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Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems

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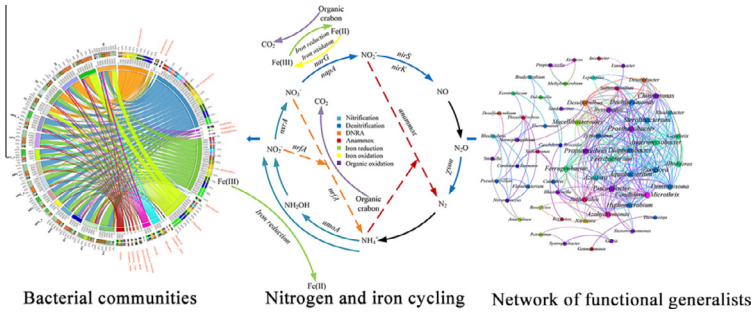
HIGHLIGHTS

- Bacterial communities and functional generalists were explored by MiSeq sequencing and qPCR.
- Anammox, *nrfA*, FeOB and FeRB genes had higher abundance in anammox bioreactors.
- Nitrification–anammox, denitrification–FeOB, and DNRA–FeRB showed co-occurrence patterns.
- Coupling of anammox, DNRA and FeRB were confirmed using correlation-based network analysis.
- Environmental factors have highly impacts on the N and Fe cycling-related bacterial communities.

GRAPHICAL ABSTRACT



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ABSTRACT

To gain a better understanding of bacterial community structures, ecological inter-correlations and functional generalists of nitrogen- and iron-cycling-related bacteria in various wastewater treatment systems (WWTSs), 16 samples collected from 3 industrial, 4 municipal and 5 anaerobic ammonium oxidation (anammox) WWTSs were used to perform metagenomic analysis. A total of 9394 to 17,130 effective reads for 16 samples were obtained from the bacterial 16S rRNA V3–V4 regions using MiSeq sequencing. Taxonomic analysis revealed that *Bacteroidetes*, *Chloroflexi*, *Proteobacteria*, and *Planctomycetes* were the dominant phyla in these samples. Furthermore, quantitative polymerase chain reaction (qPCR) was conducted and the results revealed that anammox, *nrfA*, FeOB (iron oxidizing bacteria) and FeRB (iron reducting bacteria) genes had higher abundance when *Candidatus* Brocadia was the dominant genera in anammox bioreactor. Finally, Spearman rank order coefficient correlation (SRCC) and redundancy discriminant analysis (RDA) analysis implicated that the groups of nitrification–anammox, denitrification–FeOB, and dissimilatory nitrate reduction to ammonium (DNRA)–FeRB showed positively

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correlations. Based on the co-occurrence pattern demonstrated by network analysis, the coupling of anammox, DNRA and FeRB was the noteworthy pathway for simultaneous removal of nitrogen and organic carbon. Overall, this work provides novel insights into the co-occurrence patterns and generalists of nitrogen and iron-cycling-related bacteria in wastewater treatment systems.

1. Introduction

In recent decades, biological treatment processes has been widely applied for treating industrial and municipal wastewater due to their high removal efficiency, positive energy balance and low operational cost. However, the biological treatment processes is in a period of significant change motivated by two aspects. On one hand, it is necessary to renew some wastewater treatment systems (WWTSs) that were built in the last 30 years. On the other hand, the drive from the development of technological innovation toward more sustainable including energy recover and efficiency during mainstream wastewater treatment [1]. Moreover, increasingly effluent guidelines are being implemented across the world. However, the microbial influence on nutrients removal, recovery and function of microorganisms in mainstream biological approaches remains unclear. Therefore, to gain a better understanding of the microbial community and those functional genes of anaerobic or aerobic samples in WWTSs will not only be useful for illuminating the molecular mechanisms of nitrogen and organic matter removal, but also beneficial for promoting the maneuverability and stability of different WWTSs.

