SOC was determined

using the potassium dichromate heating method, and TN was measured using the semi-micro Kjeldahl method. AN was determined using the alkaline diffusion method, AP using the sodium bicarbonate extraction-molybdenum antimony colorimetric method, and AK using the ammonium acetate extraction-flame photometry method (Cong et al., 2023). Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) (Li et al., 2018) were extracted using the chloroform fumigation method and then determined using the potassium dichromate heating method and semi-micro Kjeldahl method, respectively. Soil organic carbon fractions were measured using the improved acid extraction method as described by Rahmati et al. (2020). The highly labile fraction (H-LOC, g/kg) was extracted with 2.5 mol/L H₂SO₄, and the low-labile fraction (L-LOC, g/kg) was extracted with 13.0 mol/L H₂SO₄. The remaining fraction was considered the recalcitrant fraction (ROC, g/kg). All extracted fractions were measured using the potassium dichromate heating method.

2.3.3 Soil quality evaluation

To evaluate the effects of different fertilizer reduction treatments and the incorporation of green manure on soil quality, the overall data set method based on principal component analysis (PCA) was used (Duan et al., 2024). Thirteen indicators—pH, SOC, TN, C/N ratio, AN, AP, AK, MBC, MBN, MBC/MBN, H-LOC, L-LOC, and ROC—were included to establish the data set and further calculate the soil quality index (SQI). The calculation steps are shown in Equation 1:

$$SQI = \sum_{i=1}^n w_i \times S_i \quad (1)$$

where n is the number of soil parameters, W_i is the weight of the i -th parameter, and S_i is the score of the i -th parameter. The weights W_i were derived from PCA. First, components with eigenvalues greater than 1 were retained to determine the number of principal components. Then, factor loadings, variance percentages, and cumulative variance percentages for each principal component were obtained. Finally, the weight for each parameter (W_i) was calculated as the ratio of the communalities of the i -th indicator to the sum of the communalities of all indicators (Iheshiulo et al., 2024). For pH, the score S_i was determined using Equation 3, and for other indicators, Equation 2 was used:

$$S_i = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}} \quad (2)$$

$$S_i = \frac{X_{\max} - X_i}{X_{\max} - X_{\min}} \quad (3)$$

2.4 Data processing and analysis

Analysis of variance (ANOVA) and principal component analysis (PCA) were performed using SPSS 22.0 software. The least significant difference (LSD) method was used to test for significant differences between treatments ($p < 0.05$). Microsoft Excel 2019 and Origin 2022 software were used for data processing and graph creation.

3 Results

3.1 Wheat dry matter and yield

The reduction of different proportions of chemical fertilizers and the replanting of green manure after wheat harvest significantly affected wheat dry matter and yield formation (Table 3). The dry matter, grain yield, 1,000-grain weight, and the number of grains per spike all reached significant levels between the years (Y) ($p < 0.01$), indicating that the incorporation of green manure significantly influenced these four indicators. After the replanting and incorporation of green manure, dry matter, grain yield, and the number of grains per spike increased by 15.9–52.4%, 32.2–40.6%, and 6.1–15.5%, respectively. Before the incorporation of green manure, the yield of CF and RF6 treatments was significantly higher than other treatments. However, after the incorporation of green manure, there were no significant differences in yield among the CF, RF6, and RF12 treatments.

3.2 Changes in annual soil nutrient content

In all treatments, after the incorporation of green manure (after S3), the available soil nutrient content showed an increasing trend, while changes in pH, organic carbon, and total nitrogen content were less pronounced (Figures 1A–C). The soil alkaline nitrogen content exhibited a rapid increase during the green manure growth season (S2 to S3) and after the 175-day incorporation period (S3 to S4) (Figure 1D). The changes in available phosphorus content showed an upward trend during the 175-day (S4) and 272-day (S5) periods after the incorporation of the green manure (Figure 1E), indicating that phosphorus release from the green manure decomposition occurred slowly. The available potassium content increased rapidly after the 175-day incorporation period (S4), while during the second wheat growing season, it showed a rapid decline (Figure 1F). Compared with the wheat harvest season in 2021, the soil alkaline nitrogen content in 2022 increased by 8.0–45.9%, available phosphorus increased by 20.0–57.3%, and available potassium decreased by 8.8–14.2%.

