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ORIGINAL ARTICLE

Assessment of bacterial biomass in the highly contaminated urban Nanming River, Guiyang, SW China

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Abstract High anthropogenic N loads and abundant bacteria are characteristic of highly contaminated urban rivers. To better understand the dispersal and accumulation of bacteria, we determined contents and isotopic compositions of suspended particulate organic matter (SPOM) and bacteria in a highly contaminated urban river (the Nanming) and effluents in winter and summer of 2013. Relative to SPOM, bacterial biomass in the river was depleted in ^{13}C and ^{15}N and its C/N ratio was lower ($\delta^{13}C$: $-$ 33.2‰ ± 3.1‰; $\delta^{15}N$: $-$ 1.5‰ ± 1.2‰; C/N: 4.8 ± 0.6), while effluents showed higher ^{13}C and ^{15}N contents and C/N ratios ($\delta^{13}C$: $-$ 25‰ ± 2.1‰; $\delta^{15}N$: + 8.5‰ ± 1.1‰; C/N: 8.1 ± 1.2). Source recognition of SPOM was based on carbon isotopes because they are conservative and distinct between end-members (effluent detritus and bacterial biomass). Using a mixing model, bacterial biomass in the river was calculated to account for < 20% and < 56% of bulk suspended particulate organic nitrogen in winter and summer, respectively. An N budget showed that bacterial N was a small proportion of total nitrogen (< 7.4%) in the riverwater.

Keywords $\delta^{13}C$ · $\delta^{15}N$ · Bacterial biomass · N budget · Nanming River

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1 Introduction

Raw human wastewater usually contains high concentrations of nitrogen, phosphorus, and numerous other pollutants. Through the estuarine food web, sewage-associated contaminants transfer to aquatic consumers (McClelland et al. 1997; McClelland and Valiela 1998). Additionally, the sewage load often leads to high concentrations in the receiving river of fecal coliform bacteria that are pathogenic to humans (Han et al. 2005). The role of bacteria in carbon cycles has been examined in some aquatic ecosystems (Coffin et al. 1994). The ability of bacteria to act as links between organic and inorganic nutrients and between dissolved and particulate matter influences the production of other organisms in the microbial food web and the general cycling of nutrients in the environment. Chin-Leo and Benner (1992) found that bacterial production exceeds winter phytoplankton production in the Mississippi River plume. Because of the difficulty of isolating microbial biomass from environmental samples, direct studies on stable isotopic compositions of microorganisms have been limited (Macko and Estep 1984; Chin-Leo and Benner 1992; Coffin et al. 1994; Kelley et al. 1998).

The proportion of bacterial nitrogen in total nitrogen (TN) provides clues about the dispersal and accumulation of bacterial biomass in a receiving river. Bacterial biomass assessment also helps clarify the structure and function of aquatic ecosystems.

