Diversity of microbial plankton across the Three Gorges Dam of the Yangtze River, China

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Abstract The Three Gorges Dam (TGD) of the Yangtze River, China, is one of the largest irrigation and hydroelectric engineering projects in the world. The effects of huge man-made projects like TGD on fauna and macrophyte are obvious, mainly through changes of water dynamics and flow pattern; however, it is less clear how microorganisms respond to such changes. This research was aimed to examine differences in microbial diversity at different seasons and locations (in front of and behind the TGD). In addition, differences between particle-attached and free-living communities were also examined. The community structures of total and potentially active microorganisms in the water columns behind and

Keywords Archaea; Bacteria; Free-living; Particle-attached; Three Gorges Dam
in front of the TGD were analyzed with the DNA- and RNA-based 16S rRNA gene phylogenetic approaches over three different seasons. Clone libraries of 16S rRNA genes were prepared after amplification from extracted DNA and, for some samples, after preparing cDNA from extracted rRNA. Differences were observed between sites at different seasons and between free-living and particle-attached communities. Both bacterial and archaeal communities were more diverse in summer than in winter, due to higher nutrient levels and warmer temperature in summer than in winter. Particle-attached microorganisms were more diverse than free-living communities, possibly because of higher nutrient levels and heterogeneous geochemical micro-environments in particles. Spatial variations in bacterial community structure were observed, i.e., the water reservoir behind the TGD (upstream) hosted more diverse bacterial populations than in front of the dam (downstream), because of diverse sources of sediments and waters from upstream to the reservoir. These results have important implications for our understanding of responses of microbial communities to environmental changes in river ecosystems affected by dam construction.

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1. Introduction

The Yangtze River is the third longest river in the world with a total length of ~6300 km and a drainage area of 1.8 \times 10^6 km². The river runoff amounts to 905.1 \times 10^9 m³/y, and sediment discharge averages 348 million t/y since the mid-1980’s (Chen, 2008). For such a large river, any disturbance to the water dynamics along the water flow path is expected to cause major changes to regional climate, aquatic system health, and human activity.

The Three Gorges Dam (TGD) built in the middle reach of the Yangtze River, ~1800 km upstream from the Yangtze River estuary, is one of the largest irrigation and hydroelectric engineering projects in the world (Huang, 2001). The TGD is 185 m high and 2300 m long with a drainage area of 1080 km² and a total water storage capacity of 39.3 billion m³ (Jiao et al., 2007). One major consequence of the TGD is reduced sediment load from the Yangtze River to the East China Sea, from 348 million t/y for the pre-TGD period to <100 million t/y after the construction of the TGD (Chen, 2008). Such a dramatic decrease in sediment load is expected to have a large impact on water quality, photosynthetic activity, and aquatic system health (Jiao et al., 2007; Chen, 2008).

One study has shown that the storage of 12.4 billion m³ of water within the first 10 days of the completion of the TGD in 2003 caused 27% reduction in flow rate and a 4-fold increase in chlorophyll-a production (a measure of primary production) (Jiao et al., 2007). Such an enhanced photosynthetic activity is ascribed to reduced sediment load and increased light transparency (Jiao et al., 2007). Subsequent decay of algal biomass carried to the Yangtze River estuary may enhance consumption of oxygen and results in hypoxia in the bottom layer of the estuary.

Many other studies have demonstrated the effects of habitat fragmentation of fauna and macrophyte in the Yangtze River ecosystem caused by the TGD (Lei, 1998; Wu et al., 2003; Chen and Xie, 2009; Hu et al., 2009). In contrast, only a limited number of studies have been conducted to assess the influence of the TGD on microbial ecology. Before the TGD construction, bacterial diversity was shown to gradually change from the upstream to the downstream (Sekiguchi et al., 2002a, b). After the TGD construction and water storage in June 2003, marked changes of bacterial community structure in the Yangtze River-East China Sea estuary were observed: the overall bacterial diversity became lower, the abundance of the freshwater bacteria Betaproteobacteria decreased and the diversity of Alphaproteobacteria and Cyanobacteria increased (Jiao et al., 2007). Such observed changes were ascribed to sudden reduction of river runoff and ensuing intrusion of ocean currents (Jiao et al., 2007), little is known how the TGD construction affects the diversity of microbial communities in the immediate vicinity of the dam.

