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EDITED BY
Teresa Ferreira,
University of Lisbon, Portugal

REVIEWED BY
Elisabeth Bondar-Kunze,
Wasser Cluster Lunz, Austria
Giulia Grandi,
Ca 'Foscari University of Venice, Italy

*CORRESPONDENCE
Zhoufeng Wang,

⋈ wangzf@chd.edu.cn
Xiaohong Zhao,
xzhao@chd.edu.cn

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The sources and influencing factors of dissolved organic carbon under high-sediment environments – A case from Wuding River Basin

Manhong Xia^{1,2,3}, Nan Xue^{1,2,3}, Bo Hu⁴, Xinru Gong⁴, Zhoufeng Wang^{1,2,3}* and Xiaohong Zhao⁴*

¹School of Water and Environment, Chang'an University, Xi'an, China, ²Key Laboratory of Subsurface Hydrology and Ecological Effect in Arid Region of the Ministry of Education, Chang'an University, Xi'an, China, ³Key Laboratory of Eco-hydrology and Water Security in Arid and Semi-arid Regions of Ministry of Water Resources, Chang'an University, Xi'an, China, ⁴School of Civil Engineering, Chang'an University, Xi'an, China

River sediments are important carbon reservoirs in terrestrial-aquatic systems, and a thorough understanding of the factors that influence the sources and distribution of organic carbon in river sediments can help us understand the carbon cycling process in river ecosystems. In this study, 21 river sediment samples were collected along the upstream-downstream gradient of the Wuding River Basin. The study revealed that (1) the sources of DOC in the Wuding River Basin were spatially heterogeneous, with plankton being the largest source of DOC upstream (39%), whereas terrestrial sources were the largest source of DOC downstream (54%). (2) Influenced by geomorphic conditions and land use, the gradually increasing hydrodynamic conditions from upstream to downstream prompted the migration of surface soil organic matter and nutrients such as effective nitrogen (AN), total phosphorus (TP), and total nitrogen (TN) to the river channel, resulting in an increase in the concentration of suspended sediment, which led to the accumulation of organic carbon in the downstream section of the river. (3) Using macrogenomic techniques, microbial - driven carbon cycling processes were identified and predicted. Downstream has greater carbon - cycling potential than upstream. Upstream DOC is positively correlated with anaerobic carbon fixation and methanogenesis (p < 0.001). Downstream DOC is negatively correlated with the rTCA cycle (p < 0.001), inhibiting autotrophic fixation as microbes use available carbon. The results of this study provide data to support ecological restoration, carbon sink enhancement, and water quality assurance in highsediment rivers.

KEYWORDS

dissolved organic carbon, stable isotope, bayesian stable isotope mixing model, highsediment environments, carbon cycle

1 Introduction

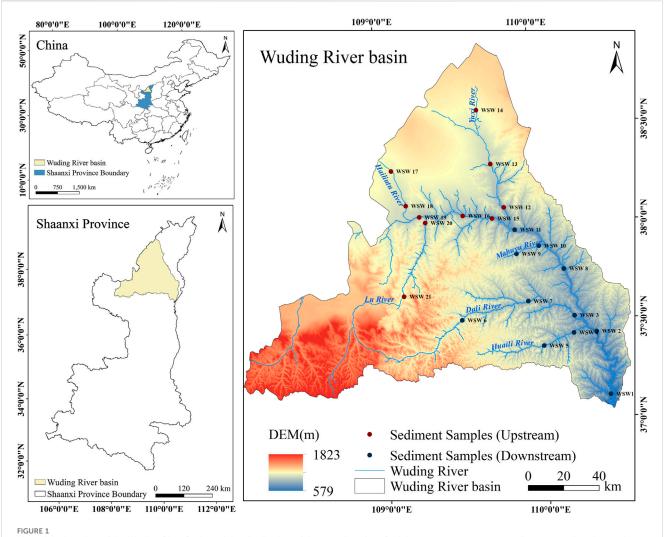
Rivers are important conduits for carbon transport in terrestrial and aquatic ecosystems. The annual dissolved organic carbon (DOC) flux of terrestrial organic carbon to the ocean through major rivers worldwide has been estimated to be 0.45–0.78 Pg (Drake et al., 2018; Ran et al., 2018). Whereas DOC in river sediments is a central carrier of this process, DOC may be buried in sediments for long periods (to form carbon sinks) (Liu et al., 2021; Smoak et al., 2013) or released by microbial decomposition into CO₂ and CH₄ (to become carbon sources) (Li Y. et al., 2025; McTigue et al., 2021). Therefore, the study of the sources of organic carbon in river sediments (vegetation apomixis, soil erosion, anthropogenic inputs, etc.) has become a focal point in the field of the global carbon cycle.

The distribution of organic carbon in river sediments is affected by a variety of environmental variables, such as pH, total nitrogen (TN) and total phosphorus (TP). Among them, pH plays an important role in the sediment organic carbon content (Zhu et al., 2024), which affects organic carbon-related microbial activity and cation exchange (Li et al., 2019), which in turn destabilizes mineral-bound organic carbon (Guo ZhiHua et al., 2014). Zhao Bin et al. studied the sediment transport system of the Yangtze and Yellow Rivers and reported that young terrestrial organic carbon (mainly from C3 plants) in the Yangtze River sediments accelerated degradation due to pH fluctuations during transport (Zhao et al., 2023). Li et al. also reported that the decomposition rate of sedimentary organic carbon was regulated mainly by pH when coastal wetlands in the Bohai Sea were studied (Li X. et al., 2025). The correlations between organic carbon and TN and TP occur because the addition of nitrogen increases the potential for carbon sequestration (Zhang S. et al., 2022), whereas the addition of phosphorus accelerates the decomposition of reactive organic carbon in sediments (Kaspari et al., 2008).

