SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 10, pp. 2234–2239, 2014

Printed in the USA

Environmental Chemistry

DIFFERENTIAL SENSITIVITY OF NITRIFYING BACTERIA TO SILVER NANOPARTICLES IN ACTIVATED SLUDGE

YU YANG,† MENGYAN LI,† CAMILA MICHELS,‡ HUGO MOREIRA-SOARES,‡ and PEDRO J.J. ALVAREZ*†
†Department of Civil and Environmental Engineering, Rice University, Houston, Texas, USA
‡Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

(Submitted 22 April 2014; Returned for Revision 21 June 2014; Accepted 26 June 2014)

Abstract: Nitrification is known as one of the most sensitive processes affected when activated sludge is exposed to antimicrobial silver nanoparticles (AgNPs). The impact of AgNPs and their released silver ions (Ag^+) on the abundance, activity, and diversity of different nitrifying bacteria in wastewater treatment plants (WWTPs), however, is poorly understood. The present study investigated the impacts of 2 sizes of AgNPs (5 nm and 35 nm) and Ag^+ ions on the nitrifier community in activated sludge, including both ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Ammonia-oxidizing bacteria were more sensitive to AgNPs than the NOB; a 5-d and 7-d exposure of activated sludge to 35 nm AgNPs (40 ppm) significantly reduced AOB abundance to 24% and 19%, respectively. This finding was confirmed further by a decrease in activated sludge ammonia oxidation activity measured by ^{14}C-labeled bicarbonate uptake. In contrast, neither AgNPs (up to 40 ppm) nor Ag^+ (1 ppm) affected the abundance of NOB. Both 5 nm and 35 nm AgNPs decreased the diversity of AOB, as indicated by denaturing gradient gel electrophoresis with ammonia monooxygenase gene (*amoA*) primers, although some unknown *Nitrosomonas* species were relatively resistant to AgNPs. The generally greater resistance of NOB than AOB to AgNPs suggests that the accumulation of bacteriostatic nitrite in WWTPs is unlikely to be exacerbated due to the accidental or incidental release of AgNPs. *Environ Toxicol Chem* 2014;33:2234–2239. © 2014 SETAC

Keywords: Silver nanoparticles Nitrification Ammonia oxidizing bacteria Nitrite oxidizing bacteria Denaturing gradient gel electrophoresis

INTRODUCTION

The number of nanotechnology enabled products has expanded rapidly during the past decade, with more than 1600 commercial products containing manufactured nanomaterials available in the market [1]. Because of their broad-spectrum antimicrobial properties, silver nanoparticles (AgNPs) are the most widely used manufactured nanomaterials in consumer products (25% of all the nanotechnology enhanced products) [1] and are commonly used in domestic, textile, medical, industrial, and environmental applications [2–4]. The rapid increase in commercial applications of AgNPs is of public concern because the unintended impacts from environmental exposure to AgNPs and associated ecological risks are not fully understood.

The widespread use of AgNPs has resulted in some releases that reach sewage systems and wastewater treatment plants (WWTPs) [5,6]. The impact of various forms of silver to activated sludge in WWTPs has been documented [7,8]; for example, concentrations of AgNPs ranging from 1 ppm to 20 ppm have been reported to upset the operation of activated sludge plants (e.g., inhibit biological nitrogen removal) [9–11]. Nitrification, which is the bio-oxidation of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-), has been suggested to be the most vulnerable step in the nitrogen cycling process [12]. A previous study by Yang et al. [13] also demonstrated the adverse effect of AgNPs on the microbial community of activated sludge, with nitrifying bacteria identified as one of the most sensitive species.

In WWTPs, 2 different groups of bacteria mediate nitrification: ammonia-oxidizing bacteria (AOB) for oxidation of NH_3 to NO_2^- and nitrite-oxidizing bacteria (NOB) for oxidation of NO_2^- to NO_3^- [14] as follows:

$$\mathrm{AOB}: NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2\,H^+ \quad (1)$$

$$\mathrm{NOB}: NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \quad (2)$$

$$\text{Overall nitrification}: NH_4^+ + 2\,O_2 \rightarrow NO_3^- + H_2O + 2\,H^+ \quad (3)$$

Nitrosomonas spp., *Nitrosospira* spp., and *Nitrosococcus* spp. are abundant in AOB communities of activated sludge [15–17]; *Nitrospira*-like bacteria, rather than *Nitrobacter* spp., are the dominant nitrite oxidizers in WWTPs and natural ecosystems [14,18]. The high sensitivity of AOB (e.g., *Nitrosomonas europaea*) to AgNPs has been reported [8,12]. The literature has not addressed, however, the relative sensitivity of AOB versus NOB to various forms of silver. Differentially inhibiting the activity of these bacteria may have environmental relevance such as changes in accumulation of toxic NO_2^-, an intermediary compound of nitrification and denitrification, and release of N_2O, a greenhouse gas. Nitrite accumulation is an important water quality concern; high nitrite levels are harmful to aquatic life and to humans (methemoglobinemia) [19–21] and can be inhibitory to bacteria at concentrations exceeding 200 mg/L [22].