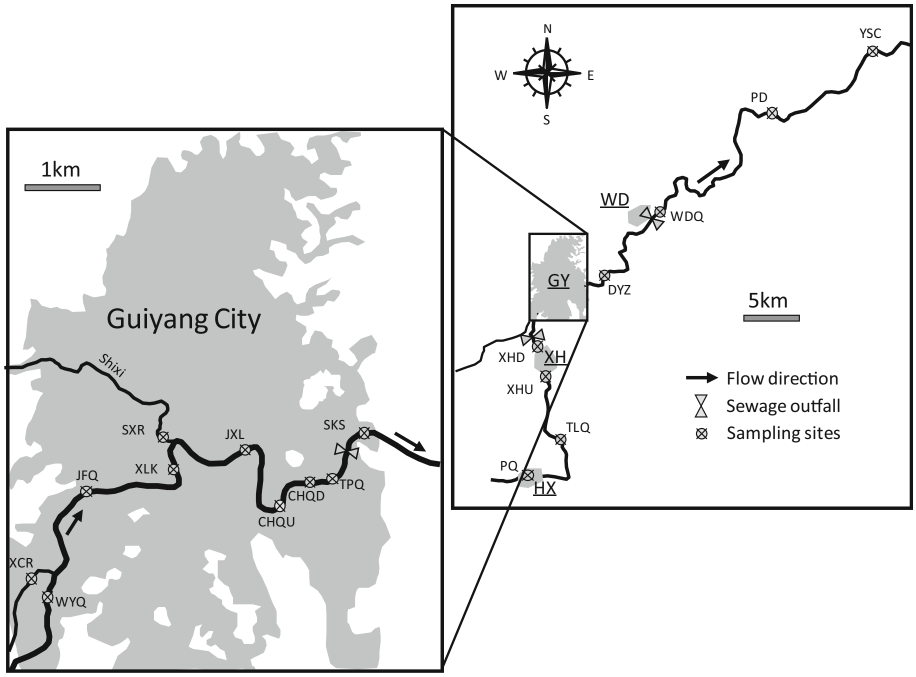

Fig. 1 Location of the sampling sites along the Nanming River. HX, XH, GY, and WD represent Huaxi, Xiaohe, Guiyang, and Wudang, respectively. Other acronyms represent sample locations described in text

2 Experimental methods

2.1 Study area

The Nanming River watershed (Fig. 1) has an area of 1433 km^2. Its headwaters are in a relatively pristine area near Huaxi. In addition to Huaxi, the Nanming River passes through two towns, Xiaohe and Wudang, and one city, Guiyang (population approximately 1.5 million, 30 km downstream of Huaxi). Around Guiyang, several man-made dams store water along the river. Downstream of Wudang, the river, running northeast, flows into the Wujiang River, a major branch of the Yangtze River.

About 450,000 m^3 of sewage effluents are discharged each day into the Nanming River without any treatment, accounting for about 40% of the annual flow of the river.[1] To address this pollution, a project of "comprehensive improvement of Nanming River water environment" with a total investment of 4.28 billion yuan has reduced Inferior V class water bodies to 7%.

The sewage outfall at Shuikousi (SKS) has been in operation since 2003. Two relatively smaller outfalls are located downstream at Xiaohe (XHD) and Wudangqiao (WDQ). The river experiences water quality problems such as low dissolved oxygen and high counts of fecal coliform bacteria (Han et al. 2005).

2.2 Sampling and analytical methods

Water samples were collected from 16 sites along the Nanming River (Fig. 1) and its two tributaries, Xiaoche River (XCR) and Shixi River (SXR), in winter and summer of 2013. In addition, sewage effluents were sampled from the SKS outfall. After sampling, water samples were immediately filtered to collect suspended particulates using Whatman glass filters (GF/F).

In order to quantify the contribution of bacteria to the suspended particulate organic matter (SPOM) in the river, microbiomes were collected using the bioassay technique reported by Coffin et al. (1989) and Kelley et al. (1998). 2 L of 0.2-μm filtered water samples collected downstream of Caihongqiao (CHQD) and at Tuanpoqiao (TPQ), as well as summer effluents, were inoculated with 20 mL of 1.0-μm filtered water samples. After being covered with black bags, the inoculated samples were incubated in situ for 48 h and then filtered through precombusted GF/F glass filters.

Suspended particulates and bacterial biomass on the filters were freeze-dried and then frozen to − 20 °C prior to elemental and isotopic determination. Nitrogen and carbon isotopic ratio samples were prepared using sealed-tube combustion and analyzed in a Finnigan MAT-252 mass spectrometer after purification with liquid nitrogen. Isotopic measurements are expressed as δ values, which are ‰ deviations from standard reference materials:

$$\delta X = \left[\left(R_{\text{sample}}/R_{\text{ref}}\right) - 1\right] \times 10^3$$

1 Cited from: How to control the environment of Nanming riverwater under the PPP path (in Chinese). Available at http://news.qx162.com/yc/2016/0802/143538.shtml.

where $X = {}^{13}C$ or ${}^{15}N$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Analysis of potassium nitrate standard (MOR2386-01, $\delta^{15}N = +1.92‰$) provided by Shoko Co., LTD, Tokyo, Japan, gave a mean (± SD) $\delta^{15}N_{air}$ value of + 1.9‰ ± 0.2‰ (n = 5). IAEA-C3 ($\delta^{13}C = -24.91‰$, cellulose) was used as a standard for $\delta^{13}C$ and the analytical precision (n = 5) was < 0.1‰.

