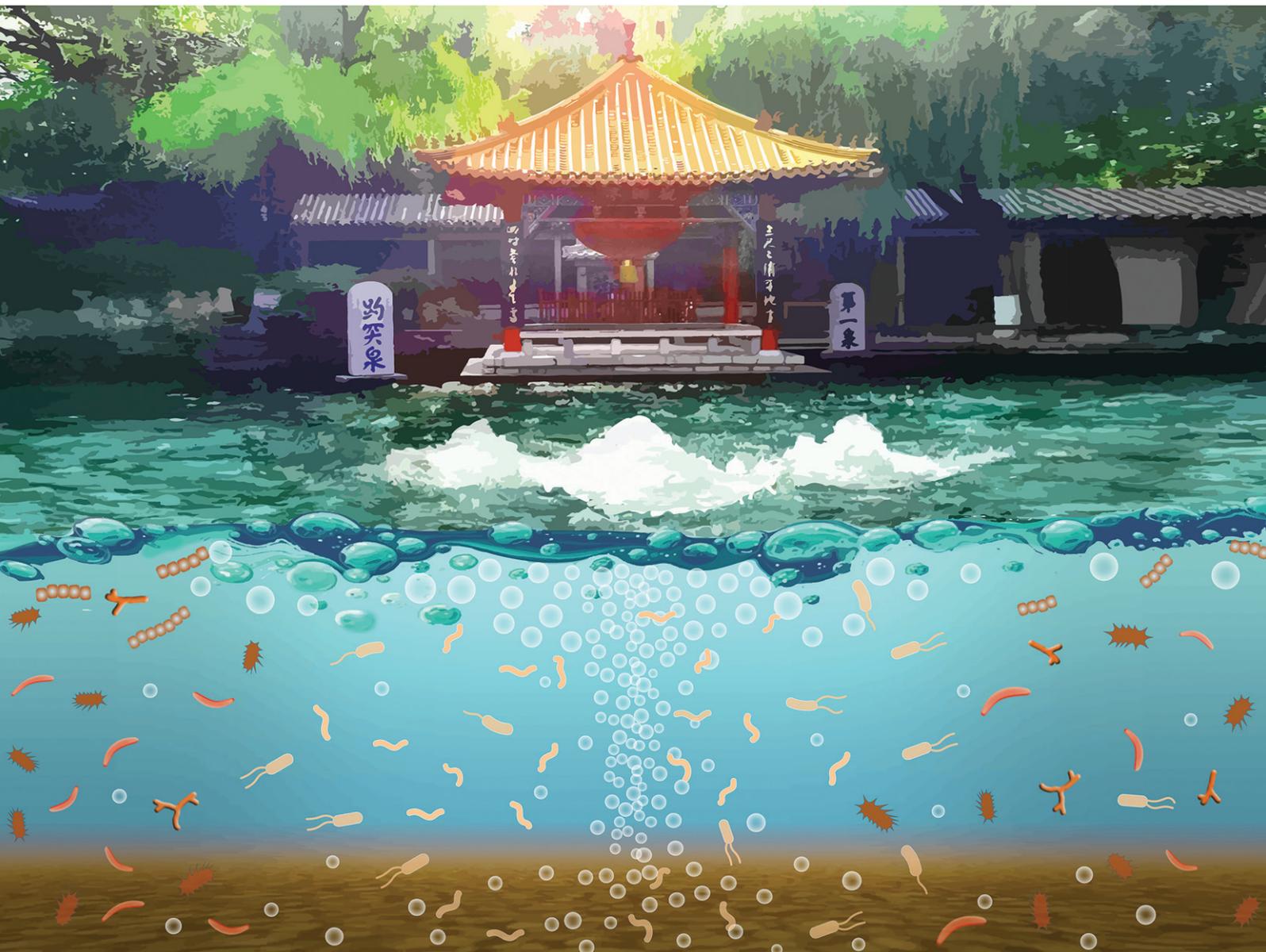


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**PAPER**

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Nanobubbles can modulate microbial communities and  
sedimentary ecosystem during the treatment of pond water



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## Nanobubbles can modulate microbial communities and sedimentary ecosystem during the treatment of pond water†

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Nanobubbles are applied in diverse water-treatment technologies. However, their underlying mechanisms are not fully understood to date. In this study, we applied metagenomic approaches to investigate the effect of nanobubble treatment on the microbial communities in the pond aquatic ecosystem by systematic analysis of the metagenomes from different samples with spatial (water body and sediment) and temporal changes. We found that nanobubbles simultaneously changed the structure and balance of the microbial communities in the whole water body and sediment system during the pond water treatment. Importantly, nanobubble treatment inhibited harmful pathogenic microbes (mainly cyanobacterial species) and altered the diversity of microbial populations in the water body, while increasing the species richness in the sediments, providing a clue for understanding the mechanism of nanobubble effects.

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### Water impact

This study demonstrates that nanobubbles can control water pollution and improve water quality by synergistically modulating the structure and balance of microbial communities in water bodies and sediment. These findings indicate that NBs with different gas compositions and treatment times can be applied to different types of sewage treatment *via* a modulation effect on the microbiome.

## Introduction

Nanobubbles (NBs), which are a type of stable gas phase suspended in the water phase with a defined diameter of less than 1000 nm,<sup>1</sup> have wide application prospects in diverse fields, *e.g.*, water disinfection, degradation of organic pollutants and decontamination of solid surfaces.<sup>2</sup> In a pond ecosystem, the external pollutants deposited on the sediments and the sediment–water interface can re-contaminate the

water body and the released nutrients (including nitrogen and phosphorus) can induce harmful algal blooms and exacerbate hypoxic/anoxic conditions. NB technology has many advantages in sewage treatment given that it is both cost effective and environmentally friendly. Firstly, the high oxidation ability of NBs, resulting from the generation of hydroxyl radicals when they collapse, can produce safer water with fewer by-products.<sup>3</sup> Secondly, due to their long-time stability and high mass transfer efficiency, NBs can more

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† Electronic supplementary information (ESI) available: The online version contains supplementary material available at additional file 1. Fig. S1: Micro/nano-bubble aeration system and the environment of the pond. Additional file 2. Fig. S2: Taxonomy analysis tree of microorganisms in water at 1st, 22nd and 67th days of NB treatment. Additional file 3. Fig. S3: Effect of NBs on the three most abundant phyla (*Actinobacteria*, *Proteobacteria* and *Cyanobacteria*) at the genus level. Additional file 4. Fig. S4: Venn diagram of microbial communities in sediment under different NB treatment days. Additional file 5. Fig. S5:

