Shifts in the metabolic function of a benthic estuarine microbial community following a single pulse exposure to silver nanoparticles

Echavarri-Bravo, Virginia; Paterson, Lynn; Aspray, Thomas J.; Porter, Joanne; Winson, Michael K.; Thornton, Barry; Hartl, Mark G. J.

Published in:
Environmental Pollution

DOI:
10.1016/j.envpol.2015.02.033

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Link to publication in Heriot-Watt University Research Portal

Citation for published version (APA):
Shifts in the metabolic function of a benthic estuarine microbial community following a single pulse exposure to silver nanoparticles

Virginia Echavarri-Bravo a, Lynn Paterson b, Thomas J. Aspray a, Joanne S. Porter a, Michael K. Winson c, Barry Thornton c, Mark G.J. Hartl a, ε

a Heriot-Watt University, Centre for Marine Biodiversity & Biotechnology, School of Life Sciences, Riccarton, Edinburgh EH14 4AS, Scotland, UK
b SUPA, Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, School of Engineering and Physical Sciences, Riccarton, Edinburgh EH14 4AS, Scotland, UK
c Biotechnology, School of Life Sciences, Riccarton, Edinburgh EH14 4AS, Scotland, UK

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abstract

The increasing use of silver nanoparticles (AgNPs) as a biocidal agent and their potential accumulation in sediments may threaten non-target natural environmental bacterial communities. In this study a microcosm approach was established to investigate the effects of well characterized OECD AgNPs (NM-300) on the function of the bacterial community inhabiting marine estuarine sediments (salinity 31‰). The results showed that a single pulse of NM-300 AgNPs (1 mg L⁻¹) that led to sediment concentrations below 6 mg Ag kg⁻¹ dry weight inhibited the bacterial utilization of environmentally relevant carbon substrates. As a result, the functional diversity changed, but recovered after 120 h under the experimental conditions. This microcosm study suggests that AgNPs under environmentally relevant experimental conditions can negatively affect bacterial function and provides an insight into the understanding of the bacterial community response and resilience to AgNPs exposure, important for informing relevant regulatory measures.

1. Introduction

The rise of nanotechnology has led to an increase in the manufacturing and use of novel materials that, at the nano-scale, exhibit different characteristics to their respective bulk materials. AgNPs are an example, and, owing to their antimicrobial properties, are being incorporated into a wide variety of applications, such as health and personal care products, and also in disinfection and anti-biofouling agents, similar, for example to chlorination. However, silver is a toxic and persistent metal that can be bio-accumulated at different trophic levels (Boisson et al., 1998; Croteau et al., 2011; López-Serrano et al., 2014). As much of the AgNPs and derived forms of silver are disposed of through domestic waste water (Blaser et al., 2008), the question arises whether accumulation in the receiving estuarine environment could negatively affect the functioning of resident bacterial communities that play an important role in biogeochemical processes, such as nutrient recycling and bioremediation (Gao et al., 2011).

Toxicity data of AgNPs have been generated using a wide variety of particle types and target organisms (e.g. different bacterial species) making comparison across multiple studies difficult. In addition, environmental conditions can affect the physicochemical characteristics of the AgNPs, and, as a result their toxicity. Thus there is an urgent need for studies that model environmentally relevant and realistic conditions (Epstein et al., 2014; Stone et al., 2014). While research in the marine environment relating to the ecotoxicity of engineered nanomaterials (ENMs) in general and AgNPs in particular has gradually increased over the last decade (Baker et al., 2014), studies relating to the effects and fate of AgNPs in marine sediments are still scarce. It is particularly important to fill this knowledge gap, because estuarine sediment is one of the environmental compartments where silver is likely to accumulate (Luoma et al., 1995). For this reason, the present study established a series of microcosms with the aim of characterizing the environmental hazard of AgNPs on the functioning of the benthic bacterial community inhabiting marine estuarine sediments. The effects of AgNPs on the degradation of environmentally relevant substrates and on the bacterial functional diversity were assessed with the Biolog EcoPlate™. Living bacterial community structure and
bacterial abundance were examined with the phospholipid fatty acid (PLFA) analysis. This is important for further understanding the potential risks of AgNPs on the ecosystem services provided by bacteria as for example nutrient recycling. The conditions in the microcosms were typically marine (salinity 31‰) as under high concentrations of chloride, silver is potentially more mobile than in fresh water (Luoma et al., 1995) and exhibit higher antibacterial activity (Gupta et al., 1998; Levard et al., 2013). The AgNPs used were NM-300, a reference nanomaterial accepted by the OECD Working Party on Manufactured Nanomaterials (WPMM) international testing programme (Klein et al., 2011).

