

Nanosilver inhibits nitrification and reduces ammonia-oxidizing bacterial but not archaeal *amoA* gene abundance in estuarine sediments.

Jessica Beddow¹, Björn Stolpe², Paula A. Cole², Jamie R. Lead^{2,3}, Melanie Sapp⁴, Brett P. Lyons⁵, Ian Colbeck¹, Corinne Whitby^{1*}.

Running title: Nanosilver inhibits estuarine nitrification.

¹School of Biological Sciences, University of Essex, Essex, CO4 3SQ, UK

²School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

³Center for Environmental NanoScience and Risk, University of South Carolina, South Carolina, 29028, USA

⁴Institute of Population Genetics, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich-Heine University, Düsseldorf, 40225, Germany

⁵Centre for Environment, Fisheries and Aquaculture Science, The Nothe, Barrack Road, Weymouth, Dorset, DT4 8UB, UK

* Corresponding author: School of Biological Sciences, University of Essex,

Wivenhoe Park, Colchester, Essex. C04 3SQ. Tel: +44 (01206) 872062 Fax: +44 (01206) 872592 Email: cwhitby@essex.ac.uk

4684 words (5 Figures, 1 Table).

Originality-Significance Statement. This is the first to report differential sensitivity of Ammonia-oxidizing bacteria (AOB) and archaea (AOA) towards AgNPs in estuaries. Our findings suggest that where AgNPs may accumulate in benthic sediments (such as downstream from wastewater treatment outflows), there is a

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13441

potential environmental risk to nitrification, especially in polyhaline sediments where ammonia-oxidation is largely driven by AOB.

Summary

Silver nanoparticles (AgNPs) enter estuaries via wastewater treatment effluents, where they can inhibit microorganisms, because of their antimicrobial properties. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are involved in the first step of nitrification and are important to ecosystem function, especially where effluent discharge results in high nitrogen inputs. Here, we investigated the effect of a pulse addition of AgNPs on AOB and AOA ammonia monooxygenase (*amoA*) gene abundances and benthic nitrification potential rates (NPR) in low-salinity and mesohaline estuarine sediments. Whilst exposure to 0.5 mg L⁻¹ AgNPs had no significant effect on *amoA* gene abundances or NPR, 50 mg L⁻¹ AgNPs significantly decreased AOB *amoA* gene abundance (up to 76% over 14 days), and significantly decreased NPR by twenty-fold in low-salinity sediments and by two-fold in mesohaline sediments, after one day. AgNP behaviour differed between sites, whereby greater aggregation occurred in mesohaline waters (possibly due to higher salinity), which may have reduced toxicity. In conclusion, AgNPs have the potential to reduce ammonia oxidation in estuarine sediments, particularly where AgNPs accumulate over time and reach high concentrations. This could lead to long-term risks to nitrification, especially in polyhaline estuaries where ammonia-oxidation is largely driven by AOB.

Keywords: Ammonia-oxidizing bacteria (AOB), Ammonia-oxidizing archaea (AOA), *amoA* gene, silver nanoparticles, ammonia-oxidation, nitrification, estuary

Introduction

A recent explosion in the use of nanotechnology in consumer products has increased concerns regarding the adverse effects of nanoparticles (i.e. particles between 1-100 nm) on the environment and human health (Nowack et al., 2011; Richardson and Ternes, 2011). Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials, because of their antimicrobial properties (Nel et al., 2006; Pal et al., 2007). Over 400 tons of AgNPs are produced globally each year, with applications in medical equipment, cosmetics, textiles, electronics, children's toys and household appliances (PEN, 2016). As a result, AgNPs or silver ions (Ag^+) may be released directly (e.g. from washing AgNP-containing textiles) or indirectly (e.g. leaching from nanosilver-enhanced products) into rivers and estuaries (Cleveland et al., 2012; Sun et al., 2014). One major potential route for AgNP entry into aquatic environments is via wastewater treatment plants (WWTPs), where ammonia-oxidizing microbial communities are important for nitrogen removal.

Ammonia-oxidation (the first and rate-limiting step of nitrification) is mediated by ammonia-oxidizing bacteria (AOB) and archaea (AOA), that possess the *amoA* gene, which codes for the alpha-subunit of the enzyme ammonia monooxygenase (AMO) (Hollocher, 1981; McTavish et al., 1993), whereby it is the AmoB subunit of AMO that is thought to harbor the active site in ammonia oxidizers (Lieberman and Rosenzweig 2005; Balasubramanian et al., 2010). It is well known that ammonia-oxidizers are sensitive to changes in their environment (e.g. pH, temperature, salinity, light) (Joye & Hollibaugh, 1995; Strauss & Dodds, 1997; Rysgaard et al., 1999; Nicol et al., 2008). Previous work has investigated the impact of AgNPs on pure cultures of ammonia oxidisers (Radniecki et al., 2011; Yuan et al., 2012; Beddow et al., 2014a) as well as wastewater sludge microbial communities (Choi

and Hu, 2009; Liang et al., 2010; Yang et al., 2014), and has demonstrated high sensitivity of AOB to AgNPs. For example, both Liang et al. (2010) and Yang et al. (2014) found population decreases in activated sludge nitrifying bacteria treated with 1-40 mg/L AgNPs. Both studies also found AgNPs to have a greater impact on activated sludge microbial communities compared to Ag^+ . Despite this work, little is currently known about the effect of AgNPs on AOB and AOA communities in receiving waters and sediments, where the fate, behaviour and toxicity of AgNPs may be vastly different to that in WWTP sludge (Fabrega et al., 2011).

From WWTPs, AgNPs may be released directly into aquatic systems via effluents at estimated concentrations of 42.5 ng L^{-1} (Gottschalk et al., 2009), or may accumulate by up to 1.55 mg kg^{-1} in biosolids and enter rivers and estuaries through agricultural runoff (Gottschalk et al., 2009; Whiteley et al., 2013). In environmental waters, AgNPs may release Ag^+ that can complex with enzymes and proteins in living cells, causing DNA damage and protein inactivation (Feng et al., 2000; Choi and Hu 2008; Auffan et al 2009; Xiu et al., 2012). AgNPs may also react with chloride ions to form Ag-chloride complexes that increases AgNP solubility, thus silver is often more mobile in seawater (Yu et al., 2013). Since dissolution kinetics of AgNPs are strongly dependent on the Cl/Ag ratio, it is possible that dissolution rates of AgNPs could be greater in marine compared to freshwaters (Levard et al., 2013). Other processes, however, can also affect AgNP dissolution rates (Levard et al., 2013). For example, in sediments, Ag^+ may bind with inorganic ligands such as S^{2-} , SO_4^{2-} and CO_3^{2-} to form Ag-complexes, which are less bioavailable and less toxic than Ag^+ (Choi et al., 2009; Levard et al., 2013; Yu et al., 2013). Changes in ionic strength, such as the presence of divalent cations (e.g. Ca^{2+} and Mg^{2+}) in natural waters, can alter AgNP surface charges and induce aggregation. Aggregated AgNPs

are more likely to deposit in benthic sediments reducing AgNP concentration in the water column (Yu et al., 2013). Aggregated AgNPs also have reduced bioavailability as a result of reduced particle adsorption to cell surfaces and reduced transport across membranes (Christian et al., 2008; Handy et al., 2008). Indeed, aggregated AgNPs with increased sizes have lower antimicrobial activities (Zhang et al., 2011). Thus, when AgNPs are released into complex ecosystems such as estuaries, changes in their surface properties and reactivity may inhibit microbial processes that are crucial for ecosystem functioning

The focus of the current study is the River Colne estuary (Fig. 1), a macrotidal, hypernutrified estuary in the United Kingdom, which demonstrates a gradient of salinity from the estuary head at the Hythe, through to the estuary mouth at Brightlingsea (Papasprou et al., 2012; Li et al., 2015). The estuary also has very high inorganic nitrogen levels in the upper estuary from inputs from the River Colne and a major sewage treatment works (Ogilvie et al., 1997; Papasprou et al., 2012; Li et al., 2015). Nitrification is an important process in the estuary and when coupled with denitrification can remove ~25% of total oxidized nitrogen before it enters the North Sea (Dong et al., 2000).

