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Grainforage rotation improved soil organic carbon fractions, carbon pool management, and chemical composition in dryland agroecosystems of the Loess Plateau China

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Research Article

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Posted Date: November 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11792759/v1

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Additional Declarations: No competing interests reported.

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Abstract

Aims Grain-forage rotation is promising for improving soil carbon sequestration and soil quality in arid agroecosystems. We tested whether potato-oat (PO), forage maize-pea (MP) and wheat-alfalfa (WA) rotations promote soil organic carbon (SOC) accumulation, optimize carbon pool management and alter SOC chemical composition on the Loess Plateau.

Methods Five cropping patterns, potato (CK1) and maize (CK2) continuous cropping, PO, MP and WA, were compared at depths (0 – 20, 20 – 40 cm) and stages (peak growth, harvest).Soil organic carbon (SOC), easily oxidizable organic carbon (EOC), dissolved organic carbon (DOC) and recalcitrant organic carbon (ROC) were measured, carbon pool management index (CPMI) calculated, and chemical structure analyzed by Fourier transform infrared spectroscopy (FTIR).

Results At harvest, surface SOC and EOC under PO were significantly higher than under other treatments, while in subsurface soil SOC and DOC under MP were significantly higher . In 0 – 20 cm, CPMI under PO rose from 100.38 at peak growth to 115.55 at harvest, whereas MP showed higher CPMI at 20 – 40 cm. FTIR revealed no differences in main chemical groups, with polysaccharide dominant; aromatic carbon proportions were relatively high under PO and MP, and aliphatic carbon proportions under continuous cropping. RDA indicated total nitrogen (TN) and pH as important environmental factors affecting SOC composition, carbon pool management and chemical structure.

Conclusions PO mainly promoted surface soil carbon accumulation and carbon pool activity, while MP enhanced deep soil carbon pool stability; both are recommended for SOC management and soil quality improvement in the Loess Plateau dryland agroecosystems.

Keywords: Grain-forage rotation; soil organic carbon; Carbon-pool management index (CPMI); Fourier-transform infrared spectroscopy (FTIR); Chemical group of soil organic carbon

Introduction

The Loess Plateau is an important ecological security barrier and dryland agricultural production base in northern China. It is also one of the regions with the most serious soil erosion and the most fragile ecological environment in the world.Long-term monocropping dominated by potato and maize· has prevailed in many farmlands in this area, which brings multiple threats to soil health (Niu et al .2020).It may increase soil bulk density and decrease soil structural stability, thereby impairing soil ecological functions (Zhang et al. 2018;Tisdall and Oades 1982;Chen et al. 2009). In addition, monocropping may reduce plant community diversity and ecosystem stability, increase the risk of surface runoff and soil erosion , and exacerbate nutrient losses, further accelerating soil degradation (Giller et al. 1997;Jia et al. 2011) .These processes may create a negative feedback loop characterized by intensified agricultural production, declining soil quality, and deteriorating ecosystem functions. Therefore, optimizing cropping mode, increasing soil carbon sequestration and improving soil quality have become critical strategies for achieving sustainable agricultural development in the Loess Plateau.

Soil organic carbon (SOC) is a fundamental component of soil ecosystem. It not only serves as an essential material basis for maintaining soil fertility and productivity but also constitutes a major component of the terrestrial carbon cycle (Morelli et al. 2022;Feng et al. 2025).Soil organic carbon is composed of different stability components. Different carbon fractions play distinct roles in soil carbon cycling because of the high heterogeneity of SOC (Leifeld and Kögel-Knabner 2005)Labile organic carbon fractions generally have relatively rapid turnover rates and are highly sensitive to tillage practices, crop types, and environmental changes (Blair et al. 1995). In contrast, recalcitrant organic carbon fractions, characterized by

greater chemical stability and longer turnover times, constitute an important component for maintaining long-term soil carbon storage (Cambardella and Elliott 1992).Some studies the stability of SOC depends not only on the size of the soil carbon pool but is also closely associated with its molecular composition and chemical properties(Basile-Doelsch and Pellerin 2020). As fundamental structural units of organic carbon molecules, functional groups whose compositional changes can directly affect the decomposition processes, microbial utilization efficiency, and long-term sequestration potential of organic carbon (Weng et al. 2022). It is demonstrated that vegetation type, cropping mode, and organic matter inputs can substantially alter the functional group composition of SOC (Guo et al. 2016;Deng, et al. 2019;Zheng et al. 2021).Therefore, characterizing the chemical structure of soil organic carbon is important for understanding the mechanisms of soil carbon stabilization under cropping systems.

Therefore, this study investigated three grain–forage rotation modes and two monocropping modes in the dryland agricultural region of the Loess Plateau. Variations in soil organic carbon (SOC) fractions and chemical structural characteristics under different cropping modes were systematically analysed by integrating SOC fractionation with Fourier transform infrared spectroscopy (FTIR). Furthermore, the regulatory mechanisms by which grain–forage rotation alters soil carbon pool activity, stability, and carbon sequestration potential were explored. The following hypotheses were put forward: (1) grain–forage rotation increases SOC content and the proportion of the active carbon pool by enhancing plant-derived organic matter inputs and promoting carbon fraction transformation, thereby resulting in greater soil carbon accumulation than the monocropping modes; (2) rotation optimizes the chemical composition of SOC and promotes the transformation of readily decomposable carbon fractions toward more aromatic and stable structures, thereby enhancing the resistance of SOC to decomposition and its stability; and (3) different grain–forage rotation modes exert distinct regulatory effects on soil carbon pool allocation and SOC structural evolution, with appropriately configured rotation modes potentially exhibiting greater carbon sequestration efficiency and a stronger capacity to improve soil quality. The results could clarify the differential carbon-sequestration effects of diverse grain–forage rotation treatments, elucidate the underlying mechanisms by which grain–forage rotation mediates the formation and stabilization of soil carbon pools, and offer a theoretical basis for developing sustainable cropping systems that promote carbon sequestration

and resource-use efficiency in arid and semi-arid regions of the Loess Plateau.

Materials and methods

Experimental site and soil characteristics

The field experiment was carried out in Xizhai Village, Xiangquan Town, Anding District, Dingxi City, Gansu Province. The site is located at 104 ° 29 ′ E, 35 ° 28 ′ N, and the altitude is about 2100 meters.This study site falls within a climatic transition zone between the northern temperate semi-humid and temperate semi-arid regions.The mean annual temperature ranges from 5.7 to 7.7 °C, and the frost free period ranges from 122 to 160 days. Annual precipitation ranges from 350 to 600 mm and is unevenly distributed, with most rainfall occurring from July to September. The average annual sunshine duration ranges from 2114 to 2433 h.The soil is classified as typical Loessial soil. The basic soil properties of 0–40 cm depth were as follows: pH 8.2, soil organic matter 10.5 g/kg, total nitrogen 0.82 g/kg, available phosphorus 22.15 mg/kg, and available potassium 125 mg/kg. The local cropping mode is predominantly characterized by continuous monocropping of potato and maize.

Experimental materials

Wheat cultivar Dingxi 40 (*Triticum aestivum* L.), maize cultivar Xianyu 335 (*Zea mays* L.), potato cultivar Dingshu 4 (*Solanum tuberosum* L.), pea cultivar Dingwan 10 (*Pisum sativum* L.), oat Dingyan 10 (*Avena sativa* L.), forage maize cultivar Yuqingzhu 23 *(Zea mays* L.), and alfalfa cultivar Gannong No. 3 (*Medicago sativa* L.) were used in this study. The crop and forage were planted manually in middle April each study year.

