Linkage between water soluble organic matter and bacterial community in sediment from a shallow, eutrophic lake, Lake Chaohu, China

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A B S T R A C T
Lacustrine sediment played important roles in migration and transformation of its water soluble organic matter (WSOM), and the source and composition of WSOM would affect water trophic status and the fate of pollutants. However, we know little about the pathway of WSOM transformation and its driving bacterial communities in lacustrine sediment. In the present study, we investigated the spatial distribution patterns of sediment WSOM and its soluble fractions across Lake Chaohu using fluorescence spectroscopy, and explored WSOM compositional structure through our proposed calculated ratios. In addition, we also analyzed sediment bacterial community using Illumina sequencing technology, and probed the possible pathway of sediment WSOM transformation under the mediate of indigenous bacteria. Our results showed that the inflowing rivers affected the spatial distribution patterns of WSOM and its five fractions (including tyrosine-, tryptophan-, fulvic acid-, humic acid-like substances and soluble microbial productions), and sediment WSOM originated from fresh algae detritus or bacterial sources. In parallel, we also found that Proteobacteria (mainly γ-Proteobacteria and δ-Proteobacteria), Firmicutes (mainly Bacilli), Chloroflexi, Acidobacteria, Planctomycetes and Actinobacteria dominate sediment bacterial community. Furthermore, these dominant bacteria triggered sediment WSOM transformation, specifically, the humic acid-like substances could be converted into fulvic acid-like substances, and further degraded into aromatic protein-like and SMP substances. In addition, our proposed ratios (P-L:H-L, A_r-P:SMP and H-L ratio), as supplementary tool, were effective to reveal WSOM composition structure. These results figured out possible pathway of WSOM transformation, and revealed its microbial mechanism in lacustrine sediment.

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Introduction
Lakes received a large amount of surface runoff by their connecting inflowing rivers at regional scales, and were deemed as sink for matter, energy and information. Lakes ecosystem played vital roles in production of organic matter (Morana et al., 2014), retention and transformation of nutri-
ents and contaminants (Harrison et al., 2012), water allocation and supply (Cui et al., 2010) and other ecological services. However, many lakes around the world had been influenced by naturally and anthropogenic activities, and suffered from eutrophication, especially for the regions with rapid economic development (Muri et al., 2017), such as lakes in the Yangtze Delta region in China, which resulted in water quality deterioration and lacustrine ecosystem degradation.

In lake ecosystem, sediment was the hot-spot for the biogeochemical processes of external nutrients and contaminants, as well as autochthonous organic matters (Gudasz et al., 2012), and which exerted important impacts on water trophic status and ecological health. Sediment buried a mass of nutrients and pollutants (especially for particulate forms) from overlying water, and recorded vast quantities of information that could reflect the lake ecosystem evolution progresses and its pollution history (Xu et al., 2017; Hillman et al., 2015). The deposited organic matter in lake sediment, especially for algal-derived organic matter in eutrophic lake, provided intact and high resolution information about water trophic status (Brenner et al., 1999; Wu et al., 2006). Hence, sediment was considered to be hotspot for the retention and transformation of nutrients and contaminants (Wu et al., 2006) in lacustrine ecosystem.

Lacustrine sediment organic matter aggregated a variety of heterogeneous aromatic and aliphatic hydrocarbon substances from terrestrial-derived and autochthonous organic matter (Leenheer and Croué, 2003). The dissolved fraction of sediment organic matter was the bioavailable part for sediment microbes, and it was operationally defined fraction of water soluble organic matter (WSOM). In sediment, the labile WSOM was preferentially biodegraded by sediment bacteria, while the refractory fractions of WSOM was accumulated and preserved in sediment (Wakeham et al., 1997; Oni et al., 2015). The transformation progresses of WSOM was critically influenced by bacterial communities in sediment (Huang et al., 2017), furthermore, these bacterial communities were considered to trigger WSOM transformation, in turn, the abundance and composition of bacterial community was also shifted by the variations of WSOM composition in lake sediment (Judd et al., 2006). However, how WSOM transformed under bacterial communities and what dominant bacteria promoted WSOM transformation in sediment were poorly understood.