The analysis of stable isotopic compositions (13C and 15N) and mixing models are effective methods for assessing organic carbon sources and degradation processes (Derrien et al., 2019). For example, Klink et al. (2022) used $\delta^{13}C-\delta^{15}N$ dual isotopes combined with Bayesian mixing modelling to quantify the significantly greater contribution of fungal residues to mineralbound organic carbon (MAOM) (42.7%) than plant residues (28.9%), revealing the dominant role of the fungal community in soil carbon pool stability (Klink et al., 2022). Similarly, Yan et al. (2023) resolved through δ^{13} C and C/N multivariate modelling that aquaculture sources were the largest source of sediment organic matter in the upstream estuarine section of a mangrove wetland (44.29%), whereas terrestrial sources contributed the greatest amount (62.87%) in the middle estuarine section of the East River of Evianfeng near the river mouth; in the downstream direction, there was a significant increase in the source of marine plankton (Yan et al., 2023). These studies confirmed the ability of isotopic techniques to detect complex carbon sources. In addition, microbial macrogenomics can reveal carbon cycling pathways (Steen et al., 2019; Zhang L.-Z. et al., 2024). Dai et al. (2021) reported that the peak abundance of carbon degradation genes at mid-altitudes susceptible to mineralization coincided with isotope fractionation features, which could enable cross-scale correlations of carbon source-metabolism pathways (Dai et al., 2021). These cases demonstrate that the synergistic application of stable isotopes and macrogenomics can more accurately and quantitatively resolve organic carbon sources. Therefore, the integration of stable isotope tracing with microbial functional gene analysis (e.g., genes encoding enzymes involved in carbon metabolism) can overcome the limitations of either approach.

The Wuding River basin is located in the hinterland of the Loess Plateau, which is one of the areas with the most severe soil erosion in the middle reaches of the Yellow River (Yang et al., 2024; Yanling and Zhijun, 2019). In recent years, with the implementation of ecological projects such as returning farmland to forest (grass) and terrace construction, the vegetation cover in the basin has increased significantly, and soil erosion has been initially controlled. However, the synergistic effects of human activities (e.g., agricultural intensification, aquaculture, and urbanization expansion) (Wen et al., 2022; Yu K. et al., 2022) and climate change (e.g., precipitation extremes) have led to a new complexity in the source-sink processes of sediment organic carbon. This complexity is reflected mainly in the increased heterogeneity of organic carbon sources and the unclear proportion of contributions from natural and anthropogenic inputs. Vegetation restoration may increase carbon sequestration by reducing soil erosion, but agricultural fertilizers or sewage discharge may also input exogenous organic carbon to rivers, altering the original carbon transport pathway (Hu et al., 2021). In addition, soil microbial community reconfiguration (e.g., changes in fungal/bacterial ratios) after fallowing may accelerate or inhibit the mineralizationhumification process of organic carbon, altering carbon stability and transport potential (Bastida et al., 2021; Liu et al., 2021). Therefore, identifying the sources of DOC in the Wuding River and its impact on microbial-driven carbon cycling processes is critical for regulating the health of high-sediment river water quality environments.

The upstream and downstream areas of the Wuding watershed have unique geomorphic types; with the upstream area crossing the Maowusu windswept beach, the surface matrix is highly permeable, forming hydrological characteristics mainly based on groundwater recharge, and the downstream area transitioning to the hinterland of the Loess Plateau, which is loosely porous, may lead to a large amount of erosion-driven organic carbon input into the river system. The organic carbon transport flux driven by soil erosion on the Loess Plateau accounts for more than 60% of the total organic carbon in Yellow River sand transport (Ke et al., 2024; Ran et al., 2014); as the core area of erosion on the Loess Plateau and the main source of coarse sediments in the Yellow River, the process of sediment organic carbon transport constitutes a key link in carbon transport in the Yellow River Basin. Moreover, in this complex environment, a detailed study of the sources and influential factors of DOC in the river sediments of the Wuding River Basin plays an important role in understanding the process of river carbon cycling in inland arid and semiarid areas. Therefore, given the unique climate, vegetation, and human activities in the Wuding River Basin, we propose the following hypotheses: Sediment DOC sources (soil, aquatic plants, anthropogenic inputs) show seasonal variations; DOC burial and degradation efficiency are controlled by mineral adsorption and nutrient components; sediment DOC concentration and composition influences microbial activity and structure, thereby regulating



Map of the location of the Wuding River Basin and the distribution of the sampling sites. Red dots represent upstream sediment sampling sites and blue dots represent downstream sediment sampling sites. The thin black line represents the boundary of the Wuding River Basin in Shaanxi Province, including the main stream and tributaries.

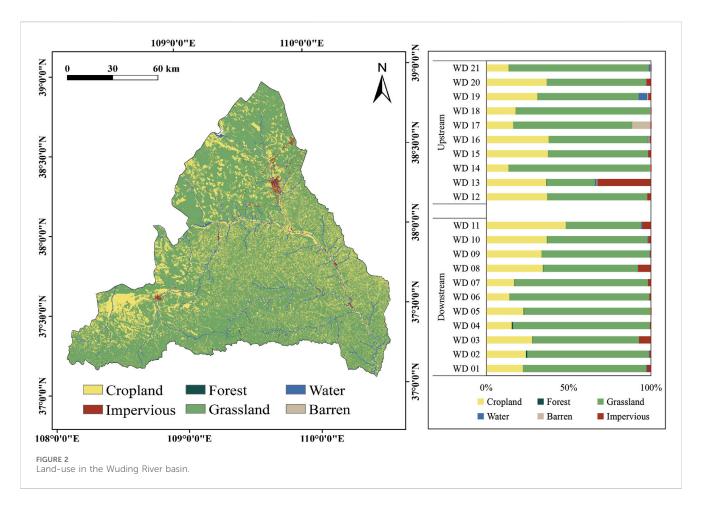
carbon cycling at the sediment-water interface. This study aims to: (1) quantify DOC source contributions using $\delta^{13}C$ and $\delta^{15}N$ mixing models; (2) identify key constraints on DOC burial and degradation; (3) reveal DOC's influence on microbially-driven sediment carbon cycling.