Experimental design and treatments

A field plot experiment was conducted with five cropping treatments, including three grain‑forage rotations and two monocropping modes. The three rotation mode treatments were potato–oat (PO), forage maize–pea (MP), and wheat–alfalfa (WA), whereas continuous potato (CK1) and continuous maize (CK2) were used as monocropping controls. All crops selected for the experiment are commonly cultivated in same area plot in the local region. Each

treatment was replicated three times, and there were 15 plots in total arranged in a randomized complete block design. Each plot was 35 m² (5 m × 7 m), with a 1m buffer zone between two plots.

All plots received basal fertilization of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) at rates of 120, 90, and 90 kg/ ha before crop establishment. Urea (46% N) was used as the N fertilizer, calcium superphosphate (16% P_2O_5) as the P fertilizer, and potassium sulfate (50% K_2O) as the K fertilizer. Field management during the growing season followed the local conventional practices. No additional fertilization or pesticide applications were performed in the growth stage.

Potato and maize were manually sown in furrows and covered with plastic film in 2024. Conventional maize was planted at a row space of 40 cm and a plant space of 20 cm, while forage maizewas planted at a row space of 40 cm and a plant space of 15 cm. Wheat was manually drilled in furrows at a row space of 20 cm.Potato and maize were manually planted in furrows and covered with plastic film using hill-drop sowing, with a row space of 40 cm and a plant space of 20 cm, in 2025. Oat, pea, and alfalfa were manually drilled in furrows at a row space of 20 cm. The seeding rates of oat, pea and alfalfa were 203, 210 and 22.5 kg • hm^{-2} respectively in two study years.

Soil sampling

Soil samples were collected from the 0–20 and 20–40 cm soil depths within each plot during the peak growth stage and at harvest. Five sampling points were randomly selected from each plot, and soil samples from the same soil depth were thoroughly mixed to obtain one composite sample per depth.Fresh soil samples were passed through a 0.25-mm sieve for the determination of dissolved organic carbon (DOC). The remaining soil samples were air-dried and subsequently passed through a 0.25-mm sieve for the determination of soil organic carbon (SOC),easily oxidizable organic carbon (EOC),recalcitrant organic carbon (ROC) and chemical structure of SOC.

Determination methods

Soil organic carbon and its fractions

Soil total organic carbon (SOC) was determined using the potassium dichromate oxidation method with external heating (Walkley and Black 1934). Easily oxidizable organic carbon (EOC) was determined using the 0.333 mol/L $KMnO_4$ oxidation method (Islam et al. 2003). Dissolved organic carbon (DOC) was extracted with 0.5 mol/L K_2SO_4 and determined accordingly (Ghani et al. 2003). Recalcitrant organic carbon (ROC) was calculated as the difference between total organic carbon and easily oxidizable organic carbon (Morelli et al. 2022).

Soil carbon pool management index

The carbon pool management index (CPMI) was calculated using CK1 as the reference soil. The total organic carbon content and carbon pool activity of CK1 were used as the reference values for total organic carbon and carbon pool activity, respectively. The CPMI was calculated according to the following equations (Blair et al. 1995) :

$$CPMI = CPI \times CPAI \times 100 \quad (1)$$

$$CPI = TOCS / TOCR \quad (2)$$

$$CPAI = CPAS / CPAR \quad (3)$$

$$CPA = CA / CNA \quad (4)$$

where CPI is the carbon pool index; CPAI is the carbon pool activity index; TOCS is the total organic carbon content of the sample; TOCR is the total organic carbon content of the reference soil; CPAS is the carbon pool activity of the sample; CPAR is the carbon pool activity of the reference soil; CA is the content of active organic carbon; and CNA is the content of non-active organic carbon, which is not oxidized by potassium permanganate, corresponding to the recalcitrant organic carbon content.

Chemical structure of soil organic carbon

The chemical structure of SOC was determined by Fourier transform infrared spectroscopy (FTIR) and KBr tabletting method. The soil samples were analyzed by FTIR spectrometer (Bruker,Germany). The spectral range was 400 – 4000 cm^{-1}, the spectral resolution was 4 cm^{-1}, and each sample was scanned 32 times. The assignment of the characteristic absorption bands in the FTIR spectra is based on the literature reported by Wu et al (2025).

Data analysis

Experimental data were organized using Microsoft Excel 2017. FTIR spectra were processed using OMNIC 8.2 software (Thermo Fisher Scientific, Madison, WI, USA), including conversion between transmittance and absorbance spectra, spectral smoothing, and baseline correction. Gaussian fitting was subsequently performed for the relevant characteristic absorption peaks, and the areas of the corresponding peaks were calculated. Statistical analyses were conducted using IBM SPSS Statistics 27(IBM Corp., USA). One-way analysis of variance (ANOVA) was performed to evaluate the effects of cropping mode, and significant differences among treatments were assessed using the least significant difference (LSD) test at $P < 0.05$. All figures were generated using Origin 2026 (OriginLab Corp., USA).

Results

Soil organic carbon and its fractions

Similar stage and soil depth dependent responses to cropping treatments were exhibited by SOC, EOC, and DOC, with greater sensitivity displayed by DOC than by SOC and EOC (Fig. 1). At the peak growth stage, higher SOC and EOC contents in the 0–20 cm soil depth were maintained by CK1 and PO, and higher DOC contents in the 0–20 cm soil depth were maintained by CK1, WA, and PO. In the 20–40 cm soil depth, greater SOC and EOC contents were observed under MP, whereas a greater DOC content was observed under PO at the peak growth stage. Obvious shifts in treatment effects on labile carbon fractions were detected at harvest. The highest SOC and EOC contents in the 0–20 cm soil depth were produced by PO, with its EOC content being 11.67% and 13.89% higher than those of CK1 and CK2, respectively. An elevated EOC content in the 20–40 cm soil depth was also obtained by PO, whereas the greatest SOC and DOC contents in the 20–40 cm soil depth were yielded by MP, with its DOC content 4.37% and 14.37% higher than those of CK1 and CK2, respectively.

Significantly higher ROC contents were detected under the CK1 treatment among all groups in the 0–20 cm soil depth at the peak growth stage. Considerably greater ROC contents were maintained under the MP and CK2 treatments relative to other treatments in the 0–20 cm soil depth at the harvest stage. Relatively higher ROC values were observed under the CK2,

MP, and CK1 treatments in the 20–40 cm soil depth at the peak growth stage. Significantly higher ROC contents were obtained under the PO treatment compared with all other treatments in the 20–40 cm soil depth at the harvest stage.