Organic C and N contents of bacterial biomass and suspended particulates were determined by elemental analyzer (PE2400II, USA) with an analytical precision of 0.1% before removal of inorganic carbon with acid.

Fractions of bacteria-derived suspended particulate organic carbon (SPOC) (F_b) in bulk SPOC were calculated using the mixing equation:

$$F_b = (X - X_e)/(X_b - X_e) \quad (1)$$

where X, X_e, and X_b are the $\delta^{13}C$ values of the sample, effluent end-member, and bacterial biomass end-member, respectively, and F_b and F_e (given by $1 - F_b$) are the respective fractions of bacterial biomass and effluent-derived SPOC in the sample.

3 Results

3.1 Carbon/nitrogen ratios

SPOC and suspended particulate organic nitrogen (SPON) contents in waters of SXR and sewage effluent were high while those from XCR were low. From Wuyanqiao (WYQ) to Xinlukou (XLK), SPOC and SPON contents decreased, suggesting little SPOM input (e.g. algae production) between the two sites; the downstream increase from upstream of Caihongqiao (CHQU) to SKS may be due to effluent feeding from SKS outfall. The highest contents of both SPOC and SPON in the Nanming River were found at SKS (Fig. 2a, c).

C/N ratios of SPOM were high at XCR (8.7) and SXR (6.4) relative to those in the mainstem Nanming. The C/N ratios of sewage effluent detritus ranged from 7.3 to 8.9 (averaging 8.1), lower than that (12.5) reported by Andrews et al. (1998) and Thornton and McManus (1994), reflecting N enrichment. Similar C/N ratios of bacterial biomass to those reported by Nagata (1986) were observed in the river (4.8).

SPOM in the river showed low atomic C/N ratios varying from 1.7 to 9.7 (averaging 4.7 ± 2.5) in winter and from 3.0 to 9.2 (averaging 5.6 ± 1.6) in summer, significantly lower than the typical value of land-derived OM (> 20; Meyers 1994), and even lower than the reported range of 6–9 for freshwater phytoplankton (e.g. Bordovsky 1965). In the domain of Guiyang (GY), the C/N ratios were especially low in winter (< 3.5) and in summer (< 5.5), comparable to planktonic bacteria (4.8).

3.2 Carbon isotopes

SPOC in SXR exhibited similar ${}^{13}C$ content to that in effluents. ${}^{13}C$ contents of SPOC in XCR were in the range of − 27.1‰ to − 25.9‰, comparable to values upstream at Pingqiao (PQ) and Tieluqiao (TLQ). The $\delta^{13}C_{SPOC}$ in sewage effluents at GY was − 25.0‰, close to that reported by Andrews et al. (1998: − 24.8‰ ± 3.2‰), slightly heavier than that by Thornton and McManus (1994: − 26.7), but lighter than that by Van Dover et al. (1992: − 22.8‰ ± 2.9‰). Bacterial biomass in the river was highly ${}^{13}C$-depleted − 33.2‰) when compared to sewage detritus.

In the river, SPOC had $\delta^{13}C$ values from − 26.6‰ to − 24.9‰ in winter (averaging − 26.0‰ ± 0.6‰) and from − 29.6‰ to − 24.4‰ in summer (averaging − 26.7‰ ± 1.5‰). The $\delta^{13}C_{SPOC}$ values at outfalls (WYQ, SKS, and WDQ) were similar to those in sewage effluents (Fig. 2b). Around GY, SPOC was depleted in ${}^{13}C$ in the downstream direction. SPOC in summer was enriched in ${}^{13}C$ relative to winter. At sampling sites beyond the domain of GY, $\delta^{13}C_{SPOC}$ values were seasonally similar.