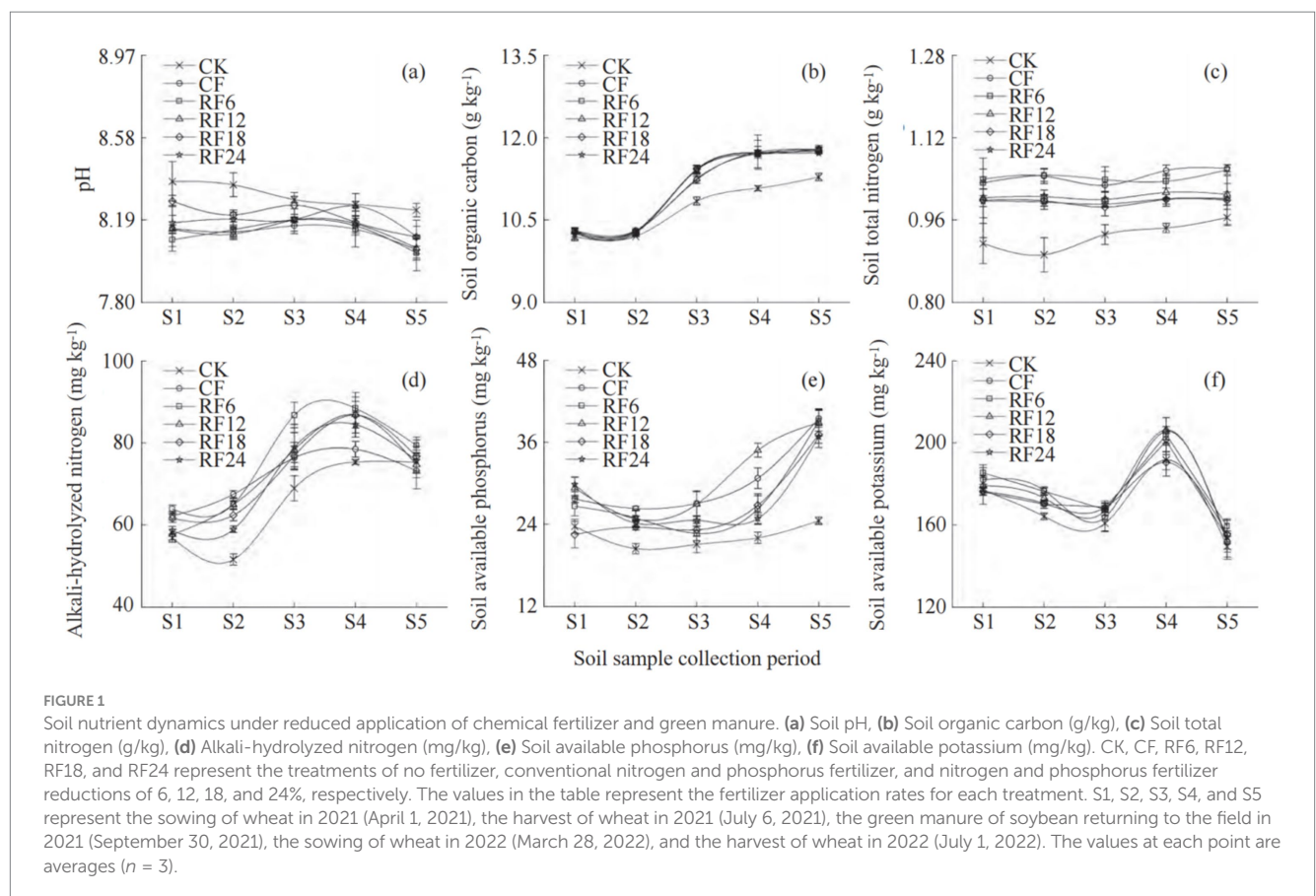
3.3 Soil organic carbon fractions and microbial biomass carbon and nitrogen contents

Compared to 2021, after the wheat harvest in 2022, the soil organic carbon content significantly increased ($p < 0.01$), with an increase range of 10.6–14.5% (Table 4). Among them, the high labile organic carbon fraction (H-LOC) increased by 34.7–44.5% ($p < 0.01$). After the return of green manure to the field, the microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents also significantly increased. The two-factor analysis of variance indicated that there were significant differences among the different fertilizer reduction treatments in all indicators, but the interaction between fertilizer reduction and the return of green manure only had a significant effect on the contents of H-LOC, MBC, and MBN ($p < 0.01$).

TABLE 3 Effects of fertilizer reduction and soybean return on wheat dry matter and yield.

Year	Treatment	Dry matter (g pot ⁻¹)	Grain yield (g pot ⁻¹)	Seeds per spike (seeds ear ⁻¹)	1,000-kernel weight (g pot ⁻¹)
2021	CK	96.81 ± 3.62 d	53.21 ± 4.44 c	17.73 ± 0.69 d	37.37 ± 0.69 c
	CF	177.68 ± 1.85 a	86.32 ± 5.61 a	23.86 ± 1.10 a	47.58 ± 1.13 a
	RF6	174.49 ± 7.31 ab	82.28 ± 2.53 a	22.80 ± 0.98 a	47.17 ± 1.28 a
	RF12	168.23 ± 3.97 bc	77.18 ± 1.23 b	21.28 ± 0.66 b	42.54 ± 0.84 b
	RF18	159.51 ± 5.10 c	75.60 ± 0.94 b	20.70 ± 0.94 bc	41.99 ± 0.45 b
	RF24	161.38 ± 2.27 c	74.88 ± 3.01 b	20.13 ± 0.66 c	42.06 ± 1.14 b
2022	CK	147.57 ± 3.87 c	90.12 ± 7.36 c	20.89 ± 1.98 c	38.89 ± 0.79 c
	CF	218.53 ± 4.70 a	115.16 ± 2.83 a	25.31 ± 1.02 a	47.95 ± 1.34 a
	RF6	214.34 ± 1.01 a	108.74 ± 0.45 ab	24.33 ± 0.35 ab	47.25 ± 1.63 a
	RF12	212.92 ± 5.86 a	108.54 ± 0.24 ab	24.00 ± 0.84 ab	46.46 ± 0.57 a
	RF18	187.63 ± 3.92 b	105.59 ± 1.65 b	23.26 ± 0.36 b	43.28 ± 0.37 b
	RF24	187.01 ± 13.28 b	101.92 ± 5.08 b	23.24 ± 0.37 b	43.09 ± 0.53 b
Two-factor ANOVA (Significance)					
Year (Y)		**	ns	ns	**
Treatment (T)		**	**	*	**
Interaction (Y × T)		**	ns	ns	**