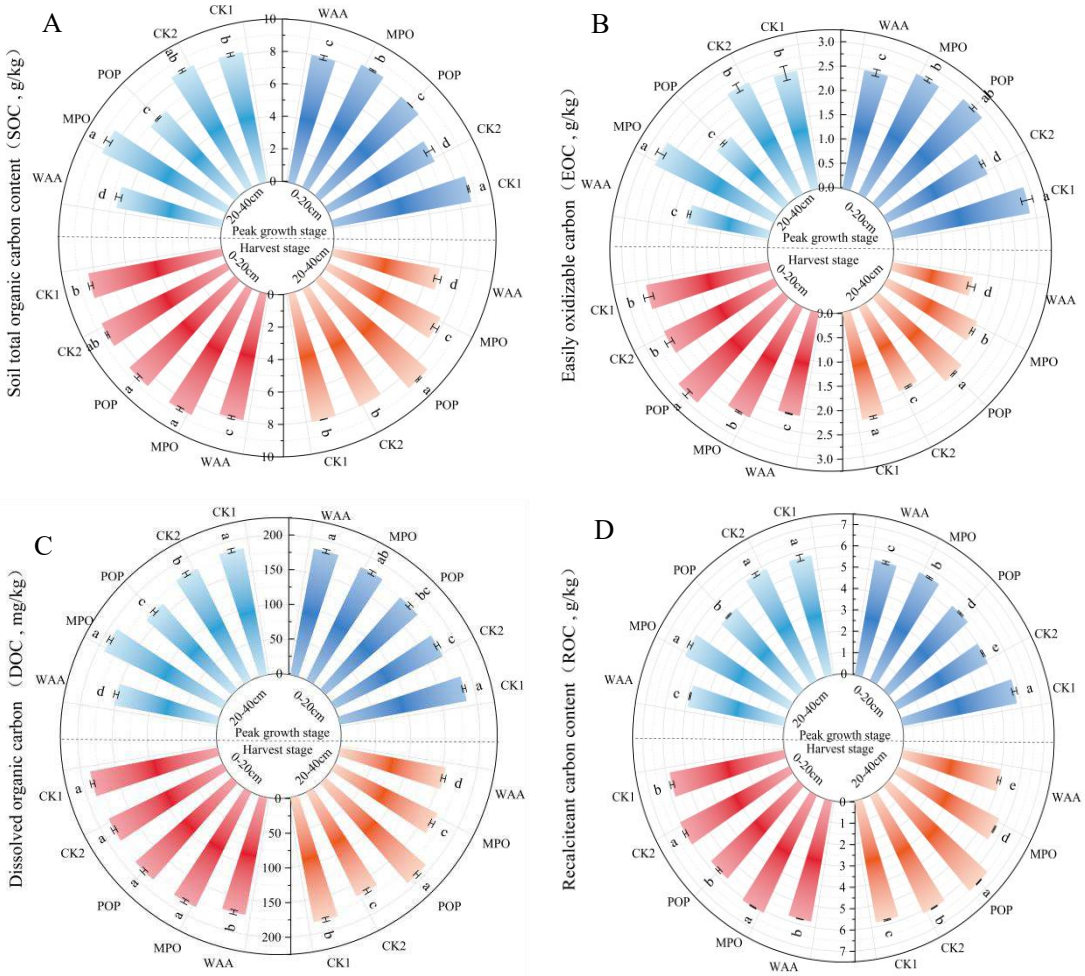


Figure 1. Soil organic carbon and its fractions content under different grain–forage rotation mode

Note: (A) Soil organic carbon (SOC) content; (B) easily oxidizable organic carbon (EOC) content; (C) dissolved organic carbon (DOC) content; and (D) recalcitrant organic carbon (ROC) content. CK1, continuous potato cropping; CK2, continuous maize cropping; PO, potato–oat rotation; MP, silage maize–pea rotation; and WA, wheat–alfalfa rotation. Different lowercase letters indicate significant differences among treatments within the same growth stage and soil depth under different cropping mode ($P < 0.05$). The same applies hereinafter.

Soil carbon pool management index

At the peak growth stage, the highest carbon pool management index (CPMI) was obtained under the PO treatment in the 0 – 20 cm soil depth. Identical ranking orders of PO > CK1 >

MP > CK2 > WA were exhibited for carbon pool activity (CPA) and carbon pool activity index (CPAI), whereas a different ranking of CK1 > MP > PO > WA > CK2 was observed for carbon pool index (CPI). In the 20 – 40 cm soil depth, CPMI followed the order MP > CK2 > CK1 > PO > WA; significantly higher CPMI values together with the maximum CPA, CPAI and CPI values were produced by MP relative to all other treatments. Low CPA (0.35 – 0.36) and CPAI values were recorded under PO and WA in this depth, leading to their markedly lower CPMI values near 70.

Distinct differences in the distribution patterns of soil carbon pool indices between the peak growth and harvest stages were observed. At harvest in the 0 – 20 cm soil depth, CPMI ranked PO > CK1 > MP > CK2 > WA, with PO showing significantly greater CPMI than all other treatments. PO also obtained the largest CPA and CPAI values, as well as 1.05 CPI, and its CPMI value increased from 100.38 to 115.55 compared with the peak growth stage, with an increase of about 15 %. WA exhibited a low CPMI of 81.80, a CPA of 0.38 and a CPAI of 0.85 at harvest. Lower overall CPMI values were measured in the 20 – 40 cm soil depth at harvest than at the peak growth stage, with the CPMI ranking CK1 > PO > MP > CK2 > WA. Treatment-specific shifts in CPMI were detected between the two sampling stages: MP showed an approximate 21.5% reduction in CPMI, while PO had an approximate 43% rise in CPMI at harvest compared with the peak growth stage. For the 20 – 40 cm depth at harvest, CK1 maintained the highest CPMI, while WA had the lowest CPMI (72.47).

Table 1. Soil carbon pool management index under different grain–forage rotation mode

Grain/Forage Growth Period	Soil depth	Treatment	CPA	CPAI	CPI	CPMI
Peak growth stage	0-20 cm	CK1	0.50±0.03 b	1.00±0.00b	1.00±0.00a	100.00±0.00a
		CK2	0.46±0.01 bc	0.93±0.05c	0.84±0.03d	77.83±2.60d
		PO	0.54±0.01a	1.10±0.09a	0.93±0.00c	100.38±2.28a
		MP	0.49±0.01bc	0.99±0.09b	0.96±0.01b	94.28±1.16b
		WA	0.45±0.02c	0.91±0.06c	0.91±0.02c	83.25±2.03c
	20-40 cm	CK1	0.43±0.04a	1.00±0.00b	1.00±0.00b	100.00±0.00b
		CK2	0.44±0.02a	1.02±.0.06b	1.03±0.00a	105.02±5.16b
		PO	0.36±0.01a	0.83±0.06c	0.86±0.01c	68.55±3.45c
		MP	0.47±0.02a	1.15±0.07a	1.06±0.03a	114.84±3.13a
		WA	0.35±0.01a	0.81±0.08c	0.82±0.02d	66.26±2.98c
Harvest stage	0-20 cm	CK1	0.44±0.03b	1.00±0.00b	1.00±0.00b	100.00±0.00b
		CK2	0.42±0.02b	0.94±0.09b	1.03±0.01a	97.19±4.78b

	PO	0.50±0.02a	1.13±0.09a	1.05±0.02a	115.55±8.59a
	MP	0.41±0.00b	0.97±0.02b	1.05±0.01a	97.89±2.21b
	WA	0.38±0.00c	0.85±0.05c	0.96±0.01c	81.80±1.22c
20-40 cm	CK1	0.39±0.01a	1.00±0.00a	1.00±0.00b	100.00±0.00a
	CK2	0.31±0.00d	0.81±0.02d	1.00±0.00b	81.18±0.97c
	PO	0.35±0.00bc	0.90±0.02bc	1.10±0.01a	98.16±2.35a
	MP	0.37±0.01b	0.95±0.02b	0.96±0.02c	90.13±1.30b
	WA	0.34±0.02c	0.87±0.05c	0.83±0.03d	72.47±3.87d

Note: CK1, continuous potato cropping; CK2, continuous maize cropping; PO, potato-oat rotation; MP, forage maize-pea rotation; WA, wheat-alfalfa rotation. Different lowercase letters indicate significant differences among treatments within the same growth stage and soil depth at $P < 0.05$. CPA, carbon pool activity; CPAI, carbon pool activity index; CPI, carbon pool index; CPMI, carbon pool management index.

