Dark carbon fixation and chemolithotrophic microbial community in surface sediments of the cascade reservoirs, Southwest China

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HIGHLIGHTS

- First evaluation of chemolithotrophic activity in surface sediment of cascade reservoirs.
- Chemolithotrophic carbon fixation was found to play an important role in carbon cycling of sediments in cascade reservoirs.
- Hydraulic residence time and relative location of reservoirs are the key control factors of activity and composition of autotrophic microbial communities in cascade reservoir sediments.

GRAPHICAL ABSTRACT
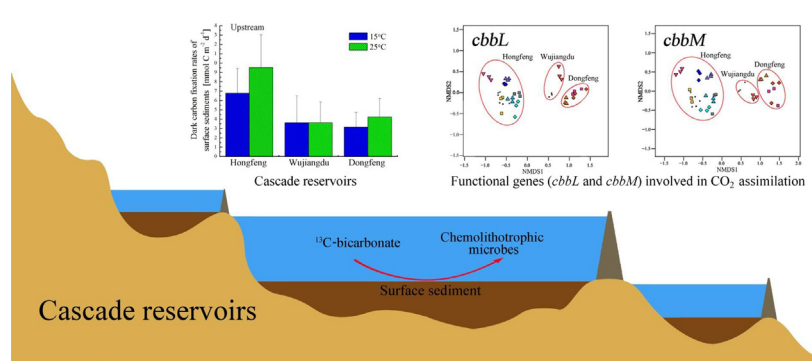
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ABSTRACT

Dark carbon fixation (DCF) by chemolithotrophic microbes can make considerable contribution to inorganic carbon fixation in aquatic ecosystems. However, little is known about the importance and diversity of chemolithotrophic microbes in cascade reservoir sediments. In this study, we determined the potential DCF rates of sediments of three cascade reservoirs in Wujiang River basin by carbon isotopic labeling. The results showed that the DCF rates of the surface sediments ranged from 1.5 to 14.7 mmol C m^{-2} d^{-1}. The ratio of DCF to mineralization rate of sediment organic matter of surface sediment was between 11.6%~60.9%. High-throughput sequencing analysis of cbbL and cbbM genes involved in Calvin Benson Cycle indicated that cbbL-carrying CO$_2$-assimilating bacteria included diverse functional groups, while cbbM type was mostly involved in sulfur oxidation. The sediments of Hongfeng (HF) reservoir, which has much longer hydraulic residence time (HRT) and locates in most upstream of a major tributary of Wujiang River, have substantially higher DCF rates. The cbbL and cbbM communities in HF were dominated by sulfur oxidizing bacteria, and were largely different from that in the other two reservoirs. Our results suggested that chemolithotrophy plays an important role in carbon cycling of sediments in cascade reservoir. Meanwhile, HRT and relative location of cascade reservoirs are the key control factors of both DCF and composition of autotrophic microbial communities in cascade reservoir sediments.

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1. Introduction

The assimilation of inorganic carbon into organic matter is one of the essential processes in ecosystems. In addition to photosynthesis, chemolithotrophic microbes can incorporate inorganic carbon and serve as an autochthonous source of organic matter in several environments (Shively et al., 1998). Chemolithotrophic microbes need the simultaneous presence of oxidized and reduced compounds that will react and provide the energy demanded to fix inorganic carbon in dark (dark carbon fixation; DCF). Sediment-water interfaces in aquatic ecosystem are often sites of intensive biogeochemical activity (Jorcin and Nogueira, 2005; Mozeto et al., 2001), oxygen is usually depleted in the upper millimeters of the sediments and reduced compounds produced in anaerobic zone can diffuse into the aerobic zone (Mozeto et al., 2001). Resultantly, surface sediments create a steep chemical gradient that provides a microenvironment with high chemolithotrophic rate (Shively et al., 1998). Ammonium and sulfur oxidation are examples of such redox reaction associated with chemolithotrophy in sediments (Francis et al., 2003; Klatt and Polerecky, 2015; Lenk et al., 2011; Vasquez-Cardenas et al., 2015). It's calculated that DCF rates were 0.01–4.0 mmol C m^{-2} d^{-1} in lake sediments (Santoro et al., 2013). Moreover, a conservative estimation showed that the global rate of oceanic DCF could be up to 0.77 Pg C per year (Middelburg, 2011). Among them, sediment chemolithotrophy (0.37 Pg C y^{-1}) accounted for 48% of the total oceanic chemolithotrophic carbon fixation rate.

Currently, seven pathways are considered to be used to fix CO_2 by chemolithotrophs (Berg, 2011; Figueroa et al., 2018; Hugler and Sievert, 2011), and the Calvin-Benson-Bassham (CBB) cycle is the most widely distributed pathway among them. The key enzyme responsible for CO_2 fixation in CBB cycle, the ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO), exists in four distinct forms (I~IV). Bacteria utilize RubisCO forms I and II (Badger and Bek, 2008), which share only 25–30% amino acid similarity (Tabita, 1988) and differ in their CO_2/O_2 substrate specificity (Badger and Bek, 2008; Tabita et al., 2007). Due to their functional significance and high degree of conservation, the large subunit RubisCO genes of form I (*cbbL*) and form II (*cbbM*) have been used as a phylogenetic marker for studying the diversity and ecology of chemolithotrophic populations in terrestrial and aquatic ecosystems (Alfreider et al., 2012; Long et al., 2015; Tourova et al., 2011). However, the genetic diversity of *cbbL*- and *cbbM*-carrying bacteria was seldom investigated in fresh water sediments (Nigro and King, 2007).

In an effort to alleviate the water shortage and increase the energy supply, over 70,000 large dams have been built in rivers worldwide (Maavara et al., 2015). China has the largest number of reservoirs in the world and numerous reservoirs are located in southwestern China (Chen et al., 2019; Lehner et al., 2011; Wang et al., 2018b). Consequences brought by damming include hydrological status variations, silt deposition, nutrients blocking and so on (Wang et al., 2011). For instance, damming has attenuated by 26% the transport of terrestrial organic carbon to the ocean (Dean and Gorham, 1998; Syvitski et al., 2005). Due to the accumulation of nutrients and settling down of microorganisms (Shi et al., 2017), damming probably will lead to strong stimulation of the DCF rates in reservoirs, together with the increase in photosynthetic primary productivity (Engel et al., 2019). Furthermore, since many reservoirs are built in cascade configuration, the consecutive damming could also influence DCF rates and result in a cascade effect (Liu et al., 2017; Shi et al., 2017). Therefore, cascade reservoirs may exhibit spatially heterogeneous DCF rates and chemolithotrophic populations. However, DCF rates and chemolithotrophic microbes have rarely been reported in reservoirs.

