Response of Prokaryotes to TOC Contamination in Dianchi Lake Sediments

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Abstract

Background: Explaining microbial consortia in sediments from the perspective of taxon, co-occurrence and function is the key to recover and maintain aquatic ecosystems. Trophic status was widely considered to be an important determinant of the lake sediment microbial community. However, little is known about the effect of a special eutrophic factor gradient on the prokaryotic community structure in situ. Within this context, we explored prokaryotic communities using an intensive field sampling from sediments in the Dianchi Lake, one of the most eutrophic lakes in China.

Results: Microbial assemblages was strongly correlated with total organic carbon (TOC). Moreover, relatively high and low TOC shaped taxonomic and functional differences in microbial assemblages. The results identified the most abundant bacteria across all samples as Proteobacteria, Nitrospirae, Chloroflexi, Firmicutes, Ignavibacteriae, Actinobacteria, Bacteroidetes, Acidobacteria, Spirochaetae and Latescibacteria. The dominant groups of archaea were Euryarchaeota, Woesearchaeota DHVEG-6, Bathyarchaeota and WSA2. Low TOC (LT) microbial assemblages displayed a major proportion of functional profiles related to some metabolisms such as carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism and metabolism of cofactors and vitamins, membrane transport. These results illustrated that TOC concentration had obvious influence on the relative abundances of KEGG orthologs. Finally, the meta-analysis results highlight that most of network parameters of the LT community were significantly greater than those of other communities, which suggesting that the LT community was larger and more complex.

Conclusion: TOC level might be a key determinant to shape taxonomic and functional construction of communities in Dianchi Lake sediment. LT community tended to establish a larger and more complex co-occurrence, which suggested that they may interact with each other strongly and exchange essential metabolites. Overall, this study could enhance our knowledge of microbial assemblages in eutrophic lake sediment and provide clues for the restoration and maintenance of sediment ecosystems.

Background

Microbial consortia harbored in the sediment are at the hub of the ecological processes, and play pivotal role in the biogeochemical cycling of nutrients, biotransformation and biodegradation of pollutants, and the restoration and maintenance of sediment ecosystems [1–3]. Understanding prokaryotic communities in lake sediment ecosystems can provide clues for ecosystem management and biodiversity preservation. It has been of particular interest to get a more comprehensive understanding of the mechanisms driving the assembly and interactions of freshwater-sediment microbial communities [4–7].

Over the years, numerous studies have shown that both variations in the physicochemical properties and in biotic factors can shape sediment ecosystems communities [8, 9]. Swan et al. found that salinity in the sediments of a California Hypersaline Lake had an effect on the distribution of bacteria and archaea [10]. Pollution levels are important environmental variables related to bacterial community in lake sediments [11]. What's more, most studies have shown the spatial variation model of prokaryotic community in lake sediment may be unique [12, 13].

All of the above conclusions are reached on the comparing inter-lake. Sediments having a longer geographical distance will be subject to distinct ambient conditions (hydrological properties, climatic influences, geological settings and catchment area). However, majority of the previous researches ignored the very significant influence of geographical distance. Thus, it can mask the impact of environmental factors and hinder the exploration of some ecological rules.

In addition, the more comprehensive mechanism of factors driving the assembly of lake-sediment prokaryotic communities remains still elusive. Some previous studies have revealed that trophic status was widely considered to be an important determinant of the lake sediment microbial community [14–17]. To date, little is known about the effect of a specific eutrophic factor gradient on the prokaryotic structure in situ. Dianchi Lake stretches 39 km length from north to south and extends 13.5 km width in west–east direction (including Caohai and Waihai on the north and south respectively). Sediments of Dianchi Lake considered as an ideal model system to address ecological principles exhibit large changes of physical and chemical parameters. Bai et al indicate that TOC is the main driving force of bacterial community variation in Dianchi Lake [5]. However, the effects of TOC gradient on variation of prokaryotic community structure in lake sediments have not been well explored.