Effects of grain – forage rotation mode on the chemical structure of soil organic carbon

FTIR spectral characteristics of soil organic carbon

FTIR spectra were acquired from 4000 – 400 $cm^{-1,}$ The characteristic absorption bands were assigned as follows: 3615 cm^{-1} for -OH stretching of alcohols and phenols; 2920 $cm^{-1}$for aliphatic -CH^2 stretching; 1633 cm^{-1} for C=C stretching of lignin-related aromatic structures; and 1027 cm^{-1} for C-O stretching of polysaccharides (Zhao et al. 2023 ;Andjelkovic et al. 2018).

Prominent absorption bands at 3615, 2920, 1633, 1434, and 1027 cm^{-1} were identified in the FTIR spectra of soil organic carbon in the 0–20 cm soil depth at the peak growth stage (Fig. 2). Similar characteristic absorption bands were detected across different treatments, which indicated that relatively consistent basic chemical structures were maintained for soil organic carbon under various cropping patterns. Obvious discrepancies were observed in the absorption intensities of characteristic bands corresponding to different soil organic carbon components nevertheless. The intensities of characteristic absorption peaks followed the sequence of PO > CK2 > WA > MP > CK1 in the 0–20 cm soil depth at the peak growth stage. The overall intensities of spectral peaks followed the sequence of CK2 > PO > WA > CK1 > MP in the 0–20 cm soil depth at harvest. These findings demonstrated that the overall carbon skeleton structure of soil organic carbon remained unchanged in the topsoil, although the relative

abundance of specific soil organic carbon functional groups was altered by different cropping patterns.

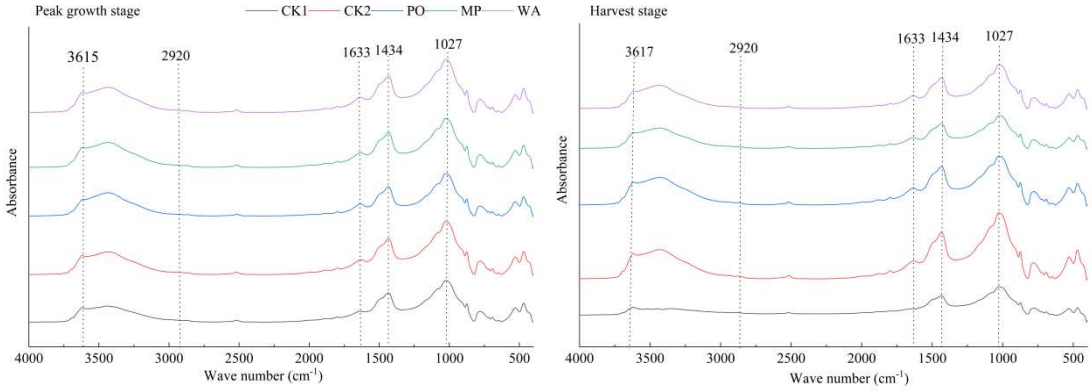


Figure 2 .FTIR spectral properties of soil organic carbon in various grain forage rotation mode

Soil organic-carbon functional groups

Dominant polysaccharide functional group, followed by aromatic groups, and lowest proportion of aliphatic groups , were detected in SOC for all experimental treatments at the peak growth stage. Minor variations in the relative peak intensities of polysaccharide compounds were observed among treatments, with the highest values recorded under CK1 and relatively lower values recorded under MP. The greatest relative peak intensities of aromatic and aliphatic compounds were obtained under MP among all treatments, while the lowest aromatic compound intensity was found under CK1 and the lowest aliphatic compound intensity was found under WA at the peak growth stage.

The highest relative abundance of polysaccharide compounds was observed under CK2 and the lowest under CK1 at harvest. Slightly higher relative abundances of aromatic compounds were detected under PO and MP at harvest. The most remarkable variation was found in the proportion of aliphatic compounds, with significantly higher values recorded under CK1 than under all other treatments at harvest.

Significant differences in the aliphatic/aromatic carbon ratio were detected among different treatments at both the peak growth stage and the harvest stage . Weak variations of aliphatic/aromatic carbon ratio were exhibited among treatments at the peak growth stage. The highest aliphatic/aromatic carbon ratio was obtained under MP.More distinct differences in the aliphatic/aromatic carbon ratio were observed among treatments at the harvest stage relative to

the peak growth stage. A remarkable increase in the aliphatic/aromatic carbon ratio was detected under CK1 and reached approximately 70% at harvest.

