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RESEARCH ARTICLE

Influence of long-term livestock manure substitution on water erosion and labile organic carbon lateral loss on subtropical sloping croplands

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Highlights

- Long-term livestock manure substitution enhances soil aggregate stability and reduces water erosion intensity.
- Annual labile organic carbon lateral loss increases significantly with long-term livestock manure substitution.
- While water erosion intensity decreases, the risk of labile organic carbon loss increases following long-term livestock manure substitution.

Abstract

The lateral transport of labile organic carbon represents a critical pathway for soil organic carbon (SOC) loss, reducing organic carbon sequestration and increasing the risk of waterbody pollution. Livestock manure application on croplands serves as a common fertilizer reduction practice to sustain crop yields, enhance SOC sequestration, and reduce water erosion. However, limited quantitative assessments have examined the effects of livestock manure substitution on labile organic carbon lateral loss and fluxes in long-term experiments. This study conducted a three-year field investigation on subtropical sloping croplands to assess the impact of livestock manure substitution on dissolved organic carbon (DOC) and particulate organic carbon (POC) loss *via* surface runoff, interflow and eroded sediments. There are four treatments: no fertilization (CK); chemical nitrogen fertilizer (SF), 40% nitrogen substitution with pig manure (PMF), and 100% nitrogen substitution from pig manure (PM). Compared to SF treatment, long-term livestock manure substitution in PMF and PM treatments significantly (P<0.05) reduced annual cumulative surface runoff fluxes by 13.5 and 21.6%, respectively. Manure applications decreased annual sediment fluxes by 12.9 and 19.1%, respectively. Soil water stable aggregates for mean weight diameter (MWD) increased significantly by 37.7 and 73.6%. Annual cumulative POC loss flux *via* eroded sediment under PMF and PM treatments increased significantly (P<0.05) by 61.1 and 47.9%, respectively. The labile organic carbon loss fluxes, including DOC and POC losses, under PMF and PM treatments increased significantly (P<0.05) by 11.9 and 31.4%, respectively. These results demonstrate that while water erosion intensity decreases due to enhanced soil aggregate stability, the risk of labile organic carbon loss increases after long-term livestock manure substitution in subtropical sloping croplands. Future research should examine labile organic carbon lateral migration under various soil types and slope gradients for livestock manure application in subtropical agricultural ecosystem croplands to better understand extreme rainfall effects.

Keywords: hydrological carbon loss, runoff and sediment, organic manure, sloping croplands

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1. Introduction

Labile soil organic carbon (SOC), comprising dissolved organic carbon (DOC) and particulate organic carbon (POC), represents a fundamental biogeochemical carbon fraction of ecosystems that undergoes lateral transport from soil to surface water bodies. Labile carbon functions as a crucial determinant of SOC sequestration globally, affecting carbon budgets and water quality in watersheds (Jacinthe *et al.* 2004; Nakhavali *et al.* 2021). DOC, which passes through a 0.45 μm filter membrane, represents a highly mobile and active carbon fraction that influences critical biogeochemical cycle processes in terrestrial and aquatic ecosystems (Ge *et al.* 2025). DOC migration from soil to water reduces SOC sequestration and potentially enhances the transport of pesticides, heavy metals, and other contaminants through runoff, affecting water quality (Chifflard *et al.* 2024). POC, exceeding 0.053 mm sieve size, contains abundant plant and biological residue produced during soil organic matter decomposition and migrates *via* sediment during water erosion (Hua *et al.* 2016; Liu *et al.* 2024). POC loss through soil erosion diminishes SOC storage and potentially serves as a transport pathway for organic and inorganic hydrophobic contaminants in the environment (Liu D *et al.* 2023). Consequently, investigating the hydrological processes of DOC and POC lateral transport in croplands is essential for understanding SOC sequestration mechanisms and protecting local aquatic environments.

The transport of DOC and POC in runoff represents a complex physiochemical process influenced by available organic carbon fractions and water movement in the soil profile. Over recent decades, numerous studies have examined the processes of DOC loss through surface runoff, leaching, and subsequent movement *via* interflow under various land use and agronomic methods across different regions (Jiang *et al.* 2022). Liu X J *et al.* (2023) demonstrated that rainfall volume was the primary factor controlling DOC loss variations between runoff events in karst ecosystems, with DOC flux in croplands being lower than in forests. Su *et al.* (2024) found that in non-karst ecosystems, DOC loss was primarily driven by rainfall volume, while POC loss correlated with maximum five min rainfall intensity. High-intensity, short-duration rainfall events resulted in greater POC than DOC loss, whereas long-duration rainfall events led to higher DOC than POC loss (Jung *et al.* 2012). Enhanced vegetation cover effectively reduces POC loss but shows limited impact on DOC loss (M´arquez-García *et al.* 2024). In the Loess Plateau region, DOC migration flux is significantly influenced by vertical interface, rainfall, and biotic factors (Ge *et al.* 2025), while POC was predominantly derived from check dam sources, constituting 87% of water erosion processes (Liu *et al.* 2024). Liang *et al.* (2025) demonstrated that straw mulching increased DOC concentration and aromatic proportions on sloping croplands during rainfall simulation in tropical agricultural regions.

The replacement of chemical fertilizers with livestock manure has become widespread to reduce chemical nitrogen fertilizer usage, enhance plant productivity, and improve SOC sequestration and fertility (Agbede and Ojeniyi 2009; Cao *et al.* 2024). Soil physicochemical and biological properties are also significantly changed by the long-term substitution of chemical fertilizers with livestock manure (Ren *et al.* 2024). Specifically, soil hydraulic characteristics, including saturated hydraulic conductivity, air permeability, and aggregate stability, show marked improvement with livestock manure application (Schlüter *et al.* 2023). The properties of soil DOC and the quantity or chemical structure of POC in the soil profile, particularly in topsoil, demonstrate significant enhancement through long-term livestock manure substitution for chemical fertilizers. Subsequently, the lateral transport of soil DOC and POC through hydrologic processes is substantially influenced by this long-term substitution.

Mountainous terrains comprise more than 69% of agricultural lands in China. In the upper reaches of the Yangtze River, sloping cropland accounts for over 65% of total cropland area (Zhu *et al.* 2009). These sloping croplands represent typical subtropical agricultural areas in China. The extensive distribution of sloping cropland has experienced severe degradation due to soil erosion, resulting in topsoil and nutrient depletion, affecting soil productivity. Nutrient losses are particularly severe during summer storms characterized by heavy precipitation on intensively cultivated agricultural fields with thin soils. During rainy seasons, nutrient losses through overland flow, leaching, or interflow are common occurrences (Zhu *et al.* 2009; Bah *et al.* 2020). While organic amendments are routinely applied to sloping croplands to mitigate water erosion and enhance soil fertility, comprehensive data regarding DOC and POC loss fluxes under livestock manure substitution remains absent. The characteristics and quantities of DOC and POC lost *via* surface runoff, interflow, and sediment under long-term livestock manure substitution remain unclear due to insufficient observational data. Limited research has examined the simultaneous effects of livestock manure substitution on hydrological DOC and POC migration. Addressing this knowledge gap would support the development of scientific strategies for organic carbon sequestration and reduce organic and inorganic composite pollution risks in watersheds. This study aims to evaluate the impact of long-term livestock manure substitution on DOC and POC losses through surface runoff, interflow, and erosion in subtropical sloping croplands. The research hypothesizes that water erosion intensity decreases while labile organic carbon loss increases under long-term livestock manure substitution in subtropical sloping croplands. The specific objectives include: (1) quantifying DOC and POC loss concentrations and flux dynamics through surface runoff, interflow, and sediment; (2) identifying key factors controlling DOC and POC loss; and (3) assessing pollution risks associated with DOC and POC losses from long-term livestock manure substitution.