2. Materials and methods

2.1. Silver nanoparticles

The silver nanoparticles used in the exposures were the NM-300 Silver Ø < 20 nm purchased from LGC Standards (JRCNM0300a) and supplied in an aqueous suspension. The concentration of AgNPs in the suspension was 10% (w/w) of the total weight. The stabilizing agents were polyoxyethylene glycerol trioleate and Tween 20 (all at 4% w/w) (Klein et al., 2011) and 7% NH4NO3 as the NH4+ source. The concentration of the NM-300 AgNPs used in the present study was analysed with transmission electron microscopy (Jeol 1200 TEM; FENAC, University of Birmingham) and the surface charge or zeta potential assessed with a Zetasizer Nano (Malvern instrument). The ionic silver content of fresh AgNPs stock suspensions was measured in Milli-Q water with an ion selective electrode (ISE, Nico 2000 Ltd).

2.2. Working suspensions

Working suspensions for dosing the microcosms were prepared at an initial concentration of AgNPs of 360 mg L⁻¹ (within the previously shown range of stability) in sterile Milli-Q water following the manufacturer’s protocol (Klein et al., 2011) and as previously reported in the literature for other AgNP dosing experiments (Bradford et al., 2009; Kermanizadeh et al., 2012). The following modifications were made in order to ensure optimal particle dispersion in the working suspension: AgNP suspensions were vigorously shaken for 4 min and ultrasonicated twice for 15 min in an ultrasonic bath (Grant XUB25) containing ice.

2.3. Microcosm set up

The microcosms were established with intertidal sandy sediments and water samples collected from Cramond, Firth of Forth estuary (Scotland, United Kingdom, coordinates 55° 58' 59.19"N, 3° 17' 50.11"W). To minimize sediment exposure time to air during low tide, the bacterial communities inhabiting sediments in the present study were collected at the extreme lower eulittoral during springtides and therefore were mostly subtidal. Oxic sediments (depth 0–10 cm) were sampled with a hand-held corer that enabled the collection of the sediments with minimal perturbation and placed in cylindrical 5 L polypropylene (PP) containers (Ø 19.5 cm). All the containers for sampling and preparing the microcosms were cleaned with Decon Neutracon and 10% v/v HNO3 and sterilized at 121°C for 15 min. The initial nominal concentration of AgNPs in water was 1 mg L⁻¹ as reported in previous studies (Bradford et al., 2009; Colman et al., 2012, 2014). To achieve this concentration 3 L of well mixed estuarine water were dosed with 8.33 ml of the initial AgNPs working suspension (360 mg L⁻¹), and then gently poured on to the surface of the sediments in each microcosm, and sediments were subsequently contaminated through precipitation of the AgNPs contained in the overlaying water. As the aqueous matrix of the AgNPs contained stabilizing agents it was necessary to investigate the effects of these separately. The dispersant or carrier control treatment was prepared similarly to the AgNPs treatment with the appropriate concentrations of sterile dispersant (JRCNM03001a). This dispersant treatment contained the same stabilizing agents as described earlier, but without AgNPs. Three different treatments (4 replicates per treatment) were established, the AgNPs treatment containing dispersant (T1), the dispersant alone or carrier control (T2), and the negative control treatment or unamended treatment (TC), the latter consisting of tanks dosed with sterile Milli-Q water instead of AgNPs solution. Continuous aeration at the surface of the sediments and along the water column with minimal sediment disturbance was achieved using a bubble-wall system to mimic aeration provided by the tide. The microcosm exposures were run at a temperature of 10°C as samples were collected in the winter (January, 2013). Salinity (31‰), temperature, pH (7.9) and dissolved oxygen (7.8 mg L⁻¹) in the water were monitored during the course of the experiment and were stable throughout. The redox potential was measured in the first millimetres (<5 mm) of the superficial sediments and the mean value was 230±14 mV. The organic content in sediments was <1%.

