Effects of sliver nanoparticles on the coupled nitrification-denitrification in suspended sediments from Taihu Lake

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Abstract. The widespread use of commercial silver nanoparticles (Ag NPs) inevitably results in their increasing release into natural waters and potentially threaten the freshwater ecosystems. During their migration, Ag NPs would interact with suspended sediments (SPS) and negatively affect the microbial communities on SPS, which play an important role in the nutrient cycling in aquatic environments. However, there is limited research focusing on the potential effects of Ag NPs on nitrogen cycling in SPS. In this study, ¹⁵N isotope tracer technique (added ¹⁵NH₄⁺) was adopted to explore the influences of different concentrations of Ag NPs on coupled nitrification-denitrification (CND) in SPS systems by determining the variation of added NH₄⁺ and the final production of CND (¹⁵N-N₂). The obtained results indicated that 10 and 5 mg/L Ag NPs inhibited the¹⁵N-N₂ production of CND by decreasing the utilization of NH₄⁺ in SPS, while 0.5 and 2 mg/L Ag NPs increased the activity of CND in SPS due to the hormesis effects. The study demonstrated the negative effects of Ag NPs on N-cycling in SPS systems and provided more evidences for evaluating the risks of Ag NPs in aquatic ecosystems.

1. Introduction

Coupled nitrification and denitrification (CND) are one of the important processes in nitrogen cycling (N-cycling) which can transform bioavailable nitrogen forms, ammonium (NH₄⁺) and nitrate (NO₃⁻), final to gaseous nitrogen. It has been reported that up to 80% of bioavailable nitrogen was removed by CND in some sediments [1]. To date, CND has been confirmed in several natural ecosystems such as soil and sediments, which required in aerobic and anaerobic conditions coexisting in close proximity [2].

Recently, nanoparticles (NPs) have been widely used due to their special characteristics such as small size effects and quantum effects. Silver nanoparticles (Ag NPs) are the most promising metal nanoparticles which are applied in the biomedical and biotechnological fields [3]. However, their increasing usage unavoidably leads to their release into aquatic environments through effluent of wastewater treatment plants or other pathways [4], and thus the potential risk of Ag NPs to freshwater ecosystems should be paid much attention to.

After their release into aquatic systems such as shallow lakes, Ag NPs are assumed to be mainly deposited in sediments [4]. It has been predicted that concentrations of Ag NPs were 0.1-900 mg/kg in sediments and even up to 2000 mg/kg in heavily polluted places [5, 6]. However, before settlement to sediments, NPs will inevitably interact with suspended sediments during their sedimentation in water systems. Suspended sediments are common in natural aquatic environments worldwide. Due to the
significance of CND in N loss of SPS systems, more attention need to be paid to this process. Therefore, considering the antibacterial activity of Ag NPs, the toxicity of Ag NPs on CND in SPS should be investigated.

2. Materials and methods

2.1. Sample collection and characterization of Ag NPs

The samples were collected at the Zhushan Bay (31.3794 N, 120.0249 E) of the Taihu Lake in May 2017. Prior to experiments, the sediment was mixed to avoid heterogeneity and wet-sieved (<0.063 mm). The overlying water was filtered through a 0.22 mm membrane. Ag NP powders were purchased from Sigma-Aldrich (St. Louis, MO, USA) and the average diameters of original Ag were less than 100 nm, confirming by images of scanning electron microscope (SEM).

2.2. Aggregation of Ag NPs

Briefly, filtered water samples were placed into sediment-free beakers, and Ag NPs stock solutions (200 mg/L) were added to yield final NP concentrations of 0.5, 2, 5, and 10 mg/L. The samples were then placed in a rotary shaker (150 rpm, 25°C), being subsequently removed at certain time intervals (2, 4, 6, 12, 16, 20, and 24 h) for aggregation analyses. The hydrodynamic diameters (HDDs) were confirmed using Dynamic Light Scattering by Malvern Zetasizer Nano (ZSP UK). The nano-aggregates were imaged by SEM (Hitachi S-4800) on a 300-mesh carbon-coated copper grid (Nanjing Zhongjing Technology Co., China) in a dust-free condition.