Recently the functional composition of a microbial community can be visualized and interpreted perfectly through number of powerful new bioinformatics tools [18]. Some particularly computational tools have obvious applications for predicting metagenomes from16S rRNA information [19]. Unfortunately, in contrast to the substantial works done on the assemblages of sediment microbial community, information on its metagenomic signatures in lake-sediment remain still fragmentary. Moreover, the specific influence of environmental factor gradient on microbial function remains largely unclear.
Microbial communities form ecological networks with each other to perform ecosystem functions [20–22]. For the purpose of maintaining homeostasis, the microbes established a stable network through nutrient cycling and niche competition processes. Thus, understanding the interactions of microbial systems is important and significant on account of it may shed light on their environmental niches, functional roles, or ecological interactions in the ecosystem. Using the analytical procedure of network analysis, a large number of studies have explored the complex links in many microbial ecosystems, including lake [23], stream soil [24] and hydrothermal vents [25]. So far, only a handful of previous works have investigated the microbial community networks in freshwater lake sediments. Nevertheless, effects of environmental factors on the interactions of microorganisms in lake-sediments have not been addressed.

In this study, we hypothesized that ascribing to differences in responses to TOC gradient, the distribution, metagenomic signatures and co-occurrence of microorganisms in Dianchi Lake sediment differ. To examine the effect of TOC level on the composition, functional characteristics and networks of archaea and bacteria, we performed a relatively high density sampling in situ at Dianchi Lake in China. Only within the lake, thirteen representative sediments were selected randomly, which there was no palpable difference in geographical distance. And then we implemented Illumina MiSeq sequencing of the hypervariable V3–V4 portion of 16S rRNA gene on the microbial communities. The objectives of the present study were to address the following three questions: (i) What is the specific mechanism of the driving force of TOC concentration on the microbial assemblages? (ii) Is there a close link between TOC gradient and functional dynamics of microbial community? (iii) Can TOC level analyze the complexity of microbial co-occurrence dramatically?

As far as we know, this is the first study to explore the composition and functional dynamics of bacteria and archaea in the TOC gradient contaminated environment and adopt network thinking and network analysis tools to research microbial communities in a lake sediment ecosystem.

Results

Variability of cardinal environmental factors of the sediments

A series of physicochemical gradients in sediments of Dianchi Lake were presented in Table S1. Some parameters of Dianchi Lake sediment existed horizontal heterogeneity. Generally, the sediment temperature values were relatively high at sites DC06 and DC07; while low at sites DC01 and DC02. The NO$_2^-$-N, and NO$_3^-$-N values were in ranges of 0.87–15.74 and 0.009–0.046 mg/kg respectively. Additionally, the TP (0.084–0.477%) content reached the highest point at site DC01 and a bottom at site DC09. The contents of TN (0.24–0.92%) also fluctuated considerably (Table 1).

Table 1: Geographical locations and physicochemical composition of sediment samples

| Samples | Longitude (E) | Latitude (N) | Depth (m) | Temp(°C) | WT (%) | TOC (g/kg) | TN (N, %) | TP (P, %) | NO$_2^-$-N (mg/kg) | NO$_3^-$-N (mg/kg) |
|---------|---------------|--------------|-----------|-----------|--------|------------|-----------|-----------|-------------------|-------------------|
| DC01    | 102°39.934'   | 25°1.167'    | 3         | 11        | 83.81  | 87.3       | 0.678     | 0.477     | 12.67             | 0.032             |
| DC02    | 102°38.807'   | 25°0.304'    | 3         | 11        | 85.97  | 131.2      | 0.92      | 0.437     | 13.59             | 0.032             |
| DC03    | 102°38.529'   | 24°58.871'   | 3.2       | 11        | 83.66  | 49.1       | 0.472     | 0.18      | 3.33              | 0.023             |
| DC04    | 102°40.742'   | 24°57.154'   | 4.2       | 16.5      | 67.11  | 32.5       | 0.24      | 0.221     | 15.74             | 0.023             |
| DC05    | 102°39.852'   | 24°55.997'   | 5         | 16        | 82.07  | 56.9       | 0.519     | 0.229     | 2.43              | 0.034             |
| DC06    | 102°41.893'   | 24°53.333'   | 5.5       | 17        | 80.45  | 45         | 0.424     | 0.22      | 9.49              | 0.009             |
| DC07    | 102°40.419'   | 24°51.799'   | 5.5       | 17        | 78.71  | 41.1       | 0.39      | 0.145     | 4.36              | 0.009             |
| DC08    | 102°45.063'   | 24°49.445'   | 6         | 15.2      | 73.67  | 36.1       | 0.343     | 0.211     | 6.51              | 0.018             |
| DC09    | 102°42.58'    | 24°49.137'   | 5.5       | 15        | 84.07  | 53.6       | 0.504     | 0.084     | 6.51              | 0.012             |
| DC10    | 102°44.603'   | 24°48.057'   | 6         | 15.2      | 83.78  | 49         | 0.448     | 0.188     | 2.6               | 0.014             |
| DC11    | 102°36.932'   | 24°46.164'   | 4.5       | 15        | 81.37  | 54.4       | 0.537     | 0.263     | 0.87              | 0.011             |
| DC12    | 102°36.785'   | 24°41.733'   | 4         | 15.1      | 75.04  | 50.4       | 0.517     | 0.305     | 6.49              | 0.014             |
| DC13    | 102°39.823'   | 24°41.324'   | 4         | 15.1      | 78.27  | 40.8       | 0.391     | 0.214     | 5.79              | 0.046             |
Significant gradient in sediment TOC (32.5–131.2 g/kg) content from each sampling site was observed. In general, the TOC contents in Caohai showed higher than those collected from Waihai. According to the contents of TOC, all the samples captured three distinct levels representing TOC gradient for low (36.1–45 g/kg), moderate (49-56.9 g/kg), and high (87.3-131.2 g/kg) concentration, respectively (Table 1).