2. Materials and methods

2.1. Study site description

The experimental site is situated in the upper reaches of the Yangtze River at an altitude ranging from 400 to 600 m in Southwest China (31°16´N, 105°28´E). The area lies within

a subtropical region; the annual rainfall from 2010 through 2012 measured 892, 1,061, and 1,080 mm, respectively, according to recorded data (Fig. 1). The soil in the region is classified as Regosol (IUSS Working Group WRB 2006) and developed in purplish shale. It exhibits a shallow profile (approximately 60 cm thick) with a typical binary structure comprising soil and bedrock. The croplands present slopes ranging between 3°–15° and depend on rainfall. Initial soil characteristics measured in 2002 indicate a loam soil with pH 8.3, soil organic matter content of 8.8 g kg^{-1}, total N of 0.6 g kg^{-1}, and saturated hydraulic conductivity of 16.8 mm h^{-1} (Zhu *et al.* 2009).

2.2. Experimental design

Field experimental plots, incorporating free-draining lysimeters (size: 8 m×4 m, slope: 6.5°, aspect: southeast to northwest), were established for the simultaneous collection of surface runoff, interflow, and eroded sediment. These free-draining lysimeters were installed in 2002 (Zhu *et al.* 2009). Each lysimeter was hydrologically isolated by concrete walls, preventing lateral leakage from adjacent plots. Collecting barrels were positioned at the soil surface and along the soil–bedrock interface to collect surface runoff and interflow, respectively. A receptacle beneath each collecting barrel facilitated the collection of water samples from surface runoff and interflow (Fig. 2).

The experiments employed a randomized block design with four treatments and three replicates. The treatments comprised: no fertilization (CK), chemical nitrogen fertilizer (SF), 40% nitrogen substitution with pig manure (PMF), and 100% nitrogen substitution from pig manure (PM). The chemical fertilizers in the SF treatment were applied once at the beginning of each wheat-growing season at rates of 130 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 36 kg K_2O ha^{-1}. Nitrogen fertilizer was applied at the beginning of each maize-growing season at 150 kg ha^{-1}. All chemical fertilizers and swine manure were manually and uniformly applied as basal fertilization. In the experiment, only mineral nitrogen fertilizer was substituted with swine manure, while mineral phosphorus and potassium remained unchanged. The mineral fertilizers and livestock manure (average total organic C, N and P contents of swine manure were 37.1, 1.9 and 0.60%, respectively) were applied as basal fertilization. Detailed information regarding fertilizer and manure application rates appears in Table 1. Winter wheat (*Triticumaestivum* L.) was cultivated from October to May, followed by summer maize (*Zea mays* L.) from June to September. A tillage depth of 20 cm was achieved using hoes with local farmers' assistance, consistent with fertilizer application. Post-tillage, all fertilizers, including swine manure, were evenly distributed, and the topsoil (0–20 cm) was harrowed using garden rakes. All fertilizers were applied to the topsoil on the planting day of wheat and maize. Crops were harvested manually.

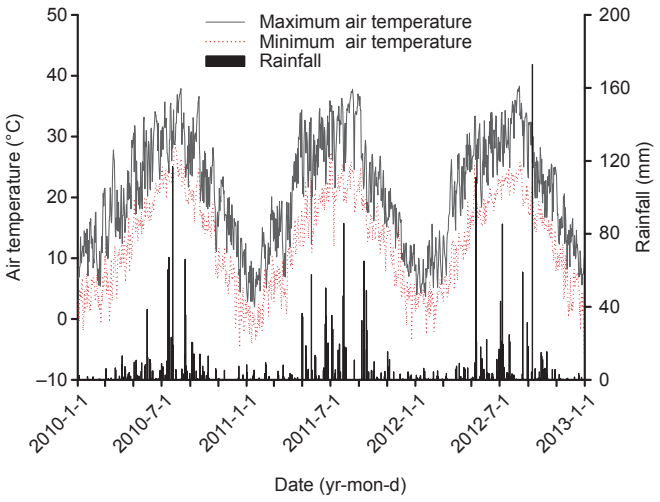


Fig. 1 Daily rainfall, along with daily air temperature recorded from 2010 to 2012.



Fig. 2 The experimental design schematic of free-draining lysimeters. A, overview of runoff plots. B, runoff collection apparatus.

2.3. Measurement of rainfall, surface runoff, interflow and sediment

Daily rainfall was automatically recorded using a tipping bucket gauge manufactured by Vaisala (R_{13}, Finland) at the meteorological observation ground of the experimental site. Surface runoff and interflow discharge were measured from 2010 to 2012 using separate five-hole diverter barrels in the runoff pond after water flow had completely ceased following rain events. Water samples for DOC analysis were collected using 500-mL polyethylene bottles after measuring water levels.

Table 1 Detailed information about the rates of chemical fertilizer and pig manure application

Growing season	Treatment[1)]	Chemical fertilizer (kg ha^{-1} yr^{-1})			Pig manure				Dissolved organic C (g kg^{-1})
		N	P	K	Amount (dry weight basis) (kg ha^{-1} yr^{-1})	Total organic C (g kg^{-1})	Total P (%)	Total N (%)	
Wheat	CK	0	0	0	0	0	0	0	0
	SF	150	39.3	30	0	0	0	0	0
	PMF	90	39.3	30	3,030.3	371.2	0.6	1.98	18.4
	PM	0	39.3	30	7,575.8	371.2	0.6	1.98	18.4
Maize	CK	0	0	0	0	0	0	0	0
	SF	130	39.3	30	0	0	0	0	0
	PMF	78	39.3	30	2,429.9	329.8	0.5	2.14	17.6
	PM	0	39.3	30	6,074.8	329.8	0.5	2.14	17.6

[1)] CK represents the control with no fertilization; SF denotes chemical nitrogen fertilizer application; PMF indicates 40% nitrogen substitution with pig manure; PM represents 100% nitrogen substitution from pig manure.

Surface runoff samples were then collected in the diverter barrels. After 48 h of settling, the water samples were discarded and the sediment samples in the diverter barrels were collected for indoor air drying. A portion of fresh sediment was used to measure DOC content, while the remaining air-dried sediment was used to determine total organic carbon (TOC), POC, and mineral-associated organic carbon (MAOC) contents. The collected sediment samples in 500-mL polyethylene bottles were dried in an aluminum box and weighed at 105°C to determine soil erosion intensity (Polyakov and Lal 2008).

2.4. Soil sampling and physico-chemical analysis methods

Following the maize harvest in mid-October of 2011 and 2012, soil samples from different layers (0–20, 20–40, and 40–60 cm) were collected in an S-shaped pattern using a stainless-steel auger within each plot. Visible stubble and straw debris were removed from the samples and air-dried for organic carbon fraction analysis. Undisturbed soil samples at the topsoil depth (0–20 cm) were collected using PVC tubes to maintain the undisturbed structure, then broken into small pieces according to the natural structure to analyze soil aggregates using the wet-sieving method (Elliott 1986).