Molecular techniques, such as polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) have been employed widely to quantify nitrifying bacteria populations and fingerprint nitrifier communities, respectively [23,24]. In the present study, we used these techniques to assess the impacts of silver on both AOB and NOB in the activated sludge community by comparing the effects of 2 different sizes of

All Supplemental Data may be found in the online version of this article.
* Address correspondence to alvarez@rice.edu.
Published online 2 July 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2678

AgNPs (5 nm and 35 nm) and Ag^+ ions after different exposure times. We quantified the abundance of AOB and NOB in the activated sludge by qPCR, performed DGGE to investigate changes in the AOB community structure, and quantified the effect on ammonia oxidation activity using a ^{14}C-labeled bicarbonate assay.

MATERIALS AND METHODS

Microcosms set-up

Activated sludge samples with a mixed liquor suspended solids at concentrations ranging from 1000 mg/L to 2000 mg/L were collected from the 69th Street WWTP in Houston, Texas. The ALS Laboratory Group characterized their chemical and physical properties (e.g., dissolved organic matter, pH, NO_3^-, NH_4^+, Cl^-, SO_4^{2-}, and PO_4^{3-}concentrations) (Supplemental Data, Table S1). In the microcosm tests, these activated sludge samples (100 mL) were exposed to 5-nm AgNPs (0.05 ppm), 35-nm AgNPs (40 ppm), or Ag^+ (1 ppm, added as $AgNO_3$) at 26 °C for 7 d. The controls without the addition of silver were treated similarly. These different silver concentrations were selected based on Yang et al. [13] to meet 2 criteria: 1) that the values be representative of concentrations measured at WWTPs [7,8], and 2) that different AgNPs be compared at equivalent concentrations, based on exerting equivalent effects; in this case, 24% to 28% reduction in activated sludge oxygen consumption, which was assessed by respirometry (Supplemental Data, Table S2). The exposed samples and controls were prepared in triplicate.

Quantifying AOB and NOB by qPCR

A 1 milliliter aliquot of an activated sludge sample was collected from each control and silver-treated microcosm at day 2, day 5 and day 7 of exposure. The activated sludge was centrifuged for 10 min at 5000 *g*, and DNA was extracted using the PowerSoil DNA isolation kit (Mobio). Quantitative PCR (qPCR) was performed using a 7500 real-time PCR system from Applied Biosystems in a 15-μL reaction mixture containing 10 ng DNA, SYBR Green Master Mix (7.5 μL), 0.3 μM of each primer, and water. The following primer sets were used: 1) for AOB, degenerate primer, *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′) and *amoA*-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′), targeting the genes encoding the ammonia monooxygenase subunit A (*amoA*) [23]; 2) for *Nitrobacteria* spp., FGPS872 (5′-CTAAAA CTCAAAG-GAATTGA-3′) and FGPS1269 (5′-TTTTTTGAGATTTGC-TAG-3′), targeting their 16S rRNA genes [25]; and 3) for *Nitrospira* spp., NSR1113F (5′-CCTGCTTTCAGTTGC-TACCG-3′) and NSR1264R (5′-GTTTGCAGCGCTTTG-TACCG-3′) [14]. Genomic DNA of *N. europaea* (ATCC 19718), extracted with the same DNA isolation kit for the activated sludge, was used as the standard for quantifying the abundance of AOB. Genomic DNA of *Nitrobacter vulgaris* (DSM 10236) was purchased directly from DSMZ, and pUC57 plasmid containing *Nitrospira* 16S DNA was synthesized by GenScript USA. The PCR reaction included an initial 10 min 95 °C denaturation, followed by 40 cycles of 1 min at 95 °C; 1 min at the respective annealing temperatures (54 °C for primer set amoA-1F and amoA-2R, 50 °C for FGPS872 and FGPS1269, and 65 °C for NSR1113F and NSR1264R); and 1 min at 60 °C.