2 Materials and methods

2.1 Study area and sampling

The Wuding River Basin, covering an area of approximately 30,000 km², is located in the northern part of Shaanxi Province, China (108°27′39″–110°34′22″E, 37°02′31″–38°55′52″N). The main channel of this water system, the Wuding River, originates from the northern foothills of Baiyu Mountain in Dingbian County, Shaanxi Province, and extends for 491 km, eventually merging into the Yellow River near Hekou Village in Qingjian County, Yulin city, Shaanxi Province, and becoming a first-class tributary of the Yellow

River as a main stream. The main tributaries in the basin are the Yuxi River, the Lu River, the Dali River, the Huaining River, the Hailiutu River, the Nalin River, and the Heimutouchuan River. The basin has an arid and semiarid monsoon climate, with an average annual temperature of 9.5°C and an average annual precipitation of 409.1 mm, of which approximately 74% occurs from June to September, and the average annual evapotranspiration is 1,200 mm (Figure 1, data sourced from http://eia-data.com/) (HE et al., 2025). The Wuding River Basin is divided into upstream and downstream watersheds based on the direction of the river's flow. The upstream region of the watershed mainly flows through the wind-sand grassland area, while the downstream mainly flows the loess hill and gully area. In this study, on the basis of remote sensing data (https://zenodo.org/records/12779975) from August 2023, a buffer zone with a radius of 5 km was created around each sampling point via ArcGIS version 10.2, and the areas of different land use types, such as cropland, forest, and grassland, were analysed and quantified within each buffer zone. The land use in the study area is dominated by grassland, cropland, and urban land, which account



for 74.7%, 23.2%, and 1.1% of the total watershed area, respectively (Figure 2).

In this study, 21 sampling sites (WD1–WD21) were deployed along the mainstem and tributaries of the Wuding River in August 2024; sampling sites WD1–WD11 were located in the downstream area, whereas WD12–WD21 were located in the upstream area (Figure 1). At each sampling site, two sediment subsamples were collected using a stainless steel Peterson grab sampler within 20–30 m north and south of the central location. These subsamples were combined in a sterilized self-sealing bag to form a single representative composite sediment sample per site. From each composite sample, three aliquots were subsequently prepared: one aliquot was refrigerated at –4°C for physicochemical parameter analysis, a second aliquot was frozen at –20°C for organic carbon isotope determination, and the third aliquot was frozen at –80°C for microbial sequencing analysis.

2.2 Laboratory analysis

2.2.1 Analysis of the physical and chemical properties of sediments

The suspended solids (SS) concentration was determined via the gravimetric method. The pH of the sediment was determined via a pH meter (FE28, Mettler Toledo Instruments (Shanghai) Co., Ltd.). The total organic carbon (TOC) and DOC (see Supplementary Text S1 for details) contents were determined via an automatic organic

carbon analyser (vario TOC cube, Elementar, Germany). The total nitrogen (TN) and available nitrogen (AN) contents were determined via an automatic nitrogen analyser (K1160, Shandong Haineng Scientific Instrument). The total phosphorus (TP) and available phosphorus (AP, see Supplementary Text S2 for details) contents were determined via an ultraviolet—visible spectrophotometer (UV-1800PC, Shanghai Mepda Instrument Co., Ltd.).

$2.2.2 \, \delta^{13}C$ composition determination

The $\delta^{13}C$ isotope ratios were determined via a MAT-251 isotope ratio mass spectrometer (MAT-251, Finnigan) and calculated as Equation 1:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \tag{1}$$

 R_{sample} and $R_{standard}$ are the isotope ratios of the sample and standard, respectively. $\delta^{13}C$ values were corrected via the national standard for stable isotopes of organic carbon GBW04407 (carbon black) based on the Vienna Peedee Belemnite (PDB) standard ($\delta^{13}C = (-22.43\% \pm 0.07\%)$), and the analytical precision of $\delta^{13}C$ was less than 0.2%.

2.2.3 Carbon cycle functional genes

After microbial DNA extraction, library construction, and Illumina sequencing, species annotation was performed using kraken2 and self-built database (Wekemo Tech Co.) to annotate

TABLE 1 Physical and chemical properties of the sediments in the Wuding River.

Class	Upstream				Downstream			
	min	Max	Avg	S.D.	min	Max	Avg	S.D.
SS (mg/L)	5	2030	450.45	645.93	1,006	247,056	46,946.6	80,817.57
рН	8.35	8.86	8.7	0.14	8.26	8.94	8.58	0.2
TOC (g/kg)	0.32	2.77	0.9	0.72	0.98	4.29	2.3	1.02
AP (mg/kg)	1.14	3.88	2.3	0.91	4.08	10.56	6.66	2.1
DOC (mg/kg)	27.78	62.06	39.35	9.13	48.91	77.45	65.53	8.56
TP (g/kg)	0.17	0.73	0.36	0.17	0.39	0.65	0.48	0.07
AN (g/kg)	0.01	0.05	0.03	0.01	0.02	0.04	0.03	0.01
TN (g/kg)	0.01	0.29	0.07	0.08	0.14	0.42	0.26	0.09
C/N	6.42	46.38	20.12	14.9	6.88	10.38	8.48	1.13

Abbreviations SS, suspended solids; TOC, total organic carbon; AP, effective phosphorus; DOC, dissolved organic carbon; TP, total phosphorus; AN, effective nitrogen; TN, total nitrogen.

and categorize the clean sequences in the samples, and Bracken to predict the actual relative abundance of species in the samples. The clean reads after quality control and de-hosting were aligned to the UniRef90 protein database using HUMAnN3 software (Franzosa et al., 2018). Based on the correspondence between UniRef90 IDs and the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/) database, the gene abundance table was generated, and then the functional abundance profiles were plotted for each sample.

2.3 Statistical modeling and data analysis

Stable carbon and nitrogen isotope three-terminal elemental Bayesian Monte Carlo (Bayesian Markov chain Monte Carlo (MCMC)) models can be used to quantify the relative contributions of potential sources to DOC. The $\delta^{13}C$ – δ^{15} N isotope mixing equations read as Equation 2 (Li et al., 2020; Yan et al., 2023):

Sources of δ^{13} C and δ^{15} N end-member values for DOC were selected based on the major land-use types within and around the Wuding River Basin and previous research in the field, including: terrestrial plant/soil sources, plankton sources, and sewage sources. The mean values of δ^{13} C and δ^{15} N measured in samples from each source were defined as the end-member values (Cai et al., 2004; Liu and Xing, 2012; Yang et al., 2023). The δ^{13} C and δ^{15} N isotope ratios of terrestrial source end elements were $-25.74\% \pm 0.40\%$ and $11\% \pm 1.46\%$, respectively (Rao et al., 2017); whereas the δ^{13} C and δ^{15} N isotope ratios of zooplankton end elements were $-23.7\% \pm 0.96\%$ and $7\% \pm 0.95\%$, respectively (Gu et al., 2017; Yan et al., 2023); and the δ^{15} N isotope values of domestic sewage sources δ^{13} C: $-25.32\% \pm 1.00\%$, δ^{15} N: $10\% \pm 0.28\%$ (Machiwa, 2010). δ^{15} N

isotope values were extracted by kriging interpolation and smoothing on the basis of data from the August 2023 contemporaneous study of Xu et al. (2023) (Xu et al., 2023).