**Prokaryotic community richness and diversity**

Perfectly unbiased profiling procedure for illustrating the shifts in relative abundance and diversity of microbial communities is provided on the basis of 16S rRNA gene amplicons generated from Illumina MiSeq sequencing. Totally, 375,170 quality-controlled sequences of the 16S rRNA gene were achieved from 13 sediment samples (Table S2). The Good's coverage for the observed OTUs was above 0.96 and the rarefaction curves (Fig. S2) showed that the number of individuals analyzed was reasonable for almost representing the diversity of the 13 samples. Trying to avoid the biases of sequencing depths among samples, 15,251 sequences were subsampled randomly in each sample and all reads (at least two sequences in one sample) were clustered into 3,312 operational taxonomic units (OTUs) with 97% similarity by high-throughput sequencing. Simpson values ranged from 0.008 to 0.036 (Table 1). The ACE estimators of microbial communities were 1,919.384–2,377.044 (Table 1, 2).

| sample | Sequences | No. of OTUs | Simpson | Chao | ACE | Shannon | Coverage |
|--------|-----------|-------------|---------|------|-----|---------|----------|
| DC01   | 26005     | 1557        | 0.009   | 2042.274 | 2084.332 | 5.889 | 0.966 |
| DC02   | 25853     | 1465        | 0.036   | 1917.700 | 2002.065 | 5.421 | 0.967 |
| DC03   | 21952     | 1424        | 0.012   | 1849.580 | 1919.384 | 5.608 | 0.969 |
| DC04   | 27606     | 1655        | 0.008   | 2100.918 | 2183.520 | 5.991 | 0.964 |
| DC05   | 26523     | 1443        | 0.013   | 2041.724 | 2108.839 | 5.508 | 0.964 |
| DC06   | 31895     | 1479        | 0.010   | 2064.937 | 2121.869 | 5.659 | 0.963 |
| DC07   | 30264     | 1366        | 0.013   | 2048.141 | 2074.313 | 5.532 | 0.963 |
| DC08   | 24990     | 1440        | 0.011   | 1989.004 | 2084.492 | 5.600 | 0.964 |
| DC09   | 33898     | 1679        | 0.008   | 2319.198 | 2377.044 | 6.010 | 0.960 |
| DC10   | 36736     | 1458        | 0.014   | 2074.249 | 2141.484 | 5.532 | 0.962 |
| DC11   | 29570     | 1280        | 0.020   | 1856.914 | 1947.488 | 5.253 | 0.966 |
| DC12   | 24665     | 1433        | 0.017   | 1994.204 | 2087.683 | 5.512 | 0.964 |
| DC13   | 35213     | 1136        | 0.016   | 1704.119 | 2105.406 | 5.122 | 0.969 |
| Total  | 375170    | 3,312       |         |       |     |         |          |

Microbial community overall variation within sediments

For the purpose of analyzing microbes in sediment, the Dianchi Lake bacterial community structure was analyzed on the basis of high throughput sequencing technology. Probing on phylum level, the dominant taxa hailing from the 13 sites were also analysed. A total of 10 bacterial phyla and 8 archaeal phyla (a relative abundance totaling > 1%) were detected across all the samples, including 46 and 13 genera, respectively. The relative abundance of bacterial and archaeal populations at phylum and genus levels was presented in (Fig. 1, DC04 was the highest. The highest Chao index occurred in DC09. The ACE estimators of microbial communities were 1,919.384–2,377.044 (Table 2).