CK, CF, RF6, RF12, RF18, and RF24 represent the treatments of no fertilizer, conventional nitrogen and phosphorus fertilizer, and nitrogen and phosphorus fertilizer reductions of 6, 12, 18, and 24%, respectively. The values in the table represent the fertilizer application rates for each treatment. Different letters in the same column represent significant differences at the 5% significance level. ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; "ns" indicates no significance ($p > 0.05$).



3.4 Soil quality evaluation

Principal component analysis revealed that there were two principal components with eigenvalues greater than 1 in 2021 and four

in 2022. The calculated weights (w_i) and soil quality index (SQI) are shown in Table 5 and Figure 2, respectively. There were no significant differences in SQI between treatments in the wheat harvests of 2021 and 2022. In 2021, the treatments showed a trend of

TABLE 4 Effects of fertilizer reduction and green manure incorporation on soil organic carbon fractions and microbial biomass carbon and nitrogen contents.

Year	Treatment	Soil organic carbon (g kg ⁻¹)	High-labile fraction (g kg ⁻¹)	Low-labile fraction (g kg ⁻¹)	Recalcitrant fraction (g kg ⁻¹)	Microbial biomass carbon (mg kg ⁻¹)	Microbial biomass nitrogen (mg kg ⁻¹)
2021	CK	10.20 ± 0.01 c	3.11 ± 0.03 d	2.08 ± 0.03 a	5.02 ± 0.02 b	60.87 ± 2.83 d	18.13 ± 2.05 c
	CF	10.31 ± 0.00 a	3.38 ± 0.02 a	1.88 ± 0.04 b	5.05 ± 0.06 ab	152.97 ± 3.45 a	40.18 ± 1.09 a
	RF6	10.30 ± 0.03 a	3.37 ± 0.00 a	1.88 ± 0.01 b	5.06 ± 0.03 ab	150.08 ± 3.85 a	39.24 ± 1.71 a
	RF12	10.30 ± 0.03 ab	3.35 ± 0.03 ab	1.88 ± 0.04 b	5.07 ± 0.04 ab	133.86 ± 3.32 b	37.87 ± 1.96 ab
	RF18	10.28 ± 0.03 ab	3.31 ± 0.02 bc	1.87 ± 0.01 b	5.10 ± 0.02 a	126.98 ± 3.87 bc	35.22 ± 1.04 b
	RF24	10.26 ± 0.02 b	3.31 ± 0.01 c	1.85 ± 0.01 b	5.10 ± 0.02 a	123.38 ± 6.08 c	35.03 ± 3.07 b
2022	CK	11.28 ± 0.07 b	4.19 ± 0.07 b	2.08 ± 0.07 a	5.02 ± 0.04 b	378.93 ± 8.81 c	47.29 ± 4.26 e
	CF	11.79 ± 0.07 a	4.80 ± 0.05 a	1.92 ± 0.01 b	5.07 ± 0.06 ab	465.11 ± 35.35 b	68.54 ± 1.37 d
	RF6	11.79 ± 0.04 a	4.79 ± 0.04 a	1.92 ± 0.02 b	5.08 ± 0.02 ab	529.11 ± 24.74 a	86.39 ± 3.93 c
	RF12	11.79 ± 0.03 a	4.79 ± 0.01 a	1.89 ± 0.01 b	5.11 ± 0.02 a	520.62 ± 32.14 a	108.48 ± 3.68 a
	RF18	11.76 ± 0.02 a	4.79 ± 0.02 a	1.88 ± 0.01 b	5.09 ± 0.04 a	510.95 ± 22.25 ab	96.36 ± 2.20 b
	RF24	11.72 ± 0.02 a	4.78 ± 0.02 a	1.86 ± 0.02 b	5.07 ± 0.02 ab	496.44 ± 18.47 ab	91.69 ± 6.94 bc
Two-factor ANOVA (Significance)							
Year (Y)		**	**	ns	ns	**	**
Treatment (T)		**	**	**	*	**	**
Interaction (Y × T)		ns	**	ns	ns	**	**

Different letters in the same column represent significant differences at the 5% significance level following the LSD test. CK, CF, RF6, RF12, RF18, and RF24 represent the treatments of no fertilizer, conventional nitrogen and phosphorus fertilizer, and nitrogen and phosphorus fertilizer reductions of 6, 12, 18, and 24%, respectively. The values in the table represent the fertilizer application rates for each treatment. ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; "ns" indicates no significance ($p > 0.05$).

