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Long-term winter wheat–rapeseed rotation configurations shape soil fertility through organic matter-mediated changes in potassium and phosphorus availability

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Abstract:

Background: Soil biological processes are fundamental to nutrient cycling and fertility maintenance in agricultural ecosystems. However, how long-term crop rotation configurations influence these processes through changes in soil organic matter (SOM) and nutrient availability remains inadequately understood. Here, we used an eight-year field experiment in the Huang–Huai–Hai Plain of China to compare seven winter wheat–rapeseed rotation configurations under a constant summer peanut system. We measured soil pH, SOM, total nitrogen (TN), total phosphorus (TP), total potassium (TK), alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), and available potassium (AK) in 2024. An integrated fertility index (IFI) was derived using principal component analysis. Treatment differentiation and associated pathways were evaluated using redundancy analysis, segmented regression, and piecewise structural equation modeling.

Results: Our results showed that long-term rotation configuration influenced SOM and available nutrient pools more strongly than total nutrient stocks. SOM ranged from 10.79 g kg⁻¹ in DRRW to 12.45 g kg⁻¹ in EW3R, whereas AK ranged from 145.57 to 178.33 mg kg⁻¹ and showed much

stronger treatment differentiation than TK. The IFI ranking identified EW3R as the highest-performing rotation system, whereas GRL ranked lowest. Redundancy analysis revealed that AK was the principal variable separating rotation systems, accounting for 96.46% of the explained variation along RDA1, while AN contributed most strongly to RDA2. Segmented regression suggested apparent non-linear patterns in the relationships of AP and AK to SOM, with breakpoints at 11.32 and 12.58 g kg⁻¹, respectively, although these breakpoints were not statistically significant in Davies tests. Piecewise SEM supported a SOM–AK–AP covariation pathway, together with positive direct effects of SOM, AN, AK, and AP on IFI.

Conclusions: These findings indicate that long-term rotation effects in this alkaline fluvo-aquic soil are likely mediated through SOM-associated changes in potassium and phosphorus availability—changes that likely reflect shifts in soil microbial habitat quality, substrate availability, and potentially microbially mediated nutrient transformation processes. The wheat-dominant EW3R configuration provided the most favorable balance between SOM accumulation and nutrient availability, highlighting the importance of rotation sequence design for sustaining soil biological functions and fertility in intensive agricultural systems.

Keywords: Crop rotation; Soil organic matter; Nutrient availability; Soil biological functions; Breakpoint response

1.Introduction

Sustaining soil biological functions and fertility is a central challenge in intensive agricultural systems worldwide. In regions where high cropping intensity relies on the long-term maintenance of soil quality and nutrient supply, the risk of nutrient imbalance, declining resource-use efficiency, and progressive soil degradation has increased substantially [1-4]. Among the major nutrient constraints in alkaline and calcareous soils, phosphorus (P) fixation and potassium (K) limitation are especially important. Phosphorus readily precipitates with Ca²⁺ and accumulates in poorly soluble forms, whereas K, despite often being present in considerable total reserves, may become adsorbed on exchange sites or fixed within clay interlayers, resulting in limited short-term availability to crops[5,6]. These constraints are intrinsically linked to soil biological processes, as microbial communities play critical roles in the mobilization, transformation, and cycling of both P and K through mechanisms such as organic acid production, enzyme secretion, and mineral weathering[7,8].

Crop rotation is widely regarded as an effective strategy for improving soil quality, stabilizing productivity, and enhancing agroecosystem resilience[9-12]. However, rotation effects are not determined by crop diversity alone; they also depend on the specific sequence configuration through which crops differ in residue inputs, nutrient demand, rooting traits, and rhizosphere processes over time[13]. A growing body of evidence shows that long-term crop diversification can enhance multiple soil functions, including organic matter accumulation, nutrient cycling, aggregate formation, and the stability of agricultural production[14-17]. Nevertheless, the benefits of diversification are often context-dependent rather than uniformly positive. The identity and relative frequency of component crops may strongly influence whether a given rotation improves nutrient retention or accelerates nutrient depletion. In cereal-based systems, the introduction of oilseed and legume crops can alter residue chemistry, biological interactions, and nutrient turnover in ways that are not fully captured by simple comparisons between monoculture and rotation[18-20]. As a result, functionally balanced rotation design may be more important than diversity alone when the objective is to sustain long-term soil fertility.

In the Huang-Huai-Hai Plain, a major grain-producing region of China, winter wheat is the dominant food crop, whereas rapeseed has attracted increasing interest because of its economic value and its potential role in system improvement through rotation[21,22]. Compared with continuous cereal systems, rapeseed is characterized by relatively high nutrient demand, particularly for potassium, and frequent inclusion of rapeseed in intensive systems may increase the risk of nutrient withdrawal if replenishment and retention are not well synchronized [23,24]. This concern is particularly relevant in alkaline fluvo-aquic soils, where phosphorus availability is often constrained by precipitation and sorption processes, and where changes in available potassium can be much more pronounced than changes in total potassium stocks[24,25]. Despite the agronomic and ecological relevance of this issue, the long-term effects of different winter wheat–rapeseed sequence configurations on soil fertility and nutrient availability remain insufficiently resolved.

Soil organic matter (SOM) is a key regulator of these processes because it influences nutrient retention, cation exchange, aggregation, microbial habitat, and the transformation and accessibility of nutrient pools[26-29]. Crucially, SOM serves as the primary energy and carbon source for soil microorganisms, and its quantity and quality directly shape microbial community composition,

activity, and metabolic functions[27,29].In alkaline and calcareous soils, SOM may also contribute to phosphorus mobilization by affecting microsite chemistry, biological activity, and competition for sorption sites—processes that are largely mediated by microbial production of organic acids and phosphatases[7,25,30]. At the same time, increasing evidence indicates that available potassium is linked not only to mineral reserves, but also to soil carbon status, biological mediation, and nutrient-use efficiency—with soil microorganisms playing key roles in K mobilization through mineral weathering and organic matter decomposition[8,24,31,32]. These observations suggest that long-term rotation effects may emerge through coordinated regulation among SOM, K availability, and P availability—processes that are fundamentally mediated by soil biological communities and their biochemical activities.

In this study, we used an eight-year field experiment in a fluvo-aquic soil, in which summer peanut remained constant while winter crop sequences differed in the relative proportion of wheat and rapeseed. We analyzed eight soil properties measured in 2024 using principal component analysis, redundancy analysis, segmented regression, and piecewise structural equation modeling. The objectives were to: (1) evaluate how different winter wheat–rapeseed rotation configurations affected soil chemical properties and integrated soil fertility; (2) identify the dominant soil variables responsible for multivariate differentiation among rotation systems; (3) determine whether SOM was associated with apparent breakpoint-dependent responses of available K and P and thereby contributed to long-term variation in soil fertility; and (4) discuss the implications of these findings for understanding the biological and biochemical processes—particularly microbially mediated nutrient transformations—that underpin sustainable soil fertility management.