PCR-DGGE

For our DGGE experiment, the gene *amoA* was selected as the molecular marker to investigate the dynamics and diversity of AOB in the activated sludge during silver exposure. The degenerate primers *amoA*-1F and *amoA*-2R were used to amplify *amoA* genes in the control and the silver-treated samples collected at day 2 day 5, and day 7 of exposure. The amplicons of *amoA* genes were then mixed with an equal volume of 2× loading buffer and loaded onto an 8% polyacrylamide gel with a denaturing gradient from 45% to 65% (where 100% denaturant corresponds to 7 M urea and 40% formamide) [23]. Gradient Maker (GM-100; CBS Scientific) helped ensure a reproducible, uniform denaturing gradient for gel casting. Electrophoresis was performed at 60 °C for 16 h at a constant voltage of 100 V using DCode Universal Mutation Detection System (BioRad). After electrophoresis, the acrylamide gel was stained with 1× SYBR Gold DNA gel stain (Invitrogen) for 0.5 h before ultraviolet imaging visualization. The optical density profiles of all gel lanes were created by 1D-Gels Tool Palette in AlphaView SA Ver 3.4.0 (ProteinSimple). Each band was excised and soaked in 200 μL DNase-free water for 2 h at room temperature [26]. The water was then removed by pipetting. A total of 30 μL DNase-free water was added to each sample, which were stored at 4 °C overnight. Polymerase chain reaction was repeated on each sample using 1 μL of the soaking water as the DNA template and *amoA*-1F and *amoA*-2R as paired primers. Polymerase chain reaction products were purified and concentrated with the QIAquick PCR Purification Kit (Qiagen) and then sequenced by Lone Star Labs. After combining the sequence reading from either side, the trimmed sequences (~490 bp in length) were blasted in the National Center for Biotechnology Information database to identify the species-specific *amoA* gene extracted from a band.

^{14}C-labeling assay of nitrification activity

To assess nitrification activity, ammonium ^{14}C-bicarbonate (2 μCi) was added to a 10 mL activated sludge sample [27,28], removed from control or silver-treated microcosms at day 0, day 1, day 3, day 5, and day 7 of exposure. Each sample was divided equally into 2 parts: 1 part containing 5 ppm of N-serve in an alcoholic solution and the other part containing the same volume of pure ethanol. After 2 h incubation at 26 °C in the dark, each sample was filtered through a nitrocellulose membrane (pore size 0.22 μm) from Millipore, and the filters were washed with 1 M hydrochloric acid (HCl) and then dried. The total radioactivity of the activated sludge was determined by combustion in a biological oxidizer (OX-600, R.J. Harvey Instrument), followed by measuring the trapped $^{14}CO_2$ in 10 mL of ^{14}C cocktail solution by liquid scintillation counting (LS 6500, Beckman) [29].

Quantifying Ag^+ release from AgNPs

After 7 d of exposure to 5-nm AgNPs, 35-nm AgNPs, or Ag^+, 1 mL of activated sludge sample was ultra-centrifuged (149 000 *g* for 3.5 h) [30,31]. The supernatant was then diluted to fit in the appropriate measuring range with 1% HNO_3. The dissolved Ag^+ concentrations were determined by inductively coupled plasma-mass spectrometry using an Elan 9000 instrument (Perkin-Elmer). For the total silver concentrations, 1 mL of the activated sludge sample was digested with 67% to 70% trace-metal grade nitric acid at 70 °C overnight and was then filtered through a 0.2 um sterile syringe filter to remove the impurities [32]. The collected solution was diluted with H_2O and then analyzed by inductively coupled plasma-mass spectrometry. The control was treated in the same way, with the control and each treatment prepared in triplicate.

Statistical analysis

The data are presented as mean ± standard error of the mean. The statistical analysis was applied to compare the significant difference between the treatments and the control. The student's *t*-test was performed when necessary, and the significance level in all calculations was set as $p < 0.05$.

RESULTS AND DISCUSSION

Exposure to AgNPs decreased AOB abundance

The higher sensitivity to AgNPs of AOB compared with NOB indicates that ammonia oxidation is a more vulnerable step during the nitrification process (Figure 1). For AOB, exposure to 35-nm AgNPs (40 ppm) caused a decrease in their abundance, whereas exposure to Ag^+ ions (1 ppm) or 5-nm AgNPs (0.05 ppm) did not have any such effect (Figure 1a), possibly due to the lower concentrations of Ag^+ and 5-nm AgNPs. Compared with the control, the 5-d and 7-d exposure to 35-nm AgNPs significantly reduced the abundance of AOB to 24% and 19%, respectively.

We did not detect Ag^+ in any of these microcosms (Supplemental Data, Table S3). Although Ag^+ is the critical determinant for the antibacterial activity of AgNPs [33], AgNPs may more strongly inhibit nitrifying bacteria than Ag^+ [9,34]. This is apparently due to the interaction between Ag^+ ions and inorganic ligands (e.g., Cl^- and S^{2-}) or organic matter, which reduces the bioavailability of Ag^+ to a greater extent than that of AgNPs, as well as mitigates toxicity [35]. This would explain the absence of free Ag^+ ions and the lack of significant effects in Ag^+-amended microcosms [13].