Variation in the relative abundance of bacteria shared by pond water and sediments over time. Additional file 6. Fig. S6: Heatmap of KEGG pathway of microorganisms in pond water. Additional file 7. Fig. S7: Heatmap of KEGG pathway of microorganisms in pond sediment. Additional file 8. Fig. S8: Heatmap of carbohydrate-active enzyme annotation in water. Additional file 9. Table S1: Base mass distribution of sequence. Additional file 10 Table S2: Obtained and processed genetic data. Additional file 11. Table S3: Relative abundance of the most abundant classes of Proteobacteria in the total community in the water samples. Additional file 12. Table S4: Shannon diversity index of microbial species in pond water after continuous NB treatment for 1, 22 and 67 days. See DOI: <https://doi.org/10.1039/d3ew00257h>

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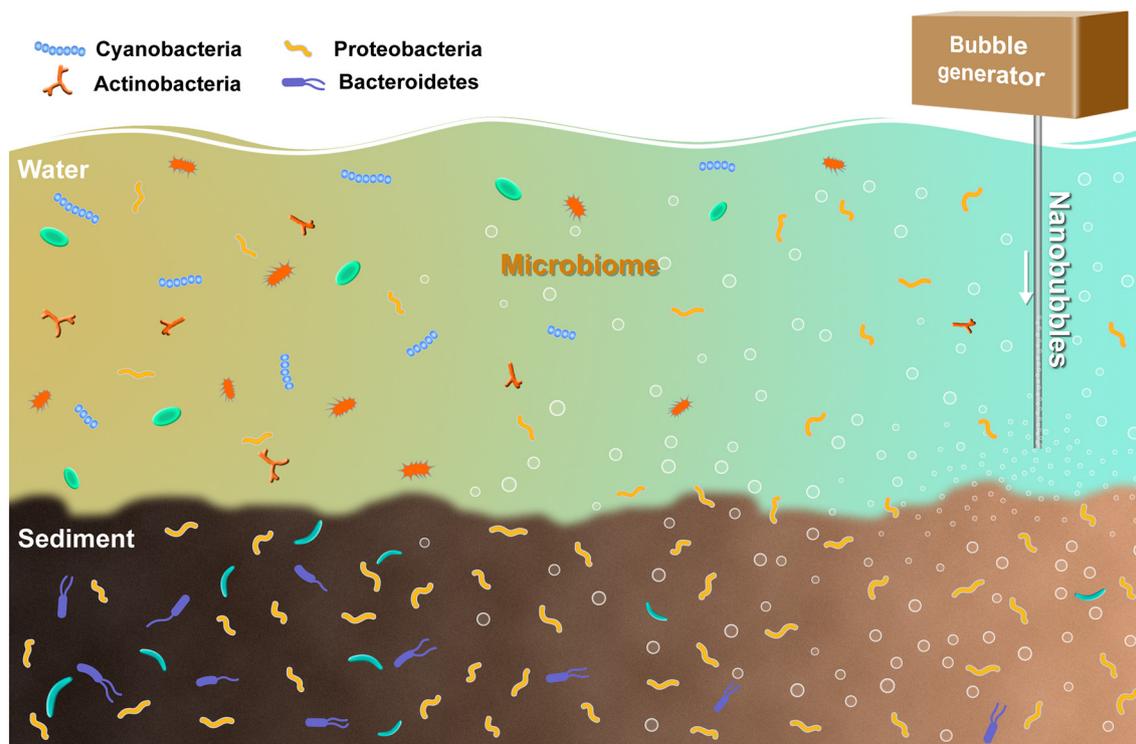
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effectively delivery oxygen to mitigate hypoxia and anoxia than conventional oxygenation methods. Thus, the lower cost and energy consumption of the NB technique enable its application in large-scale water treatment. In the past few years, NBs have become one of the main topic of research to improve the treatment efficiency in the environmental science and engineering disciplines. The ability of NBs in water purification is usually interpreted and attributed to the production of free radicals or the effective increase in the gas mass transfer<sup>3–5</sup> and the DO level in the applied interfacial microenvironment;<sup>6,7</sup> however, their underlying mechanisms have not been fully understood to date.

Recently, several studies have explored the regulatory ability of NBs on microbial communities in water systems, given that microorganisms play an important role in maintaining a healthy aquatic ecosystem.<sup>4,8–13</sup> Nghia *et al.* found that NB treatment could improve the water quality by controlling the level of *Vibrio parahaemolyticus*.<sup>14</sup> Zhang *et al.* employed *Bacillus subtilis* as a model microorganism to investigate the disinfection performance of the ozone micro-bubble technique.<sup>15</sup> Ghadimkhani *et al.* indicated that micro/nano bubbles could influence the microbial communities in polluted water, which further regulate the physicochemical characters of water, and continuously mitigate water pollution.<sup>16</sup> Although it is recognized that air or nitrogen NBs influence the activity of microorganisms in either anaerobic or aerobic conditions,<sup>3,17</sup> the comprehensive effects and

mutual changes in the water body and sediment system as a whole induced by NB treatment on the composition, diversity and dynamics of microbial communities are still limited.

In this study, we investigated the effect of NB treatment on the microbial communities in the pond aquatic ecosystem using metagenomic approaches.<sup>18</sup> Metagenomics, which is direct extraction of DNA from any environment followed by DNA sequencing and bioinformatic elucidation, is described as environmental genomics and is becoming a universal strategy to study the taxonomic and functional composition, as well as the dynamic evolution of microbial communities in response to environmental changes. The experiments were carried out at farm ponds of rural areas around Shanghai City in China during the autumn of 2018 (Fig. S1† shows images of one of the pond aquatic ecosystems). NBs were continuously introduced in the polluted pond water through a micro/nano-bubble aeration system (Fig. S1†) and the samples from the water body and sediment were collected for DNA isolation and shotgun sequencing at day 1, day 22 and day 67, respectively (Tables S1 and S2†). Computationally analyzing and comparing the metagenomes from different samples with spatial (water body and sediment) and temporal (over more than two months) changes were performed to learn about their commonalities and differences. We found that NB treatment significantly changed the composition of the microbial communities over time. Especially, the abundance of *Cyanobacteria* and *Actinobacteria* dramatically



**Fig. 1** Schematic showing the microbial community changes in the pond water and sediment induced by nanobubble (NBs) treatment. Following the continuous delivery of NBs into the contaminated pond, the water and sediment slowly become clear and depurative with a mutual change in microbial structure and diversity (especially *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*) in the water body and bottom sediment as a whole.

decreased in the water body, while there was a consistent increase in *Proteobacteria* in both the water body and the sediment (Fig. 1).