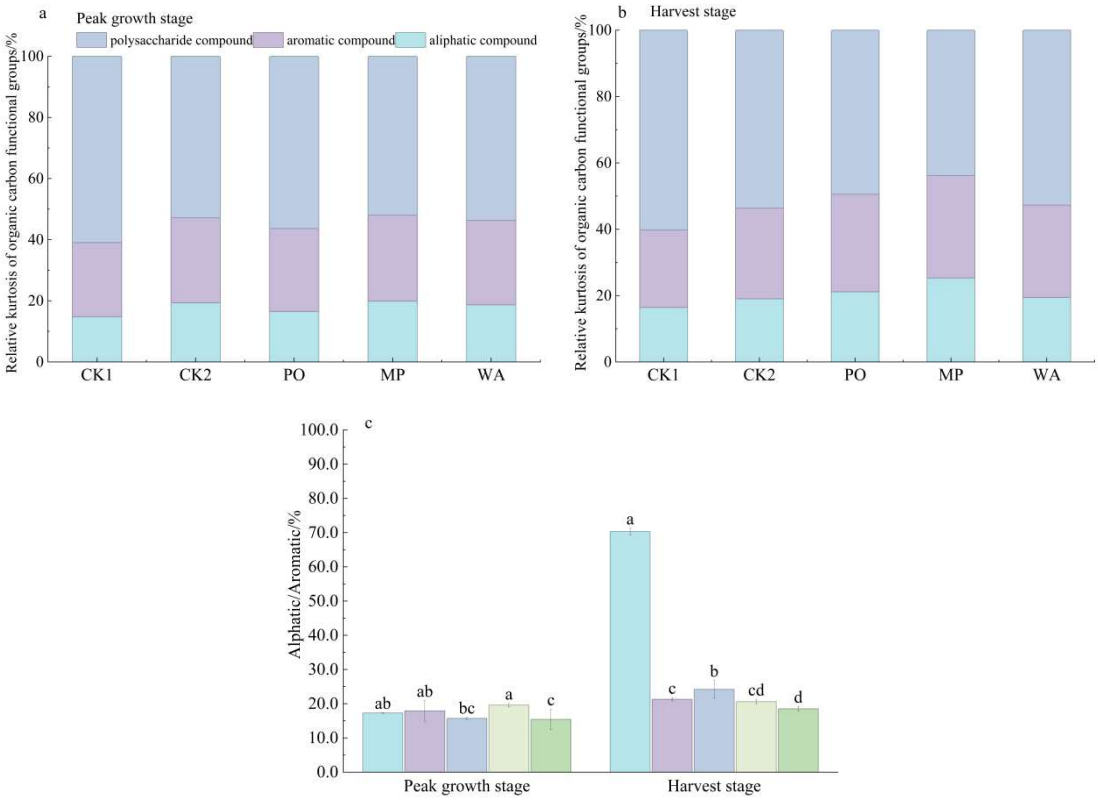


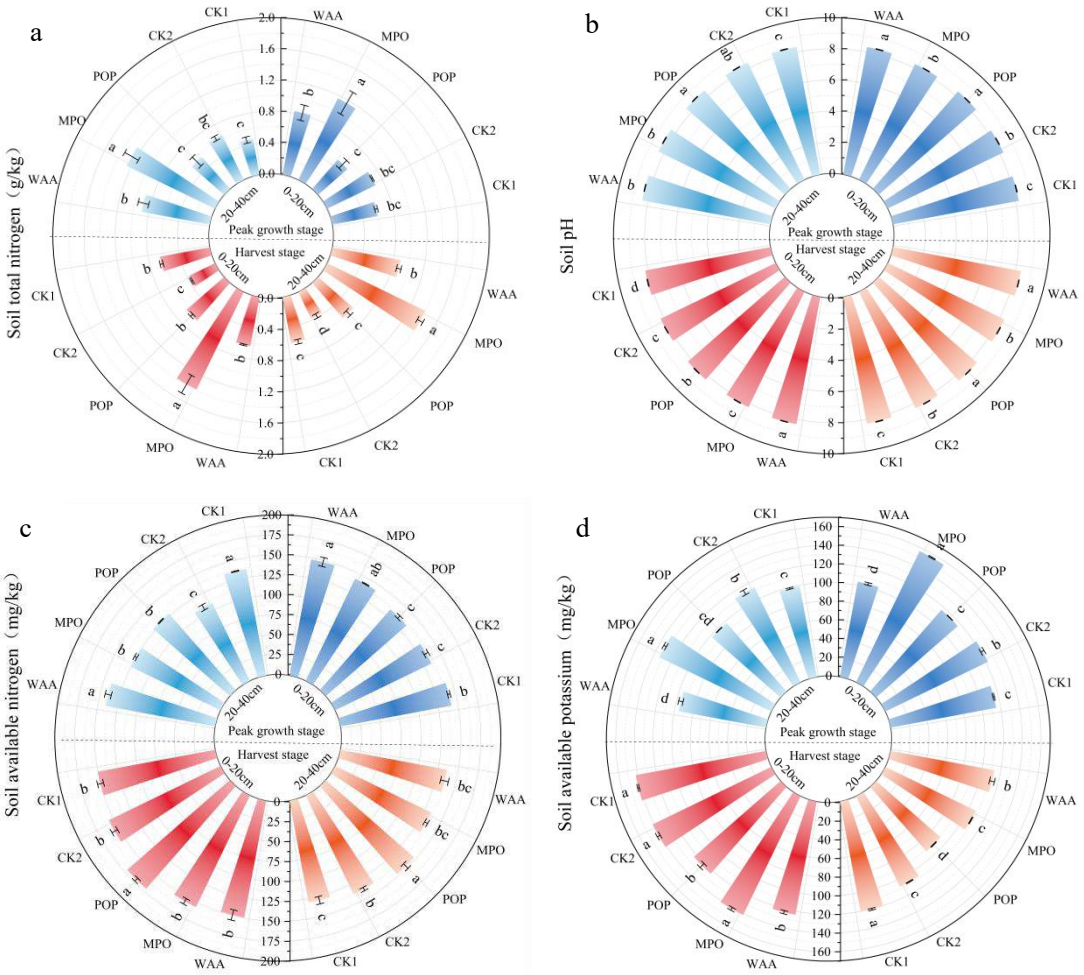
Figure 3. Soil organic-carbon functional groups

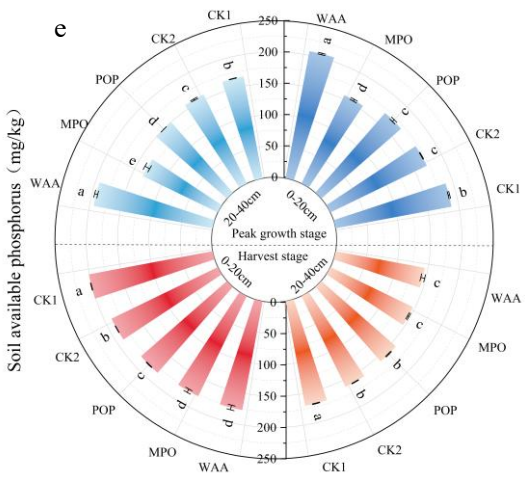
Note: (a)Relative peak intensities of soil organic-carbon functional groups at the peak-growth stage;(b)Relative peak intensities of soil organic-carbon functional groups at the harvest stage;(c) Aliphatic-to-aromatic carbon ratio of soil organic carbon

Soil key physicochemical properties

Distinct variations across growth stages and soil depths were exhibited by soil TN, pH, AN, AK, and AP under different cropping treatments. MP treatment showed the highest TN in 0-20 cm soil depth in both stages,whereas elevated TN levels were observed for both MP and WA in the 20–40 cm soil depth. Measurable differences in pH values among cropping treatments were detected for both sampling depths and both growth stages, higher pH values in the 0–20 cm soil depth were obtained under PO and WA, and the peak pH value in this depth was recorded under WA at harvest, while elevated pH levels in the 20–40 cm soil depth were sustained by PO at both the peak-growth and harvest stages. Variable AN concentrations were observed among treatments, soil depths and sampling dates; greater AN accumulation was

achieved by WA in the two soil depths at the peak growth stage, while the highest AN contents were found under PO for both soil depths at harvest. Noticeable disparities in AK concentrations across cropping treatments, soil depths and sampling dates were identified; significantly higher AK contents relative to all other treatments were obtained by the MP treatment in both soil depths at the peak-growth stage; significantly higher AK contents compared with the remaining treatments were exhibited by CK1, CK2 and MP in the 0–20 cm soil depth at harvest, and superior AK values over other treatments were recorded by CK1 in the 20–40 cm soil depth at harvest. Varied AP levels were detected among treatments over the growing cycle; increased AP accumulation was observed under WA in both soil depths at the peak growth stage, whereas the highest AP contents were maintained by CK1 across the two soil depths at harvest.





355 Figure. 4 Effects of different grain–forage rotation mode on soil physicochemical properties

356 Note: (a) Soil total nitrogen (TN) content; (b) soil pH; (c) soil available nitrogen (AN)

357 content; (d) soil available potassium (AK) content; (e) soil available phosphorus (AP) content

358 Redundancy analysis of soil physicochemical properties, soil

359 organic carbon fractions, and chemical structural characteristics

360 under different grain – forage rotation mode

361 Significant effects on soil organic carbon fractions, carbon pool management indices, and

362 organic carbon structural characteristics were exerted by soil environmental factors, as verified

363 via redundancy analysis (RDA) (Fig. 5). The first and second RDA axes explained 60.17% and

364 16.59% of the total variation, respectively, with a cumulative explanatory rate of 76.76%. Key

365 driving roles in soil organic carbon dynamics were confirmed for soil total nitrogen (TN) and

366 pH among all environmental factors, with longer vector lengths and higher explanatory

367 capacities observed for these two indicators. Positive correlations with TN and pH were

368 detected for soil organic carbon (SOC), easily oxidizable organic carbon (EOC), recalcitrant

369 organic carbon (ROC), dissolved organic carbon (DOC), carbon pool index (CPI), carbon pool

370 activity index (CPAI), and carbon pool management index (CPMI).Consistent directional

371 distribution trends with TN were exhibited by SOC, EOC, and CPMI, further verifying that soil

372 nitrogen availability acted as a core driving factor for soil organic carbon accumulation and

373 carbon pool structure optimization.

