

Longitudinal variation characteristics of stable isotope ratios of suspended particulate organic matter in the headwaters of the Qingjiang River, China

Jian Gao^{1,*}, Zehui Zhang¹, Ping Zhong², Cheng Yang¹, Mingjun Liao¹ and Yiying Jiao¹

¹ Hubei Province Key Laboratory of Ecological restoration of Lakes and Rivers and Algal Utilization, School of Civil and Environmental Engineering, Hubei University of Technology, Wuhan 430068, PR China

² Department of Ecology and Institute of Hydrobiology, Tropical and Subtropical Aquatic Ecological Engineering Center of the Ministry of Education of China, Jinan University, Guangzhou 510630, PR China

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Abstract – To determine the sources and characteristics of suspended particulate organic matter (SPOM), the spatial distribution of carbon and nitrogen and their isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were measured from upstream to downstream (*i.e.* site 1 to site 4) in the head waters of the Qingjiang River in central China. The mean annual SPOM $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values varied between sites but exhibited a unimodal pattern. The mean annual $\delta^{15}\text{N}$ increased from site 1 (2.5‰) to 3 (5.3‰), followed by a major decrease to 2.2‰ at site 4. Furthermore, the mean annual $\delta^{13}\text{C}$ varied unimodally, being the most positive at sites 1 (−21.6‰) and 4 (−22.8‰) followed by sites 2 (−24.5‰) and 3 (−26.4‰). In particular, the mean SPOM $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the tailwaters from a domestic wastewater treatment plant, which was located approximately 0.3 km upstream of site 4, were 2.2‰ and −25.6‰, respectively. The SPOM C/N values from stream water at site 4 (8.5 ± 1.5) and tailwater (6.2 ± 0.9) were similar. Collectively, the results suggested that wastewater treatment plant tailwater influenced the stable isotope values of SPOM in the stream and affected the variation trend from upstream to downstream.

Keywords: Suspended particulate organic matter / stream / stable isotope / anthropogenic pollution / tailwater

Résumé – **Caractéristiques de la variation longitudinale des rapports d'isotopes stables de la matière organique particulaire en suspension dans les eaux d'amont du fleuve Qingjiang, Chine.** Afin de déterminer les sources et les caractéristiques des particules organiques en suspension (SPOM), la distribution spatiale du carbone et de l'azote et leurs valeurs isotopiques ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) ont été mesurées d'amont en aval (c'est-à-dire du site 1 au site 4) dans le cours supérieur du fleuve Qingjiang, en Chine centrale. Les valeurs annuelles moyennes $\delta^{15}\text{N}$ et $\delta^{13}\text{C}$ de la SPOM variaient d'un site à l'autre, mais présentaient un schéma unimodal. La moyenne annuelle de $\delta^{15}\text{N}$ a augmenté du site 1 (2,5‰) à 3 (5,3‰), suivie d'une baisse importante à 2,2‰ sur le site 4. En outre, la moyenne annuelle de $\delta^{13}\text{C}$ a varié de manière unimodale, étant la plus positive sur les sites 1 (−21,6‰) et 4 (−22,8‰), suivie par les sites 2 (−24,5‰) et 3 (−26,4‰). En particulier, les moyennes $\delta^{15}\text{N}$ et $\delta^{13}\text{C}$ des SPOM dans les eaux résiduaires d'une station d'épuration des eaux usées domestiques, qui était située à environ 0,3 km en amont du site 4, étaient respectivement de 2,2‰ et −25,6‰. Les C/N valeurs des SPOM des eaux de ruissellement du site 4 ($8,5 \pm 1,5$) et des eaux résiduaires ($6,2 \pm 0,9$) étaient similaires. Collectivement, les résultats suggèrent que les eaux résiduaires des stations d'épuration des eaux usées influencent les valeurs isotopiques stables de SPOM dans le cours d'eau et affectent la tendance de variation d'amont en aval.