In this study, three karst cascade reservoirs of Wujiang River, which is located in Southwestern China and one of the earliest cascade hydroelectric development rivers in China, were chosen to study the DCF rates and community composition of chemolithotrophic microbes in the surface sediment. We hypothesize that surface sediment in cascade reservoir has strong dark carbon fixation potential and diverse autotrophic microbial populations. The objectives of this study were to evaluate the importance of DCF in the sediment of the cascade reservoir, and to investigate spatial patterns and controlling factors of the activity and community composition of chemolithotrophic microbes in cascade reservoirs.

2. Methods

2.1. Description of study area

The 1037-km-long Wujiang River (26°07′~30°22′N, 104°18′~109°22′E) is a right-bank tributary of the Yangtze River (Fig. S1), and it has an annual runoff of 53.4 billion cubic meters with a fall of 2124 m. The Wujiang River basin is located in the center of the Southeast Asian karst region, where karstification is well developed (Sweeting, 1995). Its annual average temperature is 14 °C, and the annual average rainfall is 1195 mm, occurring mostly in summer and spring. Cascade hydropower development in Wujiang River basin is one of the earliest hydropower projects in China, which is a major power source for China's West-to-East Power Transmission Project (Liu et al., 2015). Research was carried out on sediment samples collected from three reservoirs in Wujiang River basin. Main features of each reservoir are listed in Table 1. Hongfeng Reservoir (HF) locates in karst plateau, it's the first and most upstream reservoir in a major tributary of the Wujiang River basin. Wujiangdu (WJD) and Dongfeng (DF) are canyon reservoirs located on the middle reaches of the Wujiang River basin. The three reservoirs are located at an elevation between 1240 m and 760 m (Table 1), WJD is located >100 km downstream of HF and DF reservoirs. In reservoirs of the Wujiang river basin, temperature at the bottom of the water column is around 15 °C in at least half of the year, and the highest temperature of the water column could reach approximately 25 °C (Chen et al., 2019; Wang et al., 2019; Xiang, 2016).

2.2. Sediment sampling

Sampling sites (16 in total) were selected mainly according to direction of water flow and surface area of the reservoir. Sediment sampling was carried out in HF in December 2016, April 2017 and May 2017 (Named H1-H11). Sampling collection in WJD (W1, W2) and DF (D1, D2, D3) was conducted in September 2017. Detailed information of sampling sites are listed in Fig. S1 and Table S1. Sediment cores were taken in triplets in each site using a gravity corer (internal diameter 5.9 cm). No algae colonization was observed on the top of sediment cores and the overlying water was transferred to plastic carboys for later use. Sediment cores were sealed from both ends and transported vertically to the laboratory in 12 h. Sediment cores were kept in dark at 15 °C until use. The temperature at the different sampling sites is shown in Table S1.

2.3. Incubation of sediment samples

For the incubation experiment, the uppermost 2 cm depth of each sediment core was collected and homogenized. The whole process

Table 1
Main features of the cascade reservoirs sampled in this study.

Reservoir name	Year of construction	Water level (m)	Storage capacity (10^8 m^3)	Surface area (km^2)	Mean depth (m)	Hydraulic residence time (day)
Hongfeng	1960	1240	7.5	57.2	10.5	119
Wujiangdu	1979	760	21.4	21.4	44.8	49
Dongfeng	1994	970	8.6	19.1	45.2	29

was made oxygen free by flushing with pure nitrogen. A small part of the surface sediments was centrifuged and stored at −20 °C for subsequent analysis of sediment and porewater samples. The remained surface sediment samples were added into 20 ml glass vials until the depth reached 2 cm (about 7 ml in volume). Afterwards, 0.5 ml filtered overlying water and 0.5 ml 20 mM $NaH^{13}CO_3$ (99% ^{13}C; Sigma-Aldrich) were added into each vial to reach final concentration of 2 mM $NaH^{13}CO_3$. Then the vials were sealed immediately, and after gently mixed by shaking, the samples were incubated in the dark at both 15 °C and 25 °C for 4 days. Each vial was ventilated after 2 days by removing the stopper for 1 min to prevent the development of suboxic conditions in the headspace (Boschker et al., 2014). Inactivation treatment was also prepared by adding 2% (v/v) formaldehyde (final concentration) into each vial to inhibit microbial activity before incubation. Sediment organic carbon mineralization rate (CMR) was determined by incubation of the surface sediment samples with the same protocol as described above but without ventilation. At the end of the incubation, the sediment and porewater samples were collected after centrifugation, the pH values of the sediment samples were measured.

2.4. Analytical techniques

Gas samples from the vials used for CMR measurement were taken with a gas-tight pressure lock syringe (VICI, Baton Rouge, USA), after the bottles were vigorously shaken by hand, and analyzed immediately by gas chromatograph (GC). CH_4 and CO_2 were analyzed by GC equipped with flame ionization detector (FID) (HP6890, Hewlett-Packard Co., USA). CO_2 was detected after conversion to CH_4 with a methanizer (nickel catalyst at 350 °C). Porewater samples for dissolved inorganic carbon (DIC) were added to headspace vials and after acidification analyzed for DIC concentration and $\delta^{13}C$ value (Boschker et al., 2014). The anions in porewater samples were determined by ion chromatography (Dionex-ICS-90, Sunnyvale, USA). The $\delta^{13}C$ values of DIC in porewater were determined with GC-combustion-isotope ratio mass spectrometer (GC-C-IRMS) (Finnigan, Bremen, Germany). After acidification and lyophilization, the contents of organic carbon, nitrogen and sulfur of the sediment samples were determined with element analyzer (EA) (Vairo Macro CNS, Elementar, Germany), the $\delta^{13}C$ values of sediment organic carbon (SOC) were measured with EA-C-IRMS (Finnigan, Bremen, Germany). Fe (II) and Fe (III) in sediment samples were analyzed using the ferrozine reaction method (Schnell et al., 1998).