2.3.1. Sample collection and preservation for chemical and biological analysis

Water and sediment samples, collected at different time points (0, 24, 72 and 120 h), were preserved at −70°C for biological analysis and at −20°C for chemical analysis. Water samples were collected in 50 ml sterile and metal-free plastic tubes. Sediment samples were collected from the superficial (aerobic) sediments (<3 mm depth) with sterile plastic cores (5 mm diameter). The sampling areas in the sediments were delimited with an empty sterile corer in order to avoid repeatedly sampling the same spot. Three samples were taken from each tank and pooled together (total wet weight approx. 10 g). Samples collected for community-level physiological profiling (CLPP) with the Biolog Ecoplates™ were preserved at 4°C and processed within 12 h.

2.3.2. Chemical analysis

2.3.2.1. Silver analysis. Water and sediment samples were acid-digested (USEPA-3005A, 1992; USEPA-3050B, 1996). The concentration of total silver was analysed by Atomic Absorption Spectroscopy (AAS) with a Perkin Elmer AAAnalyst TM spectrometer (DL: 30 ppb). The adequacy of the digestion method was confirmed with soil reference material (Sigma Aldrich Metals in Soil, number SQC-001); the recovery of silver was between 95 and 99.6% of the expected values and always within the accepted concentrations.

2.3.2.2. Inorganic nitrogen and chemical oxygen demand (COD). The concentration of inorganic nitrogen species (ammonium, nitrite and nitrate) and COD in the overlaying water were analysed during the course of the experiment. Details of the methods are provided in the Supplementary information (SI).

2.3.3. Biological analysis of sediment samples

2.3.3.1. Community-level physiological profiles (CLPP). The Biolog EcoPlate™ (Biolog Inc., Hayward, CA) contains 31 different environmentally relevant carbon sources that are distributed in triplicate in a 96 well plate. Each well contains a single carbon substrate together with a tetrazolium dye to indicate the positive utilization (respiration) of the respective carbon source by formation of a purple coloured formazan product. Bacterial cell extraction from sediments was carried out by the addition of 10 ml of sterile water (75% sea water and 25% distilled water) to the wet sediments (2 g), vortexing and vigorous shaking (5 min). The sediment suspension
was allowed to settle down for 1 min before the collection of the bacterial suspension from the supernatant and further diluted (1:10). Each well of the Biolog EcoPlates was inoculated with 140 μl of microbial suspension extracted from sediments as described above, and incubated without agitation (Garland and Mills, 1991) for 14 days at 15°C, to enhance bacterial growth without limiting the growth of psychrophiles and psychrotrophs (Moyer and Morita, 2007). The utilization of each carbon substrate was monitored with repeated plate readings each day by measuring the optical density at 590 nm (OD590 nm) (SpectraMax®). The shift patterns of the community structure were explored with PCA on the basis of the relative abundance of each PLFA. The PLFAs included in the analysis were those regarded as relevant bacterial biomarkers and comprised ≥1% of the total PLFA biomass (estimated in mole) as recommended by Zogg et al. (1997).