Proteobacteria was the dominant bacterial group among the samples (Fig. 1), accounting for 27.4–64.4% of the total reads in each sample, followed by Nitrospirae (1.2–15.8%), Chloroflexi (3.3–8.7%), Firmicutes (0.9–11.7%), Ignavibacteria (0.5–5.7%), Actinobacteria (0.8–4.7%), Bacteroidetes (0.6–4.9%), Acidobacteria (0.8–2.9%), Spirochaetae (0.4–2.1%), Latescibacteria (0.3–1.5%). Taken together, these bacterial phyla make up more than 94.6% of reads distributed at the phylum level (Fig. 1). In the genera of bacteria, Nitrosospira (1.2–15.8%) had particularly abundant in most samples (Fig. S3). At the end of the list were Candidatus Competibacter (0.7–6.1%), Ignavibacterium (0.1–5.0%), a genus belonging to family Xanthomonadaceae Incertae Sedis (1.0–5.0%) had highly abundant in most samples (Fig. S3). At the end of the list were Candidatus Competibacter (0.7–6.1%), Ignavibacterium (0.1–5.0%), a genus belonging to family Xanthomonadaceae Incertae Sedis (1.0–5.0%) had highly abundant in most samples (Fig. S3).

The phylum level scatter of archaeal communities across the samples was also shown in Fig. 1, i.e. Euryarchaeota (3.3–32.2%), Woesearchaeota DHVEG-6 (0.6–5.5%), Bathyarchaeota (0.3–3.5%), and WSA2 (0.1–3.9%), sequences that could not be matched a known
phylum (0.1–1.0%). In addition, the phyla that could not be found in all samples included Miscellaneous Euryarchaeotic Group MEG, norank Archaea and Aenigmarchaeota. To observe the structure of archaea at the genus level, we analysed the community with higher relative abundance. As shown in Fig. S3, Methanoseta (1.0–19.7%) was the dominant archaean genus, followed by a genus belonging to phylum Woesearchaeota DHVEG-6 (0.6–5.5%), Methanoregula (0.7–6.2%), a genus belonging to family ASC21 (0.1–8.9%), Methanolinea (0.4–6.8%) and a genus of phylum Bathyarchaeota (0.3–3.5%).

**Linking sediment bacterial communities to environmental factors**

RDA was performed to depict the correlation between prokaryotic community structure and relevant environmental factors in the sediment (Fig. 2). We sought to find the most important environmental factors contributing to the distribution of microbes. The sediment environmental factors in the first and second axes accounted for 54.39% of the variance for OTU composition (RDA 1: 40.07%; RDA 2: 14.32%). Total organic carbon (TOC), total nitrogen (TN) and NO$_3^-$–N (N5) were most significantly correlated (P < 0.05 or P < 0.01) with the prokaryotic composition structure in Dianchi Lake sediment (Fig. 2 and Table S1). However, TOC was the strongest impact factor in determining prokaryotic community composition ($r^2 = 0.728, P = 0.02$) compared to all environmental variables tested. Interestingly, TOC exhibited significant negatively correlations with the community structures of almost all the samples.

**Prokaryotic β-diversity among samples**

In order to profile potential effect of TOC on the prokaryotic composition, Partial Least Squares Discriminant Analysis (PLS-DA) was performed to evaluate community similarity of all samples (Fig. 3A). The PLS-DA results at the OTU level showed that the assemblages could be parsed into four major subgroups, G1, G2, G3 and G4. Group G1 contained four samples (DC06, DC07, DC08, and DC13 from low TOC areas), Group G2 contained five samples (DC05, DC09, DC10, DC11, and DC12 from moderate TOC areas), Group G3 contained two samples (DC01 and DC02 from high TOC areas), and Group G4 contained two samples (DC03 and DC04 adjacent to the dam). The prokaryotic communities of each group were clustered together respectively, which indicated that there were significant differences in the total community structures. These distinct clustered subgroups were also proved by hierarchical cluster analysis based on OTU level (Fig. 3B). Meanwhile, Anosim ($p = 0.001$) analysis indicated that the difference among groups was greater than that within groups (Fig. S4). All these results suggested that TOC may be the most relevant abiotic factor affecting the microbial community structures in Dianchi Lake sediment ecosystems.