Based on the previous studies, we hypothesized that algae detritus deposited into sediment and replenished sediment WSOM quantity after algae bloom, and the indigenous bacteria shifted its WSOM composition. To validate the mentioned hypothesis, we investigated the spatial distribution patterns of sediment WSOM and its fractions across Lake Chaohu after algal bloom, which was a representative shallow, eutrophic lake located in the middle and lower reaches of the Yangtze River, in China. In addition, we also analyzed surface sediment bacteria abundance, community composition across Lake Chaohu. Furthermore, we probed how WSOM transformed in sediment and its driving predominant bacteria. These findings could decipher the spatial distribution patterns of WSOM and its fractions in sediment from Lake Chaohu, and would improve the understanding of the possible pathway of WSOM transformation in lacustrine sediment.

1. Materials and methods

1.1. Study area and sediment samples collection

Lake Chaohu (117.18°-117.50° E, 31.25°-31.42° N) is located in the lower reach of the Yangtze River. The lake covers approximated 770 km² with an average depth of 3 m, and the hydrological retention time is about 167 days. There are thirty-three rivers inflowing into Lake Chaohu, which has received a large amount of nutrients and pollutants from industry and agriculture around Lake Chaohu basin, and resulted in severe aquatic eutrophication and its associated algal bloom.

Twenty surface sediment (0-20 cm) samples were collected using a stainless steel grab from Lake Chaohu after algal bloom, in November, 2018. The sampling sites are shown in Fig. 1. To obtain representative samples with minimum sampling error, three replicate surface sediment samples were collected within the area of 25 m² and composited into one sample. After the stones, plant tissues, and some benthic organisms were removed from the collected sediment, the com-

Fig. 1 – Study area and sampling sites for sediment saes across Lake Chaohu.
posed sediment sample was mixed onsite and divided into two parts. In which one part was placed in sealed bags at 4°C and taken back to the laboratory, and all sediment samples were freeze-dried, gently ground and passed through 2 mm and 0.107 mm sieves for sediment physicochemical properties and their WSOM analysis. The other one was placed in pre-sterilized glass bottle at 4°C and taken back to the laboratory, then immediately stored at -80°C until microbial DNA extraction.

1.2. Sediment physicochemical properties measurements

Sediment physicochemical properties, including water soluble organic carbon (WSCOC), total phosphate (TP), total transferable nitrogen (TTN), cation exchange capacity (CEC) and pH values, were measured. WSOC contents of sediment WSOM samples were determined using the high temperature combustion method on a TOC-VPCH (Shimadzu, Japan). TP was analyzed by pre-treated at 500°C in a muffle furnace for 2 hr, then extracted by HCl, and TP content in the extracts were measured by the ammonium molybdate spectrophotometric method. TTN contents were analyzed according to the Tang’s method (Tang et al., 2018). All of the above physicochemical properties were determined in triplicate.

1.3. Optical spectra analysis for sediment WSOM

The twenty sediment WSOM samples were obtained referred to the method proposed by Ohno (Ohno and Bro, 2006). In brief, five grams of the prepared sediment samples were extracted with 50 mL of DI-H2O by shaking for 24 hr away from light at 20°C ± 3°C, and these crude extracts were filtered through pre-combusted 0.7 μm glass fiber (GF) filters (Merck Millipore, USA) and 0.45 μm polyvinylidene fluoride (PVDF) filters (Merck Millipore, USA) to get sediment WSOM samples.

Fluorescent spectra for sediment WSOM were scanned in our previous studies (Wang et al., 2019). Fluorescence spectra were scanned using a spectrophluorometer (Fl-4500, Hitachi, Japan) at excitation/emission (Ex/Em) wavelengths of 220-400 nm/250-550 nm. Simultaneously, all fluorescent excitation-emission matrix (EEM) were corrected for Raleigh scattering and inner-filter effects (Ohno and Bro, 2006), and we conducted Raman-normalizing for the corrected EEM using Raman area (Stedmon et al. 2003; Yang et al., 2013). To evaluate quantitatively the spatial patterns of sediment WSOM compositions across Lake Chaohu, we used the fluorescence regional integral (FRI) technique to divide each EEM into five regions (Appendix A Fig. S1) associated with aromatic proteins including tyrosine-like and tryptophan-like substance, soluble microbial by-products (SMP) substance, fulvic acid-like substance and humic acid-like substance (Chen et al., 2003). The volumes ($\phi_i$) of the five regions could describe the quantity for the corresponding fraction, which were calculated according to the methods reported previously (Chen et al., 2003; Guo et al., 2014; He and Fan, 2016), and the calculation and its optical fingerprint indices (BIX and HIX) were described in Appendix A.