2. Materials and methods

2.1. Experimental site

The field experiment was established in October 2016 at the Modern Agricultural Research and Development Base, Yuanyang County, Xinxiang City, Henan Province, China (34°56′ N, 113°52′ E), in the central Huang-Huai-Hai Plain. This region is a major agricultural production zone characterized by a warm-temperate continental monsoon climate, with distinct seasons, abundant solar radiation, and precipitation concentrated mainly in summer.

During the experimental period, the mean annual air temperature was 14.5 °C, the mean annual ground

temperature was 17.0 °C, the average annual precipitation was 651.1 mm, the mean relative humidity was 66%, and the annual sunshine duration was 2324 h. The annual accumulated temperature was 5300 °C, mean annual solar radiation was 113.34 kcal cm⁻², and the frost-free period was 214 days. These climatic conditions are suitable for intensive multiple-cropping systems.

The soil is a fluvo-aquic soil derived from Yellow River alluvium and classified as a Typic Haplustalf according to USDA Soil Taxonomy. Before the experiment was initiated, composite soil samples were collected from the 0–20 cm layer across the experimental area to determine baseline properties. Initial values were as follows: pH, 7.87; SOM, 10.83 g kg⁻¹; TN, 0.68 g kg⁻¹; TP, 0.65 g kg⁻¹; TK, 18.98 g kg⁻¹; AP, 28.87 mg kg⁻¹; AK, 189.67 mg kg⁻¹; and AN, 52 mg kg⁻¹. The soil texture was sandy loam, comprising 62.3% sand, 24.1% silt, and 13.6% clay.

2.2. Experimental Design and Crop Management

To isolate the effects of winter crop sequence configuration, summer peanut (*Arachis hypogaea* L.) was kept constant across all treatments, whereas the winter crop sequence varied in the relative proportion of winter wheat (*Triticum aestivum* L.) and rapeseed (*Brassica napus* L.). This design reduced variation associated with summer cropping and allowed the cumulative effects of winter sequence configuration on soil properties to be evaluated more clearly.

Seven treatments were established (Table 1; Fig. 1): AWL, continuous winter wheat; BWR, wheat → rapeseed; CWWR, wheat → wheat → rapeseed; DRRW, rapeseed → rapeseed → wheat; EW3R, wheat → wheat → wheat → rapeseed; FR3W, rapeseed → rapeseed → rapeseed → wheat; and GRL, continuous winter rapeseed. These treatments represent a gradient from wheat-dominant to rapeseed-dominant winter cropping. The crop cultivars used were ‘Zhengmai 113’ for wheat (bred by the Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou, China), ‘Shuangyou 195’ for rapeseed (bred by the Institute of Cash Crops, Henan Academy of Agricultural Sciences, Zhengzhou, China), and ‘Yuhua 23’ for peanut (bred by the Institute of Cash Crops, Henan Academy of Agricultural Sciences, Zhengzhou, China).

The experiment followed a randomized complete block design with three replicates. Each plot measured 5 m × 6 m (30 m²), and plots were separated by 1 m-wide buffer zones. Field management was standardized across treatments. Wheat was sown in mid-October and harvested in early June at a seeding rate of 225 kg ha⁻¹. Rapeseed was sown in late September and harvested in late May at a seeding rate of

6 kg ha⁻¹. Peanut was sown in mid-June after the harvest of the winter crops and harvested in late September at a seeding rate of 240 kg ha⁻¹. Fertilization, irrigation, weed control, and pest and disease management followed local high-yield recommendations and were applied uniformly across all treatments. Irrigation was applied three times during the winter crop season and twice during the peanut season by border irrigation. After harvest, crop residues were returned to the soil.

Table 1 Experimental Design of Winter Wheat-Rapeseed + Summer Peanut Rotation Systems

Treatment Code	Abbreviation	Rotation Cycle	Winter Cropping Rotation Sequence (W=Wheat, R=Rapeseed)	Summer Cropping	Wheat Proportion in Winter Cropping
A	AWL	Continuous	Wheat (continuous)	Peanut (fixed)	100%
B	BWR	2 years	Wheat→Rapeseed	Peanut (fixed)	50.0%
C	CWWR	3 years	Wheat→Wheat→Rapeseed	Peanut (fixed)	66.7%
D	DRRW	3 years	Rapeseed→Rapeseed→Wheat	Peanut (fixed)	33.3%
E	EW3R	4 years	Wheat→Wheat→Wheat→Rapeseed	Peanut (fixed)	75.0%
F	FR3W	4 years	Rapeseed→Rapeseed→Rapeseed→Wheat	Peanut (fixed)	25.0%
G	GRL	Continuous	Rapeseed (continuous)	Peanut (fixed)	0%

Note: 1. Peanut was grown as the fixed summer crop in all treatments; only the winter wheat-rapeseed rotation configuration differed among treatments
2. Wheat proportion in winter cropping was calculated as: (Years of wheat planting in rotation cycle / Total years of rotation cycle) × 100%. For continuous cropping treatments, it was calculated as (Years of wheat/rapeseed planting / Total observation period) × 100%.

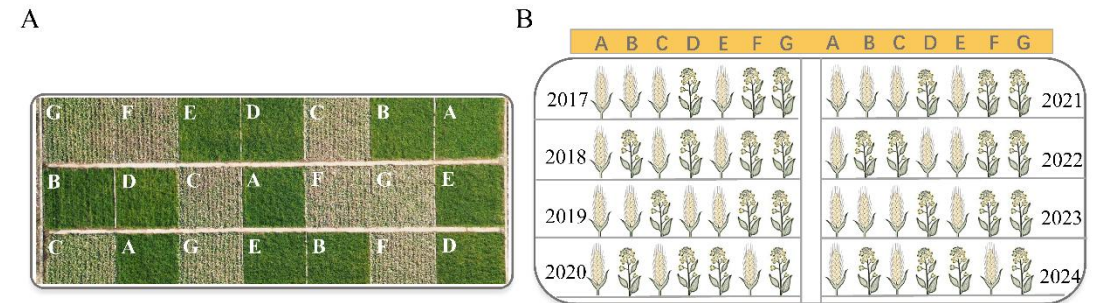


Figure 1. Spatiotemporal design of the crop rotation experiment. (A) Layout of the experimental plots (A-G), with green and brown areas representing winter wheat and winter rapeseed, respectively. (B) Temporal sequence of crop assignments from 2017 to 2024. Wheat ear and rapeseed flower icons denote wheat and rapeseed, respectively. Systems: A, AWL; B, BWR; C, CWWR; D, DRRW; E, EW3R; F, FR3W; and G, GRL.

