

# Antimicrobial effects of commercial silver nanoparticles are attenuated in natural streamwater and sediment

Benjamin P. Colman · Si-Yi Wang ·  
Melanie Auffan · Mark R. Wiesner ·  
Emily S. Bernhardt

Accepted: 21 April 2012 / Published online: 9 May 2012  
© Springer Science+Business Media, LLC 2012

**Abstract** Given the demonstrated antimicrobial properties of silver nanoparticles (AgNPs), and the key role that microorganisms play in performing critical ecosystem functions such as decomposition and nutrient cycling, there is growing concern that AgNP pollution may negatively impact ecosystems. We examined the response of streamwater and sediment microorganisms to commercially available  $21 \pm 17$  nm AgNPs, and compared AgNP impacts to those of dissolved-Ag added as AgNO<sub>3</sub>. We show that in streamwater, AgNPs and AgNO<sub>3</sub> decreased respiration in proportion to dissolved-Ag concentrations at the end of the incubation ( $r^2 = 0.78$ ), while in sediment the only measurable effect of AgNPs was a 14 % decrease in sulfate concentration. This contrasts with the stronger effects of dissolved-Ag additions in both streamwater and sediment. In streamwater, addition of dissolved-Ag at a level equivalent to the lowest AgNP dose led to respiration below detection, a 55 % drop in phosphatase enzyme activity, and a 10-fold increase in phosphate concentration.

In sediment, AgNO<sub>3</sub> addition at a level equivalent to the highest AgNP addition led to a 34 % decrease in respiration, a 55 % increase in microbial biomass, and a shift in bacterial community composition. The results of this study suggest that, in similar freshwater environments, the short-term biological impacts of AgNPs on microbes are attenuated by the physical and chemical properties of streamwater and sediment.

**Keywords** Silver nanoparticles · Microbial biomass · Microbial respiration · Enzyme activity · Environment

## Introduction

The use of silver nanoparticles (AgNPs) in consumer products is growing rapidly, as AgNPs are incorporated into clothing, containers, and dietary supplements (Project on Emerging Nanotechnologies 2010) for their antimicrobial properties (Kim et al. 2007; Lok et al. 2007; Pal et al. 2007). However, these same antimicrobial properties make AgNPs of particular concern as they enter natural and managed environments (Benn and Westerhoff 2008) as many ecosystem functions (e.g., decomposition, nutrient cycling and removal) are driven by microbial activity. In laboratory studies with plants and microbes, AgNPs have been shown to have the potential to be more toxic than equivalent concentrations of dissolved-Ag (Yin et al. 2011; Choi and Hu 2009). However, while release of dissolved-Ag into the environment is regulated, clear guidelines do not exist for AgNPs (Corley et al. 2009).

The current understanding of AgNP impacts on environmental microorganisms is largely limited to extrapolation from laboratory studies of single microbial strains grown in highly controlled culture media. In these

---

B. P. Colman (✉) · S.-Y. Wang · E. S. Bernhardt  
Department of Biology, Duke University, Durham, NC, USA  
e-mail: colmanb@gmail.com

B. P. Colman · M. Auffan · M. R. Wiesner · E. S. Bernhardt  
Center for the Environmental Implications of Nanotechnology,  
Duke University, Durham, NC, USA

M. Auffan  
International Consortium for the Environmental Implications  
of NanoTechnology (iCEINT), CEREGE UMR 7330-CNRS/  
Aix-Marseille Université, Europôle de L'Arbois,  
13545 Aix-en-Provence, France

M. Auffan · M. R. Wiesner  
Civil and Environmental Engineering Department,  
Duke University, Durham, NC, USA

experiments, highly-reactive freshly-synthesized particles are added in nutrient rich culture media at concentrations typically at or below  $100 \text{ mg L}^{-1}$  with acutely toxic effects (Yoon et al. 2007; Morones et al. 2005; Amin et al. 2009; Pal et al. 2007; Sondi and Salopek-Sondi 2004). Extrapolating from pure culture experiments to environmental microbial communities is difficult in part due to the major physicochemical differences in the media. Additionally, instead of single-strain planktonic cultures, environmental microbes are found in highly diverse communities (Girvan et al. 2005) mostly attached to surfaces in biofilms—matrices of polysaccharides, proteins, and DNA—which protect from antimicrobial solutes and particles (Costerton 2007).

However, pure culture studies can give us insight into both the potential mechanisms of AgNP toxicity toward environmental microbes, and environmental factors likely to regulate toxicity. Though there is no clear consensus on the exact mechanism of AgNP antimicrobial activity, several modes of action have been identified. Some studies suggest that  $\text{Ag}^+$  may be the primary form of Ag directly causing toxicity (Lok et al. 2006; Kim et al. 2007). While it is  $\text{Ag}^+$  that is chemisorbed on the surface of AgNPs, and  $\text{Ag}^+$  is released through oxidation and dissolution of AgNPs (Lok et al. 2007), given the many potential dissolved forms of  $\text{Ag}^+$  in systems other than deionized water, we will largely refer to  $\text{Ag}^+$  and its dissolved derivatives as dissolved-Ag.

Alternatively, AgNPs may have unique modes of toxicity caused by direct physical membrane disruption (Kim et al. 2007; Lok et al. 2006; Aruguete and Hochella 2010), stimulation of ROS production (Choi and Hu 2008), or by potentially serving as localized sources of elevated dissolved-Ag when particles are in close physical proximity to a biotic receptor (Navarro et al. 2008). Since changes in dissolution of AgNPs due to AgNP coating (Smetana et al. 2008), dissolved oxygen concentration, ionic strength (Liu and Hurt 2010; Kent and Vikesland 2011), and nanoparticle autoaggregation (Lok et al. 2007; Liu and Hurt 2010) will impact both dissolved-Ag and AgNP concentration, these processes are likely strong drivers of AgNP toxicity.

Beyond dissolution and autoaggregation, we anticipate that streamwater and sediments are likely to alter toxicity by promoting surface complexation (i.e., ligand binding leading to decreased dissolution and reactivity) by sulfides (Choi et al. 2009; Kim et al. 2010), thiols (Smetana et al. 2008; Miao et al. 2009), and other ligands. Further, we expect heteroaggregation and adsorption of organic matter on NPs (Fabrega et al. 2009; Elzey and Grassian 2010) will control the transport and fate of NPs in the environment in sediments and soils, and ultimately influence AgNP toxicity to microorganisms. Thus we would expect the extent of aggregation, dissolution, and physical separation

between microbes and AgNPs would be important determinants of their toxicity to environmental microbes (Aruguete and Hochella 2010; Lok et al. 2006; Kim et al. 2007). The interplay of these many factors then determines the probability that microbes will encounter AgNPs or dissolved-Ag, as well as the likelihood that such an encounter would prove toxic. This may in part explain the minimal impacts of AgNP exposures on microbial community composition in estuarine sediments (Bradford et al. 2009), or activity in lake sediments (Gao et al. 2011).

In this study, we considered how AgNP toxicity may be modified by two complex media—streamwater and sediments—and addressed the following questions:

Do AgNPs added to streamwater change microbial activity?

Do AgNPs added to stream sediments change the activity, abundance, or community composition of microbes?

How do the effects of AgNPs on stream microbes differ from the effects of dissolved-Ag?