The overall goal of this study was to investigate the effect of AgNPs on AOB and AOA *amoA* gene abundances and benthic nitrification potential along an estuary. Previously, Li et al. (2015) found greater AOB *amoA* gene abundance compared to AOA along the Colne estuary, suggesting that AOB are proportionally more important contributors to ammonia-oxidation in this estuary. In the Colne estuary, as Cl⁻ concentrations increase downstream, we hypothesize that AgNP toxicity will be greater towards the estuary mouth compared to the estuary head. It is also hypothesized that AOB and AOA may exhibit differential sensitivities towards

AgNPs. This study will provide important information on the potential environmental risk of AgNPs to nitrification in estuaries where complex interactions occur.

Results

Size, dissolution and aggregation behaviour of AgNPs in estuarine water

The AgNPs were more soluble in the mesohaline (Wivenhoe) water compared to the low-salinity (Hythe) water (554 versus 72 $\mu\text{g L}^{-1}$ free silver by day 25, respectively), while ultra-high purity UHP water fell approximately midway between the two (Fig. 2A and B). This variation in solubility was established quickly. For example, over the first five hours, the rate of Ag^+ release increased with increasing Cl/Ag molar ratio (from 0.11% h^{-1} in the low-salinity (Hythe) water to 0.24% h^{-1} in the mesohaline (Wivenhoe) water) (Fig. 3). AgNP particle diameter significantly increased in the mesohaline (Wivenhoe) water (60 nm \pm 0.5 nm compared to 35 nm \pm 0.2 nm in UHP) and decreased slightly in the low-salinity (Hythe) water (30 nm \pm 0.1 nm) (Table 1). Zeta potentials were determined to indicate the magnitude of electrostatic repulsion/attraction between the AgNPs in the different waters. This is known to affect particle stability, i.e. a zeta potential close to 0 indicates a greater risk of attraction, potentially leading to particle aggregation. Data suggested that the AgNPs were most stable in UHP water (-37 mV \pm 0.3 mV), but were less stable in the low-salinity (Hythe) estuarine water (-12 mV \pm 1.1 mV) and least stable in the mesohaline (Wivenhoe) estuarine water (-5 mV \pm 2.0 mV) (Table 1).

*Effect of AgNPs on AOB and AOA *amoA* gene abundance*

Whilst exposure to 0.5 mg L^{-1} AgNPs had no significant effect on AOB and AOA *amoA* gene abundance in either the low-salinity (Hythe) or mesohaline

(Wivenhoe) sediments over 14 days (Fig. 4), 50 mg L⁻¹ AgNPs caused significant reductions to AOB *amoA* gene abundance in the low-salinity (Hythe) sediments after 7 days ($F_{2,8}=9.16$, $P<0.05$) and 14 days ($F_{2,8}=6.86$, $P<0.05$) (Fig. 4A). Specifically, AOB *amoA* gene abundance in the low-salinity (Hythe) sediments exposed to 50 mg L⁻¹ AgNPs was 68% lower than controls at day 7, and 76% lower than controls at day 14 (Fig. 4A). In the mesohaline (Wivenhoe) sediments exposed to 50 mg L⁻¹ AgNPs, *amoA* gene abundance was 55% lower than controls at day 7, and 66% lower than controls at day 14 (Fig. 4B).

Overall, AOB *amoA* gene abundance was a thousand-fold greater than that for AOA at both sites (Fig. 4A-D). Furthermore, AOB *amoA* gene abundance was 37% greater in the low-salinity (Hythe) compared to the mesohaline (Wivenhoe) sediments at day 0 ($t_4=4.59$, $P=0.01$) (Fig. 4A-B). There was no significant difference in AOA *amoA* gene abundance between the two sites (Fig. 4 C-D) and, in contrast to AOB *amoA* gene abundances, exposure to AgNPs at both 0.5 and 50 mg L⁻¹ had no significant effect on AOA *amoA* gene abundance in either the low salinity or mesohaline (Wivenhoe) sediments over 14 days (Fig. 4C and D).

Effect of AgNPs on nitrification potential rates (NPRs)

In general, faster NPRs were obtained from the low-salinity (Hythe) sediments at the estuary head ($60.0 \pm 1.0 \mu\text{M NH}_4^+$ oxidized g⁻¹ dry weight sediment day⁻¹) compared to the mesohaline (Wivenhoe) sediments from mid-estuary ($42.2 \pm 7.4 \mu\text{M NH}_4^+$ oxidized g⁻¹ dry weight sediment day⁻¹) at day 0, although the difference was not significant (Fig. 5A and B). Whilst AgNPs at 0.5 mg L⁻¹ had no significant impact on NPRs in the low-salinity (Hythe) or mesohaline (Wivenhoe) sediments, NPRs were reduced over time in the low-salinity (Hythe) sediments by 20% by day 14 in

comparison to controls at day 14. AgNP concentrations of 50 mg L⁻¹ resulted in significant inhibition to NPRs in both the low-salinity (Hythe) ($F_{2,20}=265.73$, $P<0.001$) and mesohaline (Wivenhoe) ($F_{2,20}=890.37$, $P<0.001$) sediments over 14 days (Fig. 5A and B). Specifically, when exposed to 50 mg L⁻¹ AgNPs, NPRs obtained from the low-salinity (Hythe) sediments were twenty-fold lower than the controls by day 1 ($P<0.001$) (Fig. 5 A). The low-salinity (Hythe) sediment NPRs remained twenty-fold lower than controls by day 7 ($P<0.001$) (Fig. 5A). By day 14, the low-salinity (Hythe) sediment NPRs exposed to 50 mg L⁻¹ AgNPs recovered somewhat, yet remained four-fold lower than controls ($P<0.005$) (Fig. 5A).

NPRs obtained from the mesohaline (Wivenhoe) sediments exposed to 50 mg L⁻¹ AgNPs were also significantly reduced, by almost three-fold ($P<0.001$) compared to controls, by day 1, and were so low they were almost unmeasurable by day 7 ($P<0.001$) (Fig. 5B). By day 14, however, the mesohaline (Wivenhoe) sediment NPRs significantly recovered to levels similar to those prior to AgNP exposure (Fig. 5B). Killed control sediment slurries demonstrated no loss of ammonium between days 0 and 14 (data not shown).

Discussion

AgNPs are widely used in consumer products and pose a potential threat to the environment (Fabrega et al., 2011). Once released into the environment, changes in the surface properties and reactivity of AgNPs may occur, which affect their transport, behaviour and toxicity towards the *in-situ* microbial communities. AOB and AOA are important in the ecosystem functioning of estuaries, especially where wastewater effluents are released. However, little is currently known about the effects of AgNPs on estuarine AOB and AOA. Here, sediments from mesohaline

(Wivenhoe) and low-salinity (Hythe) sites in the Colne Estuary, UK, were exposed to AgNPs to determine the effect of AgNPs on estuarine nitrification and AOB and AOA *amoA* gene abundances. Exposure to 50 mg L⁻¹ AgNPs resulted in significant decreases in AOB *amoA* gene abundance in both the low-salinity (Hythe) and mesohaline (Wivenhoe) sediments, whilst AOA *amoA* gene abundances (although much lower than AOB), were not significantly affected. Whilst *amoA* gene copy numbers are an ideal indicator for AOB and AOA abundance, they cannot directly infer nitrifying activity (Wuchter et al. 2006; Li et al. 2015). Our findings therefore suggest that AgNPs may have a detrimental impact on AOB driven ammonia oxidation in estuarine environments. Previous studies have also demonstrated high sensitivity of AOB to AgNPs (Choi and Hu, 2009; Liang et al., 2010; Yang et al., 2014). For example, both Liang et al. (2010) and Yang et al. (2014) found population decreases in activated sludge nitrifying bacteria treated with 1-40 mg L⁻¹ AgNPs. Both studies also found AgNPs to have a greater impact on activated sludge microbial communities compared to Ag⁺. One possible explanation for the observed sensitivity of AOB towards AgNPs is that there are significantly different predicted structures of archaeal AmoB and bacterial AmoB. Since it is the AmoB subunit of AMO that is thought to harbor the active site in ammonia oxidizers (Lieberman and Rosenzweig 2005; Balasubramanian et al., 2010), it is possible that there are differences in ammonia oxidation functionality of this protein in AOA compared to AOB (Walker et al., 2010). Consequently, AgNPs or their ions may interact differently with the bacterial AmoB compared to the archaeal AmoB, leading to the observed differential sensitivity towards AgNPs found herein.