An air-dried undisturbed soil sample weighing 100 g was passed through an 8-mm sieve and placed in a plansifter composed of 2-, 0.25-, and 0.053-mm sieves. The plansifter was slowly submerged in distilled water and soaked for 5 min, then moved up and down at a rate of approximately 25 times per min. The residues in the 2-, 0.25-, and 0.053-mm sieves were categorized as large macro–aggregates (LM, >2 mm), small macro–aggregates (SM, 0.25–2 mm), and micro–aggregates (MI, 0.053–0.25 mm), respectively. The masses of the three aggregate types were obtained by weighing the aggregates at 60°C for 72 h, and the weight of silt/clay (SC, <0.053 mm) was obtained by subtraction. POC fractions in the LM and small macro-aggregates (SM) aggregates were measured according to the physical grouping method described by Chung *et al.* (2010). Specifically, 10.0 g samples of LM or SM aggregates were placed in a plansifter consisting of 0.25- and 0.053-mm sieves, soaked for 20 min, after which 50 small steel balls with a diameter of 4 mm were placed in the 0.25-mm sieve and moved up and down for 3 min. The particular matter residue in the 0.25 mm sieve for the LM and SM aggregates was weighed at 60°C and the organic carbon content was measured (LM-POC and SM-POC). All water samples including surface runoff and interflow were filtered through 0.45-μm filter membranes, and DOC concentrations were analyzed using a Bran+Lubbe AA_3-Auto-analyzer equipped with flow injection technology (Bran Lubbe, Norderstedt, Germany). The analysis of TOC, DOC, POC and MAOC contents in the soil or sediment were conducted using the methods presented in Hua *et al.* (2016). The key analytical procedures were as follows: fresh soil or sediment samples weighing 1.5 g were shaken with 7.5 mL distilled water and filtered through 0.45-μm filter membranes to analyze the DOC concentration in the filtrate. Air-dried samples of soil or sediment passed through 100-mesh were analyzed to determine the TOC content using the potassium dichromate volumetric method. Air-dried soil or sediment weighing 2.0 g was blended with 20 mL of 5% sodium hexametaphosphate and shaken for 18 h in a reciprocating shaker, after which the turbid liquid was poured over a 0.053-mm sieve with distilled water to ensure complete separation. All residue in the sieve was washed into an aluminum box and oven-dried at 60°C for 48 h before weighing. The organic carbon content of the residue>0.053 mm (named POC) was analyzed using the potassium dichromate volumetric method. The MAOC content was measured by subtracting the POC and DOC contents from the TOC in the soil or sediment samples (Cambardella and Elliott 1992; Liu *et al.* 2024).

2.5. Statistic analysis

The mean weight diameter (MWD) of soil aggregates was calculated as:

$$MWD=\sum D_i P_i \quad (1)$$

where D_i is the average diameter of soil aggregates (mm), and P_i is the weight percent of soil aggregates.

The DOC loss flux (Q_i) during a rainfall event was computed as:

$$Q_i=C_i \times q_i \quad (2)$$

where Q_i is the DOC loss flux of runoff (mg m^{-2}); C_i is the DOC concentration in water samples (mg L^{-1}); and q_i is the runoff depth per unit (mm).

The loss of TOC and its fractions (DOC, POC and MAOC) in sediments during an individual flow (Q_{Si}, mg m^{-2}) was

calculated as follows:

$$Q_{Si}=C_{si}\times q_{si} \quad (3)$$

where C_{si} denotes the organic carbon contents (g kg^{-1}); and q_{si} indicates the loss flux of sediment (g m^{-2}).

The annual cumulative loss load was calculated as follows:

$$Q=\sum_{i=1}^{n} Q_i \quad (4)$$

where Q represents cumulative loss (mg m^{-2} yr^{-1}) for a given year, and i=1–n (n denotes the number of runoff events). Statistical analyses were performed using the SPSS 19.0 software package (SPSS Inc., USA). Differences were evaluated using Duncan's test at significance levels (P<0.05). All figures were generated using Sigma plot 10.0 (Systat Software Inc., Chicago, IL, USA).

3. Results

3.1. Dynamics of runoff discharge and sediment flux

Surface runoff discharge dynamics under the four fertilization regimes from 2010 to 2012 are presented in Fig. 3-A. The highest average surface runoff discharge of 22.1 mm occurred in the CK treatment, significantly (P<0.05) exceeding other treatments. No significant variation was observed between the SF and manure substitution treatments (PMF and PM). The annual cumulative surface runoff discharges for CK treatment in 2010, 2011, and 2012 were 102.8, 131.2 and 184.6 mm, respectively (average of 139.5 mm). The average annual accumulation of surface runoff discharges in SF, PMF and PM treatments were 69.5, 60.4 and 54.5 mm, respectively (Fig. 3-B). Compared with SF treatment, the average annual cumulative discharges of surface runoff under PMF and PM treatments decreased notably by 13.1 and 21.6%.

The interflow discharge dynamics under the four fertilization regimes are illustrated in Fig. 3-C. The average interflow discharges per rainfall event under CK, SF, PMF, and PM treatments were 31.9, 33.5, 33.8, and 31.7 mm, respectively. The average annual cumulative interflow discharges under CK, SF, PMF, and PM treatments were 308.1, 324.0, 306.1, and 299.4 mm, respectively (Fig. 3-D), showing no significant differences among treatments. The annual accumulative sediment fluxes for CK treatment in 2011 and 2012 measured 131.2 and 184.6 g m^{-2}, averaging 157.9 g m^{-2} (Fig. 3-E). Relative to CK treatment, the average annual accumulative sediment fluxes under SF, PMF and PM treatments decreased substantially by 202, 221 and 200%, respectively. The cumulative sediment flux in PMF treatment decreased significantly by 21% compared to SF treatment (Fig. 3-F). These findings demonstrate that long-term livestock manure substitution effectively reduced soil erosion intensity while maintaining interflow levels.