Are the AgNPs physicochemically destabilized in environmental media?

We examined the responses of intact microbial communities obtained from a local stream following exposure to a commercially relevant AgNP formulation, measuring microbial biomass, microbial activity (enzyme activity, nutrient concentrations), and composition using molecular fingerprinting of the bacterial component of the microbial community.

## Methods

### Environmental media and microbial community collection

Sediment and streamwater were collected from a tributary to Mud Creek (Duke Forest, Durham, NC, USA), a small stream draining a fully forested watershed that is used as a reference site for ongoing ecological research on regional streams (Sudduth and Bernhardt 2011). Ten liters of streamwater were sampled from a calm pool into a low density polyethylene carboy (Cubatainer<sup>®</sup>, Hedwin Corporation, Baltimore, USA), and 4 L were filtered through Whatman GF/F filters (nominal pore size  $0.7 \mu\text{m}$ ) into a glass bottle to remove large colloids and algae. Streamwater had a pH of 8.1, and concentrations of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Cl}^-$  of  $8.5 \pm 1.4$ ,  $0.1 \pm 0.1$ ,  $26.3 \pm 3.2$ , and  $20.9 \pm 0.4 \text{ mg L}^{-1}$ . A total of 2 kg of sediment was collected from between 0 and 5 cm, and brought back to the lab on ice in a polyethylene bag. Sediment was then sieved to 2 mm to homogenize the sample and remove rocks, and

subsequently placed in Nytex netting to remove excess water for 1 h. Sediment was then stored in polyethylene bags at 4 °C prior to initiating experiments. Sediment had a pH of 7.0 (1:1 water:sediment), and loss on ignition of 15.4 %. The surface litter layer from the riparian area adjacent to the stream was collected for extraction of dissolved organic matter to serve as supplemental substrate for streamwater microbes.

#### Nanoparticle stock suspension characterization

The commercial AgNPs (NanoAmor, Los Alamos, New Mexico, USA) were purchased as a powder. These nanoparticles had a polyvinylpyrrolidone surface treatment added by the manufacturer to enhance their stability when placed into water, and were described as having a 10 nm size by the manufacturer. The suspensions were prepared by sonicating 257 mg/L of AgNPs in deionized water for 10 min using a Sonicator 4000 (Misonix, QSonica LLC, Newton, CT). AgNPs shape, size, and crystal structure were characterized by transmission electron microscopy (TEM; 160 kV; G2 Twin, FEI Tecnai, Hillsboro, OR, USA) and X-ray diffraction (XRD; PANalytical X'Pert PRO MRD HR diffractometer with a Cu K $\alpha$  radiation (1.5405 Å) at 45 kV and 40 mA). We confirmed that the AgNPs made of metallic silver (Ag<sup>0</sup>). No other crystalline phases were observed by XRD. These NPs have a mean TEM diameter of  $21 \pm 17$  nm ( $n > 100$ ) and a roughly spherical shape. In their stock suspensions, these AgNPs have negative surface charges (zeta potential values of  $-22 \pm 3$ ).

Before and after incubation with streamwater and sediment, the dissolution and aggregation states of the AgNPs were studied. The dissolved silver concentrations were measured by ICP-AES (inductively coupled plasma atomic emission spectroscopy) using a Jobin spectrometer. The solid and liquid phases were separated by ultracentrifugation at  $197,568 \times g$  for 2 h. The supernatants were removed and acidified immediately after preparation and stored at 4 °C prior ICP-AES analysis. Aggregation of AgNPs was quantified by following particle size distributions (mean hydrodynamic diameter) over time by dynamic light scattering (DLS) using a CGS 3 spectrometer (ALV-GmbH, Germany) equipped with a helium–neon laser (633.4 nm) and goniometer. Suspensions were analyzed at 25 °C with the photomultiplier set to a scattering angle of 90°.

#### Effects of AgNPs on streamwater microbes

A total of 30 mL of streamwater ( $8.5 \text{ mg C L}^{-1}$ ) was pipetted into each of forty 60 mL I-Chem (Thermo Fisher Scientific, Rockwood, Tennessee, USA) vials with gas tight lids fitted with rubber sampling septa. To each of

eight replicate vials, additions were made of 5 mL of leaf litter leachate ( $134 \text{ mg C L}^{-1}$ ) and then 15 mL of treatment solutions containing either AgNPs or AgNO<sub>3</sub> mixed with deionized (DI) water. This left 10 mL of headspace for gas measurements. Leachate was added to ensure adequate labile carbon supply to sustain stream microbial communities over the duration of our experiment, and was prepared by soaking 180 g leaf litter in 2 L DI water for 2 h, followed by filtration through a 0.22  $\mu\text{m}$  filter. Three concentrations of AgNPs were used (0.75, 7.5 and 75 mg Ag L<sup>-1</sup>) and these treatments were compared with negative controls that received only DI water and positive controls that received AgNO<sub>3</sub><sup>-</sup> at 0.75 mg Ag L<sup>-1</sup>. While the AgNO<sub>3</sub> treatment also increased NO<sub>3</sub><sup>-</sup>-N by  $96 \mu\text{g N L}^{-1}$ , it is unlikely that NO<sub>3</sub><sup>-</sup> would have changed microbial activity given that nitrate concentrations in the source stream vary from 0.5 to 300  $\mu\text{g N L}^{-1}$  over the course of 4 years of weekly sampling (Bernhardt, unpublished data). Concentrations of AgNO<sub>3</sub> higher than 0.75 mg Ag L<sup>-1</sup> were not used for the positive control as 0.75 mg Ag L<sup>-1</sup> was found to be sufficient to eliminate microbial respiration in previous experiments. Treatment solutions were combined with streamwater in one addition followed by vigorous shaking for 10 s. An initial headspace gas sample was then collected, and all vials were placed in the dark at room temperature on an orbital shaker at 100 rpm for 12 days. At the end of 12 days, each vial was destructively sampled for water chemistry and phosphatase enzyme activity.

Microbial respiration was measured in response to experimental treatments by comparing headspace CO<sub>2</sub> concentrations between the beginning and end of the incubation, with CO<sub>2</sub> measured on an infrared gas analyzer (Li6200; Li-Cor Environmental, Lincoln, USA). Microbial functional responses were assessed by measuring phosphatase enzyme activity using standard protocols (Saiya-Cork et al. 2002). Concentrations of NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and Cl<sup>-</sup> were measured on a Dionex ICS-2000 IC (Dionex, Sunnyvale, USA) and total ammonia and orthophosphate were measured on a Lachat QuikChem-8000 (Lachat Instruments, Loveland, USA). Total dissolved C and N were determined on a Shimadzu TOC/TN analyzer (TOC-V and TNM-1, Shimadzu Corporation, Tokyo, Japan). To determine dissolved-Ag concentrations, we took samples and centrifuged them for 1 h at 40,000 rpm ( $197,568 \times g$ ) in a L8-80 M Ultracentrifuge (Beckman, Brea, USA) to remove NPs, then analyzed acidified supernatants (5 % concentrated HNO<sub>3</sub>) on an inductively coupled plasma optical emission spectrometer (Prism ICP High Dispersion, Teledyne Leeman Labs, Hudson, USA).