2.4. Effect of chloride on the physicochemical properties of AgNPs

The influence of chloride ions on the physicochemical properties of the NM-300 AgNPs was investigated in a separate experiment. Briefly, AgNPs (final concentration: 1.5 mg L\(^{-1}\)) were exposed in triplicate in the dark to different concentrations of NaCl (0, 10 and 20 g L\(^{-1}\)), with 0.1 M HEPES, at pH 7.5 at 20°C, and continuously gently shaken at 125 rpm on an orbital shaker. Particle persistence, agglomeration and surface charge were monitored at three different time points, 0, 24 and 48 h with ultraviolet–visible (UV–vis) spectroscopy (Shimadzu 1650 UV–VIS Spectrophotometer), dynamic light scattering (DLS) and zeta-potential (Zetasizer Nano-ZS, Malvern Instruments).

2.5. Data analysis

The statistical analysis was performed with SigmaStat 2.03 (Systat Software) and IBM® SPSS® Statistics 21, and graphs were produced with SigmaPlot®. Differences between treatments were analysed with a One Way ANOVA. The total concentration of silver in water and sediments was compared at different time points with a One Way Repeated Measures ANOVA (RM-ANOVA). The effects of different concentrations of NaCl and time on the AgNPs particle size and zeta potential were analysed with a Two Way ANOVA.

2.5.1. CLPP data analysis

The average well colour development (AWCD) was estimated at each time point as described by Garland and Mills (1991). The substrate uptake rate was estimated as the slope of the AWCD curve when there was a linear relationship between OD and time. The CLPP was assessed by principal component analysis (PCA) using the absorbance values recorded in each well at a single time point (after incubating the EcoPlate™ for 14 days) as described by Garland (1996). The carbon substrates included in the PCA showed a net OD > 0.25, indicating substrate utilization.

2.5.2. PLFA data analysis

The shift patterns of the community structure were explored with PCA on the basis of the relative abundance of each PLFA. The PLFAs included in the analysis were those regarded as relevant bacterial biomarkers and comprised ≥1% of the total PLFA biomass (estimated in mole) as recommended by Zogg et al. (1997).

3. Results

3.1. NM-300 AgNPs characterization in Milli-Q water

TEM analysis showed that 300-NM AgNPs are approximately spherical (SI Fig. S1) with average size of 18.2 ± 7.3 nm (n = 105) (particle size frequency distribution in SI Fig. S2), negatively charged (−23.77 ± 5.39 mV; n = 3) and the percentage of ionic silver concentration dispersed in Milli-Q water was 2.98 ± 1.08% (n = 5).

3.2. Chemical analysis of the microcosm samples

Monitored during the course of the microcosm experiment, the concentration of total silver in water decreased significantly with a concomitant increase in sediments during the first 24 h (Fig. 1; p < 0.001). The concentration of silver in the control treatment was below the lower limit of detection and ammonium concentration decreased more slowly in the presence of AgNPs (T1; see SI Fig. S3b).

3.3. Effects of chloride on the AgNPs persistence

UV–vis spectroscopy and the DLS analysis of the effects of NaCl on AgNP stability are summarized in Figs. 2a–c and 3a, B respectively.

3.4. Biological analysis of the microcosm samples

The functional diversity and community structure were analysed at two different time points, 24 and 120 h after the beginning of the microcosm experiment based on the following information obtained from preliminary experiments: 1) AgNPs negatively affected cell viability (analysed with plate counts) after 24 h exposure but recovered after 120 h, and 2) the concentration of total silver analysed in sediments was not statistically significantly different between 24 and 120 h.

![Fig. 1.](image-url) Mean (±SD) concentrations of total silver in water and sediment samples during the course of the microcosm experiment, n = 4.
3.4.1. Effects on the community-level physiological profiles (CLPP)

3.4.1.1. Carbon substrates utilization rate. The average well colour development (AWCD) was always higher in the absence of AgNPs, see SI (Fig. S4). The utilization of D-mannitol and several amino acids (L-arginine, L-asparagine and L-phenylalanine) was inhibited after 24 h exposure to AgNPs (Fig. 4). The information obtained for all 31 carbon sources is summarized in the SI (Table S1).