In the Colne estuary, the estuary head at the Hythe has a high net input of nitrogen as a result of wastewater effluents entering the system from the nearby

wastewater treatment facility (Thornton et al., 2007; Li et al., 2015), thus ammonia-oxidation is of great importance for nitrogen transformations at this location. We demonstrated significant NPR inhibition with exposure to 50 mg L⁻¹ AgNPs, suggesting that AgNPs have the potential to reduce ammonia oxidation in estuarine sediments, particularly where AgNPs accumulate over time and reach high concentrations.

In estuaries, the structure of ammonia-oxidizing communities is complex, whereby AOA are dominant in some estuaries (Beman and Francis, 2006; Caffrey et al., 2007; Moin et al., 2009; Bernhard et al., 2010; Damashek et al., 2015), AOB are dominant in others (Abell et al., 2010; Bernhard et al., 2010), whilst other estuaries have alternating spatial trends of AOA and AOB dominance (Mosier and Francis 2008; Bouskill et al., 2012; Zheng et al., 2014). Our study and that of Li et al. (2015), found greater AOB *amoA* gene abundance compared to AOA along the Colne estuary, suggesting that AOB may have a greater contribution to ammonia-oxidation in this estuary. Our findings suggest that the accumulation of high levels of AgNPs may pose an immediate environmental risk to estuarine nitrification, and a further delayed risk to AOB abundance, especially where ammonia-oxidation is potentially predominantly driven by AOB, like in the Colne estuary. Unless communities are able to adapt and recover, this could have severe environmental risks, given that estuaries may be exposed to nanoparticles over a long duration. Furthermore, AOA communities (which were present in much lower abundances prior to AgNP exposure), may increase in abundance and become more significant drivers of ammonia oxidation in these estuaries.

It is well known that the physical and chemical properties of an environment influence nanoparticle characteristics, which in turn may affect their toxicity (Fabrega

et al., 2011; Lowry et al., 2012). In this study we hypothesized that AgNP toxicity would be greater towards the estuary mouth compared to the estuary head, yet the opposite occurred. Such differences in physicochemical factors along the estuary may help to explain the mitigation of a detrimental effect on NPRs (by 50 mg L⁻¹ AgNPs) in the mesohaline (Wivenhoe) compared to the low-salinity (Hythe) sediments, by day 14. For example, AgNPs may react with dissolved sulfide species (H₂S, HS⁻) to produce nanosilver-sulfide structures which are less bioavailable and therefore less toxic (Choi et al., 2009; Liu and Hurt 2010; Levard et al., 2013; Yu et al., 2013). Thus in sulfur-rich sediments, AgNP toxicity may be reduced. Here, sulfate concentrations were over three-fold greater in the mesohaline (Wivenhoe) sediments compared to the low-salinity (Hythe) sediments. Therefore, in the anoxic sediments, sulphate may be reduced, resulting in higher concentrations of sulphide. Thus, nanosilver-sulphide complexes would be more likely to form at the estuary mouth, decreasing toxicity and allowing the microbial communities to recover (Levard et al., 2012; Reinsch et al., 2012). Recovery of naturally occurring microbial populations exposed to carboxy-functionalised polyacrylate-capped AgNPs (1-10 nm diameter) has been demonstrated elsewhere (Das et al. 2012a, b). A possible explanation for such a recovery could be a shift in the microbial population (i.e. proliferation of more resistant populations) in response to silver exposure (Schimel et al., 2007). However, since background silver concentrations in the Colne sediments were within the lower range of those previously measured by Blaser et al. (2008) (i.e. < 0.37 mg kg⁻¹ dry weight sediment, Fig. 1), it was assumed that the *in-situ* microbiota were not pre-adapted to high silver concentrations. In addition, previous work has shown that the surface oxic layer is greater near the estuary mouth compared to the estuary head (Ogilvie et al, 1997). Thus, it is also possible that

differences in oxygen concentration along the estuary may have influenced the behaviour and toxicity of the AgNPs, which in turn influenced the growth and activity of AOA and AOB.

AgNPs often exhibit size-dependent toxicity (Sondi & Salopek-Sondi, 2004; Ma et al., 2012; Xiu et al., 2012). Here, the size and aggregation behavior of the AgNPs was strongly influenced by the estuarine waters they were dispersed in, which may have affected toxicity. We hypothesized that AgNP toxicity will be greater towards the estuary mouth compared to the estuary head. However, our findings showed that AgNP toxicity was greater in the low salinity site at the estuary head. This may be as a result of the higher salinity found at the estuary mouth causing greater aggregation of AgNPs in the mesohaline (Wivenhoe) waters compared to the low-salinity (Hythe) site, which may have reduced AgNP toxicity. Greater AgNP dissolution was also measured in the mesohaline (Wivenhoe) water compared to the low-salinity (Hythe) water, which is likely due to each water matrix inducing different chemical speciation of Ag, with either enhanced or reduced Ag^+ production (Levard et al., 2013). This is in agreement with Levard et al. (2013) who reported the formation of soluble Ag species and that the rate of release of dissolved Ag^+ increased as Cl/Ag ratio increased. High chloride concentrations in the mesohaline (Wivenhoe) water (Fig 1) are likely to have led to the formation of soluble Ag-chloride complexes (Liu & Hurt, 2010; Levard et al., 2012; Tejamaya et al., 2012)), which can explain the greater dissolved Ag found in our study. In contrast, AgNP dissolution observed in the low-salinity (Hythe) water was lower than in UHP water, despite the higher chloride concentration in the low-salinity (Hythe) water compared to UHP water. This may have been due to the formation of insoluble silver chloride species (Levard et al., 2013), or Ag-organic complexes with humic and fulvic acids (Kim et

al., 2010; Liu & Hurt 2010; Levard et al., 2012) with a higher molecular weight than the 1 kDa cut-off of the dialysis membrane that was used to separate soluble species from AgNPs. Indeed, DOC concentration was over two-fold greater at the estuary head compared to the estuary mouth (Fig. 1), suggesting that Ag-organic complexes were more likely to form at the estuary head.

In conclusion, the effects of AgNPs were studied in low salinity (Hythe) and mesohaline (Wivenhoe) sediments and AOB at both sites demonstrated sensitivity towards the AgNPs tested. Specifically, AgNPs at 50 mg L⁻¹ significantly decreased AOB but not AOA *amoA* gene abundances, and significantly decreased NPRs in both low-salinity (Hythe) and mesohaline (Wivenhoe) sediments. These findings suggest that where AgNPs are likely to accumulate in benthic sediments (such as downstream from wastewater treatment outflows), there is a potential environmental risk to nitrification, especially where ammonia-oxidation is largely driven by AOB. Future work could extend the number of sites in this estuary and in estuaries elsewhere, in order to cover more of the vastly diverse conditions AOB and AOA are subjected to, and determine whether this is the case.

Experimental procedures

Silver nanoparticles and characterisation

The AgNPs were provided as a 0.9 g L⁻¹ suspension by Dr. Paul Christian (University of Manchester, UK). The AgNPs were coated with methoxypolyethylene-glycol (mPEG), a capping agent which is commonly used to stabilize AgNPs and aid dispersion (Christian et al., 2008). The AgNPs have previously been characterized in UHP water (Beddow et al., 2014a; Beddow et al., 2014b) using a range of methods including dynamic light scattering (Zetasizer Nano ZS ZEN3600, Malvern

Instruments Ltd), transmission electron microscopy (TEM, JEOL 1200EX), flow field flow fractionation (FIFFF, AF2000 Postnova) and atomic force microscopy (AFM, XW-100, Park Systems). The AgNPs in UHP water were found to be predominantly present as single particles with an average diameter of 17-40 nm (\pm 0-10 nm) (depending on the characterization technique used) and a zeta potential of -37 mV (Beddow et al., 2014a). In the present study, the hydrodynamic diameter and zeta-potential of the AgNPs in 0.2 μ m filtered (Whatman PLC) water from the low-salinity (Hythe) and mesohaline (Wivenhoe) sites were measured by dynamic light scattering (Zetasizer Nano ZS ZEN3600, Malvern Instruments Ltd) using methods previously described (Beddow et al., 2014a; Beddow et al., 2014b). The rate by which soluble Ag was released from the AgNPs dispersed in either ultra-high purity (UHP) water or estuarine water from the two sites studied was determined over 25 days, using dialysis with a 1 kDa cut-off membrane as previously described (Beddow et al., 2014a).