2.3. Soil sampling and determination

Soil samples were collected annually in late September after peanut harvest and before sowing of the winter crops. The present study focuses on the cumulative end-point response observed in 2024 after eight years of rotation management. In each plot, five soil cores (3.5 cm in diameter) were collected from the 0–20 cm layer and combined into one composite sample. Samples were air-dried, cleaned of visible residues and stones, gently ground, and passed through a 2-mm sieve before analysis.

Soil pH was measured in a 1:5 (w/v) soil: water suspension as described by McLean[33].SOM was

determined by a standard potassium dichromate digest method. AN was measured using the alkali-solution-diffusion method, AP in soil was determined by extracting samples with 0.5 mol L⁻¹ NaHCO₃ and determining P colorimetrically using molybdate, AK was extracted with ammonium acetate, and then determined by a flame photometry, TN was determined using the semi-micro Kjeldahl method, TP was determined by HClO₄-H₂SO₄ digestion followed by the molybdenum-antimony anti colorimetric method, TK was determined using NaOH fusion followed by flame photometry[34].

2.4. Statistical analysis

Differences among treatments in individual soil properties were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at P < 0.05. Pearson correlation analysis was used to examine pairwise relationships among soil variables.

To obtain a synthetic measure of integrated fertility performance, an integrated fertility index (IFI) was constructed from principal component analysis (PCA) based on the eight soil properties measured in 2024, following previous studies[35-37]. PCA was performed on plot-level observations across all treatments after standardization of all variables. Principal components with eigenvalues > 1 were retained. The IFI was calculated as:

$$IFI = \sum_{m=1}^k \omega_m F_m \quad (1)$$

$$\omega_m = \frac{P_m}{\sum_{m=1}^k P_m} \quad (2)$$

where ω_m is the eigenvalue of the m-th principal component, and F_m is the factor score of each treatment on that component, P_m is the variance contribution of the m-th principal component. Treatment-level IFI values were then summarized to compare relative integrated fertility performance.

Redundancy analysis (RDA)[38] was used to assess multivariate differentiation in the soil property matrix among rotation treatments and to identify the variables most strongly associated with treatment separation. Segmented linear regression was applied to test for breakpoint relationships between SOM and the available nutrient pools AP and AK using plot-level observations. Breakpoints were estimated from the fitted models, and the performance of segmented and simple linear models was compared using Akaike's information criterion (AIC).

Piecewise structural equation modeling (SEM) [39-41] was used to assess direct and indirect relationships among rotation treatments, soil variables, and IFI. The analysis was based on plot-

level observations from 2024, with cropping systems represented by dummy variables. The initial model was specified according to a priori ecological relationships and simplified by removing non-significant paths ($\alpha = 0.05$) through backward elimination. Model fit was evaluated using Fisher's C statistic, with $P > 0.05$ indicating acceptable fit. Standardized path coefficients were used for interpretation. All analyses were performed in R version 4.2.2 using the packages ggplot2, vegan, segmented, and piecewiseSEM.

All statistical analyses were conducted in R version 4.2.2. The following packages were used: ggplot2 for visualization, vegan for RDA analysis[42], segmented for Piecewise linear regression[43,44], piecewiseSEM for structural equation modeling.

3. Results

3.1. Effects of long-term rotation configuration on soil properties and integrated fertility

After eight years of management, soil chemical properties differed among the winter wheat–rapeseed rotation systems (Table 2; Fig. 2). Relative to the 2016 baseline, all treatments showed higher pH, TN, and TP in 2024, although the magnitude of increase differed among systems. Soil pH increased from 7.87 to 8.21–8.30, TN from 0.68 to 0.72–0.77 g kg⁻¹, and TP from 0.65 to 0.75–0.80 g kg⁻¹. By contrast, TK varied only slightly among treatments, ranging from 17.59 to 18.92 g kg⁻¹.

Table 2 Soil chemical properties (0–20 cm) under different cropping systems in 2024 and at the baseline in 2016

Treatment	pH	SOM (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	TK (g kg ⁻¹)	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)
Baseline	7.87±0.20c	10.83±0.67b	0.68±0.05b	0.65±0.03b	18.98±0.90a	52.00±7.00b	28.87±3.88a	189.67±10.00a
AWL	8.21±0.10b	12.08±0.45a	0.76±0.02a	0.78±0.07a	18.37±0.79a	70.69±8.30a	30.83±8.47a	178.33±27.40ab
BWR	8.26±0.01ab	11.98±0.18a	0.75±0.02ab	0.75±0.02a	17.87±0.69a	76.72±3.83a	27.35±2.35a	168.73±6.98ab
CWWR	8.26±0.04ab	11.88±0.37a	0.77±0.06a	0.79±0.02a	18.48±0.05a	72.62±3.64a	28.12±1.51a	171.13±9.82ab
DRRW	8.25±0.01ab	10.79±1.61a	0.76±0.04ab	0.80±0.02a	18.49±0.39a	76.48±3.99a	29.59±2.69a	145.57±16.10b
EW3R	8.28±0.03ab	12.45±1.09a	0.76±0.05a	0.80±0.02a	18.82±0.76a	83.48±16.42a	29.64±5.46a	161.23±34.10ab
FR3W	8.30±0.01a	11.89±0.39a	0.76±0.05ab	0.77±0.02a	18.92±1.73a	79.62±8.35a	30.67±4.72a	156.07±30.60ab
GRL	8.28±0.04a	11.97±1.26a	0.72±0.07ab	0.78±0.03a	17.59±0.63a	70.21±5.92a	27.67±3.29a	156.57±21.10ab

Note: Different lowercase letters within a column indicate significant differences among treatments at $P < 0.05$.