Nitrite-oxidizing bacteria were relatively resistant to AgNPs and Ag^+ during the 7-d exposure. In our activated sludge samples, *Nitrospira*, rather than *Nitrobacter*, were the dominant NOB species; this is consistent with other studies [15]. Furthermore, NOB were 1000 times less abundant than AOB (Figure 1b and 1c). Nitrite levels in the control and the silver-treated samples remained constant during the 7-d exposure (Supplemental Data, Figure S1). Owing to the greater tolerance of NOB than AOB to AgNPs, we focused our subsequent analyses (DGGE and ^{14}C-labeling assay) on the more vulnerable AOB in the activated sludge. The lower sensitivity of NOB to AgNPs suggests that the accumulation of toxic nitrite in WWTPs is unlikely (i.e., production would be hindered to a greater extent than consumption) after incidental or accidental silver release.

Another nitrification byproduct of potential concern is N_2O, a greenhouse gas [36]. However, N_2O was not detected in any of the microcosms (Supplemental Data, Table S4), probably due to the relatively low abundance of AOB in this activated sludge microbial community [13]. Nevertheless, AgNPs (14 ppm) have been reported to enhance N_2O flux from soil (by 4.5-fold) [37], which underscores the need for further research on how AgNPs entering biological wastewater treatment systems affect such emissions.

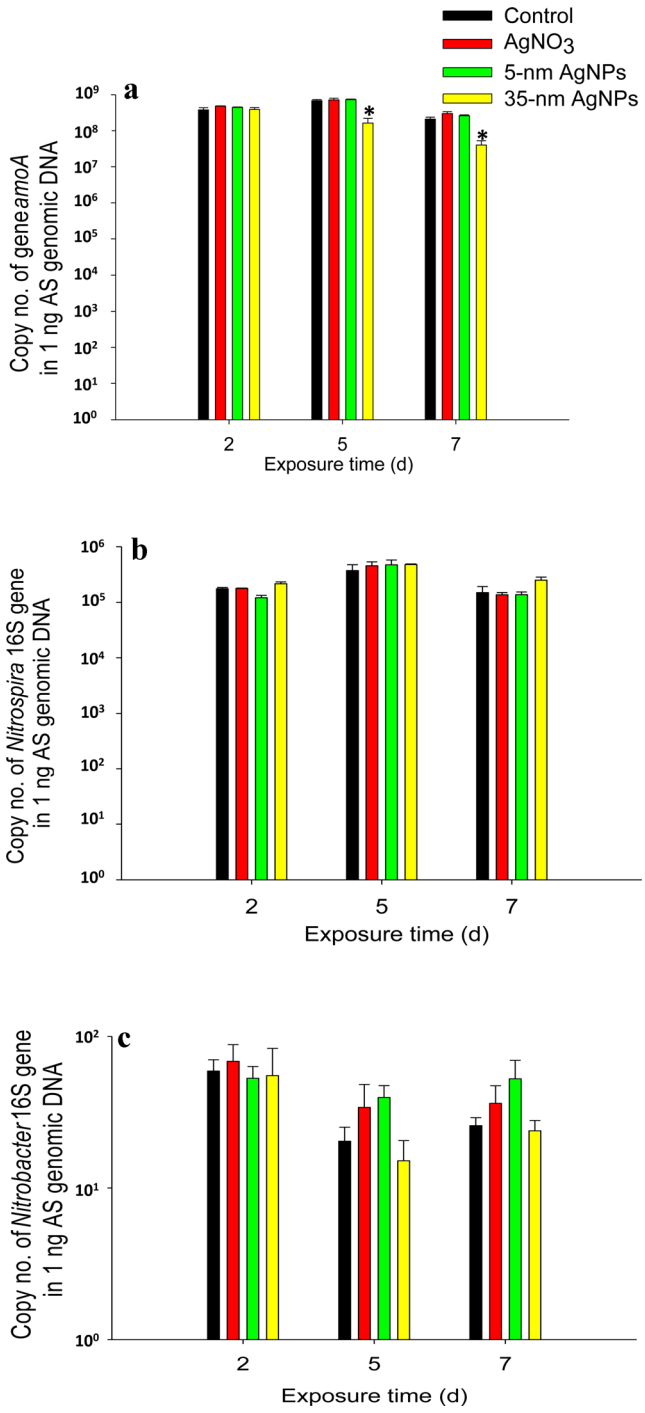

Figure 1. Abundance of ammonia-oxidizing bacteria (AOB; **a**) and nitrite-oxidizing bacteria (NOB; **b** and **c**) in activated sludge (AS) samples during the 7-d exposure to Ag^+ (1 ppm), 5-nm AgNPs (0.05 ppm), and 35-nm AgNPs (40 ppm). [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