CF > RF6 > RF12 > RF18 > RF24 > CK ($p < 0.05$), while in 2022, all fertilizer reduction treatments had higher SQIs than CK. The two-factor analysis of variance showed that both fertilizer reduction treatments and the interaction with green manure return had significant effects on SQI ($p < 0.01$).

4 Discussion

4.1 Effects of fertilizer reduction and multiple cropping of green manure on wheat yield

Nitrogen fertilizer plays a crucial role in wheat yield formation, contributing the most to yield compared to phosphorus and potassium fertilizers (Hou et al., 2023). Therefore, most studies on fertilizer reduction focus on nitrogen reduction. Due to differences in soil fertility, the potential for nitrogen reduction varies across studies. For example, in fields with high soil fertility, nitrogen reduction of 20 to 60% (Du et al., 2020; Han et al., 2021; Wang et al., 2021) had no significant effect on yield, while in fields with lower soil fertility, reducing nitrogen by 25% (Lu et al., 2021) or 50% (Bonanomi et al., 2020) resulted in yield reduction. Shi et al. (2021) provided suggestions for nitrogen and phosphorus reduction in dryland wheat through long-term experiments, recommending an application of 144 kg hm⁻² P₂O₅ when the pre-sowing soil available phosphorus level was 16.9 mg/kg. A three-year positioning experiment conducted in the early stage of this research showed that when nitrogen input was 300 kg hm⁻² and phosphorus input was

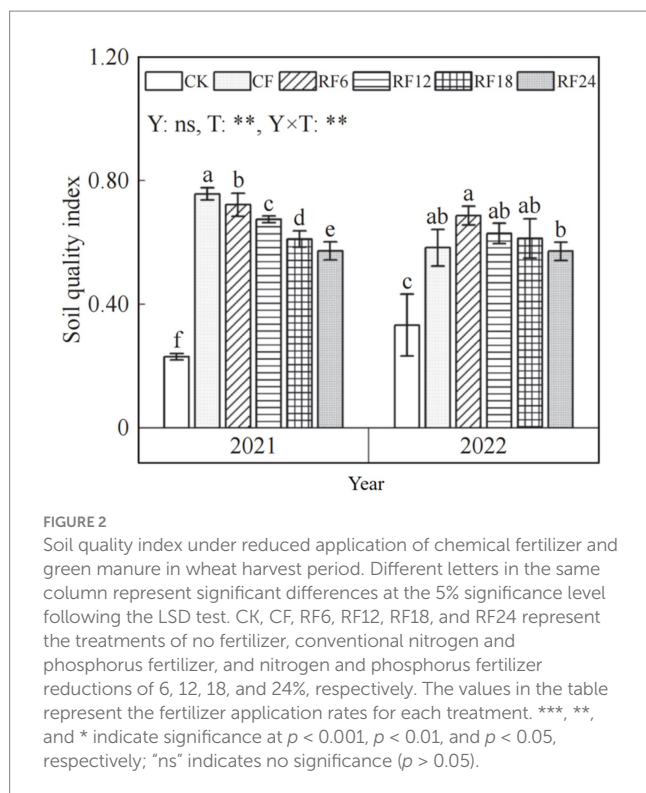
133 kg hm⁻², reducing nitrogen and phosphorus fertilizers by 6% had no significant effect on spring wheat yield, but higher reduction ratios resulted in decreased yields. The yield reduction trend became more pronounced with time, indicating that the potential for fertilizer reduction in low-fertility soils is lower. Therefore, improving soil fertility through green manure return may be an effective way to reduce chemical fertilizer input.

In this study, after multiple cropping of green manure followed by its incorporation into the soil, treatments with nitrogen and phosphorus fertilizer reductions of 6 and 12% showed no significant differences in grain yield, 1,000-grain weight, and the number of grains per spike compared to conventional fertilizer treatments. These findings are consistent with the research of Wei et al. (2024) and Qiao et al. (2021), who found that reducing nitrogen fertilizer by 15% and multiple cropping of hairy vetch increased wheat's maximum growth rate, 1,000-grain weight, average leaf area index, and total photosynthetic potential, thus increasing grain yield. The results of reducing nitrogen and phosphorus fertilizers and incorporating green manure in this study suggest that short-term incorporation of green manure does not significantly affect the number of grains per spike but mainly affects 1,000-grain weight and dry matter. This is similar to the findings of Zhang et al. (2022), who also observed this phenomenon. The reason may be that the nitrogen fixation characteristics of leguminous green manure, along with the release of nutrients during decomposition, enhanced the soil's nitrogen and phosphorus supply capacity, which affected the source-to-sink transformation in the crop. Additionally, the nutrient release cycle of green manure decomposition is relatively long, providing nutrients throughout the next crop's growing season (He et al., 2020; Amede

TABLE 5 Principal component analysis parameters of soil nutrient indices during wheat harvest in 2021 and 2022.