Sample sites and analysis

Surface sediments (6 kg from the top 0-2 cm layer of sediment) and overlying water samples (16 L from the top 50 cm of the water column) were collected in October 2011 from two sites along the Colne estuary, UK: the Hythe (51°52'47.3"N; 0°55'43.1"E), at the estuary head which has low-salinity, and Wivenhoe (51°52'8.7"N; 0°56'32.8"E), located mid-estuary which is mesohaline (Smith et al., 2007) (Fig. 1). The sampling sites were selected as they were both downstream of a large wastewater treatment facility and therefore a potential release site for AgNPs. Water salinities were determined at the time of sample collection using a portable refractometer (CETI, Belgium). To determine the background Ag⁺ content of

sediments, samples were prepared by acid digestion and analysed by ICPMS (ELAN 9000, Perkin Elmer, USA) according to the US EPA method 3051A (US EPA, 2007), by Dr. Macoura Kone, University of Alberta, Canada. Sediment slurries were prepared from 1 part sediment mixed with 2 parts overlying water. The dissolved organic carbon (DOC) concentrations of water samples filtered through 0.2 μm pore size, 47 mm diameter Millipore Durapore filters (Merck Millipore, UK), were measured using a high-temperature combustion method (Shimadzu TOC-VCSH, UK) against a set of freshly prepared glucose standards (0.1-10 mg L^{-1}). The anion/cation content of slurry pore waters were measured using a Dionex ICS-3000 (Thermo Scientific, UK) against a set of freshly prepared standards ranging from 0-500 μM (cations) or 0-200 μM (anions). Sediment dry weights (dws) were determined by drying 5 g slurry at 60°C (Hybaid oven, UK) to a constant weight.

Microcosm setup

Sediment slurries were prepared by mixing one part wet sediment with three parts overlying water from the corresponding site. Triplicate oxic microcosms containing 300 mL slurry from either the low-salinity (Hythe) (91 g dws L^{-1} slurry) or mesohaline (Wivenhoe) (130 g dws L^{-1} slurry) sites along the estuary, and AgNPs at final concentrations of 0.5 or 50 mg L^{-1} were prepared in sterile 500 mL conical flasks. AgNP concentrations were selected based on exposure models which show that predicted environmental concentrations (PECs) for AgNPs in different environmental compartments are in the range of those quoted by Maurer-Jones et al (2013) in their overview of bacterial nanotoxicity.

Triplicate control microcosms without the addition of nanoparticles were also set up. Triplicate killed control microcosms were prepared by tyndallization

(autoclaving under pressure to 121°C for 15 mins over three successive days, with periods of incubation at 20°C in between) and the viability of killed controls was checked by plating 100 µL of slurry onto R2A agar and incubating for two weeks at 20°C. Flasks were incubated at 20°C in the dark with shaking (100 rpm) for 14 days.

Nitrification potential rate (NPR) analysis

Following 0, 1, 7 and 14 days incubation, sub-samples (33 mL) from both the live and killed sediment slurry microcosms were transferred into sterile 100 mL conical flasks and further diluted with 67 mL filtered water (filtered through 0.2 µm pore size, 47 mm diameter Millipore Durapore filters (Merck Millipore, UK) into sterile glass 1L duran bottles and stored at 4°C) from the corresponding site (low-salinity (Hythe) or mesohaline (Wivenhoe)). Day 0 sub-samples were taken immediately prior to the addition of AgNPs to microcosms, and within four hours of sample collection from the estuary. Microcosm nitrification potential rates were analysed by measuring the ammonium concentration in diluted slurry sub-samples at three separate time points (0, 24 and 48 hours) as previously described (Li et al., 2015). Ammonium concentration in sediment pore water was analyzed by the indophenol blue spectrophotometric method (Pai et al., 2001). Rates of ammonium removal were determined by linear regression analysis of the concentration of ammonium with time.

Nucleic acid extraction

Following 0, 1, 7 and 14 days incubation, nucleic acids were extracted from slurry samples (1 mL) using a bead-beating method as described by Stephen et al. (1996). Extracted DNA was pelleted by centrifugation ($12,000 \times g$ for 5 min) and washed with 0.5 mL 70 % (v/v) ice cold ethanol, then air dried for 20 min at room temperature before being resuspended in 100 μ L sterile distilled water and stored at -80°C . DNA yield ranged between 0.5-1.0 μ g and DNA purity was checked at 260:280 nm using a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, UK).

Real-time qPCR analysis of AOB and AOA amoA genes

DNA standards for qPCR analysis were created from PCR-amplified low-salinity (Hythe) estuarine sediment DNA using the primer pair 1F/2R-TC (Rotthauwe et al., 1997; Nicolaisen & Ramsing, 2002) to target the AOB *amoA* gene, and the primer pair A23F/A616R (Tourna et al., 2008) to target the AOA *amoA* gene. PCR mixtures for DNA standard preparation (total 50 μ L) contained 1 μ L DNA template, and a final concentration of $1\times$ PCR buffer (containing 1.5 mM MgCl_2) (Qiagen), 0.4 μ M of each primer, 200 μ M of each dNTP and 1U *Taq* DNA polymerase (TopTaq™, Qiagen, UK). Thermocycling consisted of 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 57°C (AOB) or 55°C (AOA) for 30 s and 72°C for 1 min, with a final elongation step of 72°C for 7 min (Gene Amp® PCR system 9700 Thermocycler, Applied Biosystems, UK). Resulting amplicons were purified using a QIAquick PCR purification kit (Qiagen, UK) and quantified on a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, UK). Target abundances were calculated for each sample using the following equation, assuming a mass of 660 Da for double stranded DNA:

$$\text{Copy no.} = \frac{*6.023 \times 10^{23} \text{ (copies mol}^{-1}\text{)} \times \text{standard concentration (g } \mu\text{L}^{-1}\text{)}}{\text{MW (g mol}^{-1}\text{)}}$$

*Avogadro's constant

DNA template concentration used in q-PCR standard curves ranged from 10^1 to 10^7 target genes μL^{-1} . Q-PCR analysis was performed on triplicate microcosm samples, no template controls and standard curves, in triplicate (technical replicates), with each primer set, using a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, UK). Each 20 μL reaction contained 1 μL DNA template, 1 \times SensiFAST™ SYBR No-ROX dye (Bioline Reagents Ltd, UK) and 100 nM of each primer, prepared in BrightWhite 96-well plates (Primer Design Ltd., UK). Thermocycling consisted of 95°C for 5 min followed by 40 cycles of 95°C for 10s and 60°C for 30 s. Target genes were quantified against their corresponding standard curves using CFX Manager software (Bio-Rad Laboratories, UK) with automatic settings for Cq values and the baseline. The detection limit of the qPCR assay was ≈ 28 gene copies (≈ 33 cycles). Standard curve coefficient correlations were 0.99 and 0.97 for AOB and AOA *amoA* genes, respectively, and average efficiencies of qPCR reactions were 94.0% and 98.8% for AOB and AOA *amoA* standard curves, respectively.

Statistical analyses

Where data were normally distributed, one-way ANOVAs with Tukey's HSD post-hoc analyses and t-tests were used to test for significant differences in NPRs and gene copy numbers between samples (PASW Statistics v18, IBM, USA).