3.3 Nitrogen isotopes

$\delta^{15}N$ values of sewage effluents (+ 8.5‰) were heavier than those reported by Thornton and McManus (1994: + 2.3‰), Van Dover et al. (1992: + 3.3‰), Owens and Law (1989: + 1.4‰), and Sweeney et al. (1980: + 2.5‰). Because of large discharge fluxes from sewage outfalls (accounting for ~ 40% of river flux), effluent SPOM may be an important OM source. Similar values to those of effluent detritus were found at XCR (+ 8.3‰ ~ + 9.0‰), while lighter values were found at SXR (+ 2.4‰ ~ + 4.3‰). The $\delta^{15}N$ value of bacterial biomass in the river was − 1.5‰, the most negative of the above potential sources.

$\delta^{15}N_{SPON}$ values at most sites along the river (Fig. 2d)— + 2.5‰ ± 2.1‰ in winter and + 3.7‰ ± 2.0‰ in summer—were lighter than those in sewage effluents (+ 8.5‰) and heavier than that of bacterial biomass (− 1.5‰).

4 Discussion

Potential SPOM sources in riverwater usually include aquatic plants (e.g. phytoplankton), bacterial biomass, zooplankton, terrestrial plants (C_3 and C_4), soil OM, and effluent detritus. Data of all potential sources except

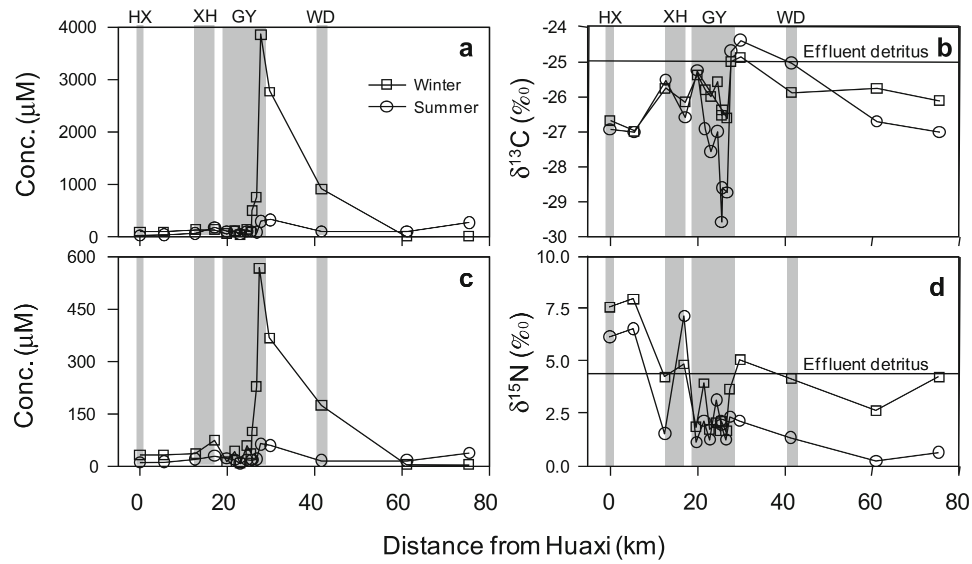

Fig. 2 Changing concentrations and isotopic compositions of suspended particulates with distance from Huaxi (HX) in winter and summer. **a**, **b**. Suspended particulate organic carbon (SPOC); **c**, **d**. Suspended particulate organic nitrogen (SPON). *XH* = Xiaohe; *GY* = Guiyang; *WD* = Wudang