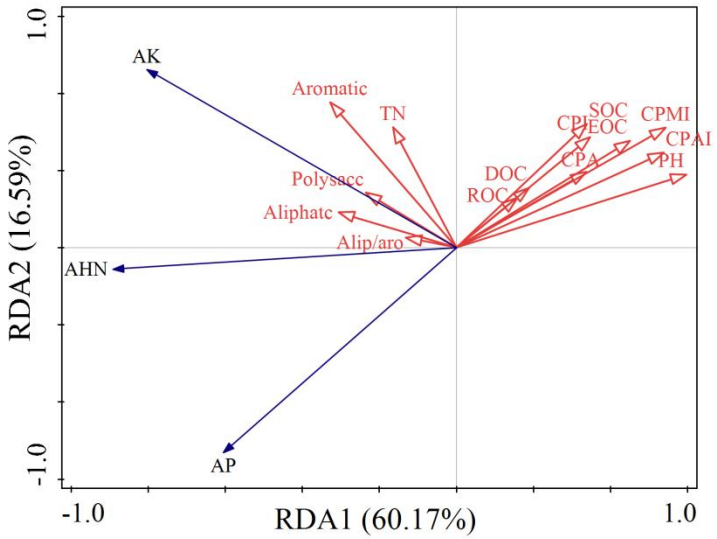


Figure 5. Redundancy analysis (RDA) of soil physicochemical properties, organic carbon fractions, and organic carbon chemical structure characteristics under different grain–forage rotation mode.

Correlation analysis among soil physicochemical properties, soil organic carbon fractions, and chemical structural characteristics under different grain – forage rotation mode

Correlation analysis showed that SOC was significantly positively correlated with EOC, DOC, ROC, CPI, CPMI, and aliphatic carbon, but significantly negatively correlated with pH. EOC was significantly positively correlated with DOC, and CPMI. DOC was significantly positively correlated with ROC, CPAI, CPI, and CPMI, while ROC was significantly positively correlated with CPMI. CPMI was significantly positively correlated with CPA, CPAI, and CPI. Regarding the chemical structure of soil organic carbon, aliphatic carbon was significantly positively correlated with aromatic carbon and showed significant correlations with DOC, CPA, CPAI, and CPI. Aromatic carbon was significantly positively correlated with AK, AP, and DOC, but significantly negatively correlated with pH. Polysaccharide carbon was significantly negatively correlated with EOC, CPAI, and CPI, but significantly positively correlated with AK and the aliphatic/aromatic ratio. The aliphatic/aromatic ratio was significantly positively correlated with aliphatic carbon and significantly negatively correlated with polysaccharide carbon.(Fig. 6).

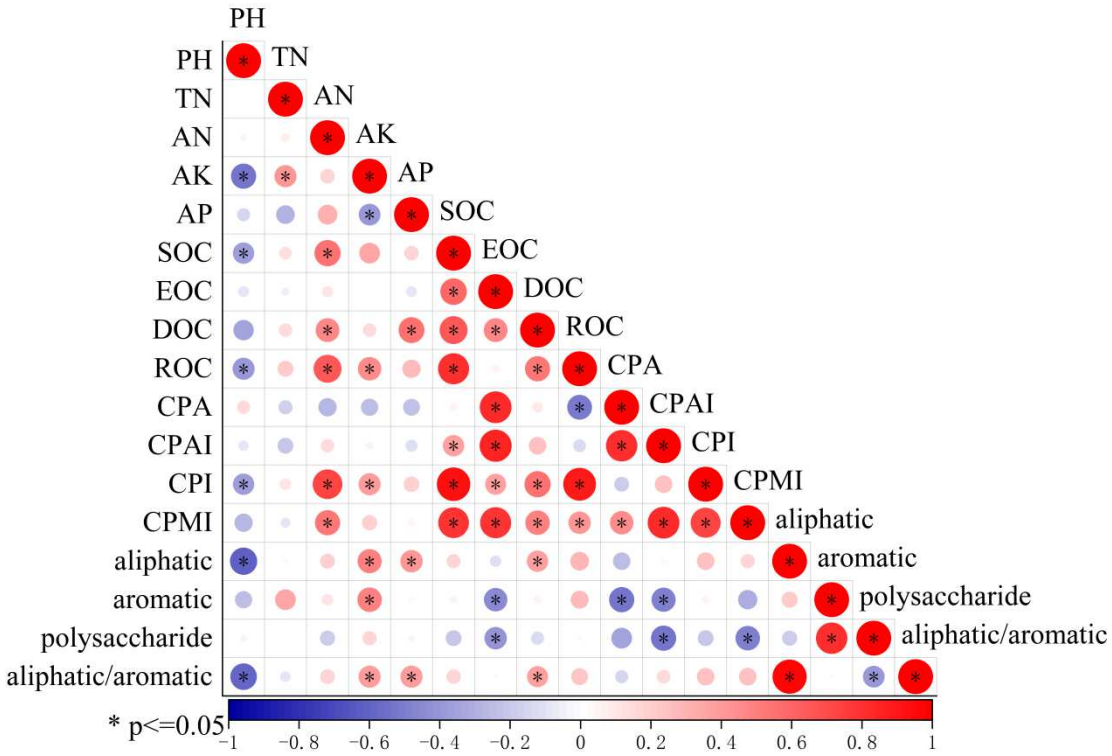


Figure 6. Pearson correlation analysis among soil physicochemical properties, SOC fractions, carbon pool management indices, and chemical structural characteristics under different grain – forage rotation mode

Discussion

Effects of grain forage rotation on soil organic carbon fractions

In this study, grain forage rotation changed the contents of soil organic carbon (SOC), EOC, DOC and ROC, and the effects of different rotation systems changed significantly with soil depth and growth stage.The PO treatment showed higher SOC and EOC contents in the 0 – 20 cm soil depth at the harvest stage, while the MP treatment showed higher SOC and DOC contents in the 20 – 40 cm soil depth, indicating clear spatial differences in the effects of the different grain grass rotation systems on soil organic carbon accumulation. Previous studies have shown that long term diversified crop rotation can increase soil organic carbon and active carbon components in the Loess Plateau, and that changes in the soil carbon pool are closely related to crop rotation duration and belowground carbon input (Fu et al. 2017;Liu et al. 2022). Diversified crop rotation generally increased soil carbon components and microbial activity in the long term rotation experiment on the Loess Plateau, an increase related to the soil carbon input and carbon transformation processes caused by different crop combinations(Fu et al.

2017). A global analysis showed that crop rotation could increase SOC content compared with continuous cropping, but the magnitude of the effect depended on soil characteristics, climatic conditions and cropping systems (Liu et al. 2022;Chen et al. 2020). Therefore, the increase in SOC under the PO and MP treatments in this study may be related to changes in soil carbon input and transformation processes caused by different crop combinations.

SOC is an important indicator of soil carbon pool status, while active organic carbon components such as EOC and DOC usually have faster turnover rates and are more sensitive to agricultural management measures than total SOC (Blair et al. 1995;Hu et al. 2023). In the present study, EOC and DOC showed relatively obvious responses to the rotation systems, while the change of SOC was relatively small, which could be used as a sensitive indicator of soil management changes with active organic carbon (Lou et al. 2011;Leifeld and Kögel-Knabner 2005). Hu et al. (2023) found that SOC, DOC, EOC, and CPMI responded differently to agricultural management practices, and DOC and EOC could quickly reflect the changes of soil carbon pools. Blair et al.(1995) proposed a carbon pool management index to comprehensively evaluate the changes of soil organic carbon content and activity. Therefore, the assessment of soil carbon pools based only on SOC content may underestimate the effect of rotation on soil carbon pool quality.