## Material and methods

### Nanobubble production and pond water treatments

A nano/micro-bubble aeration system was used to continuously insert NBs into the pond water body, and a schematic diagram of the nano/micro-bubble aeration system is shown in Fig. S1.†<sup>19</sup> The microbubble aeration system consisted of a closed-loop circuit including a tank, a centrifugal pump (20NPD04S, Nikuni pump Co., Japan), control valves, manometers, an air flow meter, and connecting pipes. All the contacting materials were made of stainless steel. Two adjustable orifice valves were placed at the inlet (valve 1) and outlet (valve 2) of the pump, and two manometers were used to measure the inlet pressure and the outlet pressure. An air valve was used to control the air flow.

### Nanobubble distribution and water quality measurement

The nanobubble aeration treatment was conducted in pure reservoir water with air as the gas source to simulate the experimental conditions. The nanobubble size distribution was measured by dynamic light scattering immediately after intermittent aeration and/or dilution using a NanoSight NS300 instrument (Malvern Panalytical, UK). Each measurement was replicated three times. During the aeration treatment, the temperature and dissolved oxygen (DO) levels of the water were measured using a YSI 556 multiparameter system (Xylem Inc., Rye Brook, NY).

### Sample collection

After continuous nanobubble exposure, pond water and sediments of ten areas nearby and far from the nanobubble injection were collected at the 1st, 22nd and 67th day, respectively. Another replicate experiment in an individual pond water system far away from the previous one was performed as the control. The collected samples were mixed and subjected to DNA extraction, fragmentation (approximately 300 bp), PE library construction and bridge-PCR. Finally, Illumina HiSeq metagenomic sequencing was performed to get the bioinformatics.

### DNA sequencing and data analysis

The raw data of gene sequences was subjected to optimization processing such as street removal, quality cutting, and decontamination. Then, the high-quality sequences were used for splicing assembly and gene prediction, followed by performing annotation of species and functions on the obtained gene sets based on NR (amino acid sequence database of non-redundant proteins), COG (clusters of orthologous groups of proteins) and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases.

**a. Annotation on species taxonomy.** The acquired gene sets were searched in the NR database using BLASTP (BLAST Version 2.2.28+, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>;  $e$ -value:  $1 \times 10^{-5}$ ), the species annotation was obtained through the taxonomic information in NR database, and then the abundance of the species was calculated using the sum of gene abundance corresponding to the species.

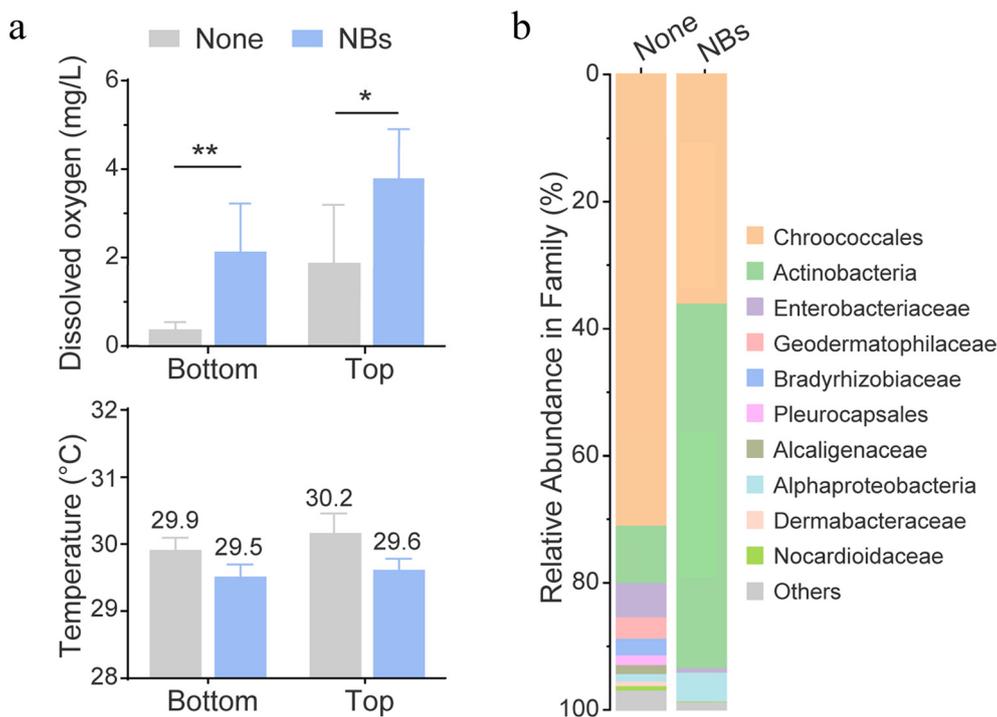
**b. COG functional annotation.** The acquired gene sets were searched in the eggNOG database (evolutionary genealogy of genes: non-supervised orthologous groups, <https://eggnog.embl.de/>) using BLASTP and the corresponding COG obtained (clusters of orthologous groups of proteins), and then the abundance of the COG was calculated using the gene abundance corresponding to the COG.

**c. KEGG functional annotation.** The acquired gene sets were searched in the KEGG database (Kyoto Encyclopedia of Genes and Genomes, <https://www.genome.jp/kegg/>) using BLASTP. The obtained comparison results were annotated using KOBAS 2.0 (KEGG Orthology-Based Annotation System, <https://kobas.cbi.pku.edu.cn/home.do>).