aquatic animals were plotted in Fig. 3, along with SPOM values from this study. Bacterial biomass was the most ^{15}N-depleted OM and had the lowest C/N ratio. This suggests that SPOM contained a fraction of N-rich bacterial biomass. Although aquatic animals, including zooplankton and benthic fauna, may also contain high contents of N, they likely contributed little as evidenced by their more positive values than phytoplankton (Gearing et al. 1984; 18. Peterson and Fry 1987). δ^{15}N values of soil OM sampled at Guiyang and reported by Liu et al. (2006) averaged + 5.7‰ ± 2.0‰. OM in surface soils (0–20 cm) in Huaxi had atomic C/N ratios between 9.3 and 19.5, and δ^{13}C values between − 26.4‰ and − 22.3‰ (Zhu 2006). In Fig. 3, soil OM lies above and to the right of the SPOM range, suggesting a minor contribution. Sediment resuspension was not important to SPOM in the river because the former showed much higher C/N ratio than the latter (Xiao et al. 2017).

The contribution of bacterial biomass to SPOM is often neglected, though bacterial biomass has been reported as contributing more than 20% to total POM (Harvey et al. 1995). In the Nanming River, SPOM C/N ratios of river-water (winter: < 3.5; summer: < 5.5) were lower than those of freshwater phytoplankton materials (6–9; Bordovsky 1965) while comparable to that of bacterial biomass (4.8), indicating that the N-rich SPOM might contain large quantities of bacterial biomass (C/N = 3.8–6.5). This is consistent with Han et al.'s (2005) finding of an

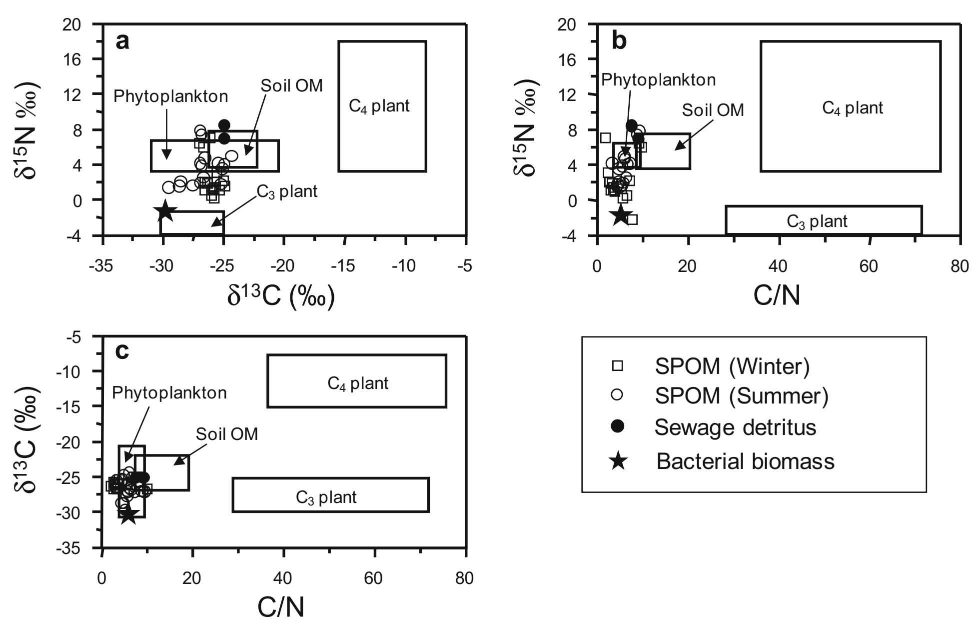

Fig. 3 Plots of δ^{15}N vs. δ^{13}C (**a**), δ^{15}N versus N/C (**b**) and δ^{13}C versus C/N (**c**). The ranges of C_3 and C_4 plants, phytoplankton, and soil organic matter (OM) are cited from Gearing et al. (1984), Peterson and Fry (1987), and Liu (2007). SPOM = suspended particulate organic matter