The R language program package MixSIAR 3.6.3 was used to quantify the contributions of sources of DOC in the sediments of the Wuding River Basin. Statistical analyses, including correlation analysis, *t*-test, and principal component analysis (PCA), were performed via Origin 2024 software (Microcal Software, Inc., Northampton, MA). Graphs for this study were plotted via ArcGIS 10.5 and Origin 2024b software.

3 Results

3.1 Physical and chemical properties of river sediments in the Wuding River basin

The SS and physicochemical properties of the river sediments in the Wuding River Basin exhibited spatial heterogeneity (Table 1). The overall variation in the SS content in the river was in the range of 5-247056 mg/L, with significantly higher SS concentrations downstream than upstream (Supplementary Figure S1). Sediment pH exhibited alkaline to strongly alkaline conditions (range: 8.26-8.94; mean \pm SD: 8.63 \pm 0.18, n = 21), with significantly higher values observed upstream (8.70 ± 0.14) than downstream (8.58 ± 0.20) (p > 0.05; Figure 3A; Supplementary Table S1). The TOC content ranged from 0.32 to 4.29 g/kg, the mean value of the upstream TOC content was 0.90 g/kg, the mean value of the downstream TOC content was 2.30 g/kg, the TOC content increased by 155.6% from upstream to downstream, and there was a significant difference between the upstream and downstream values (p < 0.05) (Figure 3B; Supplementary Table S1). The mean TP concentration was 0.36 g/kg upstream and 0.48 g/kg downstream, with coefficients of variation of 47.22% (upstream) and 14.58% (downstream), respectively, and the spatial distribution showed a trend towards downstream enrichment, where the TP concentration increased by 33.3% from upstream to downstream (Figure 3C; Supplementary Table S1). The range of AP concentrations was 1.14-10.56 mg/kg, and the mean AP concentration was 2.30 mg/kg upstream and 6.66 mg/kg

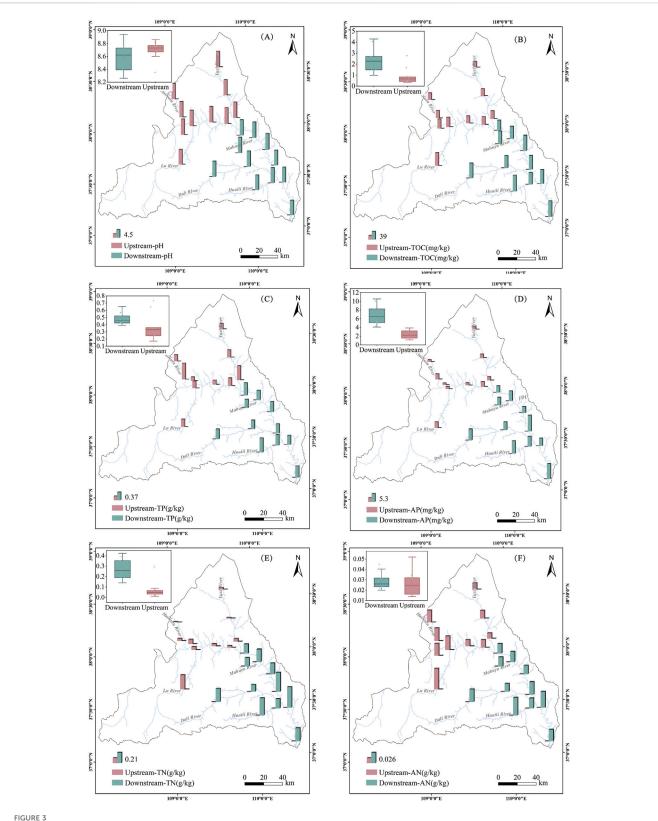
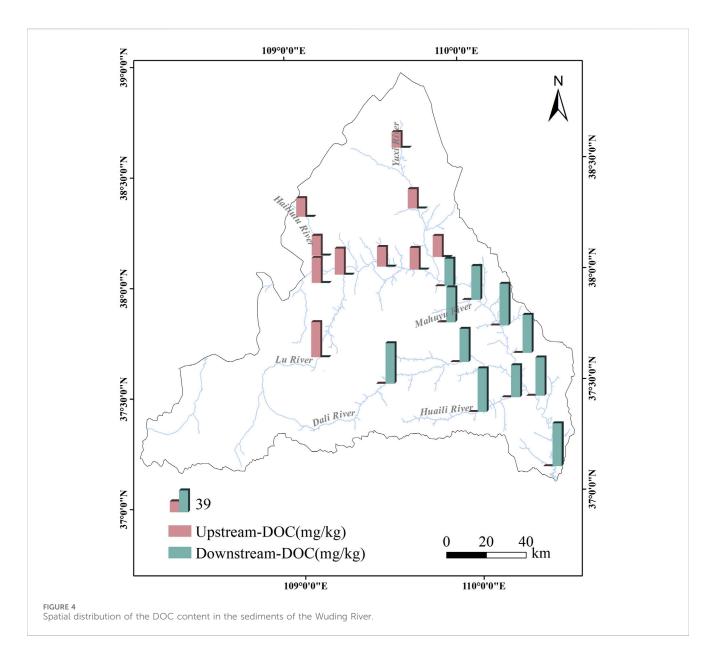


FIGURE 3
Spatial distribution of physical and chemical properties in the sediments of the Wuding River. These factors in the subplot were pH (A), TOC (B), TP (C), AP (D), TN (E), AN (F).



downstream, with significant differences (p < 0.001) between upstream and downstream AP (Figure 3D; Supplementary Table S1). The mean TN concentration was 0.07 g/kg upstream and 0.26 g/kg downstream, and the TN concentration increased by 271.4% from upstream to downstream, with a significant difference between upstream and downstream (p < 0.001) (Figure 3E; Supplementary Table S1). The mean AN content both upstream and downstream was 0.03 g/kg, and there was no statistically significant difference between upstream and downstream (p > 0.05) (Figure 3F; Supplementary Table S1). In brief, AP, TN and TOC were significantly different upstream and downstream.