Previous studies [2,3] reported that nitrogen removal in WWTSs contains various biological processes, including anaerobic ammonium oxidation (anammox), nitrification, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification. These nitrogen processes involved different 16S rRNAs and functional genes, namely anammox 16S rRNA, archaea ammonia monoooxygenase (AOA-*amoA*), ammonia monoooxygenase (AOB-*amoA*), nitrite oxidoreductase (*nxrA*), periplasmic nitrate reductase (*napA*) and membrane-bound nitrate reductase (*narG*), dissimilatory nitrate reductase (*nrfA*), copper-containing nitrite reductase (*nirK*), nitrite reductase (*nirS*), and nitrous oxide reductase (*nosZ*) [4,5]. At the same time, microbial iron cycling had been reported to play a key role in WWTSs for wastewater treatment. Microbial iron involves two processes, including iron-oxidizing (FeOB) and iron-reducing (FeRB). These two processes involved several bacterial 16S rRNAs genes, including FeOB (i.e. *Acidimicrobium* spp., *Ferrovum myxofaciens*) and FeRB (i.e. *Albidiferax ferrireducens*, *Geobacter* spp., and *Acidiphilium* spp.) [6,7]. Although the N (nitrogen) and Fe (iron) related microbial played the pivotal role in WWTSs, no attention has been paid to explore the bacterial interactions between the related microorganisms during N and Fe cycling. Moreover, the ecological linkages between bacterial community and operational parameters in different WWTSs are still unclear at present.

Currently, as the rapid development of high throughput sequencing technologies, MiSeq sequencing have been applied to explore the bacterial diversity and abundance of samples from WWTSs [8,9]. Nevertheless, the metagenomic and quantitative analysis for N and Fe cycling are few. Functional generalists, which were consisted of widely distributed bacterial genera affiliated with abundant phyla, were useful for maintaining the stability of the WWTSs. Their metabolic states have significant correlations with microbial communities and ecosystem functions. Last but not least, little is known about the co-occurrence associations among different bacteria and functional generalists in nitrogen and iron-cycling-related bacteria.

Given the above arguments, this study was performed with the following four objectives: (1) to explore the bacterial diversity and their functional generalists in 16 sludge samples from different WWTSs; (2) to elucidate the community structures of anammox bacteria and iron-cycling-related bacteria and to quantitatively analyze the absolute abundance of nitrogen and iron-cycling-related bacteria; (3) to investigate the ecological linkages between operational parameters and related bacterial community; (4) to explore the co-occurrence patterns between bacterial communities and functional generalists using network analysis.

2. Methods

2.1. Description of wastewater treatment systems and sample collection

In this study, all anaerobic and anoxic sludge samples were collected from twelve WWTSs in Shaanxi and Kunming, China. Details of processes, treatment capacity, influents, effluents, and operational parameters of the 12 WWTSs were summarized in Table S1. Among these WWTSs, IP1, IP2, and IP3 were full-scale industrial WWTSs, MP1, MP2, MP3, and MP4 were full-scale municipal WWTSs. In addition, R1 was the pilot-scale WWTSs, and R2, R3, R4, and R5 were laboratory-scale WWTSs. These WWTSs differed mainly in their influents, effluents, and operational parameters. The anaerobic/anoxic/oxic (A/A/O) process was applied in MP1, MP2, and MP4 for treating municipal wastewater. MP2 was equipped with Intermittent Cycle Extended Aeration (ICEAS) plus Anaerobic Membrane Bioreactor (AMBR) for treating municipal wastewater. IP1 was operated with Upflow anaerobic sludge blanket process (UASB) plus Orbal oxidation ditch process for treating starch wastewater. UASB plus biological contact oxidation process was employed in IP2 for pulp and paper wastewater treatment. IP3 treated landfill leachate through the UASB plus A/A/O process for COD and nitrogen removal, and MBR was further used to enhance nitrogen removal. Additionally, R1, R2, R3, R4, and R5 were operated with anaerobic ammonium oxidation (anammox) process. R1 was controlled by continuous stirred tank reactor (CSTR) for nitrogen removal. R2, R3, R4, and R5 were equipped with sequencing batch reactor (SBR), SBR, UASB, and SBR, respectively.

The sludge samples from MP1, MP2, MP3, and MP4 were collected from anaerobic and aerobic tank to compare the microbial abundance under different dissolved oxygen (DO) concentration. For others anaerobic sludge sample, they were taken from IP1, IP2, IP3, R1, R2, R3, R4, and R5. Taken together, 16 samples were obtained from 12 WWTSs. After the 16 sludge samples were collected, they were immediately fixed in 50% (v/v) ethanol aqueous solution and then stored in laboratory at −80 °C for DNA extraction.

2.2. DNA extraction, PCR amplification and Illumina MiSeq sequencing

For DNA extraction, 0.5 g wet sludge sample pellet was collected and DNA was extracted using the FastDNA® SPIN Kit for Soil (Mp Biomedicals, Illkirch, France) according to the manufacturer's protocol. Extracted genomic DNA concentrations were determined with Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA) and its quality was checked in 1.0% agarose gel electrophoresis. Microbial genomic DNA extraction was performed in triplicate for each sludge sample.