Decrease of AOB diversity by AgNPs

The DGGE results confirmed that exposure of activated sludge to 35-nm AgNPs led to a decrease in both the abundance and diversity of AOB communities. As Figure 2 illustrates, 2 (bands 4 and 6) of the 6 bands faded after the 5-d exposure, indicating bacteria harboring these *amoA*-like genes (i.e., band 1 and 2) disappeared below detection levels following exposure to 35-nm AgNPs. Moreover, only band 5 prevailed over the 7-d incubation, implying decreased diversity of the AOB community. This also suggests that AOB species containing this *amoA*-like gene (i.e., band 5) are relatively tolerant to the toxic effects generated by 35-nm AgNPs.

Similarly, exposing the activated sludge to 5-nm AgNPs also caused a decrease in the diversity of the AOB community, even though it did not decrease AOB abundance (Figure 1). Before day 5, 5-nm AgNPs had no significant effect on the AOB community. By day 7, however, we observed that band 1 vanished and band 2 diminished (Supplemental Data, Table S5).

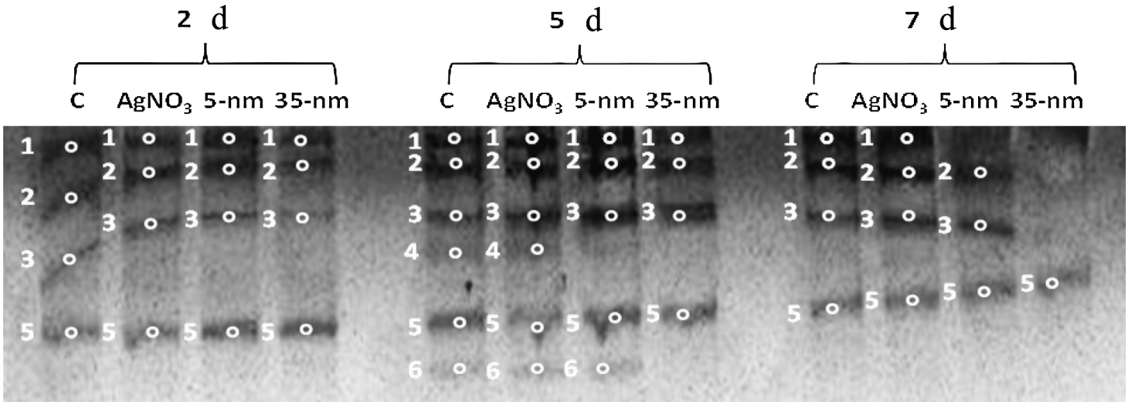

Figure 2. Denaturing gradient gel electrophoresis (DGGE) gel photograph showing the structure shift of ammonia-oxidizing bacteria (AOB) during silver exposure. The bands in each lane, marked by numbers, represent *amoA* amplified products, and their positions are indicated by white circles. The peak height of each band is given in Supplemental Data, Table S5.

A shift in the AOB community structure in activated sludge exposed to other heavy metals (e.g., Ni) has also been reported [38]. Long-term continuous loading of AgNPs at a sublethal level (0.1 ppm), however, was reported to have a negligible effect on the nitrifier community structure and their abundance [15]. For the Ag^+ treatment, the diversity of the AOB community was similar to that detected in the control at each exposure time, consistent with our previous findings identified by pyrosequencing analysis [13].

The nucleic acid sequences recovered from the bands are highly similar to the *amoA* genes from *Nitrosomonas* (Figure 3), demonstrating they are the dominant AOB species in the present study's AS samples. The prevalence of *Nitrosomonas* in ammonia oxidizing bacterial communities has been observed widely in different WWTPs, and *Nitrosomonas* spp. seem more likely to flourish in aerobic units in WWTPs [39–41]. The biased preference of primer *amoA*-1F and *amoA*-2R for *Nitrosomonas* spp. was excluded, because they have been used successfully to detect other AOB members (e.g., *Nitrosospira* and *Nitrosolobus*) in a variety of prior studies [23,42,43]. Band 3 was annotated as ammonia monooxygenase in *Nitrosomonas* sp. Nm47 (96% sequence identity) and band 6 showed high similarity (95%) to ammonia monooxygenase in *Nitrosomonas oligotropha*. All the other bands were affiliated within *Nitrosomonas*, as an unknown AOB, and band 5 could be assigned to some silver-tolerant *Nitrosomonas* based on their prevalence in all the samples. The higher tolerance of these AOB reflects the broad diversity of bacterial responses to environmental stresses and precludes generalizations about the potential impacts of AgNPs on a given phenotype (e.g., nitrifiers).