Soil property	2021				2022					
	Principal component 1	Principal component 2	Communalities	Wi	Principal component 1	Principal component 2	Principal component 3	Principal component 4	Communalities	Wi
	PC1	PC2			PC1	PC2	PC3	PC4		
pH	-0.916	0.131	0.856	0.076	-0.770	0.158	0.062	0.050	0.625	0.055
SOC	0.882	0.021	0.778	0.069	0.766	-0.113	-0.072	0.473	0.828	0.072
TN	0.874	0.369	0.900	0.080	0.542	-0.832	-0.010	0.063	0.990	0.087
C/N	-0.844	-0.382	0.858	0.076	-0.163	0.924	-0.030	0.230	0.935	0.082
AN	0.952	0.001	0.905	0.080	0.164	-0.022	0.877	0.379	0.940	0.082
AP	0.969	-0.128	0.955	0.085	0.948	-0.239	-0.079	0.000	0.962	0.084
AK	0.916	0.207	0.882	0.078	0.328	0.158	0.592	-0.579	0.818	0.072
MBC	0.985	0.006	0.971	0.086	0.914	-0.018	0.191	0.073	0.877	0.077
MBN	0.959	-0.139	0.938	0.083	0.865	0.379	0.009	-0.016	0.893	0.078
MBC/MBN	0.588	0.413	0.516	0.046	-0.824	-0.450	0.079	0.188	0.923	0.081
H-LOC	0.977	-0.034	0.955	0.085	0.958	-0.124	-0.084	-0.038	0.941	0.082
L-LOC	-0.868	0.460	0.965	0.086	-0.846	-0.184	0.149	0.371	0.910	0.080
ROC	0.361	-0.816	0.797	0.071	0.703	0.381	-0.102	0.369	0.786	0.069
Eigenvalue	9.846	1.429			6.925	2.208	1.219	1.078		
Percent variance	75.742	10.989			53.266	16.982	9.373	8.292		
Cumulative percent variance	75.742	86.731			53.266	70.248	79.621	87.913		

SOC, Soil Organic Carbon, TN, Total Nitrogen, C/N, Carbon-to-Nitrogen ratio, AN, Available Nitrogen, AP, Available Phosphorus, AK, Available Potassium, MBC, Microbial Biomass Carbon, MBN, Microbial Biomass Nitrogen, MBC/MBN, Ratio of Microbial Biomass Carbon to Microbial Biomass Nitrogen, H-LOC, Hydrophobic Light Organic Carbon, L-LOC, Labile Light Organic Carbon, ROC, Readily Oxidizable Carbon, Wi, Contribution rate of each soil property in the principal component analysis. The eigenvalue indicates the amount of variance explained by each principal component, with the percent variance and cumulative percent variance reflecting the explained proportion.



et al., 2021). This further suggests that green manure incorporation supplies additional nutrients during the growing season of subsequent crops, increasing yield and thus enhancing the potential for fertilizer reduction.

4.2 Effects of green manure decomposition on soil nutrients

This study found that incorporating green manure into soil resulted in a notable increase in soil nutrient availability, although changes in pH, organic carbon, and total nitrogen were less pronounced. Nitrogen and potassium exhibited synchronized release patterns during green manure decomposition, whereas phosphorus was released more gradually. Such findings align with prior research, including Zhou et al. (2021) and Yang et al. (2024), which demonstrated that legume green manure decomposition follows a rapid initial release phase, contributing significantly to nutrient availability within 180 days.

The decomposition and incorporation of approximately 24,000 kg/ha of green manure led to substantial increases in soil alkaline nitrogen (8.0–45.9%) and available phosphorus (20.0–57.3%) after 272 days. These results are consistent with long-term studies, such as those by Lyu et al. (2024), highlighting how legume green manure boosts nitrogen and phosphorus contents over extended periods. Conversely, available potassium levels declined (8.8–14.2%), a phenomenon explained by the activation and utilization of soil potassium reserves, as previously reported by Wang et al. (2000).

The role of green manure in improving soil quality became evident in the study's soil quality index (SQI) assessment, which showed that the incorporation of green manure compensated for nutrient deficiencies caused by reduced chemical fertilizers.

Treatments with a 12% reduction in nitrogen and phosphorus fertilizers demonstrated comparable SQI levels to conventional practices, indicating the potential of green manure to sustain soil health under lower fertilizer inputs. Previous research, such as Fan et al. (2022), supports these findings by emphasizing the interaction between legume green manure and fertilizer reduction in enhancing soil nutrient reserves.

The data demonstrate that incorporating green manure improves soil nutrient availability and mitigates the potential adverse effects of chemical fertilizer reduction. These improvements suggest that green manure plays a pivotal role in sustainable nutrient management, enabling reduced reliance on synthetic fertilizers without compromising soil fertility.