We hypothesize that (1) microbial diversity in summer is higher than in winter because of higher nutrient contents and higher temperature in warm climate; (2) differences exist in microbial diversity and community structure between water column and suspended sediment particles; (3) the TGD water reservoir traps diverse microorganisms from a variety of sediment and runoff sources from the upstream; and in contrast, the downstream river immediately below the dam would have lower microbial diversity because of reduced load and limited sources of sediments and surface runoffs. The objective of this study was therefore to test these three hypotheses by investigating microbial diversity behind (i.e., the water storage reservoir) and in front of the TGD during three different seasons in free-living and particle-attached communities.

2. Materials and methods

2.1. Field measurements and sample collections

In May, July, and December 2009, vertical profiles of water chemistry from upstream and downstream were measured near the TGD using a submersible multiple parameter probe set Horiba (U20D, Japan) on a motorized boat. The field measurements did not show any significant variations in water chemistry along the lateral (upstream and downstream by ~5 km) transect behind and in front of the TGD, however, important differences were detected across the dam. Therefore, for each season, one representative (in geochemical sense) sample behind the dam and one in front of the dam were analyzed for detailed water chemistry and microbiology. The locations for the July and December samples were the same, but the May sample behind the dam was further upstream (Fig. 1). At these three locations, there was no significant variation in water chemistry throughout the vertical profile of the water column. So for laboratory analyses, water samples were collected from the 5 m depth with a submersible pump. At each sampling location, river water samples were collected and preserved for dissolved organic carbon (DOC) analysis according to a previous protocol (Jiang...
et al., 2009a, b). Twenty litres of river water were sequentially filtered through 0.7 µm (glass fiber, Whatman, Beijing, China) and 0.22 µm (polycarbonate, Whatman, Beijing, China) filters to collect particle-attached and free-living microbial biomasses, respectively (Feng et al., 2009). For the May samples, only particle-attached microbial biomass was collected by filtering the same amount of water through 0.7 µm (glass fiber, Whatman, Beijing, China) filter. As soon as the filtration process was completed, the filters were stored in liquid nitrogen and shipped to the laboratory in a few days. Once back to the laboratory, the filters were stored at −80 °C until further analyses.

2.2. DOC measurement

DOC was measured from acidified samples by using a high temperature catalytic oxidation analyzer (Shimadzu TOC-V) (Sharp et al., 1995, 2002).

2.3. DNA extraction

For the two samples from May 2009, DNA was extracted from the biomass-containing filters to compare particle-attached microbial diversity behind and in front of the dam (TG_BD_0.7_May_09 and TG_FD_0.7_May_09, respectively), where TG stands for the “Three Gorge Dam”, BD stands for “behind the dam” (upstream), FD “in front of the dam” (downstream), and 0.7 “the 0.7-µm fraction”. The FastDNA Spin Kit for Soil (MB Biomedicals, OH, USA) was used for all DNA extraction.

2.4. RNA extraction and cDNA synthesis

For the eight samples from July and December 2009, RNA was extracted from the biomass-containing filters (TG_BD_0.7_Jul_09, TG_BD_0.2_Jul_09, TG_FD_0.7_Jul_09, TG_FD_0.2_Jul_09, TG_BD_0.7_Dec_09, TG_BD_0.2_Dec_09, TG_FD_0.7_Dec_09, TG_FD_0.2_Dec_09) using the FastRNA Pro Soil-Direct Kit (Qbiogene, Inc. CA) according to the manufacturer's protocol. The crude RNA was DNase-digested and then verified to be free of genomic DNA contamination according to our established procedures (Jiang et al., 2010a). The DNA-free RNA samples were reverse-transcribed into cDNA using the Promega AMV reverse transcription system (Promega Corporation, Madison, WI) according to the manufacturer’s instructions.