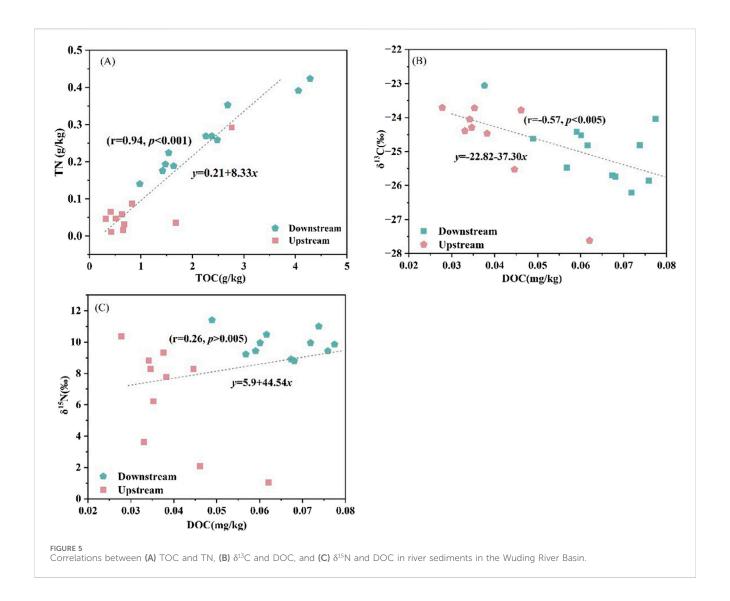
3.2 DOC geochemical characterization of river sediments in the Wuding River basin

The DOC content of river sediments in the Wuding River Basin ranged from 27.78 mg/kg to 77.45 mg/kg (mean 53.06 \pm

15.78 mg/kg). The mean DOC content was 39.35 mg/kg upstream and 65.53 mg/kg downstream, and the DOC content increased by 66.53% from upstream to downstream; the difference in the DOC content between upstream and downstream areas was significant (p < 0.001) (Figure 4).

The C/N values, δ^{13} C and δ^{15} N compositions of river sediments in the Wuding River Basin ranged from 6.42 to 46.38, -27.62-23.06, and 1.04%-11.40%, respectively. The C/N values ranged from 2.12 to 29.22 (mean 9.84) upstream and from 1.60 to 4.21 (mean 2.71) downstream; the δ^{13} C ratio ranged from -23.06-27.62% (mean 6.58% \pm 1.22%) upstream and δ^{13} C ranged from -24.04 to -26.21% (mean -25.11% \pm 0.68%); the δ^{15} N ratio ranged from 1.04% to 10.36% (mean 6.85% \pm 3.07%) upstream; and the δ^{15} N ratio ranged from 8.80% to 11.40% (mean 9.86% \pm 0.79%) downstream (Table 1).

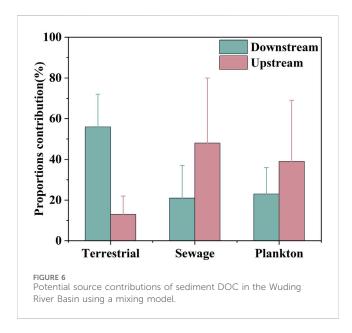
In this study, TOC and TN were significantly positively correlated (r = 0.78, p < 0.001, Spearman's rank correlation



coefficient) (Figure 5A), and the strong correlation between DOC and TN may indirectly indicate the stability of source singularity or the mixing ratio. The C/N values for the sediments in the Wuding River Basin ranged from 7.12 to 43.80 (mean 14.05), with upstream C/N values ranging from 6.42 to 46.38 (mean 20.12) and downstream C/N values ranging from 6.88 to 10.38 (mean 8.46); in this study, the mean C/N value was 13.77, indicating a mixed source. There was a significant negative correlation between $\delta^{13}C$ and C/N (Figure 5B), and in general, $\delta^{\scriptscriptstyle 13}C$ values of terrestrial higher (approximately -25 to -30%) were lower than those of aquatic organisms (approximately -20% to -25%). With increasing input of organic matter from terrestrial sources, the TOC content increases, and the proportion of terrestrial materials in sediments increases, resulting in lower δ^{13} C values. There was a positive correlation between $\delta^{15}N$ and C/N (Figure 5C), and the sediment C/N value and $\delta^{\scriptscriptstyle 15}N$ composition gradually increased from upstream to downstream of the Wuding River sediments.

3.3 Quantification of sources of DOC in river sediments in the Wuding River basin

Spatial heterogeneity was observed in the allocation of DOC sources to sediments from the Wuding River Basin. A three-terminal element mixing model was constructed with typical δ^{13} C and δ^{15} N compositions to quantify the relative contributions of three potential sources of DOC (terrestrial sources, sewage sources and planktonic sources). Figure 6 shows the results of the Monte Carlo modelling of the proportional contribution of DOC sources. In the upstream region, terrestrial sources contribute 35% ± 0.011%, sewage sources $34\% \pm 0.052\%$ and planktonic sources $39\% \pm 0.030\%$. In contrast, in the downstream region, the Terrestrial contribution increased significantly to 54% ± 0.034%. The sewage contribution decreased to 31% ± 0.018%, and the plankton contribution decreased to 18% ± 0.022%. Although the endogenous contribution remained high in the river system (34%-31%), the terrestrial contribution gradually increased as the river moved from upstream to downstream (Figure 6).