4.3 Effects of multiple cropping of green manure on soil organic carbon fractions under fertilizer reduction conditions

Green manure, as an external source of organic carbon, helps to sequester organic carbon in farmland (Liu et al., 2015). The results of this study show that reducing chemical fertilizer while incorporating green manure significantly increased the soil organic carbon content. This could be due to the addition of external carbon from green manure, which directly influenced the increase in both labile and recalcitrant soil organic carbon (SOC) fractions and promoted the decomposition and cycling of SOC (Zheng et al., 2022). In this study, the increase in SOC was significantly correlated with the increase in high-labile organic carbon (H-LOC). The improvement in wheat dry matter after green manure incorporation indirectly increased the return of straw and roots, further contributing to carbon input. The continuous three-year application of chemical fertilizers provided additional nitrogen sources for soil microorganisms, which in turn increased microbial activity and accelerated the decomposition of existing organic matter in the soil (Guo et al., 2017; Shi et al., 2021). This acceleration occurs because nitrogen fertilizers stimulate microbial biomass and activity, enhancing the breakdown of organic matter to release nutrients (Liu et al., 2022). However, this process can deplete soil organic matter stocks over time if not balanced with organic inputs such as green manure (Xu et al., 2021).

The two-factor analysis of variance results (Table 4) indicate that as the fertilizer reduction ratio increased under green manure incorporation conditions, the content of high-labile organic carbon decreased (similar trends were observed for microbial biomass carbon and nitrogen), while there were no significant differences in the low-labile and recalcitrant organic carbon fractions. This may be because, under no fertilizer or low fertilizer conditions, the distribution of organic carbon fractions tends to be stable. The corresponding labile fractions will be both sequestered and decomposed, and the turnover rate within the same soil is considered fixed. Therefore, under no or low fertilizer application, the carbon sequestration efficiency is low, while treatments with higher fertilizer applications have higher labile fractions, and their resistance to decomposition is stronger than that of the former. Some studies have suggested that the quality of organic carbon affects its sequestration in the soil, and different organic materials have different impacts on the soil carbon pool (Chang et al., 2024). Plant residues with a low carbon-to-nitrogen (C/N) ratio and low lignin content are

considered high-quality carbon sources and are more likely to form relatively stable SOC. This is because when soil nitrogen availability is high, nutrient-rich microbial communities gradually become dominant and regulate the intensity of SOC decomposition. Therefore, in this study, it is believed that the introduction of low C/N external organic matter from green manure provided carbon and nitrogen sources for soil microorganisms, which increased microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). By increasing H-LOC, this process alleviated the decomposition of other fractions, changed the turnover of soil carbon, and increased SOC content.

5 Conclusion

In this pot experiment, conducted over two wheat-growing seasons, incorporating green manure alongside a moderate reduction of synthetic nitrogen and phosphorus fertilizers effectively maintained wheat yields while improving soil quality. The study found significant increases in soil nutrient availability, with soil alkaline nitrogen and available phosphorus rising by 22.3 and 30.7%, respectively. Additionally, soil organic carbon content increased by 12.4%, driven by a boost in both labile and microbial biomass carbon fractions. These findings highlight the potential of integrating green manure with reduced fertilizer inputs as a sustainable strategy for enhancing soil carbon sequestration, improving soil fertility, and reducing dependence on chemical fertilizers. The results support the long-term viability of this approach for sustainable agricultural intensification. In summary, on irrigated gray desert soil, the return of approximately 24,000 kg ha⁻¹ of green manure, in conjunction with a 12% reduction in conventional nitrogen and phosphorus fertilizer application (N 300 kg ha⁻¹ and P₂O₅ 133 kg ha⁻¹), was able to maintain wheat yields and increase soil organic carbon content.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JZ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. WH: Conceptualization,

Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. ZW: Formal analysis, Funding acquisition, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. WG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This paper was funded by 2023 annual Shaanxi Province State Capital Operation Budget Science and Technology Innovation Special Fund Project “Guanzhong Area Green Farmland Construction Key Technology Research and Demonstration Promotion Project,” Technology Innovation Center for Land Engineering and Human Settlements, Shaanxi Land Engineering Construction Group Co., Ltd. and Xi’an Jiaotong University (Grant no. 2024WHZ0234).