We also proposed three ratios to denote WSOM compositional stucture, including the ratio of humic-like substance contribution (H-L ratio), the ratio of protein-like substance to humic-like substance (P-L-H-L), and the ratio of aromatic protein-like substance to soluble microbial production (Ar-P:SM). The ratios were calculated as the following Eqs. (1)-(3):

$$ H - L \; ratio = (\phi_III + \phi_IV) / (\phi_I + \phi_II + \phi_III + \phi_IV + \phi_V) $$

$$ P - L : H - L = (\phi_I + \phi_II + \phi_III + \phi_IV) / (\phi_III + \phi_IV) $$

$$ Ar - P : SMP = (\phi_I + \phi_II) / \phi_IV $$

where, $\phi_i$ ($i = I, II, III, IV$ and $V$) (R.U. - nm$^2$) were integrated volumes of tyrosine-like, tryptophan-like, fulvic acid-like, SMP and humic acid-like substances, and represented their quantities, respectively.

1.4. Microbial community analysis

Microbial DNA was extracted from each sediment sample using the E.Z.N.A™ Mag-Bind Soil DNA Kit (Omega, USA) according to the manufacturer’s protocols, the 16S rRNA gene (V3-V4 regions) was partially amplified through polymerase chain reaction (PCR, Bio-Rad, USA), and the gene fragments were amplified using a pair of primers with 341F (5’-CTACGGGNGGCWGCAG-3’) and 805R (5’-GACTACHVGGGTATCTAATCC-3’). The PCR system contained 15 μL of 2 × Taq master Mix, 1 μL of 10 μmol/L primer (341F and 805R), and 10-20 ng of genomic template DNA, finally added H2O to the final volume of 30 μL. PCR procedures consisted of the following cycles: 30°C at 5 min, 5 cycles at 94°C for 30 sec, 45°C for 20 sec and 65°C for 30 sec, 20 cycles at 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. The second cycle was denaturation at 95°C for 3 min, 25 cycles at 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. In DNA extraction and the subsequent PCR amplification, negative control (no sample) was performed for blank correction of contamination, and we also conducted positive control (bactillus coli) to mitigate technical artefacts during experiments.

PCR amplicons were extracted by agarose gels and purified using the Qubit™ ssDNA Assay Kit (Life, USA) according to the provided instructions. The purified amplicons were quantified on the Qubit 3.0 Fluorometer (Invitrogen, USA), and paired-end sequenced on an Illumina MiSeq platform (Illumina, USA). The above DNA extraction, PCR amplification, purification and sequencing were conducted by the Sangon Biotech (Shanghai) Company.

1.5. Bioinformatics and data statistical analysis

The raw reads were filtered, de-multiplexed and quality controlled by using the softs of Cut adapt (ver. 1.2.1), PEAR (ver.0.9.6), PRINSEQ (ver.0.20.4), USEARCH (ver.5.2.236) and Qiime (ver.1.8.0). The treated sequences with similarities larger than 97% were clustered as one operational taxonomic unit (OTU) using USEARCH (ver.5.2.236). Each OTU sequence was aligned against the RDP bacterial 16s database (http://rdp.cme.msu.edu/misc/resources.jsp) using RDP Classifier, and the bacterial taxonomic information (including phylum, class, order, family and genus) for sediment from Lake Chaohu was retrieved. Sediment bacterial richness and diversity were calculated in the Mothur (ver. 1.30.1) (Schloss et al., 2009), and the results are shown in Appendix A Table S2. Simultaneously, we analyzed the rarefaction and species accumulation curves for the twenty sediment samples were tend to be saturated (Appendix A Fig. S2), and these curves indicated that all bioinformation was valid for microbial community analysis.