To visualize the dense micron-scale particles observed in AgNP treatments, but absent in controls and the AgNO<sub>3</sub> treatment, fluorescence microscopy was used and particles

were identified as putative AgNP aggregates. Streamwater samples were imaged using a Zeiss Axio Observer wide-field fluorescence microscope using fluorescence (Carl Zeiss, Thornwood, USA). Fluorescence images were generated using the DNA intercalating fluorescent probes SYTO 9 and SYTOX Orange (Invitrogen, Carlsbad, USA), that fluoresce green ( $\lambda_{\text{ex}} = 485 \text{ nm}$ ,  $\lambda_{\text{em}} = 498 \text{ nm}$ ) and orange ( $\lambda_{\text{ex}} = 485 \text{ nm}$ ,  $\lambda_{\text{em}} = 498 \text{ nm}$ ) respectively. While these stains can be used to differentially stain microbes with intact and damaged membranes, we present images as black and white composites to better show the contrast between bacteria (bright spots), biofilm (gray areas surrounding bacteria), and particles (areas where fluorescence is blocked).

#### Effects of AgNPs on stream sediment microbes

Sediment (4 g dry weight equivalent) was weighed into each of 75 individual 60 mL I-Chem vials, to which 20 mL of treatment solution was subsequently added containing 0, 75, 125, or 250 mg Ag L<sup>-1</sup> as AgNPs (yielding concentrations of 0, 0.375, 0.625, and 1.25 mg Ag kg<sup>-1</sup> sediment), or 250 mg Ag L<sup>-1</sup> as AgNO<sub>3</sub>. All AgNP treatments were supplemented with KNO<sub>3</sub> to raise NO<sub>3</sub><sup>-</sup> concentration to 32 mg N L<sup>-1</sup>, equivalent to the concentrations in the AgNO<sub>3</sub> treatments, a level much higher than background extractable NO<sub>3</sub><sup>-</sup> in these same sediments (<0.2 mg N L<sup>-1</sup> based on KCl extractions, data not shown). Each treatment solution was added as a single injection to each of 15 replicate vials. Immediately following additions vials were mixed by vigorous shaking for 10 s. Headspace gas samples were collected for initial CO<sub>2</sub> measurements, and then vials were incubated horizontally on an orbital shaker at 100 rpm for 7 days in the dark at room temperature.

After 1, 3, and 7 days, five replicate samples were destructively sampled by first allowing samples to sit upright for 30 min, then taking 10 mL subsamples of the supernatant to filter and analyze for dissolved solutes. Two replicates per treatment and time point were frozen at -80 °C prior to enzyme and molecular analysis. Microbial biomass was measured on three replicates as described below. Before sampling supernatants and sediments on the seventh day, headspace gases were first collected for CO<sub>2</sub> analysis.

Aggregation of AgNPs in our sediment slurries was assessed through a separate set of experiments. While AgNP aggregates in streamwater were readily assessed by visual observation and DLS, aggregation in the presence of sediment was not easily observed given the high background of natural colloids from the sediment. Indirect evidence of aggregation was assessed by adding AgNP solutions to either 0.1–0.5 or 50 kDa MWCO dialysis tubes (Spectra/por Float-A-Lyzer G2 dialysis tubes;

Spectrum Labs, Rancho Dominguez, USA) which were then inserted into sediment slurries. Both types of dialysis tubing had MWCO sufficient to retain AgNPs and exclude sediments (controls with DI showed no particles inside the membrane at the end of the experiment), but allowed for differing sizes of dissolved organics and ions through to interact with the AgNPs. Prior to use, dialysis tubes were filled with and soaked in deionized water, with three changes of the water in and outside the tube. The equivalent of 4 g dry weight sediment was slurried in 22 mL of deionized water in polystyrene cylinders. Treatments all consisted of three replicates which had 9.5 mL of either AgNPs (250 ppm AgNPs) or DI water in dialysis membranes, which were then placed in the polystyrene cylinders. Experimental treatments were done in triplicate, and were incubated upright for 7 days with daily inversion to insure mixing inside and outside of the dialysis membranes. At the conclusion of the experiment, the suspension within the dialysis membrane was withdrawn using a disposable transfer pipette and transferred to polystyrene centrifuge tubes prior to particle size analysis.

#### Microbial respiration, biomass, and extracellular enzyme activity

To measure respiration for all experiments, headspace CO<sub>2</sub> concentration change was measured between the beginning and end of the incubation. Microbial biomass in sediment was determined by substrate induced respiration, which serves as an inexpensive, rapid, and sensitive index of the active microbial biomass, or biomass that can rapidly become active in the presence of abundant substrate (Fierer et al. 2003). The potential activities of three hydrolytic extracellular enzymes were measured: phosphatase (streamwater and sediment), sulfatase (sediment), and leucine aminopeptidase (sediment) using standard methods (Saiya-Cork et al. 2002).

#### Bacterial community composition

Isolation of DNA from sediment samples ( $n = 18$  total samples with 3 treatments, 3 time points, and 2 analytical replicates per treatment by time combination) was done using PowerSoil kits (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. Bacterial DNA amplification was done in triplicate using standard protocols and fluorescently labeled primers following extraction (Liu et al. 1997; Osborn et al. 2000). Resulting PCR products were composited (per treatment by time combinations), cleaned, and checked for appropriate size. Shifts in bacterial community composition were assessed using terminal restriction fragment length polymorphism (T-RFLP; Osborn et al. 2000), a culture-independent,

molecular fingerprinting method that involves cutting fluorescently labeled PCR products with a restriction enzyme to yield fragment profiles where each fragment length is associated with an operational taxonomic unit. The PCR product was digested with the restriction enzyme endonuclease *MspI* (New England Biolabs, Ipswich, USA). Digested fragments were then separated and analyzed by electrophoresis and fluorescence measurements using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, USA). Although T-RFLP data generally underestimate the richness of highly complex communities, the method has been accepted as an appropriate and efficient means of assessing overall dissimilarity in composition among bacterial communities (Osborn et al. 2000).

### Statistical analyses

All analysis of variance (ANOVA) tests were done using SigmaStat 4 (Systat Software Inc., San Jose, USA; Systat Software Inc. 2008) to test for overall differences among treatments. Holm-Sidak post hoc tests were used to isolate the differences among treatments while correcting for multiple comparisons.

To test for significant differences in microbial community composition among treatments, we used the vegan package (Oksanen et al. 2010) in R (version 2.11.1; R Development Core Team, Vienna, Austria, <http://www.R-project.org/>) to conduct analysis of variance using distance matrices (ADONIS), which is comparable to analysis of similarity (Anderson 2001; Oksanen 2010). We used Bray-Curtis distance measures and significance was calculated based on 1,000 null permutations of the data.