2.5. PCR, clone library construction, and phylogenetic analyses

Bacterial and archaeal 16S rRNA genes of the DNA and cDNA samples were PCR-amplified using the primer pairs Bac27F (5'-AGA GTT TGA TCM TGG CTC AG-3')/Univ1492R (5'-CGG TTA CCT TGT TAC GAC TT-3') and Arch21F (5'-TTC YGG GTC ATC CYG CCR GA-3')/Arch958R (5'-YCC GGC GTT GAM TCC ATT T-3'), respectively (Jiang et al., 2007). Bacterial and archaeal 16S rRNA gene clone libraries were constructed according to our previously described procedures (Jiang et al., 2009a, b; Jiang et al., 2010b). Clones were randomly selected from each clone library and were analyzed for the insert 16S
rRNA gene fragments. The 16S rRNA gene fragments of randomly selected clones were sequenced by using the specific primers (Bac27F and Arch21F for Bacteria and Archaea, respectively). Nucleotide sequences were assembled and edited by using Sequencer v.4.8 (GeneCodes, Ann Arbor, MI). Sequences were examined with Bellerophon (http://foo.maths.uq.edu.au/~huber/ bellerophon.pl) for potential chimeras, and were discarded if discovered. Operational taxonomic units (OTUs) were determined using the DOTUR program (Schloss and Handelsman, 2005) with a 97% cutoff. The sizes of clone libraries were evaluated for saturation by using rarefaction analysis (www.uga.edu/strata/ software/Software.html). One clone was chosen from each OTU for phylogenetic analysis. The selected representative clone sequences were BLAST-analyzed in the GenBank (http://www.ncbi.nlm.nih.gov). Neighbour-joining phylogenetic trees were constructed from dissimilar distance and pairwise comparisons were made with the Jukes-Cantor distance model using the MEGA (molecular evolutionary genetics analysis) program, version 4.1. Bootstrap replications of 1000 were assessed. The sequences determined in this study were deposited in the GenBank database under accession numbers HM483663-HM483846 and HQ532937-HQ533003. The diversity indices of Shannon (H'), Simpson, and ACE were calculated by using the DOTUR program. Coverage (C) was calculated as follows: 

\[
C = 1 - \frac{n_i}{N},
\]

where \(n_i\) is the number of OTU that only contains one clone in the clone library and \(N\) is the total number of clones analyzed (Jiang et al., 2007).

### 3. Results

#### 3.1. Water chemistry

For the two locations sampled in July and December, the pH values decreased from \(\sim 8.0\) in July to \(\sim 7.0\) in December, 2009; turbidity decreased from 190–480 to 0 NTU; dissolved oxygen (DO) increased from 7.4–8.0 to 8.6–9.2 mg/L; oxidation-reduction potential (ORP) increased from \(<200\) mV to \(>200\) mV. DOC concentrations in July (3–4 mg/L) were much higher than in December (1–2 mg/L).

#### 3.2. DNA-based bacterial diversity across the TGD

For the two DNA-based, May 2009 samples, the bacterial diversity, as indicated by the Shannon index, was slightly higher for TG_BD_0.7_May_09_B (3.0) than for TG_FD_0.7_May_09_B (2.9), indicating that bacterial diversity was similar across the dam (Table 2). Bacterial composition at the major group level for both samples consisted of Beta-, Gamma-, Delta-proteobacteria, Acidobacteria, Nitrospirae, Bacteroidetes, Actinobacteria, and Planctomycyes. However, minor groups Alphaproteobacteria and Verrucomicrobia were unique to TG_BD_0.7_May_09_B, and Acidobacteria and Chloroflexi were unique to TG_FD_0.7_May_09_B (Fig. 3).