2.5. Calculation of rates of dark carbon fixation and SOC mineralization

The sediment DCF rates (R_{DCF}) were determined according to the Eq. (1), which was modified from equations described previously (Boschker et al., 2014; Wang et al., 2014):

$$R_{DCF} = (C_{org} \times W \times \Delta F_{SOC})/(\Delta F_{DIC} \times S \times D) \qquad (1)$$

where C_{org} is the SOC content (%) of the sediment samples, W is the dry weight of sediment in each vial, ΔF_{SOC} and ΔF_{DIC} denote the enrichments in ^{13}C percentage contents of SOC and DIC after the ^{13}C-bicarbonate treatment, respectively. S denotes the surface area of the sediment in the vial, D is the incubation time (days).

The SOC mineralization rates (CMR) were calculated from the accumulation of total inorganic carbon (TIC) plus CH_4 during the incubation (Moodley et al., 2005; Yuan et al., 2018). TIC was defined as the sum of gaseous, dissolved and bicarbonate CO_2. Total amounts of CO_2 and CH_4 gases in the headspace of the vials were calculated based on the partial pressures using the volume of the gas space and the gas constant. The amounts of dissolved and bicarbonate CO_2 in the liquid were calculated as previously described (Stumm and Morgan, 1981; Yuan et al., 2018).

2.6. High throughput sequencing and analysis

Total sediment DNA was extracted from approximately 0.5 g sediment sample using FastDNA SPIN Kit for soil (MP Biomedicals, USA) according to the manufacturer's protocol. The quality of extracted DNA was checked by 1% agarose gel electrophoresis. The primer pair *cbbL*_K2f (5'-ACCAYCAAGCCSAAGCTSGG-3′) and *cbbL*_V2r (5'-GCCT TCSAGCTTGCCSACCRC-3′) was applied for amplification of *cbbL* genes, primer pair *cbbM*_f (5'-GGCACCATCATCAAGCCCAAG-3′) and *cbbM*_r (5'-TCTTGCCGTAGCCCATGGTGC-3′) was applied for amplification of *cbbM* genes (Alfreider et al., 2003; Tolli and King, 2005). Both forward and reverse primers contained a barcode. PCR amplification was conducted under the following conditions: 94 °C for 3 min; followed by 5 cycles of 94 °C for 30 s, 45 °C for 20 s, 65 °C for 30 s; then, 20 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s; and finished with 72 °C for 5 min. Illumina bridge-type compatible PCR primers took the place of Bar-PCR primer F for the second PCR amplification under the following reaction conditions: 95 °C for 3 min, followed by 5 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s; and finished with 72 °C for 5 min. The PCR products of appropriate size (~500 bp) were purified using Agencourt AMPure XP beads (Beckman, USA). The samples were sequenced on an Illumina MiSeq platform at Shanghai Sangon Biotech Co., Ltd.

After sequencing, original data were quality-filtered, chimera checked and clustered into operational taxonomic units (OTUs, 97% cutoff) using Usearch v9.2.64 (Edgar, 2010). Rarefaction curves and alpha diversity indices including Chao1, Shannon index and coverage were calculated in Mothur v1.30.1 (Schloss et al., 2009). Network analysis of the most abundant 100 OTUs of both *cbbL* and *cbbM* genes was carried out with QIIME v1.8.0 (Caporaso et al., 2010). Construction of Neighbor-joining phylogenetic trees from the aligned sequences were performed with MEGA7 (Kumar et al., 2016) by using p-distance. Bootstrapping (1000 replicate reconstructions) was used to estimate the reliability of phylogenetic reconstructions. The *cbbL* and *cbbM* nucleotide sequences have been deposited at the NCBI Sequence Read Archive under the BioProject number PRJNA529126.

2.7. Statistical analysis

The analysis of correlation coefficients between environmental variables and DCF rates was prepared using Origin 8.0 (OriginLab, USA). Analysis of non-metric multidimensional scaling (NMDS) was applied for illustrating the compositions of total *cbbL* and *cbbM* OTUs in sampling sites. The redundancy analysis (RDA) was applied to evaluate the influence of environmental parameters on *cbbL* and *cbbM* communities. The analysis of similarities (ANOSIM) was applied to test the differences in CO_2-assimilating communities among the reservoirs. The partial Mantel test was applied to evaluate the correlations between CO_2-assimilating communities with environmental variables. All the analyses associated with microbial communities were performed using the R package Vegan (http://cran.r-project.org/web/packages/vegan/index.html).

3. Results

3.1. Sediment biogeochemistry and dark carbon fixation in surface sediments of cascade reservoirs

The sediment organic carbon (SOC) in samples of HF reservoir ranged from 4.2% to 9.7%, much higher than that in WJD and DF reservoirs (3.1%~3.9%) (Table S2). The contents of total sulfur (TS) and nitrogen (TN), as well as the chlorion (Cl^-) and fluorion (F^-) concentrations in most of the sediment samples of HF were also higher compared with the other two reservoirs.

After labeling with ^{13}C-bicarbonate for 4 days, the $\delta^{13}C$ value of SOC in sediment samples incubated at 15 °C (−22.4‰~ − 3.1‰) and 25 °C