Mots clés : Matière organique particulaire en suspension / cours d'eau / isotope stable / pollution anthropique / eaux résiduaires

*Corresponding author: jgao13@hotmail.com

Suspended particulate organic matter (SPOM) is an important component of stream systems and is a major pathway of organic matter transport and export in a watershed (Ulseth and Hershey, 2005; Lambert *et al.*, 2017). SPOM is derived from various sources, including autochthonous algae and aquatic plants, allochthonous terrestrial plants, and faecal and animal detritus (Cummins, 1974; Ngugi *et al.*, 2017; Hou *et al.*, 2019).

The stable C isotopic values of SPOM can indicate primary productivity and CO₂ concentration (Gu *et al.*, 2006) and track the sources of organic matter (Gu *et al.*, 2011; Hou *et al.*, 2019). The stable N isotopic values of SPOM has been used to help understand various nitrogen cycling processes such as dissolved inorganic nitrogen (DIN) uptake and nitrogen fixation (Gu *et al.*, 2006) and anthropogenic pollution (Ke *et al.*, 2017; Lambert *et al.*, 2017) in aquatic environments. In general, stoichiometric C/N ratios of SPOM are higher for allochthonous terrestrial plants than for algae and aquatic plants (Anderson and Sedell, 1979; Atkinson *et al.*, 2009; Lu *et al.*, 2014). In addition, anthropogenic organic matter tends to increase the δ¹⁵N values of aquatic organisms. The nitrate δ¹⁵N value of air is close to 0‰, whereas those of septic water, wastewater, and manure are high, varying from 10‰ to 22‰ (McClelland and Valiela, 1998; Anderson and Cabana, 2006). Longitudinal variations in the δ¹⁵N characteristics of stream POM can be used to identify N sources in waterways because they provide information on the N source and major biogeochemical processes in aquatic ecosystems (Vander Zanden *et al.*, 2005; Finlay and Kendall, 2007; Ryu *et al.*, 2018; Xuan *et al.*, 2019). So the stable C and N isotopic values and C/N ratios of SPOM have been widely used in aquatic biogeochemistry to identify the sources (terrestrial, freshwater, or marine) and fate of organic matter in aquatic ecosystems based on their unique range of values for different sources (Kendall *et al.*, 2001; Usui *et al.*, 2006; Jha and Masao, 2013).

The Qingjiang River, a subtropical river in Enshi, Hubei Province, Central China, is the first large tributary and water source protection zone in the middle of the Yangtze River after it passes through the Three Gorges Dam (China GEBE, 1993; Cao and Yang, 2015). A distinct longitudinal pattern was observed in the nutrient levels in the Qingjiang River's headwater stream (Liu *et al.*, 2018). Tolerance sensibility of benthic macroinvertebrates along water flow direction was decreased from the mountains (upstream) to urban (downstream) sites in the Qingjiang River's headwater stream,

indicating water quality degradation as anthropogenic pollution (Pan *et al.*, 2018). Our study aimed to determine variations in the stable isotopes of C and N from upstream to downstream in the head waters of the Qingjiang River. We hypothesised that the δ¹⁵N values would increase, whereas δ¹³C values would decrease from upstream to downstream as the river is influenced by human activities with associated pollutions.

The total length of this river is 423 km (Cao and Yang, 2015), and the headwater stream of this river selected for this study was approximately 76.8 km. Samples were collected from upstream to downstream at four sites (sites 1–4; Fig. 1). Site 1, the most upstream site, was located in a sparsely populated residential area and is considered the most pristine site because the riparian vegetation consists of intact shrubs and forests (Tab. 1; Liu *et al.*, 2018). Site 2 was located in the middle of a village. The dominant land used here was for fields and residence, and the site was therefore likely contaminated with manure, farmland fertiliser, and other materials. Site 3 was located downstream of Wangying town, which has a population of approximately 25 000 but no wastewater treatment plant. Site 4 was located downstream of Lichuan City, which has a population of approximately 66 000 people and a wastewater treatment plant situated approximately 0.3 km upstream. Tailwater from this treatment plant flows into the river (Tab. 1; Liu *et al.*, 2018). The sewage treatment plant was built in 2010 and uses an oxidation ditch process.