Acknowledgements

This work was supported by Natural Environment Research Council (NERC) (NE/H525289/1), the Centre for Environment Fisheries and Aquaculture Science (CEFAS) and the University of Essex. The support of the NERC Facility for Environmental Nanoscience Analysis and Characterization is acknowledged. The background Ag content of sediments was determined courtesy of Ania Ulrich and Macoura Kone (University of Alberta, Canada). We thank Mr. Farid Benyahia and Mr. John Green for technical assistance. We thank Dr. Terry McGenity, Dr. Boyd McKew and Professor David Nedwell (University of Essex) for their useful comments on the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

- Abell, G.C.J., Revill, A.T., Smith, C., Bissett, A.P., Volkman, J.K. and Robert, S.S. (2010) Archaeal ammonia oxidizers and *nirS*-type denitrifiers dominate sediment nitrifying and denitrifying populations in a subtropical macrotidal estuary. *ISME J* **4**: 286-300.
- Ager, D., Evans, S., Li, H., Lilley, A.K., and van der Gast, C.J. (2010) Anthropogenic disturbance affects the structure of bacterial communities. *Environ Microbiol* **12**: 670-678.
- Allison, S.D., and Martiny, J.B.H. (2008) Resistance, resilience and redundancy in microbial communities. *Proc Natl Acad Sci U S A* **105**: 11512-11519.
- Arnaout, C., and Gunsch, C.K. (2012) Impacts of silver nanoparticle coating on the nitrification potential of *Nitrosomonas europaea*. *Environ Sci Technol* **46**: 5387-5395.
- Auffan, M., Rose, J., Wiesner, M.R., and Bottero, J-Y. (2009) Chemical stability of metallic nanoparticles: A parameter controlling their potential cellular toxicity in vitro. *Environ Pollut* **157**: 1127-1133.
- Balasubramanian, R., et al. (2010). Oxidation of methane by a biological dicopper centre. *Nature* **465**: 115–119
- Beddow, J., Stölpe, B., Cole, P., Lead, J.R., Sapp, M., Lyons, B.P., et al. (2014a) Effects of engineered nanoparticles on the growth and activity of ecologically important microbes. *Environ Microbiol Rep* **6**: 448-458.
- Beddow, J., Stölpe, B., Cole, P., Lead, J.R., Sapp, M., Lyons, B.P., et al. (2014b) Estuarine sediment hydrocarbon-degrading microbial communities

- demonstrate resilience to nanosilver. *International Journal of Biodeterioration and Biodegradation* **96**: 206-215.
- Beman, J.M. and Francis, C.A. (2006) Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahia del Tobari, Mexico. *Appl Environ Microbiol* **72**:7767-7777.
- Bernhard, A.E., Landry, Z.C., Blevins, A., de la Torre, J.R., Giblin, A.E., and Stahl, D.A. (2010) Abundance of ammonia-oxidizing Archaea and Bacteria along an estuarine salinity gradient in relation to potential nitrification rates. *Appl Environ Microbiol* **76**: 1285-1289.
- Blaser, S.A., Sheringer, M., Macleod, M., and Hungerbühler, K. (2008) Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. *Sci Total Environ* **390**: 396-409.
- Bouskill, N.J., Eveillard, D., Chien, D., Jayakumar, A., and Ward, B.B. (2012) Environmental factors determining ammonia-oxidizing organism distribution and diversity in marine environments. *Environ Microbiol* **14**: 714-729.
- Bradford, A., Handy, R.D., Readman, J.W., Atfield, A., and Mühling, M. (2009) Impact of silver nanoparticle contamination on the genetic diversity of natural bacterial assemblages in estuarine sediments. *Environ Sci Technol* **43**: 4530-4536.
- Caffrey, J.M., Bano, N., Kalanetra, K. and Hollinbaugh, J.T. (2007) Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME J* **1**: 660-662.
- Choi, O., and Hu, Z. (2008) Size Dependent and Reactive Oxygen Species Related Nanosilver Toxicity to Nitrifying Bacteria. *Environ Sci Technol* **42**: 4583-4588.

Choi, O., Deng, K., Kim, N., Ross, L., Surampalli, R., and Hu, Z. (2008) The inhibitory effects of silver nanoparticles, silver ions and silver chloride colloids on microbial growth. *Water Res* **42**: 3066-3074.

Choi, O., and Hu, Z. (2009) Nitrification inhibition by silver nanoparticles. *Water Sci Technol* **59**: 1699-1702.

Christian, P., von der Kammer, F., Baalousha, M., and Hofmann, T. (2008) Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology* **17**: 326-343.

Cleveland, D., Long, S.E., Pennington, P.L., Cooper, E., Fulton, M.H., Scott, G.I., *et al.* (2012) Pilot estuarine mesocosm study on the environmental fate of silver nanoparticles leached from consumer products. *Sci Total Environ* **421-422**: 267-272.

Damashek, J., Smith, J.M., Mosier, A.C., and Francis, C.A. (2015) Benthic ammonia oxidisers differ in community structure and biogeochemical potential across a riverine delta. *Frontiers in Microbiology* **5**:1-18.

Das, P., Williams, C.J., Fulthorpe, R.R., Hoque, E., Metcalfe, C.D., and Xenopoulos, M.A. (2012b) Changes in bacterial community structure after exposure to silver nanoparticles in natural waters. *Environ Sci Technol* **46**: 9120-9128.

Das, P., Xenopoulos, M.A., Williams, C.J., Hoque, E., and Metcalfe, C.D. (2012a) Effects of silver nanoparticles on bacterial activity in natural waters. *Environ Toxicol Chem* **31**: 122-130.

Doiron, K., Pelletier, E., and Lemarchand, K. (2012) Impact of polymer-coated silver nanoparticles on microbial communities: a microcosm study. *Aquat Toxicol* **124-125**: 22-27.

Dong, L.F., Thornton, D.C.O., Nedwell, D.B., and Underwood, G.J.C. (2000) Denitrification in sediments of the River Colne estuary, England. *Mar Ecol Progr Ser* **203**: 109-122.

El Badawy, A.M., Luxton, T.P., Silva, R.G., Scheckel, K.G., Suidan, M.T., and Tolaymat, T.M. (2010) Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticle suspensions. *Environ Sci Technol* **44**: 1260-1266.

Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S., and Lead, J.R. (2011) Silver nanoparticles: Behaviour and effects in the aquatic environment. *Environ Int A*. **37**: 517-531.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., and Kim, J.O. (2000) A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* **15**: 662-668.

Gottschalk, F., Sonderer, T., Scholz, R.W., and Nowack, B., (2009) Modeled environmental concentrations of engineered nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for different regions. *Environ Sci Technol* **43**: 9216-9222.

Handy, R., Owen, R., and Valsami-Jones, E. (2008) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* **17**: 315-325.

Herbert, R.A. (1999) Nitrogen cycling in coastal marine environments. *FEMS Microbiol Rev* **23**: 563-590.

Hollocher, T.C. (1981) Oxidation of ammonia by *Nitrosomonas europaea*. Definite 180-tracer evidence that hydroxylamine formation involves a monooxygenase. *The Journal of Biological Chemistry* **256**: 10834-10836.

Jin, T., Zhang, T., and Yan, Q. (2010) Characterization and quantification of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. *Appl Microbiol Biotechnol* **87**: 1167–1176.

Joye, S.B., and Hollibaugh, J.T. (1995) Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* **270**: 623-625.

Kim, B., Park, C., Murayama, M., and Hochella, M.F. Jr (2010) Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. *Environ Sci Technol* **44**: 7509-7514.

Kowalchuk, G.A., and Stephen, J.R. (2001) Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Ann Reviews Microbiol* **55**: 485-529.

Levard, C., Hotze, E.M., Lowry, G.V., and Brown, G.E. (2012) Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environ Sci Technol* **46**: 6900-6914.

Levard, C., Mitra, S., Yang, T., Jew, A.D., Badireddy, A.R., Lowry, G.V., and Brown, G. (2013) Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to *E.coli*. *Environ Sci Technol* **47**: 5738-5745.

Li, J., Nedwell, D.B., Beddow, J., Dumbrell, A.J., McKew, B.A., Thorpe, E.L., and Whitby, C. (2015) *amoA* gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria and not archaea dominate N cycling in the Colne estuary, United Kingdom. *Appl Environ Microbiol* **81**: 159-165.

Liang, Z., Das, A., and Hu, Z. (2010) Bacterial response to a shock load of nanosilver in an activated sludge treatment system. *Water Res* **44**: 5432-5438.

Lieberman, R.L., and Rosenzweig, A.C. (2005) Crystal structure of a membrane bound metalloenzyme that catalyses the biological oxidation of methane. *Nature* **434**:177–182.

Liu, J., and Hurt, R.H. (2010) Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol* **44**: 2169-2175.

Lowry, G.V., Gregory, K.B., Apte, S.C., and Lead, J.R. (2012) Transformations of nanomaterials in the environment. *Environ Sci Technol* **46**: 6893-6899.

Ma, R., Levard, C., Marinkos, S., Cheng, Y., Liu, J., Michel, F.M., *et al.* (2012) Size-controlled dissolution of organic-coated silver nanoparticles. *Environ Sci Technol* **46**: 752-759.

Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R., and Stahl, D.A. (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* **461**: 976-979.

Maurer-Jones, M.A., Gunsolus, I.L., Murphy, C.J., and Haynes, C.L. (2013) Toxicity of engineered nanoparticles in the environment. *Analyt Chem* **85**: 3036-304.

McTavish, H., Fuchs, J.A., and Hooper, A.B. (1993) Sequence of the gene coding for ammonia monooxygenase in *Nitrosomonas europaea*. *J Bacteriol* **175**: 2436-2444.

Moin, N.S., Nelson, K.A., Bush, A., and Bernhard, A.E. (2009) Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. *Appl Environ Microbiol* **75**: 7461-7468.

Morones, J., Elechiguerra, J., Camacho, A., Holt, K., Kouri, J., Ramirez, J., *et al.* (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* **16**: 2346.

Mosier, A.C., and Francis, C.A. (2008) Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. *Environ Microbiol* **10**: 3002-3016.

Nel, A., Xia, T., Madler, L., and Li, N. (2006) Toxic potential of materials at the nanolevel. *Science* **311**: 622-627.

Nicol, G.W., Leininger, S., Schleper, C., and Prosser, J.I. (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol* **10**: 2966-2978.

Nicol, G.W., Tscherko, D., Embley, T.M., and Prosser, J.I. (2005) Primary succession of soil *Crenarchaeota* across a receding glacier foreland. *Environ Microbiol* **7**: 337-347.

Nicolaisen, M.H., and Ramsing, N.B. (2002) Denaturing gradient gel electrophoresis (DGGE) approaches to study the diversity of ammonia-oxidizing bacteria. *J Microbiol Methods* **50**: 189-203.

Nowack, B., Krug, H.F., and Height, M. (2011) 120 years of nanosilver history: implications for policy makers. *Environ Sci Technol* **45**: 1177-1183.

Ogilvie, B., Nedwell, D.B., Harrison, R.M., Robinson, A., and Sage, A. (1997) High nitrate, muddy estuaries as nitrogen sinks: the nitrogen budget of the River Colne estuary (United Kingdom). *Mar Ecol Progr Ser* **150**: 217-228.

Pai, S.C., Tsau, Y.J., and Yang, T.I. (2001) pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method. *Anal Chim Acta* **434**: 209-216.

Pal, S., Tak, Y.K., and Song, J.M. (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**: 1712-1720.

Papaspyrou, S., Smith, C.J., Dong, L.F., Whitby, C., Dumbrell, A.J., and Nedwell, D.B. (2014) Nitrate reduction functional genes and nitrate reduction potentials persist in deeper estuarine sediments. Why? Plos One (DOI: 10.1371/journal.pone.0094111).

PEN (2016) The Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies website. www.nanotechproject.org/

Pourzahedi, L. and Eckelman, M.J. (2015) Environmental life cycle assessment of nanosilver enabled bandages. *Environ Sci Technol* **49**: 361-368

Prosser, J.I. (1989) Autotrophic Nitrification in Bacteria. In *Advances in Microbial Physiology, Volume 30*, pp.125 - 182 Academic Press Ltd, London.

Radniecki, T. S., Stankus, D. P., Neigh, A., Nason, J. A., and Semprini, L. (2011) Influence of liberated silver from silver nanoparticles on nitrification inhibition of *Nitrosomonas europaea*. *Chemosphere* **85**: 43-49.

Reinsch, B.C., Levard, C., Li, Z., Ma, R., Wise, A., Gregory, K.B., et al. (2012) Sulfidation of silver nanoparticles decreases *Escherichia coli* growth inhibition. *Environ Sci Technol* **46**: 6992-7000.

Richardson, S.D., and Ternes, T.A. (2011) Water analysis: Emerging contaminants and current issues. *Anal Chem* **83**: 4614-4648.

Rotthauwe, J.H., Witzel, K.P., and Liesack, W. (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* **63**: 4704-4712.

Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P., and Sloth, N. (1999) Effects of salinity on NH₄⁺ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* **22**: 21-30.

Schimel, J., Balsler, T.C., and Wallenstein, M. (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**: 1386-1394.

Smith, C.J., Nedwell, D.B., Dong, L.F., and Osborn, A.M. (2007) Diversity and abundance of nitrate reductase genes (*narG* and *napA*) nitrite reductase genes (*nirS* and *nrfA*) and their transcripts in estuarine sediments. *Appl Environ Microbiol* **73**: 3612-3622.

Sondi, I., and 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**: 177-182.

Stephen, J.R., McCaig, A.E., Smith, Z., Prosser, J.L., and Embley, T.M. (1996) Molecular diversity of soil and marine 16S rRNA gene sequences related to β -subgroup ammonia-oxidizing bacteria. *Appl Environ Microbiol* **62**: 4147-4154.

Strauss, E.A., and Dodds, W.K. (1997) Influence of protozoa and nutrient availability on nitrification rates in subsurface sediments. *Microb Ecol* **34**: 155-165.

Sun, Y.S., Gottschalk, F., Hungerbühler, K., and Nowack, B. (2014) Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environ Pollut* **185**: 69-76.

Tejamaya, M., Römer, I., Merrifield, R.C., and Lead, J.R. (2012) Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environ Sci Technol* **46**: 7011-7017.

Thornton, D.C.O., Dong, L.F., Underwood, J.C., and Nedwell, D.B. (2007) Sediment-water inorganic nutrient exchange and nitrogen budgets in the Colne Estuary, UK. *Mar Ecol Prog Ser* **337**: 63-77.

Tourna, M., Freitag, T.E., Nicol, G.W., and Prosser, J.I. (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ Microbiol* **10**: 1357-1364.

US EPA (2007) Method 3051A, Microwave assisted acid digestion of sediments, sludges, soils and oils. In 'Test methods for evaluating solid waste'. US Environmental Protection Agency, Washington, DC, USA. 3rd Edition.

Walker, C.B., et al. (2010). *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proc. Natl. Acad. Sci. U. S. A.* **107**: 8818–8823.

Whiteley, C.M., Valle, M.D., Jones, K.C., and Sweetman, A.J. (2013) Challenges in assessing release, exposure and fate of silver nanoparticles within the UK environment. *Environ Sci: Processes Impacts* **15**: 2050-2058.

Wuchter, C. Abbas, B., Coolen, M.J., Herfort, L., Van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G.J., Middelburg, J.J., Schouten, S., Sinninghe Damsté J.S. (2006) Archaeal nitrification in the ocean. *Proc. Natl. Acad. Sci. U. S. A* **103** (33): 12317-12322.

Xiu, Z., Zhang, Q., Puppala, H.L., Colvin, V.L., and Alvarez, P.J.J. (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Lett* **12**: 4271-4275.

Yang, Y., Quensen, J., Mathieu, J., Wang, Q., Wang, J., Li, M., Tiedje, J. M., and Alvarez, P. J. J. (2014) Pyrosequencing reveals higher impact of silver nanoparticles than Ag⁺ on the microbial community structure of activated sludge. *Water Res* **48**: 317-325.

Yu, S-J., Yin, Y-G., and Liu, J-F. (2013) Silver nanoparticles in the environment *Environmental Science: Processes and Impacts* **15**: 78-92.

Yuan, Z., Li, J., Cui, L., Xu, B., Zhang, H., and Yu, C. (2013) Interaction of silver nanoparticles with pure nitrifying bacteria. *Chemosphere* **90**: 1404-1411.

Zhang, W., Yao, Y., Sullivan, N., and Chen, Y. (2011) Modeling the primary size effects of citrate-coated silver nanoparticles on their ion release kinetics. *Environ Sci Technol* **45**: 4422–4428.

Zheng, Y., Hou, L., Newell, S., Liu, M., Zhou, J., Zhao, H. et al. (2014) Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze estuary. *Appl Environ Microbiol* **80**: 408-419.

Accepted Article

Titles and Legends to Figures

Fig. 1. Map of the sampling sites (marked with stars) along the Colne estuary, United Kingdom. Panels show the abiotic characteristics of estuarine water and sediment at the time of collection. Site 1 (the Hythe, low-salinity) was located towards the freshwater head of the estuary, just downstream of a large sewage treatment works (STW). Site 2 (Wivenhoe, mesohaline) was located further downstream of site 1, towards the marine mouth of the estuary. Ag refers to background silver concentration per kg dry weight sediment (dws). \pm represents the standard error of the mean ($n=3$). ND = Not detected.