(−21.2‰~ − 1.6‰) were higher than that in control (−29.5‰~ − 17.4‰) (Fig. 1A). Calculated according to Eq. (1), the DCF rates ranged from 1.5 to 14.7 mmol C m^{-2} d^{-2} in the reservoirs (Fig. 1B),
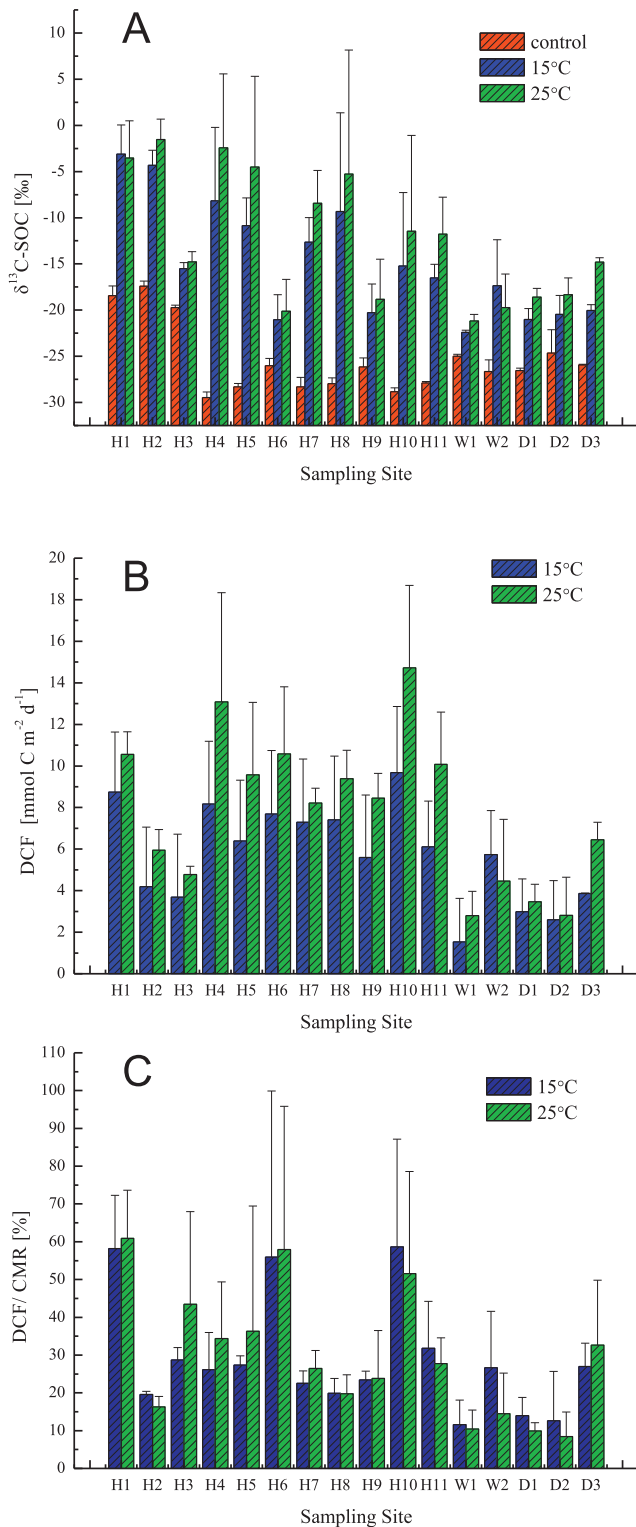
and the sediment samples usually showed higher DCF rate at elevated temperature. Most of the sediment samples of HF had higher DCF rates than that in WJD and DF reservoirs. The ratios of DCF to CMR (Fig. 1C) ranged from about 11.6% to 60.9% under both temperatures, and also were generally higher in sediment samples of HF than that in WJD and DF reservoirs.

3.2. Diversity of the cbbL- and cbbM-carrying bacterial communities in reservoir sediments

The composition of the cbbL- and cbbM-carrying bacterial communities in reservoir sediments was analyzed by high throughput sequencing. The alpha diversity indices were similar between cbbL and cbbM genes in sediment samples, however, the alpha diversity of cbbM genes were relatively higher in HF than in other two reservoirs (Table S3). The non-metric multidimensional scaling (NMDS) analysis showed that composition of both cbbL- and cbbM-carrying communities were separated based on the reservoirs (Fig. 2), especially, those populations in HF reservoir were largely different (R > 0.96, p = 0.0001) from those in the other two reservoirs (Table S4). This was in accordance