Conflict of interest

JZ, WH, ZW, YC, and WG were employed by Shaanxi Dijian Guantian Investment and Construction Co., Ltd. JZ, WH, ZW, YC, and WG were employed by Shaanxi Provincial Land Engineering Construction Group Co., Ltd. JZ was employed by Zhongshan High Standard Farmland Construction Group Baoji Co., Ltd.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Amede, T., Legesse, G., Agegnehu, G., Gashaw, T., Degefu, T., Desta, G., et al. (2021). Short term fallow and partitioning effects of green manures on wheat systems in east African highlands. *Field Crop Res.* 269:108175. doi: 10.1016/j.fcr.2021.108175
- Bonomi, G., De Filippis, F., Zotti, M., Idbella, M., Cesarano, G., Al-Rowaily, S., et al. (2020). Repeated applications of organic amendments promote beneficial microbiota,

improve soil fertility and increase crop yield. *Appl. Soil Ecol.* 156:103714. doi: 10.1016/j.apsoil.2020.103714

Carter, M. S., Sørensen, P., Petersen, S. O., Ma, X., and Ambus, P. (2014). Effects of green manure storage and incorporation methods on nitrogen release and N₂O emissions after soil application. *Biol. Fertil. Soils* 50, 1233–1246. doi: 10.1007/s00374-014-0936-5