The Pearson correlation analysis was conducted to acquire quantitative correlations between every two WSOM fractions, as well as between bacterial community and sediment WSOM composition, and result was significant if $p < 0.05$. To probe quantitative correlations for sediment WSOM transformation, stepwise regression analysis was performed using SPSS (ver.13.0). Redundancy analysis was performed to reveal the response of bacterial community composition (dominant phyla and classes) to sediment WSOM properties using CANOCO (ver.5.0).
2. Results

2.1. WSOC, TTN and TP contents for sediment in Lake Chaohu

Contents of WSOC, TTN and TP in sediment from Lake Chaohu ranged from 101.52 mg/kg to 793.22 mg/kg, from 4,918.97 mg/kg to 6,787.25 mg/kg, and from 370.28 mg/kg to 1141.67 mg/kg dry weight in sediment, with the average content of 223.99 ± 171.79 mg/kg, 5,884.27 ± 508.24 mg/kg and 637.68 ± 195.31 mg/kg (dry weight), respectively. The spatial distribution patterns of WSOC, TTN and TP across Lake Chaohu are depicted in Fig. 2, and they exhibited similar spatial distribution patterns. In Lake Chaohu, the quantities of WSOC, TTN and TP in the northwest and east parts of sediment were higher than those in the middle part, this was possibly due to the external loads input by inflowing rivers.

2.2. Fluorescent WSOM from sediment and its compositional characteristics

We further assessed the spatial variations of fluorescent sediment WSOM and its five fractions (including tryptophan-like, tyrosine-like, fluvic acid-like, SMPs and humic acid-like substances) from Lake Chaohu by using EEM-FRI technique. The total fluorescent intensities ($\phi_T$) of WSOM ranged from 1,063 R.U.·nm$^2$ to 6,937 R.U.·nm$^2$ in surface sediment from Lake Chaohu (Fig. 3A). The percent fluorescence responses of the five fractions also displayed similar spatial distribution patterns with the $\phi_T$ of WSOM (Fig. 3B-F). SMPs (35.74% ± 6.13%) and humic acid-like substances (37.73% ± 9.61%) were the dominant fractions of the WSOM, followed by the fulvic acid-like fraction (17.43% ± 0.83%), and the lowest fractions were tryptophan-like (5.37% ± 0.98%) and tyrosine-like (4.46% ± 0.79%) substances.

Fig. 2 – Spatial distribution patterns of (A) WSOC, (B) TTN and (C) TP in sediment.

Fig. 3 – Spatial distribution patterns of fluorescent sediment WSOM and its five fractions in sediment.
Moreover, we traced the origin of WSOM from sediment in Lake Chaohu through the BIX and HIX indices. The values of BIX and HIX for sediment were 0.78 - 1.26 and 1.06 - 3.85, respectively (Fig. 4A). These suggested that the algae was the major source of sediment organic matter in Lake Chaohu. In addition, we also explored WSOM composition structure by calculating the ratios of P-L:H-L, Ar-P:SMP and H-L%, and the 95% confidence intervals (95% CI) of which were 0.76 - 0.96, 0.25 - 0.30 and 0.51 - 0.58, with the mean ratios of 0.86 ± 0.21, 0.28 ± 0.05 and 0.54 ± 0.07, respectively. It was interesting that the ratios of P-L:H-L and H-L% were related to BIX and HIX indices (P < 0.05 or P < 0.001), however, no significant relationship was observed between Ar-P:SMP ratio and BIX or HIX value.

2.3. Bacterial community structure for sediment from Lake Chaohu

With the quality control of the Quantitative Insights into Microbial Ecology (QIME, version 1.6.0) pipeline, a total of 892,991 high quality 16S rRNA sequences were obtained from the twenty sediment samples in Lake Chaohu, and these 16S rRNA sequences were assigned to 82,316 OTUs after clustering at a 97% similarity level. In the present study, we depicted bacterial composition and community structures for the sediment samples at phylum and class levels (Fig. 4).