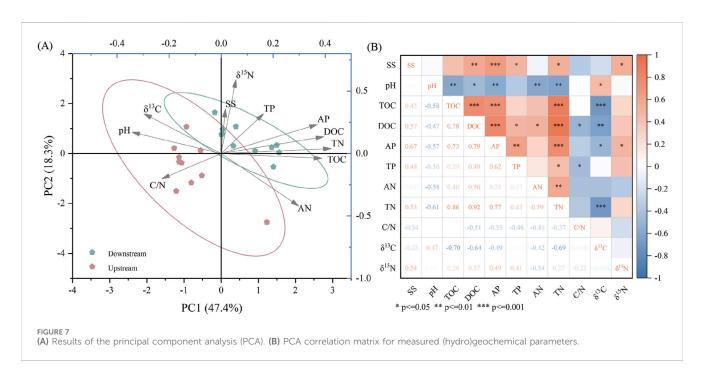
4 Discussion

4.1 Contributions of three DOC sources in river sediments in the Wuding River basin

In this study, it was found that the terrestrial contributions was significantly higher in the downstream (55.7%) than in the upstream (12.7%) (Figure 7; Supplementary Table S2). Although the land use within the 5 km buffer zone of the whole Wuding River basin is dominated by grassland and cultivated land, and there is no significant difference in the proportion of cultivated land coverage between the upstream and downstream (Figure 2), the downstream is more directly adjacent to the intensive agricultural activities and urban areas. This spatial proximity means that

downstream receiving sites are more susceptible to surface runoff input from these areas of high human activity. This spatial proximity means that the downstream is more susceptible to surface runoff input from these areas of high human activity. The contribution of terrestrial organic matter is significantly enhanced by fertilizer, sewage, and associated fulic acid exogenous organic carbon carried by surface runoff (Lambert et al., 2017; Varma et al., 2022). Wu et al. (2023) supported this view by finding that the concentration of biodegradable organic matter (BDOM) in urbanized areas was significantly higher than that in natural areas, and that the proportion of agricultural and urban land was positively correlated with the fluorescence fraction of terrestrial sources (Wu et al., 2023). It follows that the intensity of human activity, rather than the simple proportion of LU types, through enhanced surface runoff input, is the key factor driving the significantly higher contribution of downstream terrigenic sources. In addition to anthropogenic inputs, differences in DOC sources between upstream and downstream may also be influenced by other factors, such as potential differences in the specific species composition of C3 plants in vegetation communities, soil organic matter turnover processes, and changes in river hydrodynamic conditions such as water velocity through sediments and residence time of organic matter. For example, the δ^{13} C values of sediments in the Wuding River basin fall within the typical range of C3 plants (-29.5\% to -24.4\%) in both the upper (-23.06 to -27.62%) and lower (-24.04 to -26.21%) reaches (Zhuang et al., 2023). These factors may work together to result in a more significant terrigenous organic matter signal in downstream sediments.

Sewage sources contributed more to the upstream (48%) than to the downstream (20.8%). The mean $\delta^{15}N$ composition of the upstream was 6.85% (Supplementary Table S2), and the sources were usually more complex in the downstream of the river than in the upstream, e.g., the degree of humification and aromaticity of CDOM were significantly higher in the tributaries of the Qinghai



Lake Basin than in the lake itself, with the dominant role of terrestrial source input in the downstream (Yu X.-Q. et al., 2022). In addition, Zhang et al. who studied the analysis of organic matter sources in sediments from Qingpu District, Yangtze River Delta, also showed that the contribution of soil organic matter was as high as 96.8%, diluting the proportion of sewage sources (Zhang Z.-B. et al., 2022).

The contribution of organic matter from planktonic sources was greater upstream (approximately 39.3%) than downstream (approximately 23.4%) (Supplementary Table S2). The greater contribution of the plankton source (39.3%) in the upper reaches was due to two main aspects: first, the plankton source was associated with the δ^{13} C value, and when the productivity of the water body was relatively high, the plankton consumed DOC rapidly, resulting in weakened fractionation and high δ^{13} C, as the mean value of δ^{13} C in the upper reaches of the present study was -24.52% higher than that in the lower reaches of the study, which was -25.11%. Second, the geomorphological type of the upstream area is a windy sandy beach area with flat topography and slow flow, and the slow flowing waters of the river can increase the light intensity (Walks, 2007). In addition, the water quality is better, which can provide a favourable environment for the growth of plankton (Wen et al., 2020). In the downstream area, the reduced contribution of phytoplankton sources was influenced mainly by the input of organic matter from terrestrial sources. Moreover, decreased productivity and negative δ^{13} C (mean -25.11%) due to nutrient dilution or light limitation may have overlapped with C3 plant signals from terrestrial sources, which presently led to the reduced contribution of planktonic biogenic sources.

4.2 Drivers of DOC burial and degradation in river sediments in the Wuding River basin

The strong sediment transport characteristics of the Wuding River Basin lead to highly dynamic carbon exchange at the sediment—water interface. With a high sediment background, DOC regulates its source—sink function by altering environmental conditions and carbon burial efficiency, which in turn affects carbon sink potential of the basin. Therefore, in order to better understand the role of DOC in regulating the health of river water quality, it is necessary to consider the driving factors that influence the burial and degradation processes of DOC. In this study, PCA analysis showed that DOC was negatively correlated with pH, C/N, and δ^{13} C, and positively correlated with AP, TN, and TOC (Figure 7A). The generated heatmap also showed that DOC was significantly correlated with pH, TC, AP, TP, AN, TN, C/N, δ^{13} C, δ^{15} N, and other indicators (Figure 7B).

4.2.1 SS

SS, which contains a large amount of particulate organic carbon, is an important component of carbon flux (Song et al., 2023). In this study, DOC and SS were found to be significantly positively correlated (r=0.57, p < 0.01), and the concentration of suspended solids downstream of the watershed was significantly higher than that upstream, which was mainly because, during intense hydraulic erosion, the surface soil (rich in organic matter) and the deeper mineralized sediments were washed into

the river channel at the same time, and the suspended-sediment-attached microorganisms were able to degrade the particulate organic carbon in the sediment transport process, which in turn promoted the conversion of particulate organic carbon to DOC (Gao et al., 2002; Morán and Estrada, 2002). A high suspended sediment concentration is often accompanied by a high organic carbon content and low dissolved oxygen saturation. Xia et al. (2009) reported that the smaller the particle size of the suspended sediment is, the larger the specific surface area and the higher the organic carbon content (Xia et al., 2009), which further confirms the significant positive correlation between the DOC and SS contents obtained in this study.