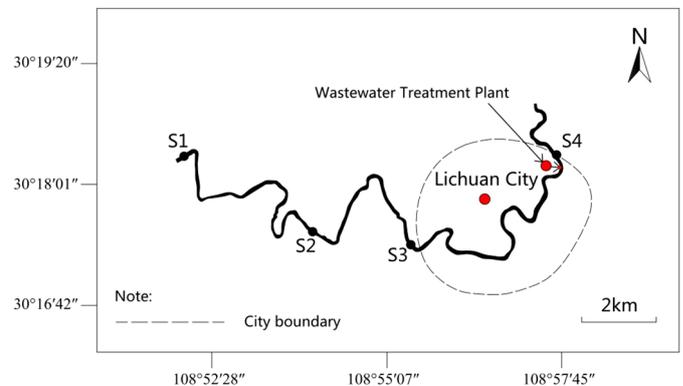


Fig. 1. Location of sampling sites in the headwater stream of the Qingjiang River. S1 to S4 denote sampling sites 1 to 4. Wastewater treatment plant located approximately 0.3 km upstream of the sampling site 4. Tailwater flowed into the stream.

Table 1. Features of sampling sites in the headwater stream of the Qingjiang River.

Sites	Land use type	Distance from the fountainhead (km)	Land used for urban, rural, industrial, mining, and residential purpose (km ²)	Description
1	Intact shrubs and forests	0.75	0.33	The most upstream site on the stream transect was located in a sparsely populated area.
2	Fields and village	18.25	1.0	Potentially contaminated with manure, farmland fertiliser, and other materials.
3	Fields, village, and town	66.65	4.64	Potentially contaminated with manure, farmland fertiliser, and other materials.
4	Urban fringe	77.55	12.16	Wastewater treatment plant located approximately 0.3 km upstream of the sampling site. Tailwater flowed into the stream.

The plant treats 20 000 tons of wastewater per day. The chemical oxygen demand (COD_{Cr}), total nitrogen (TN), ammonia–nitrogen, and total phosphorus (TP) concentrations of wastewater discharge are 60, 20, 8 (or 15 mg/L when the temperature is <12 °C), and 1.5 mg/L, respectively (China EPA, 2002).

Sampling was conducted bimonthly in odd-numbered months from May 2016 to March 2017. Water temperature (WT) was measured using a YSI metre (YSI ProPlus, Yellow Springs, OH, USA). Water samples for chemical and chlorophyll *a* (Chl *a*) were collected at 0.5-m depth below the water surface (surface water collected if the depth is <0.5 m) from three stations at each site and pooled. Approximately 2.5 L of water was stored in a cooling box and transported to the laboratory where it was analysed according to standard methods (China EPA, 2009), basically corresponding with US standards (APHA, 1998). The Chl *a* concentration was determined spectrophotometrically after sample filtration through cellulose acetate filters and extraction of the filtered materials with 90% acetone; TP and TN concentrations were determined spectrophotometrically after digestion with persulphate; the potassium permanganate method was used to determine the chemical oxygen demand (COD_{Mn}) (China EPA, 2009).

To analyse the stable isotope composition of SPOM in stream water and tailwater from domestic wastewater treatment plants, water was passed through precombusted GF/F filters, which were subsequently freeze dried. All stable isotope samples were stored in a desiccator containing dried allochroic silica gel before analysis. Thereafter, all samples were analysed using a Vario PYRO Cube elemental analyser coupled to an Isoprime-100 isotope ratio mass spectrometer at the Environmental Stable Isotope Laboratory, Chinese Academy of Agriculture Sciences. Stable isotope ratios are expressed in the delta (δ) notation, defined as parts per thousand deviations from a certified standard; $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$, where R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were a secondary standard of known relation to the international standard of VPDB and atmospheric nitrogen, respectively. The standard errors of the mean for replicates of the same tissue were 0.2‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.