3.4.1.2. Shifts in physiological profile patterns at the community level. The bacterial functional diversity was analysed with the EcoPlate™ at two different time points, 24 and 120 h after the beginning of the experiment. The PCA of absorbance (data sets measured 14 days after incubation) (Fig. 5) showed that the control (TC) and dispersant (T2) treatments closely-grouped indicating similar CLPP or functional diversity. This was not the case for the samples collected from the AgNPs (T1) treatment which exhibited higher variability. After 120 h of exposure all treatments grouped together. The carbon substrates included in the analysis and their loadings are shown in SI Fig. S5.

3.4.2. Phospholipid fatty acid analysis

3.4.2.1. Bacterial abundance. The abundance of the bacterial PLFAs was analysed with a two-way ANOVA that did not find statistically significant differences between treatments and time. The simple effects of the factor treatment were analysed with a one-way ANOVA showing statistically significant differences (p < 0.010, RM-ANOVA) within the treatment NaCl 20 g L⁻¹ between the time points 0 h–24 h and 0 h–48 h. ♦ symbolizes statistically significant differences (RM ANOVA, p < 0.05) within the treatment NaCl 10 g L⁻¹ between the time points 0 h–24 h and 24 h–48 h.

![Fig. 2. Absorbance of AgNPs in the UV-spectrum exposed to different concentrations of NaCl: a) 0 g L⁻¹ NaCl; b) 10 g L⁻¹ NaCl and c) 20 g L⁻¹ NaCl.](image)

![Fig. 3. Zeta potential (A) and average size or z-average (B) of the NM-300 AgNPs suspensions expressed as the mean ± SD (n = 3). Data points with different letters show statistically significant differences between NaCl concentrations (p < 0.05, One way ANOVA). ** symbolizes statistically significant differences (p < 0.010, RM-ANOVA) within the treatment NaCl 20 g L⁻¹ between the time points 0 h–24 h and 0 h–48 h. ♦ symbolizes statistically significant differences (RM ANOVA, p < 0.05) within the treatment NaCl 10 g L⁻¹ between the time points 0 h–24 h and 24 h–48 h.](image)
post-hoc test found that the abundance of Gram negative bacteria was significantly higher in the dispersant treatment (T2) than in the control (TC) and AgNPs (T1) treatments (p = 0.026 and p = 0.027, respectively).

3.4.2.2. Community structure. The profiling of the microbial community structure based on the PLFAs after 24 and 120 h of exposure was analysed with PCA (Fig. 7). The PCA did not show separate clusterings according to the treatments and the two sampling time points. The PLFAs included in the analysis and their loadings are shown in SI Fig. S6.

4. Discussion

Previous studies of the effects of AgNPs on bacterial communities in estuarine and freshwater sediments did not find any effects on the genetic diversity of the bacterial assemblages in estuarine conditions (Bradford et al., 2009) nor on the bacterial biomass and enzymatic activity in freshwater (Colman et al., 2012). The concentrations of total silver in sediments used were in the same order of magnitude as the ones applied in the present work. The total silver concentration originating from nanoparticles ranged from 1 to 6 mg kg⁻¹ sediment dry weight (1–6 ppm), which is within the predicted environmental concentration (PEC) of total silver in river sediments that can oscillate between 2 and 14 ppm (Blaser et al., 2008). However, the most recent predictions estimate a silver accumulation rate originating from AgNPs of 2.3 ppb per year (Sun et al., 2014). No reports of typical levels of PECs are available in the context of AgNPs in estuarine or coastal sediments. These may be of the same order of magnitude as the levels estimated by Blaser et al. (2008) due to silver discharges from waste water treatment plants (WWTPs) and tributary rivers (Flegal et al., 2007).