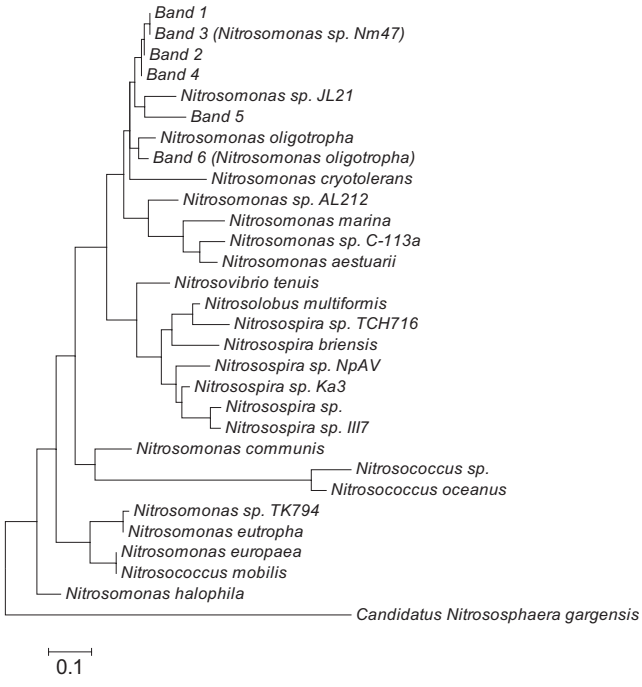

Figure 3. *amoA*-based phylogenetic tree showing *Nitrosomonas* spp. as a dominant bacterial species in the activated sludge (AS). The *amoA* sequences of the known ammonia-oxidizing bacteria (AOB) were obtained from the National Center for Biotechnology Information database, and the tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA), Ver 5.2.

Acute inhibitory effect of AgNPs on ammonia oxidation

A significant inhibitory effect on ammonia oxidation was observed mainly in the 35-nm AgNP exposed microcosms, as measured by the oxidation of assimilatory ammonium ^{14}C-bicarbonate into $^{14}CO_2$. This inhibition confirms that our data show a decrease in the abundance of the AOB community following 35-nm AgNP treatment as quantified by qPCR. The adverse effect started after the first day of exposure and increased

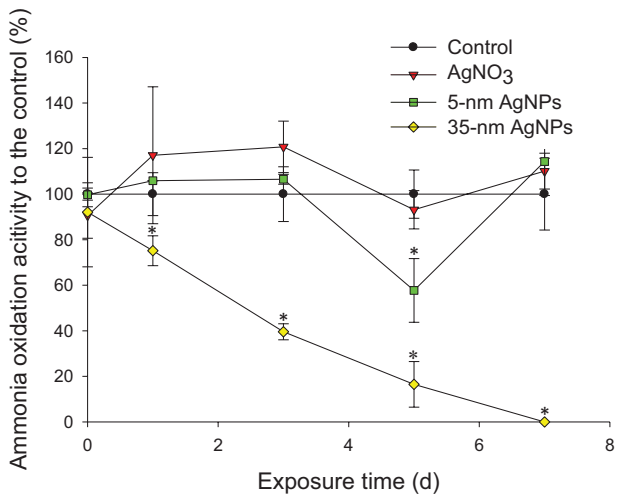

Figure 4. Effect of silver treatment on ammonia oxidation activity of activated sludge (AS). Asterisks (*) indicate significant induction compared to unexposed controls ($p < 0.05$). [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

as the exposure continued (Figure 4). A 5-d exposure to 5-nm AgNPs also significantly inhibited nitrification activity of AS, but this negative impact was alleviated by day 7. Other groups have reported inhibition of nitrification when the AS was exposed to other heavy metals (e.g., Ni, Cu, and Zn) [38,44].

IMPLICATIONS AND CONCLUSIONS

Silver nanoparticles could disrupt ammonia oxidation in WWTPs, which serve as common sinks for AgNPs released from commercial manufactured nanomaterials, and upset nitrogen removal and treatment efficiency. In agricultural soils amended with associated biosolids, silver could hinder beneficial microbial-plant interactions by decreasing both the abundance and the diversity of AOB communities. The generally higher resistance of NOB to AgNPs, however, could reduce the potential accumulation of toxic nitrite. Note that the environmental impacts of AgNPs appear to be concentration-dependent, with both sequestration of AgNPs by organic matter or inorganic ligands and development of microbial heavy metal resistance mitigating their potential effect. Overall, the potential impacts of AgNPs on the nitrification process in water and soil ecosystems underscore the importance of minimizing and intercepting incidental and accidental releases of AgNPs into the environment.