The PO treatment had higher SOC and EOC contents in the 0 – 20 cm soil depth at harvest, indicating a relatively strong positive effect of this rotation system on the surface soil carbon pool. Different crops have different root distributions, nutrient requirements and growth stages, ,and their combinations can change the process of soil carbon input and transformation , thus affecting SOC components (Huang, et al. 2003;Li et al. 2023;Christy et al. 2023). In the Loess Plateau,rotation can also affect SOC accumulation by changing soil moisture conditions and utilization processes.Chen et al.(2020) showed that there were differences in soil organic carbon, soil water content,pH and other soil properties among rotation systems on the Loess Plateau. Therefore, under the dryland conditions of the this study, the higher SOC and EOC contents in the surface under PO may be related to the different soil carbon conversion environment formed during crop growth.

MP treatment showed higher SOC and DOC contents in the 20 – 40 cm soil depth. DOC is a highly mobile active carbon component in soil,which can participate in microbial

metabolism through dissolution and transport processes into deeper soil depths (Kothawala et al. 2009;Leinemann et al. 2018).Studies have shown that DOC can migrate through the soil profiles and be retained by the mineral surface, and dissolved organic matter can participate in the formation and transformation of mineral-bound organic matter (Cotrufo et al. 2013). Therefore, the DOC content in deeper soil under MP treatment may reflect the transformation of active carbon from surface soil to lower soil under this rotation. Long-term studies in dryland soils of China have further demonstrated that TN, SOC, cropping systems, and soil texture can jointly explain changes in mineral-bound organic carbon (Cai, et al. 2021). This indicates that the effects of different rotation systems on soil carbon pool are not limited to the surface soil,but may further affect the lower soil carbon pools through carbon transport and changes in soil nutrient conditions.

ROC is arecalcitrant organic carbon, and its changes can reflect the turnover process of soil organic carbon. In this study, ROC was significantly positively correlated with SOC and DOC, indicating that there was a strong synergistic change between active carbon components. Previous studies have shown that active carbon components can be used as early indicators of soil organic carbon changes,which is closely related to microbial activity and soil carbon cycle (Ghani et al. 2003). Therefore, the effect of grain rotation on SOC may be first manifested as change of active carbon components,which in turn affects the accumulation and stability of soil carbon pool.

Effects of grain forage rotation on soil carbon pool management

The study showed that grain forage rotation altered the characteristics of soil carbon pool management,with significant differences among rotation systems detected across soil depths . Higher CPMI values were obtained under PO treatment at the vigorous growth stage in the 0 – 20 cm soil layer, and greater CPA and CPAI were also observed. CPMI increased further from 100.38 to 115.55 at harvest In the 20 – 40 cm soil layer, higher CPMI, CPA and CPAI were recorded under MP treatment.Variations in CPMI across different rotation systems were generally consistent with variations in SOC and its active components, which indicates that soil organic carbon content and carbon activity can be regulated by grain grass rotation. (Gorooei et al. 2024;Vieira et al. 2007).

CPMI is an important indicator to comprehensively evaluate the changes of soil organic carbon content and activity, The increase in CPMI generally indicates the capacity of soil carbon pool (Blair et al.1995;Vieira et al. 2007). Hu et al. (2023) found that nitrogen application increased SOC, DOC, EOC, and CPMI at the same time,indicating that changes of active organic carbon was an important part of CPMI change. Gorooei et al. (2024)also found that agricultural management and rotation systems changed CPI and CMI in semiarid areas, indicating that carbon pool management indices can sensitively reflect the changes of soil carbon pools caused by agricultural management measures. Therefore, the increase of CPMI under PO and MP treatments in the present study not only reflected the change of SOC content but also reflected the change of active soil carbon pool.

PO treatment had a relatively high CPMI in the surface soil,which was consistent with its higher SOC and EOC contents. CPMI is affected by both carbon pool capacity and carbon activity. Therefore, the simultaneous increases of SOC and EOC under PO treatment during the harvest may jointly promote the increase of CPMI (Vieira et al. 2007). MP treatment mainly showed a higher CPMI in the 20 – 40 cm soil depth, which was consistent with its higher SOC and DOC content. This indicates that the effects of PO and MP on carbon pool management showed a certain soil depth differentiation. Long-term rotation studies have shown that increased crop diversity can improve soil carbon fractions at different soil depths, and the magnitude of these effects depends on crop type and rotation duration (Fu et al. 2017;Chen et al. 2020).

In this study, CPMI was significantly and positively correlated with SOC, EOC, DOC, ROC, CPA, CPAI, and CPI, indicating that was a strong coordinated relationships between soil organic carbon content, active carbon fractions and carbon pool management indices. CPI mainly reflects changes of soil carbon pool capacity, CPA and CPAI reflect changes of active carbon pool, which together determine the change of CPMI (Gorooei et al. 2024). Therefore, the improvement of soil carbon pools caused by crop rotation may involve simultaneous changes in carbon pool capacity and activity, rather than a simply increase in SOC content.

It is worth noting that the effects of different rotation systems on carbon pool management were not completely consistent. PO treatment mainly increased CPMI and active carbon pool of the surface soil, while MP had a more significant effect on carbon pool management indices

in the subsoil. Some carbon pool indices under WA were lower than those under PO and MP, indicating that the regulation ability of different grain-forage rotations on soil carbon pools was different. Previous studies have shown that the effects of crop rotation on SOC is controlled by many factors, including crop type, rotation years , initial soil carbon level and soil environmental conditions (Huang et al. 2003;Li et al. 2023). Therefore, the changes of soil carbon pools under different grain-forage rotations may show obvious crop type dependence.

Effects of grain forage rotation on soil organic carbon chemical structure

FTIR analysis showed that soil organic carbon under different grain-forage rotation modes contained main chemical structures such as polysaccharides, aliphatic compounds and aromatic compounds, but the relative proportions of these structures were different among treatments. FTIR can be used to characterize the main functional groups in soil organic matter and compare the changes in the chemical composition of soil organic carbon under different agricultural management practices (Margenot et al. 2023;Guo et al. 2016;Deng et al. 2019;Zheng et al. 2021). Therefore, the changes of relative absorption peak intensities and chemical structure ratio between treatments in this study reflected the effects of grain-forage rotation on the chemical composition of soil organic carbon.

In this study, grain-forage rotation generally increased the proportion of aromatic structures in soil organic carbon, while the proportion of aliphatic structures in continuous cropping was relatively high. PO and MP treatments showed higher proportions of aromatic carbon, indicating that rotation could change the chemical composition of soil organic carbon and promote the chemical composition of some organic carbon to relatively complex components. Soil organic carbon is not composed of a single type of stable compound but a continuum of continuous decomposition, transformation and stability of organic compounds (Lehmann and Kleber 2015;Si et al. 2002;Cotrufo et al. 2013;Cotrufo et al. 2015). Therefore, changes in the chemical structure of organic carbon should be understood as a comprehensive result of the soil carbon transformation processe, rather than the accumulation of specific stable compounds.

The stability of soil organic carbon and the chemical composition of organic matter,

530 microbial transformation, and mineral protection processes are closely related (Córdova, et al.
531 2018;Kallenbach et al. 2016). The traditional views is that aromatic structures and relatively
532 complex organic compounds have greater chemical stability. However, recent studies have
533 shown that the long-term stability of SOC cannot be explained solely by the stubbornness of its
534 molecular structure, combined with organic mineral assemblages and microbial transformation
535 (Cotrufo et al. 2019;Liang et al. 2017). Therefore, the higher proportion of aromatic carbon
536 under PO and MP in this study can be considered as an indicator of organic carbon structure
537 change, but its contribution to SOC stability may also be affected by soil mineral protection
538 and microbial transformation processes.