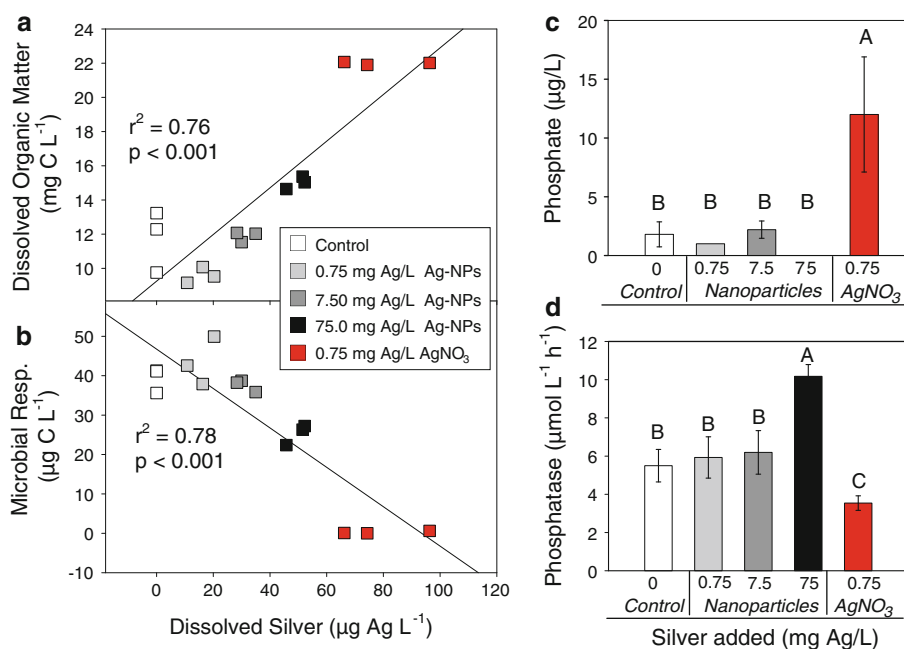
To examine differences in bacterial community composition among treatments, the ecodist package (Goslee and Urban 2007) in R was used to perform a non-metric multidimensional scaling (NMS) ordination with Bray-Curtis distance measures, a random starting configuration, and 200 runs with real data. NMS ordinations create a mapping of samples into a reduced ordination space that preserves the rank order of ecological distances among samples (i.e., presence-absence data from TRFLP profiles). NMS is unique in its lack of assumptions and its ability to present an unbiased representation of multivariate structure in reduced space. NMS ordination scores are calculated to map as directly as possible to ecological distances (McCune and Grace 2002).

## Results

### Commercial AgNPs' impacts on streamwater microbes

Addition of the commercial AgNPs slowed the decomposition of dissolved organic matter with increased dose (Fig. 1a), and produced a corresponding decrease in microbial respiration (Fig. 1b). At 75 mg Ag L<sup>-1</sup> respiration was 39 % lower than the unamended control, but was significantly higher than the positive (AgNO<sub>3</sub>) control. At 0.75 mg Ag L<sup>-1</sup>, AgNO<sub>3</sub> resulted in no detectable respiration. Concentrations of phosphate were uniformly low among the control and AgNPs treatments, averaging 1.25 µg L<sup>-1</sup>, but rose to 12 µg L<sup>-1</sup> in the AgNO<sub>3</sub> treatment (Fig. 1c). Phosphatase enzyme activity increased from an average of 5.5 to 10.2 µmol L<sup>-1</sup> h<sup>-1</sup>, from the control to the

**Fig. 1** Streamwater experiment results showing: **a** dissolved organic matter versus dissolved-Ag concentration in streamwater ( $n = 3$  per treatment), **b** cumulative respiration versus dissolved-Ag concentration ( $n = 3$  per treatment), **c** phosphate concentrations ( $n = 5$ ), and **d** phosphatase enzyme activity ( $n = 5$ ). Error bars are standard deviation. In bar graphs, capital letters denote statistically significant differences between treatments



75 mg Ag L<sup>-1</sup> treatment, respectively (Fig. 1d). It also showed a small but significant decline in the AgNO<sub>3</sub> treatment, decreasing to 3.5 μmol L<sup>-1</sup> h<sup>-1</sup>.

By the end of the experiment, aggregates of AgNPs could be observed visually in the AgNP treatments. Their hydrodynamic diameters were too large to be accurately measured by DLS. Microscopy revealed that these aggregates were colonized by microorganisms in biofilms (Fig. 2a), whereas in the control and AgNO<sub>3</sub> treatment there were no such solid and optically dense particles (Fig. 2b,c).

Over the course of the experiment dissolved-Ag concentrations increased in the lower dose AgNP treatments, and declined in the highest AgNP and AgNO<sub>3</sub> treatments. The original 250 mg Ag L<sup>-1</sup> AgNP suspension stock contained dissolved-Ag at 0.67 mg Ag L<sup>-1</sup>. By comparing the initial mass of dissolved-Ag added to the treatments with mass measured at the end of the experiment, we calculated the net production or removal of dissolved-Ag from solution for each treatment (Table 1).

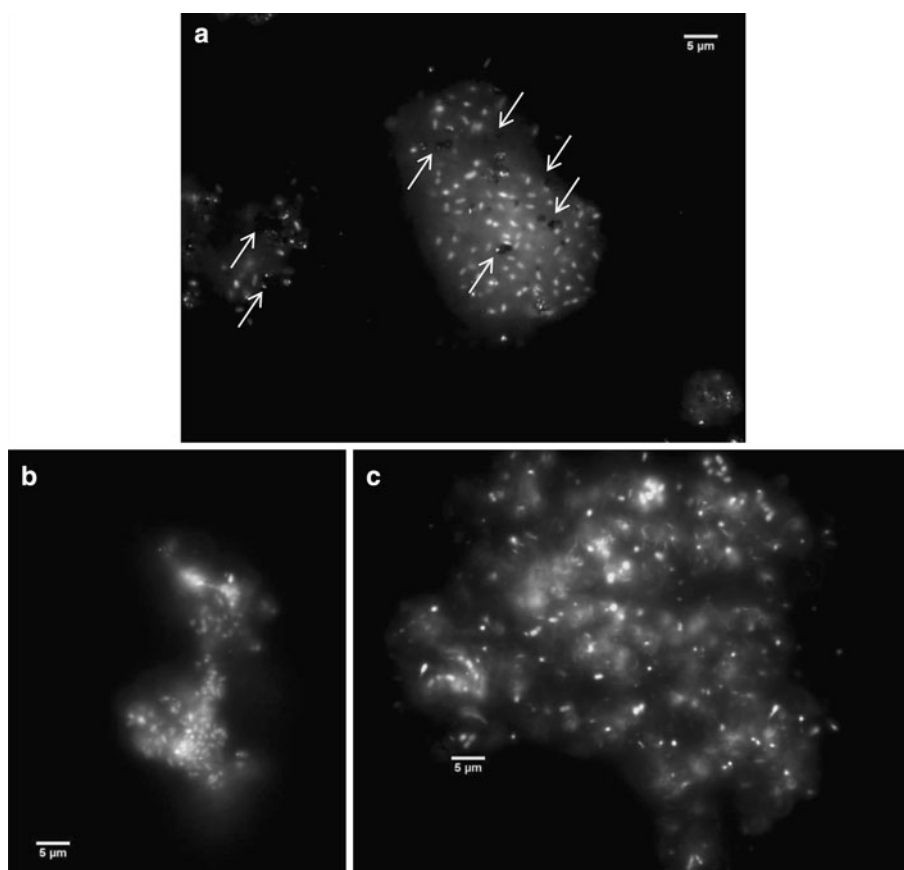
#### Commercial AgNPs' impacts on sediment microbes

In sediment there was no change in microbial biomass or respiration due to AgNPs at even the highest concentration (250 mg Ag L<sup>-1</sup>; Fig. 3a, b). In contrast, AgNO<sub>3</sub> addition

at 250 mg Ag L<sup>-1</sup> caused a 29 % decrease in respiration, though microbial biomass increased by 50 %. Orthophosphate and total ammonia both increased following AgNO<sub>3</sub> addition, but were not affected by AgNP addition (Fig. 4a, b). The only measured parameter that showed any change with AgNP addition was sulfate concentration (Fig. 4c), which was reduced by 14 % in the 250 mg Ag L<sup>-1</sup> treatment relative to controls. Once again the AgNP effects were more subtle than those of the positive control, AgNO<sub>3</sub>, which had sulfate concentrations 47 % lower than negative controls in the AgNO<sub>3</sub> treatment.

