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Distribution patterns of nitrogen micro-cycle functional genes and their quantitative coupling relationships with nitrogen transformation rates in a biotrickling filter

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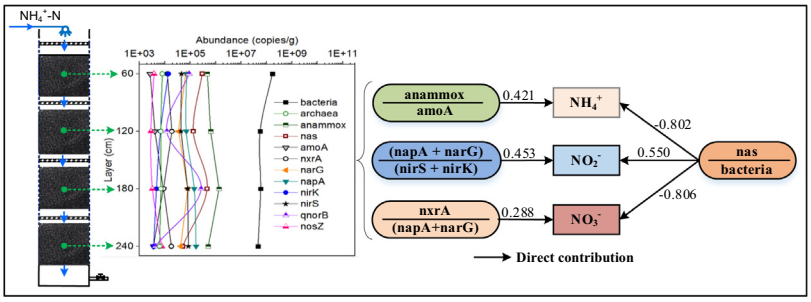
HIGHLIGHTS

- Distribution patterns of N functional genes were quantitatively assessed.
- N functional genes enriched in different depth gradients of the biofilter.
- DNRA coupled with nitrification and denitrification was a pathway for N removal.
- *Nas* showed a negative relationship with NH_4^+ removal and NO_3^- accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study explored the distribution patterns of nitrogen micro-cycle genes and the underlying mechanisms responsible for nitrogen transformation at the molecular level (genes) in a biotrickling filter (biofilter). The biofilter achieved high removal efficiencies for ammonium (NH_4^+-N) (80–94%), whereas nitrate accumulated at different levels under a progressive NH_4^+-N load. Combined analyses revealed the anammox, *nas*, *napA*, *narG*, *nirS*, and *nxrA* genes were the dominant enriched genes in different treatment layers. The presence of simultaneous nitrification, ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA) were the primary factors accounted for the robust NH_4^+-N treatment performance. The presence of DNRA, nitrification, and denitrification was determined to be a pivotal pathway that contributed to the nitrate accumulation in the biofilter. The enrichment of functional genes at different depth gradients and the multi-path coupled cooperation at the functional gene level are conducive to achieving complete nitrogen removal.

1. Introduction

The long-term intensive application of nitrogen (N) fertilizer in China has caused the contamination of surface waters (e.g., rivers, lakes, and reservoirs). Water bodies in China have been seriously polluted since the 1990s, and there have been no marked improvements in recent years (Sun et al., 2012). China has 4880 lakes, covering a total area of 83,400 km^2 and accounting for 0.8% of the country's land area. According to an evaluation of eutrophication in 121 major lakes in 2014, approximately 76.9% of the lakes were eutrophic (MWR, 2014). These lakes serve as China's main sources of drinking water. High levels of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in drinking water are also concerns for human health

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because they can poison infants by provoking methemoglobinemia (David et al., 2013). Thus, polluted drinking water must be pretreated to remove excess NH_4^+-N and NO_3^--N.

Biotrickling filter (biofilter) systems have been engineered and intensively studied as a sustainable technology for improving drinking water. Biofilters have attracted considerable interest due to the well-established advantages of these systems, such as simple design and operation, low capital and operating costs and a low requirement for energy and maintenance inputs (Van den Akker et al., 2011; Wang et al., 2015a; Wik, 2003). In a biofilter, water is distributed over a tower containing the packed media. Then, as the water trickles down, microorganisms in the biofilm degrade nitrogen via several ecological processes, such as nitrification, denitrification, and anaerobic ammonium oxidation (anammox) (Ji et al., 2012b; Wang et al., 2015b). Microbial communities exhibit substantial heterogeneity in their spatial distributions (Andrus et al., 2014). Furthermore, these differences in spatial distribution can have a considerable effect on nitrogen removal in biofilters (Ji et al., 2013); thus, investigations of the spatial distribution patterns of microbial communities can provide insight into processes mediated by microbes. The distribution patterns of the microbial community were related to the water flow and showed increased diversity with decreasing nutrient levels and increasing water residence times (David et al., 2013). An analysis of the spatial and temporal distribution of ammonia monooxygenase (*amoA*) and nitrous oxide reductase (*nosZ*) in a pilot-scale biofilter indicated that ammonia-oxidizing and denitrifying bacteria coexisted in both the anoxic and aerated areas (Gómez-Villalba et al., 2006). As noted by Gilbert et al. (2008), denitrifiers were mainly enriched near the surface of the filter, and a microbiological gradient was present along the water flow. Juhler et al. (2009) studied the abundance distribution of *amoA* in a biofilter. The results showed that the absolute abundance of the ammonium oxidation gene *amoA* was low at the biofilter outlet. Ji et al. (2013) investigated the spatial distribution of nitrogen removal functional genes in multimedia biofilters for sewage treatment. The results showed that anammox bacterial 16S rRNA (anammox) and the other nitrogen removal functional genes all were dominantly enriched at different depth gradients. Anammox, periplasmic nitrate reductase (*napA*), nitric oxide reductase (*qnorB*), and *nosZ* showed partially or mutually beneficial cooperation. The nitrite oxidoreductase (*nxrA*) and nitrite reductase (*nirK*) genes showed protocooperation, and the *amoA* and *narG* genes showed partially beneficial cooperation. The dissimilatory nitrate reduction to ammonium (DNRA) process has been described in many systems, including tropical forest soils, freshwater sediments, marine environments, coastal ecosystems, and constructed wetlands (Brunet and Garcia-Gil, 1996; Giblin et al., 2013; Silver et al., 2001; Zhi et al., 2015). The nitrate reduction coding gene *nas* is often regarded as a marker of the DNRA process (Canfield et al., 2010). DNRA, coupled with anammox and ammonia oxidation, was determined to be a pivotal pathway that contributed to NH_4^+-N and NO_3^--N removal, which is similar to a recent study that first reported that co-occurring anammox and DNRA were responsible for the intensive nitrogen loss in tidal flow constructed wetlands (Zhi et al., 2015). The DNRA process, which has been ignored in the field of biofilters to date, may be an important pathway contributing to NO_3^--N removal in biofilters and may rival denitrification in terms of its importance and contribution to the nitrogen balance.