Fig. 2. Release of soluble silver from 5 mg L^{-1} AgNP suspensions in UHP water (Δ), low-salinity (Hythe) estuarine water (\blacksquare) and mesohaline (Wivenhoe) estuarine water (\bullet), over (A) 30 hours and (B) 25 days. Each point represents the average where $n=2$. Extremities of the vertical bar on each point represent the data range.

Fig. 3. Comparison of dissolution rates as a function of Cl/Ag ratio reported by Levard et al. (2013) (\blacklozenge) with our data (\blacksquare) over the first five hours.

Fig. 4. AOB and AOA *amoA* gene abundance (mean \pm s.e.; $n=3$) in low-salinity (Hythe) and mesohaline (Wivenhoe) sediments over time in the presence of 0.5 or 50 mg L^{-1} AgNPs. Controls do not contain any AgNPs. Results that are significantly different to controls are shown by * ($p \leq 0.05$).

Fig. 5. Autotrophic nitrification potential rates (NPRs) in (A) low-salinity (Hythe) and (B) mesohaline (Wivenhoe) sediments over 14 days in the presence of 0.5 or 50 mg

L⁻¹ AgNPs. Controls do not contain any AgNPs. Results that are significantly different to controls are shown by ** ($p \leq 0.01$). Error bars represent the standard error of the mean ($n=3$).

Table 1. Characteristics of AgNPs in sterile Ultra High Purity Water (UHP) and estuarine waters that were either low salinity (Hythe) or mesohaline (Wivenhoe) (\pm standard error of the mean, $n=15$), determined by dynamic and electrophoretic light scattering (DLS) as described in Beddow et al. (2014a). Results that are significantly different to those in ultra-high purity (UHP) water are shown by ** ($p \leq 0.01$).



Low-salinity (Hythe)	
pH	7.5
Salinity	3.5 ‰
DOC	9.1 ± 0.2 mg L ⁻¹
Ag	0.37 ± 0.01 mg kg ⁻¹ dws
Cl ⁻	80 ± 9 mM
NH ₄ ⁺	19 ± 1 μM
NO ₃ ⁻	537 ± 89 μM
NO ₂ ⁻	86 ± 15 μM
PO ₄ ³⁻	221 ± 2 μM
SO ₄ ²⁻	7375 ± 737 μM

Mesohaline (Wivenhoe)	
pH	7.5
Salinity	27 ‰
DOC	3.4 ± 0.2 mg L ⁻¹
Ag	0.33 ± 0.01 mg kg ⁻¹ dws
Cl ⁻	278 ± 2 mM
NH ₄ ⁺	36 ± 1 μM
NO ₃ ⁻	227 ± 18 μM
NO ₂ ⁻	135 ± 10 μM
PO ₄ ³⁻	ND
SO ₄ ²⁻	23060 ± 193 μM

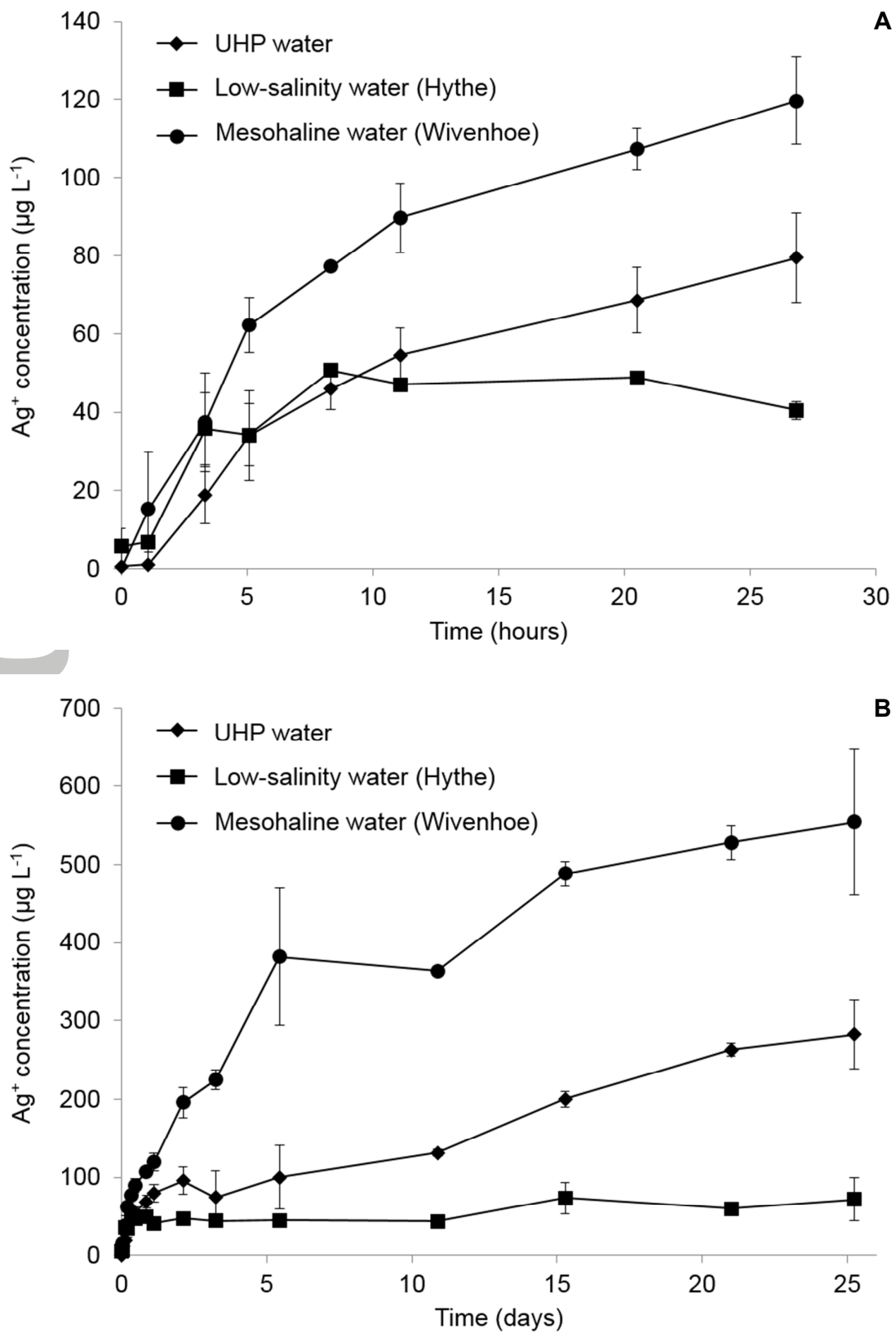


Fig. 2.