Extracellular-enzyme activities, and bacterial community composition in sediment did not change as a result of AgNP treatments, but did change with AgNO<sub>3</sub> addition. Both phosphatase and leucine aminopeptidase activities were unchanged in the highest AgNPs treatment, but were significantly lower for AgNO<sub>3</sub>, while sulfatase was low and invariable across treatments (Fig. 4d–f). Analysis of bacterial T-RFLP data (Fig. 3d) suggested that bacterial community composition in the highest AgNP treatment was indistinguishable from controls, while the AgNO<sub>3</sub> treatment showed evidence of a significant shift in community composition as measured by ADONIS. The NMS ordination corroborated the ADONIS findings and show a clear separation of AgNO<sub>3</sub> treated communities from

**Fig. 2** Micrograph showing watercolumn microbes in biofilms stained with DNA intercalating dyes, where *bright* areas are bacterial cells, *diffuse* areas are biofilm. *Dark* areas, several of which are indicated by *arrows* in panel **a** (AgNP 75 mg Ag L<sup>-1</sup>), and which are absent from both panels **b** (0.75 mg Ag L<sup>-1</sup> AgNO<sub>3</sub>) and **c** (Control) were identified as putative AgNP aggregates. Scale bars are 5 μm



**Table 1** Concentrations of total silver added, and masses of dissolved silver added and measured at the end of the streamwater and sediment experiments

| Form               | Total Ag added<br>(mg Ag L <sup>-1</sup> ) | Dissolved-Ag (µg Ag) |            |             |
|--------------------|--|----------------------|------------|-------------|
|                    |  | Added                | Final      | Net change  |
| <i>Streamwater</i> |  |                      |            |             |
| AgNP               | 0.75                                       | 0.1 ± 0.002          | 0.8 ± 0.2  | 0.7 ± 0.2   |
| AgNP               | 7.5  | 1.0 ± 0.018          | 1.6 ± 1.1  | -0.6 ± 1.1  |
| AgNP               | 75   | 10 ± 0.18            | 2.5 ± 0.9  | -7.5 ± 0.9  |
| AgNO <sub>3</sub>  | 0.75                                       | 37.5 ± 0.7           | 4.0 ± 0.2  | -33.6 ± 0.7 |
| <i>Sediment</i>    |  |                      |            |             |
| AgNP               | 75   | 4 ± 0.07             | 0          | -4 ± 0.07   |
| AgNP               | 125  | 6.6 ± 0.1            | 0          | -6.6 ± 0.1  |
| AgNP               | 250  | 13.4 ± 0.2           | 0.2 ± 0.02 | -13.2 ± 0.2 |
| AgNO <sub>3</sub>  | 250  | 5000 ± 90            | 6.6 ± 0.21 | -4993 ± 90  |

Added dissolved silver masses are calculated based on dissolved silver in 250 mg Ag L<sup>-1</sup> stock for AgNPs. Uncertainty estimates are the standard deviation for  $n = 3$  analytical replicates for dissolved silver added, and for  $n = 3$  experimental replicates for final concentrations. Net change is the final–added mass of silver (*negative* denotes a loss, *positive* denotes an increase), and shows how much dissolved silver left the dissolved phase with the error being the square root of the sum of squared errors from the final and added silver masses

control and AgNP treated communities (Fig. 3d; ADONIS: f(1,15) = 3.77; final stress was 16.4 and  $r^2$  for axes 1 and 2 were 0.73 and 0.19, respectively).

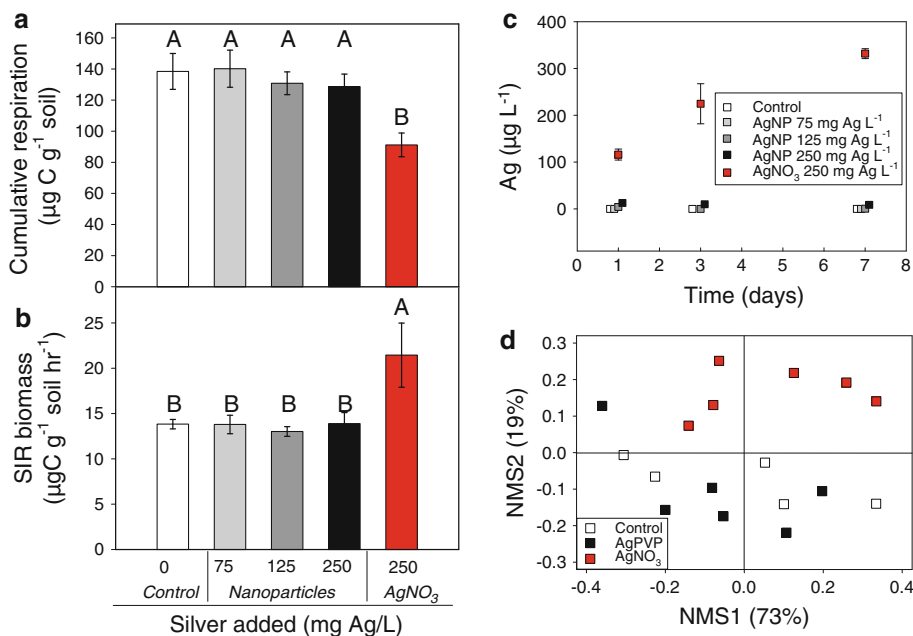
In all sediment slurry treatments, the mass of dissolved-Ag was lower at the end of the experiment than it was before treatment solutions were added to the sediment (as represented by a negative net change; Table 1), even at a

dose of 250 mg Ag L<sup>-1</sup> as AgNO<sub>3</sub> where the dissolved-Ag concentration was reduced by >99.9 %. While there was visual evidence of AgNPs aggregating in streamwater, for the sediment experiments, separate incubations were conducted (Fig. 5) in which AgNPs and sediment were isolated using dialysis membranes to assess aggregation of AgNPs when exposed to sediment associated DOM and ions (see schematic in Fig. 5b). Based on DLS measurements, no particles were formed in the dialysis tubes containing only deionized water for either the low MWCO (<0.5 kDa), or high MWCO (MWCO < 50 kDa) membrane. For AgNP suspensions contained within <0.5 kDa membranes—and thus only exposed to low molecular weight organic matter and a range of ions—particles did not aggregate, having an average hydrodynamic diameter of 23 ± 2 nm as measured by DLS (which is close to the 21 ± 17 nm diameter of the primary AgNPs measured by TEM; Fig. 5). In contrast, AgNP solutions contained within <50 kDa membranes aggregated and precipitated out of suspension, yielding a bimodal distribution with most aggregates at 1,400 ± 440 nm, and fewer aggregates at 290 ± 20 nm when particles were resuspended (Fig. 5).