4.1. Fate of AgNPs

Chloride exhibits high affinity for Ag⁺, leading to the formation of silver chloride (AgCl) salt and other chloro-complexes, and can significantly influence the persistence and toxicity of AgNPs. For this reason, in this study the effects of chloride on the AgNPs persistence were investigated. The intensity of the peak absorbance observed at 10 and 20 g L⁻¹ NaCl decreased (Fig. 2) in agreement with the results obtained by Siller et al. (2013). Similarly to Zook et al. (2011) and Kent and Vikesland (2011), the results obtained here showed that AgNPs may have dissolved due to oxidation by the dissolved O₂ in the suspension, thereby releasing Ag⁺ ions subsequently scavenged by the Cl⁻ ions (Liu and Hurt, 2010). The NM-300 AgNPs are not coated, thus at higher ionic strength there is a reduction in the electric double layer of nanoparticles. As a result, the absolute value of the surface charge (zeta potential) also decreases (as observed in our experiments, Fig 3A), and results in enhanced nanoparticle surface interaction and reduced suspension stability (Leo et al., 2013). The instability of the suspension leads to the agglomeration of the AgNPs (observed at 20 g L⁻¹ NaCl by an
increase in the average particle hydrodynamic diameter, Fig. 3B), eventually settling out (Badawy et al., 2010). The NM-300 AgNPs in the water column of the microcosm appeared to dissolve and agglomerate in a similar way to the AgNPs exposed to the highest concentration of NaCl, 20 g L\(^{-1}\). Preliminary experiments showed no differences between the persistence of the AgNPs at 20 and 30 NaCl g L\(^{-1}\) (equivalent to seawater salinity of NaCl of 21.92 and 32.87 ‰ respectively (Cox et al., 1967)) (SI Fig. S7). Therefore in the experiments addressing the effects of chloride on the persistence of AgNPs, the highest concentration of NaCl tested was 20 g L\(^{-1}\).

In the present study the initial Cl/Ag molar ratio in the water column was greater than 26,750 (Cl/Ag = 52.360) for this reason the presence of AgCl in solid form was not expected but rather the formation of soluble forms of silver chloro-complexes such AgCl, AgCl\(_2\), AgCl\(_3\)\(^2\) and AgCl\(_4\)\(^3\) (Levard et al., 2013). These soluble compounds can be present in the overlaying water and in the sediment pore water. These ionic forms of silver are bioavailable (able to cross bacterial cell membranes) and studies carried out with the Gram negative bacterium Escherichia coli (Gupta et al., 1998) show that at high concentrations they can decrease the...
bacterial resistance to ionic silver. We did not measure the concentration of sulphides in the water column as their effects on the fate of AgNPs were expected to be negligible under the microcosm conditions that were mainly marine and aerobic (Levard et al., 2012). The sulfidation of silver in sediments is not expected to occur in aerobic environments, because sulphate is the predominant form of sulphur and does not react with silver (Liu et al., 2011). Superficial sediments in the microcosms were essentially oxic with a redox potential of +230 mV measured in the first 5 mm. It is also known that other components in the water matrix, such as the type of organic matter (Al-Shaeri et al., 2013; Fabrega et al., 2009) and clays (Zhou et al., 2012), can also influence the physicochemical properties of nanomaterials. Previous studies have revealed that natural organic matter (NOM), such as humic and fulvic acids are able to reduce the dissolution of AgNPs by surrounding the AgNPs and protecting them from oxidative species (Liu and Hurt, 2010).