Forty-eight phyla were identified from sediment in Lake Chaohu at phylum level, and the relative accumulated abundances of the ten dominant phyla reached 93.51% (Fig. 4A). Proteobacteria was the most abundant phylum (37.61 ± 5.77%), followed by Firmicutes (17.57 ± 7.53%), Chloroflexi (11.44 ± 3.29%), Acidobacteria (10.28 ± 2.94%), and Planctomycetes (8.56 ± 4.92%), and the others were Actinobacteria, Verrucomicrobia, Nitrospirae, Bacteroidetes, and Aminicenantes. We also identified eight-two classes from sediment in Lake Chaohu at class level, and analyzed the eleven predominant classes with the relative abundance > 1.5%, which accounted for 80.11% accumulated abundances of the detected ones in sediment from Lake Chaohu (Fig. 4B). Gamma-Proteobacteria was the first dominant class (19.54% ± 8.66%), followed by Bacilli (13.30% ± 8.06%), and the third dominant class was delta-Proteobacteria (10.00% ± 3.37%), Anaerolineae (9.55% ± 2.92%) and Planctomycetia (8.15% ± 4.86%). The others were beta-Proteobacteria, Clostridia, Actinobacteria, Acidobacteria_Gp6, Acidobacteria_Gp17, alpha-Proteobacteria.

3. Discussions

3.1. Sources and compositions of WSOM from sediment in Lake Chaohu

There were multiple factors influenced the spatial distribution patterns and properties of sediment WSOM in Lake Chaohu, including external loads input into lake through inflowing rivers, production of algae and deposited into sediment, precipitation of particulate organic matter, biodegradation by microbes and etc. (Wang et al., 2019; Zhang et al., 2012). Chaohu
Basin Authority of Ministry of Water Resources reported (but not published) that the eleven inflowing rivers input a large amount of COD (23,085.66 tons/year), TN (16,965.52 tons/year) and TP (988.22 tons/year) into Lake Chaohu, and the urban rivers in the northwest of lake (Nanfei River, Shiwuli River, Tangxi River and Pai River) accounted for 57%, 63% and 59% of the total COD, TN and TP input loads respectively. This was the possible reasons for the spatial distribution patterns of WSOC, TTN and TP from sediment in Lake Chaohu. In addition, algae detritus and particulate organic matter (POM) deposited into sediment (Torn et al., 1997), and supplemented sediment organic matter after algal bloom in eutrophic lake. Simultaneously, the composition of sediment organic matter was varied by the sediment microbial community (Balmonte et al., 2019).

We also assessed the origin of sediment WSOM and its transformation in Lake Chaohu through the BIX and HIX indices. High BIX values (>0.8 or 1.0) indicated that DOM derived from strong autochthonous sources or aquatic biological origin (Birdwell and Engel, 2010). In addition, DOM with low HIX values (<4) was considered to be freshly produced from biological or microbial origin (Huguet et al., 2009), and these DOM were considered to be freshly produced from phytoplankton biomass, and even was deemed to be microbial mats when HIX values were less than 2.0 (Huguet et al., 2009; Hunt and Ohno, 2007). In the present study, our BIX (0.91-1.06, 95% CI) and HIX (1.19-1.77, 95% CI) values indicated sediment WSOM also showed strong recent autochthonous sources or bacterial sources (Fig. 5A). Our results were consistent with the result of Xu et al. (2017), which was identified by stable isotope δ13C and δ15N, and these further confirmed that algae was the major source of sediment organic matter, and its composition was reshaped by microbes in Lake Chaohu sediment.

Moreover, we proposed the ratios of P-L:H-L, Ar-P:SMP and H-L ratio to characterize WSOM composition, low P-L:H-L ratio (< 1) and high H-L ratio (> 0.5) indicated that humic-like substances (including fulvic acid-like and humic acid-like substances) were primary fractions in WSOM. The Ar-P:SMP ratio could reveal the composition of protein-like fraction, high Ar-P:SMP ratio (> 1) suggested the aromatic proteins (including tyrosine and tryptophan substances) dominated in protein-like, while low Ar-P:SMP ratio (< 1) showed soluble microbial productions were predominant components. Our P-L:H-L ratio (0.76-0.96, 95% CI), H-L ratio (0.51-0.58, 95% CI) and Ar-P:SMP ratio (0.25-0.30, 95% CI) indicated that the humic-like substances were slight higher than protein-like in sediment WSOM from Lake Chaohu, and SMP was the dominant components in protein-like substances. Interestingly, we found that the three ratios were significantly related to HIX values (P < 0.001, Fig. 5B-D), this demonstrated that the proposed ratios could improve the understanding of WSOM compositional structure.