**Diversity of the prokaryotic communities among groups**

Our next goal is to further explore which prokaryotes TOC has influenced. So Redundancy analysis was executed between G1 and G2 samples with the LEfSe tool. Only LDA scores of 3 or greater were shown in cladograms (Fig. 4, S5). The bacteria that were significantly enriched in group G1 included 5 groups (Fig. 4), such as a genus belonging to order MSBL5, 43F 1404R and Sva0485 respectively (including the genus and their order and family), two families Syntrophobacteraceae and Alcaligenaceae (including the family and its unknown genus). In archaea, G1 was mainly composed of two groups (Fig. 4), including Bathyarchaeota (from phylum to genus), the family Desulfobacteraceae and its unknown genus. While in the G2 samples, 6 bacterial groups were significantly enriched, including Bacteroidetes vadinHA17 (from Class to genus), Leptolina within Anaerolineaceae, Napoli_4B_65 (from order to genus), a genus Dechloromonas within Rhodocyclaceae, Desulfarculales and its family Desulfarculaceae and its genus Desulfatiglans, order Xanthomonadales and its family Xanthomonadales Incertae Sedis.

**Predicted metagenome potentials**

PICRUSt was used to predict the functional content of bacterial communities in Dianchi Lake sediment samples based on the KEGG Ortholog predictions. The relative abundances of functional profiles were different between in low TOC sites and in other relatively high TOC sites (Fig. 5). They were clustered into two groups in which one was overrepresented and the other was underrepresented in low TOC sites. The overrepresented group in low TOC sites included some metabolisms such as carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism and metabolism of cofactors and vitamins, membrane transport belonging to Environmental Information Processing, replication and repair and translation belonging to genetic information processing and Cell motility. These results illustrated that TOC concentration had obvious influence on the relative abundances of KEGG orthologs (KOs).

**Microbial interaction Network**

From the species-species association network analysis (Fig. 6), we observed that the co-occurrence patterns possessed a significantly different trend between a low TOC level and a medial TOC level. The more interactions (edges) of the LT community than the MT community was harbored despite the comparable number of vertexes. For instance, we found that the LT community network possessed a total of 146 nodes connected with 931 edges, whereas in the MT community 121 nodes and 154 edges were retrieved. Furthermore, our analysis revealed that Average degree (AD), as a robust indicator of network topology [29], was significantly higher in LT (12.8 and 2.5 respectively). The comparisons with the other topological properties widely used in network analysis were summarized in Supplementary Table S2. Interestingly,
these network parameters suggested that the LT network was more complex. LT and MT network shared only 11 common edges (Fig. S6). These results suggested LT community with lower TOC level tended to establish larger and more complex co-occurrences compared to MT community.

Discussion

To date, the environmental factors contributing to the microbial assemblages in the lake sediments are essentially unclear, although previous studies have shown that pH [26, 27], organic matter [28], nitrogen [29], phosphorus [30] and heavy metal [31] were the most important drivers of microbial communities in lake sediments. In this study, the results of RDA showed that TOC level mainly accounted for the changes of microbial community in Dianchi Lake sediment. This was consistent with the results reported in the earlier research [5]. The remarkably organic matter effect on change of sediment bacteria and archaea was also observed in the structure of microbial community in sediments of Erhai Lake [32].