abundance of bacteria in the riverwater. However, selective degradation of OM components has the potential to modify the C/N ratio. Degradation of POM by bacteria generally includes two processes: N efflux from the complex and bacterial nitrogen production (microbial immobilization of nitrogenous materials), with the dominant process depending on bulk C/N ratios. On one hand, microbial decomposition of highly nitrogenous particulates (low C/N ratio) commonly results in nitrogen efflux from the bacteria/detritus complex exceeding influx via biomass production, thereby increasing bulk C/N ratios (Herczeg 1988; Meyers 1997). On the other hand, C/N ratios of nitrogen deficient debris (high C/N ratio) tends to decrease during decay as bacterial nitrogen production supersedes efflux from the POM. In waters with high dissolved organic nitrogen (DON) concentrations such as sewage-polluted waters (7.3 mg/L DON at SKS: Fu et al. 2007), bacterial production is supported more by DON than by PON (Gardner et al. 1994; Kritzberg et al. 2004). In Nanming riverwater, therefore, biomass production through "free" planktonic bacterial biomass might be dominant and its attachment to SPOM reduce C/N ratios of SPOM.

Heterotrophic bacterial populations have been demonstrated to have carbon isotopic ratios similar to the dissolved organic carbon (DOC) they used (Coffin et al. 1989; Norrman et al. 1995; Hullar et al. 1996). At SKS, effluent-polluted riverwater was abundant and rich in DOC, with a concentration of 5.10 mg/L (Fu et al. 2007). The $\delta^{13}C_{DOC}$ value in Guiyang effluents was about − 30‰ (Liu 2007), comparable to that in a leachate close to an anthropogenic source (landfill) (van Breukelen et al. 2003). So, planktonic bacterial cells supported by this DOC might have ^{13}C contents of ~ − 30‰, which coincides with our determined $\delta^{13}C_{bacteria}$ value (− 33.2‰). Investigation results by Han et al. (2005) that the number of bacteria increased from upstream to downstream are also in good agreement with our findings that $\delta^{13}C_{SPOC}$ decreased downstream. However, Kelley et al. (1998) reported that some bacteria (e.g. nitrifying bacteria) could also use dissolved inorganic carbon (DIC) as their carbon source. Therefore, incorporation of ^{13}C-enriched DIC (− 10‰ to − 6‰; Liu 2007) into bacterial biomass can partly explain the comparatively lower winter $\delta^{13}C_{SPOC}$ values (Fig. 2b). Methylotrophic bacteria may appear in highly anoxic waters, but the observed more negative $\delta^{13}C$ values in upstream waters (within the domain of GY) should not be attributable to methylotrophic bacteria that consume strongly ^{13}C-depleted CH_4 from methanogenic reactions because CH_4 might be more abundant in upstream waters due to more anoxic environments adjacent to the effluent outfall (XHD).

During microbial immobilization, attachment to detritus of the "free" ^{15}N-depleted bacterial biomass may decrease $\delta^{15}N$ values of the microbe/detritus complex (SPOM) in waters because bacteria biosynthesis is associated with loss of ^{15}N (Macko and Estep 1984). Lehmann et al. (2002) found that after 21 days incubation, a decrease in $\delta^{15}N$ was associated with decreasing C/N ratios due to contribution from ^{15}N-depleted bacterial biomass. Bacterial biomass may thus be an important component of SPOM ($\delta^{15}N$: + 1.1‰ to + 3.9‰) as it showed isotopically depleted N ($\delta^{15}N$: − 1.5‰) within the domain of GY relative to initial SPOM.

For an isotopic ratio to be successfully used as a tracer to determine the relative contribution of two sources, it must satisfy two requirements: (1) be conservative and have a distribution in natural systems that reflects only physical mixing of materials from end-member sources; and (2) have isotopically distinct end-members. In this study, these requirements were met as effluent SPOC and bacteria cells keep their original signatures after mixing with each other in the river and the $\delta^{13}C$ value (− 25‰) of effluent detritus was significantly distinct from that of bacterial biomass (− 33.2‰).