3.2. Dynamics of DOC loss caused by alterations in surface runoff and interflow

DOC concentrations in surface water samples across all

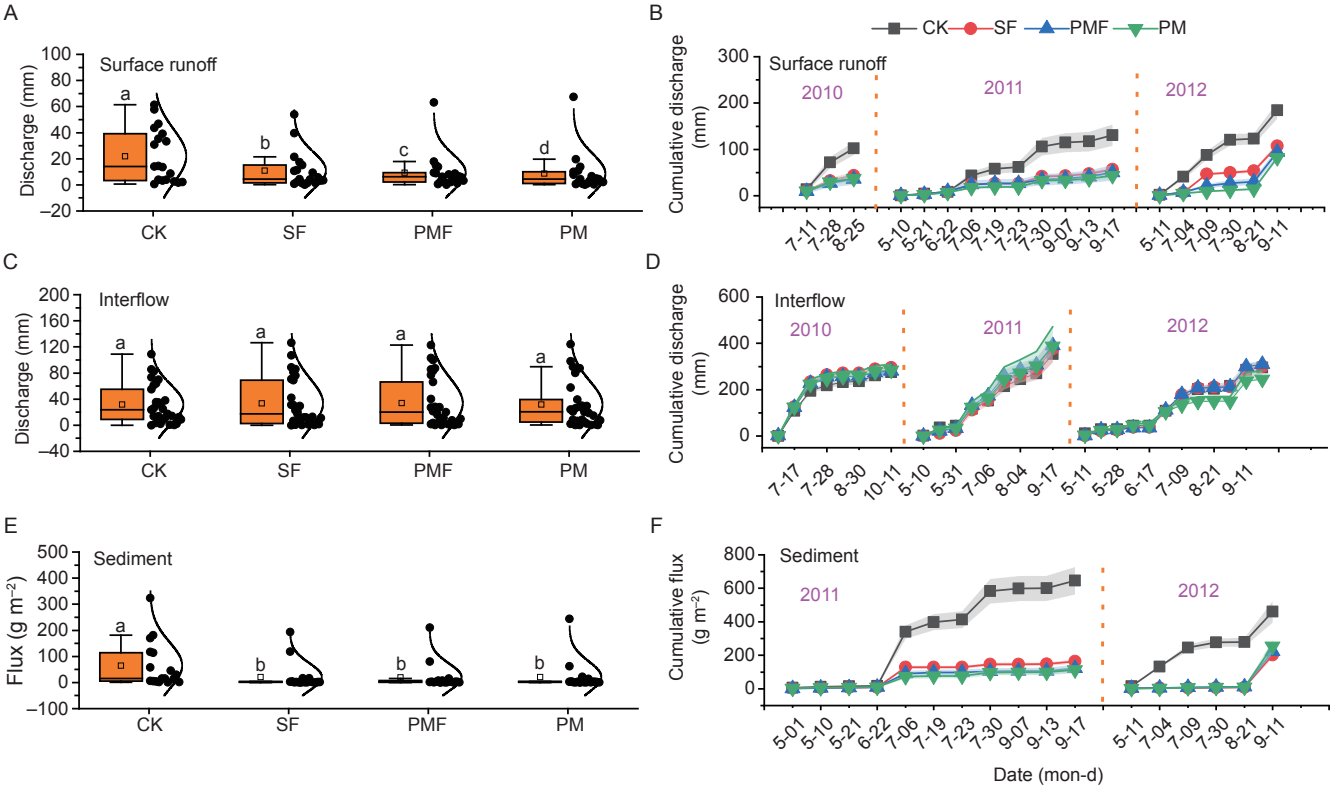


Fig. 3 Seasonal variations in runoff discharge and sediment flux (A, C and E) and cumulative runoff discharge and sediment flux (B, D and F) across different treatments during natural precipitation events from 2010 to 2012. The sediment data from 2010 were not available. CK, control without fertilization; SF, chemical nitrogen fertilizer; PMF, 40% nitrogen substitution with pig manure; PM, 100% nitrogen substitution from pig manure. The line within the box indicates the median. Values represent mean±SD (n=3). Values with different small letters indicate significant differences at P<0.05 according to Duncan's test.

treatments were monitored from 2010 to 2012 (Fig. 4-A), ranging from 2.4 to 2.9 mg L^{-1}, 2.8 to 3.9 mg L^{-1}, 3.1 to 5.4 mg L^{-1} and 2.9 to 3.1 mg L^{-1} in CK, SF, PMF and PM treatments, respectively. The CK exhibited an average DOC concentration of 2.4 mg L^{-1} in surface runoff. Compared to SF, the average DOC concentrations in PMF and PM treatments increased significantly (P<0.05) by 14.4 and 21.5%, respectively. The average DOC concentrations in interflow for CK, SF, PMF and PM treatments measured 2.1, 3.0, 3.0 and 4.6 mg L^{-1}, respectively (Fig. 4-B). Relative to SF treatment, the average DOC concentration in interflow for PM treatment increased significantly by 53.3%, while PMF treatment showed no significant variation.

During 2010–2012, the annual cumulative DOC losses through surface runoff under the control (CK) treatment were 303.6, 333.0 and 299.0 mg m^{-2}, respectively (with an average of 311.8 mg m^{-2}) (Fig. 4-C). The average annual cumulative DOC loss *via* surface runoff under the SF treatment was 187.0 mg m^{-2}, significantly lower (by 67%) than that observed in the CK treatment. The average annual cumulative DOC losses associated with interflow in the CK, SF, PMF and PM treatments were 637.2, 997.4, 1,002.4 and 1,275.2 mg m^{-2}, respectively (Fig. 4-D). Compared to the CK treatment, average annual cumulative DOC loss through interflow under the SF treatment increased significantly by 57%. Relative to the SF treatment, the cumulative DOC loss flux under the PM treatment increased significantly by 28%, while no significant difference in interflow was observed between the SF and PMF treatments.

3.3. Dynamics of organic carbon loss *via* soil erosion

The mean loss fluxes of DOC, POC, and MAOC per individual rainfall event *via* sediment under the CK treatment were 2.8, 128.9 and 368.7 mg m^{-2}, respectively, and the average annual cumulative losses of DOC, POC and MAOC through soil erosion were 23.2, 1,059.4 and 2,961.4 mg m^{-2}, respectively, from 2011 to 2012 (Fig. 5). Compared to the CK, the average annual cumulative DOC losses with sediments under the SF, PMF and PM treatments decreased significantly (P<0.05) by 148, 142 and 115%, respectively. The POC loss fluxes