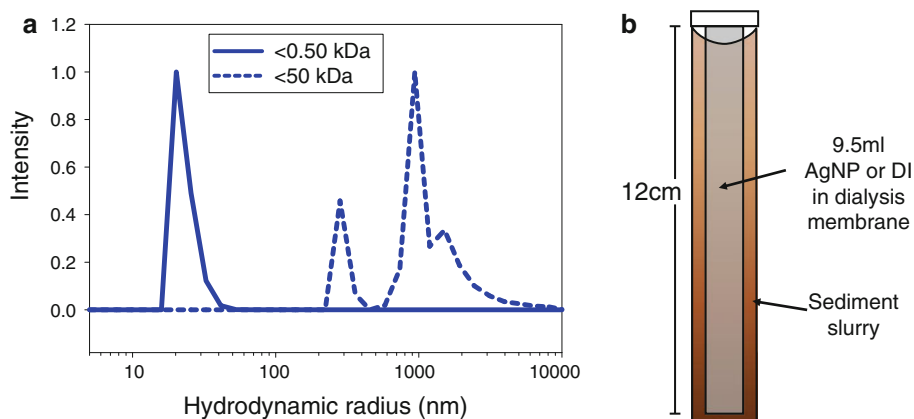
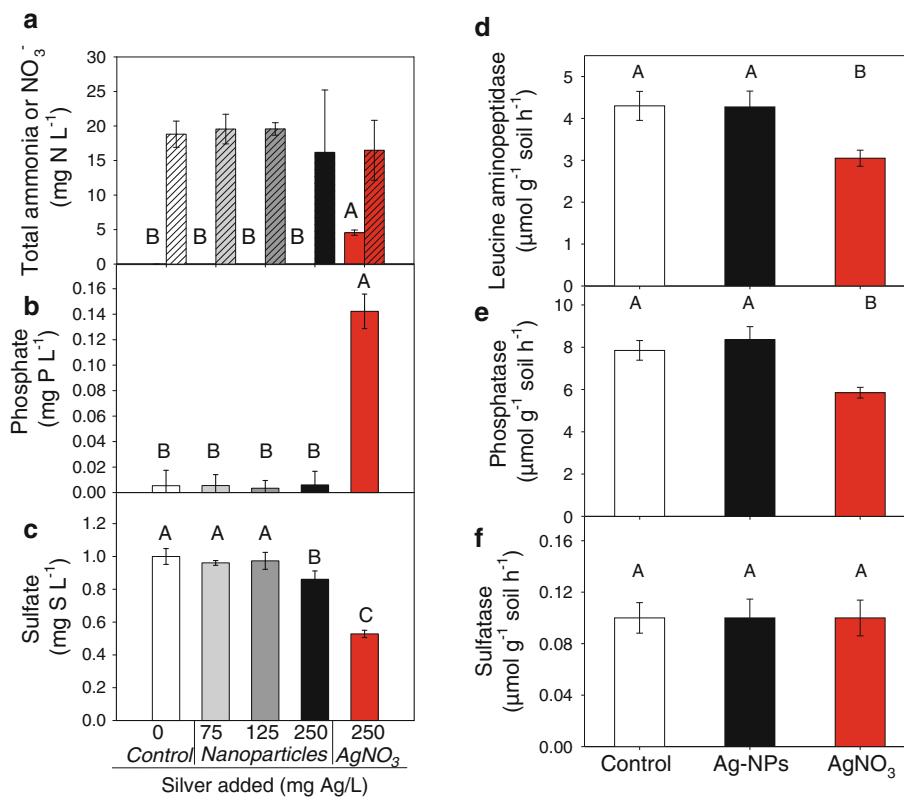
## Discussion

We assessed the response of natural microbial communities to a single pulsed addition of a high concentration of commercially produced AgNPs. While AgNPs have been reported to be highly toxic to microbes in pure culture, and have been found to have higher toxicity than dissolved-Ag added at similar doses, our experiments found that these

**Fig. 3** Results of sediment experiment showing: **a** sediment microbial respiration ( $n = 5$ ), **b** microbial biomass ( $n = 5$ ), **c** Ag concentration ( $n = 3$ ), and **d** non-metric multidimensional scaling ordination ( $n = 2$  for each of 3 days). For panels “a and b”, data were collected on day 7 of the incubation, while panels “c and d” were from days 1, 3, and 7. Error bars are standard deviation. In bar graphs, capital letters denote statistically significant differences between treatments. In panel c, data points are staggered for days 1, 3, and 7 for display purposes only



**Fig. 4** Final concentrations and activities in the sediment incubation for **a** total ammonia (open bars) and  $\text{NO}_3^-$  (hatch marked bars), **b** phosphate, **c** sulfate, **d** leucine aminopeptidase, **e** phosphatase, and **f** sulfatase. For all,  $n = 5$ , and error bars are standard deviation. Capital letters denote statistically significant differences between treatments. There were no differences between treatments in  $\text{NO}_3^-$  concentrations



**Fig. 5** **a** Distribution of hydrodynamic radius of AgNPs (measured by dynamic light scattering, DLS,  $n = 3$  experimental replicates) exposed to sediment slurry through dialysis membranes with molecular weight cut-offs of either  $<0.5\text{ kDa}$  (solid line), or  $<50\text{ kDa}$  (dashed line). **b** Diagram of experimental set up used in this

experiment. Sediment slurry (4 g dry weight sediment, 22 mL DI water) was contained in a polystyrene cylinder, to which a dialysis membrane was added containing 9.5 mL of either 250 mg  $\text{Ag L}^{-1}$  AgNPs, or DI water. Data from 250 mg  $\text{Ag L}^{-1}$  are shown, while DI water had no particles detectable by DLS (data not shown)

commercially produced AgNPs showed modest toxicity to streamwater microbes relative to  $\text{AgNO}_3$  (Fig. 1), and that AgNPs had little effect on sediment microbes (Fig. 3). These observations are consistent with our emerging conceptual understanding of NP impacts in the environment as we increasingly recognize that mechanisms such as passivation, adsorption, aggregation, and physical separation may collectively reduce the toxicity of NPs when microbes

are exposed in environmental media (Bradford et al. 2009; Gao et al. 2011).

#### The impacts of AgNPs on streamwater and sediment microbes

In streamwater, AgNPs led to decreased respiration relative to controls which was corroborated by dissolved organic C



measurements which showed higher DOC concentrations in higher AgNP treatments indicating less decomposition (Fig. 1 a, b). Interestingly, while this suggests that metabolism of DOC decreased, the activity of extracellular phosphatase (an enzyme that cleaves orthophosphate from dissolved organic phosphorous) increased in the 75 mg Ag L<sup>-1</sup> AgNP treatment (Fig. 1d). Increases in alkaline phosphatase activity suggest that some microbes were responding to either an increase in substrate availability (organophosphorous compounds) or a decrease in product concentration (in this instance, orthophosphate; Schimel and Weintraub 2003). In contrast to these impacts in streamwater, the lack of any measureable effect of sediment exposure to AgNPs on microbial respiration, microbial biomass, bacterial community composition (Fig. 3a, b, d) or extracellular enzyme activity (i.e., phosphatase, sulfatase, leucineaminopeptidase; Fig. 4d–f) leads us to conclude that this particular commercial AgNP has very limited toxicity in anoxic sediments.