Fig. 1. The δ^{13}C values of sediment organic carbon (SOC) in control and the treatments labelled with ^{13}C-bicarbonate under 15 °C and 25 °C (A). The dark carbon fixation (DCF) rates of the sediment samples incubated under 15 °C and 25 °C (B). The ratio of DCF versus CMR (carbon mineralization rate) of each sediment sample incubated under 15 °C and 25 °C(C). Error bar denotes the standard deviation (SD) of the mean (n = 3). H, W and D denote sampling sites of Hongfeng, Wujiangdu and Dongfeng reservoirs, respectively.
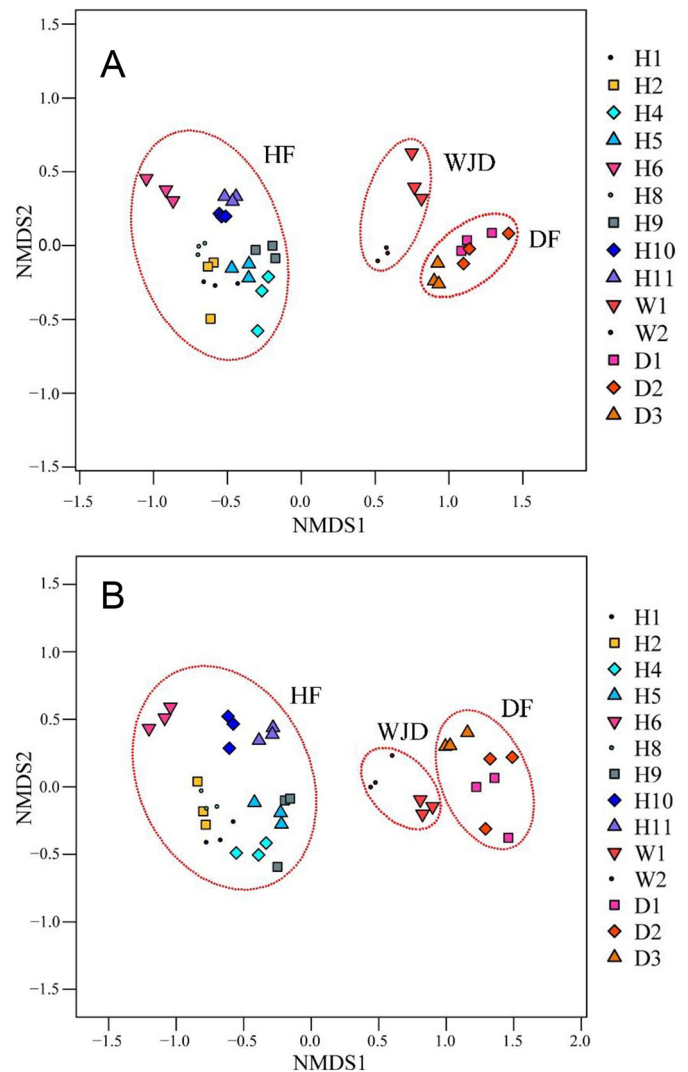
Fig. 2. Nonmetric multiple dimension scaling (NMDS) ordinations based on Bray-Curtis dissimilarity shows the compositions of total cbbL (A) and cbbM (B) OTUs in sampling sites of three reservoirs. The sampling sites located within each red cycle derive from the same reservoir. HF, WJD and DF denote Hongfeng, Wujiangdu and Dongfeng reservoirs, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the results of network analysis of the most abundant 100 OTUs of both genes (Fig. S2), which showed that samples of HF reservoir had >40 unique OTUs of each gene, even though three reservoirs shared some OTUs. Phylogenetic analysis of the most abundant 100 OTUs of both genes revealed that these OTUs for both *cbbL* and *cbbM* genes could be grouped into 17 clusters (Fig. 3 and Fig. S3). Representative OTUs of each cluster are shown in the phylogenetic trees of each gene together with sequences of known autotrophs.
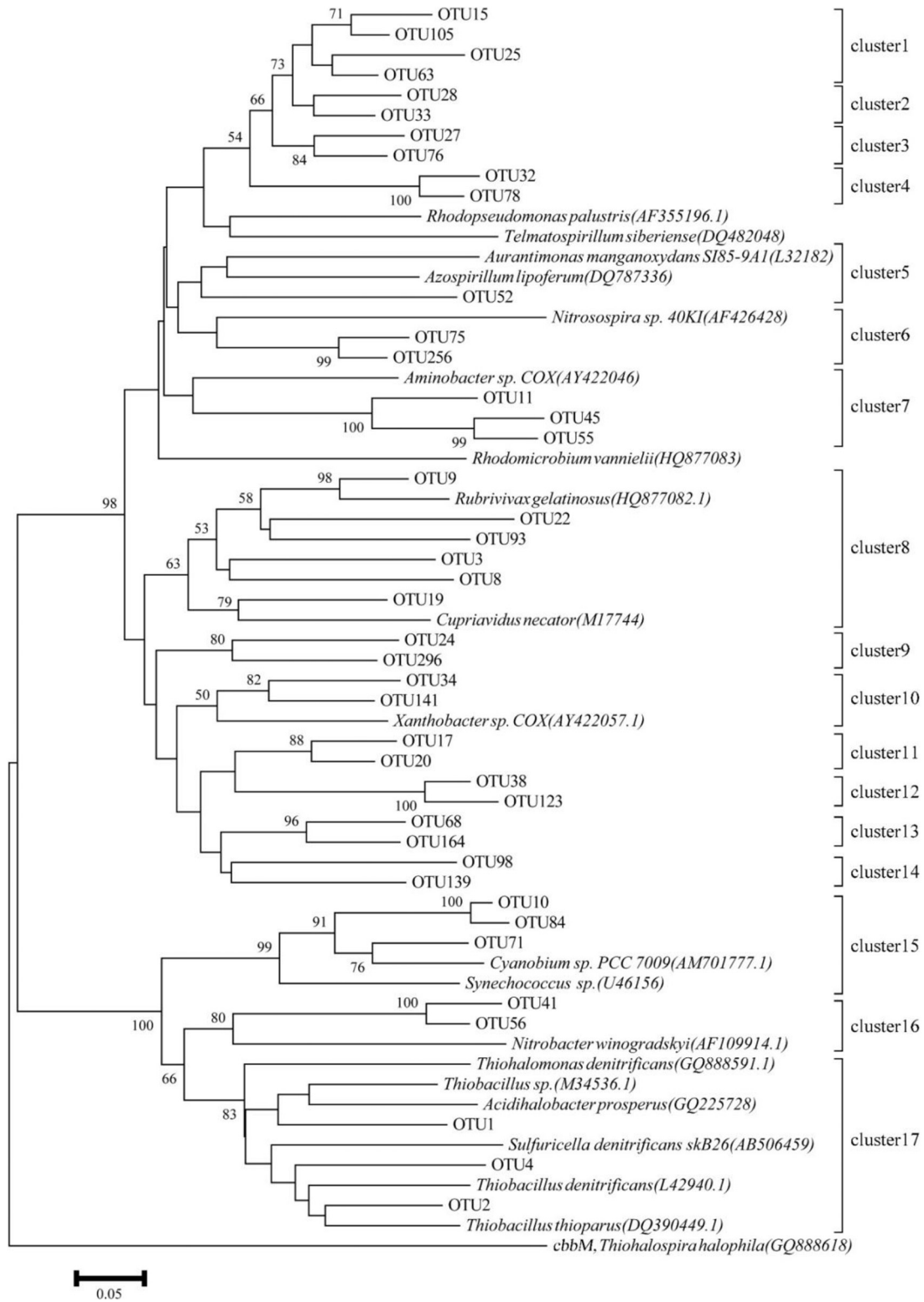