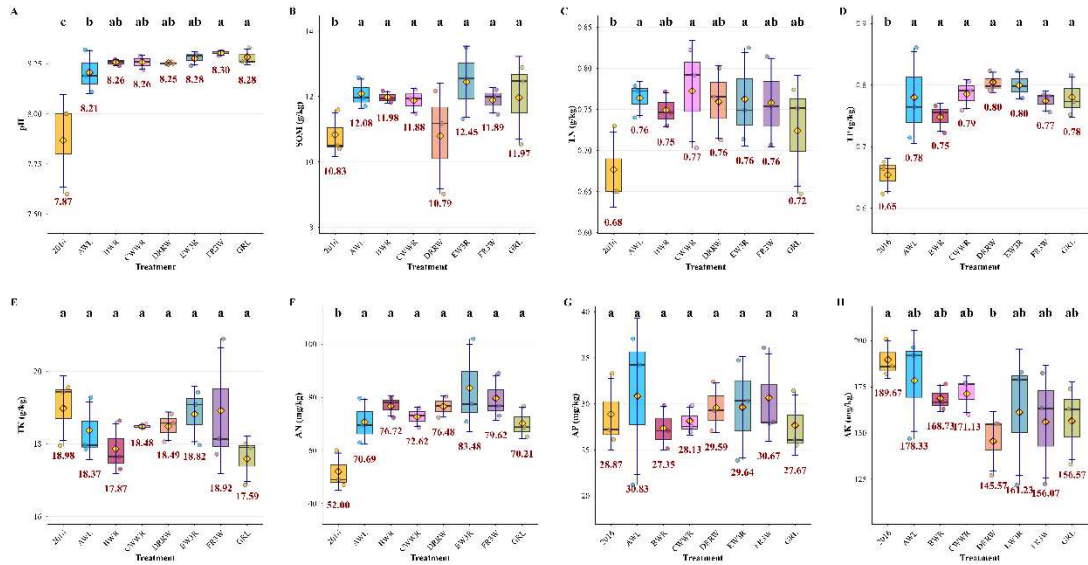


Figure 2. Soil chemical properties under different cropping systems in 2024. (A) pH, (B) soil organic matter (SOM), (C) total N (TN), (D) total P (TP), (E) total K (TK), (F) alkali-hydrolyzable N (AN), (G) available P (AP), and (H) available K (AK). Systems: baseline (2016), AWL, BWR, CWW, DRRW, EW3R, FR3W, and GRL. Different lowercase letters indicate significant differences among treatments at $P < 0.05$.

More pronounced treatment effects were observed for SOM and the available nutrient pools. SOM ranged from 10.79 g kg^{-1} in DRRW to 12.45 g kg^{-1} in EW3R. Compared with the baseline SOM value of 10.83 g kg^{-1} , all systems except DRRW showed net SOM accumulation. AN increased from 52.00 mg kg^{-1} at baseline to $70.21\text{--}83.48 \text{ mg kg}^{-1}$ in 2024, with the highest value recorded in EW3R. AP showed relatively narrow variation among treatments, ranging from 27.35 to 30.83 mg kg^{-1} . In contrast, AK showed much stronger differentiation, decreasing from the baseline level of $189.67 \text{ mg kg}^{-1}$ to $145.57\text{--}178.33 \text{ mg kg}^{-1}$ across treatments. The lowest AK value occurred in DRRW, whereas AWL maintained the highest AK level among the 2024 treatments.

The IFI provided a synthetic comparison of integrated fertility performance among treatments (Table 3). IFI values ranged from -0.7593 in GRL to 0.9721 in EW3R. AWL also showed a relatively high IFI value (0.8881), whereas CWW and FR3W showed intermediate values of 0.3120 and 0.2757 , respectively. BWR (-0.2610) and DRRW (-0.0928) ranked lower. Overall, the IFI ranking indicated that EW3R and AWL maintained more favorable integrated fertility performance than the other rotation configurations, whereas GRL showed the weakest overall condition.

Table 3. Integrated Fertility Index (IFI) and rankings of cropping systems

cropping patterns	integrated fertility index	Comprehensive ranking	cropping patterns	integrated fertility index	Comprehensive ranking
AWL	0.8881	2	EW3R	0.9721	1

BWR	-0.2610	6	FR3W	0.2757	4
CWWR	0.3120	3	GRL	-0.7593	7
DRRW	-0.0928	5	-	-	-

Note: “-” indicates not applicable.

3.2. Multivariate differentiation in soil properties among rotation systems

Redundancy analysis (RDA) showed multivariate differentiation in soil properties among the seven winter rotation systems (Fig.3; Table 4; Supplementary Table S1). The constrained model explained 23.40% of the total variance in soil physicochemical properties (constrained inertia = 132.9; total inertia = 568.0). The first two constrained axes accounted for 98.59% of the constrained variance, with RDA1 and RDA2 explaining 84.79% and 13.80%, respectively.

The variables contributing to these axes differed substantially. RDA1 was dominated by AK, which had a loading coefficient of -4.52 and accounted for 96.46% of the explained variation on this axis. AN made a much smaller contribution to RDA1 (loading = 0.85; contribution = 3.45%), whereas SOM, AP, TK, pH, TN, and TP contributed very little. In contrast, RDA2 was primarily associated with AN, which had a loading coefficient of 1.8081 and contributed 94.94% of the explained variation on this axis. AP, TK, and SOM contributed only 0.91%, 0.50%, and 0.25%, respectively.

Treatment scores further illustrated the separation among systems. Along RDA1, DRRW had the highest positive score (3.66 ± 1.99), whereas AWL had the lowest score (-3.58 ± 3.21), indicating strong separation along the AK-related gradient. FR3W (1.54 ± 3.66) and GRL (1.05 ± 2.55) were also positioned on the positive side of RDA1, whereas BWR (-1.29 ± 0.86) and CWWR (-1.96 ± 1.23) were located on the negative side. EW3R occupied an intermediate position near the center of RDA1 (0.59 ± 3.98). Along RDA2, EW3R had the highest positive score (4.02 ± 6.55), whereas GRL had the lowest (-3.57 ± 2.70), indicating stronger differentiation along the AN-associated axis.

The ordination pattern showed that AK was the main variable driving horizontal separation among the long-term rotation systems, whereas AN accounted for most of the vertical differentiation. SOM and AP were oriented in the same general direction as AN on the positive side of RDA2, but their contributions were much smaller. Overall, the ordination pattern suggested that AK was the primary variable associated with separation among the long-term rotation systems along RDA1, whereas AN accounted for most of the differentiation along RDA2.

Table 4. Loadings and contribution rates of the soil variables on the first two axes of the redundancy analysis

Soil physicochemical indicators	RDA1 loading coefficient	RDA2 loading coefficient	Contribution rate to RDA1 (%)	Contribution rate to RDA2 (%)
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pH	0.01	0.0031	0.00	0.00
SOM	-0.12	0.0930	0.07	0.25
TN	0.00	0.0031	0.00	0.00
TP	0.00	-0.0000	0.00	0.00
TK	0.03	0.1316	0.00	0.50
AP	0.03	0.1773	0.00	0.91
AK	-4.52	0.3417	96.46	3.39
AN	0.85	1.8081	3.45	94.94

Note: SOM, soil organic matter; TN, total N; TP, total P; TK, total K; AP, available P; AK, available K; AN, alkali-hydrolyzable N.