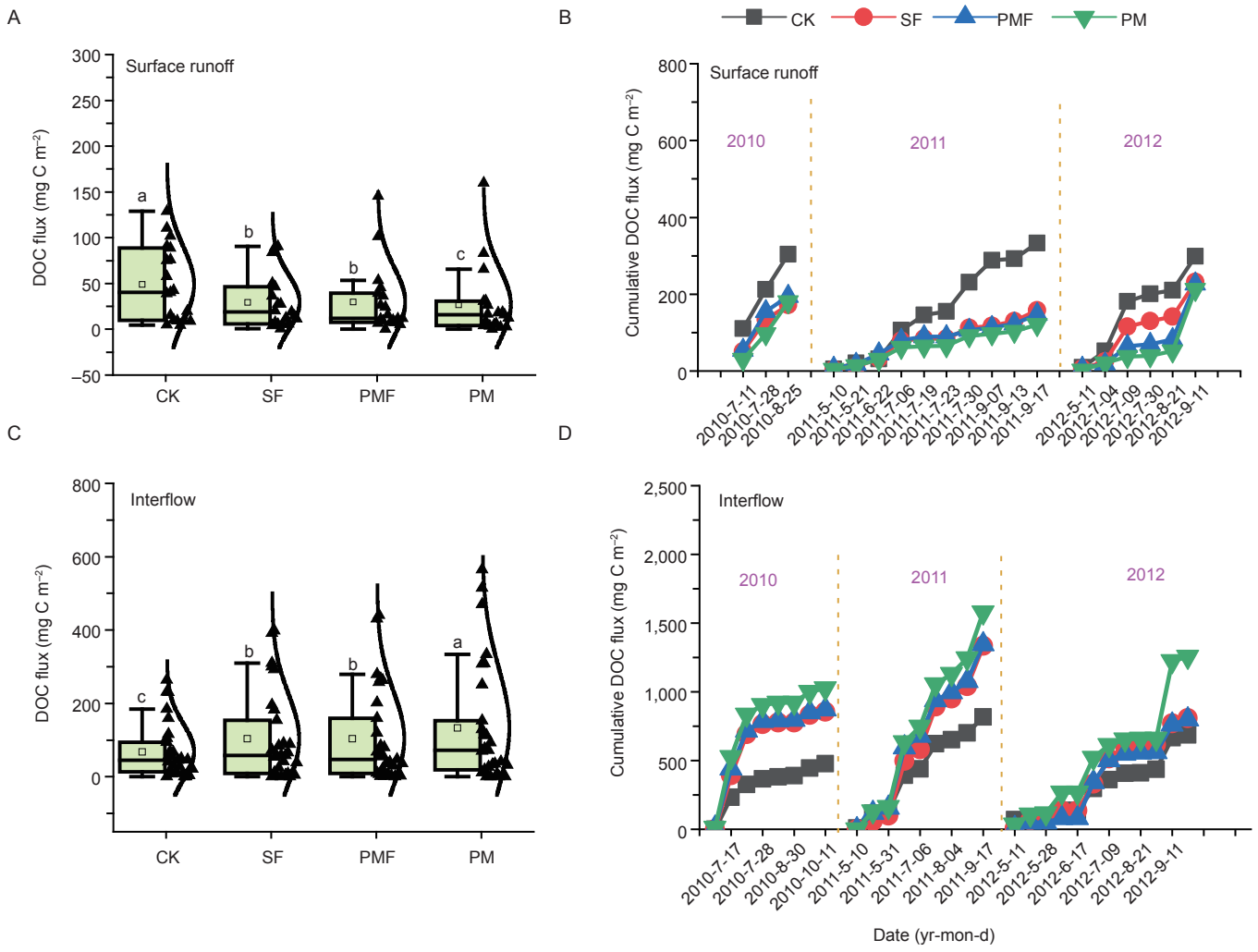


Fig. 4 Seasonal variations and cumulative dissolved organic carbon (DOC) loss fluxes *via* surface runoff and interflow across treatment groups from 2010 to 2012 during natural precipitation events. CK, control without fertilization; SF, chemical nitrogen fertilizer; PMF, 40% nitrogen substitution with pig manure; PM, 100% nitrogen substitution from pig manure. The line within each box indicates the median. Values represent mean±SD (n=3). Values with different lowercase letters indicate significant differences at P<0.05 according to Duncan's test.

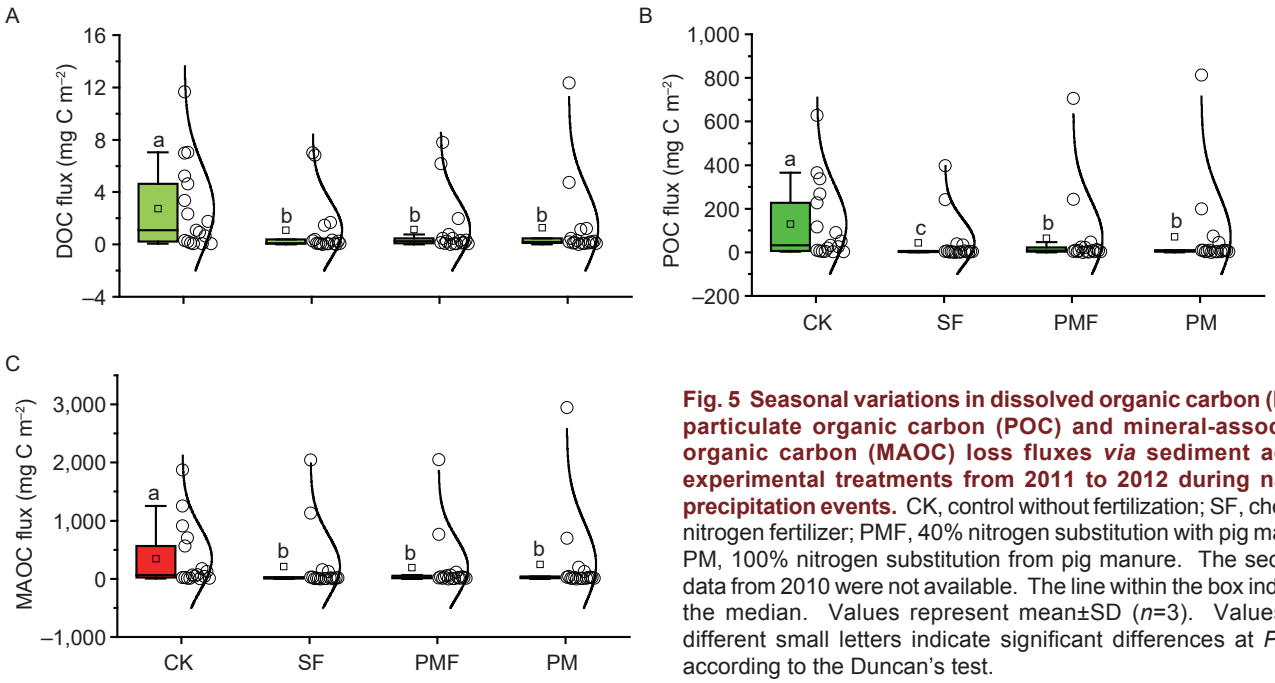


Fig. 5 Seasonal variations in dissolved organic carbon (DOC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) loss fluxes *via* sediment across experimental treatments from 2011 to 2012 during natural precipitation events. CK, control without fertilization; SF, chemical nitrogen fertilizer; PMF, 40% nitrogen substitution with pig manure; PM, 100% nitrogen substitution from pig manure. The sediment data from 2010 were not available. The line within the box indicates the median. Values represent mean±SD (n=3). Values with different small letters indicate significant differences at P<0.05 according to the Duncan's test.

under the PMF and PM treatments increased significantly by 48 and 61% compared to SF treatment. The average annual cumulative MAOC loss fluxes under the SF, PMF and PM treatments decreased significantly by 64, 82 and 41%, respectively, compared to the CK treatment (Fig. 6). Overall, the average annual cumulative TOC losses by sediment under the CK, SF, PMF, and PM treatments were 4,044.0, 2,189.0, 2,191.5 and 2,715.5 mg m^{-2}, respectively, following the order of CK>PM>PMF≈SF. The TOC loss flux under the PM treatment increased significantly by 24% compared to the SF treatment.

3.4. Contributions of labile organic carbon to total organic carbon transport

Examining the three hydrological pathways for organic carbon transport (surface runoff, interflow and eroded sediment), the average annual cumulative TOC transported within the CK, SF, PMF and PM treatments were 4,993.0, 3,373.4, 3,383.7 and 4,161.6 mg m^{-2}, respectively (Fig. 7). Compared to the SF treatment, the mean annual cumulative lateral TOC transport in the PM treatment increased significantly (P<0.05) by 23%. Additionally, the total DOC loss (including surface runoff, interflow, and sediment) constituted 19 and 35% of the TOC loss in the CK and other treatments (averaged across SF, PM and PMF treatments). POC loss comprised 21, 11, 16 and 14% of TOC loss, while their corresponding MAOC losses represented 59, 54, 48 and 51% in the CK, SF, PMF and PM treatments. Furthermore, the annual mean POC loss fluxes under the PMF and PM treatments were 553.7 and 603.1 mg m^{-2}, respectively, showing significant increases of 47.9 and 61.1%, respectively. In contrast, only the annual mean DOC loss flux under the PM treatment increased significantly by 23.4% compared to the SF treatment. Regarding labile organic carbon transport, the fluxes of labile organic carbon (including DOC and POC) loss under the PMF and PM treatments increased significantly by 11.9 and 31.4%, respectively.

