



Altitude-scale variation in nitrogen-removal bacterial communities from municipal wastewater treatment plants distributed along a 3600-m altitudinal gradient in China

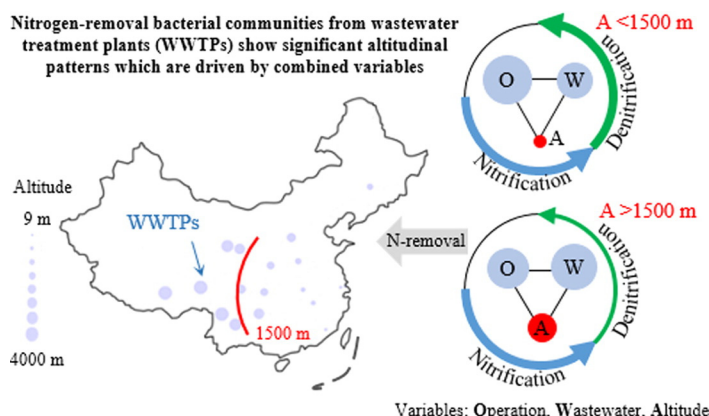
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HIGHLIGHTS

- Aimed to assess altitude-scale variation of N-removal bacterial communities in WWTPs
- Compared the quantitative relation and community composition across 18 WWTPs
- Analyzed the correlation with environmental variables
- Propose 1500-m as an altitude threshold for the quantitative relation
- Correlation between community composition dissimilarity and altitudinal distance

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial ecological information on the nitrogen removal processes in wastewater treatment plants (WWTPs) has been of considerable importance as a means for diagnosing the poor performance of nitrogen removal. In this study, the altitude-scale variations in the quantitative relationships and community structures of betaproteobacteria ammonia-oxidizing bacteria (β AOB) and nitrite-reducing bacteria containing the copper-containing nitrite reductase gene (*nirK*-NRB) and the cytochrome *cd1*-containing nitrite reductase gene (*nirS*-NRB) were investigated in 18 municipal WWTPs distributed along a 3660-masl altitude gradient in China. An altitude threshold associated with the proportions of NRB to total bacteria, NRB to β AOB and *nirK*-NRB to *nirS*-NRB was detected at approximately 1500 m above sea level (masl). Compared with the stable proportions below 1500 masl, the proportions exhibited a pronounced decreasing trend with increased altitude above 1500 masl. Spearman correlation analysis indicated that the trend was significantly driven by altitude as well as multiple wastewater and operational variables. The community structure dissimilarity of β AOB, *nirK*-NRB and *nirS*-NRB showed significant and positive correlations with altitudinal distance between WWTPs. Redundancy analyses indicated that the variation in community structures above 1500 masl were predominantly

Abbreviations: WWTPs, wastewater treatment plants; AOB, ammonia-oxidizing bacteria; β AOB, betaproteobacteria AOB; NRB, nitrite-reducing bacteria; *nirK*-NRB, the copper-containing nitrite reductase gene; *nirS*-NRB, the cytochrome *cd1*-containing nitrite reductase gene; DGGE, denaturing gradient gel electrophoresis; avsc, absolute value of Spearman correlation coefficients; masl, meter above sea level.

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associated with wastewater, followed by operation and altitude. In summary, although the variations of nitrogen-removal bacterial community in WWTPs were driven dominantly by wastewater and operational variables, altitude was also an important variable influencing the quantitative relationships and community structures of nitrogen-removal bacteria in WWTPs particularly above 1500 masl.

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1. Introduction

Microbial communities involved in nitrogen removal processes are critical in determining the performance and stability of wastewater treatment plants (WWTPs). Ammonia oxidation is the rate-limiting step in the nitrification pathway of WWTPs and is primarily performed by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Limpiyakorn et al., 2011) as well as the potential comammox organisms (Daims et al., 2015). Nitrite reduction, which is achieved by nitrite-reducing bacteria containing either the copper-containing nitrite reductase gene (*nirK-NRB*) or the cytochrome *cd₁*-containing nitrite reductase gene (*nirS-NRB*), greatly contributes to the denitrification reaction for nitrogen removal from wastewater (Shapleigh, 2006). To date, the abundance and diversity of AOB, AOA and NRB have been studied extensively in WWTPs (Lu et al., 2014; Otawa et al., 2006). Nevertheless, the study on the systematic characterization of nitrogen-removal microbial communities in WWTPs remains incomplete. For instance, the geographical or altitudinal characterization of nitrogen-removal microbial communities in WWTPs has never been considered.