## Results and discussion

### Nanobubble treatment improves water quality accompanied with microbial alterations

NB aeration treatment was conducted with air as the gas source. NBs with a size in the range of 100 to 400 nm and concentration of around  $10^7$  particles per mL, as measured by dynamic light scattering, were continuously delivered into the pond water. During the aeration treatment, the dissolved oxygen (DO) level, an important parameter in assessing water quality, and the temperature of the water were monitored. After treatment for 18 days, the DO level and microbial composition were analyzed to evaluate the effect of the NBs (Fig. 2). Consistent with previous reports,<sup>5</sup> the NB treatment could greatly increase the DO levels, while it resulted in a slight change in temperature either in the bottom or top water layers (Fig. 2a). Specially, the bottom DO level near the sediment was as high as about  $2 \text{ mg L}^{-1}$  after NB treatment. Given that the sediment layer is usually in an anaerobic state with little or no oxygen, the value of  $0.2 \text{ mg L}^{-1}$  measured in our study indicates a higher DO level, demonstrating that a lot of NBs spread to the bottom of the pond. Interestingly, corresponding to the rise in the DO level, NB treatment led to a decline in the relative abundance of *Chroococcales*, while an increase in that of *Actinobacteria* (Fig. 2b). *Chroococcales*, a family of cyanobacteria, are often proliferated by high nutrient loading and used as an indicator of declining water quality.<sup>20</sup> However, inversely to that of *Cyanobacteria*, the increase in the abundance of *Actinobacteria* indicated the improvement in the quality of the water ecosystems.<sup>21</sup> These results indicate that NB delivery could simultaneously increase the DO levels and alter the microbial communities with an improvement in the water body conditions.



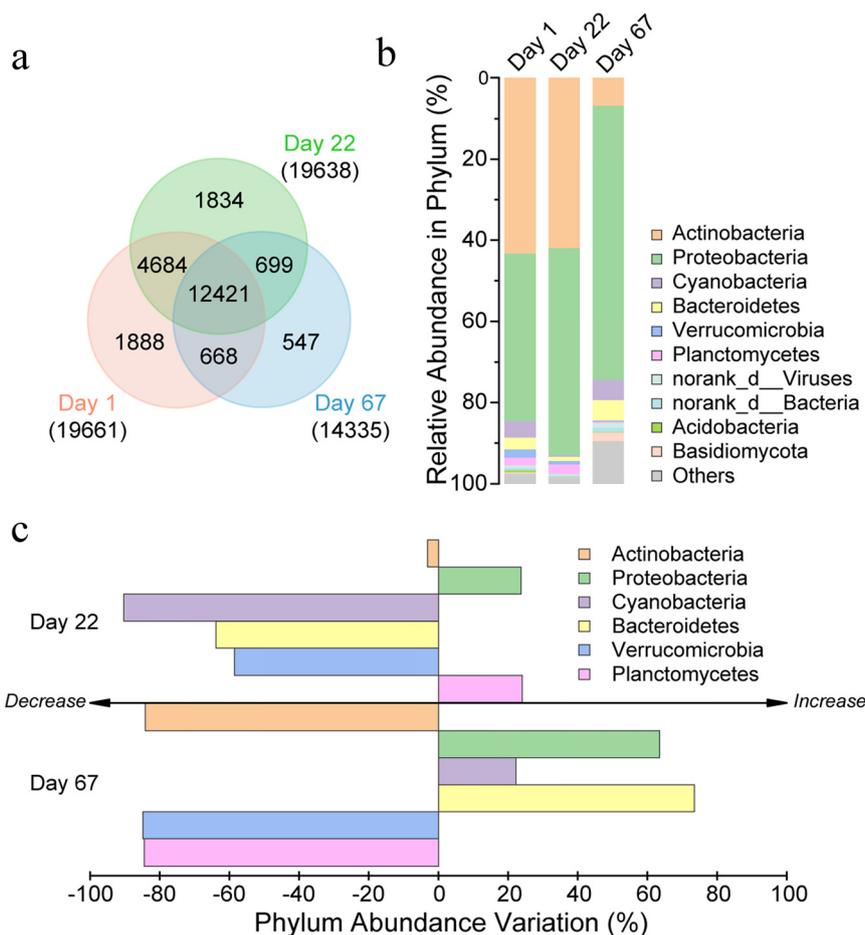
**Fig. 2** Nanobubble treatment led to water quality improvement and microbial structure changes in pond water body. (a) Dissolved oxygen level and water temperature. (b) Relative abundance of microbial communities at the family level. \* and \*\* denote  $p < 0.05$  and  $p < 0.01$ , respectively.

### NB treatment changes the composition and diversity of microbial communities in pond water body

The variation in the composition of microbial communities in the water upon NB treatment was firstly analysed at the kingdom, phylum, class, order, family, genus, and species levels, respectively. To make the experiment more reliable, we carried out another replicate experiment at an individual pond water system far away from the previous one. By comparing the gene sets with the NR database (amino acid sequence database of non-redundant proteins), the microbial community structures at day 1, 22 and 67 were obtained, as shown in Fig. 3. According to the Venn diagram, we can see that the NB treatment continuously decreased the number of species, from 19 661 on the 1st day to 14 335 on the 67th day. Among them, a total of 12 421 species exhibited no variation during the whole treatment period. After treatment for 22 days, nearly two thousand species were replaced although there was a slight change in the total numbers of the microbial community. These results can be explained by the fact that NB treatment accelerated the growth of beneficial microbes but inhibited that of the harmful or pathogenic ones to speed up the decomposition of organic matter in water and sediments to improve the water quality. With a longer treatment time, together with the water body becoming clear, more species were replaced; meanwhile, the total number of species significantly decreased. This can be attributed to the competitive inhibition resulting from the continuous increase in beneficial microorganisms in the water and the synergistic inhibition with NBs. The

corresponding taxonomy analysis tree gives the details of community structural changes over the treatment times (Fig. S2†). Notably, in this tree, potential harmful *cyanobacterial* species such as *Microcystis* decreased, but were replaced by other potentially harmful *cyanobacteria* such as *Aphanizomenon*. Although the role of specific bacteria in the regulation of microbial communication is an interesting question and needs further investigation, this result supports the finding that NBs have an effect on the microbial ecology. Based on the number of species, we further calculated the relative abundance of microbial phyla and showed the top 10 highest abundant ones in the stacked histogram (Fig. 3b). Among them, *Actinobacteria*, *Proteobacteria*, *Cyanobacteria* and *Bacteroidetes* are the top four highest relative abundance phyla with a proportion of more than 80%, consistent with other studies on the enrichment of these four phyla in aquatic ecosystems.<sup>22,23</sup> After treatment, the abundance of *Proteobacteria* increased from 43.28% (1st day) to 67.57% (day 67), while *Actinobacteria* decreased significantly with reduction in its relative abundance of nearly 90% on day 67. Interestingly, *Cyanobacteria* and *Bacteroidetes* firstly decreased in response to short-time treatment (22 days), and then increased after 67 days of stress. To clearly describe the variations, the phylum abundance on the 22nd and 67th days was calculated and compared with that on the 1st day, as shown in Fig. 3c.