For statistical analyses, we used SPSS 19.0 for Windows, and all data sets were examined for normality and transformed when needed using $\ln(x)$ before analyses (Box and Cox, 1964). Spearman correlation analysis was used to determine significant correlations between physicochemical factors and stable isotope parameters. The differences in physicochemical factors and stable isotope parameters between sites were assessed using repeated-measures analysis of variance (ANOVA) with time as the repeated factor. If a significant difference was noted, Tukey's Multiple Comparison Test post hoc tests were used to detect which treatments differed. Before performing ANOVA, we tested whether the data met the assumptions of homogeneity of variances by using Levene's test. If the assumption was not met, then we used Kruskal–Wallis nonparametric ANOVA.

The WTs were 16.5 °C in May 2016, 20.7 °C in July 2016, 18.4 °C in September 2016, 9.9 °C in November 2016, 6.1 °C in January 2017, and 9.6 °C in March 2017. The mean annual

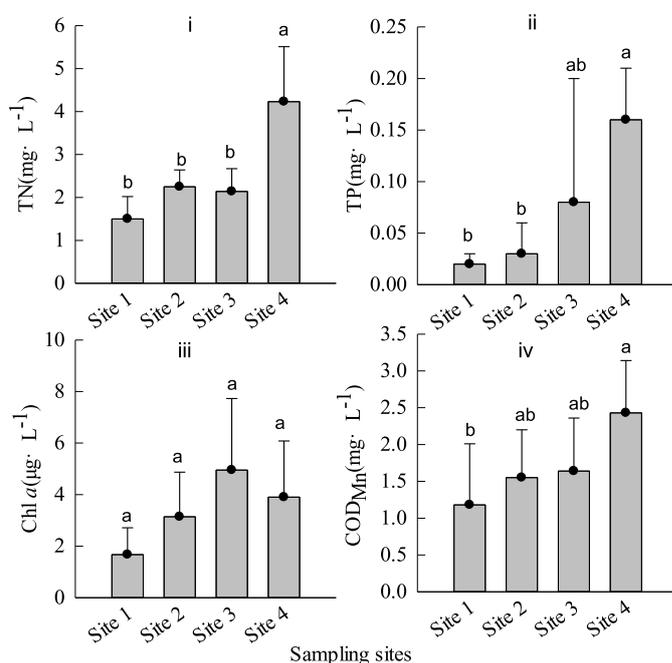


Fig. 2. Chemical and biological characteristics of the headwater stream of the Qingjiang River (mean ± standard deviation). Vertical lines indicate standard deviation. Figure i, ii, iii and iv denote TN, TP, Chl *a* and COD_{Mn} concentrations, respectively. Letters a and b indicate significant ($p < 0.05$) differences for physicochemical factors. Sampling sites that do not have a significantly different concentrations share a common letter.

concentrations of TN and TP increased from upstream to downstream (Fig. 2i, ii). TN concentrations did not differ significantly between sites 1–3, whereas it increased markedly to 4.23 mg·L⁻¹ at site 4 (one-way ANOVA, Tukey's Multiple Comparison, $p < 0.01$; Fig. 2i). TP concentrations ranged from 0.02 to 0.16 mg·L⁻¹, and higher at site 4 than at site 1 and 2 (one-way ANOVA, Tukey's Multiple Comparison, $p < 0.05$, Fig. 2ii). Chl *a* concentrations were low at all sites, ranging from 1.7 to 5.0 µg·L⁻¹ (Fig. 2iii). COD_{Mn} concentration was higher at site 4 than at site 1 (one-way ANOVA, Tukey's Multiple Comparison, $p < 0.05$, Fig. 2iv), but low values were recorded at all sites (Fig. 2iv). The high mean concentrations of TN, TP, and COD_{Mn} observed at downstream sites 2 and 3 were probably due to the nutrient contributions from village and town wastewater, agricultural areas, and livestock farming areas (Tab. 1) as by Liu *et al.* (2018). The high annual mean concentrations of TN observed at site 4 were probably due to the nutrient contributions from the wastewater treatment plants tailwater (Tab. 1, Fig. 1). Similar studies suggested that discharge from the domestic wastewater treatment plant played a crucial role in affecting nutrients dynamics of stream (Tachibana *et al.*, 2001; Jha and Masao, 2013).