#### 3.3. RNA-based bacterial diversity across the TGD at different seasons

Eight bacterial cDNA clone libraries were constructed for the July and December 2009 samples, i.e., TG_BD_0.7_Jul_09_B, TG_BD_0.2_Jul_09_B, TG_FD_0.7_Jul_09_B, TG_FD_0.2_Jul_09_B, TG_BD_0.7_Dec_09_B, TG_BD_0.2_Dec_09_B, TG_FD_0.7_Dec_09_B, and TG_FD_0.2_Dec_09_B. cDNA clone libraries exhibited overall low diversity. Systematic differences were observed at different times (July vs. December) and between particle-attached and free-living bacterial communities. Differences were also observed between the two sampling sites. When the spatial location and microbial fraction were kept constant, the July microbial communities were always more diverse than the December equivalents (Table 2). For example, the Shannon index for the TG_BD_0.7_Jul_09_B was 3.8, higher than the value of 2.4 for the TG_BD_0.7_Dec_09_B. This observation was true for both locations (behind and in front of the dam) and both 0.7 and 0.2 μm fractions (Table 2). When the

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**Table 1** Physico-chemical characteristics of river waters collected behind and in front of the Three Gorges Dam of the Yangtze River

<table>
<thead>
<tr>
<th>Sampling location (E/N)</th>
<th>Sampling time</th>
<th>Depth (m)</th>
<th>pH</th>
<th>Temp (°C)</th>
<th>Turb (NTU)</th>
<th>DO (mg/L)</th>
<th>ORP (mV)</th>
<th>Cl⁻ (mg/L)</th>
<th>DOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG_BD</td>
<td>May</td>
<td>5.0</td>
<td>8.0</td>
<td>23</td>
<td>ND</td>
<td>ND</td>
<td>7.6</td>
<td>155</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>10.1</td>
<td>8.0</td>
<td>27</td>
<td>320</td>
<td>7.4</td>
<td>157</td>
<td>12.6</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.1</td>
<td>8.0</td>
<td>27</td>
<td>390</td>
<td>7.5</td>
<td>155</td>
<td>13.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.2</td>
<td>8.0</td>
<td>27</td>
<td>430</td>
<td>7.5</td>
<td>160</td>
<td>13.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.3</td>
<td>8.0</td>
<td>27</td>
<td>410</td>
<td>7.4</td>
<td>163</td>
<td>13.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>5.0</td>
<td>7.1</td>
<td>17</td>
<td>0</td>
<td>8.3</td>
<td>260</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td>7.1</td>
<td>17</td>
<td>0</td>
<td>9.0</td>
<td>273</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0</td>
<td>7.1</td>
<td>17</td>
<td>0</td>
<td>9.2</td>
<td>277</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>TG_FD</td>
<td>May</td>
<td>5.0</td>
<td>8.0</td>
<td>18</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>July</td>
<td>2.1</td>
<td>7.9</td>
<td>27</td>
<td>480</td>
<td>8.1</td>
<td>180</td>
<td>13.7</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>7.9</td>
<td>28</td>
<td>460</td>
<td>8.0</td>
<td>180</td>
<td>14.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6</td>
<td>7.9</td>
<td>27</td>
<td>440</td>
<td>8.0</td>
<td>180</td>
<td>14.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>4.8</td>
<td>7.9</td>
<td>28</td>
<td>420</td>
<td>8.0</td>
<td>181</td>
<td>14.8</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>6.6</td>
<td>17</td>
<td>0</td>
<td>8.6</td>
<td>293</td>
<td>3.3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td>7.1</td>
<td>17</td>
<td>0</td>
<td>8.8</td>
<td>267</td>
<td>2.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* The shaded samples were used for the molecular work. The conductivity was 23 ms/m, salinity 0.15 g/L, total dissolved solid (TDS) 0 g/L, and ammonia concentration 0.100 μg/L, respectively.