Many previous studies have shown striking spatial heterogeneity in the prokaryotic community in freshwater lake sediment [26, 29, 32–35]. In the earlier research of Dianchi Lake, Bai et al indicated that the microbial communities obviously clustered into two groups (Caohai and Waihai) [5]. In the present study, the results of UPGMA cluster and PLS-DA analysis also showed that the community structure of bacterial and archaea community in Dianchi Lake sediment had obvious spatial changes. DC03 from the Caohai and DC04 from the Waihai clustered together, this may have been affected by the dam (Fig. S1) and all the other sediment samples were divided into three groups (G1, G2 and G3) according to the TOC concentration. These results confirmed that the microbial structure in Dianchi Lake sediment was positively correlated to TOC levels. To improve the accuracy of the result, we selected two representative groups G1 and G2 for the LEfSe analysis. LEfSe analysis indicated that both groups possessed their own unique biological groups.

PICRUSt analysis predict a major proportion of functional profiles in the low TOC samples related to membrane transport, carbohydrate metabolism, cell motility and the decrease of those with increasing TOC influence. In the high TOC sites, high abundance of specific genes was not observed. These results indicated the microbial community had a higher level of carbohydrate metabolism at low TOC and concentration of TOC was negatively correlated with sediment bacterial activity. Our results are consistent with those of predecessors [36]. Bacteria and meiofauna exerted a competitive relationship in the consumption of organic matter [37]. When TOC in sediment is concentrated, meiofauna dominate its utilization in virtue of its fast consuming food, whereas in diluted patches, bacteria gains control [38]. Furthermore, the sediment fauna can consume bacteria [39]. Accordingly, a strong negative relationship between sediment bacterial metabolism activity and TOC influence has been shown.

In the present study, two groups were selected to assess the relationships between TOC levels and the co-occurrence of microbial communities based on a network analysis. Certain topological indexes of the observed network were calculated to identify the co-occurrence patterns between LT and MT. Based on the co-occurrence results, LT (433 positive correlations and 498 negative correlations) interact stronger with other than MT (73 positive correlations and 81 negative correlations). In functional analysis, we found genes related to membrane transport and metabolism of cofactors and vitamins were overrepresented in LT community. This also confirmed that LT community interact with each other strongly and may exchange essential metabolites.

These findings are somewhat illogical: why are there larger and more complex interactions associated with LT networks than MT? Environmental conditions can have a serious impact on the interactions among microorganisms. MT community might primarily be colonized with higher number of phylotypes possessing a larger genome with versatile functional traits related to essential nutrients synthesis and mediate interactions. The nutrient rich status in MT could enable them to use available nutrients quickly without having to establish relationships with neighbouring members. Previous studies assumed that the streamlined genome size provide a competitive advantage for prokaryotes in a nutritionally deficient environment [43]. Such, in the case of resource scarcity, Microbes with small genomes might dominate the community. These members often lack complete access to some of the necessary genomic traits for the production of nutrient. Microbial communities can exchange metabolites in different habitats [40]. “beneficiaries” containing streamlined genome can obtain essential metabolites from the “helper” for their survival [41]. Of course, they can also, in turn, provide metabolites for their helpers. This may be the reason for the more interactions and complicated network in LT community.

Conclusions

TOC level might be a key determinant to shape sediment microbial communities in Dianchi Lake. Interestingly, the microbial community had a higher activity at low TOC and increasing concentration of TOC was negatively correlated with sediment bacterial activity. LT community interacted with each other strongly and may exchange essential metabolites. Overall, this study could enhance our knowledge of microbial assemblages in eutrophic lake sediment.
Methods

Study sites description and sampling procedure

In October 2014, sediment was sampled at thirteen locations in Waihai (DC04-DC13) randomly, while three sediment samples (DC01-DC03) were collected from Caohai. Triplicate samples retrieved from each site were packed into a 50 mL EP sterile tube then transferred on ice to the laboratory for further molecular analyses. The sampling distribution can be clearly shown in (Fig. S1). The geographic and limnological parameters including the location information, water (WT), depth, temperature (Temp), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), nitrate nitrogen (NO$_3^-$-N) and nitrite nitrogen (NO$_2^-$-N) of each sampling sites were described in detail in (Table S1).