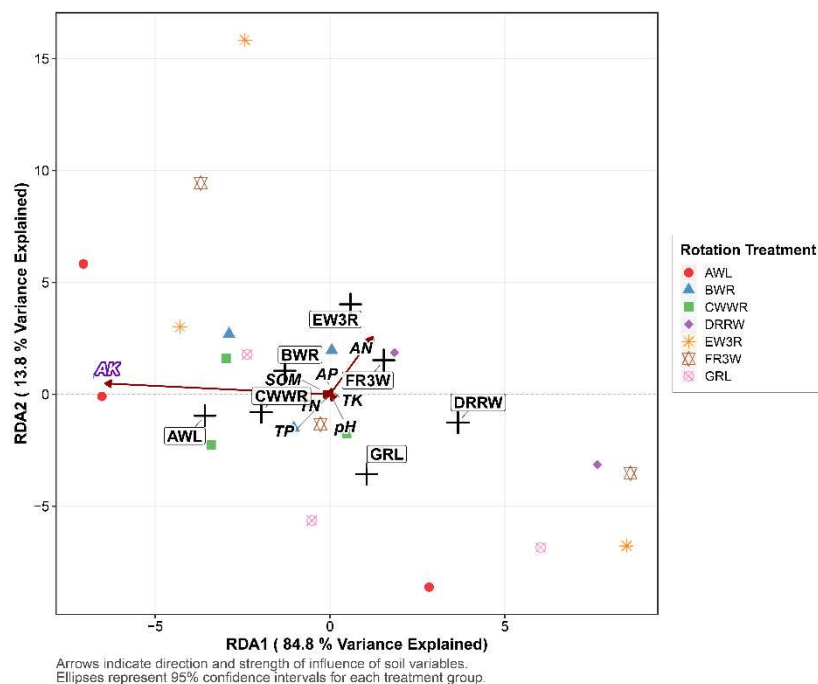


Figure 3. Redundancy analysis (RDA) of soil physicochemical properties under different cropping systems. Points represent individual soil samples, and black crosses indicate the centroids of the treatment groups. Red arrows denote soil variables, including pH, SOM, TN, TP, TK, AP, AK, and AN. Systems: AWL, BWR, CWWR, DRRW, EW3R, FR3W, and GRL. The overall RDA model explained 23.40% of the total variation in soil properties, and the first two constrained axes explained 98.59% of the constrained variation, with RDA1 and RDA2 accounting for 84.79% and 13.80%, respectively.

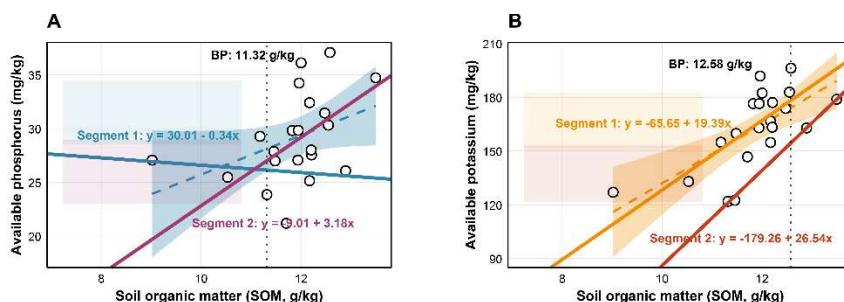
3.3. Apparent breakpoint responses of available P and K to SOM

Segmented regression analysis was used to explore potential non-linear relationships between SOM and the available nutrient pools (Fig. 4). For AP, the model suggested an apparent breakpoint at SOM = 11.32 g kg⁻¹ (95% CI: 10.85–11.79 g kg⁻¹). Below this point, SOM showed no clear relationship with AP (AP = 30.01 – 0.34 × SOM), whereas above it, the relationship appeared positive (AP = –9.01 + 3.18 × SOM). The segmented model had a substantially lower AIC than the linear model (60.66 vs. 120.01), suggesting a better fit to the data. However, the Davies test for a slope change was not significant (P = 0.89), which may reflect the limited statistical power associated with the small sample size (n = 21) and the uneven sample distribution across the breakpoint (n = 4 below, n = 17 above).

For AK, the model suggested a breakpoint at SOM = 12.58 g kg⁻¹ (95% CI: 12.21–12.95 g kg⁻¹). Below this point, AK increased with SOM (AK = –65.65 + 19.39 × SOM), and above it, the slope appeared steeper (AK = –179.26 + 26.54 × SOM). The segmented model also had a lower AIC than the linear

model (122.27 vs. 181.22). However, the Davies test was not significant ($P = 0.18$), and the high-SOM segment contained only three samples ($\text{SOM} = 12.58, 12.89, \text{ and } 13.50 \text{ g kg}^{-1}$), further limiting the statistical power of this analysis.

Taken together, these results suggest that the relationship between SOM and available nutrients may be non-linear, with the strength of the association potentially increasing at higher SOM levels. However, given the limited sample size and the non-significant Davies tests, these apparent breakpoints should be interpreted as exploratory indicators of potential non-linear patterns rather than as statistically confirmed thresholds. Across the 21 soil samples, SOM ranged from 9.02 to 13.50 g kg^{-1} , AP from 21.19 to 37.08 mg kg^{-1} , and AK from 121.9 to 196.3 mg kg^{-1} . The EW3R treatment spanned the apparent breakpoint range for both relationships. These patterns warrant further investigation with larger sample sizes and direct measurements of microbial and biochemical processes.



BP = Breakpoint

Figure 4. Segmented relationships of soil organic matter (SOM) with available P (AP) and available K (AK). (A) Relationship between SOM and AP, showing a breakpoint at 11.32 g kg^{-1} . (B) Relationship between SOM and AK, showing a breakpoint at 12.58 g kg^{-1} . Solid lines indicate the fitted segmented regressions, and shaded areas indicate 95% confidence intervals.

3.4. Direct and indirect pathways linking rotation, soil properties, and integrated fertility

The piecewise structural equation model (SEM) provided an adequate fit to the data (Fig. 5), with non-significant goodness-of-fit statistics ($\chi^2 = 12.705$, $P = 0.853$; Fisher's $C = 19.962$, $P = 0.993$). The model quantified the direct and indirect relationships among crop rotation systems, soil properties, and integrated fertility.

Several significant direct relationships were identified in the SEM (Fig. 5A). SOM had a strong positive effect on AK ($\beta = 0.755$, $P < 0.001$), and AK had a strong positive effect on AP ($\beta = 0.843$, $P < 0.001$). SOM, AK, AP, and AN all had significant positive direct effects on IFI, with standardized path coefficients of 0.268, 0.236, 0.338, and 0.183, respectively (all $P < 0.001$). In contrast, pH had a significant negative direct effect on IFI ($\beta = -0.171$, $P < 0.001$). The strong correlation between AK and AP in the SEM likely reflects their co-dependence on SOM and nutrient turnover processes rather than

a direct causal relationship between these two nutrients.