* The samples behind and in front of the Three Gorges Dam were labelled with TG_BD and TG_FD, respectively; ND: Not determined.
Table 2  Prokaryotic diversity indices estimated at 97% cutoff of OTU sequence identity in free-living and particle-attached fractions of the samples collected from the TGD of the Yangtze River (the shaded samples indicated those from the 0.2 μm fraction).

<table>
<thead>
<tr>
<th>Filter fraction (μm)</th>
<th>DNA-based</th>
<th>RNA-based</th>
<th>DNA-based</th>
<th>RNA-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling time</td>
<td>May</td>
<td>July</td>
<td>Dec</td>
<td>May</td>
</tr>
<tr>
<td>Bacterial 16S rRNA gene clone library</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone library</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.2_</td>
</tr>
<tr>
<td>Coverage (%)</td>
<td>67.80</td>
<td>19.61</td>
<td>59.38</td>
<td>89.47</td>
</tr>
<tr>
<td>No. of OTUs</td>
<td>25</td>
<td>45</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Shannon (H')</td>
<td>3.0</td>
<td>3.8</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Simpson</td>
<td>0.03</td>
<td>0.01</td>
<td>0.10</td>
<td>0.44</td>
</tr>
<tr>
<td>ACE richness index</td>
<td>84.3</td>
<td>321.5</td>
<td>71.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Chao1</td>
<td>109.5</td>
<td>318.3</td>
<td>43.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Archaeal 16S rRNA gene clone library</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone library</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.7_</td>
<td>TG_BD_0.2_</td>
</tr>
<tr>
<td>Coverage (%)</td>
<td>89.47</td>
<td>82.35</td>
<td>100</td>
<td>93.33</td>
</tr>
<tr>
<td>No. of OTUs</td>
<td>19</td>
<td>11</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Shannon (H')</td>
<td>2.4</td>
<td>1.7</td>
<td>0.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Simpson</td>
<td>0.13</td>
<td>0.32</td>
<td>1.00</td>
<td>0.22</td>
</tr>
<tr>
<td>ACE</td>
<td>26.0</td>
<td>17.7</td>
<td>0.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Chao1</td>
<td>22.7</td>
<td>14.8</td>
<td>1.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Figure 2 Neighbour-joining tree (partial sequences, ~700 bp) showing the phylogenetic relationships of bacterial 16S rRNA gene sequences cloned from the studied samples to closely related sequences from the GenBank database. One representative clone type within each phylotype is shown, and the number of clones within each OTU is shown at the end. As an example, in clone sequence TG_FD_0.2_May_09_B109, TG_FD in front of the TGD; 0.2=0.2-µm fraction; May_09 = May 2009; B109 = bacterial clone number 109. Scale bars indicate the Jukes-Cantor distances. Bootstrap values of >50% (for 500 iterations) are shown. *Aquifex pyrophilus* is used as an outgroup. Panels A, B, and C are the bacterial trees for the samples collected in May, July, December 2009, respectively. Panel B contains two subtrees, showing *Alpha*, *Beta*, *Gamma*, and *Deltaproteobacteria* and non-Proteobacteria, respectively.
Figure 2 (Continued)
spatial location and the sampling season were kept constant, the particle-attached communities were always more diverse than the free-living ones. For example, the Shannon index for TG_BD_0.7_July_09_B was 3.75, higher than the value of 1.25 for TG_BD_0.2_Jul_09_B. This observation was true for both locations and both sampling times (Table 2).