DNA extraction and PCR amplification

Total DNA was extracted immediately from the sediment using the Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's Manual. The extracted DNA purification and quantity were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked using 1% agarose gel electrophoresis. The V3–V4 regions of the 16S rRNA gene fragments were PCR amplified using the universal primers for prokaryotes (archaea and bacteria) 341F (5′- CCTAYGGGRBGCASCAG-3′) and the primer 806R (5′- GGACTACNNGGGTATCTAAT -3′) and 50 ng of DNA template under the following running conditions: initial denaturation at 98 °C for 1 min, 30 cycles of 10 sec at 94 °C, 30 sec at 50 °C, 30 sec at 72 °C, and a final elongation step for 5 min at 72 °C. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Illumina MiSeq sequencing

The purified amplicons from each triplicate sediment sample were pooled in equimolar and Illumina MiSeq sequenced (2 ×250) on an Illumina MiSeq platform (Illumina, San Diego, USA) at the Ramaciotti Centre for Genomics (UNSW) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database with project number PRJNA608846.

Sequencing data processing

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged into Raw Tags by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using CHIMERE. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU128) 16S rRNA database using confidence threshold of 70%. Plots were all visualized using the “ggplot2” package in R.

Metagenome imputation from 16S rRNA datasets

The PICRUSt [19] was employed to predict functional profiles in the Dianchi Lake sediment from 16S rRNA data. The OTU table mapped to Greengenes OTU IDs through Qiime (http://qiime.org/scripts/assign_taxonomy.html) was used as input file for PICRUSt v1.1.09 to deduce functional differences of corresponding metagenomes. 16S rRNA gene copy number was normalized according to Genome Prediction Tutorial for PICRUSt. Metagenomes were predicted against the functional database of KEGG Orthology.

Detection and visualization of microbiological interactions

Network relationships were calculated respectively according to the relative abundance of each OTU applying the R package WGCNA [42]. After calculating pairwise Spearman's rank correlations, the correlation matrix was constructed to estimate the p value. Multiple testing was performed to adjust the P-values with function mt.rawp2adjp () in package multtest [43]. The correlations with a significance threshold (Spearman's correlation coefficient ($\rho$) greater than 0.8 and p-value below 0.05) were retrieved. These final network was revealed and suitably edited using Gephi [44].

Statistical analysis
RDA (redundancy analysis) was performed by R package vegan. Taxonomic changes in Dianchi Lake sediment in response to TOC were detected using Partial least squares discriminant analysis (PLS-DA). PLS-DA was conducted with mixOmics package. LEfSe tool [45] were performed to discriminate different taxa among groups. Only the LDA scores >3.0 were identified as discriminatory groups. Other statistical analyses were conducted in R environment.

**Abbreviations**

TOC : total organic carbon  
OTUs: operational taxonomic units  
RDA: redundancy analysis  
PLS-DA: Partial least squares discriminant analysis

**Declarations**

**Ethics approval and consent to participate**  
Not applicable

**Consent for publication**  
Not applicable

**Availability of data and materials**  
The datasets generated and/or analysed during the current study are available in the NCBI Sequence Read Archive (SRA) database with project number PRJNA608846

**Competing interests**  
The authors declare that they have no competing interests

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**Authors’ contributions**  
CP analyzed and interpreted the data and written the manuscript. YP and QQ collected the data and performed the experiment. WX, CQ, YX and XL designed the study and critically revised the manuscript. All authors approved the final manuscript and agreed to be accountable for all aspects of the work.

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Not applicable

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**Figures**
Figure 1

Relative abundances of dominant bacteria and archaea at the phylum level derived from sediment samples.
Figure 2

Redundancy analysis (RDA) of dominant OTU as affected by sediment environmental variables. Colored circles represent the sediment samples at sites DC1–DC13 in Dianchi Lake Sediment, respectively.
Figure 3

(A) Partial least square discriminant score analysis the distance of microbial communities of sediment microbiota among all the samples. (B) Hierarchical clustering analysis for the similarity/diversity among the G1, G2, G3 and G4 groups.

Figure 4

LEfSe analysis (LDA) of the bacteria and archaea inhabited in Dianchi Lake sediments with different TOC levels. Microbial taxa having differentially abundant (LDA score > 3.0) between G1 and G2 samples visualized by Circos. The size of circle is proportional to the relative occurrence in each community.
Figure 5

The heat-map presenting the predicted functional profiles of the KEGG level2 pathways calculated using Bray–Curtis distance.

A
B
Figure 6

Co-occurrence patterns of phylotypes with strong pairwise correlation ($p > 0.8$, P-value $< 0.01$). The nodes represent individual OTUs; The circle diameter is proportional to the mean abundance of different phylum.

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