3.5. Soil aggregate stability, SOC fractions and correlations with organic carbon loss

The MWD of aggregates at depths between 0–20 cm under the CK, SF, PMF and PM treatments in 2011 and 2012 were 0.17, 0.28, 0.44, 0.53 and 0.18, 0.25, 0.37, 0.49, respectively. The average MWD values were 0.18, 0.27, 0.41 and 0.51, respectively (Table 2). Compared to the CK treatment, the MWD value under the SF treatment increased significantly (P<0.05) by 50%. Long-term livestock manure application in the PMF and PM treatments significantly (P<0.05) enhanced MWD values by 51.9 and 88.9% compared with the SF treatment, respectively, demonstrating improved soil aggregate stability through livestock manure application. The average soil DOC contents at 0–20, 20–40 and 40–60 cm in 2011 and 2012 within the PMF treatment increased significantly by 8.9, 27.9 and 66.8% compared to the SF treatment, respectively. The corresponding values in the PM treatment increased significantly (P<0.05) by 50.1, 39.2 and 73.4%, respectively. Compared to the SF treatment, soil POC contents at 0–20 and 20–40 cm depths under the PMF and PM treatments increased significantly (P<0.05) by 75, 108.3, 83.3, and 100%, respectively, while no significant changes occurred at 40–60 cm depth. Following the POC trend, long-term livestock manure substitution practices, including PMF and PM treatments, significantly enhanced soil MAOC contents by 13.3, 26.7 and 17.4, 41.3% compared to the SF treatment.

Further analysis revealed that the accumulative annual sediment loss flux and surface runoff discharge correlated significantly (P<0.05) and negatively with MWD values (Fig. 8), whereas no significant correlation emerged with the accumulative annual interflow discharge. These findings

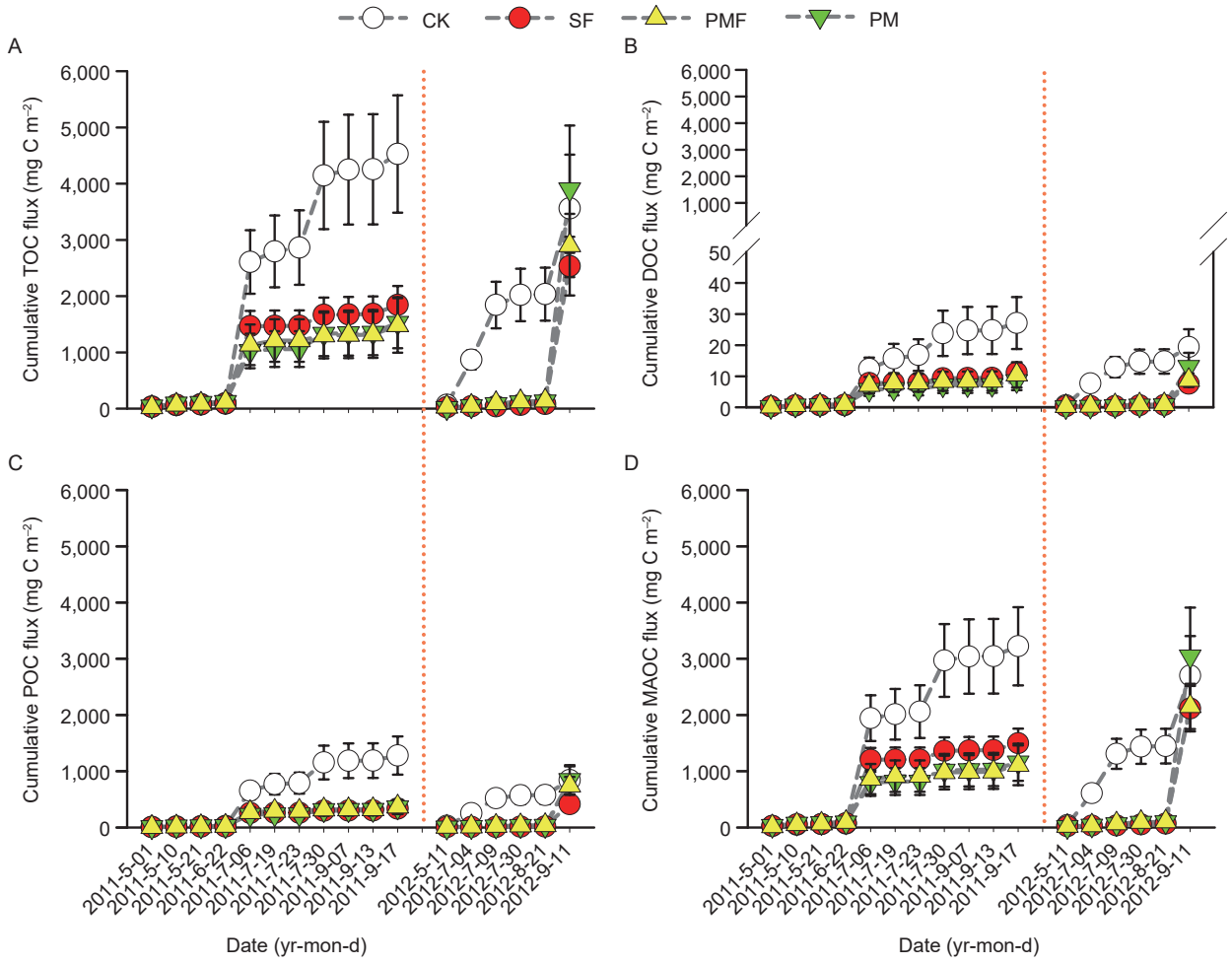


Fig. 6 Cumulative losses of total organic carbon (TOC), dissolved organic carbon (DOC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) loss fluxes through sediment transport across experimental treatments during natural precipitation events from 2011 to 2012. CK, control without fertilization; SF, chemical nitrogen fertilizer; PMF, 40% nitrogen substitution with pig manure; PM, 100% nitrogen substitution from pig manure. Sediment data from 2010 were not available. Values represent mean±SD (*n*=3).

indicate that soil aggregate stability significantly influences water loss and soil erosion. The POC content in sediment showed significant positive correlations with POC contents in both large and small soil aggregates at depths of 0–20 and 20–40 cm. Moreover, a significant positive correlation existed between the DOC content at 0–20 cm soil depth and DOC concentration *via* interflow, but not in surface runoff. A similar correlation pattern appeared between soil MAOC content and MAOC content in sediment.

4. Discussion

4.1. Long-term livestock manure substitution enhances soil aggregate stability and decreases water erosion

Soil aggregate stability significantly influences the intensity of water erosion (Six *et al.* 2004). Water erosion can be modulated by soil aggregate detachment due to raindrop impacts (Kinnell 2005; Ma *et al.* 2014). Research indicates that water erosion decreases as soil aggregate stability increases (Cantón *et al.* 2009; Lu *et al.* 2016; Zheng *et al.* 2021). Higher soil aggregate stability typically reduces soil exposure to raindrop impacts and detachment. This study revealed a negative linear correlation between soil MWD and cumulative annual sediment loss and surface runoff discharge (Fig. 8-A and B). These results confirm that soil aggregate stability reduces water erosion intensity. Additionally, long-term livestock manure application significantly enhanced MWD values by 51.9 and 88.9% compared with the SF treatment. This observation aligns with Huang *et al.* (2022), who demonstrated that swine manure addition significantly increased large macroaggregate proportion and aggregate stability compared to chemical fertilizer. Similarly, Zhu *et al.* (2024) reported MWD increases of 3.40–14.20% above the control site with increased manure application. Large water-stable soil aggregate formation strongly depends on organic matter, which serves as the primary cementing material for maintaining aggregate stability. Long-term livestock manure substitution provides abundant exogenous organic matter to soil, which microorganisms decompose to form organic and inorganic cementing materials, enhancing aggregate stability. Furthermore, humic acid functional groups, including