539 In this study, aliphatic carbon was significantly and positively correlated with aromatic
540 carbon, whereas the aliphatic/aromatic ratio was significantly and positively correlated with
541 aliphatic carbon and significantly and negatively correlated with polysaccharide carbon. These
542 results indicate clear coordinated relationships among different chemical structure components.
543 More active organic carbon components, such as polysaccharides, can be rapidly transformed
544 through microbial activity and form microbial metabolites and residues. Some of these products
545 can subsequently associate with soil minerals and form stable organic carbon (Cotrufo et al.
546 2013;Zhu et al. 2020). The microbial efficiency-matrix stabilization framework proposed by
547 Cotrufo et al. (2015) indicates that products formed through microbial transformation of plant
548 organic matter can enter relatively stable soil organic matter pools through microbial
549 assimilation and mineral association. Subsequent studies have further demonstrated the
550 important role of microbial-derived organic matter in SOC storage (Kallenbach et al. 2016).

551 The differences in the chemical structure of organic carbon between crop rotation systems
552 may be related to crop types and soil nutrient conditions. There are differences in the chemical
553 composition of organic matter from different plant sources, and there are also differences in the
554 microbial decomposition and transformation process after it enters the soil. Castellano et al.
555 (2015) pointed out that the effect of organic matter quality on SOC stability is not simply
556 determined by matrix quality but is regulated by soil carbon saturation and mineral conservation
557 processes. Therefore, the differences in the chemical structure of organic carbon between PO,
558 MP, and WA in this study may be the result of the combined effects of crop types, soil nutrients
559 and organic carbon transformation processes.

Effects of grain forage rotation on soil nutrient and environmental factors

In the present study, different grain forage rotation systems significantly altered soil TN and pH, and RDA further showed that TN and pH were important environmental factors affecting SOC fractions, carbon pool management indices, and the chemical structure of soil organic carbon. TN was closely related to SOC, ROC, DOC, CPI, and CPMI, whereas pH was clearly associated with CPMI and some organic carbon structure indices, indicating that soil nutrient status and soil acidity/alkalinity may jointly participate in soil organic carbon accumulation and structural transformation.

TN is an important nutrient factor for soil organic matter formation and microbial metabolism. Soil C and N cycles are closely linked, and N availability can affect plant growth, microbial metabolism, and organic carbon decomposition and stabilization processes (Dieckow et al. 2005;Tao et al. 2023). Cai et al.(2021) reported that TN, SOC, cropping systems, and soil texture jointly explained changes in mineral-associated organic carbon and nitrogen in dry croplands of China. Cotrufo et al. (2013) further indicated that the transformation of plant organic matter into stable SOC is jointly affected by C and N availability and microbial carbon use efficiency. Therefore, the close relationship between TN and SOC fractions and CPMI in the present study suggests that soil N availability may participate in regulating organic carbon accumulation and carbon pool formation.

In this study, MP and WA had relatively high TN in some soil depths and growth stages, and some carbon pool indices also showed relatively high values, indicating the close relationship between soil N status and organic carbon accumulation. However, the relationship between TN and SOC does not mean that the increase in N directly leads to the increase of SOC. Previous studies have shown that nitrogen inputs have different effects on soil organic carbon fractions, and these effects are affected by pH, mineral composition, and microbial processes (Wu et al. 2023;Xie et al. 2025). Therefore, the effects of TN on SOC fractions and CPMI in this study may occur indirectly by changing plant growth, microbial metabolism, and organic carbon conversion processes.

Soil pH is also an important environmental factor affecting SOC conversion. pH can

589 change microbial community composition and enzyme activity ,and then affect organic matter
590 decomposition and SOC stability (Wang, et al. 2024). Recent studies have shown that pH can
591 not only affect the priming effect of SOC decomposition but also affect the soil carbon cycle
592 by regulating microbial activity and organic matter stabilization processes (Wang and
593 Kuzyakov 2024). In addition, the formation and stabilization of stable soil organic carbon is
594 affected by soil mineral composition and mineral surface properties (Wang et al. 2024).
595 Therefore, the relationships between pH and CPMI and organic carbon structure indices in this
596 study may reflect the integrated effects of soil acidity/alkalinity on carbon pool transformation
597 and organic carbon structural evolution.

598 Conclusions

599 Compared with continuous cropping, grain-forage rotation changed soil organic carbon
600 (SOC) composition, carbon pool management index and SOC chemical composition. EOC and
601 DOC were more sensitive to changes in cropping system than total SOC, indicating that rotation
602 mainly regulated the distribution of soil carbon fractions and carbon pool activity. PO had a
603 great influence on carbon accumulation and carbon pool activity in the 0–20 cm soil depth,
604 especially at harvest, while MP had higher SOC and DOC contents and a higher CPMI in the
605 20–40 cm soil depth at the peak growth stage. The change of CPMI is mainly related to change
606 of carbon pool activity.FTIR spectra showed that rotation changed the relative proportions of
607 polysaccharides, aromatic compounds, and aliphatic compounds in soil organic carbon, but did
608 not significantly change the main chemical structure of SOC. PO and MP increased the relative
609 proportion of aromatic compounds, while potato continuous cropping showed a higher ratio of
610 aliphatic to aromatic, which was not conducive to the stabillty of SOC. RDA and correlation
611 analysis showed that TN and pH were the main environmental factors related to SOC
612 components, carbon pool management and SOC chemical characteristics. Overall, the
613 regulation of potato-oat and forage maize-pea rotation on soil carbon pool was different at
614 different soil depths and different growth stages. PO promoted carbon accumulation and carbon
615 pool activity in surface soil, especially at harvest stage. MP increased the carbon pool of deep
616 soil at the peak growth stage. Therefore, grain forage rotation has obvious potential in

regulating soil carbon components, improving carbon pool activity and enhancing SOC chemical stability in the study area.

Acknowledgements

Author contributions **Shengdong Zhou:** writing-original draft, visualization, methodology, investigation, data curation, formal analysis, conceptualization. **Yajuan Li:** supervision, validation, writing -review and editing. conceptualization, resources, funding acquisition, project administration, **Wenxia Cao:** resources, investigation, data curation.**Wenli Xiang:** supervision, validation, writing- review and editing. **Chengjun Zhang**: resources, investigation, data curation.**Qingming Shui:** resources, investigation, data curation.**Ruixiang Zang:** resources, investigation, data curation. **Xiao Li:** resources, investigation, data curation.**Juan Du:** resources, investigation, data curation.**Lizi Chen:** resources, investigation, data curation.

Funding This study was jointly funded by National Nature Science Foundation (32360351) and the earmarked fund for China Agriculture Research System (CARS-33).

Data availability The original contributions presented in this study are included in the article.Further inquiries can be directed to the author.

Declarations

AI use disclosure AI-assisted tools were used during the preparation of this manuscript for language editing, grammatical correction, sentence restructuring, improvement of clarity and readability, and assistance with the presentation and visualization of statistical results. The underlying statistical analyses, selection of statistical methods, interpretation of the results, and scientific conclusions were determined and verified by the authors. All AI-assisted output, including text and graphical presentations, was critically reviewed and validated by the authors.The authors take full responsibility for the accuracy, integrity, originality, and scientific validity of the manuscript.

Conflicts of interest The authors declare that there are no conflicts of interest regarding the publication of this article.

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