4.2. Effects of NM-300 on the bacterial abundance, community structure and function

4.2.1. Community-level physiological profile (CLPP)

The Biolog EcoPlate™ assay is widely used in ecotoxicological research to analyse the toxicity of nanoparticles and other pollutants on the bacterial metabolic diversity in different environments (Kumar et al., 2011; Rodrigues et al., 2012; Weber et al., 2008). It is regarded as an efficient method for the comparison of functional diversity, suitable for microcosm studies (Preston-MaTham et al., 2002). The results obtained showed that AgNPs inhibited the utilization of mainly amino acids (source of carbon and nitrogen) and D-mannitol (important as carbon storage), and the AWCD was always lower following exposure to AgNPs. The overall utilization and number of carbon substrates at the end of the exposure increased in all three treatments. This may be attributed to an increase in the abundance of culturable bacteria as observed in a complementary experiment (information available in the section 6 of the SI Fig. S8) showing that the time of measurement affected the total cultivable bacteria. A similar trend has been reported in previous microcosm studies developed to investigate the effects of AgNPs on marine (Doiron et al., 2012) and estuarine bacterial communities (Beddow et al., 2014), and could be explained in part by confinement in the tanks and the ex situ conditions (Lebaron et al., 2001). The increase in AWCD in the present study after 120 h of exposure was more remarkable in the dispersant and AgNPs treatments, possibly because in these treatments the concentration of ammonium was higher at the beginning of the exposures, and may have enhanced bacterial growth. Ammonium is the most relevant source of inorganic N for assimilation and also essential for bacterial growth (Neidhardt et al., 1990). The dispersant contained ammonium nitrate (NH₄NO₃) (Kermanizadeh et al., 2012) and explained the higher concentration of ammonium in the treatments containing dispersant (T1 and T2) at the beginning of the experiment. The natural concentration of ammonium in the water column of the estuary was initially low (0.02 mg L⁻¹, SI Fig. S3b), thus the addition of ammonium nitrate (NH₄NO₃) will likely have significantly increased microbial activity (Aspray et al., 2008). The PCA of the data obtained with the Biolog EcoPlate™ depicted clear bacterial metabolic fingerprint patterns for the control treatments (T1 and T2), based on the ability of bacteria to use environmentally relevant carbon sources. It revealed that the CLPP of bacterial communities exposed to AgNPs for 24 h was different (the ability to use different carbon sources decreased) compared to the communities in the negative control and dispersant (carrier control) treatments. At the end of the exposures (120 h) the samples from the three treatments grouped together showing that the communities exhibited a similar metabolic fingerprint.

4.2.2. Phospholipid fatty acid analysis (PLFAs)

Phospholipid fatty acids (PLFAs) are major components of cell membranes, and are rapidly degraded during cell hydrolysis. They can therefore be used to estimate the living biomass in soils or sediments (White et al., 1993). PLFAs with a chain length shorter than 20 carbon atoms are predominant in bacterial cells and some of them are specific to certain bacterial groups that can be used as bacterial biomarkers (Frostegård et al., 1993; Zogg et al., 1997). The PLFAs analysis is a culture-independent method applied in microbial ecology to study bacterial communities inhabiting sediments and soils (Kunihoro et al., 2014; Mayor et al., 2013; Pawlett et al., 2013). This technique was applied in the present study as a tool to quantify the abundance of living bacteria in sediments and to assess the effects of AgNPs on potential shifts in the bacterial community structure. The dispersant enhanced the growth of Gram negative bacteria (measured by the abundance of specific PLFAs) up to 8% compared to the beginning of the exposures (24 h), and supports the hypothesis that differences encountered between the control (TC) and dispersant (T1 and T2) treatment groups were due initially to the higher concentration of NH₄ in the overlaying water derived from the dispersant. These findings confirmed the importance of testing the effects of dispersants when conducting ecotoxicological tests of nanomaterials as they can mask the effects of the substance under study. Any conclusions drawn from the abundance of the PLFAs in the AgNPs treatment should be analysed cautiously as the turn-over of fatty acids in the community may not have changed at the same rate in all treatments (Frostegård et al., 2011). Furthermore, degradation rates of PLFAs may have been reduced by AgNPs inhibiting enzymatic activity in both live and dead bacteria in the same way as the utilization of some of the substrates included in the Biolog EcoPlate™ was suppressed. The PLFAs analysis suggests that the impact on the community function was not reflected at the level of the community structure. This may be due to the inhibition of activity and growth of particular bacterial groups (e.g. D–mannitol degraders), without completely removing them from the system. Ag⁻ released from the AgNPs can lead the bacterial cell to a lower metabolic state, “active but non culturable (ABNC)” (Jung et al., 2008), as a stress response, and stop cell division by causing DNA condensation (Feng et al., 2000). Even though the PLFAs analysis is considered by some as less powerful than molecular techniques in terms of resolution to describe bacterial diversity in sediments, the methodological bias is lower than PCR-based techniques (Kunihoro et al., 2014). Moreover, the results obtained with the PLFAs analysis are in agreement with a previous study developed in the same environmental compartment by Bradford et al. (2009) using a nested PCR-DGGE approach. Another hypothesis to explain the lack of differences between the AgNPs and the other treatments is based on the response of bacteria to oxidative stress: the production of fatty acids in the cytoplasmic membrane could have been stimulated in response to damage in the outer envelope as a consequence of reactive oxygen species (ROS) production induced by AgNPs, followed by oxidative stress as observed in the Gram negative bacterium Pseudomonas aeruginosa after exposure to TiO₂ NPs (Kubacka et al., 2014).