It was reported that *Proteobacteria* play an important role in nitrogen and sulfur metabolism in aquatic ecosystems<sup>24,25</sup> due to their enormous metabolic diversity and abilities of sulfur-oxidizing, nitrate-reducing and denitrifying. In our



**Fig. 3** Composition evolution of microbial communities in pond water body with NB treatment on days 1, 22 and 67. (a) Variation in the abundance of microbial species. (b) Relative abundance of top 10 bacterial phyla based on species distribution. (c) Evolutionary trends of the six most abundant microbes at the phylum level with treatment time. The x-axis represents the relative abundance variations at the phylum level, with the increased microbes shown on the right and decreased microbes on the left. Phylum abundance variation% =  $(A_n - A_1)/A_1$ , where “A” is the phylum abundance of a microbe, “A<sub>1</sub>” refers to the phylum abundance of a microbe at the first day, and “n” refers to day 22 or day 67.

studies, we found that the NB treatment significantly increased the growth of *Proteobacteria*. Specially, the relative class abundance of alpha-*Proteobacteria* and gamma-*Proteobacteria* increased from 9.72% to 19.1% and 5.34% to 17.66%, respectively (Table S3†). The increase in the proportion of *Proteobacteria* indicates that NBs could induce a more active nitrogen, sulfur cycling aquatic ecosystem. *Actinobacteria*, preferring to live in freshwater ecosystems with abundant organic substances (including polymers in dead plants, animals, fungi, etc.),<sup>26</sup> decreased dramatically after treatment, suggesting that the NB-treated water system had less organic matter. The decrease in the abundance of *Cyanobacteria* also evidences the effect of NBs on the recovery of water quality (Fig. 3c). It is well-known that *Cyanobacteria* can produce earthy/musty odorous “blooms” to reduce dissolved oxygen and secrete toxins; therefore, the composition and abundance of *Cyanobacteria* are employed as an indicator of freshwater quality.<sup>27</sup> To measure the diversity of microbial species in the pond water community, the Shannon diversity index was calculated under different

NB treatment days (Table S4†). It was found that the Shannon diversity index decreased greatly on the 22nd day and then increased after 67 days of NB treatment. This can be explained by the fact that during the first 22 days of NB treatment, the abundance of some microbial species decreased significantly, especially the species that are harmful to water quality, and the structure of the microbial community changed drastically. However, long-term treatment led to an increase in the beneficial bacterial species, followed by the recovery of the community diversity. Overall, it can be concluded from these results that NBs regulate the water body ecosystem through their impact on the composition and diversity of the microbial communities in the pond water.

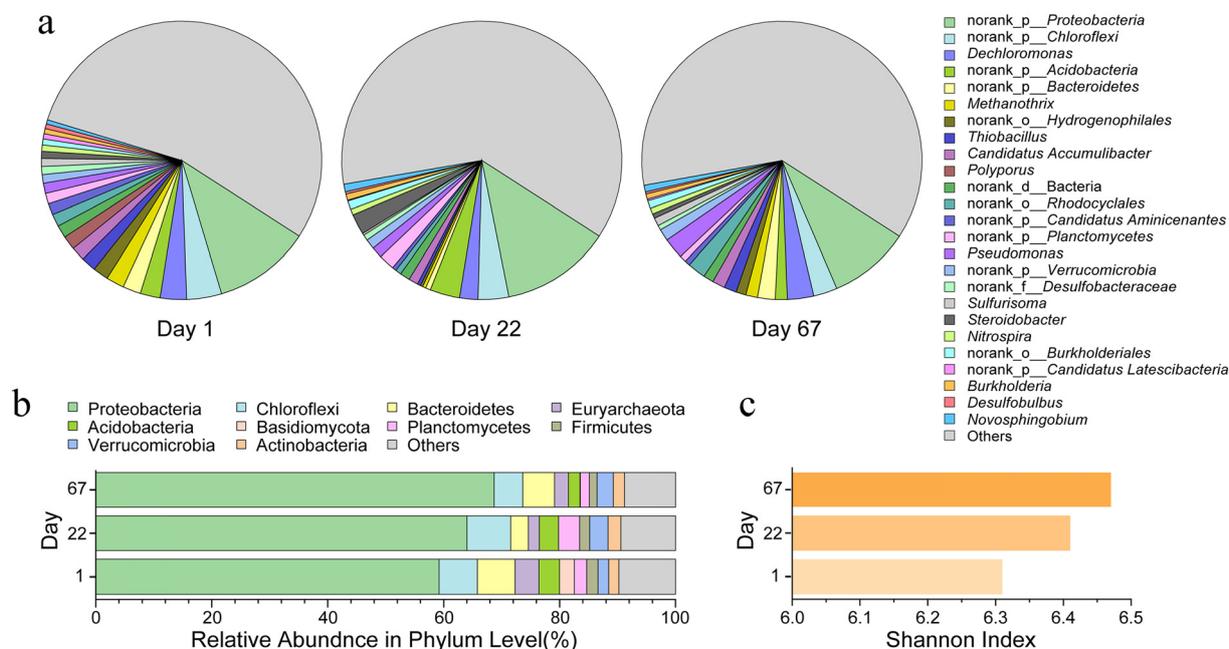
To deeply survey the effect of NBs on the microbes, we further analyzed the three most abundant phyla (*Actinobacteria*, *Proteobacteria* and *Cyanobacteria*) at the genus level (Fig. S3†). In the case of *Actinobacteria*, it can be seen that after NB treatment, the abundance of *Mycobacteriaceae* decreased significantly, a genus with many pathogenic