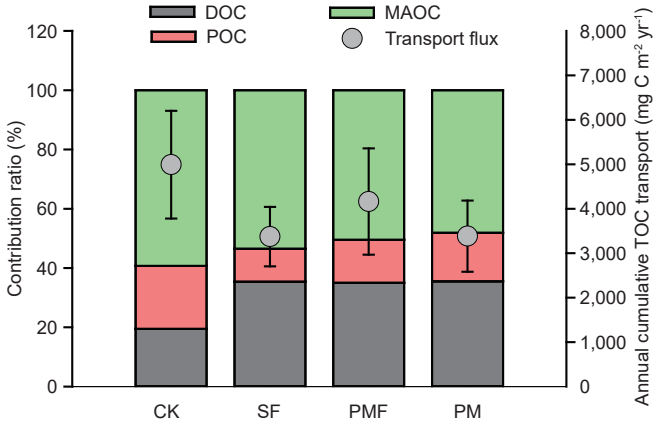


Fig. 7 The contribution ratio of dissolved organic carbon (DOC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) to total organic carbon (TOC) and annual cumulative TOC transport *via* surface runoff, interflow, and sediment across treatment variations from 2010 to 2012. Sediment data from 2010 were not available. CK, control with no fertilization; SF, chemical nitrogen fertilizer; PMF, 40% nitrogen substitution with pig manure; PM, 100% nitrogen substitution from pig manure. Values represent mean±SD (*n*=3).

Table 2 The average organic carbon fractions contents and aggregate stability in soil profiles across four treatment plots during 2011 and 2012[1)]

Soil depth (cm)	Treatment[2)]	DOC (mg kg^{-1})	POC (g kg^{-1})	MAOC (g kg^{-1})	SOC (g kg^{-1})	MWD (mm)
0–20	CK	50.9 d	1.0 c	5.1 d	6.1 d	0.18d
	SF	75.7 c	1.2 b	6.0 c	7.2 c	0.27c
	PMF	82.5 b	2.1 a	6.8 b	8.9 b	0.41b
	PM	110.5 a	2.5 a	7.6 a	10.1 a	0.51a
20–40	CK	53.3 c	0.5 b	4.5 c	5.0 c	–
	SF	56.1 c	0.6 b	4.6 c	5.2 c	–
	PMF	71.8 b	1.1 a	5.4 b	6.5 b	–
	PM	78.1 a	1.2 a	6.5 a	7.7 a	–
40–60	CK	40.4 b	0.3 b	5.0 a	5.3 a	–
	SF	40.2 b	0.5 a	4.8 a	5.3 a	–
	PMF	67.1 a	0.5 a	4.7 a	5.2 a	–
	PM	69.7 a	0.5 a	4.9 a	5.4 a	–

[1)] DOC, dissolved organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon; SOC, soil organic carbon; MWD, mean weight diameter.
[2)] CK, no fertilization; SF, chemical nitrogen fertilizer; PMF, substitution using 40% nitrogen with pig manure; PM, substitution using 100% nitrogen from pig manure.
Different lowercase letters indicate significant differences at the P<0.05 level.

aromaticity, humification, and molecular weight in macro-aggregates, increase under long-term livestock manure application, improving soil aggregate stability (Zhu *et al.* 2024). Consequently, average annual cumulative sediment fluxes and surface runoff discharge under livestock manure treatment (average of PMF and PM treatments) decreased by 11.5 and 17.4% compared to the SF treatment.

In the subtropical sloping croplands of the upper Yangtze River region, agricultural decision-makers require guidance on implementing sustainable practices for severely water -eroded soils. Livestock manure application represents a conventional method for enhancing crop yield, soil fertility, and reducing water erosion. This study demonstrates that enhanced soil aggregate stability serves as a crucial factor in reducing eroded sediment flux and surface runoff discharge under sustained livestock manure application. Specifically, the improvements in soil structure contribute to decreased water erosion intensity. While substituting chemical fertilizers with livestock manure is recommended for soil and water conservation, the long-term effects of this practice on other environmental concerns (such as agricultural non-point source pollution) involving carbon, nitrogen, and phosphorus transport warrant further investigation.

4.2. Long-term livestock manure substitution improves SOC sequestration whereas increases the risk of labile organic carbon hydrological loss

SOC sequestration represents a balance between exogenous organic carbon inputs (such as farmyard manure or crop straw) and organic carbon losses through soil heterotrophic respiration and hydrological processes. Understanding methods to reduce organic carbon losses remains crucial for enhancing SOC sequestration (Liang *et al.* 2023; Widanagamage *et al.* 2025). Recent decades have shown that long-term livestock manure application significantly increases SOC sequestration capacity, particularly regarding organic carbon quantity and biochemical stability in soil aggregates. The present study reveals significant enhancement of SOC, POC, and MAOC contents at 0–20 and 20–40 cm depths, along with increased POC contents in both large and small soil aggregates at topsoil through long-term livestock manure substitution, aligning with previous research findings (Zhou *et al.* 2010; Srinivasan *et al.* 2012; Simon *et al.* 2025).

Current literature provides limited information regarding the transport loss of labile organic carbon, particularly DOC and POC losses following long-term livestock manure substitution. Compared to organic carbon loss through soil heterotrophic respiration, the quantity lost *via* runoff and eroded sediment is generally smaller. Consequently, organic carbon flux through water erosion has often been overlooked (Berhe *et al.*, 2008; Lal 2019; Nakhavali *et al.* 2021; Hancock *et al.* 2024). The transport of DOC and POC from arable soil to water affects both SOC storage and organic carbon cycling, potentially impacting adjacent waterbody quality in agricultural watersheds (Smith *et al.* 2013). Fertilization practices, including livestock manure additions, significantly influence topsoil DOC and POC contents and agro-ecosystem hydrologic characteristics (Li *et al.* 2021). Our findings indicate that long-term livestock manure substitution substantially increased soil DOC and POC pools and their transport *via* runoff and soil erosion. The annual transport fluxes of DOC and POC reached 1,329 and 578 mg m^{-2} (mean of PMF and PM treatments) in manure-treated fields, representing significant increases of 11.3 and 54.4% compared to mineral fertilizer application alone. Additionally, POC loss contributions to SOC lateral transport increased by 39% under long-term livestock manure