Effect decomposition further clarified the relative importance of the main predictors (Fig. 5B). SOM had the largest total effect on IFI ($\beta_{\text{total}} = 0.755$), and this effect was mainly indirect ($\beta_{\text{indirect}} = 0.487$). AK had the second largest total effect ($\beta_{\text{total}} = 0.645$), with both direct ($\beta_{\text{direct}} = 0.236$) and indirect ($\beta_{\text{indirect}} = 0.409$) contributions. By contrast, the effects of AP and AN on IFI were mainly direct, with total effects of 0.338 and 0.183, respectively.

The model also retained treatment-specific relationships with intermediate soil variables. DRRW was negatively associated with SOM ($\beta = -0.461$, $P < 0.05$), whereas EW3R was positively associated with AN ($\beta = 0.518$, $P < 0.05$). The model explained substantial proportions of the variance in AP, AK, and AN, with R^2 values of 0.65, 0.53, and 0.44, respectively. Taken together, the SEM was consistent with a SOM-associated pathway involving AK and AP that was linked to variation in integrated fertility among rotation configurations.

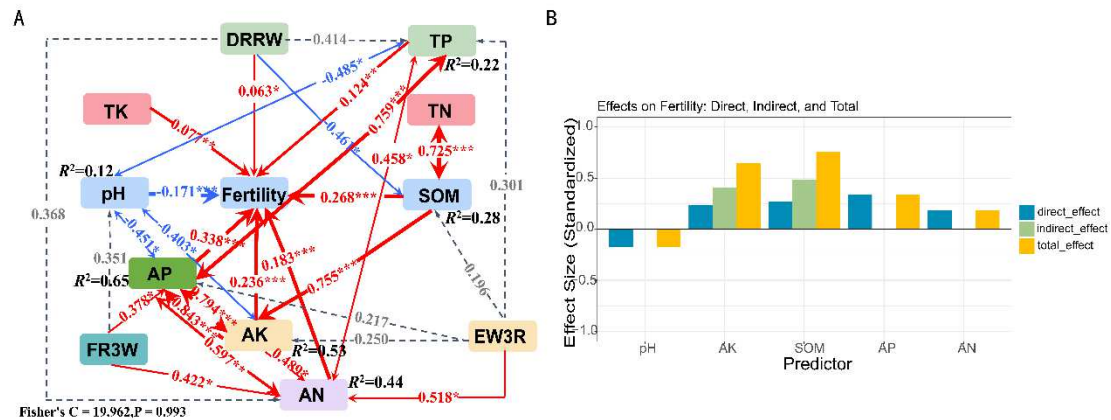


Figure 5. Piecewise structural equation model linking cropping systems, soil properties, and integrated soil fertility. (A) Structural equation model showing the direct relationships among cropping systems, soil variables, and the integrated fertility index (IFI). Numbers on arrows are standardized path coefficients. Red and blue arrows indicate positive and negative effects, respectively, and dashed arrows indicate non-significant paths. Numbers adjacent to endogenous variables indicate explained variance (R^2). (B) Direct, indirect, and total standardized effects of the main soil variables on IFI.

4. Discussion

4.1. Long-term winter rotation affected active nutrient pools more strongly than total nutrient stocks

The eight-year field experiment showed that winter crop sequence configuration influenced soil fertility in this alkaline fluvo-aquic soil, although the magnitude of response differed markedly among variables. Treatment differences were relatively small for pH, TN, TP, and TK, whereas larger differences occurred in SOM, AN, AP, and especially AK. This pattern suggests that long-term rotation effects were expressed more strongly in active nutrient pools and nutrient availability

than in total nutrient stocks. This distinction is ecologically and agronomically important because soil biological functions and crop performance depend more directly on nutrient accessibility and turnover—processes that are largely mediated by soil microbial communities—than on total elemental reserves alone.

This pattern is consistent with broader concepts of soil quality and fertility, which emphasize that agronomic performance emerges from interacting physical, chemical, and biological processes rather than from total nutrient contents alone[2,14]. In intensively managed cropland, long-term rotation effects may therefore be more detectable in variables associated with nutrient cycling and plant availability than in slowly changing total stocks. The limited variation in TK observed here, despite clear differences in AK, provides a clear example of this contrast and underscores the importance of understanding the biological processes—such as microbial mobilization of mineral-bound K and organic matter decomposition—that govern the transformation between total and available nutrient pools.

The integrated fertility index is consistent with this interpretation. Although IFI was used here as a synthetic indicator rather than a standalone endpoint, it helped identify the relative performance of the tested systems. EW3R had the highest IFI value, followed by AWL, whereas GRL had the lowest. Importantly, the superior performance of EW3R did not reflect the maximum value of every measured variable. Instead, it combined the highest SOM and AN with relatively favorable AK and AP, suggesting that balanced nutrient availability may have been more important than maximizing any single indicator. This interpretation agrees with previous studies showing that the benefits of long-term crop diversification often arise from coordinated improvements in soil and system performance rather than from uniformly larger values of individual properties[9,10,12,15].

At the same time, diversification alone did not guarantee better soil outcomes. The performance of the rotation systems depended strongly on how wheat and rapeseed were sequenced over time. EW3R, a wheat-dominant four-year sequence, produced the most favorable overall response, whereas DRRW showed lower SOM and the lowest AK. This pattern suggests that the long-term value of winter crop diversification in this system depended on maintaining a suitable balance among carbon inputs, nutrient demand, and nutrient retention—a balance that is fundamentally mediated by soil microbial communities and their biochemical activities. Because rapeseed

diversifies residue chemistry but also imposes high K demand, frequent rapeseed inclusion may intensify K withdrawal if not counterbalanced by sufficient carbon inputs and nutrient replenishment[23,24,32]. From a soil biological perspective, this highlights the importance of carbon input frequency and quality—which directly influence microbial biomass, activity, and community composition—as key determinants of soil fertility maintenance in intensive cropping systems.

4.2. Available K was the primary axis of long-term treatment differentiation

The RDA results suggested that the main multivariate difference among the rotation systems was associated with available K. AK accounted for 96.46% of the explained variation along RDA1, whereas AN contributed most strongly to RDA2. Among the measured variables, K availability therefore appeared to be the principal dimension along which the long-term winter rotation systems differed in this soil.