For the same sampling time and same fraction (either particle-attached or free-living), the bacterial population behind the dam was generally more diverse than in front of the dam (Fig. 4A). For example, the Shannon indices for TG_BD_0.7_July_09_B and TG_BD_0.7_Dec_09_B were 3.8 and 2.4, respectively, much higher than those for their corresponding in_front_of_dam counterparts (3.2 and 1.1 for TG_FD_0.7_July_09_B and TG_FD_0.7_Dec_09_B). This observation was true for both particle-attached and free-living fraction (Fig. 4A). For example, for the sample behind the dam, the free-living fraction was predominantly by Spirochaetes, Alphaproteobacteria, Bacteroidetes, and Planctomycetes (Fig. 2). Noticeably, in BRC1 (Derakshani et al., 2001) and BRC2 (Tavasoli et al., 2004) the free-living fraction; and Betaproteobacteria, Alphaproteobacteria, and Planctomycetes in the particle-attached fraction. The sample in front of the dam was dominated by Actinobacteria, Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria in the free-living fraction; and Betaproteobacteria, Gammaproteobacteria, and Actinobacteria in the particle-attached fraction (Fig. 4A). In December, more dramatic differences were observed between the two sites and between free-living and particle-attached fractions (Fig. 4A). For example, for the sample behind the dam, the free-living fraction was predominantly by Gammaproteobacteria, but Bacteroidetes and Gammaproteobacteria, Alphaproteobacteria, and Cyanobacteria were dominant groups in the particle-attached fraction. Likewise, for the sample in front of the dam, the free-living fraction was dominated by Bacteroidetes, Cyanobacteria, and Planctomycetes, but the Bacteroidetes, Alphaproteobacteria, and Firmicutes were dominant groups in the particle-attached fraction.

### 3.4 DNA-based archaeal diversity

At the group level, the May sample behind the dam had a slightly more diverse archaeal population than the May sample in front of the dam (Fig. 3). The same observation could be made at the
Figure 3 Neighbour-joining tree (partial sequences, ~700 bp) showing the phylogenetic relationships of archaeal 16S rRNA gene sequences cloned from the studied samples to closely related sequences from the GenBank database. The same algorithms as those for the bacterial tree (Fig. 2) were used. *Aquifex pyrophilus* is used as an outer group. One representative clone type within each OTU is shown, and the number of clones within each OTU is shown at the end (after the GenBank accession number). Panels A, B, and C are the archaeal trees for the samples collected in May, July, December 2009, respectively.
Figure 3 (Continued)
species level, as indicated by the higher Shannon index for the TG_BD_0.7_May_09_A (2.4) than that for TG_FD_0.7_May_09_A (2.1). Both samples consisted of Crenarchaeotal Group 1.1a, Group 1.1b, and Group 1.3b as the dominant groups. Minor groups such as Marine Benthic Group-B (MBG-B, also called deep-sea archaeal group-DSAG), Marine Benthic Group-D (MBG-D), Methanosarcina, and Natronomonas were unique to TG_BD_0.7_May_09_A; whereas Crenarchaeotal group 1.2, South Africa gold mine Crenarchaeotic group-1 (SAGC) and Methanosaeta were unique to TG_FD_0.7_May_09_A.

3.5. RNA-based archaeal community

Eight cDNA archaeal clone libraries were constructed for the July and December 2009 samples, i.e., TG_BD_0.7_Jul_09_A, TG_BD_0.2_Jul_09_A, TG_FD_0.7_Jul_09_A, TG_FD_0.2_Jul_09_A, TG_BD_0.7_Dec_09_A, TG_BD_0.2_Dec_09_A, TG_FD_0.7_Dec_09_A, and TG_FD_0.2_Dec_09_A. The July archaeal populations were much more diverse than the December populations. There was little difference in diversity between free-living and particle-attached archaeal community. The two July samples across the dam had a similar diversity at both group and species levels (Fig. 4A and Table 2), and the two December samples were both least diverse with no difference in diversity across the dam.