4.2.3. Bacterial resistance and resilience to AgNPs

The production of exopolysaccharides (EPS) by many bacterial species, such as Pseudalteromonas sp. (Gutierrez et al., 2008), can sequester metals and as a result increase the resistance of the bacterial community to AgNPs. EPS also enhance cell aggregation and reduce the cell surface area exposed to nanoparticles (Joshi et al., 2012). Separate tests with molecular techniques based on a partial sequence of the 16S rRNA gene confirmed that members of the Pseudalteromonas genus were present in the sediments of the Firth of Forth estuary (P. arctica and P. aliena). These species exhibit...
strong resistance to the NM-300 AgNPs [hibitory concentrations (IC50) > 1 mg L−1 in liquid cultures, Echavarri-Bravo unpublished]. The proliferation of bacterial species with greater resistance to the negative impacts of AgNPs as the Pseudoalteromonas spp. (they have a short generation time and tend to be ubiquitous in oxic marine environments), and bacterial functional redundancy may also explain the recovery of the metabolic profile observed with the Biolog EcoPlate™ assay at the end of the exposures. This recovery could in part also be the result of a loss in the antibacterial activity of AgNPs due to the transformation of nanoparticles into less soluble compounds, or by binding organic matter (Fabrega et al., 2009) or/and EPS (Joshi et al., 2012; Kang et al., 2013; Miao et al., 2009), thus reducing the antibacterial toxicity and/or bioavailability (as a previous study showed that AgNPs were more toxic than AgCl (Choi et al., 2008)).

5. Conclusion

The present study showed that the utilization of environmentally relevant carbon sources by bacterial communities inhabiting estuarine marine sediments was inhibited after exposure to AgNPs, and as a result the bacterial functional diversity was negatively affected. A recovery was observed after 120 h of exposure and may be explained by a number of possible factors, such as the formation of compounds less toxic than AgNPs, or by the complexation of AgNPs with natural organic matter and sediments reducing their bioavailability, or also as a result of the presence of silver resistance genes or groups of organisms more resistant to silver. Therefore the conclusion drawn from the present study is that bacterial community function is able to recover from a severe exposure to AgNPs under the experimental conditions. However whether bacterial communities may exhibit a similar recovery under continuous exposure to AgNPs and other nanomaterials remains unclear.

Acknowledgement

This project was funded by the Heriot-Watt Environment and Climate Change Theme (Grant No: A99L6401) and NERC-FENAC/2012/11/004. We are grateful to M. Stobie, S. McMenamy and H. Barras for support and advice with sample collection and chemical analysis, and P. Cyphus, and V. Goodfellow for support with biological analysis. We also acknowledge Prof. Paul Hughes for advice on statistical analysis.

Appendix A Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.02.033.

References


USEPA-3005A, 1992. Acid Digestion of Waters for Total Recoverable or Dissolved Metals for Analysis by FLAA or ICP Spectroscopy (Method 3005A) Revision 1. Environmental Protection Agency, United States.