However, few studies on DNRA in biofilters have been reported, and thus little is known about the fate of nitrogen after its transformation from NO_3^--N to NH_4^+-N via DNRA. To date, only a few studies focusing on the distribution patterns of nitrogen micro-cycle functional genes have been published, and very little is known about the DNRA process in biofilters. The lack of a quantitative link between transformation rates of NH_4^+-N and NO_3^--N and *nas* functional gene limits the ability to optimize NH_4^+-N removal and reduce NO_3^--N accumulation to reliably predict long-term effluent quality.

The overall goal of the current study was to analyze the spatial distribution of nitrogen functional genes and understand the effects of DNRA, nitrification, denitrification, and anaero ammonium oxidation (anammox) processes at the molecular level in a controlled biofilter. The following four specific objectives were pursued: (1) evaluation of the treatment performance of NH_4^+-N and NO_3^--N removal; (2) quantitative analysis the spatial distribution of functional genes involved in nitrogen removal; (3) investigation of the respective key functional genes and primary nitrogen removal pathways at different depth gradients; and (4) determination of the quantitative coupling relationships between the nitrogen transformation processes and functional genes.

2. Methods

2.1. Biotrickling filter

One laboratory-scale biofilter with dimensions of 40 cm (length) × 30 cm (width) × 240 cm (depth) (working volume of 144 L) was built (see Fig. S1 In Supplementary Information). The biofilter consisted of four treatment layers (from top to bottom: 20–60 cm, 80–120 cm, 140–180 cm, and 200–240 cm). The four treatment layers were filled with polyurethane foaming plastic with a porosity factor of 75–90%. A sieve tray was installed between each treatment layer. Forty-eight holes were evenly drilled into each sieve tray to allow for contact between the wastewater and air. The biofilter was fed with NH_4^+-N wastewater to investigate the treatment performance treating contaminated lake water. The Chemical Oxygen Demand (COD) concentration in the lake section investigated ranged from 6.0 to 25 mg/L, and the NH_4^+-N concentration in the lake section investigated ranged from 1.2 to 15 mg/L. Synthetic wastewater (see Table S1 in the Supplementary Information) was derived from Beijing groundwater, in which the NO_3^--N concentration varied from 4.9 to 5.0 mg/L throughout the study (35 week). 0.006–0.024 g glucose and 0.004–0.045 g NH_4Cl per liter were added to tap water, resulting in concentrations of 6.0–25.0 mg/L COD and 1.2–15.0 mg/L NH_4^+-N. The hydraulic loading rate was maintained at 2.0 $m^3/m^2/d$. The synthetic wastewater was prepared daily in a feeding tank and then pumped into the top treatment layers. The immobilized B350M microorganisms, which purchased from BIO-SYSTEMS Co. (USA) (Ji et al., 2012a), were placed in the four treatment layers. 40 g of B350M microorganisms was placed in each treatment layer. The biofilter was placed indoors, and influents and effluents ranged in temperature from 10.3 to 26.9 °C. The experiment began on December 28, 2012 and involved the following six stages (total 35 weeks): start-up stage (0–14 week) from December 28 to February 23; stage I (15–19 week) from February 24 to March 29; stage II (20–23 week) from March 30 to May 3; stage III (24–27 week) from May 4 to June 7; stage IV (28–31 week) from June 8 to July 12; and stage V (32–35 week), from July 13 to August 16.

2.2. Sample collection and determination

Water samples were collected from the biofilter nine times (start-up stage) and three times during each operational stage. The water samples were analyzed immediately at the Key Laboratory of Water and Sediment Sciences of Peking University. The dissolved oxygen (DO) content was measured using a DO200 dissolved oxygen meter (YSI, Yellow Springs, Ohio, USA). COD was determined using a HACH DR2800 (HACH, Loveland, Colorado, USA), and the NH_4^+, nitrite (NO_2^-), and NO_3^- were measured using a