4.2.2 pH

pH was negatively correlated with DOC (r = -0.47, p < 0.05) (Figure 7A), which was consistent with the results of a previous study (Guo ZhiHua et al., 2014). pH affects the adsorption capacity of minerals for DOC mainly by influencing the surface charge and adsorption sites of minerals in sediments (Wang Lei et al., 2017). A lower pH results in more positively charged particles and favours the adsorption of negatively charged DOC. However, higher pH limits the binding capacity of frontal clay compounds, which in turn leads to lower levels of DOC in river sediments (Abate and Masini, 2003; Vermeer et al., 1998; Zhang A. et al., 2024). The pH range in this study was just between 8.26 and 8.94, which is slightly alkaline. In addition, pH affects microbial activity and species. In weakly alkaline to alkaline environments, certain microorganisms are more capable of decomposing organic carbon, which may accelerate the decomposition rate of DOC. This is another reason for the decrease in DOC content (Rovira and Greacen, 1957).

4.2.3 TN

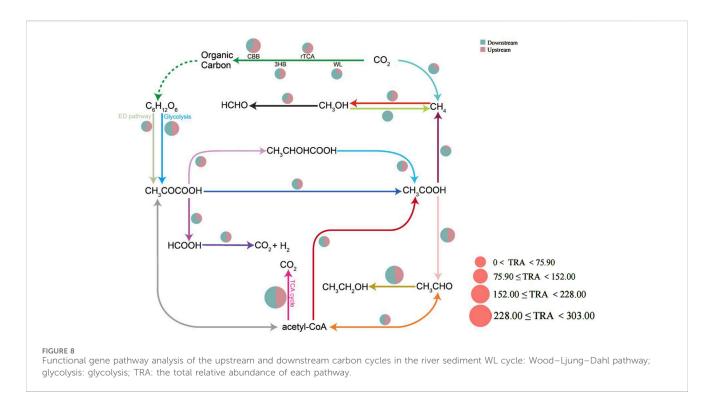
As a major nutrient in sediments, nitrogen has a very important influence on carbon fixation, distribution and accumulation as well as on plant primary productivity (Batjes, 2002). We found a strong correlation (r=0.92, p < 0.001) between the TN and TOC contents in sediments, mainly because >90% of the N in sediments is organically bound (Murphy et al., 2000), and the mechanisms of DOC and TN dynamics in sediments are usually synchronous. The effectiveness of TN directly controls the rate of DOC decomposition. The nitrogen supply can promote plant growth and thus organic carbon accumulation (Li et al., 2016).

4.2.4 C/N

C/N was significantly and positively correlated with DOC (r = -0.51, p < 0.05) (Figure 7A). The C/N value ranges from four to six and is typically less than 10. However, the C/N value at sampling points W13, W15, and W17 exceed 10, indicating that the DOC at these sampling points may influence the input of celluloserich terrestrial plants (C/N > 10). These findings indicate that the DOC at these sites may be dominated by cellulose-rich terrestrial plant inputs (C/N > 10). Terrestrial plants are composed of lignin and cellulose, which are nitrogen-poor (Yan et al., 2023).

4.2.5 AN and AP

AN was significantly and positively correlated with DOC (r = 0.50, p < 0.05) (Figures 7A,B), suggesting that changes in the content of effective nitrogen, as a plant growth limiting factor,



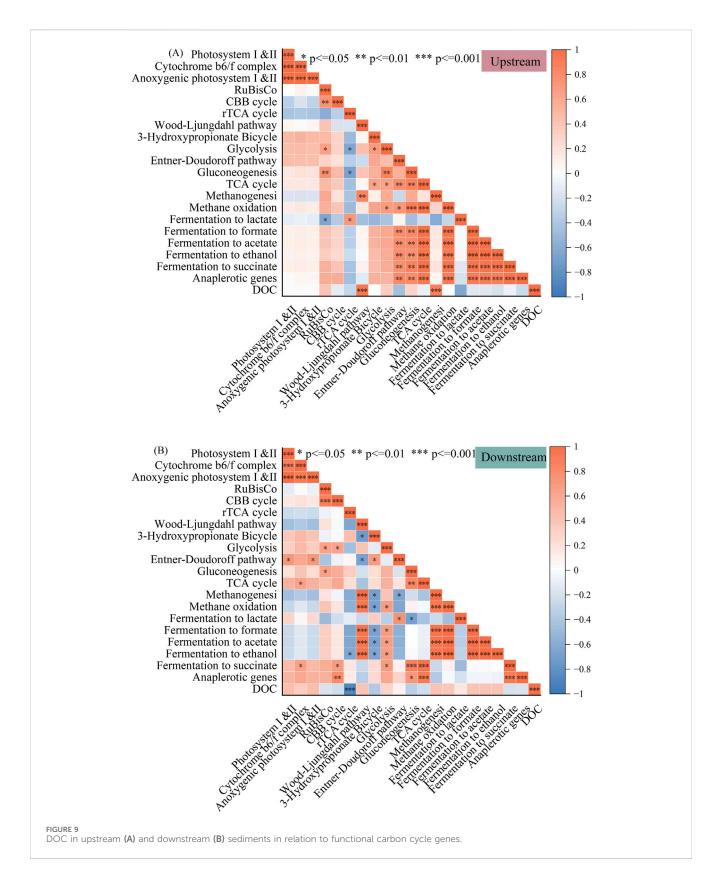
affect ecosystem organic matter output, which in turn affects DOC levels (Bei et al., 2022). Adequate AN content favours the growth and reproduction of plants, which in turn increases plant biomass (LeBauer and Treseder, 2008). Increased plant biomass means that more organic matter will enter the sediments and increase DOC levels (Cotrufo et al., 2013). Moreover, microorganisms also need nitrogen sources in the process of decomposing and transforming organic matter (Sinsabaugh et al., 2016), appropriate AN levels can maintain microbial activity and promote the decomposition and transformation of organic matter, and part of the organic carbon will be retained in the sediment during the process, causing DOC levels to rise. AP was highly and significantly positively correlated with DOC (r = 0.79 p < 0.001), and an environment with a high effective phosphorus content may promote biological activities and accelerate organic matter synthesis and accumulation. When plants grow vigorously, they will fix more carbon dioxide and synthesize more organic matter through photosynthesis, and part of this organic matter will enter the sediments in the form of apoptosis and other forms, increasing the DOC content (Huang et al., 2019). Moreover, increased microbial activity also contributes to the decomposition and transformation of organic matter (Schultz and Urban, 2008), and under suitable conditions, some organic carbon is immobilized in sediments, resulting in increased DOC contents.