strains, which may cause tuberculosis, leprosy or other chronic necrosis. However, after NB treatment, the dominant genus evolved to *Microbacteriaceae*, which exists in various terrestrial and aquatic ecosystems and only a few species are plant pathogens. The *Proteobacteria* phylum in this study mainly contained three classes, *i.e.*, beta, alpha and gamma *Proteobacteria*. In beta-*Proteobacteria*, *Polynucleobacter* is the dominant genus, and its species play a part in carbon release and is of enormous environmental relevance in freshwater habitats, and its abundance increased significantly after NB treatment. In alpha-*Proteobacteria*, after NB treatment, the abundance of *Rhodobacteraceae* increased significantly and it became the dominant genus. This genus has photosynthetic bacteria, which metabolize a large variety of organic compounds. *Caulobacteraceae*, which are chemotrophic bacteria, also increased significantly. *Methylocystaceae* almost disappeared, and given that they use methanol or methane as their sole energy and carbon source, their decrease indicated a decrease in carbon resources in the pond water. Besides these genera, the relative abundance of *Sphingomonadaceae* and *Rhizobiales* decreased, which have the ability to degrade polycyclic aromatic hydrocarbons and fix nitrogen, respectively. All these dominant genera are not pathogenic to humans. In gamma-*Proteobacteria*, *Crenothrix*, a kind of iron bacteria, which uses iron or manganese as an energy, increased. *Methylococcaceae*, a group of bacteria specialized in the utilization of methane and methanol, also increased significantly. *Pseudomonas* can degrade fats and produce lipolytic rancidity, and although this genus has some pathogenic species such as *Pseudomonas aeruginosa*, it

proportions in the total *Pseudomonas* genus decreased from 12% to 4% with NB treatment. In *Cyanobacteria*, the relative abundance of *Leptolyngbyaceae* and *Synechococcaceae* increased significantly, where *Leptolyngbyaceae* may be a major contributor to biological nitrogen fixation and *Synechococcaceae* have carbon fixation ability. Alternatively, *Pseudanabaenaceae* and *Microcoleaceae*, which can lead to bloom and harm the water environment, decreased significantly. Taken together, we can see that after NB treatment, the microorganisms that harm the water quality or are pathogenic to humans decreased, and those that promote substance circulation and maintain the water body ecological stability increased.

### Microbial community evolution together with NB treatment in pond sediment

Next, we tried to determine whether NB treatment can affect the microorganisms in the sediment ecosystem. In this case, sediment samples after different days of NB treatment were collected and analysed (Fig. 4). The fan charts in Fig. 4a show the main microbial genera in sediment on the 1st, 22nd and 67th days, respectively. It can be found that the relative abundances of the top three genera *Proteobacteria*, *Chloroflexi*, and *Dechloromonas* had little change, from 11.23%, 4.04% and 3.04% on the first day to 9.43%, 2.74% and 3.05% after 67 days of NB treatment, respectively. However, the NB treatment had a different effect on the topmost abundant phyla (Fig. 4b). NB treatment increased the relative abundance of *Proteobacteria* from 59.19% to



**Fig. 4** Structure and evolution of microbial communities in pond sediment in response to NB treatment. (a) Relative abundance of top 25 microbial communities at the genus level on days 1, 22 and 67, respectively. The microbial genera are labelled on the right. (b) Relative abundance of top 10 microbial communities at the phylum level. (c) Shannon diversity index of microbial species in the sediment after NB treatment for 1, 22 and 67 days.

68.73%, while it decreased that of *Acidobacteria* and *Basidiomycota*. Interestingly, during the about two months of treatment, *Chloroflexi* firstly increased from 6.61% to 7.53%, and then decreased to 4.93% but both *Bacteroidetes* and *Euryarchaeota* decreased significantly in the first 22 days. The Shannon diversity index showed that the treatment continuously increased the diversity of microbial species (Fig. 4c and S4†). These results indicate that NBs could modulate the microbial communities and improve their diversity in the pond sediment, thus maintaining the balance and stability of the microbial ecosystem. Overall, it can be concluded that the NBs treatment not only modulated the microbial community structure in the pond water but also could adjust the microbial composition structure in the bottom mud. Among the most abundant bacterial phyla existing in both the pond water and sediment, their dynamic changes were highly consistent (Fig. S5†). The abundance of *Proteobacteria* kept increasing with NB treatment in both the pond water and sediments, while *Bacteroidetes* decreased initially, and then increased. Given that the microorganisms released from streambed sediments may substantially affect the water quality,<sup>28,29</sup> the ability of NBs to adjust the composition structure of the microbial communities in the sediment will profoundly affect the communities in the water, helping to obtain more stable and long-term water quality improvement.

### NBs play distinct roles in the functional metabolism profile of microbial communities in the pond water and sediment

To explore the NB-induced changes and mutual influence of microbial communities in the sediments and water at the genetic level, the gene sets of microbes were searched in the eggNOG database (evolutionary genealogy of genes: non-supervised orthologous groups) and the corresponding COG acquired (clusters of orthologous groups of proteins), and then the COG abundance was calculated based on gene abundance. Functional annotation on microbial COG abundance in both pond sediments and water within 67 days was calculated and the results were displayed in a heatmap (Fig. 5). According to the COG functional annotation of microbial genes, it can be seen that (1) replication, recombination, and repair, (2) energy production and conversion, and (3) amino acid transport and metabolism were the most abundant microbial gene functions both in the water and sediments. Furthermore, the effect of NB treatment on the microbial gene expression level in the pond water and sediment ecosystem was analysed. It can be seen that in the pond water, the abundance of functional genes of amino acid transport, metabolism and energy production, and conversion decreased gradually with the NB treatment. This implies that NBs may inhibit amino acid metabolism and energy production processes of microbes in pond water.