The archaeal 16S rRNA gene clone sequences were classified into six euryarchaeotal and four crenarchaeotal major groups (Fig. 4B). These groups include Halobacteriaceae, Methanosarcinaceae, Methanosaetaceae, Methanomicrobiaceae, uncultured candidate division VALIII (Jurgens et al., 2000), unclassified Euryarchaeota, Desulfuromonadaceae, marine group I (MG-I), deep-sea archaeal group (DSAG) (Vetriani et al., 1999; López-García et al., 2001), and miscellaneous crenarchaeotal group (MCG) (Inagaki et al., 2003) (Figs. 3 and 4B). Besides the overall difference in species diversity, the relative abundance of these major groups varied between the two sampling times and between the two sites (Fig. 4B): temporally, the dominant organisms changed from MG-I in July to Desulfurococaceae in December; spatially, the minor groups were different between the two samples across the dam. In addition, minor differences existed between the free-living and particle-attached communities (Fig. 4B).

4. Discussion

4.1. Bacterial and archaeal diversity

Typical freshwater bacterial clusters include Alpha-, Beta-, Gamma-, and Deltaproteobacteria, the Cytophaga-Flavobacterium-Bacteroides (CFB) group, the Cyano bacteria, the Actinobacteria, the Verrucomicrobia, the Planctomycetes, Gram-positive bacteria, the Firmicutes, green non-sulphur bacteria, and Candidate division OP10 (Zwart et al., 2002; Hahn, 2006). Alpha-, Beta-, Gammaproteobacteria, the CFB group, the Verrucomicrobia, and the Planctomycetes are present in Columbia River (Crum et al., 1999) and they were detected in the water column near the TGD. In addition to these groups, several unique groups such as Spirochaetes, Nitrospirae, Chloroflexi, and Acidobacteria were present as minor components (Figs. 2 and 4A). Because the Yangtze River is a freshwater river, it is expected that most retrieved bacterial clones are affiliated with those typical freshwater microbes.

Obligate anaerobic methane-producing methanogens (e.g. Methanosarcinaceae, Methanosetaeaceae, Methanomicrobiaceae) were present in the archaeal clone library (Figs. 3 and 4B), despite the fact that the water column of Yanetze River was oxic. It is possible that anoxic micro-niches could be present in particles suspended in the water column. Indeed, a previous study reported the presence of methanogens within particles suspended in river waters (Crum and Baross, 2000a).

4.2. Differences in microbial communities between the three sampling seasons

Microbial diversity in the summer (May and July) was much higher than in the winter (December) (Fig. 4). Previous studies have shown that microbial diversity and community structures in rivers varied seasonally, and nutrient levels were one of the most important controlling factors for such seasonal variations (Amon and Benner, 1998; Brummer et al., 2004; Crum and Hobbie, 2005; Tirodimos et al., 2010). Indeed, the data from this study indicated that the water in the summer contained higher levels of DOC and higher turbidity than those in the winter. In more turbid
water, nutrient levels should be higher because nutrients are typically adsorbed to suspended particles.

The archaeal communities were distinctly different between the summer (May and July) and winter (December), even more so than their bacterial counterpart: the archaeal community was dominated by MG-I in the summer, but by the Desulfurococcales in winter (Fig. 4B). The temperature difference between July and December (around 10°C) did not correlate with any physiological differences between these two groups of archaea. In fact, MG-I is commonly present in the marine environment and can be dominant in deep-sea water (Karner et al., 2001). This group is also abundant in freshwater lake (MacGregor et al., 1997; Schleper et al., 1997) and Columbia River (Crump and Baross, 2000a). Therefore this group is adapted to mesophilic or cold environment. The reasons for its greater occurrence in the summer are not known.