This finding is particularly informative because total K varied only slightly among treatments, whereas AK showed strong differentiation. The contrast suggests that winter sequence configuration was more closely associated with the functional accessibility of soil K—a key aspect of soil biological functions and ecosystem functionality—than with total K reserves. In practical terms, TK was a relatively insensitive indicator of treatment differentiation in this experiment, whereas AK captured a more dynamic component of soil fertility that is shaped by biological processes such as microbial K mobilization from mineral structures and organic matter decomposition. Similar concerns have been raised more broadly, as K depletion and low nutrient-use efficiency are increasingly recognized as underappreciated constraints in intensive cropping systems, even where total soil K remains relatively high[24,31]. Our findings provide empirical field evidence supporting this concern in a major agricultural region of China.

The ordination pattern also helps explain why the highest IFI value did not simply correspond to the highest AK value. AWL maintained relatively high AK and was strongly separated along the AK-related axis, yet EW3R still ranked highest in the integrated evaluation. This indicates that AK was a dominant axis of differentiation, but not the sole determinant of overall fertility performance. Its importance depended on its interaction with SOM, AP, and AN, suggesting that K availability was an important but context-dependent variable within a broader soil nutrient network.

From a pedological and biological perspective, this pattern is plausible. In alkaline soils, the difference between total nutrient reserves and plant-accessible pools can be substantial, especially for nutrients whose short-term availability is shaped by exchange processes, residue return, and microbial mediation. Recent work has emphasized that K availability is strongly affected by interactions among carbon inputs, microbial mobilization, and soil structural conditions[8,29,32]. Microorganisms contribute to K availability through multiple mechanisms, including the production of organic acids that weather K-bearing minerals, the release of K during organic matter decomposition, and the modification of soil physicochemical properties that affect K exchange[8, 32]. Although K fractions and microbial traits were not directly measured here, the strong contribution of AK to treatment separation suggests that available K may serve as a sensitive indicator of how long-term rotation design is associated with soil fertility in fluvo-aquic soil.

4.3. SOM was associated with apparent non-linear changes in AP and AK—exploratory evidence for potential biological thresholds

The segmented regression results suggested that relationships between SOM and nutrient availability may be non-linear, with apparent breakpoints at 11.32 g kg⁻¹ for AP and 12.58 g kg⁻¹ for AK. It is important to note, however, that the Davies tests for slope changes were not statistically significant for either AP (P = 0.89) or AK (P = 0.18). This may be attributable to the limited sample size (n = 21) and the uneven distribution of samples across the breakpoints, which reduce the statistical power to detect significant slope changes. The AIC comparison, however, consistently favored the segmented models over the linear models, suggesting that the non-linear patterns may be biologically meaningful even if they do not reach conventional levels of statistical significance. Given these statistical limitations, the apparent breakpoints should be regarded as exploratory observations rather than confirmed thresholds. Nevertheless, the patterns are consistent with the broader expectation that SOM influences nutrient availability through multiple interacting mechanisms, and they generate testable hypotheses for future research. This interpretation is consistent with previous studies showing that SOM influences aggregation, pore characteristics, nutrient retention, and microbial habitat, all of which can affect the responsiveness of available nutrient pools[28,29]. The apparent breakpoints may represent SOM levels above which microbial communities become more active or shift in composition, but direct measurements of microbial

community composition and activity are needed to test this hypothesis.

The lower apparent breakpoint for AP suggests that modest SOM accumulation may already favor P availability in alkaline soil, where P is often constrained by precipitation and sorption reactions. SOM may influence these processes through several pathways, including competition for sorption sites, modification of microsite chemical conditions, and stimulation of biological activity associated with P turnover—particularly through microbial production of phosphatases and organic acids that solubilize mineral-bound P[7,25,30]. Although P fractions and phosphatase-related processes were not directly measured in this study, the apparent non-linear pattern for AP is consistent with the interpretation that SOM accumulation above a certain level may have stimulated P-mobilizing activities.

The higher apparent breakpoint for AK suggests that stronger SOM accumulation may be required before a clearer enhancement of K availability becomes evident. This may reflect the role of SOM in exchange buffering, structural stabilization, and biologically mediated nutrient release. Microorganisms contribute to K mobilization through the production of organic acids that weather K-bearing minerals (such as illite and feldspar), as well as through the release of K during the decomposition of organic residues[8,32]. However, given that the high-SOM segment for AK contained only three samples, the slope estimate for this segment should be interpreted with particular caution, and the apparent breakpoint for AK requires validation in future studies with larger sample sizes.

The treatment distribution is consistent with this interpretation. EW3R occupied the upper end of the SOM range and spanned the apparent breakpoint region for both AP and AK, whereas DRRW remained closer to the lower end and showed the weakest AK status. One plausible explanation for the relatively favorable performance of EW3R is therefore that it was associated with soil conditions in a range where SOM-related effects on nutrient availability may become more apparent. However, we emphasize that these apparent breakpoints were identified within the specific SOM gradient of this experiment (9.02–13.50 g kg⁻¹), were not statistically significant in Davies tests, and should not be extrapolated as universal critical values for agricultural management without further validation across broader soil and climatic conditions. They nevertheless provide exploratory evidence for potential non-linear relationships between SOM and nutrient availability that merit further

investigation.

4.4. A SOM-associated AK–AP covariation pattern may help explain long-term soil fertility responses

The SEM offered an integrated statistical framework for interpreting the observed relationships by linking long-term rotation configuration, intermediate soil variables, and integrated fertility performance. The model supported a strong positive relationship from SOM to AK, and a strong association between AK and AP, together with positive direct effects of SOM, AK, AP, and AN on IFI. These results suggest that long-term rotation effects on soil fertility were associated with linked variation in SOM, K availability, and P availability, rather than with isolated changes in independent nutrient variables—a finding with implications for understanding the biological and biochemical processes that govern nutrient cycling in agricultural ecosystems.

This pathway interpretation is consistent with the other analyses. The strong role of SOM agrees with the treatment-level patterns and with the breakpoint responses identified in the segmented regressions. The central position of AK is also consistent with the RDA, in which available K was the dominant axis separating the rotation systems. The SEM therefore serves as a statistical synthesis of the univariate, multivariate, and breakpoint-based evidence rather than as a standalone result. The convergence of these analyses lends support to the interpretation that SOM-associated changes in available K and P were an important feature of long-term nutrient differentiation in this experiment. The decomposition of effects further supports this view. SOM had the largest total effect on IFI, and much of this effect was indirect, whereas AK also contributed strongly through both direct and indirect pathways. This pattern suggests that SOM was an upstream variable, while AK served as an important intermediary linking SOM to broader fertility performance. Such an interpretation is consistent with growing recognition that K is not only a major nutrient, but also a variable closely linked to nutrient-use efficiency, physiological stability, and the functioning of crop–soil systems under intensive management[23,24,31].