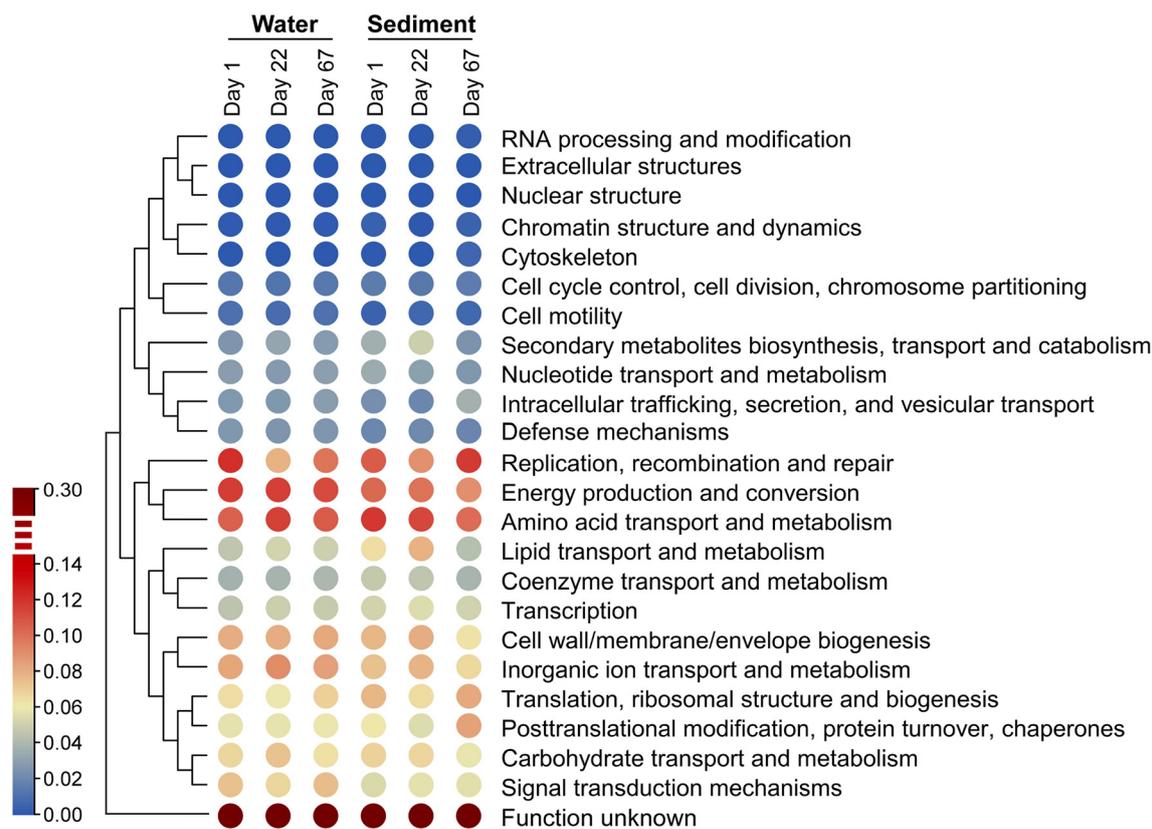


Fig. 5 Gene abundance of microbial communities in water body and sediment after NB treatment based on COG annotation. The relationship of the COG in each sample at the family level is depicted with a cluster dendrogram based on Bray-Curtis dissimilarity.

Alternatively, in the sediments, the proportion of replication, recombination, and repair gene expression of the microbes decreased initially, and then increased with the treatment of NBs, indicating that NBs can also modulate the microbial activities in the sediments. Besides, the gene abundance analysis of microbes in the water and sediments based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) annotation is shown in Fig. S6 and S7,<sup>†</sup> respectively. In the pond water, the abundance of genes related to carbon fixation pathways in prokaryotes decreased significantly, while the abundance of genes coding ribosome, bacterial secretion system or RNA degradation increased. In pond sediment, the gene abundance variations were not significant. Furthermore, the carbohydrate-active enzyme annotation also showed a significant variation with NB treatment (Fig. S8<sup>†</sup>). These results indicate that the NB treatment-induced activity changes of specific enzymes may be attributed to the microbial function in improving the water quantity. Thus, the underlying mechanism of NBs on the genes and protein function is an interesting topic for deep investigation.

## Conclusions

In this study, the biological effect of NBs on microbial communities during the pond water treatment was analysed by using the metagenomic methodology. We found that NB treatment not only inhibited the harmful microbes and altered the diversity of the microbial population in the water body but also increased the species richness in the sediment, and further balanced the microbial communities in the whole ecosystem. In the pond water, NBs increased the relative abundance of *Proteobacteria*, while they decreased the abundance of *Actinobacteria*. Given that *Proteobacteria* play an important role in nitrogen and sulfur metabolism in the aquatic ecosystem and *Actinobacteria* are active in the freshwater ecosystem, which have abundant organic substances, the variation in the abundance of both indicated that the pond water evolved from a carbon-metabolizing active system to a nitrogen and sulfur cycling active system. Moreover, the decrease in *Cyanobacteria* also supported the role of NBs in the recovering aquatic ecosystem. In the pond sediment, NB treatment significantly increased the abundance of *Proteobacteria*, while it decreased that of *Acidobacteria* and *Basidiomycota*. The consistent increase in the microbial diversity both in the water body and pond sediment upon NB treatment indicated that NBs may help maintain the balance and stability of the microbial ecosystem. Previous studies showed that NBs have an effect on sewage purification; however, their mechanisms are not fully understood to date. Due to their unique characteristics compared to macro bubbles, such as high stability and high rate of oxygen dissolution as well as the virtual disappearance of buoyancy, NBs can remain in the water and contact the sediment layer for a longer time. The superficial NBs will implode under the water pressure and release air, increasing the amount of dissolved

oxygen. Meanwhile, the depth NBs is dissolved in the sediment layer. With an increase the amount of dissolved oxygen, the aerobic microbes become more active in degrading organic matter, thus improving the water body conditions. Thus, the mutual influence and synergistic changes in the microbial communities in water and sediments may be a potential mechanism of NBs in water treatment.

## Data availability

All available data and material are original work. All data generated or analyzed during this study are included in this published article and its ESI<sup>†</sup> files. The datasets used and/or analyzed during the current study are also available from the corresponding authors on reasonable request.

## Author contributions

Yadi Wang: methodology, formal analysis, visualization, investigation, writing – original draft, review & editing. Jin Zheng: formal analysis, writing – original draft. Jie Cheng: investigation, writing – review & editing. Runlong Zhou: investigation. Xueling Li: conceptualization, supervision, writing – review & editing. Jun Hu: resources, writing – review & editing. Junhong Lü: conceptualization, methodology, supervision, resources, funding acquisition, writing – original draft, writing – review & editing.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

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## References

- 1 M. Alheshibri, J. Qian and M. Jehannin, *et al.*, A History of Nanobubbles, *Langmuir*, 2016, **32**(43), 11086–11100.
- 2 X. Bu and M. Alheshibri, The effect of ultrasound on bulk and surface nanobubbles: A review of the current status, *Ultrason. Sonochem.*, 2021, **76**, 105629.
- 3 A. Agarwal, W. J. Ng and Y. Liu, Principle and applications of microbubble and nanobubble technology for water treatment, *Chemosphere*, 2011, **84**(9), 1175–1180.