Figure 4 Bar graphs showing the frequencies of OTUs affiliated with the major phylogenetic groups in the bacterial and archaeal clone libraries for the 0.2- and 0.7-μm fractions in the waters behind and in front of the TGD of the Yangtze River. Panels A and B are for bacterial and archaeal planktons, respectively.
In contrast, the dominant group of archaea in winter, i.e., the Desulfurococcaceae species, are actually thermophilic with an optimum growth temperature $>85$ °C (Burggraf et al., 1997). In winter, the average river temperature was around 17 °C, much lower than the optimal growth temperature for this group, and even lower than their minimum temperature. At present, the reasons for its dominance in winter remained unclear. One possibility was that the Desulfurococcaceae-related microorganisms may be carried by the runoff from unknown thermal sources from the upstream. Another possibility was that these organisms may have identical 16S rRNA gene sequences as the known Desulfurococcaceae, but may have different physiological properties (Jaspers and Overmann, 2004).

4.3. Differences between the free-living and particle-attached communities

One important observation of this study was that the particle-attached communities were always more diverse than the free-living communities for all sites and sampling times. The particle-attached communities harbored some unique groups that were not present in the free-living communities (Fig. 4). This systematic difference has been observed before and has been ascribed to fundamental differences in micro-geochemical environments between sediments and water (DeLong et al., 1993; Acinas et al., 1999; Crump et al., 1999; Phillips et al., 1999; Schweitzer et al., 2001). Among these differences, sediments usually contain high levels of nutrients and even some anoxic micro-niches, even though the bulk water column may be oxic (Crump and Baross, 2000b). These nutrient-rich and diverse micro-environments in sediments would therefore support abundant and diverse microbial communities.

4.4. Differences in microbial communities between the two sampling sites

For all three sets of samples and based on both DNA and RNA approaches, the data consistently showed that the bacterial populations behind the dam (i.e., in the upstream direction) were more diverse than that in front of the dam (the downstream direction). The difference in the diversity across the dam, based on the RNA approach, was much larger than that based on the DNA approach, suggesting that metabolically active bacteria were more sensitive to environmental differences between the upstream and downstream of the dam.

The construction of the TGD and storage of a large quantity of water (up to 175 m) created major geochemical differences across the dam both visually (pictures not shown) and quantitatively (Table 1). The creation of a large reservoir immediately behind the dam substantially reduced the flow rate to the downstream river, and a large amount of sediments was trapped in the reservoir. As a result, the reservoir was expected to accumulate diverse sources of sediments and waters as well as diverse microbes associated with them. Therefore, the microbial diversity behind the dam was expected to be diverse and the bacterial diversity data supported the original hypothesis.

The reservoir water behind the dam would eventually be released to the downstream, but after a long residence time (years), microbial community within this water was expected to be less diverse. The possible development of an anoxic layer near the bottom, depletion of nutrients, and lack of water-sediment exchange in the reservoir would cause microbial death and significantly diminish microbial diversity. The observation that the DNA-based approach did not reveal significant difference across the dam, but the RNA-based approach did, supported this hypothesis. The extracted DNA from biomass should have contained both live and dead microbes carried downstream from the upstream, whereas extracted RNA should have contained live microbes only. So any cell starvation or death in the upstream reservoir would not be reflected in the DNA-based microbial population, but it would be obvious in the RNA-based community structure.

Although the results of this study suggest that the construction of the TGD alters water dynamics and geochemical environments, and may have accounted for the observed spatial differences in microbial diversity and composition, we caution that these are preliminary results. Future studies are necessary to confirm these results. In particular, sampling frequency should be increased both spatially and temporally to reveal any systematic trends. These future plans are currently underway.

5. Conclusion

In the summer, both archaeal and bacterial communities were more diverse than those in the winter, due to higher nutrient levels and higher temperatures in the summer. Systematic differences were observed between free-living and particle-attached communities, primarily because of differences in nutrient levels and micro-geochemical environments. Bacteria were more sensitive than archaea to spatial variations in geochemical conditions across the dam: the water reservoir behind the TGD hosted diverse bacterial populations from the upstream of Yangtze River; whereas the downstream river had much reduced sediment loads and reduced bacterial diversity. These results supported our hypotheses and provided a basis for formulating new questions and hypotheses for future research.

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