- Chang, F., Zhang, H., Zhao, N., Zhao, P., Song, J., Yu, R., et al. (2024). Green manure removal with reduced nitrogen improves saline-alkali soil organic carbon storage in a wheat-green manure cropping system. *Sci. Total Environ.* 926:171827. doi: 10.1016/j.scitotenv.2024.171827
- Cong, M., Hu, Y., Sun, X., Yan, H., Yu, G., Tang, G., et al. (2023). Long-term effects of biochar application on the growth and physiological characteristics of maize. *Front. Plant Sci.* 14:425. doi: 10.3389/fpls.2023.1172425
- Du, Q., Zhou, L., Chen, P., Liu, X., Song, C., Yang, F., et al. (2020). Relay-intercropping soybean with maize maintains soil fertility and increases nitrogen recovery efficiency by reducing nitrogen input. *Crop J.* 8, 140–152. doi: 10.1016/j.cj.2019.06.010
- Duan, Y., Wang, T., Lei, X., Cao, Y., Liu, L., Zou, Z., et al. (2024). Leguminous green manure intercropping changes the soil microbial community and increases soil nutrients and key quality components of tea leaves. *Hortic. Res.* 11:uhae018. doi: 10.1093/hr/uhae018
- Fan, M., Liu, X., Zhou, Y., Núñez-Delgado, A., Luo, L., Long, S., et al. (2022). Effects of the combined application of Chinese Milk vetch (*Astragalus sinicus* L.) and red mud to remediate the cadmium-polluted acidic Paddy soil. *Sustain. For.* 14:13192. doi: 10.3390/su142013192
- Guo, H., Ye, C., Zhang, H., Pan, S., Ji, Y., Li, Z., et al. (2017). Long-term nitrogen & phosphorus additions reduce soil microbial respiration but increase its temperature sensitivity in a Tibetan alpine meadow. *Soil Biol. Biochem.* 113, 26–34. doi: 10.1016/j.soilbio.2017.05.024
- Han, J., Dong, Y., and Zhang, M. (2021). Chemical fertilizer reduction with organic fertilizer effectively improve soil fertility and microbial community from newly cultivated land in the loess plateau of China. *Appl. Soil Ecol.* 165:103966. doi: 10.1016/j.apsoil.2021.103966
- He, H.-B., Li, W.-X., Zhang, Y.-W., Cheng, J.-K., Jia, X.-Y., Li, S., et al. (2020). Effects of Italian ryegrass residues as green manure on soil properties and bacterial communities under an Italian ryegrass (*Lolium multiflorum* L.)-rice (*Oryza sativa* L.) rotation. *Soil Tillage Res.* 196:104487. doi: 10.1016/j.still.2019.104487
- Hou, S., Dang, H., Huang, T., Huang, Q., Li, C., Li, X., et al. (2023). Targeting high nutrient efficiency to reduce fertilizer input in wheat production of China. *Field Crop Res.* 292:108809. doi: 10.1016/j.fcr.2023.108809
- Iheshiulo, E. M.-A., Larney, F. J., Hernandez-Ramirez, G., St Luce, M., Chau, H. W., and Liu, K. (2024). Quantitative evaluation of soil health based on a minimum dataset under various short-term crop rotations on the Canadian prairies. *Sci. Total Environ.* 935:173335. doi: 10.1016/j.scitotenv.2024.173335
- Li, L.-J., Zhu-Barker, X., Ye, R., Doane, T. A., and Horwath, W. R. (2018). Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. *Soil Biol. Biochem.* 119, 41–49. doi: 10.1016/j.soilbio.2018.01.003
- Liang, X., Jin, X., Han, B., Sun, R., Xu, W., Li, H., et al. (2022). China's food security situation and key questions in the new era: a perspective of farmland protection. *J. Geogr. Sci.* 32, 1001–1019. doi: 10.1007/s11442-022-1982-9
- Liu, H., Li, J., Li, X., Zheng, Y., Feng, S., and Jiang, G. (2015). Mitigating greenhouse gas emissions through replacement of chemical fertilizer with organic manure in a temperate farmland. *Sci. Bull.* 60, 598–606. doi: 10.1007/s11434-014-0679-6
- Liu, X., Peng, C., Zhang, W., Li, S., An, T., Xu, Y., et al. (2022). Subsoiling tillage with straw incorporation improves soil microbial community characteristics in the whole cultivated layers: a one-year study. *Soil Tillage Res.* 215:105188. doi: 10.1016/j.still.2021.105188
- Lu, J., Hu, T., Zhang, B., Wang, L., Yang, S., Fan, J., et al. (2021). Nitrogen fertilizer management effects on soil nitrate leaching, grain yield and economic benefit of summer maize in Northwest China. *Agric. Water Manag.* 247:106739. doi: 10.1016/j.agwat.2021.106739
- Lyu, H., Li, Y., Wang, Y., Wang, P., Shang, Y., Yang, X., et al. (2024). Drive soil nitrogen transformation and improve crop nitrogen absorption and utilization - a review of green manure applications. *Front. Plant Sci.* 14:600. doi: 10.3389/fpls.2023.1305600
- Mandal, U. K., Singh, G., Victor, U. S., and Sharma, K. L. (2003). Green manuring: its effect on soil properties and crop growth under rice-wheat cropping system. *Eur. J. Agron.* 19, 225–237. doi: 10.1016/S1161-0301(02)00037-0
- Qiao, J., Zhao, D., Zhou, W., Yan, T., and Yang, L. (2021). Sustained rice yields and decreased N runoff in a rice-wheat cropping system by replacing wheat with Chinese milk vetch and sharply reducing fertilizer use. *Environ. Pollut.* 288:117722. doi: 10.1016/j.envpol.2021.117722
- Rahmati, M., Eskandari, I., Kouselou, M., Feiziasl, V., Mahdavinia, G. R., Aliasgharzad, N., et al. (2020). Changes in soil organic carbon fractions and residence time five years after implementing conventional and conservation tillage practices. *Soil Tillage Res.* 200:104632. doi: 10.1016/j.still.2020.104632
- Rossi, L. M. W., Mao, Z., Merino-Martín, L., Roumet, C., Fort, F., Taugourdeau, O., et al. (2020). Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon pools. *Plant Soil* 452, 457–478. doi: 10.1007/s11104-020-04469-5
- Shi, Z., Liu, D., Liu, M., Hafeez, M. B., Wen, P., Wang, X., et al. (2021). Optimized fertilizer recommendation method for nitrate residue control in a wheat-maize double cropping system in dryland farming. *Field Crop Res.* 271:108258. doi: 10.1016/j.fcr.2021.108258
- Wang, J. L., Liu, K. L., Zhao, X. Q., Zhang, H. Q., Li, D., Li, J. J., et al. (2021). Balanced fertilization over four decades has sustained soil microbial communities and improved soil fertility and rice productivity in red paddy soil. *Sci. Total Environ.* 793:148664. doi: 10.1016/j.scitotenv.2021.148664
- Wang, J. G., Zhang, F. S., Zhang, X. L., and Cao, Y. P. (2000). Release of potassium from K-bearing minerals: effect of plant roots under P deficiency. *Nutr. Cycl. Agroecosyst.* 56, 45–52. doi: 10.1023/A:1009894427550
- Wei, J., Fan, Z., Hu, F., Mao, S., Yin, F., Wang, Q., et al. (2024). Legume green manure can intensify the function of chemical nitrogen fertilizer substitution via increasing nitrogen supply and uptake of wheat. *Crop J.* 12, 1222–1232. doi: 10.1016/j.cj.2024.07.004
- Xu, P., Wu, J., Wang, H., Han, S., Zhu, J., Fu, Q., et al. (2021). Long-term partial substitution of chemical fertilizer with green manure regulated organic matter mineralization in paddy soil dominantly by modulating organic carbon quality. *Plant Soil* 468, 459–473. doi: 10.1007/s11104-021-05130-5
- Yang, L., Zhou, J., Zamanian, K., Zhang, K., Zhao, J., Zang, H., et al. (2024). Peanut straw application rate had a greater effect on decomposition and nitrogen, potassium and phosphorus release than irrigation. *Plant Soil* 499, 193–205. doi: 10.1007/s11104-022-05614-y
- Zhang, X., Hua, Y., Liu, Y., He, M., Ju, Z., and Dai, X. (2022). Wide belt sowing improves the grain yield of bread wheat by maintaining grain weight at the backdrop of increases in spike number. *Front. Plant Sci.* 13:772. doi: 10.3389/fpls.2022.992772
- Zheng, H., Liu, D., Liao, X., Miao, Y., Li, Y., Li, J., et al. (2022). Field-aged biochar enhances soil organic carbon by increasing recalcitrant organic carbon fractions and making microbial communities more conducive to carbon sequestration. *Agric. Ecosyst. Environ.* 340:108177. doi: 10.1016/j.agee.2022.108177
- Zhou, G., Chang, D., Gao, S., Liang, T., Liu, R., and Cao, W. (2021). Co-incorporating leguminous green manure and rice straw drives the synergistic release of carbon and nitrogen, increases hydrolase activities, and changes the composition of main microbial groups. *Biol. Fertil. Soils* 57, 547–561. doi: 10.1007/s00374-021-01547-3