The one detectable difference in the sediment experiment between AgNP treatments and controls was the lower concentration of sulfate observed in the 250 mg Ag L<sup>-1</sup> treatment. While the rate of sulfate accumulation did not differ between treatments (52 µg L<sup>-1</sup> day<sup>-1</sup>, data not shown), the intercepts were different, suggesting that between treatment initiation and our first measurement, samples diverged in sulfate due to the presence of AgNPs. Given that samples were likely anaerobic for the duration of the experiment (as evidenced by N<sub>2</sub>O and CH<sub>4</sub> production, data not shown), and low sulfatase activity, the source of this sulfate is assumed to be desorption, but what drove the initial divergence is unknown.

Comparing the impact on respiration of AgNPs and dissolved-Ag concentrations (added in the form of AgNO<sub>3</sub>), our results show that dissolved-Ag had a much stronger impact on microbes in both streamwater and sediment than AgNPs (Fig. 1b, Fig. 3a). In streamwater, AgNO<sub>3</sub> at 0.75 mg Ag L<sup>-1</sup> caused respiration to drop below detection, whereas 100 fold more Ag added as AgNPs (75 mg Ag L<sup>-1</sup>) only decreased respiration by 39 % (Fig. 1a). In sediment, while AgNPs failed to have a measurable effect on anything other than sulfate concentrations (Fig. 4a–f), AgNO<sub>3</sub> exposure led to changes in almost all measured outcomes with decreased enzyme activity (Fig. 4d, e; phosphatase, leucine aminopeptidase), altered nutrient concentrations (Fig. 4a–c; phosphate, total ammonia, sulfate), and decreased microbial respiration, increased microbial biomass, and shifted bacterial community composition (Fig. 3a, b, d).

While the increased microbial biomass and decreased respiration in the sediment AgNO<sub>3</sub> treatment may at first appear contradictory, we hypothesize that these observations represent the balance between two different phenomena having to do with microbial stress ecology: stress

avoidance through decreased metabolic activity, and a shift in community composition from stress intolerant to stress tolerant microbes (Schimel et al. 2007). To avoid stresses (e.g., drought, change in ionic strength, presence of a toxin), microbes can decrease activity to wait out the stressor, then resume activity when conditions improve—so long as conditions improve before the cumulative damage of the stressor causes mortality. We suggest that silver, while it may have caused some mortality, may have led to less metabolic activity from some silver sensitive taxa. At the same time, microbes less sensitive to silver may have increased in abundance more rapidly than stress intolerant microbes that experienced mortality. This would have led to a community composition shift, as well as a net increase in microbial biomass, which is consistent with our TRFLP data showing a shift in the AgNO<sub>3</sub> treatment, but not in the control or 250 mg AgNPs L<sup>-1</sup> treatment. Upon addition of an abundant substrate (i.e., the yeast extract used to determine microbial biomass) with high concentrations of Ag<sup>+</sup> binding ligands as well as abundant energy and nutrients which could allow microbes to overcome the toxic effects of Ag, both dissolved-Ag sensitive and tolerant microbes alike became active, yielding higher microbial biomass than in the controls or AgNP treatments.

The toxicity of AgNPs may be derived in part from the release of dissolved silver (Navarro et al. 2008), thus we examined dissolved-Ag concentration in both streamwater and sediment experiments. In streamwater, respiration was proportional to the amount of dissolved-Ag at the end of the incubation ( $r^2 = 78$ ,  $p < 0.001$ ; Fig. 1a), suggesting that in streamwater, dissolved-Ag released from AgNPs could plausibly be the mechanism of toxicity of AgNPs. In sediment, concentrations were uniformly low for AgNPs with the highest concentration in the 250 mg Ag L<sup>-1</sup> AgNP treatment being  $12.6 \pm 2.2$  µg L<sup>-1</sup> on day 1, whereas the 250 mg Ag L<sup>-1</sup> AgNO<sub>3</sub> treatment had a high concentration of  $332 \pm 11$  µg Ag L<sup>-1</sup> at the end of the experiment. The lack of response in the AgNP treatments is attributed in part to the low dissolved-Ag concentration.

#### Streamwater and sediment impacts on AgNPs and dissolved-Ag

In sediment and streamwater, we hypothesize that three factors were likely at play in reducing the toxicity of silver: complexation, precipitation, aggregation, and—in sediment only—low redox potential. While examining the speciation of silver was beyond the scope of this study, it has been shown that, as Ag<sup>+</sup> or AgNPs become complexed by ligands (e.g., sulfide, thiol groups on DOM, chloride, bromide) or precipitate with anions (e.g., chloride) their reactivity and toxicity decline (Choi et al. 2009; Fabrega et al. 2009).

We found indirect evidence that complexation and/or precipitation was an important fate for Ag in both experiments. In the streamwater experiment, dissolved-Ag declined by 75 and 89 % in the 75 mg Ag L<sup>-1</sup> AgNP and the AgNO<sub>3</sub> treatments respectively. While a visible precipitate was formed in the highest AgNP treatment, there was no visible precipitate formed in the AgNO<sub>3</sub> treatment. One possible fate of dissolved-Ag is association with amines, thiol groups, and carboxylic acid groups in dissolved organic matter (Smith et al. 1997). Much of this complexed Ag would have been removed from solution during ultracentrifugation. In the sediment experiment, the mass of dissolved-Ag in all three AgNPs decreased to near or below the detection limit, while in the AgNO<sub>3</sub> treatment, the mass of dissolved-Ag dropped by over three orders of magnitude (Table 1). In sediment we suggest that both high concentrations of organic matter and potentially high concentrations of sulfide likely complexed or precipitated silver, removing it from the supernatant.

As demonstrated in these experiments, the complex mixture of organic matter, inorganic ions and particles in natural media can react with AgNPs causing aggregation. In streamwater, AgNP aggregation was observed in the AgNP treatments, with microbes growing in biofilms attached to aggregates (Fig. 2). In sediment, the dialysis membrane study showed that AgNPs aggregated when exposed to high molecular weight organic matter and ions, whereas there was little to no evidence for aggregation when exposed to only low molecular weight organic matter and inorganic ions (Fig. 5). Realistically, when AgNPs were added to sediments directly, aggregation was likely dominated by heteroaggregation rather than autoaggregation given that we added at most 5 mg Ag to 4 g sediment. While aggregation does not necessarily decrease the rate of dissolution of silver ions off of the surface of AgNPs (Liu and Hurt 2010), it has been shown to decrease toxicity (Lok et al. 2007), potentially by decreasing the number of AgNPs and the probability that an organism would encounter a AgNP, thereby reducing toxicity.

In the sediment experiment, the low oxidation reduction potential of this high organic matter sediment was likely to have influenced silver toxicity in two distinct ways: first, through promoting biological transformation of sulfate to sulfide, a strong ligand for Ag<sup>+</sup>; and second, low redox potential could foster abiotic and biotic reduction of Ag<sup>+</sup> to Ag<sup>0</sup> (below 0.2 V; Takeno 2005), which would decrease Ag toxicity.