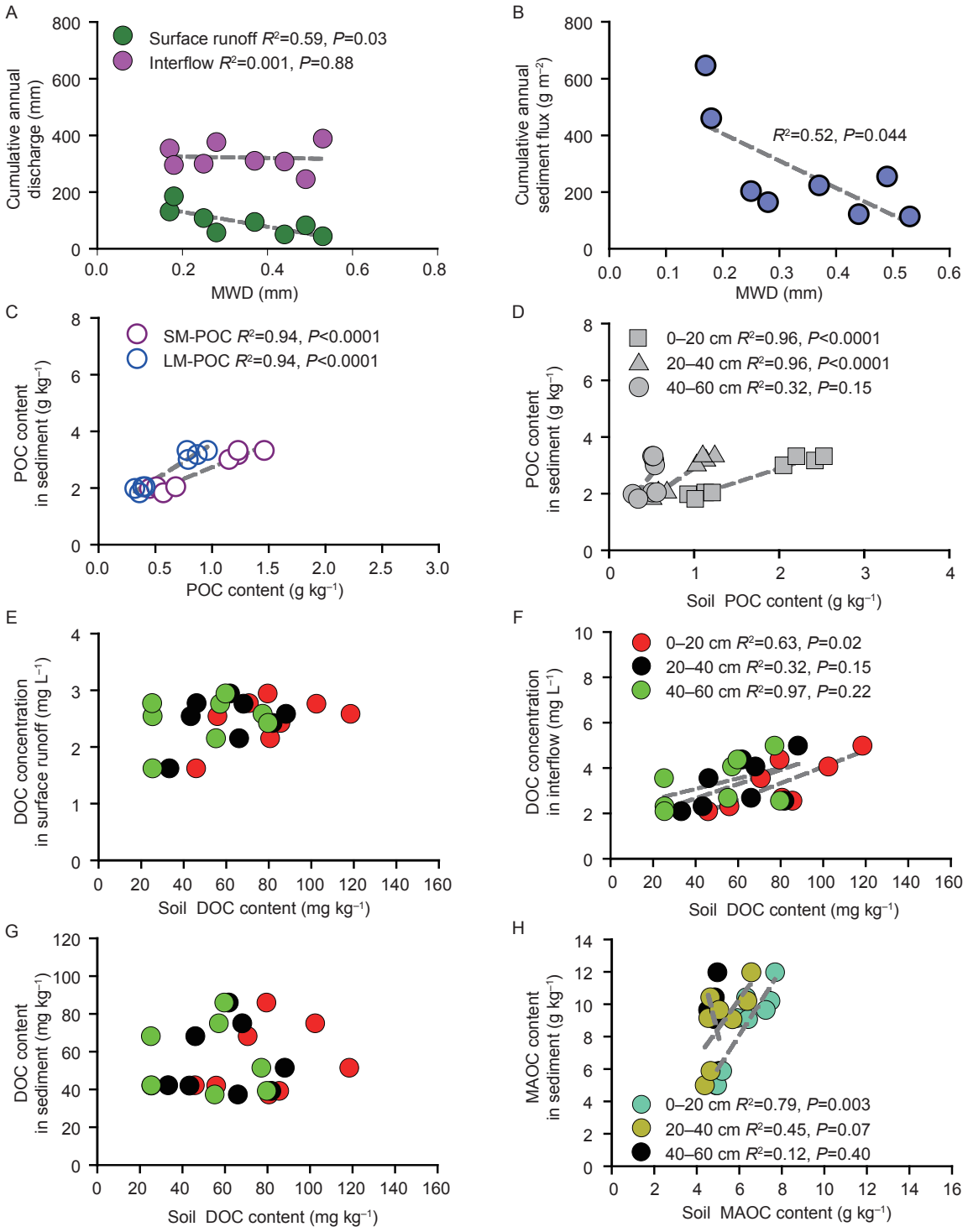


Fig. 8 Soil aggregate stability, organic carbon fractions and their relationships with organic carbon fraction losses through surface runoff, interflow, and sediment transport. No significant linear correlation was observed between soil DOC content and DOC concentration in surface runoff or sediment. MWD, mean weight diameter; SM, small macro-aggregates; LM, large macro-aggregates; POC, particulate organic carbon; DOC, dissolved organic carbon; MAOC, mineral-associated organic carbon. Values represent means (n=3).

application. This suggests intensified labile organic carbon lateral transport following sustained exogenous organic manure application. Notably, while runoff discharge and soil erosion intensity decreased significantly, DOC concentration in runoff and POC content in sediment increased markedly after manure application. The elevated risk of labile organic carbon loss primarily stems from increased exogenous total and labile organic carbon input through manure application. Furthermore, soil physicochemical and biological processes for labile organic carbon sequestration, mediated by carbon functional microorganisms and physicochemical protection, contribute to this migration risk intensification.

Labile organic carbon transport from arable soil to runoff ditches significantly affects water quality in agricultural

watersheds (Liang *et al.* 2023). DOC and POC losses *via* runoff and sediment on hill slopes can be transported through upland ditches to stream waters in hilly areas, impacting the downstream aquatic environment. Areas of sloping croplands with relatively low SOC represent crucial cultivated resources in the upper reaches of the Yangtze River. Regosols experience extensive degradation through severe water erosion due to sloping terrain and frequent rainfall events. Livestock manure application is commonly implemented to enhance SOC sequestration and increase crop yields. However, continuous long-term exogenous livestock manure application increases DOC and POC loss through runoff and erosion on hillslopes. Consequently, careful attention must be directed toward controlling DOC and POC loss *via* water erosion when applying livestock manure on sloping croplands, as this is essential for reducing organic carbon pool losses and surface water pollution risks in agricultural watersheds. Livestock manure substitution for chemical fertilizers represents a traditional agronomic method for reducing soil erosion and increasing SOC sequestration. Notably, this study demonstrates that minimizing DOC and POC loss *via* runoff and eroded sediment requires monitoring to achieve sustainable livestock manure utilization. Since the lateral transport process of DOC and POC from soil to water is crucial to the water environment, increasing SOC sequestration while limiting labile organic carbon loss under livestock manure application presents a significant scientific challenge for researchers and local environmental management strategies.

4.3. Limitations and implications

Based on these findings, we observed that enhanced SOC sequestration ability and decreased water erosion intensity can be achieved through livestock manure substitution, as demonstrated by the long-term fertilization experiment on subtropical sloping croplands. However, our three-year field datasets (2010–2012) indicate an elevated risk of labile organic carbon hydrological loss following long-term exogenous livestock manure application. Currently, approximately thirteen years later, the SOC fractions, particularly DOC and POC contents in the topsoil, should be substantially improved, suggesting increased labile organic carbon loss flux *via* runoff and sediment following persistent livestock manure application since 2012. The key trends and conclusions presented in this study remain convincing and independent of the observation period.

The processes of SOC sequestration and hydrological transport are intrinsically linked to the chemical characteristics of exogenous organic carbon input. Future research should emphasize examining the quantity and quality (e.g., chemical structure and molecular weight) of DOC and POC resulting from exogenous organic amendments input through magnetic resonance imaging and Fourier transform ion cyclotron resonance mass spectrometry technologies, thereby thoroughly analyzing the influence mechanism of livestock manure application on labile organic carbon hydrological migration. Furthermore, given global warming conditions, the frequency and intensity of extreme rainfall events in subtropical sloping croplands create complexity and unpredictability in hydrological labile organic carbon loss dynamics. The hydrological process and mechanism of organic carbon lateral transport, particularly changes in chemical organic structure and migration rates of DOC and POC *via* runoff and erosion, under various soil types (e.g., acid or alkaline Regosol) or multiple slope gradients (6.5°, 10°, 15° and 25°) during extreme rainfall events warrant investigation through artificial rainfall simulation or field observation to address future climate change. For example, soil pH influences microbial and animal abundance and diversity related to carbon cycling, while slope gradients significantly affect water erosion intensity and runoff discharge. Therefore, labile organic carbon hydrological transport processes under different scenarios require careful consideration when addressing extreme rainfall events in subtropical agricultural ecological areas.

5. Conclusion

The long-term substitution of livestock manure for chemical fertilizers enhances soil aggregate stability and reduces water erosion intensity, while showing no substantial impact on leaching discharge. Annual lateral flux of labile organic carbon loss, including DOC and POC fluxes, increases significantly with long-term livestock manure substitution, thereby elevating the risk of adjacent water body pollution. Therefore, when applying livestock manure to subtropical sloping croplands to enhance SOC sequestration, the potential environmental effects of hydrological losses of labile organic carbon require thorough consideration. Organic carbon lateral migration under different scenarios (e.g., soil types and slope gradients for organic amendment application) necessitates further investigation to improve carbon sequestration capacity and minimize waterbody pollution risk when addressing extreme rainfall and climate change in the future.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in scientific writing

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

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