The mean $\delta^{15}\text{N}$ value at upstream locations increased from site 1 to site 3, ranging from 2.4‰ to 5.3‰, but decreased to 2.2‰ at site 4 (Fig. 3i). The SPOM $\delta^{15}\text{N}$ values increased from site 1 to site 3 from upstream to downstream, reflecting anthropogenic nitrogen pollution. The primary sources of anthropogenic nitrogen were sites 2 and 3 in the form of waste

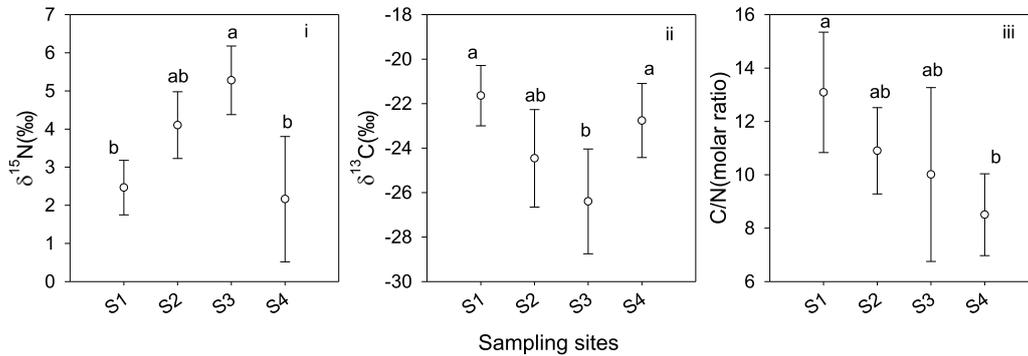


Fig. 3. Mean annual SPOM $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and molar C/N ratio in the headwater stream of the Qingjiang River. Vertical bars indicate standard deviations. Figure i, ii and iii denote SPOM $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$ values, and molar C/N ratio, respectively. Letters a, b, and c indicate significant ($p < 0.05$) differences for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N ratio. Sampling sites that do not have significantly different values share a common letter.

from livestock farming, and sanitary wastewater. The effluents are routinely discharged directly into the stream without any treatment. Hence, increases in SPOM $\delta^{15}\text{N}$ at downstream locations may be attributable to nitrogen pollution from anthropogenic organic matter. Furthermore, [Toda *et al.* \(2002\)](#) and [Ning *et al.* \(2013\)](#) have reported a significant positive relationship between the SPOM and periphyton $\delta^{15}\text{N}$ values and the percentage contribution to nitrogen loading from sewage and livestock waste. However, in the present study, unexpectedly, the SPOM $\delta^{15}\text{N}$ drastically decreased at site 4, with high concentrations of TN and TP, which was situated close to the wastewater treatment plant (only 0.3 km at the upstream location of site 4). The $\delta^{15}\text{N}$ values of stream water at site 4 ($2.2\text{‰} \pm 1.6\text{‰}$) (Fig. 3i) and wastewater treatment plant tailwater ($2.2\text{‰} \pm 0.6$) were similar in our studies. Previous studies reported that isotopic values of sewage solids varied in terms of $\delta^{15}\text{N}$ (mean, $+3.2\text{‰}$; range -1.1 to $+7.2\text{‰}$; $n = 7$) ([Van Dover *et al.*, 1992](#); [Gearing *et al.*, 1991, 1994](#); [Andrews *et al.*, 1998](#); [Ulseth, 2003](#)). Our results suggested that that sewage solids from tailwater contributed to the SPOM composition at site 4.