It is far too early to conclude from this work whether existing regulations for dissolved-Ag could serve as a basis for dealing with AgNPs, but these results suggest that this is a possible outcome of further research on this topic. Future work should seek to determine the commonalities and differences in AgNPs of different sizes and coatings

under similar environmental conditions, and should include macroscopic as well as microscopic organisms. Further studies are also needed to determine the extent to which the physical or chemical characteristics of AgNPs (e.g., size, initial coating) and the environment (e.g., DOM concentration, pH, ionic strength) influence these AgNP/environmental-matrix interactions. And finally, work with freshly suspended commercial AgNPs or synthesized AgNPs needs to be complimented by work on the “aged” forms of AgNPs (e.g., Ag<sub>2</sub>S-NPs, rather than Ag<sup>0</sup>) that may be more likely to enter the environment (Kim et al. 2010).

**Acknowledgments** The authors would like to thank Sam Johnson, Medora Burke-Scoll, Brooke Hassett, Curt Richardson, Claudia Gunsch, and Christina Arnaout for their discussions and laboratory assistance. This work was funded through the Center for the Environmental Implications of Nanotechnology (CEINT), which is supported by funding from the National Science Foundation (NSF) and the US Environmental Protection Agency (EPA). Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

## References

- Amin RM, Mohamed MB, Ramadan MA, Verwanger T, Krammer B (2009) Rapid and sensitive microplate assay for screening the effect of silver and gold nanoparticles on bacteria. *Nanomedicine* 4(6):637–643. doi:10.2217/nmm0950
- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26(1):32–46. doi:10.1111/j1442-9993.200101070ppx
- Aruguete DM, Hochella MF (2010) Bacteria-nanoparticle interactions and their environmental implications. *Environ Chem* 7(1):3–9. doi:10.1071/en09115
- Benn TM, Westerhoff P (2008) Nanoparticle silver released into water from commercially available sock fabrics. *Environ Sci Technol* 42(11):4133–4139. doi:10.1021/es7032718
- Bradford A, Handy RD, Readman JW, Atfield A, Muhling M (2009) Impact of silver nanoparticle contamination on the genetic diversity of natural bacterial assemblages in Estuarine sediments. *Environ Sci Technol* 43(12):4530–4536. doi:10.1021/es9001949
- Choi O, Hu Z (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ Sci Technol* 42(12):4583–4588. doi:10.1021/es703238h
- Choi OK, Hu ZQ (2009) Nitrification inhibition by silver nanoparticles. *Water Sci Technol* 59(9):1699–1702. doi:10.2166/wst2009205
- Choi O, Cleuenger TE, Deng BL, Surampalli RY, Ross L, Hu ZQ (2009) Role of sulfide and ligand strength in controlling nanosilver toxicity. *Water Res* 43(7):1879–1886. doi:10.1016/j.watres.2009.01.029
- Corley E, Scheufele D, Hu Q (2009) Of risks and regulations: how leading US nanoscientists form policy stances about nanotechnology. *J Nanopart Res* 11(7):1573–1585. doi:10.1007/s11051-009-9671-5
- Costerton JW (2007) *The biofilm primer*. Springer, Berlin
- Elzey S, Grassian VH (2010) Agglomeration, isolation and dissolution of commercially manufactured silver nanoparticles in aqueous environments. *J Nanopart Res* 12(5):1945–1958. doi:10.1007/s11051-009-9783-y

- Fabrega J, Fawcett SR, Renshaw JC, Lead JR (2009) Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ Sci Technol* 43(19):7285–7290. doi:101021/es803259g
- Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem* 35(1):167–176
- Gao J, Wang Y, Hovsepian A, Bonzongo JCJ (2011) Effects of engineered nanomaterials on microbial catalyzed biogeochemical processes in sediments. *J Hazard Mater* 186(1):940–945. doi:101016/j.jhazmat201011084
- Girvan MS, Campbell CD, Killham K, Prosser JI, Glover LA (2005) Bacterial diversity promotes community stability and functional resilience after perturbation. *Environ Microbiol* 7(3):301–313. doi:101111/j.1462-2920200500695x
- Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *J Stat Softw* 22(7):1–19
- Kent RD, Vikesland PJ (2011) Controlled evaluation of silver nanoparticle dissolution using atomic force microscopy. *Environ Sci Technol*. doi:10.1021/es203475a
- Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. *Nanomed Nanotechnol Biol Med* 3(1):95–101
- Kim B, Park CS, Murayama M, Hochella MF Jr (2010) Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. *Environ Sci Technol* 44(19):7509–7514
- Liu J, Hurt RH (2010) Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol* 44(6):2169–2175. doi:101021/es903557
- Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63(11):4516–4522
- Lok C-N, Ho C-M, Chen R, He Q-Y, Yu W-Y, Sun H, Tam P, Chiu J-F, Che C-M (2006) Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J Proteome Res* 5(4):916–924
- Lok C-N, Ho C-M, Chen R, He Q-Y, Yu W-Y, Sun H, Tam P, Chiu J-F, Che C-M (2007) Silver nanoparticles: partial oxidation and antibacterial activities. *J Biol Inorg Chem* 12(4):527–534
- McCune B, Grace JB (2002) Analysis of ecological communities. MjM Software Design, Glenden Beach
- Miao AJ, Schwehr KA, Xu C, Zhang SJ, Luo ZP, Quigg A, Santschi PH (2009) The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. *Environ Pollut* 157(11):3034–3041. doi:101016/j.envpol200905047
- Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* 16:2346
- Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, Sigg L, Behra R (2008) Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environ Sci Technol* 42(23):8959–8964
- Oksanen J (2010) Multivariate analysis of ecological communities in R: Vegan tutorial
- Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2010) Vegan: community ecology package. R package version 1.17-3. Available at <http://CRAN.R-project.org/package=vegan>. Accessed 12 Feb 2010
- Osborn AM, Moore ERB, Timmis KN (2000) An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environ Microbiol* 2(1):39–50
- Pal S, Tak YK, Song JM (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol* 73(6):1712–1720. doi:101128/aem02218-06
- Project on Emerging Nanotechnologies (2010) Nanotechnology consumer products inventory. <http://www.nanotechproject.org/inventories/consumer/>. Accessed April 12 2010
- Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol Biochem* 34(9):1309–1315
- Schimel J, Balsler TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88(6):1386–1394. doi:10.1890/06-0219
- Smetana AB, Klabunde KJ, Marchin GR, Sorensen CM (2008) Biocidal activity of nanocrystalline silver powders and particles. *Langmuir* 24(14):7457–7464. doi:101021/la800091y
- Smith RM, Martell AE, Motekaitis RJ (1997) NIST critically selected stability constants of metal complexes database, version 4.0. NIST Standard Reference Database 46
- Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 275(1):177–182
- Sudduth EB, Bernhardt ES (2011) Testing the field of dreams hypothesis: functional responses to urbanization and restoration in stream ecosystems. *Ecol Appl* 21(6):1972–1988
- Systat Software Inc. (2008) Sigmaplot, 11.0 edn, San Jose, USA
- Takeno N (2005) Atlas of Eh-pH diagrams, intercomparison of thermodynamic databases. Geological Survey of Japan
- Yin L, Cheng Y, Espinasse B, Colman BP, Auffan M, Wiesner M, Rose J, Liu J, Bernhardt ES (2011) More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. *Environ Sci Technol* 45:2360–2367. doi:101021/es103995x
- Yoon KY, Byeon JH, Park JH, Hwang J (2007) Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Sci Total Environ* 373(2–3):572–575. doi:101016/j.scitotenv200611007