4.3 Impact of DOC in river sediments of the Wuding River basin on microbially driven carbon cycling

In this study, a total of 20 carbon cycle reaction pathways were identified and predicted via the DiTing tool (CX et al., 2021). Among

them, the major contributor was the TCA cycle. Overall, the downstream carbon cycle potential was greater than the upstream carbon cycle potential, such as the TCA cycle, Wood–Ljungdahl pathway (WL), glycolysis (glycolysis), and organic acid metabolism pathways (Figure 8).

In this study, DOC was found to be correlated with the Wood-Ljungdahl pathway, the methanogen pathway, the rTCA cycle, and fermentation to lactate in the carbon cycle (Supplementary Figure S2). Among them, DOC in upstream sediments was significantly and positively correlated with methanogenesis (p < 0.001) (Figure 9A). This occurred for two main reasons: first, grassland apoplastic sediments are rich in easily decomposable carbohydrates, and their rapid decomposition can release a large amount of DOC in a short period (Wang et al., 2025); second, the high porosity of sandy sediments facilitates the migration and local enrichment of DOC (Zhan et al., 2023). Second, owing to the flat topography of windy sandy beach terrain, low surface water mobility, and slow pore water exchange in the sediment, a stable anaerobic layer is easily formed, and the presence of DOC in this environment can provide available substrate and reducing power for functional genes that act as methanogens, driving the process of CO₂ reduction to CH₄ (Sun et al., 2021; Zhou et al., 2019). In this anaerobic microenvironment, the concomitant decomposition of DOC and subsequent release of CO₂ drive carbon fixation via the Wood-Ljungdahl pathway. Therefore, DOC in upstream sediments was also significantly positively correlated (p < 0.001) with Wood-Ljungdahl (known as the reduced acetyl coenzyme A pathway, which is a key pathway for carbon fixation in the carbon cycle) (Figure 9A). Downstream, DOC in the sediments was significantly (p < 0.001) negatively correlated with the rTCA cycle (Figure 9B). This may be because high DOC



concentrations provide abundant substrates (McDonough et al., 2020) for heterotrophic microorganisms (which rely on readily available organic carbon) to rapidly acquire energy through catabolism (e.g., glycolysis, fermentation) (Liao et al., 2024)

without relying on the carbon fixation process of the rTCA cycle.

In summary, DOC is a key factor regulating carbon cycle pathways. DOC supports anaerobic carbon fixation and

methanogenesis, realizing carbon regeneration; downstream, human interference leads to excessive DOC, breaking the autotrophic-heterotrophic equilibrium and decreasing the efficiency of carbon fixation; and watershed management needs to pay attention to the coupling of DOC and microbial functionality.

4.4 Research limitations and management suggestions

- (1) This study only collected spatial scale samples at a single time node and failed to explore the trend of DOC content changes at different time scales. Future studies should comprehensively consider the changing patterns of DOC content at spatial and temporal scales to more fully understand the behavioural characteristics of DOC changes in different seasons and their impacts.
- (2) For the identification of DOC sources, the carbon and nitrogen isotopic signatures provided by other studies were used as the reference basis in this study. Since the carbon and nitrogen isotope values of different sources may overlap or have slight differences, this may lead to large fluctuations in the allocation ratios of organic carbon sources calculated by the MixSIAR model, resulting in uncertainty in the quantitative analysis results. Therefore, follow-up studies should focus on determining the actual carbon and nitrogen isotopic signatures of different local DOC sources to improve the assessment accuracy.

On the basis of the results of this study, the main sources of sediment DOC in the Wuding River Basin can be identified. To effectively control sediment DOC and increase the safety of river water quality, the following recommendations are proposed on the basis of the conclusions of this study: (1) Combine with soil and water conservation measures to reduce sediment inputs, and monitor the DOC dynamics in sediments to warn of water quality risks. (2) DOC input can be reduced by controlling agricultural surface pollution and restoring vegetation buffers to protect the anaerobic microenvironments in natural river sections.

5 Conclusion

In this study, we used stable carbon and nitrogen isotope techniques and metagenomic sequencing method to identify the drivers of the source and distribution of sedimentary organic matter (DOC) in the Wuding River Basin and their influence on the microbial-driven carbon cycling process. The main findings are as follows.

- The contents of DOC, AN, TP, TN and SS in the sediments of the Wuding River all increased from upstream to downstream.
- (2) Three sources of DOC in sediments from upstream and downstream of the Wuding River Basin were quantified

- based on the $\delta^{13}C$ and $\delta^{15}N$ three-terminal element mixing model.
- (3) The increased hydrodynamic forces from upstream to downstream promote the synergistic transport of soil nutrients and organic matter, resulting in the cumulative effect of the "transport-deposition" of suspended sediment and organic carbon downstream.
- (4) Microbial carbon metabolism is longitudinally differentiated: upstream DOC content drives the Wood–Ljung–Dahl pathway and methanogenesis for carbon regeneration, whereas downstream excess DOC content triggers an autotrophic–heterotrophic imbalance by inhibiting the rTCA cycle.

The research results provide a key scientific basis for carbon sink regulation and the ecological management of high-sediment rivers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

MX: Writing - original draft, Writing - review and editing, Conceptualization, Data curation, Formal Analysis, Methodology, Software, Visualization, Supervision. NX: Data curation, Investigation, Supervision, Visualization, Writing - review and editing. BH: Investigation, Project administration, Supervision, review and editing. XG: Investigation, Writing - review and editing. ZW: Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review and editing, Software. XZ: Investigation, acquisition, Project administration, Supervision, Writing - review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2025.1631894/full#supplementary-material

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