Fig. 3. Neighbor-joining tree obtained from deduced amino acid sequences of representative *cbbL* OTUs derived from high-throughput sequencing, together with reference sequences retrieved from the NCBI database. The most abundant 100 OTUs were selected for phylogenetic analysis and these OTUs were manually divided into 17 clusters based on sequence similarity. Representative OTUs of each cluster were shown in the phylogenetic tree. Accession numbers of reference sequences are given in parentheses. The *cbbM* gene from *Thiohalospira halophila* is used as outgroup. One thousand bootstrap replicates were performed, and values over 50% are indicated on nodes. The scale bar represents 5% estimated change.
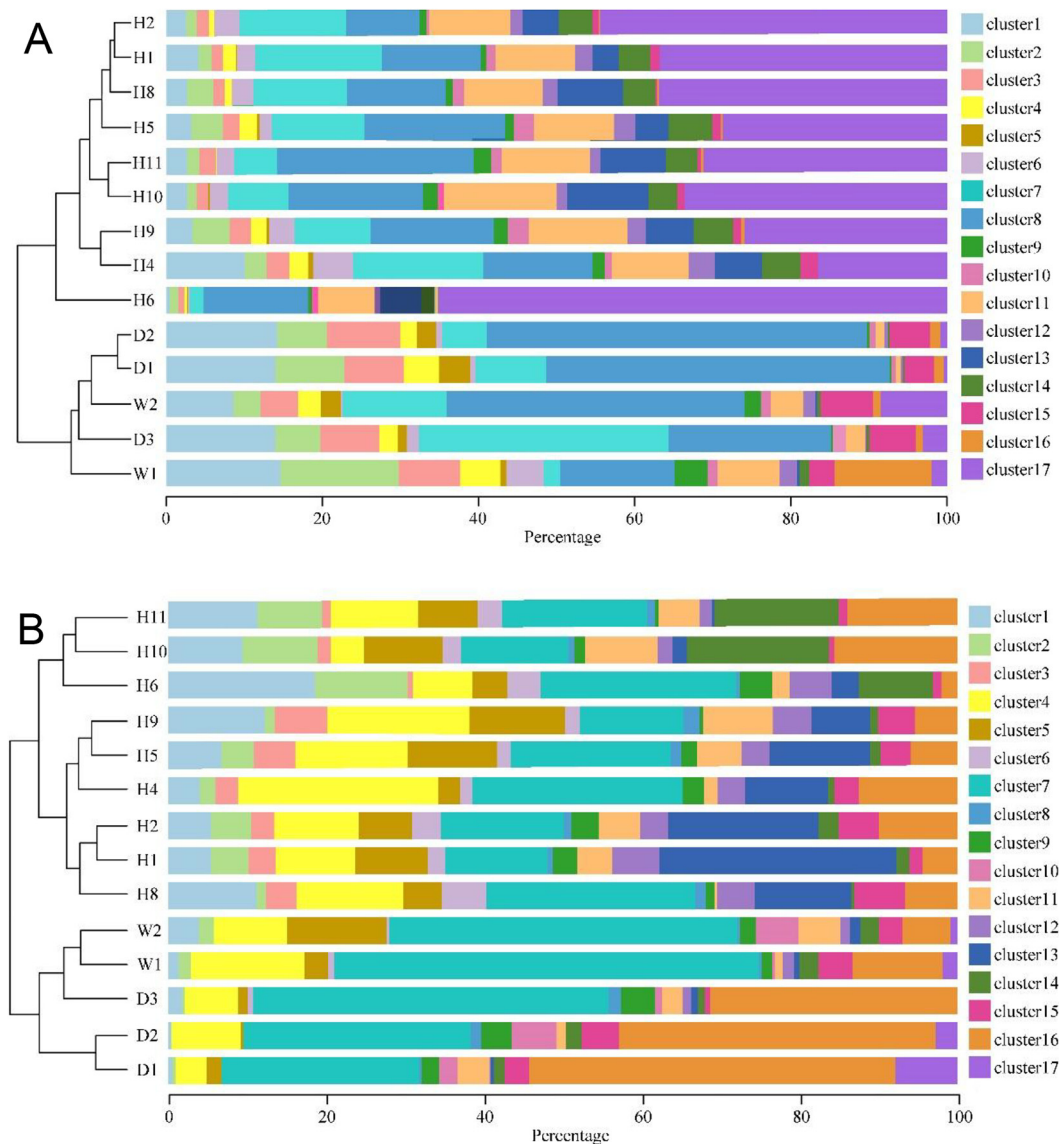
Fig. 4. Combinatorial analyses of samples clustering and the relatively abundances of *cbbL* (A) as well as *cbbM* (B) OTU clusters in each sample. The dendrograms are constructed based on Bray-Curtis dissimilarity values, the relative abundances of clusters are shown in histograms (n = 3). H, W and D denote sampling sites of Hongfeng, Wujiangdu and Dongfeng reservoirs, respectively.

The *cbbL* clusters were grouped with diverse autotrophic bacteria, such as sulfur-oxidizing bacteria (e.g. *Thiohalomonas* and *Thiobacillus*), nitrifiers (*Nitrosospira* and *Nitrobacter*), photoautotrophic purple non sulfur bacteria (*Rhodopseudomonas*, *Rhodomicrobium* and *Rubrivivax*) and other facultative chemolithotrophic bacteria (Fig. 3). In contrast, the *cbbM* clusters were almost solely affiliated with sulfur-oxidizing bacteria (Fig. S3). Most of the retrieved *cbbL* and *cbbM* sequences did not closely affiliate with known autotrophs, suggesting that these reservoir sediments may harbor numerous, novel *cbbL*- and *cbbM*-carrying bacteria. Comparison of samples by using Bray-Curtis indices resulted in a clustering pattern according to reservoir, especially for HF reservoir (Fig. 4), since the dominant clusters in HF reservoir were different from the other two reservoirs. For *cbbL* gene, phylotypes of HF reservoir were dominated by cluster 17, which affiliated with cultured sulfur oxidizers such as *Thiobacillus* and *Acidihalobacter*. While the WJD and DF reservoirs were mainly assigned to clusters 1, 7 and 8, which were distantly affiliated to photosynthetic and other chemolithotrophic bacteria. For *cbbM* gene, phylotypes of HF reservoir were composed of several relative abundant clusters while phylotypes

in the other two reservoirs were dominated by one or two clusters (clusters 7 and 16) (Fig. 4B).

3.3. Influences of environmental variables on DCF rates and composition of the autotrophic microbial community

The correlation analysis shows that DCF rates were positively correlated with reservoir hydraulic residence time (HRT), contents of SOC, TS and TN, and concentrations of Cl$^-$ and F$^-$ in sediment samples (Table 2,

Table 2
Pearson's correlation coefficients between environmental variables and DCF rates of sediment samples. DCF rates determined at 15 °C were used as input in analysis. * and ** indicate a significant correlation at the level of 0.05 and 0.01, respectively.