2.3. Laboratory incubation and exposure experiments

The incubation of SPS systems was conducted in a reactor with poly (methyl methacrylate) columns (diameter = 8 cm, h = 40 cm). The sieved sediments and water were added into columns to form 500 mg/L SPS, and the agitation rate was set as 150 rpm to guarantee the suspension of the sediment at 25 °C. All reactors were pre-incubated for 24 h to form stable system and remove residual nutrients. Next, 0.75 mg/L $^{15}\text{NH}_4^+$ (15NH$_4$Cl, 55 atm% $^{15}$N) and 10 mg/L glucose (Analytical reagent) were added to provide the trace nitrogen source and dissolved organic carbon, respectively. After 10 min of agitation to distribute added nutrients solutions, Ag NPs stock solutions were pulse injected to obtain concentrations of 0 (control), 0.5, 1, 5, and 10 mg/L, respectively. Three replicates were prepared for each group and then incubated for 24h at 25 °C being agitated at 150 rpm. At certain time intervals (0, 4, 8, 12, and 24h), 5 mL SPS water samples were collected for the determination of NH$_4^+$. Samples were also carefully flown into 20-mL gas-tight brown vials to determine the concentration of $^{15}$N-N$_2$ dissolved into water by Membrane Inlet Mass Spectrometry (MIMS).

For independent samples, a t test was performed to test differences between the Ag NPs-treated and corresponding control groups. Statistical significance was determined at a confidence level of 0.05.

3. Results and discussion

3.1. Characterization and aggregation of Ag NPs

The HDDs of low concentration CuO NPs were almost stable within 24 h whereas distinct aggregation was observed for higher concentration groups especially in 10 mg/L Ag NPs (Figure 1). Throughout the test, the NP HDDs in four groups decreased in the order of 10 > 5 > 2 > 0.5 Ag NPs, revealing the reduced stability of high concentration of NPs.
3.2. The negative effects of Ag NPs on CND in SPS

The concentrations of NH$_4^+$ in five groups were decreased during the incubation time (Figure 2) while the $^{15}$N-N$_2$ produced in SPS was increased (Figure 3), indicating the consumption of NH$_4^+$ and the occurrence of CND in SPS. More specifically, 0.5 mg/L Ag NPs dramatically stimulated the consumption of NH$_4^+$ compared with control ($p < 0.05$), and also expressed the highest $^{15}$N-N$_2$ production ($p < 0.05$), which demonstrated that the activity of CND was obviously improved by 0.5 mg/L Ag NPs. In the contrast, 10 mg/L Ag NPs significantly increased the accumulation of NH$_4^+$ ($p < 0.05$) and had the lowest concentration of $^{15}$N-N$_2$ ($p < 0.05$), exhibiting the obvious toxicity for CND. 2 mg/L Ag NPs expressed the similar performance with 0.5 mg/L groups while the increased effects were not as significant as the latter ($p > 0.05$). The same situations were also observed between 5 mg/L and 10 mg/L groups.
Figure 2. Variations of $\text{NH}_4^+$ in SPS systems with different Ag NPs exposure. The values for each sample are presented as averages with standard deviations ($n = 3$).

Figure 3. Variations of $^{15}\text{N}_2$ in SPS systems with different Ag NPs exposure. The values for each sample are presented as averages with standard deviations ($n = 3$).
The results indicated that 0.5 mg/L had significant improvement for CND by stimulating the usage of NH$_4^+$ and then produced more N$_2$. The above results could be caused by the hormesis effects of Ag NPs. Hormesis refer to the stimulation of growth by low concentration of toxins and other stressors [7]. It has been also confirmed that sublethal concentration of Ag NPs could enhance the activity of bacterial possible by improving their fitness and hinder antimicrobial applications [8]. However, high concentrations (10 mg/L) of Ag NPs had obvious toxicity for CND in SPS, and the utilization of NH$_4^+$ by nitrifying and denitrifying bacterial might be inhibited. The potential toxicity mechanisms of Ag NPs include the damage of cell membrane integrity, protein degeneration, and gene toxicity due to the released silver ion or nanoparticle effects [9].

4. Conclusions
Our study indicated that the high concentration of Ag NPs exhibited obvious toxicity for CND by decreasing the utilization of NH$_4^+$ in SPS. Therefore, the risk of Ag NPs on CND of SPS systems and other nitrogen transformation processes should be considered carefully.

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