Additionally, we noted that the SPOM $\delta^{13}\text{C}$ varied unimodally; it was most positive at site 1 ($-21.6\text{‰} \pm 1.4\text{‰}$), with low concentrations of TN and TP, and at site 4 ($-22.8\text{‰} \pm 1.7\text{‰}$), with higher concentrations of TN and TP than at the other two sites (Fig. 3ii). [Kendall *et al.* \(2001\)](#) indicated that the $\delta^{13}\text{C}$ values of C3 plants, C4 plants, and fresh water phytoplankton are -27‰ (-32‰ to -22‰), -13‰ (-16‰ to -9‰), and -30‰ (-42‰ to -24‰), respectively. The SPOM $\delta^{13}\text{C}$ in the headwater stream of the Qingjiang River ranged from -29.1‰ to -20.2‰ (Fig. 3ii), suggesting that most of the SPOM in the stream system is governed by Rubisco-mediated photosynthesis (Calvin cycle), but the contributions from C4-based (Hatch–Slack cycle) vascular plant organic matter might also be important. Particularly at site 1, maize (C4 plant) production on both sides of the stream may result in detritus entering the adjacent stream ecosystems, yielding high $\delta^{13}\text{C}$ values. The $\delta^{13}\text{C}$ values decreased from site 1 to site 3, suggesting that the contribution from instream algae sources gradually increased, whereas that from terrestrial sources decreased (Fig. 3ii). However, this observed trend in $\delta^{13}\text{C}$ values changed at site 4 (Fig. 3ii). The mean $\delta^{13}\text{C}$ values of SPOM were lower ($-25.6\text{‰} \pm 1.9\text{‰}$) in tailwater from domestic wastewater treatment plants than at site 4

($-22.8\text{‰} \pm 1.7\text{‰}$) in our study. However, other studies reported that isotopic values of sewage solids varied in terms of $\delta^{13}\text{C}$ (mean, -23.4‰ ; range -28.5‰ to -16.5‰ ; $n = 13$; [Gearing *et al.*, 1991, 1994](#); [Van Dover *et al.*, 1992](#); [Andrews *et al.*, 1998](#)). The $\delta^{13}\text{C}$ value of sewage-effected SPOM collected from site 4 in our study (-22.8‰) was similar to the literature mean value (-23.4‰) ([Gearing *et al.*, 1991, 1994](#); [Van Dover *et al.*, 1992](#); [Andrews *et al.*, 1998](#)).

Carbon to nitrogen ratios can be also used to identify organic matter sources because the molar C/N ratios of plankton and bacteria (6–7 and 4–5, respectively) are much lower than those of organic matter derived from higher plants (>20 ; [Hedges *et al.*, 1997](#); [Sarma *et al.*, 2012](#)). The molar C/N ratio of SPOM was between 6.7 and 17.6 (Fig. 3iii), with a higher ratio observed in the upper stream (13.1 ± 2.3) than in the lower stream (8.5 ± 1.5). Molar C/N ratio were also significantly negatively correlated with Chl *a* ($p < 0.05$, $n = 24$). However, the SPOM C/N ratios were similar between stream water at site 4 (8.5 ± 1.5) and tailwater (6.2 ± 0.9) in our study, suggesting that sewage solids from tailwater contributed to the composition of SPOM at site 4. Thus, these results suggested the organic matter derived from terrestrial organic matter decreased and that from plankton or bacteria increased from site 1 to site 4.

Overall, our results revealed that nutrient input increased from upstream to downstream in the headwater stream of the Qingjiang River; however, variations in SPOM $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between sites exhibited a unimodal pattern unlike variation in nutrient concentrations. The variations in the SPOM $\delta^{15}\text{N}$ exhibited no correlation with TN and TP concentrations ($p > 0.05$, $n = 24$), suggesting that different processes affect the $\delta^{15}\text{N}$ isotopic values of SPOM samples. The unimodal variations in the SPOM $\delta^{13}\text{C}$ and the negative correlations between molar C/N ratio and Chl *a* concentration suggested that organic matter derived from terrestrial organic matter decreased and that from plankton or bacteria increased from upstream to downstream. These results suggested the tailwater from the wastewater treatment plant affected the positive relationship between the SPOM $\delta^{15}\text{N}$ value and the percentage contribution to nitrogen loading from sewage and livestock waste.

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