SUPPLEMENTAL DATA

Tables S1–S5.
Figure S1. (43 KB DOC).

Acknowledgment—The authors thank H. Kaplan and F. Andrade at the University of Texas–Houston Medical School for their help with denaturing gradient gel electrophoresis. The present research was supported by a Joint US–UK research program (USEPA and UK-NERC-ESPRC) (EPA-G2008-STAR-R1).

Disclaimer—The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. The Project on Emerging Nanotechnologies. [cited 2014 January 14]. Available from: http://www.nanotechproject.org/.
2. Ip M, Lui SL, Poon VKM, Lung I, Burd A. 2006. Antimicrobial activities of silver dressings: An *in vitro* comparison. *J Med Microbiol* 55:59–63.
3. Klasen HJ. 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* 26:131–138.
4. Silver S. 2003. Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 27:341–353.
5. Gottschalk F, Sonderer T, Scholz RW, Nowack B. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. *Environ Sci Technol* 43:9216–9222.
6. Kaegi R, Voegelin A, Sinnet B, Zuleeg S, Hagendorfer H, Burkhardt M, Siegrist H. 2011. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ Sci Technol* 45:3902–3908.
7. Shafer MM, Overdier JT, Armstong DE. 1998. Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent-receiving streams. *Environ Toxicol Chem* 17:630–641.
8. Radniecki TS, Stankus DP, Neigh A, Nason JA, Semprini L. 2011. Influence of liberated silver from silver nanoparticles on nitrification inhibition of *Nitrosomonas europaea*. *Chemosphere* 85:43–49.
9. Liang ZH, Das A, Hu ZQ. 2010. Bacterial response to a shock load of nanosilver in an activated sludge treatment system. *Water Res* 44:5432–5438.
10. Choi OK, Hu ZQ. 2009. Nitrification inhibition by silver nanoparticles. *Water Sci Technol* 59:1699–1702.
11. Arnaout CL, Gunsch CK. 2012. Impacts of silver nanoparticle coating on the nitrification potential of *Nitrosomonas europaea*. *Environ Sci Technol* 46:5387–5395.
12. Yang Y, Wang J, Xiu Z, Alvarez PJ. 2013. Impacts of silver nanoparticles on cellular and transcriptional activity of nitrogen-cycling bacteria. *Environ Toxicol Chem* 32:1488–1494.
13. Yang Y, Quensen J, Mathieu J, Wang Q, Wang J, Li MY, Tiedje JM, Alvarez PJJ. 2014. Pyrosequencing reveals higher impact of silver nanoparticles than Ag^+ on the microbial community structure of activated sludge. *Water Res* 48:317–325.
14. Dionisi HM, Layton AC, Harms G, Gregory IR, Robinson KG, Sayler GS. 2002. Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR. *Appl Environ Microbiol* 68:245–253.
15. Zhang CQ, Liang ZH, Hu ZQ. 2014. Bacterial response to a continuous long-term exposure of silver nanoparticles at sub-ppm silver concentrations in a membrane bioreactor activated sludge system. *Water Res* 50:350–358.
16. Yu K, Zhang T. 2012. Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. *PLoS ONE* 7:e38183.
17. Yu L, Peng D, Pan R. 2012. Shifts in nitrification kinetics and microbial community during bioaugmentation of activated sludge with nitrifiers enriched on sludge reject water. *J Biomed Biotechnol* 2012:691894.
18. Daims H, Nielsen JL, Nielsen PH, Schleifer KH, Wagner M. 2001. In situ characterization of *Nitrospira*-like nitrite oxidizing bacteria active in wastewater treatment plants. *Appl Environ Microbiol* 67:5273–5284.
19. Philips S, Laanbroek HJ, Verstraete W. 2002. Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Re/Views in Environmental Science and Bio/Technology* 1:115–141.
20. Hannas BR, Das PC, Li H, LeBlanc GA. 2010. Intracellular conversion of environmental nitrate and nitrite to nitric oxide with resulting developmental toxicity to the Crustacean *Daphnia magna*. *PLoS ONE* 5: e12453.
21. Phipps RH, Cornfort IS. 1970. Factors effecting toxicity of nitrite nitrogen to tomatoes. *Plant Soil* 33:457–466.
22. Bollag JM, Henninger NM. 1978. Effects of nitrite toxicity on soil bacteria under aerobic and anaerobic conditions. *Soil Biol Biochem* 10:377–381.
23. Shimomura Y, Morimoto S, Hoshino YT, Uchida Y, Akiyama H, Hayatsu M. 2012. Comparison among *amoA* primers suited for quantification and diversity analyses of ammonia-oxidizing bacteria in soil. *Microbes Environ* 27:94–98.
24. Bellucci M, Curtis TP. 2011. Ammonia-oxidizing bacteria in wastewater. *Methods Enzymol* 496:269–286.
25. Degrange V, Bardin R. 1995. Detection and counting of nitrobacter populations in soil by PCR. *Appl Environ Microbiol* 61:2093–2098.
26. Li M, Mathieu J, Yang Y, Fiorenza S, Deng Y, He Z, Zhou J, Alvarez PJ. 2013. Widespread distribution of soluble di-iron monooxygenase (SDIMO) genes in arctic groundwater impacted by 1,4-dioxane. *Environ Sci Technol* 47:9950–9958.
27. Billen G. 1976. Evaluation of nitrifying activity in sediments by dark C-14 bicarbonate incorporation. *Water Res* 10:51–57.
28. Somville M. 1978. A method for the measurement of nitrification rates in water. *Water Res* 12:843–848.
29. Li D, Fortner JD, Johnson DR, Chen C, Li Q, Alvarez PJ. 2010. Bioaccumulation of $^{14}C_{60}$ by the earthworm *Eisenia fetida*. *Environ Sci Technol* 44:9170–9175.
30. Yu WW, Chang E, Falkner JC, Zhang JY, Al-Somali AM, Sayes CM, Johns J, Drezek R, Colvin VL. 2007. Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. *J Am Chem Soc* 129:2871–2879.
31. Yang Y, Zhu HG, Colvin VL, Alvarez PJ. 2011. Cellular and transcriptional response of *Pseudomonas stutzeri* to quantum dots under aerobic and denitrifying conditions. *Environ Sci Technol* 45:4988–4994.
32. Wang J, Koo Y, Alexander A, Yang Y, Westerhof S, Zhang Q, Schnoor JL, Colvin VL, Braam J, Alvarez PJ. 2013. Phytostimulation of poplars and Arabidopsis exposed to silver nanoparticles and Ag^+ at sublethal concentrations. *Environ Sci Technol* 47:5442–5449.
33. Li XH, Wei YD, Wang SM, Wang MN, Huang DW, Xiu ZL. 2012. Synthesis, biological evaluation and molecular modeling of cyclic tetrapeptide based inhibitors HDAC. *Chem Res Chinese U* 28:1011–1016.
34. Choi O, Deng KK, Kim NJ, Ross L, Surampalli RY, Hu ZQ. 2008. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res* 42:3066–3074.
35. Xiu ZM, Ma J, Alvarez PJJ. 2011. Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. *Environ Sci Technol* 45:9003–9008.

36. Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van Loosdrecht MCM. 2009. Nitrous oxide emission during wastewater treatment. *Water Res* 43:4093–4103.
37. Colman BP, Arnaout CL, Anciaux S, Gunsch CK, Hochella MF, Kim B, Lowry GV, McGill BM, Reinsch BC, Richardson CJ, Unrine JM, Wright JP, Yin LY, Bernhardt ES. 2013. Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario. *PLoS ONE* 8:e57189.
38. Yeung CH, Francis CA, Criddle CS. 2013. Adaptation of nitrifying microbial biomass to nickel in batch incubations. *Appl Microbiol Biotechnol* 97:847–857.
39. Limpiyakorn T, Sonthiphand P, Rongsayamanont C, Polprasert C. 2011. Abundance of *amoA* genes of ammonia-oxidizing archaea and bacteria in activated sludge of full-scale wastewater treatment plants. *Bioresour Technol* 102:3694–3701.
40. Gao JF, Luo X, Wu GX, Li T, Peng YZ. 2013. Quantitative analyses of the composition and abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in eight full-scale biological wastewater treatment plants. *Bioresour Technol* 138:285–296.
41. Wang XH, Wen XH, Xia Y, Hu M, Zhao F, Ding K. 2012. Ammonia oxidizing bacteria community dynamics in a pilot-scale wastewater treatment plant. *PLoS ONE* 7:e36272.
42. Onodera Y, Nakagawa T, Takahashi R, Tokuyama T. 2010. Seasonal change in vertical distribution of ammonia-oxidizing archaea and bacteria and their nitrification in temperate forest soil. *Microbes Environ* 25:28–35.
43. Oved T, Shaviv A, Goldrath T, Mandelbaum RT, Minz D. 2001. Influence of effluent irrigation on community composition and function of ammonia-oxidizing bacteria in soil. *Appl Environ Microbiol* 67:3426–3433.
44. Hu ZQ, Chandran K, Grasso D, Smets BF. 2003. Impact of metal sorption and internalization on nitrification inhibition. *Environ Sci Technol* 37:728–734.