3.2. Possible pathway of WSOM transformation in sediment and its driving bacteria

It was gradually realized that the bioavailability of WSOM relied on its sources (such as terrestrial or algae-derived organic matter), and even the refractory fractions would be metabolized in sediment (Bianchi, 2011; Baldock et al., 2004). In the present study, the quantitative correlations were observed between every two WSOM fractions (Appendix A Fig. S3 and Table 1), we found that quantities of fulvic acid-like, protein-like and SMP fractions would depend on humic acid-like substances’ quantity (Appendix A Fig. S3 A-C), simultaneously, quantities of protein-like and SMP fractions were also significantly correlated to fulvic acid-like substance’s one (Appendix A Fig. S3 D and F). These findings indicated that WSOM transformation occurred in sediment despite of its complex and heterogeneous composition. Moreover, we performed the stepwise multiple linear regression analysis to clarify the quantity relationship between the four WSOM fractions (Table 1), and these results could predict the possible...
Table 1 - Stepwise regression analysis of predicting quantitative correlations for sediment WSOM transformation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model</th>
<th>R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulvic acid-like (R.U.- nm²)</td>
<td>= 0.76 × Humic acid-like − 376.3</td>
<td>0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein-like (R.U.- nm²)</td>
<td>=0.54 × Fulvic acid-like + 28.42</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMP (R.U.- nm²)</td>
<td>=0.75 × Humic acid-like + 1.69 × Fulvic acid-like − 722.92</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SMP: Soluble microbial by-product.

fraction, and noted that the combined actions of several microbial communities triggered sediment WSOM transformation (Fig. 6). In addition, the Pearson correlations between dominant bacteria and WSOM fractions (Appendix A Table S3) confirmed the above-mentioned results, for example, Firmicutes (mainly Clostridia, P<0.05), Chloroflexi (mainly Anaerolineae, P<0.01), Acidobacteria (mainly Acidobacteria_Gp6 and Acidobacteria_Gp17, P<0.05) and Actinobacteria (P<0.01) were positively correlated to protein-like substances significantly, which indicated that these bacteria were driving forces for the production and transformation of protein-like substances. Similarly, Proteobacteria (mainly gamma-Proteobacteria, P<0.05) and Firmicutes (mainly Bacilli, P<0.05) were responsible for the fulvic acid-like substances degradation in sediment.

Fig. 6 – Redundancy analysis (RDA) of sediment WSOM fractions and indigenous bacteria (A) at phylum level and (B) at class level.

pathway of WSOM transformation in sediment, namely, humic acid-like substances were biodegraded into fulvic acid-like and SMP substances, and the fulvic acid-like substances were further degraded into SMP and protein-like substances by indigenous bacteria.

To investigate which microbes of the tested numerous microbial communities could drive the WSOM transformation in sediment, we performed redundancy analysis (RDA) of the dominant bacteria and sediment fluorescent WSOM

4. Conclusions
In the present study, we investigated the spatial distribution patterns of fluorescent WSOM and its five fractions by using EEM-FRI technique, and revealed bacterial community structure by Illumina Sequencing technology in sediment from Lake Chaohu. More broadly, the inflowing rivers input a large amount of external DOC, TN and TP loads into lake, a portion of these DOC and algae detritus deposited into sediment and supplemented WSOM in sediment. Our study showed that WSOM from sediment in Lake Chaohu exhibited the characteristics of strong recent autochthonous sources or bacterial sources, and these WSOM composition shifted by indigenous bacterial communities in sediment. We found that WSOM transformation were tightly linked to Proteobacteria, Firmicutes, Acidobacteria, Chloroflexi, Planctomycetes and Actinobacteria. In addition, our proposed ratios (P-L/H-L, Ar-P-SMP and H-L ratio) could be applied to depict WSOM composition structure. These results demonstrated that sediment stocked
lots of algae detritus and external organic matter to complement sediment WSOM quantity, then these sediment WSOM composition was reshaped by indigenous microorganism re-shaped.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.res.2020.05.023.

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