	HRT	SOC	TN	TS	F$^-$	Cl$^-$	Depth
DCF rates	0.550**	0.305*	0.515**	0.409**	0.504**	0.665**	−0.339*

HRT, hydraulic residence time; SOC, sediment organic carbon content; TN, total nitrogen content of the sediment; C/N, the ratio of SOC and TN; TS, total sulfur content of the sediment.

Table 3
Spearman's correlation of environmental variables with *cbbL* and *cbbM* community structures as determined by partial Mantel tests (Permutations: 9999). The relative abundances of OTUs were taken as input in analysis. When one environmental variable was analyzed by the partial Mantel test, the remaining environmental variables were controlled.

	cbbL genes		*cbbM* genes	
	Correlation	*p* value	Correlation	*p* value
HRT	0.674	0.001	0.675	0.0002
SOC	0.464	0.001	0.474	0.0002
TN	0.372	0.001	0.295	0.0002
TS	0.342	0.001	0.364	0.0002
F$^-$	0.210	0.003	0.250	0.0014
Cl$^-$	0.322	0.001	0.321	0.0002
Depth	0.179	0.003	0.088	0.0592

HRT, hydraulic residence time; SOC, sediment organic carbon content; TN, total nitrogen content of the sediment; C/N, the ratio of SOC and TN; TS, total sulfur content of the sediment.

$P < 0.05$). The partial Mantel test showed that these variables were also significantly correlated with the change in community structures of both *cbbL*- and *cbbM*-carrying bacteria (Table 3). Especially, HRT had the highest correlations with communities of both *cbbL* and *cbbM* genes. Besides, the influences of most of the environmental variables on *cbbM* type autotrophs were at higher significance level than that on *cbbL* type (Table 3). The redundancy analysis (RDA) showed that most of these variables were positively correlated to compositions of both *cbbL*- and *cbbM*-carrying communities in HF reservoir (Fig. 5),
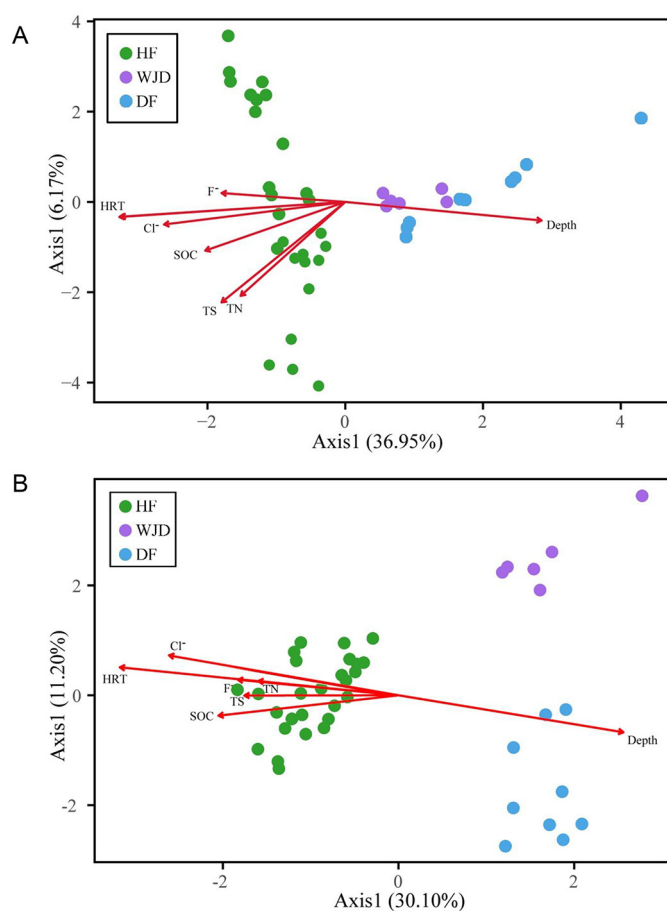

Fig. 5. Ordination diagrams from redundancy analysis (RDA) of *cbbL* (A) and *cbbM* (B) communities and environmental parameters. Arrows indicate the direction and magnitude of environmental variables associated with *cbbL* and *cbbM* community structures. Each sample is represented by colored circle. Abbreviations: HRT, hydraulic residence time; TN, total nitrogen; TS, total sulfur; SOC, sediment organic carbon.

while only depth of sampling sites was positively correlated with that in the other two reservoirs. This was consistent with the apparently higher HRT, SOC and other element contents in sediments of HF reservoir (Table 1 and Table S1).

4. Discussion

4.1. Chemolithotrophic activity in reservoir sediments

Most of the eleven cascade reservoirs in Wujiang River basin have relatively high dissolved oxygen content in the water column during the whole year (Liu et al., 2018a; Wang et al., 2018a), so that the oxygen could penetrate into the surface sediments. This was in accordance with the presence of SO_4^{2-} and NO_3^- in porewater of the sediment samples (Table S2). As a result, the surface sediments should be hotspots of DCF in these reservoirs. Meanwhile, a cascade of dams has major impacts on the river. Especially, as the first cascade reservoir in one of the major tributaries of Wujiang river, the HF reservoir could receive more allochthonous substances, because suspended particles are mainly trapped by the first cascade reservoir (Shi et al., 2017). Moreover, the much longer HRT in HF reservoir (119 days) provides conditions for higher sedimentation rate of both allochthonous and autochthonous particulate substances (Groeger and Kimmel, 1984).

For these reasons, the sediment of the HF reservoir is rich in organic carbon together with inorganic elements including F$^-$ and Cl$^-$ (Table S2), which were mainly derived from element inputs by anthropogenic activities (Li and Ji, 2016). The relatively large fluctuation in concentration of F$^-$ (0.24–1.02 mg/L) (Table S2) suggested the change in anthropogenic activities among different sampling sites in HF reservoir. After anaerobic mineralization, sediments of HF reservoir could supply abundant and spatially heterogeneous reduced inorganic electron donors. As a result, sediment samples of HF reservoir had stronger DCF rates (Fig. 1A) and greater diversity of *cbbL* and *cbbM* genes (Fig. 2 and Table S3). This could explain the positive correlation of environmental parameters (including HRT, SOC and F$^-$) with DCF rates as well as the CO_2-assimilating populations (Fig. 5, Tables 2 and 3). The higher DCF rates in HF probably could also provide support for the point of view that reservoirs with a long HRT favor an increase in primary production (Jones and Elliott, 2007; Kawara et al., 1998; Lee et al., 2015), which can result in a decrease in the release of CO_2 from the water to the atmosphere and mitigate the global warming effects of hydropower exploitation (Li et al., 2018).