From a soil biological standpoint, the strong associations among SOM, AK, and AP may reflect the integrated effects of microbial communities on organic matter decomposition, nutrient mineralization, and ion exchange processes—with SOM serving as the primary energy source for microbial activity, and microbial communities mediating the transformation of organic and mineral

nutrient pools into plant-available forms.

It is important to acknowledge that this study was based on routine soil chemical analyses and did not include direct measurements of microbial community composition, enzyme activities, or functional genes. The fitted SEM should therefore be interpreted as the most parsimonious framework supported by the measured field data rather than as direct proof of all underlying mechanisms. In particular, the strong statistical association between AK and AP in the SEM likely reflects their co-dependence on SOM and nutrient turnover processes, rather than a direct causal relationship whereby K availability directly controls P availability. Nevertheless, because the same general pattern was supported by treatment comparisons, RDA, segmented regression, and SEM, the inference of a SOM-associated AK–AP coupling pathway appears consistent with the available statistical evidence and points toward the need for future investigations of the biological and biochemical mechanisms involved—including measurements of microbial community composition, enzyme activities related to P and K cycling, and nutrient fractionation studies.

4.5. Implications for sustaining soil biological functions and fertility through rotation design

From a soil management and soil biology perspective, the present results suggest that long-term soil improvement in alkaline fluvo-aquic cropland may depend not only on maintaining total nutrient stocks, but also on sustaining the linkage between SOM accumulation and available nutrient pools—a linkage that appears to be fundamentally mediated by soil biological processes and underpins soil ecosystem functionality and resilience. In this experiment, the wheat-dominant EW3R configuration provided the most favorable overall balance among SOM, AN, AK, and AP. This suggests that moderate rapeseed inclusion within a wheat-dominant winter sequence may be more effective for sustaining soil fertility and nutrient availability than more rapeseed-intensive configurations, likely because the higher frequency of wheat—with its greater biomass and carbon input—promotes SOM accumulation. This, in turn, provides a more favorable habitat and energy source for soil microbial communities, supporting more active nutrient cycling and transformation processes.

This implication is relevant beyond the specific treatments tested here. The results are consistent with the broader idea that rotation design should be optimized not only for crop diversification, but also for how the chosen sequence influences nutrient accessibility, nutrient buffering, and the interaction between soil carbon status and available nutrients over time—interactions that are

critically dependent on soil microbial activity and biochemical processes. In particular, the sensitivity of AK to rotation configuration suggests that K management deserves more explicit attention in intensive cropping systems in which rapeseed is included regularly. Recent literature has highlighted the global importance of often overlooked K constraints and low nutrient-use efficiency in crop production [24,31], and the present results offer field-based evidence consistent with that concern. From a soil biology and fertility standpoint, our findings underscore the need to consider potassium availability as a key indicator—and potentially a target for management—when designing crop sequences for long-term soil health.

Several limitations should also be acknowledged. First, the breakpoint values identified for AP and AK should be interpreted as empirical breakpoints within this particular soil–climate–management setting rather than as universal benchmarks. Second, the dataset was based mainly on standard chemical indicators and did not directly resolve the biological and biochemical processes underlying nutrient mobilization. Third, because our study focused on cumulative end-point responses after eight years, it does not describe interannual dynamics in soil fertility, which would be valuable for understanding the temporal trajectory of rotation effects and their impacts on soil biological communities. Fourth, the pH data should be interpreted with caution given the potential influence of irrigation water quality and evaporative concentration, as discussed in Section 4.1. It is important to acknowledge that this study was based on routine soil chemical analyses and did not include direct measurements of microbial community composition, enzyme activities, or functional genes. Nevertheless, the observed breakpoint responses and the SOM–AK–AP covariation pathway provide clear statistical evidence pointing toward underlying biologically mediated processes. Future work would benefit from combining long-term rotation experiments with direct measurements of microbial community composition and activity (e.g., via phospholipid fatty acid analysis, amplicon sequencing, or metagenomics), enzyme activities (particularly phosphatases and K-mobilizing enzymes), nutrient fractions, aggregate-associated carbon, and rhizosphere processes. Future studies combining long-term rotation experiments with metagenomic and metatranscriptomic approaches are needed to elucidate the specific microbial taxa and functional genes responsible for the thresholds identified here. Such data would help test and refine the SOM-associated K–P coupling pattern identified here and elucidate the specific biological and

biochemical mechanisms involved—particularly the microbial taxa and functional genes responsible for P and K mobilization in response to different rotation regimes.

5. Conclusions

An eight-year field experiment on the Huang–Huai–Hai Plain showed that winter wheat–rapeseed sequence configuration influenced long-term soil fertility in alkaline fluvo-aquic soil. Treatment differences were expressed more clearly in SOM and available nutrient pools than in total nutrient stocks, suggesting that active nutrient status was more responsive to long-term rotation design than total nutrient reserves.

Among the measured variables, available K was the primary axis differentiating the rotation systems, whereas SOM showed apparent non-linear relationships with both AP and AK, although these patterns should be regarded as exploratory given the limited sample size. The combined evidence from segmented regression and piecewise SEM suggests a SOM-associated covariation pattern linking available K and P to integrated fertility performance, with SOM serving as a primary driver of nutrient availability. Within the tested systems, the wheat-dominant EW3R configuration showed the most favorable balance between SOM accumulation and nutrient availability.

Overall, the results suggest that functionally balanced winter rotation design—particularly sequences with higher wheat frequency and associated carbon inputs—may help improve long-term soil fertility by strengthening the linkage between SOM and available nutrient pools. These findings provide a useful basis for optimizing crop sequence design in the alkaline soils of the Huang–Huai–Hai Plain and offer insights relevant to sustainable soil fertility management in similar intensive cropping regions worldwide.

Declarations

Abbreviations

SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; IFI, integrated fertility index; PCA, principal component analysis; RDA, redundancy analysis; SEM, structural equation modeling; AIC, Akaike information criterion; ANOVA, analysis of variance; HSD, honestly significant difference; CI, confidence interval.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests

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Authors' contributions

Xiao Cui: Writing – review & editing, Writing – original draft, Formal analysis, Visualization, Software. **Jinhua Cao:** Investigation, Data curation, Writing – review & editing. **Jiacheng Zhu:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Shufen Zhang:** Conceptualization, Investigation, Project administration, Writing – review & editing. **Jiayong Shao:** Software, Visualization. **Dongfang Cai:** Investigation, Methodology. **Junping He:** Investigation, Software. **Peiyu Chen:** Investigation, Data curation. **Huiyun Shi:** Investigation, Software. **Yizi Liu:** Investigation, Data curation. **Lei Zhao:** Investigation, Data curation. **Dongguo Wang:** Investigation, Data curation

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