$\delta^{15}N$ may be not an effective and sensitive indicator of SPON origins because of nitrogen isotopic fractionation during aqueous SPON decomposition. Generally, breakdown of pure OM preferentially mineralizes ^{14}N, potentially causing an enrichment in ^{15}N in the residue (e.g. Thornton and McManus 1994). Preferential mineralization of more labile algal materials relative to terrestrial materials can also affect chemical and isotopic signatures of OM. This may cause a decrease in ^{15}N content because algal materials tend to be more ^{15}N enriched (~ + 8‰) and more labile than terrestrially derived materials (~ + 1‰) (Meyers and Ishiwatari 1993).

Table 1 N budget in riverwater upstream of Caihongqiao (CHQU)

	DIN (mg/L)	DON (mg/L)	SPON (mg/L)		Fraction of bacteria-N in total N (%)
			Sewage-derived	Bacterial biomass	
Winter	16.94	0.2	0.42 (80%)	0.11 (20%)	0.6
Summer	1.54	0.1	0.11 (44%)	0.14 (56%)	7.4

Data of dissolved inorganic nitrogen (DIN) and dissolved organic (DON) concentrations are from Liu (2007) and Xiao et al. (2017), respectively

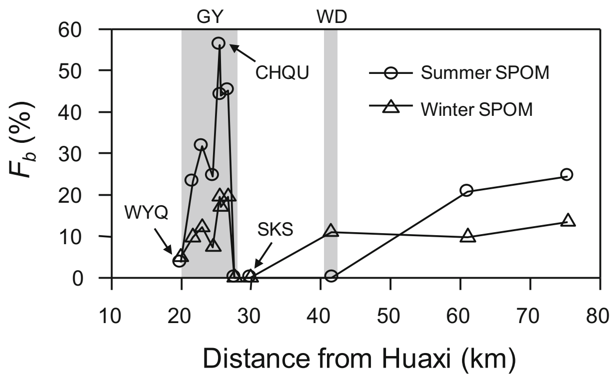

Fig. 4 Bacterial biomass contribution (F_b) to bulk suspended particulate organic matter (SPOM). GY and WD refer to Guiyang and Wudang, respectively. Other acronyms represent sample locations described in text

The calculated results by Eq. (1) at CHQU are shown in Table 1. At the site, F_b in the Nanming riverwater is 20% in winter and 56% in summer. The relatively higher F_b in summer is likely caused by the (observed) more abundant bacteria in that season (Han et al. 2005). Upstream to WYQ from CHQU, contribution of bacterial biomass decreased (Fig. 4). At CHQU, a site far from the upstream outfall, the high F_b meant a strong bacteria activity occurred, and more N immobilized by bacteria (higher production of bacterial biomass). However, even in summer, bacteria-N accounted for only a small proportion of the TN load (7.4%) in riverwater at CHQU (Table 1). On the other hand, nitrifying bacteria, which possibly used ^{13}C-enriched DIC (− 10‰ to − 6‰; Liu 2007) as their C source, might be enriched in winter compared to summer because of greater nitrification in winter (Xiao et al. 2017). These findings are in agreement with investigation results by Han et al. (2005) that bacteria were more abundant in summer than in winter and in downstream than in upstream waters. Using less negative data for $\delta^{13}C_{bacteria}$ for the winter calculation would amplify the calculated differences in F_b between winter and summer (Fig. 4).

5 Conclusion

Effluent-contaminated waters in the Nanming River contained high concentrations of bacterial biomass, which showed highly depleted ^{13}C and ^{15}N and lower C/N ratios, while isotopic values and C/N ratios of effluent detritus were higher. Carbon isotopic ratios were believed to be conservative and distinct for end-members of SPOM, and thus were an effective signature when identifying OM provenance. According to estimates from a mixing model, bacterial biomass contributed 20% and 56% to the bulk SPOM in winter and summer, respectively, at CHQU. Upstream at WYQ, the contribution of bacterial biomass decreased. However, as compared to TN in the waters, the proportion of bacteria-N remained small (< 7.4%).

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