The DCF rates of the reservoir sediments (1.5–9.7 mmol C m^{-2} d^{-1}) determined at 15 °C are in the same order as those reported in marine sediments (3–36 mmol C m^{-2} d^{-1}) (Boschker et al., 2014), but much higher than those in freshwater lake sediments (0.01–4 mmol C m^{-2} d^{-1}) (Santoro et al., 2013). Global warming might greatly stimulate the activity of chemolithotrophic microbes since the averaged DCF rate at 25 °C is 36.6% higher than at 15 °C (Fig. 1A). Based on the averaged DCF rates determined at 15 °C (5.7 mmol C m^{-2} d^{-1}), the SOC derived from chemolithotrophy in these cascade reservoirs should be around 25.0 g m^{-2} y^{-1}, close to the carbon burial flux (31.5–76.3 g m^{-2} y^{-1}) of cascade reservoirs in Wujiang river basin (Xiang, 2016). Earlier study also reported that the marine microbial DCF rates were in the same order of magnitude as the annual organic carbon burial in the ocean (Middelburg, 2011). Estimated from the global surface area of reservoirs (354,033 km^2) (Mendonca et al., 2017), the SOC derived from chemolithotrophy in global reservoirs is 0.008 Pg C y^{-1}, about 13% of the total organic carbon burial in reservoir (0.06 Pg C y^{-1}) (Mendonca et al., 2017) and twice of the amount of DCF in global deep-sea sediment (0.004 Pg C y^{-1}) (Middelburg, 2011). Moreover, the SOC derived from DCF may persist in reservoir sediment, since the organic carbon derived from autotrophic microbes is usually quite stable (Hart et al., 2013; Zhu et al., 2017). Meanwhile, the ratios of DCF versus CMR in the sediment samples even reached 60% (Fig. 1C), suggesting that chemolithotrophic microbes play an

indispensable role in the microbial food web of the sediments (Boschker et al., 2014).

Collectively, our results suggested that the autochthonous organic carbon derived from DCF could be an important component of carbon cycling in sediment of cascade reservoirs. Considering that large amount of DIC in these reservoirs of Wujiang River basin are derived from carbonate weathering (Lu et al., 2018; Wang et al., 2018a), the fixation of inorganic carbon by DCF would increase the contribution of carbonate mineral weathering to carbon sink in the freshwater ecosystems (Chen et al., 2017; Liu et al., 2018b; Wang et al., 2017).

4.2. Compositions of cbbL- and cbbM-carrying autotrophic microbial communities

According to the sequence analysis, *cbbL* phylotypes in reservoir sediments were composed of phylogenetically diverse autotrophs (Fig. 3), including obligate chemolithotrophs (eg. sulfur oxidizers and nitrifiers) (Hernandez et al., 1996; Kellermann and Griebler, 2009; Tourova et al., 2010) as well as the facultative lithotrophs and photoautotrophic purple non sulfur bacteria (Nanba et al., 2004; Oda et al., 2004; Tourova et al., 2011). In contrast, *cbbM* genes were much less diverse. Earlier study also reported that the phylogenetic breadth of form II RuBisCO is limited compared to form I RuBisCO (Kovaleva et al., 2011; Watson and Tabita, 1997). This could be explained by that form II RuBisCO, which is the more primitive form, only functions well at low oxygen and high CO_2 concentration conditions that reflect the ancient earth atmosphere. While the more modern RubisCO form I is better adapted to lower CO_2 concentrations and/or oxic conditions (Jordan and Ogren, 1981; Shively et al., 1998; Tourova and Spiridonova, 2009). These differences between forms I and II RubisCO probably could explain the variations in correlation with environmental parameters between *cbbL*- and *cbbM*-carrying communities in each reservoir (Fig. 5).

Compared with the WJD and DF reservoirs, several major phylotypes of both the *cbbL* and *cbbM* genes of HF reservoir were affiliated with the same sulfur oxidizers, such as *Thiobacillus* and *Sulfuricella* (Fig. 3 and Fig. S3), implying that they probably harbor both RuBisCO forms I and II, similar with previous findings from groundwater environments (Alfreider et al., 2009). The existence of both form I and form II RubisCO in one chemolithotrophic bacteria allows efficient CO_2 fixation in environments or the oxic-anoxic interface where the levels of oxygen and CO_2 vary considerably (Alfreider et al., 2009). Meanwhile, the facultatively anaerobic *Thiobacillus* and *Sulfuricella* are able to oxidize elemental sulfur and thiosulfate to sulfate aerobically or coupled to denitrification under anoxic conditions (Beller et al., 2006; Kojima and Fukui, 2010). These results pointed to a strong link between chemolithotrophy and the oxidation of reduced sulfur compounds in HF reservoir. This was in accordance with that the sediment samples of HF reservoir were richer in TS and SO_4^{2-}. Previous studies also reported that sulfur oxidizers predominated over other chemoautotrophs like nitrifiers (Boschker et al., 2014; Dyksma et al., 2016; Lipsewers et al., 2017) in the marine sediments.

5. Conclusions

This study provided the first evaluation of chemolithotrophic carbon fixation in surface sediments of cascade reservoirs, and gave a relatively comprehensive interpretation of the main chemolithotrophic populations by high throughput sequencing of both *cbbL* and *cbbM* genes. Our results suggested that HRT and relative location of cascade reservoirs are the key control factors of both DCF and composition of autotrophic microbial communities. These results highlighted the importance of chemolithotrophy in carbon cycle of cascade reservoir sediment, and shed light on the profound impact of cascade damming on chemolithotrophic carbon sequestration in the reservoirs. Further studies on more cascade reservoirs in different rivers are necessary for

deeper understanding of the controlling factors of community and activity of the chemolithotrophic microbes.

Declaration of competing interest

The authors declare that they have no competing financial interest associated with this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134316.

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