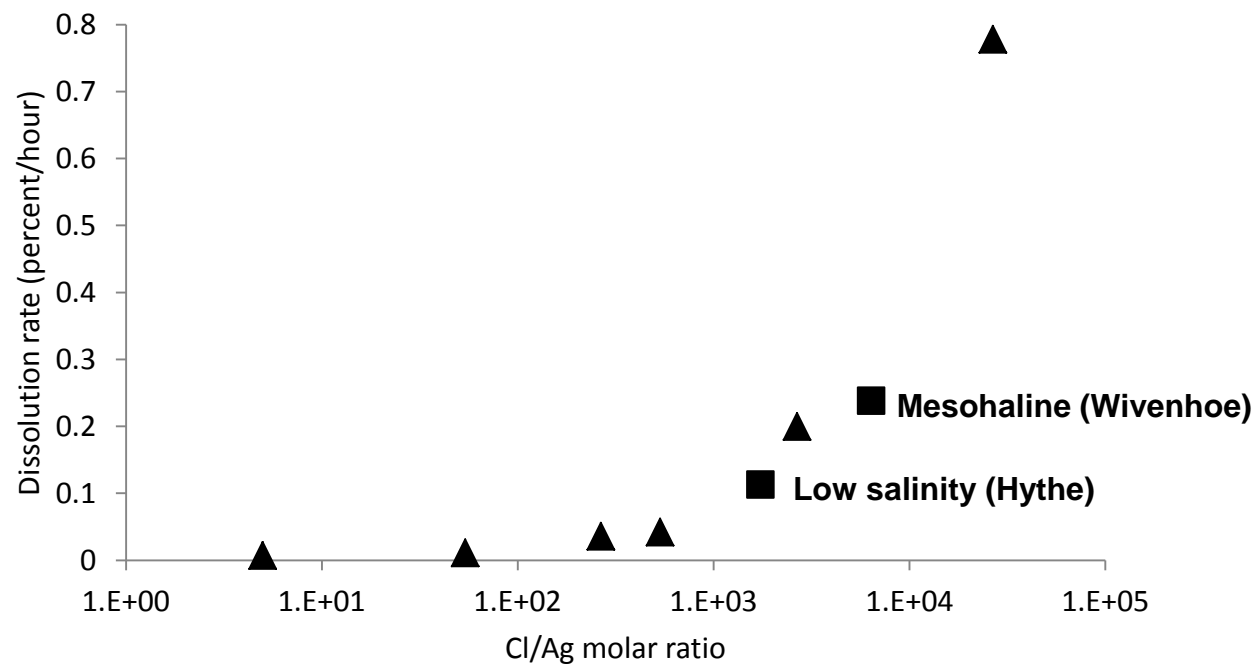


Fig. 3

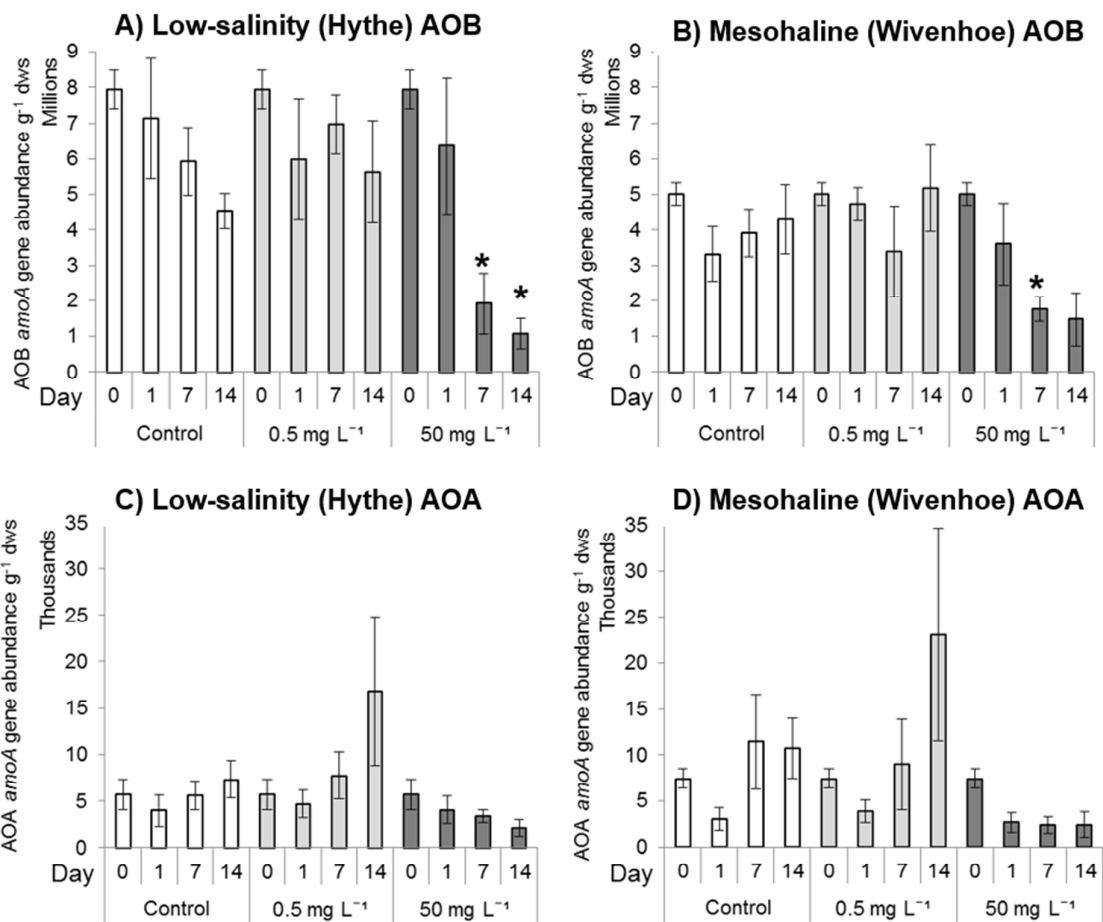


Fig. 4.

Accepted Article

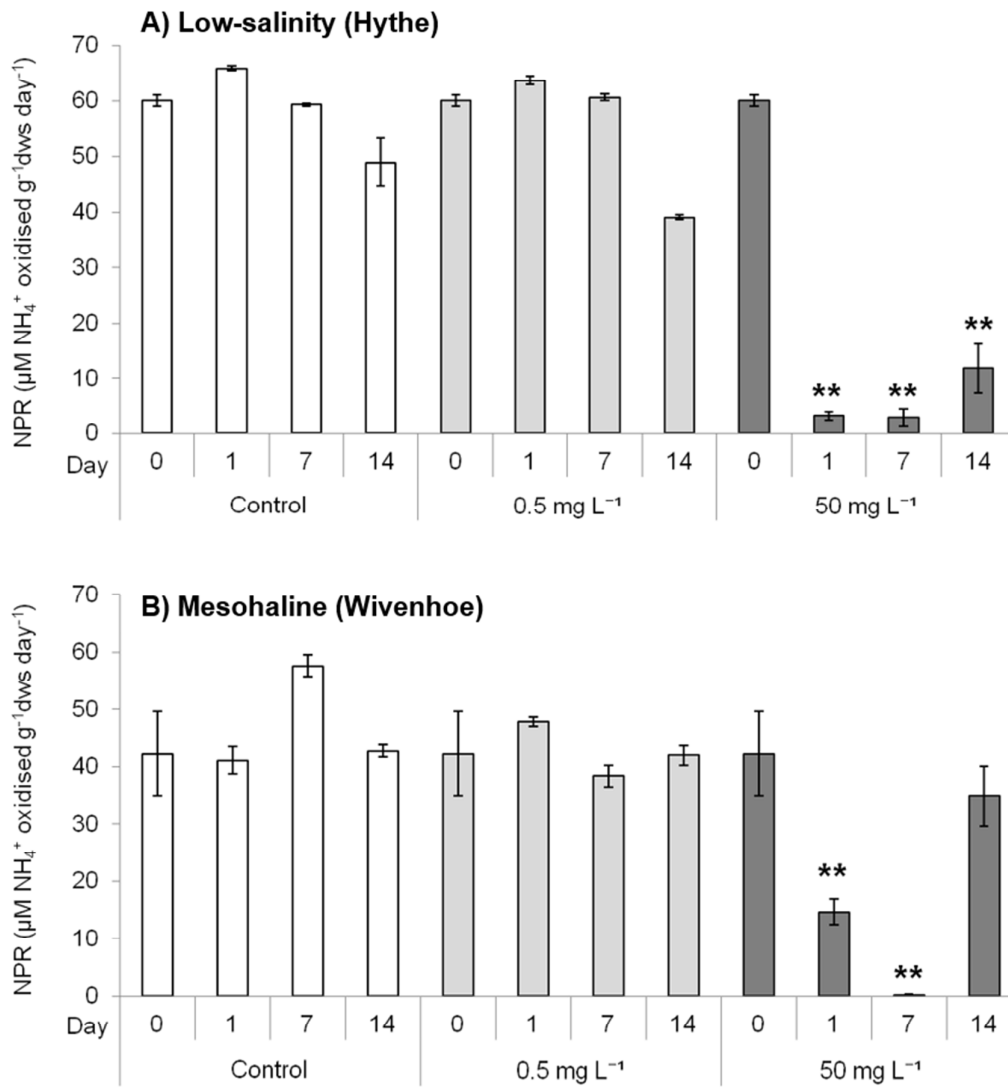


Fig. 5

Table 1.

Water	DLS diameter (nm)	Zeta(ζ) –potential (mV)
UHP	35 \pm 0.2	-37 \pm 0.3
Low salinity (Hythe)	30* \pm 0.1	-12* \pm 1.1
Mesohaline (Wivenhoe)	60* \pm 0.5	-5* \pm 2.0

Accepted Article