Efficient treatment of wastewater in WWTPs from plateaus plays an important role in the protection of the upstream regions of numerous rivers, such as the Yangtze River and the Nile. In recent years, an increasing number of WWTPs were built in the Tibetan Plateau as well as in Bolivia, with an average altitude >3000 m above sea level (masl). Unfortunately, the effluent water from these high-altitude WWTPs often failed to meet the discharge standards of several countries (Zabalaga et al., 2007), or the high-altitude WWTPs commonly performed nitrogen removal with lower efficiency than that in low-altitude WWTPs. Microbial ecological information on the nitrogen removal process is one of the important bases for diagnosing the poor performance of nitrogen removal. Nevertheless, to the best of our knowledge, the microbial community characteristics at high-altitude WWTPs have never been reported. Furthermore, whether there are altitudinal differences in nitrogen-removal microbial communities from WWTPs is an important question.

Multiple altitudinal patterns of microbial communities have been documented in natural habitats (Bryant et al., 2008; Singh et al., 2012). Unlike natural habitats, most previous studies assumed that the microbial community in WWTPs is primarily structured by deterministic effects, including operation and wastewater conditions (Wells et al., 2011). However, these studies have not taken into account the underappreciated effects of geography or altitude. According to neutral processes, quantities of local microbial communities in influent wastewater, structured by the local ambient environment (Lozupone and Knight, 2007), may also affect microbial communities in WWTPs (Sloan et al., 2006). In recent years, significant geographical differences in bacterial communities from WWTPs were revealed by few studies (Zhang et al., 2012; Zhao et al., 2014). Thus, we hypothesized that potential altitude-scale variations in the nitrogen-removal bacterial communities characterize activated sludge WWTPs.

The objective of this study was to test the hypothesis mentioned above via investigating the variations in quantitative relationships and community structures of nitrogen-removal microbial communities, represented by β -Proteobacteria AOB (β AOB), *nirS*-NRB and *nirK*-NRB, in 18 full-scale municipal WWTPs scattered along a 3660-masl altitude gradient in China (Fig. 1). Furthermore, the potential links between nitrogen-removal bacterial communities and environmental variables underlying the hypothesis were assessed using a variety of statistical approaches.

2. Material and methods

2.1. Collection of activated sludge samples

Eighteen municipal WWTPs scattered along a 3660-masl altitude gradient were selected in China (Fig. 1). All WWTPs were designed to treat municipal wastewater. The details of the 18 WWTPs, including geographic locations, wastewater variables and operational variables controlling wastewater treatment processes, are listed in Table S1. A total of 54 samples were collected from the 18 WWTPs in May, August and December of 2014. Each sample was composed of equal amounts of activated sludge from anoxic and aerobic stages in each WWTP. Activated sludge samples were stored in a dry ice box immediately after collection and then stored at -80°C in the lab. A total of 18 composite samples were obtained by mixing the three thawed samples of each WWTP with equal volumes before DNA extraction.

Genomic DNA was extracted from 54 individual samples and 18 composite samples separately using an Ultraclean DNA Extraction Kit (Mo-Bio, Carlsbad, CA) (Wells et al., 2011). DNA quality was analyzed by the 260/280 nm and 260/230 nm absorption ratios using an ND-2000 spectrophotometer (NanoDrop Inc., Wilmington, DE) and 1% agarose gel electrophoresis.

2.2. Quantitative PCR

The abundance of 16S rRNA-total bacteria, *amoA*- β AOB, *nirK*-NRB and *nirS*-NRB in 54 individual samples was quantified via quantitative PCR (qPCR). Some DNA samples were concentrated and enriched to meet the gene density of detection limits. Table S2 shows the primer sets used for qPCR. Amplification reaction followed the processes described by Cydzik-Kwiatkowska et al. (2014). Reactions were carried out on CFX96 Touch (Bio-Rad, USA). Each DNA sample was amplified in triplicate in the presence of negative and positive controls. Melting temperature of the PCR products was detected to confirm the specificity of PCR products. The standard plasmids were prepared as described by Wang et al. (2014). Standard curves for each assay were constructed



Fig. 1. Map of sampled wastewater treatment plants in China. Altitude is represented by the size of the symbols. The map was created using the online software Mapping-together (<http://c.dituhui.com>).

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