- 4 T. Temesgen, T. T. Bui and M. Han, *et al.*, Micro and nanobubble technologies as a new horizon for water-treatment techniques: A review, *Adv. Colloid Interface Sci.*, 2017, **246**, 40–51.
- 5 A. J. Atkinson, O. G. Apul and O. Schneider, *et al.*, Nanobubble Technologies Offer Opportunities To Improve Water Treatment, *Acc. Chem. Res.*, 2019, **52**(5), 1196–1205.
- 6 S. Xue, Y. Zhang and T. Marhaba, *et al.*, Aeration and dissolution behavior of oxygen nanobubbles in water, *J. Colloid Interface Sci.*, 2022, **609**, 584–591.
- 7 A. K. Patel, R. R. Singhanian and C.-W. Chen, *et al.*, Advances in micro- and nano bubbles technology for application in biochemical processes, *Environ. Technol. Innovation*, 2021, **23**, 101729.
- 8 L. Besaury, F. Marty and S. Buquet, *et al.*, Culture-Dependent and Independent Studies of Microbial Diversity in Highly Copper-Contaminated Chilean Marine Sediments, *Microb. Ecol.*, 2013, **65**(2), 311–324.
- 9 X. M. Lu, C. Chen and T. L. Zheng, Metagenomic Insights into Effects of Chemical Pollutants on Microbial Community Composition and Function in Estuarine Sediments Receiving Polluted River Water, *Microb. Ecol.*, 2017, **73**(4), 791–800.
- 10 W. Xiao and G. Xu, Mass transfer of nanobubble aeration and its effect on biofilm growth: Microbial activity and structural properties, *Sci. Total Environ.*, 2020, **703**, 134976.
- 11 Y. Sun, S. Wang and J. Niu, Microbial community evolution of black and stinking rivers during in situ remediation through micro-nano bubble and submerged resin floating bed technology, *Bioresour. Technol.*, 2018, **258**, 187–194.
- 12 W. Xiao, G. Xu and G. Li, Effect of nanobubble application on performance and structural characteristics of microbial aggregates, *Sci. Total Environ.*, 2021, **765**, 142725.
- 13 S. Yaparathne, Z. E. Doherty and A. L. Magdaleno, *et al.*, Effect of air nanobubbles on oxygen transfer, oxygen uptake, and diversity of aerobic microbial consortium in activated sludge reactors, *Bioresour. Technol.*, 2022, **351**, 127090.
- 14 N. H. Nghia, P. T. Van and P. T. Giang, *et al.*, Control of *Vibrio parahaemolyticus* (AHPND strain) and improvement of water quality using nanobubble technology, *Aquacult. Res.*, 2021, **52**, 2727–2739.
- 15 F. Zhang, J. Xi and J.-J. Huang, *et al.*, Effect of inlet ozone concentration on the performance of a micro-bubble ozonation system for inactivation of *Bacillus subtilis* spores, *Sep. Purif. Technol.*, 2013, **114**, 126–133.
- 16 A. Ghadimkhani, W. Zhang and T. Marhaba, Ceramic membrane defouling (cleaning) by air Nano Bubbles, *Chemosphere*, 2016, **146**, 379–384.
- 17 Y. Wu, H. Lin and W. Yin, *et al.*, Water quality and microbial community changes in an urban river after micro-nano bubble technology in situ treatment, *Water*, 2019, **11**(1), 66.
- 18 J. C. Setubal, J. Stoye and P. F. Stadler, *Comparative Genomics: Methods and Protocols*, Springer, 2018.
- 19 P. Li, Y. Song and S. Yu, Removal of *Microcystis aeruginosa* using hydrodynamic cavitation: performance and mechanisms, *Water Res.*, 2014, **62**, 241–248.
- 20 P. R. Deep, S. Bhattacharyya and B. Nayak, Cyanobacteria in wetlands of the industrialized Sambalpur District of India, *Aquat. Biosyst.*, 2013, **9**(1), 14.
- 21 R. Ghai, C. M. Mizuno and A. Picazo, *et al.*, Key roles for freshwater Actinobacteria revealed by deep metagenomic sequencing, *Mol. Ecol.*, 2014, **23**(24), 6073–6090.
- 22 I. H. Brümmer, A. Felske and I. Wagner-Döbler, Diversity and seasonal variability of beta-Proteobacteria in biofilms of polluted rivers: analysis by temperature gradient gel electrophoresis and cloning, *Appl. Environ. Microbiol.*, 2003, **69**(8), 4463–4473.
- 23 O. C. Betiku, K. C. Sarjeant and L. W. Ngatia, *et al.*, Evaluation of microbial diversity of three recreational water bodies using 16S rRNA metagenomic approach, *Sci. Total Environ.*, 2021, **771**, 144773.
- 24 L. Zhou, D. W. Wang and S. L. Zhang, *et al.*, Functional microorganisms involved in the sulfur and nitrogen metabolism in production water from a high-temperature offshore petroleum reservoir, *Int. Biodeterior. Biodegrad.*, 2020, **154**, 10.
- 25 A. M. Spain, L. R. Krumholz and M. S. Elshahed, Abundance, composition, diversity and novelty of soil Proteobacteria, *ISME J.*, 2009, **3**(8), 992–1000.
- 26 A. Ranjani, D. Dhanasekaran and P. M. Gopinath, An Introduction to Actinobacteria, *Actinobacteria - Basics and Biotechnological Applications*, 2016.
- 27 X. Du, H. Liu and L. Yuan, *et al.*, The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review, *Toxins*, 2019, **11**(9), 530.
- 28 Y. Park, Y. Pachepsky and E. M. Hong, *et al.*, *Escherichia coli* release from streambed to water column during baseflow periods: a modeling study, *J. Environ. Qual.*, 2017, **46**(1), 219–226.
- 29 J. K. Bradshaw, B. Snyder and D. Spidle, *et al.*, Sediment and fecal indicator bacteria loading in a mixed land use watershed: Contributions from suspended sediment and bedload transport, *J. Environ. Qual.*, 2021, **50**(3), 598–611.